



The cover features a background image of scientists in a laboratory. A large green graphic element contains a circular inset showing two women wearing face masks. The text is arranged in a structured layout with various logos and a dark green footer.

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OWSD NIGERIA NATIONAL CHAPTER
'UMUDIKE 2021' 

**5th BIENNIAL INTERNATIONAL CONFERENCE**
UMUDIKE, 2021



CONFERENCE PROCEEDINGS

THEME:
**MAKING RESEARCH COUNT IN NATIONAL DEVELOPMENT:
THE ROLE OF WOMEN SCIENTISTS**

DATE: 14TH - 17TH JUNE, 2021
VENUE: ANYIM PIUS AUDITORIUM,
MICHAEL OKPARA UNIVERSITY OF AGRICULTURE, UMUDIKE



PROCEEDING OF THE 5TH BIENNIAL CONFERENCE OF THE OWSD NIGERIA, UMUDIKE 2020

**PROCEEDINGS OF 5TH BIENNIAL
CONFERENCE OF THE ORGANIZATION FOR
WOMEN IN SCIENCE FOR THE DEVELOPING
WORLD, OWSD NIGERIA NATIONAL
CHAPTER HELD AT MICHAEL OKPARA
UNIVERSITY OF AGRICULTURE, UMUDIKE,
ABIA STATE, 14TH – 17TH TH JUNE, 2021**

PROCEEDING OF THE 5TH BIENNIAL CONFERENCE OF THE OWSD NIGERIA, UMUDIKE 2020

**ADVANCES
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PHYSICAL
SCIENCES (APS)**

WIS-APS 02 FL

COLORIMETRIC DETECTION OF Pb(II) IONS PRESENT IN INDUSTRIAL WASTEWATER USING COPPER NANOPARTICLES SYNTHESIZED BIOLOGICALLY WITH *Musa paradisiaca* LEAF EXTRACT

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ABSTRACT

This study explored the synthesis of copper nanoparticles using *Musa paradisiaca* leaf extract for the detection of Pb (II) ions present in industrial wastewater using colorimetric method with the aid of UV-visible spectroscopic technique. Characterization of the copper nanoparticle was achieved by making use of a few spectroscopic techniques which includes UV, FTIR, SEM and XRD. According to the results, the UV-vis spectroscopy, revealed maximum absorption at 271nm indicating surface plasmon absorption of copper nanoparticle. The XRD analysis of the copper nanoparticles indicated that its formation was crystalline in nature with a mixed phase structure (cubic, pentagonal and irregular). The average crystallite size of the copper nanoparticle was found to be in the range of 51.9 to 86.8 nm. FTIR analysis was carried out to ascertain the possible functional groups responsible for the reduction of copper ion to copper nanoparticle and it was found that peaks at 1733.2cm⁻¹, 1319.3cm⁻¹ and 1155.5cm⁻¹ (C=O of carbonyl functional group (aldehyde or Ketone) and C-O of alcohol were responsible for the reduction. Scanning Electron Microscopy (SEM) revealed that the morphology of the copper nanoparticle possessed cubic, pentagonal and irregular shapes of various sizes which showed smoother surfaces on magnifications while higher magnifications showed large particles which can be attributed to aggregation or clustering of smaller particles. Detection of Pb(II) ions in industrial wastewater using the colloidal copper nanoparticle was feasible because Pb(II) ions was detected even at a very low concentrations. Thus the obtained results encourage the use of economic synthesis of copper nanoparticle in the detection of Pb (II) ions present in industrial wastewater.

Keywords: Colorimetric detection, Copper nanoparticles, Industrial wastewater, *Musa paradisiaca*.

1. INTRODUCTION

Nowadays, the use of metal nanoparticles synthesized from biomaterials and their applications are on the increase due to their biological effectiveness, low cost and environmental eco-friendliness [1] compared to other methods like Laser ablation, arc discharge etc. Biologically synthesized copper nanoparticles with great catalytic activities can be applied to biosensors and electrochemical sensors [2]. Various plant extracts have been used in synthesizing copper nanoparticles such as *Nerium oleander* [3], *Euphorbia nivulia* [4], *Magnolia Kobus* [5], etc., though the potential of these plants as biological materials for the synthesis of copper nanoparticles is yet to be fully explored. *Musa paradisiaca* have been used in herbal medicine for dressing of wounds, burns, and sores. It is used as tea against worms. The concentrations of some phytochemicals and results of IR revealed that the functional groups in *Musa Paradisiaca* were alcohols, amines, alkenes, carbonyl, unsaturated/aromatic, metals and phenols. The chemical constituents were methionine, cellulose, hemicellulose, arginine, aspartic acid, glutamic acid, carbon, potassium, nitrogen and tannins [6]. The conventional physical and chemical method of synthesizing nanoparticles has resulted to serious environmental hazards, therefore there is need for alternative method of synthesizing nanoparticles that are eco-friendly, this prompted interest to

synthesize copper nanoparticles using extract from the leaves of *Musa paradisiaca* which was used in colorimetric detection of Pb(II) ions in industrial wastewater. Numerous analytical techniques have been employed to determine various ions in wastewater including electrochemical [7], titrimetry [8], chromatography [9], fluorimetry [10], and colorimetry [11][12][13][14][15]. Among these, the colorimetric method has received much attention since it shows the advantages of rapidity, simplicity, and cost-effectiveness and does not require any sophisticated instruments.

MATERIALS AND METHODS

2.1. Sample collection

Young leaves of *Musa paradisiaca* were collected from Musa plant from a farm at Michael Okpara University of Agriculture Umudike Nigeria within the latitude 05°29' North and longitude 07°33' East of rainforest and were taken for identification and authentication at the Taxonomy Section of Forestry Department of Michael Okpara University of Agriculture Umudike.

2.2. Preparation of aqueous plant extract

Fresh leaves of *M. Paradisiaca* were sliced, washed thoroughly with deionized water, air dried at room temperature for about two weeks and milled to a fine powder. About 25 grams of the powdered material

was dispersed in a 250 mL of deionized water in a 500 mL glass beaker and boiled at 80°C for 15 min and was allowed to cool. After that, the solution was filtered through Whatman No. 1 filter paper (Springfield Mill, Maidstone, Kent, England) and the filtrate was used immediately for the synthesis of cobalt nanoparticles.

2.3. Synthesis of copper nanoparticles

For the synthesis of copper nanoparticles, 100 mL of the aqueous leaves extract was added to 900 mL of 1×10^{-3} M aqueous ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) solution in a 1000 mL bottle and was stirred for about 30 mins. Within 10 minutes change in colour was observed from light brown to golden yellow indicating the formation of copper nanoparticles. The copper nanoparticles solution obtained was purified by repeated centrifugation at 4000 rpm for 15 min followed by re-dispersion of the pellet in deionized water. Then the copper nanoparticles were dried in an oven at 80°C and then allowed to cool before storing in an airtight container.

2.4. UV-visible spectroscopy analysis

The bio-reduction process of copper ions in aqueous solution was measured by sampling 1mL aliquot compared with 1 mL of de-ionized water used as blank and subsequently measuring the UV-visible spectrum of the solution. UV-visible spectrum was monitored on Cary Series UV-Visible spectrophotometer Agilent Technology, operated within the wavelength range of 200 to 800 nm.

2.5. FT-IR spectroscopy measurement

This was carried out on *M. Paradisiaca* leaves extract and on the copper nanoparticles. FT-IR measurement of the samples was performed using FTIR-Cary 630 Fourier Transform Infrared Spectrophotometer, Agilent Technology, in a transmittance method at a resolution of 8 cm^{-1} in potassium bromide (KBr) pellets in the wave number range of $4000\text{-}650 \text{ cm}^{-1}$.

2.6. Scanning electron microscopy (sem) analysis

Morphology of the nanoparticles was studied using SEM analysis using electron magnification of 80 - 150,000x (MODEL-PHENOM ProX Scanning Element Microscope manufactured by Phenom World Eindhoven, Netherlands).

2.7. X-ray diffraction (XRD) analysis

XRD (PAN analytical, Netherlands) patterns were obtained with a diffractometer (Empyrean Model, Netherlands) operated at a voltage of 45 KV and a current of 40 mA using Cu-K(alpha) radiation in a -2 configuration with a wavelength (λ) of 0.1541. The sample was made smoother and was imparted on a slide which was then charged into the machine after adjusting the machine parameters and was operated via a monitor.

2.8 Colorimetric Detection of Pb (II) Ions Using Copper Nanoparticles (CuNPs)

According to Sithara et al. [10], to confirm the practical application capability of Copper nanoparticle probe prepared from *Musa Paradisiaca* leaf extract, the concentration of Pb^{2+} in simulated wastewater sample was determined. Different concentrations of the Pb^{2+} ranging from 5 – 25 mM was used in a prepared colloidal copper nanoparticle. 2 mL of the prepared colloidal of copper nanoparticle was added to 2 mL of the different concentrations of the Pb^{2+} and was kept for 10 mins before determining different absorbance of the different concentrations with respect to different wavelength using a colorimeter.

3. RESULTS AND DISCUSSION

3.1: UV- visible spectroscopy

The reduction of copper ions present in the aqueous solution of copper sulphate during the reaction with *Musa paradisiaca* leaf extract occurred at 271nm as shown in Fig. 1. The maximum absorption obtained at 271 nm is an indication of the surface plasmon absorption of copper nanoparticles confirming their formation.

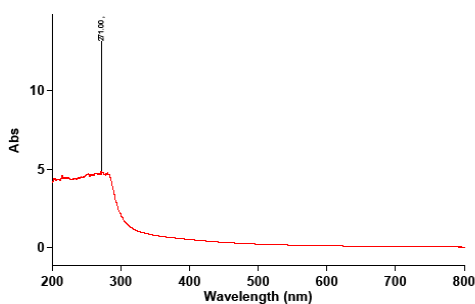


Fig 1: UV-Visible Spectrum of Copper Nanoparticles

3.2: Fourier transform infrared spectroscopy

The FTIR analysis was carried out on *Musa Paradisiaca* leaf extract before and after synthesis of the copper nanoparticles. FTIR measurements were used to identify the possible functional groups responsible for the reduction of copper ions to copper nanoparticles. Table 1 and Fig. 2

show the functional groups present in the leaf extract before synthesis while Table 2 and Fig. 3 show the functional groups present in the leaf extracts after synthesis of the copper nanoparticles. The main difference in the FTIR result of the *Musa Paradisiaca* leaf extract before and after the synthesis of the copper nanoparticles is indicated by the absence of the carbonyl functional group with peak at 1733.2 cm^{-1} on Table 2 and Fig. 3. This can be attributed to the fact that the carbonyl functional group is oxidized to the carboxylic function (COO^-). The absence of the O-M-O bond vibration which appears in the range $1000\text{-}450 \text{ cm}^{-1}$ indicated that there was no bonding between the copper and the acid. This can be explained by the fact that the

copper (II) ions were reduced to copper nanoparticles while the carbonyl in the extract was oxidized to the carboxylic ions which further dimerized. The absence of C-O at 1319.3cm⁻¹ and 1155.5cm⁻¹ equally contributed to the formation of copper nanoparticle. The FTIR spectrum of cobalt nanoparticles as shown in Fig. 3 below indicated different peaks with different functional groups as shown in Table 2.

Table 1: Functional groups of the leaf extract of *Musa Paradisiaca*.

Peaks (Wavelength in cm ⁻¹)	Functional group
3336.0	O-H of alcohol
2918.5	O-H of acid
2851.4	C-H of alkane
1733.2	C=O of carbonyl functional group (aldehyde or Ketone)
1625.1	C=C from alkene or aromatic
1461.1	C-C from alkane
1319.3	C-O from alcohol
1155.5	C-O from alcohol
1028.7	C-O from ester

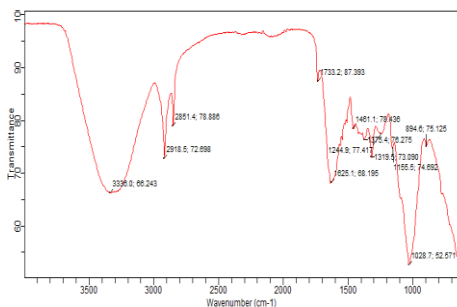


Figure 2: FTIR Spectrum of *Musa Paradisiaca* leaf extract

Table 2: Functional groups of copper nanoparticle.

Peaks (Wavelength in cm ⁻¹)	Functional group
3276.3	O-H of alcohol
2922.2	O-H of acid
2851.4	C-H of alkane
1625.1	C=C from alkene or aromatic
1438.8	C-C from alkane
1230.3	C-O from ester
1036.2	C-O from ester

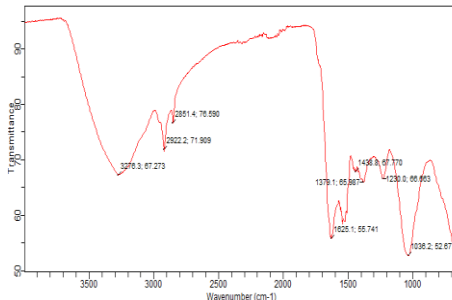


Figure 3: FTIR Spectrum of copper nanoparticles.

Comparing the functional groups in both leaf extract and copper nanoparticles, it was observed that peaks at 1733.2, 1319.3 and 1155.5 were not featured in the FTIR of copper nanoparticles indicating that these functional groups were involved in the reduction of copper ions to copper nanoparticles.

3.3 Scanning Electron Microscopy Analysis (SEM)

The SEM images of copper nanoparticles are shown in Fig. 4, 5 and 6. The morphology of the nanoparticles indicates irregular, cubic and pentagonal shapes of various sizes that are agglomerated. Further observations with higher magnifications reveal that these images possess smooth surfaces. At much higher magnification the images are seen as large particles which can be attributed to aggregation or clustering of smaller particles.

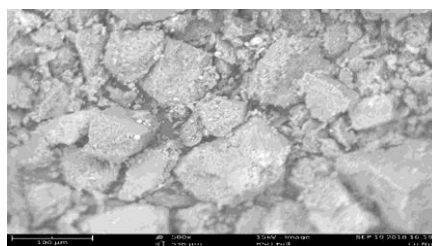


Figure 4: SEM image of copper nanoparticles

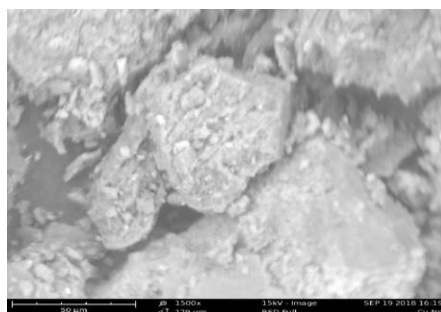


Figure 5: Zoomed SEM image of copper nanoparticles.



Figure 6: Zoomed SEM image of copper nanoparticles

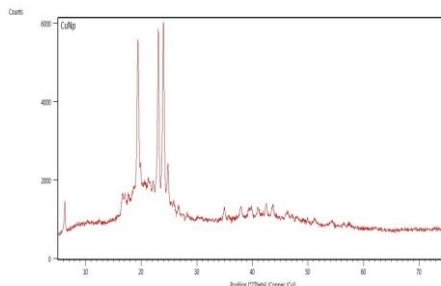


Figure 7: X-ray diffraction pattern of copper nanoparticles synthesized from *Musa Paradisiaca* leaf.

3.4 X-ray Diffraction Analysis (XRD)

Figure 7 shows the XRD pattern of copper nanoparticles biosynthesized from the leaf extract of *Musa Paradisiaca*. A number of Bragg reflections values 6.4, 20, 23, 24, 24.9, 35.2, 38, 42.4 and 43.5 within the angle range of 5.00 and 74.98 were observed. The XRD pattern indicates that the copper nanoparticles formed are crystalline in nature with a mixed phase structure (cubic, pentagonal and irregular) of copper nanoparticles. The average crystallite size of the copper nanoparticles was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Debye-Scherrer equation shown below

in equation 1.

$$D = k\lambda / \beta \cos \theta \dots\dots\dots \text{Eq.1}$$

Where D is the particle size (in nm), k is a constant equal to 0.9, λ is the wavelength of X-ray radiation (0.541), β is the full-width at half maximum (FWHM) of the peak (in radians) and θ is the Bragg angle (in degrees). The average crystallite size was found to be in the range of 51.9 to 86.8 nm.

3.5 Colorimetric Analysis

Different concentrations of Pb (II) ions with the corresponding red shift of the UV-Visible spectra are depicted in Fig. 8. The colloidal copper nanoparticles was tested with various concentration (5-25μM) of Pb (II) ions. It was observed that absorption of Pb (II) ions decreased at increased concentration of Pb (II) ions (Table 3) indicating that Pb (II) ions can be detected even at a very low concentration.

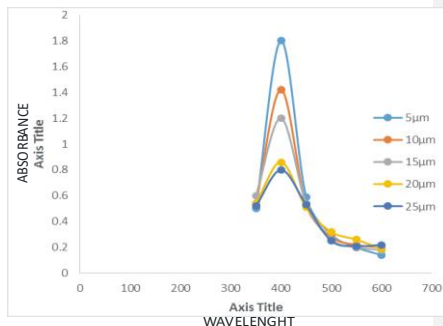


Fig.8: UV-Visible spectra of lead (II) ions

Table 3: Absorption of Pb (II) ions with colloidal cobalt nanoparticle at different concentrations

Concentration (μM)	350 nm	400 nm	450 nm	500 nm	550nm	600 nm
5	0.50	1.80	0.59	0.30	0.20	0.14
10	0.54	1.42	0.53	0.28	0.22	0.20
15	0.60	1.20	0.54	0.26	0.20	0.18
20	0.54	0.86	0.51	0.32	0.26	0.19
25	0.52	0.80	0.53	0.25	0.21	0.22

4. CONCLUSION

The characterization of the copper nanoparticle via UV, FTIR, SEM, XRD revealed maximum absorption at 271nm which is a very good indication for surface plasmon absorption for copper nanoparticle. Different

functional groups were observed in both the leaf extract of *Musa Paradisiaca* and copper nanoparticle synthesized with *Musa Paradisiaca* and it was deduced that some of the functional groups observed in the leaf extract of *Musa Paradisiaca* did not appear

in the copper nanoparticle indicating their usage in the formation for copper nanoparticle. The morphology of the copper nanoparticle indicated irregular, cubic and pentagonal shapes of various sizes with rough surfaces. The average crystalline size was found to be in the range of 51.9 to 86.8nm indicating that copper nanoparticle is within the range for copper nanoparticle. Application of copper nanoparticle in detecting Pb (II) ions in industrial wastewater was feasible because Pb (II) ions were detected even at a very low concentration.

5. RECOMMENDATIONS

Copper nanoparticle synthesized with *Musa Paradiasca* should be used in detoxifying other toxic heavy metals in industrial effluents. Application of green chemistry in nanotechnology that are eco-friendly and cost effective should be recommended for further use. Treatment of industrial wastewater with copper nanoparticle and other metal nanoparticle should be encouraged.

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WIS-APS 10

TRACE METAL PROFILE OF *Panicum maximum* (W. HILL) and *Cynodon dactylon* L. GROWN ON WASTE ENGINE OIL CONTAMINATED SOILS

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ABSTRACT

The study examined the heavy metal contents of *Panicum maximum* and *Cynodon dactylon* grown on waste engine oil contaminated soils. Waste engine oil was added to four (4) kg soil samples at different concentrations on weight basis: 0 % (control), 1 %, 3 %, 5 % and 7 % v/w oil-in-soil and allowed to stand for seven days before transplanting. The plants were harvested after 8 weeks. The dried plants and the soil samples on which they were grown were analysed for four heavy metals (Cu, Pb, Zn and Fe). The results showed that there were increased percentage reductions in the heavy metal contents of the soils. *Panicum maximum* and *C. dactylon* showed maximum reductions of 99.7% and 99.75% for Pb. The shoots and roots of the grass species and soil showed heavy metal accumulation. *P. maximum* showed maximum accumulation of Cu in roots (36.8 mg/kg) and maximum accumulation of Pb in roots and shoot while *C. dactylon* showed maximum accumulation of Pb in the shoot with the value given as 40 mg/kg. The *P. maximum* showed maximum accumulation of Fe in the shoot with the value given as 118 mg/kg. Percentage reductions were high at increased concentrations of the waste engine oil. In other words the two grass species studied possess the phytoextraction potential for phytoremediation of waste engine oil contaminated soil.

KEYWORDS: Trace metal, Concentrations, Waste engine oil, *Panicum maximum* and *Cynodon dactylon*

INTRODUCTION

Pollution is defined as an undesirable change in the physical, chemical, and biological characteristics of all the components of an environment which can threaten human health and that of beneficial organisms (Aboribo, 2001). Waste engine oil obtained in the course of servicing automobiles and generator engines is indiscriminately disposed into gutters, water drains, open vacant plots and farms in Nigeria by auto mechanics and allied artisans with workshops on the road sides and open places (Anoliefo and Vwioko, 1995). Government efforts to monitor, control, and regulate indiscriminate disposal of spent lubricating oil on agricultural lands have proven to be very difficult because of their short life span and paucity of information. Thus, contamination of agricultural ecosystems arising from discharge of used engine oil and grease is more widespread than crude oil pollution (Atuanya, 1987). Anon (1985) observed that Nigeria accounts for more than 87 million litres of spent oil annually and that most heavy metals, such as V, Pb, Al, Ni and Fe, which are below detection in unused lubricating oil, showed high values in waste engine oil.

The most important and common symptoms observed in the plants contaminated with oil and its by-products include the degradation of chlorophyll (Malallah *et al.*, 1998), increase in the production of stress-related phytohormones (Larcher, 2000), decrease in size and less production of biomass (Brandt *et al.*, 2006; Daniel-Kalio and Pepple, 2006; Adenipekun *et al.*, 2009). Depletion in the nutrient status (nitrogen and phosphorus), inhibition of microbial activities and

seed germination has also been reported in spent-oil-contaminated soils (Atlas and Bartha, 1993; Kirk *et al.*, 2005). The presence of spent lubricant oil in soil can increase bulk density and decrease water holding capacity and aeration propensity (Kayode *et al.*, 2009). Reduced nitrogen, phosphorus, potassium, magnesium, calcium, sodium and increased levels of heavy metals are common in soils contaminated with spent oil (Kayode *et al.*, 2009).

Phytoremediation is a non-destructive and cost effective *in situ* technology that can be used for the cleanup of contaminated soils. The potential for this technology in the tropics is high due to the prevailing climatic conditions which favors plant growth and stimulates microbial activity (Zhang *et al.*, 2010). Hence, the aim of this study is to determine the heavy metal contents of *P. maximum* and *C. dactylon* grown on soils contaminated with different concentrations of waste engine oil.

MATERIALS AND METHODS

Soil samples were collected from the experimental farm of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The waste engine oil was obtained as pooled engine oil from two different major mechanic workshops located in the mechanic village, Umuahia, Abia State. The plant materials were collected from bush fallow located in Umuahia metropolis. Top soil (0-10cm depth) collected from a marked area was air dried and sieved through a 2mm mesh gauge (Ogedegbe *et al.*, 2013).

Four (4) kg of the soil sample were introduced into different 4-litre perforated plastic buckets after which different concentrations (1%, 3%, 5% and 7%) of waste engine oil were added and labelled T₁, T₂, T₃ and T₄. The mixing was done gradually to ensure thorough and even mixing and the treatment were replicated three times. The untreated soil with 0% waste engine oil served as a control (T_c) (Adenipekun *et al.*, 2009). After thorough mixing, the soil samples were left under the shade for a period of seven days without planting to ensure uniformity of oil, moisture content, air content, constant temperature and effective activities of soil micro-organisms (Oyibo, 2013). The soil samples were artificially irrigated with water in the experimental farm before the transplanting of the experimental plant species and finally left for natural irrigation.

The plant species being investigated were propagated by tiller. At first, the tillers of the plants were separated differently, after selecting tillers of the same height (shoot 15 cm), the tillers were soaked in water for 2 days to improve their rooting ability (Brandt, 2003) before transplanting into different treated soil samples, each with three tillers respectively and allowed to stand for eight weeks. After eight weeks, the plant samples were harvested and soil was washed off with water after which they were separated from the shoot and placed in a well labeled separate envelop for heavy metal analysis.

Determination of Heavy Metal Concentrations in the Plant Samples

Roots and shoots samples of the plant species were oven dried at 65° C for 8 hours milled in a Thomas Willey milling machine and sieved through a 0.5 mm sieve and stored in a well labeled containers. A portion (0.2 g) of the plant samples (root and shoot) of the two plant species were weighed into 150 ml conical flask, 5 ml of the multiple nutrient extraction reagent (H₂SO₄-selenium powder salicylic acid) solution was added in each flask for digestion and allowed to stand for 20 hours. The plant samples were placed in a hot block plate at 32° C for 2 hours after which 5 ml of 75 % of perchloric acid was added to each sample and re-digested at 60° C. The digestion continued until a clear digest were seen producing a profuse perchloric fumes. The digests were allowed to cool and 50 ml of distilled water was added after which the samples were filtered and the filtrates were subjected to AAS analysis for the determination of heavy metals (Cu, Pb, Zn and Fe).

Determination of Heavy Metal Content of the Soil Samples

The soil samples were prepared for heavy metal analysis using the same digestion process as the plant samples above.

Statistical Analysis

The results were summarized using Descriptive Statistical Package of Microsoft Excel while one-way ANOVA was used to test for statistical differences among the treatments and Turkey pairwise comparisons test was performed to determine the location of significant difference (P<0.05).

RESULTS AND DISCUSSION

Some physico-chemical properties of the untreated soil (0%) sample and its texture are given in Table 1. From the table, it can be seen that the soil texture was sandy loam based on the USDA textural classes of soil i.e. 74.20 %, 15.70 % and 8.20 % for sand, silt and clay respectively. The soil pH was 5.27 indicating the acidic nature of the soil. Concentrations of phosphorus, calcium, magnesium, potassium and sodium were given as 40.30 mg/kg⁻¹, 4.20 Cmol/kg⁻¹, 3.20 Cmol/kg⁻¹, 0.043 Cmol/kg⁻¹ and 0.03 Cmol/kg⁻¹ respectively.

The percentage organic carbon was 0.57 % and within the topsoil ranges of 0.5% - 3.0% organic carbon for most upland soils. Percentage organic matter and nitrogen were 0.60% and 1.03% respectively; based on MS1517 organic fertilizer specification (2012), these values are below the requirement for optimum plant growth. Cation exchangeable capacity was moderate (7.92 Cmol/kg⁻¹), which could be attributed to the acidic nature of the soil and the soil texture type. Anion exchangeable capacity (AEC) increases at low pH, but was different in this study; having value of 1.09 Cmol/kg⁻¹, attributed to the soil texture. The soil texture at different concentrations of waste engine oil remains the same. The acidic nature of the soil was maintained but there was a gradual increase in pH. Soil nutrients (P, Ca, Mg, K, Na) were lower than control but recorded a gradual increase in their concentrations with treatments. The percent organic carbon was highest value of 2.61 % at 10 % treatment level. The higher organic carbon content of the soil recorded with the treatments may be attributed to the high carbon content of the soil. Benka-Coker and Ekundayo (2007) reported similar findings. The percentage nitrogen was lower than the control at 1% treatment level but increased higher than the control and remained constant from 3% - 7% treatment levels (0.19%). Percentage organic matter was generally higher than the control and the highest values (4.90%) were recorded in 7% treatment level. The increment could be equally due to the soil treatments with used oil. From every indication not all the soil nutrients analyzed were within the range required for plant growth. Exchangeable cation capacity and exchangeable anion were lower at different levels compared to that of the untreated soil (0 %).

Table 1: Physiochemical properties of the soil samples and its texture

Parameter	Treatments				
	0%	1%	3%	5%	7%
s					

% Sand	74.2	74.1	72.1	78.2	74.1
	0	0	0	0	0
% Silt	15.7	17.1	19.1	17.2	15.2
	0	0	0	0	0
% clay	8.20	8.20	6.20	4.20	5.20
Texture	SL	SL	SL	SL	SL
pH (H ₂ O)	5.27	5.74	5.90	6.70	6.80
P Mg/Kg	40.3	19.0	20.8	30.4	32.7
	0	0	0	1	0
% N	0.11	0.08	0.15	0.17	0.19
% OC	0.57	1.74	1.90	2.40	2.61
% OM	1.04	3.00	3.11	4.45	4.90
Ca	4.20	2.00	2.20	3.20	4.00
Cmol/Kg ⁻¹					
Mg	3.20	0.71	0.63	1.30	1.43
Cmol/Kg ⁻¹					
K	0.43	0.05	0.05	0.12	0.23
Coml/Kg ⁻¹					
Na	0.31	0.07	0.09	0.15	0.22
Cmol/Kg ⁻¹					
EA	1.09	0.31	0.45	0.61	0.75
Cmol/Kg ⁻¹					
ECEC	7.92	3.46	3.54	4.28	5.54
Cmol/Kg ⁻¹					
% BS	95.6	79.2	82.9	83.1	85.6
	3	5	1	7	7

Legend: OC = Organic Carbon, OM = Organic Matter, EA = Exchangeable Anion, ECEC = Effective Cation Exchange Capacity

The concentrations of the heavy metals in the contaminated soils increased with the increased concentrations of waste engine oil (Table 2). The 7% treatment level recorded the highest concentrations and were significantly different ($P < 0.05$) from the control (0% treatment level). The order of increment

for the heavy metals at the 10% treatment level is was Fe > Zn > Pb > Cu. Agbogidi and Egbuchua (2010) confirmed the build up of trace metals (Pb, Cr, Fe, Ni and Cd) in the soil contaminated with spent engine oil when compared with values obtained from the control plots.

Table 2: Initial heavy metal content of different concentrations of waste engine oil polluted soils one week after treatments.

	Heavy metal concentration (mg/kg) Treated Soil				
	Cu	Pb	Zn	Fe	Mean
0%	4.980	3.562	5.226	17.50	7.817
1%	25.95	16.27	50.93	134.57	56.93
3%	37.00	35.64	63.98	147.36	70.99
5%	45.15	64.46	72.85	172.67	88.78
7%	71.92	82.58	90.90	180.75	106.53
Mean	37.00	40.45	56.7	130.57	

This study showed that there was increased uptake of the heavy metals by the grasses from the soil as the waste engine concentration of heavy metals in the soil (Table 3). At 7% treatment level, *P. maximum* showed maximum reduction of 99.6% for copper while *C. dactylon* showed maximum reduction of 99.75% for lead. Considering their percentage mean reduction, the order of reduction for each plant is as follows: Cu > Pb > Zn > Fe for *P. maximum* and Pb > Cu > Zn > Fe for *C. dactylon*. The two grass species were able to reduce the heavy metals which could be as a result of low pH of the soil sample and it is prone to accumulate more heavy metals than an alkaline or a neutral soil. This agreed with the findings of Fotovat *et al* (1997) that reported that soil of low pH values tend to retain more metals when compared to soils of high pH.

Table 3: Percentage reduction of lead, zinc, copper and nickel in soil 8 weeks after planting of *P. maximum* and *C. dactylon*

Grass species	Waste engine oil Polluted soil (%)	Percentage reduction in the soil				
		Cu	Pb	Zn	Fe	Mean
<i>P. maximum</i>	0	98.8	96.7	82.2	-15.1	68.23
	1	99.0	98.2	97.6	83.9	94.67
	3	98.6	99.6	97.0	85.2	95.10
	5	98.7	99.7	96.2	88.2	95.70
	7	99.6	99.5	95.8	88.3	95.80
	Mean	98.94	98.74	93.76	66.1	
<i>C. dactylon</i>	0	98.59	97.22	85.72	-25.7	63.95
	1	98.71	99.20	98.38	83.96	95.06
	3	98.99	99.16	98.59	85.30	95.39
	5	99.03	99.48	97.77	88.20	96.12
	7	99.32	99.75	97.29	87.78	96.04
	Mean	98.92	98.96	95.55	82.92	

The concentrations of the heavy metals in the shoots and roots of the grass species and soil in which they were grown are shown in Figs.1- 4. Fig. 1 showed the uptake of copper by the root and shoot of the

grass species and soil. It was observed that the roots of *P. maximum* accumulated the highest concentration of copper (36.8 mg/kg) while the lowest accumulation was observed in the soil where *P. maximum* was grown (36.6 mg/kg).

Fig 2 showed the concentrations of the lead in soil, roots and shoots of the grass species and it was observed that the soil, roots and shoot of *P. maximum*, and the soil and shoot of the *C. dactylon* absorbed the highest concentration (40 mg/kg) of lead while the shoot of *C. dactylon* showed the lowest concentration of lead (20 mg/kg).

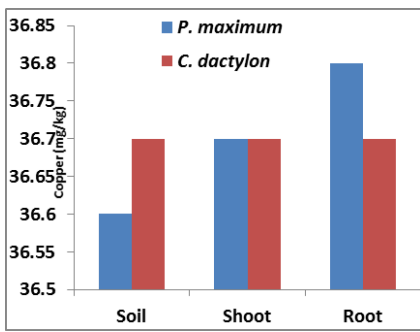


Fig 1: The End Concentrations of the Copper in Soils, Roots and Shoots of the Grass Species.

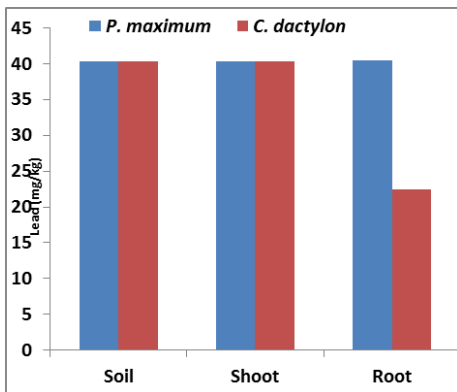


Fig 2: The End Concentrations of the Lead in Soils, Roots and Shoots of the Grass Species.

Fig. 3 showed the concentration of zinc uptake in soil, roots and shoots of the grass species; the soil of *C. dactylon* to accumulate the highest concentration of zinc (55.5 mg/kg) while the shoot of *P. maximum* had the lowest concentration (54.6 mg/kg). Fig. 4 showed the concentrations of iron in soil, roots and shoots of the grass species; the highest uptake of iron was in the shoot of *P. maximum* (118 mg/kg) while

the lowest concentration (109 mg/kg) was recorded in the root.

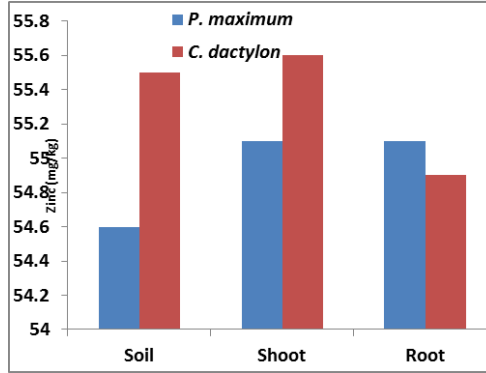


Fig 3: The End Concentrations of the Zinc in Soils, Roots and Shoots of the Grass Species.

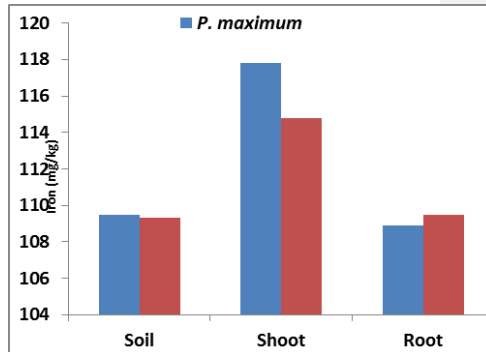


Fig 4: The End Concentrations of the Iron in Soils, Roots and Shoots of the Grass Species.

The observed accumulation of heavy metals in the shoots and roots of the grass species and soil in which they were grown have been reported. Duskor (2003) reported that plants act as solar driven pumping and filtering systems as they take up contaminants (mainly water soluble) through their roots and transport/translocate them through various plant tissues where they are metabolized. The observation was also confirmed by Frey *et al* (2000) in *Thcaspicaerule scens*, where zinc (Zn) was sequestered preferentially in vacuoles of epidermal cells in a soluble form and Sarret *et al* (2000) in *Arabidopsis halleri*, where zinc was found to be accumulated in the mesophyll cells of the leaves.

CONCLUSION

In conclusion, this study showed that there was increased uptake of the heavy metals by the grasses from the soil as the waste engine concentration increased. This was due to the phytoextracting

ability of *P. maximum* and *C. dactylon*, which involved taking up or hyper accumulating contaminants through root and storing them in the tissues of the shoots or leaves. The phytoremediation potentials of these grasses have also been established.

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HEAVY METAL CONTAMINATION AND WATER QUALITY INDEX ASSESSMENT OF BOREHOLE WATER FROM THREE LOCATIONS IN IKWUANO ABIA STATE

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ABSTRACT

Borehole water has become as essential source of water for consumption and domestic use in most African homes. However, there are serious concerns on the safety of these waters sources and suitability for consumption. This study was aimed at investigating the heavy metal contamination levels and physicochemical properties of borehole water around Health Care Centres in three locations in Ikwuano Abia state, Nigeria in a bid to ascertain possible the health risk posed by these water sources. Six samples were collected from UPHC, APHC and OPHC over a period of three months. The samples were analysed for eight heavy metals namely Magnesium (Mg), Manganese (Mn), Cobalt (Co), Cadmium (Cd), Nickel (Ni), Copper (Cu) Lead (Pb) and Zinc (Zn) as well as 10 physicochemical parameters, (B.O.D. (mg/l), C.O.D. (mg/l), Nitrogen (mg/l), Phosphorus (mg/l), Total hardness (mg/l), Magnesium (mg/l), Chloride (mg/l), Iron (mg/l), pH and conductivity ($\mu\text{s}/\text{cm}$) and compared to standard values set by WHO and ICMR respectively. The results showed all the heavy metal were within the WHO limits in all the locations except for lead which was above the recommended limit of 0.1mg/L. The physicochemical parameters were found to be within ICMR standard values except for Iron, pH, Magnesium and Biological Oxygen Demand which were above the standard values. The Water Quality Index (WQI) obtained for the water body in different locations of study were 220.13, 228.56 and 228.25 for UPHC, APHC and OPHC respectively suggesting that the water for unsuitable for consumption. Measures must be taken by the government to improve the water quality of the town and to supply clean and safe water to the public.

Keywords: Groundwater, Borehole Water Quality Index, Heavy metals, Physicochemical parameters,

INTRODUCTION

Boreholes are low-cost technology option for domestic water supply in developing countries and are generally considered as 'safe sources' of drinking water. When properly constructed and maintained, they provide consistent supplies of safe and wholesome water with low microbial load and little need for treatment of the water before drinking. Groundwater quality varies from place to place, sometimes depending on seasonal changes (Vaishali and Punita, 2013) the types of soils, rocks and surfaces through which it moves (Seth *et al.*, 2014; Thivya *et al.*, 2014). Naturally occurring contaminants are present in the rocks and sediments. As groundwater flows through the sediments, metals such as iron and Magnesium are dissolved and may later be found in high concentrations in the water (Moyo 2013). In addition, human activities can alter the natural composition of groundwater through the disposal or dissemination of chemicals and microbial matter on the land surface and into soils, or through injection of wastes directly into groundwater. Industrial discharges (Govindarajan and Senthilnathan, 2014) urban activities, agriculture (Moyo 2013) groundwater plumage and disposal of waste (Bello *et al.*, 2013) can affect groundwater quality. Pesticides and fertilizers applied to lawns and crops can accumulate and migrate to the water tables thus affecting both the physical, chemical and microbial quality of water. Heavy metals have been severally reported in potable water sources in Nigeria including surface, ground water and rain water. Heavy

metals are typically classified into essential (iron, Magnesium, zinc, copper) and non-essential (cadmium, chromium, lead and mercury), with regard to their usefulness in biological diversity. The physico-chemical and heavy metal parameters have permissible guideline recommended by World Health Organization (WHO) and Standard Organization of Nigeria (SON) (Nigerian Agency that regulate the quality of potable water resources). When the heavy metals exceed that maximum permissible concentration for potable water, such water is said to be contaminated by such heavy metal. Naturally, most heavy metals are found in variable concentration in the environment (Izah and Srivastav, 2015). Hospital water safety is a major priority and constant challenge for healthcare epidemiologists, safety officers, engineers, and administrators. Waterborne infections incur significant morbidity and mortality, and some are preventable. As with other healthcare-associated infections, occurrence of nosocomial waterborne infections erodes public confidence in healthcare facilities. Pathogens such as *Legionella* and nontuberculous mycobacteria can colonize the deep infrastructure or outlets of hospital water distribution systems, while other Gram-negative bacteria and molds tend to adhere to biofilms at or near the distal points of use (Brooke and Tara, 2014). This present study therefore was aimed at investigating the heavy metal load and physicochemical quality of borehole water used in different hospitals in Umuahia rural areas for various domestic purposes in these selected

hospitals.

MATERIALS AND METHODS

Study Area

Umudike is found in Ikwuano Local Government Area of Abia State in the south east zone of Nigeria. It is within the Qua Ibo(e) River watershed which has Anya River as the major tributary. The latter is found within the premises of Michael Okpara University of Agriculture, Umudike and flows across the National Root Crops Research Institute, Umudike.

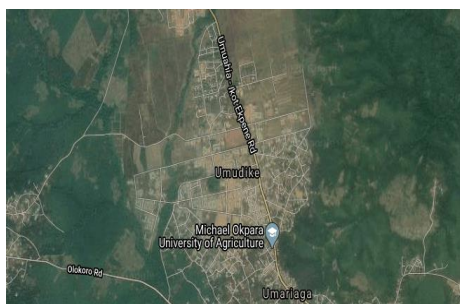


Fig. 1 Satellite image of Umudike showing the sampling areas (Source: Google Maps)

Sampling

The water samples used for this study were randomly collected from three different sites that were selected based on proximity to health centres. The three sites are Umudike Primary Health Centre (UPHC: 5° 28' 11.0" N 7° 32' 16.5" E), Amaoba Primary Health Centre (APHC: 5° 27' 16.7" N 7° 32' 26.0" E) and Primary Health Centre Oboro (PHCO: 5° 25' 52.3" N 7° 34' 10.6" E). The samples were collected in polyethylene bottles (1.5 litres capacity) which had been thoroughly washed, and filled with distilled water, then taken to the sampling site. The bottles were emptied and rinsed several time with the water to be collected. Also, the sample bottles were partially filled with the collected water and vigorously shaken to note the odour. The sample bottles were tightly covered immediately after collection and the temperature taken. They were then stored in a refrigerator at 4 °C to slow down bacterial and chemical reaction rates.

Heavy Metal Determination

The concentrations in mg/L of eight metals were determined in all the samples namely, Magnesium (Mg), Magnesium (Mn), Cobalt (Co), Cadmium (Cd), Nickel (Ni), Copper (Cu) Lead (Pb) and Zinc (Zn) with the Atomic Absorption Spectrophotometer (AAS). 2.5 ml of concentrated HNO₃ was added to 20mls of the water sample and covered with a watch glass. This was

heated gradually on hot plate at 100°C, until digestion was completed. The solution was evaporated to near dryness and cooled. 7.5ml of concentrated hydrochloric acid was then added, warm and filtered, and volume adjusted to 100ml for heavy metal determination. Heavy metal contents were quantified using AAS 2800 series. Internally added standards were used for the calibration of the AAS. The flame used for the analysis was air-acetylene mixture. Standard solutions ranging from 0.2 to 5.0 mg/l were prepared for calibration curves of the various metals. A blank analysis was performed with distilled water treated to the sample treatment.

Physicochemical Parameters Determination

The samples were analyzed for following physicochemical parameters: B.O.D. (mg/l), C.O.D. (mg/l), nitrogen (mg/l), phosphorus (mg/l), Total hardness (mg/l), Magnesium (mg/l), chloride (mg/l), Iron (mg/l), pH and conductivity (µs/cm). Weighted arithmetic index method (Brown *et al.*, 1972) was used for the calculation of water quality index (WQI) of the water body. Further, quality rating or sub index (q_n) was calculated by the following expression.

$$q_n = 100 [V_n - V_{10}] / [S_n - V_{10}]$$

Where: q_n=Quality rating for the nth water quality parameter,

V_n=Estimated value of the nth water quality parameters of collected sample,

S_n=Standard permissible value of the nth water quality parameter,

V₁₀=Ideal value of the nth water quality parameter in pure water.

Unit weight was calculated by a value inversely proportional to the recommended standard value S_n of the corresponding parameter.

$$W_n = K / S_n$$

Where: W_n=Unit weight for nth water quality parameter,

S_n=Standard permissible value of the nth water quality parameter,

K=Constant for proportionality calculated as

$$K = \frac{1}{\sum (1/S_i)}$$

The overall WQI was calculated by aggregating the quality rating with the unit weight linearly.

$$WQI = \sum q_n W_n / \sum W_n$$

Where:

q_n= Quality rating for the nth water quality parameter,

W_n=Unit weight for nth water quality parameter.

Statistical Analysis

Statistical analyses were performed using the SPSS version 22. Data was represented as means ± standard deviation (M ± SD).

RESULTS

Table 1. Heavy metal concentration in borehole water samples from hospitals in three (3) rural areas in Umuahia.

Sampling sites	Heavy metals							
	Mg (mg/L)	Mn (mg/L)	Co (mg/L)	Cd (mg/L)	Ni (mg/L)	Cu (mg/L)	Pb (mg/L)	Zn (mg/L)
UPHC	0.7777 ±0.29	0.1752 ±0.24	0.3280 ±0.01	-0.0026 ±0.00	0.0298 ±0.00	0.0022 ±0.00	0.0587 ±0.01	0.1333 ±0.00
APHC	0.9313 ±0.15	0.0007 ±0.01	0.3355 ±0.00	-0.0017 ±0.00	0.2840 ±0.01	0.0036 ±0.00	0.0586 ±0.01	0.0732 ±0.08
PHCO	0.5851 ±0.00	0.0008 ±0.00	0.3286 ±0.01	-0.0016 ±0.00	0.0350 ±0.00	0.0039 ±0.00	0.0484 ±0.02	0.1345 ±0.00
*WHO	-	0.4	-	0.003	0.07	2.0	0.01	3.0

Values are represented as mean ± S.D. Mg= Magnesium, Mn=Magnesium, Co=Cobalt, Cd=Cadmium, Ni=Nickel, Cu=copper, Pb=Lead, Zn=zinc.

Table 2. Physico-chemical parameters of borehole water samples from hospitals in three (3) rural areas in Umuahia.

Parameters	UPHC	APHC	PHCO
Electrical conductivity (µs/cm)	0.37 ± 0.297	0.357 ± 0.120	0.293 ± 0.153
pH	9.120 ± 0.566	8.843 ± 0.484	7.870 ± 0.017
Iron (mg/L)	0.326 ± 0.030	0.327 ± 0.030	0.337 ± 0.004
Total Hardness (mg/L)	35.000 ± 8.660	46.667 ± 11.547	46.667 ± 11.547
Chloride (mg/L)	2.489 ± 0.188	2.892 ± 0.259	2.465 ± 0.082
Magnesium (mg/L)	0.223 ± 0.006	0.235 ± 0.005	0.235 ± 0.003
Chemical Oxygen Demand (mg/L)	4.600 ± 0.173	4.533 ± 0.057	4.600 ± 0.173
Phosphates (mg/L)	0.257 ± 0.015	0.242 ± 0.005	0.224 ± 0.007
Nitrates (mg/L)	0.059 ± 0.002	0.064 ± 0.013	0.056 ± 0.005
Biochemical Oxygen Demand (mg/L)	5.817 ± 0.127	5.547 ± 0.393	5.733 ± 0.058

Values are represented as mean ± S.D.

Table 3. Calculation of WQI of borehole water samples from UPHC.

Parameter	Observed Value (V _n)	ICMR Standard Values (S _n)	Ideal Value (V ₁₀)	Unit Weight (W _n)	Quality Rating (q _n)	W _n q _n
Electrical conductivity (µs/cm)	0.37	300	0	0.371	0.123	0.0456
pH	9.120	7.5	7	0.219	424	92.856
Iron (mg/L)	0.326	0.3	0	0.5859	108.66	63.66
Total Hardness (mg/L)	35.000	300	0	0.0062	11.67	0.07235
Chloride (mg/L)	2.489	250	0	0.0074	0.99	0.00732
Magnesium (mg/L)	0.223	30	0	0.061	0.7433	0.0453
Chemical Oxygen Demand (mg/L)	4.600	20	0	0.0034	23	0.0782
Phosphates (mg/L)	0.257	1.0	0	0.0677	25.7	1.7399
Nitrates (mg/L)	0.059	45	0	0.0412	0.1311	0.00540
Biochemical Oxygen Demand (mg/L)	5.817	5	0	0.3723	116.34	43.313
		ΣS _n =985.5		ΣW _n = 1.7351	Σq _n = 711.357	ΣW _n q _n = 201.823

ICMR: Indian Council of Medical Research WQI = 116.32

Table 4. Calculation of WQI of borehole water samples from APHC.

Parameter	Observed Value (V _n)	ICMR Standard Values (S _n)	Ideal Value (V ₁₀)	Unit Weight (W _n)	Quality Rating (q _n)	W _n q _n
Electrical conductivity (µs/cm)	0.357	300	0	0.371	0.119	0.0441
pH	8.843	7.5	7	0.219	368.6	80.723
Iron (mg/L)	0.327	0.3	0	0.5859	109	63.863
Total Hardness (mg/L)	46.667	300	0	0.0062	15.56	0.0964
Chloride (mg/L)	2.892	250	0	0.0074	1.157	0.00856
Magnesium (mg/L)	0.235	30	0	0.061	0.7833	0.04778
Chemical Oxygen Demand (mg/L)	4.533	20	0	0.0034	22.67	0.07708
Phosphates (mg/L)	0.242	1.0	0	0.0677	24.2	1.63834
Nitrates (mg/L)	0.064	45	0	0.0412	0.1422	0.005858
Biochemical Oxygen Demand (mg/L)	5.547	5	0	0.3723	110.94	41.3029
		ΣS _n =985.5		ΣW _n = 1.6451	Σq _n = 653.17	ΣW _n q _n = 187.807

ICMR: Indian Council of Medical Research WQI = 114.1

Table 5. Calculation of WQI of borehole water samples from PHCO.

Parameter	Observed Value (V _n)	ICMR Standard Values (S _n)	Ideal Value (V ₁₀)	Unit Weight (W _n)	Quality Rating (q _n)	W _n q _n
Electrical conductivity (µs/cm)	0.293	300	0	0.371	0.0977	0.0362
pH	7.870	7.5	7	0.219	174	38.106
Iron (mg/L)	0.337	0.3	0	0.5859	112.33	65.814
Total Hardness (mg/L)	46.667	300	0	0.0062	15.56	0.0964
Chloride (mg/L)	2.465	250	0	0.0074	0.986	0.0073
Magnesium (mg/L)	0.235	30	0	0.061	0.7833	0.0478
Chemical Oxygen Demand (mg/L)	4.600	20	0	0.0034	23	0.0782
Phosphates (mg/L)	0.224	1.0	0	0.0677	22.4	1.5165
Nitrates (mg/L)	0.056	45	0	0.0412	0.28	0.0115
Biochemical Oxygen Demand (mg/L)	5.733	5	0	0.3723	114.66	42.687
		ΣS _n =985.5		ΣW _n = 1.735	Σq _n = 464.097	ΣW _n q _n = 148.40

ICMR: Indian Council of Medical Research WQI = 85.53

DISCUSSION

The heavy metal load and physicochemical parameters such as pH, electric conductivity, iron, calcium, Magnesium, fluoride, chloride, biological oxygen demand, chemical oxygen demand, nitrate and total hardness of water were analysed for the water samples collected from three hospitals in rural areas i.e UPHC, APHC and PHCO. All parameters with the mean value of the data with standard error were calculated as shown in the Table 1 and 2 above.

In this study the results for the heavy metal estimations showed that Magnesium (Mg), Cobalt (Co), Nickel (Ni), and Zinc (Zn) were all within the recommended limits set by the WHO. However, the concentration of lead (Pb) in UPHC (0.0587 ± 0.01), APHC (0.0586 ± 0.01) and PHCO (0.0484 ± 0.02) were all above the WHO recommended limit of 0.01mg/L. Possible sources of Pb pollution could result from domestic waste dump, smoking as well as house paints as the rain wash them into the soil and plumbing pipes. In children, these studies have shown an association between lead poisoning and diminished intelligence, lower intelligence quotient-IQ, delayed or impaired neurobehavioral development, decreased hearing acuity, speech and language handicaps, growth retardation, poor attention span, and anti-social and diligent behaviours (USEPA, 2017).

Biological Oxygen Demand is a measurement of the amount of dissolved oxygen (DO) required for the microorganism to performed biological decomposition of dissolved solids or organic matter in the water under aerobic conditions and provides an index to assess the

effect discharged wastewater will have on the receiving environment. The higher the BOD value, the greater the amount of organic matter or “food” available for oxygen consuming bacteria. This study showed BOD concentration of all the water samples obtained from all the locations to be higher than the standard recommended values. UPHC recorded the highest BOD levels possibly due to the high amount of waste along with rain water and from nearby waterbodies and addition of organic waste.

Chemical Oxygen Demand or COD is a measurement of the oxygen required to oxidize soluble and particulate organic matter in water. This study showed COD concentration of all the water samples obtained from all the locations to be lower than the standard recommended values. Lowest values were recorded in samples from APHC. The observed COD for the samples were observed to be lower than their respective COD levels suggesting a low microbial load as microorganisms will find it difficult to survive in the water samples since the oxygen available for their survival is limited.

Among nitrogen containing compounds, nitrate is the most common form in water. All other dissolved forms of nitrogen (nitrite, ammonia and organic nitrogen) get oxidized to nitrate over time. Nitrates are found in both ground and surface water, originating from the natural decaying process of biological matter. Nitrate has a high solubility in water and will not be filtered out like other contaminants as it seeps through the soil layers to groundwater level. The levels of nitrate in this study were observed to lower than the standard values in all

the samples obtained.

Magnesium is a mineral that naturally occurs in rocks and soil and may also be present due to underground pollution sources. Magnesium is seldom found alone in a water supply. It is frequently found in iron-bearing waters (Iyasele and Idiata, 2011). The concentration of Magnesium in all the collected samples were above the standard recommended value. High exposure to Magnesium has been associated with toxicity to the nervous system, producing a syndrome that resembles Parkinsonism. Magnesium is unlikely to produce other types of toxicity such as cancer or reproductive damage. Young children appear to absorb more Magnesium than older age groups but excrete less. This makes it particularly important for pregnant women and children to have clean drinking water (Lawson, 2011).

Chloride levels detected in the borehole water samples in the three locations were below limit (250mg/l) stipulated by ICMR. The presence of excess chlorine in water has adverse health effects; by-products of chlorination can lead to production of free radicals that lead to oxidative stress observed in most disease cases. Concentrations over 250 mg/L impart a peculiar taste to the water, thus rendering the water unacceptable for drinking purposes.

Phosphates are not toxic and therefore are not considered a direct threat to health of human's other organism. They also are not considered a serious indirect threat to water quality. Little amounts of phosphorous in surface waters is necessary for biological life, but excess of phosphorous promotes the indiscriminate growth of algae (Eutrophication). The values of phosphates for water samples from the three sampled locations were within the standard limits.

The degree of hardness of water supply is determined by the content of calcium and magnesium salts. Calcium and magnesium combine with bicarbonates, chlorides, sulphates, and nitrates to form these salts. Water can be classified into soft (75 mg/L), moderately hard (75–150 mg/L), hard (150–300 mg/L) and very hard (300 mg/L) based on hardness (Sawyer and McCarty 1967). The hardness of the water samples in this study were lower than the recommended values by ICMR. Long-term consumption of extremely hard water might lead to an increased incidence of urolithiasis, anencephaly, prenatal mortality, some types of cancer and cardiovascular disorders (Agrawal and Jagetia 1997).

Iron is one of the most troublesome elements in water supplies. Making up at least 5 % of the earth's crust, Iron is one of the earth's most plentiful resources. Rainwater as it infiltrates the soil and underlying geological formations dissolves iron causing it to seep into the aquifer that serves as source of ground water for bore holes. The observed iron content of the water samples from the three locations analysed were above

limits of specification according to ICMR.

The pH of the samples was tending towards alkalinity with values ranging from 7.8-9.1 in all the three locations which fell above ICMR standard value of 7. Deviations in the pH value of water solutions from 7 are principally due to hydrolysis of salts of strong bases and weak acids or vice versa, also dissolved gases such as carbon dioxide, hydrogen sulphide and ammonia affect pH of water (Agbabiaka and Oyeyiola, 2012)

Conductivity is defined as the ability of a solution to carry electric current. This is normally dependant on the presence of mobile ions, their concentrations, mobility, relative concentration and temperature of measurement. The electrical conductivity (EC) for all samples for all samples fell within the permissible limit set by ICMR.

Table 6. Water Quality Index (WQI) and corresponding status (Chaterjee and Raziuddin, 2002; Thakor *et al.* 2011).

Water Quality Index Level	Water Quality Status
0-25	Excellent water quality
26-50	Good water quality
51-75	Poor water quality
76-100	Very poor water quality
>100	Unsuitable for drinking

The WQI obtained for the water body in different locations of study i.e., UPHC (116.32), APHC (114.16) and PHCO (2285.53) respectively, indicate, based on the quality status shown in Table 6 above, that water is of very poor quality due to high pollution level.

CONCLUSION

The results of this study showed that most of the physicochemical parameters of the borehole water samples were above the standard limits recommended by ICMR. The water samples from the different locations sampled showed varying levels of the assayed physicochemical parameters and the observed difference in these water quality parameters can be attributed to climatic, geographic, and geologic variations between the three sampled locations as well as human activities in these areas. These observed differences can also be attributed to disparity in the hygienic condition of the boreholes. The calculated WQI for the three locations showed that the water from these boreholes were unsuitable for drinking. Based on the results for the heavy metal analyses, all assayed metals were within WHO limits except for the level of lead (Pb) which was observed to be above recommended values of WHO in the borehole water

samples from all the locations making the water unsafe for consumption as lead can cause serious adverse health effects over time. Based on these results it should be concluded that borehole water sources are unsafe hence and it is therefore necessary to treat the water from these boreholes before consumption to

prevent the spread of water borne diseases to ensure that the health of the community is protected. Measures must be taken by the government and stake holders to improve the water quality of the town and to supply clean and safe water to the public that is free from health hazards.

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INVESTIGATION OF ACTIVITY CONCENTRATIONS IN DIFFERENT CARDIAC DEVICES AFTER RADIOTHERAPY

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ABSTRACT

Two common types of Cardiac Implantable Electronic Services namely, pacemaker and defibrillators, were involved for heart pace control or monitoring. The effects of radiotherapy on patients with cardiac devices were studied using hyper-pure germanium detector. The following five (5) radionuclides ¹⁹⁸Au, ¹⁸⁷W, ¹⁹²Ir, ¹¹⁰Ag and ¹⁹⁴Ir were identified from the energy peaks of both pacemakers and defibrillators. ¹⁹⁸Au with energy peak 411.8 keV was observed to be consistent in all the studied cardiac devices after exposure to radiation. The average mean activity of the irradiated defibrillators is higher than those of the pacemakers with a factor of 2.8s. This implies that defibrillators have the higher chances of experiencing device malfunction during the course of a radiotherapy session.

Keywords: Defibrillator, Pacemaker, hyper-pure germanium detector, radiotherapy

INTRODUCTION

Cardiac Implantable Electronic Devices (CIED) are cardiovascular devices classified as either permanent pacemakers (PM) or implantable Cardioverter defibrillators (ICD). PM are used to pace the heart, coordinate the sequence of cardiac contraction and monitor heart failure whereas ICD are used to monitor cardiac rate and rhythm, deliver shock therapy and for pacing [1]. Globally, about 1 million CIED are implanted each year, yet the demand is still on the increase [2]. Similarly, there is an increase in the number of CIED patients requiring radiotherapy [2-4]. Though the impact of radiation on CIED had been known for the past two decades [3], yet the challenge still persist. Due to this challenge, the American Association of Physicists in Medicine (AAPM) recommended managing each patient individually and set the threshold dose to any part of pacemaker to be 2 Gy during radiotherapy [5]. Presently, the recommendation of AAPM has been faulted as it only pertains to pacemaker and does not involve other CIED. More so, the materials used for CIEDs then have been replaced with newer technology [3].

There is inconsistency in safe radiation doses to CIED due to different materials used by various manufacturers. Dose as low as 0.15Gy have been reported to cause clinically significant damage [6]. Another study recorded CIED defect occurring at a dose of 0.3 Gy [7]. Contrarily, other studies reported adverse damage at a very high dose [5, 8]. Meaning there is large dose gap that can cause defects in CIED. Initially, electromagnetic interference (EMI) was known to influence CIED defects the most [9]. But research has shown that EMI no longer poses threat to CIED because they are now properly shielded [2]. Another author observed that EMI induced defects still subsist but with a different damage pattern [3]. The

author further classified some CIED defects to unknown causes and recommended that all radiation safety precautions regarding CIED should be employed during radiotherapy. This confirms the study by [10] that noted that the functioning of CIED under radiotherapy is still unpredictable.

The challenge of CIED malfunctioning during radiotherapy is well documented in literature [7, 11-13]. The recorded defects are diverse and inconsistent due to various types of CIED available. A study investigated 230 patients with CIED who underwent radiotherapy; about 87% had pacemaker, 13% had defibrillators [10]. The dysfunction recorded was 7% for all categories of CIED. A similar study interrogated 69 patients, 50 patients had pacemakers while 19 had cardioverter-defibrillators. The result showed about 11% dysfunction in patients with cardioverter-defibrillators [14]. A study that investigated the impact of radiotherapy on modern CIED showed that pacemakers suffered more dysfunction than cardioverter defibrillators under the same beam energy [15-16]. The authors further noted that malfunction increases with increased dose. Another researcher observed cardioverter defibrillators experiencing more defects as compared to pacemakers [14]. Contrarily, the study subjected the cardioverter defibrillators to higher beam energy. The study by [17] has reported that cardioverter defibrillators are prone to more defects at higher beam energy. The study by [4] observed that about 2% of patient with CIED experienced damage due to the device. It was also noted that human organs and electronics devices are prone to malfunction when in field of ionizing radiation [18-19]. There is still no certainty about the exact behavior of CIED under radiotherapy. Sources of defects can be attributed to several causes such as poor knowledge of medical officials in handling

patients with CIED during radiation treatment, insufficient and inconsistent information from manufacturers on the threshold dose for the safety of their CIED, non-application of all safety rules, poor knowledge on the failure of some CIED, lack of uniform protocol in handling patients with CIED among other. Due to the random nature of CIED, this study was undertaken to verify the behavior of selected CIED under radiotherapy in order to optimize its usage and minimize the malfunction rate. Therefore constant research and discoveries are being carried out to study the effects which include the risk and rewards associated with subjecting patients with permanent cardiac devices to radiotherapy. This research intends to study selected cardiac devices and report the observations from the spectroscopic analysis in order to arrive at a conclusion on the level of radiation effect on them. Therefore, the study investigates the effects of radiotherapy on cardiac devices which include pacemakers and implantable defibrillators using a germanium detector for analysis and comparing them before and after exposure.

MATERIALS AND METHODS

There are different types of cardiac devices, implantable pacemakers and Cardiac defibrillators have been selected for this study. Radiometric analysis of 42 selected cardiac devices (23 pacemakers and 19 defibrillators) was done using PC-based high resolution gamma spectrometry system. There are also different models of pacemakers and defibrillators made by different manufacturers. The models of the cardiac device used were selected randomly as shown in Figure 1 to 9. The study focused on the effects associated with exposing patients with implanted cardiac devices (CIEDs) to radiotherapy with regards to analysing the cardiac device before and after radiotherapy with the aid of a germanium detector for gamma ray spectroscopy. Harvested spectroscopy results were analysed with the Maestro-32 software and the findings are displayed in Tables under results and discussion.



Figure1: Boston Scientific



Figure 3: Biotronik Pacemakers



Figure 4: Sorin Group Pacemaker



Figure 5: Medtronic pacemaker

Figure 8: Medtronic defibrillator



Figure 6: Biotronic defibrillator



Figure 9: St. Jude defibrillator



Figure 7: Boston Scientific defibrillator

Exposure of the Cardiac Devices to Radiation

The cardiac devices (pacemakers and defibrillators) were exposed to radiation during patient radiotherapy treatment. High purity germanium detector (HPGe) was used for collect the gamma spectra emitted by the cardiac devices. The CIEDs gamma ray spectroscopy results were then processed with masestro-32 software to obtain the activities of studied

The MAESTRO-32 software is windows based multichannel analyzer (MCA) emulation software that gives you the option of using a multi detector interface that lets you view up to eight (8) MCA windows and eight buffer windows at the same time. The MCA is defined as an instrument that sorts and counts events in real time. MAESTRO-32 can control and display an almost unlimited number of detectors either local or remote (networked). In this study, the software was used to analyse results gotten from germanium detector with the cardiac device in order to determine the number of counts or beats in real time to enable us make some calculations.

The voltage pulse produced by the detector (or by the photomultiplier in a scintillation counter) is shaped by a multichannel analyzer (MCA). The multichannel analyzer takes the very small voltage signal produced

by the detector, reshapes it into a Gaussian or trapezoidal shape, and converts that signal into a digital signal. As pulses arrive over time, the MCA memorizes a distribution of the number of pulses with respect to pulse height. This distribution, arranged in order of ascending energies, is referred to as a spectrum. This is displayed on a graph whose horizontal axis represents the number of pulses at that height. This is also referred to as a histogram. The multichannel analyzer output is sent to a computer, which stores, displays, and analyses the data. The saved spectrum is recalled and the different peaks are shown. The peak of interest is the 411 keV peak which was present in all the spectra analysed. The software displays the peak info which consists of the net/gross areas, gross/net count rates etc. Maestro-32 also displays the live time and real time in the pulse height analysis as shown in Figure 10.



Figure 10: Showing Maestro-32 spectrum analysis (peak info and pulse height analysis) of pacemaker P10 after exposure to radiation

The software package (Maestro-32) includes spectrum analysis tools such as energy calibration, peak area, net area calculation, and resolution calculation. Also, the radionuclides and their activities can be determined.

The High-Purity Germanium (HPGe) detector comprised of high-purity germanium lead shielded detector having relative efficiency of 1.81. The detector was shielded by 15-cm-thick lead wall provided with 3-mm-thick copper and 4-mm-thick tin inner lining in order to effectively reduce the natural background. All the cardiac devices were analysed with germanium detector before and after irradiation. The devices were analysed for 6400 seconds and the accumulated spectra were analysed using the Maestro-32 software. The collected and stored spectra were recalled by Maestro-32 software, the regions of interest were analysed and different peaks were identified for the cardiac devices.

The activities of all the cardiac devices exposed to radiation were calculated from the displayed peak info. The efficiency of the radiation detector must be

accounted for when determining the activity. The activity of a particular isotope is given in equation 1

$$\text{Activity(Bq)} = \frac{\text{Net Area}}{(\text{Live time})(\text{Efficiency})(\text{yield})(3.7 \times 10^4)}$$

where yield is the branching ratio fraction and live time is the actual analog to digital converter live time in seconds.

RESULTS AND DISCUSSION

Identified Radionuclides

The first analysis was to identify the radionuclides present from these energy peaks. The identification was done by comparing the identified peaks energy values with tabulated standard values of the energy of γ -radionuclides. The radionuclide present at the peak (411 keV) which was found in all the spectra obtained for all the cardiac devices after irradiation that was not present before irradiation, it was identified as ¹⁹⁸Au(Gold) which is an isotope of gold. ¹⁹⁸Au has a half-life ($T_{1/2}$) of 2.6944 days. The cardiac devices were also counted in count per seconds (cps) emission channel to trace the activity of the device. The peak info provided by the software gives the value of full width at half maximum (FWHM), the gross area and the net area as well as the gross and net count rates with the associated uncertainty. It is known that unstable elements which disintegrate and emit ionizing radiation are called radionuclides. All radionuclides are uniquely identified by the type and energy of radiation they emit, and their half-life. Radionuclides present at the different peaks when the spectra were analysed were identified and summarised in Table 1.

Table 1: Radionuclides Identified at the Different Energy Peaks

Radionuclides	Half-life ($T_{1/2}$)	E_{γ} (keV)
¹⁹⁸ Au	2.695d	411.80
¹⁸⁷ W	23.72h	685.774
¹⁹² Ir	73.831d	316.51
¹¹⁰ Ag	249.79d	657.76
¹⁹⁴ Ir	17.28h	328.455

These radionuclides are present as read from the different peaks identified by MAESTRO-32 when the devices were analysed. The radionuclide of interest is ¹⁹⁸Au at 411 keV, it became peculiar because the particular peak was consistent in all the spectra gotten when all the cardiac devices were analysed after irradiation and it has high activity compared to the other radionuclides. The gammas emitted by ¹⁹⁸Au are summarised in Table 2.

Table 2: Gammas from ¹⁹⁸Au (2.6944d) (Tori, 1999)

E _γ (keV)	Branching Ratio(I _γ)
411.802	96
675.884	0.804
1087.684	0.159

3.2 Activity of Pacemakers

The spectra from the devices were obtained before and after exposure to radiation, using the high purity shielded germanium detector which was connected to a multichannel analyzer (MCA). The spectra were then analysed using MAESTRO-32 which is multichannel analyzer (MCA) emulation software. The activities of the cardiac devices were calculated using the equation 1. The activities in Becquerel (Bq) of the 42 exposed cardiac devices (23 Pacemakers and 19 defibrillators) are presented in the Table 3 and 4.

Table 3: The pacemakers and their corresponding activities (Bq)

S/N	PACEMAKER CODE	ACTIVITY (Bq)
1	P01	4.2×10 ²
2	P21	2.1×10 ³
3	P02	2.4×10 ²
4	P04	2.0×10 ²
5	P10	1.3×10 ³
6	P11	1.2×10 ³
7	P14	9.3×10 ²
8	P03	4.8×10 ²
9	P16	1.3×10 ³
10	P05	3.4×10 ²
11	P06	2.7×10 ²
12	P18	6.9×10 ²
13	P20	1.3×10 ³
14	P22	1.1×10 ³
15	P12	2.4×10 ³
16	P07	7.9×10 ²
17	P08	1.7×10 ³
18	P09	1.2×10 ³
19	P13	1.4×10 ³
20	P15	1.0×10 ³
12	P17	2.3×10 ³
22	P19	7.9×10 ²
23	P23	8.5×10 ²
Average		1.11×10 ³

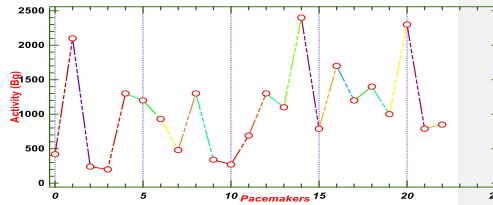


Figure 11: A graph showing the pacemakers and their activities

Activity of Defibrillators

Table 4: The Defibrillators and their Corresponding Activities (Bq)

S/N	DEFIBRILLATORS CODE	ACTIVITY (Bq)
1	D01	2.8×10 ²
2	D19	1.5×10 ³
3	D02	3.5×10 ²
4	D03	4.2×10 ²
5	D08	1.8×10 ³
6	D10	2.7×10 ³
7	D11	1.3×10 ³
8	D18	1.6×10 ³
9	D05	5.2×10 ²
10	D06	3.5×10 ²
11	D16	1.2×10 ³
12	D07	4.0×10 ⁴
13	D15	8.7×10 ²
14	D12	1.7×10 ²
15	D04	6.3×10 ²
16	D13	3.9×10 ²
17	D14	8.1×10 ²
18	D17	2.1×10 ³
19	D09	2.7×10 ³
Average		3.17 ×10 ³

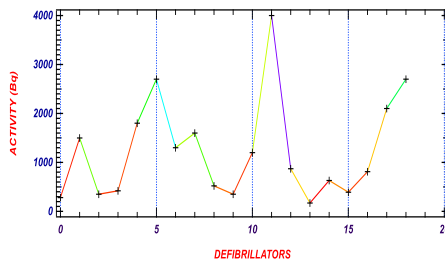


Figure 12: Graph showing defibrillators and their activities

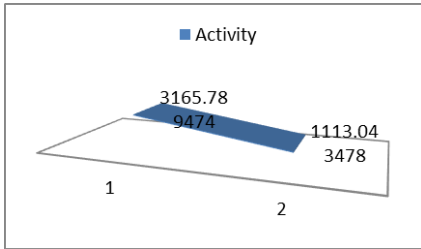


Figure 13: Comparison of Activity (1: Defibrillator, 2: Pacemaker)

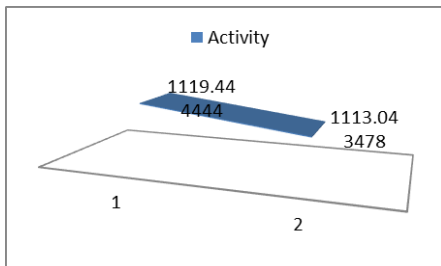


Figure 14: Modified Comparison of Activity (1: Defibrillator, 2: Pacemaker)

The statistical analysis tool used to plot activities against cardiac devices is Igor Pro 8.02. It is a scientific graphic and data analysis software for scientists and engineers. It includes a variety of built-in operations, functions, and packages for statistical analysis. They are roughly grouped into the following categories: Statistical Tests, Noise Functions/Pseudo Random Number Generators, Probability Distribution Functions (PDFs), Cumulative Distribution Functions (CDFs), Inverse CDF's, General purpose operations and functions and Procedures and Packages. The plots of the cardiac devices were done according to its serial numbers as arranged in the Tables 3 and 4, and presented in Figures 11 and 12. From Figure 11, it is observed that the pacemaker with the highest activity is P12 with serial number 15 and activity of 2.4×10^3 Bq. This means that, it is most likely to experience device malfunction during the course of a radiotherapy treatment. We can also deduce that it is the pacemaker device with the highest percentage of gold atoms by relating their activities to the decay constant of the ^{198}Au radionuclide. The pacemaker with the lowest activity is observed to be P04 with serial number 4 and having activity of 2.0×10^2 Bq. This also implies that it is the least to likely experience device malfunction during the course of a radiotherapy treatment and it contains the least percentage of gold atoms. From results in Figure 12, it is observed that the defibrillator with the highest activity is D07, having activity 4.0×10^4 Bq, which implies that, it is most likely to

experience device malfunction during radiotherapy. The defibrillator with the lowest activity is D12 with activity value of 1.7×10^2 . It is also likely having the least percentage of gold atoms. Comparing results from Tables 3 and 4, that is; comparing the mean activities of pacemakers and defibrillators, it is observed that the average mean activity of the irradiated defibrillators is higher than those of the pacemakers with a factor of 2.8, as shown in Figure 13. This implies that defibrillators have the higher chances of experiencing device malfunction during the course of a radiotherapy session. It was observed that the D07 high activity value was way too higher that all other studied defibrillators and can be seen to have abnormal value spike. This high activity value contributed to the 2.8 factor variation of the mean activities of defibrillators and pacemakers. A re-computation of mean activity value without D07 activity value shows a comparable mean activities with variation factor of 1.005, which is close to unity. With the variation factor of 1, it can be concluded that all cardiac devices are likely going to have the same response to exposure to radiation. With the variation factor of 2.8, one can conclude that device malfunction is more likely to occur with defibrillators than pacemakers. This study is in consonance with report given by [20], in the report out of all the 69 patients (50 with pacemakers and 19 with defibrillator), no patient with a pacemaker experienced a device-related malfunction while 3% of patients with defibrillator experienced device malfunctions. The malfunction may be as a result scatter radiation which is likely to occur but probability of occurrence is higher in defibrillators than in pacemakers as no patient with pacemaker had a device related malfunction.

CONCLUSION

The results from this analysis show that radiation therapy (RT) can adversely impact patients with cardiac implanted electronic devices. This implies that there is a significant risk while irradiating patients with cardiac devices although CIED malfunction from scatter RT is more likely to occur in defibrillators than in pacemakers. Radiation can cause CIED malfunction from either destruction of electrical components or partial reset of the cardiac device which would tamper with the therapy settings. Some results of malfunction include: Program reset to default values, program altered, incorrect values, failure to pace, inappropriate delivery of stimulus. Nevertheless, consequences could be serious and permanent. For instance, severe circulatory damage could potentially lead to a major catastrophic failure of the cardiac conduction system and ultimately death of the patient.

This malfunction may be due to increased use of complementary metal-oxide semi-conductors (CMOS) circuits in cardiac devices, which can be more sensitive to ionizing radiation than the bipolar semiconductor devices used previously. This increased sensitivity can lead to damage to both the hardware

and software components of the pacemaker. Malfunction may also be due to neutron capture by the device. Thus, the study concluded that cardiac devices should be relocated from direct field of radiation because devices experience malfunction as a result of destruction of electrical components due to direct irradiation.

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EFFECTS OF DIP TIME ON ELECTRODEPOSITED ZINC OXIDE THIN FILM.

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Abstract: Zinc Oxide Thin Films was successfully synthesized on Fluorine doped Tin Oxide (FTO) Glass Substrates from solutions of zinc tetraoxosulphate heptahydrate, citric acid, and sodium hydroxide using Electro deposition Technique. Time as bath parameter was varied. Four samples with Time intervals of 1 minute, 1.5 minutes, 2 minutes and 2.5 minutes were fabricated. Absorbance of the films was determined with the help of spectrophotometer. Other optical properties of the Thin Films were calculated using the appropriate equations from the literature. The deposited Thin Films have high absorbance in UV region and low absorbance in VIS – NIR region. Transmittance of the Thin Films is low in UV region and high in VIS – NIR region. Reflectance of the films is low throughout the UV – VIS NIR regions. The optimal optical thickness of 650 nm was obtained at 2.5 minutes. The band gap of the Thin Films obtained is between 2.90eV to 3.17 eV. Zinc Oxide is single crystalline.

KEYWORDS: Zinc Oxide, Optical Properties, Band Gap, Electrodeposition, XRD

INTRODUCTION

Uncertainty in energy capacity, limited fossil fuel resources, and changes in climate predicate a need for increased research and development into alternative and sustainable energy solutions. Solar energy is one solution to this problem and many variations of it exist; however, the majority of them are prohibitively expensive. Zinc oxide is a technologically important inorganic compound with the formula ZnO. Zinc and oxygen are the elements of group II and VI with atomic numbers 30 and 8 and atomic weights 65.382mol and 15.999mol respectively. In the stable form, zinc oxide takes a hexagonal wurtzite crystal structure with lattice parameters $a = 3.2458\text{\AA}$ and $c = 5.2006\text{\AA}$ ($c/a = 1.6022$) [5]. The other two forms of ZnO are zincblende and rocksalt structure. This high binding energy (~60meV) assures efficient exciton emission at room temperature under very low excitation energy [1]. ZnO materials show an n-type electrical conductivity due to its native or intrinsic defects such as oxygen vacancies and interstitial zinc atoms [2]. Intrinsic zinc oxide thin films are highly resistive in nature, but when commonly doped with Group III elements such as Ga, In or Al, they become conducting [1]. ZnO is an important semiconducting material which is widely used in the field of solar cell application because of its suitability as both an intrinsic n-type buffer layer (i-ZnO) and Al-doped top contact layer for copper indium gallium diselenide (CIGS) solar cells. ZnO thin films are deposited by the several conventional growth techniques such as DC or RF Magnetron Sputtering [14, 19, 20], Metalorganic Chemical Vapor Deposition [13, 18], Pulsed Laser Deposition and Spray Pyrolysis [7, 8, 9], Chemical Bath Deposition [3, 6, 12], Molecular Beam Epitaxy and Cathodic Electrodeposition Method. The cathodic electrodeposition method is emerging as an efficient nanotechnology for the production of ZnO

thin films and nanostructures because of its simplicity, low cost equipment, and suitable for large area substrate [1].

MATERIALS AND METHODS

All solvents and organic reagents were purchased from Sigma-Aldrich and used without further purification. Prior to the deposition of ZnO films the solutions to be used were prepared; 0.5 M of Citric acid was prepared by dissolving 0.22 g of citric acid in 100 mls of double distilled water. This solution served as precursor for oxygen. 0.5 M of Zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) solution was prepared by dissolving 0.434 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ salt in 100 mls of double distilled water and this solution served as Zn^{2+} ion precursor. 24.50 % NH_4OH solution served as both complexing agent and pH adjuster. All FTO substrates were cleaned by ultrasonic treatment in acetone for 10 minutes. The cathodic deposition of ZnO films was performed at 2.07V/SCE potential on the fluorine tin oxide (FTO) conducting glass substrates using the three electrodes setup of electrodeposition. Fig 3.1 shows the electrodeposition experimental set up containing the electrolyte, working electrode (FTO), saturated calomel electrode (SCE) and platinum foil.

During the deposition, A saturated calomel electrode (SCE) was used as the reference electrode, the platinum foil which served as the counter electrode was used as the anode while the working electrode was the FTO conducting glass substrates which served as the cathode. Dazheng DC-power supply (model: PS-1502A) and two digital multimeters; Mastech (MY-68) and DT9201ACE) were used in the setup for the electrodeposition. In this experiment, four pre-treated fluorine tin oxide (FTO) conducting glass substrates were used. Each of the 4 beakers used for experiment contains various volume of the reagents used for the

experiment. The four slides were labeled ZnO1, ZnO2, ZnO3, ZnO4, respectively. Each of the baths for deposition of ZnO contains 10 mls of ZnSO₄.7H₂O, 10 mls of Citric acid And 20 mls of ammonium hydroxide. Zinc sulphate heptahydrate was firstly measured using a 10 mls syringe, transfer to 50 mls beaker, then 10 mls of Citric acid. Lastly, 20 mls of ammonium hydroxide was also measured and transferred to the 50 mls beaker, the mixture was

stirred for 7 minutes to obtain a homogeneous mixture. The ZnO films were electrodeposited for different time intervals starting with 1 minute, 1.5 minutes, 2 minutes, and 2.5 minutes. The deposited films were annealed at 573 K. Some properties of ZnO thin films such as optical and crystal structural properties of the films were determine using spectrophotometer and x- ray diffractometer.

Table 3.1: Chemical Constituents for the Optimization of Deposition Time of ZnO Thin Films

Bath Name	Chemicals	Concentration (mol)	Volume (ml)	pH	Deposition Time (mins)	Current I (mA)	Voltage V (volts)	Temp (K)
1	ZnSO ₄ .7H ₂ O	0.5	10.00	8.6	0.5	2.59	2.60	303
	Citric acid	0.5	10.00					
	NaoH	1.0	20.00					
2	ZnSO ₄ .7H ₂ O	0.5	10.00	8.6	1.0	2.42	2.48	303
	Citric acid	0.5	10.00					
	NaoH	1.0	20.00					
3	ZnSO ₄ .7H ₂ O	0.5	10.00	8.6	1.5	2.46	1.60	303
	Citric acid	0.5	10.00					
	NaoH	1.0	20.00					
4	ZnSO ₄ .7H ₂ O	0.5	10.00	8.6	2.0	2.29	1.61	303
	Citric acid	0.5	10.00					
	NaoH	1.0	20.00					
5	ZnSO ₄ .7H ₂ O	0.5	10.00	8.6	2.5	1.43	1.60	303
	Citric acid	0.5	10.00					
	NaoH	1.0	20.00					

RESULTS

The electro deposited films are subjected to optical and structural characterization to determine their optical and structural properties. The optical properties of the film were determined using a Janway 6405 UV-visible spectrophotometer while the chemical compositions of the thin films deposited in this work were determined by phase identification of the films preformed by means of X-ray diffraction (XRD) using Bruker D8 advance X-ray diffractometer with Cu-ka line ($\lambda = 1.54056 \text{ \AA}$ in 2 θ range from 10⁰ to 90⁰).

Optical Properties

Optical properties of the deposited films are discussed in this section. The optical properties studies include: optical thickness, absorbance, transmittance, reflectance, refractive index extinction coefficient, band gap energy, optical conductivity, real dielectric constant, imaginary dielectric constant.

Figure 2 Shows the graph of absorbance against wave length (nm) for zinc oxide thin film (slide 1.0, 1.5, 2.0 and 2.5) minutes, the absorbance can be seen to increase as time of deposition increases. The absorbance is high in the UV region with numerical values between 0.62 at 320nm to 0.28 at 400nm. The value decreases slowly within VIS – NIR region with a minimal absorbance value of 0.05 for film

deposited at 1.0 minutes. This result is consistence with the findings at [15]

1. The high absorbance in the UV region makes this material important in solar energy application. Figure 3 Shows the graph at transmittance against wave length for film oxide thin film (slide 1.0, 1.5, 2.0 and 2.5) minutes. The film shows lowest transmittance in the UV region at 20% at 320, increase in the visible/ near infrared region, with an average of about 93% at 1100nm. The transmittance decreases as time of deposition increases. The high transmittance in the visible region makes zinc oxide films useful for aesthetic window glaze materials. Also, the high transmittance of the film makes it suitable for solar energy collection, because if coated on the surface of the collector it will reduce reflection of the solar radiation and transmits radiation to the collector fluids.

Figure 4 is a graph of reflectance against wave length for zinc oxide thin film. The graph of reflectance decreases gradually with increase in wave length from 11% at 320 to 20% at 1100nm, but decreases as time of deposition increases; the low reflectance makes zinc oxide thin film an important material for anti-reflection coating.

Figure 5 is a graph of refractive index of the deposited ZnO thin films plotted against wave length

the refractive index decreases gradually as wave length increases but increases as time of deposition increases. Refractive index ranged from 2.0 to 2.01, with this moderate result, it makes the material good for optoelectronic devices.

Figure 6 is a graph that shows the plot of extinction coefficient of Zinc Oxide films against wavelength; extinction coefficient of the films decreases as wavelength increases and increases as time of deposition increases, highest value of 5.4×10^{-2} was obtained at 340nm for films deposited at 2.5 minutes, and least value of 0.5×10^{-2} was obtained for films deposited at 1.0 minute.

Figure 7 shows the plot of optical conductivity against wavelength. The optical conductivity of the film slightly increases as time of deposition increases; also decreases exponentially as wavelength increases; highest value of 1105^{-1} was obtained at 300nm for film deposited at 2.5 minutes and minimal value at 0.98 of 1000nm for films deposited at 1.0 minute. This result shows that films of zinc oxide conduct Optically in UV regions more than in VIS – NIR regions of the electromagnetic spectrum.

Figure 8 shows a plot of Real Dielectric Constant against wavelength. The real dielectric constant decreases gradually as wavelength increases and decreases as time deposition increases for film deposited at 1.0 and 1.5 minutes, but increases slightly as wavelength increases for 2.0 and 2.5 minutes, peak value of 7.1 and 4.0 were obtained at the VIS region for film deposited at 1.0 minute and 2.5 minutes while the least value of 2.5 at the NIR region was obtained for the film deposited at 1.0 minutes.

Figure 9 shows a plot of Imaginary Dielectric

Constant against Wavelength. Wavelength increases as imaginary dielectric constant decreases slightly, the values increases as time of deposition increases, imaginary dielectric constant is highest in the UV region and the lowest at NIR region. The optical results are in agreement with the result at [15, 16, 17].

Figure 10 reveals the plot of absorption coefficient squared against photon energy (ev) for zinc oxide thin film. The energy gap for this film was obtained by extrapolating the linear part of the curve to the energy axis, the band gap was shown to be between 2.90ev and 3.17 the band gap values are in line with works reviewed by [15, 16, 17].

Structural Analysis.

Figure 12 shows the structural studies of the ZnO thin films deposited at 1.0 minute.

The X-ray diffraction pattern of ZnO Materials deposited on glass substrate, at fig 2 shows the crystal structure of ZnO thin films deposited at 1.0 minute. The significant peak of ZnO thin film was found at 2θ values of 56.65° with orientation along (110) plane. The films exhibits good crystallinity and the peaks are indexed for a hexagonal phase of wurtzite ZnO with lattice constant of $a = 1.64843\text{\AA}$. d- spacing ,of 1.625\AA which is in good agreement with reposed standard values (JCPDS :00-049-0577) the average crystallite size of 29.14nm was deduced by the Inverse proportional relation of the full width at half maximum (FWHM) as predated by debye –Scherer’s formula $D = \frac{0.9\lambda}{B \cos\theta}$

Where D is the crystalline size, λ is the X-ray wave length, B is the full width at half maximum (FWHM) value calculated was 0.43° and θ is the Bragg’s angle which is the diffracting angle. The crystalline size of 29.14nm confirmed that the deposited films of ZnO are in nanoscale.

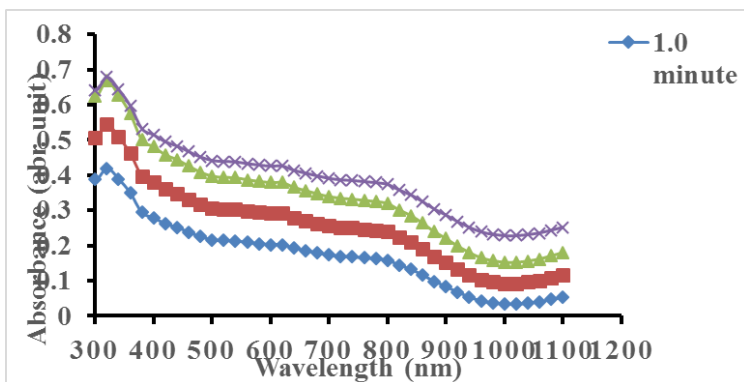


Figure 1. Plot of Absorbance (abr.unit) against Wavelength (nm)

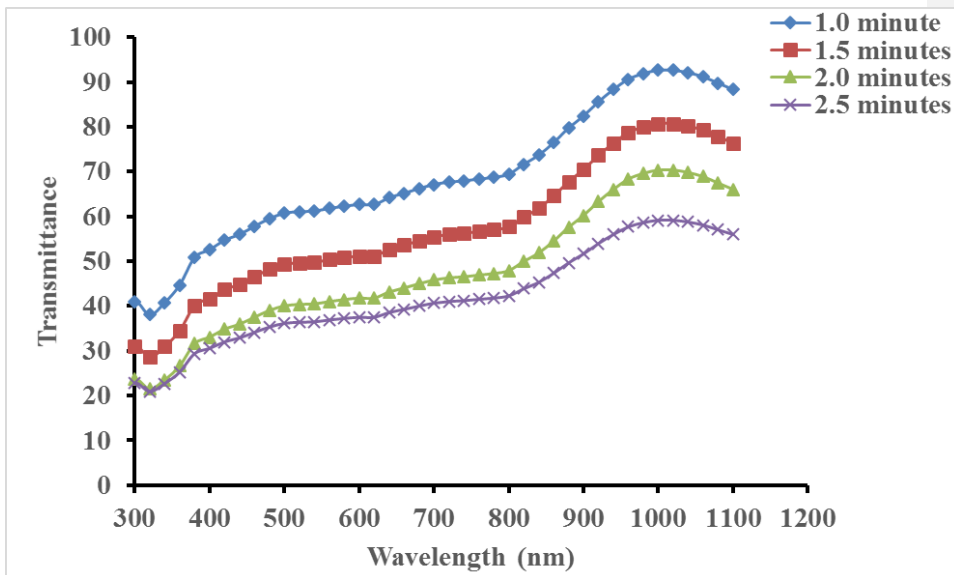


Figure 2. Plot of Percentage Transmittance against Wavelength (nm)

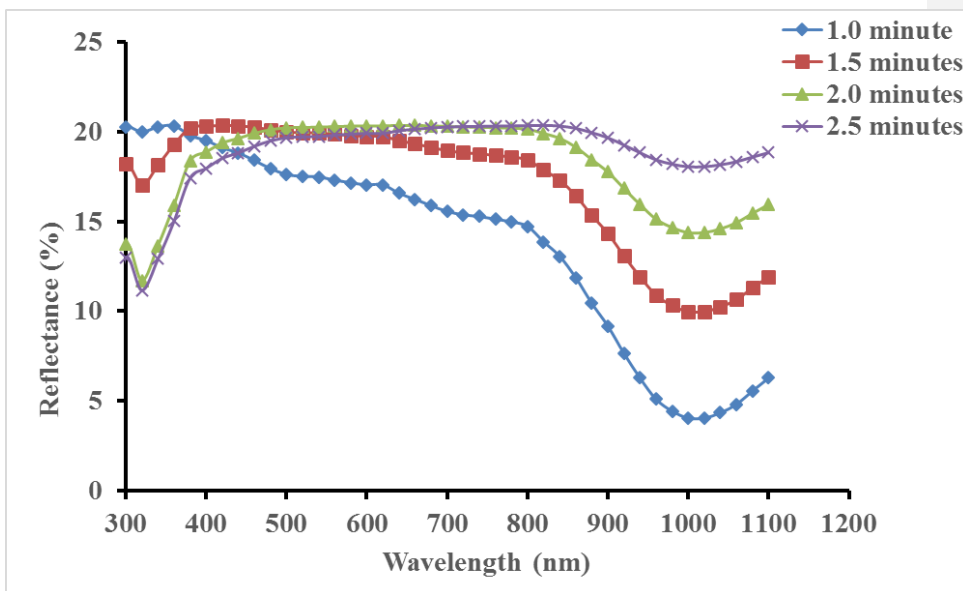


Figure 3. Plot of Reflectance (%) against Wavelength (nm)

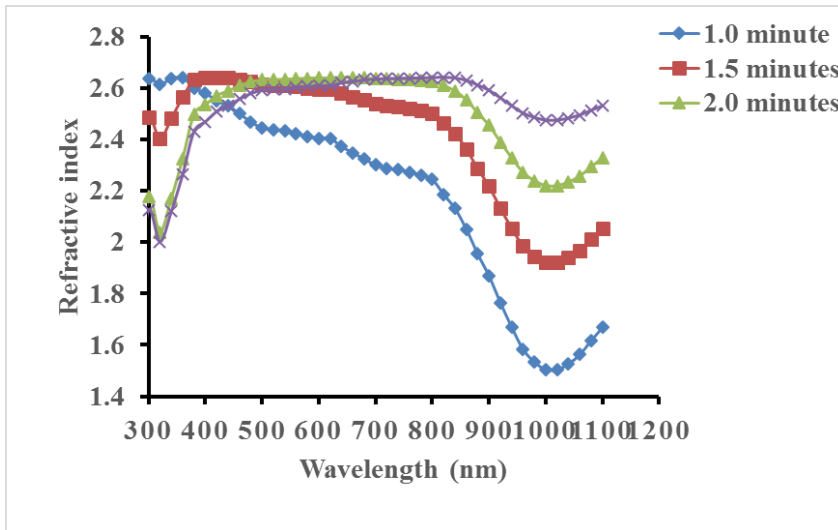


Figure 4. Plot of Refractive Index against Wavelength (nm)

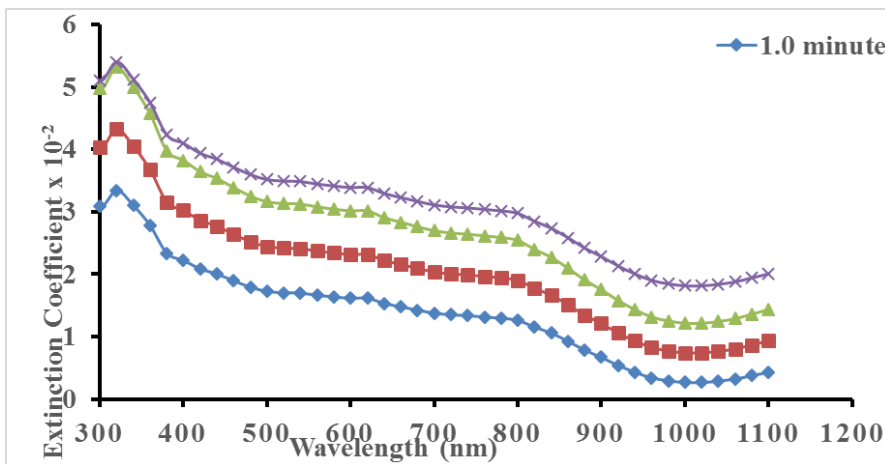


Figure 5. Plot of Extinction Coefficient (10^{-2}) against Wavelength (nm)

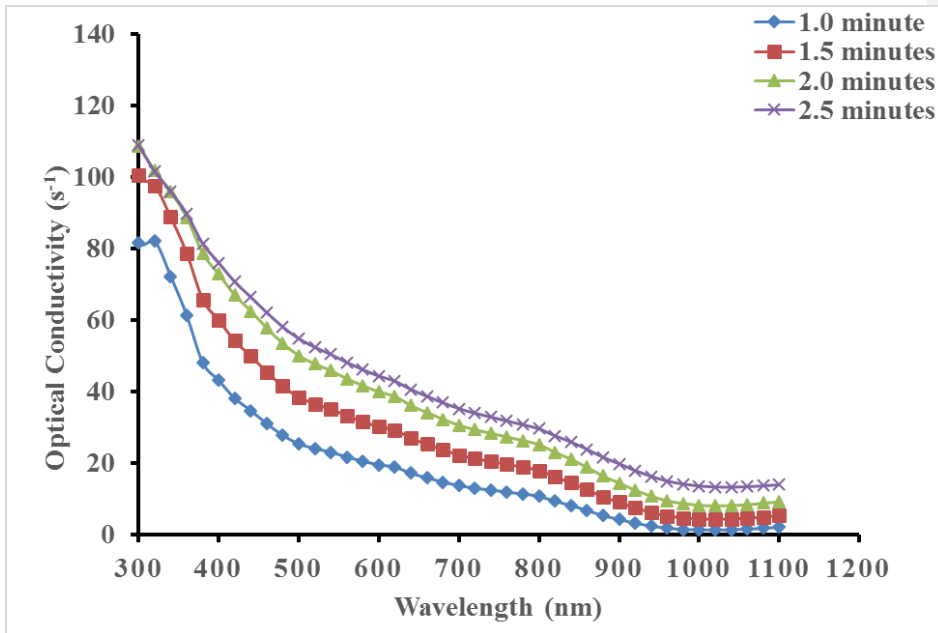


Figure 6. Plot of Optical Conductivity against Wavelength (nm)

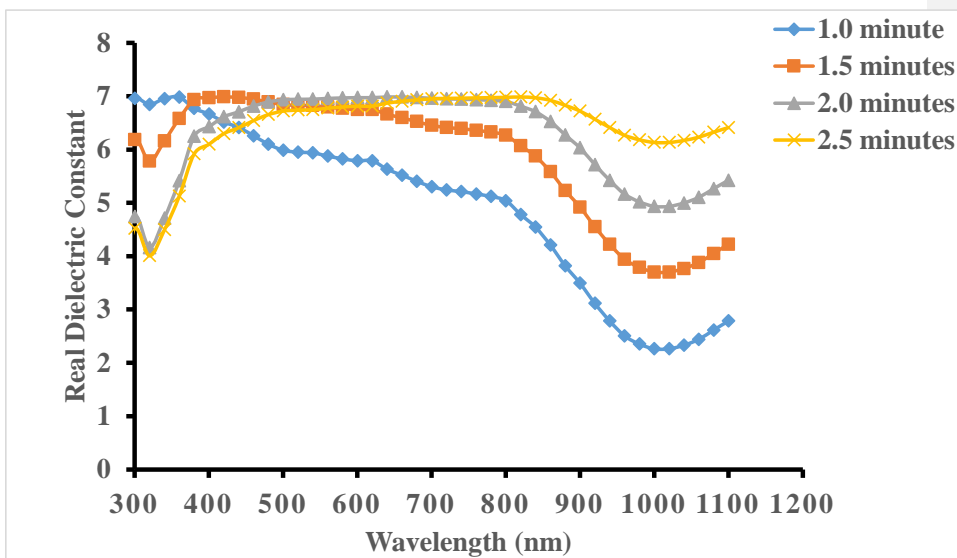


Figure 7. Plot of Real Dielectric Constant against Wavelength (nm)

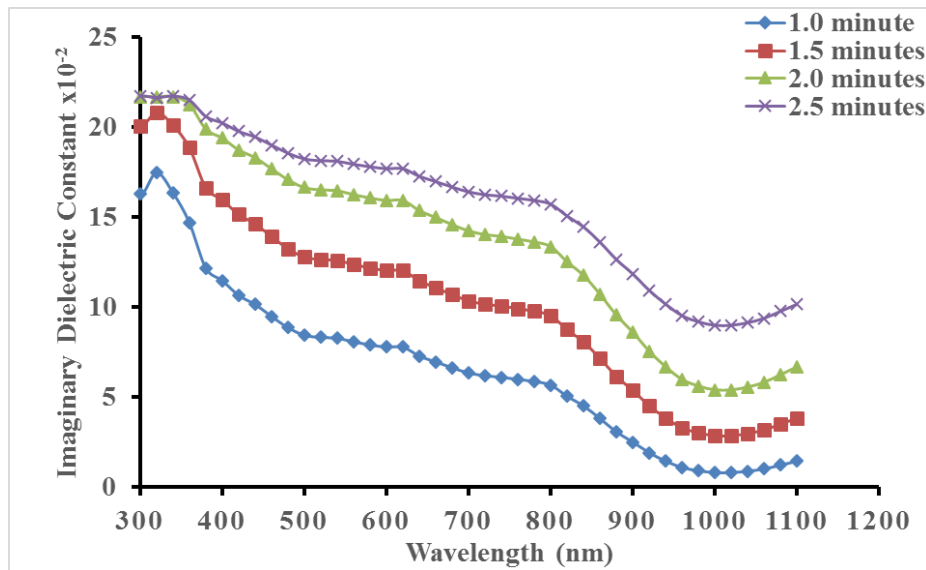


Figure 8. Plot of Imaginary Dielectric Constant against Wavelength (nm).

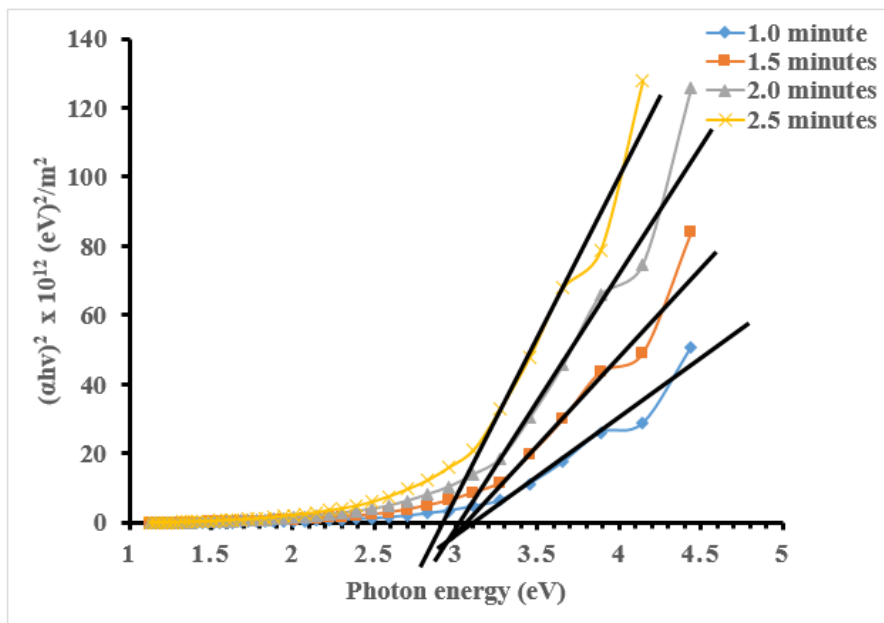


Figure 9. Plot of Absorption Coefficient Squared against Photon Energy (eV)
 Band gap values minutes: 3.17 eV, 1.5 minutes: 3.15 eV, 2.0 minutes: 3.00 eV, 2.5 minutes: 2.90 eV

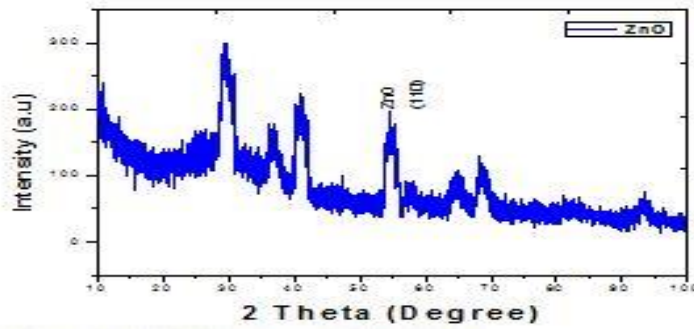


Figure 10. The XRD pattern of ZnO Thin Film

CONCLUSION

It has been shown that zinc oxide can be fabricated using electrodeposition technique. The zinc oxide was characterized using x-ray diffraction (XRD) and UV-VIS-NIR spectrophotometer for structural and optical properties respectively. The deposited thin film shows good optical properties which makes them potential materials for various industrial applications such as optoelectronic devices, solar energy application, and other industries where materials of similar optical properties are needed. The X-ray diffraction analysis of the films deposited at 1.0 minute confirmed that the deposited film is zinc oxide. The crystallite size of the deposited film is 29.14nm which suggest that the films are nano-crystalline in nature.

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ASSESSMENT OF FARMERS UTILIZATION OF YELLOW ROOT (PRO-VITAMIN A) CASSAVA IN ABIA STATE, NIGERIA

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ABSTRACT

This study was conducted among farmers in Abia State to assess the utilization of pro vitamin A (yellow root) cassava. Multi stage sampling technique was used in the study to select the communities and the respondents. Out of the seventeen local government areas(LGA) in Abia state, six were purposively selected for this study as follows; Arochukwu, Bende, Umunneochi, Umuahia north Umuahia south and Isialangwa north Local Government Areas. Two communities and twenty cassava farmers were selected in each of the LGAs. The farmers were interviewed by means of structured questionnaire to elicit information about pro vitamin A cassava utilization. Data collected were analyzed by means of descriptive statistics such as frequency distribution tables percentages, mean and ordinary least square regression analysis. A five point likert type scale was used to calculate the level of utilization of pro vitamin A cassava among the farmers. The result shows a high level of utilization of pro vitamin A cassava with the following mean scores stems(3.52), roots(4.10), fufu(3.95) garri(3.42) and abacha(3.48), slice(2.95) also pro vitamin A cassava was used for flour and chips.. The grand mean of 2.55 showed a low level of utilization of pro vitamin A cassava in the overall assessment of the utilization. The major challenges in pro vitamin A cassava utilization include lack of land, lack of fertilizer, unavailability of finance, lack of herbicides, High labour cost, high cost and inaccessibility of processing equipments, pests and climate change. It was recommended that more promotion of value addition to yellow root cassava be done for increased utilization, also yellow cassava planting materials should be less expensive and available for farmers to utilize the technology more.

Keywords ; Farmers Utilization Yellow pro vitamin Cassava

INTRODUCTION

Cassava (*manihot esculenta*) is a dicotyledonous root crop of the botanical family *euphorbiaceae*, and the major root crop grown in Nigeria. The significance of the crop in tropical agriculture has been recognized in the area of its growth potentials, human and animal food, its enrichment and fortification, industrial uses, economic of production and genetic improvement (Okereke and Ojewola 2005). Nutrition is the key for sustainable economic development and government needs a paradigm shift to adopt new technologies that will maximize the emerging opportunities for better nutrition. Many years of research and development has greatly impacted on the cassava varieties diversity on Nigerian farmers. Research showed that 100 genotypes are grown in farmer's fields in south eastern states of Nigeria (Akoroda 2011). Among root and tuber crops cassava remains the key to food security and poverty reduction in Nigeria as most households are dependent on its production, processing and marketing (Amadi *et al.*, 2011). Previous agricultural programmes in Nigeria have already emphasized high productivity and some efforts in preservation of produce, but little or no attention has been paid to improving the nutritional status of cassava as an energy giving food. Demand- driven research conducted by scientists at National Root Crops Research Institute (NRCRI) Umudike, Nigeria, has led to the development of many improved technologies on cassava with the hope to give the farmers a stable new market along with new production technologies.

Among the developed technologies were cassava stem multiplication technologies, improved cassava varieties and cassava value addition technologies (Nwakor,et al 2016). Cassava is applicable in many types of products such as foods, confectionery, sweeteners, glues, plywood, textiles, paper, biodegradable products and drugs. Cassava is utilized in animal feed and different types of food preparations: gari, fufu, lafun, kpokpogari, tapioca, abacha(FAO and IFAD 2005).The leaves constitute a good vegetable rich in protein, vitamins and minerals.(Lekule and Sarwatt, 2006). Cassava leaves and roots, if properly processed, can provide balanced diet protecting millions of African children against malnutrition. Cassava as the most important root crop grown in Nigeria has potentials in the fight against hunger and food insecurity. Recently, cassava genotypes that have high amounts of pro-vitamin A, have been developed and transferred to farmers by National Root crops Research institute and IITA, for saving thousands of people particularly children from vitamin A deficiency related diseases, such as night blindness, stunting, predisposition to common infections and even death The pro-vitamin A cassava varieties are umucass 36,umucass 37, umucass 38 and others. Egesi (2011) revealed that the next goal of the Bio cassava plus is to stack several genes conferring high level of vitamin A, iron and protein in a single variety of cassava. Since the technology was transferred, no serious study has been done on the utilization by the farmers. The study seeks to analyze

the utilization of yellow roots cassava among farmers in Abia State.

Male	34	28.3
Total	120	100

Specific Objectives of the Study were to

- 1 describe the socio economic characteristics of the respondents
- 2 identify the various yellow roots cassavas grown by farmers
- 3 analyze the level of utilization of yellow root cassava
- 4 identify the constraints militating against utilization of yellow roots cassava

Marital status

Married	90	75.0
Single	22	18.3
Divorced	8	6.7
Total	120	100

Age

21 – 30	32	26.7
31 – 40	28	23.3
41 and above	60	50.0
Total	120	100

Qualification

No formal education	2	1.7
Primary	31	25.8
Secondary	60	50.0
Tertiary	18	15.0
Others	9	7.5
Total	120	100

Household size

1-5	84	70.0
6-10	34	28.3
10 and above	2	1.7
Total	120	100

Farming experience

1-9 years	16	13.3
10-19years	46	38.3
20-29years	26	21.7
30years and above	32	26.7
Total	120	100

Cooperative membership

Yes	90	75.0
No	30	25.0
Total	120	100

Source: Field survey, 2019.

METHODOLOGY

The study was conducted among farmers in Abia Nigeria. The state is made up of seventeen LGAs. Multistage sampling technique was used for the study. In the first stage six Local Government Areas Arochukwu, Bende, Umuahia South, Isialangwa North, Umunneochi and Umuahia North were purposively selected, followed by random selection of 2 communities in each LGA. Ten cassava farmers were purposively selected in these communities. Making it a total number of 120 farmers selected for this study. A well structured questionnaire was use to elicit information from the farmers. Data collected were analyzed by means of descriptive statistics such as frequency distribution tables, percentages and mean . Also a five point likert scale type was used to analyze the level utilization of yellow root cassava among the respondents (1 +2+3+4+5 =15/5=3.0).

Results and Discussion

The result of the study on utilization of yellow root cassava among farmers in Abia state was discussed and presented here with tables and pictures.

The result in table 1 shows the socioeconomic characteristics of the respondents. The result show that majority (71.7%) of the respondents were female and majority (75.0%) were married, only but (18.3%) were single. Fifty (50%) of the respondents were below 40 years while the remaining 50% were above 40 years of age. The implication is that many young people were cassava farmers in Abia State and this will lead to increase production and utilization of yellow root in the study area. Majority (50%) of the farmers attended

Socio Economic Characteristics of the Respondents

Table 1: Distribution of Respondents According to Socio Economic Characteristics

Variable	Frequency	Percentage
Gender		
Female	86	71.7

secondary education level whereas many pass through primary (25.8%), only (15%) of the farmers had tertiary education. The implication is that the farmers were educated and this may lead to increase utilization of yellow root cassava. Majority (70%).The respondents have a moderate household size of 1-5 while (28.3%) have large household size of 6-10. Most(38.3%) of these farmers have 10-19 years farming experience..

Table 2: Distribution of Respondents According to the Varieties of Yellow Root Cassava Used

Yellow root cassava	Frequency	Percentage
Umucass 36	45	37.5
Umucass 37	14	11.7
Umucass 38	34	28.3
Umucass 44	15	12.5
Umucass 45	2	1.7
Others	10	8.3
Total	120	100
Started Time of Usage		
2014	50	47.7
2016	30	25.0
2018	40	33.3
Total	120	100

Source: Field Survey 2019

Result in table 2 shows the various varieties of yellow root cassava grown by farmers in Abia State. The result shows that the largest yellow root cassava grown among the farmers was Umucass 36 (37.5%) followed by Umucass 38 (28.3%). About (11.7%) of the respondents grew Umucass 37 while 12.5% used Umucass 44. This result showed that the farmers were growing yellow root cassava varieties but still in small proportions. Majority (47.7%) started to use Pro vitamin A cassava since 2014, while many started using it around 2018 (33.3) and others(25.0) 2016.

The result shows the highest utilization of yellow cassava roots with the mean score (4.10) for different purposes. The mean score of (3.95) shows that many used yellow root for fufu. The mean of 3.52 and 3.42 shows that there was high utilization of yellow root stems and yellow root cassava for gari. The result also shows that yellow root cassava was highly used for abacha/flakes with the mean of (3.48). Also the utilization of yellow root for slice (2.53) was moderate. There was low utilization of yellow root for flour(1.78)

bread(1.08) chinchin, doughnut and cassava ball with the mean of 1.98, 1.78 and 1.90 respectively. The grand mean of 2.55 showed a low level of utilization of Pro vitamin A cassava(yellow root cassava) among farmers in Abia state.

Table 4. presents the challenges/constraints militating against the use of yellow roots cassava in the study area which includes: lack of land, lack of fertilizer, unavailability of finance, lack of herbicide, poor yield, inaccessibility of modern processing technology, High labour cost, high cost of processing equipment, pest and diseases, climate change, storage problems, poor marketing of roots, poor marketing of stem, theft, poor processing value, fragmentation of land and inaccessibility of credit facilities. The frequency was based on multiple responses, which means that a respondents might be facing more than one challenges/constraints while utilizing yellow root cassava. However, lack of fertilizer was ranked 1st, indicating that 81.7 % of the respondents did not use yellow root cassava due to lack of fertilizer. High labour cost ranked 2nd which indicates that 71.7%, of the farmers saw high cost of labour as their problem, while unavailability of finance was ranked 3rd indicating that 65.8% of the respondents saw unavailability of money as their major constraint. Low processing value(65.0%) and inaccessibility of modern processing equipments(60.0%) ranked 4th and 5th. While high cost of processing equipments and lack of land ranked 6th an 6th position which indicated that 56.7% and 56.7% of the farmers saw these two variables as their major problems. More so, 56.7%, 52.5%, 48.3%, 42.5%, 31.7%, 23.3%, 20.8%, 15.8%, 15.0% and 13.3% of the respondents ranked lack of herbicide, theft, pest and diseases, inaccessibility of credit facilities and climate change, storage problems, poor yield, poor marketing of roots, poor marketing of stem and fragmentation of land respectively as the challenges/constraints militating against the use of yellow roots cassava in the study area.

Table 3: Distribution of Respondents According to Level of Utilization of Yellow Root Cassava

YELLOW ROOT PRODUCTS	HIU	FIU	UN	HU	NIU	TOTAL	S.SIZE	MEAN
Yellow cassava stem	54(270)	26(104)	2(6)	4(8)	34(34)	422	120	3.52
Yellow cassava root	72(360)	20(80)	0(0)	24(48)	4(4)	492	120	4.10
Yellow cassava fufu	74(370)	14(56)	6(18)	4(8)	22(22)	474	120	3.95
Yellow cassava garri	60(300)	10(40)	4(12)	12(24)	34(34)	410	120	3.42
Yellow root slice	24(120)	28(112)	24(72)	6(12)	38(38)	354	120	2.95
Yellow root abacha	32(160)	36(144)	28(84)	6(12)	18(18)	418	120	3.48
Yellow root flour	2(10)	22(88)	4(12)	12(24)	80(80)	214	120	1.78
Yellow root chips	6(12)	6(24)	16(48)	4(8)	88(88)	174	120	1.45
Yellow root leaves	0(0)	14(56)	44(132)	6(12)	56(56)	256	120	2.13
Yellow root leaves	0(0)	14(56)	44(132)	6(12)	56(56)	256	120	2.13
Cassava bread	0(0)	0(0)	2(6)	5(10)	113(113)	129	120	1.08
Yellow root cassava ball	4(20)	4(16)	44(132)	2(4)	66(66)	238	120	1.98
Yellow root cassava doughnut	0(0)	0(0)	44(132)	6(12)	70(70)	214	120	1.78
Yellow root cassava chinchin	0(0)	0(0)	48(144)	12(24)	60(60)	228	120	1.90
Grand Mean(x)								2.55

Field survey, 2019, HIU= highly in use, FIU=fairly in use, UN= Undecided, HU=highly un-use, NIU=Not in use Decision rule: ≥ 3.0 High < 3.0 = Low

Table 4: Distribution of Respondents According to Challenges/Constraints to the Utilization of Yellow Root Cassava

Challenges / Constraints	Frequency	Percentage	Rank
Lack of land	68	56.7	6 th
Lack of fertilizer	98	81.7	1 st
Unavailability of finance	79	65.8	3 rd
Lack of herbicide	63	52.5	7 th
Poor yield	25	20.8	12 th
Inaccessibility of modern processing equipments	72	60.0	5 th
High labour cost	86	71.7	2 nd
High cost of processing equipment	68	56.7	6 th
Pest and diseases	51	42.5	9 th
Climate change	38	31.7	10 th
Storage problems	28	23.3	11 th
Poor marketing of root	19	15.8	13 th
Poor marketing of stem	18	15.0	14 th
Theft	58	48.3	8 th
low processing value	78	65.0	4 th
Fragmentation of land	16	13.3	15 th
Inaccessibility of credit facilities	38	31.7	10 th

Source: Field survey, 2019 (Multiple response)

herbicides for increase in production. The study also showed that the best utilization of yellow root cassava was seen on fufu, garri and abacha production. There was high acceptability of yellow root for different purposes, Also farmers have started making living from the stems, roots and processed products of yellow root cassava.

CONCLUSION

The study on the utilization of Pro vitamin A cassava was successful and the study shows that farmers in Abia State were planting different varieties of Pro vitamin A cassava. All the farmers were aware of yellow roots and majority had used them in their farms. Some have started adding value to yellow root cassava through processing it into fufu, garri, flour etc. Some have used yellow root cassava flour for cassavita, doughnut, chinchin, akara etc. The level of utilization of Pro vitamin A or Yellow root cassava was still low among the respondents because many farmers complained of unavailability of planting materials and other inputs like fertilizer

RECOMMENDATIONS

Based on the above findings, it was recommended that;

- i. More promotion of yellow root cassava should be done for increased utilization
- ii. Promotion of value addition of yellow root cassava among farmers is needed for increased utilization
- iii. Yellow root planting materials and other inputs should be made available and affordable for farmers

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WIS-ASP 13

EFFECT OF EXPLANT TYPES AND PLANT GROWTH REGULATORS ON CALLUS GENERATION OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich.) Harms

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ABSTRACT

The effect of plant growth regulators (PGR); two auxin types [Naphthalene acetic acid (NAA) and 2,4-Dichloro phenoxy acetic acid (2,4-D)] in combination with cytokinin [6-Benzylaminopurine (BAP)] at twenty different concentrations on callus generation from the leaf, stem and cotyledon of African yam bean (*Sphenostylis stenocarpa*) was investigated. Murashige and Skoog's (MS) medium supplemented with either NAA or 2,4-D in combination with BAP was used as the culture medium. NAA in combination with BAP gave the best callus formation at a concentration of (20 mg/l NAA with 0.1 mg/l BAP) for the leaf explant and (10 mg/l NAA with 0.1 mg/l BAP) for the stem explant. 2,4-D in combination with BAP gave the best callus formation for the cotyledon explant at a concentration of (10 mg/l NAA with 0.1 mg/l BAP). At these optimal concentrations, creamy and friable calli were observed from the cultured leaf and stem explants, while the callus from the cotyledon explant was creamy and compact. After 42 days of culture initiation the calli formed from the leaf, stem and cotyledon explants weighed 0.52 g, 0.49 g and 0.51 g respectively. No callus was formed from treatment 1 (MS medium without PGR), treatment 6 (0.1 mg/l BAP) and treatment 11 (1 mg/l BAP) but at treatment 6 (0.1 mg/l BAP) roots were formed from the stem explant. Hundred per cent callus formation were observed at all the optimal concentrations. Hence, the use of leaf explant for callus generation of African yam bean is recommended.

KEYWORDS: Callus, Explant, African Yam Bean, Plant Growth Regulators.

INTRODUCTION

African yam bean (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich.) Harms is an indigenous under-utilized food legume crop in the tropics. It belongs to the family Fabaceae, subfamily Papilionoideae and genus *Sphenostylis*. It is common in Central and Western Africa, especially Cameroon, Cote D' Ivoire, Ghana, Nigeria and Togo (Potter, 1992). AYB like every other legume can derive adequate nitrogen through atmospheric fixation (Assefa and Kleiner, 1997). The protein in the tuber of AYB is more than twice the protein in sweet potato (*Ipomea batatas*) or Irish potato (*Solanum tuberosum*) (NRC, 1979) and higher than those in yam and cassava (Amoatey *et al.*, 2000). Moreover, the amino acid values in AYB seeds are higher than those in pigeon pea, cowpea and bambara groundnut (Uguru and Madukaife, 2001). The inherent lectin in the seeds of AYB has been reported to have insecticidal effects on the cowpea beetle (*Callosobruchus maculatus*) and pod-sucking bug (*Clavigralla tomentosicollis*) (Okeola and Machuka, 2001).

Legumes are the most important group of crop plants next to cereals and they are very recalcitrant to tissue culture regeneration. However, studies with other grain legumes include; black gram (*Vigna mungo*) (Saini *et al.*, 2003), soybean (*Glycine max*) (Samoylov *et al.*, 1998), cowpea (*Vigna unguiculata*) (Popelka *et al.*, 2006; Solleti *et al.*, 2008) pigeon pea (*Cajanus cajan*) (Dayal *et al.*, 2003; Thu *et al.*, 2003), adzuki bean (*Vigna angularis*) (Chitra and Padmaja, 2002)

and mung bean (*Vigna radiata*) (Avenido and Hattori, 2001; Devi *et al.*, 2004), have demonstrated the effectiveness of *in vitro* techniques as propagation tools thus offering hope for application in the propagation of African yam bean. Cultivation and utilization of AYB is limited due to the presence of anti-nutritional factors, long cooking time and low seed yield. These limitations could be overcome through genetic improvement of the crop. There is however, a dearth of information on the genetic improvement of African yam bean through conventional and biotechnological methods. Genetic transformations via tissue culture techniques offer viable crop improvement options through somaclonal variation wherein desirable changes are manifested by plantlets regenerated from different explants via a callus phase (Ogunbodede and Novak, 1998). An essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement of crop plants through genetic transformation is the availability of a reliable protocol for callus induction and plant regeneration (Wang *et al.*, 2011; Yadav and Padmaja, 2003).

The present study was designed to develop an efficient and reliable protocol for callus generation from African yam bean with a view to developing a protocol for *in vitro* regeneration of African yam bean. The objectives of the study were to determine the most suitable auxin type, the optimal concentration of plant growth regulators and the best explant type that can be used for callus generation of African yam bean.

MATERIALS AND METHODS

The experiment was carried out in the Tissue Culture Laboratory of the Physiology Division of Nigerian Institute for Oil Palm Research (NIFOR), Edo State.

The black coloured variety of African yam bean seeds were purchased from New Benin market in Benin City, Edo State. The wholesomeness and quality of the seeds were determined by soaking the seeds in distilled water. The floating seeds were regarded as non-viable ones and were discarded. Some of the viable seeds were incubated at room temperature on moistened tissue paper. After two weeks the emergent leaf and stem were cut into portions and used as explants in addition to the cotyledon (seed with the seed coat removed) of the African yam bean. Thus in this study the leaf, stem and cotyledon were used as the explants for callus generation.

Murashige and Skoog (MS) (1962) medium containing plant growth regulators at different concentrations and combinations were prepared. The pH of the individual treatment was adjusted to 5.8 and 0.3 g plant agar was added. The media in the culture tubes were sterilized by autoclaving at 120 °C (15 psi) for 20 minutes. The explants were also sterilized using 0.1 % (w/v) mercuric chloride (HgCl₂) sterilant and rinsed aseptically thrice with sterile distilled water. The sterile explants were aseptically cut into portions of about 1 cm in length and inoculated on the autoclaved solidified medium in the test tubes and McCartney bottles. The cultures were incubated in the dark tissue culture room at 25 ± 2 °C

The experiment was arranged in 2 auxins/BAP × 20 treatments × 3 replicates × 3 explant factorial in a completely randomized design. The time of response, texture of callus formed, callus colour, weight of callus formed after 42 days of culture initiation and percentage of callusing explants were the parameters evaluated.

RESULT AND DISCUSSION

The results obtained when the leaf, stem and cotyledon explants of AYB were cultured in MS medium supplemented with NAA in combination with BAP is shown in Table 1. It was observed that all the cultured leaf explants produced calli within 5-7 days at all concentrations, indicating the positive response of AYB leaf to NAA, except at treatments 1, 6 and 11 which did not produced callus. This is because treatment 1 do not contain plant growth regulator, while treatments 6 and 11 contained BAP alone at very low concentrations of 0.1 mg/l and 1 mg/l respectively. Skoog and Miller (1957) reported that both an auxin and cytokinin are usually added to culture media in order to obtain morphogenesis. A striking observation

was the formation of roots from the stem explants at treatment 6 (Figure 1a), which contained only 0.1 mg/l BAP without no auxin; as auxins are known to stimulate roots formation from shoot apices and shoot tip cultures. Bahram *et al.* (2005) found that NAA enhanced callusing from leaf of legumes. The fastest response was observed at treatment 10 in the leaf explant as callus was produced in 5 days while the slowest response was observed at treatment 19 in the cotyledon as callus was produced in 12 days. Thus NAA showed a poor response for callus generation from African yam bean using the cotyledon explants. NAA has been reported to show poor response for the callus induction from *Arachis hypogea* using the cotyledonary explant (Iqbal *et al.*, 2011). The differences in time of callus formation across the various explant types could be due to the fact that the leaf and stem contains actively dividing and meristematic cells that can easily and quickly undergo differentiation compared to the cotyledon that is a storage organ. This statement is supported with the findings of Fitch and Moore (1993). They expressed higher callus formation in meristems attributed to easier differentiation of these areas and that this may be due to the differences in the genotype and /or inherent characteristics of the cotyledon explants; as the cotyledons do not contain loose cells that are actively dividing and capable of forming friable callus. The calli formed from the cotyledon explants were all compact in texture. For the leaf and stem explants; at lower concentrations of BAP, friable calli were formed but higher concentrations of BAP gave rise to compact calli. The leaf explants resulted in green/yellow friable calli at 15 mg/l NAA, with no or little BAP (treatment 4 and 9). A creamy/white friable callus was formed at 20 mg/l NAA containing no or little BAP (treatments 5 and 10) from the stem explant. Brown/white compact callus was formed when 10 mg/l BAP in combination with NAA was used for the leaf explant. At treatment 16 (10 mg/l BAP), brown and compact calli were formed irrespective of the explant types used. Gurel *et al.* (2001) suggested that the organogenic potential is related to callus structures. The highest calli weight values obtained from the leaf (FIGURE 2A), stem (FIGURE 2B) and cotyledon (FIGURE 2C) explants were 0.52 g (at 20 mg/l NAA, 0.1 mg/l BAP), 0.49 g (at 10 mg/l NAA, 0.1 mg/l BAP) and 0.39 g (at 10 mg/l BAP) respectively. From this result it can be concluded that the best explant for callus generation from African yam bean is the leaf explant. This is in agreement with the report of Godwin *et al.* (1987). They reported that the leaf blades were better explants for callus induction and growth than either petioles or stems in tropical legume. In constrast to this result, Akande *et al.* (2009) reported that highest callus formation was recorded in stem explants.

Table 1: Effect f NAA And BAP on the Callus Generation of African Yam Bean Using Leaf, Stem and Cotyledon Explants

TREATMENT	LEAF						STEM						COTYLEDON					
	NAA	BAP	Time of response	Texture of callus	Colour of callus	Weight (g) of callus	% of callusing explant	Time of response	Texture of callus	Colour of callus	Weight (g) of callus	% of callusing explant	Time of response	Texture of callus	Colour of callus	Weight (g) of callus	% of callusing explant	
1	0	*	*	*	*	0	*	*	*	*	0	*	*	*	*	0		
2	5	7	Fr	Cr	0.13	66.67	*	*	*	*	0	10	Co	Cr	0.11	66.67		
3	10	0	6	Fr	Cr	0.47	100	6	Fr	Cr/wh	0.45	100	*	*	*	*	0	
4	15	7	Fr	Gn/yw	0.18	100	8	Fr	Cr	0.26	100	*	*	*	*	0		
5	20	7	Fr	Cr/wh	0.18	100	7	Fr	Cr/wh	0.13	100	*	*	*	*	0		
6	0	*	*	*	*	0	7	*	*	*	0	*	*	*	*	0		
7	5	6	Fr	Cr	0.39	100	8	Fr	Cr/wh	0.21	66.67	*	*	*	*	0		
8	10	0.1	7	Fr	Cr	0.24	100	6	Fr	Cr/wh	0.49	100	*	*	*	*	0	
9	15	7	Fr	Gn/yw	0.21	100	*	*	*	*	0	*	*	*	*	0		
10	20	5	Fr	Cr	0.52	100	7	Fr	Cr/wh	0.30	66.67	*	*	*	*	0		
11	0	*	*	*	*	0	*	*	*	*	0	*	*	*	*	0		
12	5	6	Fr	Cr/wh	0.34	66.67	7	Fr	Cr	0.29	100	*	*	*	*	0		
13	10	1	6	Fr	Cr	0.34	100	8	Fr	Cr	0.26	100	*	*	*	*	0	
14	15	6	Fr	Cr	0.34	100	9	Fr	Cr	0.21	66.67	*	*	*	*	0		
15	20	6	Fr	Cr	0.34	66.67	8	Fr	Cr	0.13	66.67	11	Co	Cr	0.13	66.67		
16	0	7	Co	Br	0.18	100	7	Co	Br	0.24	100	7	Co	Br	0.39	100		
17	5	7	Co	Br/wh	0.21	100	*	*	*	*	0	*	*	*	*	0		
18	10	1	7	Co	Br/wh	0.16	100	*	*	*	*	0	*	*	*	*	0	
19	15	0	7	Co	Br/wh	0.26	66.67	9	Co	Br/wh	0.08	100	12	Co	Br	0.16	33.33	
20	20	7	Co	Br	0.13	66.67	8	Co	Br	0.07	66.67	*	*	*	*	0		

* = No callus formed; NAA –Naphthalene acetic acid; BAP – Benzyl amino purine; Fr – Friable; Co – Compact; Cr – Creamy; Gn/yw – Greenish yellow; Cr/wh – Creamy white; Br – Brown; Br/wh – Brown/white.

Table 2 shows the results obtained when leaf, stem and cotyledon explants of African yam bean were cultured in MS medium supplemented with 2,4-D in combination with BAP. At treatments 7, 13 and 16 all the explants produced calli, while at treatment 1, 3, 10, 11, 18 and 19, it was noticed that the three explant types had no response. It was also observed that the stem explant produced roots at treatment 6 within 9 days of culture establishment. At treatment 7, the cotyledon had the longest time for callusing of 12 days. The calli formed from the cotyledon explants were all compact. It was observed that creamy and friable calli were formed from the cultured leaf and stem explants

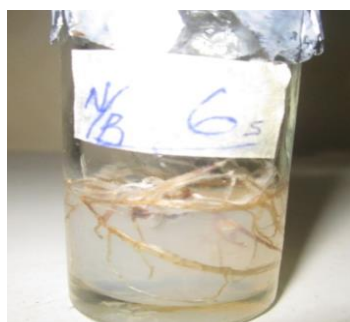
at BAP concentration of 0, 0.1 and 1 mg/l in combination with 2,4-D. Brown and compact callus was noticed at treatment 16 (10 mg/l BAP) and above for all the explants used. The highest callus mean weight of 0.51 g was recorded from the cotyledon explant (FIGURE 2C) at treatment 8 (10 mg/l 2, 4-D, 0.1 mg/l BAP), compared to that produced from the leaf and stem explants. Thus cotyledon explant could be said to have a high affinity for 2,4-D hormone. Research also revealed that 2,4-D has critical role for callus induction from the cotyledon of *Lathyrus sativus* (Misra, 1996; Misra *et al.*, 1994).

Table 2: Effect of 2,4-D And BAP on the Callus Generation of African Yam Bean Using Leaf, Stem and

Cotyledon Explants

TREATMENT	LEAF						STEM						COTYLEDON					
	2,4-D	BAP	Time of response (days)	Texture of callus	Colour of callus	Weight (g) of callus	% of callusing explant	Time of response (days)	Texture of callus	Colour of callus	Weight (g) of callus	% of callusing explant	Time of response (days)	Texture of callus	Colour of callus	Weight (g) of callus	% of callusing explant	
1	0	*	*	*	*	0	*	*	*	*	0	*	*	*	*	0		
2	5		9	Fr	Cr	0.05	66.67	*	*	*	*	0	*	*	*	*	0	
3	10	0	*	*	*	*	0	*	*	*	*	0	*	*	*	*	0	
4	15		*	*	*	*	0	*	*	*	*	0	9	Co	Cr	0.18	100	
5	20		*	*	*	*	0	*	*	*	*	0	10	Co	Cr	0.21	100	
6	0		*	*	*	*	0	9	*	*	*	0	*	*	*	*	0	
7	5		9	Fr	Cr	0.06	66.67	8	Fr	Cr	0.05	66.67	12	Co	Cr	0.28	100	
8	10	0.1	*	*	*	*	0	7	Fr	Cr	0.08	100	7	Co	Cr	0.51	100	
9	15		*	*	*	*	0	*	*	*	*	0	8	Co	Cr	0.29	66.67	
10	20		*	*	*	*	0	*	*	*	*	0	*	*	*	*	0	
11	0		*	*	*	*	0	*	*	*	*	0	*	*	*	*	0	
12	5		8	Fr	Cr	0.13	66.67	8	Fr	Cr	0.09	100	*	*	*	*	0	
13	10	1	8	Fr	Cr	0.13	100	9	Fr	Cr	0.11	100	9	Co	Cr	0.28	66.67	
14	15		*	*	*	*	0	*	*	*	*	0	9	Co	Cr	0.28	66.67	
15	20		*	*	*	*	0	*	*	*	*	0	10	Co	Cr	0.19	100	
16	0		7	Co	Br	0.26	100	7	Co	Br	0.39	100	7	Co	Br	0.16	100	
17	5		9	Co	Br	0.05	66.67	*	*	*	*	0	*	*	*	*	0	
18	10	10	*	*	*	*	0	*	*	*	*	0	*	*	*	*	0	
19	15		*	*	*	*	0	*	*	*	*	0	*	*	*	*	0	
20	20		9	Co	Br	0.05	100	*	*	*	*	0	11	Co	Br	0.08	66.67	

* = No callus formed; 2,4-D - 2,4-Diphenoxy acetic acid; BAP - Benyl amino purine; Fr - Friable; Co - Compact; Cr - Creamy; Br - Brown.



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a

b

Figure 1a and 1b: Roots Formed From Stem Explant in MS Medium Supplemented With 0.1 mg/l BAP

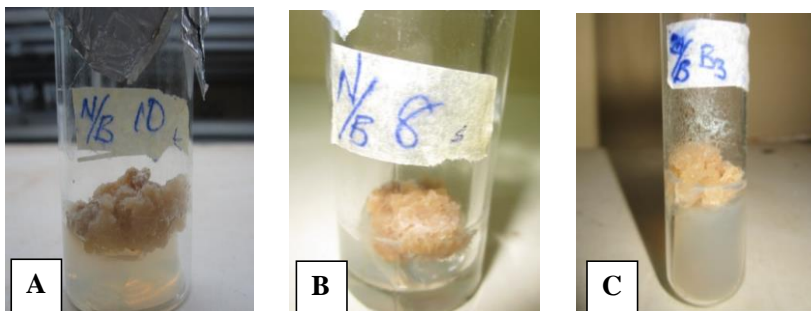


Figure 2A, 2B and 2C: The Best Calli Formed From Leaf, Stem and Cotyledon Explants of African Yam Bean
A- Callus from leaf explant; B- Callus from stem explant; C- Callus from cotyledon explant

CONCLUSION

NAA proved to be the most suitable auxin for callus generation from African yam bean leaf and stem explants when used in combination with 0.1 mg/l BAP at concentrations of 20 mg/l NAA and 10 mg/l NAA respectively; while 10 mg/l 2,4-D in combination with

0.1 mg/l BAP was most suitable for callus generation from the cotyledon explant of African yam bean. At these optimal concentrations, 100 % callusing explant were observed. Hence, the use of leaf explant for callus regeneration of African yam bean is recommended.

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WIS-ASP14

EVALUATION OF NUTRIENTS STATUS OF SOILS OF NRCRI, NYANYA SUB-STATION ABUJA FOR INCREASED CASSAVA PRODUCTION

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ABSTRACT

Soil managers recognize that soil degradation and nutrient depletion pose a serious threat to cassava production which require in depth understanding of available resources and their alternative uses and amendment's responds. Nutrient status of soils of National Root Crops Research Institute Nyanya sub-station Federal Capital Territory, Abuja Nigeria were evaluated to ascertain their present status and suggest management practices needed for an increased cassava yield. Soils were sampled at 0 – 15 cm depth and a total of 60 composite samples were collected and analyzed in laboratory. The results revealed that the soils had a mean pH value of 6.0, exchangeable Ca (9.20cmol kg⁻¹), exchangeable Mg (5.40 cmol kg⁻¹), exchangeable Na (1.55 cmol kg⁻¹), exchangeable K (0.58 cmol kg⁻¹), organic carbon (8.13 gkg⁻¹), total N (0.78 gkg⁻¹), available P (7.31 mg kg⁻¹). For increased cassava yield in the soils studied, some good management practices such as increasing the organic carbon status of the soils and application of deficient nutrient elements are recommended

Keywords: Cassava, Management, Soils.

INTRODUCTION

Cassava (*Manihot esculanta*) plays a major role in the economy of Nigeria, as it supplies more than half the calorie intake of the inhabitants (Abamet *et al.*, 2006). The country is the largest producer in the world, with an annual production rate of 59.6 million metric tons (FAO, 2017). It has diverse uses; it is principally used as human food, where it provides the major source of dietary energy for well over 200 million people in Africa. It can be processed into gari, fufu, farinhnade, mandioca, flour, chips and starch. Cassava chips, pellets and leaves are important in animal feed industry. Its starch is used in glucose, textiles and confectionery industries, as well as in food industry. It is a major source of cash income for households, as it generates cash income for a large number of them in comparison with other staple crops, thereby contributing to poverty alleviation (Onyekwere *et al.*, 2018).

The annual production rate of this important economic commodity crop by Nigeria as stated earlier is not as a result of its yield per unit area in farmers' field compared to yield obtained from countries like Brazil, China and Thailand. The production rate is due to large expanse of land subjected to its production. Presently, it has been observed that cassava yield in farmer's field in Nigeria is less than 9 t ha⁻¹. This is as a result of some factors such as declining soil fertility, pests and diseases, use of unimproved cassava varieties and weed infestation. Among all factors the most important is declining soil fertility. To get a meaningful yield of cassava from farmers' fields, the soil fertility needs to be managed to improve the soil resource base. Soil fertility management has resulted to increase in cassava root yield in experimental fields (Chairoji Wongwiwachta *et al.* 2017).

Declining soil fertility is a major problem militating

against increase in cassava yield in Nigeria (Reinhardt 2017). For Nigeria to cope with the increase in the present demand and consumption of this commodity crop, soil fertility must be tackled to enhance productivity. Making sustainable research on effort towards improving soil fertility status for yield increase is therefore necessary.

Soil fertility decline could be alleviated through fertilizer application, for fertilizer application to be effective the type and quality of fertilizer must depend on soil test result, otherwise soil abuse and low yield may be the result. The objectives of this to determine the nutrients status of soils of National Root Crops Research Institute Nyanya sub-station Federal Capital Territory Abuja, Nigeria and to determine management practices required for sustainable cassava production on these soils.

MATERIALS AND METHODS

Study area

The study area was National Root Crops Research Institute Nyanya sub-station Federal Capital Territory Abuja, Nigeria. It is located within latitude 7.62318°N and longitude 9.06733°E. The mean annual rainfall of the location is 1,404 mm, with mean annual temperature of 26.74°C and the vegetation is typical of Southern Guinea Savanna zone. The soils are derived from basement parent material.

Sampling scheme

Soil samples were collected at 0-15 cm depth, using soil auger. A total of 60 composite samples were made, labeled and then transported to the soil science laboratory of the National Root Crops Research Institute (NRCRI), Umudike, Abia State for Soil physical and chemical analysis.

Laboratory Analysis.

All the soil samples were air dried, crushed and sieved through a 2 mm mesh and re-sieved through a 0.5 mm mesh for organic carbon and total nitrogen prior to physical and chemical analysis. The samples were then analyzed using standard laboratory methods as contained in the method of soil analysis by International Soil Reference and Information Center and Food and Agricultural Organization. (ISRIC and FAO, 2002).

RESULTS AND DISCUSSION

Physical Properties

The physical properties of the soils of the study area are as shown in Table 1

Particle size distribution.

Sand had the highest fraction with a mean value of 662 gkg⁻¹, followed by silt particle size fraction with a mean value of 225 gkg⁻¹ and clay had the least fraction, with a mean value of 113 gkg⁻¹. The particle size distribution is an indication that the soils originated from coarse parent materials as reported by Onyekwere *et al* (2010).

Textural Classification

The texture of the soils is sandy loam. Generally, the textural classification of these soils agrees with optimum criterion of light medium loam sandy soil (Onyekwere *et al.*, 2009) required for unhindered anchorage and bulking of roots and tubers (including cassava) and for easy harvest. This is an indication that the soil is good for cassava production.

Table 1: Mean values of the Physical properties of the soils studied.

Parameter	Mean value
Sand (gkg ⁻¹)	662
Silt (gkg ⁻¹)	225
Clay (gkg ⁻¹)	113
Textural class	Sandy Loam

Chemical Properties

The results of some chemical properties of the soils of the study area are shown in Table 2. N, P and K are the primary nutrients most commonly demanded by crops in plant nutrition. This explains why most compound fertilizers and fertilizer requirements for the crop (cassava) is based on N, P and K and their results are shown in Table 11.

The soils had exchangeable Na above 0.2 cmolkg⁻¹ regarded as the critical value needed in soils (Onyekwere, *et al.* 2018).

Total N.

The results obtained showed that the total N of the soils studied was low with a mean value of 0.78 gkg⁻¹. The result of total N is a reflection of the organic carbon content of the soils (Onyekwere *et al.*, 2009). Positive response of cassava to applied nitrogen fertilizer is thus expected in these soils.

Available P.

Available phosphorous value of the soils was low, with a mean value of 7.13 mgkg⁻¹. This suggested that the soils will show substantial response to applied phosphorus fertilizer for cassava production.

Potassium.

The exchangeable potassium content of the soils studied was high, with a mean value of 0.58 cmol kg⁻¹. The soils had mean value that is above 0.20 cmol kg⁻¹ value regarded to be the critical exchangeable K level in the soils (Onyekwere, *et al.*, 2018). This is an indication that these soils are good for cassava production.

Soil Reaction. The soil reaction as expressed by pH (H₂O) was moderately acidic with a mean value of 6.0. Agronomically, the mean pH value of the soils is good for cassava cultivation, as it can make room for the availability of both macro and micro nutrients as well enhance the activities of microorganisms in the soils.

Organic Carbon

The Organic carbon content of the soils is low, with a mean value of 8.13gkg⁻¹ Maintenance of a satisfactory organic matter status in these soils is highly essential. Onyekwere and Ezenwa (2009) reported that incorporation of organic residues to the soil will enhance mineralization of most of the nitrogen and half of the phosphorous in the soils, if the cassava fields were unfertilized.

Calcium

The exchangeable calcium of the soils studied was high, with mean value of 9.20 cmol kg⁻¹. Soils of the study area had mean value above 4 cmol kg⁻¹ regarded as lower limit for fertile soils (Onyekwere *et.al.* 2001)

Magnesium

The exchangeable magnesium content of the soils studied was high 5.40 cmol kg⁻¹., the soils were well endowed with exchangeable magnesium.

Sodium

The exchangeable sodium content of the soils was high, with a mean value of 1.59 cmolkg⁻¹.

Table 2: Mean values of the chemical properties of the soils studied.

Parameter	Meanvalue
pH	6.0
Organic Carbon	8.13

(gkg ⁻¹)	
Total Nitrogen (gkg ⁻¹)	0.78
Exchangeable Ca (cmol kg ⁻¹ .)	9.20
Exchangeable Mg (cmol kg ⁻¹ .)	5.40
Exchangeable K (cmol kg ⁻¹ .)	0.58
Exchangeable Na (cmol kg ⁻¹ .)	1.55
Exchangeable Acidity (cmol kg ⁻¹ .)	0.14
Avaiable P (mgkg ⁻¹)	7.31

indications: That the textural classification and the soil reaction of the soils are ideal for cassava production, they can give room for the availability and uptake of both micro and macro nutrients.

That, the soils are further characterized by low organic carbon, high exchangeable Ca, Mg and Na and low primary nutrients apart from potassium. Based on these finding, incorporation of crop residue and other organic inputs and application of 90 kg N/ha and 20 Kg P₂O₅ /ha fertilizer are suggested for an increased cassava yield on the soils studied

CONCLUSION

The findings in this study gave the following

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WIS-ASP 15 FL

ASSESSING THE PREVALENCE OF MAJOR GROSS LESIONS IN EDIBLE BOVINE OFFALS IN UBAKALA SLAUGHTER SLAB, ABIA STATE, NIGERIA

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ABSTRACT

Postmortem examination was carried out on some offal of cattle slaughtered at the slab by visual inspection, palpation and incision on the organs according to procedures recommended by Food and Agricultural Organization. A total of 1,231 slaughtered cattle were sampled for various gross lesions in their offals and Three hundred and ninety nine (32.4%) were found to have gross pathological lesions. Majority of the cattle were more than 5 years (79.4%) while 19.8% were between 3-5 years and 0.8% were less than 3 years of age. The body conditions of the animals varied greatly as 37.6%, 44.1% and 18.3% had good, medium and poor body conditions respectively. The overall prevalence of lesions found in the lungs, liver, intestine and fore stomach was 72.4%, 22.1%, 25.1% and 27.3% respectively. Gross lesions of the lungs ranged from abscess (1.4%), aspiration of blood (41.2%), atelectasis (5.2%), congestion (0.3%), jaundice (0.3%), tuberculosis (7.6%), emphysema (3.1%), pneumonia (40.1%) and mixed infections (0.7%). The affected livers showed gross lesions of fascioliasis (90.9%), cirrhosis (4.5%), tuberculosis (1.1%), liver abscess (1.1%) and mixed infections (2.3%). Pimply gut (96.3%) was the major condition that affected the intestine. Other lesions were tuberculosis (1.8%), congestion (0.9%) and tumors (0.9%). This study identified major gross lesions in the lungs, liver, fore stomach (rumen and reticulum) and intestine of slaughtered cattle that are of public health concern, therefore efforts should be made at farm level to prevent cattle from acquiring these diseases, at ante mortem by removing cattle that show clinical signs of any disease from the lairage and at postmortem by condemning meat and meat products from infected cattle.

KEYWORDS: Gross lesions, bovine, edible offal, slaughter slab

INTRODUCTION

When an animal is diseased, it is usually evident in its affected internal organs popularly called offals. In Nigeria, meat along with meat offal is consumed with various delicacies. These offals are used to prepare a wide variety of meals, soups and stews. The habit of selling visibly infected meat and meat offals such as liver, lungs, intestines and heart to the general public for consumption is unhealthy because these infections can be transferred to humans (Kikiopé, 2017).

Observations and information gotten at slaughter houses can contribute to the understanding of animal diseases and pathological examination represents an important tool in making diagnosis within the slaughter line (Raji *et al.*, 2010) because the abattoir is considered as the biggest laboratory on the earth (Gracey *et al.*, 1999).

The main aim of this study is to identify the prevalent gross lesions seen in lungs, liver, fore stomach (rumen and reticulum) and intestine which constitutes the offal of cattle slaughtered at Ubakala Slaughter slab which will help in monitoring diseases/conditions that are of public health importance.

MATERIALS AND METHODS

STUDY AREA

The study was conducted at Ubakala abattoir in Umuahia South Local Government Area (LGA) of Abia State, Nigeria. The geographic location of this site is between longitude 7° 24' E and latitude 5° 10' N the geographical map of Nigeria. The slaughter house is owned by Abia State Government and managed by the State Ministry of Agriculture and Natural Resources.

STUDY POPULATION AND DESIGN

A total of 1,231 cattle were slaughtered at Ubakala during the period of four months. The animals were inspected at postmortem. The body condition of each animal before slaughter was assessed and recorded. Based on their body condition score, animals were ranked into three (3) main groups: lean, medium, and fat (Nicholson and Butterworth, 1996). The dentition of the animal was used to determine their age based on eruption and wear of the incisor teeth as described by Otesile and Obasaju (1982). Cattle were grouped into 3 groups; < 3 years, 3-5 years and > 5 years.

During the study period, postmortem examination of cattle slaughtered was made and data was recorded for each animal examined. Pictures were taken where necessary. Postmortem examination was carried out by visual inspection, palpation and systemic incision (where necessary) on the organs according to

procedures recommended by Food and Agricultural Organization (1994).

STATISTICAL ANALYSIS

Data on lesions seen in the various organs at postmortem inspection were entered into Microsoft Excel 2007 computer program. The data was analyzed using SPSS for windows version 20.0. Descriptive statistics was used to determine the frequencies and percentages of prevalence of the lesions seen in the various organs. The association and effect of different explanatory variables (age, sex and body condition score) on the prevalence and distribution of lesions were analyzed using chi-square. A statistically significant association among variables was considered to exist if p value was less than 0.05 (p<0.05) at 95% confidence level.

RESULTS

The age, sex and body condition score of slaughtered cattle as shown in table 1, 1,231 cattle were slaughtered. Result of cattle screened for pathological gross lesions during post mortem examination showed that 399 (32.4%) had gross pathological lesions. Majority of the cattle were > than 5years (79.4%) while 19.8% were between 3- 5 years and 0.8% were less than 3 years of age. More males (54.6%) were slaughtered than females (45.4%). The body conditions of the animals also varied greatly as 37.6%, 44.1% and 18.3% had good, medium and poor body conditions respectively. The overall prevalence of lesions found in the lungs, liver, intestine and fore stomach was 72.4% , 22.1%, 25.1% and 27.3% respectively.

Table 1: Frequency and percentage of the age, sex and body condition of slaughtered cattle

VARIABLES	FREQUENCY	PERCENTAGE (%)
AGE(YEARS)		
< 3	3	0.8
3 – 5	79	19.8
> 5	317	79.4
SEX		
Male	218	54.6
Female	181	45.4
BODY CONDITION		
Fat	150	37.6
Medium	176	44.1
Lean	73	18.3

TOTAL	399	100
Gross lesions found in the lungs of slaughtered cattle		

In table 2, Two hundred and eighty nine (289) lungs had various gross lesions or disease conditions. The overall prevalence of gross lesions ranged from abscess (1.4%), aspiration of blood (41.2%), atelectasis (5.2%), congestion (0.3%), jaundice (0.3%), tuberculosis (7.6%), emphysema (3.1%), pneumonia (40.1%) and mixed infections (0.7%).

The male animals were more (54.7%) prone to infections than the females (45.3%) especially abscess, aspiration of blood, congestions and pneumonia while the female were had high prevalence of atelectasis, tuberculosis, emphysema and jaundice. This difference was statistically significant at P < 0.05.

During the study period, animals that were graded to have medium body conditions had more gross lesions than animals with other body conditions. Those with relatively good body conditions had the highest prevalence of atelectasis and pneumonia but did not have gross lesion from tuberculosis while those with poor body conditions had low prevalence of atelectasis but high prevalence of tuberculosis. These differences were statistically significant (P < 0.05).

The older animals (> 5 years) had more gross lesions than the younger animals (< 3 years and 3 – 5 years) and were the only group to have tuberculosis. They were also the only group to have all the gross lesions recorded during the study period. These differences were however not statistically significant (P > 0.05).

Gross lesions found in the liver of slaughtered cattle

Gross lesions found in the liver in relation to the age, sex and body condition of slaughtered cattle are presented in Table 3. The 88 affected livers showed gross lesions of fascioliasis (90.9%), cirrhosis (4.5%), tuberculosis (1.1%), liver abscess (1.1%) and mixed infections (2.3%).

The only case of tuberculosis observed in the liver was seen in a female animal with poor body condition and greater than 5 years of age. The occurrence of cirrhosis and fascioliasis was distributed evenly among the different sexes but fascioliasis was greater in animals with medium body condition while cirrhosis occurred mostly in animals with good body conditions. Fascioliasis occurred mostly in animals older than 5 years. The difference in the prevalence of gross lesions in the liver was not significant statistically.

Table 2: Gross lesions found in the lungs in relation to age, sex and body condition score of slaughtered animals

Variables	No. Affected	Gross lesions of the lungs								
		ABS	AOB	ATE	CONG	TB	EMP	JAU	PNEU	M.I.
Sex										
Female	131 (45.3%)	0(0.0%)	46 (35.1%)	9 (6.9%)	0 (0.0%)	17 (13.0%)	6 (4.6%)	1 (0.8%)	52 (39.7%)	0 (0.0%)
Male	158 (54.7%)	4(2.5%)	73 (46.2%)	6 (3.8%)	1 (0.6%)	5 (3.2%)	3 (1.9%)	0 (0.0%)	64 (40.5%)	2 (1.3%)
Total	289 (100%)	4(1.4%)	119 (41.2%)	15 (5.2%)	1 (0.3%)	22 (7.6%)	9 (3.1%)	1 (0.3%)	116 (40.1%)	2 (0.7%)
Body condition										
Good	114 (39.4%)	2(1.8%)	56 (49.1%)	1 (0.9%)	1 (0.9%)	0 (0.0%)	5 (4.4%)	0 (0.0%)	47 (41.2%)	2 (1.8%)
Medium	120 (41.5%)	2(1.7%)	47 (39.2%)	11 (9.2%)	0 (0.0%)	3 (2.5%)	2 (1.7%)	1 (0.8%)	54 (45.0%)	0 (0.0%)
Poor	55 (19.0%)	0(0.0%)	16 (29.1%)	3 (5.5%)	0 (0.0%)	19 (34.5%)	2 (3.6%)	0 (0.0%)	15 (27.3%)	0 (0.0%)
Total	289 (100%)	4(1.4%)	119 (41.2%)	15 (5.2%)	1 (0.3%)	22 (7.6%)	9 (3.1%)	1 (0.3%)	116 (40.1%)	2 (0.7%)
Age										
< 3 years	2 (0.7%)	0(0.0%)	2 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
3-5 years	60 (20.8%)	0(0.0%)	26 (43.3%)	5 (8.3%)	0 (0.0%)	0 (0.0%)	3 (5.0%)	0 (0.0%)	26 (43.3%)	0 (0.0%)
> 5 years	227 (78.5%)	4(1.8%)	91 (40.1%)	10 (4.4%)	1 (0.4%)	22 (9.7%)	6 (2.6%)	1 (0.4%)	90 (39.6%)	2 (0.9%)
Total	289(100%)	4(1.4%)	119 (41.2%)	15 (5.2%)	1 (0.3%)	22 (7.6%)	9 (3.1%)	1 (0.3%)	116 (40.1%)	2 (0.7%)

ABS; Abscess, AOB; Aspiration of blood, ATE: Atelectasis, CONG: Congestion, TB; Tuberculosis, EMP; Emphysema, JAU; Jaundice, PNEU; Pneumonia, MI; Mixed infection

Table 3: Gross lesions found in the liver in relation to the age, sex and body condition of slaughtered cattle

Variables	No. Affected	GROSS LESIONS OF THE LIVER				
		Cirrhosis	Fascioliasis	Tuberculosis	Abscess	Mixed
Sex						
Female	46 (52.3%)	2(4.3%)	40(87.0%)	1(2.2%)	1(2.2%)	2(4.3%)
Male	42 (47.7%)	2(4.8%)	40(95.2%)	0(0.0%)	0(0.0%)	0(0.0%)
Total	88 (100%)	4(4.5%)	80(90.9%)	1(1.1%)	1(1.1%)	2(2.3%)
Body condition						
Good	30(34.1%)	2(6.7%)	28(93.3%)	0(0.0%)	0(0.0%)	0(0.0%)
Medium	37(42.0%)	1(2.7%)	34(92.0%)	0(0.0%)	1(2.7%)	1(2.7%)
Poor	21(23.9%)	1(4.8%)	18(86.7%)	1(4.8%)	0(0.0%)	1(4.8%)
Total	88(100%)	4(4.5%)	80(90.9%)	1(1.1%)	1(1.1%)	2(2.3%)
Age						
< 3 years	1(1.1%)	0(0.0%)	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)
3 - 5 years	9(10.2%)	0(0.0%)	8(88.9%)	0(0.0%)	0(0.0%)	1(11.1%)
> 5 years	78(88.6%)	4(5.1%)	71(91.0%)	1(1.3%)	1(1.3%)	1(1.3%)
Total	88(100%)	4(4.5%)	80(90.9%)	1(1.1%)	1(1.1%)	2(2.3%)

Gross lesion found in the fore stomach

Table four shows that ninety nine out of the 100 fore stomachs affected with gross lesions and diseases had paraphistomosis while one (1) was congested.

Table 4: Gross lesions found in the fore stomach (rumen and reticulum) in relation to age, sex and body condition score of slaughtered cattle

Variables	No. Affected	Gross lesions of the fore stomach	
		Paraphistomosis	Congestion
Sex	51		
Female	(51.0%)	50 (98.0%)	1(2.0%)
Male	49 (49.0%)	49 (100%)	0 (0.0%)
Total	100 (100%)	99 (99.0%)	1 (1.0%)
Body condition	29		
Good	(29.0%)	29 (100%)	0 (0.0%)
Medium	48 (48.0%)	47 (97.9%)	1(2.1%)
Poor	23 (23.0%)	23 (100%)	0 (0.0%)
Total	100 (100%)	99 (99.0%)	1 (1.0%)
Age			
< 3 years	1(1.0%)	1 (100%)	0 (0.0%)
3 - 5 years	25 (25.0%)	25 (100%)	0(0.0%)
> 5 years	74 (74.0%)	73 (98.6%)	1(1.4%)
Total	100 (100%)	99 (99.0%)	1 (1.0%)

Paraphistomosis had higher prevalence in male and younger animals than females and older animals while congestion was seen more in female and older animals. The occurrence of paraphistomosis and congestion within the various variables was not statistically significant ($P > 0.05$).

Gross lesion found in the intestine of slaughtered cattle

Pimply gut (96.3%) was the major condition that affected the intestine. Other lesions were tuberculosis (1.8%), congestion (0.9%) and tumors (0.9%). A total of 109 intestines had lesions.

Table 5: Gross lesions found in the intestine in relation to the age, sex and body condition score of slaughtered cattle

Variables	No. Affected	Gross lesions of the intestine			
		Tuberculosis	Pimply gut	Congestion	Tumor
Sex	58	1	56	0	1
Female	(53.2%)	(1.7%)	(96.6%)	(0.0%)	(1.7%)
Male	51 (46.8%)	1 (2.0%)	49 (96.1%)	1 (2.0%)	0 (0.0%)
Total	109 (100%)	2 (1.8%)	105 (96.3%)	1 (0.9%)	1 (0.9%)
Body condition	24	0	24	0	0
Good	(22.0%)	(0.0%)	(100%)	(0.0%)	(0.0%)
Medium	50 (45.9%)	0 (0.0%)	48 (96.0%)	1 (2.0%)	1 (2.0%)
Poor	35 (32.1%)	2 (5.7%)	33 (94.3%)	0 (0.0%)	0 (0.0%)
Total	109 (100%)	2 (1.8%)	105 (96.3%)	1 (0.9%)	1 (0.9%)
Age		0	0	0	0
< 3 years	0 (0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
3 - 5 years	17 (15.6%)	0 (0.0%)	16 (94.1%)	1 (5.9%)	0 (0.0%)
> 5 years	92 (84.4%)	2(2.2%)	89 (96.7%)	0 (0.0%)	1 (1.1%)
Total	109 (100%)	2 (1.8%)	105 (96.3%)	1 (0.9%)	1 (0.9%)

All the older animals with poor body conditions had tuberculous lesions on their intestine. Female animals had more lesions on their intestine and the animals with good body conditions had fewer lesions than those in the other groups. The difference in the prevalence of gross lesions in animals with different body conditions was significant ($P < 0.05$) while those of age and sex were not statistically significant.

DISCUSSION

The overall prevalence of 32.4% gross lesions in bovine offals in this study was considered to be quite high when compared with 17.8% prevalence of pathological lesion recorded by Ukwani and Kalu (2019) but lower than 86.2% gotten by Gebrehiwot *et al.*, (2015). The percentage of female animals slaughtered (45.4%) during the course of this study was also high and should be strictly discouraged because it is unethical to slaughter female animals whether pregnant or not (Abdulkadir, 2008; Riehn *et al* 2010).

The 5.2% prevalence of atelectasis in this study could have occurred as a result of overcrowding in the lairage or during transportation, inhalation of dust, exhaustion from long treks (Kebede *et al.*, 2011). Animals with medium body conditions had higher prevalence of atelectasis than those with good and poor body conditions. This could be attributed to man-handling of the animals before slaughter. In this study area, the distance between the lairage and the slaughter slab is more than 10km and these animals are made to run the distance while being flogged.

The prevalence of pulmonary congestion was highest among older male animals and this can be attributed to both improper handling of animals during slaughter and bleeding. This prevalence was lower than the 1.1% stated by Ukweni and Kalu (2019) in their study in Akure.

The overall prevalence of tuberculosis in the lungs (7.6%) in this study was lower than the 55.7%, 39.9% and 9.79% gotten by Ukweni and Kalu (2019), Opara 2005 and Ardo *et al.* 2016 respectively. Most of the animals with poor body conditions had evidence of tuberculosis. The high prevalence of tuberculosis in older animals may be due to exposure of the animals to different etiologic factors throughout their life time (Gebrehiwot *et al.*, 2015). Bovine tuberculosis is among the 7 neglected endemic zoonotic diseases in developing countries and its awareness is low (Ousman *et al.*, 2016). Therefore efforts should be made to create awareness of the disease especially among people butchers and other abattoir workers (Kalu *et al.*, 2019).

The prevalence of abscess in the liver and lungs were 1.1% and 1.4% respectively and was low when compared to the 12.5% reported by Ukweni and Kalu (2019) in bovine lungs. Abscess in the lungs may occur as a result of purulent inflammation in some parts of the body or as local abscess which have developed from purulent bronchopneumonia or a fibrinous pneumonia (Alonge, 2001).

The prevalence of emphysema was lower than the 4.71% obtained by Raji *et al.* (2010). Emphysema is a condition where there is excessive accumulation of air in the alveoli or the presence of air in the normally airless interstitial (Alonge, 2001). Interstitial emphysema may be caused by obstruction of airflow or by extensive gasping respiration during the slaughter process (FAO, 1994; Alonge, 2001). Other factors that may lead to emphysema include suffocation due to overcrowding in the lairage, insufficient rest before slaughter, improper stunning, delayed slaughter after stunning and delayed hoisting after slaughter. Exposure of animals to stress factors like dust, overcrowding and exhaustion from long treks in search of pasture and water during the dry season may also contribute to respiratory conditions (Cadmus and Adesokan, 2009; Fufa and Debele, 2003;

Kusiluka and Kambarage, 1996).

The occurrence of pneumonia in this present study (40.1%) was lower than the 8.79% and 4.5% found by Raji *et al.* (2010) and Ukweni and Kalu (2019) in their studies, respectively. Pneumonia may affect animals that are transported on foot to the abattoir because of transportation stress and starvation in addition to endemic disease such as pasteurellosis and animals having traumatically penetrated lung. The high prevalence of gross pulmonary lesion found in this study showed that the lung is one of the organs which could be affected with different gross lesions resulting in high condemnation rate (Ukweni and Kalu, 2019).

Fascioliasis has recently been shown to be a re-emerging and widespread zoonosis affecting a number of human populations (Mas-Coma *et al.*, 2005; Esteban *et al.*, 2003). Although fascioliasis rarely causes mortalities in cattle, its effects result in reduced production and condemnation of livers during meat inspection in abattoirs (Kambarage *et al.*, 1995), therefore the high prevalence of 90.9% in this study should be of grave concern to both public health and animal production.

Severe cases of paramphistomosis can lead to gastritis, anemia and cachexiation leading to carcass condemnation if there is systemic change (Gracey and Collins, 1992). The 99.0% paramphistomosis in this study may be the reasons for the presence of lesions in other organs.

Pimpily gut was the most frequently encountered gross lesion in the intestines and its occurrence was higher than 2.61% reported by Raji *et al.* (2010).

CONCLUSION

The result of this study identified major gross lesions in the lungs, liver, fore stomach (rumen and reticulum) and intestine of cattle slaughtered in Ubakala abattoir.

The most common gross lesions encountered include pneumonia, pulmonary congestion, fascioliasis and pimpily gut. Information gotten from this study will be useful in monitoring of zoonotic diseases which are acquired through the consumption of infected meat and meat product. This is because these offals are served along with various delicacies both at home and in various eateries and the consumption of these offals places the consumer at risk of acquiring diseases.

A high prevalence of gross pulmonary lesion found in this study shows that lung is one of the organs which could be affected with different gross lesions resulting in high condemnation rate.

For this reason, efforts should be made at farm level to prevent cattle from acquiring these diseases, at antemortem by removing cattle that show clinical signs of any disease from the lairage and at postmortem by condemning meat and meat products from infected cattle.

The findings of this work suggest that, meat inspection practices need some improvement. Therefore meat inspection and proper health hygiene should be encouraged so as to produce wholesome meat for human consumption. The following are hereby recommended:

Meat inspection should be done under the supervision of Veterinarians. In this way, diseased cattle can be identified either at antemortem or postmortem examinations.

Butchers should be compensated when infected cattle are condemned during the process of meat inspection because the condemnation of edible organs represents a significant economic loss to traders and the livestock industry.

Those working in the abattoir should be constantly enlightened through seminars and workshops organized by the state government on the need for only wholesome meat to be passed for human consumption to prevent the spread of zoonotic diseases.

FIGURES

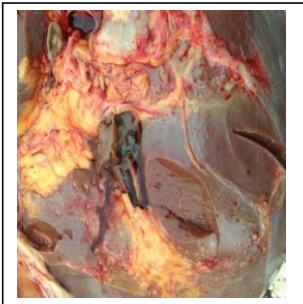


Fig 1: Adult fasciola in the bile duct mesentery



Fig 2: Immature flukes in the liver

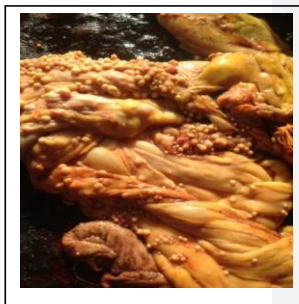


Fig 3: TB lesions in the and intestines

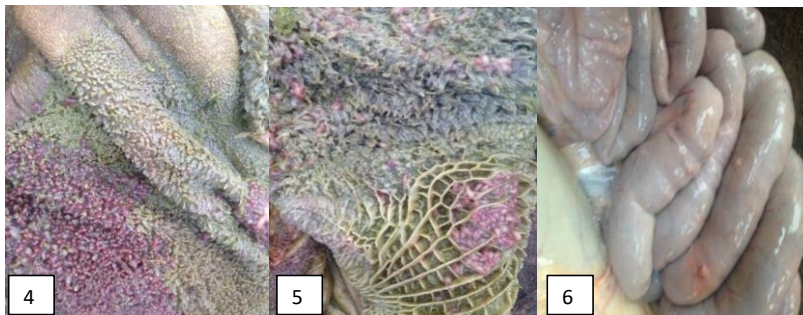


Fig 4: Paramphistomum in the rumen

Fig 5: Paramphistomum in the reticulum

Fig 6: Pimply gut.



Fig 7: Congested lungs Fig 8: Emphysema of the lungs Fig 9: Tuberculous lesions in the lungs

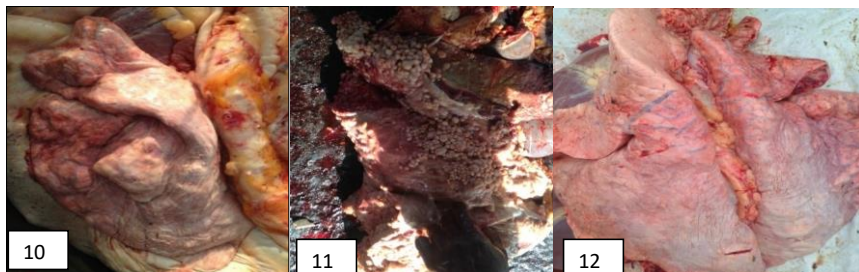


Fig 10: Atelectatic lungs Fig 11: Tuberculous lesion on the pluck Fig 12: Pneumonic lungs

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***In vitro* CONTROL OF *Phytophthora colocasiae* RACIBORSKI CAUSING LEAF BLIGHT OF TARO (*Colocasia esculenta* (L.) Schott.) WITH SELECTED BOTANICAL EXTRACTS**

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ABSTRACT

A pathogenic Oomycetous fungus was isolated from infected taro leaf tissue at the Plant Pathology Laboratory of the National Root Crops Research Institute, Umudike and identified based on morphological and cultural characters as *Phytophthora colocasiae* Raciborski. Four botanical extracts: *Azadirachta indica* A. Juss. *Eucalyptus camaldulensis* Dehn, *Senna alata* L. and *Tithonia diversifolia* (Hemsley) A. Gray were tested *in vitro* for their fungitoxic effects on radial growth and mycelia dry weight of the pathogen at 5, 10 and 20 mg/ml of each extract. Ridomil (0.67 mg/ml) and zero treatment were set along tests and all treatments were replicated thrice in a Completely Randomized Design. All the plant extracts exhibited significant but varying degrees of inhibition of radial growth and mycelia dry weight of the pathogen. Percentage inhibition increased as concentration increased. *A. indica* and *S. alata* extracts at 20 mg/ml inhibits 100 and 97.13% radial growth respectively and was significantly ($P < 0.05$) superior to 40.00 and 31.81% caused by *T. diversifolia* and *E. camaldulensis* respectively. Complete (100%) inhibition of mycelia dry weight was obtained with *S. alata* and *T. diversifolia* extracts at all the selected concentrations while *A. indica* and *E. camaldulensis* showed high inhibition only at some concentrations. These extracts have shown promising inhibitory effects against the *P. colocasiae* and were comparable to the synthetic fungicide Ridomil, therefore, their exploration in the management of TLB is strongly advocated.

Keywords: Oomycetous fungus, *Phytophthora colocasiae*, leaf blight, taro leaf, botanical extracts, fungitoxic

INTRODUCTION

Plant disease has remained a constant factor threatening food security and national economic development worldwide. Overwhelming losses of crops to some epiphytotic and their impacts both remote and recent on the course of human history were documented (Mehrotra and Aggarwal 2003; Strange and Scott, 2005). The character of agricultural practices in Nigeria, fraught with subsistence farming, low productivity, high post-harvest losses, inadequate processing and preservation facilities, dictates rising food insecurity. Yet this scenario is worsened by the outbreak in 2009 of *Phytophthora* leaf blight disease which has been devastating taro crops in Nigeria and further reducing food availability.

Phytophthora colocasiae Raciborski is the causal pathogen of the most destructive disease of taro (*Colocasia esculenta*) commonly referred to as Taro Leaf Blight (TLB) (Misra *et al.* 2008; Singh *et al.* 2012; Tarla *et al.* 2014). Named since 1900 at Java by Mariam Raciborski, *P. colocasiae* is the most important pathogen limiting taro production in various parts of the world. Yield losses a century later varies from 25- 50% in some areas in Oceania and Southeast Asia to more than 90% in other (Misra *et al.* 2011). Besides, the pathogen also causes serious post-harvest decay of corms. Symptoms typical of TLB are initially

expressed on the leaf lamina as small, water-soaked, dark brown, round to irregular necrotic lesions on the ad axial leaf lamina (Chiejina and Ugwuja, 2013) which rapidly become enlarged to 2.5-5.0 cm within few days. As the disease progresses, adjacent lesions coalesce to cover extensive leaf area showing concentric colour patterns and yellowing. Orange or reddish brown exudates also ooze out from the spot. The infected leaf dies within 7-14 days causing yield losses of up to 50% (Brunt *et al.* 2001; Misra, 2008;) in severe cases and more than 70% in extreme severe cases (Nelson *et al.* 2011).

The epidemic of taro leaf blight arrived, Nigeria in July 2009 (Bandyopadhyay, *et al.* 2011; Ugwuja and Chiejina, 2011) and almost eliminated commercial and subsistence production of taro. Similar catastrophic events were reported in Cameroon (Mbong *et al.* 2013) and Ghana (Omane *et al.* 2012). This epidemic, which occurred in South Eastern Nigeria and in the South-West and North-West districts of Cameroon caused serious losses because there were not enough produce to eat or sell. Tremendous losses attributed to this pathogen were earlier reported in Pacific Islands (American Samoa, Western Samoa, Hawaii, Fiji, and Papua New Guinea), India and China, Mauritius, Indonesia, Philippines and many other countries (Aggarawal *et al.* 1990; Misra, 1993; Onwueme, 1999;

Brunt *et al.* 2001; Jugumauth *et al.* 2001; Brooks, 2008 and Misra *et al.* 2008).

Taro is a tropical herbaceous aroid commonly known as cocoyam (Chukwu, 2015; Ukpabi *et al.* 2013; Mishra *et al.* 2008). It is an ancient traditional tuber crop grown in Nigeria (Ukpabi *et al.* 2013; Amusa *et al.* 2011) and many developing countries of Africa, Asia, Pacific Islands, West Indies, Caribbean and the Mediterranean (FAO, 2012; Manner and Taylor, 2011; Tumuhimbise *et al.* 2009; Wong, 2007; Onwueme, 1999) primarily for its underground starchy corms (Nwachukwu and Osuji, 2008). The highly nutritive leaves, petioles and flowers also serve as vegetables in some localities (Temesgen and Retta; Ukpabi *et al.* 2013; Amusa *et al.* 2011). In Nigeria, taro provides alternative source of carbohydrate to supplement yam and cassava and ranks third in importance after yam and cassava. Corm processed into flour is used for various forms of confectionery and as composite nutrient in infant meals due to its high (98.8%) digestibility (Semsek and El, 2015; Abubakar *et al.* 2008). In some localities, they are also used as adjunct in soup thickening. Besides the nutritional benefits (Alcantara *et al.* 2013) taro has great potentials as agro- industrial raw material for, pharmaceutical (Pereira *et al.* 2015; Kundu *et al.* 2012), confectionery, and brewery (Eneh, 2013), and livestock industries (Babayemi *et al.*, 2009). In Nigeria, surpluses from subsistent production find their way to the market thereby playing significant role in income generation and poverty alleviation for farmers.

Control of taro leaf blight has been attempted through cultural practices (Misra *et al.* 2007a) including removal and destruction of infected leaves, agronomic, chemical and breeding methods (Misra *et al.* 2007b; Jackson 1999; Brunt *et al.* 2001; Mishra, *et al.* 2008) in Asia and the Pacific. NRCRI Umudike reported effective fungicide management (Chukwu *et al.* 2012). While these methods have recorded some level of success, each is associated with specific limitations. Removal of leaves would quickly lead to a complete defoliation and consequent effects on yield (Tarla *et al.* 2014).

Current concerns on the accumulation of harmful chemical residues in the environment (soil, water and/or plants and animals) and the associated threats to human health (Komarek *et al.* 2010; Dhaliwal and Koul, 2011) in addition to uneconomic cost of such chemical has led to an increasing search for alternatives. Moreover, development of fungicide-resistant biotypes of pathogens has become a major constraint to the control of pathogens with synthetic chemicals (Ham *et al.* 1991; Oh and Kim, 1992). In addition, the choice of taro cultivars by farmers is driven by their tastes and marketability and some of these characteristics are lost during breeding to produce resistant cultivars (Tarla *et al.* 2014).

In view of the serious threat to food security posed by this disease viz-a-viz the scarcity of produce and lack of planting materials, loss of important genetic resources and the adverse impact on biodiversity caused by synthetic chemical, the development of environmentally sustainable control measures have become urgently necessary to boost taro production.

Plants are rich sources of bioactive principles (phytochemicals) which occur in different forms and provide viable alternatives to synthetic chemicals when extracted (Rios and Recio, 2005). Only a limited trial with plant extract has been done to control taro leaf blight (Shakywar *et al.*, 2012). Extracts of *A. indica*, *S. alata*, *T. diversifolia* and *E. camaldulensis* have been found effective in reducing the growth and development of many pathogens and have been used in the management of many other plant diseases as safe and eco-friendly alternatives to synthetic chemicals (Barra *et al.* 2010; Sule *et al.* 2010; Linthoingambi and Singh, 2013; Jimoh *et al.* 2015; Nweke, 2015).

The objective of this study was to evaluate the fungicidal effects of crude extracts of selected plants against the taro leaf blight pathogen, *P. colocasiae*. It is hoped that this information will be useful in selection of plant derived fungicides for *P. colocasiae* control.

MATERIALS AND METHODS

Location of Study

This study was conducted at the Plant Pathology Laboratory of the National Root Crop Research Institute (NRCRI) Umudike, Nigeria located within Longitude 07° 34" E, Latitude 05° 29" N and at elevation of 122m above sea level.

Collection of Plant Materials

The following plant materials were used for the preparation of plant extracts: Neem (*Azadirachta indica*) leaf, Eucalyptus (*Eucalyptus camaldulensis*) leaf, Bush candle (*Senna alata*) leaf and Mexican sunflower (*Tithonia diversifolia*) leaf. Fresh leaf samples of these plants were collected from their natural sources within Abia and Enugu States and their identity were authenticated by Mr. M.I. Nduche of the Department of Plant Science and Biotechnology Michael Okpara University of Agriculture Umudike, Nigeria.

Preparation of plant extracts

The plant materials were washed under running tap water to remove dirt, allowed to drain off water, cut into small bits and air-dried to brittleness on the laboratory benches under room temperature for two weeks. Each sample was ground into fine powder using Thomas Willy Milling Machine, sieved (with 0.5 mm mesh) to remove large particles and stored in airtight plastic containers prior to soaking following the method of Ofokansi *et al.* (2003). One hundred and fifty grammes of each pulverized sample was weighed

into 1000 ml conical flask and soaked in 600 ml of ethanol (1:4 w/v) for 48 hours after which the content of each flask was filtered into plastic tray through 4 layered cheese cloths. The trays were covered with perforated aluminum foil, placed under a fan in a sterile chamber to evaporate the ethanol. After evaporation of the ethanol, the concentrated crude extracts obtained were transferred into sterile airtight bottles and preserved in refrigerator at -20 °C until usage.

Sterilization of materials and Preparation of media

Materials such as Petri dishes, test tubes, flasks, transfer needles, cork-borers, scalpel, cotton wool and filter papers used in the laboratory during the research were sterilized in hot air Genlab oven at 160 °C for 1 hour. Potato Dextrose Agar (PDA; 200 g l⁻¹ Irish potato, 20 g l⁻¹ dextrose and 20 g l⁻¹ agar), Water Agar (WA; 20 g l⁻¹ agar), were prepared according to standard procedure (Mishra *et al.*, 2008) and autoclaved at 103 KN M⁻² and 121°C for 15 minutes .

Isolation of *P. colocasiae*

A modified isolation technique of Ugwuja and Chiejina (2011) was used. Leaves of taro plant exhibiting symptoms of the blight were collected from the research farm of National Root Crops Research Institute, Umudike (NRCRI). Thin leaf tissue fragments (2-3 mm) were excised from the periphery of lesions, surface sterilized in 0.1% mercuric chloride for 2 minutes, rinsed in three changes of sterile distilled water and plated in WA. Following incubation in Petri dishes at room temperature (27± 2 °C) for 4 days, mycelia emerging from each tissue fragment were aseptically transferred to PDA amended with 100 mg l⁻¹ Ampicillin, 100 mg l⁻¹ Penicillin, 3 mg l⁻¹ PCNB, and 3 mg l⁻¹ Nystatin. Transfers of colony growth were aseptically done from PDA cultures to clean PDA plates until pure cultures were obtained. Identification of isolates was based on observed culture growth patterns, mycelia colour and microscopic examinations of vegetative and reproductive structures with reference to relevant manuals (Drenth and Sendall, 2001; Jeffer, 2006 Ellis *et al.* 2007). Isolates were preserved in McCartney bottles agar slant for subsequent use.

Pathogenicity Test

Pathogenicity test was done for each of the organisms isolated from the infected taro leaf according to a modified method of Brooks (2008) in order to confirm the pathogen responsible for the initial TLB disease symptoms observed on the infected taro leaves as was postulated by Koch. The method of Brooks was modified thus: Agar disc inoculum of each isolate were used instead of liquid inoculum. With the aid of sterile cork borer a 5 mm agar disc was cut from the periphery of a 7-day-old actively growing culture of each isolate and placed on previously washed taro leaves laid on sterile plastic trays lined with moist

sterile cotton wool. Three replicates of discs of each isolates were made and the set up was incubated at 26 °C for 4 days on incubator.

Inhibitory effect of extracts on radial growth of *P. colocasiae*

Ethanol leaf extracts of *Azadirachta indica*, *Eucalyptus camaldulensis*, *Senna alata* and *Tithonia diversifolia* were tested for their effects on radial growth of *P. colocasiae*. The effects on radial growth were investigated on PDA. Requisite amount of each extract was weighed with a Mettler balance, suspended in sterile distilled water and added to molten PDA at 50 °C to achieve final concentration of 5 mg/ml, 10 mg/ml and 20 mg/ml. Ridomil (a standard fungicide) at 3 mg/ml was incorporated into PDA and served as positive control while the media to which nothing was added served as negative controls. Twenty millilitres of the extract- amended medium of a given concentration was dispensed into 90 mm Petri dishes. The media in the dishes were allowed to cool and solidify. They were centrally inoculated with a 5 mm mycelial disc cut from the periphery of a 7-day-old culture of *P. colocasiae* by means of a sterile cork borer. All treatments were replicated thrice and incubated on laboratory bench for 7 days at room temperature (25±4°C). Radial growth in terms of diameter of the colony in each culture dish was measured to the nearest millimetre along two equatorial axes from the back side of plates and their averages recorded for all 3 replicates of a given concentration.

Inhibition of Radial Growth

The data obtained from radial growth measurements (colony diameter) were used to deduce percentage inhibition of radial growth following the formula stated by Shovan *et al.* (2008) as follows:

$$\text{Percentage inhibition (I)} = \frac{x-y}{x} \times 100$$

Where;

x= growth of control

y = growth of extract treated plate

Inhibitory effect of extracts on mycelial dry weight of *P. colocasiae*

To determine the effect of the extracts on Mycelia dry weight of *P. colocasiae* Potato Dextrose Broth (PDB) was used. Fresh PDB was prepared using 200 g of peeled sliced potato and 20 g of dextrose in 1000 ml of distilled water. The infusion made from the potato by cooking was mixed with the dextrose-in-water, autoclaved at 121°C and 103 KN M⁻² for 15 minutes and allowed to cool. Using poisoned food technique (Dhingra and Sinclair, 1985), requisite quantities of each extract was weighed into separate 250 ml conical flask and incorporated into the broth in proper amounts to achieve final concentration of 5, 10 and 20 mg/ml of the extracts. The content of each flask was stirred thoroughly with a sterile glass rod to ensure uniform distribution of the extract. Two millimetre agar disc cut from the periphery of a seven-day- old culture of *P.*

colocasiae was introduced into each flask, shaken and incubated for 10 days at room temperature (27±.°C). After the incubation period, mycelium in each of the flasks amended with extract were harvested, weighed, oven-dried at 50 °C for 3 hours and each was compared with that of control.

Experimental Design and Statistical Analysis

The experiment was laid out with 16 treatments replicated thrice in Completely Randomized Design (CRD). Treatments were formed by the combination of 4 plant extracts and 4 concentrations of extract. Data were subjected to Analysis of variance (ANOVA) using SPSS package (IBM SPSS Statistics 22) and significant differences among means were compared using Tukey comparison test.

RESULTS

Inhibitory effect of the extracts and concentrations on radial growth of *P. colocasiae*

Results of the effects of the crude ethanol plant extracts and their concentrations on radial growth (colony diameter) (mm) and percentage inhibition (%) of *P. colocasiae* are shown in Table 1. Radial growth was significantly inhibited (P < 0.05) to various extents by all the extracts in a dosage-dependent manner compared to the control. Crude extract of *A. indica* had significantly (P < 0.05) the least mycelia growth of 0.00, 0.17 and 1.44 mm at 20, 10 and 5 mg/ml

concentrations compared to other extracts and exhibited correspondingly, the highest inhibition of radial growth, 100, 92.17 and 77.46% respectively. Crude extract of *S. alata* was next in efficacy with 0.18, 1.39 and 1.55 mm radial growths corresponding to 97.13, 78.51 and 75.58% radial growth inhibitions, while *T. diversifolia* and *E. camaldulensis* had the lowest inhibition.

Main effect of extract on radial growth

The main effect of extracts on radial growth of *P. colocasiae* is presented in Table 2. Radial growth was inhibited at various extents by all the extracts but percentage inhibition differed significantly (P < 0.05) from one extract to another. *A. indica* extract was significantly the most superior causing 67.41% inhibition, while *E. camaldulensis* extract was the least efficient with 18.51% inhibition.

Main effect of concentration on radial growth

The main effect of concentrations irrespective of individual extract on radial growth showed that all the concentrations inhibited radial growth of *P. colocasiae* except the control (0 mg/ml) which showed no inhibition (Table 3). The most efficient concentration was 20 mg/ml causing 65.54% inhibition which differed significantly (P < 0.05) from 58.34 and 49.98% inhibitions caused by 10 and 5 mg/ml respectively.

Table 1: The effects of Plant extracts and concentrations on radial growth of *P. colocasiae*

Extract rate (mg/ml)	<i>A. indica</i>		<i>E. camaldulensis</i>		<i>S. alata</i>		<i>T. diversifolia</i>	
	Radial growth (mm)	Percentage inhibition	Radial growth (mm)	Percentage inhibition	Radial growth (mm)	Percentage inhibition	Radial growth (mm)	Percentage inhibition
Control	6.38 ± 0.52 ^a	0.00 ± 0.00 ^a	6.38 ± 0.52 ^a	0.00 ± 0.00 ^a	6.38± 0.52 ^a	0.00 ± 0.00 ^a	6.38 ± 0.52 ^a	0.00 ± 0.00 ^a
5	1.44 ± 1.28 ^b	77.46 ± 2.03 ^b	5.14 ± 0.03 ^b	19.56 ± 0.42 ^b	1.55 ± 0.08 ^b	75.58 ± 0.83 ^b	3.83 ± 0.09 ^b	21.23 ± 0.91 ^b
10	0.17 ± 0.29 ^c	92.17 ± 6.56 ^c	4.94 ± 0.10 ^b	22.69 ± 1.59 ^b	1.39 ± 0.14 ^b	78.51 ± 1.96 ^b	4.64 ± 0.09 ^c	27.33 ± 1.45 ^c
20	0.00 ± 0.00 ^d	100.00 ± 0.00 ^d	4.36 ± 0.74 ^c	31.81 ± 11.64 ^c	0.18 ± 0.03 ^c	97.13 ± 0.45 ^c	5.03 ± 0.06 ^c	40.00 ± 1.20 ^d
Total	2.00± 0.55	67.41 ± 8.40	5.21 ± 0.35	18.52 ± 13.81	2.38 ± 0.19	62.86 ± 0.81	4.97 ± 0.19	22.14 ± 0.89

Values presented in the table are means of 3 replicates ± standard deviation. Means followed by the same letter within a column are not significantly different (P< 0.05) using Tukey comparison test..

Table 2: Main effect of extract on radial growth of *P. colocasiae*

Extracts	Radial Growth (mm)	Percentage Inhibition (%)
<i>A. indica</i>	2.00±0.55 ^a	67.41±8.40 ^a
<i>S.alata</i>	2.38±0.19 ^b	62.86±0.81 ^b

<i>T.diversifolia</i>	4.97±0.19 ^c	22.14±0.89 ^c
<i>E.camaldulensis</i>	5.20±0.86 ^d	18.52±13.81 ^d

Each mean represents the overall performance of a given extract irrespective of concentration. Means followed by the same letter within a column are not significantly different (P< 0.05)) using Tukey comparison test..

Table 3: Main effect of extract concentration (mg/ml) on radial growth of *Phytophthora colocasiae*

Extract (mg/ml)	Rates	Radial growth (mm)	Percentage inhibition (mm)
Control	0	6.38±0.52 ^d	0.00 ± 0.00 ^d
	5	2.99±0.37 ^c	48.46 ± 5.55 ^c
	10	2.79±0.16 ^b	55.18 ± 4.46 ^b
	20	2.39±0.21 ^a	67.24 ± 3.32 ^a

Each mean represents the overall performance of a given concentration irrespective of extract. Means followed by the same letter within a column are not significantly different (P< 0.05) using Tukey comparison test.

Inhibitory effects of extracts and concentrations on Mycelial dry weight of *P.colocasiae*

Results of the effects of crude ethanol extracts of *A. indica*, *E. camaldulensis*, *S. alata* and *T. diversifolia* at 0, 5, 10 and 20 mg/ml concentrations on mycelial weight of *P. colocasiae* grown on potato dextrose broth are shown in Table 4. Mycelial dry weight of the pathogen was similarly inhibited to varying degrees by all concentrations of the crude extracts. The treatment means were significantly (P< 0.05) different from that of the control and percentage inhibition increased as the concentration increased. *Senna alata* and *T. diversifolia* extracts were significantly superior to the other extracts in inhibiting mycelial dry weight by causing complete (100%) inhibitions of mycelia at 5, 10 and 20 mg/ml concentrations respectively, while *A.*

indica extract caused 100% inhibition only at 10 and 20 mg/ml. However, *E. camaldulensis* extract gave the least but appreciable inhibition of mycelial dry weight at 5mg/ml (30.3%), 10mg/ml (57.57) and complete inhibition at 20 mg/ml.

Main effect of extract on mycelial dry weight

Mycelial dry weight was significantly inhibited by the various extracts but percentage inhibition differed significantly (P < 0.05) from one extract to another. *A. indica* extract was significantly the most superior causing 62.37% inhibition, while *E. camaldulensis* extract was the least with 46.97% inhibition (Table 5).

Main effect of concentration on mycelial dry weight

The main effect of concentrations irrespective of individual extract on mycelial dry weight of *P. colocasiae* indicated that all the concentrations inhibited mycelial dry weight except the control (0 mg/ml) and the inhibition increases with higher concentration (Table 6). The most potent concentration was 20 mg/ml causing 100.00% inhibition which significantly (P < 0.05) differed from 89.39 and 69.95% inhibitions caused by 10 and 5 mg/ml respectively.

DISCUSSION

Germination of fungal inocula, growth of mycelium and sporulation are important physiological processes in pathogenesis. Consequently, any botanical extract which affects these aspects of a pathogen’s life cycle would invariably affect the survival of such pathogen on its host given the necessary conditions. Hence the systematic investigation and development of botanical extracts for control of plant diseases are based on established inhibition of these important physiological processes during disease development.

Table 4: The effects of Plant extracts and concentrations on mycelial dry weight of *Phytophthora colocasiae* Values presented in the table are means of 3 replicates ± standard deviation. Means followed by the same letter within a column are not significantly different (P< 0.05) using Tukey comparison test..

Extract Rates (mg/ml)	<i>A. indica</i>		<i>E. camaldulensis</i>		<i>Senna alata</i>		<i>T. diversifolia</i>	
	Mycelia weight (mg)	Percentage inhibition	Mycelia weight (mg)	Percentage inhibition	Mycelia weight (mg)	Percentage inhibition	Mycelia Weight (mg)	Percentage inhibition
0	0.33±0.12	0.00 ±0.00	0.33±0.12	0.00±0.00	0.33±0.12	0.00±0.00	0.33±0.12	0.00±0.00
5	0.17±0.11	49.49 ±32.37	0.23±0.07	30.3 ±15.03	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
10	0.00±0.00	100.00 ±0.00	0.14±0.05	57.57±0.00	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
20	0.00±0.00	100.00 ±0.00	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
Total	0.13±0.15	62.37 ±8.09	0.18±0.06	46.96±3.75	0.08±0.03	75.00±0.00	0.08±0.03	75.00±0.00

Table 5: Main effect of extract on mycelial dry weight of *P. colocasiae*

Extracts	Mycelial dry weight (mg)	Percentage Inhibition (%)
<i>A. indica</i>	0.13±0.15 ^b	62.37±6.20 ^b
<i>S. alata</i>	0.08±0.19 ^c	75.00±0.81 ^c
<i>T. diversifolia</i>	0.08±0.19 ^c	75.00±0.81 ^c
<i>E.camaldulensis</i>	0.12±0.86 ^a	46.97±13.11 ^a

Each mean represents the overall performance of a given extract irrespective of concentration. Means followed by the same letter within a column are not significantly different (P < 0.05) using Tukey comparison test..

Table 6: Main effect of concentration on mycelial dry weight of *P. colocasiae*

Concentrations (Mg/ml)	Mycelial dry weight (mm)	Percentage inhibition (%)
Control 0	0.33 ± 0.44 ^d	0.00 ± 0.00 ^d
5	0.10 ± 0.37 ^c	69.95 ± 11.85 ^c
10	0.04 ± 0.16 ^b	89.39 ± 0.00 ^b
20	0.00 ± 0.21 ^a	100.00 ± 0.00 ^a

Each mean represents the overall performance of a given concentration irrespective of extract. Means followed by the same letter within a column are not significantly different (P < 0.05) using Tukey comparison test. .

The results of the present study have shown that the four plant extracts inhibited radial growth and mycelia dry weight of *P. colocasiae* in varying degrees. This is in line with the findings of some researchers who observed varying degrees of fungitoxicity with different plant extracts used in the control of fungal pathogen (Onifade, 2002; Okigbo and Nmeke, 2005; Okigbo and Ogbonnaya, 2006; Nweke, 2015). The crude plant extracts may have differed in their activities against the pathogen probably due to the fact that extracts of different plant species and genera vary in their ability to control pathogens largely due to differences in their photochemical constituents (Edeoga *et al.*, 2005). The observed variations in the performance of the extracts could also be attributed to the age of the plants (Okigbo and Ajalie , 2005.,

Okigbo and Omodamiro, 2005). It is possible that ethanol was either not effective as a solvent for the extraction of the active principles in some of the plant whose extracts exhibited moderate inhibition or could not extract the active principles in large enough quantities.

In the radial growth test, results indicated that *A. indica* extract was the most potent in inhibiting radial growth of the pathogen while the other extracts showed high to moderate inhibitions. The result supports the observation of previous researchers (Joseph *et al.* 2008, Okigbo *et al.* 2010 and Shakywar *et al.* 2012) who found that neem leaf extract was efficient in reducing growth of *Fusarium solani* f.sp. Melongenae, yam rot pathogens (*Aspergillus niger* ,*Botryodiplodia theobromae*, *Fusarium oxysporum*, *Penicillium digitatum* and *Rhizopus stolonifer*) and *Phytophthora colocasiae* in different experiments.

However, *S. alata* and *T. diversifolia* extracts achieved complete inhibition of mycelia weight in all the concentrations tested while with *A. indica* and *E. camaldulensis* extracts complete inhibition was not observed in all concentrations.

The observed differences in the performance of these extract in the solid (PDA) and liquid (PDB) media may be attributed to one or more of such physical and chemical factors which influence the performance of active principles in mixtures (Nene and Thapliyal,1979). The factors could be atmospheric interaction, nature of test (*in vivo* or *in vitro*) type of medium (liquid or solid) particle size and redistribution or longevity of active compounds in solution.

The effective performance shown by *A. indica*, *S. alata* and *T. diversifolia* in this study corroborates the findings of other workers who demonstrated the effectiveness of these plant extracts in different trails. Sharkywar *et al.* (2012) reported similar results with leaf extract of *A. indica* as a foliar spray against *P. colocasiae*. Similarly, Sule *et al.* (2010) had earlier reported the effectiveness of *S. alata* against some fungal diseases. Jimoh *et al.* (2013) reported the effectiveness of *T. diversifolia* as foliar at 7.5 and 8.0% in the management of *Cercospora* leaf spot (CLS) and *Alternaria* leaf spot (ALS) of sesame.

Although *E. camaldulensis* extract was the least effective of the four extracts in inhibiting radial growth and mycelia dry weight of *P. colocasiae*, previous researchers have lauded its broad spectrum antimicrobial activities on a number of pathogens (Sabo and Knezevic, 2019). The potency of its leaf extracts and essential oils against *Candida* spp., (Siramon *et al.* 2013; Nasir *et al.* 2015) and various dermatophytes including *Microsporum canis*, *M. gypseum*, *Trichophyton rubrum* and *T. Verrucosum* was reported (Moghimpour *et al.* 2012). Barra *et al* (2010) also reported the efficacy of *E. camaldulensis* against *Aspergillus niger* and *Botrytis cinerea*.

The maximum inhibition exhibited by Ridomil which was used as a positive check in this trial evidently supported its usage as a standard foliar chemical in the management of taro leaf blight disease as confirmed by Misra (2008).

With respect to mycelia dry weight test, *S. alata* and *T. diversifolia* recorded no yield in PDB while *A. indica* and *E. camaldulensis* had minimal yields. This result suggests that the infective inoculum of *P. colocasiae* germinated sparingly in the liquid medium amended with the extracts of *A. indica* and *E. camaldulensis* and did not germinate at all in liquid medium of *S. alata* and *T. diversifolia* extracts. The observed inhibition of spore germination caused by *S. alata* and *T. diversifolia* in the broth and the resultant total (100%) inhibition of mycelia suggests that the activities of these two extracts may be better with liquid medium which contain ample amounts of water that holds the active principles in solution. It also suggests that these two extracts could be used as biocide- dip in pre-planting treatment for soaking planting materials such

as corms and comels in order to forestall the emergence of mycelia of the pathogen which might be hidden within them.

However, these observations and suggestions are preliminary in this line of research and require further test for confirmation. From our results it is evident that various levels of fungicidal activities were shown by the extracts against the pathogen. This may have strong correlation with the character of the active ingredient in each crude extract emphasizing therefore, the need to fractionalize each extract in order to identify the specific agents responsible for the inhibition and their minimum inhibitory concentrations. This will give us the clue on the actual quantities to be recommended to farmers for use in the control of taro leaf blight disease.

From the results of this experiment we recommend further study of these extracts with other extracting solvents and more trials in the screen house and field as biocides against the ugly scourge taro leaf blight.

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EFFECT OF NPK FERTILIZER RATES AND STAKE HEIGHT ON THE VEGETATIVE GROWTH OF WHITE YAM (*Dioscorea rotundata*) CULTIVAR YANDU IN UMUDIKE SOUTHEASTERN NIGERIA

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ABSTRACT

Field experiments were conducted at Umudike, a rainforest agro ecological zone of Southeastern Nigeria, during the 2016 and 2017 cropping seasons to study the effect of stake heights and NPK fertilizer levels on white yam cultivar *Yandu*. In each year, the experiment was laid out as a 4 x 5 factorial in a Randomized Complete Block Design (RCBD) with three replicates. The treatments comprised four levels of stake heights (0m, 1m, 2m and 3m) and five levels of NPK fertilizer levels (0 kg/ha, 200kg/ha, 400kg/ha, 600kg/ha and 800kg/ha). The use of 2m stake increased vine length, leaf area index and photosynthetic efficiency. Fertilizer best management practices required the application of the correct fertilizer at an appropriate rate, time and place

Keywords: fertilize rates, stake height, white yam, Southeastern Nigeria.

INTRODUCTION

Yam (*Dioscorea species*) is a tuber crop belonging to the family *Dioscoreaceae*. The species of economic importance include *Dioscorea rotundata*, *Dioscorea alata*, *Dioscorea cayenensis*, *Dioscorea dumetorum*, *Dioscorea bulbifera* and *Dioscorea esculenta* (Aighewi, 2014). Nigeria and Ghana together produced about 66% of the world's yam supply. The five major yam producing countries in West Africa are Benin, Cote d'Ivoire, Ghana, Nigeria and Togo. They account for 93% of world production. Nigeria alone accounts for 68% of global production (36 million tons on 3 million hectares).

As food, yam plays an important role by providing cash and dietary carbohydrate to millions of people. The crop also makes a substantial contribution to protein in the diet, ranking as the third most important source of supply (Ekanayake and Asiedu, 2003; IITA, 2013). Yam has a better keeping quality than most other tropical root and tuber crops because the tubers have an extended period of dormancy during which physiological activities are at a minimum. Yam can therefore serve as an important food security crop. 31.8% of the population in Nigeria and 26.2 % in Ghana depend on yams for food and income security. Yam tuber is usually prepared for consumption in a variety of ways which include boiling, frying, baking, processing into flour for the preparation of "amala", processing into pounded yam as well as processing into pottage (Orkwor and Ekanayake, 1998).

Traditionally, farmers produce yams on fertile soils after long periods of fallow because of the high nutrient demand of the crop. However, rising population pressure and increased demands on land for non- agricultural purposes have made soil fertility maintenance through prolonged fallows an untenable proposition, leaving maintenance of soil fertility through fertilizer usage the only viable alternative

(Law-Ogbomo and Remison, 2007). The poor tuber yields obtained from farmers' plots (IITA, 2002) suggests that soils involved in the production of yams need supplementary application of fertilizers if they are to do well. In most production systems, yam yields are reported to decline sharply when grown after short fallow of about 1-3 years duration (Watson and Goldsworthy, 1964). According to reports on trends of resource management constraints in high intensity yam growing areas in Nigeria, 72 percent of the fields had worsening soil fertility conditions whereas only 3 percent of the fields had improving fertility status (IITA, 1999). Conflicting results on the benefits of fertilizers on yam have been reported (Okwuowulu, 1995). Igwilo (1989) reported that NPK fertilizer application had no significant effect on staked plants of *Nwopoko* and *Obiaoturugo* varieties of *Dioscorea rotundata*, and reduced the yield of unstaked plants of these varieties. Law-Ogbomo and Remison, (2007) investigated the effects of five rates of NPK 15:15:15 fertilizer (0, 100, 200, 300 and 400 kg/ha) on white yam (*Dioscorea rotundata* cv. *Obiaoturugo*) and found that vine length, number of leaves and leaf area index significantly increased as fertilizer application increased, while optimum tuber yield was obtained at 300 kg/ha. Okpara *et al*, (2014) studied the effect of NPK fertilizer on white yam micro-sett and observed that mean tuber yield increased significantly in response to NPK fertilizer application up to 600kg/ ha. Staking is carried out to help twining yam stems display their leaves to attract adequate solar energy for efficient photosynthesis (Orkwor and Asadu, 1998). Tuber yield is a function of photosynthetic efficiency, crop growth duration and the harvest index. The photosynthetic efficiency of yams is closely related to the effective spread of leaf area to ensure maximum light interception (Akoroda, 1993). Staking increases light capture and therefore, enhances photosynthetic efficiency. The agroecology in which yam is cultivated is important in terms of staking requirements of the

crop as well as the availability of staking materials (Ekanayake and Asiedu, 2003). In a humid climate, where cloud can greatly limit the number of hours of sunshine, staking improves photosynthesis of plants, prevents foliar diseases, and allows the cultivation of interim crops. Staking type may be single, pyramidal or trellis. The objective of this study therefore was to evaluate the effect of NPK fertilizer rates and stake height on the vegetative growth of white yam (*Dioscorea rotundata*) cultivar *yandu* in Umudike Southeastern Nigeria.

MATERIALS AND METHODS

The study was conducted in 2016 and 2017 cropping seasons at the National Root Crops Research Institute (NRCRI), Umudike, Southeastern Nigeria. Umudike is located at Latitude 5° 29' N and Latitude 7° 33' E in the rainforest agro ecological zone of annual rainfall that ranges between 1800mm - 2200mm. It lies at about 122m above sea level. Each year, the experiment was planted on a fresh site that had been under fallow for one year for the 2016 and 2017 experiments respectively. The land used for the experiment was slashed on 2 June, ploughed on 12 June and harrowed on 14 June and ridged on 16 June, in 2016. In 2017, the land was slashed on 10 June, ploughed on 20 June, harrowed on 25 June and ridged on 28 June. The experiment was a factorial laid out in a randomized complete block design (RCBD) with three replicates. NPK fertilizer rates were at five levels (0, 200, 400, 600 and 800 kg/ha) while staking heights were at four levels (0, 1, 2 and 3m). These formed 20 treatments combinations. Each plot size measured 4m x 2m (8m²). Sowing was done at a spacing of 1m x 0.3m which gave a total of 33,333 plants per hectare. Prior to planting, the tubers were treated with insecticide and fungicide to avoid termite attack and rot of the yams. A tuber sett-size of 50g was planted per hole. Inorganic fertilizer NPK was applied at different rates using the band placement method at various rates in each plot at 8Weeks After Planting (WAP). Manual weeding was carried out at 4, 8 and 12 WAP. The plots were kept weed free manually. Data on growth parameters were taken on vine length, number of leaves per plant, leaf area, leaf area index and photosynthetic efficiency at 3 and 4 Months After Planting (MAP) from four representative plants per plot.

RESULTS

In general, stakes of 2 or 3m height at 3 and 4 MAP in 2016 produced significantly longer vine than no staking (Table 1). At 3 MAP, the 3m stake had longer vines than 1m stake, while at 4 MAP, 2m stake had longer vines than 1m stake. In 2017, the use of 2 or 3m stakes produced significantly longer vine than the use of 1m stake or no staking at 4MAP. Staking did not influence vine length at 3 MAP in 2017. In both 2016 and 2017 cropping seasons, application of NPK fertilizer did not significantly affect vine length of the white yam mini-sett at 3 and 4 MAP (Table 2).

Leaf area index value at 3 MAP in 2016 was similar for 2m and 3m stakes but these were significantly higher than that of no staking (Table 3). There were no effects of staking on leaf area index later in crop growth at 4 MAP. At 3 and 4 MAP in 2017, there were no effects of staking on leaf area index. NPK fertilizer did not significantly affect leaf area index in both cropping seasons across the different sampling dates (Table 4).

Average photosynthetic efficiency was 5.42 (mj/m) in 2016 and 54.62 in 2017 showing similar values in both years (Table 5). There were no significant effects of staking height or NPK fertilizer application or interaction of both factors in 2016. NPK fertilizer similarly did not influence photosynthetic efficiency in 2017. However, the use of 2m stake in 2017 produced significantly higher photosynthetic efficiency than no staking. There were no differences in photosynthetic efficiency with respect to the three stake lengths of 1, 2 or 3m. Interactions were also not significant.

2016	2017		
LSD (0.05) for NPK (F)	means = NS	NS	
LSD (0.05) for Staking (S)	means = NS	3.48	
LSD (0.05) for F x S	means = NS	NS	

DISSCUSSIONS

The use of 2m stake increased vine length, leaf area index and photosynthetic efficiency. Fertilizer best management practices are required the application of the correct fertilizer at an appropriate rate, time and place.

Table 1: Effect of stake length on vine length (cm) of yandu white yam cultivar at different sampling dates

Months
after

Stake height (m)	planting (MAP)	
	3	4
	2016	
0.0	108.2	171.0
1.0	115.4	188.4
2.0	164.4	219.2
3.0	173.8	205.8
Mean	140.4	196.1
LSD _(0.05)	52.4	35.4
	2017	
0.0	93.4	98.1
1.0	89.9	119.6
2.0	103.3	207.4
3.0	103.7	223.7
Mean	97.6	162.2
LSD _(0.05)	NS	42.4

Table 2: Effect of NPK fertilizer on vine length (cm) of yandu white yam cultivar at different sampling dates

NPK fertilizer (kg/ha)	Months after planting (MAP)	
	3	4
	2016	
0	155.6	185.1
200	136.5	194.4
400	118.1	188.9
600	139.6	195.9
800	152.4	216.2
Mean	140.4	196.1
LSD _(0.05)	NS	NS
	2017	
0	90.6	148.3
200	89.3	178.7
400	95.6	160.9
600	97.8	153.9
800	114.5	169.1
Mean	97.6	162.2
LSD _(0.05)	NS	NS

Table 3: Effect of stake length on leaf area index of yandu white yam cultivar at different sampling dates

Stake height (m)	Months after planting (MAP)	
	3	4
	2016	
0.0	0.204	0.195
1.0	0.283	0.521
2.0	0.463	0.748
3.0	0.465	0.545
Mean	0.354	0.502
LSD _(0.05)	0.210	NS
	2017	
0.0	0.112	0.369
1.0	0.157	0.182
2.0	0.174	0.336
3.0	0.144	0.245
Mean	0.148	0.283
LSD _(0.05)	NS	NS

Table 4: Effect of NPK fertilizer on leaf area index of yandu white yam cultivar at different sampling dates

NPK fertilizer (kg/ha)	Months after planting (MAP)	
	3	4
	2016	
0	0.438	0.368
200	0.310	0.460
400	0.348	0.767
600	0.350	0.628
800	0.322	0.288
Mean	0.354	0.502
LSD _(0.05)	NS	NS
	2017	
0	0.135	0.341
200	0.131	0.257
400	0.102	0.363
600	0.188	0.237
800	0.183	0.218
Mean	0.148	0.283
LSD _(0.05)	NS	NS

Mean 51.80 55.07 56.40 55.20 54.62

Table 5: Effect of NPK fertilizer and stake length on photosynthetic efficiency (mj/m) of yandu white yam cultivar in 2016 and 2017

NPK fertilizer (kg/ha)	Stake length (m)				Mean
	0	1	2	3	
2016					
0	50.00	54.67	58.33	56.33	54.83
200	53.67	56.33	54.67	58.33	55.75
400	50.67	54.67	57.33	45.67	52.08
600	55.33	53.00	52.67	54.00	53.75
800	55.67	59.00	54.33	53.67	55.67
Mean	53.07	55.53	55.47	53.60	54.42
2017					
0	51.33	56.67	58.00	56.67	55.67
200	52.67	52.00	57.00	56.33	54.50
400	56.67	56.33	57.67	57.00	56.92
600	48.67	55.33	54.00	52.33	52.58
800	49.67	55.00	55.33	53.67	53.42

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WIS –ASP 19

EFFECT OF SPACING AND SETT-SIZE ON THE VEGETATIVE GROWTH OF WHITE YAM (*Dioscorea rotundata*) CULTIVAR *Yandu* IN UMUDIKE SOUTH-EASTERN NIGERIA.

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ABSTRACT

Field experiments were conducted at Umudike in the rainforest agro ecological zone of southeastern Nigeria, during the 2016 and 2017 cropping seasons to study the effect of spacing and sett-size on the vegetative growth of white yam (*Dioscorea rotundata*) *Yandu* in Umudike Southeastern Nigeria. In each year, the experiment was laid out as a 4 x 4 factorial in a randomized complete block design (RCBD) with three replicates. The treatments comprised four levels of sett-size (25g, 35g, 45g and 55g) and four levels of spacing (1 m x 0.25m, 1m x 0.5m, 1m x 0.75m, 1m x 1m). The result of the study shows that sett size of 35g produced higher vine length. Closer spacing of 1m x 0.25m produced higher leaf area index and due to this, it is required for high vegetative growth and recommended in the production of *yandu* production in Umudike Southeastern Nigeria.

Keywords: spacing, sett size, vegetative growth, white yam and Southeastern Nigeria. (Remove and & fool stop)

INTRODUCTION

Yams (*Dioscorea spp*) is a tuber crop belonging to the family *Dioscoreaceae*. The species of economic importance include *Dioscorea rotundata*, *Dioscorea alata*, *Dioscorea cayenensis*, *Dioscorea dumetorum*, *Dioscorea bulbifera*, and *Dioscorea rotundata*. In west africa, *Dioscorea rotundata* is also referred to as white yam or white guinea yam. It is the most widely cultivated and the most important food and income source for millions of producers, processors and consumers in the West Africa. In 2012, world production of yam was estimated as 58.7 million tons with West Africa producing more than 92% (FAOSTAT, 2014). Yam is a monocotyledonous and annual herbaceous plant that has long climbing stems which wind themselves around supports. One single plant produces between one and five tubers of varying shapes, which may weigh 1.5 to 7kg (Ukpabi and Okoli, 2002). The leaves are heart shaped with pointed tips and spiny stem (in some varieties) which is circular in cross-section. Yam is an important staple food source in tropical countries like East Africa, the Caribbean, South America and Southeast Asia; however, West Africa remains the most important yam producing region of the World (Okonkwo, 1985).

The technique of sett-size has been developed in yam production and is based on the principle that any section of the tuber is capable of sprouting provided it has a covering portion of the skin (Onwueme, 1978). One of the problems associated with the mini-sett technology was the difficulty in obtaining the recommended 25g cut setts. (Anuebunwa *et al*, 1998), suggesting the need for use of bigger sett sizes. An increase in mini-sett size in some cultivars of *Dioscorea rotundata* can enhance their sprouting potential but agro-ecological conditions such as temperature and relative humidity also play a determining role (Ayankanmi *et al*, 2005). Plant spacing is considered important to the optimum plant population which may be reflected on optimum

plant growth and yield. Research has shown that the response of crops to spacing or plant population is affected by the yield potential of the growing environment (Paszkievicz and Butzen, 2005). For any variety at a given row width, within-row spacing may vary considerably and generally has a profound effect on total yield, as individual plants are capable of increasing their yield in proportion to the area available, within the limits of the environment (Desai and Patel, 1986). Usually, with closer spacing or increase in plant population density, the combined demand for nutrients and light results into competition for these resources unless the supply is either unlimited or has been somehow supplemented (Mkandawora and Sibuga, 2002). The objective of this study was to examine the effect of spacing and sett-size on the vegetative growth of white yam (*Dioscorea rotundata*) *Yandu* in Umudike South Eastern Nigeria.

MATERIALS AND METHODS

The study was conducted in 2016 and 2017 cropping seasons at the National Root Crops Research Institute (NRCRI), Umudike, Southeastern Nigeria. Umudike is located at Latitude 5°29' N, Longitude 7°33' E and lies at 122m above sea level in the rainforest agro ecological zone and has annual rainfall that ranges between 1800mm - 2200mm. Each year, the experiment was planted on a fresh site that had been under fallow for one year. The land used for the experiment was slashed on 3 June, ploughed on 6 June and harrowed on 8 June and ridged on 9 June, in 2016. In 2017, the land was slashed on 23 May, ploughed on 10 June and harrowed on 22 June and ridged on 28 June.

The experiment was 4 x 4 factorial, laid out in a Randomized Complete Block Design (RCBD) with three replications. The sett sizes were at four levels (35g, 45g, 55g and 65g) while the plant spacings were also at four levels (1mx0.25m, 1mx0.5m, 1mx0.75m and 1mx1m). These formed 16 treatment combinations. Each plot size measured 4m x 2m (8m²).

Planting was done at different plant spacings of 1m x 0.25m, 1m x 0.5m, 1m x 0.75m and 1m x 1m which gave plant densities of 40000, 20000, 13333 and 10000 plants per hectare respectively. Prior to planting, the tubers were treated with insecticide (Cypermethrin) and fungicide (Maconzeb) to avoid termite attack and rot of the yams. Inorganic fertilizer NPK 15:15:15 was applied at 400kg/ha using the band placement method 8WAP. Manual weeding were carried out at 4, 8 and 12 WAP. Pre-emergence herbicide (Diuron) was used immediately after planting in 2016 and 2017 at the rate of 250ml per 15L water. It was complemented with manual weeding at 12 weeks after planting (WAP). Data were taken on vine length, vine girth, leaf area, leaf area index (LAI) and photosynthetic efficiency at 3 and 4 Months After Planting (MAP) and at full maturity (8MAP) on number of tubers per plant and tuber yield (t/ha). The data obtained were subjected to analysis of variance (ANOVA) according to the procedure for a randomized complete block design using GENSTAT Discovery Edition 3 Statistical Package (2007). The comparison of treatment means for significance was done by the use of (LSD) least significant difference procedure at 5% level of probability.

RESULTS

At 3 and 4 MAP in 2016, plant spacing did not significantly influence vine length of the white yam mini-sett (Table 1). There were also no differences in vine length with respect to spacing at 4MAP in 2017, but at 3MAP, the widest spacing of 1x1m had significantly higher vine length than other spacings. Sett weight did not affect vine length at different sampling dates in 2016 (Table 2). However, at 4MAP in 2017, the smaller 35 and 45g setts produced significantly longer vines than the heavier 55 and 65g setts. In both years, leaf area index did not vary significantly with plant spacing (Table 3). At 4MAP, however, the closest spacing of 1x0.25m consistently and significantly had higher LAI than the wider spacings.

DISCUSSION

The results of this study showed that the higher leaf area index associated with the closer spacings, especially 1x0.25m spacing at 4MAP, was an indication of greater photosynthetic activity resulting in significantly greater leaf area index than the wider spacings of 1x0.75m and 1x1m. Planting the white yam mini-setts at the wider spacings of 1x1m resulted in longer vines length at 3MAP in the year 2017 but later in the year, there were no significant effect.

From this study, it appeared that *yandu* white yam cultivar production high yield could be obtained at the sett size of 35g and at plant spacing of 1m x 0.25m in Southeastern Nigeria humid agroecology.

Table 1: Effect of spacing on vine length (cm) of white yam cultivar *yandu* at different sampling dates

Plant spacing (m)	Months after planting (MAP)	
	3	4
	2016	
1x0.25	148.1	152.0
1x0.5	142.6	172.0
1x0.75	138.3	162.0
1x1	135.9	179.0
Mean	141.2	166.0
LSD(0.05)	NS	NS
	2017	
1x0.25	85.7	141.1
1x0.5	94.3	115.6
1x0.75	84.1	143.5
1x1	118.9	135.9
Mean	95.7	134.0
LSD(0.05)	28.7	NS

Table 2: Effect of sett weight on vine length (cm) of white yam cultivar *yandu* at different sampling dates

Sett weight (g)	Months after planting (MAP)	
	3	4
	2016	
35	153.8	136.0
45	142.6	172.0
55	133.0	184.0
65	135.9	172.0
Mean	141.2	166.0
LSD(0.05)	NS	NS
	2017	
35	93.8	152.2
45	101.3	151.8
55	109.1	115.0
65	78.8	117.0
Mean	95.7	134.0
LSD(0.05)	NS	37.9

Table 3: Effect of plant spacing on leaf area index of white yam cultivar *yandu* at different sampling dates

Plant spacing (m)	Months after planting (MAP)		Mean	0.144	0.155
	3	4	LSD _(0.05)	NS	0.113
	2016				
1x0.25	0.860	0.185			
1x0.5	0.830	0.102			
1x0.75	0.090	0.079			
1x1	0.060	0.048			
Mean	0.460	0.103			
LSD _(0.05)	NS	0.079			
	2017				
1x0.25	0.142	0.255			
1x0.5	0.292	0.185			
1x0.75	0.052	0.094			
1x1	0.092	0.087			

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WIS-ASP 20

CONTROL OF MAIZE STEM BORER (*Busseola fusca* F.) INFESTATION USING SELECTED PLANTS IN UMUDIKE, SOUTHEASTERN NIGERIA

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ABSTRACT

A field experiment was conducted at the Western Farm of Michael Okpara University of Agriculture, Umudike to evaluate the insecticidal potentials of *Curcuma longa* (turmeric) rhizomes, *Moringa oleifera* (Moringa) leaves, *Carica papaya* (pawpaw) seed and leaf extracts, for the control of maize stem borer, (*Busseola fusca*). A control was set up in which there was no treatment. Each treatment was replicated four times. The experiment was laid out in a Randomized Complete Block Design (RCBD). Data collected were, insect population, growth and yield parameters of maize for 2, 4, 6 and 8 Weeks After Planting (WAP). The results showed that all the treatments differed significantly ($P < 0.05$) from the control on the number of holes on the leaves per plot, on the number of holes on maize stem per plot and number of larvae per maize stem. Growth and yield of maize were significantly increased ($P < 0.05$) due to the protection given by the plant materials. The botanicals demonstrated strong insecticidal activity against maize stem borer, *B. fusca*, and they can be used as replacement for synthetic insecticides.

Keywords: *Zea mays*, *Busseola fusca*, *Carica papaya*, *Moringa oleifera*, *Curcuma longa*

INTRODUCTION

Maize (*Zea mays*) belongs to the family Poaceae, commonly known as the grass family and it originated from Mexico. Maize grows well in various agro ecologies and is unparalleled to any other crop due to its ability to adapt in diverse environments (Wudiri and Fatobi, 1992). It has emerged as a crop of global importance owing to its multiple end uses as a human food and livestock feed and serves as an important component for various industrial products. Globally, about 1016.73 million metric tonnes of maize is produced every year – the highest among major staple cereals (FAOSTAT 2013). A major portion of maize produced worldwide is used for animal consumption as it serves as a vital source of food and calories to billions of people in developing countries, particularly in Africa, and Asia (Shiferaw *et al.*, 2011).

Furthermore, it is a source of important vitamins and minerals to the human body. Along with rice and wheat, maize provides at least 30 % of the food calories to more than 4.5 billion people in 94 developing countries. Maize provides over 20 % of total calories in human diets in 21 countries and over 30 % in 12 countries that are home to a total of more than 310 million people (Shiferaw *et al.*, 2011). Maize is widely consumed as food in many parts of the world, and it is a staple food in developing countries, particularly in continents of Latin America, Asia and Africa. It is also a basic ingredient for some indigenous drinks and food products. In the developed world, maize is largely used as livestock feed and raw material for industrial products, while in developing countries it is mainly used as food (Emeasor and Dioka, 2019).

It is an important source of carbohydrate, protein, iron,

vitamin B and minerals. As food, the whole grain, freshly green or dried, may be used or may be processed traditionally by wet and dry milling methods to give a variety of food products. Preparation and uses of maize alone or in combination with other food material as staple food or snacks in Nigeria include the followings: *ogi* (in hot and cold forms), *tuwo*, *donkunnu*, *maasa*, *akple*, *gwate*, *nakia*, *egbo*, *abari*, *donkwa*, *ajepasi*, *aadun*, *kokoro*, *elekute* etc. (Abdulrahman and Kolawole, 2006)

The bulk of the concentrated feed to farm animals consist of grains, and maize is the most important and preferred one due to its low cost (compared to other cereals), low fiber content and high starch content. Maize Stover, which is the plant residue after the ear has been removed is used by many farmers in developing countries as roughage feed for livestock (Dowswell *et al.*, 1996). Silage of maize is an important feed in temperate areas (United States, Canada and Europe), and consists of entire plant, which is cut, chopped and placed in a structure for anaerobic fermentation so as to allow for storage. Maize production is constrained by insect pests such as Maize stem borer, *Busseola fusca* (Emeasor and Dioka, 2019). The insect creates small holes or 'windows' in straight lines across the newest leaves of maize. Eggs are found on the underside of the leaf; they are ~ 1mm creamy white/yellowish in colour which may darken as they develop (Ogendo, *et al.*, 2013). As the infestation develops, symptoms include: weak stems, damage to growing parts, prevention of flowering and lodging.

The stem borers make a hole into the stem and feed inside these stems. Its damage leads to "dead heart" and disruption of nutrients and water flow in the plant.

The plants become stunted and produce maize cobs that are up to 30% smaller.

Objective of the study

The objective of this study was to evaluate the insecticidal potentials of *Moringa oleifera* leaves, *Curcuma longa* rhizomes, *Carica papaya* leaves and seeds for the control of maize stem borer *Busseola fusca* on maize.

MATERIALS AND METHODS

Experimental Site

The study was conducted from June through September, 2019 cropping season at the Michael Okpara University of Agriculture Umudike Western Farm, which is located on latitude 5° 29'N and longitude 7°33'E. It has an average annual rainfall of 2177mm and temperature of 29°C – 31°C with relative humidity of 50 – 90% in the rainforest ecological zone of Southeastern Nigeria.

Source of material

The maize variety used for this study was 'Bende white' a local variety which was obtained from National Seed Service office at Umudike. This maize variety is commonly cultivated in the Umudike agro ecosystem. *Moringa oleifera* leaves, *Carica papaya* (Pawpaw) leaves and seeds, were sourced locally in Ndoro market from the University community. Turmeric rhizomes were obtained from National Root Crop Research Institute, (NRCRI) Umudike.

Experimental Layout

The treatments comprised of: *Carica papaya* leaves and seeds, *Moringa oleifera* leaves, and *Curcuma longa* rhizomes which were laid out in a Randomized Complete Block Design (RCBD) with four replications. A control was set up in which there was no treatment. The entire experimental area was 18m x 18m (324m²) with each plot measuring 3m by 5m, 0.5m inter plot and 1m intra plot spaces. Each plot had 40 stands of maize and 1 seed per hole with the planting spacing of 75cm x 25cm.

Preparation and application of plant products

The plant materials were air-dried for two weeks in a well-ventilated place. The dry *M. oleifera*, leaves, *C. longa* rhizomes, *C. papaya* seeds and leaves were ground into powder using Thomas milling machine (Model ED-5) at National Root Crops Research Institute, Umudike. The plant materials in powder form were applied onto the leaves. The treatments were separately applied at the rate of 3g/plant.

Evaluation of *B. fusca* damage on maize plants

Assessment on number of holes made by *B. fusca* on maize leaves was obtained from ten stands of maize which was selected from the two middle rows set aside for data collection for number of hole made by *B. fusca*. Each leaf of maize was assessed by counting the number of holes on the leaves. The means were obtained by adding up the number of holes gotten from the leaves of selected six maize plants and dividing by six, starting from 2 Weeks After Planting (WAP).

The number of holes made by *B. fusca* on maize stem was assessed in the field starting from 2 WAP by counting the number of holes on the stems set aside for data collection for number of holes on the stem. The number gotten from the six stems was added together and divided by six in order to get the mean number of holes on the stem. The number of *B. fusca* larvae on maize plant on a plot was carefully assessed randomly from the selected maize plants by adding the number of larvae on the leaves of maize and the number of larvae on the stems of maize plant starting from 2 WAP to 8 WAP. The assessment of the number of holes made by maize stem borer (*B. fusca*) was done by counting the number of hole on the leaves and the number of holes on the stem and by adding them together to get the number of holes per plot.

Yield Assessment

At 75 to 80 days after planting (DAP), the cobs were harvested according to the treatments and placed in medium sized polythene bags. The fresh cobs were weighed and after sun-drying for two weeks the number of grains/cob were weighed using 0.01g sensitivity weighing balance and the weight recorded.

Statistical Analysis

The data collected was subjected to statistical analysis using Analysis of Variance (ANOVA) procedure and the mean separation was carried out using Fishers' Protected Least Significant Difference (FLSD) at 5% level of probability.

RESULTS AND DISCUSSION

Results

Effect of plant products on the number of holes on maize infested with *Busseola fusca*

The effect of the plant materials on number of holes caused by *B. fusca* on maize leaves is presented in Table 1. The result indicated significant difference ($P < 0.05$) between the treatment means on number of holes per leaf of maize across the weeks under observation during the 2019 cropping season.

The result revealed that significantly higher number of holes (2.12) was recorded on the maize leaves in the control at 8 weeks after planting. The least number of holes (7.31) was recorded on the maize plant treated with *C. papaya* leaves.

Table 1 : Effect of plant products on the number of holes on maize infested with *B. fusca*

Treatments	Mean number of holes on maize leaves			
	2WAP	4WAP	6WAP	8WAP
<i>Curcuma longa</i> rhizomes	3.53	7.11	9.68	12.20
<i>Carica papaya</i> leaves	1.55	3.75	5.91	7.31
<i>Carica papaya</i> seeds	2.36	5.29	8.41	11.50
<i>Moringa oleifera</i> leaves	3.31	7.02	10.16	13.12
Control	4.58	9.73	15.18	21.12
LSD _(0.05)	0.82	1.02	1.54	2.17

Effects of plants products on number of holes on maize stem infested with *B. fusca*

The result in Table 2 shows the mean number of holes on maize stem infested with *B. fusca*. Maize plants treated with *C. papaya* leaves and seeds recorded significantly lower number of holes on maize stem. The maize plants that were treated with *Moringa oleifera* leaves and *Curcuma longa* rhizomes ranked the third and fourth respectively in controlling the number of holes per stem. The *C. papaya* leaves, *C. papaya* seed powder, *Moringa oleifera* leaves and *Curcuma longa* rhizomes performed significantly better than the control.

Effect of plant products on number of *B. fusca* larvae per stem of maize plants

The result presented in Table 3 shows the efficacy of plant products in controlling the number of larvae per stem at different time intervals. Result obtained indicated significant difference ($P < 0.05$) in number of larvae per stem between the treatment across the

different periods of application. The plant products significantly reduced the number of larvae per stem when compared with the control which recorded significantly higher number of larvae per stem during the study. At 8WAP, the mean number of larvae recorded per stem was 22.30 in the control.

Effect of plant products on number of *B. fusca* larvae per stem of maize plants

The result presented in Table 3 show the efficacy of plant products in controlling the number of larvae per stem at different time intervals. Result obtained indicated significant difference ($P < 0.05$) in number of larvae per stem between the treatment across the different periods of application. The plant products significantly reduced the number of larvae per stem when compared with the control which recorded significantly higher number of larvae during the study. At 8WAP, the mean number of larvae recorded per stem was 22.30 in the control.

Table 2: Effects of plants products on number of holes on maize stem infested with *B. fusca*

Treatments	Mean number of holes on maize stem			
	2WAP	4WAP	6WAP	8WAP
<i>Curcuma longa</i> rhizomes	0.52	1.05	1.69	1.92
<i>Carica papaya</i> leaves	0.24	0.60	0.76	0.98
<i>Carica papaya</i> seeds	0.25	0.65	1.06	1.20
<i>Moringa oleifera</i> leaves	0.45	0.86	1.18	1.40
Control	0.70	1.49	2.52	3.77
LSD _(0.05)	0.21	0.42	0.39	0.59

Table 3: Effect of plant products on the number of larvae per stem of maize plant infested with *Busseola fusca*.

Treatments	Number of larvae			
	2WAP	4WAP	6WAP	8WAP
<i>Curcuma longa</i> rhizomes	0.36	2.40	1.33	2.40
<i>Carica papaya</i> leaves	0.11	1.50	0.66	1.50
<i>Carica papaya</i> seed powder	0.22	22.90	1.66	2.90
<i>Moringa oleifera</i> leaves	0.27	2.30	1.32	2.30
Control	7.40	6.30	6.89	12.30
LSD _(0.05)	0.49	0.70	0.55	2.79

Effects of plant products on yield of maize infested with *B. fusca* during 2019 cropping season in Umudike

Table 4 shows the effect of selected plant products on

yield related parameters of maize infested with *B. fusca*. There was no significant different in the ear height between the treated plants.

Significantly higher number of maize cobs was obtained in the treated plants when compared with the control. The number of maize cobs / plot in the

different plant products compared favorably with each other except *Carica papaya* seeds that recorded significantly higher number of grains/ cob (239.60) when compared with the three treatments. The lowest number of grains/ cob (180,10) was recorded in the control.

Table 4: Effect of plant products on yield maize

Treatments	Ear height (cm)	No. of cobs/plant	No. of cobs/plot	Fresh weight of cob husk(g)	No of grains/cobs
<i>Curcuma longa</i> rhizomes	26.45	1.04	30.14	134.40	238.40
<i>Carica papaya</i> leaves	25.76	1.20	31.17	131.60	218.50
<i>Carica papaya</i> seeds	27.64	1.17	30.98	127.30	239.60
<i>Moringa oleifera</i> leaves	26.91	1.20	31.36	126.00	195.10
Control	22.62	1.05	21.08	109.90	180.10
LSD _(0.05)	1.86	0.38	1.87	10.99	40.91

DISCUSSION

The use of botanicals for the control of insect pests of crops has been found to be effective by previous researchers. The finding of this study revealed that the plant products were effective in reducing the damage to the maize plants by *B. fusca*. The results indicated that application of products of the test plant materials significantly reduced the insect population and damage caused by the maize stem borer (*B. fusca*) on maize. This is in agreement with the work of Emeasor and Dioka, (2019) who reported that that seed extracts of *C. papaya* and leaf extracts of *Cymbopogon citratus* reduced the population and damage caused by the maize stem borer, *B. fusca* on maize. Ogendo *et al.* (2013) reported similar results which showed that *Lantana camara* L., *Tephrosia vogelii* Hook and *Tagetes minuta* L. aqueous botanical extracts sprayed at 1.70% w/v, reduced the Maize stem borer loads by 53, 59 and 47%, respectively compared to the untreated control.

It can be deduced from this study that damage by stem borer on maize plant increased with time. As observed, the severity of damage in terms of number of holes on the leaves and stems, number of larvae per stem and plant height increased over the time interval between 2 WAP and 8 WAP. The damage done by the stem borer, *B. fusca* was reflected on the yield attributes. Asserfa

(1999) obtained similar results in their study which indicated that application of extracts of fruits of chinaberry (*Melia azedarach* L.), endod (*Phytolacca dodecandra* L.) and pepper tree (*Schinus molle* L.) significantly reduced the levels of leaf infestation and dead heart injury due to larvae of *B. fusca*. An increase in crop yield was also recorded.

The ear heights for the treated plots were higher than the control. The plots treated with plant products; *Curcuma longa* rhizomes, *Carica papaya* leaves, *Carica papaya* seed powder and *Moringa oleifera* leaves recorded higher number of cobs. This study corroborates Shiberu (2013) who showed that aqueous leaf extract of *Cymbopogon citratus*, and flower extract of *Chrysanthemum cinerariaefolium* at 5% concentration recorded higher mortality (75 and 58.33), respectively, against *B. fusca* within three days,

The plant products gave significant protection to the maize plant and this was reflected on all the yield parameters. This implies that maize production could be profitable by applying these botanicals. The significant reduction of infestation of *B. fusca* by the application of extracts of these plant materials could be due to the effective deterrent and suppressive activities of their active ingredients. *Carica papaya* seed powder proved the most effective. Plant products significantly

reduced maize stem borer number and damage symptoms and also improved grain yield.

CONCLUSION AND RECOMMENDATION

Maize yield improved by the application of these plant materials; *C. papaya* leaves, *C. papaya* seed, *Curcuma longa* (turmeric) rhizomes and *Moringa oleifera* leaves. The plant products demonstrated strong

insecticidal activity against maize stem borer (*B. fusca*). It does not cause environmental and health hazards. It is cheaper and readily available. The treatment of maize plants with *C. papaya* seeds, *C. papaya* leaves, *C. longa* rhizomes and *M. oleifera* leaves were effective in the control of *B. fusca*. These plant products are recommended for the control of *B. Fusca* infesting maize so as to achieve higher yield by small holder farmers. This will help to alleviate poverty

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WIS-ASP 21

**ANTIMICROBIAL USE AND CHALLENGES IN PIG PRODUCTION IN OGUN STATE, NIGERIA:
FARMERS' PERCEPTIONS AND WAY FORWARD**

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ABSTRACT

The inappropriate use of antimicrobials (AMs) in food animals has birthed proactive agendas by global health security such as the FAO, OIE and WHO to promote AM stewardship worldwide. To understand and describe pig farmers' perceptions on antimicrobial use (AMU) and challenges associated with pig production in Ogun State, Nigeria. Data on farmers' (n = 45) perceptions of drivers for AMU and challenges confronting the pig industry were collected through focus group discussions (FGDs). Antimicrobials were mainly used for prophylaxis and therapeutic purposes, and farmers perceived AMs had good and negative effects on pig production. The drivers for AMU were linked to economic benefits, farmers' previous experiences, sick animals, poor accessibility and the expensive veterinary services, lack of restrictions on AMs purchase, poor hygiene and management, inadequate biosecurity and disease preventive strategies. Challenges confronted by farmers in the pig industry were poor government funding, lack and high cost of quality feed, animal diseases, high cost of veterinary services, substandard and fake drugs, poor market (local and International) for pig products, poor infrastructure especially roads and clean water. Developing farm guidelines, policies, and training on antimicrobial stewardship as well as best farm hygiene, biosecurity and management practices are crucial. Government support through the provision of agricultural loans and improvement of veterinary services are urgently needed by pig farmers in Ogun State, Nigeria.

Keywords: *Pig farmers; perceptions; drivers; antimicrobial use; antimicrobial stewardship.*

INTRODUCTION

Antimicrobials are used in livestock production to maintain health and productivity, which enhances higher opportunities for achieving sustainable animal protein accessibility and economic gains (Uddin and Osasogie, 2017). However, the non-judicious antimicrobial use (AMU) in food animals, has attracted a global concern due to the link with increased antimicrobial resistance (AMR) in humans, usually spread via the consumption (Sadiq *et al.*, 2018). Although, presently there are no robust evidence concerning the impact of AMU in food animals on AMR in human pathogens, some studies suggest evidence of AMR transmission from food animals to humans, while other studies do not support such transmission (Ekakoro *et al.* 2019). A recent publication by Van Boeckel *et al.* (2015) projected that antimicrobial consumption in livestock will rise by 67% by 2030 globally, and nearly double in Brazil, Russia, India, China, and South Africa. The increase in AMU consumption by livestock farmers in Nigeria was projected to increase by 163% in 2030 with high impact on AMR persistence (Van Boeckel *et al.* 2015). It is therefore necessary that antimicrobial stewardship, which promotes judicious practices are widely adopted by all sectors within the animal agriculture food production system to mitigate the public health impact (Ekararo *et al.* 2019).

Understanding AMU at farm level is crucial to enable the development of efficient educational programs on AM stewardship, promotion of strategic planning or

initiatives, and policy formulation or modification for livestock production in Nigeria. Although there have been previous studies on AMU in food animals from several southwestern parts of Nigeria, information on the drivers associated with use has not been reported in pig production in Ogun State, Nigeria. Meanwhile, Ogun State is a major hub for pig production and supply of animal protein especially meat to several regions of Nigeria and the neighboring countries. We therefore in this study, used focus groups to explore farmers' perceptions on AMU and associated drivers in pig production in Ogun State, Nigeria with a view to giving insights into the current status and implications for the pig industry and public health.

MATERIALS AND METHODS

Study Location

This study was conducted in Ogun State. The state is one of the six states that made up the Southwestern Nigeria and located between latitudes 7°3.5' and 9°12' north and longitudes 3°35' and 5°27' east. Ogun State borders Lagos State to the south, Oyo and Osun states to the north, Ondo to the east and the Republic of Benin to the west. Ogun State has a total a population census estimated at 3,751,140 (NPC and NBS, 2013). The state is selected based on the relatively high livestock activities, such as pigs, catfish and poultry productions (Obayelu *et al.* 2017).

Study Design, Sample Size, and Pig Farm Recruitment:

Qualitative method using focus group discussions (FGD) was conducted to understand the perceptions of farmers relating to antimicrobial usage and practices, challenges confronting the pig production in Ogun State, Nigeria, and their recommendations on making AMs work at various stakeholders' level in the industry. All pig farms registered under the pig farmers' association, Ogun state chapter were identified and invited to participate in the study. A signed consent was obtained from the chairperson of the association.

Focus group procedure and structure

Focus group discussions (FGD) were conducted to capture pig farmers' views about AMU and issues confronting pig production in the state. The focus discussions (FD) were held during one of the association's monthly meetings- the 20th of August 2019 at about 1 pm. Prior to the FGD, a briefing on the concept of the project was provided by the lead moderator, and consent to participate were verbally sorted. The participants were made aware they could opt out of the focus groups at any time if they wish to do so. To maintain anonymity, each participant was assigned an identification number which was used throughout the discussions.

A total of 45 pig farmers present in the meeting participated., They were divided into three groups comprising of 15 volunteers recruited from a purposive sampling technique. The FGD lasted approximately 120 minutes. Three out of the four researchers served as moderators in each of the group. A panel of interview guide comprising of 14 open-ended questions was used for the discussion. The moderator's role and responsibility were to give guidance to the discussion, and asked to follow up questions while ensuring that all areas in the topic guide were addressed (Ekakoro *et al.* 2019). Participants were encouraged to explore topics in-depth, reflect, and raise their own issues. Hand written notes of essential points and audio recordings of the interviews were taken as agreed by participants. Responses in the handwritten notes and audio recordings were analyzed by researchers following several meetings and outcomes transcribed verbatim.

RESULTS

Focus group participants' demographics / characteristics

A total of 45 pig farmers, 8 females and 37 males from across the various local government areas of Ogun state participated in the three FGDs. Participants' ages ranged from 25 to 75 years old.

Objective 1: Pig farmers' perceptions in relation to AMU and practices

In this section, participants' perceptions on antimicrobials, usage and practices were asked. Antimicrobials were perceived by farmers to have both good and bad effects on production. For instance, AMs are seen to positively boost pig production, but

occasionally go wrong when there was wrong drug administration (especially over dosage or wrong route of administration). Below gives a detailed description and excerpts of the participants on their perception of the effect of AM on pig production:

.....It's both sides, either positively or negatively on production. When an animal is sick for example in the case of a pregnant animal that is sick and you use antibiotics on it, the fetus will go down, there will be premature farrowing and apart from that, the animal loses weight..... FG 3

"We are also very careful when it comes to dosing them because we don't want to lose them. The first time I injected them, I observed they were going down and I had to go and needed to go and take a refresher course on drug administration"..... FG 1

"I prefer to use herbs because it has limited or no side effects, all antibiotics have adverse side effects because they are synthetic unlike herbs that are natural"..... FG 2

Furthermore, to understand the purpose of AMU, farmers said they administer drugs commonly for prophylaxis and therapeutics purposes. Farmers declined use of AMs as growth promoters. However, accepted they use AMs in animal feeds. The preferred route of administration was via injection. The most perceived commonly used AMs by pig farmers in the state were penicillin, streptomycin, gentamycin, long-acting (LA) oxytetracyclines (20%). Farmers indicated they used majorly the broad spectrum LA oxytetracyclines (20%) to treat all diseases, nevertheless based on experiences they knew specific AMs to use for specific diseases. When asked who does the prescription and drug administration, the majority of FG participants indicated that they do self-medication and occasionally consult veterinarians. Most producers mentioned that they relied on their own experience, knowledge, judgment, and recommendations from other farmers when deciding to use antimicrobials for the pigs. However, in situations that are difficult to handle or beyond control, then they consult veterinary expertise. Other factors identified to influence farmers' decisions to use AMs were cost, composition or active ingredient and efficacy, but not companies' brand.

Some of the responses by farmers on their AMU practices are highlighted below:

..... "Infections will manifest and there are drugs you can use. With broad-spectrum like LA oxytetracyclines (20%) and the indications for use written clearly on the bottle including dosage, diseases, and withdrawal period, so you administer if it's gonna be once let it be once and if twice and if you see the disease is coming down then you give another shot, then it's off. Most of us have used LA before to treat our sick animals and it works. Also when animals do not recover, we sell them off but none of us observes the withdrawal period"..... FG 3

..... ", We do. We are confessing to you we treat our own animals. I am my own vet. I diagnose and treat my

animal because I don't have access to veterinary services" ... FG 1

"Most knowledge of AM use we have, are not from veterinarians but have been from past experience, internet, and recommendations from other farmers with similar cases. Once a drug is effective we keep buying" FG2

Objective 2: Perceptions on what drives farmers to depend on AMs and AMR

The major themes identified as drivers of AMU were: 1. when an animal becomes uncomfortable starts showing physical signs such as diarrhea, coughing, mange, loss of appetite, 2. Farmers' previous experiences, 3. Availability and accessibility of AM off counter veterinary outlets without veterinary prescriptions or restrictions, 4. Inaccessibility to veterinary services and high cost, 5. Poor farm hygiene and management practices, 6. Poor disease control and management strategies, 7. Economic - efforts to make a profit and 8. Wet season. Farmers experience more disease and AMU during the wet seasons.

Farmers said AMs are stopped as soon as an animal is active or back in good condition and according to the drug manufacturer's instructions. Participants linked the occurrence of AMR to the overuse of AMs, and lack of alternatives. One of the farmers' responses to our question on what influences them to commence and stop using AM is:

"The state of the animal, whether the sickness is mild or the animal is seriously sick. The disease conditions influence the use of antibiotics, when you notice your animal is sick you give antibiotics and when the animal is well, you discontinue the use." FG 2

Objective 3: Perceptions relating to challenges confronting pig industry in Ogun state

The major challenges ranged from lack and high cost of quality feeds, poor disease prevention and control programs, poor accessibility to veterinary services and extension officers, substandard and fake drugs, poor market for pig and pig products, poor infrastructure especially roads and clean water. The farmers emphasized there was no funding in terms of loans and government support towards pig operations in the country. Many diseases confronting pig production were mentioned. Farmers indicated they have more of disease issues during the rains. Farmers' responses to lack of funding of pig operations and animal diseases control in Ogun state were:

....." The government doesn't even give us money. There is a bias for cattle producers when it comes to funding. We fill applications to the appropriate headquarters, which usually is chaired by a person who is averse to pig rearing and we don't get the funding. Some religions are against the expansion of pig farming in the state" ---- FG 1

"Many common diseases and conditions of challenges to pig health are Mange, foot rot, helminthiasis, diarrhoea, anestrus, Africa swine fever, pneumonia, Abortion/stillbirth and piglet anaemia. These diseases

or conditions increase more whenever there was a drop in temperature" FG 1

Again, farmers expressed their displeasure about the status of veterinary extension services in the country. Some of the key responses were:

"Zero. Veterinary and extension services should go round. They should visit farms and tell us what they can offer us. They don't show up. Vet extension has gone to extinction" FG 1

Objective 4: Farmers' Proposed solutions to ensure effective use of AMs

The most common recommendations mentioned by the participants for various stakeholders' in the pig industry were extensively discussed and summarized below:

Pig producer level:

At the pig producer level, the farmers' suggested improvement in best practices in animal management (e.g. housing, quality feeding and alternatives to antibiotics), hygiene, and biosecurity should be given priorities. The need to acquire adequate knowledge on AMR stewardship and effect on animal and human health was considered important for them as well.

Veterinarian level:

The farmers recommended that veterinarians should make their services available and affordable. Proper diagnosis of diseases must be provided to them before drug prescriptions especially AMs. Development of research innovations for alternative medicine e.g. herbal therapy, and pig management techniques e.g. organic farming was mostly emphasized by the farmers. Participants suggested that more practical training and workshops on prudent AMU and animal management were needed for improving AMU in pig production. With relation to training programs, some of the responses of the participants were as follow and we quote

"Henceforth, we don't want abstract training. We are practical people; we are hands-on. Most of the training they provide is abstracts. Not everyone is educated but if you show them the technical knowhow, they will practice them on their farms" FG 1

.... The veterinarians should educate us on pig diseases, physical recognition and manifestations of these diseases. We pick information randomly from books or internet" FG 3

Government level

The need for the government to enforce quality control in relation to feeds and drug production or importation should be taken seriously. Farmers want the government to identify with them as well as provide loans and enforce policies that will enhance pig operations in the country, fund the veterinary and extension services as well as employ more vets to cover all the LGAs in Ogun state. The farmers concluded by demonstrating that the FGD was educative and that they gained some knowledge on antibiotic use. They indicated that understanding the

prudent use of antibiotics and making them work efficiently is the survival link for livestock sustainability

Discussions

This study provides an understanding of pig farmers' perception to factors that drive the use AMs in the industry. Also the various challenges confronting pig producers in the country are reported. Several drivers encouraging excessive use of AMs as self - perceived by pig farmers were poor animal nutrition due to low quality feeds and high costs of purchasing the quality ones; disease outbreak and poor preventive strategies; unrestricted accessibility to AMs off counter; high cost of and poor accessibility to veterinary services; and producer's expertise and preceding experiences, cost-benefits, all of which corroborate previous documentations by other authors (Coyné et al., 2014; Lhermie et al., 2016; Adebowale et al., 2016; Ekakoré et al., 2019).

In many countries, the high efficacy of antimicrobials especially broad spectrum AMs and their relatively low cost make their use favorable. For instance, oxytetracycline LA, a broad spectrum AM being self-reported by farmers in this study as commonly used in the industry is readily available, cheap and cost effective compared with other antimicrobials. Furthermore, farmer's intention to make high profit and avert risks influence AMU since the higher the price of the output, the higher the economic impact of the disease if not treated (Lhermie et al., 2016).

The decision making of the farmer to use AMs also depends on accessibility. In many developing countries, regulations surrounding manufacturing of drugs and accessibility present on papers but poorly implemented (Dar et al., 2016; Adebowale et al., 2016). The weak implementations of these regulations result in major sales of AMs off counter, encouraging inappropriate use such as incorrect dosage, non-observance of withdrawal periods, and reduced (Shryock, 2012). Self-medication of AMs was perceived to spur from farmers' dissatisfaction with veterinary services especially the high cost. Self-medication through acquisition of AMs off counter by livestock producers have been extensively reported by previous studies from Nigeria (Olusile et al., 2013; Adebowale et al., 2016; Ojo et al., 2016; Olosó et al.,

2019). Additionally, the predisposition to rely on personal experience sometimes leads farmers to use these drugs indiscriminately, and makes them unaware of the need to consult veterinarians, whom they all see when all obtainable means of treatment have been tried with no effect. Poor biosecurity, management and hygiene were also perceived as factors predisposing to high occurrence and persistence of diseases on the pig farms and AMU more for prophylactic than therapeutic purposes.

Lastly, various challenges confronting the pig industry reported here agrees with previous documentation by Uddin and Osasogie, 2017. The study conducted in Nigeria showed difficulty in securing institutional loans ranked top most constraint followed by cost of feed and feed ingredients and disease outbreak. Financial inadequacies and cost of feeding animals with quality feeds has resulted into slow growing pig production in the country (Uddin and Osasogie, 2017).

Conclusion

Creating positive awareness on drivers for the development continuing education on prudent antimicrobial use is needed by pig farmers in Nigeria. Training on prudent AMU is likely to be well received by pig farmers if such comes from veterinary extension officers as we observed during our interactions with the farmers. Educational programs on antimicrobial stewardship could be enhanced especially through seminars, which is the preferred means of communication suggested by the pig farmers. Scientific innovations and studies into new antibacterial drug development, possible alternative therapies to e.g. ethno veterinary, and the advancement in new animal husbandry techniques should be given priorities by the academia. Lastly, government support through the provision of agricultural loans and improvement of veterinary extension services in the country is emphasized.

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Conflict of interest

The authors declare no conflict of interest.

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WIS-ASP 22

EVALUATION OF ORGANIC MATTER AND AGGREGATE STABILITY STATUS OF AMENDED SLASH-AND-BURN SOIL IN RAINFOREST, ABIA STATE NIGERIA

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ABSTRACT

Slash-and-burn agricultural practice results in reduction of soil quality, due to surface cover removal and other biochemical alterations in the soil ecosystem. In order to investigate the effect of burning on some soil quality indices; a pot experiment was conducted at the screen house of College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike. The aim of the study was to assess the changes in organic matter and some aggregate stability indices in a slash/burn soil amended with soil inputs in rainforest zone, Abia State. The treatments were: cow dung (CD), improved cow dung mixture (ICDM), NPK fertilizer (NPK) and two controls. The two controls did not receive any amendments; they were soils collected before initiating the land burning (UBSCL) and soils collected after burning the land (BSCL). Treatments were applied at 10t/ha (equivalent of 110g) to 10kg of soil samples collected from a smallholder woman farm in Abia State and replicated five times. Mean weight diameter was 0.61mm in BSCL but was increased in UBSCL to 2.85 mm. The soil organic matter had a negative and highly significant relationship ($P \leq 0.001$; 0.68) with <0.5 mm sieve size fraction. Results obtained showed that adding amendments will improve soil organic matter and the tested stability indices of slash-and-burn soils of the study area.

Keywords: Slash/burn; Aggregate stability; Organic matter; Amendments

INTRODUCTION

In the tropical regions of the world, slash-and-burn agricultural system is still being practiced (van Vliet *et al.*, 2012; Mukul and Herbohn, 2016). The slash- and -burn agriculture as defined by Stief (2020) as the process of cutting the vegetation, setting fire on the remaining foliage and the cut down biomass; using the ashes to provide nutrients for planted crops in a particular piece of land. Cutting down of the existing vegetation and setting them on fire are the two main practices involved in slash- and -burn agriculture. Some farmers practice this system because it is cost effective; others practice it with the intention of destroying disease pathogens in the soil. By introducing fire as a means of land clearing, the farmers may save money and then channel the resources to other things that will improve their livelihood (Nathet *et al.*, 2015).

However, the use of fire on agricultural land can cause damages and changes in the ecosystem, as well as affect soil quality (Thomaz 2018). Some of the detrimental effects of slash-and-burn agricultural system include: soil free moisture vaporization, nitrogen, phosphorus, and sulphur volatilization, decline in surface soil carbon and nitrogen, soil erosion, carbon emissions, biodiversity loss, soil microbial population changes, decrease in soil fertility, and increase in water repellency (DeBano 1991; DeBano *et al.*, 1998; Certini 2005; Knicker 2007; Pellegrini *et al.*, 2018). Burning has also been reported to reduce infiltration rates, hydraulic conductivity, mean weight diameter and water stable aggregates (Are *et al.*, 2009), which are some of the indices of soil stability.

Setting fire on land affects the stability of the soil aggregates. This is because the five main aggregate formation and stabilization factors namely: microorganisms, soil fauna, environmental variables, roots and inorganic binding agents (Six *et al.*, 2004) are directly or indirectly affected by fire (Thomaz 2018). Most of the binding agents are of organic matter origin. It has been reported that organic matter plays an important role in soil stabilization (DeBano 1991). Soil organic matter, which is a complex of organic substances derived from plants, living and non-living organisms (Kalev and Toor 2018; Zhao 2018), can be reduced by land burning (Certini 2005; Ludwig *et al.*, 2018) especially those located on or near the soil surface (DeBano 1991).

Reduction in soil organic matter affects soil nutrients pool, crop productivity, soil infiltration, soil resistibility to erosion and runoff. In order to build-up soil organic matter, external soil inputs are added to the soil. The use of inputs such as cow dung are long age practices by the farmers however, the rate of organic matter build-up is usually very slow (Onwuka *et al.*, 2019). Thomaz (2018) stated that labile molecules and mucilage from organic matter have immediate effect on soil aggregation. Hence, improving cow dung to have an immediate improvement on soil organic matter and soil aggregation will be beneficial to the soil ecosystem, environment and farmers.

We hypothesized that addition of cow dung in improved form will increase soil organic matter and some aggregate stability indices. The objective of this study was to determine the cow dung, improved cow

dung mixtures and fertilizer effect on organic matter and some aggregate stability indices on a slash- and - burn land.

Material and Methods

Description of soil sampling area and preparation

The soil samples were collected from a smallholder woman farmer’s farm located at Afugiri (Latitude 5° 35’ 47”N and Longitude 7° 28’ 44” E), Abia State Nigeria in March 2019. The area falls within the Tropical Rainforest Zone of Nigeria. The dominant plant species in the study area included: black velvet tamarind (*Dialium guineense*), Okpa (*Teltracarpidum sp*) among others. The farm land was previously used for maize, cassava, vegetable and melon cultivation. After the farmer harvested her crops in October 2017, the land was left for a short fallow period of a year and five months. Within the farm, soils samples were randomly collected from ten spots using a soil auger. The samples were collected before and after burning the slashed biomass at a depth of 0-15cm. The samples were bulked to get a composite sample, which was bagged, properly labeled for the screen house experiment and pre-treatment soil analyses. The soil for the experiment and pre-treatment soil analyses were passed through a 2 mm mesh sieve size. Some of the properties of the soil are shown on Table 1

Table 1: Some selected soil properties of the soil used for the experiment

Soil properties	Value
Sand (g kg ⁻¹)	726
Silt (g kg ⁻¹)	149
Clay (g kg ⁻¹)	125
Textural class	Sandy loam
Mean weight diameter (mm)	0.70
Organic matter (%)	2.39
Water aggregates stability (%)	16.10

Description of the study area

The research was a pot experiment conducted at the screen-house of the College of Crop and Soil Sciences of Michael Okpara University of Agriculture Umudike (Latitude 05° 29’ N and Longitude 07° 33’ E). Umudike with an altitude of 122 meters above sea level falls within the Tropical Rainforest Zone of Nigeria. The mean annual rainfall is 2200 mm, distributed over nine to ten months in bimodal rainfall pattern, these are the early rains (April to July) and late rains (August to October). The relative humidity varies from 84 % to 87 %; maximum air temperature ranges from 20°C to 24°C and the monthly maximum air temperature ranges from 28°C to 35°C (NRCRI, 2019).

Treatments and treatment preparation

The treatments consisted of Cow dung (CD), Improved Cow dung mixture (ICDM), Nitrogen: Phosphorus: Potassium 15:15:15 fertilizer (NPK), a burnt soil (BSCL) which served as the first control and an unburnt soil (UBSCL) which served as the second

control. The two controls did not receive any amendment. Cow dung was sourced from the Livestock unit of Michael Okpara University of Agriculture Umudike (MOUAAU). The Cow dung was air- dried and passed through a 1mm sieve size. Improved cow dung mixture was prepared by mixing poultry manure, cow dung and composted fruit peels wastes at a ratio of 1:2:1. The composted fruit peels were sourced from farmers’ homes and local market places. The peels were composted in a composting bin for ninety days and were utilized after the compost maturity test was done. The poultry manure was sourced from the Livestock unit of MOUAAU. The N: P: K 15:15:15 fertilizer was sourced from the Ministry of Agriculture, Abia State. Some of the chemical compositions of the treatment used are shown on Table 2.

Table 2: Some chemical compositions of the treatment used in the experiment

Properties	CD	ICDM
pH(H ₂ O)	6.40	6.80
Available Phosphorus (mg kg ⁻¹)	1.72	1.83
Organic Matter (%)	24.86	26.3

Experimental Procedure and Design

The organic amendments CD and ICDM were applied at the rate of 10 t ha⁻¹ according to the recommendation of Okutu *et al.*, (2011). The grams equivalent of 10 t ha⁻¹, which was 110 g was the actual rate applied on dry basis to 10 kg dry soil in the experimental pots of 12 litre sizes. The N: P: K 15:15:15 was applied at the rate of 125 kg ha⁻¹ (0.625 g equivalent of kg/ha) recommended by Omolayo and Ayodele, (2009) for mung bean. The treatments were replicated four times in a Completely Randomized Design (CRD) to give a total of twenty pots. The experimental pots were kept moist all through the experimental period that lasted for three months. Mung bean was the test crop used and the manuscript for the mung bean data is presently under review in a Journal.

Protocols for soil properties determination

Soil organic matter was calculated after determining the organic carbon by the wet dichromate-oxidation method of Walkley and Black as modified by Nelson and Sommer (1982). The values of organic carbon gotten were multiplied by 1.72 (*Van Bemmelen Factor*). Mean weight diameter (MWD) of the water-stable aggregates was determined by the wet-sieving method of Kemper and Rosenau (1986). The formula below was used for the calculation:

$$MWD = \sum_{i=1}^n XiWi \dots\dots (1)$$

Where; MWD is the mean weight diameter of water stable aggregates, Xi is the mean diameter of each size fraction (mm), and Wi is the proportion of the total

sample mass in the corresponding size fraction after deducting the mass of stones.

The water aggregate stability was calculated as $WAS\% = \{(M_{(a+s)} - M_s) / (M_t - M_s)\} * 100$ (2) where; WAS% = water aggregate stability percentage, $M_{(a+s)}$ is the mass of the resistant aggregates in each sieve size plus sand (g), M_s is the mass of sand (g) fraction alone and M_t the total mass of sieved soil (g).

Statistical Analysis

Statistical analysis was performed using GENSTAT software package 19th Edition (GENSTAT Rothamsted Research Center, United Kingdom). Significant differences were obtained by one-way analysis of variance (ANOVA) for Completely Randomized Design (CRD) with means separated using Fisher's Least Significant Difference at probability level of 5%. Relationships among the soil properties were determined using a simple correlation analysis.

Results

Treatment effect on soil organic matter and some soil stability indices

Treatment	SOM (%) (Mean ±SEM)	WAS (%) (Mean ±SEM)	MWD(mm) (Mean ±SEM)
UBSCL	2.77±0.66	75.29±1.64	2.85±0.31
BSCL	2.42±0.29	16.13±0.95	0.61±0.07
ICDM	3.37±0.33	45.71±1.58	1.75±0.09
CD	2.98±0.45	40.33±0.11	1.56±0.32
NPK	2.81±0.04	18.82±0.35	0.70±0.10
Mean	2.87	39.26	1.49
Lsd	0.08	2.09	0.45

UBSCTL = Unburnt soil control; BSCTL= Burnt soil control; IMCD= Improved cow dung mixture; CD = Cow dung; NPK= Nitrogen; Phosphorus; Potassium; SOM=Soil organic matter; WAS= Water aggregate stability; MWD= Mean weight diameter

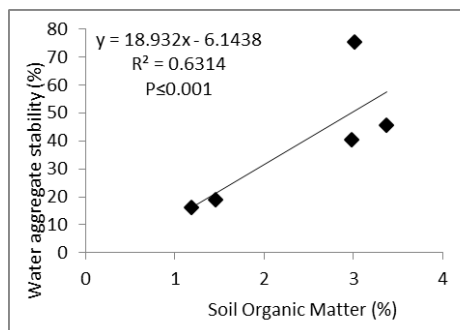
Relationship of soil organic matter with aggregate stability indices (WAS and aggregate sieve fractions)

The relationship between soil organic matter and water aggregate stability (Figure 1) was positive with a linear

Soil organic matter was significantly ($P \leq 0.05$) highest in the pots that received ICDM (Table 3) with a value of $3.37 \pm 0.33\%$ (n=5). It had percentage increases of 17.80 %, 28.19 %, 11.57% and 16.61% over UBSCL, BSCL, CD and NPK respectively. More organic matter was recorded in UBSCL than in BSCL. The value for water aggregate stability was highest in UBSCL followed by ICDM. The value of $16.13 \pm 0.95\%$ (n=5) was gotten in BSCL though, this value was not statically different from the value of $18.82 \pm 0.35\%$ (n=5) obtained in pots that received NPK. Mean weight diameter was greatly improved in UBSCL with a value of 2.85 ± 0.31 mm. Considering the amendments, ICDM had the highest value for MWD over the values obtained for CD and NPK.

Table 3: Effect of treatments on soil organic matter and soil aggregation measured as water- aggregate stability (WAS %) and mean weight diameter (MWD mm)

coefficient of determination (R^2) and a highly significant ($P \leq 0.001$) correlation coefficient (r). The R^2 was 63% and this shows that about 63% of the analyzed data fitted into the regression model. Increase in SOM, resulted to a proportional increase in WAS.



About 85% of the data fitted into the regression model of the association between SOM and >2mm size fraction (Figure 2). The association was linear and positive; the two parameters had a strongly positive correlation with a value of 0.67^{***} .

Figure 1: Relationship between soil organic matter and water aggregate stability

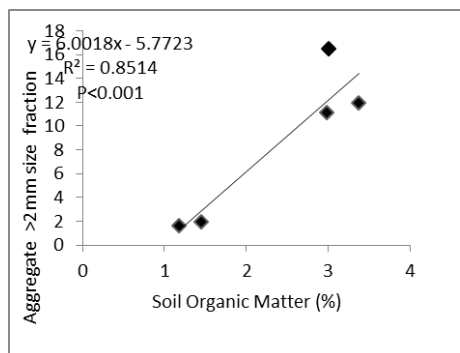


Figure 2: Relationship between soil organic matter and aggregate >2mm size fraction

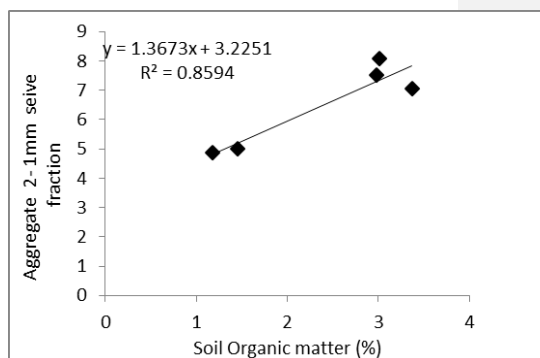


Figure 3: Relationship between soil organic matter and aggregate 2- 1mm size fraction

The relationship between SOM and 2- 1 mm size fraction was linear with R² value of 85% (Figure 3). The correlation coefficient was positive with a value of 0.22 but the relationship was not significant.

Soil organic matter had a negative but a highly significant (P<0.001; 0.68) correlation with < 0.5 mm size fraction (figure 4). The model was strongly fitted with R² being as high as 94%.

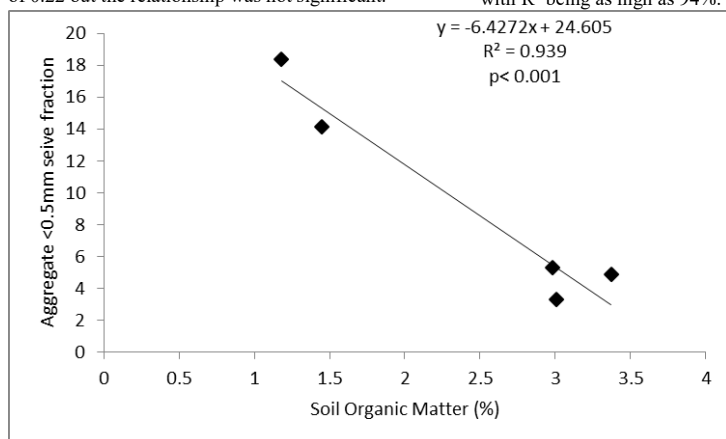


Figure 4: Relationship between soil organic matter and aggregate <0.5mm size fraction

Discussion

Soil organic matter, water aggregate stability and mean weight diameter

The increased soil organic matter recorded in pots that received ICDM could be as a result, of the readily biodegradable materials contained in the ICDM used. These biodegradable materials are easily degraded by microorganism, to result in an increase in the soil organic matter pool. The readily biodegradable fractions of the organic manure used for this study, would have come from fruits and vegetables added as part of cow dung mixture, to enhance the organic manure. Fruits and vegetables are easily degraded because they contain simple carbohydrates, small molecules of fatty acids and alcohols (Cooperband 2002; Girard *et al.*, 2013).

The low SOM recorded in BSCL which was less than that recorded for UBSCCL could be as a result of the

burning effect. When soils are subjected to burning, organic matter located in the soil and on the surface are burnt (DeBano 1991; Certini, 2005; Ludwig *et al.*, 2018). However, the finding in the present work that organic matter was reduced in the burnt soil was contrary to the findings of González-Pérez *et al.*, (2004), who reported that burning increased the soil organic matter.

Water aggregate stability and mean weight diameter show how stable the soil aggregates are to disruption. Mean weight diameter, which is the measure of soil macro-aggregates (Amezkeka 1999), was highest in UBSCCL. The increase in MWD of UBSCCL may not necessarily be from SOM (Table 3), rather from the exudates produced from plants roots (Bronick and Lal 2005), which may have bound the soil aggregates during the short fallow period. The increase in mean

weight diameter might have caused an increase in the water aggregate stability of the UBSC (Table 3). Are *et al.*, (2009) observed that there was a positive relationship between MWD and WAS. Similarly, they also observed that Alfisols subjected to burning had lower MWD and WAS values, as also observed in the present study.

Relationship among soil organic matter, water aggregates stability and sieve sizes

The positive relationship observed (Figure 1-3) among SOM, WAS, > 2 mm and 2-1 mm sieve size fractions was an indication that SOM had influence over them. The more the SOM was increased, the more WSA and > 1 mm sieve size were increased, to make the aggregates resistant to disruption. In the same vein, the negative correlation observed between SOM and <0.5 mm sieve size means that less SOM favours more

aggregates at <0.5 mm sieve size (micro aggregates).

Conclusion

The present study shows that burning reduces the soil organic matter, aggregate stability and mean weight diameter. By application of amendments in the form of improved cow dung mixture (ICDM), the lost SOM of soil subjected to slash- and-burning was increased. The study also provided information that WAS and MWD of soils not subjected to burning were higher compared to those subjected to burning in the study area. It was also observed that SOM correlated positively with WAS, > 2 mm and 2- 1 mm size fractions but negatively with <0.5 mm size fraction. If farmers must subject their farms to burning as their traditions are then, applying amendments especially ICDM in the study area will, assist in improving the soil properties studied.

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EFFECTS OF ETHIDIUM BROMIDE ON GERMINATION, GROWTH AND YIELD OF BAMBARA GROUNDNUT [*Vigna subterranea* (L.) VERDC.]

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ABSTRACT

The study was aimed at inducing genetic variability in growth and yield characters of bambara groundnut using ethidium bromide. Two accessions of bambara groundnut (TVSu 10 and TVSu 247) were obtained from the germplasm unit of International Institute of Tropical Agriculture, (IITA) and soaked in varying concentrations of ethidium bromide (EtBr): control, 0.01%, 0.02%, 0.03% and 0.04% for 3 hours after which they were rinsed with distilled water and planted in the field. The experiment was laid out in a randomized complete block design with three replicates. Growth parameters were taken at 2 weeks interval while yield parameters were taken at harvest. Result of the study revealed that EtBr inhibited germination and survival in both accessions with control plants having higher germination and survival percentage than treated plants. 0.02% EtBr induced more branches and number of leaves for TVSu 10, while 0.03% of EtBr induced taller plants and higher leaf area. However, control plants performed better than EtBr treated plants at various concentrations in TVSu 247 except for number of branches which had the highest value at 0.01% of EtBr. Treatment with 0.03% EtBr induced higher yield in TVSu 10 while 0.02% EtBr recorded highest values for TVSu 247 compared to control and other treatments. Ethidium bromide, though carcinogenic and no longer preferred for staining of DNA, promises to be a highly beneficial mutagen in creation of variability in bambara groundnut and can be applied in the genetic improvement of the crop for growth and yield related traits.

Keywords: Bambara groundnut, ethidium bromide, genetic variability, growth, yield.

Introduction

Grain legumes are the major dietary protein source for a greater part of the population in many low-income food deficit countries (LIFDCs) of the world (Linnemann and Azam-Ali, 1993). Bambara groundnut (*Vigna subterranea* (L.) Verdc.), a member of the family Fabaceae, is an indigenous crop cultivated mainly in arid and semi-arid regions of sub Saharan Africa. Bambara groundnut plays an important role in food security, especially as it is a major source of protein providing most protein requirements for low-income farmers (Collinson *et al.*, 2000). However, pod yields remain low and unpredictable (650 - 850 kg.ha⁻¹), because of the high level of variability observed in growth and development within a landrace (Squire *et al.*, 1997).

The application of conventional plant breeding techniques is time consuming and does not always lead to the development of desired variation (Cassels and Doyle, 2003). This is one of the main reasons why plant breeders moved to search for alternative ways to obtain desired variation in crops within a short period. Induced mutagenesis has immense potentials and serves as a complimentary approach in genetic improvement of crops (Mahandjiev *et al.*, 2001). It is hoped that some of these mutations may lead to the desired character to alter the genetic architecture of plant and isolate mutants with desired economic characters such as plant height, number of pods per plant, number of grain per pod, 1000 - grain weight,

grain yield, oil content and disease resistance (Javed *et al.*, 2000). The main advantage of induced mutagenesis is the possibility of improving one or two quantitative or qualitative characters without altering the rest of the plant. In several mutation derived varieties, the changed traits have resulted in increasing the yield and quality of the crop, improving the agronomic inputs and consumer acceptance (Ahloowalia *et al.*, 2004).

Chemical mutagenesis has become a widely adopted approach because it does not require special facilities. Ethidium bromide is a chemical mutagen that has been classified as intercalating agent; it binds DNA by intercalating between the base pairs of DNA strands and is an extremely useful tool that has applications in different techniques (Palchaudhuri and Hergenrother, 2007). Ethidium bromide as a mutagenic agent has been reported by few authors to induce variations in different crops; Burton and Hanna (1976) in pearl millet; Kinoshita *et al.* (1982) used ethidium bromide to induce variability in sugar beet; Hasegawa and Inoue (1982) used ethidium bromide to enhance mutagenicity created by sodium azide in pearl millet; Mehreen *et al.* (2017) induced variability in *Praecitrullus fistulosus* using ethidium bromide, colchicines and x-ray. Recently, Ajayi *et al.* (2018, unpublished data) induced drought tolerance in drought susceptible cowpea using ethidium bromide. Genetic improvement of Bambara groundnut can be achieved through induced mutagenesis using either physical or chemical mutagen. This study provided

information on creation of genetic variability using ethidium bromide on two accessions of Bambara groundnut.

Materials and Methods

Experimental Design

Seed Source

300 viable seeds of 2 accession of Bambara groundnut (TVSu-10 and TVSu-247) were obtained from germplasm unit of International institute of Tropical Agriculture (IITA) Ibadan.

Source of Mutagen and Treatment

10ml of Ethidium Bromide solution was obtained from Inqaba Biotech® South Africa, CAS number 1239-45-8mw of 394.31 formula $C_{21}H_{20}BrN_3$. Distilled water was obtained from tissue culture laboratory of National Root Crops Research Institute Umudike. The preparation was done in the undergraduate laboratory of Department of Plant science and Biotechnology, Michael Okpara University of Agriculture Umudike. The surface and the materials were sterilized with hypochlorate. Five different treatments were used for the experiment, treatment 0.01% (1ml/999ml of water), 0.02% (2ml/998ml of water), 0.03% (3ml/997ml of water), 0.04% (4ml/996ml of water) and control (1000ml of water). The seeds of the two accessions were partitioned into 5 groups each containing 60 seeds and pre-soaked in distilled water for 3 hours. After 3 hours, the pre-soaked seeds were drained and allowed to dry. The seeds were soaked for 3 hours in the different treatments already prepared. After which they were rinsed, drained and air dried.

Germination Test

After treatment, the seeds were sown in nursery bags at the screen house for germination and survival test. 50 germination bags were filled with top soil. 4 seeds were planted in each of the germination bags, 5 bags per treatment per accession. The experiment was laid out in a completely randomised design with 5 replicates. Constant watering of the plant was done at an interval of 2 days. 28 days after germination, final germination percentage and survival percentage were determined for each treatment using the formula:

For final germination percentage:

$$\frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times \frac{100}{1}$$

Survival percentage:

$$\frac{\text{Number of plants that survived 28 DAP}}{\text{Number of seeds germinated}} \times \frac{100}{1}$$

After the germination test, all treatments were taken to field as none caused less than 50% inhibition in germination.

Field Experimental Layout

The experiment was set up at the experimental field of the Department of Plant Science and Biotechnology, Michael Okpara university of Agriculture, Umudike.

The field was laid out in a completely randomized block design with 3 replicates. A field size of 30m x 30m was cleared and divided into 6 blocks (3 blocks per accession), each block measured 4.5m x 4.5m and contained 5 beds representing the five treatments. 15 beds were made per accession making it a total of 30 beds for the two accessions. 16 seeds were planted on each bed at a space of 30cm x 30cm and a distance of 0.5m was maintained between beds and 1m between blocks. At an interval of 2 weeks, the following measurements were taken; plant height number of leaves, number of branches, leaf area. At harvest, the following yield parameters were taken: number of pods/plant, weight of pod/plant, number of seeds/pod and grain yield

Plant Height (cm): this was taken at 2 and 4 weeks after planting (WAP) using the meter rule measured from the ground level (at the base of the plant) to the tip of the highest point, including the terminal leaflet.

Number of leaves/plant: this was taken at 2 and 4 weeks after planting (WAP) using counting method to count the number of leaves on each plant.

Number of branches/plant: this was taken at 2 and 4 WAP, this was done by counting the average number of branches of five healthy plants

Leaf area: This was calculated using a formula modified from Sasey and Zungu (2004) by taking the terminal leaf length and width of ten random plants at flowering stage and multiplying with a constant 0.88 ($TLL + TLW \times 0.88$).

Terminal leaflet length (TLL) [cm] Recorded 10 weeks after planting; average length of three leaves at the fourth node of five healthy plants

Terminal leaflet width (TLW) [cm] Recorded 10 weeks after planting; average width of three leaves at the fourth node of five healthy plants

Number of pods/plant: this was done by counting the number of pods per plant.

Weight of pods/plant (g): this was done by weighing the number of pods per plant using a sensitive weighing balance.

Number of seeds: this was done by counting the total number of seeds per plant.

Grain yield (g): this was taken by weighing total number of seeds per plant.

Statistical analysis

Means of each data collected was calculated and subjected to analysis of variance performed using SAS 9.1 version, the means were separated using Duncan multiple range test and significance between the means determined at ($P < 0.05$).

Results

Effect of EtBr on germination and Survival of TVSu 10 and TVSu 247

Figure 3.1 below shows the mean effect of EtBr on germination percentage of TVSu 10 and TVSu-247. The result for TVSu-10 showed that germination of

seeds was highest in the control and EtBr treatment at 0.02% with values (85%) respectively. This was followed by 0.04% (65%), 0.03% (60%) and 0.01% having the lowest germination percentage (50%). While result of TVSu 247 showed that germination of seeds was highest in the control (85%) and decreased as the concentration of EtBr increased (0.01%, 0.02%, 0.03%, 0.04% with values 80%, 50% 75% and 60%) respectively.

Figure 3.2 below shows the survival percentage of TVSu 10 and TVSu 247 respectively at 28 days after planting. For TVSu 10, 95% of the germinated control and 0.01% EtBr plants survived at 28 days after planting while the other treatments (0.02%, 0.03% and 0.04%) all had 100% survival. For TVSu 247, a high survival percentage of 100% was recorded for all control and treated plants except those treated with 0.02% EtBr which recorded 90% survival percentage.

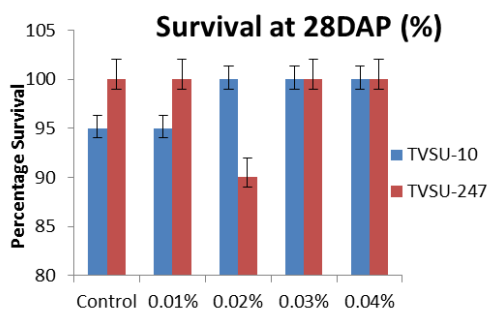


Figure 3.2: Effect of EtBr on survival at 28 days after planting on TVSu 10 and TVSu 247 (DAP = Days after planting)

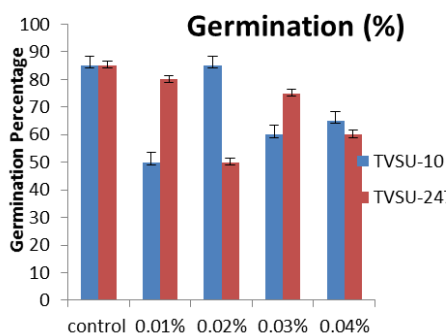


Figure 3.1: Effect of EtBr on germination percentage of TVSu 10 and TVSu 247.

Mean effect of EtBr on growth parameters of TVSu10 and TVSu 247

Table 3.1 below shows the mean effect of EtBr on growth parameters of TVSu10. A significance difference was recorded for all the growth parameters except leaf area. For plant height, 0.03% treatment induced taller plants at both two weeks and four weeks after planting respectively (22.10cm and 23.50cm) and while the least plant height was recorded at 0.01% treatment at both two weeks and four weeks after planting respectively (17.63cm and 19.20cm). At 2 and 4 weeks after planting, 0.02% recorded the highest number of branches (15.67 and 16.00) respectively while control recorded the lowest number of branches (9.33 and 10.33) respectively. At 2 and 4 weeks after planting, it was recorded that treated plants produced more leaves than the control with 0.02% having the highest number of leaves (34.00 and 37.33) respectively and control having the lowest number of leaves (20.00 and 22.00) respectively.

The result obtained for TVSu 247 showed that there was no significant difference recorded for all the growth parameters except for leaf area (table 3.1) unlike that of TVSu-10 which recorded significant difference for all the growth parameters except for leaf Area. It was observed that control did better than plants treated with the mutagen for plant height, number of leaves and number of branches at both 2 and 4 weeks after planting respectively. Control recorded the highest leaf area (8.11) while 0.01% recorded the least leaf area (4.42).

Table 3.1: Mean effect of EtBr on growth parameters of TVSu10 and TVSu 247

Accessions	Treatments	PH2WAP (CM)	PH4WAP (CM)	NB2WAP	NB4WAP	NL2WAP	NL4WAP	LA(CM ²)
TVSu 10	0.00%	19.27±3.80 ^{ab}	20.17±3.21 ^c	9.33±5.13 ^b	10.33±5.13 ^b	20.00±9.17 ^b	22.00±9.17 ^c	6.66±2.74 ^a
	0.01%	17.63±1.98 ^b	19.20±1.93 ^c	13.00±2.65 ^{ab}	14.00±2.65 ^{ab}	28.67±1.53 ^a	31.00±1.00 ^{ab}	4.69±1.04 ^a
	0.02%	19.83±4.54 ^{ab}	20.33±4.04 ^{bc}	15.67±3.51 ^a	16.00±4.00 ^a	34.00±1.53 ^a	37.33±5.03 ^a	5.84±3.04 ^a
	0.03%	22.10±1.35 ^a	23.50±1.00 ^a	10.67±4.62 ^b	11.33±4.93 ^{ab}	29.00±8.66 ^a	30.67±9.07 ^{ab}	7.54±0.78 ^a
	0.04%	21.77±2.73 ^a	23.17±2.25 ^{ab}	10.67±2.52 ^b	11.67±2.52 ^{ab}	27.00±5.20 ^{ab}	28.67±5.69 ^{bc}	5.79±3.52 ^a
TVSu 247	0.00%	20.17±3.33 ^a	21.40±3.77 ^a	11.33±1.53 ^a	12.33±1.53 ^a	28.00±1.73 ^a	38.00±6.00 ^a	8.11±3.07 ^a
	0.01%	19.50±4.33 ^a	19.97±4.05 ^a	16.67±13.43 ^a	17.33±12.86 ^a	32.00±14.53 ^a	34.00±14.53 ^a	4.42±1.58 ^b
	0.02%	17.50±2.29 ^a	19.90±3.01 ^a	14.00±1.00 ^a	15.00±1.00 ^a	30.67±2.08 ^a	32.67±2.08 ^a	6.17±1.94 ^{ab}
	0.03%	19.47±2.73 ^a	21.17±1.76 ^a	13.67±6.03 ^a	14.67±6.03 ^a	35.33±16.20 ^a	37.00±16.52 ^a	5.09±1.86 ^{ab}
	0.04%	19.37±2.49 ^a	20.47±1.76 ^a	14.00±3.61 ^a	15.00±3.61 ^a	35.67±12.50 ^a	37.00±11.53 ^a	5.21±1.25 ^{ab}

Means with the same letter are not significantly different at P<0.05.

PH =Plant Height, NB = Number of Branches, NL = Number of Leaves , LA = Leaf Area, WAP = Weeks after planting

Mean effect of EtBr on yield parameters of TVSu10 and TVSu 247

The mean effect of EtBr on yield parameters of TVSu10 is shown in Table 3.2. From the result obtained, there was no significant difference in the mean effect for all the yield parameters. However, plants treated with the mutagen produced more yield than the control with 0.03% having the highest number of pods (39.33) and 0.01% having the highest number of seeds (29.00).

significant difference was recorded for all the yield parameters except seed weight (table 3.2). Just like TVSu-10, the plants treated with mutagen produced more yield than the control with 0.02% having the highest yield for all the yield parameters: number of pods, pod weight, number of seeds and seed weight (79.33, 69.33g, 63.00 and 11.33g) respectively. Control plants recorded the least yield in all the yield parameter (10.33, 15.67g, 8.67 and 1.33g) for number of pods, pod weight, number of seeds and seed weight respectively.

The result obtained for TVSu 247 revealed that a

Table 3.2: Mean effect of EtBr on yield parameter of TVSu-10 and TVSu 247

Accession	Treatments	NP	PW (g)	NS	SW (g)
TVSu 10	0.00%	22.67±4.51 ^a	30.33±7.02 ^a	16.33±6.35 ^a	2.00±2.00 ^a
	0.01%	33.33±15.01 ^a	31.67±18.61 ^a	27.00±13.45 ^a	4.33±2.08 ^a
	0.02%	31.33±29.14 ^a	47.33±50.52 ^a	23.33±18.58 ^a	2.00±2.00 ^a
	0.03%	39.33±23.80 ^a	50.67±31.21 ^a	26.67±13.05 ^a	3.67±2.31 ^a
	0.04%	29.00±9.64 ^a	43.67±0.58 ^a	23.00±6.00 ^a	2.67±1.15 ^a
TVSu 247	0.00%	10.33±5.03 ^b	15.67±6.03 ^b	8.67±4.51 ^b	1.33±1.15 ^a
	0.01%	33.33±37.82 ^{ab}	30.33±36.95 ^{ab}	26.33±25.70 ^{ab}	1.67±2.08 ^a
	0.02%	79.33±59.28 ^a	69.33±52.65 ^a	63.00±45.04 ^a	11.33±10.26 ^a
	0.03%	48.33±45.49 ^{ab}	48.33±51.03 ^{ab}	37.67±37.42 ^{ab}	9.00±12.12 ^a
	0.04%	44.67±37.53 ^{ab}	42.33±38.89 ^{ab}	36.00±37.80 ^{ab}	5.33±6.81 ^a

Means with the same letter are not significantly different at P<0.05.

NP = Number of Pods, PW = Pod Weight, NS = Number of Seeds, SW = Seed Weight

Discussion

The result on germination and survival percentage showed that Ethidium bromide inhibited germination and seedling survival of seeds of both accessions with control plants having higher germination and survival percentage when compared with the treated plants. This was in accordance with reports of Mehreen *et al.* (2017) who stated that seed germination percentage of *Praecitrullus fistulosus* was highest in case of control plants and the second highest germination percentage (55.0%) was in case of ethidium bromide 0.10% treated plants. The reduction in seed germination with EtBr treatment dose may have been caused by a delay or inhibition of physiological processes such as enzyme activity,

hormonal imbalance, and inhibition of mitotic process and make up of the species (Kumar and Gupta, 2009; Borovsky *et al.*, 2013).

Ethidium bromide improved most of the growth parameters of TVSu 10 accession investigated. 0.03% induced the highest plant height while 0.02% induced the highest number of branches and leaves. Similar results on stem length and number of leaves was recorded by Mehreen *et al.* (2017) in *Praecitrullus fistulosus*. They observed that treated plants with ethidium bromide at 0.1% showed the higher values of plant length and number of leaves compared to the control. This result is supported by Khan *et al.* (2016) who reported that lower doses of mutagen treatments

have been found effective in altering the physiological, genetic, and chemical state of plants, and further study of the raised variants may lead to new variety development with desirable traits. The result on leaf area indicated high values from 0.03% treatment which was higher than control. This was in contrast to results obtained by Mehreen *et al.* (2017) who recorded highest leaf area for *Praecitrullus fistulosus* at 0.02% of colchicines followed by 0.05% of EtBr.

However, result on growth parameters of TVSu-247 showed that control plants performed better than EtBr treated plants at various concentrations. The control plants gave higher values for plant height, number of leaves and leaf area compared to the treated plants. This trend is not in agreement to the trend observed in TVSu-10 which indicates that variety of plants play a huge role in the mutagenic activity of mutagens (Thilagavathi and Mullainathan, 2009).

From the results on yield parameters of TVSu-10 and TVSu-247, TVSu-10 plants treated with EtBr had more yield than the control especially treatments at 0.03% which gave the highest number of pods, pod weight and number of seeds. Similarly, TVSu-247 plants treated with EtBr produced more yield than the control especially treatments at 0.02% which gave the maximum values for all yield parameters (number of pods, pod weight, number of seeds and seed weight) investigated. This result corresponds to that of Mensah *et al.* (2007) who obtained higher yield (number of fruits) in low concentration of colchicine and sodium azide (0.02%) in *Sesame indicum* L.

The general stimulatory effect of EtBr on TVSu-10 was higher than that of TVSu-247. Similar results about the effect of mutagens have been reported in

different crops such as maize (Gnanamurthy *et al.*, 2011), soybean (Satpute and Fultambkar, 2012), cowpea (Gnanamurthy *et al.*, 2013) and pigeon pea (Arimanaet *al.*, 2014). The differential responses of the two accessions to ethidium bromide could be as a result of their origins, TVSu 10 is a wild accession obtained from Nigeria while TVSu 247 is cultivated accession obtained from Gambia. Similar differential responses within accessions of the same species have been recorded in two accessions of African yam bean exposed to gamma irradiation (Ihuoma and Adesoye, 2017). It has been reported that plant genera and species and to a lesser extent, genotypes and varieties differ in their radio-sensitivity; i.e their response to radiation and chemical mutagens (Kwon and Im, 1973).

Conclusion

The result of this study revealed that EtBr had significant effect on growth and yield parameters investigated in TVSu 10 and TVSu247 accessions of bambara groundnut. The concentration of the EtBr mutagen treatment and the makeup of the varieties played a role in the influence on the plant growth and yield parameters. It was observed that concentration of EtBr at 0.02% and 0.03% induced better results in the growth and yield parameters compared to the control in both accessions except in the growth parameters of TVSu247 where control plants performed better than EtBr treated plants at various concentrations. Ethidium bromide, though carcinogenic and no longer preferred for staining of DNA, promises to be a highly beneficial mutagen in creation of variability in bambara groundnut and can be applied in the genetic improvement of the crop for growth and yield related traits.

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GROWTH CHARACTERISTICS OF DIFFERENT CHICKEN STRAINS REARED IN A HUMID TROPICAL ENVIRONMENT

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Abstract

Growth characteristics of three chicken strains using growth performance and morphometric traits were evaluated. A total of 300 day-old chicks, 100 each of Arbor Acre broiler, Noiler hybrid and Yoruba ecotype were used for the study. Growth performance traits taken were average daily feed intake (ADFI), average daily weight gain (ADWG), feed conversion ratio (FCR) and percentage mortality. Body weight (BWT) and morphometric traits – body length, breast width, keel length, shank length, wing length and drumstick length were taken at biweekly intervals. Data collected were subjected to analysis of variance using SAS analytical package. Phenotypic correlations (r_p) between BWT and morphometric traits were estimated by Pearson's Product Moment Correlations of SAS package. Significant strain effects ($p < 0.05$) were noted for the growth traits. Arbor Acre had higher ADFI (149.41 g/bird/day), ADWG (35.24 g/bird/day), heavier final BWT (3980.00 g/bird) and better FCR (4.24) compared to Noiler hybrid and Yoruba ecotype. Noiler recorded the least mortality (5.00 %). Arbor Acre also showed heavier BWT and longer linear traits in weeks 2, 6, 10 and 14 compared to its counterpart strains. Phenotypic correlation between BWT and morphometric traits ranged from moderate to high for Arbor Acre (0.414 – 0.604) and Yoruba ecotype (0.459 – 0.729). For Noiler, r_p were all high (0.674 – 0.706). All r_p estimated were positive and significant ($p < 0.01$) for the traits. The results showed genetic differences exist among the strains in their growth characteristics and their BWT can be improved by improving the linear traits.

Keywords: Strain, chicken, growth, characteristics, Noiler

Introduction

The exploitation of genetically diverse stocks in breeding programmes is a known approach for improving economically important traits in farm animals. Genetic diversity and similarity among and within strains have been determined using morphostructural differences (Rey *et al.*, 1995; Herrera *et al.*, 1996). At phenotypic level, linear body measurements can be taken and statistically translated in breeding values (Alphonsus *et al.*, 2011). Morphometric measurements are also used in evaluating the characteristics of different strains and thus give information on their suitability for selection. Tolankhomba *et al.* (2012) reported that body traits could objectively improve selection for growth by enabling breeders to identify early and late maturing animals and/or birds of different sizes. Again, there is evidence in the literature that the strain of chicken affects its feed intake, digestibility, feed conversion ratio and growth rate at different ages (Al-Marzooqi *et al.*, 2019).

Chickens have good potential for short generation, high prolificacy, high feed conversion ratio, rapid growth. Although, a lot of literature on the genetic parameters of the growth traits of commercial chicken strains is available, such is not well documented for the slow-growing local strains. Improvement in local chicken productivity would be highly valuable towards the enhancement of the socioeconomic and nutritional status of farmers (Al-Marzooqi *et al.*,

2019). Much emphasis by animal breeders has been on the improvement of traits associated with production such as growth rate, carcass yield and quality as well as egg production. This underscores the importance of evaluating strains in relation to these traits.

The objective of this study was to assess the growth characteristics of three chicken strains using growth performance and morphometric indices.

Materials and Methods

The study was conducted at the Poultry Unit of the Teaching and Research farm, Michael Okpara University of Agriculture, Umudike, which is geographically located at latitude 05°29'N. and longitude 07°33'E. It has an annual rainfall of about 2177 mm, temperature range of 18°C - 38°C, relative humidity range of 51 – 91 % depending on the season and approximately 122 m above sea level. A total of three hundred (300) day-old chicks, 100 each of the three strains – Arbor Acre broiler, Noiler hybrid and Yoruba ecotype were purchased from a reputable hatchery in Ibadan, Nigeria. The chicks were brooded in an environmentally-controlled brooder house for 2 weeks, after which they were transferred to deep litter rearing pens. Each strain was replicated 5 times with 20 birds per replicate. All routine vaccinations and medications were strictly followed. The birds were fed with diets containing 20.64 %CP, 2784 Kcal/kgME, 18.24 %CP, 3062.90 Kcal/kgME and 19.15 %CP, 3062.90 Kcal/kgME at starter, growing and finisher phases, respectively. Feed and water were given *ad*

libitum.

The growth performance traits measured were:

Initial body weight: Weight of the chicks at the beginning of the experiment.

Final body weight: Weight of birds at the end of the experiment.

Average daily feed intake (g): $\frac{\text{Quantity of feed given} - \text{leftover}}{\text{No. of birds} \times \text{No. of days of experiment}}$

Average daily weight gain (g): $\frac{\text{Final live weight} - \text{initial weight}}{\text{No. of birds} \times \text{No. of days of experiment}}$

Feed conversion ratio: $\frac{\text{Quantity of feed consumed}}{\text{Weight gained}}$

Mortality (%): $\frac{\text{No of birds dead}}{\text{Initial stock}} \times \frac{100}{1}$

Body weight and morphometric traits measured were:
 Body weight (BWT) (g): this was taken with a weighing balance to the nearest 0.01g fortnightly beginning from 2 weeks.

Body length (BL) (cm): this was measured as the length of the body from the bill to the tail near the uropigial oil gland

Breast width (BRW): BRW was the region of the largest breast expansion when positioned ventrally.

Keel length (KL) (cm): KL was measured as the length of the distance between the tip of the phalanges and the coracoids-humerus joint.

Shank length (SL) (cm): this was measured as the length of the tarso-metatarsus from the hock joint to the metatarsal pad.

Wing length (WL) (cm): this was taken as length of wing from breast region. Drumstick length (DSL) (cm): DSL was taken as length of femur bone.

Measurements in centimetres were taken with a measuring tape. All measurements were made early in the morning before feeding the birds to avoid gut fill. Experimental design was completely randomized design (CRD) with strain as the factor of interest. The statistical model applied is as given below:

$$Y_{ij} = \mu + S_i + e_{ij}$$

Where,

Y_{ij} = single observation on the j^{th} individual of the i^{th} strain

μ = overall mean

S_i = effect of i^{th} strain

e_{ij} = residual error, assumed to be independently, identically and normally distributed with zero mean and constant variance $\{iind(0, \sigma^2)\}$.

Data collected were subjected to analysis of variance (ANOVA) using SAS (2011) analytical package. Duncan's Multiple Range Test (Duncan, 1955) was used to separate significant means. Phenotypic correlations between BWT and morphometric traits were estimated using Pearson's product moment correlation of SAS (2011) analytical package. The coefficient of correlation (r) obtained from the analysis was computed using the formula:

$$r = \frac{\sum xy - \sum x \sum y / n}{\sqrt{[\sum x^2 - (\sum x)^2 / n]} \sqrt{[\sum y^2 - (\sum y)^2 / n]}}$$

Results and Discussion

Growth performance traits of the three chicken strains:

Table 1 presents the growth performance traits of the chicken strains – Arbor Acre, Noiler hybrid and Yoruba ecotype from 2 – 14 weeks. The result showed significant strain effects on the parameters measured. Arbor Acre was significantly ($p < 0.05$) superior in final weight (3980.00 g), average daily feed intake (149.41 g/bird/day), average daily weight gain (35.24 g/bird/day) and FCR (4.24) while Noiler hybrid had less mortality (5.00 %) throughout the experimental period. Strain differences in production traits of chickens have been reported. Obike *et al.* (2016) reported significant variations in final BWT, daily weight gain and FCR of Arbor Acre and Anak strains but no difference was noted in their mortality rate. Olawumi *et al.* (2012) observed superior ($p < 0.05$) FCR for Arbor Acre over Hubbard and Marshall strains from 1 week to 7 weeks of age. In an investigation on the effects of genotype on growth performance, Yakubu *et al.* (2010) reported that final BWT, average weekly BWT and weekly feed intake were affected ($p < 0.05$) by strain, with higher means recorded for Arbor Acre compared to Anak Titan. The result of a comparative study of Cobb 500 and a local strain (Omani) showed that Cobb 500 had a significantly higher feed intake and body weight gain and a better FCR than that the local strain (Al-Marzooqi *et al.*, 2019). The better FCR recorded in this study for Arbor Acre suggests superiority in its ability to convert its feed into meat compared to the other strains. Again, the lower mortality observed for Noiler hybrid showed better adaptation of the strain to the study environment.

Table 1: Growth performance traits of Arbor Acre, Noiler hybrid and Yoruba ecotype chicken strains from 2 - 14 weeks

Parameter	Arbor Acre broiler	Noiler hybrid	Yoruba ecotype	SEM
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Initial weight (g/bird)	33.33	33.33	33.33	0.08
Final weight (g/bird)	3980.00 ^a	1859.22 ^b	1468.91 ^c	30.00
ADFI (g/bird/day)	149.41 ^a	75.40 ^b	68.79 ^b	3.24
ADWG (g/bird/day)	35.24 ^a	6.24 ^b	4.98 ^c	5.22
Feed conversion ratio	4.24 ^a	12.07 ^b	13.82 ^b	1.50
Mortality (%)	13.00 ^b	5.00 ^a	14.00 ^b	1.20

^{abc} Means with different superscripts within the same row differ significantly ($P < 0.05$), SEM = standard error of mean, ADFI = Average daily feed intake, ADWG = Average daily weight gain, FCR = Feed conversion ratio

Body weight and morphometric traits of the three chicken strains:

Means plus standard error of mean of BWT and morphometric traits of Arbor broiler, Noiler hybrid and Yoruba ecotype strains in weeks 2, 6, 10 and 14 are given in Table 2. Significant strain effects ($p < 0.05$) were also observed for these traits among the strains in all the weeks measured. Arbor Acre strain. Arbor Acre had significantly ($p < 0.05$) heavier BWT, broader BRW and longer body length, keel length, shank length, wing length and drumstick length in all most the weeks. However, it compared favourably with Noiler hybrid for BL, KL, SL and DSL in week 10. Significant strain differences in linear body measurements of chickens have also been noted. Olawumi *et al.* (2012) and Yahaya *et al.* (2012) in their respective studies reported that Arbor Acre was less superior ($p < 0.05$) to Marshall and Hubbard in terms of SL and BRW and SL, neck length and thigh length. Obike *et al.* (2016) also found that Arbor Acre had heavier ($p < 0.05$) BWT, longer BL and SL at 4, 6 and

8 weeks, thigh length at 8 weeks and wing length at 2 and 8 weeks. BWT and the morphometric traits measured for each of the strains increased progressively with age, which is in line with earlier reports. The results obtained in this study connote the existence of genetic variation among the strains which can be exploited for their genetic improvement.

Phenotypic correlation between body weight and morphometric traits:

The phenotypic correlation coefficients of between body weight and morphometric traits of Arbor Acre, Noiler hybrid and Yoruba ecotype are shown in Table 3. The results showed that BWT had positive and significant ($p < 0.01$) with all the traits (BL, BRW, KL, SL, WL and DSL). The coefficients were high in Noiler hybrid ($r_p = 0.690 - 0.733$) but ranged from moderate to high in Arbor Acre ($r_p = 0.414 - 0.604$) and Yoruba ecotype ($r_p = 0.459 - 0.729$). Yahaya *et al.* (2012) reported high and positive coefficients of 0.86 – 0.97 between BWT and morphometric traits in Hubbard and Arbor Acre broiler strains. Ogunshola *et al.* (2017) obtained moderate to high positive and significant ($p < 0.01$) r_p of 0.33 – 0.79 between BWT and morphometric traits of Fulani ecotype chickens. The result of this study suggests that selection for improved body weight using the morphometric traits measured will be effective in the strains.

Conclusion

From this study, it can be concluded that significant genetic variations exist among the three strains of chicken evaluated in their growth performance as well as morphometric traits. These differences can be useful in the genetic improvement of the strains. Arbor Acre strain performed better in almost all the traits measured. The positive and highly significant phenotypic correlations observed indicate that good genetic progress can be made through indirect selection for BWT using the morphometric traits.

Table 2: Means + SEM of body weight and morphometric traits of Arbor Acre, Noiler hybrid and Yoruba ecotype chicken strains from 2 – 14 weeks

Trait	Arbor Acre broiler	Noiler hybrid	Yoruba ecotype
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Week					SEM
2	BWT (g)	211.18 ^a	124.45 ^b	110.78 ^c	4.33
	BL (cm)	10.73 ^a	9.63 ^b	8.85 ^c	0.15
	BRW (cm)	7.04 ^a	4.38 ^b	4.56 ^b	0.17
	KL (cm)	4.87 ^a	4.00 ^b	4.25 ^b	0.09
	SL (cm)	3.98 ^a	3.98 ^a	3.33 ^b	0.07
	WL (cm)	8.93 ^a	8.03 ^b	8.28 ^b	0.14
	DSL (cm)	5.50 ^a	4.70 ^b	5.11 ^a	0.08
6	BWT (g)	1102.05 ^a	480.95 ^b	419.00 ^c	26.87
	BL (cm)	20.67 ^a	16.56 ^b	15.95 ^b	0.34
	BRW (cm)	14.12 ^a	9.44 ^b	10.04 ^b	0.30
	KL (cm)	8.15 ^a	6.85 ^b	6.50 ^b	0.12
	SL (cm)	6.71 ^a	5.85 ^b	6.04 ^b	0.10
	WL (cm)	16.67 ^a	15.10 ^b	15.96 ^b	0.14
	DSL (cm)	10.85 ^a	9.21 ^b	8.52 ^b	0.18
10	BWT (g)	1570.00 ^a	997.83 ^b	883.53 ^c	19.27
	BL (cm)	23.68 ^a	23.00 ^a	21.54 ^b	0.25
	BRW (cm)	15.88 ^a	13.00 ^b	13.66 ^b	0.23
	KL (cm)	10.26 ^a	10.24 ^a	8.99 ^b	0.25
	SL (cm)	9.20 ^a	8.68 ^{ab}	8.36 ^b	0.11
	WL (cm)	22.12 ^a	20.63 ^b	17.80 ^c	0.22
	DSL (cm)	13.96 ^a	12.78 ^a	11.40 ^b	0.18
14	BWT (g)	3460.00 ^a	1724.73 ^b	1092.26 ^c	41.63
	BL (cm)	30.22 ^a	26.19 ^b	22.54 ^c	0.23
	BRW (cm)	21.42 ^a	17.23 ^b	14.23 ^c	0.17
	KL (cm)	13.98 ^a	11.07 ^b	9.66 ^c	0.09
	SL (cm)	12.08 ^a	10.14 ^b	8.93 ^c	0.09
	WL (cm)	23.92 ^a	22.57 ^{ab}	21.35 ^b	0.15
	DSL (cm)	16.48 ^a	15.23 ^b	13.32 ^c	0.13

BWT = body weight, BL = body length, BRW = breast width, KL = keel length, SL = shank length, WL = wing length, DSL = drumstick length, SEM = standard error of mean.

Table 3: Phenotypic correlations between body weight and morphometric traits of Arbor Acre, Noiler hybrid and Yoruba ecotype chicken strains

Strain	BL	BRW	KL	SL	WL	DSL
Arbor Acre	0.604**	0.590**	0.418**	0.414**	0.543**	0.527**
Noiler hybrid	0.706**	0.690**	0.700**	0.733**	0.690**	0.706**
Yoruba ecotype	0.671**	0.654**	0.459**	0.729**	0.680**	0.655**

BL = body length, BRW = breast width, KL = keel length, SL = shank length, WL = wing length, DSL = drumstick length, **correlations significant at $p < 0.01$

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GROWTH RESPONSES OF NATURAL RUBBER (*Hevea brasiliensis* Muell Arg.) SEEDLINGS UNDER DROUGHT STRESS IN UMUDIKE SOUTH EASTERN NIGERIA

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Abstract

To ensure maximum productivity, identification of drought tolerant clones of *Hevea* genotypes (*Hevea brasiliensis*) which can cope with drought stress and give better crop yield is essential. Five genotypes of NIG 800 series developed in Rubber Research Institute of Nigeria (RRIN) (NIG 800, NIG 801, NIG 802, NIG 803, NIG 804 and five exotic clones of PR 107, RRIM 628, RRIM 600, GT 1 and PB 5/51) were screen for tolerance to 26 days of drought stress in the green house. This study aimed at selecting tolerant clones for development of new promising genotypes of natural rubber. Rubber seedlings were transplanted (4 seedlings per poly bag filled with top rich soil) from the pre nursery field to the green house at one month old. After subjection to 26 days of drought stress each genotype was evaluated for five agronomic characters (plant height, number of leaves per plant, percentage plant survival, plant girth and leaf area). Phenotypic, genotypic and environmental variances as well as heritability estimate were obtained through analysis of variance. Results obtained showed that significant differences ($p < 0.05$) existed in the level of drought tolerance among the genotypes. GT1 recorded the highest plant height, number of leaves, percentage plant survival and leaf area followed by NIG 804 and PR107. From the result, it was only GT1 that was able to withstand drought stress after 26 days exposure to drought. In order to meet the ever rising global demand for natural rubber production, there is need to extend natural rubber seedlings cultivation to green house where extreme climatic conditions like drought stress and high temperature negatively influence the crop performance so as to screen tolerant genotypes and incorporating them into breeding program of rubber.

Keywords: Rubber seedlings, drought stress, genotypes, heritability, green house

Introduction

The *Hevea* genus belongs to the *Euphorbiaceae* family and comprises 11 species native to the Amazon region. Natural rubber (*Hevea brasiliensis* Willd. ex Adr. de Juss, Muell-Arg.) is the only cultivated species and the main source of natural rubber (Omokahfe *et al.*, 2008). The plant is quick growing, erect, with a straight trunk and surfaces. It is the tallest species of the genus, *Hevea*. It is a tropical crop that survives between 15°N and 10°S of the equator, where the climax vegetation is lowland, tropical forest and where the climate is hot and humid with temperature range of 25-30°C with a well distributed rainfall of 1800-2000mm per year (Korieocha *et al.*, 2015). The crop does not perform well if there is a long dry season. It also does badly if the climate is excessively humid, as this encourages leaf diseases. The rubber tree can tolerate a wide range of soil conditions from sandy to loamy soils provided the soils are deep, fertile, well drained and slightly acidic with a soil p^H of 4-6 or 4-8. The rubber tree is a perennial and deciduous crop with an economics life span of 25-30 years (Korieocha *et al.*, 2018). As global demand for natural rubber increases, a major challenge for cultivation of rubber plants is their inability to withstand unfavorable environmental conditions in the context of global climate change (Nair *et al.*, 2010). Drought remains one of the most biologically demanding and ecologically limiting factors among all environmental constraints. Drought is an extended period of dry weather characterized by shortage of water supply to plants (Acquaah, 2007, Korieocha *et al.*, 2015). Drought begins when the

readily available soil water in the root zone is exhausted (Taiz and Zeiger, 2006). Drought stress can occur at any stage of the growing process and cause complete loss of crops or serious reduction in yield. In Nigeria, a natural rubber production comes mostly from a single popular clone NIG 800 series and it occupies most of the area under rubber cultivation (Omokahfe and Alike 2003). Although it is the highest yielding clone in Nigeria, it does not exhibit good drought tolerance capacity. Sprouted seedlings are transplanted in the poly bags filled with rich top soil. In the nursery, there is no need for large scale land preparation activities instead there is need for large supply of rich top soil to fill the poly bags as well as proper field marking for easy routine movements in the field (Marattukalam and Nair, 2012). This will also lead to uniform growth of the stands. The use of large poly bags encourages plants with intact and undisturbed root system to appreciably shorten the immaturity period in the field. In spite of the above advantages that poly bag plants appear to have over plants raised on ground bed, it has continued to attract criticisms as a result of the following: The high cost of poly bags, the high labour demand for filling and transportation of the bags and the high cost of transportation. Cultivation of natural rubber in present day, faces various climatic constrains even in major growing areas and these constrains are expected to become more serious in future. Soil, atmospheric drought, high temperature and disease susceptibility are major environmental factors limiting the growth and yield of natural rubber genotypes (Mercy *et al.*,

2010). Therefore, there is need to screen natural rubber genotypes for tolerance to drought conditions especially in the greenhouse. The objective of the present study was therefore to identify and select tolerant genotypes in the green house for development of new clones of natural rubber.

Materials and Methods

Ten genotypes of natural rubber (*Hevea brasiliensis*) were screened at the green house of the National Root Crops Research Institute (NRCRI), Umudike, Abia State, for tolerance to 26 days of drought stress using five juvenile characters. The ten seedling genotypes were five RRIN developed clones (NIG 800, NIG 801, NIG 802, NIG 803, NIG 804 and five exotic clones (RRIM 600, RRIM 628, GT1, PR107 and PB5/51 from Malaysia). In the green house experiment, sprouted seedlings were transplanted from the pre nursery to the poly bags filled with rich top soil at one month old on October 10, 2017. The experiment was laid out in a completely randomized design (CRD) with three replications. Each poly bag contains 4 seedlings and the seedlings were watered twice daily (morning and evening) to enhance establishment before imposing 26 days of drought on them. Data were collected on the following five juvenile characters (plant height, number of leaves, plant girth (circumference), percentage plant survival and leaf area) at 26 days of drought stress.

Plant height was measured as the distance from the ground level to the tallest leaf using meter rule at 26 days of drought stress.

Number of leaves per plant was obtained by counting the number of leaves of each plant after which their mean was calculated and recorded.

Plant girth was obtained by measuring the diameter of the plants using slide calipers to measure the girth of plants.

Percentage plant survival was calculated by counting the number of seedlings that survived after transplanting from field to the poly bags according to (Samarappuli *et al.*, 2006).

Data on leaf area (cm²) at 26 days of drought stress was measured and calculated. Leaf Area (cm²) = L x W x K, where L is leaf length, W is maximum width of the leaf and K is a correction factor of 0.654.

All the data collected were statistically analyzed. Analysis of Variance (ANOVA) was done using the statistical analysis software (SAS, 2002) to determine if variability existed among the genotypes for various traits. Mean separation was done using Least Significant Difference (LSD) at 5% probability level following the procedure of Steel and Torrie (1990). Genetic parameters such as variance components (genotypic, phenotypic and error variances) and heritability in the broad sense was calculated according to Allard (1987) as follows:

Genotypic Variance $V_g = (MSG-MSE/r)$, Phenotypic Variance $V_p = (vg/vg+ ve)$, Environmental variance $V_e = (MSE/r)$

Where MSG, MSE, V_e and r are the Mean Squares of

genotypes, Mean Squares of error, environmental variance and numbers of replication respectively.

Results and Discussion

The mean effects of drought stress on plant height, number of leaves, percentage plant survival, plant girth and leaf area measured on 10 genotypes of rubber seedlings evaluated in the green house in 2017 were given in table 1 below. After subjection to 26 days of drought stress, plant height means per genotype varied from 21.8cm in RRIM 600 to 34.98cm in GT1. After 26 days of drought stress, GT1 recorded the highest plant height of 34.98cm followed by NIG 804 with plant height of 33.04cm. RRIM 600 recorded the least plant height of 21.8cm. Generally, there was influence of drought stress on the genotypes after 26 days of drought. Meanwhile GT1 and NIG 804 was the only genotypes that can tolerate drought period of 26 days or even more in the greenhouse. The genotype remaining genotypes had little or no tolerance to 26 days of drought in the green house. This is in line with the findings of (Samarappuli *et al.*, 2006) who reported that root growth in relation to depth may have had greater effect in the performance of rubber plants in the green house. Young plants are less likely to survive in soils with inadequate supply of water at the surface which is particularly so during the dry periods. Well developed and more efficient root system would enable the young plant to tap on a larger reservoir of water beneath the surface, thereby increasing the soil water and nutrient absorption efficiency of the plant.

Number of leaves: Number of leaves varied from 5.33 in RRIM 600 to 19.11 in GT1 after 26 days of exposure to drought stress (Table 1). As a result, GT1 had the highest number of leaves of 19.11 after 26 days of drought followed by PR107 with 18.53 while RRIM 600 exhibited the least number of leaves (5.33) at 26 days of exposure to drought stress. There were influences of drought on all the genotypes but the effects of drought stress were more pronounced in RRIM 600 and PB5/51.

Percentage Plant Survival: The percentage plant survival per genotype ranged from 25% in RRIM 600 to 66.6% in GT1 after subjection to 26 days of drought stress (Table 1). Percentage plant survival of the genotypes was generally high after establishment, before exposing them to 26 days drought, except for RRIM 600 that had 50% survival after establishment. Generally, there was much influence of drought stress on the genotypes after 26 days of drought stress but GT1 recorded the highest plant survival rate of 66.6% while RRIM 600 and PB5/51 recorded the least plant survival rate of 25%. However, GT1 and NIG 801 was the only genotypes that had high survival rate and showed more tolerance to 26 days drought while the remaining genotypes showed little or no tolerance to drought in the green house experiment.

Table 1: Mean effects of 26 days of drought stress on 10 genotypes of rubber seedlings evaluated in the green house in the year 2017.

Genotypes	Plant height	Number of leaves	Percentage plant survival	Leaf area (cm ²)	Plant girth (cm)
GT1	34.98	19.11	66.67	513.87	1.20
NIG 800	27.78	10.66	30.67	162.78	0.11
NIG 802	31.33	13.25	50.00	305.81	0.88
NIG 805	26.89	13.08	33.33	293.12	0.99
NIG 803	30.17	12.00	40.00	253.80	0.92
NIG 804	33.04	16.38	41.67	389.03	1.81
PB 5/51	26.20	6.17	25.33	73.299	0.12
PR 107	31.63	18.53	41.67	431.01	1.12
RRIM 600	21.80	5.33	30.33	84.48	0.41
RIMM 628	28.22	8.67	25.00	147.39	0.55
Means	29.20	12.32	38.47	265.46	0.81
LSD _(0.05)	10.11	4.587	32.83	50.51	0.301

The remaining genotypes showed little or no drought tolerance in the green house experiment

Leaf area: The leaf area varied from 84.48cm² in RRIM 600 to 513.87cm² in GT1 after subjection to 26 days of drought stress. GT1 produced the highest leaf area of 513.87cm² at 26 days after exposure to drought stress, followed by PR 107 with 431.01cm² and RRIM 600 produced the least leaf area of 84.48cm² at 26 days of drought stress (Table 1). There were variations among the genotypes during the drought stress period but low leaf area was recorded across the ten genotypes especially in RRIM 600. This result indicated that drought had influence on the genotypes. Since there was limited supply of water and the crops were not exposed to direct sun shine, photosynthetic process was inhibited and the leaves did not grow elaborately well (Kavar *et al.*, 2007). In 2017 green house experiment, as expected, all the traits had higher phenotypic variances than genotypic and environmental variances. Also, the environmental variances were more than genotypic variances. Consequent upon this, the broad sense heritability (h²_b %) estimates were generally low, except for leaf area and plant girth where moderate heritability (h²_b) estimates were obtained. Percentage plant survival had

the highest phenotypic variance (403.55), lower genotypic variance (7.72) compared to high environmental variance (395.83), and had a lower heritability estimate of 0.02. Leaf area had phenotypic variance of 70.64 and genotypic variance estimates of 30.10, and consequently a moderate heritability estimate of 0.43 (Table 2). The lower genotypic variances recorded, compared to environmental and phenotypic variances, suggest greater influence of the non- heritable components over and above heritable (genetic) components on the total phenotype observed (Tolessa 2017). Gain under this lower genotypic variances recorded will be very slow, and will take considerable time (Adifaiz *et al.*, 2018). Heritability is a measure of observed phenotype that is accounted for by genetic effects.

Table 2: Phenotypic, genotypic, environmental variances and heritability estimates of five juvenile traits of ten genotypes of rubber seedlings evaluated in the green house in 2017

Traits	Vp	Vg	Ve	H ² %	\bar{x}
Plant	46.332	8.7937	37.538	0.1898	30.372
Height					
Number of leaves	8.9106	1.1825	7.7281	0.1327	8.8122
Percent age					
Plant Survival %	403.56	7.7160	395.83	0.0191	60.00
Leaf Area	70.641	30.104	40.536	0.4262	26.04
Plant girth	0.5105	0.1605	0.3500	0.3144	2.283

Conclusions

From the result in the green house experiment, it was only GT1 and NIG 804 that was able to withstand drought stress after 26 days exposure to drought. The result also showed that rubber seedlings cannot withstand drought stress at nursery stage for a long period especially in the green house. The lower genotypic variances recorded, compared to environmental and phenotypic variances, suggest greater influence of the non- heritable components over and above heritable (genetic) components on the total phenotype observed. These also further explain that selection under this scenario will be very slow and will involve a lot of recurrent selection. The significant variations for the five juvenile traits will permit selection among the genotypes for tolerant.

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EFFECT OF DIETARY INCLUSION OF AIR-DRIED GINGER (*Zingiber officinale*) ON PERFORMANCE OF LAYING HENS

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ABSTRACT

This study investigated the effect of ginger on the performance, egg quality, yolk cholesterol and serum lipid profile of layers using 72 laying hens (Isa brown) in a 49 day feeding trials. Three experimental diets for laying hens were formulated such that the diets were supplemented with 0%, 0.5%, 1% air-dried and milled ginger. The diets were randomly assigned to 24 birds each replicated three times in a complete randomised design. Results showed no significant difference in feed intake and feed conversion ratio among the treatment groups ($p > 0.05$). 0.5% inclusion of air-dried and milled ginger supplement significantly ($p < 0.05$) reduced the final body weight (1743.67g/hen) relative to those in the control group (1836.67g/hen). Result showed that dietary inclusion of ginger led to a significant decrease in serum HDL but increased percentage albumen content in eggs ($p < 0.05$). There was increase in egg weight with proportional increase of the inclusion of the milled ginger whereas egg laying rate was similar across the various dietary treatment. In conclusion, inclusion of air-dried and milled ginger in layer diets led to increase in egg size and percentage albumen content but reduced yolk size.

Keywords: Ginger, Layer, Egg quality, Cholesterol, Lipid profile.

INTRODUCTION

Phytogenics are a group of natural growth promoters or non-antibiotic growth promoters, derived from herbs, spices or other plants (Thin *et al.*, 2018). Herbs exhibit an array of diverse biological activity such as antistress, adaptogenic, anti-ageing and immunomodulatory activity and provide potent anti-inflammatory, antibacterial, antiviral and antifungal benefits (Mahima *et al.*, 2012) Usefulness of phytogenics lies in some chemical substances that produce definite physiological actions in the body of the animals (Oko -& Agiang, 2009). The most important bioactive constituents include alkaloids, tannins, flavonoids, saponins and phenolic compounds. Compared with synthetic antibiotics or inorganic chemicals, these plant-derived products have proven to be natural, less toxic, residue free and are thought to be ideal feed additives in food animal production (Abd EL-Latif *et al.*, 2019).

Consumption of animal protein has been criticized due to significant contribution of dietary fat in human diet and consumer's view as regards to food quality has change in recent times to include food that provide health benefits beyond the basic nutritional needs (Adel-salam, 2010). Natural medicinal products originating from herbs and spices and their extracts have a wide range of activities (Suganya *et al.*, 2016) and is gaining importance as feed additive in animal production. Ginger is a rhizome of the plant *Zingiber officinale* consumed as a delicacy, medicine or spice; its rhizome has been reported to have a stimulating effect on peptic juices, such as bile and salivary, gastric, pancreatic and intestinal juices (Stoilova *et al.*, 2007). Research conducted in vitro showed that ginger extract can control the quantity of free radicals and the peroxidation of lipids (Al- Almin *et al.*, 2006;

Morakinyo *et al.*, 2011, Bekkouch *et al.*, 2019). Ginger has been reported to enhance animal nutrient digestion and absorption because of the positive effect on gastric secretion, enterokinesia and digestive enzyme activities (Platel & Srinivasan, 2000), it also possesses lipid lowering effects (sharma, 1996; Bekkouch *et al.*, 2019), anticarcinogenic (Aggarwal *et al.*, 2008) and antibacterial (Sudrashan *et al.*, 2010) properties.

The increase in demand for low fat poultry products is a major concern in the poultry industry. Nutritional strategies aimed at reducing the saturated fat, cholesterol contents and alteration of the lipid profile in poultry product to improve its quality using feed additives which has no adverse effect on poultry will be of great solution to the industry.

This study investigated the effect of inclusion of air-dried and milled ginger on the performance, egg quality, yolk cholesterol and serum lipid profile of layers.

MATERIALS AND METHODS

Collection and Processing of Ginger

Fresh ginger rhizome (*Zingiber officinale*) was bought from Relief Market in Owerri, Imo State and was washed in water to remove the adhering dirt. The rinds were peeled off after which they were chopped into smaller pieces using kitchen knife. It was air-dried under a shade, milled using a grinding mill machine with sieve size of 0.1 cm and then stored in a big air tight polythene bag. The air-dried and milled ginger was incorporated into three experimental diets at 0%, 0.5% and 1% respectively as shown in Table 1.

Experimental Design and Management

Seventy-two (72) laying hens of seventy-three weeks of age were randomly distributed into three dietary treatments. Each treatment has three replicates containing 8 laying hens each in a deep litter pen. Each treatment group was assigned to one of the three diets in a completely randomized design.

Fresh feed and water were served ad libitum daily. Routine management and all necessary medication were followed. Data on the laying hens were collected on the initial and final body weight. Left-over feeds were collected daily from each pen; each was weighed and subtracted from the actual feed given the previous day to estimate the feed intake. The laying hens were fed the experimental diets for one week and four days to stabilize them before the commencement of egg collection which lasted for five weeks and three days. Eggs from each replicate pen were counted and weighed daily to calculate the laying rate. Data obtained were used to compute average daily weight gain, average daily feed intake and feed conversion ratio. The economy of production was estimated as feed cost per kg egg.

Blood and Egg Sampling and Preparation

On the 49th day of the layer experiment, three eggs per replicate were randomly selected each for estimation of cholesterol level and egg quality. Each egg yolk was manually separated and extraction of cholesterol from the egg yolk was done by the method of Amadi *et al.*, (2004) and was stored at room temperature for cholesterol determination.

Egg Quality Nine eggs collected per treatment were weighed using electronic weighing scale. After weighing, each egg was broken onto a flat tray, the height of the albumen and yolk were measured using the Vernier caliper. Also, the albumen and yolk widths were measured with the same instruments at two different points. The weight of the albumen, egg yolk and egg shell were measured using the electronic digital balance and the egg yolks were preserved thereafter in a refrigerator for cholesterol analysis. Shell thickness was a mean value of three locations of the egg (air cell, equator and sharp end) using micrometer screw gauge. The egg quality indices (Shell percentage, Albumen percentage, Yolk percentage and Haugh unit) were calculated based on the data obtained.

Blood samples were taken from the wing vein of three hens per replicate without anticoagulant, each blood sample was centrifuged at 3000rpm for 10 min using 80-2 centrifuge (China) to obtain clear plasma which was carefully transferred into fresh sample bottles and appropriately labelled for estimation of serum lipid profile of the hens.

Assay on egg yolk cholesterol and serum lipid profile

Table 1:

Composition of the experimental layer diets

Egg yolk cholesterol, Total cholesterol and triglyceride were determined according to the spectrophotometric method of Allain *et al.*, (1974); high density lipoprotein (HDL) was determined using the method of Albers *et al.*, (1978) while Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) concentrations were calculated (Friedewald *et al.*, 1972).

Statistical Analysis

The mean Data were subjected to descriptive statistics and analysis of variance based on (R Core Team, 2014). Statistically significant mean was separated using the Least Significant Difference.

RESULTS

Performance of Layers

Results on the performance of laying hens fed varying dietary levels of air dried ginger are presented in Table 2

The average initial weight of the layers prior to the starting of the experiment shows no significant difference ($P > 0.05$). The final live weight of the hens was significantly different with the control birds being the highest (1836.67g), followed by T3 (1813.33g) and then T2 (1743.67g). All other parameters showed no significant differences between ginger diet birds and control. Numerically, hens fed 0.5% ginger diet had the highest hen day egg production with better feed conversion ratio, compared with other treatment groups.

Egg Quality Characteristics of the Layers

Results on the egg quality characteristics of laying hens fed varying dietary levels of air dried ginger are presented in table 3.

No significant differences were observed in the egg quality parameters with the exception of albumen weight, yolk height and albumen percentage. Egg weight and albumen weight increased with increased level of ginger in the diets. The hens fed 1% ginger diets had the heaviest egg weight, lowest shell weight and thinnest shell than the control group.

Yolk Cholesterol and Lipid Profile of Layers

Results on yolk cholesterol and serum lipid profile of layers fed varying dietary levels of air dried ginger are presented in Table 4. No significant differences were observed in the serum lipid profile of the hens except in HDL-cholesterol which was highest in control birds and lowest in birds fed 1% ginger diet. Total cholesterol, triglyceride and VLDL-cholesterol levels decreased across the treatments from T1 to T3, although the differences were not statistically significant ($p < 0.05$). Cholesterol content in the eggs was not affected by dietary treatment.

Ingredients	Ginger Inclusion Levels		
	T ₁ (0.00%)	T ₂ (0.50%)	T ₃ (1.00%)
Maize	53.00	52.50	52.00
Ginger	0.00	0.50	1.00
Soybean meal	18.00	18.00	18.00
Fish meal	3.00	3.00	3.00
Palm kernel cake	9.05	9.05	9.05
Wheat offal	10.00	10.00	10.00
Bone meal	5.00	5.00	5.00
Salt	0.25	0.25	0.25
Vitamin/Mineral (Layer) premix	0.50	0.50	0.50
L-Lysine	0.80	0.80	0.80
DL- Methionine	0.40	0.40	0.40
Calculated nutrient composition of the experimental diets (% dry matter)			
Crude Protein	17.79	17.74	17.70
Crude Fibre	4.64	4.63	4.61
Ether Extract	4.12	4.10	4.08
Ash	3.35	3.34	3.34
Metabolizable energy (kcal/kg)	2710.97	2693.81	2676.65
Feed cost (N/kg)	105.05	107.22	109.39

**Layer vitamin premix supplied the following vitamins and trace elements per kg diet: Vit A 6250IU; Vit D3 1250IU; VitE, 14.38mg; VitK3 1.25mg; Vit B, 1.88mg; Niacin 31.25mg; Calcium pantothenate 6.25mg; Vit B6 3.8mg, Vit B12 0.02mg; Chroline Chloride 250mg; Folic acid 0.63mg; Biotin 0.03mg; Mn75mg; Zn 50mg; Cu 5.31mg; I 0.94mg; Co 0.019mg; Se 0.08mg and Antioxidant 75mg

Table 2. Performance of layers fed varying dietary levels of air-dried ginger

Parameters	Percentage dietary levels of air-dried ginger			SEM
	T ₁ (0.00%)	T ₂ (0.50%)	T ₃ (1.00%)	
Initial body weight (g/hen)	1680.00	1666.67	1713.33	35.17
Final body weight (g/hen)	1836.67 ^a	1743.67 ^b	1813.33 ^{ab}	31.93
Body weight changes (g)	156.67	77.00	100.00	48.05
Feed intake (g/hen/day)	103.00	102.39	106.60	7.49
Average weekly feed intake/hen (g)	722.25	703.83	749.83	47.45
Average weekly laid egg weight/hen (g)	433.27	430.33	440.49	12.5
Feed conversion ratio (feed intake/egg mass)	1.66	1.64	1.74	0.09
Hen-day egg production (%)	46.58	47.00	46.5	6.53
Feed cost (₦/kg)	105.05	107.22	109.39	-
Feed cost/kg egg (₦)	174.73	175.48	186.69	10.04

^{a,b} Means within a row with different superscripts are significantly different (p<0.05).

SEM: Standard error of means

Table 3. Egg quality as affected by inclusion of air-dried ginger in layers' diets

Percentage dietary levels of air-dried ginger Parameters	T ₁ (0.00%)	T ₂ (0.50%)	T ₃ (1.00%)	SEM
Egg weight (g)	59.01	60.78	67.84	4.50
Yolk weight (g)	16.26	14.56	17.02	1.99
Albumen weight (g)	35.43 ^b	38.89 ^a	43.79 ^a	2.98
Albumen width (cm)	7.49	7.43	7.38	0.37
Albumen height (cm)	0.94	0.90	0.91	0.07
Yolk width (cm)	4.04	3.69	3.92	0.18
Yolk height (cm)	1.62 ^{ab}	1.77 ^a	1.46 ^b	0.12
Shell thickness (mm)	0.42	0.44	0.41	0.02
Yolk percentage (%)	27.56	23.94	24.94	1.80
Albumen percentage (%)	57.79 ^b	63.99 ^a	66.57 ^a	2.53
Shell percentage (%)	12.41	12.08	10.49	0.80
Haugh unit	97.02	95.01	93.31	2.88

a,b Means within a row with different superscripts are significantly different (p<0.05).
SEM: Standard error of means

Table 4. Serum lipid profile and egg cholesterol of layers fed diets with varying levels of air-dried ginger

Percentage dietary levels of air-dried ginger Parameters	T ₁ (0.00%)	T ₂ (0.50%)	T ₃ (1.00%)	SEM
Total cholesterol (mg/dl)	73.24	72.87	68.54	7.1
Triglyceride (mg/dl)	47.79	43.64	42.64	2.94
HDL-Cholesterol (mg/dl)	51.28 ^a	42.28 ^{ab}	42.01 ^b	2.77
LDL-Cholesterol (mg/dl)	43.70	50.43	46.08	8.05
VLDL-Cholesterol (mg/dl)	9.56	8.73	8.53	0.56
Yolk cholesterol (mg/dl)	145.70	144.06	146.52	7.79

a,b Means within a row with different superscripts are significantly different (p<0.05).
SEM: Standard error of means

DISCUSSION

The inclusion of different levels of air dried ginger in diets of layers did not negatively affect the performance of the laying hens when compared with the control group. There were no significant differences in feed intake of the hens. Hens fed with 1% air dried ginger recorded the highest feed consumption. This result is in line with the findings of Nasiroleslami & Torki (2010); Zhou *et al.*, (2011) and Wen *et al.*, (2019) who reported no significant differences in dietary consumption of broilers or laying birds fed ginger compared with the control group. Contrary to this finding, Incharoen & Yamuchi (2009) reported significant differences in feed intake among birds fed ginger compared to the control group. The slight decrease in feed intake but high hen day production recorded by layers fed 0.5% ginger diet tends to show more efficient nutrient utilization.

Significant differences (P < 0.05) were observed in the final live weight of the hens with the highest weight recorded in the control group. This suggests that whereas ginger inclusion in the diets of laying hens may not affect egg production, it certainly affects live weight. The observed live weight depression effect may be related to the effect of ginger on fat level. Since

fat is major component of fat weight, reduction in fat level will have proportionate effect on overall live weight. Also, in order to maintain egg productivity, the hens fed ginger diets may have been draining from nutrients in body reserves. Nonetheless, egg production across the three dietary treatments was not optimal because of the age of the layers.

The dietary inclusion of air dried ginger had no detrimental effect on egg quality. No statistically significant differences were observed in laying rate and egg weight. However, egg weight tended to be higher in both dietary air dried ginger groups. The egg weight was 8.83% higher in 1% air dried ginger inclusion hens and 1.77% higher in 0.5% air dried ginger inclusion hens compared with the control hens. This is in agreement with the findings of Akbarian *et al.*, (2011), Incharoen & Yamuchi (2009) and An *et al.*, (2019) who reported higher egg production and weight by hens fed ginger diets. Egg laid by hens fed diets containing ginger were higher in albumen weight, yolk height and percentage albumen compared to the control group. Nasiroleslami & Torki (2010) observed increased egg shell weight and egg shell thickness in laying hens fed with essential oil of ginger while Wen *et al.*, (2019) observed increased in haugh unit and

albumen height on hens fed ginger extract but in this present study shell weight, albumen height and haugh unit were not affected significantly by dietary ginger inclusion.

Total cholesterol, LDL- Cholesterol, VLDL-cholesterol and total triglycerides in the serum and egg cholesterol were not significantly affected by dietary inclusion of ginger. However, Significant differences were observed in HDL- cholesterol of the hens which decreased with increase in ginger inclusion level. Malekizadeh *et al.*, (2012) reported significant decrease in serum total cholesterol of laying hens fed ginger diet while Herve *et al.*, (2019) reported significant decrease in total cholesterol, LDL-cholesterol and triglycerides with increase in HDL-cholesterol among quails administered ginger essential oil which is not in agreement with the present study. Also, Prasad *et al.*, (2012) reported that ginger-juice administered to rats over a period of twenty-one days acted as a hypolipidaemic agent which reduced the total serum cholesterol level and significantly increased the serum HDL-cholesterol while LDL-cholesterol and triglycerides remained unaltered. Explanation for this apparent discrepancy is not

known, although the processing method and mode of administration may be important determinants.

CONCLUSION

Dietary supplementation of air-dried and milled ginger at different levels were associated with increase in egg weight and percentage albumen content but decrease in percentage yolk content. It is therefore concluded and recommended that inclusion of air-dried ginger up to 1.0% should be encouraged and adopted by poultry producers in diets of laying hens due to positive effect on egg weight and percentage albumen, and negative effect on yolk size since the health issues associated with egg consumption are related to the high fat and cholesterol levels in the yolk. Many consumers would prefer eggs with higher albumen, but lower yolk contents.

Further research is necessary to investigate the effect of ginger on production performance and serum lipid profile of laying hens because it is possible that other factors such as method of processing ginger, storage length after processing, route of administration, species of animal and the production state of the stock may influence the metabolic action of ginger.

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SUSCEPTIBILITY OF SOME SWEETPOTATO (*Ipomoea batatas*) VARIETIES TO BACTERIAL SOFT ROT DISEASE CAUSED BY *Erwinia* Spp AND BIOPESTICIDES CONTROL

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Abstract

The spoilage of sweetpotato tubers in transit or storage reduces the market and nutritive value. Tuber soft rot caused by the bacteria can destroy the entire tubers in few days. Pathogenicity test was conducted to investigate activities of soft rot bacteria on three sweetpotato tubers varieties *in vivo* for five weeks using cork borer method. Experiment was laid out in a Completely Randomized Design (CRD) with three replications. Biopesticide control of storage rots were evaluated using four plant extracts: *Jatropha curcas*, *Eucalyptus globolus*, *Leucaena leucocephala* and *Azadirachta indica*. Cultural characteristics and identification of the isolated pathogen was investigated. Results showed that *Eucalyptus globolus* (1.62cm) and *Azadirachta indica* (1.79cm) were more effective than *Jatropha curcas* (1.81cm) and *Leucaena leucocephala* (1.90cm) in inhibiting and reducing the radial growth and diameter of the rot pathogen (*Erwinia*). The activities of the pathogen were progressive and by the fifth week the whole tubers were rotten in the untreated (control). This was evident in the cross section of the invaded tubers within the period of investigation. Study showed that the rot organism is devastating and can be managed by the use of biopesticides (plant extracts) as a safe control measure.

Key Words: Pathogen, Biopesticides, rot organism and radial growth.

INTRODUCTION

Sweetpotato *Ipomoea batatas* (L. Lam) is an important starchy root crop of a vine of the Convolvulaceae family. Sweetpotato as food product is a source of energy, proteins, pro-vitamin A (B-carotene), vitamin C and iron. On dry matter basis, the non-carbohydrate macronutrient composition of the edible tuberous roots includes 1.4 to 8.6% protein, 3.4 to 5.9% crude fibre, 0.3 to 1.9% lipid and 1.5 to 6.3% ash (Degras, 2003). The pro-vitamin A β -carotene pigment (a dietary precursor of vitamin A) is known to be responsible for the yellow to orange coloration of the flesh of tuberous roots of some sweet potato varieties (Rodriguez-Amaya and Kimura, 2004). In Nigeria, most of the sweetpotato landraces are white fleshed roots with negligible amount of the pro-vitamin A pigment (Ukpabi *et al.*, 2012). However, Ijeh and Ukpabi (2004) had shown that a popular local yellow fleshed landrace (Ex-Igbariam) has appreciable but relatively limited quantity of β -carotene (3 μ g/g fresh root sample).

Different diseases arise after sweetpotatoes are harvested, this is because the storage organs are essentially dormant and their cells are physiologically unlike those of the growing plants. The numerous diseases which occur in transit and storage result mainly from the activities of fungi and bacteria. Amienyo and Ataga (2006) opined that such diseases considerably reduce the commercial value of the produce. *Erwinia* spp causes a wide range of plant diseases with symptoms which include wilts, blights, cankers, dieback, leaf spots, fruit spot, soft rots and discoloration of woody tissues. Opara and Asuquo (2016) reported that *Erwinia* soft rot is one of the most destructive diseases of vegetables and occurs worldwide wherever fleshy storage tissues of

vegetables and ornamentals are found. Soft rot is of considerable economic importance, since under favorable conditions it can destroy the entire tuber in few days. Harvested tubers are vulnerable to attack by these microorganisms because of their moisture content and rich nutrient. Tuber soft rot on infected sweetpotato exhibits small, cream to tan, water-soaked surface spots that progresses inward. Infected areas of the tuber can become puffy, creating blister-like lesions that often ooze a watery substance. Upon exposure to air, infected areas often turn brown or black around the rotting area. Decay can occur rapidly under moist conditions in storage due to the development of a film of water resulting in anaerobic conditions in tubers and thus predispose for soft rots (Noah *et al.*, 2016). The role of higher plants as sources of antibacterial, fungicides and insecticides and their importance in controlling different plant pathogens are gaining prominence in view of the hazards and cost of agro-chemicals. Plant extracts which are biodegradable and eco-friendly promises better alternative to agro-chemicals (Suleiman *et al.*, 2013). This work studies, the antibacterial effects of *Jatropha curcas*, *Eucalyptus globolus*, *Leucaena leucocephala* and *Azadirachta indica* as biopesticides.

The aim of this work is to determine pathogenicity test of pathogen on sweetpotato tubers, identify the causal organism of sweetpotato soft rot and evaluate some control measures against the pathogens *in vivo*.

MATERIALS AND METHODS

The experiment was conducted at the laboratory of the Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, Nigeria. The sweetpotato varieties used were Mother delight, Umuspo-1 and Umuspo-2.

Laboratory Work: The inoculation chamber and all non-glass items were sterilized by mopping with 75% absolute ethanol. All glass wares and the forceps were sterilized by autoclaving at 121°C/15psi for 15minutes prior to inoculation. The Petri dishes were placed on the chamber after sterilizing, the dissolved Nutrient Agar (NA) was sterilized by Autoclaving at 121°C/15psi for 15minutes and poured on the sterilized Petri dishes (8) approximately 15ml on each, after which it was allowed to solidify then turned upside down and kept in the refrigerator for future use.

Bio-pesticides Preparation: Plant materials used were the leaves of *Jatropha curcas*, *Eucalyptus globules*, *Leucaena leucocephala* and *Azadirachta indica*. Each of the plant leaves used was washed thoroughly in tap water and air dried at 30°C room temperature for 1hour. They were processed first into paste form by grounding with a sterile mortar and pestle to obtain 1kg paste. 70% ethanol was added to the paste and allowed to soak overnight (Amadioha, 2001).

Diseased sweet potato tubers were washed in running tap water and air dried for few minutes (30mins). Inoculation chamber was mopped with 75% Ethanol to eliminate contamination while spirit lamp was used to deter any incoming contaminant. The infected tubers were cut from the margin between the infected part and healthy part (since the pathogen move from infected to the healthy area) and subsequently placed on a sterile Petri dish. To eliminate surface saprophytes, it was washed in three exchanges of sterile water and two drops of water was added, then teased apart on a sterile Petri dish. Petri dish was allowed for 15mins for the bacteria to multiply. A sterile wire loop was used to collect the bacteria suspension and streaked onto Nutrient Agar in a zigzag fashion. The wire loop was flamed red on a spirit lamp and allowed to cool down before streaking onto the media and the growth were checked after 24hrs. The cultures were sub cultured two or three times to obtain pure culture.

CULTURAL TEST

Gram-stain: A drop of sterile water was placed on a clean microscope slide. A part of the young colony was removed with a cold sterile wire loop from the Nutrient Agar. The bacteria were smeared unto the slide, it was air-dried and heat-fixed on the slide by passing the slide 4times through a Bunsen flame. The slide was flooded with crystal violet and allowed for 60secs. It was rinsed under running water and drained off excess water. It was flooded again with iodine and was allowed for 60secs and later washed with 95% ethanol for 30secs before rinsing with water. It was dried and counter stained with safranin for 10secs, it was rinsed with water and dried then examined at x100 magnification.

Pathogenicity test: Using a cork borer of diameter 5mm, the tubers were punctured and the disc corked out, using the same cork borer the culture was also punctured and inserted inside the 2cm depth created with the cork borer and covered with the disc from the tuber. 1ml of each plant extracts were injected in the openings while a control was conducted in a similar way using sterile water instead of extract, the treated tubers were placed inside moistened water proof bags to maintain high relative humidity and extent of rot was evaluated 4 days’ intervals for 5 weeks.

RESULT AND DISCUSSION

Effect of Plant extracts

This trial supports the reports that *Erwinia* soft rot is one of the most destructive diseases of fleshy storage tissues. This agrees with the findings Opara and Asuquo (2016) that it causes a greater total loss of produce than any other bacteria. The tuber rot initially started as a white to cream colored spot and advanced to watery but dark lesion are quickly altered to deep rotten mass due the presence of rot bacteria.

Table 1 shows that in Umuspo 1 variety, *E. globolus* extract gave the best result when compared with the control sterile water followed by *J. curcas* and there was significant difference at $P \leq 0.05$ among all the five treatments. However, in Umuspo 2 variety, the best treatment in inhibiting the disease diameter was *A. indica* (1.39cm) followed by *J. curcas* (1.57cm) while the control had the poorest (1.63cm). while among the mother delight variety *E. globolus* and *A. indica* did the best (1.37cm) followed by *J.curcas* (1.40cm) while the control had the least (1.59cm).

Table 1: Influence of plant extracts on *Erwinia* inhibition diameter on three varieties of sweet potato

Umuspo 1		Umuspo 2		Mother Delight
Extracts	Dia cm	Dia cm	Extracts	Dia cm
<i>J. curcas</i>	1.39	1.57	<i>J. curcas</i>	1.40
<i>E.globolus</i>	1.36	1.61	<i>E.globolus</i>	1.37
<i>L.leucocephala</i>	1.50	1.62	<i>L.leucocephala</i>	1.51
<i>A.indica</i>	1.49	1.39	<i>A.indica</i>	1.37
Control	1.53	1.63	Control	1.59
LSD	0.18			0.11

Similar observation was made by Amadioha (2004), that extracts of *Azadirachta indica*, *Cymbopogon citratus* and *Ocimum gratissimum* effectively checked charcoal rot of potato caused by *Rhizoctonia solani*. in this study. Also, Opara and Nwankwo (2015), found that some medicinal plants contain antimicrobial agents and phytochemical properties including Azadirachtin, Oleoresin, Zigingiberene, Zingerone, and allicin. Certain essential oils obtained were outstanding as good antibacterial agents that were comparable to the synthetic penicillin and their essential food components including carbohydrate,

protein, fat, fiber and ash make them safe and useful to both plants and human.

From **Table 2** below, the combined analysis indicated that the highest radial diameter was recorded by Umuspo 1 (2.01cm) when compared to Umuspo 2 (1.69cm) at the 5th week.

Table 2: Combined analysis showing Influence of the disease on radial diameter of three sweet potato varieties for 5 weeks.

Wk	Mean Disease Diameter		
	Var: Umuspo 1	Var: Umuspo 2	Var: Mother Delight
1	1.18	1.22	1.15
2	1.28	1.27	1.28
3	1.57	1.50	1.48
4	1.78	1.59	1.62
5	2.01	1.69	1.74
Mean	1.56	1.46	1.46
LSD	0.18	0.10	0.11
P=0.05			

Generally, disease incidence was highest at the 5th week compared to week 1 and there was significant difference between week 1 and week 5 in the three varieties tested and by the 5th week the whole tubers were completely rotten. The result showed that Umuspo 1 is most susceptible to *Erwinia* soft rot with the mean (1.56cm) than Umuspo 2 and Mother delight with the mean (1.46cm).

Cultural Characteristics

The result of the cultural test showed that 24hrs after inoculation, different types of colonies were formed, yellow, cream and blueish colonies but the one that was more prevalent or more frequent was the creamy mucoid colony which was suspected to be *Erwinia* spp. When dilution plating was used to obtain pure

colonies, the real mucoid creamy was obtained.

Gram-reaction

The result of Gram-reaction test obtained showed that the pathogen colonies obtained after staining were reddish in color showing negative bacteria and when observed under microscope it was more or less rod shaped and movement was towards end otherwise polar movement was observed under microscope (Goszczyńska *et al.*, 2000).

Pathogenicity Test

After 7 days of inoculation, the three varieties of sweet potato tubers showed the signs of rot changing from milky to dark brown color and after 5 weeks, it was accompanied with a pungent smell, water-soaked and soft.

Some works on tuber rots have also been reported by previous workers using plant botanicals to reduce the disease incidence. Example, in the work done by Opara and Agugo (2014), they determined the inhibition potentials of some indigenous medicinal plants and their essential oils against some plant pathogens associated with sweet potato soft rot *in vitro* and *in vivo*. From their result, it showed that in the *in vitro* trials that all the extracts and oils assayed inhibited growth of the pathogens in culture to varying degree when compared with that of the control (sterile water). They found that *Z. officinale* oil was most effective in the inhibition of *Erwinia* pathogen. In this trial *A. indica* and *E. globolus* were found to be effective in inhibiting soft rot.

CONCLUSION AND RECOMMENDATION

In this study, *E.globolus* and *A. indica* are found to be more effective than *J.curcas* and *L. leucocephala*. This work shows the potency of the antibacterial activities of the test plant extracts *E. globolus* and *A. indica* in reducing or inhibiting the growth of *Erwinia in vivo*. The plant extracts contain some antimicrobial agents or phyto-chemicals which include Azadiractin in *A. indica*.

E. globolus and *A.indica* are recommended for farmers for the control of soft rot of tubers in storage

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AN EVALUATION OF EXCHANGEABLE AND NON- EXCHANGEABLE POTASSIUM UPTAKE FROM SOIL TO PLANTS IN SOUTH WESTERN NIGERIA

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Abstract

Potassium (K) is abundant in most soils, but the vast majority is unavailable to plants. A trial was carried out to evaluate the contribution of fixed K to the supplying capacities of South Western Nigerian soils to plant uptake of maize (*Zea mays*) with two treatments Plus K and minus K using 100 g NPK, 100 g NP fertilizers. These treatments were applied in a complete randomized design (CRD) and was replicated three times in the green house. Six composite soil samples were collected from 0 – 30 cm depth from Ife, Modakeke, Ife-Ijesha, Edun-Abon, Ede and Ibodi communities. Twelve (12) composite soil samples were collected from 0 – 30 cm depth from South Western Nigeria, where 4 samples were collected from Ondo, Ogun and Oyo States respectively. The results indicated that there was significant difference between K uptake and K fertilizer. The result showed that the levels of mean exchangeable K (41%), fixed K (2.61%) and total K (289%) respectively vary in the Southwestern Nigeria soils and that both exchangeable and non- exchangeable K source contribute to the K supply to the plant. The K uptake response to fertilizer was inversely proportional to the level of exchangeable and non-exchangeable K in the soils. Generally, there was response by the maize plant in response to K fertilizer application.

Keywords: Fixed, Potassium (K), Contribution, Supplying.

Introduction

Potassium is one of the essential elements for plant growth and development. It is one of the primary nutrients with Nitrogen and phosphorus which are required in large amounts (Lakrudzala 2013). Owing to the importance of K in life of plant, it has been studied extensively (Ndukwe et al., 2012).

It plays significant roles in the physiological processes of protein formation, transportation of water, nutrients and carbohydrates, photosynthesis, N utilization, stimulation of early growth and in insects and disease resistance. (Rehm and Schmitt, 2002; Lakrudzala 2013). Also, it promotes the transportation of assimilations, control of stomata opening, enzyme activation in plants especially those responsible for energy transfer and formation of sugars, starch and protein as well as promotion of microbial activities. (Al-Zubaidi et al., 2008, Yawson et al., 2011).

Potassium exists in four forms in the soil, exchangeable K, solution K, Non-exchangeable K, structural K (sparks, 2000). Exchangeable K is the form that is electrostatically bound to outer surface of clay mineral and humic substances and which is readily exchangeable with cations and available to plants. Non-exchange or fixed K represents the portion held between adjacent tetrahedral layers of dioctahedral and trioctahedral micas, vermiculites and integrate clay minerals such as chloritized vermiculite and which is sparingly or moderately available to plants (Taiwo et al, 2018) while structural K consists of the potassium that is bounded within crystal structures of soil mineral particles (Timothy et al, 2003). Soluble K is the form of K that is readily available for plant growth (water soluble). Held on clay mineral (feldspars and mica) particles exchange sites (Daniel

K and Carl R, 2018).

Soil potassium distribution and availability could be altered due to change in soil management and land use practices. For instance, it has been reported that increased addition of organic manures yielded a corresponding increase in exchangeable K content of Ultisol (Mbah, 2008). Also, variation in available K distribution have been indicated in soils of varying land use system (Raheb and Heidari 2011; Ayeles, 2013).

Potassium fixation is a widespread phenomenon in most soil and accounts significantly for the availability of applied K to plants. Although this phenomenon is a direct consequence of the presence of 2:1 clay minerals, recent studies have shown that this phenomenon also occurs in tropical soils with insignificant content 2:1 silicate clays. Also, the knowledge of potassium status of soils provides useful information for rational K fertilizer management. Therefore, this study investigated the potassium fixation capacities as well as the potassium status of some soils. It has been observed that some soils that test high in K may respond to K fertilization contrary to expectation. This is an indication that there are other forms of K other than the exchangeable K contributing to K needs of crops. Non-exchangeable K has been shown to also contribute significantly to plant K uptake. This has been ascribed to the fixed K. Hence, this trial was to investigate the contribution of fixed k to k⁺ supplying capacity of South Western Nigeria soils. Also there is little information on the availability of K to growing plants in soils. Therefore, need to investigate the ability of the soil to supply k to plants and the relative contributions of exchangeable and non- exchangeable k form to k uptake using maize

(*Zea mays L.*) as a taste crop.

Materials and Method

Composite Soil Samples were collected randomly from 0-30 cm depth from Southwestern Nigeria. Four of these samples were collected from, Ondo, Ogun and Oyo States respectively. Soil samples were collected based on the land use of the areas hence, soil samples were collected from locations where fertilizer have not been applied. All sampling and analysis were done according to the Tropical Soil Biology and Fertility (TSBF) programme handbook of method as described by Anderson and Ingram (1989). Exchangeable K in soil was determined by using neutral Ammonium acetate (1N NH⁴OAC) solution as extractant, and subjecting the extract to flame analyzer. Then the exchangeable K in the filtrate was determined with the flame photometer (Spencer, 2010). IN HNO³ was used to determine the fixed K in the soil samples. The total K was extracted by 5ml of HF and 0.5ml of HCl104. The extracted K was determined with flame photometer.

Each soil sample was replicated 3 times in a bucket contain 2 kg of soil each in green house given a total of 36 plots. Before planting, the soils were treated with plus K (+k) and Minus K (-k) solutions. Hence there were 2 treatment per soils. The plus K solution contained N, P, and K while the minus K contained N & P but no K. The levels of NPK applied were 100, 50, 100g respectively as NaNO₃ Na₂ HPO₄.12H₂O and KC before the treatment were mixed with the soil before planting was done. Each treatment was replicated 3 times giving a total of 24 Pots/State and a total of 72 pots /SW. The K supplying and fixing capacity of the 12 Soil samples were determined by four cropping in the green house by planting 2 plant/pot.

At planting 75 percent of the field moisture capacity (FMC) was maintained by adding water to pots till harvest to maintain the quantity initially added. 5 maize seeds were planted in each pot and were later thinned down to 2 plants per pot. Harvesting of the maize seedlings was carried out between three to four weeks after emergence in each sequence. Four cropping were done with each one preceding the other after harvest, and in all cases watering was discontinued two days before harvesting. The plant dry matter yield were collected and weighed for the various pots and for the four cropping periods.

Result and Discussion

Pre cropping soil K status of the area

Table 1 shows soil K status before planting, the total K of the soils varied from 5, 10 *cmol kg⁻¹* for Efon-Alaye and Ede samples to 127.89 *cmol kg⁻¹* for Iyin-Ekiti and Ado-Ekiti samples. The fixed K of the soils varied from 0.03 *cmol kg⁻¹* for Efon-alaye sample to 1.38 *cmol kg⁻¹* for Ikare and Modakeke samples. The exchangeable K of the soils varied from 0.13 *cmol kg⁻¹*

¹ for Iperu and Ilaro samples to 0.56 *cmol kg⁻¹* for Ise-Ijesha and Modakeke samples.

Soils with high total K content also had high exchangeable and fixed K content. Examples of these soils are Ado-Ekiti, Ikare, Edun-Abon and Modakeke samples. Conversely, soils with low total K also showed low exchangeable and fixed K content. Examples of such soils are Efon-Alaye, Ikperu, Ilaro and Owode samples. This is with an exceptional case of Ede sample which had very low total K content but high exchangeable and fixed K. some of the soils had higher exchangeable K than fixed K, e.g. Ife, Efon-Alaye and Owode samples. While some soils had higher fixed K than exchangeable K, e.g. Modakeke, Edun-Abon, Ikare, Ado-Ekiti and Iyin-Ekiti samples. The Efon-Alaye soil sample was generally poor in K content. It had very low total K (4.48 *cmol kg⁻¹*), very low fixed K (0.03 *cmol kg⁻¹*) and relatively low exchangeable K (0.24*cmol kg⁻¹*). It is interesting to note that Ibodi and Ede soils had comparable levels of fixed and exchangeable K but differed in the amount of total K. Ibodi soil had a total K level of 38.37 *cmol kg⁻¹* while Ede soil had 5.1 *cmol kg⁻¹*.

Table 1: Composite soil Samples from the Locations and the Soil K Status

Soil No.	Soil Name/ Locations	Exch. K(<i>cmol kg⁻¹</i>)	Fixed K(<i>cmol kg⁻¹</i>)	TotalK (<i>cmol kg⁻¹</i>)
1	Ife	0.35	0.24	40.92
2	Modakeke	0.61	1.46	53.07
3	Ise-Ijesha	0.56	0.74	47.96
4	Ibodi	0.45	0.31	38.37
5	Edun-Abon	0.46	0.87	51.16
6	Ede	0.45	0.31	5.10
7	Ikare	0.38	1.38	31.97
8	Efon-Alaye	0.24	0.03	4.48
9	Ado-Ekiti	0.50	0.91	143.88
10	Iyin-Ekiti	0.18	0.86	127.89
11	Owode	0.15	0.07	40.29
12	Odogbolu	0.17	1.10	35.17
13	Ilaro	0.13	0.08	36.45
14	Iperu	0.13	0.10	
				37.73

PLANT DRY MATTER YIELD

Table 2 shows average plant dry matter yield for both the pots into which K was added (plus pots) and those pots into which no K was added (minus pots).

Generally, for all the soil samples, there was decrease in yield as the time of cropping increased. The dry matter yield of the first cropping for all the K pots was the highest with a mean weight of 1.89g, while the yield of the fourth cropping was the lowest with a mean weight of 0.58g. The same trend occurred in the yield of the cropping for all the plus K pots. The mean yield of dry matter for the first cropping was 2.44g while that of the fourth cropping was 0.73g. The yield

obtained from the plus K pots was generally higher than the yield obtained from minus K pots being 1.66g for plus K and 1.18g for the minus K treatments. This suggests that there was an inadequate amount of available K in the soil and hence a high response to fertilizer application in the plus K pots. As in table 3 the response mean was 41 percent. The least response was observed on Ado-Ekiti soil sample. And the

highest response was obtain from Odogbolu and Ilaro soil samples. Ado-Ekiti had high quantities of all K fractions while Odogbolu and Ilaro had low. Ibodi soil and Ede soil which had comparable levels of exchangeable K and fixed K (Table 1), give approximately the same dry matter yield.

Table 2: Average Dry Matter Yield (g/pot of 2 plants).

Soil Name	-K Pots Cropping					+ Pots Cropping				
	1 st	2 nd	3 rd	4 th	Ave.	1 st	2 nd	3 rd	4 th	Ave.
Ife	1.60	0.82	1.10	0.66	1.05	2.87	1.18	1.60	0.73	1.60
Modakeke	1.95	0.94	1.80	0.70	1.35	1.90	0.90	3.60	0.89	1.82
Ise-Ijesha	1.53	1.34	2.82	0.71	1.60	2.27	1.45	3.60	1.08	2.10
Ibodi	1.80	1.09	1.60	0.61	1.28	2.50	1.32	2.89	0.93	1.91
Edun-Abon	1.95	1.22	2.19	0.62	1.50	2.15	1.47	3.24	0.89	1.94
Ede	1.82	0.68	2.27	0.62	1.35	1.23	1.03	2.82	1.19	1.57
Ikare	1.55	0.74	0.59	0.39	0.82	1.87	1.33	0.65	0.40	1.06
Efon-Alaye	1.60	0.42	1.20	0.47	0.92	2.40	1.30	1.41	0.45	1.39
Ado-Ekiti	3.25	0.93	1.0	0.58	1.44	3.07	1.29	0.79	0.64	1.45
Iyin-Ekiti	2.32	0.77	0.56	0.44	1.02	2.95	1.05	1.08	0.85	1.48
Owode	1.75	1.06	0.87	0.63	1.08	3.02	1.55	1.99	0.52	1.77
Odogbolu	1.78	0.77	0.68	0.50	0.93	3.23	1.55	2.28	0.45	1.88
Ilaro	1.40	0.53	0.42	0.45	0.70	2.26	1.81	1.40	0.19	1.42
Iperu	2.17	1.36	1.82	0.67	1.50	2.47	2.09	1.63	1.05	1.81
Mean	1.89	0.90	1.35	0.58	1.18	2.44	1.38	2.07	0.73	1.66

Table 3: Dry Matter Yield Response

Soil Name	Crop Average	Response		
		A- K Pots	+K Pots	% Response
Ife	1.05	1.60	0.52	52
Modakeke	1.35	1.82	0.35	35
Ise-Ijesha	1.60	2.10	0.31	31
Ibodi	1.28	1.91	0.49	49
Edun-Abon	1.50	1.94	0.29	29
Ede	1.35	1.57	0.16	16
Ikare	0.82	1.06	0.29	29
Efon-Alaye	0.92	1.39	0.51	51
Ado-Ekiti	1.44	1.45	0.007	0.7
Iyin-Ekiti	1.02	1.48	0.45	45
Owode	1.08	1.77	0.64	64
Odogbolu	0.93	1.88	1.02	102
Ilaro	0.70	1.42	1.03	103
Iperu	1.50	1.81	0.21	21
Mean	1.18	1.66	0.41	41

Note: % Response $\frac{(+K - (-K))}{(-K)} \times \frac{100}{1}$

Average Potassium Concentration in Tissue (%)

As shown in Table 4, the average K content of maize tissue (%) decreased from the first to the fourth

cropping. However, the amount in the plus K pots was higher than in the minus K pots. This explains why K deficiency showed up in some of the minus K Pots very quickly. For instance, the mean K content of the minus K pots was 0.79 percent while the mean of the plus K pots was 2.61 percent. This marked difference confirmed the earlier suggestion that the minus K pots contained inadequate amount of K. it also confirmed the high response to fertilizer application in the plus K pots.

In both the minus K pots and plus K pots, the tissue K content decrease with successive cropping. The percent K content was highest in tissue of first cropping and was least in the fourth cropping. A gradual decrease was observed in the K content in the minus K pots cropping but in the plus K pots the decrease in K content was very slight from the first cropping to the third cropping.

This is due to the fact that there was still a good amount of the added K fertilizer. However, the fourth cropping of the plus K pots showed a pronounced decline in percent K content in tissue. This is attributed continuous cropping due to K fertilizer application in the soil. In many cases in the soil, it may be the first cropping after fallow that will enjoy luxury consumption of K and the following crops will suffer as a result of insufficient amount of K in the soil.

Table 4: Potassium Concentration in Tissue (%)

Soil Name	-K Pots Cropping	+ Pots Cropping
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	1 st	2 nd	3 rd	4 th	Ave.	1 st	2 nd	3 rd	4 th	Ave.
Ife	1.17	0.67	0.38	0.14	0.59	3.03	3.73	3.06	0.80	2.66
Modakeke	2.30	1.13	0.85	0.17	1.11	2.94	5.04	2.96	0.96	2.97
Ise-Ijesha	2.68	2.25	0.85	0.21	1.50	3.14	3.81	2.69	1.08	2.68
Ibodi	2.14	1.17	0.31	0.15	0.94	4.42	3.88	3.35	1.01	3.16
Edun-Abon	1.83	1.96	0.79	0.20	1.20	3.50	4.40	3.10	1.09	3.02
Ede	1.89	1.48	0.46	0.17	1.00	2.60	4.90	3.44	0.98	2.98
Ikare	0.84	0.35	0.33	0.15	0.42	2.56	3.69	3.27	0.96	2.62
Efon-Alaye	1.25	0.96	0.67	0.17	0.76	2.68	3.06	3.79	1.02	2.64
Ado-Ekiti	2.32	1.04	0.88	0.40	1.16	3.74	3.92	4.92	1.00	3.39
Iyin-Ekiti	1.35	0.65	0.44	0.17	0.65	2.87	2.25	1.81	0.94	1.97
Owode	0.89	0.46	0.23	0.13	0.43	2.92	2.33	1.06	0.75	1.77
Odogbolu	0.66	0.29	0.25	0.20	0.35	2.51	4.50	1.40	0.56	2.24
Ilaro	0.79	0.42	0.35	0.21	0.44	2.67	3.54	2.17	0.58	2.24
Iperu	0.77	0.85	0.19	0.20	0.50	2.56	3.98	1.67	0.75	2.24
Mean	1.49	0.98	0.50	0.19	0.79	3.01	3.79	2.76	0.89	2.61

Potassium Uptake by Plants

Table 5 shows the average K uptake by maize plants from both plus K and minus K pots. The K uptake of the maize plants was highest in the Ise-Ijesha and Ado-Ekiti soil samples, followed by Edun-Abon, and Modakeke soil samples. And the least K uptake was observed in the Ilaro, Odogbolu and Owode soil samples. However, the Ibodi and Ede soil samples which had similar levels of exchangeable K and fixed K (Table 1) also showed approximately equal K uptake by the plants.

The uptake was highest for the first cropping, it reduced gradually in the second and third cropping but dropped sharply in the fourth cropping (Table 5). The mean of the total K uptake in the first cropping was 28.4mg K/pot, the second cropping was 9.7, the third cropping was 8.9 and that of the fourth cropping was 1.1mg K/pot. This result indicates that at the fourth cropping the soil K available to the crop had become very low.

For all the soil samples and in all the four successive cropping, the K uptake was higher in plus K pots than in minus K pots. The mean of the average of the four cropping for the soil samples in minus K pots was 12.2mg K/pot and that of plus K pot was 47.4mg K/pot. The wide difference between K uptake in minus K pots and plus K pots shows that K fertilizer application markedly increased the K supply to the plant from soil. Also the K uptake by the maize plants seems to reflect the level of soil available K much better than dry matter yield or K concentration in the crop tissue. This is because generally the plants showed high response to fertilizer application, and the K uptake response was much higher than dry matter yield response. The mean uptake response was 289

percent while the mean dry matter response was 41 percent. The response to K application both in dry matter yield and K uptake seems to indicate the K supplying capacity of the soil samples. For instance, the highest response in K uptake was observed in the Ilaro soil (903%) and Odogbolu soil (1002%) (Table 6); and the highest response in dry matter yield was also observed in these two soil samples (Table 3). The Ado-Ekiti soil showed the least response both in terms of uptake and dry matter yield, and table 1 showed that Ado-Ekiti soil contained high level of fixed K plus exchangeable K while Ilaro and Odogbolu soils contained low levels of fixed and exchangeable K. this suggests that the lower the level of fixed and exchangeable K, the greater the response to K fertilization in these soils.

Conclusion

The results showed that the levels of exchangeable form of K, fixed K and total K vary in the South western Nigerian soils and that the total K is much high than the exchangeable K and the fixed K.

There was insufficient available K in the soils, and if continuous cropping is done on these soils without K fertilizer application, K deficiency will show up in crop growth and yield. From the results of the dry matter yield, generally, there were response to K fertilization in the area.

Result of the study indicates that both exchangeable and non- exchangeable sources of K contribute to the soil K supply for plant uptake, under intensive cultivation. Hence, both the exchangeable form and the non- exchangeable form of K may serve as an index in assessing the plant available soul K status of South Western Nigerian soils.

Table 5: Potassium Uptake (mg k/Pot of 2 Plants)

Soil Name	-K Pots Cropping					+ Pots Cropping				
	1 st	2 nd	3 rd	4 th	Ave.	1 st	2 nd	3 rd	4 th	Ave.
Ife	18.7	5.5	4.1	0.9	7.3	87.0	44.0	49.0	6.4	46.6
Modakeke	44.9	10.6	15.4	1.2	18.0	55.8	45.4	106.5	8.5	54.1
Ise-Ijesha	41.0	30.2	24.1	1.5	24.2	71.2	55.3	96.8	11.7	58.7
Ibodi	38.4	12.7	5.0	0.9	14.3	111.0	51.2	96.9	9.4	67.1
Edun-Abon	35.8	23.9	17.3	1.3	19.6	75.3	64.6	100.6	9.7	67.1
Ede	34.3	10.1	10.4	1.0	14.0	32.0	50.4	96.9	11.6	47.8
Ikare	13.1	2.6	19.7	0.7	9.0	47.9	49.0	21.3	3.8	30.5
Efon-Alaye	20.0	4.0	8.0	0.8	8.2	64.3	39.8	53.5	4.6	40.5
Ado-Ekiti	75.5	9.7	8.8	2.3	24.1	114.9	50.5	38.8	7.2	52.9
Iyin-Ekiti	31.4	5.0	2.5	0.7	9.9	84.0	23.6	19.6	8.0	33.9
Owode	15.5	4.9	2.0	0.8	5.8	88.1	36.2	21.1	4.6	37.5
Odogbolu	11.7	2.3	1.7	1.2	4.2	81.1	69.8	31.8	2.5	46.3
Ilaro	1.1	2.2	1.5	0.9	3.9	60.3	64.1	30.3	1.6	39.1
Iperu	16.7	11.5	3.4	1.4	8.3	63.3	83.2	27.2	7.8	45.4
Mean	28.4	9.7	8.9	1.1	12.2	74.1	51.9	56.5	7.0	47.4

Table 6: Potassium Uptake Response.

Soil Name	Crop		Response	% Response
	Average			
	- K Pots	+ K Pots		
Ife	7.3	46.7	5.38	538
Modakeke	18.0	54.1	2.01	201
Ise-Ijesha	24.2	58.7	1.43	143
Ibodi	14.3	67.1	3.69	369
Edun-Abon	19.6	62.5	2.19	219
Ede	14.0	47.8	2.41	241
Ikare	9.0	30.5	2.39	239
Efon-Alaye	8.2	40.5	3.94	394
Ado-Ekiti	24.1	52.9	1.20	120
Iyin-Ekiti	9.9	33.9	2.42	242
Owode	5.8	37.5	5.47	547
Odogbolu	4.2	46.3	10.02	1002
Ilaro	3.9	39.1	9.03	903
Iperu	8.3	45.4	4.47	447
Mean	12.2	47.4	2.89	289

Note: % Response = $\frac{(+k) - (-k)}{(-k)} \times 100$ (- k) 1

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WIS ASP 31

EVALUATION OF THE GROWTH AND ANATOMY OF *AXONOPUS COMPRESSUS* AND *Eleusine indica*, GROWN ON CRUDE OIL CONTAMINATED SOIL

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ABSTRACT

This study assessed growth and anatomy of *Axonopus compressus* and *Eleusine indica* grown on crude oil contaminated soil. The experiment was a factorial arrangement (3x4) in a completely randomized design (CRD). The treatment levels were 0%, 1%, 2%, 3% and 4% crude oil concentration (volume/ weight) in the soil. The study observed a significant ($P<0.05$) reduction in plant height, number of leaves, stem girth and fresh and dry weight of the two plants grown on the crude oil treated soils (1%, 2%, 3% and 4%). Some anatomical changes were observed in the treated leaf, root and stem samples (1%-4% v/w). Disorder in the arrangement of epidermal cells in the leaves and distortion of cells of roots and leaves were observed. The results of the study were presented in tables and charts. The anatomical changes in the leaves, stems and roots of the plants could be a viable tool for phytomonitoring of crude oil pollution in the environment.

Keywords: Plant Growth, Anatomy, *Axonopus compressus*, *Eleusine indica*, Crude oil, Contamination.

INTRODUCTION

Various researchers have carried out detailed studies on the effects of crude oil on the various growth parameters of plants such as germination, seedling growth and development, leaf size and number, stomatal distribution, fruit development, stem height and girth, root development and elongation, leaf and stem anatomy and general morphology of plants (Omosun *et al.*, 2010; Baishya and Kalita, 2015; Adewole and Moyinoluwa, 2012). Although plants require certain heavy metals for growth, excessive quantity of these metals can become toxic to plants.

Crude oil contaminated soil causes stressful conditions that interfere with plants uptake of water and gaseous exchange, resulting in an anaerobic condition (Omosun *et al.*, 2009). Osuagwu and Edeoga (2012), reported that the mineral concentration, as well as the photosynthetic apparatus of *Gongronema latifolium* was adversely affected by water stress. Crude oil contamination of soil has been reported to cause anatomical changes in most plants. In a study carried out by Omosun *et al.* (2008) on the effect of crude oil contaminated soil on the anatomy of *Amaranthus hybridus*, it was reported that epidermal cells were irregularly shaped, the anticlinal cell walls were curved as compared to those of the non- treated *A. hybridus* which were straight.

Uwalaka and Osuagwu (2015), reported a reduction in the number and size of intercellular air spaces as well as cortical cell of *Pueraria phaseoloides* and *Centrosema pubescens* treated with crude oil. Omosun *et al.*, (2009), observed that the distribution of stomata in the upper and lower epidermis of the leaves of *Mucuna jaspodea* and *M. veracuz* reduced with increase in level of pollution.

Not all plants can withstand the hazardous effects of crude oil contamination, hence the choice of these grasses (*A. compressus* and *E. indica*) as they are considered suitable to degrade and metabolise

contaminants due to their extensive fibrous root system which offers increased rhizosphere zone (Ogbo *et al.*, 2009).

This study is therefore aimed at assessing and comparing the effect of crude oil contamination on the growth and anatomy of *A. compressus* and *E. indica*.

MATERIALS AND METHODS

DESIGN OF THE EXPERIMENT: The design used for the study was completely randomized design. The treatments were different crude oil concentrations (volume per weight) in the soil (0%, 1%, 2%, 3%, and 4%) with two plant species *Axonopus compressus* and *Eleusine indica*. Each treatment was replicated four times.

COLLECTION OF SAMPLES: The soil samples with no prior history of crude oil contamination were obtained from top soil (0-15cm), within the campus of Michael Okpara University of Agriculture, Umudike. *Axonopus compressus* and *Eleusine indica* plants were obtained within the surroundings of College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, and identified by the taxonomy unit of the Department of Plant Science and Biotechnology, MOUAU.

SOIL TREATMENT AND PLANTING: Soil samples were air-dried, sieved and homogenized. 5kg of soil samples were weighed into 5litre plastic bucket already perforated. Each bucket was treated with 50ml, 100ml, 150ml, and 200ml of crude oil to obtain 1%, 2%, 3%, and 4% of crude oil contamination. Each treatment including the control (0%) was replicated four times.

The grasses (*A. compressus* and *E. indica*) of equal height were transplanted into the already perforated buckets. Three *A. compressus* plants were planted per bucket and three *E. indica* plants were planted per bucket.

RECORDING OF GROWTH PARAMETERS: The

growth parameters recorded were, plant height, number of leaves, stem girth, plant fresh weight and dry weight. Plant height was measured from the soil level to the top-most leaf with the use of a meter rule. Number of leaves were obtained by visual counting (Anoliefo and Vwioko, 2001). The stem girth was obtained using a vernier caliper. Fresh and dry weight were measured at 12 weeks after planting. Dry weight was determined by oven drying at 80°C for five days. The recording of the growth parameters was done every two weeks except the fresh and dry weight which were obtained at the end of the experiment (12 WAP).

ANATOMICAL STUDIES: AT 12 twelve weeks after planting (12 WAP), some mature and fresh samples of the two plants (*Axonopus compressus*, and *Eleusine indica*) were collected. These plant parts were fixed in FAA (formalin, Acetic acid and alcohol) in ratio of 1:1:18 respectively. The root, stem and leaves were washed in water and sectioned with a Sipcon Rotary Microtome. The sections were first stained with drops of alcian blue for three minutes and washed off, then counterstained with safranin solution for about two minutes and then dehydrated with pure xylene at intervals of few seconds and finally mounted on slides using Canada balsam (Edeoga *et al.*, 2008).

EPIDERMAL PEELS: The epidermal peels were obtained using the method of Edeoga *et al.* (2008), where fresh leaves of the plants were boiled in a concentrated nitric acid over a water bath for 2-3 minutes. The samples were then carefully washed in water. The epidermal peels were stained using safranin solution for about two minutes and washed off with water before mounting in glycerine.

PHOTOMICROGRAPHY: Photomicrographs and

observation of slides of the anatomical sections and epidermis were taken using Noval Digital Microscope (scope image 9.0) at the Histology laboratory of the College of Veterinary Medicine, Michael Opkara University of Agriculture, Umudike.

STATISTICAL ANALYSIS:

The data obtained were subjected to analysis of variance (ANOVA). The treatment means were separated using Least Significant Difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

Effects of the various concentrations of crude oil contamination on the growth (Plant height, number of leaves and stem girth) of the two plants. The heights of the two plants were affected by the crude oil contamination of the soil as shown in figures 1&2. It was observed that the height of plants grown on the control (0%) were significantly higher ($P < 0.05$) than those grown on treated soils (1%, 2%, 3%, 4%). However, there were no significant difference between 3% and 4%.

Crude oil contamination of soil affected the number of leaves of the two plants as indicated in figures 3&4. It was observed that number of leaves were significantly higher ($P < 0.05$) in control compared to 1%, 2%, 3%, and 4% treatment levels. There were significant differences among treatment levels, however no significant difference between 2% and 3%.

This study showed that the stem girth of the two plants were affected by crude oil contamination. Plants grown on the untreated soil had significantly ($P < 0.05$) larger stem girth than those grown on crude oil treated soils as shown in figures 5&6.

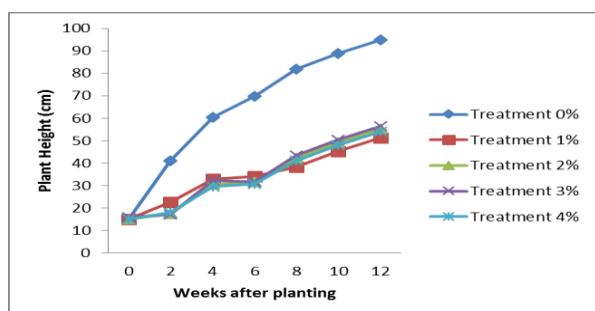


Figure 1: Effect of various concentrations of crude oil contamination on the plant height of *A. compressus*

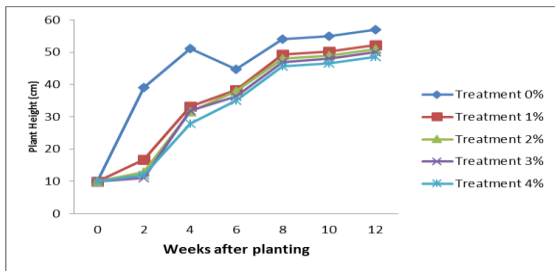


Figure 2: Effect of various concentrations of crude oil contamination on the plant height of *E. indica*

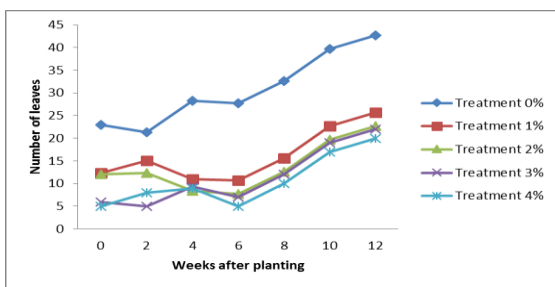


Figure 3: Effect of various concentrations of crude oil contamination on the number of leaves of *A. compressus*

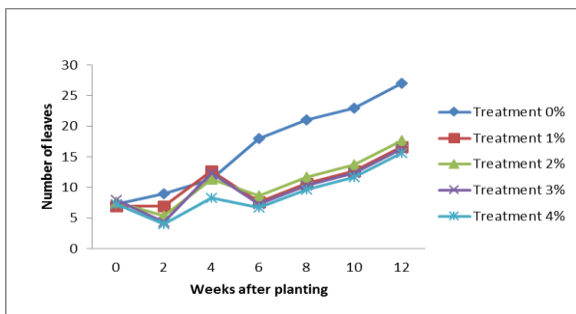


Figure 4: Effect of various concentrations of crude oil contamination on the number of leaves of *E. indica*

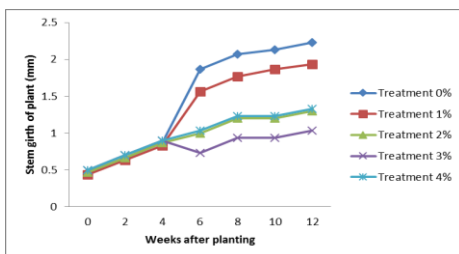


Figure 5: Effect of various concentrations of crude oil contamination on the stem girth of *A. compressus*

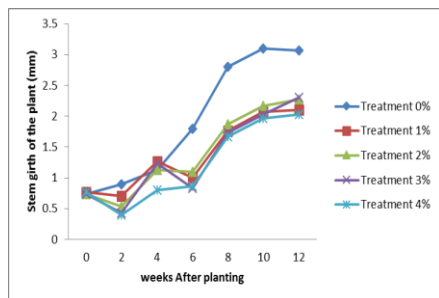


Figure 6: Effect of various concentrations of crude oil contamination on the stem girth of *E. Indica*

Effects of the various concentrations of crude oil contamination on the leaf, stem and root anatomy of *A. compressus*.

The epidermal peels of the two plants were affected by crude oil contamination of the soil as shown in plates 1 - 4. It was observed that the silica bodies of the epidermal cells of the control (0%) were arranged in a

straight line while those grown on treated soils (4%) were scattered.

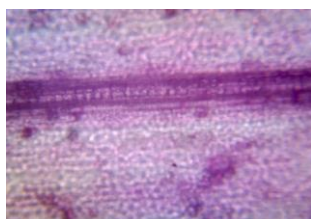


Plate 1: *A. compressus* leaf 0% x40

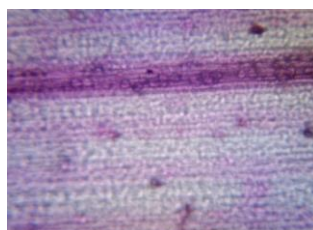


Plate 2:

A.



Plate 3: *A. compressus* leaf 0% x400

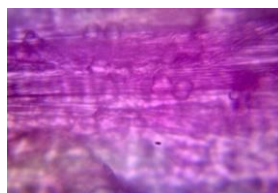


Plate 4: *A. compressus* leaf 4% x400

Plates 1 and 3: Leaf epidermis of *A. compressus* grown on 0% (control) showing normal arrangement of epidermal cells with silica bodies arranged in a straight line. Plates 2 and 4: Leaf epidermis of *A. compressus* grown on 4% crude oil polluted soil showing a disorder in the arrangement of epidermal cells and silica bodies.

The anatomy of the root and stem of the two plants shows a reduction in intercellular air spaces and thicker parenchyma cells on contaminated plants as shown in plates 5 - 12.

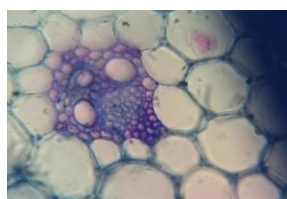


Plate 5: *A. compressus* stem 0% X400



Plate 6: *A. compressus* stem 2% X400

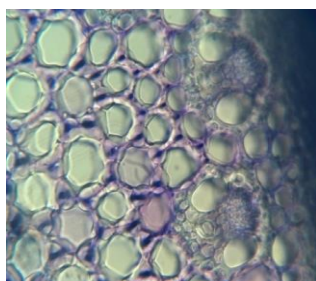


Plate 7: *A. compressus* stem 3% X400

Plate 8: *A. compressus* stem 4% X400

Plate 5: *A. compressus* stem grown on 0% crude oil polluted soil (control) showing larger parenchyma cells. Plate 6: *A. compressus* stem grown on 2% crude oil polluted soil showing reduced parenchyma cells. Plate 7: *A. compressus* stem grown on 3% crude oil

polluted soil showing reduced parenchyma cells. Plate 8: *A. compressus* stem grown on 4% crude oil polluted soil showing much reduced parenchyma cells and thicker cell wall.

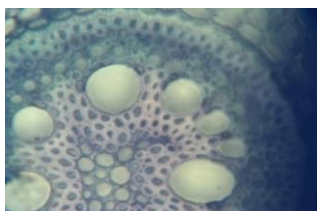


Plate 9: *A. compressus* root 0% X400

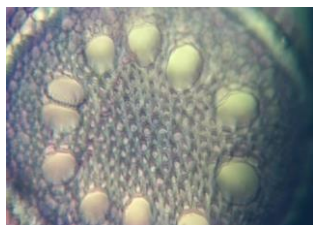


Plate 10 *A. compressus* root 2% x400

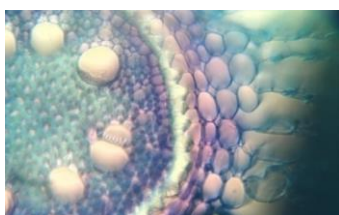


Plate 9: *A. compressus* root grown on 0% crude oil polluted soil (control) showing number of intercellular air spaces. Plate 10: *A. compressus* root grown on 2% crude oil polluted soil showing reduced intercellular air spaces and parenchyma cells due to increased levels of

crude oil contamination. Plate 11: *A. compressus* root grown on 3% crude oil polluted soil showing reduced parenchyma cells. Plate 12: *A. compressus* root grown on 4% crude oil polluted soil showing greatly reduced parenchyma cells.

DISCUSSION

The results from the effects of crude oil contamination on plant growth (figures 1 - 6) show that the values of the growth parameters decreased as concentration of contamination increased. This observation is in agreement with the work of Quinones-Aguilar *et al* (2003) who reported that the crude oil formed a

hydrophobic layer over the root, which limited water and mineral absorption. The crude oil contamination caused a progressive decrease in the heights of plants with increase in contamination when compared to the control (0%) (Figures 1 & 2). This report agrees with Omosun *et al* (2008) who reported a depression in height, number of leaves and leaf area of *A. hybridus*

treated with different crude oil concentration. This progressive decrease is as a result of changes in soil condition which interferes with water uptake and gaseous exchange leading to a condition of physiological drought. According to Agbogidi *et al.*, 2007; Ogbuehi *et al.*, 2011) the reduction in plant height could be due to reduction in the nutrient contents of the soil as petroleum products have been reported to reduce Nitrogen availability in soil.

In this study, a progressive reduction in number of leaves of crude oil-treated plants as compared to the control was observed (figs. 3&4). This observation is in agreement with the work of Adedokun and Ataga (2007), who asserted that the contamination of soils with crude oil significantly affected the plant height, leaf production and biomass of *V. unguiculata*. A similar effect was reported by Olayinka and Arinde (2012), who studied the effect of spent engine oil on germination and the seedling growth of *A. hypogea*. Crude oil contamination affected the stem girth of plants as compared to the control (figs. 4&6). This is in agreement with the report by Firi-Appah *et al.* (2014) that crude oil pollution affected the stem girth of *H. esculentus*. The observation by Agbogidi and Ilondu (2013) also supports it as they reported that spent engine oil pollution had a deleterious effect on the stem girth of *Moringa oleifera*.

In this present study, disorder in the arrangement of the epidermal cells as the level of contamination increased and this report is in line with the report of Omosun *et al.* (2009). The most visible change observed in this present study was in the arrangement of the silica bodies of the leaf epidermis. The silica bodies were observed to be scattered and fewer in the polluted leaves as compared to the control. This observation can be attributed to an adaptation mechanism by the plants to withstand drought stress. According to Evert (2006),

the epidermal cells of grasses are arranged in parallel rows with combinations of diverse cell types, some of which are specific for silicon (Si) deposition and are called Silica cells. These develop into structures of various shapes and properties called silica bodies. Plant silica bodies have been implicated in reducing stressors such as fungal infection, herbivory, wear and drought (Chen *et al.*, 2012; Hunt *et al.*, 2008; Massey and Hartley 2009; Chen *et al.*, 2011). However, no previous study has reported the effect of crude oil specifically on the silica cell arrangement of grasses. The reduction in parenchyma cell and cell wall thickness of the treated *A. compressus* stem and root is in agreement with the work of Omosun *et al.* (2008). This could be as a result of physiological drought which caused the cell to be smaller and the cell wall thicker in order to reduce water loss.

CONCLUSION

This study observed that the two plants were affected by crude oil contamination. The effects observed were reduction in plant height, number of leaves, stem girth, fresh and dry weight and some damages on the cells and tissues of the epidermal layers of leaves, roots and stems. These observed effects increased with increase in the level of contamination, hence confirms earlier reports that crude oil has adverse effects on plants.

RECOMMENDATION

The anatomical changes in the leaves, stems and roots of the plants could be a viable tool for phytomonitoring of crude oil pollution in the environment. However, caution should be applied in the disposal of the experimental materials (soil and plants). These should be buried or discarded into the dump site, away from agricultural sites in order to avoid the reintroduction of the contaminants into the soil and possible absorption by plants.

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WIS-ASP 32 FL

SEED BORNE FUNGI DISEASES OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa*) AND THEIR CONTROL WITH *Monodora myristica* AND *Piper guineense*

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ABSTRACT

African Yam bean is one of the underutilized crops that have the potentials to broaden man's food sources. Information on the potential seed pathogens and their control are almost lacking, therefore, this study was carried out to identify fungi associated with African yam bean and their control with selected botanicals. Fungi were isolated using the agar plate method. Five seeds from each sample from the different locations were plated on prepared and solidified Potato Dextrose Agar (PDA) in Petri dishes. The seeds were incubated for 5 days, each fungi colony was transferred to fresh PDA in Petri dishes to obtain pure cultures of fungi for identification. Evaluation with *Monodora myristica* and *Piper guineense* for antifungal activity against seven seed borne fungi was also carried out. Experimental design was completely randomized design with three replicates. Data were analyzed using ANOVA at $\alpha_{0.05}$. The result shows that *Aspergillus tamarii*, *Penicillium*, *Rhizopus*, *Aspergillus ochraceus*, *Aspergillus niger* and *Aspergillus flavus* and *A. Fumigatus* are the cause of seed borne deterioration of African yam bean in the areas sampled as pathogenicity test of the various isolates from infected seeds showed that they were virulent; causing deterioration of African yam bean seeds examined. Seed treatment with *M. myristica* and *P. guineense* inhibited the growth of various fungi between 13.0 – 63.0% and 15.9 – 59.0%, respectively compared to the control. Therefore, their medicinal potentials can be exploited in the management of seed associated fungi of African yam bean.

Keywords: African yam bean, Seed borne fungi, Botanicals, antifungal

Introduction

African yam bean (*Sphenostylis stenocarpa*) locally known as *Akidi* in Igbo dialect is a perennial or climbing herb from a tuberous rootstock with the stem often reddish, glabrous or sparsely puberulus, and woody near the base (Ghadsingh and Mandge 2012). It is one of three taxa used by humans from the large genus *Sphenostylis* E. Meyer (Leguminosae; Papilionoideae; Phaseoleae) comprising 7 species that occur in dry forests and in open or forested savannas in tropical and southern Africa. It is the most widely distributed and morphologically variable species in the genus and by far the most important economically (Ghadsingh and Mandge 2012).

African yam bean (AYB) originated in Ethiopia, but both wild and cultivated types now occur in tropical Africa as far north as Egypt and also throughout West Africa from Guinea to southern Africa). It is cultivated in Nigeria mainly for seed and also grown for tubers in Coted'Ivoire, Ghana, Togo, Cameroon, Gabon, Democratic Republic of Congo, Ethiopia, parts of east Africa, Malawi and Zimbabwe (). Seeds also take the same time as tubers from planting to harvest, normally from April/July to December. In some areas of Nigeria, it is also cultivated as a minor crop in (Utter, 2007; Klu *et al.*, 2001).

It is grown either for the seeds or the tubers or both in the different areas where it is grown. The roots develop starchy tubers that serve as organs of perennation when the above ground parts die back during the dry season (Klu *et al.*, 2001). The tuberous roots contain more protein than sweet potatoes, Irish potatoes or cassava roots and above ground part produces good yields of up to 2000 kg/ha of edible seeds (Klu *et al.*, 2001). The leaves are also utilized as a spinach/cooked

vegetable (Ghadsingh and Mandge 2012). African yam bean seeds are high in vitamin C, dietary fiber, vitamin B6, potassium, and manganese; while being low in saturated fat, sodium, and cholesterol (Utter, 2007). The AYB tubers are regarded as important source of starch and protein in tropical Africa, and the plant is potentially important as a pulse legume. The amino acid content of the protein is similar to that of the soybean, though rather higher in histidine and isoleucine. The energy content of the seeds per 100 g dry matter is 1, 640 KJ (Utter 2007). AYB products generally have a lower glycemic index than other legume products, which means that they will provide a more sustained form of energy.

All varieties of pulses are excellent source of easily digestible protein, there are several factors which are responsible for its low production. Among them, diseases play an important role. Numerous examples exist in literature on plant diseases occurring as a result of planting seeds that were contaminated with pathogens. Seed-borne diseases have been found to affect the growth and productivity of crop plants (Ghadsingh and Mandge 2012). Presence or absence of seed borne fungi on seed surface is one of the important aspects that determine the quality of seed. Pathogen free healthy seed is urgently needed for desired plant populations and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops (Ghadsingh and Mandge 2012). A substantial part of food insecurity comes from inadequate management of seed-borne pathogens that cause diseases either on the fields or induces spoilages on stored produce (ARS, 2015). Seeds are the foundation of many crop productions and the most

important input to ensure good production. Good quality seeds should in addition to having genetic purity, uniform size, shape, colour and uniformity and conformity to the standards of the particular cultivar; must be disease-free, viable, free from admixtures of seeds of other crops and weeds and inert materials (Ramey *et al.*, 2017). The benefits of using high yielding cultivars may be nullified by dangerous seed-borne diseases. Infected seeds are responsible for some of the most severe crop losses in the world today. Crop losses still occur particularly in developing and under developed tropical countries due to use of infected seeds (Chiejina, 2006).

The seed-borne pathogens could remain viable for long periods if the seeds were kept under good storage conditions until planting time. These pathogens may cause failure of the seeds to germinate or may infect the germinating seedlings and the mature plants in future (Adewale and Odo, 2013). Farmers in Nigeria still keep seeds from one cropping season to another thus carrying a potential source of inoculum detrimental to crop production (Vincelli and Williams, 2017).

Using seed treatments to control seed-borne pathogens is often very effective for disease control. Although chemical seed treatments have important benefits, they also pose certain risks. One risk is accidental exposure of workers who produce or apply seed treatments. Non-chemical seed treatment does not have any negative impact on the environment, ensure workers and consumer safeties compared to chemical seed treatments and are expected to be one of the fastest growing seed treatment segments (Kana *et al.*, 2012). Not much is known about the impact of seed-borne diseases on the yield and quality of African yam bean crops grown in Nigeria (Ramey *et al.*, 2017). A careful review of the available literatures on plant pathology shows that very little work is being undertaken by researchers on African yam bean and its associated seed-borne pathogens. The study of seed borne fungi associated with AYB to enhance proper identification and management of the fungi that could be limiting its production due to use of infected seeds as inputs and possible seed treatments of plant origin is necessary.

Therefore, the objectives of the study are to:
identify seed borne pathogens associated with African yam bean seeds
determine the effect of isolated seed-borne pathogens on African yam bean seeds and its germinability.
determine the effect of selected botanicals on fungi infecting African yam bean seeds.

Materials and methods

Location of the experiment, sources of seed and botanicals

The experiment was conducted at the Department of Plant Health Management Laboratory, College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike. The native African yam bean

seeds were bought from three (3) different markets in Umuahia and its environs namely: Ngoro, Orié Ugba, and Ubani, all in Ikwano and Umuahia North Local Government Areas. Uziza (*Piper guineense*) and Ehuru (*Monodora myristica*) seeds were purchased from the Orié Ugba market in Umuahia.

Isolation of fungi from African yam bean seeds

Fungi were isolated using the agar plate method. Five seeds from each sample from the different locations were plated on prepared and solidified Potato Dextrose Agar (PDA) in Petri dishes. The seeds were incubated for 5 days, each fungus colony was transferred to fresh PDA in Petri dishes to obtain pure cultures of fungi which were identified. Different fungal colonies were counted and recorded.

Identification of isolated fungi

The isolated fungi were identified using morphological appearance of fungi colonies on PDA medium and by making slides from fungal colonies; mounting on the Olympus reflected light optical microscope and using mycological reference books and research articles (Barnett and Hunter, 1999; Alexopoulos *et al.*, 2002; Samson *et al.*, 2004).

Pathogenicity test

Healthy AYB seeds were surface sterilized using 10% sodium hypochlorite and rinsed in three changes of sterile distilled water before the seeds were inoculated with spore suspension of the identified fungi. The inoculated seeds were placed on two layers of a moistened filter paper and incubated for 7 days. The effects of moulds was observed and recorded. The control was inoculated with sterile water. This was laid out in a complete randomized design with three replicates.

Effect of isolated fungi on African yam bean seed's germinability

Five (5) healthy seeds of African yam bean were washed in three changes of sterile distilled water and then inoculated with each of the identified fungi and plated on moistened filter paper as described above. The seed were incubated for ten days. The percentage seed germination of the inoculated seeds was recorded. The control was inoculated with sterile water. The experiment was laid in complete randomized design with three replicates.

Preparation and determination of effect of botanicals on fungi infecting seeds

The seeds of Ashanti pepper (*Piper guinensis*) and *Monodora myristica* obtained were dried properly and ground to fine powder for use in this study. Twenty gram (20g) of the powder was suspended in 100 ml of sterile water for 12 hours; filtered with sterile muslin cloth into clean conical flasks. Clean and healthy AYB seeds were surface sterilized with 10% sodium hypochlorite and rinsed in three changes of sterile water, then blotted dry with sterile paper towel. The seeds were suspended in the plants extract for three hours and then air dried for one hour. The extract

treated seeds were inoculated with spore suspension of 5-day old culture of the identified fungi and then plated on PDA; this was incubated for 6 days. The control was inoculated with sterile water. The percentage fungi colonization on treated seeds was recorded and percentage fungal growth reduction was calculated using the formula below as reported by Atehnkeng *et al.*, (2008):

$$\text{Percentage reduction} = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

Control

Statistical analysis

Data were analyzed using analysis of variance of the SAS (version 9.4, SAS Institute, Cary, NC). Means were separated and compared using Fishers Least Significant Difference (F – LSD) at 5 % probability level.

Results

Percentage incidence of fungi isolates

Six (6) fungal genera belonging to *Aspergillus* (*A. tamarii*, *A. Ochraceus*, *A. niger*, *A. flavus*, *Rhizopus*

sp., *Fusarium sp.*, *Cladosporium*, *Phomopsis sp.* and *Penicillium sp.*) were encountered in the AYB seed from three different markets visited. *A. flavus* and *A. tamarii* were encountered in the three locations (Table 1). *A. flavus* and *A. tamarii* had the same percentage incidence in all the three markets. *A. Ochraceus* had the highest (52.38%) disease incidence in Orié Ugba market followed by Ubani market (47.61%), while in Ndoro market, no *A. Ochraceus* was recorded (Table 1). In Ubani market, *Rhizopus* was not isolated but recorded 54.54% incidence in Orié Ugba and 45.45% in Ndoro market. *A. niger* was encountered only in Ubani market 100% (Table 1).

In Ubani market, only *Fusarium sp.* (100%) was recorded. *Cladosporium, sp.* was only recorded in Ndoro market (100%). In Ubani market, *Phomopsis sp.* (60.00%) had the highest disease incidence followed by Orié Ugba market (40.00%), while no *Phomopsis* incidence was encountered in Ndoro market. No incidence of *Penicillium spp.* was recorded in Ubani and Ndoro markets while Orié Ugba had 100% disease incidence (Table 1),

Table 1: Percentage incidence of fungal Isolates

Markets	<i>Aspergillus tamarii</i>	<i>Aspergillus ochraceus</i>	<i>Rhizopus sp.</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Fusarium sp.</i>	<i>Cladosporium sp.</i>	<i>Phomopsis sp.</i>	<i>Penicillium chrysogenum</i>
Ubani	33.33	52.38	0.00	100.00	33.33	100.00	0.00	60.00	0.00
Orieugba	33.33	47.61	54.54	0.00	33.33	0.00	0.00	40.00	100.00
Ndoro	33.33	0.00	45.45	0.00	33.33	0.00	100.00	0.00	0.00

Effects of Isolated Fungi associated with AYB on seeds and it’s Germinability

Rhizopus sp. had the highest colonization percentage of 90% followed by *Aspergillus flavus* (86.7%), while *Penicillium oxalicum* had the least 13.3% and was significantly different at P=0.05 from the colonization of *Rhizopus sp.* On the effect of fungi on AYB germinability, *Penicillium oxalicum* had the highest seed germination of 70% followed by *Aspergillus tamarii* (60%), while *Penicillium montanense* had the least germination of 10% (Table 2).

percentage of 26.5% and was significantly (P >= 0.05) lower than the control. For seeds treated with *Piper guineense*, *Rhizopus sp.* had the highest colonization percentage of 59% which was lower than the control (75%) and they were not significantly different at P >= 0.05, while *Aspergillus fumigatus* had the lowest colonization percentage 15.5% and was not significantly different (Table 3)

Effect of *Monodora myristica* and *Piper guineense* on fungi associated with African Yam Bean Seeds

For seeds treated with *Monodora myristica*, *Rhizopus* had the highest colonization percentage of 63% which was lower than the control (75%) (i.e. seed inoculated with only *Rhizopus sp.* without treatment) and they were not significantly different at P >= 0.05. *Aspergillus niger* had the lowest colonization

Table 2: Effects of Isolated Fungi associated with African Yam Bean seeds and it's Germinability

	Colonization n (%)	Germination n (%)
<i>Rhizopus sp</i>	90.0	0.0
<i>Aspergillus flavus</i>	86.7	20.0
<i>Aspergillus tamaritii</i>	80.0	60.0
<i>Aspergillus niger</i>	76.7	20.0

<i>Aspergillus ochraceus</i>	63.3	20.0
<i>penicillium montanense</i>	56.7	10.0
<i>Clodosporium sp</i>	53.3	40.0
<i>Aspergillus fumigatus</i>	46.7	20.0
<i>Penicillium chrysogenum</i>	46.7	20.0
<i>Penicillium oxalicum</i>	13.3	70.0
Control	0.0	60.0
LSD (P=0.05)	4.1	0.0

Table 3: Effects of *Monodora myristica* (Ehuru) and *Piper guineense* (Uziza) on Fungi associated with African Yam Bean Seeds

	<i>Aspergillus tamaritii</i>	<i>Penicillium chrysogenum</i>	<i>Rhizopus sp</i>	<i>Aspergillus ochraceus</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>
<i>Ehuru</i>	40.0	38.5	63.0	32.5	26.5	43.0	31.0
Control	82.0	80.0	75.0	72.0	63.0	61.0	39.0
LSD (0.05)	16.6	16.1	23.5	18.4	15.4	31.1	28.9
<i>Uziza</i>	46.0	39.5	59.0	32.5	45.5	39.0 ^a	15.5
Control	82.0	80.0	75.0	72.0	63.0	61.0 ^a	39.0
LSD (P=0.05)	16.7	11.7	22.6	16.0	12.2	28.7	26.9

DISCUSSION

The results showed that a total of nine fungal species were isolated using the agar plate method which in most cases was found to be superior than blotter for the isolation of seed mycoflora. Seven (7) out of the nine fungal species (*Aspergillus tamaritii*, *A. ochraceus*, *A. niger*, *A. flavus*, *A. fumigatus*, *Rhizopus sp.* and *Penicillium. chrysogenum*) caused deterioration of African yam bean seeds studied. These results are in line with the findings of Obani and Ikotun (2014), and Salami and Popoola, (2007) who had reported similar organisms in other seeds/grains studied.

The occurrence of these fungi in AYB has been reported by other authors in Rivers and Enugu States (Nwachukwu and Umechuruba, 2001; Ugwuoke and Ukwueze, 2010). The result indicated high and low incidences of various fungal species. The high incidence may be attributed to colonization ability, rapid germination of spore, fast hyphal invasion, highly competitive nature, their nutrient composition and their ability to utilize a wide variety of substrate. Similar results have also been reported from seeds other than African yam bean, e.g. sunflower, egusi and groundnut seeds (Embaby and Abdel-Galil, 2006 Muthii *et al.*, 2014).

For seed treatment with botanicals, results obtained in this study confirmed the importance of the two plant studied species as having antifungal properties both in the in vivo experiment. The present investigation is an important step in protecting seeds from fungal contamination with seed protectants which are eco-friendly for the management of important seed borne fungi. Therefore, use of naturally available chemicals for plant protection will play a prominent role in development of future commercial pesticides for crop protection and disease management strategies. Much work has been done on the use of plant extracts against the plant-pathogenic fungi (Okigbo *et al.*, 2005; Ogbebor *et al.*, 2007; Mondali *et al.*, 2009). This shows that these botanicals contain bioactive ingredients reported to exhibit physiological activity against most microorganisms and are inhibitory to the growth of these pathogens (Fleischer *et al.*, 2008). Results showed that treated seeds yielded less growth of seed-borne fungi than the untreated seeds which is in close conformity with the findings of (Tripti *et al.*, 2012) and other authors. The extracts of the seed of two (2) plant species *Monodora myristica* (*Ehuru*) and *Piper guineense* (*Uziza*) reduced the growth of these organisms significantly in culture when compared with the control experiment indicating the presence of

anti-fungal substances in the test plants. The antifungal activities of the plant extracts studied were supported by many other investigators mention above. Both plants' extracts considerably inhibited the growth of these organisms on inoculated seeds, however, *Piper guineense* inhibited the fungi growth more than *Monodora myristica*. The difference in efficiency of the extract of the plants may be due to the presence of varying active ingredients in the two extracts studied (Obani and Ikotun, 2014). Various authors have reported anti-fungal activities of uziza leaf (Ogbebor *et al.*, 2007; 2008; Obani and Ikotun, 2014). Both plants are environmentally friendly and finance implications to obtain them are quite less, which implies that they can serve as good alternatives to chemical fungicides considering their safety and degradable qualities (Amadioha and Nwazuo, 2019).

Conclusion

The African yam bean is an underutilized crop that has received little attention from researchers despite its numerous potentials to broaden man's protein-based food sources. Improvement efforts on this crop to enhance production of nutritious food for the world's poor to a large extent depend on the identification of potential pathogenic fungi diseases and their effective

control measures. Knowledge of the potential pathogens especially those associated with the seeds therefore will play a significant role in healthy crop production and improvement programs of AYB. Although both of the plant extracts considerably inhibited the growth of these organisms on inoculated seeds however, *Piper guineense* inhibited the fungi growth more than *Monodora myristica*. Both plants are environmentally friendly and have fewer financial implications to obtain, which implies that they can serve as a good alternative to chemical fungus considering their safety and degradable qualities.

Recommendation

This study determined the efficacy of two plant extracts (*Monodora Myristica* and *Piper guineense*) in the control of seed borne fungi of AYB. The results showed that they were effective in the reduction of seed borne fungal disease of AYB and did not inhibit the germination of the seeds nor significantly affect the seed adversely. This could translate to better performance of (AYB seeds) after storage. Therefore, the increase in values of African Yam bean seeds and reduction in the risk of disease infection of the seeds can be achieved by the use of *Monodora Myristica* and *Piper guineense* extracts for seed storage.

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EVALUATION OF THE EFFECT OF COLCHICINE ON THE GROWTH AND YIELD OF CUCUMBER (*Cucumis sativa* L.) VARIETY 'NATIONAL PICKLING'

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ABSTRACT

This study is aimed at evaluating the dose effects of colchicine on the germination, vegetative growth and yield of *C. sativa* seeds of cucumber variety 'national pickling' were obtained from Seed Council of National Root Crops Research Institute (NRCRI) Umudike, Abia State. The experimental design was completely randomized design (CRD) with three replicates and the experimental site was at the University farm land opposite the Post Graduate School at Michael Okpara University of Agriculture, Umudike, Abia state. Four different concentrations of colchicine mutagen were prepared in the following percentages 0.02%, 0.04% 0.06% and 0.08% and a control treatment with 0% mutagen. After germination test, the seeds were transplanted to the farm land and watered regularly. The germination, growth parameters and yield were recorded. The data collected was subjected to analysis of variance (ANOVA). The means were separated with LSD test at 5% level of significance. From the result of germination percentage, it was observed that colchicine at lower doses did not inhibit the seed germination of *C. sativa* but showed little inhibition at higher doses. The results obtained for plant height, stem girth and number of leaves at 8 weeks after planting indicated that colchicine had a positive effect on *C. sativa*. The results also revealed that colchicine at higher doses significantly induced ($p < 0.05$) number of branches, number of flowers and fruit yield of *C. sativa* than the control. Therefore, treatment of seeds of *C. sativa* with colchicine at 0.08% is ideal for improved fruit yield.

Keywords: Colchicine, effect, yield, cucumber, mutation.

INTRODUCTION

Cucumber (*Cucumis sativa* L.) is an important vegetable crop in the Cucurbitaceae family that has been cultivated by man for over 3,000 years (Adetula and Denton, 2003; Okonmah 2011). Remison (2005) noted that Cucumber originated in Northern India and is widely distributed throughout the world. Cucurbits are composed of 118 genera and 825 species. There exist three main varieties of cucumber; slicing, pickling and burpless. Within these varieties, there are several new different cultivars. Many cultivars of cucumber exist with varying shapes, skin colors and carotene content (Simon, 1992). According to FAO (2006), the most important cucurbits in terms of world total production are water melon (*Citrullus lanatus* L.), cucumber (*Cucumis sativa* L.) and melon (*Cucumis melon* L.).

Cucumber has ascertained as one of the most important market vegetables in the world. The production of the fruit in Nigeria however, is very low due to limited use. Adetula and Denton (2003) noted that cucumber is produced mainly in the Northern states of Nigeria. The development is rapid and takes a shorter time from planting to harvest than for most crops (Wehner and Maynard, 2003).

It is a necessity therefore, to increase the production of cucumber in order to supplement the high intake of carbohydrate, especially in the Southeastern Nigeria, where there is dearth and over dependence on the Northern part of the country for its supply for salad vegetables and fruit, resulting to relative higher price. The increase in the production of cucumber can be

achieved through genetic engineering, mutation breeding and hybridization.

Stanys *et al.* (2004) stated that colchicine, is a hormonal plant product extracted from the seeds and bulbs of the plant *Colchicum autumnale* L., is anti-mitotic agents used to induce polyploids in living organisms especially plants. According to Petersen *et al.* (2003), colchicine acts by binding to the dimmers, preventing the formation of microtubules, and consequently, spindle fibers during cell division. Colchicine is known to inhibit mitosis in a wide variety of plant and animal cells by interfering with the orientation and structure of the mitotic fibres and spindle (Finnie and Staden, 1994).

Bragal (1995) has also reported that because of the many positive results obtained from using colchicine as a mutagen for genetic manipulation, it is now frequently used in breeding plants to create genetic variability. Several high yielding variants of crop plants have been developed using colchicine (Bragal, 1995). Eunice *et al.* (2015) reported the effect of colchicine on plant morphology and growth traits of *Vigna unguiculata*. They observed that minimum dose of colchicine is effective to enhance vegetative growth of *Vigna unguiculata*. There were similar reports done on Sesame (Mensah *et al.*, 2007) and *Trigonella foenumfraeum* (Datta and Biswa, 1998).

MATERIALS AND METHODS

Source of materials

The seeds of cucumber (*Cucumis sativa* L.) variety 'National pickling' was obtained from National Seed Council of Nigeria located at National Root Crops

Research Institute (NRCRI), Umudike, Abia State. Colchicine, nose mask, hand gloves, tissue paper, distilled water, Petri dishes, beakers, filter paper and weighing balance were obtained from the Department of Plant Science and Biotechnology laboratory at Michael Okpara University of Agriculture, Umudike, Abia State.

Study area

The experiment was conducted between 19th of September 2019 to November 2019 within the premises of Michael Okpara University of Agriculture, Umudike. Umudike is in the rainforest belt of Nigeria and lies on latitude 05° 29'N and longitude 07° 33'E 245mm (N.R.C.R.I., 2016). Annual average rainfall varies from 1900 mm to 2,400 mm and is 123m above sea level. Minimum and maximum temperatures are 21°C and 34°C respectively (N.R.C.R.I., 2016).

Experimental design

The experiment was conducted using a completely randomized design (CRD) with three replications at the University farm land opposite the School of Post Graduate Studies, Michael Okpara University of Agriculture, Umudike, Abia State.

Colchicine preparation and induction

Before the treatment, seeds were pre-soaked in water for three (3) hours, then the seeds were removed and air dried for 30 minutes. During the period of pre-soaking, the floating seeds were removed to eliminate non-viable seeds

Five different beakers were used for colchicine preparation for cucumber seeds treatment of which one beaker out of the five served as control. Four different concentrations of colchicine mutagen were prepared in the following percentages 0.02%, 0.04% 0.06% and 0.08%. The following grams of colchicine (0.02g, 0.04g, 0.06g, and 0.08g) were weighed out using the sensitive weighing balance and then dissolved in 100ml of distilled water.

After the preparations, the already dried seeds were soaked into freshly prepared concentrations for four (4) hours with intermittent shaking for providing uniform treatment to the soaked seeds. After the treatment time, the seeds were removed from the colchicine concentrations and washed thoroughly with distilled water to remove the residual chemicals on the seeds. Five Petri dishes were used for the germination test. The Petri dishes were labelled accordingly (0.0% (control), 0.02%, 0.04% 0.06% and 0.08%) and then ten seeds were placed on each Petri dish.

Measurement of growth parameters

The following growth parameters were measured at 14 days interval after planting.

Germination percentage (%): this was done at 14 days after planting. Germination percentage (%) was calculated as follows:

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times \frac{100}{1}$$

Plant height (cm): This was measured from the soil level to the apex of stem (meristematic tip) using a meter rule.

Number of leaves: This was calculated by visual counting of the number of leaves of the plant.

Number of branches: This was by visual counting of the number of branches of the plant.

Stem girth (mm): This was done using the vernier caliper.

Number of flowers: this was by visual counting of the flowers of the plant.

Number of fruits (yield): this was by visual counting of the fruits of the plant.

Leaf area (cm²): the leaf area was calculated by using Blanco and Folegatti (2014) formula below:
Leaf area of cucumber = 0.88 (L x W) – 4.27

Statistical Analysis

The data collected was subjected to analysis of variance (ANOVA). The treatment means were separated with LSD test at 5% level of significance. The results of the analysis were expressed as means and standard deviation.

Results

Result for effect of colchicine on germination

The result in Table 3.1 showed the effect of colchicine on the germination percentage of cucumber (*Cucumis sativa*) var. 'national pickling'. From the result, it was observed that the germination percentage of the seed was highest at the T1 (0.02%) with value (60%), followed by the control and T3 (0.06%) with value (40%) respectively. The least with value 20% was recorded for T2 (0.04%) and T4 (0.08%) respectively. This suggests that colchicine had varying effects on the germination percentage of *C. sativa*. At (0.02%) concentration it had the best result compared to the control but as concentration increased, the germination percentage reduced.

Table 3.1: Effect of colchicine on the germination percentage of *C. sativa*

Treatment (%)	Germination percentage %
Control 0.00	40
T1 0.02	60
T2 0.04	20
T3 0.06	40
T4 0.08	20

Results for effect of colchicine on plant height

The result from Plant height revealed the effect of

colchicine on plant height of *C. sativa* 8 weeks after planting. As shown in the Fig. 3.1 below the plant height at 2 weeks after planting (WAP) showed no significant difference ($p>0.05$) among all the treatments. The result at 4, 6 and 8 WAP showed that colchicine significantly improved the plant height of *C. sativa*. Plant height was maximum at T4 (0.08%)

with value 42.87 cm and was lowest at T2 (0.04%) with value 17.16 cm. This revealed that colchicine has no specific dose for improving the plant height of *C. sativa*. The result of the analysis of variance showed that the control treatment was significantly different ($P < 0.05$) at 4, 6 and 8 WAP compared to the colchicine treatment.

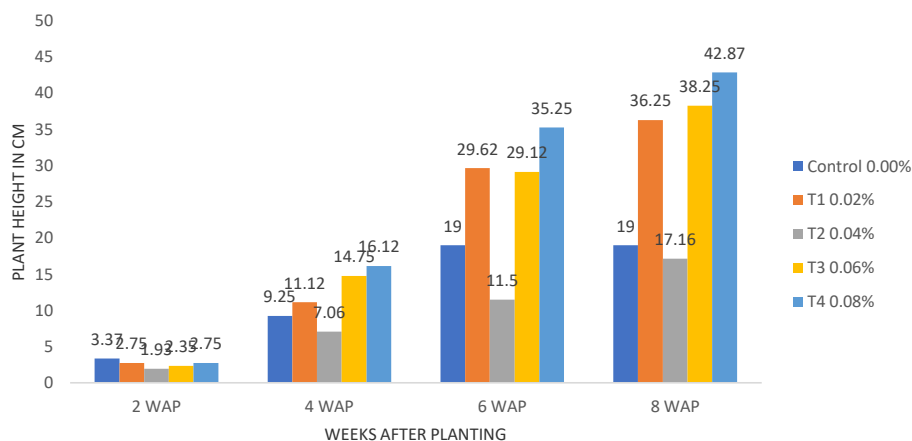


Fig. 3.1: Effect of colchicine on the plant height of *C. sativa* 8 weeks after planting ($P<0.05$)

Results for effect of colchicine on stem girth.

The result from stem girth revealed the effect of colchicine on the stem girth of *C. sativa* 8 weeks after planting. As shown in the Fig. 3.2 below the mean stem girth at 2 WAP showed no significant difference ($p>0.05$) among all the treatments. The result showed that stem girth of *C. sativa* was best at T4 (0.08%) followed by T1 (0.02%) at 4, 6 and 8 WAP. The result showed that the control 0.00% and T3 0.06% had the same values at 8 WAP while T2 0.04% had the least value. The result for stem girth was significantly different ($P < 0.05$) among the treatments at 4, 6 and 8 WAP.

Result for effect of colchicine on number of leaves

Fig. 3.3 showed the effect of colchicine on the number of leaves of *C. sativa* 8 weeks after planting. The result in the Fig. 3.3 below showed that there was no significant difference ($p>0.05$) in the number of leaves at 2 WAP. However, there was significant difference ($p<0.05$) observed in subsequent weeks after planting. Colchicine treatments at 0.08% and 0.02% showed better results than the other treatments at 4, 6 and 8 WAP. Maximum number of leaves was recorded at T4 (0.08%) with 55 leaves and was least at T2 (0.04%)

with 31 leaves.

Results for effect of colchicine on some growth and yield parameters

The result from plant yield showed the effect of colchicine on some growth parameters and yield of *C. sativa* 8 weeks after planting. Results gotten from Table 3.2 below indicated that colchicine at higher doses significantly increased ($p<0.05$) the number of branches compared to the control. The result on number of flowers revealed that high and low concentration of colchicine (T4 (0.08%) and T1 (0.02%)) produced more flowers than the control. The yield was significantly increased by the colchicine treatment at 0.08% as shown in Table 3.2 below. From the results on leaf area, it was shown that T1 (0.02%) recorded the highest leaf area and the least was recorded at T2 (0.04%). The result of the analysis of variance showed that the treatment significantly affected ($p<0.05$) the leaf area with regards to the control.

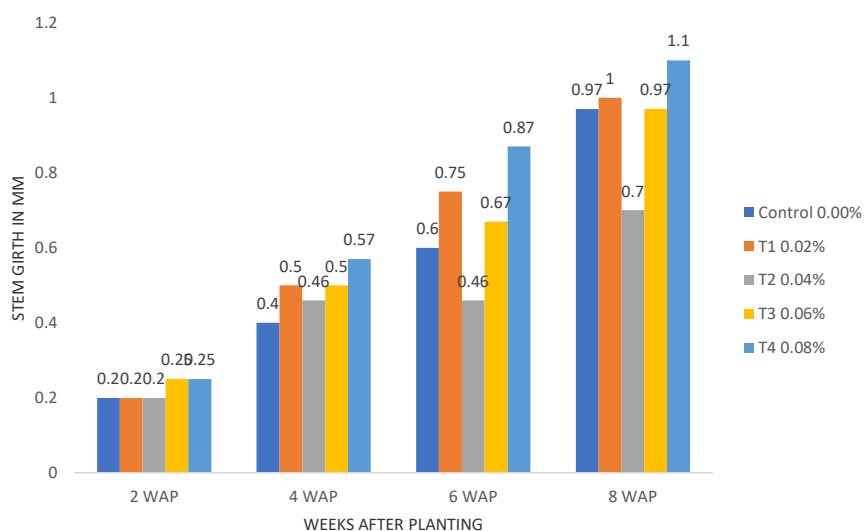


Fig. 3.2: Effect of colchicine on the stem girth of *C. sativa* 8 weeks after planting

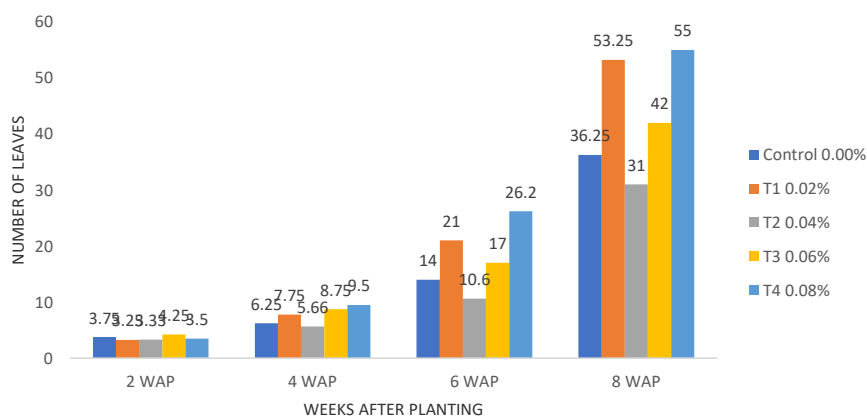


Fig. 3.3: Effect of colchicine on the number of leaves of *C. sativa* 8 weeks after planting.

Table 3.2: Effect of colchicine on the growth and yield parameters

Treatment %	No. of branches	No. of flowers	No. of fruits (yield)	leaf area (cm ²)
Control 0.00	3.66 ± 1.15 ^b	15.50 ± 11.8 ^b	3.00 ± 1.41 ^b	115.14 ± 31.0 ^b

T1 0.02	5.50 ± 1.29 ^b	23.75 ± 12.9 ^b	8.50 ± 0.70 ^a	123.08 ± 16.5 ^b
T2 0.04	4.50 ± 2.12 ^a	12.50 ± 2.12 ^a	2.00 ± 1.41 ^b	104.19 ± 3.73 ^a
T3 0.06	5.25 ± 2.87 ^a	20.00 ± 15.2 ^b	12.50 ± 0.70 ^a	118.56 ± 41.0 ^b
T4 0.08	6.25 ± 1.70 ^b	24.00 ± 13.2 ^b	28.00 ± 1.41 ^b	117.30 ± 24.4 ^b

Values are mean ± SD, values with different subscript on the same column are significantly different at P≤0.05.

Discussion

From the results above, colchicine showed promising potentials for improving growth and yield qualities of cucumber. Colchicine application at lower doses improved seed germination of *C. sativa* but showed little inhibition at higher doses. This suggests that colchicine had varying effects on the germination percentage of *C. sativa*. This agrees with the study of Eunice *et al.* (2015) which recorded that germination percentage of *Vigna unguiculata* seeds were reduced with increasing concentration of colchicine treatment especially in the C1 (first generation of seeds treated with colchicine) than C2 (second generation of seeds treated with colchicine). The treatment with the lowest dose (0.002%) of colchicine showed promising germination potential. This was in agreement with the respective researches of Mensah *et al.* (2005) and Mensah and Akomeah (1997) which reported reduction in percentage germination with increasing mutagen (colchicine) concentration in Sesame with the effects more pronounced in C1 (first generation of colchicine treatment) than C2 (second generation of colchicine treatment). However, studies by Udensi and Ontui (2013) on pigeon peas recorded a contrary result even though they recorded a prolonged period for the emergence of seedlings. Chemical mutagens have been reported to have inhibitory effects on seeds leading to low percentage germination (Dhakhnamoorthy *et al.*, 2010; Pande and Khetmalas, 2012).

The results obtained for mean plant height at 8 weeks after planting indicated that colchicine improved the plant height of *C. sativa*. Plant height was best at the treatment with highest dose (0.08%) of colchicine. Similarly, data obtained for stem girth and number of leaves of *C. sativa* showed that treatment with highest dose (0.08%) of colchicine performed better than the control. It was also observed that lower dose of colchicine produced good results. This result agrees with that of Amiril *et al.* (2010) who observed that maximum number of leaves per branch of Jimsonweed (*Datura stramonium* L.) was observed in the treatment containing the highest dose of colchicine (0.5g) in time period of 48hr. Generally, the number of leaves

increased with regards to the treatment of colchicine concentration or incubation time. This result however, contradicted with the result obtained by Amiril *et al.* (2010) who reported that the plant height of Jimsonweed (*Datura stramonium* L.) was best in the control and in the treatment with the lowest dose of colchicine. The minimum plant height was observed at the treatment with the highest dose of colchicine as well. Similarly, Eunice *et al.* (2015) reported the effect of colchicine on plant morphology and growth traits of *Vigna unguiculata*. In that study it was observed that the number of leaves and length of longest branch in the C1 generation was highest in the control and was followed by the treatment with the lowest dose of colchicine. The plant height and number of primary branches was highest in the treatment with the lowest dose of colchicine. This indicated that minimum dose of colchicine effectively enhanced vegetative growth of *Vigna unguiculata*. Results reported on Sesame (Mensah *et al.*, 2007) and *Trigonella foenumgraecum* (Datta and Biswa, 1998) was in accordance with the result above.

The results obtained from the number of branches indicated that colchicine at higher doses induced more branches than the control and there was significant difference (p<0.05) in the number of branches. This result conformed to the results reported by Amiril *et al.* (2010) who observed that the result of number of branches per plant of Jimsonweed (*Datura stramonium* L.) showed maximum number of branches in the treatment containing the highest dose of colchicine. The minimum number of branches recorded in the control was in agreement with reports from Mensah *et al.* (2007) who indicated that colchicine treatments enhanced the number of branches in sesame (*Sesame indicum* L). Increased branching (bushy habit) has been reported with colchicine treatment in other studies (Hewawasam *et al.*, 2004; Ndukwu and Obute, 2006).

In the present study, the result on number of flowers revealed that high and low concentrations of colchicine (T4 (0.08%) and T1 (0.02%)) produced more flowers than the control and there was no delay

in the days to flowering. The yield was significantly increased by the colchicine treatment at 0.08% as shown in our result. This was in contrast to the report by Eunice *et al.* (2015) who reported that colchicine treatments in the C1 generation with high concentration delayed flowering suggesting that the chemical may have interfered with maturity and early flowering. Hormones (e.g. florigen) may have also been induced or inhibited to initiate or impede flowering (Eunice *et al.*, 2015). The result showing higher yield of *C. sativa* with regards to colchicine treatment was in agreement with Dhakhanamoorthy *et al.* (2010) who recorded that yield per plant of *Jatropha curcas* was enhanced in some of the colchicine treatments. Early flowering and fruit maturity may be due to the physiological changes caused by the mutagen (Dhakhanamoorthy *et al.*, 2010).

Bragal (1995) has reported that because of the many positive results obtained from using colchicine as a mutagen for genetic manipulation, it is now frequently used in breeding plants to create genetic variability. Several high yielding variants of crop plants have been developed using colchicine (Bragal, 1995).

In Conclusion

From the study colchicine induced marked vegetative growth leading to the formation of large plants and a greater number of leaves and branches per plant. High number of flowers per plant in some of the colchicine treated cucumber in the study was also recorded. Yield of *C. sativa* was significantly increased by the colchicine treatment.

Further research should be carried out using higher doses in order to ascertain a suitable dosage for high yield.

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WIS-ASP 34

EFFECT OF SELECTED PLANT POWDERS AND THEIR ASHES ON COWPEA WEEVIL (*Callosobruchus maculatus* Fab.) INFESTING STORED COWPEA AT UMUDIKE, NIGERIA

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ABSTRACT

A laboratory study was conducted at the Agronomy laboratory of Michael Okpara University of Agriculture, Umudike to evaluate the efficacy of some medicinal plant powders and their ashes in the management of cowpea weevil (*Callosobruchus maculatus*). The plant materials used were: *Vernonia amygdalina*, *Occimum gratissimum*, and *Moringa oleifera* at different concentrations as ashes and powders. A total of sixteen treatments were used which were: *Vernonia amygdalina* powder (2.0g, 4.0g, 6.0g), *Occimum gratissimum* powder (2.0g, 4.0g, 6.0g), *Moringa oleifera* (2.0g, 4.0g, 6.0g) *Vernonia amygdalina* ash (1.0g, 2.0g, 3.0g), *Occimum gratissimum* ash (1.0g, 2.0g, 3.0g) and *Moringa oleifera* ash (1.0g, 2.0g, 3.0g) all replicated three times. All the plant extracts considerably reduced up to 50% oviposition weight loss and adult emergence of the cowpea weevil compared to the control (No extracts). The mortality of the cowpea weevils was recorded at 1, 2, 3 and 4 week after infestation (WAI) and the results indicated that the mortality increased with increasing concentration of the plant extracts. The highest dosage 6.0 of *V. amygdalina* powder was the most effective as it caused 30.0, 33.0, and 40.3% mortality at 1, 2, and 4 WAI respectively and this was significantly different from the control which recorded lowest mean mortality of 3.3, 6.0, 5.0 and 2.0 at 1, 2, 3 and 4 WAI respectively. It also significantly ($P < 0.05$) showed the strongest effect on oviposition (43.30%) compared to the control (162.70%). This was followed by 4.0g *V. amygdalina* Powder (50.70%). Also 6.0g of *V. amygdalina* powder had the lowest weight loss (21.5%) followed by the 4.0g of *V. amygdalina* Powder (25.2%) while the control showed highest weight loss of cowpea seeds (59.8%). Few adults emerged on seeds treated with 6.0g of *V. amygdalina* Powder gave 15.67 and 23.0 respectively at 1 and 2 WAI respectively. Results from this experiment revealed that these plant extracts can be used in the management of cowpea weevil infestation in storage and therefore is recommended to local farmers as an alternative to chemical pesticides which are not readily available and also are associated with health hazards and environmental contamination.

Keywords: Plant Powders, Cowpea weevil (*Callosobruchus maculatus*), Stored cowpea, Ashes.

Introduction

Cowpea (*Vigna unguiculata* L. Walp) belongs to the family *Fabaceae*. It is grown in the semi-arid and tropical zones covering Africa, Asia, Europe, United States and Central South America (Asante *et al.*, 2001). It is an important leguminous crop providing plant protein for humans and animals (Okosun and Adedire, 2010). Cowpea can be consumed boiled as porridge or boiled and eaten with stew. It serves as cover crops (Onwueme and Sinha, 1991). It generates a triple benefits such as; additional income, additional nutrient rich food and increased soil fertility (Onwueme and Sinha 1991).

Cowpea production is greatly constrained by a number of pests attack both in the field and store such as *Callosobruchus maculatus*, *Ootheca mutabilis*, *Maruca testutalis*, *Riptorius dentipes*, *etc.* It has been reported that both quantitative and qualitative losses has risen due to insect pest attack on cowpea (Emeasor *et al.*, 2005). For instance, the cowpea aphids, *Aphis craccivora* can cause direct losses by sucking and indirectly through transmission of viral diseases (Emeasor *et al.*, 2005). *Ootheca mutabilis* are leaf feeders, the adult feed and make abrasive holes on the leaf lamina, they transmit the cowpea yellow mosaic virus diseases as well as the cowpea mottle virus (Ekesi, 2001).

Chemical control is an effective strategy that has been used extensively in daily life in the control of cowpea pests (Egho and Emosairue, 2010). However, the wide spread use of synthetic insecticide has led to many negative consequences both in the environment and human health and this has resulted in the search for other alternative control measures that are cheap, less hazardous and environmentally and human friendly such as botanicals (Cosimi *et al.*, 2008). Safe storage of grains and food products against insect damage is a serious concern to entomologist and food producers. It has been estimated that about 9-40% of the world's grain production is lost to post harvest insects and mites infestation (Hodges, *et al.*, 2014). Though spectacular progress in pest control has been made with chemicals, this measure is no longer favourable. The adverse effect of chemicals on ecological system and human life has stressed the need to develop alternative measure for controlling various insect pests. Some plants such as *Azadirachta indica* (A Juss), *Cassia fistula* (L) and *Chrysanthemum coronarium* (L) have shown insecticidal, antecedent, repellent and growth regulating properties (Saxena, 2011). Hence the objective of this study is to evaluate the efficacy of some medicinal plant powders and their ashes in the management of storage pest of cowpea (*Callosobruchus maculatus*).

MATERIALS AND METHODS

Experimental site

The laboratory study was conducted in 2017 at the Crop Science Laboratory, College of Crop and Soil Sciences, Michael Okpara University of Agriculture Umudike which is located at latitude 05° 29'N, longitude 7° 33'E and altitude of 122 m above sea level in the rain forest agro-ecological zone of South Eastern Nigeria with average annual rainfall of 2200mm, relative humidity of 60% - 83% and temperature range of 21.2 °C -33.6°C (NRCRI, 2016).

Insect culture

The test bruchid (*C. maculatus*) adults that were used for the study was collected from naturally infested stored cowpea purchased from the local market within the environment. Adult weevils are 1/8 inch long, reddish brown slightly elongate beetles compared to the other members of this family with typical rounded appearance. They were brought to the laboratory and cultured on a brown cowpea variety IAR 48 in a plastic buckets covered with Muslin cloth held with rubber bands to ensure aeration and prevent escape of the weevil, it was kept inside laboratory cupboards at ambient temperature and relative humidity (28±2°C and 75±20% R.H). The insects were allowed to oviposit for 5 days and then sieved out and left for about 35 days for the emergence of freshly *C. maculatus* before the start up of the experiment.

Source of cowpea seeds used

Cowpea seeds (variety IAR 48) that was used for the study was sourced from Umudike Seed Innovation Center while the botanicals tested were sourced within the environment. The different plant materials used include *V. amygdalina* (Bitter leaf), *M. oleifera* (Moringa) and *O. gratissimum* (Scent leaf).

Treatments and Experimental Design

The treatments doses were: *V. amygdalina* powder (2.0g, 4.0g, 6.0g) and *V. amygdalina* ash (1.0g, 2.0g, 3.0g). *M. oleifera* powder (2.0g, 4.0g, 6.0g) and *M. oleifera* ash (1.0g, 2.0g, 3.0g). *O. gratissimum* powder (2.0g, 4.0g, 6.0g) and *O. gratissimum* ash (1.0g, 2.0g, 3.0g) and Control (No extract).

The experiment was laid out in a Completely Randomized Design (CRD) with 3 replications.

Preparation of plant materials

The fresh leaf materials were washed: air dried in a well-ventilated area for about 7 days. The dried materials were divided into two, one part burnt into ashes and other part milled in to powdered form. Each material was kept in separate plastic bag until when needed. Each of the powder and ash extracts were weighed using sensitive weighing balance into different dosages of 2.0g, 4.0g and 6.0g while the ashes were weighed at 1.0g, 2.0g and 3.0g and added to 20g wholesome clean cowpea seeds in 250ml plastic vials covered with nylon mesh with tight

fittings and perforated lids for aeration.

The different powders and their ashes were mixed with 20g of cowpea seeds. Ten newly emerged adults of *Callosobruchus maculatus* (5 males and 5 females); were released into each container containing the clean seeds and placed on the laboratory bench. A control treatment was set up in which neither powders nor ashes was added. Each treatment was replicated three times.

Data collection

Mortality count

The following data were collected; *C. maculatus* mortality was taken at 7, 14, 21 and 28 days after infestation. After which the percentage mortality was calculated using the formula.

$$\text{Percentage Mortality} = \frac{\text{Total number of dead insects}}{\text{Total number of insects released}} \times 100$$

Oviposition

The bruchids were allowed to mate for 7 days after which the seeds were carefully examined using magnifying lens to count the number of eggs laid.

Adult Emergence

The number of adult bruchid emerged was counted weekly from the first date of emergence. Each one counted was removed till 14 days after first emergence.

Percentage seed weight loss: This was calculated using the formular

$$\text{Seed weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times \frac{100}{1}$$

Data obtained were subjected to analysis of variance. Significant differences between means were determined using Least Significant Differences (LSD) at (P < 0.05)

Results

Effect of different levels of plant extracts on adult mortality of cowpea seed beetles (*Callosobruchus maculatus*) at 1, 2, 3 and 4 weeks after infestation.

Table 1 shows the percentage mortality of adult *C. maculatus* exposed to the different doses of plant extracts at 1, 2, 3 and 4 weeks after infestation (WAI). The different concentrations of the test plants exhibited varying degrees of insecticidal activities killing adult *C. maculatus* more than the control. There were significant differences (p < 0.05) in the mortalities of *C. maculatus* in the treated plates compared to the untreated plate (control) except at 2 and 4 weeks after infestation (WAI). At 1 and 2 WAI, the highest dose of *V. amygdalina* (.6.0) Powder was the most effective as it caused 30.0, 33, and 40.3% mortality at 1, 2, and 4 WAI, respectively which was significantly higher (p < 0.05) than what was obtained from other concentrations. This was followed by 4.0g *V. amygdalina* powder that caused 26.7%, 31.0%,

40.0% and 30.3% mortality at 1, 2, 3 and 4 WAI respectively and then followed by 1.0g of *M. oleifera* ash that caused 6.0%, 16.7% and mortality 10.0% at 1, 2, and 4 WAI, respectively. However, control showed the lowest mean mortality of 3.3%, 6.0%, 5.0% and 2.0% at 1, 2, 3 and 4 WAI, respectively.

Table 1. Effect of Different Doses of Plant Extracts on Adult Mortality of *Callosobruchus maculatus* (Cowpea seed beetle) at Weeks 1, 2, 3 and 4 After Treatment.

Concentration of plant extract	Adult mortality At week 1	Adult mortality At week 2	Adult mortality At week 3	Adult mortality At week 4
Control	3.3	6.00	5.00	2.00
1.0 <i>M. oleifera</i> Ash	6.0	16.7	30.0	10.0
1.0 <i>O. gratissium</i> . Ash	6.7	23.3	26.7	23.3
1.0 <i>V. amygdalina</i> . Ash	10.0	26.7	30.0	20.0
2.0 <i>M. oleifera</i> Ash	6.7	23.3	30.0	13.0
2.0 <i>O. gratissium</i> . Ash	10.0	26.7	26.7	30.0
2.0 <i>V. amygdalina</i> Ash	13.3	30.0	33.0	20.0
3.0 <i>M. oleifera</i> Ash	10.0	30.0	35.0	20.0
3.0 <i>O. gratissium</i> . Ash	13.3	30.3	30.0	16.7
3.0 <i>V. amygdalina</i> . Ash	16.7	31.3	33.3	16.7
2.0 <i>M. oleifera</i> Powder	13.3	20.0	20.0	26.7
2.0 <i>O. gratissium</i> . Powder	16.7	23.3	26.7	26.7
2.0 <i>V. amygdalina</i> Powder	23.3	26.7	26.7	16.7
4.0 <i>M. oleifera</i> Powder	16.7	23.3	23.3	26.7
4.0 <i>O. gratissium</i> . Powder	20.0	26.7	30.0	10.0
4.0 <i>V. amygdalina</i> Powder	26.7	31.0	40.0	30.3
6.0 <i>M. oleifera</i> Powder	20.0	23.3	30.0	13.3
6.0 <i>O. gratissium</i> . Powder	20.0	30.0	30.0	13.3
6.0 <i>V. amygdalina</i> . Powder	30.0	33.0	40.3	13.3
MEAN	15.1	26.0	29.8	19.1
LSD (0.05)	9.8	NS	11.6	NS
Coefficient of variation (%)	39.3	27.0	23.5	43.3

Table 2 represents the percentage oviposition and weight loss at different doses of plant extracts. From the results, mean number of eggs on seeds treated with all the plant extracts were significantly ($p < 0.05$) different from that of the untreated seeds (control). It was observed that 6.0g of *V. amygdalina*. powder was able to reduce the number of eggs up to (43.30%) compared to other treatments. This was followed by 4.0g of *V. amygdalina*. powder (50.70%). However, the untreated control showed the highest number of

eggs (162.70%). The different doses of the treatments reduced significantly weight loss of cowpea seeds. The highest dose of *V. amygdalina* powder (6.0g) gave the lowest weight loss (21.5%) followed by the 4.0g of *V. Amygdalina* Powder (25.2%). However, untreated control recorded the highest weight loss of cowpea seeds (59.8%). Generally there was significant ($p < 0.05$) difference between weight loss obtained in the treated and untreated seeds.

Table 2. Effect of different levels of plant extracts on oviposition and weight loss.

Concentration of plant extract	Oviposition	Weight loss (%)
Control	162.70	59.8
1.0 <i>M. oleifera</i> . Ash	85.70	48.2
1.0 <i>O. gratissium</i> A	64.00	39.8
1.0 <i>V. amygdalina</i> Ash	55.30	26.5
2.0 <i>M. oleifera</i> . Ash	67.30	41.4

progeny emerged on seeds treated with the highest dosage, 6.0g of *V amygdalina* powder was significantly lower (15.67 and 23.0) at 1 and 2 WAI, respectively. This was followed by seeds treated with 4.0g of *V amygdalina* powder dosage which recorded 17.0 and 23.3 adult emergence at 1 and 2 WAI, respectively. The highest number of adult emergence were 58.00 and 62.00 at 1 and 2 WAI, respectively was recorded on the untreated control. All the treatments showed significant ($p < 0.05$) effects on the weevil emergence when compared with the control at 1 and 2 WAI.

Effect of different levels of plant extracts on mean progeny emergence of *C. maculatus* at 1 and 2 week after infestation.

The effects of application of varying levels of plant extract on emergence of adult *C. maculatus* on cowpea seeds (Table 3) showed significant ($p < 0.05$) effects between the treated and untreated plots. The mean

Discussion

The results from this study showed that the tested extracts were effective in reducing the damage from *C maculatus* on the treated cowpea seeds. Both the powders and their ashes significantly ($p < 0.05$) reduced oviposition, adult mortality, adult emergence as well as seed weight loss.

Table 3 Effect of different levels of plant extracts on mean progeny emergence of *C maculatus* at 1 and 2 weeks after infestation

Concentration of plant extract	Adult emergence at week 1	Adult emergence at week 2
Control	58.00	62.00
1.0 <i>M. Oleifera</i> Ash	40.67	60.30
1.0 <i>O. gratissium</i> Ash	34.00	38.00
1.0 <i>V. amygdalina</i> Ash	22.33	29.70
2.0 <i>M. Oleifera</i> Ash	26.33	33.30
2.0 <i>O. gratissium</i> Ash	25.00	26.70
2.0 <i>V. amygdalina</i> Ash	22.33	24.70
3.0 <i>M. Oleifera.</i> Ash	23.33	26.70
3.0 <i>O. gratissium</i> Ash	23.00	34.70
3.0 <i>V. amygdalina</i> Ash	21.33	24.70
2.0 <i>M. Oleifera</i> Powder	35.67	50.30
2.0 <i>O. gratissium</i> Powder	36.00	38.00
2.0 <i>V. amygdalina</i> Powder	28.00	29.70
4.0 <i>M. Oleifera.</i> Powder	34.00	33.30
4.0 <i>O. gratissium</i> Powder	24.67	26.70
4.0 <i>V. amygdalina</i> Powder	17.0	23.30
6.0 <i>M. Oleifera</i> Powder	27.67	26.70
6.0 <i>O. gratissium</i> Powder	25.33	24.70
6.0 <i>V. amygdalina</i> Powder	15.67	20.00
MEAN	28.65	33.30
LSD _(0.05)	5.595	13.92
Coefficient of variation (%)	11.80	8.42

The results have shown the ability of these plant extracts in the management of the cowpea weevil. The insecticidal effects of these plant extracts might be attributed to their inherent properties such as: repellence, fumigant effect, stomach poison effect, mechanical action, starvation and desiccation (Dales, 1996). Earlier literature indicate the importance of plant extract in protecting seeds by way of direct mixing of the plant powders and ash on seeds during post -harvest storage (Ngamo *et al.*, 2007). The

findings obtained in this study agreed with the earlier reports that powdered plant parts could adequately protect stored grains against storage insects (Paul *et al.*, 2009). Higher doses of the treatments used favoured high mortality of *C. maculatus* as well as duration of exposure of the pest to treatments. This reduction in adult emergence could either be due to egg mortality, or larval mortality or even reduction in the hatching of the eggs. It has been reported that the larvae which hatch from the eggs of *Callosobruchus*

sp must penetrate the seeds to survive (FAO, 1999). The larvae would be unable to do so unless the eggs are firmly attached to the seed surface. It could also be as a result of feeding deterrence resulting in the death of the insects or ovicidal action leading to reduced adult emergence as has been the case with other plant materials investigated as it was also observed in this study. The reduction in seed weight loss could be as a result of the reductive effect recorded in adult emergence. As the progenies emerged, more dead adult insects were observed than live ones which could be due to the bitter taste of *V. amygdalina*. leaves and the biochemical constituents present which invariably deterred feeding. The findings in this experiment are similar to those of Raja *et al* (2000) who reported that leaf extracts of *A. Indica* and *Jathropa curcas* showed oviposition deterrent and antifeedant activity as well as reduction of adult emergence of *C maculatus*. The results also agreed with (Okwu, 2000) that plants possess phytochemicals in different concentrations that protect the plants from external influence.

Conclusion and Recommendations

The results of the study revealed that the cowpea seed beetle, (*C. Maculatus*), can be managed by the use of the ash and powder extracts of *Vernonia amygdalina*, *Occimum gratissum*, *Moringa oleifera*. The use of higher concentrations of these extracts is highly recommended for more effective control in small farm storage, as the cost of these botanicals are low and easily available when compared with the losses incurred in untreated seeds hence more seeds would be available for use as food and for sale by the farmer as grain infestation would be reduced. Consumers would also get more value for their money, as well as enjoy cowpea seeds that are free from beetle infestation and chemical pesticides throughout the year. More study is required to ascertain the effectiveness of other *V. amygdalina* plant parts like roots, stem and stem barks, compare their effectiveness as a sole treatment and in combinations.

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EFFECT OF SOME BOTANICAL EXTRACTS IN THE MANAGEMENT OF MAIZE STEM BORER AT UMUDIKE SOUTH EASTERN NIGERIA

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Abstract

The Maize Stalk borer (*Busseola fusca*. Fuller) is one of the major insect pests of maize in Nigeria and can cause losses between 10-70% in maize if not controlled. A field experiment was conducted in 2018 cropping season at the Teaching and Research Farm of the College of Crop and Soil Sciences Michael Okpara University of Agriculture Umudike Nigeria to evaluate the efficacy of the aqueous extracts of three botanicals in six treatment combinations namely: *Azadirachta indica* (Neem), *Allium sativum* (Garlic) and *Capsicum frutescense* (Hot pepper) against maize stem borer. The experiment consisted of 7 treatments which included: *A. indica* extract, *A. sativum* extract, *C. frutescense* extract, *A. indica* + *A. sativum* extract, *A. indica* + *C. frutescense* extract, *A. indica* + *C. frutescense* + *A. sativum* extract and Control (no extract). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replicates. Parameters evaluated were stemborer population density, plant height, number of cobs, number of cobs damaged, number of leaves damaged, number of stems damaged and the weight of cobs. Results from data analysis indicated that the botanical extracts used reduced damage from stem borer on the maize plant. A significant difference ($P < 0.05$) was recorded in the stem borers population between the treatments and the control at 8 and 12 weeks after planting. Result also showed that lower cob damage was recorded in all the treated plots (1.67, 2.03, 1.57, 1.77, 1.87, 1.03) than the control which recorded (4.50). The application of *A. indica* + *C. frutescense* + *A. sativum* gave the highest number of cobs (9.83) and cob weight (0.77kg) than the control which recorded (1.87 and 0.42kg) respectively. Results from this study revealed the potential of these botanical extracts in the management of maize stem borer and therefore is recommended to local farmers for use in the management of maize stem borer.

KEYWORDS: Maize, Stem borer, plant extracts.

INTRODUCTION

Maize (*Zea mays* L.) belongs to the grass family Poaceae and originated from Mexico (Matsuoka 2002). Maize is one of the world's three primary cereal crops after wheat and rice (Joseph, 2011). It is a staple food in many regions of the world and an important source of carbohydrate, protein, iron, minerals, vitamin B and vitamin C (CGIAR, 1996). It is one of the most widely-grown crops around the world in both temperate and tropical regions (CIMMYT, 1999). It is the third leading crop of the world after rice and wheat (Shiferaw *et al.* 2011). In Africa maize forms part of the diet for 50% of the population, and consumption can be as high as 328 grams per person per day (Nuss and Tanumihardjo, 2010). Maize has three major uses: as food, as feed for livestock and as raw material for industry (FAO, 2007). Africans consume maize in different forms which includes; porridges, pastes, corn meal etc. Green fresh maize on cob is eaten baked, roasted or cooked. Every part of the maize plant is important, the grain, leaves, stalk, tassel, and the cob can all be used to produce a large varieties of food and non-food products (IITA, 2009).

The worldwide production was 817 million tonnes in 2018 (FAOSTAT, 2018), more than rice (499.1 million tonnes) or wheat (726 million tonnes). The largest maize producer in Africa is Nigeria with nearly 8 million tonnes (IITA 2009). In Nigeria, Maize is grown for its grain which contains 65% carbohydrate. 10-12

percent protein and 4-5 percent fat (Kopsell *et al.*, 2009). It is also an essential source of various major phytochemicals such as carotenoids, phenolic compounds and phytosterols (Kopsell *et al.*, 2009).

The poor performance of maize in Africa in general, and Nigeria in particular, could be as a result of poor soil fertility, and the prevalence of numerous insect pests and diseases (Neupane, 1995). Insect infestation results in weight losses and quality deterioration which constitute a threat to food security especially in developing countries like Nigeria. *Busseola fusca* is considered to be one of the most important pest of maize and sorghum in sub-Saharan Africa (Paul-Andre, *et al.* 2014). The average yield loss of maize caused by this pest in Nigeria was estimated between 20-50% (Addis, 2016). Damage is caused by the caterpillars, which first feed on the young leaves and later enter into the stems, killing the growing points of the plant and causing dead-heart that disrupts the flow of nutrients to the grain (Pascal, 1998). Grain damaged by the stem borers become susceptible to infection by moulds such as *Aspergillus flavus* which produce aflatoxin, a toxic by-product extremely poisonous to people and which can lead to cancer (Setamou *et al.*, 2000). There are several options available for the management of these insect pests. Farmers depend heavily on pesticides because of their quick effect (Schreinemachers *et al.* 2017). These conventional pesticides control measures adequately control the pest but the indiscriminate use of

these pesticides for the control of insect pest leads to phytotoxicity, environmental pollution and insects resistance which results to severe yield losses. Under these circumstances it becomes necessary to find out some eco-friendly alternative methods for insect pests management. Plant materials with insecticidal properties provide small holders in whose hands, the main bulk of maize production lies, with locally available and biodegradable control strategy (Shiberu, 2013). Plant-derived pesticides are one of the alternatives to chemical pesticides and are considered environmentally friendly. The efficacy of plant-derived pesticides is highly demonstrated not only in the grain storage insects (Valencia *et al.*, 2006) but also to control various field insect pest species. Several studies showing the potentials of plant-derived pesticides to control *B. fusca* were reported during the last two decades. (Valencia, *et al.*, 2006; Wink, 2012; Shiberu, 2013). This study is therefore geared at evaluating the effectiveness of three medicinal plant extracts (*Azadirachta indica* (Neem), *Allium sativum* (Garlic) and *Capsicum frutescense* (Hot pepper) and their combination in the management of (*Busseola fusca*) in maize field.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Teaching and Research Farm of the College of Crop and Soil Sciences of Michael Okpara University of Agriculture, Umudike. Umudike is located at latitude 05° 29'N, longitude 7° 33'E and altitude of 122 m above sea level in the rain forest agro-ecological zone of South Eastern Nigeria with average annual rainfall of 2046mm

The seeds of maize variety, 'Bende White' were obtained from National Agriculture Seed Council. The medicinal plant materials used were obtained from the local market within the environment and these included garlic bulbs (*Allium sativum*), fresh chilli pepper fruit (*Capsicum frutescens*) and neem leaves (*Azadirachta indica*). The field was cleared of vegetation and ploughed using spade. The experimental design that was used was Randomized Complete Block Design (RCBD) with seven treatments, replicated three times and this gave a total of 21 beds. The experimental plots size was 2m x 3m and separated from each other by 1m between rows and 50cm within rows. The seeds were sown directly to the beds at a spacing of 50cm x 50cm

The treatments combinations were as follows; *A. indica* extract, *C. frutescens* extract, *A. sativum* extract, *A. indica* extract + *C. frutescens* extract, *A. indica* extract + *A. sativum* extract, *A. indica* + *C. frutescens* + *A. sativum* extract and the control. Other practice such as weeding was done with the use of hoe at two weeks intervals.

Extract Preparation

The plant materials used were washed with sterile water, oven dried and pulverized. One hundred grammes

of each of the materials was weighed out using a scale, and then transferred into separate buckets. A liter of hot water was added and mixed thoroughly after which it was allowed to stay overnight. The following day, the plant extract were separated by decanting through a muslin cloth to obtain aqueous extract. 20 mls of vegetable oil and two table spoon of liquid soap were added to the prepared extracts to increase adhesion. For the extract mixture, equal volume of each of the different plant material was used for the application. This process was repeated at every application.

Treatment Application

Spraying of the plant extract was done early in the morning before sunrise between 6:30 am and 8 am on a two weekly interval starting from four weeks after planting. Foliar application was done using manually operated hand sprayer of 2 litre capacity. The following data were collected starting from 4 weeks after planting: Plant height, Pest population, No of cobs, Cob weight, Cob damage, Percentage leaf damaged and stem damage..

Data Analysis

The experiment was laid out in a Randomized Completely Block Design (RCBD) and the data obtained were subjected to analysis of variance (ANOVA). Least (LSD) test was used for mean separation at 5% level of significance.

RESULT AND DISCUSSION

Effects of medicinal plant extracts on the population of maize stem borer.

The result of the effect of medicinal plant extracts on the population of maize stem borer are presented in Table 1. The result showed that there was no significant difference in the number of stem borers at 4 WAP. However, there was a significant ($P < 0.05$) reduction in the number of stem borers at 8 (1.80, 3.23, 5.33, 2.33, 2.77, 2.00) and 12 (1.40, 1.53, 2.43, 2.87, 1.53, 1.03) WAP in the treated plots than the untreated plots which had a higher number of stem borers population (20.17 and 19.67) respectively. Among the 3 plant extracts, the plots treated with *Allium sativum* recorded the lowest number of maize stem borers (1.80) and (1.40) at 8 and 12 WAP respectively. This was closely followed by plots treated with the combination of the three extracts (*A. indica*+*A. sativum*+*C. frutescense*) (2.00 and 1.03) and this is adjudged to be the best among the treatments.

Effects medicinal plant extracts on the maize plant height (cm)

The result from the study (Table 2) shows that there was a significant ($P < 0.05$) increase in the maize plant height at 8 and 12 WAP on the treated plots when compared with the untreated plots. The highest record on plant height was obtained when the three extracts were combined together at 8 and 12 weeks (214.20 and 385.30 respectively) while the control recorded (116.80 and 175.90).

Table 1: Effect of plant extracts on the population of Maize stem borer

Treatments	4 WAP	8 WAP	12 WAP
<i>Allium Sativum</i>	8.67	1.80	1.40
<i>Capiscum frutescense</i>	8.97	3.23	1.53
<i>Azadirachta. Indica</i>	9.03	5.33	2.43
<i>A. indica</i> + <i>A. sativum</i>	8.70	2.33	1.53
<i>A. indica</i> + <i>C. frutescense</i>	9.07	2.77	2.87
<i>A.indica</i> + <i>A.sativum</i> + <i>C.frutescennnnse</i>	8.87	2.00	1.03
Control (distilled water)	8.60	20.17	19.67
Mean	8.84	5.38	4.35
LSD (0.05)	NS	1.70	1.38

Table 2: Effect of plant extracts on Maize plant height (cm²)

Treatments	4 WAP	8 WAP	12WAP
<i>Allium Sativum</i>	51.10	187.50	241.60
<i>Capiscum frutescense</i>	60.20	166.40	209.30
<i>Azadirachta. indica</i>	45.80	148.00	226.90
<i>A. indica</i> + <i>A. sativum</i>	53.70	192.00	260.10
<i>A. indica</i> + <i>C.frutescense</i>	51.90	168.60	249.60
<i>A. indica</i> + <i>A. sativum</i> + <i>C. frutescense</i>	58.10	214.20	385.30
Control (distilled water)	48.40	116.80	175.90
Mean	52.70	170.50	249.80
LSD (0.05)	NS	40.53	32.80

Effect of plant extracts on the yield parameters of Maize

Result from the data analysis revealed that (Table 3) revealed that the plant extracts were able to increase the number of cobs per plot, weight of cobs per plot as well as reduce the number of cobs damaged, number of leaves damaged and number of stem damaged per plot (Table 3). All the treated plots were significantly difference (P < 0.05) from the untreated plots. The mixed extract gave the highest number of cobs (9.83) and cob weight (770.0g) when compared with the

single extracts and this also is significantly (P<0.05) difference from the untreated plots which gave the lowest cobs number and weight. (1.87 and 0.42 respectively). The higher cob number and weight obtained in this study might be as a result of reduction in the number of leaf and stem damage which was as result of the reduction in the number of leaf and stem damage by stem borer in plots treated with plant extracts. The number of cobs, leaves and stem damaged were all recorded higher in the untreated plots while the treated plots had the lowest damage.

Table 3: Effect of plant extracts on the yield and yield parameters of maize/plot

Treatment	No. of Fresh					
	Cobs /plot	Cob Wt(kg/ nlot)	No. of Cobs Damaged	No. of Leaves damaged	Percentage of leaves damaged	No. of Stems damaged
<i>Allium Sativum</i>	6.13	0.6	1.67	7.20	29.4	2.80

<i>Capiscum frutescense</i>	3.97	0.54	2.03	10.10	47.0	4.67
<i>Azadirachta. Indica</i>	4.03	0.45	1.57	9.13	73.1	6.33
<i>A. indica</i> + <i>A. sativum</i>	5.97	0.64	1.77	7.67	28.5	2.23
<i>A. indica</i> + <i>C. frutescense</i>	4.93	0.56	1.87	9.68	52.1	3.50
<i>A. indica</i> + <i>A. sativum</i> + <i>C. frut</i>	9.83	0.77	1.03	4.80	17.2	1.77
Control (distilled water)	1.87	0.42	4.50	15.43	89.3	8.67
Mean	5.25	0.57	2.06	9.14	48.10	4.28
LSD (0.05)	1.14	88.10	2.02	4.99	17.08	1.23

Discussion:

Results from this study revealed the potential of these botanical plant extracts in the management of maize stem borer. It revealed that applying the extracts singly are effective in the borer management while their mixtures are more effective suggesting the synergistic effects of these medicinal plants when combined. Oparaek (2004) showed that medicinal plant extracts exhibits varying degree of efficacy on the control of field pests. Pepper has been reported to be effective in repelling insects due to the presence of a chemical called capsaicin. Capsaicin is the primary substance that controls hotness in pepper. It irritates and also distasteful to the insects, animals and people (Kathleen 2010). The significant reduction in the population of stem borer in the *Allium sativum* treated plots could be attributed to the antifeedant, insecticidal and repellent properties of the plant which is as a result of its active ingredient Allium (Koch and Lawson, 1996). Apart from Allium, *Allium sativum* contains sulphur compounds which deter insects from feeding on plants (VijaYakshme et al., 2009). While neem has a chemical component called azadirachtin which is responsible for the insect pest reduction (Bhattacharyya et al. 2017). These and many other phytochemical components are present in these plants which is the reason for their efficacy and ability to help in the management of the insect pest. The low yields recorded in the untreated plots might be as a result of the feeding damage by the stem borer on the stalks and leaves of the plants which creating extensive holes in the maize plant which reduced photosynthetic activities of the plants and thereby reducing the yield.

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The increase in the plant height of the maize in the treated plots could be as a result of the reductions in the population of the stem borers due to the extracts applied. These results agreed with the report of Asawalam (2006) and Isman (2008) who reported that plant extracts from different parts of *A. indica*, *Allium sativum*, *C. longa*, *M. paradisiacal*, *O. gratissimum*, *V. amygdalina* and *X. aethiopica* have insecticidal activity against insect pests of mungbean. The result also agrees with Emeasor et al., (2017) who reported that the plant derived insecticides used for the control of *Podagrica. uniforoma*, and *Podagrica. sjostedti* in okra field were effective and significantly reduced the population of the insect pests. The results are also in line with the result obtained by Stoll (1988) on chilli pepper (*Capiscum frutescense*) in mixtures with garlic (*A. sativum*), onion (*Allium cepa*) bulb extracts and lemon grass (*Cymbopogon citratus*) leaf extracts were found very effective against some leaf eating insect pests of crops.

CONCLUSION AND RECOMMENDATIONS

The three plant extracts used in this study showed varying degrees of reduction in the population of maize stem borer in the study hence the significant results recorded in increase in plant height, number of cobs and fresh cob weight and a decrease in cob, leaves and stem damaged in the treated plots than the untreated plots. It is therefore recommended that these plant extracts be used by resource - poor farmers with limited access to synthetic pesticides for the management of maize stem borer. In-depth research efforts should be encouraged at studies on half-life and formulation of biopesticides against stemborers

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**EFFECT OF HAIR DRESSING SALON EFFLUENT ON THE JUVENILES OF AFRICAN CATFISH
(*Clarias gariepinus*)**

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ABSTRACT

The toxicity of hair dressing salon effluent on *Clarias gariepinus* juveniles was studied using static bioassay method between May and August, 2018. This study investigated, histopathological changes, behavioural response in *Clarias gariepinus* juveniles. The fish specimens were distributed randomly into; T₁ (200ml/L), T₂ (400ml/L), T₃ (600ml/L), T₄ (800ml/L), and T₅ (0%), all in three replicates, in a completely randomized experiment. Mortality increased with increased effluent concentrations; 250.96 ml/L was recorded as LC₅₀ for 96 hours. Various behavioural changes were observed before mortality. Histopathological changes were observed in treated fishes while the livers and gills of the control fishes showed normal histological appearance. It was concluded that hair dressing salon effluent had some negative effect on histopathology and behavioural response of *Clarias gariepinus*. Therefore, it is recommended that the effluent should be properly treated before being discharged into the environment.

Keywords: Environmental pollution, salon effluent, histopathology; *Clarias gariepinus*

Introduction

Environmental pollution has become one of the most important problems of the world (Chandran *et al.*, 2005). One of the most critical problem of the developing countries is improper management of vast amount of wastes generated by various anthropogenic activities (Fakayode, 2005). A change in the quality of water by the presence of toxins/ contaminants makes it potentially harmful to life forms, instead of sustaining them (Agrawal *et al.*, 2010). The entry of toxicant into aquatic media may affect the water quality parameters which in turn leads to changes in the haematological variables of fish and other aquatic lives (Carvalho and Fernandes, 2006; Karitha *et al.*, 2010). Increase in volume and diversity of solid wastes and sewage generation has led to eutrophication of water bodies, destruction of fisheries and decrease in the aesthetic and recreational value of water resources (Oduwole, 1997) Biological changes in fish that are related to the exposure or to the effect of contaminants are called bio-markers and their use has led to good result in environmental risk assessment.

Beautification is one of man's body care process that involves the heavy and prolific use of surfactants, which are primary ingredients of body care products (Chude and Ekpo, 2010). The result is a proliferation of hair dressing among other body care fashion process. Wastes from these salons have therefore become a very common source of pollution to the aquatic environment especially in Sub-Saharan Africa; where there is little or no prohibition for violators (Chude and Ekpo, 2010). Interestingly, it is this very same property that affects aquatic life adversely. They alter the properties of fish gill and consequently changes the fish normal uptake of ions from the water (Ndu, 2004).

Effluents from hair dressing salons cause tissue

damages and pathological degradation as the fish show haematological responses to toxicants; and generally such degradation of histological changes occurs in the gills, liver, hearts and kidney of aquatic lives. (Ndu, 2004). Because the liver of fish can be considered a target organ to pollutants, alterations to its structure can be significant in the evaluation of fish health (Myers *et al.*, 1998). These effluents at high concentrations were found to affect the swimming pattern, skin coloration, feeding rate and general behaviour of fish which suggest that fish can tolerate low concentrations of pollutants (Dahunsi *et al.*, 2011). The exact changes can vary depending on the toxicant type, species of fish, water quality and length of exposure (Jee *et al.*, 2005; Monteiro *et al.*, 2005). There is paucity of information of the effect of hair dressing salon on living aquatic biota.

This research therefore aims at evaluating the potential effects of hair salon effluent on the juvenile of *Clarias gariepinus*.

Materials and Methods

Equipment

Plastic containers (25 litres), formalin, Prodder juveniles (*Clarias gariepinus*), meter rule, water, hair dressing salon effluent.

Study Area

The work was carried out in the laboratory of Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike between May and August 2018.

Effluent collection

Raw effluents were collected in batches from different hair dressing salons in Umuariaga, Ikwuano L.G.A, Abia State, Nigeria. They were collected from the discharge points and transported to the laboratory using clean containers. The collected effluents were

filtered, pH determined and then stored at 4°C until they were ready for use. During the test, all the samples were pooled together to avoid variability in concentration.

Physico-chemical properties

Chemical oxygen demand (COD), total dissolved solids (TDS), electrical conductivity and alkalinity, pH, temperature, were analyzed, using the methods of APHA (1998).

Experimental Design

Healthy and active juvenile *Clarias gariepinus* of unspecified sexes had an average weight of 1.5kg; length 14cm were obtained from the fish farm of Michael Okpara University of Agriculture, Umudike. They were kept at the frequently disinfected and well ventilated Animal House of the Department of Zoology and Environmental biology, Michael Okpara University of Agriculture, Umudike. The fishes were acclimatized for 2 weeks. The fish had unrestricted access to water, fed twice daily with commercial fish meal (pellets) and the water changed every two days. The tanks were covered with net. The design for this study was completely randomly designed. The acute toxicity test (LC₅₀) had four concentrations (800ml/l, 600ml/l, 400ml/l, 200ml/l and control) of the test substance (hair dressing salon effluent). Each treatment had 3 replicate. The acute test had 7 fishes per tank. Range Finding

Four plastic tanks were used during the range finding which was filled with 20 litres of water. The fish (20 each) were introduced into the rectangular tank and allowed to acclimatize to the environment for two hours before the toxicant was applied. The effluent was applied to each of the four plastic tanks at the concentration of 400ml/l, 300ml/l, 200ml/l, and 100ml/l respectively. The range finding was observed from 24 hours.

Acute Toxicity Test

After the acclimatization period, range finding test followed suit to determine the definite concentrations to be used for the acute evaluation test. Twelve plastic rectangular tanks were labelled A-E in 3 replicates. The test solution (Effluent) was thoroughly mixed by pouring into a big plastic container before exposing the test organisms into the labelled tank. The varying concentrations used were A=800ml/l, B=600ml/l, C=400ml/l, D=200ml/l and E=20 litres of water, serving as the control experiment. 7 fishes were exposed to each concentration including the replicates. The experiment was monitored for 96 hours during which the following parameters were evaluated: behavioural responses, total number of death (mortality) after 96 hours. The percentage mortality at 96 hours, calculation of the LC₅₀ which is the concentration at which half or 50% of the test organism died on exposure using probit analysis. Since the organisms were exposed for 96 hours, the 96.h LC₅₀ was determined from the graph of percentage

mortality against concentration.

Water Quality Analysis

Water quality parameters (Dissolved oxygen, pH, temperature, total dissolved substance, electric conductivity and alkalinity) in the aquaria were checked bi-weekly for the 28 days study period. Dissolved oxygen was measured by using DO meter. (Milwaukee MW 600, SN: 15532). pH values were measured with a pH meter (PH-008(1)). Water temperature was determined during dissolved oxygen measurement using the DO meter. Electrical conductivity was measured using an electric conductivity meter. Total dissolved solid was measured using a TDS meter (Model: EC-1)

Histopathological Analysis

Alter 96 hours, the gill and liver of the fish from each treatment were exercised and preserved in 10% formalin. The samples were processed for histological examination using standard histological techniques and then viewed using a photomicroscope.

Ethical Issues

At the end of the experimentation, all test organisms were humanely destroyed and disposed of in an appropriate manner by burying them.

Statistical Analysis

Results were summarized and expressed as mean ± standard error of mean (SEM). Statistical analysis was done using analysis of variance (ANOVA). LSD was used to identify significant differences between control and treated groups at <0.05

Results

Table 1: Physicochemical characteristics of hair dressing salon effluent.

This shown in table 1. The data obtained have some of its values conforming to FMENV 2011 specifications for maximum limits allowed for effluent discharge into water bodies while the value for total dissolved solids do not conform to the standard.

Paramet ers	Experimental Values				FMEN V 2011 FMEV N 2011
	T1	T2	T3	T4	
pH	3.83	4.73	5.73	6.23	6.50- 8.50
DO (mg/L)	7.23	7.16	6.86	6.63	10.0
TDS (mg/L)	208.66	172.66	131.66	89.00	500.0
EC (us/cm)	416.33	338.33	263.66	181.33	ND

DO=Dissolved oxygen, TDS=Total dissolved solid
 ND=Not detected, EC=Electric conductivity.
 Behavioural Response of test Organism

Clarias gariepinus exhibited distress behavioral response due to the effect of the hair dressing saloon effluent. This was noticed by the sudden change in the organism response to the environment such as erratic swimming, occasional gasping for breath, a frequent surfacing which increases as the concentration

increases. All these are indications that the concentration have become hypoxic and have induced brain dysfunction in the test organisms due to low oxygen supply. As the experiment progressed, some of the test organisms were seen to get weaker evident by reduction in movement, their ventral surface were subsequently turned upwards while those that couldn't tolerate the concentration any longer went into a state of motionlessness. Normal behavior were however observed in the control.

Table 2: Behavioural response of *Clarias gariepinus* during exposure to acute concentrations of hair dressing salon effluent.

Behaviour	Concentrations				
	0.00	200ml/l	400ml/l	600ml/l	800ml/l
Erratic swimming	-	-	+	+	+
Gasping for breath	-	+	+	+	+
Loss of reflex	-	+	+	+	+
Opercula beating	-	-	-	+	+
Frequent surfacing	-	-	+	+	+
Motionlessness	-	+	+	+	+
Hyperventilation	-	-	+	+	+
Light	-	-	-	+	+

Legend: Absent (-), Present (+)

Mortality

The result of the acute toxicity shows minimal mortality rate in the lower concentration while, the maximum mortality was observed in the highest concentration.

Table 3: Mortality rates of *C. gariepinus* exposed to varying concentrations of hair dressing salon effluent.

Exposure Period	Control	800ml/l	600ml/l	400ml/l	200ml/l
	R ₁ R ₂ R ₃	R ₁ R ₂ R ₃	R ₁ R ₂ R ₃	R ₁ R ₂ R ₃	R ₁ R ₂ R ₃
12 Hours	---	---	---	---	---
24 Hours	---	2 1 2	---	---	---
48 Hours	---	4 2 1	3 2 1	---	---
72 Hours	---	1 2 4	4 4 6	---	---
96 Hours	---	- 2 -	- 1 -	-- 1	---

Table 4: Percentage mortality

Total mortality	0/21	21/21	21/21	1/21	0/21
% mortality	0%	100%	100%	4.8%	0%

Table 5: LC₅₀ values for hair dressing effluent (with 95% confidence limit) estimated by Probit analysis

Time (hrs.)	LC ₅₀ Values (ml/L)	Lower limit (ml/L)	Upper limit (ml/L)
48 Hours	380.344	338.24	483.50
72 Hours	250.96	299.52	272.31

96 Hours	250.96	229.52	272.31
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This table shows the probit analysis for the hair dressing effluent studied for 96hrs. From the result it shows

Both limits (lower and higher) of the concentrations for which were effective within specific time range. At 95% confidence level, the LC₅₀ for the test substance was 380.34 ml/l, 250.96 ml/l and 250.96 ml/l at 48, 72

and 96 hrs. respectively.

Histological examinations

The results of the histological examinations are shown in plates 1-6. The liver histology are shown in plates 1-3 while Gill histology are shown in plates 4-6.

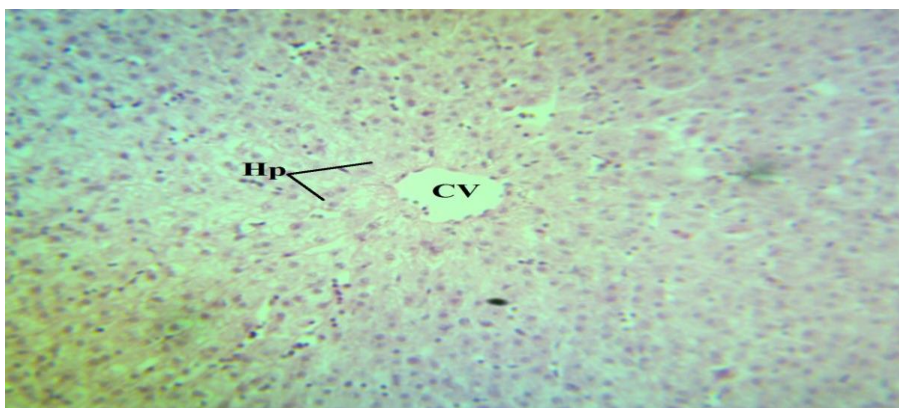


Plate 1: Photomicrograph of liver showing normal histologic architecture in control treatment: central vein (CV) and hepatocytes (HP). X400.

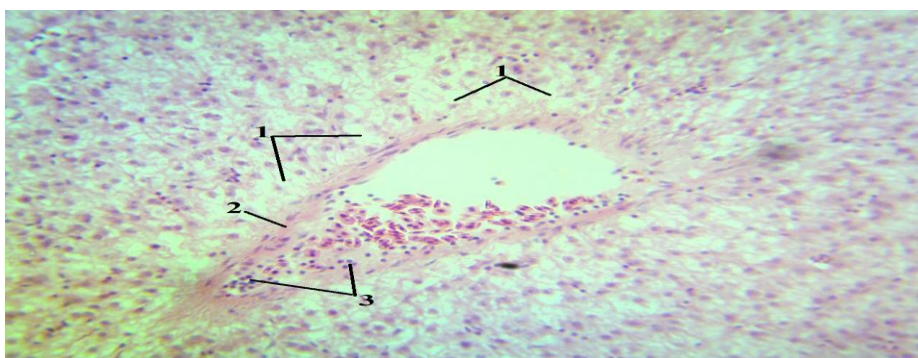


Plate 2: Photomicrograph of liver showing hydrophobic degeneration of hepatocytes (swelling of cells) (1), thick fibrous connective tissue (fibrosis) lining the central vein (2), moderate congestion and mononuclear cell infiltrations (3), in 200ml/L. H&E, X400.

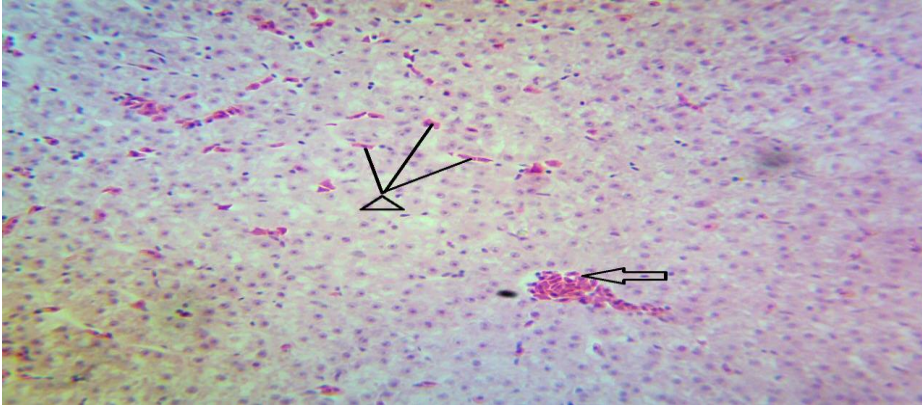


Plate 3: Photomicrograph of liver showing diffused marked congestion of central vein (arrow) and sinusoids (arrow head), in 100ml/L. H&E, X400.

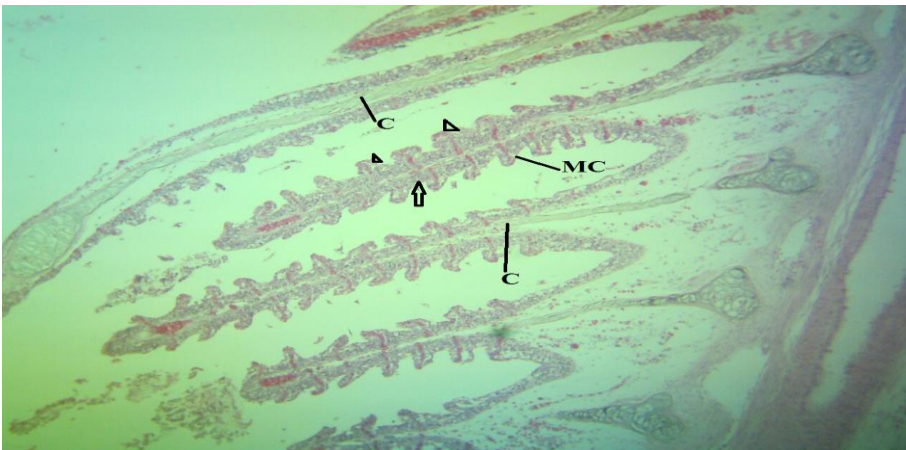


Plate 4: Photomicrograph of gill showing normal histologic architecture in Control group: primary lamella (PL) secondary lamella with the epithelial covering (arrow head) and mucous cell (MC). In the central core is a mass of hyaline cartilage (C) and blood vessels and is covered with stratified epithelium.H&E, X400.

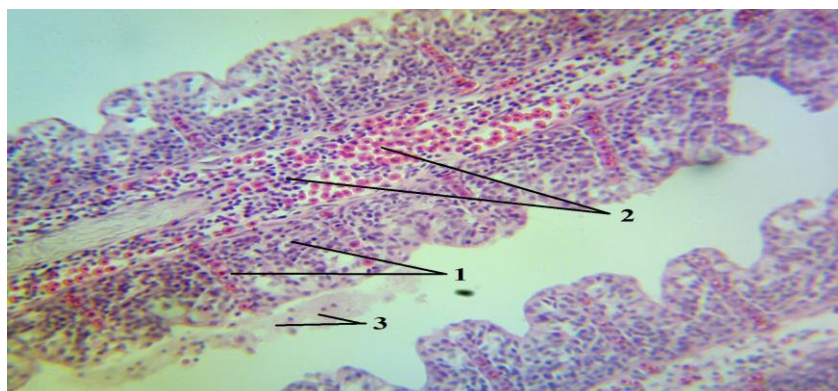


Plate 5: Photomicrograph of gill showing swelling and fusion of the epithelial cells with necrosis and adhesions of gill lamellae(1),sloughing of epithelial cells (arrowhead), marked proliferation of mucous cells (2), congestion of blood vessels (arrow) and haemorrhages (H)and infiltration of mononuclear inflammatory cells, in 200ml/L. H&E, X400.

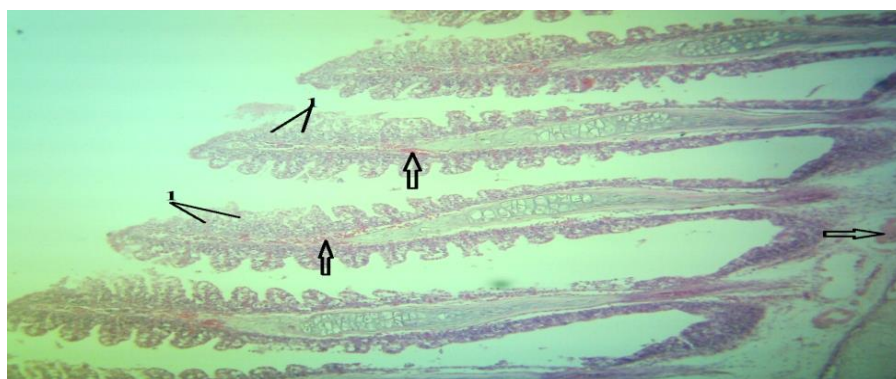


Plate 6: Photomicrograph of gill showing swelling of epithelial cells and adhesions of gill lamellae (1), moderate proliferation of mucous cells, congestion of blood vessels and infiltration of mononuclear inflammatory cells (2), and excessive production of mucus (3), in 100ml/L. H&E, X400.

Discussion

This work shows that hair dressing salon effluent low in total dissolved solids (TDS), higher dissolved oxygen and high electrical conductivity which shows the effluent to be toxic for discharge into our immediate environment. This agrees with the findings of Dahunsi and Oranusi (2013) that the observed characteristic features may have resulted from the organic loads in the waste water. The abnormalities (gasping for breath and frequent surfacing) observed prior to mortality are indications of depleted oxygen content (hypoxias) due to higher demand for oxygen. The erratic swimming, restlessness, increase in opercular beating, gasping for air as well as accumulation of mucus on the fish body is as a result of skin irritation, respiration rate impairment or a response to altered locomotion activity, an indication of the effect of toxicant on the nervous system.

Fish exposed to very low concentration of toxicant may not react quickly, rather become very adapted to the new environment (Ayoola *et al.*, 2008). This was essentially the case with test organisms in treatments 1 and 2 where abnormal behaviours were not observed. However, the stressful and erratic behaviour of the fish in T1 and T2 gives a signal to respiratory impairment. This may be a consequence of the high effluent levels or toxicity effect of the gills. This agrees with the work of Ogaga *et al* (2015).

From this study, it is obvious that the effluent inhibits activity of enzyme ACHE, which is present in the synaptic regions and mediates transmission of impulses by breaking acetylcholine into acetic acid and choline. The acetylcholine at neural and neuromotor regions upon accumulation causes "hyper excitability", which in turn might also influence behavioural pattern and may lead to death of fish. Das

and Mukherjee (2003) and Tiwari *et al* (2012). The 96-h LC₅₀ value for the acute test was 250.96 ml/L which means that at this concentration of the effluent in natural aquatic environment, some of the population will become dead.

Histological investigations of the liver tissues in plate 1 showed normal histologic architecture of central vein (CV) and hepatocytes in control treatment (untreated fishes). Plate 2 shows hydropic degeneration of hepatocytes (swelling of cells), thick fibrous connective tissue (fibrosis) lining the central vein, moderate congestion and mononuclear cell infiltrations of the liver which showed a progressive architectural distortion at varied concentrations and period of exposure.

Plate 3, shows diffused marked congestion of central vein (arrow) and sinusoids (arrow head) of the liver. These are in agreement with the submission of the Srivastava and Srivastava (1994) who reported that teleost accumulated lead (pb) both directly from diet and indirectly from the aqueous medium through an active food chain by the surface lamellae. The most generally encountered type of degenerative changes was congestion, mononuclear cell infiltration and sinusoids.

Gill showing swelling and fusion of the epithelial cells with necrosis and adhesions of gill lamellae, sloughing of epithelial cells (arrowhead), marked proliferation of mucous cells, congestion of blood vessels (arrow) and haemorrhages (H) and infiltration of mononuclear inflammatory cells as observed in plate 5 resulted from excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification.

Gill showing swelling of epithelial cells and adhesions of gill lamellae moderate proliferation of mucous cells, congestion of blood vessels and infiltration of mononuclear inflammatory cells and excessive production of mucus as observed in plate 6 were as a result of the fish trying to cope with the challenge of the toxicants.

High accumulation of several components of hair dressing salon effluent in the liver is a pointer to the fact that, liver plays a major role in the accumulation and detoxification. Frieberg *et al* (1971) submitted that fishes are known to possess sequestering agents (metallothionein), the bioaccumulation of these trace element in the liver tissue reaches a proportion in

which the function of the liver is impeded. Thus, resulting in a progressive degeneration of the liver cells syncytial arrangement.

Therefore, necrosis became evident as the concentration increases and this may be due to the inability of the fishes to regenerate new liver cells. It was also observed that the histopathological changes in the liver caused metabolic problems. An increase in the degree of damages done to the gills of the fishes held in 200 and 100ml/L hair dressing salon effluent was generally related to important degenerative and necrotic processes, this observation was in line with the submission of Chang *et al* (1998) and Pacheco and Santos (2002).

The histological alterations identified within the hepatocytes may have been the results of various biochemical lesions. Irregular shaped central vein and infiltration may be attributed to the accumulation of lipids and glycogen due to liver dysfunction as a result of exposure to the toxicants, this is in conformity with the submission of Fanta *et al* (2003). Pacheco and Santo (2003) also described increased level of vacuolation of the hepatocytes as a signal to the degenerating process that suggest metabolic change, possibly related to exposure to contaminated water. Therefore, the histological changes observed in the liver indicated that the fish were responding to the direct and the cumulative effects of the contaminants as much as other effects such as stress. Such information confirmed that histopathological alterations are good biomarkers for both field and laboratory assessment, particularly in tropical areas that are naturally subjected to a multiplicity of environmental variations or depletion due to chemical contamination. Ajani, F and Awogbade, A.A (2012)

Conclusion

In conclusion, exposure of *Clarias gariepinus* juvenile to even low concentrations (100ml/L) of hair dressing salon effluent could result in change in behavioural pattern histological degradation and death. In view of the toxicity effect of this effluent, it can be inferred that, indiscriminate discharge of effluents can induce damages to the tissue and organ, which might make all the living entities in polluted environment vulnerable to diseases, and eventually lead to death. Therefore, there is need for the adoption of proper effluent treatment technology which would ensure proper treatment of industrial effluents prior to their discharge into the environment.

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WIS-ASP 37

ENHANCING CATTLE DUNG NUTRIENT AVAILABILITY THROUGH BIOMIMICRY FOR SMALLHOLDER FARMERS TO COMBAT CORONAVIRUS PANDEMIC EFFECT ON CROP PRODUCTIVITY IN NIGERIA

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Abstract

Soil applied cattle dung has great potential to increase soil nutrients and crop yields. However, it takes a long time for it to decompose when compared to other organic manure. The objective of this study was to apply the principle of biomimicry to cattle dung, by reducing the size fraction and determining its effects on selected soil properties, lettuce growth parameters and nutrients-uptake, nitrogen and carbon isotopes in the shortest possible time. The pot experiment was conducted at the greenhouse facility of Rothamsted Research, North Wyke Devon United Kingdom. The treatments were: Above 4 mm sieve mesh cattle dung fractions (CDA4), Cattle dung fractions retained at 4mm sieve mesh (CD4), Cattle dung fraction that passed through 4mm sieve mesh (CDL4) and a control (no amendment). They amendments were applied at 3.50 g (equivalent of 20t ha⁻¹) to 300 g air-dried soil in a growth pot. The treatments were replicated five times in Completely Randomized Design with lettuce (*Lactuca sativa L.*) as the test crop. Phosphorus soil content was significantly increased by applied CD4 with value of 7.51±0.16 g kg⁻¹ when compared with control that had 5.83±0.14 g kg⁻¹. More enriched ¹⁵N were recorded in CD4 with value of 5.60‰. Lettuce heights were increased by applied CD4 at 5 and 6 weeks after planting. Reducing the cattle dung size fractions to CDL4 and CD4, increased most of the soil properties and lettuce growth parameters measure within the short period of six weeks.

Keywords: cattle dung; biomimicry; particle- sizes; nutrient availability; stable isotopes

Introduction

The outbreak of coronavirus disease 2019 (COVID-19) in the world is of great concern because of its impact on global health, food security, economy among others. 'World Health Organization' (2020) described Covid-19 as an infectious disease caused by novel coronavirus. In Nigeria most of the State Governments are putting up measures that will curtail the spread of the virus. Some of them included: restricted movement except for those on essential duties like health workers. Others are inter-States borders closures and in some States total lockdown with no movement. All these safety measures are good but they will in a long run have a multi-faceted negative effect on food security. This is because the main producers of food in Nigeria, the smallholders' farmers are also restricted from going to cultivate their farms even with the onset of raining season. According to FAO (2020), the restriction on movement will lead to fertilizer shortage, disruption of the food chain and food production.

It is important that the farmers are assisted, to overcome the pressures that will come on them and the nation from food demand during and after the pandemic. One of the ways to achieve this is by encouraging the farmers to add external soil inputs, which would release its nutrients very fast to boost crop production. Inorganic fertilizer is an ideal soil input to be used, unfortunately its scarcity and high cost (FEPSAN, 2011) even before the disease outbreak is regrettable. In place of mineral fertilizers, animal manure that is readily available can be used as an alternative source of nutrients for crops (Mubarak *et al.*, 2009). Cattle dung as one of the most available

animal manure has been reported to improved soil fertility through increasing soil organic matter, cation exchange capacity, improved soil structure indices and enriched heavy nitrogen isotope (Bakayoko *et al.*, 2009; Mubarak *et al.*, 2009; Rasoulzadeh & Yaghoubi, 2010; Szpak 2014). However, the challenge with this dung is its coarse nature, which results to its slow rate of decomposition, to release the needed nutrients by crop (Onwuka *et al.*, 2019).

Microorganisms acts on cattle dungs to break it down for mineralization and nutrient release (Gallant 2016). This natural process takes a longer time for nutrient release. If this process of decomposition must be hasten to have high yield turnover, then the coarse cattle dung must be reduced into smaller particle sizes. Particle size reduction of cattle dung is based on the concept of Biomimicry. Biomimicry is a paradigm of technological research or ecological innovation (Dicks 2016; Blok & Gremmen 2016); its principle is based on mimicking the natural ecosystem or processes to provide a bio- friendly solution to mankind. Reducing cattle dung into smaller sizes has been reported to speedily increase the soil nutrients and crop yields within a short duration (Aryanpour *et al.*, 2017). This is because the smaller particles have large specific surface areas and high reactivity (Chen *et al.*, 2010).

Nitrogen is an important plant primary nutrient which is added to the soil through animal manure and the N isotopic composition of animal manure, has been reported to be high (Chalk, *et al.*, 2014; Szpak, 2014). Nitrogen isotope is useful in determining the total nitrogen input, output and fractionation processes

within the plant (Dominquez & Sampedro 2008). To monitor the effect of cow dung particle sizes on soil nutrients and uptake in this study, lettuce (*Lactuca sativa* L) was used as the test crop. Lettuce is a leafy vegetable that belongs to the Compositae family (Asteraceae) (Jeong *et al.*, 2015). It is a short duration crop and has high ascorbic acid, polyphenols, carotenoids contents, which make it a healthy and medicinal crop (Said *et al.*, 1996). The hypothesis of this work is to test whether reducing cattle dung size will improve soil nutrients and nutrient uptake in lettuce used as a test crop. Hence the objectives of the work is to apply the principle of biomimicry through fractionization of cattle dung and determine its effects on selected soil properties, lettuce growth parameters, nutrient-uptake, nitrogen and carbon isotopes in the shortest possible time.

Materials and Methods

Description of the experimental site

The study was a pot experiment conducted at the greenhouse facility of Rothamsted Research Center, North Wyke, Devon, United Kingdom (50°45'N, 3°50'W). The greenhouse temperature was adjusted to day/night temperature of 20°C/12°C until harvest; Relative air humidity 60-65%; and day length of 12-h light/ 12-h darkness (Jeong *et al.*; 2015) were determined. These were done to mimic the temperature, relative humidity requirement for lettuce used for the experiment.

Soil sampling and preparation

An acid 5.41 Creden loam soil (Cc 243 subgroup and series name; Cranfield University, 2018) was collected from a fallow field previously grown with wheat at

Skittle Alley field, Devon United Kingdom (50°47'36.64"N, 3°57'4.87"W). Soil samples were randomly collected from twenty spots within an area of 20m by 20m, from a depth of 0 to 30 cm. The sampled soils were bulked to form a composite sample, used for the experiment and pre-treatment soil analysis. The soil was air-dried and passed through a 2 mm sieve mesh. The soil texture was classified as loam (Sand, 38%; Silt, 38%; clay, 24%). The pH_(water) was 5.42, which is classified as strongly acidic (Soil Science Division Staff, 2018). The organic matter content was 3.9%, total nitrogen was 0.2% (dry weight). The other soil properties have been submitted for publication in a journal.

Treatment and treatment preparation

In order to facilitate the decomposition of the cattle dungs when applied to the soil, the biomimicry technology by the way of reducing the dung particle size was employed. The treatment comprised of the various particle size fractions of the cattle dung. The sizes were above 4 mm sieve mesh sized (CDA4), Cattle dung fraction retained at 4mm sieve mesh (CD4), Cattle dung fraction that passed through 4mm sieve mesh (CDL4) and a Control (received no amendment). The Cattle dung (CD) was collected from the Farm Platform of Rothamsted Research, North Wyke, Devon, UK and was air dried. The cattle dung was passed through a set of two sieve sizes with nominal aperture size of 5 mm and 4 mm. Local prototype of the two sieve sizes were developed for easy dissemination to the smallholder farmers, in Nigeria. The chemical characterization is shown on Table 1.

Table 1: Chemical characterization of the amendment used for the experiment

Properties	Units	CDA4	CD4	CDL4
pH _(water)	-	8.88	8.98	8.98
EC	mS/cm	3.93	4.09	2.99
OM	%	72	73.4	75.6
TN	g kg ⁻¹	2.0	2.00	2.00
TP	g kg ⁻¹	0.70	0.70	0.70
TK	g kg ⁻¹	2.60	2.60	2.60

EC= Electrical conductivity; OM= Organic matter, TN =Total nitrogen, TP=Total phosphorus, TK= Total potassium

Note that the values for TN, TP and TK for the cattle dung sizes were same because they were all subjected to same process of determination, which involved grinding with ball mill to reduce their sizes.

Experimental procedure

The air-dried soil (300g) was weighed into plastic Terracota pot with drainage hole (11cm height, 13 cm top diameter and 9cm base diameter). The treatment were applied at the rate of 20t ha⁻¹(gram equivalent of 3.5), replicated five times and the pots were arranged in a Completely Randomized Design (CRD). The test crop lettuce (*Lactuca sativa*; variety webbs wonderful) was seeded directly into the pots and after one week of germination, they were thinned down to two plants per

pot. The treatments were applied at one week after the thinning. The reason for applying the treatments as the crops grew was to mimic the application methods of most smallholder farmers in Nigeria. The experiment was established in August 2017. The plants were watered every other day for the period of seven weeks that the experiment lasted.

Plant parameters measured

The following plant parameters were measured: plant height (cm) and number of leaves counted at 2, 3, 4, 5 and 6 weeks after treatment application (WAT). Others measured were top biomass and root dry matter yields at harvest.

Plant preparation for biomass dry matter weight, macro and micro nutrient uptake

The plant materials above and below ground were harvested, washed with de-ionized water and placed into an oven for 30 min at 105°C to deactivate the enzymes. Afterwards, they were oven dried to a constant weight for 48 hours at 65°C for shoot and root dry matter yield. The oven dried materials were fine-ground in a plant ball mill, for macro and micro nutrient concentration determination while the nutrients uptake was calculated. The following nutrients concentration in the harvested biomass; N, P, K, Ca, Mg, S, Fe, Zn and Cu were determined by digestion process in aqua regia (1M HCl: 1 M HNO₃; 80:20 V/V) in open tube digestion blocks and passed through Inductively Coupled Plasma- optical emission spectrometry (ICP-OES). The nutrients (N, P, K, Ca and Mg) uptake was calculated by multiplying the concentration values of the nutrients in the plants by the weight of the top biomass dry matter yield. The contribution of the individual treatments to the biomass by method of Peng *et al.*, (2011) was calculated with the equations below:

$$\begin{aligned} \text{Contribution}_{\text{Soil}}(\%) &= \frac{\text{Biomass Control} \times 100}{\text{Biomass CDA4} + \text{CD4} + \text{CDL4}} \quad (1) \\ \text{Contribution}_{\text{CDA4}}(\%) &= \frac{\text{Biomass CDA4} - \text{Biomass Control}}{\text{Biomass CD4} + \text{Biomass CDA4} + \text{Biomass CDL4}} \times 100 \quad (2) \\ \text{Contribution}_{\text{CD4}}(\%) &= \frac{\text{Biomass CD4} - \text{Biomass Control}}{\text{Biomass CD4} + \text{Biomass CDA4} + \text{Biomass CDL4}} \times 100 \quad (3) \\ \text{Contribution}_{\text{CDL4}}(\%) &= \frac{\text{Biomass CDL4} - \text{Biomass Control}}{\text{Biomass CD4} + \text{Biomass CDA4} + \text{Biomass CDL4}} \times 100 \quad (4) \end{aligned}$$

Stable isotope analysis of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) in soil

The soil total nitrogen, total carbon, stable isotopes of delta 15Nitrogen ($\delta^{15}\text{N}$) and delta 13Carbon ($\delta^{13}\text{C}$) values were determined using the stable isotope measurements method. Here a NA2000 Elemental Analyser (Carlo Erba, Milan, Italy) linked to 20-22, Isotope Ratio Mass Spectrometer (Sercon, Crewe, UK) situated at Rothamsted Research North Wyke, Okehampton, UK, was used to perform the determination of the isotopes. The $\delta^{13}\text{C}$ results were expressed relative to the reference standard, Pee Dee Belemnite (PDB). The results of $\delta^{15}\text{N}$ were expressed relative to atmospheric nitrogen as (‰) using the

Farquhar *et al.*, (1982) equation below:

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000$$

Where δ = the heavy isotope of carbon or nitrogen, R = ratio of heavier to lighter isotope for the sample and the standard ($^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$). The values 0.1 and 0.2‰ were the analytical precisions measurement for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Nitrogen isotope ratios are reported relative to AIR. ‘AIR’ or atmospheric nitrogen gas is used as the reference standard with a very constant 15N composition of 0.366% (Junk and Svec, 1958).

Determination of some soil properties at pre-treatment and harvest of lettuce

Soil samples were analyzed before treatment application and at the end of experiment. At the end of the experiment, the soil in each pot was removed, thoroughly mixed and sampled in triplicate for analysis. Total P, K, Ca, Mg, S, Zn and Cu of the soil samples were digested in aqua regia (1M HCl: 1 M HNO₃; 80:20 V/V) and passed through ICP-OES. Soil pH was measured with deionized water at a soil: water ratio of 1:2.5, using a pH meter (pH Meter 3320 Jenway). The soil particle size distribution was determined at Natural Resource Management, a trading division of Cawood Scientific Ltd (report number 95761-18).

Statistical Analysis

One –way analysis of variance was done to test the effect of the treatments using GENSTAT software package 18th Edition (GENSTAT Rothamsted Research Center, United Kingdom). Significant differences were obtained by one-way analysis of variance (ANOVA) for experiment in Completely Randomized Design (CRD) with means separated using Fisher’s Least Significant Difference at probability level of 5%. Any differences between the mean values at $p < 0.05$ were considered statistically significant.

Results

Treatments effect on lettuce growth parameters

Applied CDL4 significantly ($P < 0.05$) increased lettuce height at 2 and 3 weeks after planting (WAP) (Figure 1). At 4 WAP, significant ($P < 0.05$) increase in lettuce was recorded on the soils that received CD4 and CDL4. The soils that received CD4 significantly ($P < 0.05$) increased heights at 5 and 6 WAP.

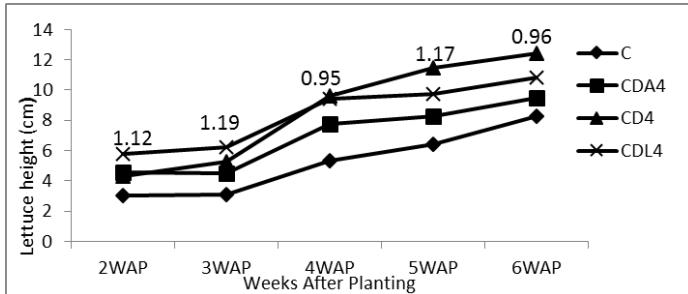


Figure 1: Effect of treatments on lettuce height at weeks after planting. Values of LSD at P<0.05 showed in plot area

The number of leaves were significantly (P<0.05) more in pots that received CDL4 at 2, 3 and 4 WAP (Figure 2). At 5 and 6 WAP, the pots that received CD4 had more number of leaves with values of 11 and 12 respectively.

significantly (P<0.05) higher root dry matter yield over the control (Figure 3). Cattle dung applied at less than 4mm particle size had the highest top biomass dry matter yield of 0.698 g/pot. This value was statically at par with the value of 0.676 g/pot gotten from soil that received CD4.

The three treatments CDA4, CD4 and CDL4

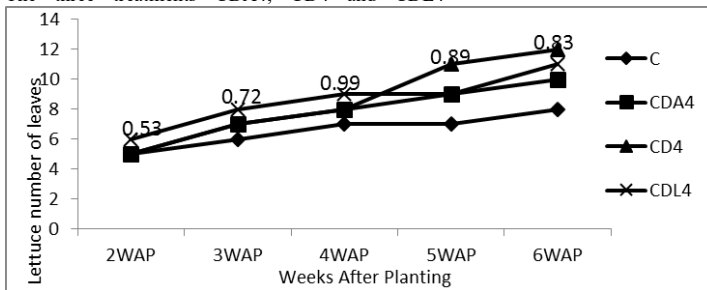


Figure 2: Effect of treatments on lettuce leaves numbers at weeks after planting. Values of Lsd at P<0.05 are showed in plot area

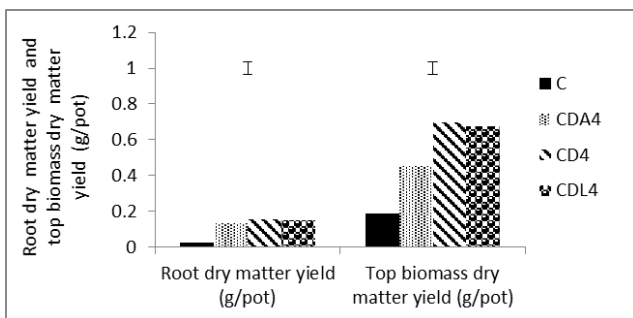


Figure 3: Effect of treatments on lettuce root and top biomass dry matter yields at the end of the experiment. Vertical bars represent Lsd at P<0.05.

Contribution of individual treatment to lettuce total biomass

The contribution of each treatment to lettuce biomass is shown on Table 2. The inherit soil fertility which

represented the control contributed 10.76% of the total biomass; CDA4 contributed 19.51%, while CD4 and CDL4 contributed 38.41% and 42.53% respectively.

Table 2: Contribution of treatments to lettuce biomass

Treatment	Calculation based on equation	Values (%)
C	Equation 1	10.76
CDA4	Equation 2	19.51
CD4	Equation 3	38.41
CDL4	Equation 4	42.53

Nutrients concentration in the soil and nutrient uptake by lettuce at the end of the experiment

There was a significantly (P<0.05) total nitrogen increases of the mean values of amendments over the mean value of the control (Table 3). Cow dung applied as CDL4 had the most significant total nitrogen mean

value. However, the mean value of 26.45±0.38 (g kg⁻¹) recorded for CDL4 was statistically at par with the mean value of 25.23±0.25 (g kg⁻¹) obtained for CD4. There was no significant difference recorded among the treatments, however, there exists a significant difference between the treatments and the control. No significant differences were recorded among the treatments for total potassium. The mean value of 9.98±0.52 (g kg⁻¹) and 4.93±0.37 (g kg⁻¹) obtained from pots treated with CDA4 for total calcium and magnesium respectively, were significantly (P<0.05) higher than the mean values of the other treatments. The control had the highest mean value recorded for total sulphur. Meanwhile there were significant differences among the treatments and between the treatments and the control.

Table 3 Treatments effect on soil macro- nutrients at the end of the experiment

Treatment	Total N (g kg ⁻¹)	Total P (g kg ⁻¹)	Total K (g kg ⁻¹)	Total Ca (g kg ⁻¹)	Total Mg (g kg ⁻¹)	Total S (g kg ⁻¹)	Total C (g kg ⁻¹)
C	20.01±0.00	5.83±0.14	20.11±0.85	6.58±0.27	2.23±0.12	0.31±0.54	184.81±0.02
CDA4	22.21±0.31	7.18±0.21	25.23±1.27	9.98±0.52	4.93±0.37	0.18±0.14	185.63±0.01
CD4	25.23±0.25	7.51±0.16	26.64±2.83	7.80±0.21	2.54±0.16	0.14±0.01	186.12±0.01
CDL4	26.45±0.38	7.32±0.03	27.49±2.68	7.03±0.27	2.73±0.36	0.14±0.19	187.75±0.01
Grand mean	23.48	6.96	24.86	7.85	3.15	0.19	186.07
LSD (p<0.05)	0.03	0.02	Ns	1.50	0.89	0.04	ns

Total N= Total nitrogen; Total P= Total phosphorus; Total K = Total potassium; Total Ca= Total calcium; Total Mg= Total Magnesium; Total S= Total Sulphur and Total C= Total carbon

The control pots had significantly (P< 0.05) higher mean values for total iron (Table 4), whereas CDL4 had significantly (P< 0.05) higher mean values for total copper over the other treatments. There was no

significant difference observed among the treatments for total zinc. The pH was significantly (P< 0.05) increased by applied CDA4 and CD4 respectively.

Table 4 Treatments effect on some soil micro- nutrients and pH at the end of the experiment

Treatment	Total Fe (mg kg ⁻¹)	Total Cu (mg kg ⁻¹)	Total Zn (mg kg ⁻¹)	pH
C	0.24±0.01	0.22±0.80	0.06±0.12	5.44±0.04
CDA4	0.17±0.01	0.24±0.68	0.07±0.05	5.56±0.01
CD4	0.12±0.02	0.24±0.20	0.07±0.04	5.56±0.01
CDL4	0.12±0.05	0.25±0.25	0.07±0.11	5.54±0.02
Grand mean	0.16	0.24	0.07	5.53
LSD (p<0.05)	0.05	0.18	Ns	0.01

Total Fe= Total iron; Total Cu= Total copper; Total Zn= Total zinc

The result for some nutrient-uptake (N, P, K, Ca and Mg) by the lettuce plant is shown on Table 5. The highest mean value for N- uptake was recorded in pots that were treated with CDL4, and this was followed by those treated with CD4. The percentage increase nitrogen which, pots that were treated with CDLA had over pots treated with CD4, CDA4

and C were 1.47%, 32.27% and 77.21% respectively. Similarly, pots that received CDLA had significantly higher P and K uptakes respectively. The highest significant mean values of 5.51±0.38 (g kg⁻¹) and 5.51±0.38 (g kg⁻¹) were gotten from CDA4 for the uptakes of Ca and Mg individually

Table 5 Treatments effect on some nutrient's uptake by lettuce plant

Treatment	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)	Ca(g kg ⁻¹)	Mg (g kg ⁻¹)
C	0.31±0.01	0.33±0.05	3.22±0.61	1.55±0.21	0.76±0.10
CDA4	0.88±0.00	1.00±0.06	11.08±0.72	5.51±0.38	5.51±0.38

CD4	1.34±0.01	1.44±0.14	17.55±0.02	4.90±0.39	1.77±0.15
CDL4	1.36±0.00	1.78±0.09	18.08±0.51	4.47±0.33	1.52±0.16
Grand mean	0.97	1.14	12.48	4.12	1.63
LSD (p<0.05)	0.46	0.56	4.93	1.08	0.56

N= Nitrogen uptake; P=Phosphorus; K= Potassium uptake; Ca= Calcium uptake and Mg= Magnesium uptake
 3.5 Treatment effect on soil stable carbon and nitrogen isotopes at the end of the experiment

The applied CDA4 had the highest mean value (-27.69‰) of depleted $\delta^{13}\text{C}$ (Figure 4) and C had the highest enriched $\delta^{13}\text{C}$ (-27.51‰) mean value. However, all the mean values were not statistically different from one another. All the pots that received the amendments had significantly (p<0.05) enriched $\delta^{15}\text{N}$ values, over the treatment with CDA4 having the highest mean value of 5.60‰.

Discussion

The finer cattle dung particle size fractions CD4 and CDL4 had increased lettuce heights, number of leaves, root and top biomass dry matter yields, total N, P, K, Ca and Mg uptakes, soil contents of N, P, K and pH. Similar findings of increase in crop yields and soil properties by cattle smaller size fractions were recorded by El-Nagar & Mohamed (2019). The reason for this increase could be that the grinding process of the cattle dung, created smaller particles with large surface area making it highly reactive (Chen *et al.*, 2010). More surface area will result to larger contact with the nutrients and hence faster nutrient dissolution rate for uptake. Finer fractions have also been reported to retain nutrients and water (Duarte *et al.*, 2019) which would be release for crop uptake. This also may be another reason why CDL4 and CD4 were able to improve lettuce yield as well

as contributed more to the lettuce biomass. Coarse manure particles have been reported to increase pH, Ca, Mg, Fe and Mn (Zhoa *et al.*, 2012), this may be the reason why CDA4 had more of these nutrients than the other treatments.

The amendments had higher $\delta^{15}\text{N}$ values than the control; this could be as a result of the cattle dung in the other treatments. Manure has been reported to have $\delta^{15}\text{N}$ enrichment in the soil and subsequently in plants (Szpak 2014). Soils that received manure amendments contain more $\delta^{15}\text{N}$ than the non-amended soil, because the lighter $\delta^{14}\text{N}$ volatilizes easily from the amended soil leaving the soil with heavy $\delta^{15}\text{N}$ (Högberg 1997, Petersen *et al.*, 1998).

More depleted $\delta^{13}\text{C}$ was observed in the cattle dung amended pots than in the control, which had more $\delta^{13}\text{C}$. Dungait *et al.*, (2010) stated that feeding cattle with naturally ^{13}C - enriched C_4 forage such as maize will produce a convenient source of natural abundance ^{13}C labelled dung. The depletion could be as a result of the amended soils contributing to the SOC (though not determined in the present study). Carbon isotopic technique helps to quantify the contribution of C_3 and C_4 to SOC (Shi *et al.*, 2017).

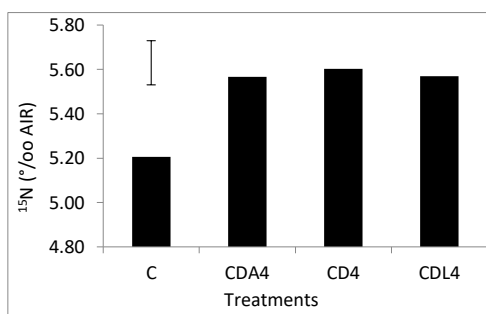
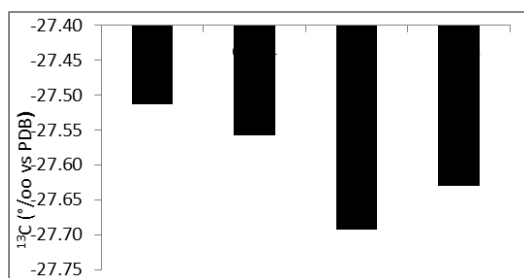


Fig 4: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition of lettuce shoot after harvest. Vertical bar represents LSD at ($P < 0.05$). PDB = Pee Dee Belemnite is the reference standard of $\delta^{13}\text{C}$. AIR³ or atmospheric nitrogen gas is used as the reference standard for measuring $\delta^{15}\text{N}$

Conclusion

This study evaluated the use of the principle of biomimicry to hasten nutrient release for crop production. This is aimed at helping the smallholder farmers meet the challenge of food production during and after covid-19 pandemic. The smaller cattle dung sizes of between 4.00 and <4.0 mm sieve size fractions improved the soil TN, TP, TK and their uptakes, which translated into increased lettuce growth, number of leaves, top biomass and root dry weight yields. Reducing the size of cattle dung also led to enriched $\delta^{15}\text{N}$ and depleted $\delta^{13}\text{C}$. Based on the results obtained, reducing cattle dung size before applying to the soil

will enhance crop growth. However, further field work is recommended.

Acknowledgment

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STUDIES ON THE PRODUCTION POTENTIALS OF TURMERIC INTERCROP USING LIVE MULCH SPECIES AS AN ALTERNATIVE MULCH IN SOUTH EASTERN NIGERIA

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ABSTRACT

A study was carried out in 2014 and 2015 to investigate the production potentials of Turmeric using live mulch on Turmeric production in South Eastern Nigeria. Treatments consisted of one variety of Turmeric (Maro variety), two varieties of orange fleshed sweet potato (Umuspo1 and Umuspo3), two varieties of egusi melon (large seed and small seed) and two varieties of cowpea (Ifebrown and Akidiani) Elephant grass (*Panicum maximum*) as grass mulch (sole) for turmeric and zero mulch as control. There were fourteen treatment combinations laid out in a Randomized Complete Block Design with three replicates. The results of the experiment showed increase in plant height, number of tillers and leaves. Depression of growth occurred due to Umuspo1 and Akidiani variety. The highest mean yield of turmeric was achieved in grass mulch (10.6 t/ha), while the highest mean yield of turmeric intercrop in both years was obtained from Egusimelon+T (8.53 for EMS and 8.35 for EML) t/ha. Turmeric intercropped with Akidiani+Turmeric and Umuspo3+Turmeric produced 7.42 and 7.12 t/ha yield respectively while the least mean yield of Turmeric intercrop was obtained in Umuspo1+T (3.99 t/ha) and IfeB +T (3.47 t/ha). The competitive indices were more than unity. The live mulch species gave a higher mean LER of between 1.05-1.42 except intercropping turmeric and Ifebrown which gave a low mean land equivalent ratio of 0.90 (LER < 1). The highest average LERs of 1.4-1.42 were obtained from turmeric + Egusimelon and Turmeric+Akidiani mixture. The mean competitive ratio was higher (1.00-1.22) in Egusimelon varieties intercropped with turmeric whereas the lowest were obtained from intercropping cowpea (0.01-0.06) and orange fleshed sweet potato varieties (0.03-0.04) showing high competitive ability of the component crops. The intercrop yield obtained in Umuspo1 is 17% higher than that of Umuspo3 which is not statistically difference from each other, on that premise Akidiani, Egusimelon and Umuspo3 are therefore recommended for intercropping as live mulch with Turmeric.

Keywords: Production, Turmeric, Intercrop, Live Mulch.

Introduction

Turmeric (*Curcuma longa* L. Lam) is a herbaceous perennial belonging to the family Zingiberaceae and a native of south Asia particularly India. (Ouma and Jeruto, 2010). Turmeric whose active ingredients curcumin is widely used as coloring agent for curries and is used in cooking many dishes. Mulching is one of the agronomic practices for turmeric production which is done immediately after planting and it induces the emergence of turmeric shoots. Incorporating cover crops like orange fleshed sweet potato (*Ipomoea batatas*L), melon and vegetable cowpea (*Vigna unguiculata*) into the system as live mulch provide live cover and minimize soil erosion (Eke-Okoro, 1997). It also helps to retain soil moisture, balances soil temperature and provides organic matter and nutrients as it decomposes.

Intercropping is a multiple cropping practice involving growing two or more crops in proximity (Gomez and Gomez, 1994). Land equivalent ratio (LER) is a concept in agriculture that described the relative land area require under sole cropping to produce same yield. It is used as a measure of land used competitive ratio and efficiency.(Taha and EL-Mahdy, 2014). Competitive ratio (CR) is proposed as a measure of intercrop competition to indicate the number of times by which one component crop is more competitive than the other.(Khonde *et al.*, 2018). In our farming systems mulching is not a common practice, introduction of different live mulch species in turmeric inter cropping

system will help to smother weed, improve soil fertility and increase yield. But there is a dearth of information on the use of live mulch cover crops as an alternative mulching system in turmeric production. Therefore the objective of the experiment is to determine the effect of live mulch cover crops on the productivity of turmeric.

Materials and Method

The experiment was conducted in the research farm of National Root Crops Research Institute(NRCRI) institute in 2014 and 2015 cropping season. The existing vegetation was cleared ploughed harrowed and ridged. Plot size was 3m x 4m with plant spacing of 30cm x 50cm in a randomized complete block design (RCBD) Treatments comprised of Fourteen combinations as follows

- Sole turmeric +grass mulch (SGM)
- Sole turmeric + 0 mulch (control)
- SoleUmuspo 1(OFSP)
- Sole Umuspo 3(OFSP)
- Sole Ifebrown (Cowpea)
- Sole Akidiani (Cowpea)
- Sole Egusimelon (Largeseed)
- Sole Egusimelon (Small seed)
- Turmeric intercropped with Umuspo1 (Umuspo1+T)
- Turmeric intercropped with Umuspo3 (Umuspo3+T)
- Turmeric intercropped with Ifebrown (IFEB+TUM)
- Turmeric intercropped with Akidiani (AKD+TUM)
- Turmeric intercropped with Egusimelon large seed (EML+TUM)
- Turmeric intercropped with Egusimelon small seed

(EMS+TUM)

Management and cultural practices were done. Growth data was collected at 4,8,12 and 16 weeks after planting (WAP) while yield data was recorded during harvest at five months after planting and was subjected to analysis of variance using General Statistical Package. Mean separation was carried out using least significant difference (LSD) $p < 0.05$.

Land equivalent ratio was calculated thus;

$$\frac{\text{Sole crop yield of crop A} + \text{Sole crop yield of crop B}}{\text{Intercrop yield of crop A} + \text{Intercrop yield of crop B}}$$

Competitive ratio (CR) was calculated as the ratio of individual LERs of the component crops (Willey and Rao, 1980; Assim *et al.*, 2008).

Results and Discussion

Effect of treatment on plant height was significant in 2015, (Fig 1) this gave the highest plant height at EML+T+TUM and SGM while Ifebrown+Turmeric gave the lowest plant height in both years during the growth period of this study, (Figs, 1 and 2). This was due to the compression effect of this cowpea variety on turmeric stands, the same observation was also similar with Umuspo1 + Turmeric mixture. Number of leaves in figures 3 and 4 were significantly affected by live mulch which increased profusely as growth increased. (Vandermeer, 2009). In 2015, number of leaves were reduced due to compression effect of Umuspo1+T and IFB+T at 12 and 16 WAP. This could be as a result of change in soil factors and change in rainfall pattern which occurred in 2015. This could have stimulated the growth of component crop (Obasi, 2019). Tiller production was more in SGM in 2014 and EML+TUM in 2015 while the Umuspo1+TUM and

IfB+TUM gave the least tiller number. (Figs 5 and 6) The highest mean weight in tonnes per hectare was achieved in grass mulch turmeric which yielded 38.5% and 54.7% in 2014 and 2015 respectively over the control. (Table 1) This revealed that grass mulch (*Panicum maximum*) used in this experiment undergone some decomposition and mineralization which further enriched the soil fertility thereby increasing the soil nutrient pool available for the crops. This is in line with the report by Chukwu and Agugo (2003). Land Equivalent Ratio (LER), and competitive ratio (CR) showed very high productivity in the system. It was observed that all the combinations were above and equal to unity ($LER \geq 1.0$) except when Ife Brown (0.80) was intercropped with turmeric in 2014. This could be due to difference in cultural practices and higher intra specific competition involved in the system.

Furthermore, the observation made during this study (Table 2) showed that higher mean yield of grain (AKD, EMS and EML in the system) was obtained in sole (2tonnes) than intercrop (1tonne) Umuspo1 and Umuspo3 gave mean tuber yield of 12.1 and 11.2 in sole and 10.3 and 8.80 tonnes per hectare in intercrop respectively. This showed that higher yield can be achieved in sole crop than in mixture due to less intra row specific competition effect on nutrient requirement (Obasi and Muoneke 2011). The mean intercrop yield obtained in Umuspo1 is 17% higher than that of Umuspo3 which is not statistically difference from each other. The study revealed that intercropping turmeric and cover crop species is of benefit in turmeric production. This also depict compatibility of the component crops which was observe more on Akidiani, Egusi melon and Umuspo3 which are therefore recommended for intercropping as live mulch with turmeric.

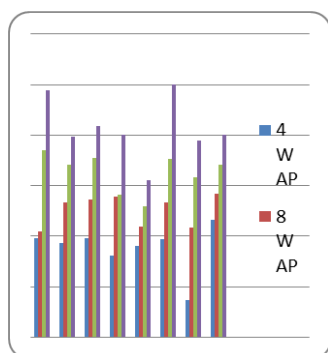


Fig 1 Effect of live mulch on plant height of Turmeric (2014)

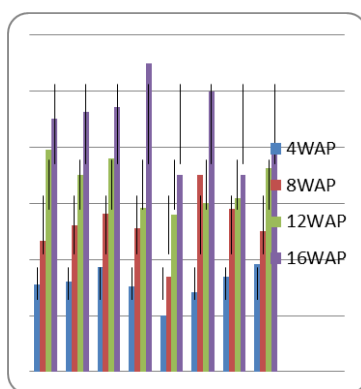


Fig 2 LSD (0.05) Effect of live mulch on plant height of Turmeric (2015)

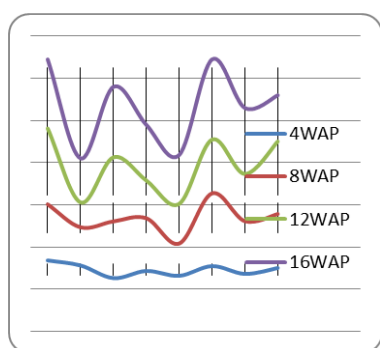


Fig 3 LSD(0.05)
Effect of live mulch on number of leaves of Turmeric (2014)

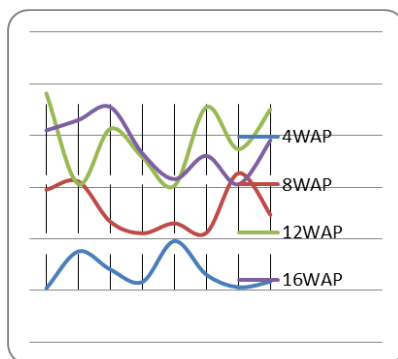


Fig 4 LSD (0.05) Effect of live mulch on number of leaves of Turmeric (2015)

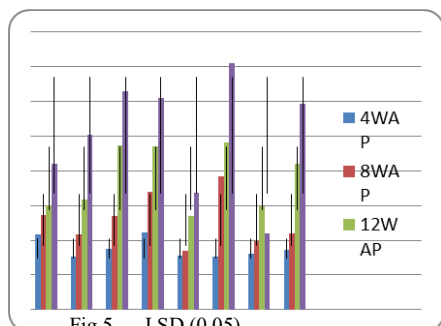


Fig 5 LSD (0.05)
Effect of live mulch on tiller number of Turmeric (2014)

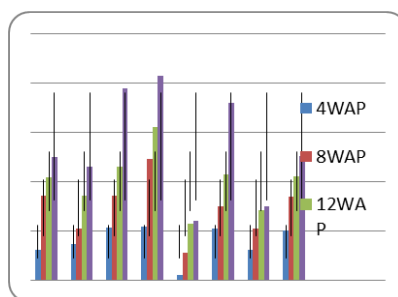


Fig 6 LSD (0.05)
Effect of live mulch on tiller number of Turmeric (2015)

Conclusion and Recommendation

Sole grass mulch gave the highest yield while Egusi (melon) varieties are the most compatible with turmeric, followed by Umuspo3 and Akidiani. Ife brown and Umuspo1 suppressed the growth of the component crops due to their spreading habits. The LERs in the intercrop were greater than unity (LER>1) indicating yield advantage in terms of land usage except IfB+TUM which gave a lower value of 0.90.

This showed low productivity. Competitive ratio (CR) recorded higher mean values (1.00-1.22) in Egusi (melon) /turmeric mixture, depicting strong compatibility. Egusi (Melon) varieties, Umuspo3 and Akidiani, were compatible with turmeric in mixture and are therefore recommended for higher yield and productivity.

Table 1: Effect of Live Mulch on Productivity and Yield (t/h) of Turmeric

Treatments	LER			CR			Turmeric Yield in tonnes per hectare		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
0 mulch	-	-	--	--	--	--	8.52	6.13	7.34
AKD + T	1.33	1.50	1.42	0.06	0.11	0.09			

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							8.11	6.72	7.42
Ems + T	1.36	1.40	1.40	1.41	1.03	1.22			
							9.03	8.02	8.53
Eml + T	1.60	1.24	1.42	1.04	0.81	1.00	9.23	7.42	8.33
IFB + T	0.80	1.01	0.90	0.01	0.03	0.02	3.46	3.48	3.47
SGM	-	-	-	-	-	-	11.77	9.48	10.63
Umuspo1 + T	1.10	1.00	1.05	0.01	0.07	0.01	3.15	4.83	3.99
Umuspo3 + T	1.02	1.40	1.21	0.08	0.56	0.32	6.19	8.05	7.12
LSD 0.05	0.52	0.39	-	1.23	1.20	-	1.17	1.92	-

Table 2: Yield in tonnes per hectare of live mulch cover crops

Treatments	Sole		Mean	Intercrop		Mean
	2014	2015		2014	2015	
AKD +T	2.58	2.23	2.41	1.21	1.60	1.41
EMS+T	2.53	2.56	2.50	1.16	2.80	1.98
EML+T	1.88	2.22	2.05	1.22	1.41	1.32
IFE+T	1.50	1.91	1.70	1.37	1.77	1.57
Umuspo1+T	12.4	11.7	12.1	9.79	10.9	10.3
Umuspo3+T	12.0	10.3	11.2	8.50	9.10	8.80
LSD	0.11	0.34	-	0.28	0.33	-

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EVALUATION OF GINGER (*Zingiber officinale*) RHIZOME EXTRACT AS ABIOTIC ELICITOR OF COCOYAM SECONDARY METABOLITES

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ABSTRACT

Plants respond to biotic and abiotic stimuli such as stress, pests and predators by producing secondary metabolites. Moreover, these metabolites are produced in limited amount and may not be adequate in plant defence. However, there are reports on successful use of biotic and abiotic elicitors in culture room to enhance secondary metabolite production. In this experiment, we made attempt to increase the quantities of secondary metabolites produced in cocoyam plant by using extract of ginger rhizome as abiotic elicitor. Extract of ginger was evaluated for elicitation of secondary metabolite production in cocoyam plants, in a screen house experiment. The experimental design was Randomized Complete Block with three replicates. The results of the study showed that 1% ginger extract applied for 3 days at a rate of 5 ml/day increased the concentrations of phenols, alkaloids, flavonoids, tannins and anthocyanins by (0.04, 0.03, 0.19, 0.03 and 0.03 mg/100ml) respectively, 5% gave an increment of (0.08, 0.07, 0.31, 0.02, 0.04 mg/100ml) respectively while the control which is the vehicle gave the following increases (0.00, 0.03, 0.09, 0.01 and 0.02) respectively. These increases in secondary metabolites levels were observed to be dose dependent and significantly higher than that given by the control. In addition, preliminary phytochemicals analysis of acetone extract of ginger rhizome gave the following results (%), phenols (0.712±0.3), tannins (0.31±0.4), flavonoids (1±0.3) and alkaloids (66.55±0.2). The results of the study suggest ginger as a potential biotic elicitor which can further be explored.

Introduction

Secondary metabolites are organic products of plants secondary metabolism which include phenols, alkaloids, flavonoids, tannins and anthocyanins. They are not directly involved in normal plant growth and development but are very important for their survival and provide potent defense in plants. Plants can be sensitised to increase the biosynthesis of their secondary metabolites by exposing them to elicitors which are compounds that can stimulate the plant defence and promotes secondary metabolism. (Beckers and Conrath 2007). Eliciting technique involves introducing into the plant cell medium or directly onto the plant a substance (elicitor) that can trigger stress thereby enhancing the production of secondary metabolites (Narayani and Srivastava, 2017). Increasing the secondary metabolites profile of tissue culture plants via elicitation has been reported by several authors as an effective strategy for biotechnological production of bioactive compounds in plants (Mu, *et al.*, 2009; Naik, *et al.*, 2016). Elicitors can be biotic or abiotic, biotic elicitors are substances of biological origin such as polysaccharides and microorganisms while abiotic elicitors are physical, chemical or hormonal factors used to enhance secondary metabolite produced. Both biotic and abiotic elicitors have been found effective in inducing the production of secondary metabolites and defensive enzymes in a screen house and tissue culture experiment (Upadhyay, *et al* 2016, Mu, *et al.* 2009). Eliciting by inducing osmotic stress is reported to be an effective abiotic means of enhancing secondary metabolite production in plant (Liu and Cheng, 2008). In this experiment we used concentrated ginger extract to induce osmotic stress in order to elicit secondary

metabolite produced in cocoyam plant in screen a house experiment. This experiment seeks to evaluate the effect of varying concentrations of ginger extract applied directly on cocoyam leaves will have on the concentrations of secondary metabolites produced by the cocoyam plant.

Materials and Method

Present investigation was carried out in screen house of National Root Crops Research Institute Umudike. Soil was collected from the upper 0-15 cm layer and was sterilized by heating for 12 hours. The sterilized soil was filled in 5 kg capacity plastic pots and kept in screen house and the experiments were laid out in a completely randomized design with three replications. The pots were watered and left for two days to maintain appropriate moisture for proper seed germination. Cocoyam (*Colocasia esculenta*) corms were sown three per pot and pots were watered regularly as at when required to maintain optimum moisture. Forty days after germination, the top leaves were collected.

Fresh ginger (*Zingiber officinale*) rhizomes were sliced and shade dried until constant weight was obtained, then milled into fine powder. One hundred grams of each powder was soaked in 300ml of acetone for 48 hours, filtered and the filtrate was left for all the solvent to completely evaporate, then stored in cool dry place until when required to be used. Two concentrations 1% and 5% were constituted from the extract with distilled water to which 2 drops of tween 20 was added and then filled into labelled spray bottles. Each group was sprayed with 10ml of the respective formulation for 3 days. Group 1 and 2

received 1% and 5% extract formulation respectively while the control, group 3 received distilled water to which two drops of tween 20 was added. The treated plants were left for a period of three days after which the top leaves were collected from all the groups for phytochemical analysis. Both the leaves collected

before and after treatment were freeze dried in a lyophilizer at a temperature of -40^oC until crispy dry. They were crushed into powder using laboratory mortar and pestle. Phytochemical analyses were conducted on both sets of collected leaf samples.

Results and Discussion

Table 1: Effect of ginger extract on phytochemical level of cocoyam leaf

Treatments	Alkaloids mg/100g	Flavonoids mg/100g	Tannins mg/100g	Phenols mg/100g	Anthocyanins mg/100g
Group1 B	0.47 ^d	1.15 ^d	0.43 ^a	0.65 ^c	0.16 ^c
Group1 A	0.5 ^{bc}	1.34 ^b	0.46 ^a	0.69 ^b	0.19 ^{ab}
Group2 B	0.46 ^d	1.16 ^d	0.45 ^a	0.65 ^c	0.17 ^{bc}
Group2 A	0.53 ^a	1.47 ^a	0.47 ^a	0.73 ^a	0.21 ^a
Group3 B	0.48 ^{cd}	1.13 ^{cd}	0.45 ^a	0.63 ^c	0.16 ^c
Group3 A	0.51 ^{ab}	1.22 ^c	0.46 ^a	0.63 ^c	0.18 ^{bc}

Abbreviations: B - Before treatment; A – After treatment.

Table 2: Comparative rise in secondary metabolite levels

Treatments	Alkaloids mg/100g	Flavonoids mg/100g	Tannins mg/100g	Phenols mg/100g	Anthocyanins mg/100g
Group1	0.03	0.19	0.03	0.04	0.03
Group2	0.07	0.31	0.02	0.08	0.04
Group3	0.03	0.09	0.01	0.00	0.02

Table 3: Secondary metabolite levels in ginger

Phytochemical Constituents	Content
Phenols (mg/100g)	0.712±0.3
Tannins (mg/100g)	0.031±0.4
Flavonoids (%)	1±0.3
Alkaloids (%)	66.55±0.2

By the third day after applying treatment, the plants in groups 1 and 2 appeared wilted with group 2 plants wilting more than group 1. Wilting suggests osmotic pressure on the plant, however the leaves of the control, group 3, remained fresh.

Comparative analysis of secondary metabolite in the cocoyam leaf before (B) and after (A) treatment is represented in table 1. The evaluated metabolites are alkaloids, flavonoids, phenols, tannins and anthocyanins. From table 1, metabolite concentration after treatment with the elicitor is observed to be significantly (p<0.05) higher than the concentration before treatment for most metabolites in groups 1 and 2. However the increase in concentration of flavonoids is observed to be higher than the other metabolites with an increase of 0.19 and 0.31mg/100ml respectively for group 1 and 2 (Table 2), but no significant increases in concentration of metabolites were observed for plants in group 3. This method of increasing the concentration of metabolites by foliar application of exogenous elicitor is in agreement with the experiment of Perez-Balibrea *et al.* (2011) where the concentration of secondary metabolites in broccoli sprouts were increased by spraying with varying concentrations of exogenous elicitors.

Tables 1 and 2 show the treatment effects were dose dependent with 5% treatment giving a higher rise than the 1%. This report agrees with the findings of Namdeo *et al.* (2002) who reported ajmalicine accumulation higher in cells elicited with higher concentration (5%) of elicitor extract than lower concentration (0.5%).

Worthy of note is concentration of tannins before and after treatment which were not significantly (p>0.05) different groups, 1 and 2, suggesting that elicitation with ginger extract has no effect on tannins production in cocoyam plant. Reports on elicitation by other scientists equally shows different classes of secondary metabolites respond to different elicitors (Perez-Balibrea *et al.*, 2011; Upadhyay *et al.*, 2016; Mu *et al.*, 2009). The higher accumulation of secondary metabolites in the groups treated with the extract than the untreated supports the claim that secondary metabolites accumulation may represent chemical adaptations to environmental stress (Isa, 2019).

The result of secondary metabolite content in ginger, table 3, shows that the metabolites present in the elicitor were not same as metabolites elicited therefore suggesting elicitation to be a product of biological process in plants. This may support the hypothesis of Thakur and Sohal (2013) that elicitation involve the triggering of transduction mechanism in plant which may activate the primary immune response.

Conclusion and recommendation

Elicitation enhances secondary metabolism in plants

although the exact mechanism of elicitation is not exactly understood. The findings of this study has shown a necessity in creating more awareness to agriculturists and other plant scientists that increasing the net yield of plants that are either constantly jostled

or jabbed by diseases could be achieved by the process of elicitation. Therefore the need to explore the potential of elicitation of plant secondary metabolites for a sustainable agriculture.

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PRODUCTIVITY, PROXIMATE AND PHYTOCHEMICALS CONTENTS OF *Pleurotus ostreatus* (Jacq.Ex. Fr) P. Kumm. CULTIVATED ON CARBONIZED SAWDUST

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ABSTRACT

Mushrooms require carbon for their growth and development more than any other mineral element. The bulk of this carbon is found in the substrates where they grow. This study was therefore conducted to determine the influence of carbonized sawdust (CS) on the fructification and some myco-chemical constituents of *Pleurotus ostreatus* fruit bodies. A given quantity (2.5kg) of dry sawdust was carbonized at 15, 25 and 35mins and made into 5 replications of 500g each, including control of non-carbonized sawdust. The time of carbonization served as treatment. Actively growing mycelium (50g spawn) of *P. ostreatus* was used in each case to inoculate substrate, stuffed in 2.5liter transparent plastic bucket. Treatments were replicated in a completely randomized design (CRD). Results showed that 15 and 25 mins CS produced primordia within the shortest duration of 14days. Control produced the highest number of fruit bodies (140) with smallest pileus diameter (3.78±1.06), which resulted to low fruit body yield (141.33g) and biological efficiency (5.65%) when compared to those treatments at 15 mins (524.83g and 20.99%), 25mins (376.89g and 15.08%) and 35mins (513.42g and 20.54%). Sawdust carbonization triggered a significantly ($p \leq 0.05$) decreased amount of ash from 4.28±0.03 in control to 3.53±0.06 in 25mins; crude protein from 20.17±0.01 in control to 19.50±0.13 in 15mins and ether extract from (5.18±0.03 control - 3.71±0.02 in 35mins CS); Finally, CS significantly ($p \leq 0.05$) decreased the concentration of studied phytochemicals in the fruit bodies when compared to control. Therefore, commercial oyster mushroom growers targeting bigger sized, crude fibre and carbohydrate rich-fruit bodies should adopt sawdust carbonization techniques for more yield and profit maximization.

Keyword: Basidiomycota, Carbonized, Sawdust, Fructification

INTRODUCTION

Mushroom is a general term applied mainly for the fruiting - bodies of macro - fungi that belong to the division *Basidiomycota* and a few members of the division *Ascomycota*. They are saprophytes and are known to grow on a wide variety of substrates and habitats. Mushrooms which may be epigeous or hypogeous, can be large enough to be seen with the unaided eyes and can be picked by hand (Chang and Miles, 2004). There are edible and poisonous mushrooms and both categories possess nutrient and medicinal values.

According to Okwulehie and Odunze (2004a), mushrooms generally contain low oil and fat, and because of the low content of oil and fat in mushrooms, they are recommended as good supplements for patients with cardiac problems.

The ever-growing need for cheap nutritious foods and the lack of protein in developing countries have led to the development of mushroom cultivation initiative (Zodrazil, and Burnnert, (1997).).

Mushrooms are rich sources of carbohydrates, proteins, vitamins, and minerals. Mushrooms grow on decay organic matter rich in lignin, cellulose and other complicated carbohydrates, and on large quantities of agro-industrial wastes that are produced worldwide often causing environmental and health problems. Mushroom cultivation represents the only current economically viable biotechnology process for the

conversion of waste plant residues from forests and agriculture (Wood and Smith, 1997).

According to Wood and Smith, (1987), mushroom cultivation represents the only current economically viable biotechnological process for the conversion of waste plant residues from manufacturing industries, forests and agricultural farmlands into protein rich food (mushrooms) and other valuable intermediate or finished products. Conversion of lignocellulose into food and feed rich in protein by fungi offers an alternative means for developing unconventional sources of proteins as food/feed (Mane *et al.*, 2007). Hence, the use of different agricultural wastes such as banana leaves, Alam grass (*Imperata cylindrica*) straws, bagasse, husk, pods, pulp, waste paper, corn cobs etc. as substrates is a good development.

Many researches have been carried out to evaluate the use of different substrates in the cultivation of oyster mushroom especially *Pleurotus ostreatus*. Baysal *et al.* (2003) conducted an experiment involving the cultivation of *P. ostreatus* on waste paper with addition of chicken manure, peat, and rice husks.

Agro-wastes are ordinarily not of importance to humans. They are unsightly and constitute nuisance to the society. Agro-wastes also cause forest fires leading to the destruction of lives and properties. Mushrooms are known to grow on a wide variety of substrates and habitats (Adesina *et al.*, 2011). The agricultural wastes are converted into edible biomass in the form of fruit-

bodies.

Carbonization of substrate has been reported to be an effective means of growing mushroom (Reuben *et al.*, 2018). Biochar derived from carbonizing substrates have been used to improve the carbon composition of mushroom and has also been used as soil amendment to improve soil productivity, carbon storage, and filtration of percolating soil water (Lehmann and Joseph, 2009). Hence, it is necessary to study and document the outcome of using carbonized sawdust as substrate for the growth and yield of *P. ostreatus* fruit bodies.

The aims of this investigation were to assess the yield of *Pleurotus ostreatus* fruit-bodies grown on carbonized sawdust; to determine the effect of the carbonized sawdust on the micro-chemical contents of the *Pleurotus ostreatus* fruit-bodies and to ascertain the influence of carbonized sawdust on the fruiting duration of *pleurotus ostreatus* fruit-bodies. Others were to evaluate the effect of carbonization on some macro-morphological characteristics of *Pleurotus ostreatus* fruit-bodies, determine the influence of carbonized sawdust on the yield and biological efficiency of *Pleurotus ostreatus* fruit-bodies and to ascertain the effect of carbonization on some myco-chemical composition of *Pleurotus ostreatus* fruit-bodies.

MATERIALS AND METHODS

The study was conducted at the mushroom house of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located between longitude 7° and 7°05'05"E and latitude 5° and 5°25'05"N; with humid tropical climate. Rainfall is bi-modally distributed with peaks between July and September of each year. Annual rainfall is approximately 170mm, spread between April and November each year. (Onyeizu *et al.*, 2017).

Pure mycelia culture of *Pleurotus ostreatus* was obtained from the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia state.

The sawdust used for this study was obtained from Timber Main market located along Ikot-Ekpene road Umuhia, Abia State.

Substrate Carbonization and treatments:

Sawdust measuring 2500g each was carbonized by Pyrolysis, separately in a heated metallic drum at 15, 25 and 35mins at 180°C. Another sawdust measuring 2500g served as control. During heating, the substrate was stirred regularly using a wooden stirrer to ensure uniform circulation of heat. The three levels of carbonized sawdust were allowed to cool, after which they were moistened with tap water (It is not better to moisten and allow to cool). It seems that you are describing the process of biochar production.

Allowing it to cool will attract oxygen that will cause continuous burning leading to the material being more alkaline with less carbon. according to the modified method of Chang and Miles (2004). Palm test method was used to determine optimum moisture content of the sawdust. The treatments were sawdust carbonized for 15 minutes, 25 minutes, 35 minutes and a control with sawdust that was not carbonization.

Preparation of Cropping Room

Before the substrate inoculation, the cropping room floor was scrubbed with water and detergent. The walls were rid of cobwebs and disinfected with Detol disinfectant. The room was well illuminated and ventilated by opening the window panes. This equally maintains the daytime temperature at 27±2°C and improves ventilation in the room (Okwulehie and Odunze, 2004).

Substrate Pasteurization

All the substrates were separately stuffed into Bagco bags, codified and lowered into metallic pasteurization columns and were pasteurized for 3hours at 100°C. After that they were allowed to cool overnight.

Substrates Inoculation and Experimental design

The treatments were replicated four times in a Completely Randomized Design (CRD). The substrate and their combination dispensed in 2-5 litre perforated transparent plastic buckets perforated were inoculated following the method of Okwulehie and Okwujako, (2008). Actively growing spawn of *Pleurotus ostreatus* were sprayed in layers of the substrate.

Fruiting and Harvesting

Fruiting of the mushroom started on the different carbonized substrates from the 14th day after inoculation. The first flush was harvested from the 25-30th September 2019, and the second flush was harvested from the 2nd-7th of November 2019. The experiment lasted for 31 days.

Measurement parameters

(i). Yield/Number of fruit-bodies

The fruit bodies were counted directly after splitting them from their bunch from each bucket.

(ii). **Pileus diameter (cm):** This was measured in centimetre (cm) with a transparent plastic ruler from one edge of the pileus across the stipe to the other edge.

(ii). **Stipe length (cm):** Similarly, the length of the stipe was also measured in centimetre (cm) using a transparent plastic ruler.

(iii) Fresh and dry fruit-bodies:

The fresh and dry weight of the fruit-bodies were obtained by weighing the fruit bodies using a digital scale (Model, 2000) made in the USA.

The fresh fruit-bodies were put in brown envelopes and dried in a hot air oven ,at 80°C for 48 h.

Determination of Biological Efficiency:

The biological efficiency (BE) i.e., the percentage yield of fresh mushroom fruit bodies per dry weight (g/kg) substrate was calculated using the formula recommended by Chang and Milles (2004), viz:

$$B.E = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times \frac{100}{1}$$

Determination of the Phytochemical composition

(i). Flavonoids Flavonoids in the dry samples was determined following the methods of Harborne, (1973). Ten (10) g of the sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was transferred into a crucible of known weight, evaporated to dryness over a water bath and dried in an electric oven to a constant weight.

The flavonoid content was expressed in percentage thus:

$$\% \text{ Flavonoids} = \frac{\text{weight of residue}}{\text{weight of sample}} \times \frac{100}{1}$$

(ii). Alkaloids

To estimate the amount of alkaloids, 5g of the dry powdered sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4 hrs. This was filtered and the extract was concentrated by evaporating in boiling water to one quarter of the original volume. Concentrated Ammonium hydroxide (NH₄OH) was added drop-wise to the extract until the precipitation was complete. The suspension was allowed to settle and the precipitate was collected by filtration and weighed (Harborne, 1973). The Alkaloids were expressed in percentage as:

$$\% \text{ alkaloids} = \frac{\text{weight of residue}}{\text{weight of sample}} \times \frac{100}{1}$$

(iii). Tannins

Tannins were determined according to the method of Harborne, (1973). A measure (0.5g) of the sample was dispensed into a flask containing 10ml of 2m HCl and shaken for 5mins and transferred into a volumetric flask and made up to 50ml. The mixture was filtered and 5ml of the filtrate was introduced into a test tube. 3ml of 0.1 NHCl and 3ml of 0.008m of potassium ferro-cynide (K₃F[CN]₃) were added. The absorbance was read at 720nm within 10mins.

(iv). Saponins

A measure of 0.1 g of the powdered samples were boiled with 5 ml of distilled water for 5 minutes and decanted while still hot. The filtrate was used for frothing and emulsion tests.

Frothing test: One milliliter (1 ml) of the filtrate was diluted with 4 ml of distilled water and the mixture shaken vigorously and observed on standing for suitable froth.

$$\% \text{ saponins} = \frac{\text{weight of residue}}{\text{Weight of}} \times \frac{100}{1}$$

sample 1

(v). Phenols

The Phenolics content of the sample (fat-free sample), two (2g) of the sample was de-fatted with 100ml of diethyl ether, using soxhlet apparatus for 2 hours. To extract the phenols component of the sample, the fat-free sample was boiled with 50ml of either for 15mins. Five millilitres of the extract was Pipette into a 50ml flask into which 10ml of distilled water, 2ml of ammonium hydroxide (NH₄OH) solution and 5ml of concentrated amyl alcohol had been added. The mixture was made up to mark and left to react for 30 min. for colour development. The absorbance of solution was read using a spectrophotometer at 505nm wave length (Harborne, 1973).

STATISTICAL ANALYSIS

Data collected was analyzed by Analysis of Variance (ANOVA) as described by Steel and Torrie (1984). Turkey's Honestly Significant Difference (HSD) test at probability level of 5% was used to separate the means and standard deviation (means ±SD)

RESULTS AND DISCUSSION

RESULTS

The result of fruiting body and fruiting duration of *P. ostreatus* using 15mins CS, 25mins CS, 35mins CS and Control is presented in Table 1. The results showed that control started producing fruit bodies after 17days with a total of 140 fruit bodies 15- and 24-minsCS produced fruit body primordia after 14days with a total of 103 and 122 fruit bodies respectively while 35mins CS produced fruit body primordia, after 19days with a total of 138 fruit bodies

Table 1: Fruiting duration and fruit body number of *P. ostreatus*.

Substrate	Fruiting Duration	FBN
Control	17	140
15 Mins CS	14	103
25 Mins CS	14	122
35 Mins CS	19	138
Mean	16	125.75

FBN = Fruit body number, CS= Carbonized Sawdust

The results of the morphological characters of *P. ostreatus* fruit bodies using 15mins CS, 25mins CS, 35mins CS and Control is presented in Table 2. The results showed that Pileus diameter (PD) of fruit bodies harvested from 35mins CS was highest at 5.48±1.82, followed by those of 15mins CS (5.33±3.06). Fruit bodies with the smallest PD were obtained at control (3.78±1.06) followed by 25mins CS (4.03±2.18). Stipe-length (SL) was significantly (P>0.05) high in fruit bodies obtained from 35mins CS at 3.46±1.63, followed by 15mins CS (2.82±0.81) while control and 25 mins CS were 2.25±0.55 and 2.37±0.78 respectively. It was also observed that fruit bodies from 35mins CS had the highest weight

(4.90±0.00) while the lowest was obtained at 25mins CS at 3.10±0.00.

Table 2: Morphological characters of *P. ostreatus* fruit bodies

Substrate	PD (cm)	S.L (cm)	WT (g)
Control	3.78±1.06 a	2.25±0.55 a	3.70±0.00 b
15 Mins CS	5.48±1.82 b	3.46±1.63 c	4.90±0.00 d
25 Mins CS	4.03±2.18 a	2.37±0.78 a	3.10±0.00 a
35 Mins CS	5.33±3.06 b	2.82±0.81 b	3.80±0.00 c

Means followed by the same alphabet within column are not significantly different by Turkey's

HSD test (p<0.05), means±SD (n=3). PD= Pileus diameter, S.L= stipe length, WT= weight, CS=carbonized sawdust

The results of yield and Biological Efficiency of *P. ostreatus* fruit bodies using 15mins CS, 25mins CS, 35mins CS and Control is presented in Fig.1. The result showed that 2500g of sawdust carbonized at three-time interval levels; including control, produced varying amount of *P. ostreatus* fruit bodies as follows. Control produced the lowest quantity of fruit bodies at 141.33g and 5.65% representing yield and biological efficiency respectively. This was followed by 25mins at yield (376.89) and biological efficiency of 15.08%. The fruit body yield produced at 35mins CS produced of 513.42g and BE of 20.54% while 15mins produced the highest amount of fruit bodies at yield of 524.83g and BE of 20.99%.

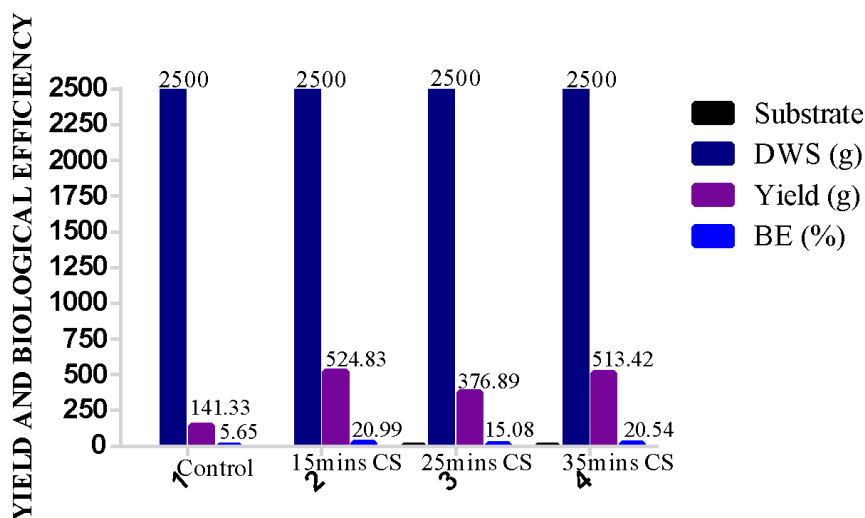


Fig. 1: Yield and biological efficiency of *P. ostreatus* fruit bodies.
DWS= Dry weight substrate, BE= Biological efficiency, CS= Carbonized sawdust

Table 3: Proximate composition of fruit bodies

Substrate	MC	DM	ASH	CP	EE	CF	CHO
Control	10.27± 0.04 ^c	89.73±0.04 ^a	4.28±0.03 ^c	20.17±0.01 b	5.18±0.03 c	3.64±0.02 a	56.47±0.13 a
15Mins CS	9.85±0.01 ^a	90.15±0.01 ^c	3.77±0.04 ^b	19.50±0.13 a	4.32±0.12 b	4.71±0.01 d	57.85±0.21 a

25Mins CS	10.05±0.04 ^b	89.84±0.05 ^b	3.53±0.06 ^a	19.70±0.09 ^a	4.08±0.06 ^b	4.21±0.04 ^c	58.43±0.01 ^a
35Mins CS	10.17±0.17 ^b	89.84±0.05 ^b	3.67±0.02 ^b	19.79±0.01 ^a	3.71±0.02 ^a	3.83±0.04 ^b	60.35±2.26 ^a

Means followed by the same alphabet within column are not significantly different by Tukey's HSD test (p≤0.05), means ± SD (n=3). MC = Moisture content, DM=dry matter, CP= crude protein, EE= Ether extract, CF= Crude fibre, CHO = Carbohydrate, CS= carbonized sawdust

Table 4: Phytochemical composition of fruit bodies

Substrate	FLV.	ALK.	TAN.	SAP.	PHEN.
Control	1.81±0.01 ^c	0.58±0.02 ^c	1.87±0.04 ^b	0.85±0.00 ^c	0.44±0.02 ^b
15 Mins CS	1.73 ±0.0 ^{a,b}	0.49±0.01 ^b	1.74±0.02 ^a	0.81±0.02 ^{b,c}	0.34±0.03 ^{b,c}
25 Mins CS	1.70±0.00 ^a	0.44±0.02 ^{a,b}	1.79±0.01 ^{a,b}	0.76±0.01 ^{a,b}	0.31±0.01 ^a
35 Mins CS	1.76 ±0.00 ^{a,b}	0.37±0.01 ^a	1.82±0.03 ^{a,b}	0.73±0.01 ^a	0.23±0.01 ^a

Means followed by the same alphabet within column are not significantly different by Turkey's HSD test (p≤0.05), means±SD (n=3).

FLV.=Flavonoids, ALK.=Alkaloids, TAN.= Tannins, SAP.= Saponins, PHEN.= Phenols, CS= Carbonized sawdust

The result of phytochemical composition of fruit bodies of *P. ostreatus* using 15mins CS, 25mins CS, 35mins CS and Control is presented in table 4. The results of Phytochemical composition of fruit bodies indicated that flavonoids content of fruit bodies from control was recorded at 1.81±0.01 while those of 15; 25 and 35 mins CS were found at 1.73±0.01, 1.76±0.00 and 1.76±0.00 respectively. Alkaloids were found at 0.58±0.02 in fruit bodies from control but 0.49±0.01, 0.44±0.02 and 0.37±0.01 respectively. Tannins content was recorded at 1.87±0.04, 1.74±0.02, 1.79±0.01 and 1.82±0.03 in fruit bodies from control 15, 25 and 35 mins CS respectively. Highest amount of Saponins was found in control (0.85±0.00) and 15 mins CS (0.81±0.02) while lowest was recorded in fruit bodies from 25 (0.76±0.01) and 35 mins CS (0.73±0.01). Phenols content was high in fruit bodies from control (0.44±0.02) compared to those of 15, 25 and 35 mins CS which were not significantly different at 0.05 probability

DISCUSSION

Shah *et al.* (2004) recorded longer fruiting time of *P. ostreatus* with saw dust, wheat straws and other agro-wastes combinations. They reported that the longest fruiting duration was 35days while the shortest was 27days. The short period of commencement of fruiting recorded in this work especially at 15 and 25 mins CS, could be due to the fact carbonizing sawdust within this time frame could provide the optimum biotic and abiotic environment required to trigger early fruiting, possibly by lowering the lignocellulosic composition of the sawdust.

The period of 15 and 25mins CS produced the smallest number of fruit bodies at 103 and 122. This justifies the claims by Stamets (2000) which noted that substrates with low lignocellulosic content produce fruit bodies earlier and for a shorter time compared to those with high lignocellulosic content which produce fruit bodies later but fruit for a longer time.

Results of morphological characters of fruit bodies indicated that Pileus diameter (PD) of fruit bodies harvested from 35mins CS was highest at 5.48±1.82, followed by those of 15mins CS (5.33±3.06). The mean pileus diameter of fruit bodies from all levels of CS substrates; including control was higher than the values reported by Okwulehie *et al.*, (2018), with uncarbonized substrates. The large PD obtained in this study could be due to high amount of carbon in the CS substrate. According to Okwulehie *et al.*, (2018), large mushroom cap diameter is a desirable market quality and growers must ensure the production quality fruit bodies to maximize profit. Stipe-length (SL) was significantly (p>0.05) high in fruit bodies obtained from 35mins CS at 3.46±1.63, followed by 15mins CS (2.82±0.81) while control and 25 mins CS were 2.25±0.55 and 2.37±0.78 respectively. The large stipe length and fruit body weight could also be attributed to high carbon content of fruit bodies. Therefore, carbonizing sawdust at different time intervals could greatly influence fruit body morphology, especially in oyster mushrooms.

Results showed that 15mins CS produced the highest number of fruit bodies at yield of 524.83g and BE of 20.99%. This shows that heating sawdust for 15mins provides optimum carbon requirement for yield of oyster mushroom. Osunde *et al.* (2019) however, reported that improved growth and high yield obtained from carbonized comcob was due to higher nitrogen content and a lowered C/N ratio. Generally, high fruit body yield was recorded at all levels of CS, except in control; when compared The overall yield and biological efficiency of *P. ostreatus* fruit bodies as observed in this experiment were significantly higher than those obtained by Shah *et al.*, (2004) who cultivated *P. ostreatus* on sawdust amended with different agro-wastes, Nwoko *et al.*, (2017) who grew *P. ostreatus* on trees logs, Okwulehie and Okwujiako, (2008), Sharad, (2013), Okoi and Iboh, (2015) who in

their separate investigations cultivated oyster mushrooms on different agro-waste components. his experiment recorded low biological efficiency. Rip, (2010) reported that biological efficiency of 100% and above is a comparatively favourable fruit body yield and mostly obtained by experienced commercial mushroom growers.

Chang and Miles (2004) maintained that high CHO contents of mushrooms is due to the high carbon and lingo-cellulosic compositions in the substrate where they grow; which mushrooms were able to break down using extracellular enzymes. The observed slight reduction in flavonoids concentration was probably due to heat of carbonization which may lead to loss of some volatile bioactive compounds in the substrates. Flavonoids act as anti-carcinogens, anti-bacterials (Hilang and Ferraro, 1992). Alkaloids have powerful effect in animal physiology and are important in pharmaceutical industries, for drug manufacturing (Edeoga and Erieta, 2001). Edeoga and Erieta (2001) also recorded that alkaloids are stimulants and acts by prolonging the action of several hormones. saponins are implicated in the prevention of parasitic fungal diseases (Edeoga and Erieta, 2001) while tannins have been used as anti-tumour agents and perform a wide range of anti-infective actions (Haslam, 1996).

Phenols content was high in fruit bodies from control (0.44±0.02) compared to those of 15, 25 and 35 mins CS which were not significantly different at 0.05 probability level. Although, there seems to be

reduction in the concentrations of flavonoids, saponins, phenoles, tannins and alkaloids in all fruit body samples obtained from various levels of CS, when compared to control; photochemical values recorded in this experiment were higher than those reported by Okwulehie *et al.* (2007).

CONCLUSION

In this experiment, substrate carbonization caused a slight decrease in the amount of ash, crude protein and ether extract but increased but enhanced crude fibre and carbohydrate contents.

Finally, substrate carbonization significantly ($p \leq 0.05$) decreased the concentration of all the studied phytochemicals in the fruit bodies as they were found to be higher in control.

It is pertinent to recommendations therefore that mushroom growers should carbonize their sawdust substrate between 15 and 25 mins at 100°C for early primordial formation.

Commercial mushroom growers targeting bigger sized and attractive *P. ostreatus* fruit bodies should adopt substrate carbonization, especially at the threshold of 15mins. Production of crude fibre and carbohydrate-rich *P. ostreatus* fruit bodies should be increased by use of carbonized sawdust, but should not be encouraged in the production of protein-rich mushrooms. Adoption of carbonized sawdust in the production of phytochemicals-rich *P. ostreatus* fruit bodies should not be encouraged as most volatile phytochemicals are likely lost via carbonization.

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PRELIMINARY EVALUATION OF SOME AGRO WASTES ON SOIL MICROBIAL POPULATION AND TUBER YIELD OF COCOYAM (*Colocasia esculenta*) IN UTISOL OF SOUTH EASTERN NIGERIA

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ABSTRACT

This study was conducted to evaluate the short-term effects of some agro organic waste sources of nutrients on soil microbial population, plant height and tuber yield of cocoyam (*colocasia esculenta*), using randomized complete block design in four replications. The trial involved five treatments: non-fertilized (control), cassava peel (CP), empty palm bunch (EPB), saw dust (SD) and rice mill waste (RMW). Soil samples were collected from the surface soil (0.20 cm) for microbial analysis before and after treatments at monthly intervals for 4 months. Cultural morphology and biochemical identification were carried out using standard microbiological techniques. Results obtained showed that plot amended with 5 t/ha saw dust (SD) induced the highest bacterial population throughout the 4 month of sampling, followed by plot amended with 5 t/ha cassava peel (CP), 5 t/ha rice mill waste (RMW) and 5 t/ha empty palm bunch (EPB) while control showed the lowest. Fungal population recorded maximum in plot amended with 5 t/ha CP, followed by 5 t/ha SD, 5 t/ha RMW and 5 t/ha EPB while control recorded the minimum fungal population. Cocoyam yield showed that SD gave the highest yield of 8.6 t/ha, followed by cassava peel (CP) 7.8 t/ha, rice mill waste (RMW) 7.8 t/ha, empty palm bunch (EPB) 5.5 t/ha and control, 3.6 t/ha. About 5 t/ha saw dust agro waste was recommended for improvement of soil quality, promotion of soil biological and microbial activities, growth and yield of cocoyam production in an ultisol of South eastern Nigeria.

Keywords: Agro wastes, cocoyam, microbial population, soil, yield

INTRODUCTION

Agro-wastes are waste of various agricultural activities which includes manures, budding, plant stalks, leaves and vegetable matter. These agro wastes contain essential nutrients needed to improve soil fertility, plant growth and yield. Many researchers have demonstrated the efficacy of some of these agro-wastes in improving soil microbial activity and yield of crops (Ayeni and Adeleye, 2011). However, proper utilization of these organic wastes by farmers is still poor despite their high nutrient composition (Ayeni and Adeleye 2011), and improvement of physical, chemical and biological properties in the soil (Yu *et al.*, 2015). Increasing the sustainability of cropping systems involves the reduction of agrochemical and fertilizer inputs through the reliance on soil ecosystem processes and biological interactions for the provision of plant nutrients (Drinkwater and Snapp 2007). Extensive use of chemical fertilizers can lead to decrease in soil organic carbon and soil quality (Kumar *et al.*, 2018). Therefore, there is need to consider soil microbial processes as they are crucial for plant nutrient supply given their central role in soil organic matter decomposition and nutrient dynamics. Thus the need to utilize plant residues such as Cassava peel, Empty palm bunch, saw dust and Rice mill waste to maintain a satisfactory level of soil fertility, and this can be practicable and adopted by farmers. The objective of this study is therefore to investigate the effects of agro organic wastes sources of nutrient on microbial activity, growth and yield of cocoyam

(*colocasia esculenta*) in an ultisol of south eastern Nigeria.

MATERIALS AND METHODS

The study was carried out on a loamy soil at the agricultural experimental field of National Root Crops Research Institute Umudike, Nigeria (longitude 07° 33' E, latitude 05° 29' N and altitude 122 M). Umudike is in the low-land humid tropics of south eastern Nigeria. The research plots were established in April 2018, and the trial was set-up as a randomized complete block design (RCBD), with four replications. Each plot size measured 15 m² (3 m x 5 m); with 1m spacing between the plots. The test plant used in the experiment was cocoyam (*Colocasia esculenta*). Treatments comprised Control (without fertilization), cassava peel at 5 t/ha, Saw dust (SD) at 5 t/ha, empty palm bunch (EPB) at 5 t/ha and Rice mill waste (RMW) at 5 t/ha; and were uniformly distributed. The treatments were allowed to incubate for one week before planting the test crop. Cocoyam (*Colocasia esculenta*) was planted on the mounds at a depth of 5cm and spacing of 0.7 m between rows and 0.25 m within row, respectively.

Soil sampling was conducted in accordance with the method of Saeki and Toyota (2004). Soil samples were collected from the study site before treatment and four times after treatments at monthly intervals beginning from May 2018 to August 2018. Soil auger was used to collect top soil sample from depth of 0-20 cm at four randomly selected locations in each of the plots.

Composite samples were collected from each plot and stored in a sterile polypropylene bag and kept cool using coolers during field sampling. The composite samples were homogenized and sieved twice using meshes (2.0 and 0.2 mm) in order to remove stone and plant debris. Samples collected were subsequently processed for microbial analysis within 24 - 48 hour.

Microbiological analysis

One gram (1 g) of each of the soil samples was weighed and agitated in 9 mL of distilled water, to dislodge the organisms from the soil particles. An aliquot of 1 mL was serially transferred from each sample into series of test tubes containing sterilized distilled water to obtain dilution of 10⁻¹ and 10⁻¹⁰. For total heterotrophic bacteria, aliquot (0.1 mL) of 10⁻², 10⁻⁴, 10⁻⁶ and 10⁻⁸ was inoculated into nutrient agar plated in triplicates. The plates were all incubated aerobically at room temperature (30^o C) for 24 hours. The resulting colonies were counted and recorded as colony forming units per gram (CFU/g) using colony counter. The counts were characterized based on cultural characteristics, staining reaction and biochemical tests.

Plant height measurement

Measurement was done after one month of planting, with 95% germination; three plants were selected and labeled specifically for data collection, at monthly interval. Plant height was measured using a meter rule from the surface of the soil to the tip of the tallest leaf (Nwafor *et al.*, 2010)

Tuber yield determination

Cocoyam yield was determined through the weight of tubers collected from two middle rows of each plot (expressed in t/ha), and was done at the end of the growing season 4 months after planting (MAP).

Statistical analysis

The data were subjected to an analysis of variance (ANOVA Statistics SPSS 5), using a GENSTAT and the significant effects between individual-factor level and interaction means were separated using Duncan new multiple range test (DNMRT) at P≤0.05 level.

RESULTS AND DISCUSSION

The results reveal that soil microbial population, plant height and cocoyam tuber yield were significantly affected by the different amendments. Total heterotrophic bacterial population recorded during the growing season showed highest bacterial population to occur at the second month (June) of sampling. Also,

plot amended with 5 t/ha saw dust recorded the highest bacterial count while the minimum was recorded in control plot. A significant variation in bacterial population was observed between treated plots and control P≤0.05 (Table 1). Saw dust (SD) provided organic substrates that proliferated bacterial population and activities in the soil, as they breakdown soil organic matter and multiply in the soil, which accounted for the highest bacterial count that peaked in the second month of sampling. The increase in SD amended plot might be as a result of suitable conditions which acted as a good substratum for microbial activity. Similar short-term increases have been reported by (Dinesh *et al.*, 2010), and attributed to the supply of organic Carbon substrates. Other researchers have also shown that incorporation of organic amendments such as poultry manure increased soil microbial population, enzymatic and microbial diversity (Girvan, *et al.*, 2004; Iwoh, *et al.*, 2020). SD promoted biological and microbial activities, which accelerated the breakdown of organic substances in the soil thereby raising carbon content and enzyme activities. Further increase was recorded with plot amended with 5 t/ha cassava peels (CP), which also might be attributed to the symbiotic and mycorrhizal relationships with soil microorganisms. Mader *et al.* (2002) reported that soils under organic farming had enhanced microbial functional diversity in comparison with the soils of conventional farming.

Total heterotrophic fungal populations during the four months of sampling under different treatments showed that fungal count was highest at 1st month and gradually reduced at 2nd, 3rd and 4th months. Fungal count with maximum value was recorded in plot amended with 5 t/ha cassava peel while minimum value recorded in control plot. Cassava peel (CP) promoted fungal growth in the soil with a significant variation between the treated plot as well as control plot at P≤0.05 (Table 2). The increased might be as a result of adequate supply in organic carbon content for microbial growth and utilization. Studies showed that increased soil pH in the acidic range caused a shift towards dominance of the bacterial community, while fungal communities were unaffected (Pennanen, 2001). Fungal population in soil at harvest was low as compared to bacterial population. Yevdokimov *et al.*, (2008) found that the addition of increasing amounts of N fertilizer increased soil fungal biomass significantly. Alternatively, it is possible that fungi did not react as fast as bacteria to the addition of Carbon substrates with the organic fertilizers.

Table 1: Effect of different treatment applications on total heterotrophic bacterial mean count (CFU/g) in 2018 cropping season

Treatments	Sampling period			
	May	June	July	August
Control	81.33x10 ⁶ ±3.05 ^a	81.33x10 ⁶ ±2.00 ^a	81.33x10 ⁶ ±2.51 ^a	81.33x10 ⁶ ±1.62 ^a

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CP	102.43x10 ⁶ ±1.52 ^d	112.43x10 ⁶ ±2.5 ^d	110.43x10 ⁶ ±1.73 ^d	106.00x10 ⁶ ±2.30 ^c
EPB	82.43x10 ⁶ ±2.71 ^b	88.63x10 ⁶ ±1.42 ^b	87.43x10 ⁶ ±1.72 ^b	84.00x10 ⁶ ±2.10 ^b
SD	99.33x10 ⁶ ±1.15 ^c	126.66x10 ⁶ ±2.18 ^c	122.66x10 ⁶ ±1.20 ^c	103.66x10 ⁶ ±2.20 ^c
RMW	95.66x10 ⁶ ±1.70 ^c	105.00x10 ⁶ ±2.20 ^c	108.00x10 ⁶ ±1.32 ^c	97.33x10 ⁶ ±1.63 ^d

Values show mean of triplicates analysis ± standard deviation. Figure with different superscripts down the column were significantly different according to Duncan new multiple range test at P≤0.05.
 CP =Cassava peel; EPB= Empty palm bunch; SD= saw dust; RMW= Rice mill waste

Table 2: Effect of different treatment applications on total heterotrophic fungal mean count (CFU/g) in 2018 cropping season

Treatments	Sampling period			
	May	June	July	August
Control	13.33x10 ² ±1.19 ^a	14.33 x10 ² ±1.15 ^a	13.33x10 ² ±1.52 ^a	13.33x10 ² ±1.17 ^a
CP	28.33 x10 ² ±1.25 ^d	25.66 x10 ² ±1.25 ^d	24.16x10 ² ±1.25 ^d	22.61x10 ² ±1.19 ^{dc}
EPB	19.00 x10 ² ±1.16 ^b	18.66 x10 ² ±1.55 ^{cb}	16.26x10 ² ±1.35 ^c	14.28x10 ² ±1.45 ^b
SD	26.66x10 ² ±1.73 ^c	23.66x10 ² ±1.35 ^c	22.43x10 ² ±1.42 ^c	20.61x10 ² ±1.35 ^c
RMW	20.53x10 ² ±1.65 ^c	18.00x10 ² ±1.17 ^{bc}	18.10x10 ² ±1.31 ^b	16.19x10 ² ±1.65 ^{cd}

Values show mean of triplicates analysis ± standard deviation. Figure with different superscripts down the column were significantly different according to Duncan new multiple range test at P≤0.05.

The effect of the applied amendment on the soil microbial status was quite in agreement with their effect on plant height and tuber yield of cocoyam. Plot amended with sawdust recorded the highest plant height followed by cassava peel, rice mill waste and palm bunch, while the least was from control plot (Table 3). Similar observation was noted by Adekayode and Olojugba (2010), who recorded 4% increase with application of saw dust ash on the growth of maize. Yield of cocoyam recorded also showed same sequence of increases as plot amended with dust waste recorded the highest yield followed by cassava peel, rice mill waste and empty palm bunch, while control recorded the lowest value (Table 4). The increase in cocoyam yield resulting from the treatments is in line with results obtained by other authors who reported the importance of both their individual and combined use not only in terms of yield, but also in terms of improvement of soil production characteristics and preservation of soil ecological status (Cerny *et al.*, 2010; Manqiang *et al.*, 2009). Manqiang *et al* (2009) also stressed the importance of substituting costly mineral fertilizers with manure and other organic substrates as part of the improvement of soil biological productivity and crop yield.

Table 3: Effect of treatments on plant height (cm) of cocoyam at 4 MAP in 2018 cropping season

Treatments	Plant height			
	May	June	July	August
Control	6.5	9.5	14.1	14.3

CP	10.9	29.7	36.8	36.7	28.52
EPB	8.6	26.5	37.3	37.5	27.47
SD	11.2	32.6	40.9	40.9	31.40
RMW	10.8	30.4	38.6	38.7	29.62

CP = Cassava peel; EPB= Empty palm bunch; SD= saw dust; RMW= Rice mill waste

Table 4: Effect of treatments on tuber yield (t/ha) of cocoyam at harvest in 2018

Treatments	Tuber yield (t/ha)
Control	3.6
CP	7.8
EPB	5.5
SD	8.6
RMW	7.8

CP = Cassava peel; EPB= Empty palm bunch; SD= saw dust; RMW= Rice mill waste

CONCLUSION

The present investigation showed that all the agro organic amendment applied had great impact on soil microbial population, Beneficial effects occurred even when the organic agro waste amendments were only a small portion of the total amounts supplied to the soil

throughout the 4 months, suggesting that a shift to more sustainable production systems could significantly improve soil fertility while maintaining crop yield, and these would help provide a better understanding of the importance of these organic fertilizers in promoting soil microbial activities, growth and yield of crops. We therefore recommend

the application of 5 t/ha saw dust agro wastes for successful integrated cocoyam production system in order to promote biological and microbial activities, soil fertility, growth and yield of cocoyam production in an ultisol of Southeastern Nigeria under the agro environmental conditions specified in this study.

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ASSESSING THE EFFECT OF COVID-19 PANDEMIC ON *Chrysichthys nigrodigitatus* (SILVER CATFISH) CAPTURE IN LAGOS STATE, NIGERIA

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Abstract

The study focused on the perceived effect COVID-19 on silver catfish capturing in Epe LGA of Lagos State, Nigeria. It analyzed the capture methods, catches and sales of Silver catfish from the wild among artisanal fisher folks in Epe lagoon Lagos State; Using structured interview guides, data were collected from 96 respondents in Ebute Afuye, Oluwo market and marine fishing communities, and analyzed using descriptive statistics. Majority of the respondents (88.54%) were aware of the COVID-19 Pandemic, with the media (i.e. TV, radio and newspaper) constituting their major (83.33%) source of information. Majority (77.08%) of the respondents admitted COVID-19 Pandemic affected their business negatively. Although all the respondents reported that the pandemic did not affect the size of their catch, neither did it increase the cost of fish (82.29%) as there were movement restrictions. The major impact noted by respondents was that they were unable to sell, leading to income loss, which adversely affected their sustenance in the pandemic period. The study concludes COVID-19 pandemic has negatively impacted the fishing communities at Epe fishing communities because they were not able to sell their catches and majority of the fisher folks are on full time fishing.

Keyword; Silver catfish, Fisher folks, COVID-19 Pandemic

Introduction

The COVID-19 pandemic has led to a dramatic loss of human life worldwide and presents an unprecedented challenge to public health, food systems and the world of work. The economic and social disruption caused by the pandemic is devastating: tens of millions of people are at risk of falling into extreme poverty, while the number of undernourished people, currently estimated at nearly 690 million, could increase by up to 132 million by the end of 2020. (WHO, 2020) Corona virus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first confirmed case of COVID-19 in Nigeria was on 27th February 2020. By 8th June 2020, 12801 cases have been confirmed with 361 deaths. Thirty-six states including the Federal Capital Territory (FCT) have reported at least one confirmed case (NCDC 2009). The Nigerian Government in response to COVID-19 outbreak put a lot of interventions in place. This includes the following: international travel bans, domestic air travel bans, closure of schools, universities, and religious places, ban on social and cultural activities, and general restriction of movements. This culminated in a lockdown (stay at home) order imposed by the Federal Government on States with very high infection rates while State Governments locked down their states to control the infection rates in their states at different times as the epidemic trajectory increases.

Capture fisheries sector still remains dominant for a number of species and vital for domestic and international food security. Developing countries, mainly in Asia, are by far the predominant producers

with China, Indonesia, India, Viet Nam and Peru being the key producers in 2018. About 89 percent of fish production is directed to human consumption. World apparent per capita fish food consumption has significantly grown during the last few decades, from 9 kg in the 1960s to about 20.3 kg in 2017. At the global level, fish accounts for about 17 percent of the world population's intake of animal proteins and provides about 3.3 billion people with almost 20 percent of their average per capita intake of animal proteins and 5.4 billion people with 10 percent of such proteins. (FAO, 2020) Capture fisheries in the world's inland waters produced 11.6 million tonnes in 2016, representing 12.8 percent of total marine and inland catches. Nigeria is a major producer with 39 758 tonnes in 2016. Inland catches are also an important food source for several countries in Africa, which accounts for 25 percent of global inland catches (FAO, 2018). Nigeria still remains Africa's largest fish importer of sea and some fresh water fish. Nigeria Population Figure in 2015 was 187.3 Million, the total fish demand was 3.25m Mt and Fish Production from Aquaculture is 316,727.00 Mt, Captured fisheries is 694,867.00 there is huge deficit in local production, only 806,000 Mt tons of fish are imported to meet the annual demand in Nigeria. (NBS, 2017-2018).

The Lagos lagoon comprises a network of nine lagoons of shallow waters (Ibe, 1988), which covers an area of 208 km² (Ekundayo and Akpata, 1978) Nigerian fishing industry comprises of three major sub sectors namely the artisanal, industrial and aquaculture. The artisanal covers the operations of small-scale canoes, fisheries operating in the coastal areas, creeks, lagoons, inshore water and the inland

rivers. The artisanal fishery is characterized by low capital outlay, low operational costs, low technology application and it is labor intensive (Adedokun *et al.*, 2006, Bolarinwa, 2014). Artisanal fishery could be defined as the sector that employs traditional methods in operational activities which include capturing, preservation and distribution of fish and fish products (Pauly, 2006). The small-scale fisheries sector remains the backbone of fish production in Nigeria, contributing an annual average of 81.94 per cent in 2002 and 82.10 per cent in 2003 of the total fish production in spite of the low technological development (FAO, 2004). Fish plays a vital role in feeding the world's population and contributing significantly to the dietary intake of millions of the populace (Ajao, 2011). Economically fish provides an important source of food and income for both men and women and fishing has an important social and cultural position in riverine communities.

Chrysichthys nigrodigitatus (Lacepede 1803) is a Bagridae mud silver catfish which occurs in most of the major rivers in Africa including Nigeria; the catfish is endemic to inland and coastal waters of Africa: Western, Southern and Central Africa, (Emmanuel & Osibona 2013). The silver catfish, is one of the most economically abundant species found in the Lagos lagoon complex. *C. nigrodigitatus* (Lacépède) occurs in most of the major rivers and coastal zones of Africa including Nigeria, Senegal, Gambia, Ivory Coast, Liberia, Zaire, and Gabon (Ezenwa, 1981). It makes significant contribution to the artisanal fisheries of the lagoons, 15,871 metric tonnes of *C. nigrodigitatus* was captured in 2015 (NBS, 2017-2018), the fish is caught with drag net, hook and line, bottom-set gillnet, and bottom-set traps (Bamboo and PVC pipes) because of the bottom dwelling habit. Although *C. nigrodigitatus* is a commercial important fish that is widespread in Africa tropical waters (Boulenger, 1909-1915; Adite *et al.*, 2006; Adite *et al.*, 2017) where they grow and spawn actively, and also constitute one of the dominant catches of capture fishery by artisanal fishermen, Owing to its economic importance and suitability for culture, considerable research has been devoted to the study of several aspects of the species in Nigerian waters (Ikusemiju and Olaniyan, 1977; Ezenwa, 1981; Anyanwu, 1991; Ekanem, 2000; Offem *et al.*, 2008).

This study focused on the perceived effect of Covid-19 on *Chrysichthys nigrodigitatus* (silver catfish) capture, the socioeconomic analysis of artisanal fisher folks. It analyzed the capture methods, catches and sales of Silver catfish from the wild among artisanal fisher folks in Epe lagoon Lagos, Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted in selected fishing communities in Epe which has a maximum depth of about 6 m lies between longitude N 06° 33.710' E 004° 03'.710' and latitudes N 06° 31.893' E 003° 31.912'.

The lagoon is a freshwater ecosystem sandwiched between two lagoons, Lekki lagoon (freshwater) in the east and Lagos lagoon (brackish) in the south with the Osun River being the main river discharging into the lagoon. The climate is classified as tropical with dry season occurring between December and April and the rainy season concentrated between May and November. *Chrysichthys nigrodigitatus*, Silver catfish is captured throughout the year but mostly abundant between April and June. Epe is a town and Local Government Area (LGA) in Lagos State, Nigeria, located on the north side of the Lekki Lagoon. It is a Yoruba town located next to the Lagos lagoon with 294 rural and 24 semi-urban communities. The lagoon is over 50 km long and varies from 3 km to 13 km wide. (Patrick *et al.*, 2019) Thus, its banks can be mostly seen from either side. It is fairly shallow and is not plied by ships but by smaller barges and boats. This makes it possible for subsistence fishing to be carried out in it. Epe is known for its fish market which feeds off the hard work of those men and women whose lives depend on the lagoon – and the fish inside it. Both men and women engage in fishing. While more men than women go deep offshore to catch fish, the women also have their fair share of deep fishing activity. Epe lies on the north bank of the coastal Lagos lagoon and is a collecting point for the export of fish. It is no surprise then that fishing is the major occupation of its locals, including men, women and children. Structured interview guides were used to elicit information from 120 fisher folks from 3 communities; Ebute Afuye, Oluwo market and marine fishing communities, 96 questionnaires were retrieved after the exercise and analyzed using descriptive statistics. Data were collected on socioeconomic characteristics of the fisher folks, capture methods of silver catfish and major constraints to fishing activities.

Analytical procedures

Descriptive statistics: This involves the use of tables, frequencies and percentages to describe the socioeconomic characteristics of the respondents, capture methods and constraints to capturing silver catfish in the study area.

RESULTS AND DISCUSSION

Social Economic characteristics of respondents

Table 1, shows that: Of the total 96 respondents, 89.58% were found to be males while 10.42% were female. Male dominated the fishing activities. Age is an important socioeconomic characteristic because it affects productivity and output. It was obtained from the survey that 39.58% of the respondents' ages in the study area are between 41-50 years, 26.04% are between 30-40 years, 23.96% are between 51-60, while 10.42 are above 60 years. This implies that most people engaged in fish catching were still active and physically fit to paddle the canoes and set traps. The implication was that the respondents are within the productive and economic active age, and could be able to increase fish catch and improve livelihood of the

families. The finding was in agreement with those of Olaoye (2010), who found that most of the fisher folks are in their economic active ages to undertake strenuous task associated to the fishing enterprise and also in agreement with Ifejika (2007), who reported that 60% of the fish farmers in Borgu Local Government Area of Niger State were within the productive age group of 41-50 years and that this has positive correlation with acceptance of innovation and risk taking and Aminu *et al* (2017) who found most 61.7% of the fisher folks were below 46 years.

determining the profit levels of artisanal fisher folks, the more the experience, the more fisher folks understand the system, conditions, trends terrains, prices, etc. Dey *et al.*, (2002) agreed that experience is crucial and contributes to the success of Asian fisher folks.

A larger proportion of the fisher folks (93.75%) were married, while 6.25% were separated. This implies that majority of the fisher folks shoulder a lot of family responsibilities. Fisher folks who had formal education; secondary school accounted for 50%, primary school 33.33% , Tertiary Education 6.25% of the sampled respondents, while the remaining 10.42% had no formal education. This implies that respondents were relatively educated and this would have positive consequences on their capacity to exploit latent opportunities in the fishing activities. This finding disagreed with the assertion of Olaoye *et al.* (2012) who reported that 60 percent of fisher folks were uneducated and with the general opinion that most fisher folks are illiterates or semi illiterates, most of who dropped out of school system.

Half (50%) of the respondents had household size of 5-8 persons, 34.38% had between 1-4 persons, 5.21% had 9-12 persons, while 5.21% had 13 and above. This is likely to boost the availability of family labour in the sampled communities. In fact, the intensity of artisanal fisheries has been found to have a direct relationship to household size (Akanni, 2010). This is also in line with the assertion of Adegbite & Oluwalana (2004), who opined that the higher the household size, the more likelihood for labour efficiency on fisher folks. From the survey 73.96% of the fisher folks were on full time, while 26.04% were on part time. Having the majority of the fisher folks in full time fishing shows the enterprise is a profitable one and it will enable them to carter for the families.

For those involved in part time fishing 10.42% were involved in produce markets while 10.42% were also involved in other things. Farmers' years of production experience dictates efficiency and effectiveness in agricultural production, from the results it shows that fisher folks with 28.13% had 20-25years experience, 28.13% also had above 30 years' experience were the majority while 11-15years and 16-20years had 11.46% respectively, while 10.42% had 1-6 years in the study area. The years of farming experiences suggests that fisher folks in the study area have over a decade experience in fish capturing, indicating that they were quite experienced. The fisher folks had considerable years of fishing experience in the study area. This is substantiated by the findings of Olaoye (2010), who observed that fishing experience is important in

BACKGROUND INFORMATION

Table 1

		Frequency	Percentage
Gender	Female	10	10.42
	Male	86	89.58
	Total	96	100.00
Age	30-40 years	25	26.04
	41-50 years	38	39.58
	51-60 years	23	23.96

	Above 60 years	10	10.42
	Total	96	100.00
Marital Status	Single	0	.00
	Married	90	93.75
	Widow /Widower	0	.00
	Separated	6	6.25
	Divorced	0	.00
	Total	96	100.00
Level of Education	Primary Education	32	33.33
	Secondary Education	48	50.00
	Tertiary Education	6	6.25
	No formal Education	10	10.42
	Total	96	100.00
Family Size	0	5	5.21
	1-4	33	34.38
	5-8	48	50.00
	9-12	5	5.21
	13 and Above	5	5.21
	Total	96	100.00
Fishing Status	Full time	71	73.96
	part time	25	26.04
	Total	96	100.00
	Arable crops	11	11.46
	Cash crops	0	.00
	Livestock	0	.00
	Poultry	0	.00
	Fish farming	0	.00
	Produce market	10	10.42
	Others	10	10.42
Total	96	100.00	
Fishing Experience	1-5 years	0	.00
	6-10 years	10	10.42
	11-15 years	11	11.46
	16-20 years	11	11.46
	20-25 years	27	28.13
	26-30 years	10	10.42
	Above 30 years	27	28.13
	Total	96	100.00

CAPTURE PRACTICES

Table 2 shows the capture practices. From this table, majority (60.42%) of the fisher folks are aware silver catfish can be caught in brackish water, while (39.58%) are aware it can also be caught in fresh water bodies. Silver catfish is available throughout the year but there are months of abundance, majority 38.54% agreed it is more abundant in the month of April-June, 27.08% agreed it July-September, 17.71% agreed its October-December, while 16.67 agreed its January-March.

The sex determination of *C.nigrodigitatus* and size through its morphometric characteristics very important for the purposes of propagating the fish in aquaculture, 72.92% indicated that the males are bigger while 27.08 said the females are bigger. Majority 59.38% of the fisher folks do not have access to Extension Officer, while 40.63% have access. Access to extension agent is very important fish enterprise. Items used in capturing silver catfish net 100%, hook 88.54%, traps 67.71%, bamboo 69.79%, while PVC is 12.50%

Table 2 Capture Practices

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		Frequency	Percentage
Type of Water	Fresh water	38	39.58
	Brackish water	58	60.42
	Marine water	0	.00
	Total	96	100.00
Month of Abundant Silver fish	January - March	16	16.67
	April-June	37	38.54
	July - September	26	27.08
	October-December	17	17.71
	Total	96	100.00
	Sex of Bigger Silver Fisher	Male	70
Female		26	27.08
Total		96	100.00
Access to Extension Officer	Yes	39	40.63
	No	57	59.38
	Total	96	100.00

Table 3. Items Used

	Yes		No	
	Count	Percentage	Count	Percentage
Net	96	100.00	100	100.00
Hook	85	88.54	11	11.46
Bamboo	67	69.79	29	30.21
Trap	65	67.71	31	32.29
PVC	12	12.50	84	87.50

Table 4 Effects of Covid-19 on Captured Fishery

		Frequency	Percentage
Awareness of Covid19	Yes	85	88.54
	No	11	11.46
	Total	96	100.00
Source of information on Covid19	Media; TV, radio, Newspaper	80	83.33
	Social Media	5	5.21
	Church	5	5.21
	Mosque	6	6.25
	Mosque	0	.00
	Others	0	6.25
	Total	96	100.00

Table 4 shows the effect of covid-19 on captured fisheries; from the table 88.54% were aware of the covid-19 pandemic while 11.46% were ignorant of the virus. Awareness of covid-19 also afford you the opportunity to raise more awareness about COVID-19 and promote hygienic behavior which will protect you from the infection, UNICEF has supported the Lagos State Government through motorized campaigns in 20 local government areas. The campaigns worked alongside advocacy with influencers in the communities, interpersonal engagement, and mass awareness activities at major human interface locations such as markets and motor parks. 83.33% got

their information on Covid19 from the Media (television, radio, newspaper) with this information adult and children became change champions within their communities as they help raise awareness on curbing the spread of the coronavirus.

Majority of the fisher folks 77.08% agreed Covid19 affected their business and caused a major economic shock. While 22.92% said it didn't affect their business, many small businesses are financially fragile, during the covid-19 pandemic most businesses had widely varying beliefs about the likely duration of COVID-related disruptions. 54.17% of the fisher folks revealed that the pandemic affected them negatively because their expectations of their business at that critical point in time when both the progression of COVID-19 and the government's response were quite uncertain, with disruptions in captured fisheries supply chain being a huge factor. There were no movement of vehicles to convey fish to major fish markets and consumers. The impacts also varied across industries, with retail, arts and entertainment, personal services, food services, and hospitality businesses all reporting employment. Furthermore 100% of the fisher folks were not engaged in any form of business during the pandemic because of the total lockdown. Also 100%

said covid-19 did not affect the size of their catch, because majority of the fisher folks live around the fishing communities and might as well take a walk to the Lagoon. 82.29% said the lockdown did not increase the cost of fish while 17.71% agreed that it increased the cost of their fish, this disparity might be that some of the fisher folks beat the lockdown to move their catches to their consumers. 72.92% of the fisher folks express their views that they didn't gain any experience during the lockdown while 27.08% agreed that they gained a lot of experience which are saving money for the raining days, being a member of a cooperative society and helping each other at all times.

Conclusion and Recommendation

The study concludes COVID-19 pandemic has negatively impacted the fishing communities at Epe because they were not able to sell their catches and majority of the fisher folks are on full time fishing. The results suggest that the pandemic had already caused massive dislocation among small fisher folks just several weeks after its onset and prior to the availability of government aid through the Coronavirus Aid on palliatives of food commodities.

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STORAGE CONDITIONS AND PACKAGING MATERIALS: IMPACT ON THE MOISTURE CONTENT OF LOCALLY GROWN Cucumber (*Cucumis sativus* L.) IN OWERRI, IMO STATE

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Abstract

The storage conditions and packaging materials of locally grown Cucumber fruits (*Cucumis sativus* L.) were evaluated for extended shelf life and quality in a 3 x 4 factorial experiment; with a completely randomized design (CRD), using three different storage conditions, namely - wet river sand (WRS), refrigeration (R) and control (ambient condition). Also, four (4) packaging materials; perforated polyethylene bags (PPB), perforated corrugated cartons (PCC), perforated plastic containers (PPC) and control (no packaging) were evaluated, three (3) weeks after storage (WAS) in triplicates. After two (2) weeks of storage, the moisture content (MC) of fruits stored in PCC with WRS performed significantly better ($p < 0.05$) than fruits stored in PPC with WRS (89.990 % and 89.590 % respectively). The control (fruits stored at ambient conditions) in cartons had the least MC (85.887 %). At 3 WAS, the MC of fruits stored with (WRS) in (PCC) performed significantly better ($p < 0.05$) than the fruits stored with (WRS) in (PPC) (89.193 % and 88.267 %) respectively. The MC for the control (No packaging) was least (83.557 %). The control fruits were unmarketable after 14 days of storage with defects like large yellow patches, wrinkles due to moisture loss, with undesirable off taste. All cucumber fruits stored at refrigeration conditions were also unmarketable after three (3) weeks of storage, as they were frozen and had off taste due to chilling injury. Cucumbers stored with WRS in PCC exhibited best features as they remained dark green at three weeks (WAS). The atmosphere modification provided by wet-river sand resulted in the enhanced and acceptable market quality of the fruits after three (WAS).

Keywords: Cucumbers, Storage conditions, Packaging materials, Moisture content.

Introduction

According to Bruhn (1995), eating quality fruit or vegetable is a combination of characteristics, attributes and properties that lead to enjoyment in horticulture; while consumers say that appearance and freshness are most important in initial purchase of any horticultural produce. They select products that are the appropriate colour, size and shape with proper firmness and consumers also expect horticultural produce on sale to be safe with desirable taste. Appropriate colour, shape and size are important quality criteria developed from adequate postharvest handling. A characteristic taste is desirable, as it indicates ripeness and reflects eating quality. Scars, scratches mark lower quality rating.

Attitude studies indicate that consumers tend to prefer locally grown produce, both due to perception of higher quality and to support the local economy (Zagory, 1999). Horticultural produce is viewed as a healthy food choice. In each of the last 10 years, 70% or more consumers responding to Food Marketing Institute's annual survey indicate that they have increased produce consumption to obtain a healthier diet (Bruhn, 1995). Consumers view fruits and vegetables as good sources of vitamin, minerals and fiber; helpful in calorie control a possible cancer preventative.

Kader (1983) pointed out that losses in quantity and quality affect cucumber between harvest and consumption. The magnitude of postharvest losses in fresh cucumber is an estimated 5 to 25% in developing countries, depending upon the cultivar and handling condition. To reduce these losses, producers and

handlers must first understand the pre-harvest, biological and environmental factors involved in deterioration; and secondly use the postharvest techniques that delay senescence and maintain the best possible quality.

Fresh cucumbers are living tissues that are subject to continuous change after harvest. While some changes are desirable, most are not. Post-harvest changes in fresh cucumber cannot be stopped, but they can be slowed within certain limits. Senescence is the final stage in the development of plant organs, during which a series of irreversible events lead to breakdown and death of the plant cells. Fresh cucumbers are high in water content and are subject to desiccation (wilting, shriveling) and to mechanical injury.

Storage temperature and humidity are most important as they influence the senescent phases of fresh vegetables by regulating the rate of all associated physiological and biochemical processes (Salunkhe and Desai, 1984). The objectives of this study were to evaluate the optimum storage conditions, vis-a viz the packaging materials, for the extension of the shelf-life of locally grown cucumbers.

Materials and Methods

Experimental site – Cucumbers (*Cucumis sativus*) were grown from January to April in a sandy loam shallow soil at the Horticultural Farm of Imo State Ministry of Agriculture and Natural Resources Owerri; with a monthly rainfall of 200-270cm per annum, minimum temperature of 21.14°C, maximum

temperature of 29.49°C with relative humidity of 89-93% and soil pH was 6.0-7.0.

Agronomic History of Study Area – The soil had unconsolidated parent material within 1m of the soil surface. Formerly cultivated with cassava, the soil was left grass fallow for one year. The soil was treated against nematodes with Furadan 3G after which basal fertilizer application of poultry manure was applied after making the beds. Seeds were sown in well prepared raised beds with width of 100cm at spacing of 50 by 50cm². Cucumbers were harvested early in the morning, packed unwashed and transported the same morning to the laboratory.

Cucumbers used in the experiment were manually graded and sized with an average weight, length and diameter of 80.4g, 136mm and 29.4mm respectively. The fruits were sorted to eliminate bruised, punctured and damaged ones after which they were washed with clean water and air dried. They were later packaged in perforated polythene bags.

Experimental Design – the experiment was carried out in a 3 x 4 factorial arrangement with parent completely randomized design (CRD), to test the effects of the treatments on the moisture content of cucumber fruits during storage for three (3) weeks with three (3) replications.

Treatments – the cucumber fruits were subjected to three different storage conditions – wet river sand (temp. 20-25°C; RH. 85 – 90%); refrigeration (temp. 4-5°C; RH. 90 – 95%) and the control (ambient condition – temp. 30 – 35 °C; RH. 70 – 80 %); using four (4) packaging materials, namely, perforated polyethylene bags, perforated corrugated cartons, perforated plastic containers and the control (no packaging).

Data Collection – Data on moisture content were collected on a four (4) day interval. Moisture content was determined according to the methods described by Abdel-Razek *et al.* (2005), using the formula:

$$\% \text{ Moisture Content} = \frac{\text{Difference in weight} \times 100}{\text{Weight of sample}}$$

Analysis of variance (ANOVA) was carried out using Genstat (2000) statistical analysis software. Data interpretation was based on Wahua (1999), where significant differences were observed at 5% level of probability. Least significant difference (LSD) was used to separate and test the means for differences.

Results and Discussion

Table 1 shows the effect of packaging materials and storage medium on moisture content of cucumber fruits during a three (3) weeks storage period. At the commencement of the storage, there was no significant difference between the storage or packaging materials; neither was there any between the interactions. After one (1) week of storage, cucumber fruits stored in paper cartons with wet-river sand performed significantly better (p<0.05) with highest moisture content (90.88 %) than fruits stored with polythene in the refrigerator (90.81 %). The least moisture content (88.50 %) was exhibited by fruits stored at ambient temperature with no packaging. After two (2) weeks of storage, cucumber fruits stored in paper cartons containing wet-river sand had moisture content of 89.99 % and performed significantly better (p<0.05) than the cucumber fruits stored with wet river sand in plastic containers with 89.59 %. The least moisture content (85.89 %) was exhibited by fruits stored at ambient temperature in cartons. After three (3) weeks of storage, fruits stored in paper cartons with wet-river sand performed significantly better (p<0.05) than the fruits stored with wet river sand in plastic containers with moisture content of 89.19 and 88.27 % respectively. The least moisture content (83.56 %) was exhibited by fruits stored at ambient temperature without packaging.

Postharvest practices include the management and control of variables such as temperature and relative humidity, the selection and use of packaging, and the application of such supplementary treatments as fungicides (FAO, 2009). According to El-Ramady *et al.* (2015), all fruit-vegetables, except peas and sweet corn, are susceptible to chilling injury if exposed to temperatures below 5 °C e.g., cantaloupe, lima bean, snap bean; 7.5 °C e.g., pepper; 10 °C such as cucumber, soft-rind squash, eggplant, okra, chayote; or 12.5 °C e.g., tomato, muskmelons other than cantaloupe, pumpkin, hard-rind squash. This explains why the fruits stored in paper cartons containing wet-river sand performed significantly better (p<0.05) than the other storage media because, even though, cucumber storage is best in a condition that adopts low temperature and high humidity (Abdul *et al.*, 1999), the average temperature and humidity combination offered by the refrigerator did not favour the storage quality of the cucumber but rather subjected it to chilling injury due to the temperature.

Table 1: Effect of Packaging Materials and Storage Medium on Moisture Content of Cucumber fruits during a three (3) weeks storage period

Storage Medium (Control)	Packaging Materials				Mean
	No Packaging	Carton	Plastic	Polythene	
At 0 Week					
After					

Storage					
Ambient temperature (Control)	91.593	91.593	91.593	91.593	91.593
Refrigerator	91.593	91.593	91.593	91.593	91.593
Wet River Sand	91.593	91.593	91.593	91.593	91.593
Mean	91.593	91.593	91.593	91.593	
LSD _(0.05) for S. Med.	NS				
LSD _(0.05) for P. Mat	NS				
LSD _(0.05) for	NS				
Interaction					
At 1 Weeks After					
Storage					
Ambient temperature (Control)	88.500	89.193	88.853	89.247	88.948
Refrigerator	90.253	90.450	90.580	90.807	90.522
Wet River Sand	89.867	90.883	90.567	90.307	90.406
Mean	89.540	90.176	90.000	90.120	
LSD _(0.05) for S. Med.	0.2098				
LSD _(0.05) for P. Mat	0.2422				
LSD _(0.05) for	0.4195				
Interaction					
At 2 Weeks After					
Storage					
Ambient temperature	85.947	85.887	86.370	86.033	86.059
Refrigerator	89.347	88.367	87.147	89.007	88.467
Wet River Sand	88.467	89.990	89.590	88.613	89.165
Mean	87.920	88.081	87.702	87.884	
LSD _(0.05) for S. Med.	0.1245				
LSD _(0.05) for P. Mat	0.1437				
LSD _(0.05) for	0.2490				
Interaction					
At 3 Weeks After					
Storage					
Ambient temperature (Control)	83.557	84.880	84.787	84.623	84.462
Refrigerator	87.033	86.133	86.440	86.287	86.473
Wet River Sand	86.447	89.193	88.267	87.407	87.828
Mean	85.679	86.736	86.498	86.106	
LSD _(0.05) for S. Med.	0.3590				
LSD _(0.05) for P. Mat	0.4145				
LSD _(0.05) for	0.7179				

Key:

S. Med. = Storage Medium

P. Mat. = Packaging Material

The temperature and humidity combinations particularly the temperature, offered by the wet river sand in carton ensured that the fruits stored in that condition, remained fresh till the third week of storage.

The variation observed in moisture contents among the cucumber fruits in different packaging materials and

storage media may be due to different humidity regimes provided by these storage media and packaging materials. According to Gorny (2001), due to the high moisture content of cucumber fruits, there is the tendency for large amounts of water loss, wilting, dryness, sensory quality decline and loss in edibility when they are placed in circumstances of

relatively low humidity under the action of moisture evaporation. Although Gorny *et al.* (1998) suggested that fresh produce must be kept in a moist atmosphere as low as possible to prevent water loss; the rate of water loss depends on temperature and duration of storage and exposure to lower oxygen (O₂) and/or high Carbon IV oxide (CO₂) concentrations.

Conclusion:

Low temperature reduces the cucumber metabolism and the high humidity keeps the moisture content of cucumber so as to keep the cucumber fresh. It is an

important means to maintain the fruit and vegetable quality. Storage medium greatly affected quality of cucumber. However, among the treatments investigated in this work, the best low cost and available postharvest treatment combination for managing cucumber quality was provided by storage in perforated cartons with wet river sand. The fruits subjected to this treatment had good visual appearance, good taste (subject to a palatability test conducted); and low moisture loss. They were of high quality, which could translate to good marketability.

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GROWTH AND YIELD RESPONSES OF FLUTED PUMPKIN (*Telfairia occidentalis*) AS INFLUENCED BY LONGITUDINAL SEED DIVISION

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Abstract

Fluted pumpkin has an inherent potential of producing more than one seedling from an embryo. This research was launched to investigate whether there is advantage in using half and quarter seed divisions in the production of fluted pumpkin and to compare the agronomic performances of whole seed, half seed, and quarter seed. The study was carried out in randomized complete block design with three replications at National Root Crop Research Institute, Umudike. Responses to treatments were evaluated in vine length, number of leaves, number of branches, stem girth, and fresh vegetable weight at 6 weeks after sowing (WAS), 10WAS and 14WAS. There were significant treatment effects on all the attributes measured at 6, 10 and 14 WAS except number of branches at 14 WAS and fresh vegetable weight at 6 WAS. The results showed that whole seeds gave the highest values of vine length, number of leaves, number of branches, leaf area, stem girth and fresh vegetable weight at harvest. Half seeds gave a higher value than quarter seeds. However, when compared their mean values, half seeds could be comparable to whole seeds in terms of number of branches, number of leaves and stem girth. Quarter seeds could not compare to half seeds as there was a big difference between their means. Hence the use of half seed as planting material could be beneficial especially during the scarcity period.

Keywords: Seed division, polyembryony, *Telfairia occidentalis*, seed multiplication, growth and yield.

INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis* Hook. F.) belongs to the family *cucurbitaceae*, which consists of 90 genera and 750 species (Onovo *et al.*, 2009). It serves as one of the natural sources of amino acids for human consumption (Okonwu *et al.*, 2018) which compared favourably with those of important legumes such as soybean and groundnut, also that protein and oil contents of the seed at 30.1 and 47%, respectively.

Incorporating boiled fluted pumpkin seeds in diets results to good growth and showed no detectable toxicity in rats (Ejike *et al.*, 2010). The seeds can be used to supplement energy and protein requirements. It ranks highest in net income of common tropical leafy vegetables in South Eastern Nigeria (Agbugba and Thompson, 2015).

Recent studies have shown that *Telfairia occidentalis* leaf is rich in minerals (such as iron, potassium, sodium, phosphorus, calcium and magnesium), antioxidants, and vitamins. Leaves of *T. occidentalis* can be beneficially used in heart diseases, hypertension, hypoglycemia, diabetes and even in fatal cases of meningitis. They have been effectively used in lowering blood cholesterol, increasing hemoglobin and preventing blood clotting (Iweala and Obidoa, 2009).

The female plant is preferred by farmers to the male plant because it produces seeds for planting (Akoroda, 1990). The seeds of *T. occidentalis* are the only known important propagation materials since the plant cannot

be easily propagated vegetative. The seeds quickly lose their germination potentials especially when the seeds are poorly developed due to biochemical transformation that usually occur in the seed at different moisture levels (Tairu and Bolounduro, 2006). The seeds of *T. occidentalis* are very delicate to handle because they do not undergo maturational drying and cannot be dried without damage. Also due to high moisture content of the seeds, it cannot be stored for a long period of time and still be viable (Tairu, 2003).

One characteristic feature of fluted pumpkin is its exhibition of the polyembryonic trait. Polyembryony is the simultaneous emergence of two or more seedlings from one germinated seed. It is the division of one sexually produced embryo into many, and the resulting ones are genetically identical to each other, but distinct from their mother (Onovo *et al.*, 2009). Polyembryonic seed is an important feature due to commercial multiplication (Duarte *et al.*, 2013). This trait may confer great benefits, *Telfairia occidentalis* plants may have increased production and competitiveness because a seed may produce two to four (Onovo *et al.*, 2009) normal plants favoring production because of the increase of number of plants per surface unit (Michel *et al.*, 2018). Other benefits are lower production costs because with the same number of seeds, farmers can have more plants per unit area. So, to plant a unit area will require less seed that will result in lower storage and transportation costs.

Different forms of polyembryony have been reported

to occur in *T. occidentalis*. It was suggested that the occurrence of polyembryony is natural and that multiple seedlings were observed to develop in two areas of the seeds of the crop (Odiyi, 2003). The occurrence of multiple embryos in the embryonic axis and cotyledons makes it possible to obtain more plants from one seed. This may help to overcome shortage of planting materials. Hence this study aims at comparing the agronomic performances of seed divisions (whole, half and quarter seeds) in fluted pumpkin and to investigate whether there is advantage in using half and quarter seed divisions in the production of fluted pumpkin.

MATERIALS AND METHODS

The study was conducted at the National Root Crops Research Institute (NRCRI) Umudike, Abia state from May to August 2015. Umudike is located on latitude 5°29'N, longitude 7°32'E and 122m above sea level. The annual rainfall is 1500–1900mm with temperature ranges between 27–30 °C and the soil is sandy loam ultisol. The experimental design used was Randomized Complete Block Design (RCBD) with three replications. Treatments consist of three different seed sizes (whole seed, half seed and quarter seed). There were 3 plots in a replication and each plot comprises of 6 beds of 2m × 2m each, furrow size in between beds 50cm and furrow size in between replications 1m. Each of the treatments was assigned to a plot in a replication.

The pods of fluted pumpkin were obtained from Ikwuano in Abia state, Nigeria. The seeds were separated into whole seeds, half seeds and quarter seeds, before planting. Half seeds were gotten by dividing the seed longitudinally into two halves and each having half section of the embryo while quarter seeds were a single seed cut longitudinally into four and each having quarter section of the embryo.

The nursery box measuring 1m × 1m and 10 cm deep was filled with saw dust and then watered to moisten. After wetting the saw dust, the *Telfairia* seeds (quarter seeds) were then sown randomly. The seedlings were watered until they reached transplanting stage 3 weeks after nursery establishment when most of the seedlings have emerged. Transplanting took place in the evening at spacing of 25 cm x 25 cm apart and a total of 16 seedlings per bed.

Whole and half seeds were sown directly into the field at 1m x 1m and 50 cm x 50 cm respectively. A total of 4 and 9 seeds were sown per bed for whole seeds and half seeds respectively. Weeding started two weeks after planting and repeated as at when due.

Data for growth and yield attributes were recorded at 6, 10 and 14 weeks after sowing (WAS). Six plants were selected randomly from whole seed, half seed, and quarter seed respectively for data collection from each plot. From the sampled plants, averages of the following attributes were recorded: vine length,

number of leaves per plant, number of branches, stem girth, leaf area, and fresh vegetable weight.

The data collected were subjected to analysis of variance (ANOVA) using GenStat Discovery Edition 7 (GenStat, 2012), and the means that were significantly different were separated using Least Square Difference (LSD). Where significant differences were observed, LSD at 5% level of probability was used to separate and test the means for difference.

RESULTS AND DISCUSSION

The coefficient of variation (CV) obtained showed that the attributes had low, moderate and high CV estimates. The CV is used to compare the degree of precision or closeness from one data series to the other. It showed that attributes like fresh vegetable weight at 6 WAP had the highest CV (47.1%) unlike the stem girth at 14 WAP that had the lowest CV (2.1%).

There were significant treatment effects on all the attributes measured at 6, 10 and 14 WAS except number of branches at 14 WAS and fresh vegetable weight at 6 WAS. This suggests that differences in treatments (whole seed, half seed and quarter seed) which were brought about by seed longitudinal division were responsible for variation in these attributes.

The effect of seed division on establishment counts and percentage survival of *T. occidentalis* is shown on Table 1. The three treatments (whole, half and quarter seeds) had 76.7, 66.3 and 38.6 percentage survival respectively. Whole seed had the highest survival rate, followed by half seed and the least survival rate was gotten from quarter seed. The reason might be that the divided seeds had lower food reserves and also exposed to harsher condition in the environment (pest and diseases) than whole seeds especially as no fungicides or pesticides application were involved (Willie and Okoronkwo, 2016). Schippers (2000) reported that the larger the seeds the better the chances of having good germination and establishment.

The effect of seed division on vine length and number of leaves at 6, 10 and 14 WAS were presented in Table 2. Whole seeds produced the longest vine (49.9cm, 88.4cm and 133.1cm respectively), followed by half seeds (43.6cm, 78.7cm and 103.0cm respectively). However, the shortest vines were observed in quarter seeds (7.8cm, 39.5cm and 58.0cm respectively). Whole seed produced the highest number of leaves (36.0, 47 and 102 respectively) followed by half seed (32.2, 43.4 and 86.3 respectively) and quarter seed had the least number of leaves (13.2, 24.7 and 56.8 respectively).

Table 1. Effect of seed division on establishment count and percentage survival of *T. occidentalis*.

Treatments	Establishment count	Percentage survival
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		(%)	LSD _(0.05)	2.03	19.16
Whole seed	3.07	76.7	CV (%)	32.6	31.7
Half seed	5.97	66.3			
Quarter seed	6.17	38.6			

Table 2. Effect of seed division on vine length and number of leaves of *T. occidentalis* at 6, 10 and 14 WAS

Treatments	Vine Length (cm)			Number of Leaves		
	6WAS	10WAS	14WAS	6WAS	10WAS	14WAS
Whole seed	49.9	88.4	133.1	36.0	47.9	102.3
Half seed	43.6	78.7	103.0	32.2	43.4	86.3
Quarter seed	7.8	39.5	58.0	13.2	24.7	56.8
LSD _(0.05)	19.04	28.21	30.49	10.70	13.90	20.77
CV (%)	24.4	11.8	12.4	21.5	22.4	15.0

Table 3. Effect of seed division on leaf area and number of branches per plant of *T. occidentalis* at 6, 10 and 14 WAP

Treatments	Leaf area per plant (cm ²)			Number of branches per plant		
	6WAS	10WAS	14WAS	6WAS	10WAS	14WAS
Whole seed	249.0	292.0	322.0	12.14	12.78	26.1
Half seed	199.0	265.0	262.7	10.81	12.81	17.5
Quarter seed	69.0	131	177.6	4.44	8.06	17.4
LSD _(0.05)	69.3	94.1	21.97	3.736	2.236	NS
CV (%)	18.4	8.5	5.9	20.9	10.4	21.1

Table 4. Effect of seed division on stem girth and Fresh vegetable weight of *T. occidentalis* at 6, 10 and 14 WAS.

	6WAS	10WAS	14WAS	6WAS	10WAS	14WAS
Whole seed	1.930	2.273	2.847	338.0	703.3	1066.5
Half seed	1.543	2.127	2.540	259.4	685.3	938.4
Quarter seed	0.503	1.220	1.620	60.6	245.4	646.2
LSD _(0.05)	0.6041	0.3372	0.1448	240.7	251.6	184.3
CV (%)	17.8	12.6	2.1	47.1	10.4	3.9

The effect of seed division on leaf area and number of branches per plant at 6, 10 and 14 WAS is shown in Table 3. There was no significant variation between whole seeds and half seeds at 6 and 10 WAS while at 14 WAS, significant variation existed in leaf area. Whole seeds had 249, 292 and 322 cm leaf area, half seed had 199.0, 265.0, and 262.7 cm, while quarter seed had 69.0, 131.0 and 177.6 cm mean per plant at 6, 10 and 14 WAS respectively.

The result indicates that at 6 and 10 WAS, Whole seeds and half seeds did not significantly differ from one another in respect to number of branches. Quarter, half and whole seeds had 4.44, 10.81 and 12.14 per plant respectively at 6 WAS. At 10 WAS, whole seeds recorded 12.78, half seeds recorded 12.81 while quarter seeds recorded 8.06.

Table 4 which presents the effect of seed division on stem girth and fresh vegetable weight per hectare at 6, 10 and 14 WAP depicts that, whole seed produced plants with the biggest stem girth (1.93 cm, 2.27 cm

and 2.85 cm respectively) while quarter seed produced plants with the smallest stem girth (0.50 cm, 1.22 cm and 1.62 cm respectively). Effect of seed division on fresh vegetable yield per hectare at 6, 10 and 14 WAS, indicates that whole seeds produced plants with largest fresh vegetable yield per hectare (338.0kg, 703.3kg and 1066.5kg respectively) while quarter seed produced plants with least fresh vegetable weight per hectare (60.6kg, 245.3kg and 646.2kg respectively). There was no significant variation between whole seeds and half seeds at 10 WAS. The effects of seed size on growth and fresh vegetable weight were based on the fact that whole seed contains more biomass for the seedling to feed during early growth than half seed and half seed contains more biomass than quarter seed (Willie and Okoronkwo, 2016).

Whole seeds performed better than other treatments while half seed performed better than quarter seed. This shows that the size of seeds highly influenced all the attributes studied. According to Dhillon and Kler

(1976) seed size in cereal and legumes appears to influence the grain yield. The size of the seed is one of the components of seed qualities which affect the performances of crop (Ojo, 2000, Adebisi *et al.* 2011).

Whole seed gave the highest values of vine length, number of leaves, number of branches, leaf area, stem girth and fresh vegetable weight at harvest. However, half seed gave a higher value when compared to a quarter seed. These results are in line with Riface *et al.* (2004) who reported that seed size had significant effect on yield and yield component. The technology of obtaining multiple seedlings from *T. occidentalis* should be properly investigated because of the inherent potential of producing genetically identical materials for use in work requiring homozygosity (Mezie, 2006).

However, Bradbeer (1992) recognized the use of seed sizes as a compensation for variation in environmental condition and the enhancement of the productive capacity in a plant. He also observed that large seeds were preferred by growers of *T. occidentalis*, since they germinate more vigorously and gave rise to large seedlings which may mean greater herbage yield. The result showed that though seedling of fluted pumpkin can be multiplied through longitudinal seed division, whole seed is better than half seed and half seeds better than quarter seed if compared individually. However, if looked at another point of view, let's say from production and profit point of view. "2 half seeds = 1 whole seed". If whole seeds

mean values from the growth and yield parameters in this study are divided into two, the values obtained will be far below the mean values obtained from the half seeds in this study. For example, the fresh vegetable weight kg/ha of whole seed at 6, 10 and 14 WAS were 338.0, 703.3 and 1066.5 respectively. If these values are divided by two, we will have 169.0, 351.65 and 533.25 respectively. Comparing these values (69.0, 351.65 and 533.25) with the mean values of half seeds obtained also in fresh vegetable weight (259.4, 685.3 and 938.4 for 6, 10 and 14 WAS respectively), one would observe that 2 half seeds values were far better than 1 whole seed. This is also applicable to all the growth parameters studied in this work. Therefore, there is advantage of multiplying fluted pumpkin through seed division by half.

CONCLUSION

Whole seed gave the highest values of vine length, number of leaves, number of branches, leaf area, stem girth and fresh vegetable weight at harvest. Half seed gave a higher value than quarter seed. However, when compared the mean values of whole seeds, half seeds and quarter seeds in all the parameters measured, it was observed that whole seeds were slightly higher than half seeds, and half seeds could be comparable to whole seeds in terms of number of branches, number of leaves and stem girth. Quarter seeds could not compare to half seeds as there was a big difference between the means. Hence the use of half seed as planting material could be beneficial especially during the scarcity period.

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CHEMICAL PROPERTIES AND HEAVY METALS CONCENTRATION IN SOILS AND CASSAVA ROOTS FROM COMMUNITIES AROUND MICHAEL OKPARA UNIVERSITY OF AGRICULTURE UMUDIKE, ABIA STATE.

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ABSTRACT

The study was conducted to investigate the concentration of some heavy metals (Cr, Cd, Pb and Zn) in soils and cassava roots from communities around Michael Okpara University of Agriculture Umudike (Umuariaga, Umudike, Amoba and Amawom). Soil samples and cassava roots were collected from 4 communities around the University and control (samples were collected from Agricultural land in Ndoru village, about 30 Km away from the host communities), the samples were analyzed in the laboratory using standard methods, data obtained were analyzed using analysis of variance (ANOVA) as contained in the GENSTAT 2015 version. Result obtained showed that the soil pH increased significantly ($p < 0.05$) at the communities around the university when compared with control sample. Umudike recorded highest pH value of 6.58 while control had 4.87, Available Phosphorus were higher at Umudike (39.3 mg/kg) and lower at control with 15.9 mg/kg. Apart from exchangeable acidity that was higher at control with 1.78 cmol/kg. Chromium, Cadmium, Lead and Zinc concentration were above WHO permissible limits and significantly ($p < 0.05$) higher in the soil samples and cassava roots from studied sites than control.

Keywords: Heavy metals, concentration, soil properties, cassava root

INTRODUCTION

Heavy metal concentration in soil is associated with biological and geographical cycles and actions such as agricultural practices, industrial activities and waste disposal methods (Eja *et al.*, 2003). The knowledge of heavy metal accumulation in soils, the origin of these metals and their possible interactions with soil properties are priority in many environmental monitoring (Qishlaqi and Moore, 2007). The accumulation of heavy metals in agricultural soils is of increasing concern due to food safety issues and potential health risks, as well as its detrimental effects on soil ecosystem (Alloway and Aynes 1997). Most of the agricultural lands within the communities around the university (Michael Okpara University of Agriculture Umudike) are becoming a dumping ground for technological waste, especially used electronics and other harmful material from the university computer village and student hostels within the school and its host communities. It is a known fact that some of these products/materials contain hazardous metals such as iron, lead, copper, cadmium, mercury, chromium, arsenic, zinc and so on. Increase in human population, business activities and technological improvement in most communities around the University have increased the quantity of wastes deposited on agricultural lands due to poor waste management. It has been a common practice in these communities to burn those wastes to reduce it or get rid of the wastes before cultivating their land, thus generating heavy metal rich ashes. These ashes will be dissolved in rain water and leached into the soil, causing contamination of underground water or washed away by run-off water into streams and rivers, thereby contaminating the environment. Cassava is an important staple crop and in the tropical world it ranks fourth in importance after rice, wheat and maize

(ANU, 2007). In sub-Saharan Africa (SSA), cassava is currently the major staple food for over 40% of the population and for an estimated 500 million people in the tropics, Boadi *et al.* (2008). In most of the communities around MOUAU, cassava and its product (garri, fufu, tapioca, starch and flour) serves as major source of calories for their families because of its high starch content. Therefore evaluation of heavy metals in the soils and cassava roots from communities around the university will go a long way in suggesting remedial measures to avoid the soil being used to grow crops, which when consumed will lead to transfer of heavy metals into animal and human body. Hence the objective of the study is to evaluate heavy metal concentration in agricultural land and cassava roots from communities around Michael Okpara University of agriculture Umudike. Therefore, there is high tendency of heavy metal poisoning when contaminated cassava roots are consumed human or animal.

Materials and Methods

The study was carried out in communities around Michael Okpara University of Agriculture Umudike; Umuariaga, Umudike, Amoba and Amawom all in Ikwuano LGA of Abia State South Eastern Nigeria. Umudike is located at latitude 05° 27'N and longitude 07° 32'E, Umuariaga is located at latitude 05° 28'N and longitude 07° 32'E, Amoba is located at latitude 05° 28' N and longitude 07° 33'E, Amawom is located at latitude 05° 28'N and longitude 07° 34'E while control (Ndoru) is located at latitude 05° 44'N and longitude 07° 56'E

Sample collection

Soil samples and cassava root were collected from agricultural lands in Umuariaga, Umudike, Amoba,

Amawom and control sample was picked from farm land in Ndoru., Ndoro is about 40 Km away from the University. The samples were collected four (4) each from the communities and control at depth of 0-30cm with soil auger, making a total of 20 samples. The samples were labelled and transported to laboratory for chemical and heavy metal analysis.

Laboratory analysis

Soil pH was determined in H₂O using glass electrode pH meter at a soil liquid ratio of 1:2.5 as explained by Udo *et al.* (2009). Soil Organic Carbon was determined by Walkley and Black wet oxidation method and modified by Udo *et al.* (2009). Organic Matter was determined by calculation using the conventional Van Bellema factor of 1.724. Total Nitrogen, Available Phosphorus, Exchangeable Bases (Ca, Mg, K and Na) and Exchangeable acidity were determined according to Udo *et al.* (2009). Effective Cation Exchange Capacity (ECEC) and Percentage Base Saturation (BS) were calculated by summation of TEB + EA and $\{(ECEC - EA) \div ECEC\} \times 100$, respectively. Heavy metals in soil and cassava root (Zn, Cr, Pb and Cd) were determined with Atomic Absorption Spectrophotometer (AAS) as described by Udo *et al.*, (2009).

Data analysis

Data collected from the laboratory analysis were subjected to analysis of variance (ANOVA) using GENESTAT software package, and Fishers least significant difference (LSD) were used to compare the means at 5% probability level.

RESULTS AND DISCUSSION

Table 1: chemical Properties of Soils from studied sites

Location	Depth (cm)	pH	Av.P	TN	OM	Ca	Mg	K	Na	EA	ECEC	BS
			mg/kg	%			cmol/kg					%
Umuariaga	0-30	6.17	38.2	0.435	4.63	13.73	6.77	0.574	0.443	0.83	22.41	96.31
Umudike	0-30	6.58	39.3	0.465	4.76	14.2	7.03	0.561	0.425	0.77	22.99	97.00
Amoba	0-30	6.05	36.1	0.407	4.56	13.3	6.42	0.515	0.420	0.82	21.46	96.18
Amawom	0-30	5.88	32.0	0.393	4.44	12.95	6.2	0.535	0.389	0.87	21.04	95.84
Control	0-30	4.87	15.9	0.122	1.76	4.55	1.15	0.134	0.104	1.78	7.71	77.78
LSD (0.05)		0.09	1.46	0.008	0.20	0.37	0.20	0.005	0.001	0.09	0.59	1.78

Note: Av.P = Available phosphorous, TN = Total Nitrogen, OM = Organic Matter, Ca = Calcium, K = Potassium, Na = Sodium, EA = Exchangeable acidity, ECEC = Effective Cation Exchange capacity, BS = Base saturation, LSD = Least significant difference

Heavy metal in soil

heavy metal in soils from the the studied sites (Umuariaga, Umudike, Amoba and Amawom) (Table2) shows that Chromium (Cr) value ranged from 129.80 to 95.03 mg/kg with Umudike soil given highest value of 129.80 mg/kg followed by 116.95 mg/kg and 109.03 mg/kg from Umuariaga and Amoba soil. The value of Cr obtained across the studied sites were significantly higher than control and above WHO permissible limits in soil. While that of Amawom was

Chemical properties of soil from the studied sites and control (table1) shows that pH value ranged from 6.58 to 4.87, thus the pH of the soil is slightly acidic. Low or moderate pH tends to favour faster release of metals in the soil solution due to proton competition for exchange sites of clay minerals, organic matter or through proton promoted dissolution of soil minerals (Igwe and Nwachukwu, 2016). pH of soil affects process of metal uptake into root of cassava. Also, the retention of metals to root organic matter is weaker at low pH resulting in more available metal in soil solution for root absorption (Ano *et al.*, 2007). Therefore the slightly acidic nature of the soils may have favoured heavy metal uptake by cassava. Available phosphorous content across the studied sites were very high when compared with control. Total nitrogen, Organic matter, Calcium, Magnesium, Potassium and Sodium concentration were moderately higher at the studied sites than control. The result showed a significant (p<0.05) increase in the chemical properties of soil tested when compared with control samples. Increase in most of the soil chemical properties (pH, OM, TN, Av.P, Ca, Mg, K, and Na) recorded across the 4 communities around MOUAU maybe as a result of indiscriminate dumping of wastes on agricultural lands within these communities from students and dissolved ashes from burnt wastes that leached into the soil during raining season. Apart from Exchangeable acidity that was higher at the control sample, every other chemical properties were higher across the 4 communities sampled. The result obtained from this study is in agreement with the findings of Eja *et al.* (2003) where they observed a similar increase in most chemical properties of soil collected from agricultural lands around industrial estates.

higher than control but below permissible limit. Mean concentration of Cadmium (Cd) value ranged from 91 to 67 mg/kg. The highest value was recorded from Umuariaga sample 91 mg/kg followed by Umudike and Amoba (81.9 and 70.3 mg/kg). while control was very minute that it was not detectable (ND). Lead (Pb) concentration was higher in the studied sites than control. Pb ranged from 183.1 to 0.13 mg/kg, Umuariaga gave the highest value of 183.1 mg/kg followed by 177.8 mg/kg and 174.95 mg/kg from

Umudike and Amoba samples. Amawom had 170.02 mg/kg which is above WHO permissible limits while control samples had the lowest value of 0.13 mg/kg. Zinc (Zn) value ranged from 229 to 2.44 mg/kg and was higher in the studied sites than control. The result shows that zinc concentration in the soil were above WHO permissible limits. The increase in heavy metal in the soil may be attributed to increase in indiscriminate dumping of refuse from business centers within and around the university and increase in student hostel within the communities around the university.

Table 2: Concentration of heavy metals from studied sites

Location	Depth (cm)	Cr	Cd	Pb	Zn
.....mg/kg					
Umuariaga	0-30	116.95	91	183.1	222.1
Umudike	0-30	129.8	81.9	177.8	229
Amoba	0-30	109.0	70.3	174.95	209.6
Amawom	0-30	95.03	67.05	170.02	206.5
Control	0-30	ND	ND	0.13	2.44
LSD (0.05)		0.56	0.78	3.41	5.16
WHO/FAO Permissible Limit (1996)		100	0.8	85.0	50.0

Note: Cr = Chromium, Cd = Cadmium, Pb = Lead, Zn = Zinc, ND = Not detectable, LSD = Least significant difference, WHO = World Health Organization, FAO= Food and Agriculture Organization.

Table 3: Concentration of heavy metals in Cassava root from studied sites

Location	Cr	Cd	Pb	Zn
..... mg/kg				
Umuariaga	6.85	5.45	18.45	38.3
Umudike	7.25	6.1	17.3	42.8
Amoba	6.01	5.05	17.52	36.2
Amawom	5.75	5.25	16.3	37.4
Control	ND	ND	ND	0.03
LSD (0.05)	0.08	0.32	0.26	0.76
WHO/FAO Permissible Limit (1996)	1.30	0.02	2.0	0.60

Note: Cr = Chromium, Cd = Cadmium, Pb = Lead, Zn

= Zinc, ND = Not detectable, LSD = Least significant difference, WHO = World Health Organization, FAO= Food and Agriculture Organization.

Heavy metal concentration in cassava root.

Chromium (Cr) and Cadmium (Cd) concentration in cassava roots from the studied sites were significantly (p<0.05) higher than the control (table3). The result shows that Cr and Cd values were above WHO maximum permissible limits in plants. Concentration of Zinc (Zn) and Lead (Pb) in cassava roots from studied sites were higher when compared with control. Zinc and Lead often occur in an easily soluble form in the soil, thus they are readily available for plant uptake. Heavy metals (Cr, Cd, Pd and Zn) accumulation by cassava shows that cassava can grow on contaminated soil without showing any negative symptoms on the root or shoot system. Studies shows that moderate or low pH affects process of metal uptake into the root of plants (Qishlaqi and Moore, 2007). The high concentration of heavy metals recorded on the studied sites maybe as a result of increase in number of business centers that uses heavy metal contained materials.

Conclusion and Recommendations

Plants will definitely take up available elements in the soil, even when the elements are toxic and not essential to them. The study revealed that heavy metals (Cd, Cr, Pb and Zn) were found to be very high and above WHO permissible limit in the soil and cassava roots of most communities around Michael Okpara University of Agriculture Umudike as a result of increase in students population/business center that uses materials that contains heavy metals and indiscriminate disposing of waste materials that contains heavy metal on agricultural lands within the communities around the university by students and business center owners. Therefore, there is high tendency of heavy metal poisoning when contaminated cassava roots are consumed human or animal. Cassava is a common food for human and livestock in these host communities. It is therefore recommended that special space (land) far from agricultural lands should be mapped out for disposing of wastes from business centers and private hostels within the communities around the university.

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OKRA GROWTH AND HEAVY METAL UPTAKE IN SOIL CONTAMINATED BY LEAD (Pb) MINING ACTIVITIES IN TWO COMMUNITIES OF EBONYI STATE, NIGERIA

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ABSTRACT

We evaluated the growth of Okra over a three month period and its uptake of Lead (Pb), Cadmium (Cd), Iron (Fe) and Zinc (Zn) in pots using soils collected within a 2 km radius of Lead (Pb) mines in two villages of Ebonyi State, Nigeria. Treatments in the study were distance from mines and status of the mines. Soil was collected at a single depth of 0 -20 cm at distances of 10, 20, 40m and 2km from mine pits comprising two active mines, one abandoned mine while a non- mine soil was the control. Okra planted in mine impacted soil exhibited zero or poor germination followed by rapid death of seedlings. Plants grown in soils 20 - 40 meters away from mines exhibited stunted growth and zero flowering, while those grown in soils of abandoned mine pits exhibited relatively good growth, flowering and fruiting. There was significant Pb uptake (5.0 - 15.4 mg/kg) by Okra at 12 weeks of growth, which exceeded allowable limits of 0.1 – 10 mg/kg Pb for food crops. The uptake of Fe, Cd and Zn were not significant and were however within acceptable limits of 20,000 mg/kg Fe; 0.1mg/kg Cd; and 90 mg/kg Zn. Soil metal levels in mining communities pose a potential risk to residents, as growing crops may eventually take up toxic levels of such heavy metals into their edible portions. Hence, it is advised that crop production be discontinued in mining communities, especially those with active mines.

Keywords: Pb mining, soil metals, Okra growth

INTRODUCTION

The soil is a natural source of background levels of heavy metals like lead, zinc, cadmium, arsenic, nickel, copper etc. Heavy metal contamination however often results from anthropogenic activities (Vaaramaa *et al.*, 2010), through which such metals can reach man directly through or from plant and animal sources. Minute quantities of heavy metals may constitute toxic concentrations and therefore be hazardous to plants, animals and ultimately to humans via the food chain.

Commercial lead mining began in Enyigba, Ebonyi State, Nigeria nearly hundred years ago. Many farming communities in the area are fast turning into mining towns owing to the economic importance attached to such mines by the residents. Enyigba mining community lies in the Northeastern fringes of south east Nigeria, classified as a derived savanna (Nwagbara *et al.*, 2014). In the region, Okra, *Abelmoschus esculentus* (L Moench) is a widely cultivated vegetable grown for its fruits which are harvested and eaten when they are young and succulent. Nutritionally, it is reported that Okra contains vitamin C, proteins and Carbohydrates and they also have antioxidant and antidiabetic properties (Adetoye *et al.*, 2008).

It is reported that most crops are affected by toxic substances in the soils resulting in dehydration or impaired nutrient uptake by plants as a result of the presence of such toxins in soils. Wong *et al.* (2001) maintained that high soil acidity favours soil availability and absorption of heavy metals. Metal accumulation in plant tissues is influenced by inherent properties of plants which differs between species, ecotypes and in plant parts (Cobb *et al.*, 2000). Plants on the other hands have different specific root response mechanisms to environmental conditions (Fitz and

Wenzel, 2006).

The chemical effect of lead on man is enormous, lead is a neurotoxin that changes the nervous system leading to brain disorders at concentrations > 10µ/dL (Nwachukwu and Pulford, 2008). It has very pronounced effect on children, like delayed cognitive functions. Language and reading skills problems, spatial and visual motor skills are all affected by high lead contamination in the body especially of prepubescent children. Therefore, given the involvement of children in the business of Pb mining in Ebonyi State, a study of the effect of the metal on crop growth and Pb uptake by widely grown food crops becomes essential.

In this study we investigated the effect of mine status (active and inactive), distance from mines on the growth and performance of Okra planted in a mine impacted soil, and also determined the plant's uptake of selected heavy metals (Pb, Cd, Fe and Zn) at maturity.

MATERIALS AND METHODS

The study evaluated Okra uptake of Pb, Cd, Zn and Fe during a three-month growing period in a pot experiment. The experiment was conducted at the Greenhouse Facilities of Michael Okpara University of Agriculture Umudike. Three mine pits were sampled (of which one mine pit was abandoned since 1974), and also a non mining area that is two kilometers away from any mine pits of Enyigba.

The coordinates of the locations were Isiagu mine at Enyigba (6° 11' 40"N 8° 8' 38"E); Enyigba Mine pit at Enyigba (6° 11' 47"N 8° 8' 17"E); Abandoned mine (6° 11' 16"N 8° 8' 22"E) and finally, an adjacent

farmland situated two kilometres from the mines at Enyigba (6° 11' 53"N 8° 8' 13"E) which served as a control (non- mine impacted soil).

Soil sampling and treatment

Soil was collected at a single soil depth of 0 – 20 cm at the centre of each pit, distance of 20 meters from the pit and then 40 metres from the pit using soil auger. Sampling was in a radial fashion at each distance from the pit centre, each in triplicates, while the control area was sampled in a radial manner at 20 metres apart following the downward slope of the land. These gave a total of thirty six soil samples. All the sampled soils were subjected to routing laboratory analysis for pH, hydraulic conductivity, porosity, particle size distribution, some major nutrient contents, Effective cation exchange capacity, % Base Saturation, Exchangeable acidity, and selected heavy metals. For the pot experiment, about 2 kg of bulk soil was also collected from each sampling point for cultivation of Okra in the greenhouse.

Experimental Protocol and design

Plastic pots of 18cm diameter x 22cm height were used for the experiment. About 2kg of air-dried composite soil was weighed into the pots. The pots were arranged as Split-split plot in Completely randomised design with the 'status of the mine' being the main factor and 'time of sampling'(time of sampling was either before planting or after 12 weeks of plant growth) and 'distance from the mine pit' being the sub plot and sub-sub plot factors respectively, each replicated three times. During a twelve week cultivation period, agronomic parameters measured on the Okra plants were germination %, age to first flowering, fresh harvest weight and oven dried weight of whole plant.after twelve weeks of growth. The soils used for the pot experiment were also analysed at the end of 12 weeks of Okra growth.

Plant nutrient -uptake analysis procedure

Whole Okra plants (including roots) were carefully uprooted after 12 weeks of growth, cleaned by gently washing away soil particles with distilled water, and weighed at Soil Science laboratory, Michael Okpara University of Agriculture Umudike, Nigeria. The cleaned plants were placed individually on laboratory tissue to absorb excess water from rinsing, and each was weighed to obtain the fresh weight. Each whole plant was then put in labelled large brown envelopes and dried in the oven at 60 °C for 48 hours. After oven drying, each plant sample was ground to powder with a porcelain mortar and pestle, and the powdered samples were stored in clean stoppered glass receptacles. Plant metal uptake was calculated as concentration of metal mg/kg in whole plant x dry weight of the plant material analysed.

Heavy metal transfer factors were also assessed by using the relative concentration of contaminants in

soils and plants calculated on the basis of dry weight Kumar and Edwards (2009) given as:

$$PCF = C_{plant} / C_{soil}$$

$$C_{if} = C_{io-1} / C_{in}$$

Where C_{io-1} is the mean concentration of metals from the soil sampling sites (at least five sampling sites and C_{in} is the average concentration of tested (as a reference value) elements in the earth's crust.

Statistical Analysis

The data generated were subjected to Analysis of Variance (ANOVA) for experiment in Split-split plot design in Completely Randomised Design using GENSTAT package (2010). The significant means were separated using Fischer's Least significant Difference (0,05).

RESULTS AND DISCUSSION

Growth of Okra

Many of the Okra plants failed to grow in the active mine soils, especially in samples collected from the centre of the pit. Those that continued to survive were in soils 20 meters and 40 meters away from the mine pit (Plate 1). Several studies show that crops grown in heavy metal-contaminated soils take up high quantities of these metal ions into plant parts, and this uptake is affected by metal species, pH and cation exchange capacity, organic matter content, soil nutrients and ions in the rhizosphere, soil texture, crop type, species and age of plants (Nwachukwu and Pulford, 2008). The abandoned mine soils and the adjacent farmland had all the plants still growing in them at the end of the experiment. The non mine impacted farmland soil had the highest nitrogen uptake by Okra plants while Isiagu mine soils recorded the least nitrogen uptake.



Plate 1. Okra growth in soil impacted by Pb mining

In effect the bioavailability of heavy metal is very highly complex as it involves physicochemical parameters of soil, microbiology of soil and biotic and abiotic interactions involving any range of synergy, mutualism and or antagonistic reactions (Nwachukwu and Pulford, 2011).

Table 1: Average Fresh weight of Okra 12 weeks after planting.

	Enyigba mine	Isiagu mine	Abandoned mine	Adjacent Farm
Distance (m)	Fresh wt(g)	Fresh wt(g)	Fresh wt(g)	Fresh wt(g)
0	0	0	12.07	13.62
20	11.45	5.60	13.90	14.92
40	7.47	6.55	12.47	16.52

The crops planted in the mine pit soils died soon after germinating. Shafiq and Iqbal (2005) found that germination of seeds are inhibited by heavy metal concentration of soils. The different distances away from the mine pits showed that the plants were affected by the level of heavy metal toxicity inside the pits. Results suggest that a combined effect of acidity and heavy metal toxicity was responsible for the rapid death and poor performance of the crops in the vicinity of the mines. The crops were unable to take up needed water and plant nutrients after germination owing to reverse osmotic pressure as a result of the high acidity and toxic heavy metal concentration of the mine soils (Akciil and Koldas, 2006). Okra seedlings that died out after germination were not subjected to laboratory analysis to understand their levels of heavy metal uptake. The adjacent farm land had the healthiest plants that survived throughout the planting period as well as having the highest plant weight at harvest followed by the abandoned mine soils (Figure 1).

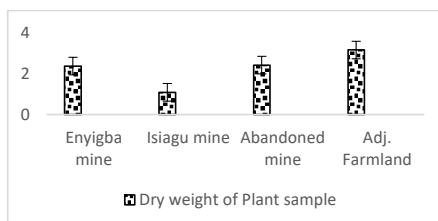


Figure 1: Dry weight (g) of the Okra crops grown in study soils after 12 weeks of growth
Dry weight of Okra plants from the adjacent farmland was significantly highest followed by the Okra plants from the abandoned mine soils.

PLANT TISSUE ANALYSIS

It should be noted that eight out of the thirty-six plots had no plants to be sampled at the end of planting period because of the death of the okra plants planted in such pots. At the end of the 12 weeks, plants that survived were sampled by harvesting whole plants. This resulted in unequal sample size for statistical analysis. Okra plants were analysed for the plant nutrient uptake, and in spite of metal contamination, Okra plants took up significant amounts of N, K, P, Ca, Mg, Na, (Table 2).

Table 2: Okra nutrient content after 12 weeks of growth in Pb mine impacted soils

	N	P	K	Ca	Mg	Na
Enyigba 0m	na	na	na	na	na	na
Enyigba 20m	1.60	0.33	0.32	1.40	0.43	0.18
Enyigba 40m	0.91	0.27	0.13	1.40	0.47	0.16
Isiagu 0m	na	na	na	na	na	na
Isiagu 20m	0.7	0.23	0.14	1.07	0.34	0.12
Isiagu 40m	0.81	0.1	0.08	0.35	0.12	0.06
Abandoned 0m	0.92	0.25	0.29	1.37	0.43	0.20
Abandoned 20m	1.2	0.21	0.22	0.934	0.223	0.21
Abandoned 40m	0.65	0.093	0.19	0.53	0.18	0.17
Adj. farmland 0m	1.1	0.28	0.22	1.23	0.47	0.22
Adj. farmland 20m	1.63	0.36	0.34	1.17	0.36	0.17
Adj. farmland 40m	1.8	0.35	0.34	1.67	0.53	0.23

na – data not available as a result of zero germination or death of plant

In addition, significant concentrations of heavy metals Fe, Cd, Zn, and Pb (mg/kg) were also found in the tissue of Okra (Table 3).

Table 3: Heavy metal uptake by Okra after 12 weeks of growth

Mines/distances	Pb mg/kg	Cd mg/kg	Zn mg/kg	Fe mg/kg
Enyigba 0m	na	na	na	na
Enyigba 20m	5.00	2.80	4.30	1.99
Enyigba 40m	2.90	1.50	3.80	0.61
Isiagu 0m	na	na	na	na

Isiagu 20m	3.40	0.86	2.56	3.60
Isiagu 40m	15.40	3.75	1.20	3.00
Abandoned 0m	2.60	0.76	1.61	4.50
Abandoned 20m	1.85	3.97	3.00	0.92
Abandoned 40m	2.20	4.41	3.60	1.60
Adj. farmland 0n	1.60	1.37	2.60	3.20
Adj. farmland 20m	0.87	0.64	2.70	3.70
Adj. farmland 40m	0.44	0.40	1.40	1.70

na – data not available as a result of zero germination or death of plant

According to Bowen (1979), the normal levels of these heavy metal is given as: 14.1 mg/kg, 0.1 mg/kg and 90 mg/kg for Lead, Cadmium and Zinc respectively .The levels of heavy metal uptake by Okra is shown in Table 4.

Table 4: Mean concentration of Pb, Cd, Zn, and Fe in Okra after 12 weeks of growth

Mean levels of heavy metals in plants	Abandoned mine	Enyigba mine	Adj. farmland	Isiagu mine
Lead (mg/kg)	2.21	3.84	0.95	8.22
Cadmium (mg/kg)	3.05	2.03	0.93	2.02
Zinc (mg/kg)	2.74	4.02	2.22	1.96
Iron (mg/kg)	2.33	1.16	2.87	3.34

From the plant concentration factor however, the results show the order of PCF as Cd>Fe>Zn>Pb in Okra plant samples. The plant concentration factor (PCF) was calculated based on the equation of Liang *et al.* (2011)

Table 5: Plant Conversion Factor (PCF)

Nutrients	Abandoned mine	Enyigba mine	Adj. farmland	Isiagu mine
Lead	0.13	0.07	0.19	0.11
Cadmium	2.22	1.06	0.50	1.21
Zinc	0.17	0.20	0.22	0.15
Iron	0.27	0.12	0.47	0.43

In Isiagu mine (pH 2.5) with highest soil lead levels (80.7 mk/kg), the plant lead uptake at 40 meters (0.11µg/kg) was not significantly different from the abandoned mine soils (pH 5.5) at 0 meters with a soil lead content of 20.2 mg/kg and a plant lead uptake that is higher at 0.13mg/kg.

Uptake of Nutrient Iron (Fe) by the Okra plants planted in the mine soils: As with Pb, Cd and Zn, higher quantities of iron was taken up in soils nearer to the mine site, declining as one moved away from the mine pits. At the centre of the mine (zero), Fe uptake was 3.8 mg/kg, 2.5 mg.kg at 20 meters away and only 1.6 mg/kg at 40 meters away from the mine.

CONCLUSION

As shown by this study, okra plants showed significant levels of heavy metal uptake owing to the fact that it was grown on contaminated soils. Results also show that gradual reduction of heavy metal levels through natural attenuation can help in reclaiming mine areas so that agricultural activities can also be carried out in the vicinity of abandoned mines. Unlike in the active mine pits, soils from the abandoned mine was able to sustain Okra cultivation though the performance was lower than the adjacent farm land, therefore the point source of the heavy metals derive from within the mine pits. The farther away from the mine pit a farm is, the lower the levels of heavy metals available in the soil

for plant uptake.

That the abandoned mine soils contained higher levels of plant nutrients like phosphous, calcium, magnesium, sodium and organic carbon did not translate to better Okra crop growth during the study This could be as a result of the effects of heavy metals in the soils together with unfavourable pH level of the mine soils. High zinc content in mine soils may have led to poor root conditions and therefore higher heavy metal uptake which led to the poor quality performance of the plants in the mine soils.

As expected, Pb had significantly the highest concentration of the four metals evaluated since the mines in Enyigba are very rich in lead oxides as the major minerals. Lead poisoning of the food chain by plant uptake in crops like vegetables, fruits and water sources in Enyigba is therefore a potential threat. At 0.015 mg/kg Pb uptake in Enyigba mine soils, crops grown in the vicinity of mines in that location are very high and unsafe for human consumption. In adjacent farm lands however, the mean lead plant uptake was significantly lower at 0.0025 mg/kg. Lead uptake from Enyigba mine soils at 40 meters away from the mine pits were also low and within permissible levels. Nevertheless, planting of crops around the mine sites are not recommended.

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LAND USE EFFECTS ON SOIL QUALITY INDICES IN A RAIN FOREST ECOLOGY, AKWA IBOM STATE, NIGERIA

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ABSTRACT

Soil quality indices of land cultivated to cassava/maize, leafy vegetable and fallow land were evaluated in Ikot Ekpene LGA in Akwa Ibom State, Nigeria. Soil quality indices evaluated were soil texture, bulk density, hydraulic conductivity, total porosity, soil pH, electrical conductivity, organic carbon, available phosphorus, total Nitrogen, organic matter, exchangeable bases, exchangeable acidity, and total microbial count using standard laboratory methods. Eighteen composite soil samples were collected from 0-20 cm and 20-40 cm soil depths across the three land use types. Laboratory analyses showed that the surface soil was significantly higher in pH, electrical conductivity and base saturation under vegetable land use than that of cassava/maize and fallow land. Exchangeable Ca and Mg, exchangeable acidity, ECEC, and base saturation were also significantly higher ($p < 0.05$) in vegetable land use than in cassava/maize or fallow land use. However, exchangeable acidity was significantly higher in fallow land. Microbial population in 0 – 20 cm soil depth of all three land use types were 21.45, 26.31 and 23.3 cfu/g in cassava/maize land use, fallow land, and vegetable land respectively. At 20–40 cm soil depth, there was reduction in total microbial population being 17.3, 24.21 and 20.14 cfu/g in cassava/maize, fallow and vegetable land use respectively. Soil texture of all land use types were loamy sand except the fallow land which was sandy loam. Based on the MVIT model, vegetable cropped land had the highest soil quality rating of 54 % > cassava/maize (45.5 %) = fallow land (45.5%). Generally, the three land use types were rated moderate (medium) in soil quality.

Keywords: Land use, soil quality, soil quality indices

INTRODUCTION

Soils form a fundamental base to productivity of agricultural and natural ecosystems. Soil has both inherent and dynamic qualities (USDA, 2006), and the concept of soil quality was developed to characterize the usefulness and health of soils as means of evaluating sustainable soil management practices (Ouedrago *et al.*, 2001). Soil quality index (SQI) is increasingly proposed as an integrative indicator of environmental quality, food security and economic variability (Lal, 1999). A soil's inherent properties cannot be managed or adjusted, as they are determined by factors such as climate, topography, vegetation, parent materials and time. Soil quality is a dynamic interaction between various physical, chemical and biological soil properties of soil, and it is influenced by many factors such as land use, land management, the environment and socio-economic priorities (Sharma *et al.*, 2011). SQI includes an intrinsic part covering the inherent capacity of the soil for plant growth (Carter, 2002), and a dynamic part influenced by the soil user or manager (Ball and De la Rosa, 2006).

Land use is the purpose to which a land is applied such as engineering/construction, urban development, landfill/dumpsite, forest reserve or agricultural production (USDA, 2006). Soil quality is considered a key element of sustainable agriculture (Warkentin, 1995) because it is essential to support and sustain crop, range and woodland production and helps maintain other natural resources such as water, air, and wildlife habitat. Therefore, the long term use of any land has a direct effect on the potential of that land for crop productivity (Carter, 2002).

Measurable soil qualities may be physical, chemical or microbiological (Nortcliff, 2002). USDA (2006) suggested key physical, chemical and biological indices to represent a minimal dataset to characterize soil quality for sustainable crop production. The indices are: Physical indicators: bulk density, infiltration (porosity), hydraulic conductivity, aggregate stability, water holding capacity, top soil, rooting depth and particle size distribution. Chemical indicators: soil pH, cation exchange capacity, effective cation exchange capacity, exchangeable cations, electrical conductivity, total nitrogen, available phosphorus and base saturation (Moura *et al.*, 2009). Biological indicators: total microbial population count, total organic carbon, organic matter, and mineralizable nitrogen (Idowu *et al.*, 2008; Karlen and Cambardella, 1996). All these soil properties influence crop production.

Soils of Akwa Ibom vary in quality due to the different parent materials from which they are formed. These includes coastal plain soils, false bedded sandstones, clay-shales, upper coal measure and lower coal measures (Akamigbo and Ukaegbu, 2003). The soils of Ikot Ekpene Area of Akwa Ibom State are loose and highly weathered, supports intensive agricultural activities in the agro-ecological zone. It has good drainage, coarse sandy loam over sandy loam texture, low to medium moisture holding capacity, very acidic pH (4.7-4.9), very low nutrient status and low % base saturation (Akamigbo and Ukaegbu, 2003).

Increasing demand for land leading has led to continuous cultivation in the rain forest ecology of

Akwa Ibom State. In order to balance productivity and environmental protection while achieving self sufficiency in food production soil quality index should be investigated for sustainable land management systems.

This study aimed to

assess selected soil quality indicators for cassava/maize, fallow, and vegetable land uses in Ikot Ekpene area, Akwa Ibom State, Nigeria determine soil quality rating of the different land use types using Multiple variable indicators transform (MVIT) model.

MATERIALS AND METHODS

Land which had been continuously cropped to cassava/maize intercrop and vegetable for more than 5yrs, as well as fallow land were the three land uses evaluated in the study. Each land use was selected at three different locations at 0-20 cm and 20 - 40 cm depths. Soil was collected from each land use type with a soil auger, sampled randomly at three sampling points per location and bulked to form composite samples. Thus, the experiment was a randomized complete block design of 3 (land use) x 2 (depths) x 3 (replications) making a total of 18 composite soil samples. Laboratory analyses of soil properties were according to standard procedures. Particle size distribution was determined by hydrometer method according to the procedure of Gee and Or (2002), Soil pH was measured in 1:2.5 soil : water, Hydraulic conductivity was determined by the constant head permeameter method (Stolte, 1997); bulk density was calculated as described by Anderson and Ingram (1993), organic carbon was determined using wet oxidation method of Walkley and Black as modified by (Nelson and Sommers, 1982); total nitrogen ((Bremmer and Mulvaney, 1982); available phosphorus was extracted with Bray-1 solution and was determined by method of (Murphy and Riley, 1962). Exchangeable potassium and sodium were extracted using 1N ammonium acetate (NH₄OAc) and then determined using flame photometer, while

exchangeable magnesium and calcium were extracted using ethylene diamine tetra acetic acid (EDTA), (Thomas, 1982) and exchangeable acidity was measured titrimetrically using 1N KCl against 0.05N sodium hydroxide.

The model used for the assessment of soil quality in the study area was Multiple variable indicator transform (MVIT) method of Smith *et al.* (1993) used by Oluwatosin *et al.* (2008). In the model, soil quality indicators were transformed on the basis of their ability to attain a critical level or range. Any indicator that is equal to or above the threshold level for crop production was scored 1 and any one below the critical level was scored zero. The Enwezor *et al.*(1989) rating for soil data interpretation was used as critical level. The scores were further integrated into percentage quality rating using the formula below.

$$\text{Percentage (\%)} \text{ quality rating} = \frac{\text{Number of Indicator that attain critical level}}{\text{Total Number of indicator assessed}} \times 100 \dots\dots\dots (5)$$

Soil quality classes were:
 >65 % - high
 35- 65 % - medium
 < 35 % - low (Smith *et al.*, 1993; Oluwatosin *et al.*, 2008).

RESULTS AND DISCUSSION

The soil physical properties which are typically responsible for aggregation and prevention of erosion are presented in Table 1. There was no significant effect of cultivation or fallow on bulk density, saturated hydraulic conductivity or total porosity. This confirms that the soils of Akwa Ibom are naturally high in organic matter and are well drained soils (Akamigbo, 2003). Deforestation however has been known to decrease mean weight diameter (MWD) by 52 % and increased sand content by 25.2 % (Ayoubi *et al.*, 2011).

Table 1: Soil physical indicators of the three land use practice in the study area

Land use	Sand (%)	Silt (%)	Clay (%)	Texture	BD (gcm ⁻³)	Ksat (cm/hr)	TP (%)
Surface soil (0-20 cm)							
Cassava/maize	83.20*	2.70ns	14.10ns	LS	1.67ns	4.8ns	63.0ns
Fallow	84.73ns	4.00ns	11.30ns	LS	1.35ns	4.4ns	50.7ns
Vegetable	89.80	3.33ns	6.90ns	LS	1.49ns	13.0ns	56.0ns
LSD (0.05)	5.56	4.14	8.71		0.46	14.8	19.9
Subsurface soil (20-40 cm)							
Cassava/maize	83.20*	2.67*	14.13*	LS			
Fallow	77.00*	6.40*	16.60*	SL			
Vegetable	88.47*	3.33*	8.20*	LS			
LSD (0.05)	1.76	1.61	1.34				

BD- Bulk density (gcm⁻³); Ksat – Hydraulic conductivity (cm/hr); TP – Total porosity (%)

*=significant; ns = not significant

Table 2a: Selected soil chemical indicators of three land use practice in the study area

Land use	Soil pH (H ₂ O)	EC (dsm ⁻¹)	OC (%)	AV. P (mg/kg)	Total N (%)
Surface soil (0-20 cm)					
Cassava/maize	5.9*	0.09ns	2.6ns	41.7ns	0.08ns
Fallow	5.6*	0.08ns	2.3ns	48.3ns	0.10ns
Vegetable	7.6*	0.23ns	2.9ns	55.7ns	0.11ns
LSD (0.05)	0.2	0.47	1.5	14.7	0.06
Subsurface soil (20-40cm)					
Cassava/maize	5.9	0.06	1.8ns	41.7*	0.08ns
Fallow	5.56	0.05	2.3ns	48.7ns	0.10ns
Vegetable	7.9	0.07	3.1ns	54.7ns	0.12ns
LSD (0.05)	0.6	0.01	2.3	8.1	0.07

EC = Electrical conductivity (dsm⁻¹); OC = Organic carbon (%); AV. P = Available phosphorus (mg/kg); Total N = Total Nitrogen (%)

*= significant; ns = not significant

Table 2b: Selected soil chemical indicators of three land use practice in the study area

Land use	Exch. Mg	Exch. Ca	Exch. K	Exch. Na	EA	ECEC	Base Saturation (%)
(cmol/kg)							
Surface soil (0-20cm)							
Cassava/maize	2.1ns	6.3ns	0.1*	0.06ns	1.8	10.4ns	82.1*
Fallow	2.2ns	6.8ns	0.1*	0.06ns	2.1	11.4ns	81.4*
Vegetable	2.4ns	7.2ns	0.2*	0.07ns	0.7*	10.6ns	93.0*
LSD (0.05)	1.0	3.1	0.09	0.01	0.5	4.0	7.6
Subsurface soil (20-40 cm)							
Cassava/maize	2.1*	6.3*	0.1*	0.06ns	1.8	10.4ns	82.1*
Fallow	1.9*	5.3*	0.1*	0.05*	2.1	9.4ns	77.7*
Vegetable	3.4*	10.1*	0.2*	0.07*	0.9*	10.6ns	93.7*
LSD (0.05)	0.83	2.5	0.07	0.01	0.5	2.9	7.6

OM = Organic matter; ;Exch . Mg = Exchangeable Magnesium; EA = Exchangeable Acidity; Exch .Ca =Exchangeable Calcium; Exch.K=Exchangeable Potassium; Exch. Na = Exchangeable Sodium

SOIL QUALITY RATING OF THREE LANDUSE PRACTICE IN THE STUDY AREA

Multiple Variables Indicator Transform (MVIT)

In using the Multiple Variables Indicator Transform (MVIT) method of Oluwatosin *et al.* (2008), any indicator that is equal (within) to or above the critical range was scored 1 and any one below the critical range was scored zero. The values of the selected soil quality indicators were then compared with the critical range.

The results of soil quality rating based on MVIT are shown in Table 3. In the surface soil of cassava/Maize plot, electrical conductivity, organic matter, available P, exchangeable Ca, Mg, and base saturation were above or within critical range, while soil pH, total N, K, and Na were limiting indicators to cassava cultivation in the surface soil (Table 3).

Table 3: Multiple variable indicators transform (MVIT) surface soil (0-20 cm)

Soil quality indicators	Critical range	Land use types		
		Cassava	Fallow	Vegetables
pH (H ₂ O)	6.5-7.0	0	0	1
Electrical Conductivity (ds/m)	<0.95	1	1	1
Organic Matter (%)	1.7-2.6	1	1	1
Total Nitrogen (%)	0.15-0.2	0	0	0
Available Phosphorus (mg/kg)	7-20	1	1	1
Exchangeable Calcium	5-10	1	1	1
Exchangeable Magnesium	1-3	1	1	1
Exchangeable Potassium	0.3-0.6	0	0	0
Exchangeable Sodium(cmol/kg)	0.3-0.7	0	0	0
ECEC (cmol/kg)	12-25	0	0	0
Base Saturation (%)	40-60	1	1	1
Soil quality rating (%)		54.5 %	54.5%	63%

ECEC – Effective cation exchange capacity; Classification; high =>65%, medium =35-65%, low =<35%

Based on the soil quality rating, pH, total nitrogen, exchangeable potassium, and sodium were rated below

the critical range. Generally, the vegetable plot had the highest soil quality rating, the vegetable plot had the highest soil quality rating of 63.0% compared to the fallow and cassava/maize plots with 54.5% each in both surface and subsurface soils. The variation in soil quality between Cassava/maize, vegetable and fallow plot, was the variation in soil pH, but other indicators such as electrical conductivity, organic matter, total N, available P, Ca, Mg, Na, K, ECEC and base saturation

were similar. Also texture of the soil common to all the land use type was loamy sand in the soil surface and slight variation of loamy sand to sandy loam in the subsurface soil. The high soil pH, in the vegetable plot compared to that of cassava/maize and fallow plots could be attributed to the application of lime and base-bearing fertilizers by the farmers, further confirming that land use types strongly influences soil quality (Teshahunegn, 2014).

The observations in the subsurface soil were similar to that of surface soil.
Table 4: Multiple variable indicators transform (MVIT) subsurface soil (20-40 cm)

Soil quality indicators	Critical range	Land use types		
		Cassava	Fallow	Vegetables
pH (H ₂ O)	6.5-7.0	0	0	1
Electrical Conductivity (ds/m)	<0.95	1	1	1
Organic Matter (%)	1.7-2.6	1	1	1
Total Nitrogen (%)	0.15-0.2	0	0	0
Available Phosphorus (mg/kg)	7-20	1	1	1
Exchangeable Calcium	5-10	1	1	1
Exchangeable Magnesium	1-3	1	1	1
Exchangeable Potassium	0.3-0.6	0	0	0
Exchangeable Sodium(cmol/kg)	0.3-0.7	0	0	0
ECEC (cmol/kg)	12-25	0	0	0
Base Saturation (%)	40-60	1	1	1
Soil quality rating (%)		54.5 %	54.5%	63%

ECEC – Effective cation exchange capacity; Classification; high =>65%, medium =35-65%, low =<35%

Total N, soil pH, exchangeable K, Na, and ECEC were the indicators that reduced the soil quality rating of the study areas. These observations confirm the findings of of Ibia and Udo (2009), that the soils of the study area are acidic, low in total N, exchangeable bases and cation exchange capacity.

The sum total of all the variables under consideration resulted in the soil quality index (Table 5). The results showed that in fallow plot, total N, exchangeable K scored below 0.6 in both surface and subsurface soils. This is an indication of deficiency of those nutrients in

the soil. Soil texture, soil pH, organic carbon and available P scored between 0.6 and 1.0 indicating fair to adequate soil quality. Other parameters under consideration also scored between 0.6 to 1.0 in both surface and subsurface soil .in vegetable plot, total N, and exchangeable K scored below 0.6, while soil texture, soil pH, organic carbon and available P scored between 0.6 to 1.0 in both surface and subsurface soil. The soil under cassava/ maize land use is therefore rated poor, while those under vegetable cultivation or fallow are rated fair.

Table 5: Summary of soil quality ratings in the study area

Parameters	Fallow		Cassava/maize		Vegetables	
	0 – 20cm	20 – 40cm	0 - 20cm	20 – 40cm	0 – 20cm	20 – 40cm
Soil texture	0.6	0.8	0.6	0.6	0.6	0.6
Soil pH	0.6	0.6	0.6	0.6	1	1
Organic carbon	0.8	0.8	0.8	0.6	0.8	0.8
N	0.2	0.2	0.8	0.8	0.2	0.4
P	1	1.0	1	1.0	0.1	1.0
K	0.2	0.2	0.2	0.2	0.2	0.2
Aggregate	0.51	0.55	0.98	0.47	0.54	0.56
SQI	FAIR	FAIR	BEST	POOR	FAIR	FAIR

This shows that total N and exchangeable K were the most limiting soil properties that contributed to the reduction in soil quality, as shown by Ibia and Udo

(2009), that the total N and exchangeable K are the most deficient nutrient elements in the soil of the study area.

CONCLUSION

The results of this study showed that the soils in the study area have high sand and moderate clay content, and were all loamy sand except fallow land which had sandy loam at 20-40 cm depth. The soils generally had high values of hydraulic conductivity, available P, and organic matter. In rating the three land use types, MVIT rated cassava/maize land, fallow land and vegetable land as moderate (medium) at both depths, cassava/maize land poor at 20-40cm depth, fallow land was rated fair at 0-20cm soil depth while vegetable land was rated fair at both 0-20cm and 20-40cm depths.

In spite of the clear advantages of using soil quality as an indicator of sustainable land management, it has not been widely adopted due to a number of limitations such as controversial results in using different models. In conclusion, soil quality is a necessary but not sufficient or absolute indicator of sustainable land management. Other parameters such as cultivation practices, cropping pattern and fertilizer use must also be considered and integrated in order to determine whether a soil is being, or can be managed sustainably.

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EFFICACY OF *Chromolaena odorata* L. (Asteraceae) OIL AGAINST *Anopheles gambiae* and *Culex quinquefasciatus*

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ABSTRACT

Insect-borne diseases are a global problem especially in tropical countries, hence the continuous search for effective, safe and environment friendly control strategies. The objective of this research was to determine the insecticidal potential of oil from the leaves of *Chromolaena odorata* against adults of *Anopheles gambiae* and *Culex quinquefasciatus*. Extraction of oil was done using the hydro-distillation method in a Clevenger apparatus. The oil was analysed using Gas Chromatography- Mass Spectrometry (GC-MS). Forty components were obtained, representing sesquiterpene (78.3%) and monoterpene (10.8%) hydrocarbons as well as oxygenated sesquiterpenes (9.0%). Stock solution was prepared using 20% ethanol as the diluent. Concentrations of the oil: 0.15, 0.30, 0.45 and 0.60 %v/v were used and each concentration was replicated four times. The control (2ml of 20% ethanol) also had four replicates. Whatman No1 filter paper was separately impregnated with different concentrations of the test oil and placed inside the WHO insecticide susceptibility test tubes. Twenty adults each of the test mosquitoes were separately exposed to the oil concentrations, to ascertain the knockdown and insecticidal effects for 30 and 60 minutes, respectively. Results showed that the oil was potent against the two mosquito species tested, because it exhibited knockdown as well as insecticidal effects. After 60 minutes of exposure, 100% mortality was observed for both test species. *Cx. quinquefasciatus* was more susceptible than *An. gambiae* and this difference in susceptibility was significant ($p < 0.05$) as revealed by t-test. *C. odorata* should therefore be considered for the production of a potent natural insecticides *An. gambiae* and *Cx. quinquefasciatus*.

Keywords: Efficacy, *Anopheles gambiae*, *Culex quinquefasciatus*, *Chromolaena odorata*.

Introduction

Morbidity and mortality due to mosquito-borne diseases has remained a scourge and these disease are transmitted through the bite of infected female mosquitoes. These diseases could be viral, or parasitic in nature (Radhika *et al.*, 2011). Symptoms of mosquito-borne diseases range from very mild to very severe and in severe cases, serious complications may occur, making mosquito-borne diseases a significant public health concern. Mosquito-borne diseases, such

as yellow fever, lymphatic filariasis, dengue fever, viral encephalitis and malaria, mostly affect third world and tropical countries (Service, 2012). Inadequate health services in these countries and rising instances of drug resistance are some of the factors that enhance the dissemination of mosquito-borne diseases (Vontas *et al.*, 2012).

Mosquito-borne diseases continue to plague the world, in spite of concerted efforts to control vector species (WHO, 2017). Malaria is a mosquito-borne disease that is caused by the parasitic protozoan, *Plasmodium* spp. and transmitted by female mosquitoes of the genus *Anopheles*. It is one of the leading causes of death for children under five years worldwide (WHO, 2019). In 2018, an estimated 228 million cases of malaria and death of 435, 000 due to the disease occurred worldwide and most of these were in the WHO African region and Nigeria accounted for 25% (WHO, 2019). Female *Anopheles* mosquitoes are also capable of transmitting parasites responsible for lymphatic filariasis (WHO, 2013). Female mosquitoes of the species *Culex* are also incriminated in the transmission of filarial nematodes responsible for lymphatic filariasis (Tuno *et al.*, 2010). According to the World Health Organization (WHO) report of 2018, the global baseline estimate of people affected by lymphatic filariasis was 25 million men with hydrocele and over 15 million people with lymphoedema. At least 36 million people remain with these chronic disease manifestations (WHO, 2018). All of these are a cause for serious concern.

Some degree of success at mosquito vector control has been achieved using synthetic insecticides, but this too has recorded some drawbacks or limitations, such as the development of resistance with time by the vectors to the insecticides (Rozendal, 1997; NPIC, 2008). Other limitations include the detrimental effect of these synthetics to public health and the environment (Maksymiv, 2015). Another factor of concern is the fact that these synthetic insecticides are often costly and unaffordable, considering the poor populace that are worst hit especially in tropical countries, notably in Africa, South of the Sahara (Ndakidemi *et al.*, 2016). These demerits have resulted in the search for suitable alternatives and this search has been directed in recent times extensively to the botanicals that seem to hold solution to vector control challenges (Ubulom *et al.*, 2019; Umohata *et al.*, 2020).

Chromolaena odorata L (Asteraceae) is a tropical weed that proliferates at a fast rate (Rajmoha and Logankumar, 2011, Mandal and Josh, 2014). It is not habitat specific but has preference for wastelands (Vaisakh and Pandey, 2011). This plant, locally called 'awolowo' by the people of Akwa Ibom state in Southern Nigeria, is used in traditional medicine as a wound healing and a local antiseptic agent. Vaisakh and Pandey (2011), have reported on the anti-diarrheal, astringent, anti-plasmodial, anti-hypertensive, anti-inflammatory, diuretic and anti-pyretic properties of *C. odorata*. Leaves are traditionally reported as cough remedy (Vijayaraghavan *et al.*, 2017). *C. odorata* moved originally from Southern Mexico to Argentina and the Caribbean and is now widespread in sub-tropical and tropical areas all over the world.

The objective of this study was to determine the efficacy of the essential oil from the leaves of *Chromolaena odorata* against *Anopheles gambiae* and *Culex quinquefasciatus*.

Materials and methods

Plant Collection and Identification

The plant, *Chromolaena odorata*, was collected in February 2018, from the Medicinal Plant Farm of the Faculty of Pharmacy, University of Uyo, Nigeria. The plant materials were authenticated in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

Plant Preparation and Extraction of Oil

Plant preparation and extraction were carried out in the department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. The leaves of *C. odorata* were collected, washed and chopped before shade drying on laboratory tables. The shade dried specimen was pulverised using a manual grinder and weighed using a Triple Beam balance (MB-2610g).

Oil extraction was done by hydro distillation using the procedure described in the British Pharmacopoeia (2018). The hydro-distillation method using a Clevenger apparatus was used in the extraction process. The pulverized leaves of *C. odorata* weighing 2500g, were introduced into 5 litre capacity round-bottom flask to half its volume and distilled water was added to cover the plant materials. Thereafter, the round bottom flask was placed on a 10 litre capacity heating mantle and connected to Clevenger apparatus. The set up was allowed to boil for five hours. During this process of boiling, the water vaporized with the oil from the plant materials and condensed at the condenser. Thus, on the condenser, oil rich and water rich layers were formed. The procedure was done three times and oil collected into well stopped small glass containers. The oil was dried over sodium sulphate and stored in a refrigerator at a temperature of 4°C prior to

further use.

Chemical Analysis of the Oil by Gas Chromatography- Mass Spectrophometry (GC-MS)

Chemical analysis of the essential oil from the leaves of *C. odorata* was determined by GC-MS as described by Elekwa *et al.*, 2011

The oil was subjected to GC-MS analysis on an Agilent system consisting of a model 7890N gas chromatograph, a model mass detector Triple Quad 7000A in EI mode at 70eV (m/z range 40–600amu) and an Agilent Chem Station data system. The GC column was an HP-5 MS fused silica capillary with a 5% phenyl-methyl polysiloxane stationary phase (30m x 250µm x 0.25µm). The carrier gas was helium with a column head pressure of 9.7853 psi and flow rate of 1.2ML/min. Inlet temperature and Most Significant Digit (MSD) detector temperature was 250°C.

The components were identified by comparison of their mass spectra with library data of the GC-MS system as well as by comparison of their retention indices (RI) with the relevant literature data of Adams, 2007.

Experimental Mosquito Species

Mosquito species used for this research were *Anopheles gambiae* and *Culex quinquefasciatus*. These mosquito specimens were provided by the Malaria Vector Research Laboratory and Insectary of the Department of Animal and Environmental Biology, Faculty of Science, University of Uyo. Adult mosquitoes of each species were separately transferred into insect cages where they were fed and maintained on a 10% sucrose solution as described by the World Health Organization (WHO, 2005) and Onyido *et al.* (2009). They were left for 24 hours before the commencement of the experiment.

Knockdown and Insecticidal Testing

Insecticidal potential of *C. odorata* oil was separately tested against *An. gambiae* and *Cx. quinquefasciatus* in the laboratory of the Department of Animal and Environmental Biology, Faculty of Science, University of Uyo. The experiment was conducted at an ambient temperature of 25 ± 2°C and 80 ± 5% Relative Humidity (RH). A stock solution of the oil was prepared using 20% ethanol as the diluent. From the stock solution four different concentrations (0.15, 0.30, 0.45 and 0.60 % v/v) of the oil were used for the study in four replicates. The control which consisted of 2 ml of 20% ethanol also was replicated four times. Twenty adult mosquitoes of each species were separately exposed to each test concentrations and their respective replicates. The same was done for the control experiments. The adult mosquitoes were collected from the insect cage and transferred into different transparent WHO insecticide susceptibility test tubes using an aspirator.

The insecticidal potential of the plant essential oil was then tested by separately impregnating Whatman No 1 filter paper with different concentrations of the oil, using a micropipette. The filter paper was then placed in the WHO approved Insecticide susceptibility test tubes as described by WHO, 2016. Observation of the repellent, knockdown and insecticidal effects of the oils on the adult mosquitoes were observed for 30 minutes at intervals of 5 minutes for knockdown and for 60 minutes at interval of 10 minutes for mortality. The results obtained were recorded accordingly.

Statistical Analysis

Data collected were analysed using one-way Analysis of Variance (ANOVA) and student t- test using the graph pad Prism 6.0.

RESULTS AND DISCUSSION

Chemical composition of the oil of *C. odorata*
GC-MS analysis of *C. odorata* essential oil led to identification of chemical components, representing 98.1% of the oil (Table 1). The oil was rich in sesquiterpene hydrocarbons and monoterpene hydrocarbons (78.3% and 10.8%, respectively) and oxygenated sesquiterpenes (9.0%). Sesquiterpene hydrocarbons present in the oil of *C. odorata* have been reported previously by Jija and John (2011) who detected sesquiterpenes in the oils of the four species of *Salvia* they studied. They attributed the insecticidal activities observed in their study against mosquito larvae to the abundance of sesquiterpenes in the oils.

Table 1: Constituents of Chromoleana oil Analysed using Gas Chromatography-Mass Spectrometry (GC-MS)

Constituents	LRI ^a	COL ^b	Constituents	LRI ^a	COL ^b	Constituents	LRI ^a	COL ^b
α-pinene	941	4.7	α-neo-clovene	1452		δ-cadinene	1524	10.6
Sabinene	977	0.5	α-humulene	1455	3.4	Elemol	1550	0.6
		3.0	trans-cadina-1(6),4-		0.7			0.2
1-octen-3-ol	981		diene	1475		(E)-nerolidol	1564	
		0.5			1.0	caryophyllene		1.0
Limonene	1032		γ-muurolene	1478		oxide	1582	
(Z)-β-ocimene	1042	0.2	germacrene D	1482	20.0	Viridiflorol	1591	0.7
		1.2	Bicyclosquiphellan		0.8	humulene		0.2
(E)-β-ocimene	1052		drene	1491		epoxide II	1607	
trans-		2.7			3.6	humulane-		0.2
pinocarveol	1141		Bicyclogermacrene	1496		1,6-dien-3-ol	1616	
pregeijerene	1288	15.8	α-muurolene	1499	1.1	1-epi-cubenol	1629	0.7
α-copaene	1377	3.2	germacrene A	1505	1.0	γ-eudesmol	1632	0.5
β-bourbonene	1385	0.8	δ-amorphene	1505	0.3	T-cadinol	1641	1.7
β-		11.1			0.2			0.4
caryophyllene	1419		(E,E)-α-farnesene	1508		β-eudesmol	1651	
β-copaene	1430	0.4	trans-γ-cadinene	1514	0.6	α-cadinol	1652	2.4
cis-muurola-		0.2			0.4			
3,5-diene	1448		Cubebol	1515				

COL: *Chromoleana odorata* leaves; LRI: linear retention Index; b: Order of elution on HP-5ms capillary column; identification by comparison of the mass spectral and retention index data

Repellent and Knockdown effects of Test oil on *Anopheles gambiae* and *Culex quinquefasciatus*

When the test oil was introduced into the WHO insect susceptibility test tubes, it was observed that the two mosquito species all flew upwards the test tubes in a bid to escape from the tube but for the presence in each case of untreated net at the top of the tube, with a mesh size small enough to prevent escape of the test mosquitoes, but wide enough to allow for passage of air and ventilation of the experimental set up. This was an indication that the test oil has repellent effect on the different mosquito species. The test organisms, *An. gambiae* and *Cx. quinquefasciatus* avoided contact with the filter paper where the test oil (*C. odorata*) was applied by restlessly exhibiting frantic attempts to

escape from the WHO susceptibility test tubes. This is in agreement with Bouda *et al.* (2001), who reported that the essential oil of *C. odorata* exhibited insect repellent and insecticidal property against the beetle *Sitophilus zeamais*, a stored product pest.

At the end of 30 minutes observation period and at the highest concentration of 0.60% v/v, the percentage of *An. gambiae* species knocked down by the test oil was 97.50% while that of *Cx. quinquefasciatus* was 100 %. Mosquitoes in the control treatment were not knocked down throughout the 30 minutes exposure period (Table 2).

The knockdown effect of the test oil against the experimental mosquito species reported in this study

corroborated the reports of other researchers who also observed a knockdown effect of botanical oils against their experimental mosquito species. Uniyal *et al.* (2014) stated that *Aedes aegypti* exhibited a 100% knockdown when 23 different essential oils were used

in the study. Furthermore, Yoon *et al.* (2015), observed that *Citronella* exhibited a knockdown effect on mosquitoes when 5% *Citronella* oil was tested using 20 human volunteers.

Table 2: Knockdown Effect of *C. odorata* on *An. gambiae* and *Cx. quinquefasciatus*

Conc. (%) v/v	No of Mosq. Exposed	Exposure period (Min)					
		5	10	15	20	25	30
<i>An. gambiae</i>							
0.15	80	3.50±0.29	5.50±0.29	9.00±0.58	15.50±0.29	17.50±0.29	20.00±0.00
0.30	80	6.50±0.29	9.00±0.58	15.50±0.29	18.50±0.29	20.00±0.00	-
0.45	80	8.50±0.29	12.00±0.58	16.50±0.29	18.50±0.29	20.00±0.00	-
0.60	80	11.50±0.29	14.50±0.29	16.50±0.29	18.50±0.29	20.00±0.00	-
Ctrl	80	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Cx. quinquefasciatus</i>							
0.15	80	3.50±0.29	5.50±0.29	9.00±0.58	15.50±0.29	17.50±0.29	20.00±0.00
0.30	80	6.50±0.29	9.00±0.58	15.50±0.29	18.50±0.29	20.00±0.00	-
0.45	80	8.50±0.29	12.00±0.58	16.50±0.29	18.50±0.29	20.00±0.00	-
0.60	80	11.50±0.29	14.50±0.29	16.50±0.29	18.50±0.29	20.00±0.00	-
Ctrl	80	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Insecticidal Effects of Test Oil on *Anopheles gambiae* and *Culex quinquefasciatus*

The effect of time on mortality of *An. gambiae* and *Cx. quinquefasciatus* exposed to *C. odorata* is shown in Table 3. Hundred percent mortality was observed for the four concentrations (0.15, 0.30, 0.45 and 0.60 % v/v) at the end of the exposure period of 60 minutes.

The effects of the varying concentrations of *C. odorata*, on the mortality of *An. gambiae* and *Cx. quinquefasciatus* are shown in Table 4. Data revealed 100% mortality for *An. gambiae* at the 40th minute of exposure to 0.45 % v/v concentration of the oil. Significant difference (p< 0.05) was observed in mortality of *An. gambiae* with time in all concentrations. Hundred percent mortality was observed for *Cx. quinquefasciatus* at the 30th minute for all concentrations (0.15 - 0.60% v/v). Analysis of variance revealed significant difference (p<0.05) in mortality of *Cx. quinquefasciatus* with time in all concentrations.

The essential oil of the leaves of *C. odorata*, in this study has insecticidal potential against *An. gambiae* and *Cx. quinquefasciatus*. This potency is attributable to the chemical constituents of the oil. The insecticidal activity as exhibited here was a function of both concentration and exposure period. *C. odorata* showed increased insecticidal activity against *An. gambiae* and *Cx. quinquefasciatus* with an increase in concentration of *C. odorata* essential oil. Results of the insecticidal efficacy of *C. odorata* oil against *An. gambiae* and *Cx. quinquefasciatus* corroborate the findings of Udebuani *et al.*, 2015 who reported high mortality of *Periplanata americana* with increasing concentrations of *C. odorata* plant powders and extracts. Thus, the mortality data of this study were concentration and time dependent.

Table 3. Effect of time on the Mortality of *Anopheles gambiae* and *Culex quinquefasciatus* exposed to *Chromolaena odorata* oil

Time of Exposure	Concentration (% v/v)			
	0.15	0.30	0.45	0.60
<i>Anopheles gambiae</i>				
10 minutes	5.50±0.65 ^d	6.50±0.65 ^d	8.50±0.65 ^c	9.50±0.65 ^d
20 minutes	8.50±0.65 ^c	12.50±0.65 ^c	14.50±1.94 ^b	15.50±0.65 ^c
30 minutes	17.50±0.65 ^b	16.50±0.65 ^b	18.75±0.48 ^a	18.50±0.29 ^b
40 minutes	18.00±0.58 ^b	18.50±0.65 ^a	20.00±0.00 ^a	20.00±0.00 ^a
50 minutes	20.00±0.00 ^a	20.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a
60 minutes	00.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a
Mean	14.92±1.21	15.67±1.03	16.96±0.94	17.25±0.81
p Value	0.000*	0.000*	0.000*	0.000*
<i>Culex quinquefasciatus</i>				
10 minutes	4.50±0.29 ^c	7.50±0.29 ^c	11.00±0.58 ^b	12.50±0.29 ^c
20 minutes	14.50±0.29 ^b	16.50±0.29 ^b	17.00±0.58 ^b	17.50±0.29 ^b
30 minutes	20.00±0.00 ^a	20.00±0.00 ^a	20.00±0.00 ^a	20.00±0.00 ^a
40 minutes	00.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a
50 minutes	00.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a
60 minutes	00.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a	00.00±0.00 ^a
Mean	16.50±1.20	17.33±0.96	18.00±0.70	18.33±0.58
p Value	0.000*	0.000*	0.000*	0.000*

*significant at p≤ 0.05

Table 4: Effect of varying concentrations of *C. odorata*, on the mortality of *Anopheles gambiae* and *Cx. quinquefasciatus*

Concentration(% v/v)	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
<i>An. gambiae</i>						
0.15	5.50±0.65 ^b	8.50±0.65 ^b	17.50±0.65 ^b	18.00±0.58 ^b	20.00±0.00	00.00±0.00
0.30	6.50±0.65 ^b	12.50±0.65 ^a	16.50±0.65 ^{ab}	18.50±0.65 ^b	20.00±0.00	0.00±0.00
0.45	8.50±0.65 ^a	14.50±1.94 ^a	18.75±0.48 ^a	20.00±0.00 ^a	00.00±0.00	00.00±0.00
0.60	9.50±0.65 ^a	15.50±0.65 ^a	18.50±0.29 ^a	20.00±0.00 ^a	00.00±0.00	00.00±0.00
Mean	7.50±0.50	12.75±0.85	17.81±0.33	19.13±0.30	20.00±0.00	20.00±0.00
p Value	0.003*	0.004*	0.043*	0.012*	NA	NA
<i>Cx. quinquefasciatus</i>						
0.15	4.50±0.29 ^d	14.50±0.29 ^b	20.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
0.30	7.50±0.29 ^c	16.50±0.29 ^a	20.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
0.45	11.00±0.58 ^b	17.00±0.58 ^a	20.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
0.60	12.50±0.29 ^a	17.50±0.29 ^a	20.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Mean	8.88±0.82	16.38±0.34	20.00±0.00	20.00±0.00	20.00±0.00	20.00±0.00
p Value	0.000*	0.001*	NA	NA	NA	NA

Comparative Susceptibility of Experimental Mosquitoes to the Test oil

Data on susceptibility of test mosquitoes to the oil was analysed using t-test. Results obtained revealed that *Cx. quinquefasciatus* was more susceptible to the oil than *An. gambiae* (Table 5). At the highest concentration, mean values observed for *An. gambiae* were 16.20, on exposure to *C. odorata*, while 17.54, was observed for *Cx. quinquefasciatus* on exposure to

C. odorata. From analysis of variance a p-value of 0.047 was obtained and this indicated significant (p<0.05) difference in susceptibility of the test mosquitoes to *C. odorata*. The observed differential susceptibility of the test mosquitoes to the test oil might be attributable to the difference in the genetic makeup of the test mosquito species. This is substantiated by the reports of Bradley *et al.* (2018) and Derciliano *et al.* (2019), on the relationship

between genetic variation of mosquito species and susceptibility to insecticide.

Table 5: Susceptibility of Experimental mosquitoes to the oils of *C. odorata*

Species of Mosquitoes	Mean mortality
	<i>C. odorata</i>
<i>An. gambiae</i>	16.20±0.50
<i>Cx. quinquefasciatus</i>	17.54±0.44
Mean	16.87±0.34
p Value	0.047*

*significant at $p \leq 0.05$

Conclusion

Results obtained from this study revealed that *C. odorata* oil has repellent, knockdown and insecticidal potentials against *An. gambiae* and *Cx. quinquefasciatus*. However *Cx. quinquefasciatus* was

found to be more susceptible to the test oil. Essential oil obtained from the leaves of *C. odorata*, is biodegradable and potentially suitable for use in integrated vector control program and therefore should be further explored for elucidation of its active principles.

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CO-INFECTION OF HEPATITIS B SURFACE ANTIGEN (HBsAg) AND HUMAN IMMUNODEFICIENCY VIRUS (HIV) AMONGST PATIENTS IN BENIN CITY, EDO STATE

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ABSTRACT

The Human Immunodeficiency Virus (HIV) and hepatitis B virus (HBV) co-infection is a global health challenge, with approximately 10 % of all HIV infected patients living with chronic HBV. This study was conducted to ascertain the occurrence and co-infection of hepatitis B surface antigen (HBsAg) and Human Immunodeficiency virus (HIV) amongst patients attending Government Hospital Benin City, Edo state. The study was conducted on 323 blood samples randomly collected from patients attending Government Hospital Benin City using the Rapid test strip to test for HIV and the Enzyme linked immunosorbent assay method (ELISA) to test for Hepatitis B surface antigen (HBsAg) virus. A total of 127 (39.3 %) patients tested positive to HBsAg and 52(6.8 %) tested positive to HIV, there was a coinfection of 22(6.8 %) between HBs Ag and HIV. Age group 30-39 had the highest occurrence with 38(11.8 %) patients testing positive to HBsAg and 17(5.3 %) positive to HIV. In the demographic factor, females had the highest prevalence rates with 91(28.2 %) positive to HBsAg and 42(13.0 %) positive to HIV. This study confirms occurrence of co-infections of HIV and HBV in the study population. There is a need for health promotion awareness campaign to educate the general public on the danger, mode of transmission and risk factors associated with HBV infection and the need for vaccination.

Keywords: Hepatitis B Virus, Human Immunodeficiency Virus, Co-infection, Risk factors.

INTRODUCTION

Hepatitis B infection is a serious and common infectious disease of the liver, affecting millions of people throughout the world (Pouti *et al.*, 2006). The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC) (Thio *et al.*, 2002). Infection occurs very often in early childhood when it is asymptomatic and often leads to the chronic carrier state. More than 2000 million people alive today have been infected with HBV at some time in their lives. Every year there are over 4 million acute clinical cases of HBV, and about 25% of carrier, while 1 million people a year, die from chronic active hepatitis, cirrhosis or primary liver cancer (WHO, 2001).

Human immunodeficiency virus (HIV) is the virus that can lead to acquired immunodeficiency syndrome or AIDS if not treated. Unlike some other viruses, the human body can't get rid of HIV completely, even with treatment (CDC, 2019). Human Immunodeficiency Virus attacks the body's immune system, specifically the CD4 cells (T cells), which help the immune system fight infections (CDC, 2019). No effective cure currently exists, but with proper medical care, the infection can be managed.

Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) co-infection is common due to their shared transmission routes (Bonacini *et al.*, 2004). Approximately, 10 % of all HIV infected patients worldwide are estimated to have chronic HBV co-

infection (Ranjbar *et al.*, 2011). However, wide regional variations are observed with co infection prevalence rates estimated to be 5–10 % in areas such as North America, Europe and Australia compared to higher prevalence rates of 20–30 % in areas such as Sub-Saharan Africa and Asia (Singh and Wong, 2009). These statistics are of significant importance in Sub-Saharan Africa where over 70 % of the world's 36.9million people infected with HIV live (UNAIDS, 2014). The population prevalence of HIV/HBV co-infection in Africa is thought to reflect the population prevalence of hepatitis B surface antigen (HBsAg). Several studies in Nigeria have recorded prevalence rates of HBsAg ranging from 7.5% to 44.7% from one location to another (Chukwuka *et al.*, 2004; Bukbuk *et al.*, 2005) and the prevalence of HIV/HBV co-infection among Nigerian children was 8.3% (Rawizza *et al.*, 2010).

Although, the specific mechanisms by which HBV interacts with HIV to influence disease progression are not clearly understood, HIV infected individuals have been found to be about six (6) times more likely to develop HBV infection than their HIV negative counterparts (Gatanaga *et al.*, 2000). Additionally, the progression rate and complications such as liver fibrosis, cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC) and mortality due to liver pathology arising from HBV infection are accelerated in patients with HIV co infection (Thio *et al.*, 2002).

The aim of this research is to determine the current

prevalence of Hepatitis B surface antigen (HBsAg) virus and co infection with HIV among the general population in Benin City, Edo state.

MATERIALS AND METHODS

Study Design: This study is a population-based, descriptive cross sectional design. A questionnaire comprising of multiple questions regarding patient demographic, risk factors was collected from each consented patient.

Study Area: The study was conducted among people attending a selected Government hospital located in Benin City. Benin City, the capital of Edo State is within the south-south of Nigeria lying between 6° 20' North latitude and 5° 37' East longitude. It is situated approximately 40km north of the Benin River and 320km by road East of Lagos. The city has a humid climate and two (2) climate seasons; the rainy and dry season. The rainy season is between April and October with average rainfall of 250cm. The dry season lasts from November to March and also a cold humid and dusty harmattan period between December and January. The average temperature ranges between 22 °C in the rainy season and 28°C in the dry season. The total human population density in 2015 was 1,200/km².

Sample Collection: A total of 323 blood samples were collected from consenting patients All materials required for the collection of blood samples was assembled and was labelled with the patients' identification number and date. Blood samples (5ml) was collected via venipuncture with the assistance of laboratory scientist from consenting patients into EDTA (Ethylene Diamine Tetraacetic Acid) bottles immediately. The blood samples in the EDTA bottles were centrifuged at 1000rpm for 10minutes. The plasma was collected into clean container and was stored by freezing at -4°C in the freezer till when needed for HBV and HIV test.

HIV Test

The HIV 1/2 Human Immunodeficiency Virus Rapid Test Strip was used for the detection of antibodies to HIV ½ from the subject's plasma. During testing 2-3drops of plasma specimen of subjects was dropped on the strip placed on a flat surface and allowed to stand for 5-10mins before taking the results. If the specimen contains antibodies to HIV 1 and/or HIV 2, a coloured line will appear in the test line region indicating a positive result. If the specimen does not contain HIV 1/HIV 2 antibodies a coloured line will not appear in the test line region indicating a negative result

according to the manufacturer's procedure. Laboratory examination of Hepatitis B Surface Antigen (HBsAg) All study subjects were screened for HBsAg by ELISA diagnostic kits (Bioneovan, China). All assay protocols, cut-offs and interpretation were according to the manufacturer's instructions.

Data Analysis

Result from analysis and data from questionnaire was reduced to percentage and presented on tables and graphs. Statistical analysis was done using SPSS version 16 and P-value less than 0.05 was taken statistically significant.

RESULTS

A total of 323 subjects participated in the study, where 127(39.3%) were positive to HBsAg and 52(16.1%) to HIV and there was a coinfection of 22(6.8%). Table 1 showed the age related prevalence, the age range of 30-39 had the most participants of 98(30.3%) with 38(11.8%) and 17(5.3%) positive to HBsAg and HIV respectively. The age range 20-29 had the second highest number of samples collected at 89(27.6%) with 32(9.9%) tested positive to HBsAg and 15(4.6%) HIV. The age range 0-9 had the least number of attendant with prevalence of 2(0.6%) to HBsAg and 0(0.0%) for HIV. Out of a total of 323 participants, 239(74.0%) were females while 84 (26.0 %) were males. The female gender had HBsAg and HIV occurrence of 91(28.2%) and 42(13.0%) respectively, while the males 36(11.1%) were positive to HBsAg and 10(3.1%) to HIV.

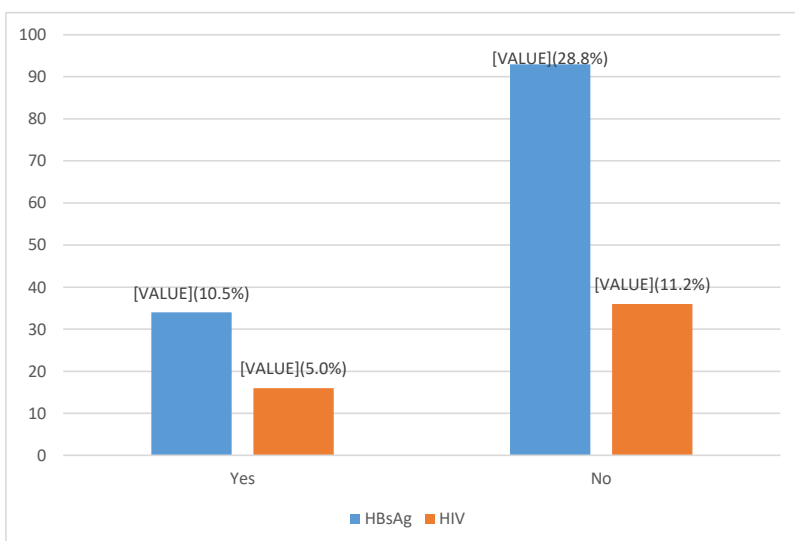
The occupational related prevalence showed that out of 77(23.8%) traders, 31(9.6%) tested positive to HBsAg and 9(2.8%) to HIV. Out of 72(22.3%) students, 29(9.0%) were tested positive for HBsAg and 13(4.0%) tested positive for HIV. Out of 8(2.5%) medical practitioners, 3(0.9%) were HBsAg positive and 1(0.3%) tested positive to HIV (Table 2). Marital prevalence showed that out of the 89(27.6%) single patients, 33(10.2%) tested positive to HBsAg and 15(4.6%) tested positive to HIV. 227(70.3%) patients indicated as married and 92(28.5%) tested positive to HBsAg with 36(11.1%) testing positive to HIV. The population of widowed participants 6(1.9%) with 2(0.6%) positive to HBsAg. The level of education demography showed that out of the 63(19.5%) participants with primary school education, 28(8.7%) were reactive to HBsAg and 8(2.5%) to HIV. Figure 1 shows the related prevalence of pregnancy status to HBsAg and HIV. A total of 34(10.5%) who were pregnant tested positive to HBsAg.

Table 1: Age related occurrence of Hepatitis B surface antigen (HBsAg) and Human immunodeficiency virus.

Age	No Examined	HBsAg Positive (%)	HIV Positive (%)	Co-infection (%)
0-9	4	2(0.6)	0(0.0)	0(0.0)
10-19	15	6(1.9)	4(1.2)	2(13.3)
20-29	89	32(9.9)	15(4.6)	6(6.7)
30-39	98	38(11.8)	17(5.3)	8(8.2)
40-49	42	15(4.6)	8(2.5)	3(7.1)
50-59	47	23(7.1)	3(0.9)	1(2.1)
>60	28	11(3.4)	5(1.5)	2(7.1)
Total	323	127(39.3)	52(16.1)	22(6.8)

Table 2: Demography of the occurrence of Hepatitis B surface antigen (HBsAg) and Human immunodeficiency virus (HIV)

Parameters	No Examined	HBsAg Positive (%)	HIV Positive (%)	Co-infection (%)
Sex				
Male	84	36(11.1)	10(3.1)	5(6.0)
Female	239	91(28.2)	42(13.0)	17(7.1)
Total	323	127(39.3)	52(16.1)	22(6.8)
Occupation				
Trader	77	31(9.6)	9(2.8)	3(3.9)
Student	72	29(9.0)	13(4.0)	6(8.3)
House wife	22	10(3.1)	7(2.2)	4(18.2)
Medical Practitioner	8	3(0.9)	1(0.3)	1(12.5)
Self employed	38	15(4.6)	3(0.9)	2(5.3)
Civil servant	28	10(3.1)	5(1.5)	2(7.1)
Unemployed	10	2(0.6)	2(0.6)	0(0.0)
Retired	21	7(2.2)	4(1.2)	1(4.8)
Others	47	20(6.2)	8(2.5)	3(6.4)
Total	323	127(39.3)	52(16.1)	22(6.8)
Marital Status				
Single	89	33(10.2)	15(4.6)	6(6.7)
Married	227	92(28.5)	36(11.1)	16(7.0)
Widowed	6	2(0.6)	1(0.3)	0(0.0)
Divorced	1	0(0.0)	0(0.0)	0(0.0)
Total	323	127(39.3)	52(16.1)	22(6.8)
Level of education				
Primary	63	28(8.7)	8(2.5)	4(6.3)
Secondary	127	51(15.8)	26(8.0)	8(6.3)
Tertiary	129	44(13.6)	17(5.3)	9(7.0)
None	4	4(1.2)	1(0.3)	1(25.0)
Total	323	127(39.3)	52(16.1)	22(6.8)



DISCUSSION

Human Immunodeficiency Virus and hepatitis are major global infections with several countries investing heavily on their control and treatment. The prevalence of Hepatitis B Surface Antigen (HBsAg) in this study was 39.3%. In comparison with earlier studies carried out in Nigeria, this figure is higher than 26.5% reported in Gombe (Mustapha and Jibril, 2004), 11.9% reported in Ibadan (Otegbayo *et al.*, 2008), 15.5% reported in Benin city (Ojide *et al.*, 2020) but lower than 46.7% in North Eastern Nigeria (Olokoba *et al.*, 2008), 51.9% in Lagos (Iwalokun *et al.*, 2006) and 70.5% in Kano (Nwokedi *et al.*, 2006). Comparing these highlighted rates with the occurrence could be because of ignorance or poor medical check-up attitudes of infected persons and HBsAg seropositivity indicates a carrier state or an active infection.

In this study there was a mixed infection of 6.8% to HBsAg and HIV which is higher than 0.6% reported in Benin city (Ojide *et al.*, 2020) and 3.6% in Borno (Bello and Olabode, 2011). In this study and other reported studies have shown the endemicity of the viruses and their co-infection in our environment which is an emerging concern in the clinical management of patients because of increased mortality and accelerated hepatic disease progression. It is known that the two viruses share similar routes of transmission, therefore, there is need to intensify efforts to get HIV infected individuals routinely screened for HBV and HIV in Nigeria as recommended by expert guidelines developed in United State and Europe (Sulkowski, 2011). Such guidelines, if adopted, will lead to early detection of cases of co-infection and proper adjustment of

management and treatment strategies. Evidence has shown that HBV can infect lymphocytes and produce a protein capable of activating HIV-1 replication (Seto, 1989). In addition, it has recently been found that HIV positive patients with HBV infection are at increased risk of liver related mortality (Thio *et al.*, 2002).

Individuals in the age group 30-39 years had the highest HBsAg 11.8% which is in contrast to the work of Mustapha and Jibril, 2004 who reported a prevalence of 41.6% in age group 40-49 years. Also Ojide *et al.* 2020 recorded a high prevalence of HBsAg 16.4% among age group 31-40. The higher prevalence reported is not surprising as the older subjects could have been more exposed to risk factors of HBV such as drugs injection and unprotected sex.

The sero-prevalence of HBsAg in pregnancy was 10.5 % in our study which was found to be lower than 14.9% and 12.3% recorded by Frempong *et al.*, 2019 and Ofori and Agyeman 2016 respectively but less than the work of Rouet *et al.*, 2004 who reported a sero-prevalence of 9.0% among HIV-positive pregnant women. This discrepancy might be due to sample size and participants were not all HIV positive pregnant women.

It was deduced in this study that females are more infected with HBsAg 91(28.2%) than males 36 (11.1%). This is in contrast with the findings of (Adebola *et al.*, 2016) who found that prevalence of HBV was higher in males than females. However, HIV prevalence was found to be higher in females (13.0%) than males (3.1%) this is in agreement with the finding of (Munirah *et al.*, 2018) which also revealed a larger

occurrence of HIV infection in females than males. This could be as a result of sexual promiscuity among females where they have more than one sex partner.

Occupational prevalence showed that traders had highest number of HBsAg (9.6%) positivity this could be as a result of limited health education and information. It could also be as a result of the fact that most of these traders had not been vaccinated as most of the traders who participated in the study agreed on never been vaccinated. Students showed highest HIV positivity this is in agreement to the findings of

(Awofala and Ogundele, 2016) which showed that young people recorded the most prevalence of HIV in the country.

CONCLUSION

There is a need for health promotion awareness campaign to educate the general public on the danger, mode of transmission and risk factors associated with HBV infection especially among youth populace. There is therefore, a serious need to consider anti-HBV therapy in addition to antiretroviral therapy in those with co infection.

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THE OCCURRENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Citrobacter* SPECIES ISOLATED FROM WASTEWATER IN UMUAHIA, ABIA STATE, NIGERIA.

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Abstract

A large part of antibiotics consumed today ends up in wastewater and may exert selective pressure for or maintain resistance among other microorganisms. This study determined the occurrence and antimicrobial susceptibility profile of *Citrobacter* species isolated from wastewater in Umuahia, Abia State, Nigeria. Twenty (20) wastewater samples were collected from four locations (five from each) in Umuahia and inoculated after serial dilution onto eosin methylene blue agar (EMB). The isolates were identified following standard microbiological procedures. The antimicrobial susceptibility testing was done using Kirby Bauer disc diffusion method with standardized commercial impregnated discs. The results of this study showed 19 isolates (95%) of the *Citrobacter* species. The antibiotic sensitivity pattern showed that all the isolates were sensitive to Ofloxacin 19(100%), followed by Gentamicin 15(78.9%) and then Nitrofurantoin and imipenem 14(73.7%) respectively. All 19(100%) isolates were resistant to the cephalosporins (Ceftazidime and Cefuroxime) and the β -lactamases (Ampicillin and Augmentin) used in this study. Cotrimoxazole and Meropenem had 84.2% resistance recorded against each of them. The multiple antibiotic resistance (MAR) index of the isolates ranged from 0.4-0.9 showing that the isolates were multidrug resistant (MDR). The use of downstream rivers contaminated with multidrug resistant bacterial pathogens poses public health risk as these MDR species may also transmit resistant genes to other pathogenic and commensal organisms found in the water bodies.

KEY: *Citrobacter*, antibiotic, resistance, wastewater

INTRODUCTION

Citrobacter species belong to a group of facultative, anaerobic, Gram-negative bacilli within the family Enterobacteriaceae. They are frequently found in water, soil, sewage, food, and the intestines of animals and humans (Bae *et al.*, 2010; Liu *et al.*, 2017). Previously recognized as environmental contaminants or colonizers with low virulence, they are found to be opportunistic pathogens causing a wide spectrum of infections involving the urinary tract, liver, biliary tract, peritoneum, intestines, bone, respiratory tract, endocardium, wounds, soft tissue, meninges, and the bloodstream (Khorasani *et al.*, 2008; Kumar *et al.* 2013, Rajan and Rajan, 2013; Leski *et al.*, 2016).

Municipal wastewater refers to a mixture of domestic and individual sewage and run off into wastewater treatment plants that are responsible for treatment. Wastewater provides an enabling environment for the existence and proliferation of microorganisms including pathogens (Karzenewska, 2011). It is characterized by the presence of different microorganisms which depend on their sources and also other factors like condition and health of producer coupled with other environmental factors (Bawaic *et al.*, 2016). Numerous factors determine the microbial composition of wastewater and they include the type of industry discharging the sewage, presence of hospital, pharmaceutical industries and other health facilities, presence of animal breeding farms and the health condition of the populace (Kim and Aga, 2007).

Waste water contains antibiotics, disinfectants and

metals which can form a selection pressure for antibiotic resistance even in low concentrations (Karkman *et al.*, 2018). It can act as a reservoir and environmental suppliers of antibiotic resistance and has also been proposed to be hotspots for horizontal gene transfer which enables the spread of resistance genes between different bacterial species (Moges *et al.*, 2014; Fouz *et al.*, 2020). Untreated, hospital and pharmaceutical wastewaters are characterized by high levels of clinically relevant antimicrobial resistant bacteria (ARBs) and antimicrobial resistant genes (ARGs) (Wang *et al.*, 2015; Cahill *et al.*, 2019). In developing countries like Nigeria, wastewaters generated from various sources do not get appropriate treatment before being released to the nearby rivers and streams (Onuoha *et al.*, 2016). There is need therefore to identify the antibiotic resistance status of our wastewaters to effectively combat the threat posed by antibiotic resistance to the prevention and treatment of both human and animal infections hence the present study.

MATERIALS AND METHODS

Study area

Umuahia is the capital city of Abia State in South-Eastern Nigeria coordinating latitude 5° 32'N and longitude 7° 29'E on the Northern and Eastern hemispheres respectively, located along the rail road that lies between Port-Harcourt to the South and Enugu city to its North.

Collection of Samples

Twenty (20) wastewater samples (five from each location) were collected using sterile container from four waste water channels located in Health Centre Road (H1-H5), Afara (A1-A5), Umudike Junction (U1-U5) and Ehimiri (E1-E5), all in Umuahia metropolis. The samples were transported to the laboratory within an hour of collection for laboratory analysis.

Bacterial Isolation

A ten-fold serial dilution up to 10⁻⁵ was carried out as described by Gurung *et al.* (2009). 0.1ml aliquots of the appropriate dilutions of the waste water were inoculated onto MacConkey agar (Rapid Labs, UK) and Eosin Methylene Blue (EMB) agar (Rapid Labs, UK), using a sterile glass rod. The plates were incubated at 37°C for 24h.

Purification and maintenance of the microbial isolates

Pure cultures of the *Citrobacter* spp isolates were obtained by sub-culturing in fresh medium using the streak plate method. Discrete colonies were maintained in culture by transferring onto nutrient agar slants and stored in the refrigerator at 4°C for further analysis.

Identification of the isolates

The bacterial isolates were identified using standard microbiological protocols, which include cultural, morphological and biochemical as described in Bergey’s Manual of Determinative Bacteriology.

Antimicrobial susceptibility testing

Few colonies of overnight culture on nutrient agar plates were re-suspended in sterile normal saline to match the turbidity of 0.5 McFarland standard for sensitivity testing as described by Cheesbrough (2006). The sensitivity of standard inocula of the

isolates to the following antibiotics Cefazidime(30µg), Cefuroxime (30µg), Cefepime (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Ampicillin (25µg), Augmentin (30µg), Nitrofurantoin (300µg), Aztreonam, Cotrimoxazole (25µg), Imipenem and Meropenem (Rapid Labs, UK) were determined on Mueller Hinton agar using the modified Kirby Bauer technique as described by Cheesbrough (2006). After incubation, the diameter zone of inhibition was measured in millimeters and interpreted using the CLSI (2014) interpretative chart. All intermediate sensitivity results were regarded as resistant. Multidrug resistance was defined as resistance to three or more classes of drug (Magiorakos *et al.*, 2012). The multiple antibiotic resistance (MAR) index was determined using the method described by Krumperman (1983) using the formula: a/b, with “a” being the number of antibiotics to which an organism is resistant to and “b” being the total number of antibiotics tested.

Results

Table 1: Prevalence of *Citrobacter* spp from different waste water channels

Location	Total no of samples collected	Positive isolates (%)
Health centre Road (H1-H5)	5(100)	5(100)
Umudike (U1-U5)	5(100)	5(100)
Ehimiri (E1-E5)	5(100)	5(100)
Afara (A1-A5)	5(100)	4 (75)
Total	20(100)	19(95)

Table 2: The antibiotic susceptibility pattern of the *Citrobacter* species isolated from wastewater

Class	Antibiotics	Code	No of isolates	
			Sensitive (%)	Resistance (%)
Cephalosporins	Ceftazidime	CAZ	0(0)	19(100)
	Cefuroxime	CRX	0(0)	19(100)
	Cefepime	FEP	10(52.6)	9(47.4)
Aminoglycosides	Gentamicin	GEN	15 (78.9)	4 (21.1)
	Fluoroquinolones	Ciprofloxacin	CPR	9(47.4)
		Ofloxacin	OFL	19 (100)
β- lactams	Ampicillin	AMP	0(0)	19 (100)
	Augmentin	AUG	0(0)	19 (100)
Monobactam	Aztreonam	ATM	11 (57.9)	8 (42.1)
Nitrofurans	Nitrofurantoin	NIT	14 (73.7)	5 (26.3)
Sulfonamide	Cotrimoxazole	COT	3 (15.8)	16 (84.2)
Carbapenem	Imipenem	IMP	14(73.7)	5 (26.3)
	Meropenem	MER	3 (15.8)	16 (84.2)

Table 3: The antibiotic susceptibility profile and the MAR index of the *Citrobacter* spp isolates

Isolate Id	Resistance profile	No of resistance	MAR index
H1	CAZ, CRX, FEP, CPR, AMP, AUG, ATM, COT, MER	9	0.7
H2	CAZ, CRX, FEP, CPR, AMP, AUG, ATM, COT, MER	9	0.7
H3	CAZ, CRX, FEP, GEN, CPR, AMP, AUG, NIT, ATM, COT, MER	11	0.9
H4	CAZ, CRX, FEP, CPR, AMP, AUG, ATM, COT, MER	9	0.7
H5	CAZ, CRX, FEP, CPR, AMP, AUG, ATM, COT, IMP	9	0.7
U1	CAZ, CRX, AMP, AUG, COT, IMP, MER	7	0.5
U2	CAZ, CRX, GEN, CPR, AMP, AUG, COT, MER	8	0.6
U3	CAZ, CRX, AMP, AUG, NIT, COT, MER	7	0.5
U4	CAZ, CRX, AMP, AUG, IMP, MER	6	0.5
U5	CAZ, CRX, GEN, CPR, AMP, AUG, COT, MER	8	0.6
E1	CAZ, CRX, FEP, AMP, AUG, NIT, ATM, COT, MER	9	0.7
E2	CAZ, CRX, FEP, AMP, AUG, MER	6	0.5
E3	CAZ, CRX, AMP, AUG, COT, MER	6	0.5
E4	CAZ, CRX, AMP, AUG, COT, MER	6	0.5
E5	CAZ, CRX, CPR, AMP, AUG, COT, MER	7	0.5
A1	CAZ, CRX, AMP, AUG, COT, IMP	6	0.5
A3	CAZ, CRX, FEP, GEN, CPR, AMP, AUG, NIT, ATM, COT, MER	11	0.9
A4	CAZ, CRX, FEP, CPR, AMP, AUG, NIT, ATM, COT, IMP	10	0.8
A5	CAZ, CRX, AUG, AMP, MER	5	0.4

DISCUSSION

This study investigated the occurrence and antimicrobial susceptibility profile of *Citrobacter* species isolated from wastewater in Umuahia, Abia State, Nigeria. *Citrobacter* spp although generally known to be opportunistic pathogens has been implicated as contaminants in fruits and vegetables especially when untreated wastewater are used for irrigation (Adegun *et al.*, 2019). It is the etiologic agent of a wide variety of infections including urinary tract infections, gastroenteritis, wound infections, brain abscesses (Rajan and Rajan, 2013; Leski *et al.*, 2016). High prevalence (95%) of *Citrobacter* spp was reported in this study. This attests to its ubiquity in the environment, which makes their response to antibacterial agents relevant in public health. *Citrobacter* spp has been isolated from humans and animals, sewage, soil and water. (Bac *et al.*, 2010; Liu *et al.*, 2017). A previous study identified *Citrobacter* spp as one of the frequently identified bacteria in waste water (Moges *et al.*, 2014).

The susceptibility test showed that *Citrobacter* spp isolated in this study had 19(100%) sensitivity to Ofloxacin followed by Gentamicin 15 (78.9%), Nitrofurantoin and Imipenem (73.7%) each. This shows that these antibiotics can be used in the treatment of probable infections that may arise due to these organisms. This pattern of resistance might be as

a result of low usage of these antibiotics in both human and veterinary medicine. The work of Eze *et al.* (2018) corroborates this work reporting 100% sensitivity to Ofloxacin and Gentamicin respectively and 97.67% to Nitrofurantoin but differed in recording 100% sensitivity to Ciprofloxacin as against 47.4% recorded in this study. Cefepime recorded 52.6% susceptibility which contrasts the observation of Maraki *et al.* (2017) who recorded 94.8% sensitivity of the *Citrobacter* spp to the antibiotic.

The isolates showed marked resistance (100%) to the Cephalosporins with the exception of Cefepime (a fourth generation cephalosporin) and the Penicillins/ β -lactamase inhibitor (Ampicillin and Augmentin). This result is in agreement with the work of Tesfaye *et al.* (2019) who although worked with smaller sample size, reported 100% resistance to Ampicillin and Augmentin and 75% resistance to the cephalosporins used in their study. Although a further test is required to confirm Extended spectrum beta-lactamases (ESBLs) in the *Citrobacter* spp used in this work, the high record of resistance against these antibiotics is worrisome. Increased resistance of bacteria to these antibacterial agents has continued to pose a global threat (Eze *et al.*, 2018)

The organisms also recorded high resistance (84.2%) against Cotrimoxazole and Meropenem respectively.

This might be as a result of misuse and abuse. In Nigeria, Cotrimoxazole is one of the antibiotics that is normally used without prescription for the treatment of cough and diarrhea and being one of the cheapest drugs makes it easier for people to access it. The increased resistance to Meropenem is of great public health concern since it is used in the treatment of bacterial meningitis, nosocomial infections and other mixed bacterial infections (Cahill *et al.*, 2019). This result however, is out of line with a previous study which reported 0% resistance against carbapenems ((Imipenem and Meropenem), 27.4% against 3rd generation cephalosporins (Ceftazidime), 3.2% against Cefepime and 12.9% against Ciprofloxacin (Liu *et al.*, 2017). Liu *et al.*, 2018 also recorded similar results. The organisms in this study recorded 47.4% resistance to Cefepime, 52.6% resistance to Ciprofloxacin, 26.3% and 84.2% resistance to Imipenem and Meropenem respectively.

High prevalence of multidrug resistant *Citrobacter* spp was reported in this work. All (100%) the isolates were resistant to at least 3 classes of antibiotics. This is in line with earlier reports by Moges *et al.* (2015), Leski *et al.* (2016), Liu *et al.* (2017) and Tesfaye *et al.* (2019). Multiple antibiotic resistance (MAR) index is a measure of extent of resistance to antibacterial agents and gives indirect suggestion of the level of risk

associated with the probable source of organisms. MAR index of >0.2 indicates isolates recovered from sources with high risk where strict observation of prescription and usage are lacking (Adenaike *et al.*, 2013). The MAR index recorded for all isolates in this study is a pointer to the level of abuse and over use of antibiotics in human and veterinary medicine as well as in animal farming in a developing country like Nigeria. The Health centre road and the Afara waste water channels recorded higher MAR index than Ehimiri and Umudike Junction. This could be attributed to the fact that the Health centre road wastewater channel could be termed a hospital waste water effluent while Afara is a downstream effluent of the Health centre road.

Conclusion

This study recorded increased occurrence of *Citrobacter* spp in wastewater. While this confirms the ubiquitous nature of this organism, their high resistance to most of the commonly used antibiotics emphasizes the role of waste water, especially untreated waste water in dissemination of antibiotic resistance genes. Exhibition of multiple antibiotic resistance should be a public health concern since these organisms can find their way to the food chain through waste water discharged into the natural waters, soils and when used for irrigation purposes

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HEAVY METAL CONTAMINATION AND WATER QUALITY INDEX ASSESSMENT OF BOREHOLE WATER FROM THREE LOCATIONS IN IKWUANO ABIA STATE.

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Abstract

Borehole water has become as essential source of water for consumption and domestic use in most African homes. However, there are serious concerns on the safety of these waters sources and suitability for consumption. This study was aimed at investigating the heavy metal contamination levels and physicochemical properties of borehole water around Health Care Centres in three locations in Ikwuano Abia state, Nigeria in a bid to ascertain possible the health risk posed by these water sources. Six samples were collected from UPHC, APHC and OPHC over a period of three months. The samples were analysed for eight heavy metals namely Magnesium (Mg), Magnesium (Mn), Cobalt (Co), Cadmium (Cd), Nickel (Ni), Copper (Cu) Lead (Pb) and Zinc (Zn) as well as 10 physicochemical parameters, (B.O.D. (mg/l), C.O.D. (mg/l), Nitrogen (mg/l), Phosphorus (mg/l), Total hardness (mg/l), Magnesium (mg/l), Chloride (mg/l), Iron (mg/l), pH and conductivity ($\mu\text{s}/\text{cm}$) and compared to standard values set by WHO and ICMR respectively. The results showed all the heavy metal were within the WHO limits in all the locations except for lead which was above the recommended limit of 0.1mg/L. The physicochemical parameters were found to be within ICMR standard values except for Iron, pH, Magnesium and Biological Oxygen Demand which were above the standard values. The Water Quality Index (WQI) obtained for the water body in different locations of study were 220.13, 228.56 and 228.25 for UPHC, APHC and OPHC respectively suggesting that the water for unsuitable for consumption. Measures must be taken by the government to improve the water quality of the town and to supply clean and safe water to the public.

Keywords: Groundwater, Borehole Water Quality Index, Heavy metals, Physicochemical parameters,

Introduction

Boreholes are low-cost technology option for domestic water supply in developing countries and are generally considered as 'safe sources' of drinking water. When properly constructed and maintained, they provide consistent supplies of safe and wholesome water with low microbial load and little need for treatment of the water before drinking. Groundwater quality varies from place to place, sometimes depending on seasonal changes (Vaishali and Punita, 2013) the types of soils, rocks and surfaces through which it moves (Seth *et al.*, 2014; Thivya *et al.*, 2014). Naturally occurring contaminants are present in the rocks and sediments. As groundwater flows through the sediments, metals such as iron and Magnesium are dissolved and may later be found in high concentrations in the water (Moyo 2013). In addition, human activities can alter the natural composition of groundwater through the disposal or dissemination of chemicals and microbial matter on the land surface and into soils, or through injection of wastes directly into groundwater. Industrial discharges (Govindarajan and Senthilnathan, 2014) urban activities, agriculture (Moyo 2013) groundwater plumage and disposal of waste (Bello *et al.*, 2013) can affect groundwater quality. Pesticides and fertilizers applied to lawns and crops can accumulate and migrate to the water tables thus affecting both the physical, chemical and microbial quality of water. Heavy metals have been severally reported in potable water sources in Nigeria

including surface, ground water and rain water. Heavy metals are typically classified into essential (iron, Magnesium, zinc, copper) and non-essential (cadmium, chromium, lead and mercury), with regard to their usefulness in biological diversity. The physico-chemical and heavy metal parameters have permissible guideline recommended by World Health Organization (WHO) and Standard Organization of Nigeria (SON) (Nigerian Agency that regulate the quality of potable water resources). When the heavy metals exceed that maximum permissible concentration for potable water, such water is said to be contaminated by such heavy metal. Naturally, most heavy metals are found in variable concentration in the environment (Izah and Srivastav, 2015). Hospital water safety is a major priority and constant challenge for healthcare epidemiologists, safety officers, engineers, and administrators. Waterborne infections incur significant morbidity and mortality, and some are preventable. As with other healthcare-associated infections, occurrence of nosocomial waterborne infections erodes public confidence in healthcare facilities. Pathogens such as *Legionella* and nontuberculous mycobacteria can colonize the deep infrastructure or outlets of hospital water distribution systems, while other Gram-negative bacteria and molds tend to adhere to biofilms at or near the distal points of use (Brooke and Tara, 2014). This present study therefore was aimed at investigating the heavy metal load and physicochemical quality of borehole

water used in different hospitals in Umuahia rural areas for various domestic purposes in these selected hospitals.

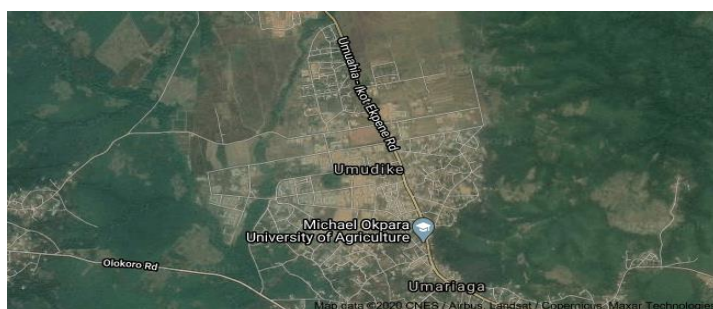
Materials and Methods

Study Area

Umudike is found in Ikwuano Local Government Area

of Abia State in the south east zone of Nigeria. It is within the Qua Ibo(e) River watershed which has Anya River as the major tributary. The latter is found within the premises of Michael Okpara University of Agriculture, Umudike and flows across the National Root Crops Research Institute, Umudike.

Fig. 1 Satellite image of Umudike showing the sampling areas (Source: Google Maps)



Sampling

The water samples used for this study were randomly collected from three different sites that were selected based on proximity to health centres. The three sites are Umudike Primary Health Centre (UPHC: 5° 28' 11.0" N 7° 32' 16.5" E), Amaoba Primary Health Centre (APHC: 5° 27' 16.7" N 7° 32' 26.0" E) and Primary Health Centre Oboro (PHCO: 5° 25' 52.3" N 7° 34' 10.6" E). The samples were collected in polyethylene bottles (1.5 litres capacity) which had been thoroughly washed, and filled with distilled water, then taken to the sampling site. The bottles were emptied and rinsed several time with the water to be collected. Also, the sample bottles were partially filled with the collected water and vigorously shaken to note the odour. The sample bottles were tightly covered immediately after collection and the temperature taken. They were then stored in a refrigerator at 4 °C to slow down bacterial and chemical reaction rates.

Heavy Metal Determination

The concentrations in mg/L of eight metals were determined in all the samples namely, Magnesium (Mg), Manganese (Mn), Cobalt (Co), Cadmium (Cd), Nickel (Ni), Copper (Cu) Lead (Pb) and Zinc (Zn) with the Atomic Absorption Spectrophotometer (AAS). 2.5 ml of concentrated HNO₃ was added to 20mls of the water sample and covered with a watch glass. This was heated gradually on hot plate at 100°C, until digestion was completed. The solution was evaporated to near dryness and cooled. 7.5ml of concentrated hydrochloric acid was then added, warm and filtered, and volume adjusted to 100ml for heavy metal determination. Heavy metal contents were quantified using AAS 2800 series. Internally added standards were used for the calibration of the AAS. The flame

used for the analysis was air-acetylene mixture. Standard solutions ranging from 0.2 to 5.0 mg/l were prepared for calibration curves of the various metals. A blank analysis was performed with distilled water treated to the sample treatment.

Physicochemical Parameters Determination

The samples were analyzed for following physicochemical parameters: B.O.D. (mg/l), C.O.D. (mg/l), nitrogen (mg/l), phosphorus (mg/l), Total hardness (mg/l), Magnesium (mg/l), chloride (mg/l), Iron (mg/l), pH and conductivity (µs/cm). Weighted arithmetic index method (Brown *et al.*, 1972) was used for the calculation of water quality index (WQI) of the water body. Further, quality rating or sub index (q_n) was calculated by the following expression.

$$q_n = 100 [V_n - V_{10}] / [S_n - V_{10}]$$

Where: q_n=Quality rating for the nth water quality parameter,

V_n=Estimated value of the nth water quality parameters of collected sample,

S_n=Standard permissible value of the nth water quality parameter,

V₁₀=Ideal value of the nth water quality parameter in pure water.

Unit weight was calculated by a value inversely proportional to the recommended standard value S_n of the corresponding parameter.

$$W_n = K / S_n$$

Where: W_n=Unit weight for nth water quality parameter,

S_n=Standard permissible value of the nth water quality parameter,

K=Constant for proportionality calculated as

$$K = \frac{1}{\sum (1/S_i)}$$

The overall WQI was calculated by aggregating the quality rating with the unit weight linearly.

$$WQI = \frac{\sum q_n W_n}{\sum W_n}$$

Where:

q_n = Quality rating for the n^{th} water quality parameter,
 W_n =Unit weight for n^{th} water quality parameter.

Statistical Analysis

Statistical analyses were performed using the SPSS version 22. Data was represented as means ± standard deviation (M ± SD).

Results

Table 1. Heavy metal concentration in borehole water samples from hospitals in three (3) rural areas in Umuahia.

Sampling sites	Heavy metals							
	Mg (mg/L)	Mn (mg/L)	Co (mg/L)	Cd (mg/L)	Ni (mg/L)	Cu (mg/L)	Pb (mg/L)	Zn (mg/L)
UPHC	0.7777 ±0.29	0.1752 ±0.24	0.3280 ±0.01	-0.0026 ±0.00	0.0298 ±0.00	0.0022 ±0.00	0.0587 ±0.01	0.1333 ±0.00
APHC	0.9313 ±0.15	0.0007 ±0.01	0.3355 ±0.00	-0.0017 ±0.00	0.2840 ±0.01	0.0036 ±0.00	0.0586 ±0.01	0.0732 ±0.08
PHCO	0.5851 ±0.00	0.0008 ±0.00	0.3286 ±0.01	-0.0016 ±0.00	0.0350 ±0.00	0.0039 ±0.00	0.0484 ±0.02	0.1345 ±0.00
*WHO	-	0.4	-	0.003	0.07	2.0	0.01	3.0

Values are represented as mean ± S.D. Mg= Magnesium, Mn=Magnesium, Co=Cobalt, Cd=Cadmium, Ni=Nickel, Cu=copper, Pb=Lead, Zn=zinc.

Table 2. Physico-chemical parameters of borehole water samples from hospitals in three (3) rural areas in Umuahia.

Parameters	UPHC	APHC	PHCO
Electrical conductivity (µs/cm)	0.37 ± 0.297	0.357 ± 0.120	0.293 ± 0.153
pH	9.120 ± 0.566	8.843 ± 0.484	7.870 ± 0.017
Iron (mg/L)	0.326 ± 0.030	0.327 ± 0.030	0.337 ± 0.004
Total Hardness (mg/L)	35.000 ± 8.660	46.667 ± 11.547	46.667 ± 11.547
Chloride (mg/L)	2.489 ± 0.188	2.892 ± 0.259	2.465 ± 0.082
Magnesium (mg/L)	0.223 ± 0.006	0.235 ± 0.005	0.235 ± 0.003
Chemical Oxygen Demand (mg/L)	4.600 ± 0.173	4.533 ± 0.057	4.600 ± 0.173
Phosphates (mg/L)	0.257 ± 0.015	0.242 ± 0.005	0.224 ± 0.007
Nitrates (mg/L)	0.059 ± 0.002	0.064 ± 0.013	0.056 ± 0.005
Biochemical Oxygen Demand (mg/L)	5.817 ± 0.127	5.547 ± 0.393	5.733 ± 0.058

Values are represented as mean ± S.D.

Table 3. Calculation of WQI of borehole water samples from UPHC.

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Parameter	Observed Value (V_n)	ICMR Standard Values (S_n)	Ideal Value (V_{10})	Unit Weight (W_n)	Quality Rating (q_n)	$W_n q_n$
Electrical conductivity ($\mu\text{s/cm}$)	0.37	300	0	0.371	0.123	0.0456
pH	9.120	7.5	7	0.219	424	92.856
Iron (mg/L)	0.326	0.3	0	0.5859	108.66	63.66
Total Hardness (mg/L)	35.000	300	0	0.0062	11.67	0.07235
Chloride (mg/L)	2.489	250	0	0.0074	0.99	0.00732
Magnesium (mg/L)	0.223	30	0	0.061	0.7433	0.0453
Chemical Oxygen Demand (mg/L)	4.600	20	0	0.0034	23	0.0782
Phosphates (mg/L)	0.257	1.0	0	0.0677	25.7	1.7399
Nitrates (mg/L)	0.059	45	0	0.0412	0.1311	0.00540
Biochemical Oxygen Demand (mg/L)	5.817	5	0	0.3723	116.34	43.313
		$\Sigma S_n=985.5$		$\Sigma W_n=1.7351$	$\Sigma q_n=711.357$	$\Sigma W_n q_n=201.823$

Research **WQI = 116.32**

ICMR: Indian Council of Medical

Table 4. Calculation of WQI of borehole water samples from APHC.

Parameter	Observed Value (V_n)	ICMR Standard Values (S_n)	Ideal Value (V_{10})	Unit Weight (W_n)	Quality Rating (q_n)	$W_n q_n$
Electrical conductivity ($\mu\text{s}/\text{cm}$)	0.357	300	0	0.371	0.119	0.0441
pH	8.843	7.5	7	0.219	368.6	80.723
Iron (mg/L)	0.327	0.3	0	0.5859	109	63.863
Total Hardness (mg/L)	46.667	300	0	0.0062	15.56	0.0964
Chloride (mg/L)	2.892	250	0	0.0074	1.157	0.00856
Magnesium (mg/L)	0.235	30	0	0.061	0.7833	0.04778
Chemical Oxygen Demand (mg/L)	4.533	20	0	0.0034	22.67	0.07708
Phosphates (mg/L)	0.242	1.0	0	0.0677	24.2	1.63834
Nitrates (mg/L)	0.064	45	0	0.0412	0.1422	0.005858
Biochemical Oxygen Demand (mg/L)	5.547	5	0	0.3723	110.94	41.3029
		$\Sigma S_n=985.5$		$\Sigma W_n=$ 1.6451	$\Sigma q_n =$ 653.17	$\Sigma W_n q_n =$ 187.807

ICMR: Indian Council of Medical Research **WQI = 114.16**

Table 5. Calculation of WQI of borehole water samples from PHCO.

Parameter	Observed Value (V _n)	ICMR Standard Values (S _n)	Ideal Value (V ₁₀)	Unit Weight (W _n)	Quality Rating (q _n)	W _n q _n
Electrical conductivity (μs/cm)	0.293	300	0	0.371	0.0977	0.0362
pH	7.870	7.5	7	0.219	174	38.106
Iron (mg/L)	0.337	0.3	0	0.5859	112.33	65.814
Total Hardness (mg/L)	46.667	300	0	0.0062	15.56	0.0964
Chloride (mg/L)	2.465	250	0	0.0074	0.986	0.0073
Magnesium (mg/L)	0.235	30	0	0.061	0.7833	0.0478
Chemical Oxygen Demand (mg/L)	4.600	20	0	0.0034	23	0.0782
Phosphates (mg/L)	0.224	1.0	0	0.0677	22.4	1.5165
Nitrates (mg/L)	0.056	45	0	0.0412	0.28	0.0115
Biochemical Oxygen Demand (mg/L)	5.733	5	0	0.3723	114.66	42.687
		ΣS_n=985.5		ΣW_n= 1.735	Σq_n = 464.097	ΣW_nq_n = 148.40

ICMR: Indian Council of Medical Research **WQI = 85.53**

Discussion

The heavy metal load and physicochemical parameters such as pH, electric conductivity, iron, calcium, Magnesium, fluoride, chloride, biological oxygen demand, chemical oxygen demand, nitrate and total hardness of water were analysed for the water samples collected from three hospitals in rural areas i.e UPHC, APHC and PHCO. All parameters with the mean value of the data with standard error were calculated as shown in the Table 1 and 2 above.

In this study the results for the heavy metal estimations showed that Magnesium (Mg), Cobalt (Co), Nickel (Ni), and Zinc (Zn) were all within the recommended limits set by the WHO. However, the concentration of lead (Pb) in UPHC (0.0587 ± 0.01), APHC (0.0586 ± 0.01) and PHCO (0.0484 ± 0.02) were all above the WHO recommended limit of 0.01mg/L. Possible sources of Pb pollution could result from domestic waste dump, smoking as well as

house paints as the rain wash them into the soil and plumbing pipes. In children, these studies have shown an association between lead poisoning and diminished intelligence, lower intelligence quotient-IQ, delayed or impaired neurobehavioral development, decreased hearing acuity, speech and language handicaps, growth retardation, poor attention span, and anti-social and diligent behaviours (USEPA, 2017).

Biological Oxygen Demand is a measurement of the amount of dissolved oxygen (DO) required for the microorganism to performed biological decomposition of dissolved solids or organic matter in the water under aerobic conditions and provides an index to assess the effect discharged wastewater will have on the receiving environment. The higher the BOD value, the greater the amount of organic matter or “food” available for oxygen consuming bacteria. This study showed BOD concentration of all the water samples

obtained from all the locations to be higher than the standard recommended values. UPHC recorded the highest BOD levels possibly due to the high amount of waste along with rain water and from nearby waterbodies and addition of organic waste.

Chemical Oxygen Demand or COD is a measurement of the oxygen required to oxidize soluble and particulate organic matter in water. This study showed COD concentration of all the water samples obtained from all the locations to be lower than the standard recommended values. Lowest values were recorded in samples from APHC. The observed COD for the samples were observed to be lower than their respective COD levels suggesting a low microbial load as microorganisms will find it difficult to survive in the water samples since the oxygen available for their survival is limited.

Among nitrogen containing compounds, nitrate is the most common form in water. All other dissolved forms of nitrogen (nitrite, ammonia and organic nitrogen) get oxidized to nitrate over time. Nitrates are found in both ground and surface water, originating from the natural decaying process of biological matter. Nitrate has a high solubility in water and will not be filtered out like other contaminants as it seeps through the soil layers to groundwater level. The levels of nitrate in this study were observed to be lower than the standard values in all the samples obtained.

Magnesium is a mineral that naturally occurs in rocks and soil and may also be present due to underground pollution sources. Magnesium is seldom found alone in a water supply. It is frequently found in iron-bearing waters (Iyasele and Idiata, 2011). The concentration of Magnesium in all the collected samples were above the standard recommended value. High exposure to Magnesium has been associated with toxicity to the nervous system, producing a syndrome that resembles Parkinsonism. Magnesium is unlikely to produce other types of toxicity such as cancer or reproductive damage. Young children appear to absorb more Magnesium than older age groups but excrete less. This makes it particularly important for pregnant women and children to have clean drinking water (Lawson, 2011).

Chloride levels detected in the borehole water samples in the three locations were below limit (250mg/l) stipulated by ICMR. The presence of excess chlorine in water has adverse health effects; by-products of chlorination can lead to production of free radicals that lead to oxidative stress observed in most disease cases. Concentrations over 250 mg/L impart a peculiar taste to the water, thus rendering the water unacceptable for drinking purposes.

Phosphates are not toxic and therefore are not considered a direct threat to health of human's other organism. They also are not considered a serious indirect threat to water quality. Little amounts of

phosphorous in surface waters is necessary for biological life, but excess of phosphorous promotes the indiscriminate growth of algae (Eutrophication). The values of phosphates for water samples from the three sampled locations were within the standard limits.

The degree of hardness of water supply is determined by the content of calcium and magnesium salts. Calcium and magnesium combine with bicarbonates, chlorides, sulphates, and nitrates to form these salts. Water can be classified into soft (75 mg/L), moderately hard (75–150 mg/L), hard (150–300 mg/L) and very hard (300 mg/L) based on hardness (Sawyer and McCarty 1967). The hardness of the water samples in this study were lower than the recommended values by ICMR. Long-term consumption of extremely hard water might lead to an increased incidence of urolithiasis, anencephaly, prenatal mortality, some types of cancer and cardiovascular disorders (Agrawal and Jagetia 1997).

Iron is one of the most troublesome elements in water supplies. Making up at least 5 % of the earth's crust, Iron is one of the earth's most plentiful resources. Rainwater as it infiltrates the soil and underlying geological formations dissolves iron causing it to seep into the aquifer that serves as source of ground water for bore holes. The observed iron content of the water samples from the three locations analysed were above limits of specification according to ICMR.

The pH of the samples was tending towards alkalinity with values ranging from 7.8-9.1 in all the three locations which fell above ICMR standard value of 7. Deviations in the pH value of water solutions from 7 are principally due to hydrolysis of salts of strong bases and weak acids or vice versa, also dissolved gases such as carbon dioxide, hydrogen sulphide and ammonia affect pH of water (Agbabiaka and Oyeyiola, 2012).

Conductivity is defined as the ability of a solution to carry electric current. This is normally dependant on the presence of mobile ions, their concentrations, mobility, relative concentration and temperature of measurement. The electrical conductivity (EC) for all samples for all samples fell within the permissible limit set by ICMR.

Table 6. Water Quality Index (WQI) and corresponding status (Chaterjee and Raziuddin, 2002; Thakor et al. 2011).

Water Quality Index Level	Water Quality Status
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0-25	Excellent water quality	assayed physicochemical parameters and the observed difference in these water quality parameters can be attributed to climatic, geographic, and geologic variations between the three sampled locations as well as human activities in these areas. These observed differences can also be attributed to disparity in the hygienic condition of the boreholes. The calculated WQI for the three locations showed that the water from these boreholes were unsuitable for drinking. Based on the results for the heavy metal analyses, all assayed metals were within WHO limits except for the level of lead (Pb) which was observed to be above recommended values of WHO in the borehole water samples from all the locations making the water unsafe for consumption as lead can cause serious adverse health effects over time. Based on these results it should be concluded that borehole water sources are unsafe hence and it is therefore necessary to treat the water from these boreholes before consumption to prevent the spread of water borne diseases to ensure that the health of the community is protected. Measures must be taken by the government and stake holders to improve the water quality of the town and to supply clean and safe water to the public that is free from health hazards.
26-50	Good water quality	
51-75	Poor water quality	
76-100	Very poor water quality	
>100	Unsuitable for drinking	

The WQI obtained for the water body in different locations of study i.e., UPHC (116.32), APHC (114.16) and PHCO (2285.53) respectively, indicate, based on the quality status shown in Table 6 above, that water is of very poor quality due to high pollution level.

Conclusion

The results of this study showed that most of the physicochemical parameters of the borehole water samples were above the standard limits recommended by ICMR. The water samples from the different locations sampled showed varying levels of the

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ANTIOXIDANT PROPERTIES OF AQUEOUS EXTRACT OF *Newbouldia laevis* LEAVES, STEM BARK AND ROOT WITH SOME ENZYMES LINKED TO ARTHRITIS - *IN VITRO*

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Abstract

This study examined effects of aqueous extract of *Newbouldia laevis* (*N.l*) leaves, stem bark and root on some enzymes linked to arthritis in rat's serum together with antioxidant properties - *in vitro*. The aqueous extracts of *Newbouldia laevis* leaves, stem bark and root were separately prepared in weight/volume, with pulverized sample of 10 mg/ml concentration. Total phenol, total flavonoid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and NO radical scavenging abilities, ferric reducing power, Fe²⁺-induced lipid peroxidation (LPO) as well as acetylcholinesterase (AChE), butyryl-cholinesterase (BChE) and arginase activities were determined *in vitro* using standard methods. The result revealed stem bark aqueous extract had highest total phenol (4.71 mgAE/g), the root had highest total flavonoid contents (12.12 MEQ/g) and the leaves had highest reducing property (6.15 mgAAE/g). All the samples exhibited strong antioxidant properties. Also, the extracts inhibited AChE activity dose-dependently. However, the root extract (IC₅₀= 3.55 µg/ml) had higher inhibitory activity than stem bark (IC₅₀= 5.41 µg/ml) and leaves extract (IC₅₀= 6.68 µg/ml). While BChE activity was inhibited by the extracts concentration dependently (0-20 µg/ml), both root (IC₅₀= 9.12 µg/ml) and stem bark extracts (IC₅₀= 9.02 µg/ml) had the highest inhibitory effect than the leaves (IC₅₀= 11.04 µg/ml) respectively. The root extracts (IC₅₀= 1.53 µg/ml) had highest arginase inhibitory effect than leaves (IC₅₀= 2.42 µg/ml) and stem bark (IC₅₀= 4.07 µg/ml) respectively. Thus, the enzymatic modulatory and antioxidant effects of extracts could be possibly the underlying mechanisms by which the plant elicits its anti-arthritis effects in folklore and employed in the management of arthritis.

Keywords: *N.l*-leaves; *N.l*-stem bark; *N.l*-root; Enzymes; Arthritis

Introduction

Arthritis could be referred to as joint inflammation or the stiffness and swelling of joints, including lupus and rheumatoid arthritis (Rothschild, 1988). The disease encompasses three basic interrelated processes such as inflammation, synovial proliferation and joint tissue destruction as well as cell oxidative damage due to free radicals released by platelets recruitment and actions of cytokines (Gory, 2000). Oxidative stress is an imbalance between free radicals and antioxidants in the body (Veselinovic, 2014). Inflammation is a defence mechanism in the body, the immune system recognizes damaged cells, irritants, and pathogens, and elicit the healing process (Veselinovic, 2014). Acute inflammation result into chronic inflammation and chronic inflammation eventually causes several diseases and conditions, including cancers and rheumatoid arthritis (Gory, 2000). Long-term inflammation can last for several months and even years (Gory, 2000). The efferent vagus nerve regulate inflammation through acetylcholine (ACh) a neurotransmitter, which known as cholinergic anti-inflammatory pathway (van Maanen et al., 2009). ACh is known to exert its effects on immune cells via the triggering of various signalling mechanisms that interact with each other to achieve the anti-inflammatory effects (van Maanen et al., 2009).

Increased acetylcholinesterase activity is associated with decreased ACh anti-inflammatory effect, thus promote inflammatory conditions (van Maanen et al., 2009). Endothelial function improvement is seen as a crucial approach in the management of arthritis sufferers (Sodergren et al., 2010). Impaired endothelial function is associated with adjuvant-induced arthritis rats model. As observed with this model there is positive correlation between endothelial dysfunction and severity of arthritis (Prati et al., 2011). A body of evidence has reported vascular arginase upregulation as a mechanism contributing to endothelial dysfunction in arthritic rats. Therefore, the use of arginase inhibitors could be a good strategy for endothelial dysfunction associated arthritis (Prati et al., 2012).

Newbouldia laevis is a tropical rainforest plant also called "Tree of Life" with medicinal value ranging from anti-inflammatory, antioxidant, anti-microbial, anti-fungi, analgesic and wound healing properties (Idu et al., 2009). *N. laevis* is widely used in African folk medicine for the treatment of malaria and fever, stomachache, coughs, sexually transmitted diseases, rheumatoid arthritis, tooth ache, breast cancer, and constipation (Idu et al., 2009). Scientific reports on the phytochemical constituents of the plant revealed the

presence of alkaloids and phenylpropanoids in the root, flavonoids, and tannins in the leaf (Germann *et al.*, 2006). In consideration of side effects associated with the use of conventional drugs and the efficacy of the plants in folklore against arthritis, this study is designed to assess and compare the antioxidant properties of aqueous extract of *Newbouldia laevis* leaves, stem bark and root with some enzymes linked to arthritis *in vitro*.

Material and Methods

Material Collection and preparation of sample

Different part of *Newbouldia laevis* were collected from Isaba area in Ikole, Ekiti state, Nigeria. Collected Parts of the plant were taken to Plant science Laboratory at Federal University Oye Ekiti (FUOYE) for identification. The plant parts (stem bark, leaves and root) used were collected fresh and sun dried and blended into fine powder using electric blender, the powders were further sieved and kept in plastic airtight containers for subsequent use.

Chemicals and Reagents

Acetylthiocholine, butyrylthiocholine, thiobarbituric acid (TBA), were purchased from Sigma (St Louis, MO, USA). All other chemicals used were of analytical grade and glass distilled.

Methods

Extraction of the samples

0.5g of the powders was weighed using weighing balance. To prepare the extract, 0.5grams of each pulverized plant materials (leaves stem bark and root) was put in 50ml of distilled water inside a bottle and then placed in oscillator for vigorous shaking for 6hrs followed by filtration.

Handling and use of animals

The handling and use of animals were in accordance with the NIH guide for the care and use of laboratory animals. The use of animal in this study was duly approved by the Animal Ethics Committee of our institution. In this experiment, twenty (20) male wistar strain albino rats weighing 180–200g were purchased from the breeding colony of the Department of Biochemistry, Federal University of Technology Akure, Nigeria. Rats were maintained at 25 °C, on a 12-h light/12-h dark cycle, with free access to food and water. They were acclimatized under these conditions for 2 weeks before the commencement of the experiment. The preparation of tissue homogenates was as reported by Okeke *et al.* (2018).

Biochemical Assays (In Vitro)

The effect of the extracts on cholinesterase [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] activities was determined as reported by Okeke *et al.* (2018). Arginase activity was assessed by measuring the concentration of urea produced by the reaction of Ehrlich's reagent as described by Okeke *et al.* (2018).

The effects of the extracts on nitric oxide radical scavenging ability is according to the method of Moncada (1989). The DPPH* (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging ability of the extracts were evaluated as described by Gyamfi *et al.* (1999). The lipid peroxidation assay was carried out using a modified method as described by Okeke *et al.* (2018). While the total phenol and flavonoid content was determined according to the method of Oboh *et al.* (2016). The reducing property of aqueous extracts were determined by assessing the ability of the extracts to reduce FeCl₃ solution as described by Okeke *et al.* (2018).

Data analysis

The results of two replicates were pooled and expressed as mean ± standard deviation (SD). A one-way analysis of variance and the least significance difference test were carried out (Okeke *et al.*, 2018).

Results

The effect of *N.L* leaves, stem bark and root extracts on the activities of AChE, BChE and arginase, *in vitro* was investigated and the results were presented in Figures 1A, 1B and 1C respectively with their IC₅₀ values in Table 1. The result revealed the extracts inhibited AChE dose-dependently. However, the root extract (IC₅₀= 3.55 µg/ml) had a higher inhibitory activity than stem bark (IC₅₀= 5.41 µg/ml) and leaves extracts (IC₅₀= 6.68 µg/ml). The BChE activity inhibition by the extracts were in a concentration dependent manner (0-20 µg/ml), however, both root (IC₅₀= 9.12 µg/ml) and stem bark extracts (IC₅₀= 9.02 µg/ml) had the highest inhibitory effect than the leaves (IC₅₀= 11.04 µg/ml) respectively. While the root extracts (IC₅₀= 1.53 µg/ml) also had highest arginase inhibitory effect than leaves (IC₅₀= 2.42 µg/ml) and stem bark (IC₅₀= 4.07 µg/ml) respectively. Also, the result of Figure 2A, revealed that the extract scavenged NO radicals with the stem bark extract (IC₅₀= 1.44 µg/ml) was more potent than the root and leaves as shown in Table 3. Also, all the extracts scavenged DPPH radicals as shown in Figure 2B, with the root extract demonstrating the highest potency (Table 3). Also the incubation of rat brain tissue homogenates in the presence of Fe²⁺ caused a significant increase in the thiobarbituric acid reactive substance (TBARS) produced (Figure 2C); the extracts inhibited TBARS produced in a concentration-dependent manner, however, leaves extract of *N.L* (IC₅₀= 6.14 µg/ml) had the highest inhibitory effect on TBARS produced in the brain tissue than root and stem bark as shown in Table 3. Table 2 shows the total phenol and flavonoid contents as well as the reducing property of *N.L* leaves, stem bark and root aqueous extracts. The stem bark aqueous extract had highest total phenol (4.71 mGAE/g), the root had highest total flavonoid contents (12.12 mQE/g) and the leaves had highest reducing property (6.15 mgAAE/g) as shown in Table 2.

Table 1.0: IC₅₀ values for AChE, BChE, and Arginase inhibitory activity of *N.L* Leaves, Stem bark and Root.

Samples	IC ₅₀ Values (µg/ml)		
	AChE	BChE	Arginase
<i>N.L</i> Leaves	6.68±0.46	11.04±1.19	2.42±0.16
<i>N.L</i> Stem bark	5.41±0.36	9.02±0.08	4.07±19
<i>N.L</i> Root	3.55±24	9.12±0.65	1.53±0.36

Values represent mean ± standard deviation, number of sample replicate n=3.

Samples	Total Phenol	Total flavoured	Reducing Property
	<i>N.L</i> Leaves	3.36±0.19	5.15±0.32
<i>N.L</i> Stem bark	4.71±0.19	11.42±0.15	3.74±0.24
<i>N.L</i> Root	3.85±0.21	12.12±0.07	0.49±0.11

Values represent mean ± standard deviation, number of sample replicate n=3.

Table 3.0: IC₅₀ values of NO radical scavenging ability and Lipid peroxidation inhibitory effect of *N.L* Leaves, Stem bark and Root.

Samples	IC ₅₀ Values (µg/ml)		
	NO	LPO	DPPH
<i>N.L</i> Leaves	1.75±0.07	6.14±1.16	7.76±0.29
<i>N.L</i> Stem bark	1.44±0.03	8.19±0.0	6.31±0.24
<i>N.L</i> Root	1.70±0.14	7.60±0.49	5.79±0.24

Values represent mean ± standard deviation, number of sample replicate n=3.

Table 2.0: Total Phenol, Total Flavonoid, Reducing property of *N.L* Leaves, Stem bark

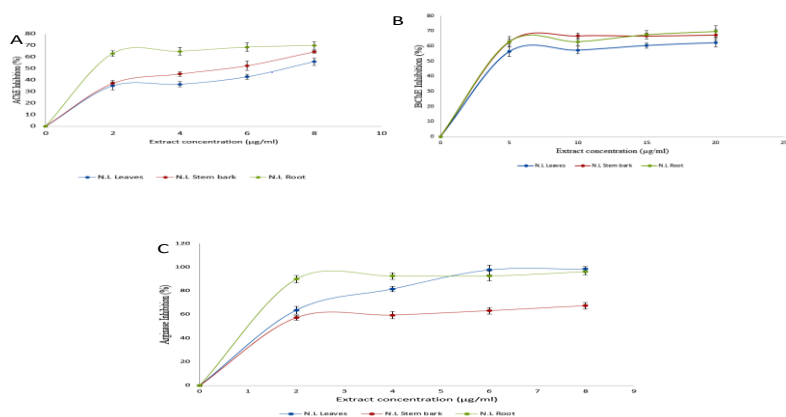


Figure 1: (A) AChE (B) BChE (C) Arginase Inhibitory Effect of *N.L* Leaves, Stem bark and Root Extract – in vitro

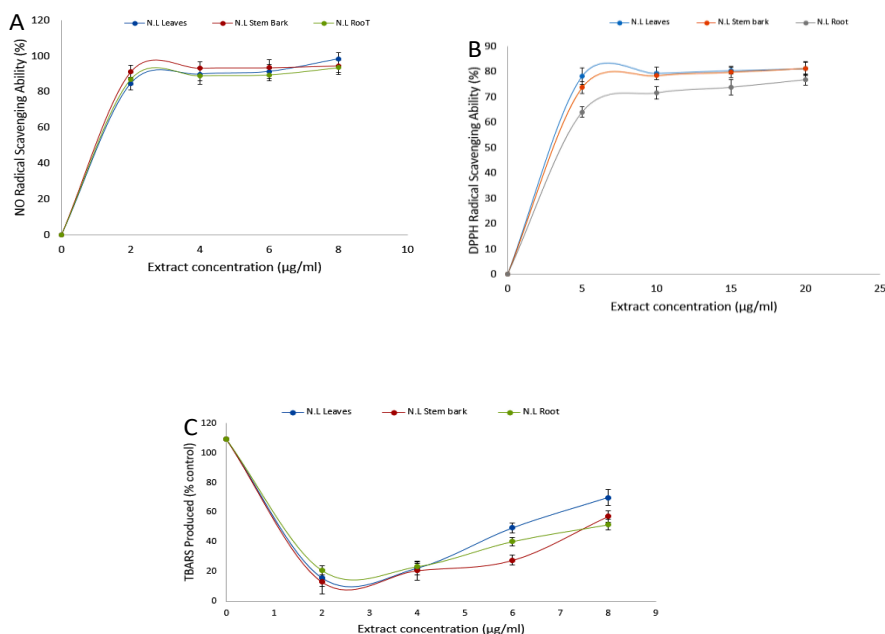


Figure 2: (A) NO (B) DPPH Radical Scavenging Abilities (C) Fe²⁺-induced lipid peroxidation inhibitory effect of *N.L* Leaves, Stem bark and Root Extract – *in vitro*

Discussion

The ability of plant foods to give potential health promoting effects are being well explored in order to proffer cheap and complementary source of therapy for different pathological conditions such as arthritis, erectile dysfunction, diabetes, hypertension and neurodegeneration (Oboh, 2006, Adefegha *et al.*, 2017). These benefits have largely been associated with their bioactive phytochemicals contents including alkaloids, phenolics, flavonoids, bioactive peptides and amino acids (Adefegha *et al.*, 2017; Okeke *et al.*, 2018). Therefore, owing to this numerous health promoting benefits and little or no side effects medicinal plants had gained more advantage over synthetic drugs. Both AChE and butyrylcholinesterase (BChE) perform functions related to the transmission of nerve impulses in cholinergic synapses, haematopoiesis, inflammatory markers, production and coordination of movement, and memory (Jaime *et al.*, 2006). The cholinergic anti-inflammatory pathway is mediated by acetylcholine and also regulates the levels of serotonin, dopamine and other neuropeptides

and thus modulates the immune response as well as neurotransmission (van Maanen *et al.*, 2009; Bennett *et al.*, 2019). The progressive deterioration of cholinergic innervation leads to signaling impairment (Bennett *et al.*, 2019). NO is implicated in mediating acetylcholine inhibition of platelets (Bennett *et al.*, 2019). This occurs due to high level acetylcholinesterase (AChE) activity associated platelets endothelial dysfunction in RA. The inhibition of AChE promotes the inhibition of platelets activation due to accumulation of acetylcholine level that ensures anti-platelets activity (Bennett *et al.*, 2019). According to the finding of this work, as represented in Figures 1A and 1B, the root extract had higher inhibitory activity than stem bark and leaves extracts and in BChE activity both root and stem bark extracts had the highest inhibitory effect than the leaves respectively. This inhibitory effect of these extracts could be related to the phytochemical compounds present in the extract. Also, as could be observed in Table 2, which confirms the fact that the extracts contain plant secondary metabolites such phenolic compounds and flavonoids

which are potent inhibitors of cholinergic enzymes and possesses antioxidative, anti-inflammatory, and antithrombotic effects against arthritis and other dietary related diseases. Thus, this could be an aspect of the mechanisms by which the plant carries out its antiarthritic functions in folklore.

The inhibition of arginase activity has considered an important approach in the management of arthritis and sedentary and lifestyle related diseases (Adefegha *et al.*, 2017). The result of the in vitro findings on arginase activity revealed that the root extracts also had highest arginase inhibitory effect than leaves and stem bark respectively. Thus, phytochemical distribution in different parts of the plant could be responsible for difference in the inhibition observed as shown in Figure 1C. The inhibition of arginase activity will in turn promote the production of nitric oxide (NO) which will enhance the inactivation of platelets and enhance endothelial function in preventing and management of arthritis (Adefegha *et al.*, 2017; Bennett *et al.*, 2019). As observed in the findings of this study, all the extracts scavenged NO and DPPH

radicals (Figures 2A and 2B), and inhibited iron induced lipid peroxidation (Figure 2C and caused reduction of ferric to ferrous as shown in Table 2. The reason for this observed effects could be due to phenol and flavonoid compounds (Table 2) present in the plant extracts. This proves the ability of the extracts to prevent or combat oxidative stress due to free radicals in disease conditions. Study has shown that intake of dietary antioxidants and certain antioxidant micronutrients particularly beta-cryptoxanthine and supplemental zinc in diet with fruits and cruciferous vegetables possesses protective role against the development of RA (Cerhan *et al.*, 2003).

Conclusion

The results of this study undeniably suggest all the extract modulated arginase and cholinergic enzymes activities as well as exhibited strong antioxidant properties which could be possible mechanism by which the plant elicit its anti-arthritis function in folkloric medicine. Thus, the extracts could serve as an effective promising therapeutic agent for treatment of chronic inflammatory diseases such as arthritis.

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MATERNAL AUTONOMY AND HEALTH CARE UTILIZATION IN NIGERIA

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Abstract

Maternal health care utilization has been shown to prevent most maternal deaths and health issues faced by women in Nigeria. There is a dearth of information on maternal autonomy and health care utilization in Nigeria therefore this study examined the effect of maternal autonomy on maternal health care utilization in Nigeria. Data on 10193 married women, living in Nigeria were sourced from Nigeria Demographic and Health Survey 2013. Data were disaggregated into six Geo-political zones namely North Central, North West, North East, South East, South-South, and South West. Information on socio-economic characteristics, level of maternal autonomy, maternal health care services, and women's autonomy was assessed. Data were analysed using descriptive statistics, composite score analysis and logit regression. The results revealed that 48.8% of women are within 25-34 years while 54.6% of the women have low level of autonomy. Household size is significant at 5% and increase in household size leads to higher likelihood of maternal health care utilization. The study recommend the need to empower and enlighten women to achieve higher levels of autonomy due to its effect on their health through education, seminars, enlightenment programs and trainings on the need for maternal healthcare utilization.

Keywords: Autonomy, Health care Utilization, Women, Nigeria

Introduction

Women autonomy can be defined as a concept with several dimensions which include both control over resources (that is, physical, human, intellectual, and financial) and ideologies (such as, beliefs, values, attitudes, internal strength, self-esteem, and self-confidence) (Pradhan, 2003). Researchers also identified some of the direct measures of women autonomy which include access to and control over resources, participation in economic decisions, self-esteem, and mobility (ability to move freely without restrictions) (Adhikari *et al.*, 2011). Women autonomy play vital role in the utilization of maternal health care services which has great effect on their health. Good health is a basic requirement for living a life that is productive both socially and economically, also the health status of women affect their ability to work, and thus supports the welfare of the household, including the children's development (Asenso-Okyere *et al.*, 2011). It has been shown in research that both individual level of autonomy and community level of autonomy can affect a woman's decision to seek medical care (Adjiwanu and Legrand, 2014). At the individual level, low autonomy can influence women's health through lower opportunities to engage in paid employment, availability of domestic violence and low access to health care services (Fainlyson and Downe, 2013). At the community level, low autonomy can influence women's health through cultural beliefs. Some African cultures believe that women leaving husband's houses to seek medical care is unacceptable (Shamaki and Buang, 2014).

Studies have examined the relationship that occurs between women's autonomy and utilisation of any or

all of the maternal health care services, and have found association between them (Bhandari *et al.*, 2017; Hagos *et al.*, Lowe *et al.*, 2016; Tiruneh *et al.*, 2017). Women's autonomy is also an important factor in the utilization of maternal health care services in Nigeria. Some studies have shown that some women do not benefit from health care services neither do they derive any joy of motherhood, as a result of their young age, lack of autonomy and inexperience (Baral *et al.*, 2010). Also, some women need to take permission from their husband/partners or mother-in-laws before decisions pertaining to their health can be taken, including maternal health care services and care for their babies as a result of their lack of needed autonomy or decision making power to seek health services (Baral *et al.*, 2010). It has been shown that there is a relationship between women's autonomy and the utilization of health care services (Asweto *et al.*, 2014). This problem of lack of autonomy by some married women in relation to utilization of health care services has adversely affected the health status of maternal women because of their inability to access health care centre for prompt medical attention except they take permission from their spouses which sometimes might not be timely. Some African cultural beliefs and practices encourage maternal women's low autonomy and this has adversely affected the health of the women and also some cultural practices (especially northern Nigeria) does not encourage their women going to health centres for medical care, they consider it unacceptable (Shamaki and Buang, 2014).

Research Objectives

The general objective of the study is to determine the effect of maternal autonomy on the utilization of health care services by women in Nigeria. In achieving this

objective, the following are the specific objectives:
 To determine the level of maternal autonomy in Nigeria.
 To identify the maternal health care service utilization in Nigeria.
 To examine the effect of maternal autonomy on maternal health care service utilization in Nigeria.

Justification

One of the subject matters in sustainable development goals (SDG) is to ensure healthy lives and promote well-being for men, women and children at all ages. Maternal health is the crux of the third sustainable development goal (ensuring healthy lives) and it is important that the goal is being achieved and the target is 2030. This study will help policy makers to focus on improving maternal autonomy especially women in the rural areas through seminars, trainings, enlightenment programmes and so on. This will increase the knowledge of the women and encourage them to utilize maternal health care services which invariably will decrease the maternal deaths and morbidity in the country. In order to bring the sustainable development goal to reality, the study aims at providing information on how maternal autonomy affects health care service utilization since increase in the autonomy could be an efficient way to maximize the utilization of maternal health care services in Nigeria which will help improve the health of the women and reduce maternal mortality. The study will also assist policy makers in making policies that will empower women to stand on their rights in making decisions pertaining to their health.

Some studies have attempted to examine the barriers to the utilisation of maternal health care services, some from demographic and economic perspectives (Titaley *et al.*, 2010; MCNamee *et al.*, 2009 ; Amin *et al.*, 2010) while some others from sociocultural and behavioural perspectives (Titaley *et al.*, 2010; Islam *et al.*, 2014; Deo *et al.*, 2015; Akeju *et al.*, 2016). Few studies have been carried out on the effect of maternal autonomy on utilization of healthcare services in Nigeria and the factors influencing maternal autonomy and health care service utilization in Nigeria. In some other studies, autonomy was used as a dimension to measure the welfare of women or poverty level of women, but in this study, autonomy was used as a major variable, not as a dimension, having effect on maternal health care utilization. Most of the studies on maternal autonomy and health care utilization were carried out in rural areas, but this study was carried out across the six geopolitical zones and place of residence (rural and urban) of the respondents in Nigeria. This study aims at filling the gap in knowledge.

METHODOLOGY

Scope of study

The scope of study for this research was Nigeria. Nigeria lies on the west coast of Africa between

latitudes 4°16' and 13°53' north and longitudes 2°40' and 14°41' east. It occupies approximately 923,768 square kilometres of land stretching from the Gulf of Guinea on the Atlantic coast in the south to the fringes of the Sahara Desert in the North. The territorial boundaries are defined by the republics of Niger and Chad in the north, the Republic of Cameroon on the east, and the Republic of Benin on the west. Nigeria is the most populous country in Africa and the 14th largest in land mass. The country's 2006 Population and Housing Census placed the country's population at 140,431,790 (DHS 2013).



Source: DHS, 2013
 Figure 1: Map of Nigeria showing the six Geo-Political Zones

Source of Data

The study made use of secondary data. The data used was Nigeria Demographic and health surveys (DHS) data set 2013. The 2013 Nigeria Demographic and Health survey (NDHS) was implemented by the National Population Commission. It is the fifth in the series of Demographic and Health surveys conducted so far in Nigeria.

Sampling Procedure

The sample for the 2013 NDHS was nationally representative and covered the entire population residing in non-institutional dwelling units in the country. The sample was stratified and was independently selected from the sample frame. The sample was designed to provide population and health indicator estimates at the national, zonal and state levels. The 2013 NDHS sample was selected using a stratified three-stage cluster design. 15,545 women were interviewed in the urban area while 23,403 were interviewed in the rural area. The data used for this study was based on the information available on women on NDHS 2013.

Data Extraction

The analysis for this study focussed only on married women or women living with a male partner of reproductive age (15–49 years) who had a live birth within 5 years before the survey. The NDHS data was sorted and cleaned for the purpose of this study. Information on maternal autonomy which is the main crux of this study was only available for 10193 women. Thus this study used the information that was available.

Analytical Tools

The analytical tools used for the study are descriptive statistics, composite score analysis and logit model.

Descriptive statistics

The data collected on socioeconomic characteristics and maternal health care service utilization in Nigeria was analysed using descriptive statistics. The data analysed were age of women, educational status of women, occupation of household head, educational status of household head, wealth index, and type of residence. The descriptive statistical tools used include percentages, table, histogram and frequency distribution.

Composite Score Analysis

The data collected on the level of maternal women’s autonomy in Nigeria were analysed using composite score analysis. Four main questions that have to do with decision making involving women were used to capture women’s autonomy. The questions are:

- Who usually makes decision about health care?
- Who usually makes decisions about household purchases?
- Who makes decisions about visits to relatives?
- Who makes decisions on how partners’ earnings will be used?

Responses to all these questions were measured using the following: respondent alone, respondent and husband alone, respondent and other person, husband alone and someone else. The categorisation into high, intermediate and low level of autonomy was achieved using composite score analysis as used by Pooja *et al.*,(2019). A binary scale of 1=yes, 0=no, was used to rate the respondents. A woman’s autonomy can score a maximum of 4 and minimum of 0.

High autonomy= between 4 points to (mean + standard deviation) points

Intermediate autonomy= between upper and lower categories

Low autonomy= between (mean – standard deviation) points to 0 points.

Logit model

The data collected on the effects of maternal autonomy of the utilization of health care services were analysed using Logit model. It is a uni/multivariate technique

which allows for estimating the probability that an event will occur or not through prediction of a binary dependent outcome from a set of independent variables (Roopa, 2000). The logit model gives parameter estimates which are asymptotically efficient, consistent, normal and the model is known to produce results that are statistically sound (Gujarati and Porter, 2009).

$$Z_i = \log \frac{P_i}{1-P_i} = b_0 + \sum \beta_k X_{ij} + \epsilon_i$$

$\log p_i / 1 - p_i$ = the log-odds of the logit model

P_i = probability that a woman utilize health care services

$1 - p_i$ = probability that a woman does not utilize health care services

$$E(Y/X) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \dots + \beta_k X_k + \epsilon_i$$

Dependent variable Y= Health care utilization

Indicators of maternal health care utilization are:

Number of antenatal care visits= (< 4 visits= 1, ≥4 visits=0)

Attendants during antenatal care visits= (unskilled=1, skilled= 0)

Assistance during delivery= (unskilled=1, skilled=0)

Place of delivery= (Home=1, health facility=0)

Independent /explanatory variables (X_s) are the indicators of women autonomy which are:

- X_1 =Decision about respondent’s health care (respondent alone=1, otherwise=0),
- X_2 =Decision on major household purchases (respondent alone=1, otherwise=0),
- X_3 =Wealth index (poor=1, otherwise=0),
- X_4 =Decision about visits to family/relative (respondent alone=1, otherwise=0),
- X_5 =Decision on how earnings will be used (respondent alone=1, otherwise=0),
- X_6 = Age of women
- X_7 = Years of formal education

Ethical permission

No ethical permission gotten because secondary data was used for this study and not primary data where ethical permission is needed.

Limitations of the study

The limitation experienced in this study is as a result of missing responses on women’s autonomy compared to other information in NDHS data.

RESULTS

Table 1: Distribution of women according to their socio economic characteristics

Variable	North central	North East	North West	South East	South- South	Total
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Age							
15-24	6.17	7.42	7.22	2.35	2.76	3.87	29.79
25-34	9.29	7.92	7.41	6.18	6.49	11.48	48.77
35-49	3.8	3.43	3.25	3.11	3.1	4.76	21.45
Household size							
1-5persons	8.9	6.73	5.01	5.99	6.12	12.82	45.57
6-10persons	8.67	7.91	6.06	8.12	5.6	6.89	43.25
> 10persons	1.68	4.13	0.57	3.76	0.63	0.39	11.16
Respondents educational status							
No formal	5.62	10.1	10.76	0.47	0.53	1.55	29.03
Primary	5.11	3.97	3.53	3.17	3.57	5	24.65
Secondary	6.14	3.75	3.11	6.47	6.79	10.03	36.29
Tertiary	2.38	0.94	0.47	1.54	1.46	3.52	10.31

Source: Author's computations, 2013 DHS * Figures in Percentages

Table 2: Distribution of level of maternal autonomy across geopolitical zones

Level of maternal autonomy	North Central	North East	North West	South-South	South West	south East	Total
Low	10.08	13.99	15.46	4.55	5.41	5.12	54.61
Intermediate	8.81	4.71	2.26	7.46	13.44	6.25	42.92
High	0.37	0.06	0.12	0.34	1.26	0.27	2.46
Total	19.26	18.77	17.88	12.35	20.10	11.65	100

Source: Author's computations, 2013 DHS * Figures in Percentages

Table 3 Distribution of respondents' attendance during delivery across geopolitical zones

Maternal Health Care Utilization	NC	NE	NW	SE	SS	SW	ALL
Type of health attendant							
Nurse	14.34	11.13	11.17	8.91	9.15	16.23	73.4
Auxiliary midwife	0.61	2.4	0.7	0.65	0.48	1.74	6.57
Doctor	9.07	3.15	3.53	4.91	5.32	13.12	39.1
Community extension worker	1.67	3.59	1.33	0.2	0.61	0.49	7.89
Traditional birth worker	0.14	0	0	0.33	0.44	0.92	1.83
community village worker	0.22	0.14	0.01	0.13	0.14	0.14	0.77

Source: Author's computation, DHS 2013 * Figures in Percentages

Table 4 Distribution by number of antenatal care visits

Antenatal care visits	NC	NE	NW	SE	SS	SW	TOTAL
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Greater than 4 visits (≥4)	15.8	13.28	12.47	10.74	10.76	19.29	82.35
Less than 4 visits (<4)	3.45	5.48	5.41	0.9	1.59	0.81	17.65

Source: Author's computations, 2013 DHS data * Figures in Percentages

Table 5: Distribution according to place of delivery across Geopolitical zones

Place of delivery	North Central	North East	North West	South East	South- South	South West	Total
Respondent's home	6.57	12.37	13.30	1.01	1.42	1.68	36.36
Other home	0.30	0.15	0.12	1.08	3.22	2.31	7.17
Government hospital	5.58	3.55	3.62	1.91	2.93	4.29	21.89
Government health centre	2.41	1.71	0.65	2.23	2.79	4.20	13.98
Government health post	0.36	0.56	0.04	0.02	0.04	0.06	1.08
Other public sector	0.00	0.02	0.00	0.01	0.00	0.00	0.03
Private hospital/clinic	3.89	0.36	0.15	5.18	1.85	7.51	18.94
Other private medical	0.13	0.05	0.00	0.21	0.10	0.07	0.55
Total	19.26	18.77	17.88	11.65	12.35	20.10	100

Source: Author's computation, 2013 DHS data * Figures in Percentages

Table 6 Distribution of respondents according to assistance during delivery

Type of health assistance	NC	NE	NW	SE	SS	SW	ALL
Nurse	3.21	1	1.38	1.73	1.96	6.02	15.3
Auxiliary midwife	10.54	4.56	3.81	8.39	6.99	14.25	48.53
Doctor	0.69	0.8	0.14	0.99	0.66	2.74	6.01
Community extension worker	1.83	1.49	0.27	0.22	0.6	0.39	4.81
Traditional birth worker	0.7	2.4	4.39	0.85	2.97	1.5	12.81
Community village health worker	4.07	7.3	4.56	0.52	0.86	1.04	18.36
No health assistance	0.02	0.02	0.01	0.00	0.00	0.00	0.05

Source: Author's computations, 2013 DHS * Figures in Percentages

Table 7 Distribution by Women's Check-up after Delivery

Check-up after delivery	North Central	North East	North West	South East	South- South	South West	Total
No	5.97	10.21	11.90	3.91	3.10	4.13	39.23
Yes	13.28	8.58	5.97	7.73	9.25	15.97	60.77
Total	19.26	18.77	17.88	11.65	12.35	20.10	100

Source: Author's computations, DHS 2013 * Figures in Percentages

Table 8: Effects of maternal autonomy on health care utilization of women in Nigeria

Maternal health care utilization	Coefficient	Z	P>z	Standard Error	Marginal effects
Explanatory variables					
Decision on health by respondents alone	-1.9833*	-1.66	0.097	1.1935	-0.0886
Decision on health by respondents and partner	-2.0421*	-1.73	0.084	1.1837	-0.0913
Decision on health by husband alone	-1.7132	-1.45	0.148	1.1829	-0.0766
Decision on visits to relatives by respondent alone	1.163	1.58	0.115	0.7373	0.0519
Decision on visit to relatives by respondent and partner	1.3484*	1.85	0.064	0.7275	0.0603
Decision on visits to relatives by husband alone	1.5818**	2.17	0.03	0.7295	0.0707
Sector					
Rural	-0.4237***	-4.04	0.000	0.1049	-0.0184
Years of formal education	-0.019	-1.3	0.195	0.0147	-0.0009
Household size	0.2998*	1.92	0.055	0.0157	0.0013
Respondents' occupation					
Services	0.31718*	1.94	0.052	0.1633	0.0109
Traders	-0.2859**	-2.16	0.031	0.1326	-0.0129
Farming	-0.5187***	-3.27	0.001	0.1585	-0.026
Visit to health facilities	0.2247**	2.43	0.015	0.0925	0.0041
_cons	3.6514***	3.52	0.000	1.0374	
Chi-square	105.01				
Probability	0.000				
Pseudo R-square	0.0280				

Source: Author's computations, 2013 DHS data

DISCUSSION

Basic socio-economic characteristics of respondents

Age of respondents across geopolitical zones

The table 4.1 above shows that 29.79% of the women across geopolitical zones fall within the age category 15-24 years, 48.77% are within 25-34 years while 21.45% are within 34-49 years. The minimum age is 15 years while the maximum age is 49 years. The overall mean age is 30 with a standard deviation of 6.95. The highest mean age is found in South West while the lowest is found in South East. Majority of the women in South West fall within the productive age 25-34.

Age of respondents according to place of residence

Figure 2 showed the age of respondents across sectors. In the rural area, 18.73% of the women fall within the age 15-24 years, 24.13% fall within 25-34 years, 10.86% fall within 35-49 years. Majority of the women in rural area fall within the age 25-34. This is in accordance with the result obtained in a study carried out by Adeoti and Akinwande (2013). In the urban area, 11.06% fall within age 15-24 years, 24.63% fall within the age 25-34 years while 10.59% fall in the age 35-49 years. The result below shows that most of the maternal women in the urban and rural area fall within the productive age 25-34 years.

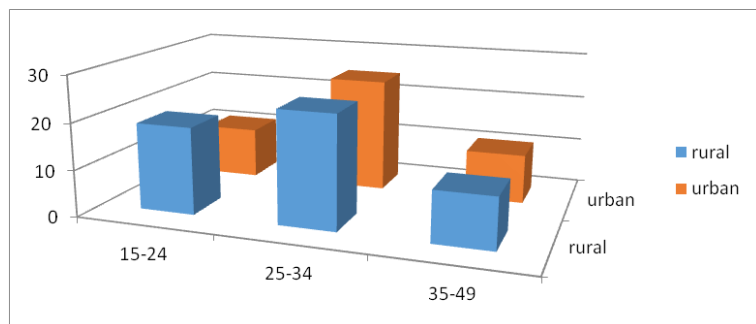


Figure 2: Distribution of age group according to place of residence.
Source: Author's computation, 2013 DHS data *figures in percentage

Household size across geopolitical zones

From the table 1 above, 45.57% of the women across geopolitical zones are members of households with 1-5 persons, 43.25% have 6-10 members and 11.16% have greater than 10 persons in their household. Majority of the respondents are found in household size with 1-5 persons. In the south west region, most of the women are found in household size of 1-5 persons. In North East, North West and South East, majority of the respondents have household size of 6-10 persons while north central, South South, and South West have majority of their respondents in household size of 1-5 persons.

Educational status of women across geopolitical zones

The table 1 above shows the distribution of women according to their educational status across the six geopolitical zones. 29.03% of the women have no formal education, 24.35% have primary education, 36.29% have secondary education and 10.31% have tertiary education. Majority of the women in north central, south east and south west have secondary education while majority of women in north east, north-west have no formal education. This study (using 2013 DHS) shows that the highest percentage of women with tertiary education is found in the South West and the lowest is found in the North West.

Level of Maternal Women Autonomy across geopolitical zones and place of residence

This section shows the level of maternal women autonomy across geopolitical zones and sector. Maternal autonomy level is grouped into low, intermediate and high. From the results in the table 2 above, 54.61% of the maternal women have low level of autonomy, 42.92% of the respondents have intermediate autonomy and 2.46% of the respondents have high autonomy. Majority of the respondents have low autonomy. Also, most of the respondents in North Central (NC), North East(NE) and North West(NW) have low maternal autonomy while majority of the respondents in South-South (SS), South West(SW) and

South East(SE) have intermediate maternal autonomy. This shows that women in the North region are worse of in relation to autonomy on how they spend money, their health and even visit to relatives. Their level of autonomy is lower in relation to those in the south. This is in line with Adeoti and Akinwande (2013); Oladokun et al., (2018) findings, which shows that women in the northern region have lower autonomy when compared to those in the southern region.

Level of maternal women autonomy according to place of residence

In urban sector described in the figure 5 below, 21.04% have low level of maternal autonomy, 23.62% have intermediate level of maternal autonomy and 1.61% of the respondents have high autonomy while in the rural sector, 33.57% have high maternal autonomy, 19.30% have intermediate autonomy while 0.85% have high maternal autonomy. From the result, it is observed that women in rural sector have lower maternal autonomy as compared with women in urban area. This could be as a result of lack of formal education of the women. Majority of the women in urban sector have intermediate level of maternal autonomy and most of the women in rural sector have low level of maternal autonomy.

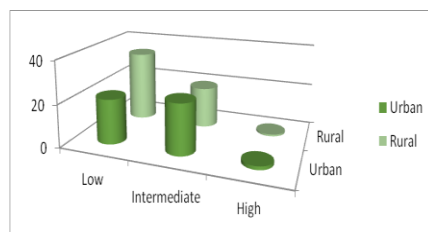


Figure 5: Distribution of level of maternal autonomy according to place of residence
Source: Author's computation, 2013 DHS data

Maternal Health Care Service Utilization across geopolitical zones and place of residence
Attendance during delivery across geopolitical

zones

The table 3 above explains the type of attendants that attended to the maternal women before and during child birth across geopolitical zones. 73.4% of the women were attended to by a nurse, 6.57% by auxiliary midwife, 39.1% by a medical doctor, 7.89% by a community extension worker, 1.83% by a traditional birth worker and 0.77% by a community village worker. From the results, majority of the women across the geopolitical zones were attended to by a nurse. None of the women in the North East and North West were attended to by a traditional birth worker. South West has higher percentage of women that were attended to by a medical doctor.

women were attended to by a nurse, 2.61% by an auxiliary midwife, 23.58% by a medical doctor, 1.35% by a community extension worker, 1.02% by a traditional birth worker, 0.18% by a community village health worker while in the rural sector, 36.53% were attended to by a nurse, 3.96% by an auxiliary midwife, 15.52% by a doctor, 6.53% by a community extension worker, 0.81% by a traditional birth worker and 0.59% by a community village health worker. From the result, more of the women in the urban sector were attended to by doctors as compared to those in the rural sector. Also, more of the women in rural sector were attended to by community extension worker, auxiliary midwife and community village worker as compared to the urban sector. Majority of the women in the rural area were attended to by a nurse. This finding is in accordance to the result obtained in a study carried out by Oladokun 2018; Adeoti and Akinwande (2013).

Attendance during delivery according to place of residence

Figure 6 reveals that in the urban sector, 36.86% of the

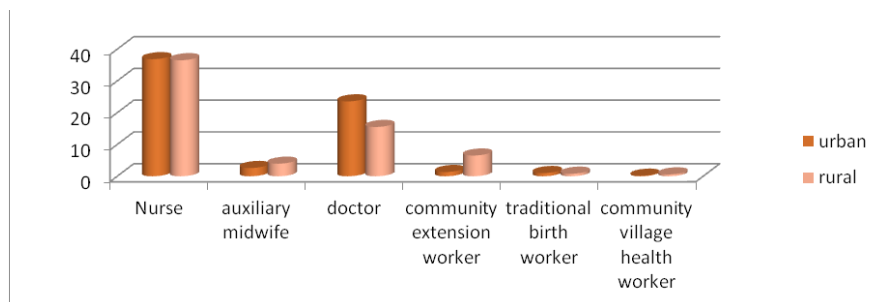


Figure 6: Distribution of attendance during delivery according to place of residence
Source: Author's computations, 2013 DHS data *figures in percentages

Antenatal Care visits across geopolitical zones

As shown in the table 4 above, 82.35% of the respondents had greater than four visits to the antenatal care while 17.65% of the respondents had less than four visits to the clinic for antenatal care. This indicates that majority of the respondents had greater than four visits to the antenatal care. South-West has the highest percentage of women who had greater than four (4) antenatal care visits while North Central has the lowest. North Central has the highest percentage of women who had less than 4 antenatal care visits while South East has the lowest. The result shows that most of the women had antenatal care visits.

respondents in the urban sector. It is also shown that more than half of the respondents received antenatal care during pregnancy. This finding is similar to one of the recent research of one of the recent studies conducted in Ethiopia which showed that more than half of the respondents received antenatal care during pregnancy (Tsegay et al., 2013).

Antenatal Care visits according to place of residence

Figure 7 below shows that in the urban sector, 41.14% of the women went for antenatal visits more than four times and 5.14% went for antenatal less than four times while in the rural sector, 41.21% of the women went for antenatal more than four times and 12.51% went for antenatal less than four times. The result shows that more of the women in the rural area went for antenatal clinic less than four times as compared to the

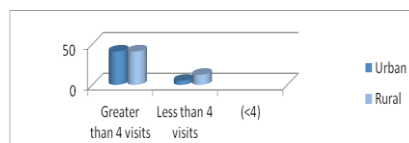


Figure 7: Distribution of antenatal care visits according to place of residence
Source: Author's computations, 2013 DHS data *figures in percentages

Place of Delivery across geopolitical zones

Shown in the table 5 above is the distribution of respondents according to their place of delivery. It shows that 36.36% delivered in their homes, 7.17% delivered in other people's home, 21.89% delivered in government hospital, 13.98% delivered in government

health centre, 1.08% delivered in government health post, 0.20% delivered in other public sector, 18.94% delivered in private hospital and 0.55% delivered in other private medical sector. From the result, most of the respondents in the North Central, North East, North West, had their delivery in the home while majority of the respondents in the South East and South West had their delivery in private hospital.

Place of Delivery according to place of residence

Figure 8 shows that, in the urban sector, 9.42% of the respondents had their delivery in the home, 3.23% at other homes, 13.11% in government hospitals, 6.64%

in government health centre, 0.34% in government health post, 0.01% in other public sector, 13.3% in private hospital, 0.23% in other private hospital while in the rural area, 26.94% of the respondents had their delivery in the respondent's home, 4.11% at other homes, 8.78% at government hospital, 7.34% at government health centre, 0.74% at government health post, 0.02% at other public sector, 5.64% at private hospital, 0.16% at other private hospital. A related study carried out by [unintelligible] showed that respondents who reside in urban areas utilized institutional place of delivery more than those in the rural areas.

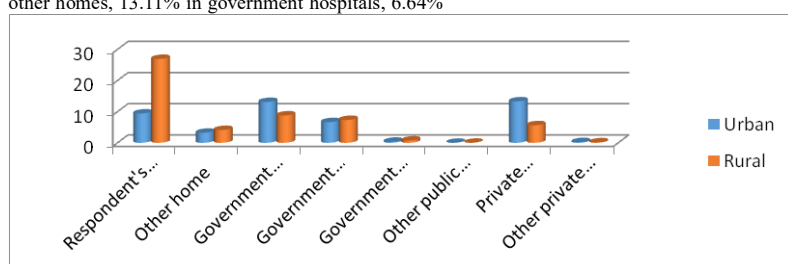


Figure 8: Distribution of place of delivery according to place of residence
Source: Author's computations, 2013 DHS data

Assistance during delivery across geopolitical zones

The table 6 above explains the type of health assistants that attended to the maternal women. It shows that 15.30% of respondents were assisted by a nurse, 48.53% were assisted by auxiliary midwife, 6.01% were assisted by a doctor, 4.81% were assisted by community health workers, 12.81% were assisted by traditional birth workers, 18.36% were assisted by community village health workers and 0.05% had no health assistance at all.

respondents went for check-up after delivery while 39.23% did not go for check-up. Majority of the respondents in the North Central, South East, South South and South West went for check-up after delivery, while most of the respondents in the North East and North West did not go for check-up after delivery.

Type of Health Assistance according to place of residence

The figure 9 below shows that in the urban sector, 9.94% of the maternal women were assisted by nurse, 30.19% by an auxiliary midwife, 3.46% by a doctor, 1.09 by a community extension worker, 3.98% by a traditional birth worker, 4.5% by a community village health worker and 0% had no assistance while in the rural sector, 5.37% of the respondents were assisted by a nurse, 18.35% by an auxiliary midwife, 2.55% by a doctor, 3.72% by a community extension worker, 8.83% by a traditional birth worker, 13.85% by a community village health worker and 0.05% had no assistance. From the result, majority of the respondents in the urban area were assisted by an auxiliary midwife as compared to those in the rural sector.

Women's check-up delivery according to place of residence

The figure 10 below shows that in the urban sector, 13.14% of the respondents did not go for check-up after delivery and 33.14% went for check-up while in the rural sector, 26.10% of the respondents did not go for check-up and 27.63% went for check-up after delivery. The highest number of respondents that went for check-up after delivery are the ones that reside in the urban sector as compared to the maternal women in the rural sector.

Respondent's Check-up after Delivery across geopolitical zones

Shown in the table 7 above is the distribution of respondents according to their check up after delivery across geopolitical zones. It shows that 60.77% of the

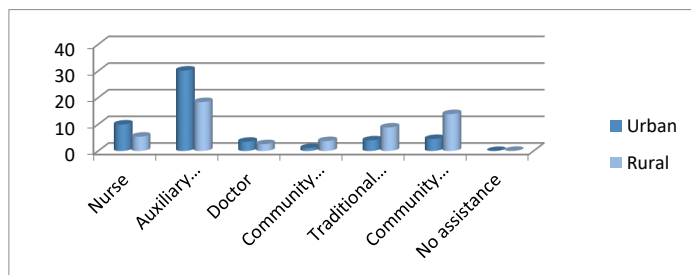


Figure 9: Distribution of health assistance according to place of residence
Source: Author's computations, 2013 DHS data

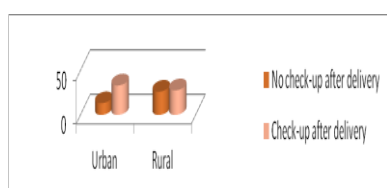


Figure 10: Distribution of respondents according to place of residence
Source: Author's computations, 2013 DHS data

Effect of Maternal Autonomy on Maternal health care utilization

Indicated in the table below is the parameters estimated for the logistic regression model for effects of maternal autonomy of health care utilization of maternal women in Nigeria. The result of the logit model reveals that the log likelihood is -1822.5248, pseudo R² is 0.0280 and Chi-square value was 105.01. Thirteen variables were considered in the model, nine variables were significant. The significant variables are decision on health, decision on visits to relatives, sector, household size, respondent's occupation and visit to health facilities.

Decision on health by respondents alone

Decision on health by respondents alone was significant at 10% and negatively related to maternal health care utilization. This shows that a unit increase in decision on health related issues by respondent alone leads to a decrease in the maternal health care utilization. For women who are involved in making decisions regarding their health alone, the probability of the women utilizing maternal health care services decreases by 8.86%. This result is in contrast with the findings Hou and Ma (2013) in a related study carried out in Pakistan which shows that there is positive association between women's decision making power and maternal health care utilization.

Decision on health by respondent and husband

Decision on health by respondent and husband was significant at 10% and negatively related to health care

utilization. A unit increase in decision on health related issues by respondents and partners lead to a decrease in the maternal health care utilization. For women who make decisions on health related issues with their husbands, the likelihood of the women utilizing maternal health care services reduces by 9.13%. This could be as a result of lack of formal education of the husband and can also be a function of the inadequate knowledge the husband has on maternal health care and maternal mortality.

Decision on visits to relatives by respondent and husband

Decision on visits to relatives by respondents and husband was significant at 10% and positively related to maternal health care utilization. A unit increase in decision by respondents and partners on visit to relatives leads to an increase in the maternal health care utilization. This finding is related with the research carried out by Baral *et al* (2010), which shows that maternal health care utilisation is influenced by maternal autonomy. Respondents who make decisions regarding their visits to relatives with their husband have 6.03% likelihood of utilizing maternal health care services.

Decision on visits to relatives by husbands alone

Decision on visits to relatives by husband alone was significant at 10% and positively related to maternal health care utilization. A unit increase in decision on visits to relatives by husbands alone leads to an increase in their maternal health care utilization. For women whose husbands make decisions alone regarding visit to relatives have 1.5818 likelihood of utilizing maternal health care services. This study is in relation to a study on the relationship between maternal autonomy and health care utilisation carried out by Bloom *et al* (2000), which shows that freedom of movement was significantly associated with the use of maternal health care services by women.

Place of residence

Rural sector was significant at 1% and negatively related to maternal health care utilization as compared

urban sector. This implies that respondents who reside in rural areas are less likely to utilize maternal health care services. The respondents in rural sector have 0.4237 probability of not utilizing maternal health care services. This could be as a result of lack of knowledge of importance of maternal health care services. It could also be due to inadequate functional medical centres in the rural sector when compared with those in the urban sector.

Household size

Household size was significant at 10% and positively related to maternal health care utilization. This implies that a unit increase in household size leads to increase in the respondent's maternal health care utilization. The likelihood of women who have increased household size to utilize maternal health care services is increased by 0.2998. The marginal effect is 0.13%.

Occupation of women

Service was significant at 5% and a unit increase in service leads to increase in maternal health care utilization. This means respondent who engaged in services utilized maternal health care more than respondents who are not working. Respondents who engage in services as occupation are more educated and enlightened. They know the usefulness of seeking medical attention during pregnancy to reduce mortality. For respondents who are engaged in services, the likelihood of utilizing maternal health care services increases by 0.31718.

Agriculture as an occupation is significant at 1% and negatively related to maternal health care utilization. This means a unit increase in respondents who engaged in farming leads to a decrease in maternal health care utilization. This could be as a result of inadequate enlightenment of farmers on the need to seek medical attention during pregnancy and for child delivery to prevent mortality. Also, farmers engage in farming and find it difficult to use health services at the appropriate hours unless services open for 24 hours when they are back from the farm. For respondents

who are farmers, the likelihood of utilizing maternal health care services reduces by 2.6%.

Trading was significant at 1% and negatively related to maternal health care services. A unit increase in respondents who engage in trading as occupation leads to a decrease in respondent's maternal health care utilization. The likelihood of the respondents utilizing maternal health care services reduces by 0.2859.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

This study provides information on the effects of maternal autonomy on health care utilization of women in Nigeria.

Women in rural Nigeria have lower maternal autonomy as compared with their counterparts in urban Nigeria.

For health care utilization during antenatal care visits, 82.35% of the respondents had greater than four visits to the antenatal care while 17.65% of the respondents had less than four visits to the clinic for antenatal care. Respondents who make decisions alone, those whose husbands make all the decisions, and those who make decisions with their husbands make lesser use of maternal health care services.

Policy Recommendation

The following policies were recommended based on the findings from this study.

National and international agencies including Non-governmental organisations, should engage in enlightenment programs for communities on the need for women to utilize maternal health care services.

Autonomy contributes much to the utilization of health care services by maternal women. Therefore, efforts should be made by governmental organisations, non-governmental organizations and other institutions to give women more chance and opportunities to be a part of decision-making especially on issues relating to their health. Seminars, trainings, workshops, and the likes should be organised for women in the rural sector to enlighten them on the need to utilize maternal health care services.

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WIS-BPH 21FL

DIETARY INTAKE AND BLOOD PRESSURE OF ADULTS IN RURAL COMMUNITIES OF ABIA STATE, NIGERIA

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ABSTRACT

Hypertension is a growing public health problem and diet has been identified as a major modifiable risk factor. The aim of this study was to assess the relationship between dietary intake and blood pressure of adults. The study was a descriptive cross-sectional survey of 429 adults (≥ 20 years) randomly selected from rural communities in Ikwuano LGA of Abia State. A structured questionnaire was used to assess sociodemographic characteristics. Overweight and obesity were determined from anthropometric measurements, dietary intake was assessed by 24 hour recall and blood pressure was measured and defined following standard procedures. Pearson correlation coefficient was used to assess the association between dietary intake and blood pressure levels. The overall prevalence of hypertension and obesity were 26.8% and 11%, respectively. There was a positive correlation between systolic pressure and sodium intake ($r = 0.112$, $p = 0.021$) ($p < 0.05$). Vitamin C intake was negatively correlated with systolic pressure ($r = -0.139$, $p = 0.004$) ($p < 0.05$) and diastolic pressure ($r = -0.100$, $p = 0.038$) ($p < 0.05$). The result has significant clinical and public health importance suggesting that diets rich in Vitamin C and low in sodium could contribute to prevention of hypertension in adults. Therefore, educational campaigns regarding dietary approaches to solving hypertension should be encouraged.

Keywords: Adults, Hypertension, Nutrient intake, Rural.

INTRODUCTION

High blood pressure is a problem worldwide, the single most important cause of death and second major cause of disability next to childhood malnutrition, and so remains a major global health burden (1, 2). Hypertension is a growing public health problem and diet has been identified as a major modifiable risk factor. Cardiovascular diseases are complications from high blood pressure resulting in about 7.5 million deaths and 57 million disability-adjusted life years (DALYs) worldwide, both accounting for about 12.8 and 3.7% of global deaths and DALYs, respectively (3, 4). Nigeria, currently with a population of over 160 million, is the most populous African country (5), and the prevalence of hypertension in the country hugely contributes to the overall burden in Africa (6). Okubadejo *et al.* (7) in 2019 estimated hypertension prevalence of 55% in Nigeria. Hypertension is a chronic disease which has been reported to be common mostly among elderly and young adults (1).

Nutrition is an important factor in health and disease (8), as such, the incidence and severity of hypertension are affected by nutritional status and dietary intake (9). Studies have also shown that excessive weight gain, salt intake, smoking of cigarettes, drinking of alcohol and physical inactivity are the major predisposing factors for hypertension (10, 11, 12, 13). It is therefore evident that dietary intake, dietary pattern and lifestyle of an individual are important elements in the prevention, management and treatment of hypertension (13, 12). Nevertheless, few studies on the

relationship between dietary intake and blood pressure in Africa, especially in Nigeria are available, thus creating an insight in this area is of great importance.

MATERIALS AND METHODS

Study population

Data used in this study was from a cross-sectional survey conducted in two rural communities (Amaba and Amawom) in Abia State, Nigeria. A total of 429 respondents were selected using multistage sampling. The study was approved by the Ethics Committee of the Federal Medical Centre, Umuahia. Oral informed consent was obtained from the adults before participating in the study.

Data collection

A validated questionnaire was used to obtain information on socio-demographics, anthropometric (height and weight) and blood pressure measurements as well as dietary intake. The questionnaire was pre-tested in another community, not included in the final analysis. Trained research assistants administered the questionnaire and carried out anthropometric measurements.

Socio-demographic characteristics

A standardized questionnaire was used to obtain information on age, gender, marital status, educational levels, monthly allowance and occupation.

Anthropometry measures

Height was measured with a portable calibrated measuring rod and recorded to the nearest 0.1 cm. Body weight was measured using a calibrated portable scale (Model HN-289-E) with a maximum capacity of 120 kg. The participants removed their shoes and heavy clothing prior to weighing, and weight was taken to the nearest 0.1 kg (14). Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters and used as proxy measure for overweight and/or obesity (14).

Blood pressure measurements

Blood pressure of the respondents were taken using an Andron digital automatic blood pressure sphygmomanometer (KD-595). Three blood pressure measurements were taken on the left arm of the subject in a seated position at 5-minute intervals between readings. The cuff was wrapped around the arm, after which the push button was pressed to take the readings. Blood pressure readings displayed on the electronic device were recorded in millimetres of mercury (mmHg) for systolic BP (SBP) and diastolic BP (DBP). The initial first reading was discarded and the mean of the last two readings were recoded for each participant. The values of SBP and DBP were related to the reference standard and used to classify participants as having normal (120/80mmHg) or high blood pressure ($\geq 130/89$) (15).

Dietary intake data

Twenty-four (24) hour recall was used to collect information on nutrient intake. Participants were asked to recall all the foods and drinks they had consumed in the past 24 hours prior to data collection. This was done by asking the participants to list all the foods which they consumed for breakfast, lunch dinner, in-between meals and bedtime snack alongside the quantity eaten. The quantities, were estimated by showing the respondents household equipment such as cups, slices, serving spoons, models etc. (16). Nutrient intake was analysed using Nutri-survey software (17).

Statistical analysis

Data entry, cleaning and statistical analysis were performed using SPSS version 20. Descriptive statistics were performed to characterize the participants. while Pearson correlation analysis was used to establish association between dietary intake and blood pressure. A p-value of ≤ 0.05 was regarded as statistically significant.

RESULTS

Table 1 shows the characteristics and dietary intakes of participants. There was a significant difference in the sex ($p=0.000$), education ($p=0.001$), income ($p=0.002$) and marital status ($p=0.000$) of participants.

Table 2 shows the anthropometric status and blood pressure classification of the participants. About 47% of the population had normal blood pressure (120/80mmHg), while 15.4% had stage 1 hypertension or mild hypertension (130-139/90-99mmHg) and 11.4% were morbidly hypertensive or had stage 2 hypertension ($\geq 140/100$ mmHg). The prevalence of overweight and obesity were 17% and 11%, respectively.

Table 3 shows the nutrient intake of the participants compared to Recommended Nutrient Intake (RNI). The mean energy intake in kilocalories was significant between males and females ($p = 0.038$). In addition, the result showed the respondents consumed below the recommended nutrient intakes for all the nutrients for adults (FAO/WHO/UNU, 2004), except for zinc and Vitamin C.

Table 4 shows the relationship between nutrient intake and blood pressure. It was observed that there was a significant positive correlation between systolic pressure and sodium ($r = 0.112, p =0.021$) ($p<0.05$), and vitamin C had significant negative correlation with systolic pressure ($r = -0.139, p = 0.004$) and diastolic pressure ($r = -0.100, p = 0.038$) ($p<0.05$).

Table 1: Characteristics of study population

Characteristics	All	Males	Females	X ²	p-value
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PROCEEDING OF THE 5TH BIENNIAL CONFERENCE OF THE OWSD NIGERIA, UMUDIKE 2020

	n (%)	n (%)	n (%)		
Sex	439 (100)	227 (52.9)	202 (47.1)		0.000*
Age range					
20-30 years	254(59.2)	138(60.8)	116(57.4)		
31-40 years	94(21.9)	50(22)	44(21.8)		
41-50 years	28(6.5)	14(6.2)	14(6.9)	1.04	0.904
51-60 years	24(5.6)	11(4.8)	13(6.4)		
61 and above	29(6.8)	14(6.2)	15(7.4)		
Religion					
Christianity	423(98.6)	224(98.7)	199(98.5)	0.02	0.886
Traditional	6(1.4)	3(1.3)	3(1.5)		
Marital status					
Single	248(57.8)	154(67.8)	94(46.5)	19.90	0.000*
Married	181(42.2)	73(32.2)	108(53.5)		
Household size					
1-3	137(31.9)	81(35.7)	56(27.7)		
4-6	190(44.3)	99(43.6)	91(45)	4.88	0.181
7-9	82(19.1)	36(15.9)	46(22.8)		
10 and above	20(4.7)	11(4.8)	9(4.5)		
Highest educational level					
No Formal Education	15(3.5)	4(1.8)	11(5.4)		
Primary Education	23(5.4)	12(5.3)	11(5.4)	16.20	0.001*
Secondary Education	189(44.1)	85(37.4)	104(51.5)		
Tertiary Education	202(47.1)	126(55.5)	76(37.6)		
Occupation					
Trader	114(26.6)	40(17.6)	74(36.6)		
Farmer	15(3.5)	7(3.1)	8(4)		
Artisan	149(34.7)	92(40.5)	57(28.2)	21.68	0.000*
Civil Servant	34(7.9)	18(7.9)	16(7.9)		
Unemployed	117(27.3)	70(30.8)	47(23.3)		
Monthly income (Naira)					
Less than 30,000	281(65.5)	133(58.6)	148(73.3)		
30,000-50,000	108(25.2)	63(27.8)	45(22.3)		
51,000-70,000	18(4.2)	16(7)	2(1)	16.76	0.002*
71,000-100,000	9(2.1)	7(3.1)	2(1)		
100,000 and above	13(3)	8(3.5)	5(2.5)		

Table 2: Anthropometric status and blood pressure classification of the participants

Variables	Frequency (n)	Percentage (%)
Blood pressure		
Normal	210	49.0
Pre-hypertension	104	24.2
Stage 1 hypertension	66	15.4
Stage 2 hypertension	49	11.4
Total	429	100
Systolic mean ± SD	121.26 ± 19.87	
Diastolic mean ± SD	77.33 ± 12.81	
BMI		
Underweight	17	4.0
Normal weight	292	68.1
Overweight	73	17.0
Obesity	47	11.0
Total	429	100
Height mean ± SD	1.65 ± 0.09	
Weight mean ± SD	64.88 ± 11.08	

Table 3: Nutrient intake and adequacy of the participants

Nutrients	RNI		Male Mean ± SD	Female Mean ± SD	Adequate intake	P-value
	Male	Female				
Energy (Kcal)	1950-2859	1800-2150	1581.22 ± 657.61	1457.13 ± 575.65	153(35.7)	0.038*
Carbohydrate (g/day)	55-75	55-75	272.78 ± 134.04	251.03 ± 110.86	103(24)	0.070 ^{NS}
Protein (g/day)	58-62	50-53	47.30 ± 21.18	44.36 ± 19.04	120(28)	0.133 ^{NS}
Fat (g/day)	56-75	49-63	30.17 ± 18.60	27.37 ± 19.64	52(12.1)	0.129 ^{NS}
Vitamin C (mg/day)	45	45	52.97 ± 69.47	49.57 ± 65.26	26(6.1)	0.603 ^{NS}
Sodium (mg/day)	1200-1500	1200-1500	1202.12 ± 773.64	1156.30 ± 943.51	93(21.7)	0.581 ^{NS}
Potassium (mg/day)	4700	4700	1037.16 ± 839.75	1015.09 ± 637.09	7(1.6)	0.762 ^{NS}
Calcium (mg/day)	1000-1300	1000-1300	310.22 ± 447.34	278.95 ± 272.66	12(2.8)	0.390 ^{NS}
Magnesium (mg/day)	400-420	310-420	230.06 ± 247.45	234.33 ± 281.19	24(5.6)	0.867 ^{NS}
Iron (mg/day)	27	23-59	13.35 ± 8.66	12.46 ± 7.49	26(6.1)	0.255 ^{NS}
Zinc (mg/day)	6.2-6.6	4.3-4.7	12.83 ± 8.09	12.31 ± 7.84	33(7.7)	0.504 ^{NS}

* = Significant NS = Not significant, RNI=WHO/FAO (2004)

	SBP	DBP	energy	protein	fat	CHO	Vitamin	Na	K	Ca	Mg	Fe	Zn
SBP	1												
DBP	0.760**	1											
Energy	0.028	-0.004	1										
Protein	0.043	0.027	0.594**	1									
Fat	0.003	-0.018	0.562**	0.474**	1								
CHO	0.024	-0.001	0.938**	0.407**	0.382**	1							
Vitamin	-	-	0.162**	-0.062	0.039	0.180**	1						
C	0.139**	0.100*											
Na	0.112*	0.062	0.510**	0.226**	0.345**	0.467**	0.075	1					
K	-0.030	-0.012	0.558**	0.477**	0.218**	0.517**	0.126**	0.304**	1				

Ca	-0.006	0.007	0.338**	0.474**	0.204**	0.266**	0.130**	0.137**	0.680**	1				
Mg	-0.019	0.016	0.280**	0.342**	0.094	0.252**	0.009	-0.006	0.468**	0.509**	1			
Fe	0.043	0.017	0.604**	0.591**	0.294**	0.548**	0.027	0.290**	0.474**	0.470**	0.626**	1		
Zn	0.060	0.055	0.657**	0.591**	0.325**	0.601**	-0.072	0.333**	0.523**	0.384**	0.623**	0.825**	1	

Table 4: Relationship between nutrient intake and blood pressure

*Correlation is significant at 0.05 levels (2-tailed)

Ca = Calcium CHO = Carbohydrate Fe = Iron Zn = Zinc Mg = Magnesium

Na = Sodium K = Potassium SBP = Systolic blood pressure DBP = Diastolic blood pressure

DISCUSSION

In this cross-sectional survey, we sought to assess dietary intake of adults in Ikwuano Local Government Area of Abia State and determine whether there was an association with blood pressure. Given the paucity of data on the study population, the findings have important implications. The findings demonstrate that dietary intake of adults in rural areas have associations with blood pressure.

The high prevalence of young adults, and educational levels could be attributed to the presence of a tertiary institution (Micheal Okpara Federal University of Agriculture Umudike) in the local government area. Despite the presence of a tertiary institution in the study area, unskilled labour (artisan) and income below current minimum wage were prevalent. This could be attributed to the lack of jobs in the country and the fact that the study was carried out in rural communities (18).

As regards blood pressure, over one quarter of the respondents (26.8%) were hypertensive (stages 1 and 2), with another 24.2% having elevated blood pressure (pre-hypertension). This however, was lower than 48.5% and 52% prevalence of hypertension found among university workers in Nigeria and professional nurses in Eastern Cape, South Africa, respectively, who were hypertensive (combination of stages 1 and 2) (19, 20), but higher than 9% and 9.6% reported among hypertensive road transport workers in Ibadan (21) and hypertensive fire fighters in Rivers state, respectively (22). Result from this study was also higher than 13.0 % found among Ghanaians who were hypertensive but similar to 22% found among Ghanaians with elevated blood pressure (23). The disparities in the prevalence of hypertension might be due to differences in physical activity pattern and socioeconomic status of the different population groups studied. The hypertension prevalence in this study was consistent with the systematic review and meta-analysis by Adeloye *et al.* (24) which found hypertension among rural adults to be 26.4%. Hence, this calls for urgent public health intervention in Nigerian adults.

The difference in energy intake between male and female participants, corroborates results of the first nationwide survey evaluating food intake in Brazil which showed that men consumed 23% more calories and nutrients than women, irrespective of region or location of residence (25). In another study of urban

population from eight Latin American countries (26), men reported higher energy intake than women, independent of age and country. Gender differences in energy intake reported in our study is consistent with the well-established sex differences in energy intake due to differences in physiological composition between males and females (27).

An inverse but significant correlation was observed between systolic blood pressure and vitamin C, which is consistent with the result of a study on black and white women in USA (28). The study found an inverse association between vitamin C and systolic and diastolic blood pressure. Similarly, Juraschek *et al.* (29) in a review of randomized trials evaluating the effect of vitamin C on blood pressure, reported a significant reduction in systolic blood pressure by -3.84mmHg. Result of this study, is consistent with findings in other studies, which reported an inverse association between fruits (an abundant source of vitamin C) and hypertension (30, 31, 32). This association may be attributed to the antioxidant properties of vitamin C which protect the lining of the blood vessels from damage caused by oxidative stress and increased availability of nitric oxide, a gaseous signalling molecule that helps relax arterial walls. Thus, Angelo *et al.* (2015) reported that the higher the vitamin C status of the body, the lower the blood pressure (33). Furthermore, a positive correlation was observed between sodium intake and systolic blood pressure. This corroborates with results of Hajjar *et al.* (34) which found a strong association between sodium and systolic blood pressure. Other studies in South America and China reported that excess dietary sodium predisposes to high blood pressure and changes in sodium intake is associated in general with corresponding changes in arterial blood pressure (35, 36, 37).

The cross sectional nature of this study is a limitation as it did not permit assessment of causal relationship between dietary intake and blood pressure. There is also a possibility of recall bias using the 24-hour recall, but this was minimized with the use of research assistants in the administration of the questionnaires. Despite these limitations, the study contributes to the body of knowledge regarding dietary intake and blood pressure of adults.

Conclusion

The result suggests that diets rich in vitamin C and low in sodium could contribute to the prevention of

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hypertension in adults. Therefore, educational campaigns regarding dietary approaches to solving hypertension, weight management and control should be intensified in rural communities.

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PREVALENCE OF UROPATHOGENS AND ASSOCIATED RISK FACTORS IN URINARY TRACT INFECTION IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC IN A TEACHING HOSPITAL IN AWKA, NIGERIA

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Abstract

Urinary tract infection (UTI) is one of the common problems encountered in pregnancy, and it is associated with significant maternal and fetal risks. The prevalence of UTI varies worldwide. This study was undertaken to determine the prevalence of uropathogens and associated risk factors in UTI in pregnant women in Awka, Nigeria. This was a cross sectional study that involved 269 pregnant women. Clean-catch mid-stream morning urine specimens were collected and examined macroscopically, microscopically and bacteriologically. Information on Socio-demographic characteristics and some clinical factors were collected using pre-designed questionnaires. Statistical analysis was done using SPSS version 22 and the p-value was set at $p \leq 0.05$. A total of 118 isolates were obtained and the prevalence of UTI in the study population was 39.8%. The most predominant bacterium was *Enterococcus faecalis* 34(29%), followed by *Escherichia coli* 21(17.8%), *Klebsiella pneumoniae* 15(12.7%), *Staphylococcus aureus* 10(8.4%), *Staphylococcus saprophyticus* 7(6.0%), *Enterobacter cloacae* 6(5.1%), and *Staphylococcus epidermidis* 4(3.4%). *Citrobacter freundii*, *Pseudomonas aeruginosa* and *Proteus mirabilis* occurred at frequency of 3(2.5%) each while *Pseudomonas oryzihabitans* and *Morganella morganii* occurred at 2(1.7%) each. *Candida* species were also isolated, including *Candida albicans* 4(3.4%), *Candida krusei* 3(2.5%) and *Candida guilliermondii* 1(0.8%). None of the socio-demographic variables were statistically associated with UTI. Teachings on personal hygiene during every antenatal visit can help prevent UTI in pregnant women.

Keywords: Uropathogens, Mid-stream urine, Antenatal, Socio-demographic variables

Introduction

Urinary tract infections (UTIs) are infections that result when microbial count of culture of midstream urine specimens is greater than 10^5 cfu/ml (Getachew *et al.*, 2012); with or without clinical symptoms such as dysuria, fever, urgency, flank pain and hematuria (Donkor *et al.*, 2019; Boye *et al.*, 2012; Cheesbrough, 2006). Collee *et al.* (1989) stated that these high counts which are fairly constant in serial specimens from the same patient, reflect bacteria multiplication in the urine *in vivo* and are accepted as indicating significant bacteriuria. UTI could be both community and hospital acquired infections (Chaudhary and Mahadeva, 2013). Community acquired urinary tract infection (CA-UTI); occurs in the community or within less than 48 hours of hospital admission and was not incubating at the time of hospital admission (Kabugo *et al.* 2016). Hospital acquired UTI (nosocomial UTI) could also be defined as the onset of UTI in patients, 48 hours after admission (Donkor *et al.*, 2019).

UTI is one of the common bacterial infections that complicate pregnancy (Ahmed *et al.*, 2016) hence the reason for choice of pregnant women as the study group in this research. In pregnancy it may be symptomatic, commonly manifested as urethritis, cystitis or pyelonephritis; or it may remain asymptomatic (Onoh *et al.*, 2013). Pregnancy however, enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to other adverse obstetric outcomes such as prematurity, low-birth weight and higher fetal mortality rates (Getachew *et al.*, 2012). The significance of UTI in pregnancy has been widely evaluated at several studies from different countries

such as Ethiopia, Ghana, and Nigeria (Alemu *et al.*, 2012; Labi *et al.*, 2015; Onoh *et al.*, 2013; Akobi *et al.*, 2014).

In Nigeria, the prevalence of UTI among antenatal patients has been reported in Benin, Ebonyi and Nassarawa State as 13.8%, 55% and 62.67% respectively (Alfred *et al.*, 2013; Onuoha and Fatokun, 2014; Ajide *et al.*, 2016). The predisposing determinants of high prevalence of UTI in pregnancy include induced ureteral dilation, urinary stasis, reduced immune function, and presence of vesicoureteric reflex and difficulty with hygiene due to a distended pregnant belly (Onoh *et al.*, 2013; Ajide *et al.*, 2016; Ahmed *et al.*, 2016). It has also been reported that up to 70% of pregnant women develop glycosuria, which encourages bacteria growth in urine (Alemu *et al.*, 2012; Getachew *et al.*, 2012).

Uropathogens which are organisms that cause UTI are those from the normal vaginal, perineal and fecal flora (Onoh *et al.*, 2013). They include *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter* species, *Streptococcus agalactiae* and *Candida* species (Oli *et al.*, 2010; Ahmed *et al.*, 2016; Donkor *et al.*, 2019). The predominant organisms that cause UTIs during pregnancy are *E. coli* which accounts for 80%-90% of infection (Getachew *et al.*, 2012; Onoh *et al.*, 2013).

There is paucity of data on the prevalence of UTI and its associated risk factors in Awka, the capital City of Anambra State. Although a study has been carried out in Awka metropolis, reporting on the bacteriological aetiology and

incidence of UTIs among pregnant women (Obiogbolu *et al.*, 2009), the associated risk factors were not evaluated. This study was therefore aimed to determine the prevalence, uropathogens and associated risk factors of both asymptomatic and symptomatic UTIs in antenatal patients at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka.

Materials and Methods

Study site

The study was conducted at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka, Anambra State, Nigeria. It serves as a referral center for Hospitals in Anambra State and beyond. The analysis of specimens collected was done at Medical Laboratory Department of Regina Caeli Hospital and Maternity, Awka. Further confirmation of isolates was done at Evans Biomedics (Nig), Clinical and Analytical Laboratory, Oyiogbo; Rivers State, Nigeria.

Study design

The study with reference number (COOUTH/AA/VOL.1.010) was approved by the ethical committee of the hospital (COOUTH). This study was a cross sectional study which was carried out over a period of nine months from 11th of March to 31st December, 2016.

Study population

The study population comprised of pregnant women attending antenatal clinic (ANC) as outpatients at COOUTH. The study participants included those women who have not taken antibiotics within 2 weeks before presentation at the clinic and also were willing to participate in the study. Slovin's (1960) formula (modified by Andale, 2015) was used to calculate the appropriate sample size from the population which was calculated as 269 participants. The 269 pregnant women were randomly selected on presentation at ANC. Socio-demographic variables (age, marital status, education level, residence/address and other clinical information such as parity, gravidity, trimester, history of catheterization, and history of UTI) were obtained from the patients using pre-designed questionnaires.

Specimen collection and analysis

Clean-catch midstream (morning) urine were used for the study. Sterile leak-proof specimen bottles (which have been previously labeled) were distributed to the women after they were instructed on how to collect such specimens by the matron in charge of ANC (which involves the initial cleaning of the urethral area with clean water) and the importance of clean catch midstream urine in the study. The specimens received from patients were transported to the laboratory in specimen box containing ice pack (Emiru *et al.*, 2013) and laboratory analysis undertaken immediately within 1-2 hours of collection (Cheesbrough, 2006). The urine specimens were examined macroscopically for colour and turbidity as described by Nwankwo *et al.* (2020) and Kabugo *et al.* (2016). Microscopically, specimens were analyzed using wet preparation to detect presence of pus

cells (i.e white blood cells), red cells, casts, parasites, yeast cells, crystals, bacterial cells (since the urine were freshly collected) as described by Cheesbrough (2006) and Ince *et al.* (2016).

The specimens were cultured on Cystine Lactose Electrolyte Deficient (Rapid Labs) media with a loop that holds an inoculum of 0.002ml and incubated aerobically at 37°C for 18–24 hours. Following incubation, significant bacterial growth was taken as any count of uniform colonies equal to or in excess of 10⁴ CFU per milliliter of urine (Aker *et al.*, 2014; Mina *et al.*, 2017). The colony count/bacterial numbers were estimated using a simple Mathematical method (Cheesbrough, 2006). Isolated organisms were identified based on their colony morphology, Gram- stain reactions and biochemical tests including motility, catalase, coagulase, sugar fermentation, urease, oxidase, Triple Sugar Iron (TSI), citrate utilization, indole, litmus milk decolorization, and Eosin Methylene Blue (EMB) agar tests. The bacterial isolates were further confirmed by culturing in Chromagar Orientation. The fungal isolates, in addition to direct microscopy of wet unstained preparation and germ tube test were confirmed to species level using Chromogenic Candida Agar (CCA). Polymicrobial growth was considered significant only after complete evaluation of clinical symptoms of UTI, history of UTI, the patient's age, catheterization, pus cells, and bacterial count (Bajpai *et al.*, 2014).

Statistical Analysis

Data were coded, entered and analyzed using Statistical Package for Social Science (SPSS), version 22, EPI Info® version 7.2.1.0. Study findings were explained in words and tables. Results/ proportions for categorical variables were compared using percentages, 2x2 tables and chi square test. In all cases, P-value ≤ 0.05 was taken as statistically significant.

Results

Out of the 269 specimens examined for UTI, 107 showed significant microbial growth giving an overall prevalence of 39.8%. The result of macroscopy and microscopy urinalysis has been reported (Nwankwo *et al.*, 2020). Of all considered risk factors, none was statistically associated with UTI. However, greater prevalence of UTI was observed among those women within the age group of 16-20 years (50%), rural dwellers (41%), no formal education (55.6%) and with history of catheterization (43%) (Table 1). Gram positive, Gram negative bacteria including *Candida* species were identified in this study (Tables 2, 3 and 4). A total of 118 isolates were obtained out of 107 specimens that yielded significant microbial growth (Table 5). Some specimens showed polymicrobial growth 11(4.09%), non-significant and no growth 40(14.87) {Fig.1}. Of all the isolates, bacteria were more prevalent 110(93.2%) than yeast 8(6.8%) which were *Candida* species. Gram-positive and Gram-negative bacteria occurred at equal proportion in this study 55(45.6%); though the enterobacteria had the highest prevalence of 84(71.2%) compared to other isolates 34(28.8%).

Table 1: Prevalence of UTI in relation to socio-demographics and clinical variables of study population at COOUTH

Variables	Description	No tested (%)	Negative UTI No(%)	Positive UTI No (%)	P-value
Age (years)	16-20	4(1.5)	2(50)	2(50)	0.94
	21-25	52(19.3)	30(58)	22(42)	3
	26-30	96(35.7)	57(59.4)	39(40.6)	
	31-35	85(31.6)	52(61)	33(39)	
	>35	32(11.9)	21(66)	11(34)	
Marital status	Married	266(99)	160(60)	106(40)	0.65
	Other	3(1)	2(66.7)	1(33.3)	2
Residence	Urban	171(64)	104(60.8)	67(39.2)	0.44
	Rural	98(36)	58(59)	40(41)	6
Educational status	None	9(3.3)	4(44.4)	5(55.6)	0.39
	Primary	4(1.5)	3(75)	1(25)	1
	Secondary	72(26.8)	39(54.2)	33(45.8)	
	Tertiary	184(68.4)	116(63)	68(37)	
Symptoms of UTI	Yes	48(18)	28(58)	20(42)	0.44
	No	221(82)	134(61)	87(39)	5
	No	221(82)	134(61)	87(39)	
Gravidity	1-3	232(86)	138(59.5)	94(40.5)	0.74
	4-6	35(13)	12(34.3)	23(65.7)	6
	7-9	2(1)	1(50)	1(50)	
Parity	Nulliparous	82(30.4)	49(60)	33(40)	0.99
	Monoparous	103(38.3)	62(60.2)	41(39.8)	2
	Multiparous	84(31.2)	51(60.7)	33(39.3)	
Trimester	1 st	22(8.2)	13(59.1)	9(40.9)	0.97
	2 nd	90(33.4)	55(61)	35(39)	6
	3 rd	157(58.4)	94(60)	63(40)	
History of catheterization	Yes	35(13)	20(57)	15(43)	0.41
	No	234(87)	142(60.7)	92(39.3)	2
History of UTI	Yes	58(22)	39(67)	19(33)	0.13
	No	211(78)	123(58.3)	88(41.7)	9

Table 2: Morphological and Biochemical Characterization of Gram Positive Bacterial Isolates

S/No	Colony Characteristics on CLED medium	Gram Reaction	Catalase	Coagulase	Sucrose	Glucose	Lactose	Fructose	Maltose	Mannitol	Litmus milk medium	Growth on Blood agar	Colony Appearance Chromagar Orientation	Isolate Identified as
1.	Deep yellow colonies	Positive cocci in clusters	+	+	+	+	+	+	+	+	NT	Cream with β -hemolysins	Golden and opaque	<i>Staphylococcus aureus</i>
2.	Yellow to white colonies	Positive cocci in clusters	+	-	+	+	+	+	+	+	NT	NT	Pink, opaque and small	<i>Staphylococcus saprophyticus</i>
3.	Whitish discrete colonies	Positive cocci in clusters	+	-	+	+	+	+	+	-	NT	Small, whitish with β -hemolysins	Clear and translucent	<i>Staphylococcus epidermidis</i>
4.	Small yellow colonies	Positive cocci in pairs and short chains	-	-	+	+	+	+	+	+	+	White with β -hemolysins	Torquoise blue	<i>Enterococcus faecalis</i>

Fermentation tests:
 + = Sugar fermented with acid production
 - = Sugar not fermented
 + = Positive
 - = Negative
 NT = Not tested

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Table 3: Morphological and Biochemical Characteristics of Gram Negative Isolates

S/ No	CLED Appearance on Media	Appearance on EMB Agar	TSIA												Appearance on Macconkey Agar	Growth on Blood Agar	Appearance on Chromagar Orientation			Isolate Identified as:				
			Motility	Indole	Urease	Simmon citrate	Oxidase	Sucrose	Fructose	Maltose	Lactose	Manitol	Glucose	Slope			Butt	H ₂ S	Gas		Dark	pink	to	
1.	Yellow opaque colonies with deeper coloured centre	Blue-black colonies with green metallic sheen	+	+	-	-	-	d	+	+	+	+	+	+	Y	Y	-	d	Pinkish colonies	NT	Dark reddish	pink	to	<i>Escherichia coli</i>
2.	Large mucoid yellow or yellow-white colonies	Dark purple colonies	-	-	+	+	-	+	+	+	+	+	+	+	Y	Y	-	+	Pinkish and large	NT	Dark blue without halo	metallic with or without brown		<i>Klebsiella pneumoniae</i>
3.	Blue-grey colonies	Colourless colonies	+	-	+	-	-	-	-	-	-	-	+	R	Y	+	+	Colourless colonies	NT	Clear without halo	with or without brown		<i>Proteus mirabilis</i>	
4.	Green colonies with rough periphery	Colourless colonies	+	-	-	-	+	-	-	-	-	-	-	R	R	-	-	Colourless colonies	Light with green pigmentation	pink dark	translucent	and	<i>Pseudomonas aeruginosa</i>	
5.	Yellowish-white, mucoid/moist colonies	Pinkish colonies	+	-	-	D	-	+	+	+	+	+	+	Y	Y	-	+	Pinkish /brick red colonies		Dark blue	/metallic		<i>Enterobacter cloacae</i>	
6.	Translucent or colourless opaque-grey colonies and moist colonies	Colourless colonies	+	+	+	+	-	+	+	+	-	+	+	Y	Y	-	+	Colourless colonies	Large, whitish with α(partial) hemolysis	Violet interior with cream burder	purple with		<i>Citrobacter freundii</i>	
7.	Opaque/grey discrete colonies	Colourless colonies	+	+	+	-	-	-	+	+	-	-	+	R	Y	-	d	Colourless colonies	NT	Clear with halo	with brown		<i>Morganella morganii</i>	
8.	Green colonies	Purple /grey colonies	+	-	-	-	-	-	-	-	-	-	+	R	Y	-	-	Wrinkled pinkish colonies with serated	NT	Cream translucent	and		<i>Pseudomonas oryzihabitans</i>	

edges

S= Serial, CLED = Cystine Lactose Electrolyte Deficient, R = Red-pink (alkaline reaction), Y = Yellow (Acid reaction)
EMB = Eosin Methylene Blue agar, d = different strains give different reaction, TSI = Tripple sugar iron agar, NT= Not tested
H₂S = Hydrogen sulphide, + = Positive, - = Negative

Table 4: Morphological and Biochemical Characteristics of *Candida* Isolates

S/ No	Appearance on CLED Agar	Gram reaction	Fermentation						Growth on Sabouraud's Dextrose Agar (SDA)	Growth on Chromogenic Candida Agar (CCA)	<i>Candida</i> Isolate
			Glucose	Maltose	Sucrose	Lactose	Fructose	Germ tube			
1.	Small whitish and moist colonies	Positive cells of <i>Candida</i>	+	+	-	-	+	+	Small, whitish and moist	Light green coloured colonies	<i>Candida albicans</i>
2.	Small whitish and moist	Positive cells of <i>Candida</i>	+	-	-	-	+	-	Small, whitish and moist	Light pink coloured colonies	<i>Candida guilliermondii</i>
3.	Small whitish and moist	Positive cells of <i>Candida</i>	+	-	-	-	+	-	Small, whitish and moist	Purple fuzzy colonies with spreading pale edges	<i>Candida krusei</i>

CLED = Cystine Lactose Electrolyte Deficient
 + = Positive
 - = Negative

Table 5: Frequency of isolates

Isolated uropathogen	No.	(%)
<i>Enterococcus faecalis</i>	34	29.0
<i>Escherichia coli</i>	21	17.8
<i>Klebsiella pneumonia</i>	15	12.7
<i>Staphylococcus aureus</i>	10	8.4
<i>Staphylococcus saprophyticus</i>	7	6.0
<i>Enterobacter cloacae</i>	6	5.1
<i>Staphylococcus epidermidis</i>	4	3.4
<i>Citrobacter freundii</i>	3	2.5
<i>Proteus mirabilis</i>	3	2.5
<i>Pseudomonas aeruginosa</i>	3	2.5
<i>Pseudomonas oryzihabitans</i>	2	1.7
<i>Morganella morganii</i>	2	1.7
<i>Candida albicans</i>	4	3.4
<i>Candida krusei</i>	3	2.5
<i>Candida guilliermondii</i>	1	0.8
TOTAL	118	100

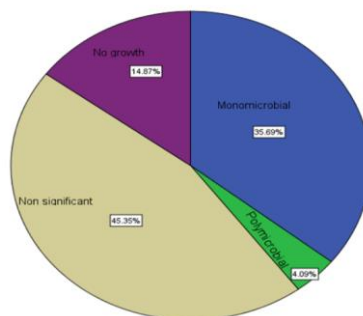


Figure 1: Characteristic growth pattern of urine culture among antenatal patients at COOUTH

Discussions

The overall prevalence of urinary tract infection in this study was 39.8% which was found concordant with 39%, 49.4% observed in two different locations (Mehdipatnam and Kanataka) in India (Ahmed *et al.*, 2016; Manjula *et al.*, 2013); 40% and 47.5% observed in Ilorin and Ibadan both in Nigeria (Ajayi *et al.*, 2012; Okonko *et al.*, 2009) among the same study group.

Lower prevalence of 10.1% and 21% was reported by Donkor *et al.* (2019) at Ghana and Mordi *et al.* (2015) at Benin, Nigeria. Higher prevalence (55% and 62.67%) compared to that observed in this study was also reported in Nigeria at Afikpo, Ebonyi State and Karu, Nasarawa State respectively (Onuoha and Fatokun, 2014; Ajide *et al.*, 2016). These variations may be explained by the fact that there are differences in geographical locations, social habits of the community, drainage system, standard of personal hygiene and education (Okonko *et al.*, 2009; Alemu *et al.*, 2012; Manjula *et al.*, 2013).

Bacterial count as demonstrated in polymicrobial infection in this study was up to 10^4 cfu/ml and this is in line with the findings of Akter *et al.* (2014). Our result 11(10.3%) of polymicrobial growth is consistent with that of Bajpai *et al.* (2014) (9.7%) but different from that of Akter *et al.* (2014) who reported polymicrobial prevalence of 2.26% in their study findings.

The frequency of isolates in this study revealed a changing trend in the bacterial profile found in bacteriuria among women attending antenatal clinic. From most previous studies apart from Labi *et al.* (2015) that reported that *Enterococcus faecalis* 4(26.7%) was predominantly isolated among pregnant women in Ghana, it has been very uncommon to recover *Enterococcus faecalis* as the most prevalent isolate as seen in this work. Most findings from other studies have reported *E. coli* as the most frequently isolated pathogen both in Nigeria, Ethiopia, Ghana and Iran (Onoh *et al.*, 2013; Alemu *et al.*, 2012; Donkor *et al.*, 2019; Mina *et al.*, 2017). Enterococci are hardy, facultatively anaerobic Gram positive cocci in pairs or short chains that can grow and survive in many environments (Olawale *et al.*, 2011). This organism has been noted as a significant bacterial isolate from women with UTI in pregnancy (Onoh *et al.*, 2013). This study has recorded the predominance of enterobacteria compared to other isolates and this could be explained by the fact that they are intestinal normal flora, which might enter the urethra through poor personal hygiene such as washing or cleaning perineum backwardly (Almushait *et al.*, 2013). The equal proportion of Gram positive and Gram negative bacteria observed in this study was because Gram positive cocci were the most predominant. With the exception of *Enterococcus faecalis*, the Gram negative bacteria were the most frequently isolated organisms. The high prevalence of Gram negative enterobacteria could be as a result of unique structures which aid the attachment to the uroepithelial cells and prevent bacteria from urinary bladder lavage, allowing them to invade the tissues and multiply giving rise to invasive infection and pyelonephritis in pregnancy (Onoh *et al.*, 2013). Secondly this dominance observed in Gram negative enterobacteria could also be attributed to an increase in levels of amino acids and lactose during

pregnancy which particularly encourages *E. coli* growth (Boye *et al.*, 2012). *Pseudomonas oryzihabitans* 2(1.7%) isolated in this study has been reported as a rare pathogen from clinical specimens (Qian and Wang, 2011) though it has been incriminated in some studies by Qian and Wang, (2011) who reported 11 cases of *P. oryzihabitans* in hospital setting in China, while Topkaya *et al.* (2007) and Sunita, (2013) reported a case each of UTI caused by *P. oryzihabitans* in Turkey and India respectively. *Candida* species with the prevalence of 8(6.7%) isolated in this study in addition to numerous bacteria was in agreement with the study at India (Bajpai *et al.*, 2014) that observed *Candida* species (9.3%); in addition to bacteria isolated in their study. A lower prevalence (2.5%) was reported in the same scenario by Okonko *et al.* (2009). *Candida* being a normal flora in the vagina and perineum, it could be understood that under favorable humid condition, and by a certain way of multiplication and ascending of the agent to urethral meatus, infection can be initiated at this point (Almushait *et al.*, 2013). Candiduria observed in this study could also be as a result of the higher incidence of vaginal candidiasis in pregnant women when compared to the non -pregnant subjects (Oli *et al.* 2010).

Urinary tract infection affects all age groups and both sexes (Okonko *et al.*, 2009). The higher prevalence of UTI among younger women in this study which decreased steadily among the older women could be as a result of increased sexual activity usually observed in younger than older women (Ajide *et al.*, 2016). However this study didn't capture the sexual activity within the subjects which is one of the limitations of the study. The higher prevalence of positive urine cultures which was observed among women from rural areas 40(41%) could be that the women were not enlightened and not aware of the need for proper personal hygiene. The highest prevalence of positive urine cultures among those with no formal education 5(55%) could be attributed to lack of awareness/poor knowledge of practicing personal hygiene in pregnancy and in agreement with a study at Nnewi, Nigeria (Oli *et al.*, 2010). The higher prevalence of UTI (though not statistically significant) among those with history of catheterization is in agreement with findings of Emiru *et al.*, (2013); among pregnant women at Felege Hiwet Referral Hospital, Bahir Dar, North West Ethiopia. Other studies that reported a statistically significant relationship between the use of catheter and UTI prevalence include Getachew *et al.*, (2012) and Alemu *et al.*, (2012). The non-significant relationship observed in this study could be because in normal clinical practice, introduction of catheter is usually done aseptically and the patients were normally placed on antibiotics throughout the period of catheterization. Also the reason could be because the period of hospitalization was not determined in this study. Pregnant women with history of UTI had a

lower prevalence of UTI, 19(33%) in this study. This finding is in agreement with the study at Ethiopia (Alemu *et al.*, (2012) that also reported a lower prevalence of 13(6.4%) among those with UTI history. This lower prevalence observed in this study could be as a result of effective treatment of the previous infection, which ruled out the possibility of any resistant strains. However, the relationship between history and prevalence of UTI as observed in this study was not statistically significant. This finding however varied from those of other researchers (Getachew *et al.*, (2012), Emiru *et al.*, (2013), and Almushait *et al.*, (2013) who reported higher prevalence of UTI among those with previous history of UTI.

All the risk factors of UTI evaluated in this work were associated with a higher prevalence of UTI but none was statistically significant which is in agreement with studies by Onoh *et al.* (2013) and Almushait *et al.* (2013) among pregnant women at Federal Medical center and Ebonyi State University Teaching Hospital Abakaliki Ebonyi State, Nigeria and Abha General Hospital Saudi Arabia. There is a possibility that perhaps UTI among these women were predisposed by other associated risk factors of UTI including maternal anaemia, poor personal hygiene, previous use of contraceptives, and increased sexual activity during pregnancy among others as observed by Al-mushait *et al.* (2013) and Emiru *et al.* (2013). These associated risk factors should therefore be critically examined in

further research studies.

CONCLUSION

The overall prevalence of UTI in this study is 39.8%. *Enterococcus faecalis* was the most frequently isolated bacterium from urine of pregnant women attending antenatal clinic at COOUTH Awka. From this study, it was also discovered that *Pseudomonas oryzihabitans* was incriminated in urinary tract infection (UTI) in pregnancy. This pathogen has not been reported to cause urinary tract infection in Nigeria. Previous research findings have reported only *Candida albicans* as aetiologic agents of UTI, but from our study it was obvious that non-*Candida albicans* species (*C. krusei* and *C. guilliermondii*) were now involved as causative agents of UTI in pregnancy in the study area. There was no significant statistical association between the prevalence of UTI and other factors like maternal age, symptoms of UTI, address/residence, educational and marital status, gravidity, parity, trimester, history of catheterization and UTI. Considering the high prevalence in this study, it is vital to introduce screening of pregnant women for UTI as part of antenatal care.

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WIS-BPH 25

EVALUATION OF BENEFICIAL EFFECTS OF METHANOL EXTRACT OF *Sansevieria liberica* AGAINST DIHYDROTESTOSTERON ESTRADIOL-VALERATE INDUCED BENIGN PROSTATIC HYPERPLASIA IN MALE WISTAR RATS

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ABSTRACT

Sansevieria liberica (SL) has been reported to exhibit various pharmacological effects in the treatment of many diseases. However the effectiveness of the plant against benign prostatic hyperplasia (BPH) has not been reported. This study was conducted to evaluate the effect of methanol extract of SL leaves in improving BPH. Thirty male rats, weighing between 180 - 197g were divided into five experimental groups; Normal control, Disease control, BPH + finasteride, BPH treated with 200 and 400mg/kg of SL respectively. BPH was induced by daily subcutaneous injection of a mixture of 5mg/kg dihydrotestosterone and 0.5mg/kg estradiol varelate. Biomarkers evaluated include body and relative organ weight, prostate specific antigen, testosterone, dihydrotestosterone, Aspartate transaminase, alanine transaminase and antioxidant markers. The percentage body weight of all the experimental groups increased significantly ($p < 0.05$). No significant change was observed in ALT and AST. The relative prostate weight, testosterone, PSA and DHT of the diseased control group significantly increased ($p < 0.05$) compared to that of the normal control groups. Treatment with SL or finasteride significantly ameliorated these parameters in comparison with diseased control rats. Catalase, SOD, GST and GSH significantly decreased in untreated BPH rats compared to normal rats ($p < 0.05$). Finasteride or SL treatments increased the levels of the antioxidant parameters. Histological studies showed a considerable improvement in the prostatic histology of the diseased groups administered with the plant extract. These findings suggest that methanol extract of SL leaf may suppress the development of BPH and could be useful in its treatment and management.

Keywords: Benign prostate hyperplasia, prostate specific antigen, *Sansevieria liberica*, finasteride, dihydrotestosterone estradiol varelate

Introduction

Benign Prostatic Hyperplasia (BPH) is a noncancerous enlargement of the prostate gland which occurs in aging male at 45 years and above. It is clinically characterized by bladder out flow obstruction, hyperplastic epithelial modifications together with stromal growth and slowly progressing process of micro and macronodular appearance (Song *et al.*, 2016). The effect of prostate enlargement may lead to urethral constriction, weak urinary stream, incomplete bladder emptying, nocturia, dysuria and overt bladder outlet obstruction (Patel and Parsons, 2014). As the prostate gland expands, it squeezes the urethra and causes the muscles around the urethra to contract, making it difficult to urinate freely. Prostate gland is mainly influenced by Dihydrotestosterone (DHT), an active metabolite generated by the enzymatic conversion of testosterone by steroid 5 α -reductase although other metabolites may play a role in health and disease (Lepor *et al.*, 2012; Iweala and Ogidigo, 2015). Hence hormones affect the development and progression of BPH since the development and growth of the prostate gland very closely depends on androgen receptor stimulation.

Prevalence of BPH varies across different ethnicity and country. It increases as patient age increases that is

from 14.8% in younger males aged 40 to 36.8% in males aged 80 and above (Lee *et al.*, 2017). Several synthetic drugs such as steroid 5 α -reductase inhibitors (finasteride) and α -adrenoceptor antagonists such as alfuzosin and terazosin have been used in the treatment of BPH (Gravas and Oelke, 2010). Many plants have also been identified as a source of essential chemotherapeutics which has protection against BPH and other degenerative diseases. As the population of men with BPH increases, phytotherapeutic management and treatment has become increasingly important.

Sansevieria liberica Gerome and Labroy (Agavaceae) is a perennial plant with thick rhizomes widely distributed in the tropical, subtropical, and temperate zones of the world. The plant is commonly called "mother-in-law tongue," "African bowstring," and "Leopard lily." Local names in Nigeria include "Mooda" (Hausa; north), "Ebube-agu" (Igbo; south-east), "Okonno" (Efik; south-south), and "Oja ikoko" (Yoruba; south-west). In Nigeria, preparations of the leaves and roots are used traditionally in the treatment of convulsion (Adeyemi *et al.*, 2007). The ant-snake venom activities (Akar *et al.*, 2019), anti-inflammatory activities (Eze *et al.*, 2011), anticancer activity (Akindele *et al.*, 2015) and anti-hypertensive

(Ikewuchi *et al.*, 2011) activities of *S. liberica*. has been reported. Also are the diuretic effect (Omodamiro and Jimoh, 2017) and anti analgesic (Umuokro *et al.*, 2008) activities. The aim of this study is evaluate the beneficial effect of *S. liberica* against dihydrotestosterone estradiol-valerate induced benign prostatic hyperplasia in male wistar rats

Materials and Methods

Chemicals

Dihydrotestosterone (DHT) and estradiol valerate (ESV) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Finasteride (Gideon Richer, Hungary) and Olive oil (Goya En Spana S.A.U, Spain) were purchased from Grace and Mercy Pharmacy, Umuahia, Abia State, Nigeria., Testosterone ELISA kit (Cayman, Ann Arbor, MI, USA), Prostate Specific Antigen (PSA) (Novatein Biosciences, Woburn, MA, USA) were used for the study. All other chemicals and reagents used were of analytical grade.

Collection and Identification of plant material

The fresh leaves of *Sansevieria liberica* were collected from Amaekpu in Ohafia Local Government Area, Abia State, Nigeria. The plant material was identified by Mr. Ibe Ndukwe, a taxonomist in the herbarium section of the department of forestry and environmental management, Michael Okpara University of Agriculture, Umudike. A voucher specimen was deposited at the herbarium of the same department.

Extraction of plant materials

The leaves of SL were air dried and milled to a fine powder with a grinding machine at National Root Crops Research Institute, Umudike, Umuahia, Abia State. About 2,500g of the plant material was weighed into a Winchester bottle after which 2.5L of 80% methanol was added until it covered the surface of the leave powder. The mixture was shaken every two hours for 48 hours after which it was filtered through whatman's filter paper (No. 1) into a pre-weighed glass beaker. The filtrates were put into a water bath to concentrate at 40°C and then lyophilized (Ilshinbiobase, Dongducheon-si, Korea). A total of 85g of extract powder (*Sansevieria liberica* concentrate) was obtained. The extract was put in a sample bottle, tightly covered and stored in a refrigerator (4°C) until ready for use.

Experimental Animals

Adult 9 weeks male Wistar rats (180-197g) were purchased from the animal breeding center of University of Nigeria Nsukka. They were housed in laboratory animal cages of six rats per cage under controlled standard conditions of light (12/24 h), 60 - 70% humidity and temperature (25±2°C). Food pellets and tap water were provided continuously. All experiments for Care and Use of Animals was approved by the Ethics committee of the Department

Biochemistry, Michael Okpara University of Agriculture, Umudike Nigeria.

Acute Toxicity Test

Extract of *S. liberica* was subjected to acute toxicity testing to determine the safe dose for BPH studies according Organization for Economic Co-Operation and Development guidelines (OECD). Thirty five mice (20–25 g) of both sexes were divided into 7 groups of 5 mice each and kept in animal cages with feed and water. After 7 days of acclimatization, they were administered graded doses (1000, 2000, 3000, 4000, 5000,6000mg/kg) of *S. liberica* extract. Observation was made closely for toxicity signs and number of death in each group during the first 30 min and hourly for 3 h, then during the first 24 h for delayed toxicity or mortality. The (LD₅₀) determination was calculated using the method of Enegeide *et al* 2013 The LD₅₀ of the test substance is said to be greater than 5000 mg/kg and hence has a high degree of safety.

Hormonal Induction of Benign Prostatic Hyperplasia

A mixture of dihydrotestosterone (DHT) and estradiol valerate (ESV) dissolve in 100ml of Olive oil was used to induce BPH in rats (Kalu *et al.*, 2016). The dose was formulated as 5mg/kg of Dihydrotestosterone (DHT) and 0.5mg/kg of Estradiol Valerate (ESV) and was given by subcutaneous injection every other day for 28days. Finasteride serves as a standard BPH drug. A stock of finasteride and *S.Liberica* extract (200 and 400mg/kg.bw) was prepared and stored in an air-tight, dark container in refrigerator.

Experimental Design and Treatments

Thirty male rats, 9 weeks of age and weighing between 180 - 197g were divided into five experimental groups of six rats each. group 1(the normal control group were not induced with BPH. Groups (2,3,4, and 5) rats were induced with BPH

Group 1: Normal control (Vehicle - 1mg/kg of olive oil orally and 1mg/kg olive oil by subcutaneous injection)

Group 2: BPH control – 1mg/kg oil orally

Group 3: BPH rats – 3mg/kg finasteride orally

Group 4: BPH rats – 200mg/kg *S. liberica*

Group 4: BPH rats – 200mg/kg *S. liberica*

The body weights of the rats were recorded on a daily basis. At the end of 28 days rats were fasted over night. On the 29th day, they were sacrifice by cervical dislocation and blood collected by cardiac puncture into EDTA and plain bottles according to their groups for biochemical analysis. Organs (heart, liver, kidney, spleen, and prostate) were harvested and weighed. The prostate was subjected to histopathological examination.

Measurement of body weight and Relative organ weight

The body weights of the animals were measured and recorded on daily basis throughout the period of the experiment and prior to dissection, a final body weight was recorded. Body weight variation in the experimental animals was described as weight gain. The percentage body weight of the rats was calculated as;

$$F_1 / F_0 \times 100$$

Where F_1 Final Body weight of the rat

F_0 Initial Body weight of the rats

The relative organ weight was measured as the ratio of total weight of the organ and final body weight multiply by 100.

Measurement of Serum Alanine Aminotransferase and Aspartate Aminotransferase Blood samples collected in plain bottles were coagulated at room temperature for 45 min, and then the serum was separated by centrifugation (at 3500 rpm for 15 min). The serum level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the method of Reitman and Frankel (1957) to assess the liver function.

Determination of Serum Prostate specific antigen (PSA):

The serum Prostate Specific Antigen (PSA) levels were determined with a PSA ELISA kit according to the manufacturer's instructions ELISA kit (CusabioBiotech, Wuhan, China.) (Strowell *et al.*, 1991). The PSA ELISA is a solid-phase, noncompetitive immunoassay based on the direct sandwich technique. Following the step by step instructions, Calibration curves were constructed for each assay by plotting the absorbance versus the concentration of each calibrator. The PSA concentration of the samples was then read from the calibration curve and expressed as milliliters in the serum

Dihydrotestosterone assay

Blood samples were allowed to clot into a serum separator tube for two hours at room temperature before centrifugation for 20 min at $1000 \times g$. Serum levels of DHT were assayed by Enzyme- Linked Immunosorbent Assay (ELISA) using commercially available kits (Santa Cruz Biotechnology, Santa Cruz, California, USA). Briefly, triplicate aliquots of standards and samples were put into separate wells pre-incubated with DHT- specific biotin-conjugated polyclonal antibody solution. Afterwards, avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. Same procedure was applied with the standard. Then, 3,3',5,5'-tetramethyl-benzidine substrate solution was added to each well and the reaction was stopped by adding a 0.16 M sulphuric acid solution. The reading

of the colour change of each well was assessed by spectrophotometric at the absorbance of 450 nm wavelength using analytical grade laboratory reagents. The final concentration of DHT was calculated by comparing the O.D. of the samples to the standard curve

Measurement of Testosterone Level

Testosterone content was measured with an assay kit (Enzo Life Sciences, Farmingdale, NY, USA) using the following method. Serum (100 μ L) was mixed with testosterone enzyme immunoassay (EIA) antibody (50 μ L) and cultured for 1 h. Thereafter, 50 μ L of conjugate was added and the solution was cultured for 1 h. After washing with washing solution, 200 μ L of pNpp substrate solution was added and allowed to for 1 h. 50 μ L of stop solution, solution of trisodium phosphate in water, was then added to stop the reaction and the absorbance was measured at 405 nm wave length. Testosterone content was calculated using a testosterone standard curve.

Determination of antioxidant Enzymes

Determination of Catalase Activity

Catalase activity was determined according to the method of Higashi and Peters (1963). The reaction mixture contained 250 μ L of 0.01 M phosphate buffer (pH 7.0), 25 μ L of tissue homogenate, and of tissue homogenate, and 100 μ L of 2M H₂O₂. The reaction was stopped by the addition of 500 μ L of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a1: 3 ratio). The absorbance was read at 620 nm and expressed as micromoles of H₂O₂ consumed/min/mg protein at 25 ° C.

Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was determined using the method of Winterbourn *et al.*, (1986). An aliquot of 200 μ L of supernatant was added to the reaction mixture containing 500 μ L of 100mM TRIS/HCl (pH 7.8), 200 μ L of 75 mM nitro-blue-tetrazolium, 200 μ L of 2 μ M riboflavin, and 200 μ L of 6 mM EDTA. The absorbance was read at 560 nm. One unit of SOD is defined as the quantity required to inhibit the rate of nitro-blue-tetrazolium reduction by 50%. The enzyme activity is expressed as units/mg protein.

Estimation of Reduced Glutathione

Glutathione (GSH) content was estimated according to the method described by Sedlak and Lindsay (1968), This involves its reaction with DTNB (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically. About 500 μ L of prostate homogenate were added to 500 μ L of 10% trichloroacetic acid and centrifuged (Remi cold centrifuge) at 2,000 g for 10 min at 4 ° C. An aliquot of 33 μ L of supernatant was added to the reaction mixture containing 66 μ L of 100 mM of

DTNB and 900 µL of 0.1 M phosphate buffer (pH 7.4). The absorbance was read at 412 nm.

Glutathione transferases

Glutathione transferases activity was measured spectrophotometrically at 340 nm by the method of Habig *et al.*(1974) with CDNB (1-Chloro-2,4-dinitro benzene) as substrate. One unit of enzyme activity was defined as the amount of enzyme that catalyses the formation of one mole of 2,4-dinitrophenol GSH product per minute. Specific activity was expressed as moles of GSH conjugate formed per minute per milligram of protein.

Histopathological examination

Prostate tissues were rapidly excised, washed with cold normal saline, blotted with filter paper, weighed on electronic balance (Ohaus, USA) and fixed in 10% buffered-paraformaldehyde for 24 h, washed with 70% ethanol, dehydrated with a graded alcohol series (70%, 80%, 90% and 100% absolute alcohol), embedded in paraffin, sectioned at 5µm thickness, After dewaxing and rehydration, prostate section were mounted on slides and then stained with hematoxylin for 1min, washed in flowing water for 30s, then stained with eosin (Sigma-Aldrich, St. Louis, MO, USA) for 30s according to standard protocol. The

slices were again washed with 94% ethyl alcohol two times for 30 s, treated with absolute alcohol for 30 s, washed with xylene for 30 s and washed with flowing water for 30 s (Jang *et al.*, 2010). The stained tissue slices were viewed under light microscope (Olympus, Tokyo, Japan) for histological examination, photographed and interpreted by a consultant pathologist.

Statistical analysis

Statistical analysis was performed by using the Statistical Package for the Social Sciences software (SPSS Institute, Armonk, NY, USA). Data are expressed as mean and standard deviation values, and statistically significant differences among groups were evaluated by using one-way-ANOVA (analysis of variance). Statistically significant differences among group means were tested at p < 0.05 by using Duncan's multiple range tests.

Results

Toxicity study revealed the non toxic of the plant extract, The rats treated with different doses of *S. liberica* did not show any drug induced physical sign of toxicity during the whole experimental period and no death was recorded up to 5000mg/kg body weight.

Table 1: Initial and final body weight, body weight gain and Percentage change in body weight

Group	Initial body weight(g)	Final body weight (g)	body weight gain(g)	% change in body weight
Group I	180.30 ± 2.81 ^b	223.41±1.64 ^a	43.11 ±3.71 ^a	23.91
Group II	197. 42 ± 3.22 ^a	245.62±1.53 ^b	48.20± 3.22 ^b	24.41
Group III	191.11 ± 3.19 ^a	221.66±1.80 ^b	30.55 ± 4.1 ^b	15.90
Group IV	190.30 ± 2.14 ^a	238.63±1.15 ^c	48.33 ± 2.54 ^c	28.23
Group V	184. 78 ± 2.11 ^b	236.94±1.60 ^c	52.16 ±1.98 ^c	25.40

Values are Mean ± SD. n = 5. The letters in the column indicate statistical differences by Duncan multiple range test (P<0.05). Values with different superscript along a column are significantly different at P< 0.05 compared to control. Group 1- Normal control, . Group 2-Disease control, . Group 3- BPH+ Finasteride, . Group 4- BPH + . 200mg/kg *S. Liberica*, Group 5- BPH + 400mg/kg *S. liberica*

3.1 Body weight gain and Percentage change in body weight

The body weight changes of all the experimental animals are shown in table 1. The result showed a progressive and significant increase (P<0.05) in the body weight of all the animals. However the percentage body weight gain of disease control rats (24.41%) is higher compared to finasteride treated rats (15.90%).

Table 2: The effect of methanol extract of *S. Liberica* on Relative in rats organ weight

Group	Liver	Heart	Kidney	Prostate	Spleen
Group II	3.80 ± 1.44 ^a	0.36± 3.21 ^a	2.80 ± 2.15 ^a	0.35 ± 3.00 ^a	0.39 ± 0.13 ^a
Group II	3.81± 0.63 ^a	0.38± 2.10 ^a	2.81 ± 3.14 ^a	1.23 ± 1.39 ^b	0.40 ± 0.17 ^a
Group III	3.83± 0.75 ^a	0.39± 3.11 ^a	2.79 ± 2.14 ^a	0.68 ± 2.15 ^a	0.38 ± 0.12 ^a
Group IV	3.89± 3.40 ^a	0.37± 0.10 ^a	2.82 ± 1.10 ^a	0.95 ± 1.16 ^a	0.42 ± 0.10 ^a
Group V	3.87± 4.01 ^a	0.36± 1.35 ^a	2.87 ± 1.92 ^a	0.45 ± 1.73 ^a	0.41 ± 0.30 ^a

Values are Mean ± SD. n = 5. Values with different superscript along a column is significantly different at p < 0.05 compared to control. Group 1- Normal control, . Group 2-Disease control, . Group 3- BPH+ Finasteride, . Group 4- BPH + . 200mg/kg *S. Liberica*, Group 5- BPH + . 400mg/kg *S. liberica*

R
relative organ weight of Rats

As shown in Table 2, at the end of the study, the relative prostate weights of the rats that had been treated with methanol extract of *S. liberica* differed from those of the untreated BPH models. Specifically, the relative prostate weight in the BPH control group increased significantly (P<0.05) when compared to normal control rats. Upon the administration of 200 and 400mg/ kg of *S. liberica* or Finasteride (0.5mg/kg body weight) for 4 weeks, the relative prostate weights were lower than those in the BPH group. The relative weight of liver, heart, kidney and spleen were within normal range throughout the study, irrespective of the group considered and no statistically significant difference was observed.(P>0.05)

Assessment of serum Testosterone, DHT and PSA Levels

Figure 1,2 and 3 depict the effect of treatments on Testosterone, DHT and PSA Levels. Animals in the BPH controlled group showed a significant increase in DHT (8.54±1.14) testosterone, (8.33±1.11 ng/ml) and PSA (7.27±1.51 ng/ml) serum level of testosterone in comparison with group 1(P<0.05 vs. group). Treatment with *S. liberica* or finasteride brought about a significant decrease of both parameters (p<0.05 vs. untreated BPH), with 400mg/kg *S. Liberica* showing a better reduction than finasteride. The BPH induced animals that were untreated for 28 days of the experimental period indicated insignificant decrease in those parameters.

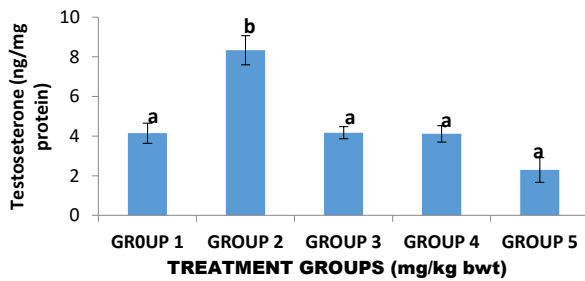


Figure 1: Testosterone level in serum of rats. Value mean ± SD

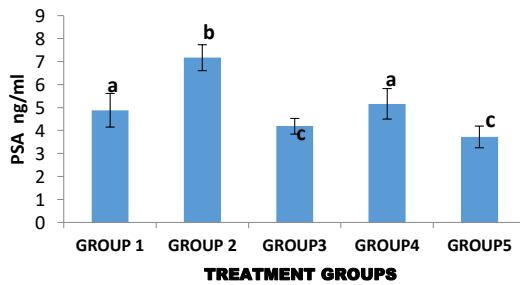


Figure 2 : PSA in serum of rats. Value mean ± SD **c mean...

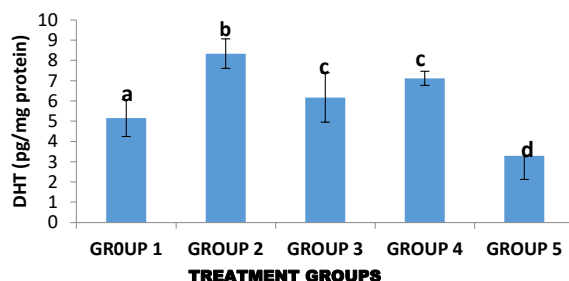


Figure 3: DHT in serum of rats. Value mean \pm SD^{a-d}
^{a-d} mean with different superscripts are significantly different at P<.05 for each group

Group 1-Control, Group 2- BPH control, Group 3- BPH + Finasteride
 Group 4- BPH+ 200mg/kg SL, Group 5- BPH +400mg/kg SL . SL - Sansevieria liberica

Table 3: The effect of treatment with methanol extract of *S. Liberica* on ALT and AST in rats

Group	ALT(U/L)	AST (U/L)
Group I	50.29 \pm 2.77	47.33 \pm 2.56
Group II	52.05 \pm 1.26	49.19 \pm 2.11
Group III	51.33 \pm 1.50	42.62 \pm 1.88
Group IV	53.11 \pm 1.22	41.85 \pm 2.10
Group V	56.73 \pm 1.37	42.22 \pm 2.14

Values with different superscript along a column is significantly different at p < 0.05 compared to control.

Group 1- Normal control, Group 2-Disease control, Group 3- BPH+ Finasteride,
 Group 4- BPH + 200mg/kg *S. Liberica*, Group 5- BPH + 400mg/kg *S. liberica*

In table 3, no significant difference was observed in the levels of ALT and AST in all the experimental groups (P> 0.05).

Table 4: The effect of treatment with methanol extract of *S. Liberica* on Antioxidant levels

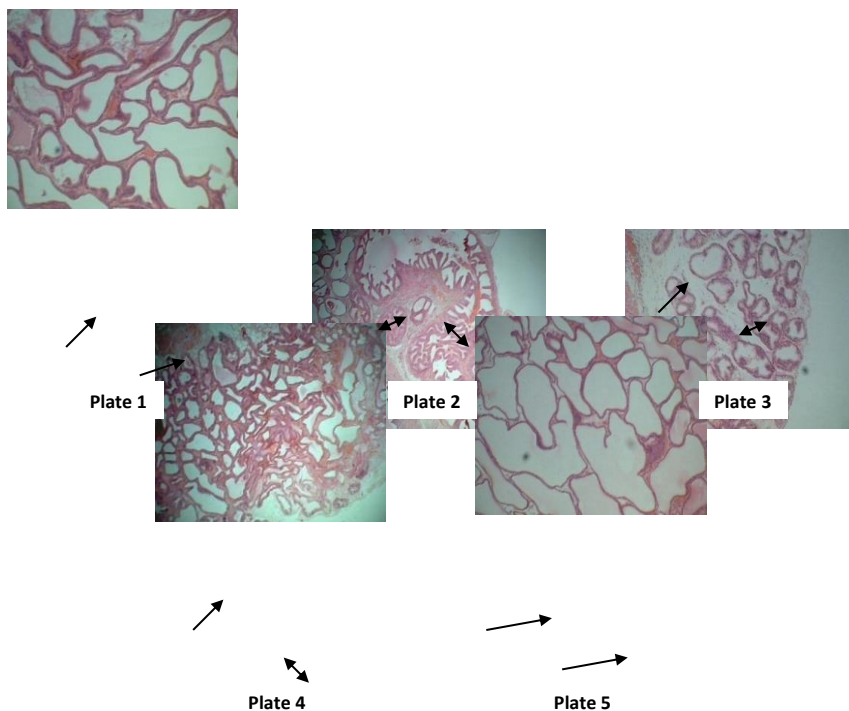
Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH (μ moles/mg protein)	GST (U/mg protein)
Group I	14.12 \pm 1.23	10.98 \pm 1.22	8.55 \pm 2.10	5.71 \pm 1.20
Group II	3.44 \pm 1.11	4.77 \pm 0.18	3.79 \pm 1.67	1.99 \pm 1.11
Group III	9.49 \pm 1.13	7.33 \pm 1.17	8.10 \pm 1.98	3.12 \pm 2.10
Group IV	10.98 \pm 1.17	7.99 \pm 1.56	7.87 \pm 2.14	5.40 \pm 1.44
Group V	9.66 \pm 1.33	10.10 \pm 1.32	7.99 \pm 1.88	5.62 \pm 1.97

Values are Mean \pm SD. n = 5. Values with different superscript along a column are significantly different at p < 0.05 compared to control. Values are Mean \pm SD. n = 5. Group 1- Normal control, . Group 2-Disease control, . Group 3- BPH+ Finasteride, . Group 4- BPH + . 200mg/kg *S. Liberica*, Group 5- BPH + . 400mg/kg *S. Liberica*. SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione, GST: Glutathione transferease

Effects on Antioxidant Parameters

There was oxidative stress in response to BPH induction. Induction of BPH resulted to significant decrease in the activity of the enzymes; SOD, CAT,

GSH and GST as shown in Table 4. However, BPH rats treated with the extract of *S. Liberica* or standard drug, significantly (p < 0.05) showed improvement in the activities of the enzymes compared to non treated



Histological plates: Photomicrographs of prostate samples from different experimental groups.

Plate 1: Normal -vehicle-treated group

Plate 2: BPH control group

Plate 3: BPH treated with finasteride.

Plate 4: BPH treated with 200mg/kg *S. Liberica*

Plate 5: BPH treated with 400mg/kg *S. Liberica* (x40)

DISCUSSION

The percentage body weight of all the experimental animals increased significantly at the end of 28 days. BPH animals administered *S. Liberica* did not show any statistical difference in weight when compared to that of normal control group. In BPH control group, the body weight significantly increased. The increased in body weight of untreated BPH rats may be associated with increased abdominal pressure and inflammation due to enlarged prostate size. Hence treatment with finasteride or extract of SL did not show reduction of the weight of BPH induced animals. Parikesit *et al.* (2016) reported that increase in weight in BPH subject may be associated with altered endocrine status. In the present study, the animals with BPH showed an increased in relative prostate weight in comparison to normal control groups. Previous studies by Nahata *et al.* (2011) on animals with BPH have also shown an increased relative prostate weight. Hence increase in relative prostate weight in male animals is used as an important marker in the detection of pathological changes in the development of BPH

(Veeresh Babu *et al.*, 2010). Finasteride or extract of *SL* used to treat BPH result to decrease the relative prostate weight. Treatment with *S. Liberica* (200 and 400mg/kg) showed a reduction in prostate weight compared to BPH group. This suggests that the plant extract have the potentials of shrinking the prostate tissue. Testosterone is a major secretory product in the cells of the testis and it is converted to dihydroxytestosterone by the enzyme 5α – reductase in androgen target cells before binding with high affinity to the intracellular androgen receptor. Testosterone and DHT play a key role in the development of male reproductive organs, and these hormones are commonly associated with BPH (Shin *et al.*, 2012). In the present study treatment of BPH induced rats with 400mg/kg of *S. Liberica* extract was effective in slowing the progression of BPH induced by testosterone. Hence the extract was able to reduce the levels of testosterone and DHT by inhibiting 5α – reductase enzymes resulting in the reduction of prostate size (Nickel *et al.*, 2011). These findings are consistent with those of a previous study by (Moon *et*

et al., 2017) in reducing testosterone and DHT level by inhibiting enzyme 5 α – reductase. However, PSA levels have been found to increase in BPH and prostate cancer (Wang *et al.*, 2016). Therefore a reduction in PSA level indicates the effectiveness of methanol extract of *S. Liberica* in the management and treatment of BPH. Increased prostate size in BPH induces inflammation, cause oxidative stress which leads to the release of oxygen free radicals (Bostanci *et al.*, 2013). These conditions may be responsible for the reduced activities of antioxidant enzymes in BPH controlled group and also to the low activity of superoxide dismutase and catalase. Free radical scavenging enzymes such as sod etc are the main line of cellular defence against oxidative injury. Antioxidants played an effective defence role in inhibiting and scavenging free radicals. t providing supported to defense mechanism of the body against risk of free radical mediated deadly diseases. Oxidative stress in BPH is coupled to a decrease in the antioxidant status, which can increase the deleterious effects of free radicals (Udensi and Tchonunou, 2016). Antioxidant enzymes act as scavengers that remove free radicals. Reduced activities of these enzymes may lead to deleterious effects and accumulation of superoxide anion (O) and hydrogen peroxide (H₂O₂), which in turn generate hydroxyl radicals (OH). (Minciullo *et al.*, 2015) In the present study, *S. Liberica* extract or finasteride treated BPH induced groups showed a significant increase in SOD and CAT activities of rats. This means that the extracts may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes. This result clearly shows that *methanol extract of S. Liberica* contain a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of superoxide radicals and hydrogen peroxide radical. The increased antioxidant capacity of *S. Liberica* could possibly provide mechanism for protective effect against development of BPH. The

pretreatment of rats with *S. Liberica* ameliorated the effect of testosterone as shown by the reduction of prostate weight, testosterone, and PSA. Interestingly, *S. Liberica* enhanced antioxidant defense mechanisms. Tissue distribution of some of the amino-transferases is used diagnostically by measuring the release of specific enzymes during cellular necrosis and tissue damage. The transaminases (AST and ALT) are well-known enzymes used as biomarkers to predict possible toxicity to the liver (Rahaman *et al.*, 200). Elevation in serum activities of these enzymes to rats suggested damage to the liver cells. In this study, no significant differences was observed in ALT and AST of untreated BPH induced rats, BPH induced animals treated with finasteride or *S. Liberica* (200 and 400mg/kg) when compared with the normal rats. This is an indication of the safety of methanol extract of *S. Liberica* on the liver function in the treatment of BPH. Histological changes including the epithelial thickness, and lumen area were restored after treatment with SL or finasteride.

Conclusions

In conclusion, oral administration of SL at a dose of 200 and 400mg/kg in a BPH rat model decreased the prostate weight as well as PSA, testosterone, and DHT levels in the serum and prostate. These findings indicate that 400 mg/kg SL may effectively slow the progression of testosterone-induced BPH possibly through enhancement of antioxidant defense mechanisms

Thus, SL could be a potential phytotherapeutic agent in the management of BPH in men. although more research studies including clinical trials are needed to confirm these findings.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest

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***Combretum dolichopentalum* Extract Normalized Biochemical and Haematological Parameters in Carbon tetrachloride Induced- Stress in Rats**

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Abstract

Background: The aqueous extract of *C. dolichopentalum* is employed in Nigeria to stabilize the uterus after parturition. **Objective:** The ability of the ethanol extract of *Combretum dolichopentalum* (EECD) to confer protection on rats destabilized by moderate concentrations of carbon tetrachloride (CCl₄) was evaluated. **Materials and methods:** Fifty rats were assigned to 5 groups of 10 rats each. The test organisms after acclimatization were handled accordingly: Groups 1 and 2 respectively were maintained on food and water only throughout the study. Group 3, 4, and group 5 were pre-treated with 250 mg/kg and 500 mg/kg body weight of EECD and 50 mg/kg of silymarin for 28 respectively. All groups except group 1 were intoxicated to 0.2 ml/kg body weight of CCl₄. Administered via intraperitoneal route on day 29. Serum pipetted from the blood of the rats after cardiac puncture was assayed for antioxidant enzymes, blood buffer, Lipid peroxidation product, biochemical as well as haematological parameters of the test organism were determined. Standard biochemical techniques were employed for the assay and results were subject to statistical analysis using one way ANOVA. **Results:** Administration of CCl₄ at 0.2 ml/kg b.w slightly increased the oxidizing species as indicated in the concentration of malondialdehyde in the rats; it increased the Iron and zinc concentrations and also the haematological parameters except the white blood cells. However, this was corrected by pre-treatment with the ethanol extract of *C. dolichopentalum* dose dependently. *C. dolichopentalum* has been reported to possess a wide array of flavonoids, alkaloids, saponins and tannins. The presence of these phytochemicals *C. dolichopentalum* could be responsible for reducing the increased MDA and normalization of altered Iron and Zinc concentrations in the rats. **Conclusion:** These characteristics portends that the crude

ethanol extract of *C. dolichopentalum* could be employed to correct minor oxidative perturbation induced by CCl₄ intoxication.

Keywords- *C.dolichopentalum*, Flavonoids, Carbon tetrachloride, Antioxidant, Pharmacological.

Introduction

Ageing and various degenerative diseases associated with it are attributable to the injurious attack of reactive oxygen species on cellular constituents, including chromosomes, connective tissues and mitochondrial DNA (Nita and Grzybowski, 2016). Univalent reduction of oxygen gives rise to damaging oxidative species (reactive oxygen species, (ROS). ROS can damage many different kinds of cellular macromolecules including lipids, proteins and DNA. Damage to DNA can lead to irreversible loss or alteration of genetic information in post mitotic cells. This produces many different oxidatively modified purines and pyrimidines, including the most commonly measured 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), as well as single and double strand breaks in both rats and human DNA and chromosomes (Brand, 2010., Cui *et al.*, 2012., Quinlan *et al.*, 2014). Protein modifications elicited by direct oxidative attack on lysine, arginine, proline or threonine and by secondary reaction of cysteine, histidine or lysine residues with carboxyl compounds can lead to the formation of protein carboxyl derivatives.

Biomolecules most susceptible to oxidative damage in cells, are unsaturated fatty acids of cell membranes and this susceptibility increases as a function of their double bonds. Abstraction of hydrogen from unsaturated fatty acids by oxygen centred radicals mainly initiates lipid peroxidation, this is followed by the formation of hydroperoxides. A variety of derivatives including various carbonyl products results from the degradation of hydroperoxides (Antonio *et al.*, 2014., Nita and Grzybowski, 2016). Such unsaturated carbonyls include deoxyosones, dienals, enals, hydroxylenals, 2-ketoaldehydes, trienals and various reductions that are really reactive and toxic to most cellular and extracellular biomolecules (Antonio *et al.*, 2014., Nita and Grzybowski, 2016). Many carbonyls react readily even at neutral pH and room temperature, with important biochemical groups such as amino, thiol or hydroxyl. A secondary functional group of the carbonyls increases their reactivity potential and may induce irreversible reaction products, or result in cross-linking reactions (Antonio *et al.*, 2014. Antonio *et al.*, 2014 reported that 4-hydroxyl alkenals could react with nearly all amino acids under appropriate conditions. Due to its reactivity, the carbonylic products, particularly α,β -

unsaturated carbonyls of lipid peroxidation, are implicated in various types of cell damage, including depletion of glutathione, protein modification, disturbance of calcium homeostasis, cell membrane destruction, tissue injuries, enzyme inhibition and disruption in DNA, RNA, and protein synthesis (Zarkovic, 2003., Nita and Grzybowski, 2016., Hongqiao and Henry, 2017). Besides, endogenously produced reactive oxygen species, ROS can also be induced exogenously. Contact through ingestion, inhaling, or skin with toxic substances released either from industrial plant or home -made chemicals present in certain cleaners, dry-cleaning agents, pesticides, and refrigerants etc carbon tetrachloride. The dose, duration, and administration are factors that determine the degree of harm caused when exposed to carbon tetrachloride (ATSDR, 2005).

Exposure of CCl₄, to a variety of specie, causes haematological disorders, centrilobular hepatic necrosis besides other symptoms (Rahmouni *et al.*, 2017). This study is determined to demonstrate the effect of the ethanol extract of *C. dolichopentalum*, on some biochemical and haematological parameters in CCl₄ oxidative stress as *C. dolichopentalum* has been demonstrated to have a wide pharmacological active Phytochemical constituents capable of scavenging free radicals (Ujowundu *et al.*, 2011., Ujowundu *et al.*, 2015a., Ujowundu *et al.*, 2015b., Rahmouni *et al.*, 2017).

2.0 MATERIALS AND METHODS

2.1 Collection and authentication of plant material: *C. dolichopentalum* leaves were harvested from Obinze in Owerri West Local Government Area of Imo state. The fresh plant was authenticated by Mr. A. Ozioko, of the Bioresource Development and Conservation Program (BDCP), Research Centre at the University of Nigeria, Nsukka, Enugu State, Nigeria.

2.2 Preparation of plant extract: *C. dolichopentalum* leaves were plucked from their stems washed with water and allowed to air dry at room temperature. The dried samples were pulverized (using electric blender) and stored in an airtight container. Three hundred grams of the pulverized *C. dolichopentalum* powder was extracted with 1.75 L of 80 % ethanol by maceration for 2 days, this was done in three separate cans of 100 g each and then pulled together. The residue was removed by filtration using a sieve followed by a Whatman No 1 filter paper. The concentration of the extract was carried out using a rotary evaporator under

mild temperature and reduced pressure and labeled ethanol extract of *Combretum dolichopentalum* (EECD).

2.3 Experimental animals: The experimental animals, Wistar albino rats of male specie weighing 150-200 g, were purchased from the Animal House of the Department of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. The animals were acclimatized for 7 days at room temperature in metal cages under 12/12 hour light and dark and were maintained *ad libitum* on water and rat pellets (Pfizer Feeds, Aba, Nigeria). This study was carried out in accordance with laws and regulations for handling experimental animals as was approved by the Department of Biochemistry, FUTO.

2.4 Experimental Design: Fifty (50) Wistar albino rats weighing between 150 and 200 g were used for this prophylactic study. The rats were assigned to 5 groups of ten rats each after 7 days acclimatization. This study was designed as shown in Table 1.

Table 1: Experimental treatment

Groups	Group Identity	Treatments
1	Normal control (NC)	feed + water
2	Positive control (PC)	feed, water + CCl ₄
3	Treated group (T ₂₅₀)	250 mg/kg body weight of EECD + CCl ₄
4	Treated group (T ₅₀₀)	500 mg/kg body weight of EECD + CCl ₄
5	Silymarin group	50 mg/kg body weight of Silymarin + CCl ₄

All groups received food and water *ad libitum* for 4 weeks. On day 29, 0.2 ml/kg body weight of CCl₄ in liquid paraffin (2:1) was administered intraperitoneally to all groups (except normal control). The CCl₄ was allowed to act on the animals for 2 days. The rats were sacrificed and blood collected by cardiac puncture after overnight fast and light anaesthesia with dimethyltetrachloride. Liver tissue of the animals were obtained, washed in 1.15 % KCl buffered solution and dabbed with paper, weighed and prepared for homogenization.

2.5 Blood collection: Blood samples of each animal were collected by cardiac puncture into EDTA bottles for haematological parameters and plain bottles for biochemical parameters. Blood sample collected into plain bottles were allowed 45 minutes to clot at room temperature, thereafter centrifuged at 600 x g for 15 minutes; the serum collected was used to assay various biochemical parameters.

2.6 Biochemical Assays: SOD activity was assayed according to the method of Xin *et al.*, 1993 This method studies the activity of SOD as it converts superoxide to hydrogen peroxide in the

presence of a detector iodinitrozoium violet. The activity of GPx was determined according to the method of Paglia and Valentine, 1967. **Principle:** GPx catalyzes the oxidation of GSH by hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized (GSSG) glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance of NADPH at 340 nm is measured in a spectrophotometer. The concentration of MDA was determined according to the method of Wallin *et al.*, 1993. Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex which in acid solution, absorbs maximally at 532 nm. Bicarbonate was determined according to the methods of Tietz 1987) using CO₂ gas electrode. Atomic absorption spectrophotometer was used to determination iron and zinc concentrations. An automated haematology analyzer machine (Mindray BC 2300, USA) was used for the haematological analyses.

2.7 Statistical analysis: Results were subject to statistical analysis using one way ANOVA.

RESULTS AND DISCUSSION

The toxicity of carbon tetrachloride has been elucidated both from the biochemical and pathological view points and as a result the data available provides particular insight into mechanisms of toxicity especially on liver. The key organ which plays crucial role in regulating various physiological processes in the body is the liver. It is associated with detoxication, metabolism, secretion, storage, and synthesis of metabolites. This is the reason, injury to the liver due to hepatotoxic agents may be of grave consequences (Shahani, 1999). Most hepatotoxic agents destroy liver cells mainly by initiating lipid peroxidation and other oxidative damages (Dianzani, 1993). The liver is the major target organ of CCl₄ toxicity owing to its high content of cytochrome P-450 (Södergren *et al.*, 2001), however it is also known that the red blood cell is metabolically active towards pharmacologically active endogenous and exogenous substances, it is evident that the RBC displays moderate cytochrome P-450-like activity, not excluding its ability to catalyse various other transformations of a range of drugs (Cossum, 1988).

Trichloromethyl free radicals ($\cdot\text{CCl}_3$ or $\cdot\text{CCl}_3\text{OO}$) is produced from CCl₄ mediated by hepatic microsomal cytochrome P-450 (Preethi, and Kuttan, 2009), which in turn initiate lipid peroxidation process (Adewole *et al.*, 2007., Adewole *et al.*, 2012). Carboxylic products, such as the α , β –unsaturated carbonyls of lipid peroxidation, are very reactive, thus inflicts various

types of cell damage, such as cell membrane destruction, depletion of glutathione, disturbance of calcium homeostasis, enzyme inhibition, decreased DNA, RNA and protein synthesis, protein modification, retardation of respiration, and tissue injuries (Ujowundu *et al.*, 2015b., Nita and Grzybowski 2016., Rahmouni *et al.*, 2017).

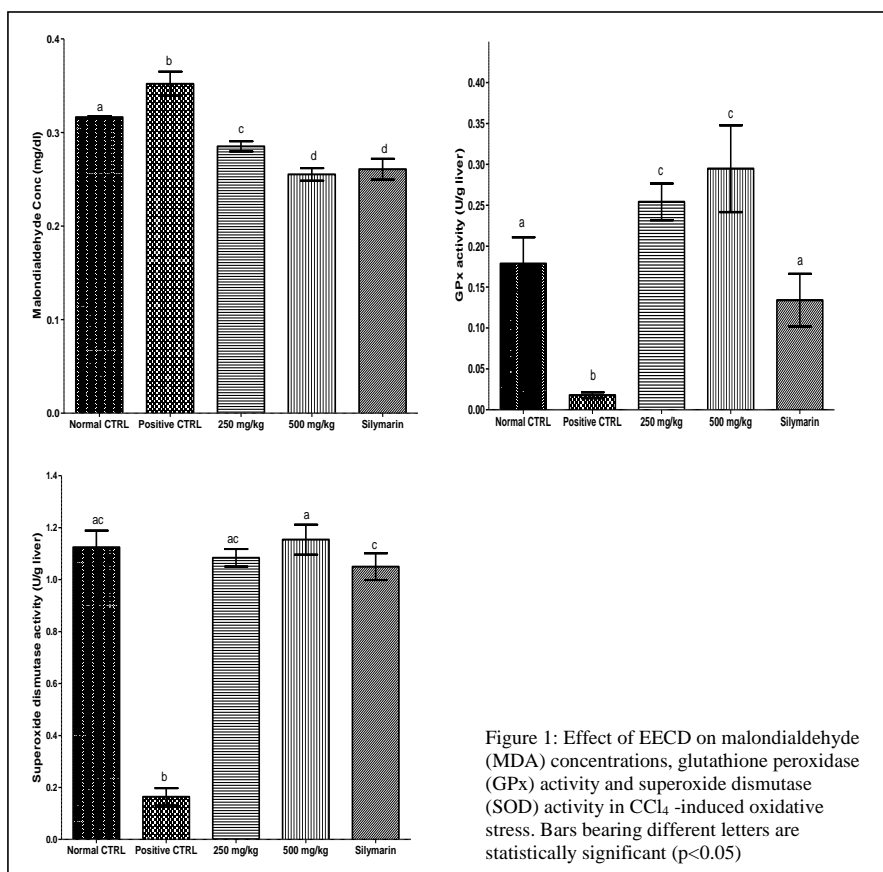


Figure 1 shows a significant ($p<0.05$) increase in MDA concentrations in the positive control group compared to the normal and the EECD treated groups. However there was no significant difference ($p>0.05$) between the 500 mg/kg b.w and the Silymarin treated groups. Pre-treatment with EECD before intoxication with CCl₄ showed decreased MDA concentrations. The anti-lipid peroxidative activity of the EECD as shown by the reduction of CCl₄ induced oxidative stress might be as a result of the extract's ability to prevent the penetration of CCl₄ into the

liver cells, thus preventing the activation of the toxicants, since activation of CCl₄ is a prerequisite for lipid peroxidation to occur (Kamel *et al.*, 2011).

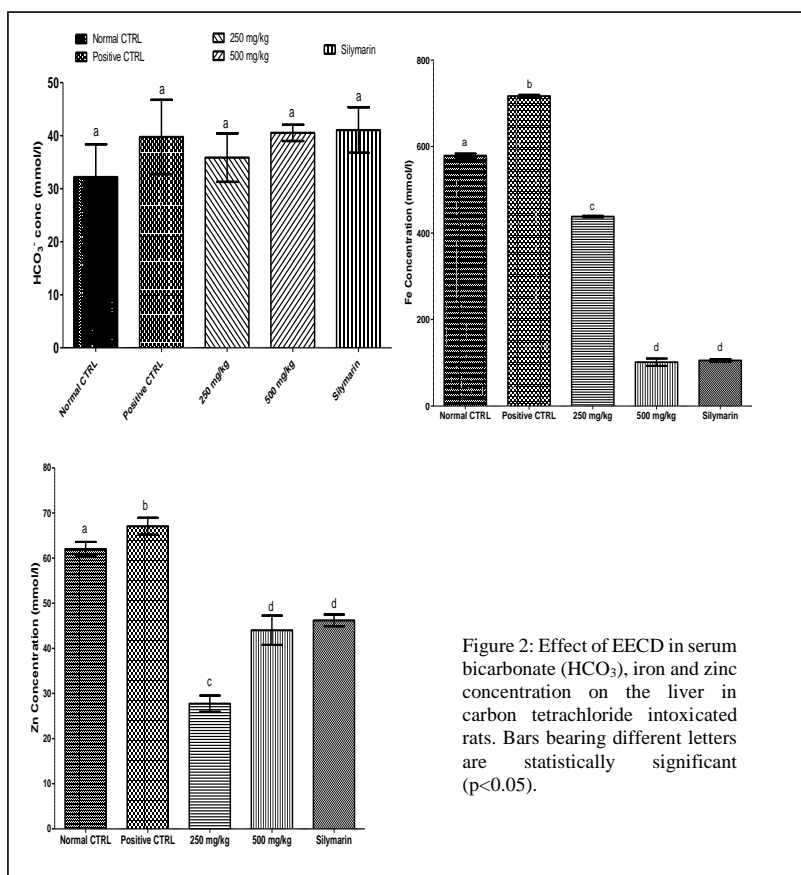


Figure 2: Effect of EECD in serum bicarbonate (HCO₃⁻), iron and zinc concentration on the liver in carbon tetrachloride intoxicated rats. Bars bearing different letters are statistically significant (p<0.05).

Figure 1 reveals that GPx activity of the positive control was reduced significantly ($p > 0.05$) when compared to the normal, EECD treated groups. But there was no significant difference ($p > 0.05$) between the 250 mg/kg b.w and 500 mg/kg b.w of the extract. The most important enzyme for the extraperoxisomal inactivation of H₂O₂ is Glutathione peroxidase (a selenium-containing enzyme) (Kaplowitz *et al.*, 1996). It is observed that a reduction in GSH will in turn reduce the activity of GPx as is evident in the intoxicated but untreated group (positive control). This is because GSH is a substrate for GPx as it provides the electrons needed to reduce H₂O₂ or hydroperoxides (Boelsterli, 2007). Ujowundu, 2017) had reported the *in vitro* evaluation of free radical-scavenging potentials of ethanol extract of *C. dolichopentalum* leaves. Correction

of this anomaly was achieved probably due to the free radical scavenging activity of the extract. Ujowundu *et al* (Ujowundu *et al.*, 2012., Ujowundu *et al.*, 2014), reported similar restoration of GPx activity after intoxication with CCl₄ and subsequent treatment with plant extract.

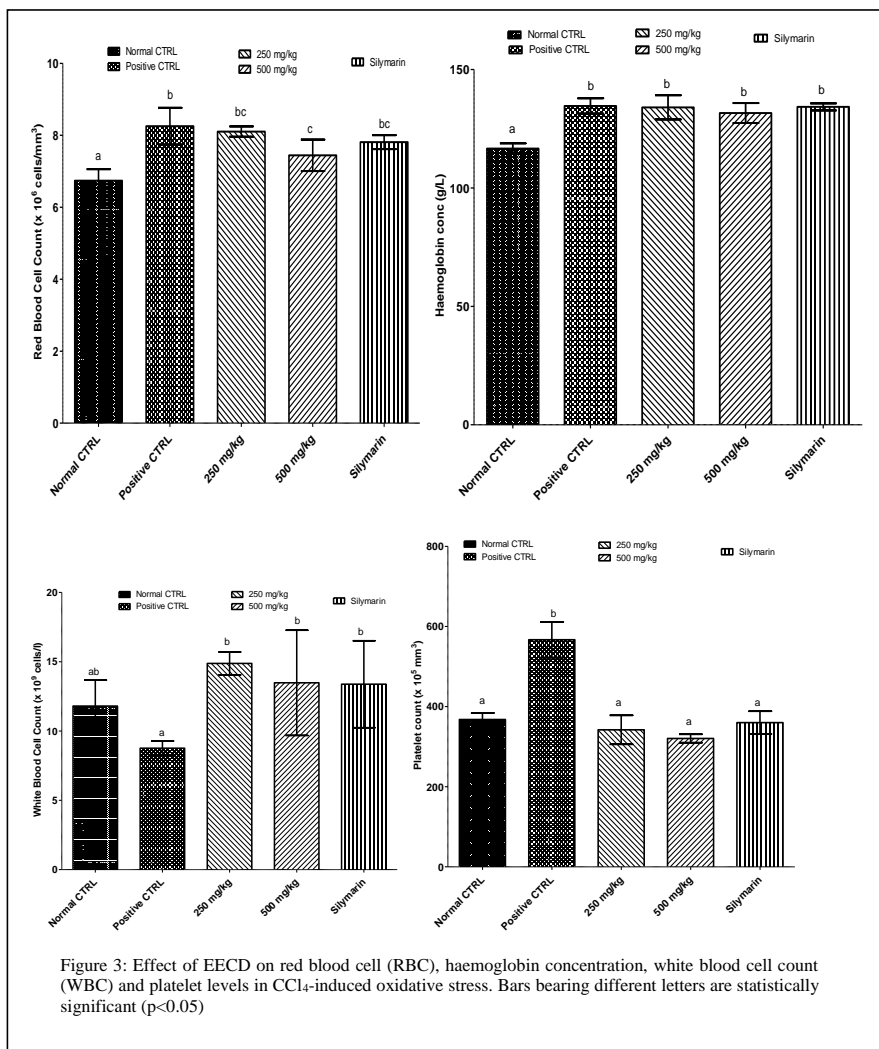


Figure 1 reveals a significant (P<0.05) reduction in SOD activity of the positive control group, when compared to those of the normal control, 250 mg/kg and 500 mg/kg b.w EECD treated group. Also revealed was the non significant difference (p>0.05) between the normal and 250

mg/kg b.w EECD treated group. There are evidences, which support the assumption that removal of superoxide by Superoxide dismutase (SOD) is a detoxication reaction (Gunawardhana *et al.*, 1993). Basically the more susceptible SOD to the preoxidants, is the cytosolic form (Cu, Zn - SOD (Wheeler *et al.*, 2001) whereas the mitochondrial form of SOD can be induced under oxidative stress, the activity of SOD decreased significantly ($P < 0.05$) in the group of rats intoxicated with CCl_4 only. This decrease in SOD activity might be ascribed to the depletion of these antioxidants enzymes. In enzyme catalysed reaction, a short initial pre-steady state or burst, where the enzyme – substrate complex is formed and unbound enzyme decreases, the rate of turnover (enzyme activity) is low in this state, following to the principle of mass conservation. Total enzyme is the summation of the bound and unbound enzyme (Biswagner, 2008). As unbound enzyme reduces, the enzyme activity decreases. This may account for the observed decreases in the activities of SOD and GPx in oxidatively stressed systems.

The pH of blood needs to be maintained around (7.35–7.45), for this is required of life, and relatively small variability in pH value of blood can portend severe metabolic consequences. The main buffers in blood are haemoglobin in the erythrocytes, and bicarbonate ion (HCO_3^-) in the plasma. Bicarbonate buffer system is the primary buffer of blood and also makes up the alkali reserve (Chatterjea and Shinde, 2007). Bicarbonate buffers neutralize strong and non-volatile acids (such as HCl, H_2SO_4 , and lactic acid) entering the Extracellular fluid (ECF), converting these acids into weak and volatile acids; H_2CO_3 thus formed is diffusible, and eliminated by diffusion of CO_2 through the alveoli of the lungs. Figure 2 shows a non-significant ($p > 0.05$) increase in the serum bicarbonate concentration of the positive control group compared to the normal. Also observed was a non significant ($p > 0.05$) difference between the positive control and both the EECD and silymarin treated groups. A significant ($P < 0.05$) increase in $[\text{HCO}_3^-]$ was observed in the CCl_4 treated group which depicts a condition which might lead to alkalosis. The condition is associated with an absolute or relative increase in $[\text{HCO}_3^-]$. Increase in the alkali reserve is the most frequent cause of clinically observed alkalosis. However, maintenance of acid- base balance was not observed in the treated group, but an increase in alkali reserve in a dose dependent fashion was shown in the treated groups. That is to say that, the EECD and the silymarin groups may have initiated a compensatory mechanism that raised pulmonary respiration. This increased ventilation would result in loss of CO_2 and reduction in $[\text{H}_2\text{CO}_3]$ with sequel increase in $[\text{HCO}_3^-]$, increasing NH_3 formation, H^+

excretion compared to K⁺ excretion in distal tubule and HCO₃⁻ reabsorption. The treated groups rather portend exposure to metabolic acidosis (a reduction in plasma level of HCO₃⁻).

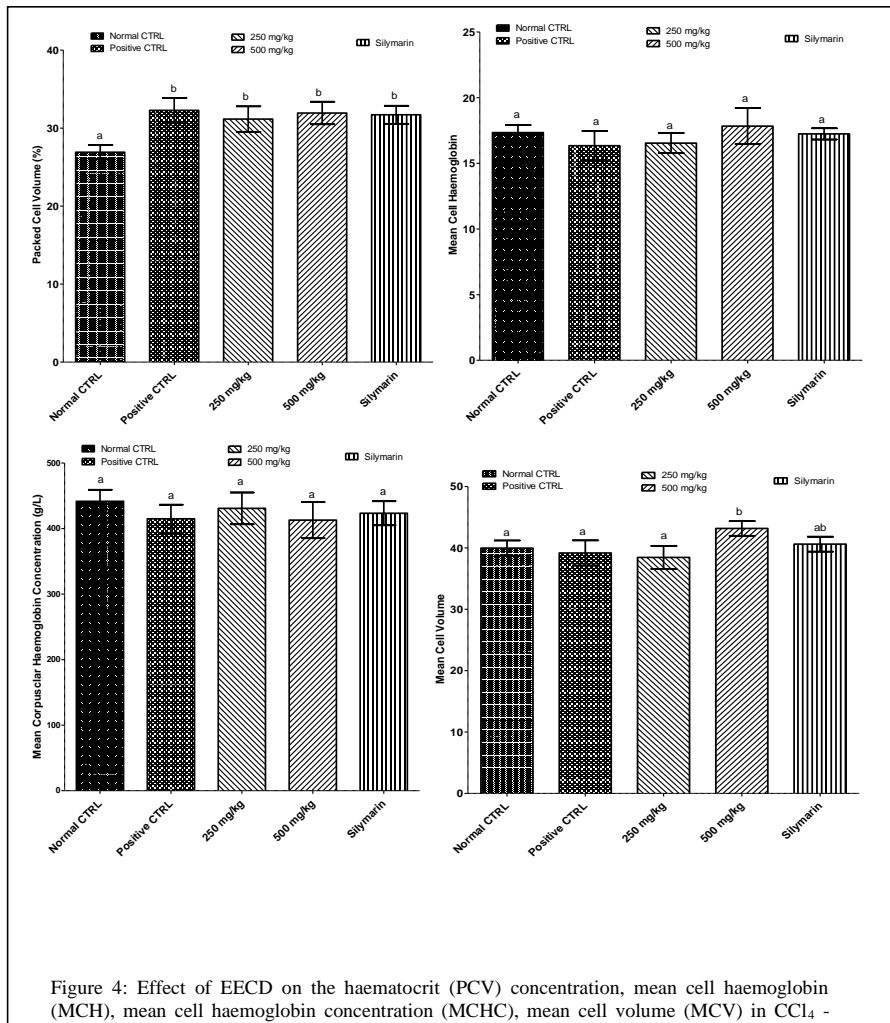


Figure 4: Effect of EECD on the haematocrit (PCV) concentration, mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV) in CCl₄ - induced oxidative stress. Bars bearing different letters are statistically significant (p<0.05)

Result shown in figure 2 reveals a significant ($p < 0.05$) increase in iron concentration of the positive control compared to the normal and both the EECD and silymarin treated groups. Intracellular redox active transition metals notably Fe^{2+}/Fe^{3+} and Cu^+/Cu^{2+} have been implicated in catalyzing oxidative stress due to their Fenton activity (Carr *et al.*, 2000 Boesterli, 2007) Thus, iron chelators like desferroxamine (DFO) preclude binding of H_2O_2 to catalytically active iron and thus inhibit the formation of hydroxyl radicals (Saad *et al.*, 2014). The results as shown in Figure 2 recorded a significant ($P < 0.05$) increase in iron concentration of the positive control group compared to the normal. Within the cells, the highest concentration of free, redox-active iron is found in the lysosomes. If an oxidant damages the lysosomal membrane, then iron leaks out into the cytosol, where it can participate in Fenton reactions and greatly aggravate oxidative damage (Boesterli, 2007).

Treatment with both concentrations of EECD caused a significant ($P < 0.05$) reduction of iron concentrations of the liver in a dose dependent manner. If lysosomal degradation of metalloprotein is inhibited, then oxidant stress induced cell injury can be greatly diminished. Because intracellular iron and other catalytically active transition metals are potentially dangerous to a cell, they are either tightly bound to proteins or low-molecular ligands or sequestered in specific pools (Boesterli, 2007). It appears that EECD enhanced the sequestering of iron judging from the lower concentration of iron in the treated group compared to the normal.

Figure 2 shows that zinc concentration was significantly ($p < 0.05$) elevated in the positive control group compared to the normal and the treated groups at 250 mg/kg b.w and 500 mg/kg b. w including the silymarin group. However a non significant ($p > 0.05$) difference was observed between the 250 mg/kg and 500 mg/kg b.w EECD treated groups. Zinc is an important constituent of proteins, yet would be highly toxic if the free concentrations of zinc in the cell increased beyond a certain critical level. A significant ($P < 0.05$) increase in zinc concentration was recorded in the positive control group (Figure 2) compared to the normal. This indicates that toxicants could interfere with normal zinc homeostasis. Toxicants indirectly displace zinc from certain key proteins or directly by causing bioavailability of exceedingly high intracellular zinc concentration which induces a stress response. This can either activate or disrupt signaling pathways. If via multiple parallel pathways, xenobiotics can shift the overall equilibrium towards an unfavourable response (Andrews, 2000). Increasing cellular $[Zinc^{2+}]$ levels activates the metal sensor, MTF-1 (metal-response-element-binding transcription factor) which

transmigrates in the nucleus and activates genes involved in the homeostasis of metals, such as those of metallothionein (MT), metal efflux carriers and antioxidant enzymes (enzymes involved in GSH synthesis). Pretreatment with the EECD prevented the disruption of zinc homeostasis significantly ($P < 0.05$).

Figure 3 shows a significant ($P < 0.05$) increase in the RBC count of the positive control group compared to the normal, and the 500 mg/kg EECD treated group. However, there was no significant ($p > 0.05$) difference among the positive control, 250 mg/kg EECD treated and silymarin groups. The total mass of red blood cells (RBC) in the circulatory system is regulated within a narrow limit, thus adequate red cells are always available to provide sufficient transport of oxygen from the lungs to the tissues. The cells do not become so numerous that they can impede blood flow. Any condition that causes the quality of oxygen transported to the tissues to decrease ordinarily, increases the rate of RBC production as seen in the CCl_4 -intoxicated rats of positive group in this study. Destruction of major portions of bone marrow by any means: various diseases of the circulation that cause decreased tissue blood flow might result in hypoxia. Hypoxia causes a marked increase in erythropoietin production and the erythropoietin in turn enhances RBC production until the hypoxia is relieved (Alleyne *et al.*, 2008). Compared to the positive control, the treated group was able to offer little protection against secondary polycythaemic condition. The silymarin group however offered a better protection.

Figure 3 shows a significant ($P < 0.05$) increase in the haemoglobin concentration of the positive control, 250 and 500 mg/kg EECD and silymarin treated groups compared to the normal. A non significant ($p > 0.05$) difference was also observed between the positive control, 250 and 500 mg/kg EECD and silymarin treated groups. Compared to the normal control, the positive control group showed a significant ($p < 0.05$) increase in haemoglobin concentration. Many compounds of diverse structure including insecticides, carcinogens and others, when administered to a mammal can result in marked increase in hepatic δ -ALA synthetase (δ -amino laevulinic acid synthetase; the main rate – limiting enzyme in the synthetic pathway of porphorins). This occurs because most of the drugs are metabolized by cytochrome P450. During metabolism of CCl_4 , consumption of haem by cytochrome P450 is greatly increased; this in turn diminishes the cellular concentration of haem, leading to derepression of δ -ALA synthetase with a corresponding increase in ratio of haem synthesis (Chatterjea and Shinde, 2007). Furthermore, the increase in RBC may also result in an increase in the synthesis of hemoglobin to fill the RBC (Guyton and Hall, 2011). Hemoglobin is measured to detect

anaemia and its severity. This is because hemoglobin carries oxygen from the lungs to the tissues.

Figure 4 shows a significant ($P < 0.05$) increase in the haematocrit concentration of the positive control, 250 mg/kg, 500 mg/kg EECD and silymarin treated groups compared to the normal. Also a non significant ($p > 0.05$) difference was observed among the positive, 250 mg/kg, 500 mg/kg EECD and the silymarin treated groups. The haematocrit (HCT) or the packed cell volume (PCV) is used to calculate the mean cell hemoglobin concentration (MCHC) and mean cell volume (MCV). These red cell indices are used in the investigation of anemia when it is not possible to measure hemoglobin, to diagnose polycythaemia vera, and to monitor its treatment. PCV values are increased in all forms of polycythaemia. The PCV values of the intoxicated group are significantly higher than those of the normal control. PCV is that property of the whole blood occupied by red cells. Thus if the positive control group recorded a high RBC and Hb values, it is only rational that the PCV values of the intoxicated group increased. This may not be as a result of healthy condition, but only a response to poor delivery of oxygen from the lungs to the tissues.

Figure 4 shows a non-significant ($p > 0.05$) reduction in the concentration of MCH of the positive control group and 250 mg/kg EECD treated groups compared to the normal. Similarly a non significant ($p > 0.05$) fluctuation in MCH concentration was observed between the normal control group 500 mg/kg EECD and silymarin treated groups. Figure 4 shows a non-significant ($p > 0.05$) reduction in the MCHC concentration of the positive group compared to the normal and a non significant ($p > 0.05$) difference was observed among the EECD at 250, 500 mg/kg b.w and the silymarin treated groups. Figure 4 shows a non-significant ($p > 0.05$) drop in the MCV levels of the positive control group compared to the normal and the 250 mg/kg EECD treated group. However, a significant ($P < 0.05$) increase was observed in the 500 mg/kg EECD treated group compared to the positive control group.

There was also a drop in the MCHC, MCV and MCH values in the positive control group compared to the normal. Low MCHC values are found in iron deficiency anemia and other conditions in which the red cells are microcytic and hypochromic. The MCV values reflect the size of the red blood cell, while MCH and MCHC reflect the hemoglobin content of the blood cells. RBC indices are therefore used to diagnose types of anemia. Anemia is defined based on cell size (MCV) and amount of hemoglobin concentration. The values obtained for RBC count

in the EECD treated rats indicate that the extract tend to balance the irregularities in the RBC cell count.

Figure 3 shows a significant ($P < 0.05$) reduction in WBC count of the positive control group compared to the normal, the EECD at 250 and 500 mg/kg as well as the silymarin treated groups. However, there was a non significant ($p > 0.05$) difference among the EECD treated at 250 mg/kg, the 500 mg/kg and the silymarin groups. The liver harbors a large percentage of resident macrophages (Kupffer cells) and specific resident T cells ($\gamma\delta$ T cells) that have been implicated in certain forms of immune cell – mediated toxicity induced by xenobiotics. The results of this study showed a drop in the white blood cells (WBC) count. A significant decrease was recorded in the WBC of the positive control group compared to that of the normal. The effects of xenobiotics which can modulate the function of immune system at several levels can have two opposing consequences. If xenobiotics push the normal function of the immune system out of balance, the result can be either an immuno-suppression or an immuno-overestimation. Either direction portends danger and result in toxicity; immunosuppression can result in a diminished resistance against infections. CCl_4 is bio-activated in the liver and induces centrilobular necrosis (Laskin and Laskin, 2001). Thus CCl_4 could have suppressed the maturation and development of immune cells, and thus caused an immunosuppression. Phagocytes are involved in the scavenger pathway for cholesterol uptake. Phagocytes detect, oxidize, engulf and digest extra circulating LDL-cholesterol especially when the amount of LDL-cholesterol in the bloodstream is excessive (Schaefer, 2002).

Figure 3 shows a significant ($P < 0.05$) increase in the platelet level of the positive control group compared to the normal, both the EECD and the silymarin treated groups. However, there was a non significant ($p > 0.05$) difference among the normal, the silymarin, the 250 and 500 mg/kg b.w EECD treated groups. Platelet counts are used to investigate abnormal skin and mucosal bleeding, thrombocytopenia and thrombocytosis. This study showed a significant increase in platelet numbers, probably caused by myeloproliferative diseases, such as polycythaemia vera, following tissue injury. The polycythaemic condition agrees with this study since the intoxicated rats (PC) showed increases in RBC, which defines polycythaemia vera. But pretreatment with EECD alleviated this increase. Platelets are tiny cells in the body that help in forming blood cloth. Platelets play important role in blood coagulation, haemostasis and blood thrombus formation. The action of platelets, though quite beneficial in initiating the reaction to injury may actually be harmful in conditions such as coronary occlusion (Cleveland Clinic,

2021). In that case, platelet function may delay reperfusion and help to cause re-occlusion of the vessel.

This study is in line with the work done by Gilberto (Gilberto *et al.*, 2003) who used Gossypitrin against the hepatotoxicity of carbon tetrachloride. Aqueous extract of *Podophyllum hexadrum* and *X. aethiopica* was also shown to exhibit antioxidant against CCl₄ induced stress (Ganie *et al.*, 2011., Adewale *et al.*, 2014).

CONCLUSION

The presence of phytochemically active constituent in *C. dolichopentalum*, suggest that *C. dolichopentalum* could be pharmacological responsible for correcting the imbalances in the disrupted system in the test organisms.

Conflict of interest: The authors declare no conflict of interest

SIGNIFICANCE STATEMENT

This study discovered an increase in alkali reserve in a dose dependent fashion in the groups treated with ethanol extract of *C.dolichopentalum*, this implies that EECD could be employed to neutralize strong and non-volatile acids in cases of increased plasma acidity, Pre-treatment with EECD alleviated an increase in platelet concentration as they can be harmful in conditions such as coronary occlusion. In that case, platelet function may delay reperfusion and help to cause re-occlusion of the blood vessels.

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The authors declare no conflict of interest

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CHEMICAL COMPOSITION AND EFFECT OF METHANOL EXTRACT OF *Loranthus micranthus* ON THE ELECTROLYTE INDICES OF CASTOR OIL INDUCED-DIARRHOEIC RAT

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ABSTRACT

Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). The phytochemical compositions of methanolic extract of *Loranthus micranthus* and the effect on electrolyte indices of castor oil induced-diarrhoeic rat was evaluated. A total number of twenty five rats were randomly divided into five groups comprising of five rats per group. Group 1, Diarrhoeic rats induced with castor oil and normal saline (2ml/kg) only, Group 2, Castor oil induced diarrhoeic rats treated with the standard drug (loperamide), while Groups 3, 4 and 5 were induced with castor oil and administered 100, 500 and 1000mg/kg of *Loranthus micranthus* leaf extract orally using oropharyngeal cannula. After six hours of administration, the rats were anesthetized and blood samples were obtained for electrolytes studies. Data obtained was analyzed using GraphPad and result expressed as mean \pm standard error of the mean. One-way analysis of variance (ANOVA) following Turkey post hoc test was used for data comparison. The extract showed the presence of flavonoids, tannins, alkaloids, saponins, glycosides, minerals such as potassium, calcium, sodium, magnesium, copper, zinc, iron and biomolecules. There was a significant ($p < 0.05$) decrease in the level of potassium in groups 4 and 5 when compared with the untreated (negative control) group. The plant extract was more effective at dose level 1000mg/kg. The study shows that the ameliorative potential of *Loranthus micranthus* on electrolyte imbalance caused by castor oil induced diarrhoea.

Keywords: *Loranthus micranthus*, Electrolyte Indices, Castor Oil Induced-Diarrhoeic Rat

INTRODUCTION

Diarrhoea is a disorder characterized by discharge of semisolid or watery faecal matter from the bowel three or more times in a day (Suleiman *et al.*, 2008). It involves increase in fluidity, volume and frequency of bowel movements, wet stools and abdominal pain, accompanied by increased secretion and decreased absorption of fluid, and loss of water and electrolyte (Navaneethan and Giannella, 2008). Diarrhoea can be very serious in infants and elderly people because of the risk of severe dehydration which can be fatal. Global statistics estimate that in 2016, diarrhoea was the eighth leading cause of death among all ages and the fifth leading cause of death among children younger than 5 years (GBD, 2016).

Antimotility agents used in the management of diarrhea have emerged but none has found a place in the routine management of diarrhea due to their side effects after prolonged use (Gale, 2009). The search for an alternative remedy especially for developing countries from traditional herbal medicines was introduced by World Health Organization to overcome the side effects of antimotility agents. Several plants have been reported to be useful in the management of diarrhea (Musa *et al.*, 2008; Osarenwindae *et al.*,

2009). Mistletoe, which consists of about 1400 species around the world, belongs to the class Magnoliopsida, the largest family of this mistletoe is *Loranthaceae* which has 75 genera and over 900 species (Judd *et al.*, 2002). Among them, six major genera are found in Nigeria, namely: *Tapinanthus*, *Agelanthus*, *Globimetula*, *Phragmanthera*, *Englerina* and *Loranthus* (Omolaja and Gamaye, 1998). Local names of mistletoe are as follows: 'afomo' (yoruba speaking area in Nigeria), 'apari (igbo)' and 'Kauci' (hausa) (Oluwole *et al.*, 2013).

This plant, *Loranthus micranthus* is a parasitic plant because it grows on various host trees, example *Pentaclethra macrophylla*, *Kola accuminata*, *Baphia nitida*, *Persea americana*, *Azdirachta indica* and *Irvingia gabonensis* (Osadebe and Ukwueze (2004). It is used in folkloric medicine in the treatment of epilepsy, hypertension, headache, infertility, cancer and rheumatism, and has been reported to have antidiabetic, antimicrobial (Osadebe and Ukwueze, 2004), and immunomodulatory activities (Osadebe *et al.*, 2006). This study is aimed at determination of the phytochemical components of methanol extract of *Loranthus micranthus* and its effects on electrolyte balance of castor oil induced diarrhea rats.

MATERIALS AND METHODS

Plant material

The leaves of *Loranthus micranthus* were obtained from Umuahia, identified by a Botanist, Dr. Garuba Omosun, Department of Plant Science and Biotechnology, College of Natural Sciences; Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Preparation of extract

The leaves were washed and air dried at room temperature for 7 days after which they were ground to coarse powder using an electric blender (model MS-233, China). Some quantities of the flour were used for phytochemical, proximate and mineral analysis while the remaining quantities of the flour (2 kg) were extracted for 48 h with methanol in a Soxhlet extractor as described by Jensen (2007). The extract was collected and dried at low temperature (40°C) to obtain a resinous dark-green extract which was used for animal experiments and for GC-MS analysis.

Phytochemical analysis

The alkaloid content of the flour was determined using the method of (Harborne, 1973). Tannins and saponins were determined using the method of AOAC (1990). Flavonoids were determined using the method of Boham and Kocipai (1994). The method of Sofowora (1993) was used for the determination of cardiac glycosides and oil. Terpenoids were assayed for following the method of Ferguson (1956).

Proximate analysis

The method of AOAC (1990) was used for the determination of moisture, ash, protein, lipid, and crude fiber contents of the flour. Total carbohydrate content was obtained by difference.

Mineral analysis

An Atomic Absorption Spectrophotometer (Analyst 200, Perkin Elmer, Waltham, MA, USA) was used to analyze the Ca, Mg, Zn, Cu, Pb and Fe contents of the flours; the flame photometric method was used for the analysis of sodium and potassium (AOAC, 1990); the Molybdate method (Onwuka, 2005) was used for the analysis of phosphorous while the method of AOAC (1990) was used for the analysis of Mn contents of the flour.

Animal experiments

Twenty five (25) mature inbred apparently healthy male and female albino rats of the wistar strain (100-150g) were procured from the animal house of the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The animals were acclimatized to their feed (Vital feed®, Nigeria) and water (which they had access to *ad libitum*) for two

weeks before commencement of the experiment. The study protocol was approved by the Department of Biochemistry, Research Ethics Committee, Michael Okpara University of Agriculture, Umudike. The animal study was carried out in accordance with the established institutional guidelines and the NIH guidelines on the use of experimental animals.

Induction of diarrhoea and experimental procedure

Diarrhoea was induced in rats by oral administration of castor oil (2 ml/kg) as previously described by (Awouters *et al.* 1978) as modified by (Mukherjee *et al.* 1998). The rats used were fasted for 12 hours prior to the experiment (during which time the animals were given free access to drinking tap water). Two hours before beginning the experiment, water was removed and the rats were divided into five groups of (five) 5 animals each. Diarrhoea was induced to all experimental animals. Groups 1 served as negative control and was given Castor oil and normal saline (2ml/kg), Group 2 animals served as the positive control and were treated with loperamide, used as standard drug (5 mg/kg). Group 3 animals were treated with a low dose of *Loranthus micranthus* plant extract (100mg/kg), Groups 4 animals received a higher dose of *Loranthus micranthus* plant extract (500mg/kg), while Groups 5 received the highest dose of *Loranthus micranthus* plant extract (1000mg/kg).

All administration was done orally using oropharyngeal cannula, after six hours of administration, the rats were anesthetized with 2% sodium pentobarbital (75 mg/kg) intraperitoneally. Venous blood was obtained via the orbital and transferred into plain tubes for electrolytes studies.

Electrolytes studies

Sodium, potassium and chloride were estimated with Easylyte® analyzer Medica Corporation, Bedford, USA.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism Version 5 for windows (GraphPad® Software, San Diego, CA, USA). Results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) following Turkey post hoc test was used for data comparison. Results were considered significant when $P < 0.05$.

RESULTS

The result obtained from the phytochemical composition analysis, mineral composition analysis, proximate analysis of the methanol extract of *Loranthus micranthus* leaves and the electrolyte profile analysis of castor oil induced-diarrheic albino wistar rat are summarized in the tables below. Table 1 shows the phytochemical component of the plant extract and their concentrations, Table 2 shows

the mineral component present in the plant extract at mg/100g, Table 3 shows the proximate composition of the extract at mg/100g while, Table 4 shows the result of the electrolyte indices on the castor oil induced diarrheic-albino wistar rat. As shown in the table, there was an increase in sodium and chlorine and significant ($p < 0.05$) decrease on the level of potassium when compared with the untreated (negative control group) after treating with standard drug, extract at dose level 100mg/kg, 500mg/kg and 1000mg/kg. There was change on the electrolyte profile of the experimental rat when it was treated with 1000mg/kg of the plant extract.

Table 1: Phytochemical contents of *Lmicranthus*

Phytochemical	Percentage composition
Alkaloid	++
Tannin	++
Saponin	++
Flavonoid	+++
Cardiac glycosides	+
Terpenoids	+

Values in the table

are reported as means \pm SEM of triplicate experiments

Key:

+ = low concentration

++ = high concentration

+++ = very high concentration

Table 2: Mineral contents of *L micranthus*

Mineral	Composition (mg/100 g)
Calcium	700.15 \pm 0.01
Magnesium	395.21 \pm 0.02
Potassium	987.81 \pm 0.03
Sodium	20.16 \pm 0.01
Zinc	3.02 \pm 0.09
Iron	110.25 \pm 0.03
Copper	2.21 \pm 0.06

Values in the table are reported as means \pm SEM of triplicate experiments

Table 3: Proximate composition of *L micranthus*

Mineral	Composition (mg/100 g)
Moisture Content	7.15 \pm 0.01
Crude Fat	3.25 \pm 0.02
Crude Protein	13.31 \pm 0.13
Crude Fibre	14.66 \pm 0.01
Total ash	8.51 \pm 0.30
Carbohydrates	53.12 \pm 0.31
Energy value (kcal/100g)	310.97 \pm 0.19

Values in the table are reported as means \pm SEM of triplicate experiments

Table 4: Effect of *L micranthus* leaf extract on markers of renal function in rats

Groups Parameters	Control (Negative)	2 (100mg/kg)	3 (100mg/kg)	4 (500mg/kg)	5(1000mg/kg)
Sodium (mEq/L)	102.36 \pm 1.00	125.25 \pm 0.96*	102.50 \pm 1.29	105.73 \pm 3.73	110.25 \pm 0.96
Potassium (mEq/L)	10.38 \pm 0.49	5.30 \pm 0.48*	9.87 \pm 0.16	8.50 \pm 0.33*	7.10 \pm 0.08*
Chloride (mEq/L)	97.86 \pm 1.03	127.25 \pm 1.08*	98.50 \pm 1.73	107.50 \pm 5.07	113.50 \pm 4.20*

Values are mean \pm standard error of the mean (SEM). * $p < 0.05$ versus negative control (Group 1)

DISCUSSION

The result of the mineral compositions of the leaf extract showed the presence of potassium, which was the most abundance mineral element followed by calcium, magnesium, iron, zinc and copper. Potassium is a principle component of the ECF and contribute to electrolyte balance, calcium is reported to be essential in blood clot formation and cofactor in some enzyme catalysis (Vasudevan *et al.*, 2011). Magnesium helps to maintain osmotic pressure, iron is essential for blood formation (Thomas and Krishnakumari, 2015) and zinc is vital for cellular differentiation, immunity and protein synthesis (Pathak and Kapil, 2004). The presence of these mineral element also can also be one of the reason the plant is useful in the management and treatment of various diseases.

The proximate analysis of the leaf extract showed the presence of carbohydrates which is the most abundant of the macromolecules and functions to supply energy to the brain, muscles and the blood (Emebu and Anyika, 2011). Protein was also present and functions in maintaining body development, formation of hormones, enzymes, maintenance of fluid balance, immunity (Emebu and Anyika, 2011). Crude fiber functions to aid absorption (Abolaji *et al.*, 2007). Ash content was found to be 8.51mg/100g, this suggests a high content of minerals in the plant. Mineral matter may also contribute to pharmacological effect of the leaf extract.

Electrolyte disturbances are involved in many disease processes, and are an important part of patient

management in medicine (Balci *et al.*, 2013). Common causes of hyponatremia may be diarrheal illness, intoxication via excessive consumption, and enemas (Walls *et al.*, 2018). As potassium levels get higher (hyperkalemia), individuals may begin to experience nausea, vomiting, and diarrhea (Walls *et al.*, 2018). Our results show that on castor oil induced-diarrheic rat had significantly decreased ion concentrations of potassium when compared to untreated (negative control). There was also significant ($p < 0.05$) increase in the level of sodium and chloride and significant ($p < 0.05$). These changes were most significantly observed at the dose level of 1000mg/kg. Serum electrolytes in patients with diarrhea show hyponatremia, hypokalemia,

hypochloremia, and metabolic alkalosis (Bockenbauer and Zieg, 2014). Hence from the result it was observed that following administration of extracts from *Loranthus micranthus*, the level of sodium and chloride was significantly increased and potassium decreased. The administration of the extract at dose level 1000mg/kg significantly ($p < 0.05$) increased the level of sodium, chloride and decreased the level of potassium but did not completely ameliorate the electrolyte balance caused by diarrhea.

CONCLUSION

The study shows that the extracts from *Loranthus micranthus* had ameliorative effect on electrolyte imbalance caused by castor oil induced diarrhea.

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EFFECT OF n-HEXANE EXTRACT OF *Emilia sonchifolia* LEAF ON SODIUM ARSENITE INDUCED HEPATOTOXICITY IN ALBINO RATS

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Abstract

The present study was conducted to investigate the effect of n-hexane extract of *Emilia sonchifolia* leaf on sodium arsenite-induced hepatotoxicity in rats. 28 animals were divided into four groups of 7 rats each. Group 1 was used as the control, group 2 was orally treated with 250mg/kg body weight *Emilia sonchifolia* extract (ESE), group 3 was intraperitoneally administered 2.5mg/kg body weight sodium arsenite (Na₂AsO₃) and group 4 was administered 2.5mg/kg body weight Na₂AsO₃ and 250mg/kg body weight ESE. The respective doses were administered to the rats daily for 14 days. The result of group 3 showed that sodium arsenite significantly increased the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). Malondialdehyde (MDA) concentration significantly increased while reduced glutathione (GSH) concentration significantly decreased. The activities of catalase (CAT) and glutathione peroxidase (GPx) were significantly decreased compared to the control. In group 2 (ESE alone) the activities of ALT, AST, and ALP significantly increased while there was a reduction in GGT activity. MDA concentration was increased non-significantly while GSH level increased significantly. Treatment with Na₂AsO₃ and ESE in group 4 significantly reduced the activities of ALT, AST, ALP, and GGT. MDA concentration decreased while the GSH level increased significantly. There was also a significant increase in the CAT activity when compared with Na₂AsO₃ only treated group. This suggests that *Emilia sonchifolia* showed some level of protection against sodium arsenite-induced liver injury in rats though some level of toxicity was observed at the dose tested.

Keywords: *Emilia sonchifolia*, hepatotoxicity, sodium arsenite, malondialdehyde.

INTRODUCTION

Inorganic arsenic compounds are heavy metal toxicants that have been recognized as human carcinogens (Jan *et al.*, 2015; Palmieri *et al.*, 2015). Among them is sodium arsenite which is the most hazardous inorganic arsenic compounds for human and animal health (Chung *et al.*, 2014). Human populations are exposed to arsenic and its compounds through occupational or environmental processes (Ramanathan *et al.*, 2003). Arsenic is used in industrial processes such as manufacturing of glass, semiconductors, various dyes, and additives to metal alloys. They are also used as insecticides, herbicides, fungicides, and algacides (El-Demerdash *et al.*, 2009). Exposure of humans to arsenic is associated with several diseases such as diabetes (Lai *et al.*, 19994), hypertension (Chen *et al.*, 1995), and cancer (Chen *et al.*, 1990). The toxicity of sodium arsenite involves the generation of reactive oxygen species (ROS) such as peroxy radicals, superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide within cells (Ellinsworth, 2015; Flora *et al.*, 2007). These ROS inhibit the activities of enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) leading to alterations in cell's antioxidant defences, thereby resulting in oxidative stress or disturbed antioxidant/pro-oxidant ratio (Liu *et al.*, 2001). Several studies have demonstrated that the liver is the primary organ for

arsenic metabolism (Hughes *et al.*, 2003). Antioxidants can donate electrons and scavenge free radicals resulting in the prevention of cell injuries (Sacidnia *et al.*, 2013). Goudarzi and colleagues reported that ROS produced by arsenic reduces the cellular content of GSH and the activity of antioxidant enzymes including SOD, CAT, GPx, glutathione reductase, and glutathione-S-transferase (GST) (Goudarzi *et al.*, 2018). Humans have been using herbs and plant products in the management of diseases since time immemorial. Frequent consumption of foods that are of plant origin has numerous health benefits rooted in their physiological effects because of their phytochemical and nutritional constituents (Hunter *et al.*, 2002). *Emilia sonchifolia* (Family: *Asteraceae*) commonly known as lilac tassel is an annual weed seen in most tropical and subtropical regions worldwide. It is used in ethnomedicine against inflammation, eyesores, convulsion, cuts, wounds rheumatism, and insect bites (Sophia *et al.*, 2011). Sophia *et al.* (2011) also reported the protective effect of *Emilia sonchifolia* herb against high protein diet-induced oxidative stress in the liver of rats. The present study evaluated the hepatoprotective effect of n-hexane extract of *Emilia sonchifolia* on sodium arsenite-induced hepatotoxicity in male rats to understand its role in the management of hepatic disorders and help in the validation of its hepatic protective claims by local traditional medicine users.

Materials and methods

Collection of plant materials

Fresh leaves of *Emilia sonchifolia* was obtained from Umuariaga in Ikwuano Local government area of Abia State and was authenticated by Dr N. K. Ibe of the Forestry and Environmental Management Department, Michael Okpara University of Agriculture, Umudike. The leaves were washed with clean water, air-dried and milled into a coarse powder.

Preparation of extract

The powdered plant sample was soaked in n-hexane for 48 hours with constant stirring at room temperature. The extract was filtered first with a muslin cloth and then filtered using Whatman filter paper number 42. The filtrate was concentrated by evaporation using a water bath at 40°C and was stored in a refrigerator at 4°C until when needed.

Animals

Twenty-eight male albino rats weighing 34-69.0g were obtained from the animal house of Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were housed in standard cages and were acclimatized for two weeks at the animal house of the Department of Biochemistry Michael Okpara University of Agriculture Umudike, Abia State. They were maintained under standard conditions of 12 hours light and dark cycle and were given feed and water *ad libitum*.

Experimental design

The animals were randomly divided into four groups of seven rats each. Group 1 was used as the control and was given distilled water only. Group 2 was orally treated with 250mg/kg body weight *Emilia sonchifolia* extract (ESE), group 3 was intraperitoneally administered 2.5mg/kg body weight sodium arsenite (Na₂AsO₃) and group 4 was administered 2.5mg/kg body weight Na₂AsO₃ and 250mg/kg body weight ESE. The respective doses were administered to the rats daily for 14 days. Twenty-four hours after the last treatment, all the rats were sacrificed by cervical dislocation, and blood was collected by cardiac puncture into a non-heparinized sample bottle. The blood was allowed to clot for about two hours and was centrifuged at 3000 g for 30 minutes. The supernatant (serum) was aspirated with Pasteur pipettes into a clean tube and was used for biochemical assay.

Determination of serum liver enzymes activities

ALT, AST, GGT, and ALP activities were assayed according to the methods of Reitman and Frankel as outlined in the Randox kit (Reitman *et al.*, 1957).

Determination of antioxidant enzymes activities

Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as contained in the Randox kit. The activity of catalase was assayed

by the method of Sinha (1972). Glutathione peroxidase activity was assayed according to the method of Paglia and Valentine (1967).

Estimation of reduced glutathione

The reduced glutathione level was determined by the method of Exner *et al.* (2000).

Assessment of lipid peroxidation

Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al.* (1993).

Statistical analysis

The results obtained were statistically analyzed using the Statistical Product and Service Solutions 20.0 and the results expressed as mean ± standard deviation. The mean was compared for statistical significance using Duncan's multiple comparison *post hoc* test (least significant difference). Significant differences in the result were established by one-way analysis of variance (ANOVA), and the acceptable level of significance was $P < 0.05$ for all the results.

RESULTS

Liver enzyme assay

As shown in Table 1, exposure to Na₂AsO₃ alone induced a significant ($p < 0.05$) increase in the activity of AST, ALT, ALP, and GGT when compared with the control. When rats were exposed to Na₂AsO₃ and ESE, activities of these enzymes decreased significantly ($p < 0.05$) compared to sodium arsenite alone treated group. ESE alone increased the activities of ALT, AST, and ALP while GGT activity was significantly decreased compared to the control.

Assessment of lipid peroxidation

Malondialdehyde (MDA) level was used to measure the extent of lipid peroxidation in the liver of rats. The result shows that the MDA level in the serum of rats exposed to Na₂AsO₃ alone was significantly ($p < 0.05$) increased compared to the control. Treatment with Na₂AsO₃ and ESE significantly ($p < 0.05$) decreased the MDA level in comparison with Na₂AsO₃ alone treated group. MDA level increased non-significantly ($p < 0.05$) in the group treated with ESE alone compared with the control as shown in Table 2.

Effect of ESE on Na₂AsO₃ induced changes in reduced glutathione (GSH) concentration

The result of Table 3 shows a significant ($p < 0.05$) decrease in GSH level of rats exposed to Na₂AsO₃ compared to the control. Treatment of Na₂AsO₃ exposed rats with ESE increased GSH levels significantly ($p < 0.05$). ESE alone also increased the GSH level significantly compared to the control.

Effect of ESE on Na₂AsO₃-induced changes in antioxidant enzyme activity

As shown in Table 4, Na₂AsO₃ significantly (p<0.05) decreased the activities of CAT and GPx. There was a non-significant decrease in the activity of SOD compared to the control while treatment with ESE significantly (p<0.05) increased the activity of CAT

while SOD and GPx activities were slightly increased compared to the group treated with Na₂AsO₃ alone. ESE alone had a significant effect on CAT and non-significant effect on SOD and GPx activities.

Table 1
Effect of sodium arsenite and *Emilia sonchifolia* leaf extract on serum AST, ALT, ALP, and GGT activity of control and treated rats.

GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
GRP1 (control)	65.51±5.36 ^a	36.70±3.82 ^a	23.94±1.03 ^a	2.59±0.28 ^a
GRP2 (ESE)	76.58±5.64 ^b	47.56±2.79 ^b	36.40±3.58 ^c	1.90±0.20 ^b
GRP3 (Na ₂ AsO ₃)	87.98±8.96 ^c	70.68±6.77 ^c	32.00±3.03 ^b	4.70±0.24 ^c
GRP4 (ESE+Na ₂ AsO ₃)	68.32±6.97 ^{ab}	44.10±5.13 ^b	24.20±1.30 ^a	2.55±0.24 ^b

* Values are expressed as means± standard deviation; n = 7 for each treatment group
* Values with a different superscript in the same rows are significantly different, p<0.05.

Table 2
Effect of sodium arsenite and *Emilia sonchifolia* leaf extract on serum malondialdehyde levels of control and treated rats.

GROUPS	MDA (mg/ml)
GRP1 (control)	0.42±0.07 ^a
GRP2 (ESE)	0.61±0.26 ^a
GRP3(Na ₂ AsO ₃)	1.42±0.15 ^b
GRP4(ESE+Na ₂ AsO ₃)	0.59±0.07 ^a

* Values are expressed as means± standard deviation; n = 7 for each treatment group
* Values with the same superscript in the same rows are not significantly different, p<0.05.

Table 3: Effect of sodium arsenite and *Emilia sonchifolia* leaf extract on serum glutathione activity of control and treated rats.

GROUPS	GSH (mg/dl)
GRP1 (control)	1.31±0.13 ^b
GRP2 (ESE)	1.63±0.39 ^c
GRP3(Na ₂ AsO ₃)	0.87±0.20 ^a
GRP4(ESE+Na ₂ AsO ₃)	1.29±0.06 ^b

* Values are expressed as means± standard deviation; n = 7 for each treatment group
* Values with the same superscript in the same rows are not significantly different, p<0.05.

Table 4: Effect of sodium arsenite and *Emilia sonchifolia* leaf extract on serum SOD, CAT, and GPx activity of control and treated rats.

GROUPS	SOD (U/L)	CAT (U/L)	GPx (U/L)
GRP1 (control)	1.09±0.04 ^a	8.32±0.51 ^c	22.13±1.14 ^b
GRP2 (ESE)	1.09±0.44 ^a	3.50±0.32 ^b	21.55±0.85 ^b
GRP3 (Na ₂ AsO ₃)	1.04±0.07 ^a	0.94±0.14 ^a	16.28±0.59 ^a
GRP4 (ESE+Na ₂ AsO ₃)	1.06±0.03 ^a	3.22±0.30 ^b	16.97±0.51 ^a

* Values are expressed as means± standard deviation; n = 7 for each treatment group
* Values with the same superscript in the same rows are not significantly different, p<0.05.

Discussion

Arsenic is commonly found in the environment and has been suggested to be very toxic and carcinogenic. The liver is the primary site for arsenic metabolism. In the liver, arsenic is methylated to methylarsonic and dimethylarsenic acid (DMA) which reacts with molecular oxygen to form dimethylarsenic radical and superoxide anion. Dimethylarsenic radical reacts with O₂ to form dimethylarsenic peroxy radical (Gholamine *et al.*, 2019). Damage of liver cells could result to increase in the concentration and subsequent activities of liver enzymes in the extrahepatic tissues as seen in various hepatic disorders. We investigated the effect of n-hexane extract of *Emilia sonchifolia* leaf on sodium arsenite-induced hepatotoxicity in Wistar rats. Our findings show that Na₂AsO₃ significantly increased the activities of serum liver function marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) compared to the control. This is an indication that Na₂AsO₃ caused a loss of functional integrity of the hepatocellular membrane and

subsequent increase in membrane permeability led to the cellular leakage of these enzymes into the blood and consequently elevated levels in systemic circulation in line with findings of Turk *et al.*, (2018) and Gholamine *et al.*, (2019). Treatment of rats with Na₂AsO₃ and ESE led to a significantly reduced ($p < 0.05$) activities of these enzymes in comparison to the group treated with Na₂AsO₃ alone. This indicates that the extract has a protective effect on Na₂AsO₃-induced hepatotoxicity. Arsenic-induced oxidative stress is by the disruption of the prooxidant-antioxidant balance (Yamanaka *et al.*, 1991). Sodium arsenite in this study significantly ($p < 0.05$) increased the level of malondialdehyde (MDA) compared to the control. This is an indication of lipid peroxidation. Treatment with the extract significantly reduced the MDA level compared to Na₂AsO₃ alone treated group. Reduced glutathione (GSH) acts as a nonenzymatic antioxidant by direct interaction of its thiol group with ROS or it can serve as a cosubstrate for glutathione-S transferase (GST) in biochemical conjugation of xenobiotic (Oyagbemi *et al.*, 2010). In this study, treatment with Na₂AsO₃ alone resulted in decreased GSH levels. This indicates the overutilization of GSH during oxidative stress in the liver because GSH acts as an electron donor for the conversion of arsenic (V) to arsenic (III). GSH may also be oxidized due to interaction with free radicals generated by sodium arsenite resulting in the oxidation of thiol group of GSH to GSSG which is the Oxidized form of glutathione (El-Dermerdash *et al.*, 2009). Treatment

with ESE increased the level of GSH. Enzymatic antioxidants such as SOD, CAT, and GPx play a major role in protecting the body against oxidative stress by inactivating ROS to keep only a small amount necessary to maintain normal cell function. Oxidative stress occurs when the production of ROS overwhelms the defensive mechanism of these antioxidants (Oyagbemi *et al.*, 2010). SOD converts superoxide anion to hydrogen peroxide (H₂O₂) which is then removed by catalase. Catalase converts H₂O₂ to O₂ and H₂O (Usoh *et al.*, 2005). The decrease in the activities of SOD, CAT, and GPx suggests the accumulation of superoxide anion, H₂O₂, and OH⁻. Treatment with ESE increased the activities of these enzymes. This suggests that the extract can protect the liver against sodium arsenite-induced oxidative damage. Administration of the extract alone significantly increased the activities of AST, ALT, and ALP. This suggests possible toxicity at the dose tested.

Conclusion

The findings from our study show that ESE at the dose tested showed some level of toxicity in the liver of rats that took the extract alone and appeared to offer hepatoprotection in rats that were exposed to sodium arsenite and were treated with the extract. However, further study is needed to clarify the possibility of toxicity at high doses.

Conflicts of Interest

No conflicts of interest were disclosed by the authors

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WIS-BPH 35

IN- VITRO EVALUATION OF THE EFFECT OF AQUEOUS EXTRACTS OF DIFFERENT SPECIES OF GARDEN EGG (SOLANUM SPP) FRUITS ON ENZYMES LINKED TO ERECTILE DYSFUNCTION IN MALE WISTAR RAT

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ABSTRACT

Garden egg which belongs to the family of Solanum spp has been found useful in ethnomedicine. This preliminary study investigates the possible ameliorative potential of garden egg fruits consumption on erectile dysfunction using an in vitro model. Fresh matured varieties of the garden egg [S. aethiopicum, (PGW), S. kumba (PG) and S.gilo (PW)] which could be found at all seasons were used to determine the inhibitory activity of the aqueous extracts (w/v) on arginase an enzyme associated with erectile dysfunction as well as angiotensin-1-converting enzyme (ACE) linked to hypertension using wistar rat's penile tissue. The phytochemical screening and minerals analyses were also determined the vitamin c content, iron (II) chelation, ferric reducing antioxidant property (FRAP), total antioxidant property [2,2'-azinobis-3-ethylbenzotiazoline-6-sulfonicacid (ABTS*) and scavenging activity using [1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO*) and hydroxyl (OH*)], copper-induced, iron-induced and quinolinic-induced lipid peroxidation were assessed. The results revealed that the garden egg fruits extract scavenged the free radical species as well as reduced malondialdehyde (MDA) produced during peroxidation in the male tissue with an increasingly antioxidative potential in ascending order of the fruit varieties PW>PGW>PG. However, S. aethiopicum, (PGW) and S. kumba (PG) exhibited better inhibition of arginase and ACE enzymes activities. In conclusion the presence of essential minerals as well as other phytochemicals with antioxidant property could be the mechanism in which erectile dysfunction and hypertension could be managed using the garden egg fruits

Keywords: garden egg; antioxidant; erectile dysfunction; hypertension

INTRODUCTION

Erectile dysfunction has been linked to infertility with approximately 50% incident rate according to Dessars and Cochaux, (1999). This male infertility affected 10 to 15% of couples in the world, with elevated data from Africa of 30 to 50% according different regions in Africa (Dessars and Cochaux, 1999). Single-ingredient monotherapeutic drugs(s) such as vigra for treating erectile dysfunction and other related diseases fail to address the heterogeneity of complications associated with these drugs which also come with its side effects such as increased in cardiac function and hypertension (Nwanna et al., 2015). Currently scientists are searching for a single constituent or product from plant-based to treat and in the management of erectile dysfunction with multiple therapeutic functionality in order to combat the multi-interrelated ailments as well as counteract the need of intake of multiple drugs. Nutraceuticals from garden egg fruits with the least adverse effects is effective in promoting holistic healthy state of being (Nwanna et al.,2019). Solanum spp from tropical origin predominate in West Africa such as kumba, aethiopicum, and gilo were reported by Nwanna et al. (2016) to have rich polyphenol content which could be used in the treatment of type 2 diabetes mellitus and peptic ulcer (Anosike et al., 2011). However, this

present study focuses on the phytochemicals, mineral composition, antioxidant activity and also to assess the in-vitro effect of these garden eggs fruits Solanum kumba, aethiopicum, and gilo water extracts on enzymes linked to erectile dysfunction and hypertension using rat's penis tissue.

MATERIAL AND METHODS

Sample Collection and Preparation

Matured fruits of garden egg Solanum varieties (gilo, aethiopicum and kumba) were gotten from Erekesa market Akure in Ondo state, Nigeria. The identification of the samples was carried out at the Crop, Soil, and Pest management (CSP) Department, Federal University of Technology, Akure, Nigeria the herbarium is IFE-17718 for Solanum kumba, IFE-17719 for Solanum aethiopicum and IFE-17720 for Solanum gilo. The eggplant fruits were washed air dried, sliced into tiny piece and ground into fine powder and thereafter lyophilized in order to preserve freshness and subsequently kept for further analysis. All the chemicals used were with analytical grade.

Methodology

Phytochemical screening of the garden egg fruits (Dried weight) such as saponin, tannin, flavonoid and alkaloid were carried out using the method of

AOAC,(1990) and Odebiyi and Sofowora, (1999).

Mineral analyses

The minerals namely, Ca, Cu, Mg, Fe and Zn, were determined in aliquots of the solutions of the ash by established AAS procedures using a Perkin-Elmer atomic absorption spectrophotometer (model 372) Perkin-Elmer (1982) while Na and K were determined using flame photometry. The values reported are means of triplicate readings of the samples with their standard deviations.

Determination of Fe²⁺ - Chelating ability was carried using

The Fe²⁺ chelating ability of the extracts were determined using a modified method of Minotti and Aust (1987) method with a slight modification (Puntel et al., 2005). Freshly prepared 500 µmol L⁻¹ FeSO₄ (150 µL) was added to a reaction mixture containing 168 µL of 0.1 mol L⁻¹ Tris-HCl (pH 7.4), 218 µL saline and the extracts (0 – 100 µL). The reaction mixture was incubated for 5 min, before the addition of 13 µL of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe²⁺ chelating ability was subsequently calculated.

Determination of ferric reducing antioxidant activity

The reducing power of the extract was determined by assessing the ability of the extract to reduce FeCl₃ solution as described (Oyaizu,1986). Briefly, appropriate dilution of the extract (2.5 mL) was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 2.5 mL 10% trichloroacetic acid was added. This mixture was centrifuged at 353 x g for 10 min. five milliliters of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant power was expressed as mg ascorbic acid equivalent/g of the sample.

Determination of Vitamin C content

Vitamin C of the garden egg extract was determined using the method of Benderitter et al., (1998) Briefly, 75 µl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg CuSO₄·5H₂O in 100 ml of 5M H₂SO₄) were added to 500 µl reaction mixture (300 µl of an appropriate dilution of the extracts with 100 µl 13.3% trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 h at 37 °C, then 0.5 ml of 65% H₂SO₄ (v/v) was added to the medium, and the absorbance was measured at 520 nm. The vitamin C content of the extracts was subsequently calculated.

Determination of 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) scavenging ability

The ABTS scavenging activity of the extract was determined according to the method described by Re et al. (1999). The ABTS was generated by reacting an ABTS aqueous solution with K₂S₂O₈ (2.45 M/l final conc.) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. 0.2 ml of the appropriate dilution of the extract was then added to 2.0ml of ABTS solution and the absorbance was read at 732 nm after 15 min. The TROLOX equivalent antioxidant capacity was subsequently calculated.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging ability

The radical scavenging ability of the extracts against DPPH free radical was evaluated as described by Gyamfi et al., (1999). Appropriate dilution of the extract was mixed with 1 ml of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min, and the absorbance was measured at 516 nm. The DPPH radical scavenging ability was subsequently calculated with respect to the reference, which contained all the reagents without the test sample.

Hydroxyl (OH) radical-scavenging ability

The method of Halliwell and Gutteridge (1981) was used to determine the ability of the extracts to prevent Fe²⁺/H₂O₂ induced decomposition of deoxyribose. The extract 0 to 100 mL was added to a reaction mixture containing 120 mL of 20 mg deoxyribose, 400 mL of 0.1 M phosphate buffer, 40 mL of 500 mM of FeSO₄, and the volume was made up to 800 mL with distilled water. The mixture was incubated at 37°C for 30 minutes and the reaction was then stopped with the addition of 0.5 mL of 28% trichloroacetic acid. This was followed by addition of 0.4 mL of 0.8% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 minutes. The absorbance was measured at 532 nm in a spectrophotometer.

NO radical scavenging ability (NO·)

The scavenging effect of the extract on nitric oxide radical (NO) was measured according to the method of Mercocci et al 1994. One hundred to 400 mL of the extract was added in the test tubes to 1 mL of SNP solution (25 mM) and tubes were incubated at 37°C for 2 hours. An aliquot (0.5 mL) of the incubated mixture was removed and diluted with 0.3 mL Griess reagent (1% sulfanilamide in 5% H₃PO₄ and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately measured at 570 nm against the blank (distilled water). The NO scavenging ability was subsequently calculated and reported as % NO radical scavenging ability.

Determination of lipid peroxidation

Male albino rats were decapitated via cervical dislocation and the reproductive tissue (whole penis)

was rapidly dissected, placed in normal saline (0.9 g NaCl in 100mL distilled water) on ice and weighed and later homogenized with phosphate buffer pH 7.4 (1:5 w/v), with about 10-up and down strokes at approximately 1,200 rev/min in a Teflon-glass homogenizer. The homogenate was centrifuged for 10 min at 3,000 g to yield a pellet that was discarded and the supernatant was used for lipid peroxidation assay Ohkawa et al (1979). 100µL of the tissue supernatant was mixed with a reaction mixture containing 30 µL of 0.1 M Tris-HCl buffer (pH 7.4), quinine solution (0.001–13 µM) and 30 µL of 250 µM freshly prepared FeSO₄ as well as 30 µL of 250 µM freshly prepared Copper solution, these solutions serve as the pro-oxidant. The volume was made up to 300 µL with distilled water before incubation at 37 °C for 2hours. Subsequently, 300µL of 8.1 % sodium dodecyl sulphate (SDS), 500 µL of acetic acid/HCl buffer (pH 3.4) and 500 µL of 0.8% thiobarbituric acid (TBA) were added to the reacting mixture. This mixture was incubated at 100 °C for 1 h and Thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm using a spectrophotometer. Malondialdehyde (MDA) was used as standard and TBARS produced was reported as MDA equivalent.

Determination Angiotensin-I converting enzyme (ACE) activity

Appropriate dilution of the aqueous extract (0-25 µL) and ACE solution (50 µL, 4 mU) was incubated at 30 °C for 15 min. The enzymatic reaction initiated by adding 150 µL of 8.33 mM of the substrate Bz-Gly-His-leu in 125 mM TrisHCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37°C, the reaction was arrested by adding 250 µL of 1 M HCL. The BzGly produced by the enzymatic reaction was extracted with 1.5 mL ethyl acetate. Thereafter the mixture was centrifuged to separate the ethyl acetate layer; then 1mL of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was re-dissolved with 1mL of distilled water and its absorbance was measured at 228 nm using Cushman and Cheung (1971) method. The ACE inhibitory activity was also expressed as percentage (%) inhibition.

Determination of Arginase activity

Arginase activity was determined by the measurement of urea produced by the reaction of Ehrlich's reagent according to the modified method Kaysen and Strecker (1973). The reaction mixture contained in final concentration 1.0 mM Tris-HCl buffer, pH 9.5 containing 1.0 mM MnCl₂, 0.1 M arginine solution and 50 µL of the enzyme preparation in a final volume of 1.0 mL. The mixture was incubated for 10 min at 37 °C. The reaction was terminated by the addition of 2.5 mL Ehrlich reagent (2.0 g of p-dimethylaminobenzaldelyde in 20.0 mL of concentrated hydrochloric acid and made up to 100 mL with distilled water). The optical density reading

was taken after 20 min at 450 nm. The control experiment was performed without the test sample and the arginase inhibitory activity was expressed as percentage inhibition. Protein content was determined using Lowry et al (1951) method and used for the calculations.

Statistical analysis

The results of the replicate readings were pooled and expressed as means ± standard deviation. Student t-test was performed and significance was accepted at P≤0.05 with graphpad prism 6.0. While the IC₅₀ (concentration of extract that could cause 50% inhibition) was determined using Microsoft Excel 2013 linear regression analysis Zar (1984).

RESULTS and DISCUSSION

The qualitative screening of the phytochemicals in the fruits dried weight revealed the presence of five chemical constituents such as saponin, tannin, flavonoids, terpenoids, and alkaloids as shown in (Table 1) this study further confirms the report by Anosike et al (2011) on the phytochemicals in the local specie of *Solanum aethiopicum*. These chemical compounds found in the fruit have been reported to have various biological properties such as antioxidant, anti-diabetes, neuroprotective ability, anti-cholesterol as well as low glycemic index (GI) (Nwanwa et al., 2019a and Nwanwa et al.,2019b). Zinc (Zn) happened to be one of the minerals elements found in addition to Na, K, Fe and Mg (Table 2). Zn is an essential trace element which plays an important role in macromolecules like DNA and RNA found useful in protein synthesis, cell division, stability of biological membranes related to sexual maturation and reproduction (Roy et al., 2013). Phenolic phytochemicals in fruits and vegetables have been shown to have positive health effects by reducing free radicals generation (Nwanwa et al., 2016) which make then a potent antioxidant agents as shown in (Table 3) their ability to reduce Fe³⁺ to Fe²⁺ with no significant difference in three varieties and in its chelating ability (Table 4) shows the complexity of the activity of various forms of the chemical compounds found in these garden eggs fruits at different concentration. Also it would be observed that these garden egg fruits extracts are rich source of vitamin c (Table 3), a water soluble vitamin although *S.gilo* (plain white) had the highest concentration followed by *S.kumba* (Plain green) while the fruits total antioxidant ability (Figure 1) against ABTS as well as DPPH radicals revealed that *S. kumba* had the highest ability followed by *S. aethiopicum* while *S. gilo* had the less total antioxidant ability, the observation is majorly due to *S.kumba* and *aethiopicum* having abundant polyphenol and flavonoid content as characterised and quantified from the HPLC analysis (Nwanwa et al., 2016). The extracts scavenged radical species (OH and NO) in a concentration dependent manner which could be due to the fruit rich vitamin c as well as polyphenol

and flavonoid with more of double bonds which was able to quench the reactive state during oxidative stress as seen from the IC₅₀ in Table 4. While the antioxidative potential of the garden egg fruit helps in maintaining the balance of the level of nitric oxide (NO) produced in the biological system. In addition to this observed activities, different pro-oxidants such as copper, iron and quinolinic were used to induced oxidative stress in-vitro, since brain happened to be most sensitive and fragile part of the body this necessitate the incubation of rat brain tissue homogenates in the presence of iron(II), quinolinic, and copper these caused a significant increased (p<0.05) in the malondialdehyde (MDA) produced. However, introduction of garden egg aqueous extracts inhibited the MDA produced in a concentration dependent manner with IC₅₀ values less than 1.55 mg/mL (Table 4).

Increase in lipid peroxidation of cell membranes causes damage which induces oxidative stress which contributes to the causation of injuries in brain as well as other tissues such as the penile tissue thus the extract was restored the antioxidant status as seen in the results which is largely due to fruit rich phytochemicals and its rich vitamin c content. These oxy-radicals are toxic to the tissues if the concentration is much but they are also responsible for various inflammatory responses and signalling including erection of the penis however, excess nitric oxide (NO) when it accumulated in the acidic environment could reacts with oxygen to form nitrite ions and could induce mutagenic reactions (Yin et al., 2007) thus causes damage to the tissue. This study clearly shows that the diet of these eggplant fruits could bring about the desire biological functions in erectile dysfunction.

Table 2: Showing minerals composition of the garden egg sample (% dry weight)

Sample	Na	K	Fe	Zn	Mg
PGW	30.1±3.51 ^a	45.5±2.75 ^a	1.07±0.05 ^a	0.68±0.05 ^a	3.50±1.28 ^a
PG	22.5±2.97 ^b	40.2±2.61 ^b	1.07±0.05 ^a	0.84±0.07 ^b	3.98±1.28 ^b
PW	27.4±1.81 ^a	41.6±2.56 ^b	0.84±0.05 ^b	0.66±0.04 ^a	3.77±1.21 ^a

Values represent means of triplicate. Values with the same letter along the column are not significantly different (p<0.05).

Table 3: Showing vitamin C content and reducing property (FRAP) of the garden egg

Parameters	Vit C (mg/ml)	FRAP (mg/100gAAE)
PGW	95.01±4.27 ^a	75.04±0.93 ^a
PG	134.01±5.24 ^c	75.05±0.92 ^a
PW	154.01±7.99 ^b	74.04±0.82 ^a

Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different (P >0.05).

The extracts inhibited arginase and angiotensin-1-converting enzyme as shown in (Table 6) from their IC₅₀ values in a concentration dependent manner. Garden egg fruit diet due to its rich phytochemicals could be of immense importance in the management of hypertension as this would help to reduced elevated diastolic and systolic blood pressure as reported by in-vivo experiment by Nwanna et al (2016). It would be of note that elevated arginase (an enzyme involved in fertility) activity is also linked with angiotensin-II-induced arterial thickening, fibrosis, and stiffness (Nwanna et al.,2015) However, controlling or modulating it activity through dietary regime with garden egg fruit would reduce the elevated blood pressure because hypertension is a well-established risk factor for erectile dysfunction.

Finally, from the study garden egg fruit diet is rich in essential minerals and phytochemicals which could be used as a natural plant derived source to boost fertility, erectile function as well as manage oxidative stress and hypertension.

Table 1: Showing phytochemical screening of the garden egg samples

Treatment	PGW	PG	PW
SAPONIN	+	+	+
TANNIN	+	+	+
FLAVONOID	+	+	+
TERPENOID	+	+	+
ALKALIOD	+	+	+

presence + Key: PGW: Solanum aethiopicum PG: Solanum kumba PW: Solanum gilo

Table 4: Showing the IC₅₀ of the antioxidant properties (mg/mL) of the aqueous extracts of the garden egg on DPPH, NO, OH and Fe-chelating ability

Parameters	DPPH*	NO*	OH*	Fe-chelating
PG	22.71±2.01 ^a	3.52±0.10 ^b	2.55±0.05 ^b	2.77±1.01 ^a
PGW	19.71±2.01 ^a	4.87±0.15 ^a	1.39±0.10 ^a	2.68±0.98 ^a
PW	20.78±2.12 ^a	5.39±0.26 ^b	2.98±0.15 ^b	2.98±1.27 ^a

Value represent mean of triplicate readings. Values with the same letter along the same column are not significantly different (p>0.05).

Table 5: IC₅₀ for the inhibition of Quinolinic, Copper-induced and Fe²⁺ induced lipid peroxidation in rat brain by aqueous extracts garden egg species (mg/ml).

Parameters	Quinolinic-Induced	Copper-induced	Iron-induced
PGW	0.75±0.30 ^a	1.43±0.40 ^a	0.87±0.10 ^a
PG	0.72±0.10 ^a	1.34±0.22 ^a	0.76±0.20 ^a
PW	0.87±0.10 ^b	1.54±0.40 ^a	1.14±0.10 ^a

Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different (P < 0.05).

Table 6: IC₅₀ inhibition of Angiotensin-1-converting

enzyme and Arginase (µg/ml)		
Parameter	Angiotensin-1-converting enzyme	Arginase
PGW	0.51±0.02 ^a	2.77±0.08 ^a
PG	0.41±0.01 ^a	2.38±0.07 ^a
PW	0.99±0.02 ^b	1.25±0.05 ^b

Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different (P < 0.05).

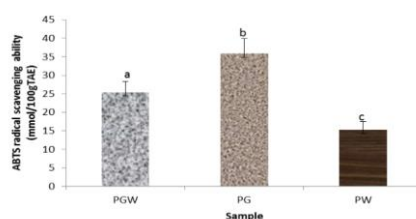


Figure 1: Showing the total antioxidant ability (ABTS). Value represent means of triplicate readings. Bar with different letters are significantly different (p<0.05).

CONCLUSION

The antioxidant properties of the garden egg fruit extracts with inhibition of arginase and angiotensin-1-converting enzymes suggest that the fruit diet could be promising in the management of hypertension and erectile dysfunction thus improve fertility. However, in-vivo study on fertility boosting using the garden egg fruit is encouraged. Solanum kumba which had the best output from this study is recommended.

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MICROBIAL BURDEN OF SOME TABLETS OBTAINED FROM THE PATENT MEDICINE STORES IN ABA

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Abstract:

The microorganisms associated with the contamination of some across the counter tablets obtained from different locations in the market and patent medicine stores on the street in Aba metropolis were determined. The samples of tablets investigated were sealed and unsealed Septrin, Flagyl, and Paracetamol, respectively. The unsealed tablet samples had a higher rate of contamination than the sealed tablets. The microorganisms isolated were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* sp., *Aspergillus* sp., *Penicillium* sp., and *Mucor* sp. The samples obtained from the patent medicine stores on the street had higher contamination of 40.6% microbial occurrence whereas the samples obtained from the patent medicine stores in the market had a lesser contamination of 36% microbial occurrence. The prevalent organism was *Bacillus* sp. Among the tablets analyzed, Paracetamol had a higher level of contamination than the other samples tested. Adherence to good manufacturing practices and strict observance of dispensing guidelines would reduce the high rate of microbial contamination and the associated health hazards.

Keywords: sealed and unsealed tablets, contamination, patent medicine stores, microorganisms.

INTRODUCTION

Drugs are used in a variety of ways in the prevention, treatment, and diagnosis of diseases. In recent years, manufacturers of pharmaceuticals have improved the quality of pharmaceuticals such that today such products contain only minimal bioburden (Mugoyela and Mwambete, 2010). A study by Beveridge (1977) revealed that drugs are usually products of chemicals or biological substances used for the treatment of health disorders and they are usually composed of organic substances while some are composed of various plant extracts from various species of plants. Also, Itah *et al.* (2004) in their findings stated that pharmaceutical products of various forms and dosage are susceptible to contamination by a variety of microorganisms during manufacturing and use.

The microbial quality of pharmaceuticals is influenced by the environment and quality of raw materials used during formulation (Akerle and Ukoh, 2002). A study by Akerle and Ukoh (2002) also shows that most raw materials for pharmaceutical products support some forms of microbial growth, depending on the nutritive properties and moisture content. Hence, dry tablets are capable of undergoing some forms of microbial spoilage or degradation. The more serious problem of microbial contamination of tablets is where there are no obvious signs of spoilage. Therefore, there is a need to know the microbial content of all drugs and medicines whether they are sterile or non sterile (Mugoyela and Mwambete, 2010). Solid dosage forms, mainly tablets and capsules constitute a large proportion of the medicines which are dispensed in modern dispensaries, though many are now presented in blister packs (sealed) but most developing countries including Nigeria still have instances where such

medicines are supplied in bulk containers, with the prescribed amount being drawn from these containers (Mugoyela and Mwambete, 2010). Contamination of pharmaceuticals with microorganisms irrespective of being harmful or objectionable or non-pathogenic can bring about changes in their physical characteristics (Mugoyela and Mwambete, 2010; Islam *et al.*, 2015; Al-Kafl *et al.*, 2015).

A study by Itah *et al.* (2004) reported that with the exception of preparations which are terminally sterilized in their final containers, the micro flora of the final products may represent contaminants from the raw materials, from process operating personnel and packaging of the final product. From the research on microbiological stability of tablets stored under tropical conditions by Bos *et al.* (2002), it was observed that the nature of raw material and storage condition plays important role in the prevention of microbial contamination of pharmaceutical products.

Handling of the tablets also constitute to their contamination. Dispensers use a range of applicators which include spoons, plates, brushes, pads and spatulas, and it is a common practice when one goes to the open stores to purchase these tablets. Carstensen and Rhodes (2000) reported that mishandling may result in a serious health hazard following ingestion of highly contaminated drugs/solid dosage forms by patients whose immunity is already compromised by illness. The presence of microbes in drugs not only makes them hazardous from the infectious standpoint but may also change the physical, chemical and organoleptic properties of the drugs, alter the contents of active ingredients, or convert them to toxic products (Mugoyela and Mwambete, 2010). This work seeks to

determine the microbial content of unsealed Flagyl, Septrin and Paracetamol marketed in Aba metropolis, Abia State, Nigeria and also to check for the microbial indicators of poor quality control in the drugs.

MATERIALS AND METHODS

Study area and sources of samples

The study area was the new market (Ahia Ohuru) and some selected patent medicine stores in the streets of Aba metropolis. Aba is a big city comprising the higher, middle and lower classes of individuals. The selected drugs tablets of study are the common and cheap ones namely: Septrin, Flagyl, and Paracetamol. These are affordable even by the poorest of the poor. Three different patent medicine stores in the market (Ahia Ohuru) and three different patent medicine stores on the streets of Aba were randomly selected.

Sample collection

Tablet samples were obtained from the drug sellers in the market and streets. These tablets include the sealed (blistered) and unsealed (counting) Septrin, Flagyl and Paracetamol. For the sealed samples, a sachet of 10 tablets of Septrin, a sachet of 10 tablets of Flagyl and a sachet of 12 tablets of Paracetamol were obtained from each store. While for the unsealed samples, twenty tablets of each sample from each of the stores were collected using a sterile polythene bags.

Preparation of culture media and materials

The culture media used in this work were Biotech Culture Media of the MacConkey Agar, Nutrient Agar and Sabouraud Dextrose Agar (SDA). They were obtained in hydrated powdered forms and prepared based on the manufacturer's instructions. After preparation, they were sterilized by autoclaving at 121°C for 15 minutes and the pressure of 15 pounds. The media were allowed to cool down to about 40°C and were then poured into sterilized Petri dishes aseptically (Cheesbrough, 2006). The SDA medium was allowed to cool to about 35°C-37°C before adding chloramphenicol to inhibit the growth of bacteria. This medium was also dispensed into sterile Petri dishes using an aseptic technique. These medium were allowed to solidify before sample inoculation. The tablets were crushed to powder using sterile laboratory mortar and pestle (Obi and Nwannunu, 2010). One gram of each of the crushed/ powdered tablets samples (Flagyl, Septrin and Paracetamol) were suspended/dissolved in 9ml of sterile distilled water which were serially diluted up to 10⁻⁴ dilution factor (Itah *et al.*, 2004).

Inoculation of samples

0.1ml aliquot of each tablets suspension was measured and spread on Nutrient Agar, MacConkey and SDA plates (all in duplicate plates) and were also labeled accordingly for easy identification (Obi and

Nwannunu, 2010). The bacterial plates were incubated at 37°C for 24-48 hours while the fungal plates were incubated at 24°C for 72 hours. The isolates from crowded primary culture plates were purified by repeated sub-culture using the streak plate technique (Joanne *et al.*, 2008). Using a sterile inoculating loop, a small portion of each species was collected and transferred to the freshly prepared Petri dishes containing the medium on which it grew and then streaked out over the surface of the Agar.

RESULTS

The results of both sealed and unsealed Septrin, Flagyl and Paracetamol at the time of administration which were sold in the market and street's patent medicine stores are shown in Tables 1 – 4.

Table 1: Bacterial load of tablets obtained from patent medicine stores in the market

STUDY POINTS	SAMPLES	BACTERIAL COUNTS	
		NA cfuml ⁻¹	MA cfuml ⁻¹
Store 1	Sa	3.7×10 ⁵	1.5×10 ⁵
	Sb	1×10 ⁵	2×10 ⁵
	Fa	3.25×10 ⁵	2×10 ⁵
	Fb		
	Pa	1.7×10 ⁶	8.25×10 ⁵
	Pb	1.5 ×10 ⁵	2×10 ⁵
Mean counts		4.41 ×10 ⁵	2.63 ×10 ⁵
Store 2	Sa	1.5×10 ⁵	2.5 ×10 ⁵
	Sb		
	Fa	2.5 ×10 ⁵	1 ×10 ⁵
	Fb		
	Pa	4.25 ×10 ⁵	3.25 ×10 ⁵
	Pb		
Mean counts		1.38 ×10 ⁵	1.13 ×10 ⁵
Store 3	Sa		
	Sb		
	Fa	5.25 ×10 ⁵	3 ×10 ⁵
	Fb		
	Pa	3.83 ×10 ⁵	3.75 ×10 ⁵
	Pb	4.5 ×10 ⁵	2.5 ×10 ⁵
Mean counts		2.26 ×10 ⁵	1.54 ×10 ⁵

Key: NA –Nutrient Agar; MA – Macconkey Agar; CFU/MI – Colony Forming Unit Per Milliliter; Sa – Unsealed Septrin; Sb –Sealed Septrin; Fa – Unsealed Flagyl; Fb – Sealed Flagyl; Pa – Unsealed Paracetamol; Pb –Sealed Paracetamol.

Table 2: Bacterial load of tablets obtained from patent medicine stores in Aba streets.

STUDY POINTS	SAMPLES	BACTERIAL COUNTS	
		NA CFUML ⁻¹	MA CFUML ⁻¹
Store 1	Sa	2.75×10 ⁵	2.5×10 ⁵
	Sb	-	-
	Sa	4.75×10 ⁵	2.5×10 ⁵
	Fb	1×10 ⁵	2×10 ⁵
	Pa	1.38×10 ⁵	7.25×10 ⁵
	Pb	9×10 ⁵	7.5×10 ⁵
Mean counts		5.22×10 ⁵	3.63×10 ⁵
Store 2	Sa	1.33×10 ⁵	1.25×10 ⁵
	Sb	2.5×10 ⁵	3×10 ⁵
	Fa	3.25×10 ⁵	1.5×10 ⁵
	Fb	-	-
	Pa	9.25×10 ⁵	5.75×10 ⁵
	Pb	3.5×10 ⁵	1.5×10 ⁵
Mean counts		3.31×10 ⁵	2.17×10 ⁵
Store 3	Sa	3.5×10 ⁵	2.5×10 ⁵
	Sb	2×10 ⁵	1.5×10 ⁵
	Fa	4.5×10 ⁵	-
	Fb	-	-
	Pa	5.25×10 ⁵	3×10 ⁵

Pb
Mean counts 2.54×10⁵ 1.17×10⁵

Key: NA – Nutrient Agar; MA- Macconkey Agar; CFU/ML - Colony Forming Unit Per Milliliter; Sa - unsealed septrin; Sb: Sealed septrin; Fa – unsealed Flagyl; Fb - Sealed Flagyl; Pa – unsealed Paracetamol; Pb- Sealed Paracetamol.

DISCUSSION

This research shows that most of the tablets analysed had both bacterial and fungal contamination especially the unsealed tablets. The bacteria isolated from this work are *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus* sp. while the fungal isolates are *Aspergillus* sp., *Penicillium* sp. and *Mucor* sp which is in conformity with the results obtained by Mugoyela and Mwambete, Akerele and Uko; Obi and Nwannunu who did similar work.

It was observed from table 1 and table 2 (which shows the bacterial load of tablets obtained from patent medicine stores in the market and bacteria load of tablets obtained from patent medicine stores in Aba streets) that table 2 has the highest occurrence of bacterial contamination of 3.01×10⁵ cfu/ml while table 1 has the lowest occurrence of 2.23×10⁵ cfu/ml. therefore, the bacterial load of tablets obtained from patent medicine stores in the market in this work contradict the research result by Ogbonna (1980) which stated that the number of organisms in the atmosphere depends on the activity in the environment and the amount of dust disturbed.

Tables 3: Percentage occurrence of microbial isolates from tablets obtained from patent medicine stores in the market.

Samples	<i>Staph. aureus</i>	<i>E. Coli</i>	<i>Bacillus</i> sp.	<i>Aspergillus</i> sp.	<i>Penicilium</i> sp.	<i>Mucor</i> sp.
Store 1						
Sa	+	+	+	+	-	+
Sb	-	-	+	-	-	-
Fa	-	+	+	+	+	+
Fb	-	-	-	-	-	-
Pa	+	+	+	+	+	-
Pb	-	-	+	-	-	-
Total	33	50	83	50	33	33
Store 2						
Sa	-	-	+	+	-	+
Sb	-	-	-	-	-	-
Fa	+	-	+	-	+	+
Fb	-	-	-	-	-	-
Pa	-	+	+	+	+	+
Pb	-	-	-	-	-	-
Total	17	17	50	33	33	50
Store 3						
Sa	-	-	-	-	-	-
Sb	-	-	-	-	-	-

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Fa	-	+	+	-	+	-
Fb	-	-	-	-	-	-
Pa	+	+	+	+	+	+
Pb	-	-	+	-	-	-
Total	17	33	50	17	33	17

Key: NA – Nutrient Agar; MA- Macconkey Agar; CFU/MI - Colony Forming Unit Per Milliliter; Sa - unsealed septrin; Sb: Sealed septrin; Fa – unsealed Flagyl; Fb - Sealed Flagyl; Pa – unsealed Paracetamol; Pb- Sealed Paracetamol.

Table 4: Percentage occurrence of microbial isolates from tablets obtained from patent medicine stores on the street.

	<i>Staph. aureus</i>	<i>E. coli</i>	<i>Bacillus</i> sp.	<i>Aspergillus</i> sp.	<i>Penicilium</i> sp.	<i>Mucor</i> sp.
Store 1						
Sa	+	-	+	-	+	+
Sb	-	-	-	-	-	-
Fa	-	+	+	+	+	+
Fb	-	-	+	-	-	-
Pa	-	+	+	+	+	-
Pb	-	-	+	-	-	-
Total	17	33	83	33	50	33
Store 2						
Sa	+	+	+	-	+	+
Sb	-	-	+	-	-	-
Fa	-	+	+	+	+	-
Fb	-	-	-	-	-	-
Pa	-	+	+	+	+	+
Pb	-	-	+	-	-	-
Total	17	50	83	33	50	33
Store 3						
Sa	+	+	-	-	-	+
Sb	-	-	+	-	-	-
Fa	+	-	-	+	+	-
Fb	-	-	-	-	-	-
Pa	+	-	+	+	+	+
Pb	-	-	+	-	-	-
Total	50	17	50	33	33	33

Key: NA – Nutrient Agar; MA- Macconkey Agar; CFU/MI - Colony Forming Unit Per Milliliter; Sa - unsealed septrin; Sb: Sealed septrin; Fa – unsealed Flagyl; Fb - Sealed Flagyl; Pa – unsealed Paracetamol; Pb- Sealed Paracetamol.

The presence of high levels of contaminants in some of the tablets analyzed could be attributed to unhygienic practices and non-adherence to good manufacturing practice. The poor state of the manufacturing environment, dirty filling equipment, unhygienic handling of the products and lack of microbiological in-house control might have also contributed to the high microbial load in some samples (Itah *et al.*, 2004).

Highest mean percentage occurrence of *Bacillus* in Tables 3 and 4 can be traced back to the raw material used in the production of these tablets since this organism is present in the water and un-sterile air in the manufacturing environment which needs fumigation or filtration. The manufacturing equipment may be handicapped by a number of designed faults and products made in these conditions might reasonably be expected to be contaminated with microorganisms such as aerobic spore bearers and

Gram positive cocci (Itah *et al.*, 2004).

The occurrence of *Escherichia coli* in the tablets analyzed is another source of great concern with respect to hygienic practices in an environment. Joanne *et al.* (2008) reported that *E. coli* has the human colon as the natural habitat and its presence in water and food is a strong indication of faecal contamination.

Adebayo (1999) reported that organisms found in contaminated environment include fungi and bacteria such as *staphylococcus* sp., *Aspergillus* sp., *Mucor* sp. *Penicillium* sp. whose presence on the tablets signifies spoilage are common environmental contaminants which ordinarily are not pathogenic in the environment but becomes pathogenic and cause opportunistic infection when taken into the system. Mishandling of these tablets in the hands of untrained personnel could result in serious health hazards following ingestion of highly contaminated drugs by patients whose immunity is already compromised by illness (Akerlele and Ukoh, 2002).

Obuekwe *et al.* (2000) reported that unsealed drugs are subject to unrestricted handling and are therefore,

potentially susceptible to postproduction contamination by microorganisms from both the handlers and the environment.

CONCLUSION

From the results obtained in this research work, it is obvious that the most contaminated are the unsealed tablets. This is because of their frequent exposure to the environment and frequent direct contact of the tablets by the dispensers which predisposes the tablets to microbial contamination.

Tablets that do not have direct contact with the body like the sealed tablets can not be easily contaminated and if there is any contamination, the source will be traced back to the raw materials used for the production or it could also mean that the preservatives added during production are not sufficient to eliminate the organisms that survive treatments during production. Therefore, mishandling of these tablets in the hands of untrained personnel will result in serious health hazards following ingestion of highly contaminated drugs by patients that have depressed immunity.

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PHYSICOCHEMICAL PROPERTIES OF *MORINGA OLEIFERA* SEED OIL AND ITS ANTIHYPERLIPIDAEMIC EFFECTS ON MALE WISTAR ALBINO RATS

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Abstract

Hyperlipidaemia is the major contributing factor in complications associated with obesity, diabetes mellitus, and cardiovascular diseases. This study evaluated physicochemical properties of *Moringa oleifera* seed oil and its effects on the body weight and lipid profile of male Wistar albino rats. The physicochemical properties and lipid profile were analysed using standard methods. Thirty rats randomly divided into 5 groups comprising 6 rats each were used for the experiment. Group 1 served as the normal control and received 2 ml/kg of normal saline, group 2 – 4 were treatment groups that received 2, 1 and 0.5 ml/kg of *M. oleifera* seed oil respectively while group 5 served as the standard control and received 1 ml/kg of olive oil. The administration of normal saline, *M. oleifera* seed oil and olive oil were done orally for 28 days. Body weights of the rats were taken every 2 days. Animals were sacrificed on 29th day and blood sample were collected for lipid profile analyses. The results showed that *M. oleifera* seed oil has moderately high specific gravity, acid value, free fatty acid, iodine and peroxide values, saponification value, and low level of unsaponifiable matter. The *M. oleifera* seed oil significantly ($P < 0.05$) decreased total cholesterol level in a dose dependent manner when compared with normal control. The findings of this study show that *M. oleifera* seed oil could prevent hypercholesterolemia and maintain healthy lipid profile.

Keyword: *Moringa oleifera* seed oil, lipid profile, physicochemical properties, body weight, olive oil.

Introduction

There is persistent surge in the number of people with cardiovascular disease, stroke, hypertension, obesity and type II diabetes among other medical conditions that negatively impair one's health and quality of life due to abnormal lipid level in the blood and tissues. Abnormal lipid profile mostly occur due to high blood levels of cholesterol and low density lipoprotein which are responsible for atherosclerosis and various cardiovascular diseases (Nelson, 2013). Diets, drugs and medicinal plants restore abnormal lipid levels including total cholesterol and low density lipoprotein (LDL) in blood and tissues to normal values are considered to possess antihyperlipidaemic properties and could prevent associated health consequences that usually result to morbidities and mortalities (Joshi and Jain, 2014). Drugs like statins, carnitine, fibrates, cholesterol absorption inhibitors, and bile acid sequestrants are common antihyperlipidaemic agents used to maintain normal lipid profile but their use are associated with adverse health effects such as diarrhoea, hyperuricemia, muscle aches, and altered liver function (Kumar *et al.*, 2008; Seriki *et al.*, 2015). Due to adverse health effects and ineffectiveness of many available drugs for the management of hyperlipidaemia, there is increasing interest in the use of medicinal plants in the management of dyslipidaemia and various parts of *Moringa oleifera* have demonstrated promising antihyperlipidaemic activities.

Moringa oleifera commonly known as drumstick or horse radish in English is a member of *Moringaceae* family widely distributed across Asia and West Africa mostly in the south eastern part of Nigeria (Naznin *et al.*, 2008). It is a rich source of many essential elements, antioxidant vitamins and phytochemicals, nitrile, mustard oil glycosides; β -sitosterol and thiocarbamate glycosides associated with antihypertensive and antihyperlipidaemic activities of many medicinal plants (Anwar *et al.*, 2003; Gheith and El-Mahmoudy, 2019). *Moringa oleifera* is a highly rich medicinal plant whose roots, leaves, flowers, green pods, and seeds have been used for medicinal purposes (Saini, 2015). *Moringa oleifera* seeds have high oil yield of about 40% with high level of monounsaturated fatty acids mostly oleic acid and are commonly used in biodiesel production which could be of commercial value (Moffjur *et al.*, 2014; Leone *et al.*, 2015). The seeds are rich in bioactive compounds with established antimicrobial and coagulation activities (Kansal and Kumari, 2014). Moringa seed oil, commercially known as "Ben oil" has high content of oleic acid, sterols and tocopherols (Lakshmi Priya *et al.*, 2016). The oleic acid, a monounsaturated fatty acid is the major fatty acid composition of the *M. oleifera* seed oil accounting for 70% of its total fatty acid composition whereas its polyunsaturated fatty acids content is below 1% and with these properties the oil is relatively stable and less prone to oxidative rancidity (Anwar and Bhangar, 2003; Faisal *et al.*, 2018). The oil is edible and can be

used in cooking, lubricant, cosmetic and in perfumes in addition to biodiesel production (Fahey, 2005; Lakshmi Priya *et al.*, 2016). Despite the high oil content in *M. oleifera* seeds there has not been sufficient studies on the health implications of its consumption rather focus has been on its various applications for economic gain. Therefore, this study was designed to evaluate physicochemical properties of *M. oleifera* seed oil and its effects on the body weight and lipid profile of male Wistar albino rats.

Materials and Methods

Collection and identification of *Moringa oleifera* seeds

Moringa oleifera seeds were collected from National Root Crops Research Institute, Umudike and identified by Prof. G.C. Osuagwu at the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike.

Experimental Animals

Thirty (30) male Wistar albino rats weighing 78 – 135 g were purchased from the Department of Zoology, University of Nigeria Nsukka and acclimatized for 7 days at the Animal House of the department of Biochemistry, Michael Okpara University of Agriculture, Umudike with free access to clean water and feed (vital grower).

Preparation of plant materials

Moringa oleifera seeds were hand-picked to sort out the spoilt seeds and any possible contaminants. The seeds were air dried at room temperature until a constant weight was obtained and then dehulled, further dried and pulverised with grinder and stored into an air tight container for the experiment.

Plant sample extraction

A quantity, 100 g of pulverised *M. oleifera* seeds were soaked in 400 ml of hexane overnight to dissolve the oil content in the pulverised seeds. The mixture was filtered using Whatman No 1 filter paper to separate the residue from the filtrate. The oil in the filtrate was extracted using soxhlet extractor as described by Awais *et al.* (2015).

Experimental Design

The 30 rats after 7 days of acclimatization were randomly divided into 5 groups comprising 6 rats each and used for the experiment. Group 1 served as the normal control and received 2 ml/kg of normal saline, group 2 – 4 were treatment groups that received 2, 1 and 0.5 ml/kg of *Moringa oleifera* seed oil respectively while group 5 served as the standard positive control and received 1 ml/kg of olive oil. The administration of normal saline, *Moringa oleifera* seed oil and olive oil were done orally for 28 days. Body weights of the rats were taken every 2 days with electronic weighing balance to ascertain their weight

gains. Blood samples were collected from the rats on the 29th day for lipid profile analyses.

Biochemical analysis

The total serum cholesterol concentrations in the rats was determined enzymatically using the method of Allain *et al.* (1974) as outlined in QCA commercial kit for cholesterol determination. Triacylglycerol (TAG) concentration in the serum was quantified by enzymatic hydrolysis according to the method of Albers *et al.* (1978) and explained in the Randox commercial kit. The high density lipoprotein (HDL) concentration was determined by the precipitation method outlined by Friedewald *et al.* (1972) while the serum low density lipoprotein (LDL) was calculated using the equation described by Friedewald *et al.* (1972)

Statistical analysis

The data obtained were statistically analysed using one way analysis of variance (ANOVA) with the aid of Statistical Products and Service Solutions (SPSS) version 22. The means were separated using Duncan Multiple range test and results presented as mean \pm standard deviation. The statistical significance was established at $P < 0.05$

Results

The data in Table 1 show the physicochemical properties of *M. oleifera* seed oil which indicated that the oil has moderately high specific gravity, acid value, free fatty acid, iodine and peroxide values, unsaponifiable matter, low level of saponification value.

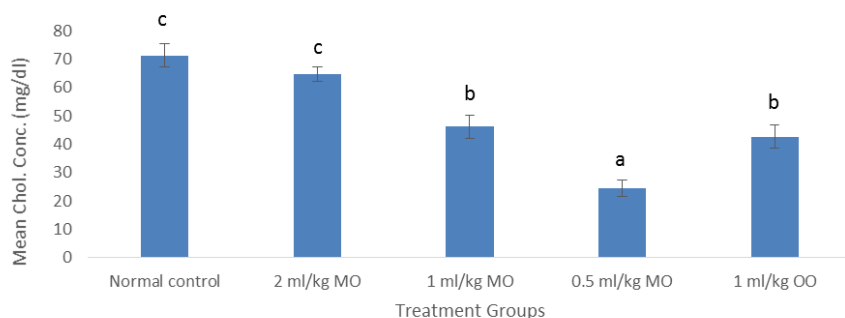
Table 1: Physicochemical properties of *Moringa oleifera* seed oil

Oil properties	Mean Values
Specific gravity (g/cm ³)	0.84 ± 0.18
Acid value (mg EqKOH/g oil)	16.41 ± 0.01
Free fatty acid (mg KOH/g)	8.22 ± 0.03
Iodine value (g I ₂ /100g)	68.41 ± 0.01
Peroxide value (MeqO ₂ /kg oil)	13.41 ± 0.02
Saponification value (mg KOH/g oil)	168.22 ± 0.03
Unsaponifiable matter (%)	15.81 ± 0.01

Value are presented as mean ± standard deviation (n = 3)

concentrations of rats administered graded doses of *Moringa oleifera* seed oil, olive oil and normal saline (normal control) respectively. The total cholesterol concentrations observed in the rats administered 1 and 0.5 ml/kg *M. oleifera* seed oil and 1 ml/kg olive oil respectively showed significant (P < 0.05) decreases when compared with the normal control. However, there was no significant (P > 0.05) decrease in the total cholesterol concentration observed in the rats administered 2 ml/kg *M. oleifera* seed oil relative to the normal control. Also, the total cholesterol concentrations in the rats administered 0.5 ml/kg *M. oleifera* seed oil was significantly (P < 0.05) lower when compared with the total cholesterol concentration recorded in all other rats administered graded doses of *M. oleifera* seed oil and olive oil respectively.

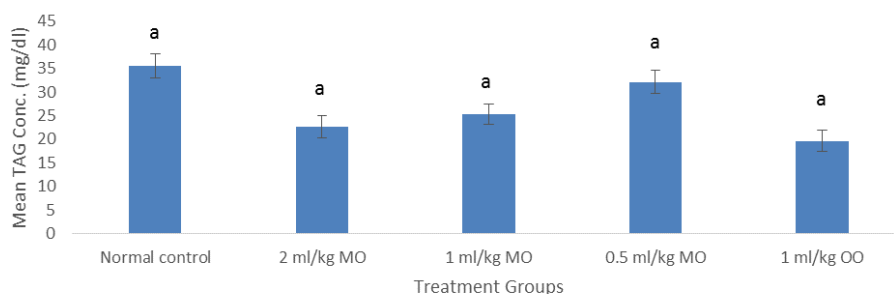
The data in Fig.1 show the total serum cholesterol



OO = Olive oil; MO = *Moringa oleifera* seed oil
 Each bar represent mean ± standard deviation (n = 6)
 Bars with different superscripts are significantly different (P < 0.05)

Fig. 1: Cholesterol levels in rats administered *M. oleifera* seed oil

The triacylglycerol (TAG) concentrations of rats administered graded doses of *M. oleifera* seed oil and olive oil showed no significant (P > 0.05) decreases in the TAG concentrations in the rats when compared with normal control (Figure 2). The no significant (P > 0.05) decreases in the TAG concentrations of the rats administered *M. oleifera* seed oil were observed to be inversely proportional to the dose of *M. oleifera* seed oil administered



OO = Olive oil; MO = *Moringa oleifera* seed oil
 Each bar represent mean ± standard deviation (n = 6)
 Bars with different superscripts are significantly different (P < 0.05)

Fig. 2: Triacylglycerol levels in rats administered *M. oleifera* seed oil

The result in Figure 3 show the high density lipoprotein (HDL) concentration of rats administered *M. oleifera* seed oil and olive oil respectively. The concentration of HDL observed in the rats administered *M. oleifera* seed oil inverse dose dependent on significant ($P > 0.05$) when compared with the normal control. Similarly, there was no

significant ($P > 0.05$) differences observed in the HDL concentrations of rats that received *M. oleifera* seed oil and administered olive oil with the HDL observed in the rats that received 1 ml/kg *M. oleifera* seed oil been no significantly ($P > 0.0$) high than the rats that received equivalent dose of olive oil

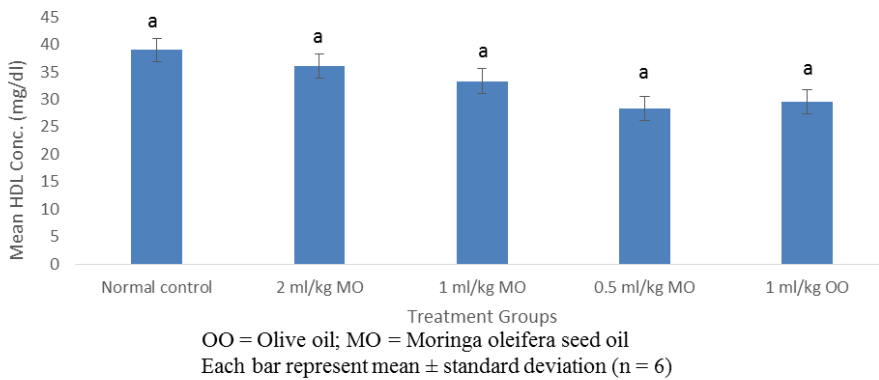


Fig. 3: High density lipoprotein (HDL) levels in rats administered *M. oleifera* seed oil

The results of the low density lipoprotein (LDL) concentrations in rats administered *M. oleifera* seed oil and olive oil respectively showed no significant ($P > 0.05$) decrease when compared with the normal control. Rats administered 1 ml/kg *M. oleifera* seed oil

had the least amount of LDL when compared with other groups of rats administered *M. oleifera* seed oil while rats administered 1 ml/kg olive oil had the least LDL concentration when compared with normal control rats and rats administered *M. oleifera* seed oil respectively

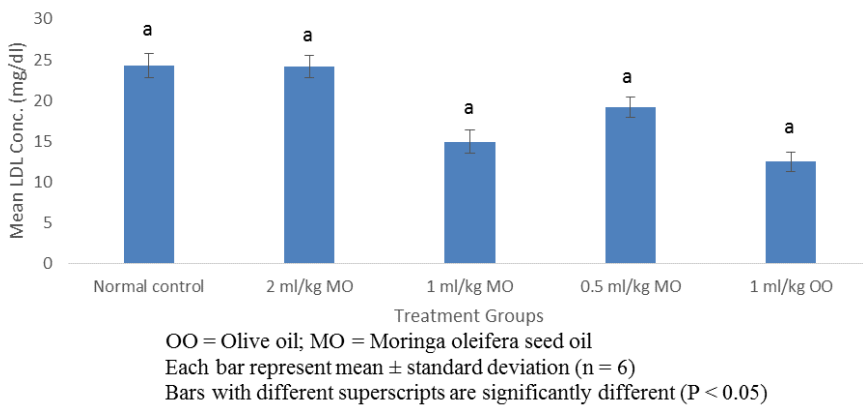


Fig. 4: Low density lipoprotein (LDL) levels in rats administered *M. oleifera* seed oil

The body weight of rats administered *M. oleifera* seed oil, olive oil and normal saline (normal control) respectively showed no significant ($P > 0.05$) difference after week 1 (Figure 5). However, at week

2, rats administered 1 ml/kg olive oil showed significant ($P < 0.05$) increase in body weight when compared with the normal control while the rats administered *M. oleifera* seed oil showed no significant ($P > 0.05$) increase in body weight relative to the normal control. At week 3, there was no

significant ($P > 0.05$) difference observed in the body weight of rats administered 0.5 ml/kg *M. oleifera* seed oil and 1 ml/kg olive oil respectively when compared with the normal control. The body weight of rats administered 1 and 2 ml/kg *M. oleifera* seed oil respectively at week 3 showed no significant ($P >$

0.05) decrease relative to the normal control. At week 4, the body weight of all the rats administered *M. oleifera* seed oil and olive oil respectively decrease non-significantly ($P > 0.05$) relative to the normal control.

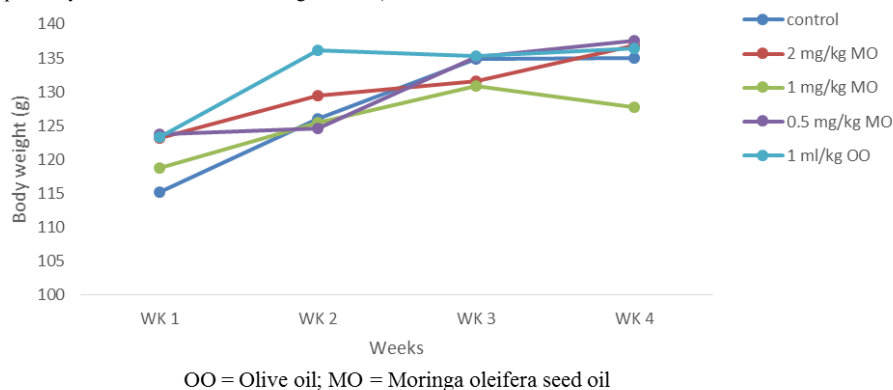


Fig. 5: Body weight of rats administered *M. oleifera* seed oil

Discussion

This study evaluated physicochemical properties of *M. oleifera* seed oil and its effects on lipid profile of male Wistar albino rats. This was done to ascertain the quality of the oil and possible protective roles it could play in maintaining healthy lipid profile to prevent obesity, cardiovascular diseases and other health complications associated negative lipid profile.

Acid value of oil gives the degree of its free fatty acids (FFAs) content released from hydrolysis or oxidative reactions. The moderately high acid value of *Moringa oleifera* seed oil could be attributed to the high free fatty acids content in the oil that might have been released from the n-hexane extraction of the oil. The acid value of *M. oleifera* seed oil recorded in this study is closer to 20.5 mgKOH /g oil reported by Pereira *et al.* (2016) from n-hexane extraction of *M. oleifera* seeds and suggested that n-hexane solvent interaction with the oil elicited release of free fatty acids. The high acid values in the oil show that its triacylglycerol content has been partially hydrolyzed, thereby making the oil relatively unstable which is in agreement with earlier report by Bianca *et al.*, 2012 that oils with low acid values have good stability against oxidative rancidity and vice versa.

The peroxide value of a given oil gives the extent of its unsaturated fatty acid constituents and primary oxidation of the oil which usually result to the rancidity of the oil. The moderately high peroxide value of *Moringa oleifera* seed oil observed in this study could be attributed to its richness in unsaturated fatty acids and suggest that the oil could be prone to

oxidative rancidity that could alter its quality if stored for long. Oils with very high peroxide values have very short shelf life, deficient in fat soluble vitamins and can be easily be recognized by their off-flavour. Oils with high peroxide value are usually considered unsafe for consumption (Gotoh and Wada, 2006). The high iodine value of *M. oleifera* seed oil indicates that the oil has saturated fatty acid and high unsaturated fatty acid contents. This suggests that the oil may prevent accumulation of cholesterol and low density lipoprotein in the body when consumed in right quantity. Also, the low saponification value of *M. oleifera* seed oil indicates low triacylglycerol content in the oil due to low ester linkages and may not be of good commercial value in cosmetic production (Akbar *et al.*, 2009). The low saponification value of *M. oleifera* seed oil further suggests that it is rich in long chain fatty acids, as Ramzya *et al.* (2014) has previously reported that oils with low saponification values contain high amount of long chain fatty acids. The high percentage of unsaponifiable matter in the *M. oleifera* seed oil is an indication that the oil may not be suitable for soap production unless they possess desirable properties like moisturization potentials, flavour, conditioning and vitamins that will improve the texture and acceptability of the soap.

Cholesterol when present in high amount can be deposited on the arteries and increase the risk of heart attack or stroke by preventing smooth flow of blood through the altered arteries but persistent low level of cholesterol could predispose one to cancer, depression, and some other medical conditions (Su-Min *et al.*, 2018). The significant reductions in the

total cholesterol levels of the rats administered moderate and low (1 and 0.5 ml/kg) *M. oleifera* seed oil relative to the normal control rats could be attributed to the cholesterol lowering effects of the *M. oleifera* seed oil. The *M. oleifera* seed oil are rich in polyunsaturated fatty acids and possibly other non-polar bioactive components that speed up the rate of cholesterol uptake from the blood, its catabolism and slows down its synthesis in the body leading to the prevention of excess blood cholesterol level. This decreased HDL level observed in rats, suggest the dose dependent significant decrease in the total cholesterol level may not be due to enhanced transport of cholesterol from the blood stream to the liver for breakdown rather it is due to decrease food intake (Raveh *et al.* 2001). This will invariably reduce the risks associated with deposition of excess cholesterol in the blood and blood vessels. The blood cholesterol lowering effects of *M. oleifera* seed oil observed in this study suggest that individuals suffering from hypercholesterolaemia could drastically reduce their cholesterol level by consuming appropriate amount of *M. oleifera* seed oil. The *M. oleifera* seed oil could have lower cholesterol by the induction of increased biliary cholesterol excretion in the rats. The effects of *M. oleifera* seed oil of blood cholesterol level is inversely related and indicated that the *M. oleifera* seed oil is more effective in reducing blood cholesterol level at lower doses and administration of higher doses of *M. oleifera* seed oil should be avoided to maximize its therapeutic effects and prevent its wastage. Maintenance of optimum blood cholesterol level is necessary to stay healthy, thus indiscriminate consumption of *M. oleifera* seed oil by healthy individuals because of its cholesterol lowering effect should be discouraged as it could impair normal biochemical functions mediated by cholesterol in the body. This is because cholesterol apart from its role in maintenance of membrane fluidity plays key roles in the synthesis of some hormones, and lipid rafts required for protein sorting, cellular signalling, and apoptosis (Jacobson *et al.*, 2007).

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The no significant decreases observed in the serum triacylglycerol (TAG), high density lipoprotein (HDL), and low density lipoprotein (LDL) levels of rats administered *M. oleifera* seed oil further support the healthy lipid profile maintaining ability of the *M. oleifera* seed oil. The ability of the *M. oleifera* seed oil to reduce serum LDL showed that it could maintain health heart functions by preventing deposition of LDL-cholesterol on the arterial walls which causes atherosclerosis, heart failure and stroke in the absence of the medical intervention. The oil prevents hyperlipidaemia and promotes improved health conditions of individuals that consume right amounts of it and could play vital roles in the management of hyperlipidaemia or dyslipidaemia by effectively maintaining sufficient level of HDL that will transport LDL from arterial walls to the liver for metabolism and detoxification. This prevents atherosclerosis and associated cardiovascular disorders. The *M. oleifera* seed oil demonstrated comparable hypolipidaemic effect on the rats comparable to olive oil and could effectively serve as a good replacement for it. The hypolipidaemic effects of *M. oleifera* seed oil exhibited in this study could attributed to its rich unsaturated fatty acid content as demonstrated by its high peroxide value.

Oil and plant extracts with antihyperlipidaemic effects as demonstrated by *M. oleifera* seed oil could be used in the management of obesity and prevention of its associated health consequences to improve quality of life and life expectancy of obese patients which are in agreement with the earlier report of Mahmoud *et al.* (2017) that obesity reduces life expectancy. The findings of this study show that *M. oleifera* seed oil does not have any negative effects on the body weight and possesses antihyperlipidaemic properties that could prevent hypercholesterolemia and maintains healthy lipid profile.

Conflict of interest

Authors have declared no conflict of interests.

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HEPATOPROTECTIVE EFFECTS OF *Peperomia pellucida* BUTANOL LEAF EXTRACT ON ATRAZINE-INDUCED TOXICITY IN ALBINO WISTAR RATS.

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Abstract

Herbicides play significant roles in environmental health hazard. Hepatoprotective effects of *Peperomia pellucida* leaf extract was assessed in atrazine-induced toxicity in albino rats.

Rats were divided into 5 groups of 5 animals each. All animals except the normal control (Group 1), were intoxicated with a single intraperitoneal injection of atrazine at the dose of 120 mg/Kg bodyweight. Group 2 received only the toxicant. Groups 3 & 4 were treated with 200mg/kg and 400mg/kg bodyweight of *Peperomia pellucida* leaf extract respectively, while Group 5 was the standard control and treated with 100mg/kg bodyweight of Vitamin C. All treatments were administered by intubation for 15 days after intoxication. The normal group (Group 1) received standard pelletized diet and water only. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Serum bilirubin were assessed using standard procedures. Elevated levels of liver enzymes and serum bilirubin were significantly reduced by low dose butanol leaf extract of *P.pellucida*, but not by the higher dose. The extent of reduction was quite comparable to that of the standard vitamin C. Low dose *Peperomia pellucida* butanol leaf extract possesses hepatoprotective effect which may be due to its versatile composition of minerals and phytochemicals. Previous studies by the group showed the plant is rich in potassium and sodium ions and is an effective antioxidant.

Keywords: *Peperomia pellucida*, Herbicides, Liver function Tests, Toxicity, Ethnomedicine.

INTRODUCTION

The problem of providing basic health care needs for her citizenry had constantly been a major cause of concern for most developing countries. The World Health Organization (WHO 2013) estimated that more than half of the world's population do not have access to sufficient health care needs. To ensure use of safe, effective and quality products, and to guarantee patients' safety, she encourages upgrading skills and knowledge in the use of traditional medicine for the populace. In Africa, Middle East, Latin America and Asia, 70-95 per cent of the population still use traditional medicine (TM) for primary healthcare. About a 100 million people are believed to use traditional, complementary or herbal medicine in the European Union (EU) alone — as high as 90 per cent of the population in some countries (WHO 2011). Considering the rigorous steps and cost involved in new drug development, and the fact that modern medicine is desperately short of new treatments (Rinaldi, Shetty 2020), researchers are increasingly searching herbal remedies for complementary treatments of all diseases.

Oxidative stress is a situation whereby steady-state reactive oxygen species (ROS) concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents (Lushchak 2014).

The liver is an important organ of toxicity studies. It is in fact the major organ responsible for metabolism of various endogenous and exogenous compounds. During metabolism of xenobiotics, free radicals are produced, causing deleterious effects on the liver as

well as on other organs (Casas-Grajales and Muriel 2017). This is because most of the substances absorbed by the intestine pass first through the liver where toxins may accumulate. Generation of oxidative stress that leads to hepatic damage is a common mechanism by which toxic substances (like atrazine) harm the liver (Hasanein and Emamjomeh 2019).

Atrazine (ATZ), a triazine-based herbicide extensively used in agriculture worldwide, is a well-known environmental pollutant that is reported to induce toxic effects on several tissues of mammalian experimental models (Adesiyun *et al.* 2011). It is known to cause hepatic damage in rats and pigs (Santa Maria *et al.* 1987; Campos- Pereira *et al.* 2012) as well as induce oxidative stress in several experimental models (Abarikwu *et al.* 2012; 2014). Atrazine has been previously reported to induce oxidative stress in rat tissues and blood at a dose of 120 mg/kg body weight within 16 days of treatment (Farombi *et al.* 2013) and 300mg/kg body weight within 7-21 days (Singh *et al.* 2011).

Peperomia pellucida also called Silver bush, Shiny bush, pepper elder, rat ear or 'Ewe rinrin' belongs to the family Piperaceae. It is a herbaceous plant found in the tropics. It is indeed a lesser known plant as it grows mostly along bush paths, around fences and in moist shady places, on seasonally flooded river banks and wooded rocky hillsides, up to 1100m altitude. It is particularly common on damp hard surfaces such as walls, roofs and steep gullies. It may also grow as an epiphyte on fallen and dead tree trunks. The plant species have a long history of ethnomedicinal use

which depends on the region(Oloyede *et al.* 2011). In Guyana, the plant has been used to lower cholesterol and has been used as a cough suppressant, diuretic, emollient and treatment of cardiac arrhythmia in the Amazon region (Bojo *et al* 1994, Nadine 2004). However, within our locality, there is still paucity of data regarding usage and safety of this herb following usage.

This present work therefore aims at investigating the hepatoprotective effects of butanol leaf extract of *peperomia pellucida* on atrazine-induced oxidative stress in wistar albino rats. Butanol extract was used because previous findings showed that butanol fraction of *P. pellucida* were more active and exhibited better medicinal effect than the crude extract and other fractions of the plant in a comparative study (Khan and

Omoloso 2002).

MATERIALS AND METHOD

Collection and Preparation of Plant Extracts

Aerial parts, stems, leaves and flowers of *Peperomia pellucida* were collected, weighed and air-dried for 23 days to a constant weight and after drying, the leaves were separated from the stems and then pulverized using a milling machine. The pulverized samples were weighed and kept for further analysis. About 200g of the fine powder was wrapped and placed in the soxhlet apparatus for solvent extraction of the plant using butanol (Oloyede *et al.* 2011). The residual mixture was evaporated to slurry with the aid of a water bath at 100°C for 3 hours. The slurry was weighed with analytical balance to give 9.25g and was stored in a petri dish and kept in a refrigerator until required.



Figure 1 : *Peperomia pellucida*, the Miraculous plant.

Experimental design

Twenty five emale Albino Wistar rats weighing 100±2g were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka in Enugu state, Nigeria and acclimatized for 14 days (2 weeks). The animals were housed in aluminum cages with metal netting top. They were maintained on standard pelletized diet and water *ad libitum*. They weighed averagely 150±3g after acclimatization/before experimentation. The animals were subsequently divided into five groups of five animals each, according to the range of their body weights. The animals were handled following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) which were approved by the Federal University of Technology, Owerri Animal Ethics Committee. The animals were grouped as follows :

GROUP I: Normal control of five rats (Not induced with atrazine)

GROUP II: Negative control of five rats (induced with 120mg/kg body weight atrazine and untreated)

GROUP III: Atrazine-induced rats treated with 200mg/kg body weight of *Peperomia pellucida* leaf extract.

GROUP IV: Atrazine-induced rats(5) treated with

400mg/kg body weight of *Peperomia pellucida* leaf extract.

GROUP V: Standard control of oxidatively- stressed five rats treated with 100mg/kg body weight of vitamin C.

At the end of the treatment period (23 days), the animals were fasted for 24 hrs. and then sacrificed following mild anaesthetics with dichloromethane. Blood samples were collected via ocular puncture and allowed to clot. Serum separated and analyzed for the biochemical parameters.

Biochemical Studies

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined using Reitman and Frankel method (1957). The De Ritis ratios were calculated by the formula AST/ALT (Botros and Sikaris 2013). Alkaline Phosphatase (ALP) activity was determined by King and Armstrong (1934) method. The conjugated and total bilirubin were determined using the Max Discovery Tm Total Bilirubin Assay Kit and Diazyme's Direct Bilirubin Vanadate Oxidation assay by the colorimetric method described by Jendrassik and Grof (1938).

Statistical Analysis

All data were expressed as mean ± SD. Comparison of the data between test and control groups of animals were analyzed using one-way Analysis of variance (ANOVA). P-values less than or equal to 0.05 were taken as statistically significant.

RESULTS

The results of the effects of *P. pellucida* on atrazine-induced oxidatively-stressed albino wistar rats were as presented in the figures below. Figure 2 showed the effect of butanol leaf extract of *Peperomia pellucida* on alanine aminotransferase (μ/l) activity of albino rats. ALT activity was significantly elevated in the negative control group as a result of atrazine administration. There was a decrease in ALT concentration as shown in group III (200mg/kg Extract) when compared to the negative control, while the group IV (400mg/kg Extract) produced no difference. Also, the concentration of ALT decreased significantly (P<0.05) in the standard control (Vit.C) group when compared to the negative control. AST enzyme activity of the rats was shown in figure three (3). No significant effect was seen in AST activity of all the animal groups, although an increase was observed in group IV. The De Ritis ratios were 2.41, 1.40, 1.90, 1.86 and 2.12 for the normal control(Group I); negative control(Group II); 200mg/kgExtract(Group III); 400mg/kgExtract(Group IV) and Vitamin C(Group V) respectively. Furthermore, atrazine administration resulted in significant elevation of ALP activity which was significantly reduced in the low-dose extract (Group III) and standard Vitamin C (Figure 4). Elevation of total bilirubin concentration occurred in the negative control group as a result of atrazine administration (Figure 5). This was equally reduced in groups III and IV although non-significantly. A significant reduction of total bilirubin concentration was observed in group V when compared to all others.

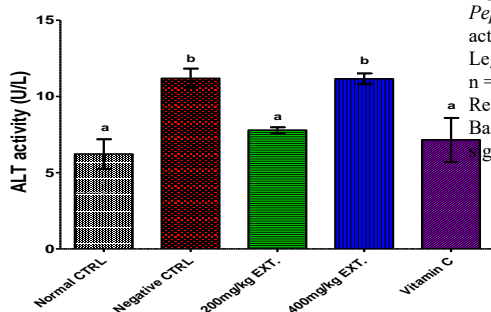


FIGURE 2 : The effects of butanol leaf extract of *Peperomia pellucida* on the Alanine Aminotransferase (μ/l) activity of atrazine-induced hepatotoxic rats.

Legend:
n = 5 for each group
Results are represented as Mean ± SD
Bars with different superscript letters are statistically significant (p<0.05).

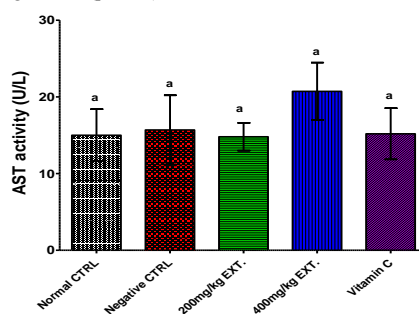


Figure 3: The effects of butanol leaf extract of *Peperomia pellucida* on the Aspartate Amino Transferase (μ/l) activity of atrazine-induced hepatotoxic rats.

Legend:
n = 5 for each group
Results are represented as Mean±SD
Bars with different superscript are statistically significant (p<0.05).

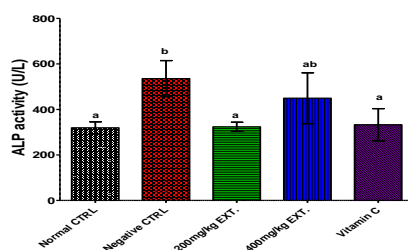


Figure 4: The effects of butanol leaf extract of *Peperomia pellucida* on the Alkaline Phosphatase (μ/l) activity of atrazine-induced hepatotoxic rats.

Legend:
n = 5 for each group
Results are represented as Mean±SD
Bars with different superscript is statistically significant (p<0.05).

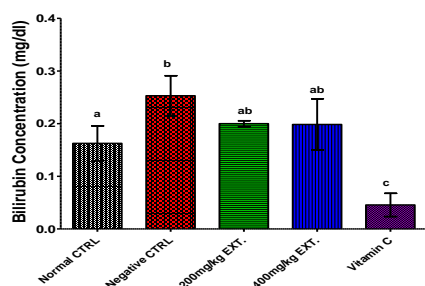


Figure 5: The effects of butanol leaf extract of *Peperomia pellucida* on the Total Bilirubin (mg/dl) concentration of atrazine-induced hepatotoxic rats.

Legend:

n = 5 for each group

Results are represented as Mean ± SD

Bars with different superscript are statistically significant (p<0.05).

DISCUSSION

Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase are periodically produced by hepatic and other cells of the body. The present study investigated the propensity of atrazine to induce liver stress and its possible attenuation by *Peperomia pellucida* in liver of female wistar albino rats. Most frequently, liver damage occurs as parenchymal vacuolar degeneration, necrosis of hepatocytes or disorders in the activity of metabolic enzymes (Philips et al 1987). In our case, the later was the situation. Atrazine administration led to increased levels of liver marker enzymes (ALT and ALP) as well as total bilirubin. The significantly higher ALT activities in animals exposed to atrazine (120mg/kg body weight) when compared to control groups were due to the leakage of aminotransferase (AT) enzymes from injured liver cells. These results were similar to that of Jestadi *et al.* 2014 who obtained a similar result in a study of effects of short term exposure of atrazine on the liver and kidney of normal and diabetic rats in India. ALT is known to be more specific for hepatic injury because it is usually present mainly in the cytosol of the liver and in low concentrations elsewhere (Campos-Pereira *et al.* 2012). The elevation of ALT in our study may be attributed specifically to the injury of liver cells caused by atrazine. Aspartate aminotransferase concentration was not significantly affected in our study. This differs from some previous assessment of effect of toxicant on liver enzymes where AST values were elevated (Zilva et al 1988, Jestadi et al 2014; Emejulu et al 2016). AST has not been a very reliable marker of liver integrity because it is also present in abundance in other organs. AST is known to be a mitochondrial enzyme found in the heart, liver, skeletal muscle, and kidney and is normally present in plasma (Fowler *et al*

2012).

De Ritis ratio is a very useful indicator of liver diseases. It is usually up to 1.7 in women, 1.3 in men, about 2 in children and greater than 2 in neonates (Botros and Sikaris 2013). Generally, it is regarded as normally less than 1 (0.8). When the AST/ALT ratio is equal to one (i.e ALT is equal to AST), it is suggestive of acute viral hepatitis or drug-induced liver toxicity. As the ratio increases to 2.0 or higher or ALT level exceeds 300U/L, it is indicative of alcoholic liver disease. It is usually 1 or less, in non-alcoholic fatty liver disease and ratios greater than 1 can be seen in end-stage liver failure, alcoholic hepatitis, cirrhosis, hepatocellular carcinoma etc. since the ratio increases as fibrosis advances(Mak 2019). In our study, the De Ritis ratio was generally high (>2) in both the normal and standard controls, this was not very clear and infact, we had the lowest ratio in the negative control group. This may seem to differ from the above findings but the fact that it was the finding in the normal control animals showed that the value may be relatively higher in these laboratory animals.

An increase in liver enzyme concentration as it appeared in blood serum revealed that they were leaked into the blood. ALT was significantly (p<0.05) higher and this can result to liver disease, hepatitis and other liver malfunctions. According to Roschester (2010), any rise in serum bilirubin and conjugated bilirubin above 10mg/dl is abnormal and an indication of liver damages and diseases. It then follows that the increases observed in ALT, AST, ALP and serum bilirubin in our study was an indication of liver damages and oxidative stress induced by the toxicant atrazine.

Mechanism of herbicide toxicity has been usually associated with increased lipid peroxidation of the liver (Sharma *et al* 2005; Datta *et al* 1994). Therefore, the results indicated that atrazine might have generated free radicals that reacted with membrane lipids and induced liver damage through distortion of membrane structures (Sharma *et al* 2005). The present study revealed that *P. pellucida* treatment obviously resulted in decreased lipid peroxidation in the liver and protected it from atrazine - induced oxidative stress. The plant had earlier been found to lower cholesterol and very useful in treatment of cardiac arrhythmia in the Amazon region (Bojo *et al* 1994, Nadine 2004). These activities may be due to the presence of many phytochemicals and minerals that have been found in the plant.

The study also demonstrated that there was a significant increase in the level of liver enzymes of atrazine untreated group when compared to normal control (p<0.05). Oral administration of *P. pellucida* to the oxidatively- stressed rats significantly reduced

their ALT, ALP levels as well as serum bilirubin. At low dose of 200mg/kg body weight, the level of the enzymes showed significant decreases when compared to the intoxicated untreated group. At high dosage of 400mg/kg body weight of the extract, the effect was not pronounced. The positive control group treated with Vitamin C also showed a significant reduction of the enzyme activity when compared to the untreated negative control group and was seen to be more effective in reducing the liver enzymes activity than the groups treated with *P. pellucida* butanol leaf extract.

CONCLUSION

This study has demonstrated that there was evidence of liver damage shown by increased concentrations of liver enzymes in the serum of rats induced with atrazine. These effects could lead to edema, cirrhosis and vasculature of liver tissues. However, the effects were ameliorated with administration of low-dose butanol leaf extract of *Peperomia pellucida*.

CONFLICT OF INTEREST : The authors declare that they have no conflict of interest.

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TOXIC EFFECTS OF *Cannabis sativa* LOCAL GIN FORMULATION (MONKEY TAIL) AND TRAMADOL IN ADULT MALE WISTAR RATS: A COMPARATIVE INVESTIGATION

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Abstract

In this study, the toxic effects of *Cannabis sativa* local gin formulation (CSLGF) and tramadol were evaluated and compared. Acute toxicity values were determined for both agents while 25 male rats assigned to 5 groups of 5 rats each were used for the comparative toxicity investigation. While the rats in group 1 were administered 0.2 ml distilled water each and served as the control, those in groups 2 and 3 were administered 25 and 50 mg/kg of tramadol respectively. The rats in groups 4 and 5 also received 25 and 50 mg/kg of CSLGF respectively. Treatment was via the oral route, administered daily and lasted for 21 days. At the end of treatment, the rats were sacrificed by cervical dislocation and blood was collected by cardiac puncture into both EDTA and plain bottles for haematological and biochemical analyses. Result of acute toxicity (LD₅₀) studies revealed an LD₅₀ value of 109.52 mg/kg body weight for CSLGF and 141.42mg/kg body weight for tramadol. CSLGF and tramadol significantly lowered body weights in the rats when compared with control (p<0.05). Of the two test substances, only CSLGF significantly reduced red blood cells (RBCs) count, packed cell volume (PCV) and haemoglobin concentration with increase in platelets values (p<0.05). Both agents significantly increased white blood cells (WBCs) count, aspartate aminotransferase (AST) concentration, alanine aminotransferase (ALT) level, alkaline phosphatase (ALP) concentration and total bilirubin concentration but decreased the concentrations of total protein and albumin (P<0.05). Serum creatinine and urea were also significantly increased in the CSLGF treated rats (P<0.05) but remained unchanged in the tramadol groups. Electrolytes concentrations were not significantly altered following treatment with both CSLGF and tramadol (P>0.05), however glutathione, superoxide dismutase and catalase activities were all significantly lowered in these test rats with concurrent rise in MDA concentrations (p<0.05). We therefore conclude that CSLGF and tramadol may be sources of systemic toxicity in rats with CSLGF showing higher level of toxicity than tramadol.

Keywords: *Cannabis sativa*, tramadol, alcohol, blood

INTRODUCTION

The use of medicinal plants for the management of diseases has become even more relevant and acceptable globally and may be why over 80 percent of the world's population currently relies on herbal medicine for the management of diseases (Ijioma, 2016), a situation attributable to increasing availability of these plants across the world's fauna (Anyanwu and Nwosu, 2014). Unfortunately only few of current medicinal plant studies have addressed the discourse on plants toxicity, making information on the toxic effects of most currently used medicinal plants to be scanty. Another issue of concern is the various forms in which these medicinal plants are formulated. In Africa and Nigeria in particular, the common practice is to use local gin (alcohol) as a base or vehicle without taking into account the toxicity responses which may triggered by either the alcohol base, the medicinal plant in use or both. Systemic toxicity due to alcohol consumption has been reported (Zhou et al., 2017; Teixeira et al., 2014). In southern Nigeria, it is a common practice to soak pulverized *Cannabis sativa* leaves into local gin for consumption after 2-3 days of

extraction. The resulting solution commonly called "monkey tail" is believed to be an effective agent for the management of body pains.

Cannabis sativa is reportedly the most widely used illicit drug globally and has been associated with a number of mental health problems (Degenhardt et al., 2010; Van Andel, 2000; Kumar et al., 2001). Painful muscle cramps, chronic pain in the extremities, tingling and prickling of the fingers of the hands and toes of the feet, as well as ataxia, tremors and intestinal dysfunctions are some reported toxicity manifestations of *Cannabis sativa* (Turner and Agrawal, 2020). On the other hand, Tramadol is a centrally acting synthetic drug with well established and widely reported analgesic activity (Dhanjal et al., 2009; Lee et al., 1993; Raffa et al., 1992). Although, a controlled drug, tramadol in most local communities in Nigeria has become an over the counter product, attesting to its ready availability and ease of unauthorized purchase. With recent reports on the toxic responses and even deaths following tramadol use (Jones et al., 2016; Matthiesen et al., 1998; Shadnia et al., 2008; Belin et

al., 2017), the reassessment of the toxic potentials of the agent has become a subject of research interest. This study therefore, investigated the toxicity potentials of *Cannabis sativa* local gin formulation (CSLGF) and tramadol in adult male rats, with a view to evaluating the degree of toxicity of each agent using common toxicity indicators and comparing same.

MATERIALS AND METHOD

Collection and identification of sample

The leaves of *Cannabis sativa* were purchased from Igbogene Epie, Yenagoa Local Government Area of Bayelsa State, while Tramadol[®] was purchased from a patent medicine store in Umuahia, Umuahia north Local Government Area of Abia State, Nigeria and was identified by a Pharmacologist at the Federal Medical Center (FMC), Umuahia, Abia State, Nigeria. A sample of the plant material was taken to the Department of Forestry and Environmental Management, Micheal Okpara University of Agriculture, Umudike where it was identified as *Cannabis sativa*. A sample specimen was deposited in the herbarium of the Department of Physiology and Pharmacology, Micheal Okpara University of Agriculture Umudike with voucher number MOUAU/VPP/18/021).

Preparation of extract and tramadol stock solution

The leaves were air-dried and milled into fine powder. Twenty (20) grams of the fine powder was weighed and was dissolved in 100ml of local gin (Ogogoro) and allowed to stand for 48 hours within which intermittent stirring was carried out. The resulting solution was then filtered to obtain a filtrate which was kept for use. The weight of the dried residue was determined to be 14.70g. Hence the weight of extract in 100 ml of the local gin was 5.3g, representing a concentration of 0.053 g/ml (53 mg/ml) and is hereafter referred to as *Cannabis sativa* local gin formulation (CSLGF). For Tramadol[®], 100 mg was dissolved in 100 ml of distilled water, representing a stock concentration of 1 mg/ml. The two agents were preserved in a refrigerator for use.

Animals and experimental design

A total of sixty one (61) male Wistar rats (150-180 g) were used for the study. Thirty six of the rats were used for acute toxicity evaluation of the prepared CSLGF and tramadol while the remaining 25 were used for the main study. The rats were purchased from the laboratory animal house of the College of Veterinary Medicine, Michael Okpara University Umudike and were allowed to acclimatize for two weeks before commencement of the experiment. The rats were kept under normal standard environmental conditions and were allowed access to feed (Vital Finisher mash) and water *ad libitum*. Animal experiments were carried out in accordance with the United States guidelines for care and use of experimental animals (1998; NRC, 2010) and as approved by the ethical committee of the

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Acute toxicity (LD₅₀) evaluations of the extract and tramadol

DETERMINATION OF ACUTE TOXICITY (Median Lethal Dose, LD50)

PRINCIPLE- Different doses of test of substance was administered to a group of

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METHOD

The method used by Orieko *et al.* (2019) was adopted with little modification. For each test substance, two test phases were carried out on 18 rats used. In the first phase, 3 groups of 3 rats each were administered 10, 50 and 100 mg/kg of the extract as treatments for groups 1, 2 and 3 respectively. Following mortalities recorded, the test proceeded to the second phase. In this phase, 3 rats assigned to 3 groups of 3 rats each were used. The first group was administered 120 mg/kg of the extract, the second received 200 mg/kg while the third got 400 mg/kg. All treatments were done via the oral route. The same protocol was adopted in the case of tramadol. After 24 hours, mortalities recorded were used to calculate the acute toxicity value of the test substances using Lorke's formula stated as:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$ was employed.

Where: LD₅₀ = Lethal Median Dose

D₀ = Highest dose that gave no mortality

D₁₀₀ = Lowest dose that produced 100% mortality

Sub-acute toxicity evaluation of CSLGF and Tramadol

Twenty five male wistar rats assigned to 5 groups of 5 rats each were treated according to the order below:

Group 1: 0.5ml of water (control)

Group 2: 25mg/kg of tramadol

Group 3: 50mg/kg of tramadol

Group 4: 25mg/kg of CSLGF

Group 5: 50mg/kg of CSLGF

Treatments were oral and lasted for period of 21 days.

At the end of the period, the animals were sacrificed by cervical dislocation and blood was collected from each animal by cardiac puncture into EDTA for haematological study and also into plain bottles for all biochemical studies including liver and renal function tests and antioxidant enzyme assays. Body weights of the rats were measured at the beginning of treatment and on the day of sacrifice.

2.6. Determination of haematological, biochemical and antioxidant values

Haematological values including red blood cells count

(RBCC), packed cell volume (PCV), haemoglobin (Hb), white blood cells count (WBCC), platelets count (PLTC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined at once for each blood sample in an automated haematology analyser (BC-2300, Mindray Company, China). Biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, bilirubin, urea, creatinine, sodium, potassium, chloride and bicarbonate concentration were determined using a commercial test kits following standard procedures prescribed by the producer Randox Laboratories, UK. Superoxide dismutase (SOD) activity was determined by the method of sigma described by Sun, *et al.*, (1988) while reduced glutathione activity was determined in accordance with the method used by Kanu *et al.* (2016).

Statistical analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) and the difference between the samples mean were analyzed by Tukey *post-hoc* test using R-statistics software version 3.03. All results were presented as mean values ± standard deviations (mean ± SD). P-values ≤ 0.05 were considered statistically significant.

RESULTS

3.1. Acute toxicity values of CSLGF and Tramadol
 Zero percent and one hundred percent mortalities were observed in groups treated with 100 mg/kg and 120 mg/kg of CSLGF, producing an acute toxicity value of 109.52 mg/kg body weight (Tables 1 and 2). For tramadol, zero percent and one hundred percent mortalities were observed following the administrations of 100 mg/kg and 200 mg/kg body weight, yielding an acute toxicity value of 141.42mg/kg body weight (Tables 3 and 4).

Table 1. Phase 1 result of acute toxicity evaluation of the Cannabis-local gin formulation

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Clustering and calm
2	50	0/3	Clustering and calm
3	100	0/3	Clustering, depression and death

Table 2. Phase 2 result of acute toxicity evaluation of the Cannabis-local gin formulation

Group	Dose (mg/kg)	No. of death	Observation
1	120	3/3	Clustering, depression

			and death
2	200	3/3	Clustering, depression and death
3	400	3/3	Clustering, depression and death

$$LD_{50} = \sqrt{A \times B}$$

A= Maximum dose that produced no mortality
 B= Minimum dose that killed all animals in a group
 $LD_{50} = \sqrt{100 \times 120}$
 $LD_{50} = \sqrt{12,000}$
 $LD_{50} = 109.52\text{mg/kg}$

Table 3. Phase 1 result of acute toxicity evaluation of Tramadol

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Clustering and calm
2	50	0/3	Clustering and calm
3	100	0/3	Clustering, depression and death

Table 4. Phase 2 result of acute toxicity evaluation of Tramadol

Group	Dose (mg/kg)	No. of death	Observation
1	120	1/3	Clustering and calm
2	200	3/3	Clustering, depression and death
3	400	3/3	Clustering, depression and death

$$LD_{50} = \sqrt{A \times B}$$

A= Maximum dose that produced no mortality
 B= Minimum dose that killed all animals in a group
 $LD_{50} = \sqrt{100 \times 200}$
 $LD_{50} = \sqrt{20,000}$
 $LD_{50} = 141.42\text{mg/kg}$

Effect of CSLGF on body weight changes in rats
 Treatment with CSLGF and tramadol significantly lowered body weight gains in the experimental rats when compared with control ($p < 0.05$) with the

severity of fall been higher in the high dose CSLGF treated group and all groups treated with tramadol (Table 5).

Table 5. Results on the effects of *Cannabis sativa*-local gin formulation on body weight changes in male rats

Parameters	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	Monkey tail (25mg/kg)	Monkey tail (50mg/kg)
Weight on day 0 (g)	195.16±15.87 ^a	177.86±11.99 ^a	264.30±9.09 ^a	195.92±82.79 ^a	231.32±3.2.43 ^a
Weight on day 21 (g)	229.96±13.31 ^a	183.04±16.29 ^b	265.52±7.83 ^c	242.40±8.00 ^a	231.66±8.11 ^a
Weight gain(g)	34.80±3.37	5.18±1.20	1.22±0.08	46.48±4.30	0.34±0.09
% Gain	17.83±2.39	2.91±1.04	0.46±1.31	23.72±3.88	0.15±0.05

Values represent the mean ± SD for N = 5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other ($p > 0.05$).

Effects of CSLGF and Tramadol on the haematological values of rats

Haematological values including RBC, PCV and Hbwere significantly lowered in all groups treated with CSLGF ($p < 0.05$)but did not significantly differ from the control in groups treated withtramadol

($p > 0.05$). Platelets and WBC counts were also significantly increased in all groups treated with both CSLGF and tramadol when compared with control($p < 0.05$) as was the case also withMCH andMCHC values (Table 6).

Table 6: Effects of CSLGF and tramadol on the haematological values of male rats

	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	CSLGF (25 mg/kg)	CSLGF (50 mg/kg)
RBC (x10 ¹² /L)	8.00±0.16 ^a	7.88±0.37 ^a	7.62±0.36 ^a	6.84±0.11 ^b	5.93±0.20 ^c
PCV(%)	48.52±0.43 ^a	47.14±1.62 ^a	46.06±2.50 ^a	41.56±1.52 ^b	38.68±1.20 ^b
Hb (g/dL)	13.48±0.36 ^a	12.98±0.67 ^a	12.80±0.74 ^a	11.01±0.16 ^b	10.91±0.39 ^b
WBC(x10 ⁹ /L)	9.58±0.92 ^a	13.48±0.52 ^b	13.86±0.10 ^b	14.96±1.67 ^b	15.60±2.23 ^b
Platelets(x10 ⁹ /L)	672.80±31.73 ^a	823.80±9.86 ^b	940.60±27.03 ^c	965.00±35.30 ^c	941.80±35.30 ^c
MCV(fl)	60.68±0.75 ^a	59.84±1.36 ^a	60.46±1.15 ^a	60.78±2.01 ^a	65.25±0.40 ^b
MCH (pg)	16.88±0.59 ^a	16.48±0.38 ^a	16.80±0.45 ^a	16.24±0.15 ^a	18.42±0.19 ^b
MCHC (g/L)	47.30±1.10 ^a	46.42±1.68 ^a	45.31±1.92 ^a	37.59±2.49 ^a	35.42±2.68 ^a

Values represent the mean ± SD for N = 5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other ($p > 0.05$). PCV, Packed Cell Volume; Hb, Haemoglobin; RBC, Red Blood Cells; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Volume; WBC, White Blood Cell.

Effect of CSLGF and tramadol on liver function parameters in rats

Oral administrations of CSLGF and tramadol significantly lowered total protein and albumin concentration but increased the concentrations of AST, ALT, ALP and total bilirubin in the treated rats. These observed biochemical changes were however more severely reduced in the CSLGF treated animals than those administered tramadol (Table 7).

significantly increased in all rats administered CSLGF when compared with control ($P < 0.05$) but serum concentrations of sodium, potassium, chloride and bicarbonate were all lower than control values in the CSLGF and tramadol treated rats rats (Table 8).

Effect of CSLGF and tramadol on renal function parameters in rats

Serum concentrations of creatinine and urea were

Effect of CSLGF and Tramadol on antioxidant parameters in rats

Serum concentrations of antioxidant enzymes including GSH, SOD and catalase fell below control values following treatment with higher doses of CSLGF and tramadol ($p < 0.05$) with concurrent rise in MDA concentration ($p < 0.05$). The results on

antioxidant parameters are presented in table 9.

Table 7: Effect of CSLGF and Tramadol on liver function parameters in male rats

	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	CSLGF (25mg/kg)	CSLGF (50mg/kg)
Total Protein (g/dL)	7.86±0.27 ^a	7.62±0.13 ^a	7.16±0.21 ^b	6.52±0.19 ^c	6.10±0.24 ^d
Albumin (g/dL)	4.64±0.45 ^a	3.27±0.45 ^b	3.15±0.06 ^b	2.87±0.26 ^b	2.75±0.19 ^c
Globulin (g/dL)	3.22±0.23 ^a	4.35±0.32 ^b	4.01±0.19 ^b	3.65±0.11 ^a	3.35±0.05 ^a
AST (U/L)	39.20±2.28 ^a	113.60±8.08 ^b	149.20±2.78 ^c	161.40±6.15 ^d	185.40±5.73 ^c
ALT (U/L)	33.60±2.51 ^a	80.60±2.07 ^b	121.80±2.49 ^c	128.00±5.83 ^c	155.00±9.46 ^d
ALP (U/L)	104.00±4.53 ^a	161.60±6.66 ^b	207.60±6.47 ^c	244.00±6.89 ^d	257.60±8.17 ^c
Total Bilirubin (µmol/L)	0.41±0.05 ^a	0.45±0.10 ^a	0.59±0.16 ^a	1.03±0.13 ^b	1.27±0.05 ^c
Direct Bilirubin (µmol/L)	0.28±0.02 ^a	0.11±0.05 ^b	0.24±0.06 ^a	0.37±0.04 ^c	0.49±0.07 ^d
Indirect Bilirubin (µmol/L)	0.14±0.04 ^a	0.34±0.07 ^b	0.35±0.11 ^c	0.65±0.12 ^d	0.78±0.02 ^d

Values represent the mean ± SD for N = 5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p > 0.05). AST, Aspartate amino transferase; ALT, Alanine amino transferase; ALP, Alkaline phosphatase.

Table 8: Effect of CSLGF and tramadol on renal function parameters in male rats

	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	CSLGF (25mg/kg)	CSLGF (50mg/kg)
Creatinine (mg/dL)	0.46±0.05 ^a	0.28±0.04 ^b	0.38±0.01 ^c	0.64±0.02 ^d	0.70±0.02 ^c
Urea (mg/dL)	18.58±0.83 ^a	22.14±1.11 ^b	19.99±1.37 ^a	26.82±0.66 ^c	27.07±0.36 ^c
Sodium (mEq/L)	144.06±2.72 ^a	140.38±3.94 ^a	141.82±1.19 ^a	141.56±1.96 ^a	140.64±3.03 ^a
Potassium (mEq/L)	4.89±0.15 ^a	4.64±0.12 ^b	4.84±0.13 ^a	4.24±0.43 ^c	4.05±0.12 ^c
Chloride (mEq/L)	95.25±0.75 ^a	83.54±0.48 ^b	81.18±0.81 ^c	83.62±0.55 ^b	82.50±1.12 ^b
Bicarbonate (mMol/L)	26.60±2.22 ^a	22.74±1.39 ^a	23.96±1.42 ^a	23.12±1.20 ^a	23.54±0.70 ^a

Values represent the mean ± SD for N = 5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p > 0.05).

Table 9: Effect of CSLGF and tramadol on antioxidant parameters in male rats

	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	CSLGF (25mg/kg)	CSLGF (50mg/kg)
GSH (IU/L)	47.30±1.10 ^a	46.42±1.68 ^a	45.31±1.92 ^a	37.59±2.49 ^b	35.42±2.68 ^b
SOD (IU/L)	25.53±1.32 ^a	25.27±0.37 ^a	24.93±0.70 ^a	24.33±1.07 ^a	22.76±1.15 ^b
CAT (IU/L)	16.77±2.32 ^a	16.43±0.92 ^a	13.88±0.79 ^b	14.95±0.78 ^a	13.23±0.85 ^b
MDA (mmol/L)	0.62±0.07 ^a	0.73±0.16 ^a	0.74±0.13 ^a	1.37±0.16 ^b	2.05±0.16 ^c

Values represent the mean ± SD for N = 5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p > 0.05). GSH, reduced Glutathione; SOD, Superoxide Dismutase; CAT, Catalase; and MDA, Malondialdehyde

DISCUSSION

In this study, the toxicological effects of a CSLGF and tramadol in male Wistar rats were investigated. The low acute toxicity values obtained for both substances suggest narrow margins of safety associated with the use of these agents. CSLGF was also found to have

lower acute toxicity value than tramadol and suggests further that CSLGF may have higher acute toxicity effect than tramadol. Toxic responses like dizziness, drowsiness, nervous problems, gastritis and liver diseases have been associated with the use of *Cannabis sativa* (Turner and Agrawal, 2020). In the

current study, the mortalities observed following the administration of CSLGF may be attributed to either the quantity of alcohol or phytochemical agents consumed by the rats or both. Toxicity effects in rats due to alcohol administration have been reported (Teixeira *et al.*, 2014). Tetrahydrocannabinol too, a major toxic component in *Cannabis sativa* has been implicated in Cannabis induced toxicity in rats (Turner and Agrawal, 2020). Various degrees of toxicity including deaths have also been reported due to tramadol intake (Salah *et al.* 2018; Youssef and Zidane, 2015). Therefore, the rats that died during the acute toxicity tests may have been administered toxic doses of CSLGF and tramadol.

The gross weight loss observed in the rats due to administered CSLGF suggest that the formulation may have enhanced the metabolism of fat and at the same time decreased the storage of same in the rats. The agent may have lowered intestinal food transit and appetite in the rats leading to weight loss. These physiological changes in the body are established causes of body weight loss in animals (Cummings *et al.*, 2007; Drucker, 2007).

The values of haematological parameters have been used to assess the toxicity effects of substances (Akomas *et al.*, 2015). In this study, the administration of CSLGF at the doses used caused significant fall in the values of RBC parameters, an effect which may be attributed to the alcohol content of the formulation. Haematotoxicity may manifest as decline in the number of RBCs in circulation (Oshilonyah *et al.*, 2015). The observed fall in these parameters may be due to destruction of red blood cells, suppression of erythropoietic mechanisms in the bone marrows and inadequate absorption of folic acid in the gastrointestinal tract, occasioned by consumed alcohol. These physiological changes have been implicated in alcohol-induced anaemia (Zhou *et al.*, 2017). The observed haematotoxicity of CSLGF may also be due to the toxic effect of tetrahydrocannabinol, a major constituent in *Cannabis sativa* known for its ability to cause prolonged suppression in bone functions in rats (Turner and Agrawal, 2020). The insignificant effect shown by tramadol on the treated rats suggests that anaemia may not be part of the toxicity effects of tramadol treatment. However, the elevations in white blood cells count following treatment with both CSLGF and tramadol may be associated with liver inflammations caused by these treatment agents. Elevation in white blood cells count due to liver inflammation is well established (Chung *et al.*, 2016). Rise in WBCs count may also be due to the activation of body's defense mechanism and immune system, a positive response for survival due to cell mediated immune response.

The observed increase in platelets number following treatment with both tramadol and CSLGF is consistent

with existing data (Gautheir, 2005; Obembe *et al.*, 2015) and suggests that thrombocytopenia is one of the toxicity manifestations of tramadol and CSLGF. High platelets counts have been implicated in the aetiology of thrombosis, cardiovascular shock and myocardial infarction (Ijioma, 2016; Randal, *et al.*, 2004) which are complications associated with alcohol and narcotics addiction.

The Liver exerts incontrovertible influence on the functions of many organs in the body and is prone to substance-induced injury due to its central role in the metabolism of numerous substances in addition to its portal location within the body (Jones, 1996). Alterations in liver biomarkers are therefore used to access the toxicity levels of substances including plant extracts (Akomas *et al.*, 2015). The decreased concentrations of serum total protein and albumin with increase in globulin levels in the tramadol and CSLGF treated rats suggest that these agents may have negated liver functions. The liver is the site for the synthesis of these proteins and decline in serum total protein concentration may be an early sign of liver dysfunction (Thepa and Wall, 2007). Increase in total bilirubin concentration, ALT level and AST concentration may be due to hepatic injury and hepatotoxicity (Oshilonyah *et al.*, 2015b). The elevated serum ALP concentration suggests possible biliary tract obstruction and further liver damage (Akomas *et al.*, 2015; Shahjahan *et al.*, 2004) due to metabolites generated following repeated administrations of tramadol and CSLGF. Studies have shown that metabolites may cause greater toxicity effects than their parent substances and may affect not just the liver but also other organs like the kidneys (Goeringer *et al.*, 1997). This may be the reason for the observed elevated urea and creatinine concentrations in the current study. Increase in serum urea and creatinine concentrations have been reported in rats treated with tramadol (Atici *et al.*, 2005). The fact that serum electrolytes concentrations were not significantly affected suggests that kidney problems due to tramadol and CSLGF may be limited to the impact of elevated urea and creatinine concentrations (Murray, 1997)

Lipid peroxidation is considered a useful biomarker for measuring oxidative stress status (Akomas *et al.*, 2015), hence high MDA values with falls in the levels of antioxidant enzymes like glutathione, superoxide dismutase and catalase are indicative of increasing oxidative stress while low MDA values with increase in the levels of these enzymes suggests improved body antioxidant defense line and increase in general well-being (Kanu *et al.*, 2016; Bekhe *et al.*, 2011). Antioxidant agents decrease oxidative stress by scavenging free radicals in biological systems (Ijioma *et al.*, 2019). In the current study, treatment with tramadol and CSLGF caused fall in body's antioxidant enzymes concentrations with increased lipid

peroxidation. This result is consistent with existing literature on tramadol toxicity (Ismail *et al.*, 2010), but oxidative stress due to CSLGF may be linked with toxicity effects of the toxic components of *Cannabis sativa* and the alcohol used as vehicle.

Conclusion

Results of this study have shown that both CSLGF and tramadol are potential sources of systemic toxicity,

having demonstrated significant haematotoxic, hepatotoxic and nephrotoxic effects and possessing acute toxicity values which indicate very narrow margins of safety and high toxicity potentials in the rats used. Although the two substances under study may have induced toxicity via similar mechanisms, *Cannabis sativa* local gin formulation (CSLGF) popularly called monkey tail, caused a higher level of toxicity than tramadol

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RENOPROTECTIVE EFFECT OF METHANOL EXTRACT OF *Justicia carnea* LEAVES ON GENTAMICIN-INTOXICATED ALBINO RATS.

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ABSTRACT

The renoprotective effects of methanol extract of *Justicia carnea*(JC) leaves on gentamicin (GEN)-intoxicated albino rats were evaluated. The animals were grouped into five groups of five rats each according to bodyweight. Nephrotoxicity was induced in the rats intraperitoneally with 80 mg/kg b.w of gentamicin. Group 1 served as normal control (NC) without induction, group 2 served as the gentamicin-intoxicated control (GIC) without treatment, groups 3-5 were intoxicated with 80 mg/kg of gentamicin and treated with 200, 500 and 1000 mg/kg b.w of methanol extract of *Justicia carnea* leaves respectively. After 10 days of extract administration, the animals were sacrificed under mild anesthesia and the serum collected for biochemical analyses. Urea and creatinine concentrations showed a significant ($p<0.05$) increase in the GIC when compared with the NC, while a significant ($p<0.05$) decrease was observed in urea and creatinine concentrations in groups treated with 200mg/kgb.w, 500mg/kgb.w and 1000mg/kgb.w of JC extracts when compared with GIC. In contrast, there was a significant ($p<0.05$) increase in uric acid concentration in the GIC when compared with the NC, but a significant ($p<0.05$) reduction was only observed in the group treated with 200mg/kgb.w of *Justicia carnea* when compared with the GIC. The activity of superoxide dismutase (SOD) and reduced glutathione (GSH) levels showed a significant ($p<0.05$) increase in the GIC when compared with NC, but treatment with 200, 500 and 1000 mg/kg b.w significantly ($p<0.05$) decreased the SOD activity and GSH concentration when compared with GIC. Malondialdehyde (MDA) concentration increased significantly ($p<0.05$) in the GIC when compared with NC, while treatment with different concentrations of *Justicia carnea* extract significantly ($p<0.05$) reduced the concentration of MDA. In the histopathology evaluation of the kidney, the rats in NC group showed normal renal histomorphology against GIC which showed degeneration and necrosis in the cortex and outer medullar, but administration of different concentrations of *Justicia carnea* extract showed a mild widespread degeneration of the renal tubules. The result of this study showed that different concentrations of *Justicia carnea* extract ameliorated the toxic effect seen in the podocytes caused by gentamicin intoxication. This suggests that *Justicia carnea* extract may exhibit protective effects on the podocytes, which may be attributed to the antioxidants present in the plant.

INTRODUCTION

Medicinal plants are used as herbal medicine for prevention and treatment of diseases. It ranges from traditional and popular medicines of every country to the use of standardized and titrated herbal extracts (Adesina, 2005). It has become a popular form of health care even though several differences exist between herbal and conventional pharmacological treatments. Herbal medicine needs to be tested for efficacy using conventional trial methodology and several specific herbal extracts has been demonstrated to be efficacious for specific conditions (Alabi *et al.*, 2017). It is recognized as the most common form of alternative medicine (Ogbonna *et al.*, 2011). The World Health Organization (WHO estimates that 80% of the world's population relies on these alternative plant base medicines as their primary medical intervention especially in the developed countries where modern medicines are predominantly used (WHO, 1991). Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases, this practice being an alternative way to compensate for perceived deficiencies in orthodox pharmacotherapy (Ogbonnia *et al.*, 2011).

Traditionally, several species of *Justicia carnea* have been used in the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, kidney diseases, rheumatism and arthritis (Badami *et al.*, 2003; Correa 2012). Ukpabi-ugo *et al.*, 2019 also reported the hepatoprotective effect of *Justicia carnea* on CCl₄-induced hepatotoxicity; and it was also reported to possess anti-inflammatory, anti-allergic, anti-tumoral, anti-viral and analgesic activities (Radhika *et al.*, 2013). Species of *Justicia* found in India such as *Justicia traquebarensis* and *Justicia wynaadensis* have been reported to have cardio protective properties and antioxidant activity respectively (Radhika *et al.*, 2013). A survey among the Igbo local populace in Nigeria revealed that the plant under study is locally called 'Ogwu Obara' meaning blood tonic (Onyeabo *et al.*, 2017). The deep purple juice from the leaves of this plant is extracted neither by soaking or boiling in water which can be drunk as tea. In other localities in Nigeria, raw leaves are chewed and used together with 'nchuanwu' as culinary vegetables to garnish yam porridge.

Renal failure connotes the inability of the kidney to excrete nitrogenous waste and maintain fluid and electrolytes homeostasis (Abdul, 2000). Renal failure can be acute or chronic kidney failure (Onyeabo *et al.*, 2017).

Gentamicin, an aminoglycoside antibiotic with spectrum of activities, is vastly used in the treatment of gram negative bacterial infections (Martins *et al.*, 2007), but its usefulness is limited due to its serious side effects such as nephrotoxicity. Gentamicin is known to generate reactive oxygen species associated with an increase in lipid peroxidation and decrease in antioxidant enzyme activity in the kidney (Bandayet *al.*, 2008). It has been showed that up to 30% of people who receive a course of gentamicin treatment develop some symptoms of nephrotoxicity (Ali *et al.*, 2011).

Despite these scientific and medicinal values, there is paucity in information on the renoprotective effect of methanolic extract of *Justicia carnea* leaves. Therefore this study was designed to evaluate the reno-protective effects of the methanolic extract of *Justicia carnea* leaves on gentamicin-intoxicated albino rats.

Materials and methods

Collection and identification of plant material

The fresh leaves of *Justicia carnea* were collected from Umuariaga Village, Umudike, Abia State, Nigeria. It was identified by the Taxonomic unit of the Department of Plant Science and Biotechnology in Michael Okpara University of Agriculture, Umudike (MOUUA), Abia State, Nigeria with the voucher number MOUUA/VPP/2018/04.

Preparation of leaves extract

Justicia carnea leaves were pulverized into coarse powder using an electric blender and weighed. One hundred and twenty (120) grams of *Justicia carnea* was dissolved in 600ml of methanol with constant stirring for 24 hours to facilitate the extraction. The solution was filtered with Whatman No. 4 Filter paper and the filtrate was concentrated to a semi-solid residue in a water bath at 40°C. The residue obtained was dissolved in DMSO₄ and water. A stock solution was prepared with the concentration of 1g of plant extract in 1ml of DMSO₄ and 9ml of water.

The volume of stock to be administered was calculated based on the individual body weight of each animal, using the formula:

$$\text{Volume} = \frac{D \times P}{C}$$

where; D = dose to be administered, P = body weight of animal in kg,

C = concentration of the stock (Ukpabi-ugo *et al.*, 2016)

Experimental design

Twenty-five (25) albino rats (110 – 140 g) proven maturity were housed in clean cages contained in well

ventilated standard housing condition. The animals were fed with normal commercial rat feed (Vital Feeds) and water. The animals were kept in a well ventilated stainless steel cages and left under laboratory conditions for and were acclimatized for one week before the commencement of the experiment. After acclimatization period, the rats were weighed, randomized and divided into five groups of five rats each on the basis of bodyweight

Group 1- Normal control receiving only normal rat feed and water

Group 2 was administered 80mg/kgb.w of Gentamicin only

Group 3 was administered 80mg/kgb.w of Gentamicin and 200mg/kgb.w of *Justicia carnea* extract

Group 4 was administered 80mg/kgb.w of Gentamicin and 500mg/kgb.w of *Justicia carnea* extract

Group 5 was administered 80mg/kgb.w of Gentamicin and 1000mg/kgb.w of *Justicia carnea* extract

At the end of the treatment period, all the rats were anaesthetized using chloroform-saturated chamber, dissected and blood samples were collected by cardiac puncture.

Preparation of drugs (Gentamicin)

The volume of Gentamicin administered was calculated using the formula,

$$\text{Quantity} = \frac{\text{Dose (mg/kg)} \times \text{Body weight(g)}}{\text{Concentration}}$$

(Ukpabi-ugo *et al.*, 2016)

Induction of nephrotoxicity

Nephrotoxicity was induced by administering 80mg/kgb.w of Gentamicin to the animals every day for 10 days (Ramaswamy and Perumal, 2004).

Blood collection

After the 10 days dose treatment, the rats were weighed, anaesthetized and sacrificed. Thereafter, blood samples were collected by cardiac puncture in EDTA-free bottles for biochemical assay.

Serum urea determination

Serum urea was estimated by the Urea method of Weather burn (1967).

Serum creatinine determination

Serum creatinine was estimated by the method of Bowers and Wong (1980).

Superoxide dismutase(SOD) activity determination

The method of Beauchamp and Fridovich (1971) was used.

Determination of Catalase(CAT) activity

Catalase activity was determined by the method of Aebi (1984).

Determination of serum reduced Glutathione (GSH) Concentration

Reduced glutathione was determined by the method of Ellman (1959).

Determination of Malondialdehyde (MDA) Concentration

Malondialdehyde concentration was determined by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al.*, 1993.

Histology of the kidney

At the end of the experiment, the rats were weighed

and anaesthetized with chloroform in order to dissect them and harvest the kidney. Adherent tissues were trimmed off the harvested kidneys and were immediately fixed in 10 % formalin to avoid autolysis and they were processed and embedded in paraffin wax to provide a hard support for sectioning. Every third section was mounted in glass slide and stained with Hematoxylin and Eosin and subjected to photomicrography

Statistical analysis

Descriptive statistics were carried out on the data generated using Statistical Package and Service Solutions (SPSS). Results were expressed as the Mean ± standard deviation (SD). One way Analysis of Variance (ANOVA) was used to separate means with Post-Hoc multiple comparison (option - LSD). Statistical significance was accepted at P values less than 0.05 (p<0.05).

RESULTS

Effect of methanol extract of *Justicia carnea* on the serum kidney markers(urea, creatinine and uric acid) The result of effect of methanolic extract of *Justicia carnea* in Table 1 below showed that there was a significant(p<0.05) increase in the concentration of urea and creatinine in the group treated with gentamicin only when compared with normal control. However, a significant (p<0.05) decrease was observed in the concentration of urea and creatinine in the groups treated with different concentrations of *Justicia carnea* (200mg/kgb.w, 500mg/kgbw and 1000mg/kgb.w) when compared with the gentamicin-intoxicatedcontrol . On the other hand, there was a significant (p<0.05) increase in the concentration of uric acid in the gentamicin-intoxicatedcontrol when compared with the normal control but a non-dose dependent significant (p<0.05) reduction was only observed in the group treated with 200mg/kgb.w, while 500mg/kgb.w and 1000mg/kgb.w shows no significant(p>0.05) difference when compared with the Gentamicin-intoxicatedcontrol.

Table 1: Effect of methanol extract of *Justicia carnea* on the serum kidney markers(urea, creatinine and uric acid)

GROUPS	TREATMENT	UREA (mg/dl)	CREATININE(mg/dl)	URIC ACID (mg/dl)
1	CONTROL	15.81±1.96 ^b	0.86±0.10 ^b	6.38±0.97 ^b
2	GEN-INTOXICATED CONTROL	27.29±0.10 ^a	2.78±0.10 ^a	11.62±2.52 ^a
3	GEN +200mg/kg bw	24.14±1.60 ^{ab}	1.74±0.16 ^{ab}	8.80±0.59 ^{ab}
4	GEN+ 500mg/kg bw	21.22±1.21 ^{ab}	1.85±0.04 ^{ab}	9.95±0.39 ^a
5	GEN+ 1000mg/kgbw	13.25±0.56 ^{ab}	0.78±0.11 ^{ab}	10.12±1.46 ^a

Values are expressed as mean±SD. Mean values with different superscript in a column are significantly different (p<0.05). GEN= Gentamicin

Effect of methanol extract of *Justicia carnea* on some serum antioxidant enzymes, non-enzymatic

antioxidant and oxidative stress markers (SOD, CAT, GSH, MDA).

The result of effect of methanol extract of *Justicia carnea* (Table 2) showed that there was a significant ($p < 0.05$) increase in the activity of superoxide dismutase (SOD) and reduced glutathione (GSH) concentration in the gentamicin-intoxicated control when compared with the normal control, but treatment with different concentrations of *Justicia carnea* extract (200, 500 and 1000mg/kg b.w) significantly ($p < 0.05$) decreased the SOD activity and

GSH concentration when compared with the gentamicin-intoxicated control. On the other hand, malondialdehyde (MDA) concentration increased significantly ($p < 0.05$) in the Gentamicin-intoxicated control when compared with the normal control, while treatment with different concentrations of *Justicia carnea* extract significantly ($p < 0.05$) reduced the concentration of MDA when compared with the Gentamicin-intoxicated control.

Table 2: Effect of methanol extract of *Justicia carnea* on some serum antioxidant enzymes, non-enzymatic antioxidant and oxidative stress markers (SOD, CAT, GSH, MDA).

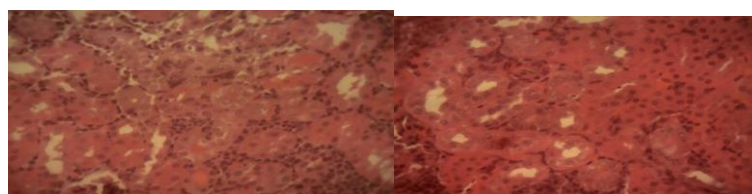
TREATMENTS	SOD (U/g protein)	CAT (KU/g protein)	GSH (µg/ml)	MDA (nanomole/g protein)
1 CONTROL	26.87±0.98 ^b	20.02±1.26	51.84±2.68 ^b	1.00±0.23 ^b
2 GEN-INTOXICATED CONTROL	19.36±3.62 ^a	18.65±1.62	41.15±1.36 ^a	2.04±0.18 ^a
3 GEN+200mg/kg bw	23.50±0.63 ^{ab}	18.14±0.55	42.38±3.32 ^a	1.60±0.04 ^{ab}
4 GEN+500mg/kg bw	23.23±0.39 ^{ab}	17.80±0.79 ^a	43.82±2.30 ^a	1.48±0.05 ^{ab}
5 GEN+1000mg/kg bw	24.53±0.62 ^{ab}	20.05±1.35	53.15±2.55 ^{ab}	1.36±0.08 ^{ab}

Values are expressed as mean±SD. Mean values with different superscript in a column are significantly different ($p < 0.05$). GEN= Gentamicin

Histopathology results

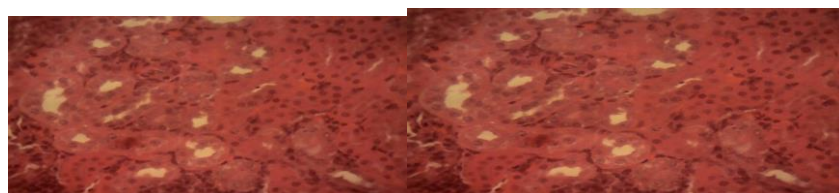
Histopathological examination of the kidney tissues showed that the normal control group (Group I) had normal renal histomorphology compared with Gentamicin-treated control that showed mild

multifocal areas of renal tubular degeneration and necrosis (black arrow) in the cortex and outer medullar but treatment with different doses of *Justicia carnea* minimize these renal tubular degeneration and necrosis in the cortex and outer medullar.



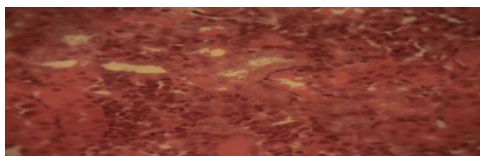
Normal control

Gentamicin-Intoxicated control



GEN+200mg/kg bw J.C

GEN+ 500mg/kg bw J.C



GEN + 1000mg/kg bw.I.C

DISCUSSION

There are certain factors which affect the functionality of the kidney, resulting in kidney failure. Kidney failure may arise from the destruction of the tubules by drugs. This affects the main functions of the kidney which are glomerular filtration, tubular reabsorption and secretion. The functional capacity of nephrons of animals is evaluated by the renal function indices such as creatinine, urea and uric acid (Yakubu *et al.*, 2003). Gentamicin has a nephrotoxic potential as indicated by the impairment in the renal function, which was reflected in the significant increases in serum creatinine, serum urea, and uric acid concentration when compared with the normal control (Pratibha *et al.*, 2009).

Creatinine is a nitrogenous product produced from metabolism of creatine phosphate by creatine kinase in the skeletal muscles and filtered out of the blood by the kidney, and hence is considered as one of the most reliable indicators of efficiency of renal function (Baba *et al.*; 2011). In this study, there was a significant increase in the concentration of creatinine in the Gentamicin-intoxicated control group when compared with the normal control. It has been documented that Gentamicin-induced nephrotoxicity results in an increase in serum creatinine, accompanied by an increase in blood urea nitrogen levels and severe proximal renal tubular necrosis (Ermed *et al.*, 2000; Al-mejed *et al.*, 2002). Furthermore, significant increase in blood creatinine is associated with distinct renal structural damage (Chaware *et al.*; 2011). A significant decrease was observed in the concentration of creatinine in the groups treated with different concentration of *Justicia carnea* extract when compared with the Gentamicin-intoxicated group. This may suggest that of *Justicia carnea* leaves extract may ameliorate the toxic effects on the kidney as a result of flavanoids and phenols constituents of *Justicia carnea*, which play important roles in protecting biological systems (Okwu, 2004). Gupta and Prakash (2009) also reported that green leafy vegetables are particularly important in promoting health because of their nutritive contents.

Urea is a major nitrogenous product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid and in kidneys. It is filtered out of blood by glomeruli so the most frequently determined clinical indices for estimating renal function depends on the concentration of urea in the serum (Gowda *et al.*,

2010). There was a significant increase in the concentration of urea in the Gentamicin-intoxicated group when compared with the normal group. This agrees with Hazilawati *et al.* (2009) who reported that rats with acute kidney injury due to Gentamicin overdose showed elevated urea and creatinine. However, a significant decrease was observed after treatment with different concentration of *Justicia carnea* leaves extract. We therefore, propose that *Justicia carnea* leaves may have some protective effects against gentamicin-induced toxicity. Uric acid is the final oxidation product of purine metabolism and is normally excreted (Johnson *et al.*, 2011). Therefore, elevated serum uric acid concentrations are seen in patients with reduced glomerular filtration rate (GFR) (Christinet *et al.*, 2015). In this study, there was a significant increase in the concentration of uric acid in Gentamicin-intoxicated control when compared with the normal control. This agrees with the result of Ahmed *et al.* (2016) who reported that gentamicin elevated the serum levels of creatinine, urea and uric acid, as well as the MDA level in the renal tissue and produced degenerative changes in glomeruli and tubules associated with increased expression of apoptotic markers and decreased expression of anti-apoptotic markers. But a significant reduction was observed in the group treated with different concentrations of *Justicia carnea* extract when compared with the gentamicin-intoxicated control. *Justicia carnea* is rich in phenols and flavonoids and studies have shown that phenols and flavonoids are capable of inhibiting xenobiotic-induced nephrotoxicity in experimental animal models due to their potent antioxidant or free radical scavenging activities (Paya *et al.*, 1993).

Antioxidant enzymes are sensitive to severe damage to cells (Valko *et al.*, 2007). They participate in inactivation of reactive oxygen species which can cause harm to the cell. Superoxide dismutase is an important antioxidant defense in nearly all living cells exposed to oxygen species. In this study, there was a significant increase in the activity of superoxide dismutase in the gentamicin-intoxicated control group when compared with the normal control, suggesting renal damage. Gentamicin is known to generate reactive oxygen species associated with increase in lipid peroxidation and decrease in oxidation enzyme activity in the kidney (Banday *et al.*, 2008). However, a significant decrease observed on treatment with different concentrations of *Justicia carnea* extract when compared with the gentamicin-intoxicated

control. This may suggest that the presence of bioactive components of *Justicia carnea* were able to avert the gentamicin-induced toxicity (Paya *et al.*, 1993).

Reduced glutathione (GSH) is a tripeptide that plays a major role in kidney detoxification. It is an important antioxidant in plants and is capable of preventing damage to important cellular components caused by reactive oxygen species such as peroxides, lipid peroxide and heavy metals (Pompella *et al.*, 2003). It can be regarded as an endogenous protective agent against nephrotoxics. In this study, there was a significant increase in the concentration of GSH in the gentamicin-intoxicated control when compared with the normal control. This may be as a result of accumulation of reactive oxygen species and its induced changes in the cellular system (Karthikey and Rani, 2003). So, it is an indication of oxidative stress. However, treatment with different concentrations of *Justicia carnea* extract significantly decreased the concentration of GSH when compared with the Gentamicin-intoxicated group. This indicates that the extract may have enhanced glutathione synthesis and regeneration of GSH (Nasir, *et al.*, 2013). It may also be due to the ability of the extract to stimulate these antioxidant enzymes to counteract the ROS produced by gentamicin (Mahmoodzadeh *et al.*, 2017). Pratibha *et al.* (2009) reported that gentamicin administration to rats appears to enhance the production of O₂⁻ anions and unstable OH[•] radicals.

Malondialdehyde (MDA) is an organic compound which occurs as a natural marker for oxidative stress. It results from lipid peroxidation of polyunsaturated fatty acids (Davey *et al.*, 2005). The degree of lipid peroxidation can be estimated by the amount of malondialdehyde. In this study, there was a significant increase in the concentration of MDA in the

gentamicin-intoxicated control when compared with the normal control. The mechanism for the generation of renal toxicity with gentamicin may be due to the production of reactive oxygen species (ROS) and (NOS) reactive nitrogen species in renal cells (Christo *et al.*, 2011), which can lead to inflammation and apoptosis of mesangial cells (Ali *et al.*, 2011). Meanwhile, treatment with different concentrations of *Justicia carnea* extract significantly reduced the concentration of MDA when compared with the gentamicin-intoxicated control. *Justicia carnea* has been reported to possess antioxidant activity (Ukpabi-ugo *et al.*, 2018). It may be hypothesized that gentamicin treatment is likely to produce free radicals indicating oxidative damage at the cellular level of the renal cortex thus, the possible mechanism of renoprotection of *Justicia carnea* may be attributed to its antioxidant and free radical-scavenging properties.

Histopathological examination revealed that the rats in the normal control group showed normal renal histomorphology, compared to the gentamicin-intoxicated group that showed mild multifocal areas of renal tubular, degeneration and necrosis in the cortex and outer medullar but administration of different concentrations of *Justicia carnea* extract showed a mild widespread regeneration of the renal tubules indicating that the extract may have healing and regenerating efficacy on the podocytes and may be able to prevent kidney damage.

In conclusion, methanolic extract of *Justicia carnea* leaves was able to confer protection to the kidney of experimental rat via antioxidant action against gentamicin-induced toxicity and hence may be used as an adjuvant in the treatment of acute renal injury or diseases in man.

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EFFECT OF *Sphenostylis sternocarpa* and *Telfairia occidentalis* ON OXYGEN AFFINITY OF SICKLE HAEMOGLOBIN (HbS)

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ABSTRACT

Sickle cell disease is characterized by mutation in the gene encoding β -subunit of haemoglobin (Hb) which results in the substitution of valine for glutamic acid at the sixth position of the β -globin subunit. Under low oxygen tension or deoxygenation, sickle haemoglobin (HbS) polymerizes and aggregates into fibers leading to a drastic decrease in the red cell deformability. HbS in deoxygenated state polymerizes into fibers. Oxygen affinity of HbS is dependent on its rate of polymerization thus substances that increase the affinity of HbS for oxygen will be beneficial for sickle cell disease therapy. We screened the effect of two plant extracts (*Sphenostylis stenocarpa* seed and *Telfairia occidentalis* leaf) on HbS oxygen affinity. Haemolysate was prepared from blood collected from sickle cell patients. The effect of the extracts on oxygen affinity were measured as an index of absorbance changes in haemolysates treated with different concentrations of extracts. *Sphenostylis stenocarpa* seed extracts (SSSE) at 40 μ l increased the concentration of oxyhaemoglobin from 0.041mM to 0.043mM while at 10 and 20 μ l, the concentration of oxyhaemoglobin increased from 0.041mM to 0.042mM at 540 and 576nm. *Telfairia occidentalis* leaf extract (TOLE) decreased the concentration of oxyhaemoglobin at 540 and 576nm band from 0.041mM to 0.036mM, 0.034mM and 0.033mM respectively. SSSE increased the affinity of Hb for oxygen and thus can prevent HbS polymerization. Although TOLE decreased the affinity of Hb for oxygen, previous studies show that it possesses anti-sickling properties through other mechanisms. The effects of the various concentrations of the extracts on HbS also show that none of the extracts oxidized Fe²⁺ to Fe³⁺ as there was no absorbance at 630nm band which characterizes the methaemoglobin (Fe³⁺) species.

Keywords: Sickle cell disease, haemoglobin, *Telfairia occidentalis*, *sphenostylis sternocarpa*

INTRODUCTION

Sickle cell disease is a genetic disorder arising from a point mutation in the β -globin gene which leads to the replacement of glutamic acid by valine at the sixth position of the β -chain of haemoglobin. In deoxygenated state, Sickle haemoglobin (HbS) is poorly soluble and when this is prolonged, intracellular gelation occurs that results in polymerization of HbS into fibers (Piccin *et al*, 2019). This causes the deoxygenated HbS to rigidify and distort in erythrocytes of patients with sickle cell anaemia (SCA) and is the basis for the pathogenesis of SCA (Hofrichter *et al*, 1974). Rigid sickle RBCs prematurely breakdown, damage endothelia, and occlude vasculature, leading to a cascade of haemolysis, ischemia, inflammation, and endothelial injury. Patients are afflicted with intensely painful episodes, susceptibility to infection, end-organ injury, and early mortality (Hoban *et al*, 2016). In some areas of sub-saharan Africa, up to 2% of all children are born with sickle cell anaemia. It affects 2.3% of the world population and is responsible for about 2% death in children below the age of 5 in sub-saharan Africa. In Nigeria, the prevalence of SCA is about 20 per 1000 births, that is, about 150,000 children are born annually with SCA (WHO, 2006). Haemoglobin

transports oxygen from the lungs to the tissues and in doing this, it alternates between the tense or T-state (deoxygenated Hb) and the relaxed or R-state (oxygenated Hb) (Safo *et al*, 2014). UV-Visible spectra of oxyHb absorbs at 540 and 576nm bands. Synthetic allosteric effectors such as aromatic aldehydes (Abdulmalik *et al*, 2005), isothiocyanates (Park *et al*, 2003), acyl salicylates derivatives (Klotz *et al*, 1973; Walder *et al*, 1980), disulfides (Garel *et al*, 1972) and maleimides (Benesch *et al*, 1960) can bind to haemoglobin and regulate the equilibrium between the two states. In 1987, Mozzarelli, Eaton and Hofrichter suggested that increasing oxygen affinity could be one of the therapeutic approaches for controlling gelation. Herbal remedies and medicinal plants products from indigenous flora have been used in the traditional management of SCD and proper and intense scientific investigations on these plants could be of remarkable help in developing effective and safer drugs for SCD treatment (Atabo *et al*, 2016). Plants that increase the oxygen affinity of HbS can be potential therapeutic agents in the management of sickle cell disease because increased oxygen affinity decreases the fraction of deoxyHbS that is susceptible to polymerization (Nakagawa *et al*, 2014). *Telfairia occidentalis* has been reported to possess free radical

scavenging activity (Fasuyi, 2006), blood level boosting activity (Toyin *et al.*, 2008), antimicrobial activity (Oyewole *et al.*, 2012) and anti-sickling activity (Atabo *et al.*, 2016).

MATERIALS AND METHODS

Equipments, chemicals and reagents: JENWAY 6405 UV-Visible spectrophotometer, water bath, methanol.

Collection and extraction of plant materials: *Telfairia occidentalis* leaf and *Sphenostylis sternocarpa* seed were purchased from Oriugba market in Umuahia North Local Government Area of Abia State. The plants were identified and authenticated by Dr. K.K. Ibe of Forestry and Environmental Management Department, Michael Okpara University of Agriculture, Umudike. The plants were shade-dried and milled into coarse powder. *Telfairia occidentalis* leaf was extracted with distilled water by cold maceration for 48 hours with intermittent shaking. *Sphenostylis sternocarpa* seed was extracted with aqueous methanol (25% methanol in H₂O) by cold maceration for 72 hours with intermittent shaking. At the end of the extraction, the crude extracts were filtered using whatman filter paper number 42. The filtrates were concentrated by evaporation using water bath at 45°C and stored in the refrigerator at 4°C till further use.

Blood collection and ethical approval: Blood (2ml) was obtained from homozygous sickle cell disease patients attending Federal Medical Centre (FMC), Umuahia. Approval by the Health Research Ethical Committee (HREC) was obtained. The HbSS status of the patients was confirmed. Their blood samples were characterized by haemoglobin electrophoresis on cellulose acetate gel at pH 8.6.

Blood Sample preparation: The blood samples were collected in sodium ethylene diamine tetracetic Acid (EDTA) tubes and thoroughly mixed by gently rolling the bottle. 3mls of distilled water was added to 10µl of the blood to get the haemolysate that was used.

Treatment of the haemolysate with the extracts

This was done according to the methods described by Ezeawgula and Onwubiko (2016). A stock solution of 10mg/ml of the extracts was prepared. The haemolysate was put in four different test tubes and different concentrations of the extracts were added (the control contains haemolysate not treated with the extracts, the test groups were treated with 10, 20 and 40µl of the extracts), the mixtures were incubated at room temperature for 10 mins and the absorbance was read at 300 – 700nm using JENWAY 6405 UV/Visible spectrophotometer. The spectrophotometer was calibrated using distilled water. The concentration of oxyHb of the two bands was calculated using Beer-lamberts law.

Results:

The effects of the various concentrations of the extracts on the sickle haemoglobin are shown in figure 1 to 6 below. 10µl of SSSE increased the concentration of oxyHb from 0.041mM to 0.042mM at 540nm and 576nm bands (fig 1). 20µl of SSSE also slightly increased the concentration of oxyHb from 0.041mM to 0.042mM at 540nm and 576nm (fig 2) while 40µl of SSSE increased the concentration of oxyHb from 0.041mM to 0.043mM at 540nm and 576nm (fig 3). There was no absorbance at 630nm band which characterizes the methaemoglobin (Fe³⁺) species. This implies that SSSE slightly increased the affinity of sickle Hb for oxygen at the various concentrations tested. *Telfairia occidentalis* leaf extract at 10, 20 and 40µl decreased the concentrations of oxyHb from 0.041mM to 0.036mM, 0.034mM and 0.033mM respectively at 540nm and 576nm bands (Fig 4-6). This implies also that TOLE slightly decreased the affinity of sickle haemoglobin for oxygen. Notwithstanding, Atabo and his colleagues reported that aqueous extracts of various parts of *Telfairia occidentalis* possess anti-sickling activities by stabilizing the membrane of sickle erythrocytes and reversing sickled erythrocyte (Atabo *et al.*, 2016). There was no absorbance at 630nm band which implies that the extract was not able to oxidize Fe²⁺ to Fe³⁺ which is characteristic of methaemoglobin.

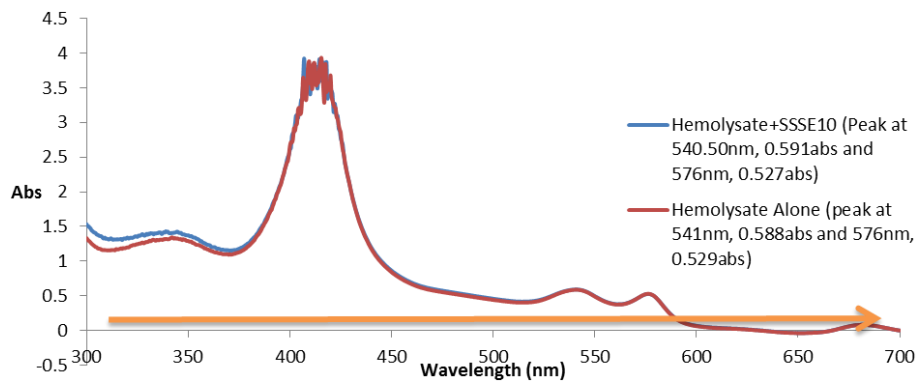


Fig 1 Effect of 10µl of *sphenostylis sternocarpa* seed extract on Haemoglobin S oxygen affinity

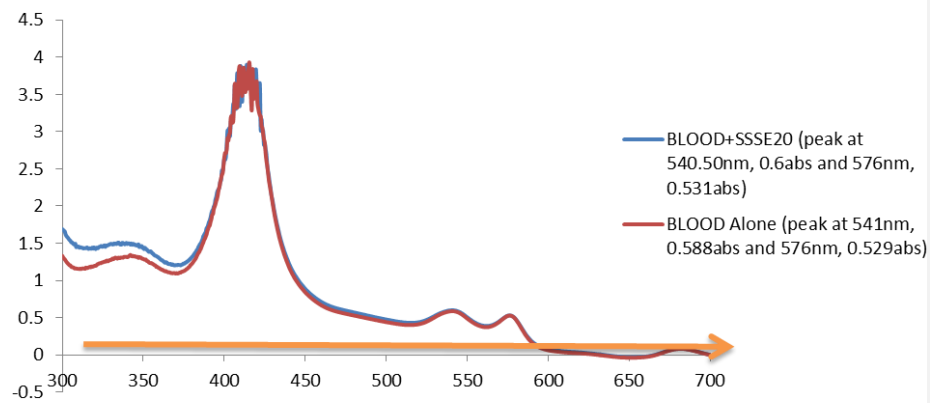


Fig 2 Effect of 20µl of *sphenostylis sternocarpa* seed extract on Haemoglobin S oxygen affinity

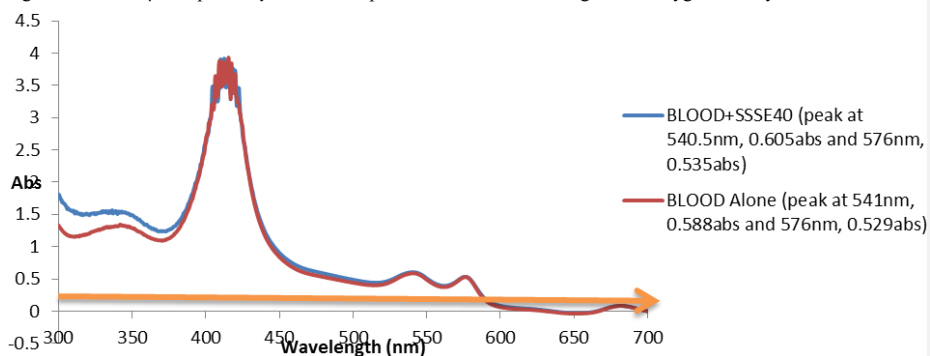


Fig 3 Effect of 40µl of *sphenostylis sternocarpa* seed extract on Haemoglobin S oxygen affinity

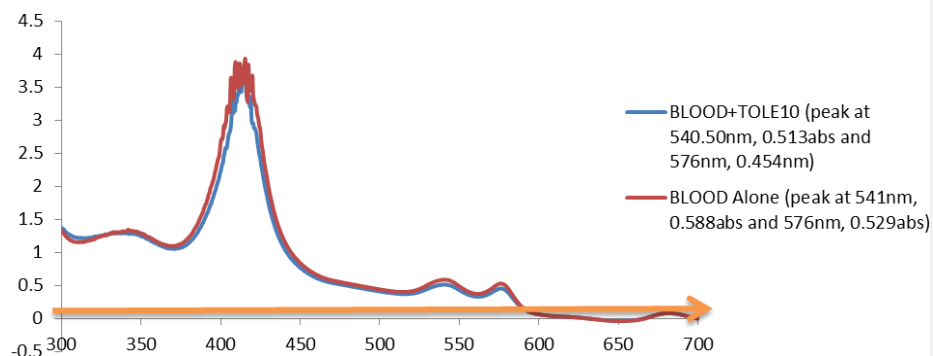


Fig 4 Effect of 10µl of *Telfairia occidentalis* leaf extract on Haemoglobin S oxygen affinity

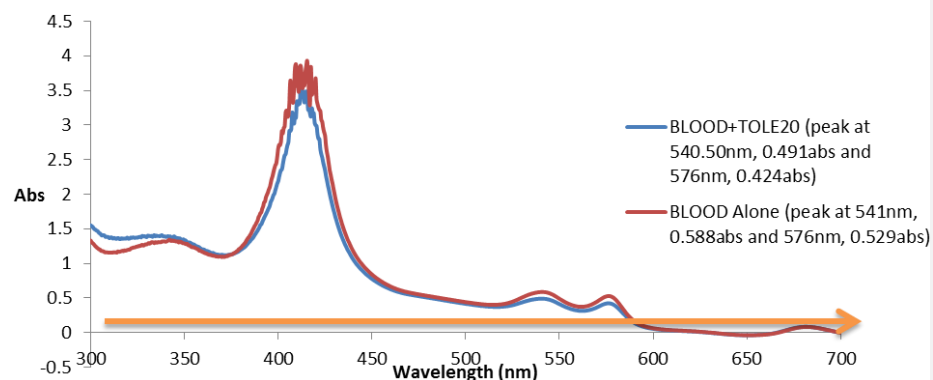


Fig 5 Effect of 20µl of *Telfairia occidentalis* leaf extract on Haemoglobin S oxygen affinity

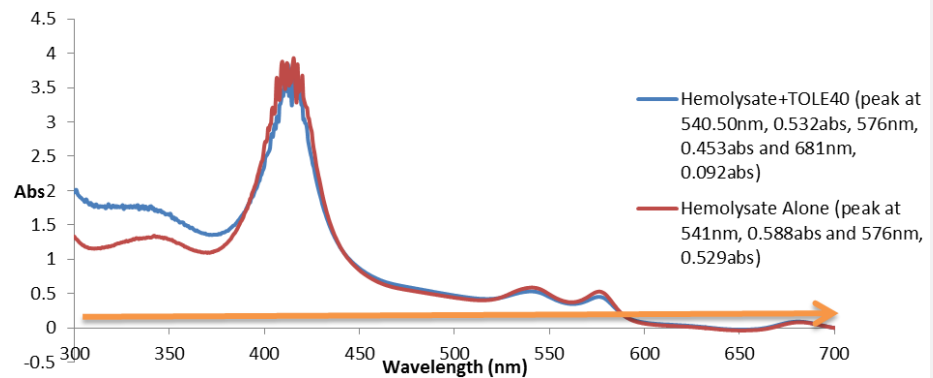


Fig 6 Effect of 40µl of *Telfairia occidentalis* leaf extract on Haemoglobin S oxygen affinity

Conclusion:

We evaluated the effect of *sphenostylis sternocarpa* seed and *Telfairia occidentalis* leaf extracts on sickle Hb oxygen affinity. At the concentrations tested, SSSE Slightly increased the oxygen affinity of Hb in

the haemolysate while TOLE slightly decreased the oxygen affinity of Hb in the haemolysate. There was no absorbance at 630nm band. Our findings suggest that SSSE can be a therapeutic agent in the treatment of patients with sickle cell anaemia.

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ASSESSING THE RISK OF LASSA FEVER USING GEOGRAPHIC INFORMATION SYSTEM

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ABSTRACT

Lassa Fever Virus (LFV) is a zoonotic virus pathogen that causes acute viral haemorrhagic fever in humans and constitutes a major health problem, particularly in West Africa. Epidemiological studies have revealed that the rodents *Mastomys natalensis* are the reservoir for LFV and have been implicated in various outbreaks. A six-month study was conducted (August 2019 – January 2020) to evaluate the environmental risk factors associated with Lassa Fever in Umuahia South Local Government Area of Abia state, Nigeria, where two cases of Lassa Fever have been reported using Geographic Information System (GIS). A comprehensive literature search was performed to identify the environmental factors in the transmission of LFV. Epidemiological criteria chosen included: waste dumpsites, rural settlements, administrative boundaries and vegetation cover of Umuahia South. ArcGIS 10.7.1 (2019 edition) software was used for data analysis. Spatial data analysis performed included proximity analysis, overlay operations and point distance analysis. Results showed a sparse to clustered settlement pattern of the area, the location of waste dumpsites within settlement area, and 13 % (11/85) of the settlements fell within the 500 m buffer range from the dumpsite. The point distance analysis indicated wrong siting of dumpsites. The spot 5 satellite imagery showed a vast vegetation cover and an undeveloped area with poor road networks highlighting the rurality of study area. This study highlighted the importance of the application of GIS for mapping and analysing epidemiological data to predict disease outcomes and planning control strategies.

INTRODUCTION

Lassa Fever Virus (LFV) is a zoonotic pathogen that causes acute viral haemorrhagic fever known as Lassa Fever (LF) in humans, and constitutes a major health problem, particularly in West Africa (Fichet-Calvet and Rogers, 2009; WHO, 2020). West African countries reported to be endemic for LFV include Sierra Leone, Guinea, Liberia, and Nigeria (Yun and Walker, 2012). LFV infection is estimated to cause 300,000 illnesses and 5000 to 10,000 deaths annually in West Africa (CDC, 2015; Mylne *et al.*, 2015; Buba *et al.*, 2018). Clinical signs include a broad spectrum of diseases ranging from mild febrile illnesses to severe cases of vascular bleeding, encephalopathy, multi-organ failure, and death (CDC, 2015), and the virus is reported to persist in body fluids, including semen even after recovery (WHO, 2020).

First discovered in 1969 in the town of Lassa in Borno state, Nigeria (Adewuyi *et al.*, 2009), since then the virus has continued to cause notable outbreaks across Nigeria. Recently, in 2019, the Nigerian centre for disease control (NCDC) reported an unusual spike in laboratory-confirmed cases of LF. From January 1, through September 2019, a total of 3931 suspected cases were reported from 23 states of the federation. Of this figure, 714 were confirmed positive, 18 probable and 3176 negative (NCDC, 2019). The percentage distribution amongst the 23 states in Nigeria include: Edo (38%), Ondo (31%), Bauchi (7%), Nasarawa, Ebonyi (7%), Plateau (5%), Taraba (6%), Adamawa, Gombe, Kaduna, Kwara, Benue, Rivers, Kogi, Enugu, Imo, Delta, Oyo, Kebbi, Cross River, Zamfara, Lagos and Abia have recorded at least

one confirmed case across 92 Local Government Areas. Hence since the onset of the 2019 outbreak, there have been 153 deaths in confirmed cases (NCDC, 2019; Dan-Nwafor *et al.*, 2019).

Epidemiological studies have revealed that *Mastomys natalensis* “multimammate” rats are the primary reservoir host of LFV and indigenous to most of Sub-Saharan African countries (Buchmeier *et al.* 2001). Sero-prevalence has been reported to be as high as 60%-80% in “multimammate” rats (Demby, *et al.*, 2001; Mylne *et al.* 2015). Once infected, the rat excretes virus in urine and excreta for an extended period, maybe for the rest of its life and also, has the capacity to transmit virus to its offspring (Demby, *et al.*, 2001).

The Multimammate rats are all year round breeder and peri-domestic rodents, which readily colonize human homes and waste dumpsites (Richmond and Baglole, 2003; Abul, 2010; Burrell *et al.*, 2016). Transmission of LFV to humans occurs most commonly through ingestion of poorly stored foods directly contaminated with rat urine or faeces, or inhalation of rodent faeces believed to be aerosolised (Baize, *et al.* 2001; Bausch *et al.*, 2001). Recent studies have demonstrated that 80 % of LF outbreaks are associated with interactions between the *Mastomys* rodents and human populations, whereas human-to-human transmission of approximately 20% of cases occurs mainly in health care facilities (Iacono *et al.*, 2015; Mylne *et al.*, 2015).

Dumpsites constitute major public health threat (Abul,

2010; Bassey *et al.*, 2015) and have been associated with LF outbreaks in Nigeria (Ekechi *et al.*, 2020). The United Nations Environment Program Agency (UNEP, 2006) stated that wastes that are not managed properly, especially solid wastes from households and communities constitute a serious health hazard which can lead to the spread of diseases (UNEP, 2006).

The capacity of these reservoir rats to survive for a long time in the environment (forest, farmlands, human dwellings and in wastes dumpsites), provides a basis for the application of geographical information systems (GIS) as a spatial epidemiological tool to determine the environmental risk factors associated with LF infection. (Benema *et al.*, 2019). Geographic information systems (GIS) are “automated systems for the capture, storage, retrieval, analysis, and display of spatial data” (Clarke, 1986). Mapping has long been used in epidemiological investigations when analysing associations between location, environment, and disease (Ward and Carpenter, 2000).

The very recent confirmed case of LF in Abia state, reported on the 7th of January 2020 (<https://punchng.com/one-dies-as-lassa-fever-hits-abia-again/>), underscores the need to ascertain the main determinants for the disease reoccurrence and exposure sources. Hence, this study aims to use GIS to assess the environmental risk factors implicated in the transmission of LF in Umuahia South of Abia state. The objectives are to: Identify environmental risk factors associated with the vector of Lassa Fever virus (multimammate rat) in Umuahia South Local Government Area. To use GIS software to delineate potential hotspot areas for LF outbreak.

It is believed that the spatial data generated will aid in the ongoing surveillance activities in Abia state and reduce the risk of future outbreaks.

MATERIALS AND METHOD

Study Area: Abia is a state in the South Eastern part of Nigeria, located between longitude 7°30'E and latitude 5°25'N and the capital is Umuahia. Abia state consists of 17 local government areas including Umuahia South. Umuahia South has an area of 140km² and a population of 138,570 (NPC, 2006). Geographically, Umuahia South is located within longitude 7°22'-7°33'E and latitude 5°26'-5°34'N in the rainforest belt. Agriculture is a major practice in this area with rural dwellings rearing food crops and livestock (Hoiberg, 2010).

Criteria for Study Area: The index patient of LF outbreak in Umuahia in 2018 (<https://punchng.com/abia-commissioner-confirms-lassa-fever-outbreak-in-umuahia/>), was from Umuahia South. Also, the most recent report of LF case in Jan 2020; was from Umuahia South Local Government Area (<https://punchng.com/govt-fmc->

[confirm-lassa-fever-case-in-abia/](https://punchng.com/govt-fmc-confirm-lassa-fever-case-in-abia/)). Hence, the decision to assess the risk of LFV transmission in Umuahia South Local Government Area, Abia State.

Study Design: Spatial epidemiology methods were used to conduct a six-month study on the environmental risk factors associated with the LFV vector *Mastomys natalensis* from August 2019 to January 2020 in the study area. A comprehensive literature search was performed to identify the environmental factors that influence the transmission of LFV via the *Mastomys* rat. Consequently, a field visit was made to objectively assess the study area. Spatial analysis was performed using GIS technology and epidemiological criteria included human settlements, waste dumpsites and bushes/forest in the study area.

Secondary Data Acquisition: Secondary data sourced from internet (<https://grid3.gov.ng/state/abia>) include; Umuahia south boundary map, Umuahia south ward map, Umuahia south dumpsite data, Umuahia south settlement data, Federal Medical Centre coordinate data, Umuahia south health facilities data, SPOT5 Satellite Imagery of Umuahia North and South. Geospatial data were imported into ESRI's ArcGIS 10.7.1 (2019 edition) GIS software.

Spatial Analysis: GIS operations performed include; i. Proximity analysis-buffering operations around waste dumpsites ii. Overlay operations environmental risk factors iii. Point distance analysis. All spatial analysis was performed at the GIS laboratory, Lagos State Ministry of Science and Technology, Nigeria.

RESULTS

The study area Umuahia south is divided into ten (10) wards with Ubakala A having the largest landmass, covering an expanse of 25.27909 km², followed closely by Ubakala B covering an expanse of 17.01112 km² and Ogbodi Ukwu having the smallest landmass covering an expanse of 3.289 km² (Fig. 1). There are 85 settlements in Umuahia south with Ubakala B having the highest number of settlements 24.7% indicating a greater number of population live there, AhiaUkwu A having 17.6% whereas Ubakala A and AhiaUkwu B had 3.5%, characterized by a very sparse settlement sandwiched with a vast vegetation cover. (Fig. 1).

Figure 2 shows the satellite imagery of Umuahia south and Umuahia North. The thin yellow line indicates the various ward boundaries, and a vast vegetation cover surrounds the clustered settlements in Umuahia South in contrast to the extensive greyish background of Umuahia North (built up area), highlighting the extent of urbanization between Umuahia South and Umuahia North.

Figure 3 shows Avonkwa Olokoru open market

dumpsite, which provides a favourable breeding habitat for rats. Also, the index case (patient zero) in 2018, and the recent January 2020 patient of LF resided in Avonkwu Olokoro.

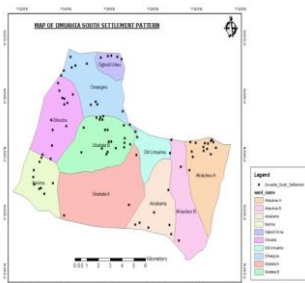


FIGURE 1: Map layer of Umuahia South wards and settlements.

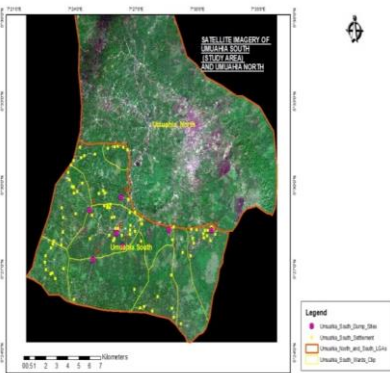


FIGURE 2: Satellite imagery of Umuahia South and Umuahia North showing the contrast between the rural settlements in the south and urban area in the north.

Of the 10 wards that make up Umuahia South, only 5 wards have waste dumpsites. Two dumpsites located in Ubakala B, and one at the border between Ubakala B and Ohiocha. The Abia state dumpsite is very close to the border of Ubakala B and Ubakala A which is farther away from the settlements in Ubakala A (Fig. 4). Also, 27 health facilities were present across the 10 wards, Ubakala B has 10 health facilities and one health facility exists in Ubakala A and Old Umuahia. All the health facilities were in proximity with the clustered settlements as indicated in Ubakala B and AhiaUkwu A. The Federal Medical Centre (FMC) is located in Umuahia North. (See Fig. 4).

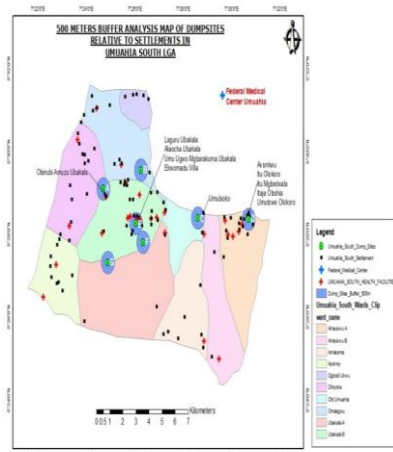


FIGURE 4: Map layer of the 27 Health Facilities and proximity analysis of a 500 m buffering operation from the 7 waste dumpsites in Umuahia South.

Given the travel distance of *Mastomys* rats, which ranges between 150-500 m (FAO, 1994), a proximity analysis of a 500 m buffering operation from the waste dumpsites was carried out, to assess the number of settlements that fall within this buffer distance. Analyses shows one settlement falls under dumpsite A, four settlements fall under dumpsite E, one settlement (Umubioko) falls under dumpsite C, and five settlements falls under dumpsite D. (See Fig. 4). Furthermore, 1500 m buffering from the dumpsites included 11(13%) settlements under dumpsite D including Avonkwu market Dumpsite amongst other dumpsites. (See Fig. 5). Point distance analysis of 7 waste dumpsites is shown in Table 1.

TABLE 1: Point distance analysis of the waste dumpsites in Umuahia South LGA.

DUMPSITES	POINT DISTANCE (m*)
Umugwo and Lapuru Dumpsite	994
Obinubi Amuzu Dumpsite and Amaibo Dumpsite	2988
Obinubi Amuzu Dumpsite and Abia state Dumpsite	3296
Abia state Dumpsite and Lapuru Dumpsite	2840
Alaocha village Dumpsite and Avonkwu Market Dumpsite	3877



FIGURE 3: Avonkwu Market Dumpsite.

The overlay operations of the various relevant risk factor maps (waste dumpsites, rural areas, bushes), including the buffer zones (500 m and 1500 m), represents a good visual indicator of probable hot spots for LF outbreak across the wards in Umuahia South. Suggested hotspot wards include Ubakala B, AhiaUkwu A, Ubakala A, Omaegwu, Ohiocha, Old Umuahia, and boundary wards between Umuahia South and Umuahia North. (See Fig. 6).

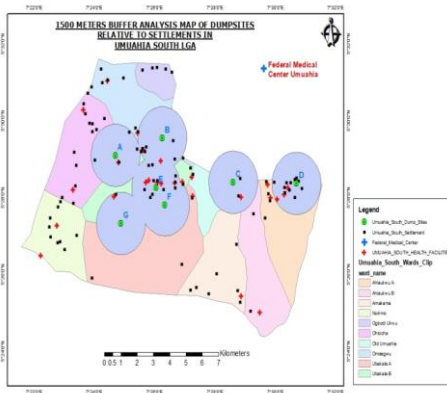


FIGURE 5: Map layer of proximity analysis of a 1500 m buffering operation from the waste dumpsites in Umuahia South LGA.

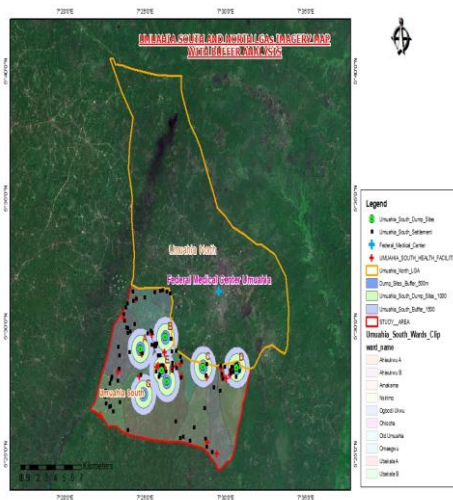


FIGURE 6: An overlaying of all the map layers to form a single map including buffer analysis.

DISCUSSION

Traditionally, spatial epidemiology involves the use of maps as a visualization tool which allows epidemiologists to determine associations between location, environment, and disease (Bhatt and Joshi, 2012). In this study, our spatial data indicates rurality of study area, which is reflected by the clustered and sparse human settlements (Fig. 1), the presence of vast vegetation cover including farmlands, forest and bushes (Fig. 2) and poor sanitation (Fig. 3) (Chigbu, 2013, Abdulwakeel, 2017). In addition, the satellite imagery (Fig. 2), gives a powerful visual contrast between urbanized Umuahia North and the rural settlements of Umuahia South, this imagery accentuates the rurality of the study area. Studies have demonstrated that rural areas characterised by these environmental factors provide a conducive breeding ground for *Mastomys* rat (Richmond and Baglole, 2003; Adebayo *et al.*, 2015). This suggest that the study area could be an ecological niche for rats including *Mastomys* rat, thus providing a zoonotic transmission potential for LFV (Mylne *et al.*, 2015). Furthermore, bush burning is a traditional agricultural practice (Ambe *et al.*, 2015), usually observed during the dry season (December-April), which corresponds with the peak of human cases of LF in Nigeria (WHO, 2020). Since bush burning leads to the displacement of rats into human dwellings, which increases the likelihood of direct and indirect contact with the LFV (Asogun *et al.*, 2012; Bonwitt *et al.*, 2017; WHO, 2020). We postulate, that the presence of the vast vegetation cover, is an important risk factor to the transmission of LFV infection in the study area. The presence of government owned primary health

care facilities in the study area is a good indication of existing health services in the localities. However, these primary health facilities are majorly used for maternity care and usually poorly equipped, also, other aspects of health care including management of infectious disease outbreaks are usually not covered (Ehiri *et al.*, 2005; Tobin *et al.*, 2013). The World Health Organisation (WHO, 2017) indicates that confirmatory tests for LF are carried out in a biosafety level 3 or 4 laboratory. Lack of LF expertise and diagnostic capacity undermines active detection of LF patients, leading to a possible under-reporting of the disease.

Open waste dumpsites serve as a potential source LFV transmission in Nigeria (Ekechi *et al.*, 2020), for the following reasons; the possible presence of rats, the prolific potential of *Mastomys* rat, mobility which includes travel distance (150 -500 m) and the potential for indirect transmission of diseases to humans (FAO, 1994). In this study, eleven settlements (13 %), including Avonkwu where the recent LF outbreak was reported fell within the 500 m buffer zone. Given the mobility and travel distance (150 -500 m) of *Mastomys* rats, these settlements may be considered as hotspots for LF outbreaks. Furthermore, settlements that fell within the 1000 m and 1500 m distance buffer zone from the dumpsites, maybe delineated as medium to low risk zones, taking into account that multimammate rats are prolific breeders if unchecked, with a gestation cycle of 23 days and litter size of up to 20 pups. Population explosion could result in further emigration of *Mastomys* rats to other locations possibly influenced by excessive competition for resources in one area. In addition, the National

Environmental Standards and Regulations Enforcement Agency (NESREA), recommends dumpsites to be not less than 2000 m apart (NESREA, 2019). In this study, the point distance analysis between the seven dumpsites in Umuahia south, indicated that only Umugwo and Lapuru dumpsites which were 994 m apart, and did not meet the NESREA standards. Consequently, these settlements are at increased risk of LF infections (FAO, 1994).

CONCLUSION

In this study, the environmental risk factors associated with the reservoir host of LFV was assessed using GIS. Spatial data generated include open waste dumpsites, settlement patten and SPOT5 Satellite Imagery of Umuahia North and South, which accentuates the rurality of the study area. The spatial analysis, which include the proximity around the waste dumpsites, overlay operations of environmental risk factors and the point distance analysis, enabled the identification of probable hotspots of LF outbreak in various wards of Umuahia south. These include Ubakala B, AhiaUkwu A, Ubakala A, Omaegwu, Ohiocha, Old Umuahia, and boundary wards between Umuahia South and Umuahia North.

RECOMMENDATION

Further studies to assess the prevalence of the RNA LF virus in rats within the locality is recommended. In addition, a one health collaborative study which will include human and veterinary epidemiologists among other professionals is recommended to enable a comprehensive risk assessment study of LF in Abia state.

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ANALYSIS OF ANTIBIOTIC RESISTANCE OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CLINICAL AND ENVIRONMENTAL SAMPLES

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ABSTRACT

The emergence of multiple antibiotic resistance in bacteria and the indiscriminate use of antibiotics contributes to the dissemination of resistant pathogens in the environment which may cause problems in therapy and is a serious public health issue. This study was conducted to determine the occurrence of *Pseudomonas aeruginosa* isolates in certain clinical and environmental samples as well as to determine the susceptibility patterns of these isolates to some commonly used antibiotics. The organisms from the clinical and environmental samples were isolated using standard microbiological techniques, identified using the standard cultural morphological and biochemical characteristics and the antibiotics susceptibility was determined using disc diffusion method. Fourteen isolates were obtained from the 60 samples analysed. Results showed that the clinical and soil isolates were 100% resistant to amoxicillin, augmentin, pefloxacin, chloramphenicol, streptomycin, sparfloracin and septrin. It was also observed that all the clinical isolates except the isolate from Wsi, and the environmental isolates showed no resistance to gentamycin. Result also showed that isolates from wound swab (WSI) had 100% resistance to the drugs, those from other clinical samples and soil samples had 87.5% resistance while water samples isolates had 75% resistance. The diameter of the zone of inhibition by gentamycin ranged from 17mm – 19mm for clinical isolates and 15mm-17mm for the environmental isolates. Zone diameter of 26mm and 29mm were produced by streptomycin against the isolates from water produced by Streptomycin against the isolates from. Therefore, the determination of antimicrobial susceptibility of isolates from clinical and environmental sources is crucial for optimum antimicrobial therapy of infected patients.

Keywords: *Pseudomonas aeruginosa*, Resistance, Antibiotics, Environments, susceptibility.

INTRODUCTION

The emergence of bacterial resistance to most of the commonly used antibiotics is of considerable medical significance (Poole, 2004). It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Jamshald *et al.*, 2008). Infections with antibiotic resistant bacteria make the therapeutic options for infection treatment extremely difficult or virtually impossible in some instances (Deb-Madal *et al.*, 2011).

Pseudomonas aeruginosa, a gram negatives bacterium, is a ubiquitous aerobe that is present in water, soil and on plants (Todar (2002). Naturally, this organism is endowed with weak pathogenic potentials. However, its profound ability to survive on inert materials, minimal nutritional requirement, tolerance to a wide variety of physical conditions and its relative resistance to several unrelated antimicrobial agents and antiseptics, contributes enormously to its ecological success and its role as an effective opportunistic pathogen (Gales *et al.*, 2001). *Pseudomonas aeruginosa* is responsible for 16% of nosocomial pneumonia cases, 12% of hospital acquired urinary tract infections, 8% of surgical wound infections, and 10% of bloodstream infections. It is the third most common pathogen associated with hospital acquired catheter-associated urinary tract

infections (Ogunnusi and Adeyinka, 2016).

Pseudomonas aeruginosa is considered the most challenging pathogen due to its resistant rate which is high to antimicrobial agents. (Dubois *et al.*, 2001). It flourishes in different ecological niches including soils, rivers, waste water, plant, animal and human. Environment containing antibiotic residues exert selection pressure and contribute to the appearance of resistant bacteria (Ogunnusi and Adeyinka, 2016). However, it is believed that *Pseudomonas aeruginosa* is generally environmentally acquired and that person to person spread occurs only rarely (Harbour *et al.*, 2002). As such, contaminated respiratory care equipment, irrigating solutions, catheters, infusions, cosmetics, dilute antiseptics, cleaning liquids, and even soaps have been reported as vehicle of transmission (Jokelike *et al.*, 1992, Berrouane *et al.*, 2000, Dubios *et al.*, 2001, Ezeador *et al.*, 2020).

Efflux pumps are common components of multidrug-resistance in *Pseudomonas aeruginosa* isolates, and prevent accumulation of antibacterial drugs within the bacterium, extruding the drugs from the cell before they have the opportunity to achieve an adequate concentration at the site of action. The efflux pumps often work together with the limited permeability of the *Pseudomonas aeruginosa* outer membrane to produce resistance to β -lactams, fluoroquinolones, tetracycline, chloramphenicol, macrolides, TMP and

aminoglycosides (Livermore, 2002). The multidrug efflux systems of *Pseudomonas aeruginosa* are composed of three proteins that are structurally and functionally joined. *Pseudomonas aeruginosa* possess both outer membrane and a cytoplasmic membrane, which flank the periplasmic space. The tripartite efflux system is required for effective removal of compounds across both membranes of the cell (Livermore, 2002).

Massive quantities of antibiotics are being prepared and used each day. As a result of this, an increasing number of diseases are resisting treatment due to the spread of drug resistance resulting from drug misuse. Patients with *Pseudomonas* infections may inherently develop resistant to many classes of antibiotics as a result of misuse and improper disposal of drug in the environment and this may cause difficulty in treatment and may lead to life-threatening diseases and possibly death.

This study is aimed at determining the antibiotic susceptibility pattern and rate at which *Pseudomonas aeruginosa* occurs in clinical and environmental samples.

MATERIALS AND METHODS

Sample collection: A total of 60 samples were collected for the study of which 30 samples were of clinical origin constituting of 15 samples of urine and 15 wound swabs. Also 30 environmental samples were randomly collected from water and soil. Urine and wound swab samples were aseptically collected with sterile containers and swab sticks respectively while the water samples were collected in sterile containers from two rivers around Michael Okpara University of Agriculture, Umudike. The soil samples were collected from a mechanic workshop and soils around the river banks in a sterile specimen bag.

Isolation procedure: Streak plate method of inoculation was adopted for the isolation of the test organism from the clinical samples while pour plate method was adopted for isolation from the environmental samples. Nutrient agar and MacConkey agar were the media used for the isolation. After inoculation, all plates were incubated at 37°C for 24 hours. Different colonies were further purified to obtain pure cultures. Pure colonies obtained were stored on agar slants for further identification. The isolation procedure follows the method of Cheesbrough (2006).

Identification of the isolates: Isolates were identified using colony morphology, pigment formation, mucoidy, positive oxidase test, positive catalase test, growth at 42°C on nutrient agar, mortality test and gram reaction (Cheesbrough, 2006).

Antibiotic Susceptibility Test: The agar disc diffusion method of Kirby-Bauer modified based on National Committee for Clinical Laboratory Standard

(2000) was adopted to carry out the susceptibility test for the *Pseudomonas aeruginosa* isolates. The pure isolates of *Pseudomonas* were evenly spread on prepared Mueller-Hinton agar using sterile wine loop. Thereafter, the clinically pertinent 8 antibiotic discs with the following drug contents; Augmentin (Au), gentamycin (Cu), Pefloxacin (PEF), Streptomycin (S), Chloramphenicol (CH), Sparfloxacin (SP), Amoxicillin (AMX) and Septrin (SXT) were placed on the plates aseptically using sterile forceps. The plates were left on the bench for 30 minutes to allow for diffusion of the antibiotics and were incubated at 37°C for 24 hours. After 24 hours, clinical interpretation (resistant ® and sensitive(s)) of the size of the zone was evaluated based on the MIC susceptibility value as determined by the diameter from the zone of inhibition.

Statistical analysis

The data's generated were analysis by descriptive statistics and presented in tables.

Results:

A total of fourteen *Pseudomonas aeruginosa* isolates were recovered from the sixty samples (23.3%). Two isolates (20%) were recovered from the ten water samples while three isolates (25%) were recovered from the ten soil samples. Out of the twenty one urine samples, six *Pseudomonas aeruginosa* isolates were recovered (29%). Three isolates were recovered from the seventeen wound swabs (18%). All the fourteen organisms produced distinct greenish pigment on culture media. They were Gram negative and rod-shaped. They were oxidase and catalase positive and produced grape-like smell. On MacConkey agar, the isolates grew but did not turn pinks showing that they were non-lactose fermenters. The isolation rate of *Pseudomonas aeruginosa* from clinical and environmental samples is shown in table 1.

Table 2: shows the zone of inhibition diameter of *Pseudomonas aeruginosa* isolated from clinical samples. The result showed that *Pseudomonas aeruginosa* isolate from urine samples were inhibited by gentamycin (17-19mm) but no activity was observed among the isolates against augmentin, pefloxacin, chloramphenicol, streptomycin, sparfloxacin, amoxicillin and septrin. The result of the inhibitory activity of the antibiotic against the isolates reveals that two of the isolates (Ws₂ and Ws₃) were inhibited by gentamycin (19mm and 18mm respectively). There was no activity observed with augmentin, pefloxacin, streptomycin, chloramphenicol, sparfloxacin, amoxicillin and septrin against all the isolates.

The diameter of the zone of inhibition of *Pseudomonas aeruginosa* isolates from environmental samples is shown in table 3. *Pseudomonas aeruginosa* isolates from water were inhibited by streptomycin

(29mm and 26mm) and gentamycin (15 and 17mm) but showed resistance against augmentin, pefloxacin, chloramphenicol, sparfloracin, amoxicillin and septrin. The isolates from soil were resistant to all the antibiotic except gentamycin. The isolates exhibited no activity against augmentin, pefloxacin, streptomycin, chloramphenicol, sparfloracin, amoxicillin and septrin.

Table 4 shows the antibiotic susceptibility pattern for both the clinical and environmental isolates. *Pseudomonas aeruginosa* isolates from water were sensitive to gentamycin and streptomycin only while the three soil isolates were sensitive to gentamycin but resistant to the other drugs. The table also showed the susceptibility pattern of the clinical isolates. Two of the isolates from wound swabs were sensitive to gentamycin and the whole isolates were resistant to the other antibiotics. The isolates from urine showed resistance to all the antibiotics used except

gentamycin.

Table 1: Isolation rate of *Pseudomonas aeruginosa* from clinical and environmental samples

Source of sample	Site of collection	No. of samples examined	No. of positive isolates	(%)
Clinical	Urine	21	6	29%
	Wound swab	17	3	18%
Environmental	Water	10	2	20%
	Soil	12	3	25%
Total		60	14	

Table 2: Diameter of the zone of inhibit of the clinical isolates

Antibiotics	Isolates/zone of inhibition diameter (mm)									
	U ₁	U ₂	U ₃	U ₄	U ₅	U ₆	WS ₁	WS ₂	WS ₃	
Au	-	-	-	-	-	-	-	-	-	-
CN	19	18	17	19	17	18	-	19	20	
PEF	-	-	-	-	-	-	-	-	-	
S	-	-	-	-	-	-	-	-	-	
CH	-	-	-	-	-	-	-	-	-	
SP	-	-	-	-	-	-	-	-	-	
AMX	-	-	-	-	-	-	-	-	-	
SXT	-	-	-	-	-	-	-	-	-	

Key: U₁, U₂, U₃, U₄, U₅, U₆ = urine isolates; WS₂, WS₃ = wound swab isolates, Au = augmentin, CN = gentamycin, PEF = pefloxacin, S = streptomycin, CH = chloramphenicol, SP = sparfloracin, AMX = amoxicillin, SXT = Septrin

Table 3: Diameter of the zone of inhibition of the environmental isolates

Antibiotics	Isolates					
	Zone of inhibition diameter (mm)					
	W ₁	W ₂	S ₁	S ₂	S ₃	
Au	-	-	-	-	-	
Cn	15	17	20	19	21	
PEF	-	-	-	-	-	
S	29	26	-	-	-	
CH	-	-	-	-	-	
SP	-	-	-	-	-	
AMX	-	-	-	-	-	
SXT	-	-	-	-	-	

Key: W₁, W₂, = water isolates; S₁, S₂ and S₃ = soil isolates, Au = augmentin, CN = gentamycin, PEF = pefloxacin, S = streptomycin, CH = chloramphenicol, SP = sparfloracin, AMX = amoxicillin, SXT = Septrin

Table 4: Antibiotic susceptibility pattern of the clinical and environmental isolates

Antibiotics	Susceptibility pattern													
	clinical isolates							Environmental isolates						
	U ₁	U ₂	U ₁	U ₂	U ₃	U ₄	WS ₁	WS ₂	WS ₃	W ₁	W ₂	S ₁	S ₂	S ₃
Au	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CN	S	S	S	S	S	S	R	S	S	S	S	S	S	S
PEF	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S	R	R	R	R	R	R	R	R	R	S	S	R	R	R
CH	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SP	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AMX	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SXT	R	R	R	R	R	R	R	R	R	R	R	R	R	R
percentage	87.5%	-	-	-	-	-	100%	87.5	87.5	75%	75%	87.5	87.5	87.5

Key: R = resistant, S = Sensitive, U₁ – U₆ = urine isolates, WS₁ – WS₃ = wound swab isolates; W₁ and W₂ = water isolates, S₁ – S₃ = soil isolates, Au = augmentin etc.

Discussion

Multiple antibiotic resistances in bacterial population is currently one of the greatest challenges in the effective management of infections. Antimicrobial drugs have been proven to be remarkably effective for bacterial infections control. However, it was soon evidenced that bacterial pathogens were unlikely to go down easily and some pathogens rapidly became resistant to many antibiotics (Cheesbrough, 2006).

In this study, several clinical and environmental samples of urine, wound, water and soil were examined for the presence of multidrug resistant *Pseudomonas aeruginosa*. Fourteen isolates of *Pseudomonas aeruginosa* were recovered and tested for antibiotic resistance against eight antibiotics.

The result of this study showed that there was higher occurrence of *Pseudomonas aeruginosa* in wound than in urine. This harmonized with the findings of Anuratha *et al.* (2008) who reported that *Pseudomonas aeruginosa* was the most frequent of isolates obtained from burn wound. Evaluation of environmental samples showed that *Pseudomonas aeruginosa* was more frequent in soil. The isolates showed some degree of resistance to all the antibiotics tested. This agrees with the findings made by Manikandan *et al.* (2011) which reported multidrug resistance by bacteria isolated from UTI. This is also in line with the result of Uchenna (2005) who reported multidrug resistance by Gram negative bacteria. The result also goes along with the work of Gales *et al.* (2001) who reported presence of multidrug resistance of *Pseudomonas aeruginosa* against various antibiotics which includes those used in this study. Achan *et al.*, (2011) reported complete resistance to amoxicillin by *Pseudomonas aeruginosa*. According to Harbottle *et al.* (2006), the

overuse of antibiotic has become the major factor for the emergence and dissemination of multi antibiotics resistance strain of several bacteria. In these regards, *Pseudomonas aeruginosa* antibiotic resistance was raised from both the intrinsic and acquired resistance. Multidrug resistance often reflects not one, but a combination of resistance mechanisms (Fred, 2006). Efflux pumps are common components of multidrug resistant *Pseudomonas aeruginosa* isolates and they prevent accumulation of antibacterial drugs within the bacterium, extruding the drugs from the cell before they have the opportunity to achieve an adequate concentration at the site of action (Fred, 2006).

All the isolates showed different levels of resistance to the antibiotics except gentamycin. This is in solidarity with the report of Ogunnusi (2016) and Frilla (1994) which says that amino glycosides such as gentamycin can be used to treat *Pseudomonas aeruginosa* infection. The highest resistance was observed in clinical isolates. This agrees with the findings of Shahid and Malik (2004) who reported that 96% of clinical *Pseudomonas aeruginosa* isolates were multidrug resistant and the majority (71.4%) were resistant to five or more antibiotics. The high level of resistance to these antibiotics might be attributed to the emergence of new bacterial resistance mechanism as a result of improper and extensive use of these antibiotics, antibiotic discharge in various amounts in the environment, indiscriminate use of antibiotic in medical, veterinary and agricultural practices (Diab *et al.*, 2002).

Conclusion

This study has shown that there are multidrug resistant *Pseudomonas aeruginosa* in clinical and environmental samples with the clinical isolates being

more resistant than those from the environment. Since gentamycin inhibited almost all the isolates, it could serve as a drug of choice in treatment of diseases caused by *Pseudomonas aeruginosa*.

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ACUTE TOXICITY EVALUATION AND NEPHRO-PROTECTIVE EFFECT OF *IRVINGIA GABONENSIS* LEAF EXTRACT IN *TRYPANOSOMA BRUCEI BRUCEI* INFECTED WISTAR RATS

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ABSTRACT

In this study, the ameliorative effect of *Irvingia gabonensis* leaf extract (IGLE) on renal function and kidney histology of *Trypanosoma brucei brucei* infected adult Wistar rats was evaluated. Extract prepared from the plant material was subjected to acute toxicity test. For the main study, thirty wistar rats assigned to 6 groups of 5 rats each were used. Rats in group 1 were uninfected and served as the normal control while those in groups 2-6 were infected with the parasite and thereafter treated orally. Group 2 was treated with normal saline (2 ml/kg) and served as the negative control while group 3 was treated with a standard drug. Groups 4, 5 and 6 were treated with 200, 400 and 800 mg/kg of IGLE respectively. At the end of 7 days treatment, the rats were sacrificed to collect blood and kidneys for renal function and histopathological studies. Results obtained indicated an acute toxicity (LD₅₀) value above 5000 mg/kg body weight. Serum urea and creatinine concentrations were significantly higher in the negative control group when compared with the normal control (P<0.05). Treatment with extract significantly lowered the elevated urea and creatinine values in the test groups (P<0.05), while serum electrolytes concentrations including sodium, chloride, bicarbonate and potassium were not significantly altered across the treatment groups (P<0.05). Kidney histopathology showed significant degenerations, necrosis, oedema, haemorrhage and tissue infiltrations following infection. However, treatment with the extract significantly ameliorated the pathological impact of the infections. We therefore conclude that *Irvingia gabonensis* leaf extract may be safe for use as food and medicine and may be of further value in the management of renal function complications and histopathological changes associated with *Trypanosoma brucei brucei* infection.

Key words: Histopathology, *Irvingia gabonensis*, kidney, renal function

Introduction

Trypanosomiasis is one of the current tropical diseases with global life-threatening effects on humans and other animals. The disease, caused by *Trypanosoma* species, is known to have adversely affected well-being and productivity across Africa (Onditi *et al.*, 2007; Adewoga *et al.*, 2010). In Nigeria, the African canine trypanosomiasis caused mainly by *T. brucei brucei* and naturally transmitted through tse-tse fly bites has particularly been identified as one of the tropical diseases capable of limiting animal health, thus affecting human lives indirectly (Ezeokonkwo *et al.*, 2010). Parasitic diseases (inclusive of trypanosomiasis) are particularly dangerous because the parasites accumulate in body organs over time and secrete toxins with pharmacologically established toxicity on body systems, affecting mostly blood and causing severe damage to tissues and organs (Ohad and Baneth, 2012). It is established that following infection with *Trypanosoma*, anaemia may result due to the massive destruction of the erythrocytes by the organisms (Nwoha and Anene, 2011). Disruption of body biochemical compositions and decline in the strength of the immune system are among common outcomes of *Trypanosoma* infection. Other physiological changes in animals may include pyrexia, immune-suppression, emaciation, hair loss and oedema of lower jaw, fore and hind limbs (Nwoha *et al.*, 2013; Gobert *et al.*, 2000).

The renal system is a target of several parasitic

infections. It has been reported that of 342 parasites that infect humans, 20 are associated with the kidney with only Schistosomes, filariae, plasmodia, and leishmanias currently known to cause significant clinical impact on the kidneys (Barsoum *et al.*, 2013). Disruption of renal functions due to parasitic infections may result to complications such as imbalances in body electrolytes, uremia and immune depression. These complications are reportedly more severe in individuals with pre-existing renal problems (Reza *et al.*, 2014). A source of major concern is the fact that drug interventions aimed at effective treatment of parasitic diseases have not achieved desired treatment goal (Geerts *et al.*, 2001), making the search for alternative management strategies like herbal medicine an area of great interest.

The fact that African flora is host to numerous plants means a number of plants with medicinal values may be found here. This may be why traditional medicine is officially recognized as an integral part of, or complementary to the healthcare delivery system (Ogbadoyi *et al.*, 2007). However, scientific validation of the effectiveness of medicinal plants is considered a major step in new drug discovery (Ijioma *et al.*, 2014). *Irvingia gabonensis* is only one of the numerous medicinal plants under investigation.

Irvingia gabonensis is an economic tree, native to most tropical forests in west and central Africa. The plant is usually called "African mango" or "bush

mango” because the trees bear mango-like fruits (Matos *et al.*, 2009). Extracts from *Irvingia gabonensis* is leaves have been used traditionally for the management of diseases like diarrhoea (Lowe *et al.*, 2000), pain, hyperglycaemia (Ngondi *et al.*, 2005) and microbial infections (Ngondi *et al.*, 2005, Fajimi *et al.*, 2007). Phytochemical agents identified in *I. gabonensis* include saponins, tannins, cardiac glycosides, phenols and flavonoids (Adedapo *et al.*, 2009, Fadare and Ajaiyeoba, 2008).

Due to paucity of information on the impact of *trypanosoma brucei brucei* on the kidney and the absence of literature on the ameliorative potentials of *Irvingia gabonensis* leaf extract in rats, this study was therefore designed to investigate the local anti-parasitic claims on the plant material and to provide background data for future studies.

Materials and methods

Collection of plant materials and authentication

Fresh leaves of *Irvingia gabonensis* plant were collected from a bush fallow at Umudike, Ikwuano Local Area, Abia State, Nigeria and were identified by a botanist in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A specimen sample of the plant material was given a voucher number MOU/ZE/18/003 and was preserved in the herbarium of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike.

Preparation of extract

The method used by Oshilonyah *et al.*, (2015) was adopted with little modification. The collected leaves were dried under shade on a laboratory bench for 14 days, pulverized into powder for extraction and 100 g of the powdered material was introduced into the extraction chamber of the soxhlet extractor. Extraction was done using ethanol as a solvent with the temperature maintained at 65 °C for 48 hours. At the end of the period, the extract in solution was concentrated to dryness at low temperature (40 °C) in a hot air oven to obtain a solid dark green extract with characteristic aromatic odour which weighed 9.87 g and represented a yield of 9.87%. The extract so prepared was preserved in a refrigerator until use and is hereafter referred to as *Irvingia gabonensis* leaf extract (IGLE).

Acute toxicity evaluation

Thirty five adult rats (120-140 g) assigned to 7 groups of 5 rats each were used for acute toxicity study of the extract according to the method used by Ijoma *et al.*, (2017) with little modification. The groups tagged 1, 2, 3, 4, 5, 6 and 7 were assigned single oral dose of IGLE in the order: 500 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg, 5000 mg/kg and 6000 mg/kg body weight respectively. The rats were

returned to their respective cages after treatment and were allowed access to feed and water *ad libitum*. The number of deaths recorded in each group within 24 hours and a further 7 days was noted and used to calculate the LD₅₀ value of IGLE using Karber’s arithmetic technique.

Animals, inoculation, treatment and collection of samples for analysis

Thirty wistar rats obtained from the animal house of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike and divided into 6 groups of 5 rats each were used for the study. Each was housed in a separate aluminum cage and was provided standard feed (Vital Feed, Nigeria) and water *ad libitum* but starved for 12 hours before commencement of experiment. The experiment was carried out in accordance with International regulations for care and use of laboratory animals. A minute quantity (0.3 ml) of solution previously prepared by dilution in normal saline of blood collected from the tail vein of mice infected with *T. brucei brucei* was administered to all rats in groups 2 to 6 via the intraperitoneal route. The rats in group 1 were uninfected and served as the normal control group. After 11 days, the rats were tested to confirm their infection status. The Load of infection for each rat was determined by viewing under the microscope and carrying out parasite counts on smears made from each rat’s blood (wet mount technique). The animals were thereafter assigned treatment according to the order below:

Group 1: Normal control

Group 2: Infected rats with no treatment

Group 3: Infected rats treated with standard drug (Diminazene aceturate, 2.5mg/kg)

Group 4: Infected rats treated with IGLE (200mg/kg)

Group 5: Infected rats treated with IGLE (400mg/kg)

Group 6: Infected rats treated with IGLE (800mg/kg)

Treatments lasted 7 days before animals were sacrificed by cervical dislocation to collect blood by cardiac puncture into plain blood bottles for the determination of renal function parameters including urea, creatinine and electrolytes concentrations. The abdominal cavity of each rat was opened using scissors and forceps to harvest kidney tissues which were transferred immediately into sample bottles containing 10% formalin for histopathological studies.

Determination of renal function parameters

Renal function parameters including urea, creatine and electrolytes (Na⁺, K⁺, Cl⁻ and HCO₃⁻) were determined in serum using commercial test kits in accordance with standard protocols outlined by the producer, Randox Laboratories, UK.

Preparation of slides for histopathological studies

Kidney tissues were processed in accordance with the protocol used by Orieko *et al.*, (2019). The tissues were dehydrated, embedded in paraffin wax and sectioned to 5µ thick in a rotary microtome (Heitz 150

Rotary microtome, Cambridge model). Sections were stained using the haematoxylin and Eosin (H&E) technique. Images were viewed using a light microscope and captured with a moticam camera attached to a computer.

Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) and results were presented as means ± standard error of mean (SEM). Significant differences were assessed at 95% level of significance between the infected untreated group and each IGLE treated group using Duncan and LSD (Post Hoc) tests. P values less than 0.05 were considered

significant. Computer software package, SPSS version 21 was employed.

Results

Result of acute toxicity study

No mortality was observed in any of the treatment groups assigned graded doses of IGLE within the acute toxicity study period of seven days, even at the highest dose of 5000 mg/kg body weight administered. The animals in all groups remained physically agile and had stable disposition through-out the period, indicating an acute value greater than 5000 mg/kg body weight (Table 3.1).

Table1: Result of acute toxicity evaluation of IGLE

Dose (mg/kg)	Number of Deaths	Percentage mortality	Dose Difference (DD)	Mean Death (MD)	DD X MD
500	0	0	500	0.00	0.00
1000	0	0	1000	0.00	0.00
2000	0	0	1000	0.00	0.00
3000	0	0	1000	0.00	0.00
4000	0	0	1000	0.00	0.00
5000	0	0

LD₅₀>5000 mg/kg body weight

Effect of IGLE on renal function parameters

Treatment with IGLE significantly lowered the elevated urea and creatinine values in the *T. brucei brucei* infected rats when compared with the infected (untreated) group (p<0.05), but serum electrolytes

concentration were not significantly altered following treatment (p>0.05). These results are presented in table 3.2.

Table 3.2: Renal function parameters of treated rats

Group	Treatment	Urea (mg/dl)	Creatinine (mg/dl)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mEq/L)
1.	Normal control	16.72±1.34*	0.73±0.16*	132.65±5.68	4.83±0.29	90.14±4.86	20.44±2.08
2.	Infected (untreated)	49.24±3.19	1.72±0.24	137.51±4.71	4.77±0.24	103.22±5.84	23.27±2.21
3.	Infected + Diminazene (2.5 mg/kg)	21.84±2.04*	0.82±0.19*	137.03±3.97	4.86±0.26	93.56±4.12	20.72±1.98
4.	Infected + IGLE (200 mg/kg)	36.33±2.87*	0.97±0.17*	135.41±4.22	4.79±0.31	92.19±4.55	19.41±1.87
5.	IGLE (400 mg/kg)	32.80±3.71*	0.83±0.20*	136.70±5.02	4.92±0.25	93.50±4.72	21.04±1.93
6.	IGLE (800 mg/kg)	25.72±3.60*	0.85±0.18*	134.61±3.39	4.79±0.34	92.89±4.03	20.62±2.15

Values represent the mean ± SEM for N =5. Values in the same column marked * are significantly different from the infected untreated group (p<0.05)

Effect of IGLE on kidney histopathology

No pathological changes were observed in the section of group 1(normal control) kidneys. The renal tubules, glomerulus and renal blood vessels (vein and artery)

all appeared normal (Plate 1). Different histological presentations were however observed in the infected untreated rats (Group 2), wheresections of the kidney tissue showed faint outlines of the nephrons with

inflammatory infiltration(IF) oedema(O),degenerating glomerular tuft (DG), necrosis (N), haemorrhage (HR) and disruption of renal cortex (Plate 2). Treatment with both the standard drug and the extract offered only

mild ameliorative effects but did not return the kidney architecture to normal (Plates 3-6).

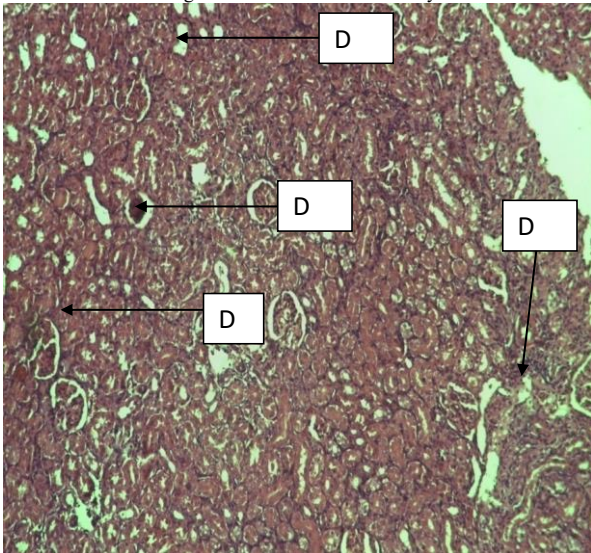


Plate 1: Normal control section of the kidney tissue showing normal histological features and no pathological features (H&E,100x)

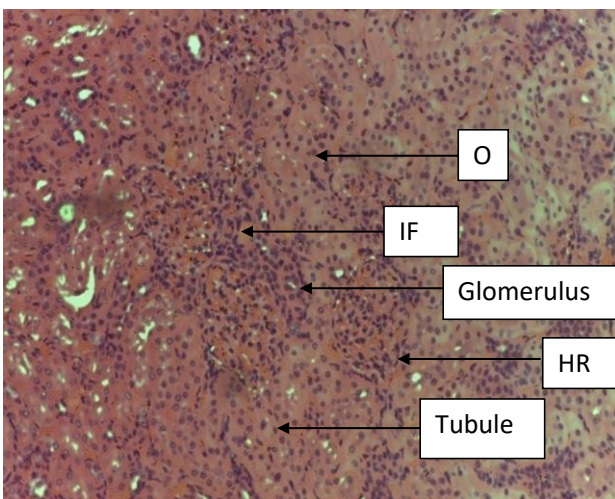


Plate 2a: A section of infected untreated kidney showing faint outlines of the nephrons with inflammatory infiltration(IF),Oedema(E),Haemorrhage (HR) and disruption of renal cortex (H&Ex100).

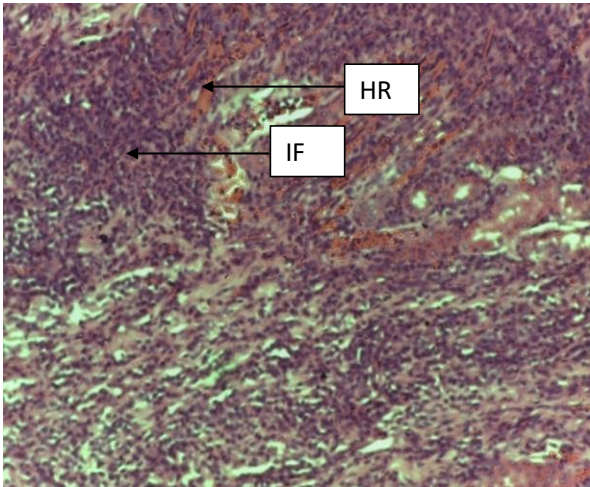


Plate2b: A section of infected untreated kidney showing faint outlines of the nephrons with heavy inflammatory infiltration(IF),Edema(E), Haemorrhage (HR) and disruption of renal cortex (H&Ex100).

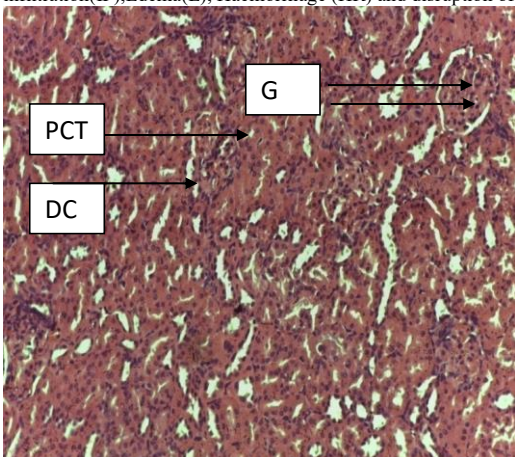


Plate 3: Section of standard drug treated kidney showing the glomerulus (G), with clear outlines,proximalconvoluted tubules(PCT) and distal convoluted tubules (DCT).(H&Ex100).

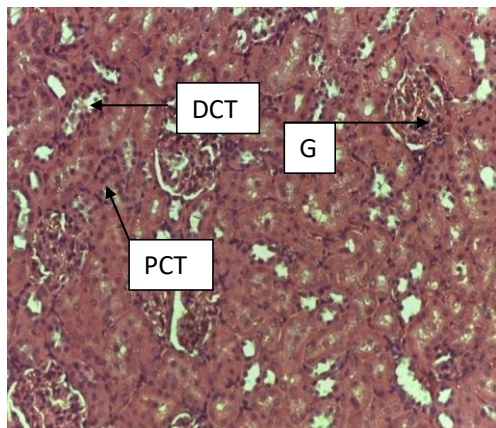


Plate 4: Section of 200 mg/kg IGLE treated group showing the glomerulus(G), with clear outlines,proximalconvoluted tubules(PCT) and distal convoluted tubules (DCT).(H&Ex100).

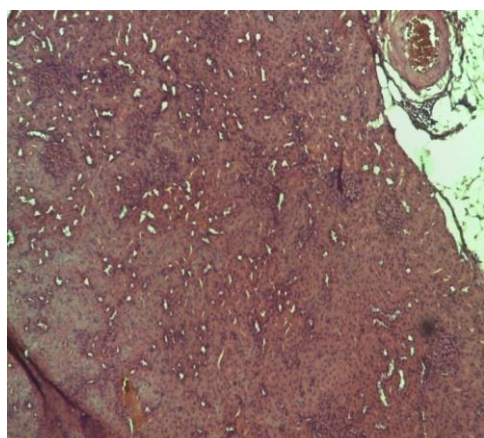


Plate 5: Section of 400 mg/kg IGLE treated group showing the glomerulus(G), with clear outlines,proximalconvoluted tubules(PCT) and distal convoluted tubules (DCT).(H&Ex100).

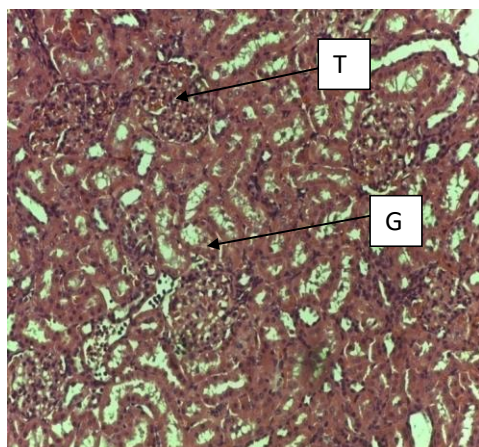


Plate 6: Section of 800 mg/kg IGLE treated group showing the glomerulus(G), with clear outlines,proximalconvoluted tubules(PCT) and distal convoluted tubules (DCT).(H&Ex100).

Discussion

The ameliorative effects of *Irvingia gabonensis* leaf extract on kidney function parameters and histology in rats infected with *Trypanosoma brucei brucei* infected rats have been investigated with results indicating significant amelioration and improvement in the infected rats.

The fact that no mortality was observed during the acute toxicity test period of the extract even at 5000 mg/kg body weight suggests that IGLE may be safe for use as food and medicine. This may be why over the years *Irvingia gabonensis* has been used as both food and medicine without any report of toxicity (Adedapo *et al.*, 2009). International standards for acute toxicity evaluation had stipulated that mortality is the expected outcome of acute toxicity evaluation and where no such occurs within a population treated with a dose range at which mortality is expected, then the test agent may be seen as being free of acute toxicity (OECD, 2001). Therefore, treatment with IGLE as was carried out in this study may have caused no form of toxicity in the treated rats. Similar conclusions were made in other studies carried out to determine the acute toxic effects of plant extracts (Orieke *et al.*, 2019, Ijioma *et al.*, 2015).

The kidney is the most important route of urea and creatinine excretion and as a result, serum urea and creatinine concentrations have long been used as a barometer for renal function with values higher than normal suggesting possible renal trauma and or failure (Shivaraj *et al.*, 2010; Corbette, 2008). Therefore, the significant increase in urea and creatinine concentrations in the infected rats suggests that infection with *Tbrucei brucei* may have impaired kidney functions in the infected rats. This finding is consistent with the results of other studies which

showed high serum urea and creatinine values following infection with parasites (Ohad and Baneth, 2012; Thomas and Tange, 1985). The amelioration observed in the groups treated with the extract occasioned by significantly lowered urea and creatinine values also suggests that IGLE may contain substances with antioxidant potentials. Antioxidants are known to limit stress on body organs via free radical scavenging pathways (Ijioma *et al.*, 2019). Phytochemical agents such as polyphenols and flavonoids, and vitamins C and E usually present in most leafy vegetables are known to possess strong antioxidant properties and as such are responsible for the antioxidant effects of most plants used as food and medicine. These bioactive substances have been reported to be present in *Irvingia gabonensis* leaf extract (Adedapo *et al.*, 2009; Fadare and Ajaiyoba, 2008).

The inflammatory infiltrations, oedema, degenerating glomerular tuft, necrosis, haemorrhage and disruption of renal cortex observed in the infected rats compliments the results of the renal function tests and suggests higher degree of damage to the kidneys due to the infection. Recent studies have shown that toxicants and toxins released by parasites can cause varying degrees of renal degeneration (Brzoska *et al.*, 2003; Thomas and Tange, 1985). Varying degrees of kidney necrosis, inflammations and haemorrhage have also been reported following histological evaluations of the kidneys of albino rats infected with parasites (Abdellaoui *et al.*, 2006). The mechanisms of histopathology in this study due to infection with *T. brucei brucei* may not be different from that of other reported microbial infections (Brzoska *et al.*, 2003; Abdellaoui *et al.*, 2006), even as the ameliorative effects offered by treatment with IGLE may not be unconnected with the already stated antioxidant

properties of the extract.

Conclusion

Results of this study have shown that *I. gabonensis* may be safe to use as food and medicine and IGLE may be of value in the management of renal function

complications and histopathological changes due to *Trypanosoma brucei brucei* infection, having significantly lowered elevated urea and creatinine concentrations and significantly cleared necrosis, inflammations and haemorrhage in the kidneys of infected rats.

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COMMUNITY BURDEN OF INTESTINAL PARASITES AND IT'S PUBLIC HEALTH CONCERNS IN OBIZI, AMAKAMA OLOKORO, UMUAHIA SOUTH L.G.A, ABIA STATE, NIGERIA

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ABSTRACT

Intestinal parasitic infections are the leading causes of morbidity and mortality in sub-Saharan Africa. This could be due to the dominant factors such as poverty, poor hygiene and absence of basic infrastructures. This study, conducted between December 2017 and May 2018 was done to determine the community burden of intestinal parasites in Obizi, Amakama-Olokoro, Umuahia-South L.G.A Abia State. Ethical permission was received from Umuahia South Local Government Authority, (AD/ 69/ VOL.11/S5/456), as well as the traditional ruler of Obizi. Informed consent was given by respondents before the study commenced. Data collection involved obtaining fecal samples from 284 consenting individuals, analyzed using formol ether concentration technique and with the aid of questionnaires for demographic data of respondents. Statistical analysis was done using the Pearson's Chi-square through the PAST package to determine significance. Results from the study showed 96 persons (33.8%) were infected and ten parasites were isolated, with *Ascaris lumbricoides* (15.5%) recording the highest prevalence and *Fasciola hepatica* (0.7%) and *Entamoeba coli* (0.7%) the lowest prevalence. Mixed infections occurred. The combination of Hookworm and *Taenia saginata* occurred the most (1.8%). Females had the highest rate of infection (40.3%); also the age group (11-20) years were more infected (76.9%). Students recorded the highest prevalence (50.9%). Fever, fatigue, blood in stool, diarrhea were the symptoms of ill health reported. This study has revealed a community burden of intestinal parasites which is of great public health concern. Public enlightenment and deworming through mass drug administration (MDA) are recommended.

Keywords: Community Burden, Intestinal Parasites, Public Health, MDA

INTRODUCTION

Sub-Saharan Africa is specifically susceptible to intestinal parasitic infections (Akinboye, 2016). Some people are more prone to infections than others; for example, a person who is already sick or has compromised immune system is more at risk of infections. Individuals who lack potable water are also at the risk of getting infected with parasitic infections. Also, a person who loves to swim in ponds, rivers, stagnant waters or lakes and ingest the water, is also at risk (Akinboye, 2016).

Disability-adjusted life years (DALY) analyses of the global burden of disease (BOD) of neglected tropical diseases (NTDs) have analytically demonstrated the dominance and wide epidemiological spread of intestinal nematode infections (INIs) with an approximately 5.19 million DALYs (Hotez *et al.*, 2014). The bulk are accounted for by hookworm diseases (3.23 million), then ascariasis (1.32 million), and finally trichuriasis (0.64 million) (Hotez *et al.*, 2014). The burden of intestinal infection appear to vary greatly within major global regions, which is impressive with regards to the *Ascaris lumbricoides* and *Trichuris trichiura* are acquired by ingestion of soil contaminated with human faeces containing infective eggs (Morenikeji, *et al.*, 2009). Others are transmitted from animals to man (Zoonoses) such as giardiasis. People are also infected through; rain, water runoff, animals, and human migration amidst inadequate

public sanitation and poor infrastructure (Pullan, *et al.*, 2014).

Many of these intestinal nematodes are transmitted when people ingest contaminated vegetables containing eggs or cysts while others are infected by consuming under-cooked meat such as in the case of Trichinellosis.

In developing countries intestinal parasitic infections have become a major public health issue with the increasing case of insurgency, insecurity, and poor nutrition, lack of adequate infrastructure, poor housing and unhygienic environmental conditions. Although intestinal parasitic infections have become neglected by so many including government agencies, researchers have continued to conduct studies on different aspects of parasitic disease prevalence with so much reference to intestinal parasitic infections. The main aim of this work is to determine the community burden of intestinal parasites in Obizi and their public health concerns, through the following objectives; to ascertain prevalent rate with respect to Sex, Age and occupation and to determine the most prevalent symptoms associated with these parasites.

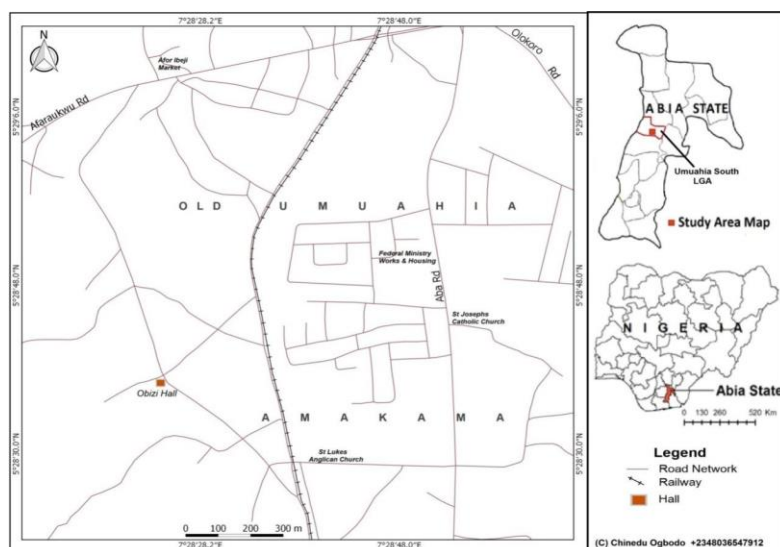
METHODOLOGY

Study Area

The research was carried out in Obizi, Amakama Olokoro, Umuahia-South L.G.A, Abia State in the

Eastern part of Nigeria. Obizi is located between latitude ($5^{\circ}28'30''N$) and longitude ($7^{\circ}28'48''E$). The community consists largely of peasant farmers and traders, who live in close proximity to each other with houses built very close to each other just like a typical

rural settlement. The community is in the rain forest zone, where rainy season starts from April to October and dry season between September to March. Infrastructures such as Health care centre and accessible roads were absent (Egwu., *et al* 2018).



Source: GIS Map by Chinedu Ogbodo, 2020
 Fig 1: Map showing the Location of Obizi Hall in Umuahia South LGA, Abia State.
 Ethical Approval

Permission was sought for and received from the Umuahia South Local Government Authority, (AD/69/ VOL.11/S5/456), the village head and the Chairman of the community union. Following discussions with the community Chairman, an awareness campaign for the study was created. Health workers from the University Medical Centre assisted in the Public Health Awareness programme. Days before the commencement of the study, public health awareness campaigns were carried out through the town crier. Permission was granted based on the agreement that patient anonymity will be maintained and that every finding will be treated with utmost confidentiality for the purpose of this research only. Informed consent of the participants was also sought and received before being included in the study. For the children, consent was received from their parents before their inclusion in the study. This is a cross sectional study of persons who willingly gave their consent for themselves or their children to be included in the study spanning the period of December, 2017 to May, 2018.

Collection of Faecal Samples

Participants were given wide-mouthed sample bottles for collection of faecal samples. These bottles were properly labeled on return for identification.

Faecal Analysis

Faecal analysis was done using the formol-ether concentration technique. A little quantity of faeces was emulsified in 15ml of 10% formol saline and mixed well. Thereafter 3ml of the sample was poured into a centrifuge tube and 7ml of ethyl acetate added to make it up to 10ml. The sample was centrifuged at 1,500rpm for 5mins. The lipid and aqueous phase were decanted carefully and the resultant pellet was emulsified in normal saline, dropped onto a slide and viewed under the microscope using x10 objective lens and x40 to confirm. (Cheesbrough, 2009).

Statistical Analysis

Data analysis was done using frequency distribution. The results were tested with Pearson’s Chi-square to determine variability in the distribution of categorical variables of each study outcome, with an α -level of $P \geq 0.05$ indicating statistical significance using the Paleontological statistical software.

RESULTS

Overall Prevalence of Intestinal Parasites

Ascaris lumbricoides had the highest prevalence 44 (15.5%) However, *Fasciola hepatica* and the non-pathogenic *Entaemoba coli* had the lowest prevalence of 2 (0.7%) (Table 1).

Table 1: Overall Prevalence of Intestinal Parasites n=284

Intestinal Parasites	Number infected	%
<i>Hookworm</i>	11	3.9
<i>Ascaris lumbricoides</i>	44	15.5
<i>Taenia saginata</i>	10	3.5
<i>Schistosoma mansoni</i>	12	4.2
<i>Enterobius vermicularis</i>	3	1.1
<i>Fasciola hepatica</i>	2	0.7
<i>Trichuris trichuria</i>	4	1.4
<i>Entamoeba coli</i>	2	0.7
<i>Chilomastix mesnili</i>	5	1.8
<i>Balantidium coli</i>	3	1.1
Total	96	33.8

Prevalence of Single and Mixed Infections of Intestinal Parasitic Infection

Ascaris lumbricoides recorded highest prevalence of single infection among intestinal parasites 41 (14.4%). Whereas Hookworm and *Taenia Saginata* recorded the highest prevalence of mixed infection 5 (1.8%) *Fasciola hepatica* and *Schistosoma mansoni* also *Balantidium coli* and *Entamoeba coli* all recorded lowest prevalence of mixed infection 2 (0.7%) (Table 2).

DISCUSSION

Community burden of intestinal parasites was established in study population. *Ascaris lumbricoides* recorded the highest prevalence of 14.4%. This is similar to the report of Nduka *et al.*, (2006) who recorded a prevalence of (17.88%) in Abia State. This value is also similar to studies by Awobode *et al.*, (2016) who recorded a prevalence of (11.4%) in Oyo State. The prevalence value of (14.4%) is high compared to other authors who recorded low prevalences. These include Colman *et al.*, (2013) who recorded a prevalence of (6.3%) among prison inmates in Maiduguri; Anosike *et al.*, (2004) who recorded a prevalence of (6.2%) among nomadic Fulanis in south-east; Amaechi *et al.*, (2013) who recorded a prevalence of (8.2%) in rural communities in Abia State, Nigeria. The result however varied with those of other authors who reported higher prevalences such as

Ukpai and Ugwu (2007) who recorded a prevalence of (36.7%) among primary school children in Ikwuano, Abia state; Auta *et al.*, (2013) (30.7%) among school children in Gwagwudu, Kaduna; Sigh and Muhammad (2017) (80.0%) among Noma patients in Sokoto; Morenikeji *et al.*, (2009) who recorded a prevalence of (48.4%) in south-west Nigeria; Agbolade *et al.*, (2003) (62.8%) in Ijebu, Ogun State while Mordi and Ngwodo (2007) recorded a prevalence of (30.0%) in Edo State. The prevalence recorded in this work could be as a result of the transmission pattern of *Ascaris lumbricoides* and could be because of poor personal hygienic practices.

Ascaris lumbricoides infection appears to be the most prevalent intestinal helminth parasitic infection in most studies especially in the tropics. This could be accredited to the micro-polysaccharide feature of the embryonated egg. This enables the parasite to withstand harsh environmental conditions and also enables the parasites to adhere easily to a wide range of surfaces, hence they can adhere to door handles, dust, fruits, vegetables, money and enhance easy transmission (Opara *et al.*, 2012). *Schistosoma mansoni* had a prevalence of (4.2%). A lower prevalence of (0.05%) was reported by Colman *et al.*, (2013) among prison inmates in Maiduguri while Mordi and Ngwodo (2007) reported a higher prevalence of (11.7%).

Hookworm recorded a prevalence of (3.9%) (Table 2). This may be attributed to faecal pollution of the environment and inadequate sewage disposal system. The prevalence of (3.9%) seem to be moderately low. This is lower than what was reported by Amaechi *et al.*, (2013) who recorded a prevalence of (5.1%) and Colman *et al.*, (2013) who recorded a prevalence of (6.47%). The result of (3.9%) however, contradicts with the reports of Agbolade *et al.*, (2004) who recorded a higher prevalence of (16.6%); Nduka *et al.*, (2009) a prevalence of (14.8%) and Morenikeji *et al.*, (2009) a prevalence of (15.6%). *Taenia saginata* recorded a prevalence of (3.5%). This compares with the results of Awobode *et al.*, (2016) who also reported a low prevalence of (1.2%) and Houmsou and Amuta (2009) who reported a prevalence of (3.69%) among school children in Markurdi. *Chilomastix mensnili* recorded a low prevalence of (1.8%), though it is a non-pathogenic intestinal protozoa. *Trichuris trichiura* recorded a low prevalence of (1.4%). This is similar to the result of Ukpai and Ugwu (2007) who also reported a low prevalence of (2.3%) and differed from the result of Morenikeji *et al.*, (2009) who reported a higher prevalence of (21.8%) in their work. *Enterobius vermicularis* recorded a prevalence of (1.1%). *Balantidium coli* recorded the lowest prevalence of (1.1%). Mixed infections were observed. The highest combination was that of Hookworm and *Taenia saginata* (1.8%), followed closely by that of *Ascaris lumbricoides* and *Taenia*

saginata (1.1%). *Entamoeba coli* and *Fasciola hepatica* occurred only as mixed infections. The combination of *Entamoeba coli* and *Balantidium coli* was (0.7%) while that of *Fasciola hepatica* and *Schistosoma mansoni* was (0.7%). The presence of mixed infections buttresses further the community burden of these intestinal parasites. The highest prevalence of intestinal parasites was recorded among female participants (40.3%) while the males recorded a prevalence of (20.4%). The prevalence of intestinal parasites is not dependent on gender as supported by the statistical analysis. This means that both sexes can acquire the parasites with equal exposure. The high prevalence value recorded among the female participants could be accredited to the fact that females are exposed to a lot of outdoor chores, gathering infected vegetables, taking care of infected family members and friends etc. This result is similar with Singh and Muhammad (2017) who recorded higher prevalence among females (53.33%) than males (46.67%). Morenikeji *et al.*, (2009) recorded higher prevalence of intestinal parasites among females (57.8%). There is a significant difference between age and prevalence of parasitic infections. Age dependence in prevalence was observed as the age group (11-20) years which were predominately young school children had the highest prevalence of intestinal parasites (76.9%). This is due to their frequent unhygienic practices such as eating unwashed vegetables and fruits obtained on their way home from school, eating with unwashed hands, poor toilet facilities in schools and homes, edaphagy (soil eating habits), lack of hand washing habits. This is consistent with results reported by other parasitologist. Auta *et al.*, (2013) recorded highest prevalence between (13-

15) years. Ukpai and Ugwu (2007) recorded high prevalence rates among the following age groups (5-7), (8-10) and (11-13) years with prevalence of (54.7%), (54.3%) and (57.8%) respectively. Students recorded the highest prevalence (50.9%), followed by farmers (38.9%), then traders (32.1%) and civil servants recorded the lowest prevalence of (0.4%). Statistical analysis confirmed that the prevalence of intestinal parasites with respect to occupation is significant among the various occupations. This implies that school children which were mostly classified as students were involved in unhygienic practices. Farmers are also exposed as a result of occupational hazards experienced in the farm. This is because there is a deficit of well-constructed toilet systems in most homes in rural communities. This agrees with Nduka *et al.*, (2006) who recorded highest prevalence of intestinal parasites among students (46.27%) and farmers (38.48%) in Ishiagu. A greater percentage indicated they felt tired easily (fatigue) 75 (82.4%), participants who ascertained they had recurrent fever had a prevalence of 89 (81.0%).

CONCLUSION AND RECOMMENDATION

The results from this work have shown that intestinal parasitic infections have great impact on the community burden of parasitic diseases especially among rural dwellers and have become a public health concern. I recommend an increased public health education for the masses and deworming through mass drug administration (MDA). I advocate for an adequate, well-equipped and functional health care system and diagnostic centre for most rural dwellers to be executed by the government.

Table 2: Prevalence of Single and Mixed Infections of Intestinal Parasites n= 284

Single Infection Parasites	Number Infected	%
<i>Ascaris lumbricoides</i>	41	14.4
<i>Hookworm</i>	6	2.1
<i>Taenia saginata</i>	2	0.7
<i>Schistosoma mansoni</i>	10	3.5
<i>Enterobius vermicularis</i>	3	1.1
<i>Fasciola hepatica</i>	0	0.0
<i>Trichuris trichuria</i>	4	1.4
<i>Chilomastix mensnili</i>	5	1.8
<i>Entamoeba coli</i>	0	0.0
<i>Balatidium coli</i>	1	0.4
Total	72	25.4
Mixed Infectin		
AS + T	3	1.1
H+ T	5	1.8
FH+ SM	2	0.7
BC+ EC	2	0.7
Total	24	8.5

Key: AS= *Ascaris lumbricoides*, T= *Taenia saginata*, H= Hookworm, SM= *Schistosoma mansoni*, FH= *Fasciola hepatica*, BC= *Balatidium coli*, EC= *Enterobius vermicularis*

Prevalence of Intestinal Parasites with Respect to Sex

Intestinal parasitic infection was higher in females 40.3% than in males 20.4% (Table 3).

Table 3: Prevalence of Intestinal Parasites with Respect to Sex

Gender	No. Examined	Number Infected	(%)	P-value
Male	93	19	20.4	0.20
Female	191	77	40.3	
Total	284	96(33.8%)		

Prevalence of Intestinal Parasites with Respect to Age

The age group (11-20) years recorded the highest prevalence of 76.9%, while the age group (61-70) years recorded the lowest prevalence 18.2% (Table 4).

Table 4: Prevalence of Intestinal Parasites with Respect to Age

Age (years)	No. Examined	Number Infected	(%)
1-10	10	6	60.0
11-20	26	20	76.9
21-30	22	18	18.8
31-40	20	4	20.0
41-50	50	15	30.0
51-60	48	10	20.8
61-70	88	16	18.2
71-80	20	7	35.0
Total	284	96(33.8%)	

Prevalence of Intestinal Parasites with Respect to Occupation

Students recorded the highest prevalence of infection 50.9%, followed by farmers with prevalence of 38.9%, traders with a prevalence of 32.1% and civil servants were the least infected 2.3% (Table 5).

Table 5: Prevalence of Intestinal Parasites with Respect to Occupation

Occupation	Number examined	Number Infected	Percentage of Infection (%)	P-value
Farmers	108	42	38.9	0.00
Traders	78	25	32.1	
Civil servants	43	1	2.3	
Students	55	28	50.9	
Total	284	96	33.8	

Signs and Symptoms of Ill-health Reported

The signs and symptoms of ill-health reported were fever (38.7%), Fatigue (32.2%), nausea (28.5%) and diarrhoea (17.6%). (Table 6.)

Table 6: Signs and Symptoms Reported

Symptoms	Yes	%	No	%	No Response	%
Fever	110	38.7	102	35.9	72	25.4
Fatigue	91	32.0	60	21.1	133	46.8
Blood in Stool	5	1.8	269	94.7	10	3.5
Nausea	81	28.5	92	32.4	111	3.9
Diarrhoea	50	17.6	95	33.5	139	48.9

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PHYTOCHEMICAL ANALYSIS OF *Cynodon dactylon* AND THE EFFECT OF ITS AQUEOUS EXTRACT ON SOME BIOCHEMICAL PARAMETERS OF WISTAR RATS

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Abstract

The study determined the phytochemical composition of *Cynodon dactylon* and effect of its aqueous extract on some biochemical parameters of Wistar rats. Sixteen (16) adult male Wistar rats were randomly divided into four groups (1-4) of four rats each. Group 1 (control) was administered water only. Groups 2-4 were administered 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of aqueous extract of *C. dactylon* respectively. Phytochemicals analysis revealed: saponins (1.25 ± 0.01 %), flavonoids (4.15 ± 0.15 %), steroids (0.87 ± 0.02 %), alkaloids (5.00 ± 0.01 %) and tannins (1.62 ± 0.05 %). After 21 days daily administration of the extract, there was no significant difference in the relative-liver weight, relative-kidney weights, AST, ALT, total bilirubin, conjugated bilirubin, creatinine and bicarbonate levels in all the groups compared to their controls. Sodium and potassium concentrations were significantly ($p < 0.05$) higher in test groups. Liver and kidney histology did not show visible disruption of their architecture. Aqueous extract of *C. dactylon* showed minimal signs of toxicity at the administered concentrations.

Keywords: Biochemical, *Cynodon dactylon*, Histology, Phytochemical

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Introduction

Plants have remained the primary source of medicine for management and treatment of diseases. Majority (80%) of the world population source their medicine from plants (Rahman, 2014). More than 20% of drugs used globally are sourced from herbal plants (Wachtel-Galor and Benzie, 2011; Savadi et al., 2020). Medicinal plants contain important bioactive compounds (essential oils, phytochemicals and so on) that are very effective in managing various ailments (Andrade et al., 2019); hence, their wide exploitation by pharmaceutical industries (Al-Snafi, 2016). These bioactive compounds have been reported to possess different pharmaceutical properties (Oyenihi and Smith, 2019). Plant-derived medicines are believed to be less toxic than synthetic drugs (Andrade et al., 2019).

Cynodon dactylon Pers is a very important medicinal plant used in traditional healthcare. It belongs to the family of "Poaceae" and commonly known as Bermuda grass, bahama grass, dhub; chiaawar sarki (Hausa), kooko igba (Yoruba). It is a perennial grass that grows as common weed in Africa, Asia, Australia and Europe (Venkatachalam et al., 2018). In addition to its use as medicine, it is planted on fields to check erosion and also to feed live stocks. The plant is used by herbalists to treat wounds (Mangathayaru et al., 2009), snake bites, gout and rheumatism (Chopra et al., 1999). Its root decoction is used as medicine to treat syphilis and urinary tract disorders (Auddy et al., 2003). The antimicrobial activity (David et al., 2020), Antidiabetic and antidiarrhoeal activity (Rahman, 2015) has been reported. However, its toxicological effect has not been reported despite its varied uses in ethnomedicine. The aim of this study is to assess its

phytochemical content as well as effect of *C. dactylon* aqueous extract on some biochemical parameters of Wistar rats.

Materials and Methods

Plant material

Fresh matured leaves of *C. dactylon* were harvested around evening hours from a natural population at Umuariaga, Oboro, Ikwuano LGA, Abia State. The plant was identified by Mr. Ibe, K. Ndukwe, a Forester in the Department of Forestry and Environmental Management Michael Okpara University of Agriculture, Umudike (MOUUA).

Sample preparation

The leaves were sorted and cleaned to remove dust particles and debris. The plant sample was air-dried under shed in the Biochemistry Laboratory, Michael Okpara University of agriculture, Umudike. The dried samples were ground with the aid of a Thomas milling machine. One hundred grammes of the powdered sample was weighed out using a top loading balance (ScoutTM-pro spu402), soaked in 700 ml of distilled water for 72 hours and thereafter, filtered with a muslin cloth. The filtrate was then evaporated slowly at 45°C in a water bath to get a solid extract which was stored in a refrigerator until it was ready for use.

Phytochemical analysis

Determination of alkaloids, saponins and flavonoids contents were determined according to the method described by Harboume (1973). Phenols and tannins were determined by the method of AOAC (1990) and AOAC (2006) respectively.

Animal grouping

Sixteen male Wistar rats weighing (52-74) g were acquired from the Animal Breeding Unit of the College of Veterinary Medicine, University of Nigeria Nsukka (UNN). After two weeks of acclimatization, they were weighed and divided into four groups of four rats each. An oral gavage was used to administer different concentration of the aqueous extract to the groups as follows:-

Group 1: Control fed with the normal rat feed and water.

Group 2: 100 mg/kg body weight of *C. dactylon*

Group 3: 200 mg/kg body weight of *C. dactylon*

Group 4: 400 mg/kg body weight of *C. dactylon*

The rats were weighed at the beginning and before sacrifice. After 21 days daily administration of aqueous extract of *C. dactylon*, blood samples were collected from the rats by cardiac puncture. Blood was collected into plain and EDTA bottles for biochemical parameters. Portions of organs (liver and kidney) were also excised and stored in 10% formalin for histology.

Determination of Relative organ weights

The relative organ weights (liver and kidney) of the animals in each group was calculated using the formula given below: Relative organ weight = $\frac{\text{Weight of organ}}{\text{Weight of rat}} \times 100$

Biochemical analysis

Method described by Reitman and Frankel (1957) was employed for determination of aspartate amino transferase (AST) and alanine amino transferase activity (ALT). Alkaline phosphatase (ALP) activity was determined by the method described by Deutsche Gesellschaft fur Klinische Chemie. Serum urea was determined using the urease-Berthelot method described by Weatherburn (1967). Serum creatinine was determined using the method described by Henry (1974). Determination of serum Sodium was determined using the method first described by Maruna (1958) and Trinder (1951). Potassium concentration was determined using the method described by Terri and Sesin (1958). Determination of serum chloride was carried out using the colorimetric method described by Schoenfeld and Lewellen (1964) while determination of serum bicarbonate was carried out using the method described by Forrester *et al.*, (1976).

Histology

The method described by Bancroft and Steven (1990) was employed.

Statistical Analysis

Descriptive statistics were carried out on the data generated. Results were expressed as mean ± SEM (Standard Error of Mean). One way analysis of variance (ANOVA) was used to separate means with LSD multiple range test. All statistical analysis was done using IBM SPSS version 20. Data from the test groups were compared with their respective controls and differences at p< 0.05 were considered to be significant.

RESULT

Phytochemical composition of *Cynodon dactylon*

The result of the phytochemical composition of *C. dactylon* is as shown in Table 1. The result showed the presence of appreciable quantities of saponins, flavonoids, steroids, alkaloids, phenols and tannins. Alkaloids were the most abundant phytochemical detected while phenols were the least.

Table 1: Phytochemical composition of *Cynodon dactylon*

Phytochemical	Composition (%)
Saponins	1.25 ± 0.01
Flavonoids	4.15 ± 0.15
Steroids	0.87 ± 0.02
Alkaloids	5.00 ± 0.01
Phenols	0.46 ± 0.02
Tannins	1.62 ± 0.05

Values are expressed as mean ± standard error of mean (SEM) of duplicate determinations.

Relative organ weight

The effect of aqueous extract of *C. dactylon* on relative organ weight is as presented in Table 2. The result obtained indicated a non-significant effect of the extract on this parameter.

Table 2: Effect of *Cynodon dactylon* aqueous extract on relative organ weights of Wistar rats

Group	Dose (mg/kg)	Liver	Kidney
1 (Control)	---	4.65 ± 0.11	0.84 ± 0.01
2	100	4.78 ± 0.26	0.99 ± 0.20
3	200	4.30 ± 0.38	0.93 ± 0.03
4	400	5.00 ± 0.11	0.81 ± 0.02

Values are expressed as mean ± standard error of mean (SEM) of duplicate determinations.

Liver function

Table 3 shows the result of the effect of *C. dactylon* on some liver function parameters. Significant changes were not observed for all the parameters studied.

Table 3: Effect of *Cynodon dactylon* aqueous extract on some liver function parameters of Wistar rats

DOSE mg/kg)	AST (U/I)	ALT (U/I)	ALP (U/I)	TOTAL BILIRUBI N (mg dl ⁻¹)	CONJUGATED BILIRUBIN (mg dl ⁻¹)
Control	11.25 ± 3.35	16.00±3.34	144.90±12.76	1.11±0.39	0.88±0.37
100	8.75±1.18	18.50±5.70	156.55±1.59	1.22±0.36	0.90±0.26
200	8.50±1.19	9.50±0.96	185.30±14.35*	1.99±0.53	1.30±0.25
400	12.00±2.35	16.25±5.31	139.18±2.38	0.81±0.11	0.44±0.17

Values are expressed as mean ±standard error of mean (SEM) of duplicate determinations. * = Significantly different.

Kidney function

The observed effect of *C. dactylon* aqueous extract on urea and creatinine concentration is depicted in Table 4. Urea concentration increased as the dose increased from 100 to 400 mg/kg extract. However, the urea concentration obtained for rats administered 100 mg/kg body weight of extract was significantly (p<0.05) lower than the control. Creatinine concentrations were statistically similar (p>0.05).

Table 4: Effect of *Cynodon dactylon* aqueous extract on urea and creatinine concentration of Wistar rats

Group	Urea (mg/dl)	Creatinine (mg/dl)
Control	49.34±6.75	1.91±0.53
100	27.81±7.97*	1.91±0.55

200	66.73±3.68	0.91±0.25
400	66.63±5.45	1.25±0.31

Values are expressed as mean ±standard error of mean (SEM) of triplicate determinations. * = significantly different from control.

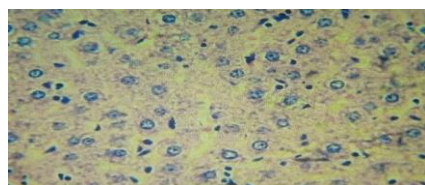
Serum electrolyte

The effect of *C. dactylon* aqueous extract on serum electrolyte concentration is presented in Table 5. Observed increase in sodium and bicarbonate concentrations in the rats were not significant (p>0.05). Potassium concentration for all the treated rats was significantly (p<0.05) higher compared to the control. Rats administered 100 mg/kg extract, had a lower concentration (p<0.05) compared to the control.

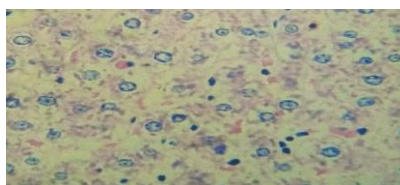
Table 5: Effect of *C. dactylon* aqueous extract on serum electrolyte concentration of Wistar rats

Dose (mg/kg)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Control	142.50±5.26	6.93±1.00	93.50±15.02	29.50±2.08
100	192.53±15.45*	12.05±1.29*	74.00±6.68*	30.75±6.70
200	217.25±17.19*	11.20±2.90*	94.50±20.37	31.00±4.08
400	188.75±43.28*	10.23±1.83*	92.75±19.24	33.75±4.50

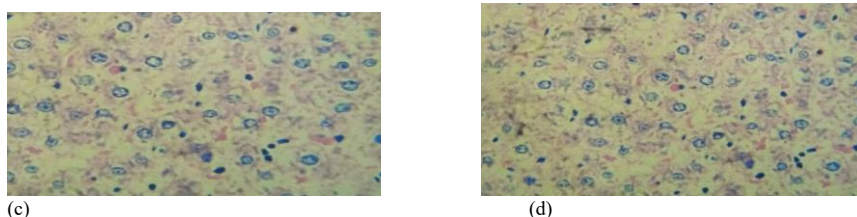
Values are expressed as mean ±standard error of mean (SEM) of triplicate determinations. * = significantly different from control.



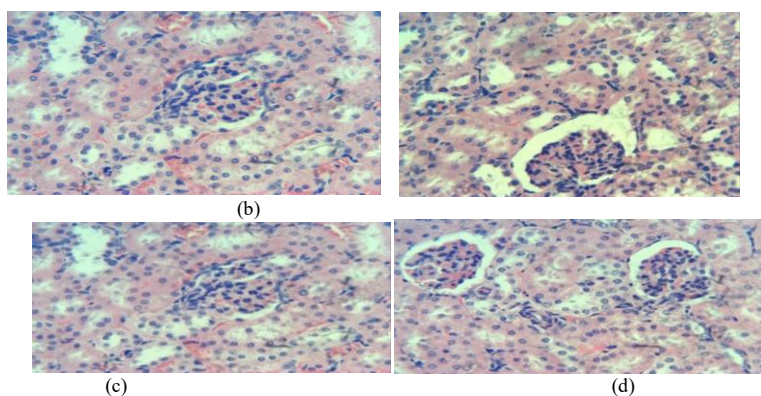
(b)



(a)



(c) (d)
Figure 1: Effect of aqueous extract of *Cynodon dactylon* on liver histology of Experimental rats. (a), (b), (c) and (d) represent liver histology of rats from control, 100 mg/kg, 200 mg/kg and 400 mg/kg groups. (H&E ×400)



(b) (d)
(c) (d)
Figure 2: Effect of aqueous extract of *Cynodon dactylon* on kidney histology of Experimental rats. (a), (b), (c) and (d) represent kidney histology of rats from control, 100 mg/kg, 200 mg/kg and 400 mg/kg groups. (H&E ×400)

Discussion

Cynodon dactylon contains important phytochemicals (Table 1) that are known to perform vital functions in plants. Alkaloids possess analgesic, hypotensive, and sedative potentials (Osugwu & Eme, 2013); Flavonoids, phenols and tannins are phenolic compounds (Omaga & Azeke, 2014) with antioxidant properties (Akhbari et al., 2012). Saponins have hypotensive (Omaga & Azeke, 2014); antifungal (Osugwu & Eme, 2013); and antihypercholesterolemic (Soetan & Aiyelaagbe, 2009) properties. Flavonoids are well known for their antihyperglycaemic activity (Muriithi et al., 2015); antioxidant properties and ability to suppress tumor progression (Osugwu & Eme, 2013). Steroids have anti-inflammatory properties, Tannins have anti-inflammatory and wound healing property (Osugwu & Eme, 2013). The presence of these phytochemicals lend support to the use of *C. dactylon* for treatment of various health disorders in ethnomedicine.

Relative organ weight differences is a key pointer to the effect of an extract in animal models even when there are no morphology changes (Bailey et al., 2004). It is therefore used to assess toxicity (Timbrell, 2002). Increase in organ/body weight ratio is usually attributed to the presence of inflammation while a decrease signals cellular constriction (Moore and

Dalley, 1999). The non-significant increases in liver and kidney body weight ratios in this study could be an indication that the extract was not toxic (Schmidt et al., 2007). Result of this study corroborated that of Kaid et al. (2019).

Liver enzyme assays and bilirubin concentrations are suggestive of the functionality and cellular integrity of the liver (Shivaranj et al., 2009). Cytosolic enzymes like AST, ALT and ALP leak into the blood following injury to the hepatic cells (Kwo et al., 2017). Aspartate amino transferase (AST) and ALT are intracellular liver enzymes that serve as biomarkers of toxicity (Oh and Husted, 2011). Alkaline phosphatase is located in the biliary duct of the liver (Nyblom, et al., 2006). When the biliary duct is obstructed, ALP levels increase in the serum. Bilirubin is a by-product of haem catabolism whose accumulation results in jaundice. The non-significant ($p < 0.05$) difference in AST and ALT in this study could be an indication of low toxicity of the extract. However, the significant ($p < 0.05$) increase in ALP activity observed in the 200 mg/kg extract could be an indication of infiltrative diseases of liver and bone or biliary obstruction (Sharma et al., 2014).

Urea, creatinine and electrolyte (sodium, potassium, chloride and bicarbonate) concentrations were

measured to assess the functionality of the kidney. The kidney excretes toxic metabolites, produces renal enzymes and main normal acid-balance (Oh and Hustead, 2011). Abnormal functioning of the kidney leads to accumulation of nitrogen breakdown products that are easily measured in the form of blood urea and creatinine (Oh and Hustead, 2011). Urea and creatinine are excreted in the kidney as end products of protein metabolism in the liver (Bunel *et al.*, 2017) and nonenzymatic breakdown of phosphocreatinine in the muscle (Hosten, 1990) respectively. Results from this study indicated that the urea and creatinine values were statistically similar. However, the significant decrease in serum urea concentration observed in this study for 100 mg/kg extract group could be a result of alterations in the secretory and excretory functions of the kidney.

Serum electrolyte concentrations are part of the assessment used to diagnose disease state in patients. Sodium and potassium are the commonly assessed electrolytes. The significant increase in sodium and potassium concentrations indicated that the extract may have affected the reabsorptive function of the kidney (Boone and Deen, 2008). This has lent credence to its reported *diuretic properties* (Arua *et al.*, 2013).

The liver and kidney histology in this study showed the absence of clear hepatic or renal damage in all the rats.

Conclusion

The result of this study showed that *C. dactylon* contained important phytochemicals. Also, aqueous extract of *C. dactylon* showed low toxicity to liver and kidney at the doses administered.

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PREVALENCE OF BIOFILM PRODUCING *STAPHYLOCOCCUS AUREUS* OBTAINED FROM DIFFERENT FOOD PROCESSING SURFACES IN UMUAHIA

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Abstract

In this study, a total of 200 food contact surfaces and meat processing surfaces were investigated for biofilm producing *Staphylococcus aureus*. Samples obtained from the swabbed surfaces were cultured on mannitol salt agar using the streak plate technique. The isolates were identified by standard biochemical characterization procedures and further assessed for their susceptibility to antibiotics using the disc diffusion method. Assay for biofilm production was carried out by means of Congo Red agar. A total of one hundred and ten (110) *S. aureus* isolates were isolated from the different swabbed surfaces. The prevalence of *S. aureus* in meat processing surfaces and food processing surfaces was 61.0% and 49.0% respectively. Drug susceptibility profile of the isolates revealed varying degree of susceptibility and resistance to the test antibiotics. All isolates from the two surfaces exhibited 100% resistance to ceftazidime and augmentin while showing high susceptibility to the fluoroquinolones-ofloxacin, ciprofloxacin and levofloxacin. Result of biofilm production showed that 26(42.6%) of the total 61 and 18(36.7%) of the total 49 *S. aureus* isolates isolated from the meat and food processing surfaces respectively possessed biofilm formation potentials. The result obtained from this study calls for caution and need for improved hygiene among food and meat retailers to forestall the emergence of multidrug-resistant *S. aureus* isolates with potentials to form biofilms.

Keywords: Antimicrobial resistance, antibiotic susceptibility profile. Biofilm, *Staphylococcus aureus*

Introduction

The surfaces that foods make contact with play important roles in transmission of microorganisms in food processing plants. Staphylococcal food borne disease is one of the most common food borne disease worldwide resulting from the contamination of food by *Staphylococcus aureus* enterotoxins (Kadariya *et al.*, 2014). This species can produce many different enterotoxins which are stable to heat and have varying degrees of toxicity to the human body (Balaban and Rasooly 2000; Hennekinne *et al.*, 2012). Infections caused by *Staphylococcus* species are of concern owing to the fact that these species of bacteria show remarkable resistance to an array of antibiotics thus making such infections almost impossible to treat (Jasmine *et al.*, 2007). In certain environmental conditions, *S. aureus* has been reported to vary its phenotypic and genotypic makeup to suit its immediate surroundings thus biofilm formation is one of such recognized phenotypic changes (Archer *et al.*, 2011). Biofilm is a structured community of bacterial cells enclosed in self-produced polymeric matrix adherent to an inert or living surface (Donlam, 2002). They can form on both biotic and abiotic surfaces. One of the functions of biofilm is to protect the microorganism from host defenses and impede delivery of antibiotics. Biofilm formation in *S. aureus* involves initial adherence to a solid substrate, after which cell to cell adhesion occurs; the bacteria then multiply to form a multilayered biofilm encased in extra polymeric substances (EPS).

In the food industry, biofilms increase bacterial resistance to environmental stresses including

cleaning, disinfection, and inhibition, enabling these microorganisms to persist on surfaces and processing equipment, compared to planktonic cells (Kostski *et al.*, 2012; Laird *et al.*, 2012; Bridier *et al.*, 2015). Poor hygiene practices in food processing plants may result in the contamination of food products with pathogens, which means a serious risk for the health of consumers. The complete elimination of pathogens from food processing environments is a difficult task, in part because bacteria can attach to food contact surfaces and form biofilms, where they survive even after cleaning and disinfection (Brooks and Flint, 2008).

The consequence of acquisition of the biofilm phenotype in *S. aureus* is that infection with the organism becomes extremely difficult to treat and can thus bring about an increase in morbidity and mortality as well as exacerbate the risk of transmission of infection to food handlers and consumers.

Moreover the ability of *S. aureus* to adhere and form biofilms improves their survival and growth in food processing plants, providing an additional physiological advantage as a causative agent of food borne diseases (Hennekinne *et al.*, 2012). The present study was carried out to determine the prevalence of biofilm producing *Staphylococcus aureus* obtained from food contact surfaces in Umuahia metropolis.

Materials and methods

Sample collection and processing

The samples used in this investigation were collected by swabbing the surfaces (including tables, knives) of

different food preparatory and meat processing centres within Umuhia Abia State with a sterile cotton swab moistened with sterile physiological saline. The swabs were cultured on Mannitol salt agar plates by simple streaking and the plates incubated at 37°C for 24 hrs. Upon establishment of growth, bright yellow colonies were purified by streaking out on nutrient agar plate.

Biochemical characterization of isolates

Standard microbiological techniques were performed for biochemical characterization of the purified isolates. Biochemical tests which included Gram Staining, coagulase, catalase, and DNase tests were carried out according to standard procedures as described in Cheesbrough (2000).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2009) on Mueller Hinton agar (Hardy Diagnostics, USA). Discrete colonies from a 24 hr nutrient agar plates were suspended in sterile normal saline in a test tube to achieve a bacterial suspension equivalent to 0.5McFarland turbidity standard. A sterile cotton swab was dipped into the bacterial suspension and used to inoculate the entire surface of a Mueller Hinton agar plate, creating a lawn of each bacterium. The antimicrobial susceptibility discs were placed on the surface of the inoculated plate with flame sterilized forceps. The plates were incubated in an inverted position for 16–18 hrs at 35-37°C. The diameter of the

zones of inhibition produced by the antibiotic disks was measured to the nearest millimeter (mm).

Biofilm formation

Biofilm formation potentials of the isolates was evaluated by subsequently streaking out the respective isolates on Congo red agar (CRA) plates followed by incubation at 37°C for 24 hrs. The macroscopic characteristics of the *S. aureus* isolates on the CRA were observed. Crusty black colonies, with dry filamentous appearance, were recorded as positive for biofilm formation whereas the smooth pink colonies were non-producers (Arciola *et al.*, 2001).

Results

A total of 61 isolates of *S. aureus* were isolated from meat processing surfaces in different meat markets. While for the food contact surfaces, a total of 49 *S. aureus* isolates were isolated (Table 1). The antibiogram revealed that 100% of the isolates were resistant to Augmentin and Ceftazidime each while Erythromycin and Azithromycin showed 70.5% and 50.8% resistance levels respectively. Most of the isolates from both the food and meat processing surfaces showed similar susceptibility patterns to Levofloxacin (87.8% and 83.6%) Ofloxacin (83.7% and 81.9%) and Ciprofloxacin (79.6% and 78.7%). (Fig. 1 and 2). However 26 (42.6%) samples from food contact surfaces were strong biofilm producers while 35 were non biofilm producers. Similarly, 18 (36.7%) of the 49 isolates from the food contact surfaces had potential for biofilm formation (Table 2).

Table 1: Prevalence of *S. aureus* on the contact surfaces.

*MSA = Mannitol salt agar

Growth on MSA plates	No of isolates (%)	
	Food processing surfaces	Meat processing surfaces
No (%) of samples yielding <i>S. aureus</i>	49 (49.0)	61 (61.0)
No of samples with CONs	14 (14.0)	23 (23.0)
No of samples without growth	51 (51.0)	39 (39.0)

CONs = Coagulase Negative *staphylococci*

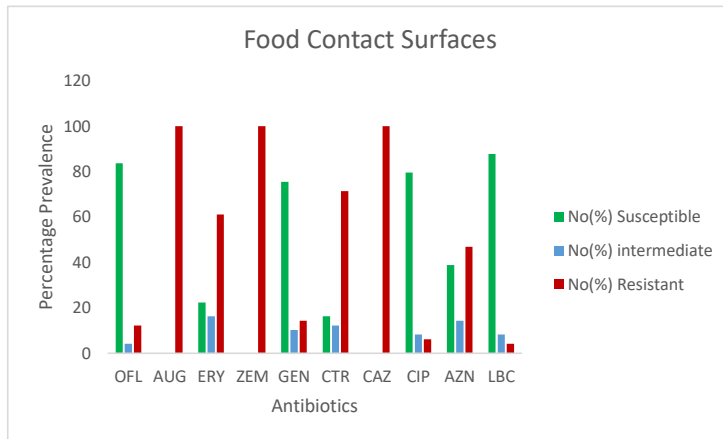


Fig 1: Antibiogram of *S. aureus* isolates from food contact surfaces
 *OFL: Ofloxacin, AUG: Augmentin, CAZ: Cefazidime, AZN: Azithromycin, GEN: Gentamicin, CTR: Ceftriaxone, ERY: Erythromycin, CIP: Ciprofloxacin, ZEM: Cefepime, LBC: Levofloxacin.

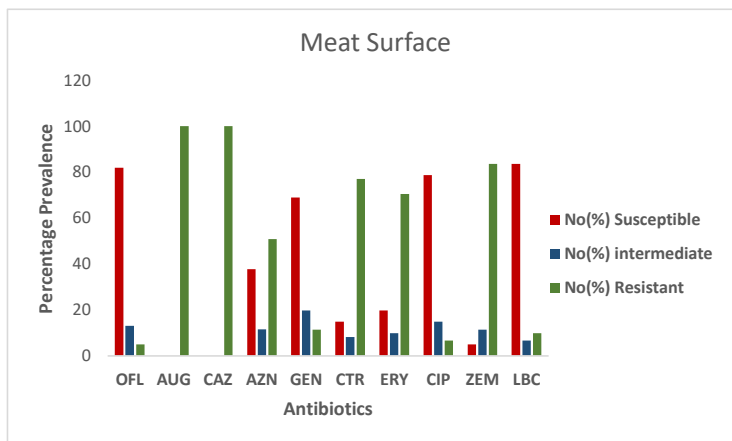


Fig 2: Antibiogram of *S. aureus* isolates from Meat Processing surfaces
 *OFL: Ofloxacin, AUG: Augmentin, CAZ: Cefazidime, CRX: Cefuroxime, GEN: Gentamicin, CTR: Ceftriaxone, ERY: Erythromycin, CIP: Ciprofloxacin, ZEM: Cefepime, LBC: Levofloxacin.

Table 2: Biofilm formation by the *S aureus* isolates (Congo red method).
 Colony appearance on CRA
No (%) positive to Biofilm Formation
 Food Meat processing

	processing surfaces	surfaces
Pink / red (negative)	31 (63.3)	35 (57.4)
Black colonies (positive)	18 (36.7)	26 (42.6)
Total Number of Isolates	49	61

*CRA = Congo red agar.

Discussion

S. aureus is a common and potent pathogen in humans. Colonization of food processing surfaces by *S. aureus* represents a potent and increasingly prevalent risk factor for subsequent *S. aureus* infection in a variety of healthy populations.

A total of one hundred and ten (110) *S. aureus* isolates were recovered from the different swabbed surfaces. The prevalence of *S. aureus* in meat processing surfaces and food processing surfaces was 61.0% and 49.0% respectively. These results suggested that the prevalence of *S. aureus* in food processing surfaces was at a relatively low level compared with that of meat processing surfaces.

The prevalence rate observed in this study is in agreement with earlier findings of Chairat *et al.* (2015) and El Bayomi *et al.* (2016) with 46.2% and 37.5% colonization rate. Our results are however, contrary to the reports of Asiimwe *et al.*, (2017) on similar study where 20.3% prevalence was recorded. Also, *S. aureus* was associated with 11.7% of samples studied in a related study by Gounadaki *et al.*, (2008) from meat processing facilities (Gounadaki *et al.*, 2008). The contamination level of food contact surfaces with *S. aureus* suggests that the handling as well as cleaning and disinfection of food industry facilities must be improved.

Assessment of antibiotic resistant pattern of the isolates from the meat processing surfaces as shown in fig 2 reveals that 100% of the isolates were resistant to Augmentin and Ceftazidime each while Erythromycin and Azithromycin showed 70.5% and 50.8% resistance levels respectively. Gentamycin recorded 68.9% susceptibility levels against the isolates.

Most of the isolates from both the food and meat processing surfaces were susceptible to levofloxacin (87.8% and 83.6%) ofloxacin (83.7% and 81.9%) and ciprofloxacin (79.6% and 78.7%) as can be seen from figures 1 and 2 above. The increased sensitivity profile of the isolates to the flouroquinolones and gentamicin is in line with earlier studies by Gowrishankar *et al.*, (2012). This result also supports the notion of Onanuga *et al.*, (2005) that non-beta-lactam antibiotics are preferred drugs for the treatment of staphylococcal infections. It could however, further be seen from the figures above that isolates are gradually showing reduced sensitivity

(intermediate category) to flouroquinolones and gentamycin. The high level of resistance observed for augmentin and other cephalosporins agrees with previous reports of Wertheim *et al.* (2005) and Nkwelang *et al.*, (2009). *S. aureus* has developed multidrug resistance worldwide, although reported prevalence rates indicate that wide variations exist regionally (Andre *et al.*, 2008; Al-Ashmawy *et al.*, 2016). Aydin *et al.* (2011) reported that 25.3% of *S. aureus* strains showed multidrug resistance. In addition, Al-Ashmawy *et al.* (2016) found that (84.1%) isolates exhibited high resistance levels, with resistance to three or more antimicrobials.

From this study, it was observed that 26(42.6%) of the total 61 and 18(36.7%) of the total 49 *S. aureus* isolates isolated from the meat and food processing surfaces respectively (Table 2) possessed biofilm formation potentials. This characteristic may allow them to adhere to different points on various food processing materials. This observation is in accordance with Sharvari and Chitra, (2012) and Ramakrishna *et al.*, (2014) who found that staphylococci biofilm producers were more resistant to commonly used antibiotics. Ferreira *et al.* (2014) and Pinto *et al.* (2015) both reported that *Staphylococcus* isolates have a significant incidence of biofilm formation in the food industry.

This study hence alerts to the risk that the *S. aureus* contamination in meat and food processing surfaces could pose as the food chain is considered a potential route of transmission of antibiotic-resistant bacteria to humans. The resistance to antimicrobials also raises high concern as an emerging problem in the food environment.

CONCLUSION

Biofilm formation can cause public health problems and economic losses, associated with food contamination by the pathogen resistant to hygiene treatments of food contact surfaces. Despite the fact that the analyzed *S. aureus* strains were susceptible to most of the tested antibiotics, a substantial contribution of biofilm forming strains among them may cause problems in chemotherapy of infections, particularly in the dose assessment. In this light, it is obvious that the information about bacterial species prevailing in food environment, their susceptibility and resistance patterns and on their biofilm formation ability is vital for both health care providers for the

implementation of infection prevention and control plans and for physicians in building up adequate antibacterial therapies.

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CHROMIUM 6⁺ COMPOUND INDUCED CARDIOTOXICITY IN ADULT MALE WISTAR RATS.

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ABSTRACT

Chromium 6⁺ compound (Cr[VI]) poses serious threat to life due to its ability to induce systemic toxicity. In this study, the cardiotoxic effect of the agent was evaluated in adult male wistar rats. The rats were intoxicated with the agent in 3 different phases. In the first phase (sub-acute), sixty rats were assigned to 6 groups of 10 rats each treated with specific doses of Cr[VI]. Group 1 received no treatment and served as the control, but groups 2, 3 and 4 were administered 10, 20 and 30 mg/kg b.wt of the agent via the oral route, respectively. Groups 5 and 6 received intraperitoneal administration of Doxorubicin at doses 15 mg/kg and 20 mg/kg body weight respectively (two days before sacrifice). Treatment lasted 21 days, after which the rats were sacrificed and blood collected for biochemical studies. Heart tissues were also harvested for further biochemical tests and a part preserved in formalin for histopathological evaluation. The procedure was repeated in the second phase but for 60 days (chronic study). In the 3rd phase, diets for groups 2, 3 and 4 were supplemented with 0.01%, 0.02% and 0.03% of Chromium 6⁺ compound, respectively, but treatments for groups 1, 5 and 6 remained unchanged. For all animals, heart homogenates and sera were assayed for indices of oxidative stress, myocardial infarction and inflammation. The results indicated a significant decrease in antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and Catalase activities when compared with control ($P < 0.05$). A significant increase was observed in the levels of cardiac troponin, creatinine-kinase, C-reactive protein, aspartate-transaminase, lactate-dehydrogenase, malondialdehyde and nitric-oxide compared to the control ($P < 0.05$). The effects of Cr[VI] was similar to that of Doxorubicin except that no significant increase was observed in MDA concentration ($P > 0.05$) when compared with control. Histopathologic evaluation of heart samples showed a dose-dependent pulmonary oedema and hyaline necrosis, which manifested as displacement of adjacent myocytes in Cr[VI] treated rats, just as Doxorubicin did. We, therefore, conclude that Chromium 6⁺ compound has shown potential for cardiotoxicity induction. However, there is need for further evaluation of this finding.

Keyword: Chromium 6⁺, Cardiotoxicity, Doxorubicin, Potassium dichromate

Introduction

Chromium (Cr) is an element required by the body in trace amounts (Onyedikachi *et al.*, 2019b). Cr has varying oxidation states and solubility. The most important and stable forms are the trivalent [Cr(III)] and hexavalent [Cr(VI)] forms (Carvalho *et al.*, 2014; Homaet *et al.*, 2016). It is implicated in lipid and glucose metabolism and widely used for industrial activities like photo production, chrome plating, paint making, welding, electroplating, tanning of leather etc. (Bagchi *et al.*, 2007; Garcia-nino *et al.*, 2015; Onyedikachi, 2019). Exposure to Cr(VI) is possibly through inhalation, dermal contact and ingestion of contaminated water from the waste site. The Cr(VI) forms are more hazardous to health because they are powerful oxidizing agents which get absorbed easily across the membranes through non-specific anion carriers. When ingested in toxic doses, Cr(VI) affects the respiratory, gastrointestinal, hepatic, renal, neurological and reproductive systems negatively and alters general development which may eventually result in death (OEHHA, 2009; ATSDR, 2012; Garcia-nino *et al.*, 2015). The health effects of Cr, just like other trace elements and chemicals of concern, may vary with the route of exposure (Carvalho *et al.*, 2014; Avila *et al.*, 2016; Paris *et al.*, 2018; Onyedikachi

2019a, Onyedikachi 2019b). Respiratory exposure has been associated with lung, nasal and sinus cancer (NIOSH, 2013). High levels of Cr has been found in tissues collected from workers exposed to Cr(VI) (Zhang *et al.*, 2011). The variation in Cr(VI) toxicity mechanisms within the biological systems is attributable to their easy passage through the cell membrane and its relative reduction (ATSDR, 2000; Nduka and Orisakwe, 2010).

Intracellularly, Cr(VI) is reduced through some reactive intermediates like Cr(IV) and Cr(V) to a more stable and non-toxic form (Cr(III)) aided by some reductants like Vitamin C, Glutathione and Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-dependent flavoenzymes. Some ingested Cr(VI) are also reduced to Cr(III) by the gastrointestinal fluid, thereby inducing secretion of acidic gastric juice in the intestine. Organ toxicities of Cr(VI) compounds like potassium dichromate ($K_2Cr_2O_7$) are through increased production of oxidative stress (Sengupta *et al.* 1990; Stohs and Bagchi 1995; Harris and Shi 2003; Wang *et al.*, 2006). Exposure of cells in culture to $K_2Cr_2O_7$ and sodium chromate can cause lipid peroxidation, DNA damage, protein oxidation and alteration in the activities of

antioxidant enzymes (Bagchi *et al.*, 2001, Bagchi *et al.*, 2002). Cellular toxicity multiplies through cell division; while this can be reversible and short-termed (toxicity) for some tissues, cardiotoxicity is usually irreversibly and permanent. Thus, these unique characteristics of the cardiac tissue could expose the heart to irreparable damage from stress sustained from intoxication, especially during chemotherapy (Soudani *et al.*, 2011).

Doxorubicin is a drug with anti-tumour activity (Carvalho *et al.*, 2014). It slows and stops the growth of cancer cells by blocking the enzyme-topoisomerase, which is involved in the cell division and growth of cancer cells. However, doxorubicin is known to induce cardiotoxicities, even though its benefits clinically are dose-dependent (Bashkir *et al.*, 2015). The cardiotoxic effect of doxorubicin occurs following either acute or chronic clinical use and usually manifests as dilated cardiomyopathy (Carvalho *et al.*, 2014). The effect occurs within 2 to 3 days, often resulting in the induction of apoptosis, necrosis, and oxidative stress giving rise to irreversible cardiotoxicity (Hardaway, 2019). Cr is reportedly present in industrial raw materials and effluents (Bagchi *et al.*, 2007; Bacon *et al.*, 2013; Garcia-nino *et al.*, 2015; Mitra *et al.*, 2017; Onyedikachi *et al.*, 2018; Onyedikachi *et al.*, 2019b; Nur-E-Alam *et al.*, 2020). Chromium has shown evidence of being able to induce skin, oral, developmental, reproductive and respiratory traumas (OEHHA, 2009; NIOSH, 2013; Garcia-nino *et al.*, 2015; Das *et al.*, 2015; Nur-E-Alamet *et al.*, 2020), but there is paucity of data on its cardiotoxic effects. This study was therefore designed to investigate the cardiotoxic effect of $K_2Cr_2O_7$ on the morphological and functional integrity of the cardiac system of male wistar rats.

Material and methods

Drugs and chemicals

Potassium dichromate (BDH, UK) was purchase from Imesco Chemicals and Reagent, Aba. Doxorubicin and sterile water (Pfizer Drugs, USA) was purchased from Grace and Mercy Pharmacy, Umuahia, Nigeria. Distilled water was obtained from the Biochemistry Laboratory, Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.

Animals and experimental design

One hundred and eighty adult male Wistar albino rats (120-140 g) obtained from the Animal House of the College of Veterinary Medicine, Michael Okpara University of Agriculture, were used for the study. The animals were thereafter conveyed to the animal house of the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike where the study was carried out. The animals were housed in aluminium cages, allowed access to feed and water *ad libitum* and acclimatized for 14 days but starved for 12 hours before commencement of experiments. Good

housing condition (12h light and 12h dark) was maintained for all animals all investigations involving the experimental animals were carried out in accordance with international guidelines for care and use of laboratory animals as reported by Orieket *et al.*, (2019).

Sixty of the rats were used for each of the 3 phases of the study.

Experimental design for the first phase was according to the order below:

Group 1: Control

Group 2: 10 mg/kg body weight of $K_2Cr_2O_7$ [Cr(VI)] compound

Group 3: 20 mg/kg body weight of $K_2Cr_2O_7$ [Cr(VI)] compound

Group 4: 30 mg/kg body weight of $K_2Cr_2O_7$ [Cr(VI)] compound

Group 5: 15 mg/kg body weight doxorubicin (2 days before sacrifice)

Group 6: 20 mg/kg body weight doxorubicin (2 days before sacrifice)

Treatment lasted 21 days before animals were sacrificed by cervical dislocation, and blood was collected by cardiac puncture into plain bottles and later centrifuged to obtain sera for the biochemical analysis. Liver samples were also collected, homogenized, and centrifuged to obtain supernatant on which biochemical analysis was carried out. In the second phase of the experiment, the animals were also treated as stated above but for a duration of 60 days (chronic study). All analysis, including biochemical and histopathology carried out in the first phase, were also repeated in the second and 3rd phases. However, in the 3rd phase, treatment was modified as shown below and also lasted for a period of 60 days.

Group 1: Control

Group 2: 0.01% of $K_2Cr_2O_7$ [Cr(VI)] compound supplemented in feed

Group 3: 0.02% of $K_2Cr_2O_7$ [Cr(VI)] compound supplemented in feed

Group 4: 0.03% of $K_2Cr_2O_7$ [Cr(VI)] compound supplemented in feed

Group 5: 15 mg/kg body weight doxorubicin (2 days before sacrifice)

Group 6: 20 mg/kg body weight doxorubicin (2 days before sacrifice)

Preparation of heart homogenate and collection of supernatant

The heart samples were removed and rinsed in normal saline, and 1 g was weighed out, sliced and ground with laboratory mortar and pestle in 5 ml of phosphate buffer solution. The resulting solution was centrifuged at 2200rpm for 25minutes in a cold centrifuge at 4°C. The supernatant was collected, labelled and preserved in the freezer until analysis. The serum samples were used formalondialdehyde (MDA), superoxide

dismutase (SOD), glutathione peroxidase (Gpx), catalase (CAT) and nitric oxide (NO) analysis. On the other hand, the heart homogenates were used to assess Creatine kinase-MB C-Reactive Protein, Lactate dehydrogenase (DH), troponin, part of the organ (heart) were excised was fixed in 10% formalin for a histopathology examination.

Blood Antioxidant Assay

Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as contained in the commercial Randox kit. The activity of SOD in the plasma was estimated based on the inhibitory effect of SOD on the reduction of nitroblue tetrazolium dye by superoxide anions generated by the photo-oxidation of hydroxylamine hydrochloride. The absorbance was measured with a visible spectrophotometer and used to calculate the enzyme activity. Glutathione peroxidase (GPx) assay was carried out according to the method of Paglia and Valentine (1967). It catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. The reaction of GSH and disulfide dinitrobenzoic acid produces a 5-thionide dinitrobenzoic acid anion, which exhibits a stable yellow colour. The activity of GSH-PX was calculated by measuring its absorbance at 412 nm. The activity of catalase was assayed by the method of Sinha (1972). Dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of per chromic acid as an unstable intermediate. The chromic acetate formed was measured at an absorbance at 590nm. The activities of these antioxidant enzymes are expressed in U/ml and were determined in strict accordance with the instructions of commercial kits.

Estimation of lipid peroxidation (Malondialdehyde)

Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallinet *al.* (1993). The mixture was boiled for 40 minutes in a water-bath and then cooled in cold water. Then 0.1ml of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at wavelength 532nm and 600nm against a blank.

Troponin test (Cardiac Specific)

Troponin test was carried out according to the method of Melanson *et al.* (2007). It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes microtiter wells coated with monoclonal anti-TnI antibodies. Two other monoclonal antibodies are in the antibody-horseradish peroxidase (HRP) conjugate solution. The test sample reacts with antibodies. After 15 minutes of incubation, the colour changes from blue to yellow. The concentration of troponin-I is directly proportional to the colour intensity of the test sample, which is

measured spectrophotometrically at 450nm. The optical density was read at 450 nm with a microtiter well reader

Biochemical Assay

Lactate dehydrogenase analysis was carried out according to method used by Jung *et al.* (2000). The activity of LDH in the serum was determined. LDH catalyzes L-lactate dehydrogenation to produce pyruvate and 2,4-dinitrophenzone. The reaction is brownish-red in colour in magnetic solution. A standard curve was prepared to calculate the results. C-reactive proteins were analyzed using the method of Anderson and McCarthy (1950). A turbid metric immunoassay for determining C-reactive protein in human serum is based on the principle of an agglutination reaction. It is measured at 630nm wavelength. The increase in turbidity corresponds to the concentration of CRP in the specimen. Assay for Creatinine Kinase was carried out according to method of Henry (1974). Aspartate transaminase (AST) assay was according to method of Reitman and Frankel (1975) using Randox commercial kits. Nitric oxide (NO) assay was carried out according to method used by Dessy and Ferron (2004).

Preparation of slides for histopathological evaluation

Tissue sections of the heart were collected for histopathological studies. The samples were fixed in 10% phosphate-buffered formalin for a minimum of 48 hours prior to tissue preparation. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the tissue-containing wax blocks were cut into 5µm thick sections with a rotary microtome, floated in a water bath and incubated at 60°C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80% and 70%). The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant, DPX.

Slide Examination The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x400 magnifications.

Statistical Analysis

The least significant difference (LSD) was used to compare differences in each sample within treatments. Data were reported as mean Standard error (S.E). One way analysis of variance (ANOVA) was also used to determine significant difference between groups, considering a level of significance of less than or equal

to ($P < 0.05$) by using SPSS. Significant difference was assessed using Duncan at ($P < 0.05$).

RESULT

The results of the absolute and relative heart weight of

rats treated orally and by supplemented diet with $K_2Cr_2O_7$ were presented respectively on Table 1.1 and Table 1.2 respectively. The absolute and relative heart weight of the experimental rats indicated significantly different from the control ($p < 0.05$).

Table 1.1: Effect of $K_2Cr_2O_7$ oral treatments on the body weight, absolute and relative organ weights of the hearts of rats(g)

	Absolute	Relative	Final	Initial
Control	0.57±0.03	0.33±0.06	168.73±1.48	126.4±1.75
10mg/kg Cr	0.58±0.05	0.37±0.02	156.80±2.66	138.25±1.84
20mg/kg Cr	0.55±0.10	0.35±0.02	155.75±3.56	137.75±3.58
30mg/kg Cr	0.59±0.12	0.38±0.01	153.23±3.97	135.75±0.85
15mg/kg dox	0.58±0.06	0.38±0.01	159.50±2.73	126.40±1.24
20mg/kg dox	0.57±0.07	0.35±0.01	163.5±2.15	124.00±1.68

Each value represents the mean ± SE; *Significantly different from the control; ($p < 0.05$).

Table 1.2: Effect of supplemented $K_2Cr_2O_7$ diet on the body weight, absolute and relative organ weights of the hearts of rats (g).

	Absolute	Relative	Final	Initial
Control	0.54±0.01	0.34±0.02	161.73±0.03	123.40±0.03
10mg/kg Cr	0.60±0.01	0.38±0.01	156.73±0.02	122.28±0.02
20mg/kg Cr	0.47±0.03	0.29±0.01	161.43±0.02	132.55±0.01
30mg/kg Cr	0.59±0.01	0.37±0.12	160.25±0.03	136.20±0.02
15mg/kg dox	0.47±0.02	0.30±0.13	156.43±0.02	125.70±0.02
20mg/kg dox	0.47±0.01	0.30±0.01	156.15±0.03	122.83±0.01

Each value represents the mean ± SE; *Significantly different from the control; ($p < 0.05$).

The results for the oral administration of distinct doses of $K_2Cr_2O_7$ for 21 and 60 days and also, by supplementation in diet presented on Tables 2, 3 and 4 respectively. To assess the cardiotoxic effect of hexavalent Chromium using Quantitative assay of the oxidative stress biomarkers (Malondialdehyde

(MDA), Superoxide dismutase (SOD), Catalase(CAT), Gluthathione peroxidase(GPx), Cardiac function biomarkers (Troponin(Trop), Creatine kinase(CK), Aspartate Transaminase(AST), Lactate Dehydrogenase(LDH) C-Reactive Protein(CRP) and Nitric Oxide(NO)).

Enzymic, Antioxidant, Myocardiac and Inflammatory Activities of male Wistar Albino Rat treated orally with K₂Cr₂O₇ and Doxorubicin for 21 days

	LDH (IU/L)	AST (IU/L)	CK (IU/L)	Gpx(mg/dl)	CAT (IU/L)	SOD (IU/L)	MDA (mg/ml)	TROP (mg/dl)	CRP (mg/dl)	NO(µg/ml)
CONTROL	3.94±0.22 ^a	69.6±2.32 ^a	30.99±2.96 ^a	2.47±0.12 ^a	4.31±0.16 ^a	1.12±0.01 ^a	0.30±0.08 ^a	2.57±0.10 ^a	0.73±0.10 ^{ab}	0.43±0.02 ^a
10Cr mg/Kg	8.03±0.30 ^b	85.28±1.87 ^{ab}	36.09±0.55 ^a	2.15±0.12 ^{ab}	2.98±0.29 ^b	0.85±0.28 ^a	0.15±0.02 ^{ab}	3.57±0.18 ^b	0.99±0.03 ^{bc}	0.46±0.01 ^b
20 Cr mg/kg	13.78±0.40 ^c	95.88±1.04 ^{ab}	45.12±1.43 ^b	1.93±0.07 ^{bc}	2.24±0.27 ^c	1.12±0.00 ^a	0.49±0.03 ^b	3.47±0.12 ^b	1.06±0.06 ^{bc}	0.42±0.01 ^{ac}
30 Cr mg/kg	16.83±0.59 ^d	114.35±3.38 ^c	58.16±0.79 ^c	2.63±0.14 ^d	2.49±0.12 ^{bc}	1.13±0.00 ^a	4.39±0.13 ^c	3.32±0.08 ^b	1.33±0.03 ^c	0.41±0.01 ^c
15DOX mg/kg	4.28±0.14 ^a	98.08±0.92 ^{ab}	31.21±1.30 ^a	1.62±0.20 ^c	3.52±1.12 ^b	1.12±0.01 ^a	0.31±0.08 ^b	1.97±0.06 ^c	0.56±0.09 ^a	0.43±0.01 ^{ac}
20DOX mg/kg	4.84±0.23 ^a	97.45±1.94 ^{ab}	48.38±2.02 ^b	2.56±0.08 ^d	6.31±0.15 ^a	1.12±0.01 ^a	0.59±0.17 ^{ab}	2.29±0.08 ^a	0.90±0.25 ^{ab}	0.41±0.01 ^a

Values are presented as Mean± Standard error of the mean. Different alphabets indicate a significance difference (P≤0.05). LDH means Lactate Dehydrogenase; CRP: C-Reactive Protein; TROP: Troponin; CK: Creatinine Kinase; MDA: Malondialdehyde; Gpx: Glutathione peroxidase; AST: Aspartate Transaminase; NO: Nitric Oxide; CAT (Catalase); SOD (Superoxide dismutase); Dox (Doxorubicin).

Table 3: Antioxidant, Myocardiac and Inflammatory Activities of male Wistar Albino Rat treated with K₂Cr₂O₇ and Doxorubicin (Dox), Orally and Intraperitoneally for 60 days. (Phase 2)

	LDH (IU/L)	AST (IU/L)	CK (IU/L)	Gpx (mg/dl)	CAT (IU/L)	SOD (IU/L)	MDA (mg/ml)	TROP (mg/dl)	CRP (mg/dl)	NO(µg/ml)
Control	107.66±0.34 ^a	62.71±0.81 ^a	130.77±0.63 ^a	3.69±0.10 ^a	3.31±0.14 ^a	1.20±0.13 ^a	1.36±0.04 ^a	2.29±0.18 ^a	1.00±0.00 ^a	0.82±0.03 ^a
10mg/kg Cr	144.66±2.21 ^b	82.00±1.12 ^b	133.97±0.74 ^b	3.59±0.12 ^a	2.29±0.07 ^b	0.96±0.05 ^b	1.99±0.11 ^b	3.75±0.16 ^b	2.13±0.13 ^b	1.08±0.07 ^{bc}
20mg/kg Cr	158.44±0.76 ^b	87.00±1.36 ^b	144.86±0.49 ^a	2.88±0.09 ^b	2.67±0.17 ^c	0.93±0.03 ^b	3.42±0.14 ^c	3.71±0.18 ^b	3.29±0.18 ^c	1.16±0.02 ^d
30mg/kg Cr	183.29±1.70 ^c	121.38±2.16 ^c	154.88±1.20 ^{ab}	2.56±0.12 ^b	1.80±0.14 ^d	0.73±0.03 ^c	6.37±0.13 ^d	3.13±0.13 ^c	4.00±0.00 ^d	1.45±0.06 ^d
15mg/kg Dox	113.08±2.33 ^d	53.13±0.69 ^d	133.76±1.22 ^{ab}	3.49±0.13 ^a	3.25±0.12 ^a	1.07±0.02 ^d	1.39±0.09 ^a	1.75±0.16 ^d	1.38±0.18 ^c	0.98±0.05 ^a
20mg/kg Dox	116.72±0.88 ^d	65.57±1.59 ^a	136.35±1.05 ^{ab}	3.38±0.15 ^a	3.00±0.14 ^a	1.03±0.02 ^d	1.74±0.07 ^b	2.43±0.20 ^d	2.14±0.14 ^b	0.76±0.08 ^c

Values are represented as Mean± Standard error of the mean. Different alphabets indicate a significance difference (P≤0.05). LDH means Lactate Dehydrogenase; CRP: C-Reactive Protein; TROP: Troponin; CK: Creatinine Kinase; MDA: Malondialdehyde; Gpx: Glutathione peroxidase; AST: Aspartate Transaminase; NO: Nitric Oxide; CAT (Catalase); SOD (Superoxide dismutase); Dox (Doxorubicin).

Table 4: Enzymic, Antioxidant, Myocardiac and Inflammatory Activities of male Wistar Albino Rats treated with K₂Cr₂O₇ and Doxorubicin, supplemented with diet and Intraperitoneally treatments respectively for 60 days (Phase 3)

	LDH (IU/L)	AST (IU/L)	CK (IU/L)	Gpx (mg/dl)	CAT (IU/L)	SOD (IU/L)	MDA(mg/ml)	TROP (mg/dl)	CRP (mg/dl)	NO (µg/ml)
CONTROL	60.74±0.37 ^a	83.42±9.09 ^a	8.80±0.15 ^a	3.72±0.15 ^a	147.00±1.52 ^a	1.72±0.13 ^a	1.24±0.03 ^a	0.02±0.01 ^a	31.17±0.60 ^a	4.20±0.06 ^a
10Cr mg/kg	57.01±0.64 ^b	111.69±11.48 ^b	7.78±0.29 ^b	4.38±0.09 ^b	137.33±1.76 ^b	1.67±0.09 ^a	1.47±0.02 ^b	0.05±0.00 ^b	32.00±0.32 ^b	4.97±0.09 ^b
20Cr mg/kg	52.9±1.11 ^c	83.77±7.86 ^a	6.73±0.14 ^c	5.14±0.04 ^d	148.33±1.20 ^a	1.52±0.04 ^{ab}	1.33±0.02 ^a	0.05±0.01 ^b	30.13±0.58 ^a	5.97±0.14 ^c
30Cr mg/kg	64.93±0.93 ^d	104.44±8.65 ^c	6.20±0.15 ^c	4.91±0.01 ^d	135.67±1.20 ^b	1.33±0.09 ^{bc}	1.26±0.02 ^a	0.02±0.01 ^a	26.87±0.55 ^d	6.60±0.31 ^c
15DOX mg/kg	63.17±0.44 ^d	96.90±3.68 ^d	7.87±0.19 ^b	4.63±0.06 ^c	170.67±3.84 ^c	1.19±0.01 ^c	2.07±0.04 ^c	0.06±0.00 ^b	31.97±0.70 ^b	5.70±0.38 ^d
20DOX mg/kg	63.70±0.75 ^d	97.30±3.33 ^d	8.60±0.55 ^{ab}	4.96±0.03 ^d	190.67±4.98 ^d	1.16±0.02 ^c	2.3±0.06 ^d	0.10±0.01 ^c	33.40±0.66 ^c	6.43±0.49 ^c

Values are represented as Mean ± Standard error of the mean. Different alphabets indicate a significant difference (P ≤ 0.05). LDH means Lactate Dehydrogenase; CRP: C-Reactive Protein; TROP: Troponin; CK: Creatinine Kinase; MDA: Malondialdehyde; Gpx: Glutathione peroxidase; AST: Aspartate Transaminase; NO: Nitric Oxide; CAT (Catalase); SOD (Superoxide dismutase); Dox (Doxorubicin).

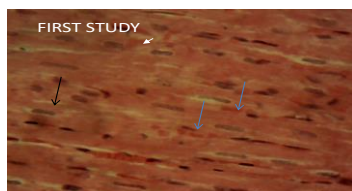


PLATE 1: GRP 1 HISTOPATHOLOGY HEART

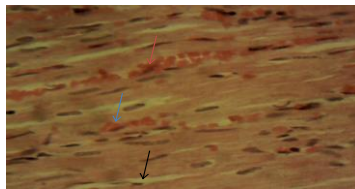


PLATE 2: GRP 2 HISTOPATHOLOGY HEART

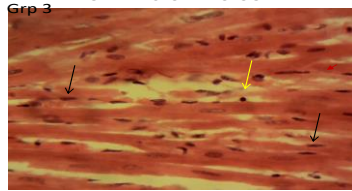


PLATE 3: GRP 3 HISTOPATHOLOGY HEART

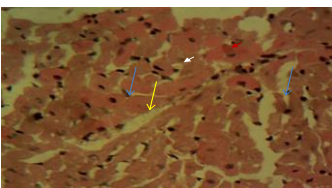


PLATE 4: GRP 4 HISTOPATHOLOGY HEART

Ex400. Myocyte nuclei (black/yellow arrow); Capillary (red/blue arrow); Pericytes (white arrow) H and

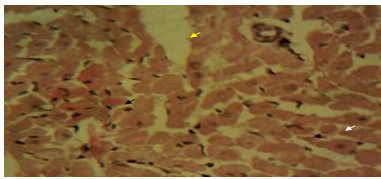


PLATE 5: GRP 5 HISTOPATHOLOGY HEART
Second Study Grp 1

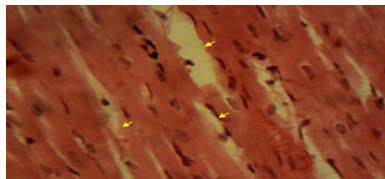


PLATE 6: GRP 6 HISTOPATHOLOGY HEART

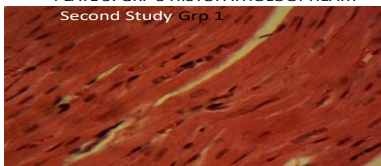


PLATE 7: GRP 1 HISTOPATHOLOGY HEART

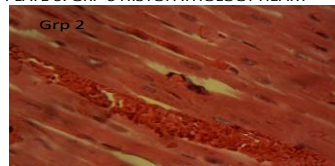


PLATE 8: GRP 2 HISTOPATHOLOGY HEART ³⁶

H and Ex400.
Myocyte nuclei (black/yellow arrow); Cappilary (red/blue arrow); Pericytes (white arrow)

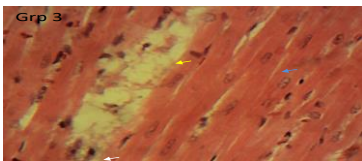


PLATE 9: GRP 3 HISTOPATHOLOGY HEART

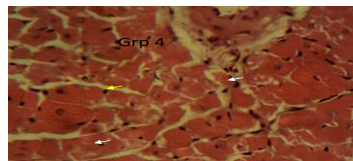


PLATE 10: GRP 4 HISTOPATHOLOGY HEART

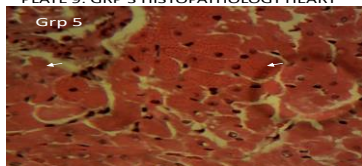


PLATE 11: GRP 5 HISTOPATHOLOGY HEART

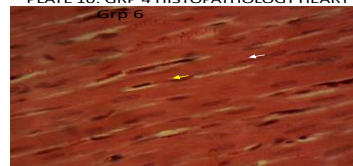


PLATE 12: GRP 6 HISTOPATHOLOGY HEART ³⁷

H and Ex400.
Myocyte nuclei (black/yellow arrow); Cappilary (red/blue arrow); Pericytes (white arrow)

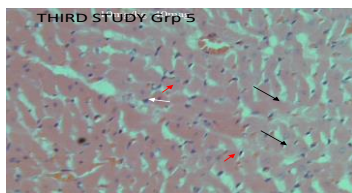


PLATE 13: GRP 2 HISTOPATHOLOGY HEART

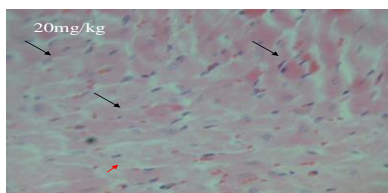


PLATE 14: GRP 3 HISTOPATHOLOGY HEART

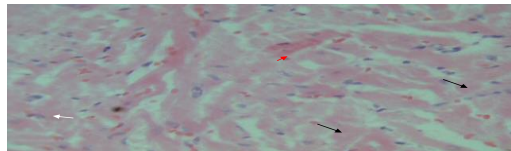


PLATE 15: GRP 4 HISTOPATHOLOGY HEART ³⁸

H and Ex400. Myocyte nuclei (black/yellow arrow); Cappilary (red/blue arrow); Pericytes (white arrow)

4.0 DISCUSSION

There was a significant difference in the absolute and relative heart weight of rats fed $K_2Cr_2O_7$ (Table 1), the absolute and relative heart weight of all the experimental rats were statistically unchanged. The results for the oral administration of different doses of $K_2Cr_2O_7$ and also supplementation in diet are presented in Tables 2, 3 and 4, respectively. Risks associated with Cr(VI) have been reported and are attributable to industrial pollution (Langard and Vigander, 1983; Davies, 1984; ATSDR, 2000; Lee *et al.*, 2001; Soudaniet *al.*, 2011; Paris *et al.*, 2016; Onyedikachiet *al.*, 2019b). Although there was no mortality recorded during the study, Poisonous effects of Cr(VI) have been confirmed following prevalent lipid peroxidation levels in the heart of Wistar albino rats treated with $K_2Cr_2O_7$ (Fatima *et al.*, 2005; Pedraza-Chaverri *et al.*, 2008). Organ and body weight changes may be ways of measuring the toxicity of exogenous compounds (Soudani *et al.*, 2011). Treatment with $K_2Cr_2O_7$ decreased bodyweights and reduced feed intake in the treated rats, which may be due to the palatability of feed or other associated effects compared to the control groups (Soudani *et al.*, 2012).

The dysfunctions on the cardiovascular system have also been reported concerning individuals who have been exposed to Cr(VI) compound via the different ingestion pathways (Jenkins *et al.*, 1994; De Lassece *et al.*, 1999; ATSDR, 2000; Lee *et al.*, 2001; Soudaniet *al.*, 2011). The results in this study indicated that Cr(VI) induced toxicity on the cardiovascular system of male Wistar albino rats through the two methods of exposure used, i.e. oral administration of different doses of $K_2Cr_2O_7$ and by supplementation in diet presented in Tables 2, 3 and 4. The prevalence of malondialdehyde in Cr(VI) treated rats is attributable to the peroxidation of lipids and increase in ROS production, which occurred as a result of oxidative stress. Oxidative stress-induced through the treatment degrades polyunsaturated lipids or fatty acids in the perturbed biological system of the rats resulting in increased levels of MDA (mg/ml), which is a biomarker and product of oxidative stress (Dourerdjou and Koner, 2008; Ayala *et al.*, 2014). Oxidative stress plays a significant role in the deleterious effect of many heavy metals and many other xenobiotics (Anane and Creppy, 2001). Doxorubicin also has been proven to generate cardio-dysfunctions through the induction of free radicals such as oxyanions, hydroxyl radicals and hydrogen peroxide (Teraoka *et al.*, 2000; Bhardwaet *al.*, 2011; Hardik *et al.*, 2013). Several studies have shown that increase in MDA is one of the striking effects of cardiac muscles dysfunction as observed in the cardio dysfunction induced by doxorubicin (Richard *et al.*, 2011; Colaket *al.*, 2012; Jiang *et al.*, 2016).

In all the treatments, there were marked elevation in MDA (mg/ml) levels following both Cr and Dox

administration (Fatima *et al.*, 2005; Pedraza-Chaverri *et al.*, 2008), resulting in antioxidant scavenging effects which predispose the biological system to a high risk of diseases/ ailments. This histology result showed sections of the heart collected from the animals in this study with distinct structures of the cardiac muscle cells/myocytes. The control had a normal heart structure while the Cr(VI) treated groups showed mild tissue oedema (yellow arrow), manifesting as displacement of adjacent myocytes by clear spaces. The sections of the heart collected from the animals in these groups showed mild oedema and multifocal areas of hyaline necrosis (white arrow), involving mainly the myocardium and occasionally the endocardium. The affected myocytes show loss of striation, fragmentation of the myocytes, intense eosinophilic staining of the sarcoplasm (myocytes cytoplasm) with nuclear pyknosis and karyorrhexis. Relatively normal myocytes with normal nucleus (black arrow).

Antioxidants, on the other hand, are symbolic as the defence system that mops out the toxicity result from free radicals. The cardio-Enzymatic antioxidants such as reduced glutathione and other enzymes like SOD, GPx and Catalase are the primary antioxidants (McDonough, 1999; Hayyanet *al.*, 2016). However, the cardiac tissues have lower levels of these enzymes compared to other tissues e. g. liver (Husain and Somani, 1997; Meister and Anderson, 1983). In this study, both the Cr groups in the various treatment concentrations and doxorubicin indicated that these antioxidants were significantly impaired. This is because the antioxidant enzymes SOD (mg/ml), GPx (mg/dl) and CAT (IU/L) are limited, and thus, the effects of oxidant molecules in tissues like the heart are affected, resulting in oxidative cell injury due to the overwhelming activities of free radical scavengers (Gutteridge, 1995; Soudani, 2011). These enzymes work together to mop out active oxygen species, but minor deviations in concentrations lower than their physiological levels might expose cellular lipids, proteins and DNA to a porous defence system (Bagchiet *al.*, 2002; Abarikwuet *al.*, 2016). Hence, the observed decrease in antioxidant enzyme activities as seen in this study could be attributed to the compromised defence mechanism by both Cr(VI) and doxorubicin toxicity in the heart. El-Demerdash (2004) demonstrated that there was a decrease in the antioxidant power of rats exposed to heavy metals due to Cr (VI) and doxorubicin treatments. The results of GPx, Cat and SOD (mg/ml resp.) as seen in table 2, 3 and 4 indicated that the defence system was compromised, and this may be attributed to the overwhelming oxidant production arising from $K_2Cr_2O_6$ and doxorubicin treatments resulting in cardio-damages as seen in the histopathological results as shown on the plates (Krishnamurthy *et al.*, 2015; Abarikwuet *al.*, 2016; Ankita *et al.*, 2019). The histopathology results presented in Plates 1-15

indicated that there were similarities in the cardiac morphology of both group 1(control)and group 2(10mg/kg) rats as the sections of the heart collected from the animals in these groups revealed the normal cardiac histo-architecture for laboratory rodents owing to the ability of the antioxidant to withstand the impact of the treatments. This may be because the biological defence system tried to expunge the ravaging effect of the oxidants. The myocardium was composed of elongated cardiac muscle cells arranged in overlapping spiral patterns, surrounded by a rich network of blood vessels and capillaries embedded in the connective tissue matrix. Also, the histopathology results for hearts samples collected from groups 3 and 4 (20mg/kg and 30mg/kg Cr) showed the typical structures of the cardiac muscle cells/ myocytes. However, mild tissue oedema (yellow arrow); manifesting as displacement of adjacent myocytes by clear spaces, were observed. This observation indicates that as Cr VI concentrations increased in the treatments, the biological defence system was disturbed, resulting in an imbalance in the antioxidant system.

The results for AST(IU/L), troponin(mg/dl) and CK-MB(IU/L), C-reactive protein(mg/dl) showed a marked increase as seen in Table 2, 3 and 4. AST activities, as seen in this study, indicates that cardiotoxicity was induced owing to the production of free radicals, which in turn caused an elevation in AST in serum, indicating myocardial injury (Gaze, 2007; Dringenet *al.*, 2000). Photomicrographs of the heart collected from the animals in groups (2, 3, 4, 5 and 6) showed oedema and multifocal areas of myocytolysis (red arrow) and hyaline necrosis (white arrow). The lesion tends to involve mainly the myocardium and occasionally the endocardium. The affected myocytes show loss of striation, fragmentation of the myocytes, intense eosinophilic staining of the sarcoplasm (myocytes cytoplasm) with nuclear pyknosis and karyorrhexis, indicating that the cardiotoxic effect of both the Cr and Dox groups induced myocardial injury.

Troponin is useful as a biomarker used in the diagnosis of different heart anomaly especially in the indication of myocardial infarction (Roberton *et al.*, 2010). Toxic substances such as alcohol, cocaine, methamphetamine or chemotherapy may lead to cTn release by various mechanisms; also, multiple processes, including both ischemic and non-ischemic mechanism, may contribute to such release. Elevated troponins have been implicated in predicting diastolic dysfunction (Kilickapet *al.*, 2009).

CRP is recognized as an acute-phase protein originating from the liver, thus increasing the release of interleukin-6 by T-cells and macrophages. It is a very sensitive marker of systemic inflammation and tissue damage (Pepys *et al.*, 2003). The significant

increase in the CRP concentration may be attributed to mild tissue injury (Lauet *al.*, 2005).

The CK-MB isozyme is distributed basically in the cardiac myocyte, and its increase is an indicator of myocardial dysfunction (Wagner *et al.*, 2003). Likewise, the impairment of cardiac functions could be a result of impairment of myocardial architecture. Evidence of cardiomyocyte death(cardio-necrosis) was obtained in our study as shown in the increased CK(IU/L) and also, histopathology results which revealed foci of myocyte loss, interstitial fibrosis, perivascular fibrosis and nuclear condensation.

The marked increase in LDH(IU/L) activities as seen on tables(2, 3 and 4) is suggestive that there was tissue breakdown due to the treatments with K₂Cr₂O₆ and Dox. LDH levels may be used as a tumor marker. Although, some other non-cancer conditions that can raise LDH levels include hypothyroidism, anaemia, and meningitis(Jung *et al.*, 2000).The results also showed an increase in cardiac troponin, which could be a result of thechanges in the cardiac morphology as observed in the histopathology photomicrographs. Nitric oxide is produced virtually in all the cells in the cardiac system. It also regulates cardiac functions via vascular-dependent and independent event. It is a gaseous signalling molecule (radical gas). It functions in the regulation of coronary vessel tone, thrombosis, proliferation, inflammation, as well as potent support incellular angiogenesis. The decrease in NO suggests that there was vasoconstriction in the cardiac system,which led to a decrease in blood circulation, thus resulting in the pathogenesis of inflammation. Although,under normal physiological conditions, NO depicts an anti-inflammatory effect (Sharmahet *al.*, 2007) and may result in an increase in blood pressure which puts the heart at serious risk as the fine-tuning and optimization of cardiac pump functions is impeded (Dessy and Ferron, 2004).However, an increase in NO values was observed as doses of Cr(VI) compound increased(table 2.3), and this is suggestive that toxicity varies based on the mode of administration. Generally, the effect was more significant in the oral treatment than in the supplemented diet.The histo- architecture results also show that both treatment modes resulted in multifocal areas of oedema, myocardial degeneration and necrosis.

Conclusion

We therefore conclude that Cr(VI) compound has the potential to reduce significantly the body's antioxidant defense line due to fall in the levels of antioxidant enzymes such as GSH, SOD and CAT and increase in levels of troponin, CK, CRP, AST, LDH, MDA and NO. Evidence of pulmonary oedema and hyaline necrosis in the cardiac region was also observed following histopathologic examination heart tissues. Findings from this work therefore indicate that there is

an urgent need for the government, regulatory bodies, standard organization, policymakers and other concerned stakeholders to make recommendations and policies that would help mitigate the health risk associated with the exposure of industrial workers and the general public to industrial chemicals and effluents.

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ATTITUDE AND PSYCHOLOGICAL SUPPORT OF HEALTH CARE PERSONNEL AS PREDICTORS OF MENTAL WELL-BEING AMONG PATIENTS WITH TERMINAL ILLNESS IN JALINGO TOWN TARABA STATE NIGERIA

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ABSTRACT

This study examined attitude and psychological support as predictors of mental wellbeing among patients with terminal illness in Jalingo town. The ex post facto research design was employed for the study. The instruments used were attitude scale, the psychological support scale and mental wellbeing scale. A total of 270 respondents took part in the study. Simple and Multiple Regression Analysis were used for data analyses. Findings revealed that attitude predicted mental wellbeing. Findings also indicated that psychological support predicted mental wellbeing. Results further showed that attitude and psychological support jointly predicted mental wellbeing. Based on these findings, it was recommended among other things that health care personnel should give more time and energy to help terminally ill patients cope with the illness and treatment.

KeyWords: Attitude, Psychological Support, Mental Well-being, Terminal Illness

Introduction

Providing care by the health care personnel through preventing and relieving suffering can have a positive influence on the mental wellbeing of patients until death (World Health Organization, 2012). To live with an incurable disease can limit one person's life and lead to a feeling of being trapped and unable to liberate from the condition. If the person is able to cope with the condition; the feeling of freedom can be present despite the disease. With professional and well functioned caring, it is possible for the patient to increase the feeling of mental wellbeing even though the limitations of the incurable disease still exist (Dahlberg & Segesten, 2011).

Mental wellbeing is a feeling that is subjective and can be described as a condition that appears when one person is relieved from pain and suffering (Torunn Bjork & Breievne, 2006). Care for terminally ill patients can be stressful, but previous study shows that, psychologist, doctors and registered nurses (RN) and other health care personnel were strongly committed to care for the patients in best possible way at the end of life (Johansson & Lindahl, 2009). It is important to be aware of how the work situation can affect the psychologist, doctors, nurses and other health care personnel which also can lead to lack of quality in caring for the patients and aware of this fact could lead to better care for the patients (Dahlberg & Segesten, 2010).

Providing palliative care means to prevent and relieve suffering by increasing mental wellbeing. It also includes caring for the patient's psychological and physiological needs (World Health Organization, 2012). A previous study shows that patient's mental wellbeing is important in palliative care; is an essential part of health care personnel all along the line but

especially when caring for terminally ill patients because of their need of expressing their thoughts and feelings (Hench, Danielsson, Strang, Browall & Melin-Johansson, 2013).

There is a link between well-being and attitude (Tedeschi & Calhoun, 2006). The characteristic reaction of an individual under different situations that is enduring and consistent is called attitude (Costa & McCrae, 2008) individual behavior reflects the person's attitude. Evidence has pointed to the robustness of attitude in the explanation of mental wellbeing (Costa, & McCrae, 2008; David & Suls, 2012). This has been admitted and applied in psychology, sociology and management. Health care personnel positive attitude are more likely to be warm, pleasant, kind, and cooperative; they may experience positive changes in their interpersonal relationships with friends and family during the adverse effects of terminal illness and also tend to be dependable, responsible, efficient, productive, organized, planful, able to delay gratification, reliable, responsible, and ethical (McCrae & John, 2012). As a result, patients will tend to cope better with terminal illnesses.

O'Brien and Moorey (2010) Speculated that positive attitude is associated with better emotional adjustment to terminal illness. O'Brien and Moorey used the term "positive attitude" to describe people who have optimism about survival, belief in their ability to affect the disease, and determination to adjust to their illness. An optimistic attitude has been attributed to improved well-being in people with life-threatening illness. In a correlational study to determine the relationship between treatment-specific optimism (the belief that a specific treatment will cure one's illness) and depressive symptoms with advanced cancer patients, Cohen, de Moor, and Amato (2001) found that

optimism and mental wellbeing was associated with fewer depressive symptoms. The authors extrapolated that when people have optimism and mental health about the effectiveness of their treatment, they may adjust better to their illness, even if they are aware that the treatment is not likely to cure them, than those who are pessimistic.

Similarly, when Thomsen, Rydahl-Hansen, and Wagner (2010) conducted a review of attitudes factors relevant to coping with terminal illness, they found that creating meaning and positive reframing attitudes of negative aspects of illness were important for mental well-being. They also reported that some studies had described participants as being able to be lifted out of the suffering by experiencing joy and pleasure from positive attitudes.

The studies of mental health personnel attitude reviewed provided possible ways that people with terminal illness are able to experience mental well-being. Mental well-being in one or more dimension seems to enhance over-all well-being or at least diminish distress in another dimension. Furthermore, the attitude people take to their life and the way they see the world seems to impact their ability to experience mental well-being in terminal illness.

Psychological support is the comfort given to patients by the psychologist, doctors, nurses and other health care personnel including our families, friends, coworkers and others (Onyishi, Okongwu & Ugwu, 2012). These psychological supports could help patients cope with varying life challenges.

Psychological support is emotionally beneficial to the mental well-being of the terminally ill patients (Cohen & Wills, 2005). While the relationship between psychological support and well-being is likely reciprocal, in that patients who are healthy and happy may elicit positive social relations, there is a growing belief on the concept that psychological support leads to increased mental well-being and general health (Seeman, Berkman, Charpentier, Blazer, Albert & Tinetti, 2012; Walen & Lachman, 2011). A wealth of research has focused on the positive outcomes of Physicians, Nurses, psychologist as well as other practitioners need to create an environment in which the patient feels comfortable and safe to relate and communicate. This therapeutic relationship plays a vital role to patients and their families and they do rely on health staff for the emotional journey. Oh and Kim (2010) have shown that psychological issues can influence terminal illness recovery with patients that experience psychological distress such as anxiety and depression often experiencing increased physical side effects and more difficulty managing their self-care and experiencing an overall reduced mental health. Vodermaier et al (2009) state that relatively brief but validated questionnaires would seem to be the tools of choice for routine screening of terminally ill patients

emotional distress. An advantage to systematic screening of cancer patients for emotional distress is that it is likely to promote equal access to psychological services, where as a system based only on physician or patient initiated referrals might fail to identify and/or overlook a substantial proportion of emotionally distressed patients who are in need of supportive treatment. Cancer in particular is known to be a highly stressful experience associated with emotional difficulties (Lin & Bauer- Wu, 2003).

Psychological support is the active, holistic care of patients with advance progressive illness and their families. Care is focused on the total patient encompassing body, mind and spirit. Throughout the continuum of the disease, psychological support recognizes and addresses the needs of the patient and necessitates access to information, patient autonomy, and choice. Some of this needs maybe emotional, psychological, cultural, intellectual, spiritual, social and or physical. This care optimizes mental health by anticipating, preventing, alleviating suffering (WHO, 2002).

Psychological support care involves bereavement and grief support both for the family and other caregivers during the life of the patient and continuing after the death of the patient. Psychological support aims at maximizing quality of life for the dying patient psychological support is more than a physical experience, the effect of cultural and spiritual, emotional and mental wellbeing on the dying process asks for a style of care that doesn't look at death from a medical point of view rather it should be viewed as a process. Most psychological support patients desire an end of life experience that is peaceful and dignified, where they can exercise their own autonomy and remain in control to the greatest extent possible (Breier-Mackie, 2001).

Psychological support has strong influences on many aspects of well-being, including physical well-being, psychological well-being, life satisfaction and resilience. Psychological supports are known to increase mental well-being and decrease levels of stress (Taylor, Sherman, Kim, Jarcho, Takagi & Dunagan, 2004).

Attitude and psychological support are emotionally beneficial to the mental well-being of terminally ill individual (Cohen & Wills, 2005). While the relationship between health care personnel attitudes, psychological support and mental well-being is likely reciprocal, in that people who are healthy and happy may elicit positive social relations, there is a growing belief on the concept that positive attitudes and psychological support leads to increased mental well-being and health (Seeman, Berkman, Charpentier, Blazer, Albert & Tinetti, 2012; Walen & Lachman, 2000).

In spite of the potential for distress and suffering caused by the challenges of terminal illness, some

patients describe themselves as happy and satisfied with their lives (Sahlberg-Blom, Ternstedt, & Johansson, 2001); they are able to maintain mental well-being (McMillan & Weitzner, 2000). Blinderman and Cherny (2005) interviewed people with terminal illness and found that while they had considerable existential concerns, many were not in distress. The researchers attributed this finding to the early timing of psychological support palliative care, attitudes, effective coping strategies and religious beliefs.

Terminal illness, on the average, has a negative impact on individuals' mental well-being, physical well-being, and relationship well-being and life satisfaction. A positive relationship between terminally ill rates and indices such as mortality, heart disease, mental health, heavy drinking, life satisfaction and the use of mental health services have been portrayed (McKee-Ryan, Song, Wanberg & Kinicki, 2006). However, these relationships have been criticized on a number of grounds, including its inability to allow generalization to an individual level.

Therefore, this research is interested in the attitude and psychological support of health care personnel in predicting mental wellbeing among the terminally ill in Jalingo town. This is because one variable alone may not provide the explanation we need regarding mental well-being among the terminally ill hence the combination of the two independent variables. Therefore, it is more commendable to examine the predictive power of attitudes and psychological support on mental well-being of patients with terminal illness. This is because evidences abound that over the years these two factors have been found to correlate positively with mental well-being:

Objectives:

- (i) To assess the influence of health care personnel attitudes on mental well-being of patients with terminal illness in Jalingo town Taraba state Nigeria.
- (ii) To determine whether health care personnel's psychological support will predict mental well-being among patients with terminal illness in Jalingo town Taraba state Nigeria.
- (iii) To ascertain the joint influence of attitudes and psychological support of health care personnel on mental health of terminally ill patients in Jalingo town Taraba state Nigeria.

H1 Attitude of health care personnel will significantly predict mental well-being among patients with terminal illness in Jalingo Taraba state Nigeria.

H2 Psychological support will significantly predict mental well-being of patients with terminal illness in Jalingo town Taraba state Nigeria.

H3 Attitude and Psychological support of health care personnel as predictors of mental well-being among patients with terminal illness in Jalingo town Taraba state Nigeria.

Method

Research Design

This study employs the ex-post facto research design. The method is expected to show relationships amongst the variables in the study. The predictor variables are attitude and psychological support; the criterion variable is mental well-being which is measured as a single or composite construct.

Study Population

The population of terminally ill patients in Jalingo town for the period of this study was 1,203 (Taraba State Ministry of Health, 2016). A breakdown of the population showed that male patients with terminal illness were 756 while females were 447.

Sampling Technique

The researcher used multistage sampling technique for data collection. Three qualitative sampling techniques of purposive, convenience and criterion sampling were used to sample patients with terminal illness for inclusion into the study. Purposive or judgmental sampling involves purposely selecting patients judged to be typical of the population or particularly knowledgeable about the subject. Convenience sampling involves selecting the most readily available patients as participants. Selection of terminally ill patients started with those who were present and readily available to participate in the study. Criterion sampling involved consciously selecting the personnel from the target population who met the selection criteria of:

- i. Patients with terminal illness residing in Jalingo Local Government Area of Taraba state.
- ii. Those who are currently on palliative care and undergoing one form of treatment.
- iii. Consenting and willing to participate in the study.
- iv. Expressing readiness to share their lived experiences via responding to questionnaires.
- v. Demonstrate the ability to competently respond to the questionnaire.

Sample Size Determination

The population of terminally ill patients in Jalingo town for the period of this study was 1,203 (Taraba State Ministry of Health, 2016). Taro Yamane (1967) Formula was used to determine sample size for this study.

Formula

$$n = \frac{N}{1 + N(e)^2}$$

Where:

n = Estimated sample size.

N = Total number of the population

1 = Constant value

e = Estimated level of confidence interval

$$n = \frac{1203}{1 + 1203(0.05)^2}$$

$$n = \frac{1203}{1 + 1203 \times 0.0025}$$

$$n = \frac{1203}{1 + 3.0075}$$

$$n = \frac{1203}{4.0075}$$

n = 300

Participants

The total numbers of participants for the study were three hundred selected using convenience sampling selecting the most readily available patients as participants. Selection of terminally ill patients started with those who were present and readily available to participate in the study. Out of this 165 were male and 135 were female. The age range of the participants is between 15 and 76 years.

Instruments

In order to collect data for the study, the researcher made use of three validated instruments with subscales. Which include; Attitudes scale, Psychological Support and Mental Well-being. A modified version of the Risser (1975) Attitude Scale by Hinshaw and Atwood (1982) was used to elicit the research data designed to evaluate patients’ attitudes towards health workers and originally contained three subscales of Technical-Professional (TP) domain, Educational Relationship (ER) domain and Trusting Relationship (TR) domain. Jefferson Scale of Psychological Support is a 5-item instrument developed by Gregory et al (2006) item intended to measure patient’s perceptions of his/her physician’s empathic concern and understanding. The 12-Item Mental Well-being Scale (GHQ-12) developed by Goldberg and Williams (1988) consists of 12 items, each one assessing the mental status over the past few weeks. (See appendix I).

Reliability

Pilot study was carried out using 30 samples on patients with terminal illness in Ardo-Kola Local Government of Taraba State. A reliability coefficient of .71, .60, and .62 was obtained for attitude scale, psychological scale and mental wellbeing respectively and this confirmed a good reliability of the instrument. (See appendix II)

Procedure

Letter of introduction from department of psychology Benue State University was submitted to the management of the health institutions and approval was received with the ethical permission from the ethical committees of the health institutions. The researcher made contact with the terminally ill during their follow up to the health centre. The researcher sought the assistance of the health centre and solicits their cooperation towards ensuring that the questionnaires were distributed. This was done with the assistance of the management. Terminally ill who came to enroll and follow up in the centre were given questionnaire to respond and return it while returning their forms of enrollment and follow up. The

instruments were given to the participants with the help of the guidance/interpreter for those that cannot read or write. The purpose was explained to them and was assured of confidentiality that their response will not be used against them; they were informed that no answer is right or wrong. Therefore, they should feel free to express their opinion.

The questionnaires contained information regarding the aim of the study, respondents consent and an introduction letter soliciting the organization/ respondents’ cooperation. two hundred and seventy questionnaires were used for the final analysis. This is because some respondents did not return their questionnaires while others were discarded due to incomplete filling of the questionnaires. Finally, all measures in the present study were collected on a single questionnaire within three months.

Data Analysis

Responses to questionnaires were coded and entered into the Statistical Package for Social Sciences (SPSS) version 20.0. SPSS was used in the statistical analyses. The analysis included reliability assessment of the scale (Cronbach alpha) for pilot study. Simple and Multiple Regression Analysis was used to determine the individual and joint influence of independent variables on dependent variable.

RESULTS

Hypotheses I

This hypothesis states that attitude of health care personnel will significantly predict mental wellbeing among patients with terminal illness in Jalingo Town.

Table 1: Simple Regression Analysis showing the influence of attitude of healthcare personnel on mental wellbeing among patients with terminal illness

Variables	R	R ²	F	β	t	p
Constant	.436	.190	49.179		8.440	.000
Attitude				.436	7.013	.000

Dependent Variable: Mental Wellbeing

The results presented in table 1 above showed that attitude of healthcare personnel significantly predict mental wellbeing among terminally ill patients ($R = .436 = R^2 = .190(F(1, 208) = 49.179, t = 8.4480, p < .05)$). This suggest that attitude of healthcare personnel contributed 19.0% to variation in mental wellbeing. This finding implies that higher level of positive attitude of healthcare personnel is likely to bring about higher degree of mental wellbeing among terminally ill patients. Therefore, this hypothesis has been accepted and the null hypothesis rejected.

Hypotheses II

This hypothesis states that psychological support will significantly predict mental wellbeing among patients with terminal illness in Jalingo town.

Table 2: Simple Regression Analysis showing the influence of psychological support on mental wellbeing among terminally ill patients

Variables	R	R ²	F	β	t	P
Constant	.247	.061	13.550		10.217	.000
Psy. Support				.247	3.681	.000

Dependent Variable: Mental Wellbeing

The results presented in table 2 above indicated that psychological support significantly predicted mental wellbeing among terminally ill patients ($R = .247 = R^2 = .061$ ($F(1, 208) = 13.550, t = 10.217, p < .05$). This means that psychological support contributed 6.1% to variation in mental wellbeing. This finding implies that higher level of psychological support is likely to leads to higher level of mental wellbeing

among terminally ill patients. Therefore, this hypothesis has been accepted and the null hypothesis rejected.

Hypotheses III

This hypothesis states that attitude of healthcare personnel and psychological support will jointly influence mental wellbeing among terminally ill patients in Jalingo town.

Table 3: Multiple Regression Analysis showing the joint influence of attitude and psychological support on mental wellbeing among terminally ill patients

Variables	R	R ²	F	β	t	p
Constant	.437	.191	24.4		7.680	.000
			80	.442	5.771	.000
Attitude				-.009	-.117	.907
Psy. Support						

Dependent Variable: Mental Wellbeing

The results presented in table 3 above revealed that there was a significant joint influence of healthcare personnel attitude and psychological support on mental wellbeing among terminally ill patients ($R = .437 = R^2 = .191$ ($F(2, 207) = 24.480, p < .05$). This means that healthcare personnel attitude and psychological support jointly contributed 19.1% to variation in mental wellbeing among terminally ill patients. The results further showed that healthcare personnel attitude significantly makes the strongest unique contribution to explaining mental wellbeing, when the variance explained by psychological support is controlled for ($\beta = .442, p < .05$). The Beta value of psychological support made no significant contribution ($\beta = -.009, p > .05$) lower than attitude indicating that it made no unique contribution to mental wellbeing when attitude of healthcare personnel is controlled for. Therefore, this hypothesis has been accepted and the null hypothesis rejected.

presented in the previous chapter, all the hypotheses formulated for the study were supported.

Hypothesis 1 result of the analysis found a positive correlation between attitude and mental well-being. This finding supports the work of Shimmack et al. (2004) who found attitude to be the predictor of mental well-being. In a related development, this finding corroborates with those of Vitterse (2001); Brakko and Sabol (2006); Chen, Tu and Wang (2008); Joshanloo and Afasharia (2011), who in their separate studies linked attitude to relate with mental well-being. This finding lends credence to the work of McCrae and Costa (2008) that showed in their study that positive attitude increases the probability of positive experiences in ill situations, and this, in turn, is directly related to mental well-being.

DISCUSSION

In the present study the mental wellbeing scale patterning to terminally ill were tested. The mental wellbeing was regressed on the attitude factors and psychological support factors. The Four- Way- Anova, simple and multiple regression analysis conducted revealed that all the predictor variables jointly and independently predicted mental well-being. However, there was no interplay and significant interactive effect of religion, age, sex and marital status on the mental wellbeing where marital status was not found to predict mental well-being. Based on the findings

Even though the results of multiple regression and correlation showed that attitude correlates or predicts mental well-being, this does not support Sheikh’s (2004) view. He failed to find a significant correlation between these two variables — which the non-significant result may be due to the nature of participants he used. Also, the results support the hypothesis that psychological support will significantly predict mental well-being among terminally ill. This result corroborates with earlier research findings (e.g., Young, 2006; Yeung & Fung, 2007; Au, et al., 2009) that psychological support significantly predicted mental well-being. This finding lends credence to the work of Calvete and Connor-Smith (2006) who found psychological support from

the health personnel to reduce the impact of psychological problems among patients. This finding also corroborates with Dollete et al. (2004) who also found that psychological support could act as a protective factor that could decrease psychological problems.

Hypothesis 3 results of the multiple regression analyses confirmed that attitude and psychological support jointly predicted mental well-being. Supported by Cohen and Wills, 2005 which states that attitude and psychological support are emotionally beneficial to the mental well-being of terminally ill patients, the relationship between health care personnel attitudes and psychological support is likely reciprocal, in that patients who are happy may elicit positive social relations. There is a growing belief on the concept that positive attitudes and psychological support leads to increased mental well-being and health.

These findings indicate that the combination of an individual's health care personnel attitude and psychological support determine the mental wellbeing of patients with terminal illness. Furthermore, multiple regression analyses showed a significant interaction effect of attitude and psychological support on the mental well-being of patients with terminal illness.

Limitations of the Study

This study contributes significantly to our understanding of the contributions of attitude and psychological support to mental well-being among patients with terminal illness in Jalingo town, there are some factors that limit the generalization of the results. One limitation of this study is that it focused on just Jalingo town. The replication of the current study in other areas of human functioning in the state or nation may be important in generalizing the results. Another limitation of the study is on the number of the variables studied. Other variables such as socioeconomic, family background and general wellbeing could also contribute to mental well-being of the terminally ill beyond the effect of attitude and psychological support. This study was based on an ex post factor design. The exploration of attitude, psychological support and wellbeing does not allow inferring causality from the present data. Therefore, these results need to be replicated to generalize the findings,

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preferably in a study with a longitudinal designed.

Finally, all measures in the present study were collected on a single questionnaire within three months. A longitudinal study may help us to establish cause and effects relationship

Conclusion

i. Despite the limitations of the present study, it expands our knowledge in many ways. Therefore, we can conclude, based on the findings of the study, that: Attitude significantly relates to mental well-being of patients with terminal illness in Jalingo town. Also, attitude was found to predict mental well-being of patients with terminal illness in Jalingo town.

i. Psychological support significantly relates to mental well-being of patients with terminal illness in Jalingo town. The study concluded that health care personnel psychological support predicted mental well-being.

ii. It was also concluded, based on the findings, that attitude and psychological support jointly influence mental well-being among patients with terminal illness in Jalingo town.

iii. And also, the results of the incidental findings presented in Table 4.1 concluded that no significant interactive effect of religion, age, sex and marital status on mental wellbeing of patients with terminal illness in Jalingo town. This further implies that religion, age, sex and marital status are not co-determinants of mental wellbeing of patients with terminal illness in Jalingo.

RECOMMENDATION

More attention is needed to discover the significant factors that influence mental well-being of terminally ill patients.

In future the current study could be replicated using larger groups of terminally ill patients from a greater variety of backgrounds and different states, to determine whether patients with terminal illness in different areas of Nigeria experience mental well-being differently.

The present study could encourage future longitudinal studies, where researchers do not only assess the mental well-being of patients with terminal illness, but also the long-term effects that high/low levels of mental well-being the terminally ill have on an individual's life.

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APPENDIX I

Attitude Scale

A modified version of the Risser (1975) Attitude Scale (AS) was used to elicit the research data. The version implemented here is the one produced by Hinshaw and Atwood (1982) which compared to the original Risser scale differs at the 7th item of the “technical-professional” subscale where the phrase “over the telephone” was deleted. This version was psychometrically tested in five studies with a total of 600 patients, primarily medical-surgical inpatients. The results showed stable internal consistency estimates in the different studies with the average coefficients alpha values reported being 0.79, 0.78 and 0.88 for the three subscales respectively. The AS was designed to evaluate patients’ attitudes towards health workers and originally contained three subscales with a total number of 25 items defined as follows:

1. Technical-Professional (TP) domain contains seven items concerning technical issues on care and measurement of the nurses behaviors.
2. Educational Relationship (ER) domain contains seven items concerning nurses attitude with patients, the exchange of information between the nurse and patient.
3. Trusting Relationship (TR) domain approaches eleven interpersonal relationship situations between nurses and patients the verbal and nonverbal communication that occurs between the nurse and client. Each question is assessed on a five point Likert-type measurement scale ranging from “Strongly agree” (=1) to “Strongly disagree” (=5). The negative sentences are assessed in reverse, and the higher the AS score is, the higher is the patient satisfaction with the nursing care provided.

Psychological Support Scale:

Jefferson Scale of Psychological Support is a 5-item instrument developed by Gregory et al (2006) item intended to measure patient’s perceptions of his/her physician’s empathic concern and understanding. Patients are required to response to each item on their physicians empathy by using a 5-point Likert-type scale (from = 1strongly disagree to 5 = strongly agree). It takes a few minutes to answer the scale. It has an item total correlation of 0.90 thus making it reliable for use.

General Health Questionnaire (GHQ-12): The 12-Item Mental Wellbeing Scale

(GHQ-12) developed by Goldberg and Williams (1988) consists of 12 items, each one assessing the mental status over the past few weeks using a 4-point Likert-type scale (from 0 to 3). The score was used to generate a total score ranging from 0 to 36. The positive items were corrected from 0 (always) to 3 (never) and the negative ones from 3 (always) to 0 (never). High scores indicate worse health.

APPENDIX II

Reliability

Pilot study was carried out using 30 samples on patients with terminal illness in Ardo-Kola Local Government of Taraba State. A reliability coefficient of .71, .60, and .62 (see appendix II) was obtained for attitude scale, psychological scale and mental wellbeing respectively and this confirmed a good reliability of the instrument.

In the current pilot study conducted by the researcher, the attitude instrument has a Cronbach’s alpha of .71 which indicates that the test items are reliable. With regards to validity the total variance explained is 76.247 which mean that the test items measured 76.2% of the variable of interest.

Psychological support scale for pilot study conducted by the researcher has a Cronbach’s alpha of .60 indicating that the test items are reliable with the validity total variance explained is 63.046 which means that the test items measured 63% of the variable of interest.

The pilot study conducted using the mental wellbeing questionnaire has a Cronbach’s alpha of .62 which indicates that the test items are reliable. With regards to validity the total variance explained is 77.652 which indicate that the test items measured 77.7 % of the variable of interest.

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PHYTOCHEMICAL SCREENING AND NUTRITIONAL ANALYSIS OF *Irvingia gabonensis*
(FRUIT MESOCARP)

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ABSTRACT

Irvingia gabonensis, the African bush mango is a tropical evergreen crop grown for its edible fruits and seeds. The fruit is a drupe with rich pulpy fibrous mesocarp (edible portion) mostly consumed and enjoyed as a seasonal fruit delicacy by African women and children. The usefulness of fruits lie in their chemical constituents. This study focused on the phytochemical, proximate and mineral analysis of the fruit mesocarp. The fruit with intact pericarp, without pericarp and fruit stored for three days were compared. The mineral concentrations (Ca, Mg, K,) were determined with atomic absorption spectrometer while sodium was determined with flame photometer. Phytochemical analysis revealed the presence of alkaloids, flavonoids tanins and saponins while the nutrient present include protein, carbohydrate, vitamins and minerals. The fruit with intact pericarp have higher nutrient and phytochemical constituents more than fruit without pericarp followed by the stored one. Vitamins were found to be 284.7mg (Vitamin A) in fruit without pericarp, 30.15mg (Vitamin C) in fruit with intact pericarp, and 17.1mg (Vitamin E) in fruit with intact pericarp. The mineral were present as (potassium) 169.4mg, (sodium) 98.67mg, (Calcium) 22.86mg, (Magnesium) 10.4mg and fruit with only the mesocarp have low values of the major constituent which are made up of vitamin and minerals. However, the fruits were found to be rich in vitamin and mineral especially in fruit with intact pericarp and poor in major constituents- protein, carbohydrate and fat.

Keywords: *Irvingia gabonensis*, Phytochemical, Proximate, Pericarp, Mesocarp

INTRODUCTION

Irvingia gabonensis (family of Irvingiaceae) is one of the forest tree species that has immense domestic usefulness to rural and urban dwellers in many countries in west and central Africa where it occurs naturally (Onyekwelu, 2014). It is a large tree which have dense compact crown of evergreen large leaves with edible seeds and sweet edible fruit pulp (Obianime and Uche 2010). *Irvingia gabonensis* has two varieties: the fruit with sweet edible scanty fibrous pulp, fluted or cylindrical holes and fruits with bitter in edible very fibrous pulp and buttressed whole (Obianime and Uche 2010). It is has other common names like Dika fruit, African bush mango, wild mango and sweet mango (Leakey et al., 2001; Okolo, 2000). Out of 171 indigenous woody plants of economic importance identified within the forest zone of Nigeria, *Irvingia gabonensis* ranks amongst five principal fruit trees grown for their edible fruits and seeds (Atangana et al., 2001). The fruits are ellipsoidal drupe, 4 - 7cm long, with a fleshy mesocarp, very juicy fibrous pulp when ripe, green when unripe and yellow or light orange when ripe (Etebu and Tungbulu 2015 ; (Ogunsina et al., 2012).The dicotyledonous seeds are consumed as food additive (soup thickener) and additive for flavouring (Okolo CO 1995; Obianime et al., 2009). The pulp, kernel and fruit are edible by man and animals, although bitter and acrid, with turpentine flavor (Atangana et al., 2001). *Irvingia gabonensis* fruits are rich in oil and can be used in making bread, chocolate, cheese, butter, soap and feed cake (Mgbemena et al., 2019). The sweet pulp can be juiced or used for making smoothie, jelly, jam and wine

(Mgbemena et al., 2019; Okolo CO 1995).

It is a multi – purpose fruit which is widely used for foods and traditional medicine. It serves as an important source of livelihood and income for many farmers (Sawab et al., 2018). *Irvingia gabonensis* is one of the wild edible fruit commonly found in the market during rainy season in Southern and Eastern Nigeria but the problem of poor storage facilities and poor processing makes the fruit and seeds unavailable all the year round. Consumption of the fruit mesocarp is wide spread in African countries where it is enjoyed as a seasonal fruit delicacy (Ogunsina et al., 2012). However, emphasis appeared to be focused on the seed more than the fruit. The reasons adduced for this consumption pattern is that the mesocarp spoils within a few days of harvest and the seeds have greater utility value since it is a preferred soup thickener with drawing ability which is an essential quality of staple food like garri, fufu and cereal flours (Archinewhu and Ogbonna 1995). Consequently, more work have been done on the seed especially with respect to its oil than on the fruit. In spite of the extended value of the seed (kernels), the mesocarp is still widely consumed especially amongst children and women.

The usefulness of plants (fruits and vegetables) lies in their chemical constituents. It has been observed that some plants have the ability to produce and accumulate some phytochemicals which have found usage in traditional and ethnomedicinal formulations (Cowan 1999). Fruits and vegetables possess a wide range of phytochemicals (flavonoids, saponins, and alkaloids) with established antioxidant and

pharmacological activity (Onimawo et al., 2003). Hence, the objectives of the study are to qualitatively and quantitatively analyse the sample for nutritional components, to quantitatively carry out the phytochemical screening on the sample and to evaluate the nutritional value of the drupe with intact pericarp and without the pericarp.

MATERIALS AND METHODS

The test sample *Irvingia gabonensis* were obtained from the horticulture Umuahia Central Market and its botanical identity was authenticated by the Field officer, Horticulture Unit, National Root Crop Research Institute Umudike (NRCRI), Laboratory services Unit, NRCRI Umudike.

PROXIMATE ANALYSIS

Proximate chemical composition of each fruit sample was carried out to determine the moisture, ash, fat, crude fibre, protein and carbohydrate contents of each sample according to the method of AOAC (1999) method.

ESTIMATION OF MINERAL ELEMENTS

The sodium and potassium contents were determined by Flame photometry (Jenway Ltd Dunmow, Essex, UK). Estimation of calcium and magnesium were by atomic absorption spectrophotometry as described by Okoronkwo et al., (2014).

Estimation of Sodium Contents

A 2.0 g of each sample was ashed in a furnace at the temperature of 450°C for 3h. The ashed sample was dissolved with 5 ml of 30% HCL before the solution was made up to 50ml with deionized water in a volumetric flask. This was filtered and aspirated into the enablizer of the flame photometer and the electron emission reading taken at 539 nm wavelength. The standard was prepared using sodium salt, and the concentration of the test sample extrapolated from the standard reading ($7 = 10\text{ppm}$)

Estimation of Potassium Contents

A 2.0 g of each sample was ashed in a furnace at the temperature of 450°C for 3h. The ashed sample was dissolved with 5 ml of 30% HCL before the solution was made up to 50ml with deionized water in a volumetric flask. This was filtered and aspirated into the enablizer of the flame photometer and the electron emission reading taken at 767 nm wavelength. The standard was prepared using potassium salt, and the concentration of the test sample extrapolated from the standard reading ($16 = 20\text{ppm}$).

Estimation of Magnesium and Calcium contents

Determination of calcium and magnesium was by the Versante complexometric (EDTA) titration as described by James, (1995). 20 mls of the digest was dispersed into a conical flask, a pinch each of hydroxylamine, hydrochloride and potassium cyanide (masking agents) was added to it. Then 10 mls of ammonia was added to get the pH at 10.0. An indicator dye, Erichrome black T, was added and the mixture was titrated against 0.02N EDTA solution. Titration was done from a mauve colour to a permanent deep blue colour and was for a combined Ca and Mg ions. A second titration was conducted to determine Ca alone. A 10% NaOH solution was used to raise the pH to 12.0 while solochrome black blue was used as indicator in place of Erichrome.

DETERMINATION OF VITAMINS

Vitamin C

Vitamin C was determined using titrimetric method. A 20 g portion of each processed sample was homogenised in 50mls of 6% trichloroacetic acid /EDTA extractant solution, the homogenate was centrifuged at 300xg for 10mins and the supernatant was decanted and its volume was recorded. 20 mls of the extract was dispersed into a conical flask and 10 mls of 30% of potassium iodide was added to it, shaken to mix well and 5 mls of 1% starch solution was added as indicator. The mixture was titrated against 0.05m Cuso4 solution and titration was done to bluish end point. A reagent blank was prepared using 20 mls of the extract solution for titration.

Vitamin A

The method of Association of Vitamin Chemist was used to determine vitamin A. 10 g of each processed sample was dispersed in 30 ml of absolute alcohol, then 3 mls of 56% potassium hydroxide solution was added to it and the mixture was transferred to a separation funnel. 50 mls of ether was used to wash the mixture thus extracting the Vitamin A. The vitamin A extract was washed with 4 x 50ml distilled water. The extract was then evaporated to dryness and dissolved in 10 mls of 190 propyl alcohol to obtain standard vitamin A. The absorbance was measured at 325nm.

Pytochemical Analysis/Screening.

Phytochemical screening and quantitation of flavonoids, phenols, saponins, alkaloids, tanins and hydrogen cyanide was done using the method of AOAC (1999).

Table I: Qualitative phytochemical screening of *Irvingia gabonensis*.

Sample	Alkaloid	Flavonoid	Saponin	Cyanogenic glycoside HCN	Tanin	Starch
Without Pericarp A	+	+	+	+	+	-
With Pericarp B	+	+	+	+	+	-
Stored for 3 days	+	+	+	+	+	-

Note: Key: (+) = Present; (-) = Absent

Table II: The percentage phytochemical composition of *I. gabonensis*

Sample	Tanin %	Flavonoid %	Saponin %	Phenol %	HCN Mg/Kg	Alkaloid %
A	0.28 ± 0.0	0.23 ± 0.01	0.29 ± 0.03	0.26 ± 0.02	13.15 ± 0.02	0.23 ± 0.02
B	0.88 ± 0.006	0.32 ± 0.06	0.53 ± .0005	0.48 ± .0005	28.92 ± 0.3	0.46 ± 0.02
C	0.28 ± 0.06	0.23 ± 0.01	0.25 ± 0.02	0.22 ± .0005	12.73 ± 0.1	0.20 ± 0.02

Mean + Standard deviation of triplicate determination

Table III: The percentage proximate composition of *I. gabonensis*.

Sample	Protein	Fat	Fibre	Ash	Moisture Content	Carbohydrate	Dry Matter
A	5.13 ± 0.6	0.34 ± 0.1	2.39 ± 0.03	2.3 ± 0.01	72.45 ± 0.01	17.38 ± 0.01	27.54 ± 0.01
B	12.72 ± 0.3	3.45 ± 0.03	5.12 ± 0.03	5.24 ± 0.01	68.75 ± 0.01	4.72 ± 0.01	31.25 ± 0.01
C	5.77 ± 0.5	0.33 ± 0.06	2.37 ± 0.02	2.37 ± 0.01	76.64 ± 0.2	12.52 ± 0.01	23.36 ± 0.2

Mean + Standard deviation of triplicate determination

Table IV: The Mineral and Vitamin content of *I. gabonensis*

Sample	Ca	Mg	K	Na	Vit A 1u/100g	Vit C mg/100g	Vit E 1u/100g
A	17.37 ± 3.3	6.4 ± 1.1	86.67 ± 0.4	13.41 ± 0.02	284.7 ± 0.4	26.99 ± 0.8	13.9 ± 0.7
B	22.86 ± 5.0	10.4 ± 2.3	169.4 ± 2.0	98.67 ± 0.04	175.5 ± 0.3	30.51 ± 1.7	17.17 ± 1.2
C	17.37 ± 2.3	5.8 ± 1.1	86.93 ± 0.4	13.64 ± 0.3	282.0 ± 0.1	24.05 ± 0.8	14.97 ± 0.5

Mean + Standard deviation of triplicate determination

RESULTS AND DISCUSSIONS

Table 1 shows the phytochemical screening results of the fruit mesocarp without pericarp (A), with pericarp (B) and stored for 3 days (C). The results revealed the presence of tannins, saponins, alkaloid, HCN, flavonoids and the absence of starch in the samples. These compounds are known to be biologically active and possess established antimicrobial activities against

bacteria, fungi and some viruses (Cowan 1999 and O.O Igbinsosa et al., 2009).

In Table II, the quantity of specific phytochemicals in the test fruit sample is shown. The result shows that there are more of these constituent in the fruit mesocarp with pericarp (B) which contains 196%, 100%, 48% more tannins, alkaloids and flavonoid

respectively than sample A and C. Similarly, saponin, phenol and hydrocyanic acid content of the fruit with its skin show an increase from 0.29 to 0.53, 0.26 to 0.48 and 13.15 to 28.92% which represent a percentage increase by 82.76, 76, 92 and 119.72% respectively. However, there are more of saponin, phenol, HCN and flavonoid respectively in the content of the fruit with its skin than the one without skin and the one stored for a few days. The high content of saponin in sample B is in agreement with the work of Mgbemena et al., 2019. The flavonoids and saponins have nutritional significance in man. Saponin is believed to be useful in the control of human cardiovascular diseases (Adeyeye 2017). Flavonoids are known to protect plants against damaging effect caused by UV-radiation, antimicrobial infection and through their oxidative properties absorb oxygen radicals that cause skin oxidation (Oakenfull et al., 1986; Mgbemena et al 2019). The Hydrocyanic acid (HCN), which is a product of hydrolysis of cyanogenic glycoside is known to be toxic to man while tannins are able to affect the availability of proteins. However, what matters is the quantity of these phytochemicals present in the test fruits.

In contrast to the above, a reduction in most of the constituent level was obtained in the fruit when allowed to store for 3 days. There was no change on the tannin content while the alkaloid and flavonoid reduced from 0.23 to 0.20% and 0.25 to 0.23% respectively. This represents a percentage decrease of 13.04% and 8.0%. Similarly, the HCN, Phenol and Saponin reduced by 3.19%, 15.85% and 13.79% respectively. Reduction in HCN level may be due to volatilization in addition to possible microbial activity on the fruit. Fermentation (dry or wet) is known to cause reduction in HCN (Balago pdan *et al* 1988). The loss in other phytochemical parameters may have been apparent staining from the increased moisture content on storage (a sign of spoilage or overripe). Generally, the composition that are shown in the result indicate that the so called anti-nutrient parameters (HCN and phenol) were contained at safe levels since it takes up to 50mg/kg of HCN in food to become unacceptable

(Kink and Sawyer 1989).

Table III shows the proximate composition of the test fruit sample. The moisture contents in fruit mesocarp without pericarp (A), with pericarp (B) and stored for 3 days (C), were 72.45 ± 0.01 , 68.75 ± 0.01 , and 76.64 ± 0.2 respectively. High moisture content was seen in the samples and the major constituents – protein, fat and carbohydrate were poor. High moisture content in fruits is an index of its water activity, measure of stability and susceptible to microbial contamination (Brooker 2005; Ponnusha et al 2011). Also, among the three samples higher values were obtained in protein, fibre and fat in the sample with pericarp.

Table IV revealed the results of the mineral and vitamins content. The mineral under study comprised four major minerals (Ca, Mg, Na and K) and Vitamins A, C and E. The result shows that the percentage of Ca, Mg, Na and K were high in sample B followed by sample A and C. The fruit sample contains much vitamin A (284.78%) with an average content of vitamin C (30.51%); the mineral K (165.4%) and Mg (98.67%) respectively. The mineral percentage of K and Mg present in this study is slightly different in Onimawo et al., 2003 report. Therefore, the fruit is rich in vitamin A, with an average content of vitamin C and vitamin E. These vitamins possess antioxidant activity and thus are medicinal especially when eaten raw against when eaten cooked where the antioxidants effect is lost due to cooking. (Onimawo et al., 2003).

Conclusion and Recommendation

The proximate composition and phytochemical constituents of *Irvingia gabonensis* showed that *I.gabonensis* fruit is rich in vitamins more than other nutrients and contain high moisture content. It is recommended that further studies should be carried out with a view to recognizing the specific alkaloids, flavonoids, etc, a knowledge that will obviously open for increased utilization of the fruit in a more diversified way such as food and pharmaceutical industries.

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SERUM BIOCHEMICAL PROFILE OF MONOSODIUM GLUTAMATE-INTOXICATED ALBINO RATS TREATED WITH ETHANOL EXTRACT OF *MUCUNA SLOANEI* SEEDS.

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ABSTRACT

This study assessed the serum biochemical parameters of monosodium glutamate intoxicated albino rats treated with *Mucuna sloanei* ethanol seed extract (MSEE). Thirty (30) female albino rats used were divided into 6 groups (n= 5) and treated as follows; Group A:(Control) distilled water only, Group B: 8000 mg/kg monosodium glutamate (MSG), Group C: 400 mg/kg MSEE; and Groups D, E and F: 200, 400 and 800 mg/kg body weight of MSEE in addition to 8000 mg/kg body weight of MSG respectively. Treatment lasted for 30 days and blood was collected for assay of renal function (creatinine, urea) as well as liver function (AST, ALT, ALP, total protein, albumin and bilirubin). Both renal and hepatic biomarkers were significantly (P<0.05) elevated in MSG intoxicated rats compared to the control. Serum creatinine and urea were significantly (P<0.05) increased from 18.18±1.92 and 0.55±0.05 to 29.40±1.44 and 1.30±0.18 respectively in MSG intoxicated rats compared to the rats given only distilled water. The extract, at 800 mg/kg caused significant and highest (P<0.05) reduction in the renal parameters. Also, rats intoxicated with MSG had significantly (P<0.05) higher AST, ALP, ALT and bilirubin levels (43.67±4.26; 67.54±2.50; 35.67±1.45 and 1.03±0.14) compared to the rats in the control group (23.67±2.40; 59.68±3.36; 16.33±1.86 and 0.70±0.09). There were dose-dependent and significant (P<0.05) reductions in the levels of these biomarkers in intoxicated rats treated with MSEE. We conclude that MSEE has both nephroprotective and hepatoprotective effects on MSG intoxicated rats.

Keywords: *Mucuna sloanei* seeds, nephroprotective, hepatoprotective, monosodium glutamate intoxication

INTRODUCTION

There is a steady rise in global prevalence of renal (Gyurasova *et al.*, 2019) and hepatic (Zhu *et al.*, 2012) diseases, attributed to lifestyle such as use of all forms of food additive, including monosodium glutamate, used to improve the flavour of foods (Eweka and Adjene, 2007). High concentrations of monosodium glutamate in the central nervous system may cause neuronal necrosis and damage of the retina and circumventricular organs. Studies have also shown that MSG produces oxygen derived free radicals (Adrienne, 1999) and its chronic administration leads to retinal degeneration, endocrine disorder, addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease and Alzheimer's disease. (Adrienne, 1999; Eweka and Adjene, 2007).

However, some plants are known to reverse these deleterious effects, and one of such plants is *Mucuna sloanei*, which is known to have some medicinal properties (Nwosu, 2011). The seeds are contained in a pod containing two or three seeds and have a characteristic three-layered appearance, appearing like the eyes of a large mammal or like hamburger leading to names like "Ox-eye bean" or "Hamburger

bean". *Mucuna* seeds are usually toasted before grinding and flouting to supplement as thickener in sauce or soups (Waryekeche *et al.*, 2003). *Mucuna Sloanei* seed is used by the Igbo community in Sub-Saharan Africa as soup thickener (Ukachukwu *et al.*, 2002). Traditionally, the seeds have been used as a diuretic, purgative and for soothing haemorrhoids (Standley, 1926). The seeds are also used to make black dye and to produce oil that can be used for various purposes such as soap making. The constituents of *Mucuna sloanei* seeds include crude proteins, carbohydrates, fat, crude fibers, moisture, ash, phosphorus, magnesium, calcium, sodium, iron, manganese, copper, tannins, glycosides, zinc and L-Dopa (Tuleun *et al.*, 2008; Nwosu, 2011). Its medicinal properties include antidiabetic and antiparkinson activities (Molloy *et al.*, 2006), anti-oxidant and antimicrobial effects (Rajeshwar *et al.*, 2005), aphrodisiac, antineoplastic, anti-epileptic, anti-dementia effects (Poornachandra *et al.*, 2005) and antihelminthic property (Jalalpure, 2007).

Renal and hepatic biomarkers are routinely deployed to study the state of these organs in a living organism. Liver function tests may help to explain the extent of damage on the liver using some serum enzymes like

the aspartate aminotransferase (AST), alanine aminotransferase (ALT) as well as bilirubin levels (Zhu *et al.*, 2012) while creatinine and urea are the common biomarkers routinely used to assess renal function (Gowda *et al.*, 2010). The aim of this study, therefore, is to evaluate the effect of *Mucuna sloanei* ethanol seed extract on renal and hepatic function of MSG intoxicated rats using known biomarkers.

MATERIALS AND METHODS

Collection and preparation of *M. sloanei* ethanol seed extract (MSEE)

Mucuna sloanei seeds were obtained from Amokwo Ugwu Nkpa in Bende LGA in Abia State and authenticated at the Department of Crop Science, College of Crop and Soil Science, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A voucher specimen was deposited in the herbarium of Veterinary Physiology and Pharmacology laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The seeds were toasted, de-hulled and ground. A portion (500 g) of the powder was extracted in 2.0 litres of 98 % ethanol for 48 hours agitated intermittently every 3-hours using cold maceration method, after which it was sieved using Whatman's® filter paper (No 4.) and dried in a hot air oven set at 40°C. Calculated amount of the extract was weighed and reconstituted in distilled water to give the required different doses of 200, 400 and 800 mg/kg body weight and given per os using oral gavage daily for 28 days.

Experimental animals

Thirty female albino rats (*Rattus norvegicus*) of Wistar strain with an average weight of 113 g were obtained from the Animal House of the department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. They were housed in aluminum cages under standard conditions (temperature 26 °C; photoperiod- 12 hours day light and 12 hours darkness; humidity: 45-50%) and allowed to acclimatize for two weeks before the commencement of the experiment. They were given free access to feed (Grower pellets, Vital Feeds®, Nigeria) and clean tap water. All experiments were carried out in accordance with the guidelines of the National Institute of Health, USA for ethical treatment of laboratory animals.

Experimental design

Thirty (30) mature female albino rats were randomly divided into six groups (n=5) and dosed once per day for 28 days as follows; Group A (Control) received 1 ml of distilled water as negative control; Group B received 8000 mg/kg b.w of MSG only; Group C received 400 mg/kg b.w of the extract only and Groups D-F received 8000 mg/kg b.w of MSG in addition to

different doses of the extract (200, 400 and 800mg/kg b.w) respectively. Monosodium glutamate (Ajinomoto®) was procured from Ubani market Umuahia, Abia State. A toxic concentration of 500 mg/ml was formulated to be given to all the groups at the dose of 8000 mg/kg except Group A (distilled water only) and Group C (Extract only) *pe ros* using oral gavage daily for 28 days .A previous acute toxicity study (LD₅₀) showed that *Mucuna sloanei* extract was not toxic with LD₅₀ of 4000 mg/kg b.w. (Oguwike *et al.*, 2017). On day 29, the rats were sacrificed by inhalation anaesthesia using chloroform. Blood was collected by cardiac puncture into plain bottles, allowed to clot and then centrifuged at 3000 rpm for 10 minutes to obtain sera which were labeled appropriately

Serum biochemical assays:

Serum creatinine levels was determined by the modified Jaffe method (Blass *et al.* 1974). Serum urea level was determined by the modified Berthelot-Searcy method (Lamb and Price 2008). The concentrations of the electrolytes were determined in the serum following standard procedures as described in the respective assay kits. The serum enzymes, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total proteins and albumins assays were carried out according to the method of Reitman and Frankel (1957).

Statistical Analysis

Data obtained from this study were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0 for Windows. Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $P < 0.05$. Post Hoc multiple comparisons for differences within groups were established using least significant difference (LSD). Results were presented as Mean \pm S.E.M.

RESULTS

Kidney function tests results

Treatment with monosodium glutamate caused increases in serum levels of urea and creatinine, from 18.18 ± 1.92 and 0.55 ± 0.05 to 29.40 ± 1.44 and 1.30 ± 0.18 respectively. *M.sloanei* caused a significant ($P < 0.05$) dose-dependent reduction in serum urea and creatinine levels, with the highest reduction in rats treated with MSEE at 800 mg/kg (25.35 ± 0.79 and 0.79 ± 0.05), respectively (Table 1). Sodium, potassium and chloride ion levels were significantly ($P < 0.05$) higher in rats treated with MSEE at 800 mg/kg compared to both control and MSG only group. There were no significant differences ($P > 0.05$) in bicarbonate ion levels across the groups (Table 1).

Table 1: kidney function biomarkers of rats intoxicated with MSG and treated with MSEE

Means with different superscript letters (a-d) in the same column are significantly different from each other (P<0.05).

	UREA (mg/dl)	CREATININE (mg/dl)	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mEq/L)	HCO ₃ ⁻ (Mmol/L)
Control	18.87±1.92 ^c	0.55±0.05 ^d	102.96±2.46 ^b	5.35±0.21 ^{ab}	84.68±6.82 ^c	24.48±0.45 ^a
MSG only	29.42±1.44 ^a	1.30±0.18 ^a	104.44±7.62 ^b	4.81±0.28 ^b	90.17±3.54 ^c	23.80±0.61 ^a
Extract only (400mg/kg)	14.06±0.87 ^d	1.08±0.06 ^{ab}	108.53±10.73 ^b	5.85±0.48 ^a	95.20±8.06 ^{bc}	22.33±0.54 ^a
MSG + Extract (200mg/kg)	23.48±0.58 ^b	0.88±0.03 ^{bc}	112.74±3.53 ^{ab}	5.11±0.05 ^{ab}	105.87±1.71 ^{ab}	23.09±0.87 ^a
MSG + Extract (400mg/kg)	25.35±0.68 ^b	0.81±0.04 ^{bcd}	114.26±3.67 ^{ab}	5.39±0.16 ^{ab}	106.82±1.81 ^{ab}	22.51±1.18 ^a
MSG + Extract (800mg/kg)	25.35±0.79 ^b	0.79±0.05 ^{cd}	130.03±3.59 ^a	5.66±0.15 ^{ab}	116.90±2.44 ^a	22.20±1.00 ^a

Liver function tests results.

Rats intoxicated with MSG had significantly (P<0.05) higher AST, ALP, ALT and bilirubin levels (43.67±4.26; 67.54±2.50; 35.67±1.45 and 1.03±0.14) compared to the rats in the control group (23.67±2.40; 59.68±3.36; 16.33±1.86 and 0.70±0.09) respectively (Table 2). These were significantly (P<0.05) lower (33.33±5.13; 59.75±0.38; 26.00±0.58 and 0.85±0.04)

respectively in rats treated with MSEE (800 mg/kg). Serum albumin and total protein levels were significantly (P<0.05) lower in MSG intoxicated rats (3.25±0.14 and 6.19±0.20) compared to the control (4.83±0.25 and 8.48±0.19). These were significantly (P<0.05) reduced in rats treated with MSEE at 800 mg/kg (4.43±0.27 and 7.74±0.10). (Table 2).

Table 2: Liver function biomarkers of rats intoxicated with MSG and treated with MSEE

GROUPS	AST(U/L)	ALP(U/L)	ALT(U/L)	T.PROTEI N(mg/dl)	ALBUMIN (mg/dl)	BILIRUBIN (mg/dl)
1-Control	23.67±2.40 ^{bc}	59.68±3.36 ^b	16.33±1.86 ^c	8.48±0.19 ^a	4.83±0.25 ^a	0.70±0.09 ^b
2-MSG only	43.67±4.26 ^a	67.54±2.50 ^a	35.67±1.45 ^a	6.19±0.20 ^c	3.25±0.14 ^c	1.03±0.14 ^a
3-Extract only (400mg/kg)	20.00±1.15 ^c	59.75±0.38 ^b	16.00±1.15 ^c	7.40±0.17 ^b	4.01±0.23 ^b	0.77±0.06 ^b
4-MSG + Extract (200mg/kg)	31.33±2.40 ^b	66.26±1.86 ^{ab}	26.00±1.53 ^b	7.54±0.36 ^b	4.11±0.10 ^b	0.82±0.01 ^{ab}
5-MSG + Extract (400mg/kg)	28.00±2.08 ^{bc}	67.02±2.32 ^{ab}	26.00±1.15 ^b	7.42±0.13 ^b	4.27±0.03 ^{ab}	0.87±0.05 ^{ab}
6-MSG + Extract (800mg/kg)	33.00±5.13 ^b	67.06±1.96 ^{ab}	26.00±0.58 ^b	7.74±0.10 ^b	4.43±0.27 ^{ab}	0.85±0.04 ^{ab}

Means with different superscript letters (a-d) in the same column are significantly different from each other (P<0.05).

Discussion

Monosodium glutamate intoxicated rats had renal and hepatic damage seen as increased levels of renal and hepatic markers. Studies have shown that MSG produces oxygen derived free radicals (Singh and Ahluwalia, 2003) and it has been established that MSG intake initiates renal damage by oxidation and high concentrations at the dose of 1.6 mg/kg of body weight may cause an adverse effect on the central nervous system, hepatic and renal functions while inducing neuronal necrosis and damage to the retina (Pavlovic *et al.*, 2007). *Mucuna Sloane* iethanolic seed extract elicited dose dependent protective effect in the damage caused by MSG intoxication which was seen as reductions in levels of the renal and hepatic biomarkers in the serum.

Serum levels of ALT and AST which are the two diagnostically important transaminases as well as ALP have not only been used as good bio-indicators of the functionality and cellular integrity of the liver but to also assess the functional health status and the internal environment of the organism (Lavanaya *et al.*, 2011). Normally, an elevation in their serum levels may be indicative of an inflammation or damage to the hepatocytes or liver dysfunction (Sood, 2006) especially whenever the liver undergoes such pathological conditions as cirrhosis or subjected to abnormal onslaught that accompany the presence of toxins or usage of some drugs (Crook, 2006). Thus, MSG caused elevations in these biomarkers as a result of damage to the hepatocellular

tissues, leading to release of these biomarkers into the blood. The reduction in these biomarkers seen in rats treated with MSEE suggests that the extract has a hepatoprotective ability.

Creatinine and urea are routine biomarkers of renal damage in clinical practice (Ihedioha *et al.*, 2019). While creatinine is a product of the catabolism of muscle creatine, urea is produced from the breakdown of protein (Gowda *et al.*, 2010). Both biomarkers were elevated in MSG intoxicated rats, which is an indication renal damage. This was reversed in MSEE treated rats, suggesting that the extract has the ability to protect the kidney damage caused by MSG intoxication.

Mucuna sloanei seed extract, on its own, has been reported to have no deleterious effect on renal and hepatic biomarkers in rats (Ugwu *et al.*, 2018). This was also corroborated in this study, where rats given only MSEE did not have increased levels of renal and hepatic biomarkers. This suggests that it is safe (Ugwu *et al.*, 2018).

Conclusion

Monosodium intoxication in rats resulted in increased levels of serum renal and hepatic biomarkers, which suggest renal and hepatocellular damage. These increases were subsequently, ameliorated by *M. sloanei* ethanol seed extract at the different doses. We conclude, therefore, that MSEE ameliorates renal and hepatic damage caused by MSG intoxication in rats.

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MICROBIAL DIVERSITY OF COMMERCIAL MANUAL BLENDERS WITHIN PORT HARCOURT METROPOLIS

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ABSTRACT

Blending is an old popular practice of processing food products by human populace, especially in commercial markets. A survey of commercial manual blenders was carried out within Port Harcourt metropolis using spread plate technique. The microbial count revealed Oil Mill market ($4.6 \pm 0.5 \times 10^5$ CfU/ml) as the lowest and Borokiri market ($5.0 \pm 0.3 \times 10^5$ CfU/ml) the highest. Plasmid profiling indicated 80% of the isolates had high molecular weight of 10kb plasmids and 20% had no plasmid. Amplicons from Sanger sequence subjected to metagenomic analysis software showed a phylogenetic tree with *Ochrabacterium intermedium*, *Pseudomonas aeruginosa*, *Candida tropicalis* and *Pichia kudriavzevii* as the prevalent identified organisms. These results revealed the presence of enteric pathogens which might have originated from the poor hygiene practices during food handling, the environs where the operation is carried out and their indiscriminately waste disposal pattern. Therefore public health professionals need to educate these operators on the importance of good hygiene practices, proper HACCP, the danger of being vectors of food borne infections and its diverse implications.

KEYWORDS: Enteric pathogens, hygiene practices, medium scale grinders, phylogenetic tree, plasmid profiling.

INTRODUCTION

Food blending is an important part of food processing, storage, preserving and preparation for consumption. They are used at home for processing and preparation of food products, in other places such as market so as to reduce the labour while preparing food at home (Bankole *et al.*, 2013). In Nigeria, this practice is commonly used in local markets for grinding of food products such as crayfish, melon seeds, tomatoes, pepper and other food products purchased by consumers (Bankole *et al.*, 2013; Adu-Gyamfi *et al.*, 2012). This practice leads to contamination of food products during processing in the market because of the haste exhibited by the machine operators after each activity, inability / improper cleaning of the machine prior to carrying out a new task. The diversity of microorganisms usually found as contaminants of food processors include *Salmonella* sp., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* sp. amongst others (Ackah *et al.*, 2011; FAO/WHO, 2005). The presences of these organisms not only reduce the shelf life of the food products processed, it could also cause food-borne diseases and food spoilage leading to a high socio-economic burden for consumers (Ackah *et al.*, 2011; FAO/WHO, 2005; Amoah *et al.*, 2006). Microbial diversity associated with commercially used blenders is crucial for improving public health especially in developing countries such as Nigeria. An investigation of the microorganisms associated with commercial blenders and their plasmid characteristics was carried out in this study.

METHODS

Sample Collection

A total of 100 commercial manual hand-blenders were sampled for the study. Sterile swab sticks were used to collect swab samples from 20 manual hand-blenders used in each of the following five markets: Mile 1, Mile 2, Borokiri, Rumuokoro, and oil mill markets.

Microbial Assessment

The swabbed samples were plated on nutrient agar and saboraand dextrose agar (SDA) according to prescribed standards (BAM, 2005). Nutrient agar plates were incubated at 37°C for 24h, while SDA plates were incubate at 25°C for 72h.

Identification of Isolates

Axenic isolates with distinct colonies were subjected to confirmatory biochemical tests as described by Agwa *et al.* (2015).

Molecular Identification

DNA extraction from the microbial isolates

ZR fungal/bacterial DNA mini prep extraction kit was used for the DNA Extraction. A heavy growth of the axenic culture was suspended into a ZR Bashing Bead Lysis tubes containing 200 microlitre of isotonic buffer with 750 microlitre of lysis solution and processed at maximum speed for 5 mins. The mixture was centrifuged at 10,000xg for 1 min, the recovered supernatant of about 400 microlitres was transferred to a collection tube, and re centrifuged in a Zymo-Spin IV spin Filter tube at 7000 x g for 1 min. To the filtrate was added about 1200 microlitres of fungal/bacterial DNA binding buffer and 800 microlitre of the mixture was then centrifuged at 10,000xg for 1 min. The other

volume remaining was centrifuged, DNA Pre-Wash buffer of 200 microlitre was added to Zymo-spin IIC in a new collection tube, recentrifuged at 10,000xg and 500 microlitre of fungal/bacterial DNA Wash Buffer was also recentrifuged at 10,000xg for 1 min. A clean 1.5 microlitre centrifuge tube was used to collect the Zymo-spin IIC column, to the column matrix about 100 microlitre of DNA elution buffer was added and centrifuged at 10,000xg microlitre for 30 secs to elute the DNA which was stored at -20°C for other downstream reaction.

DNA quantification

The Nanodrop 1000 spectrophotometer was used to quantify the extracted genomic DNA.

16S rRNA Amplification

The 27F and 1492R primers were used to amplify the 16s rRNA region of the rRNA genes of the bacteria isolates on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles. The PCR mix template compositions are: the extracted DNA, the X2 Dream taq Master mix containing (DNTPs, taq polymerase, MgCl) and the primers at a concentration of 0.4M. The PCR initial denaturation was carried out at 95°C for 5 mins; denaturation at 95°C for 30 secs; annealing at 52°C for 30 secs; extension at 72°C for 30 secs for 35 cycles and final extension at 72°C for 5 mins. Agarose gel of about 1% was used to resolve the product at 120V for 15 mins and subsequently a UV trans illuminator was used to visualize the amplified DNA.

Internal Transcribed Space (ITS) Amplification

The ITS1(TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) primers were used to amplify the ITS region of the rRNA genes of the fungal isolates on an ABI 9700 Applied Bio systems thermal cycler using a final volume of 50 microlitres for 35 cycles. The PCR mix template compositions are: the extracted DNA, X2 Dream taq Master mix containing (DNTPs, taq polymerase, MgCl) and the primers at a concentration of 0.4M. The PCR initial denaturation was carried out at 95°C for 5 mins; denaturation at 95°C for 30 secs; annealing at 52°C for 30 secs; extension at 72°C for 30 secs for 35 cycles and final extension at 72°C for 5 mins. Agarose gel of about 1.5% was used to resolve the product at 120V for 15 mins and subsequently a UV trans illuminator was used to visualize the amplified DNA.

Sequencing

Sanger-Sequencing was done using the Big Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

Phylogenetic Analysis

The bioinformatics algorithm Trace edit was used to edit the obtained sequences. Using BLASTN, from the National Center for Biotechnology Information (NCBI) data base similar sequences were downloaded

and Crustal X was used to align these sequences. Neighbor-Joining method in MEGA 6.0 was used to infer the evolutionary history (Saitou and Nei, 1987). The evolutionary history of the taxa analyzed was used to infer the bootstrap consensus tree from 500 replicates (Felsenstein, 1985). The Jukes-Cantor method was used to compute the evolutionary distances (Jukes and Cantor, 1969).

Plasmid Isolation

The plasmids were isolated via the HiPura Plasmid DNA Minirep purification spin kit procured from HiMedia Pvt. Ltd. Mumbai, India. The plasmid curing and separation was carried out using the method of Esimone *et al.* (2010); extraction using the modified method of Agbagwa *et al.* (2012) and the method of Odeyemi *et al.* (2015) was adopted in plasmid characterization from the blenders to ascertain the size of the plasmids and its quality.

Statistical Analysis

The One-way Analysis of Variance was used to determine the difference in heterotrophic bacteria counts observed from the swab samples collected from the different sources at a 95% confidence interval and a p-value less than 0.05 was considered significant using the Epi Info v7 software (CDC, USA).

RESULTS

There was no significant difference ($p > 0.05$) in the mean heterotrophic bacteria count of the swab samples from the various sources. The least count was observed in Oil Mill market ($4.6 \pm 0.5 \times 10^5$ CfU/ml) and the highest count was found in Borokiri market ($5.0 \pm 0.3 \times 10^5$ CfU/ml) as shown in Table 1.

Table 1: Total Heterotrophic Bacteria Count (THBC)

Sample Source	Mean THBC (10 ⁵ cfu/ml)
Borokiri	5.0 ± 0.3
Oil Mill	4.6 ± 0.5
Mile I	4.7 ± 1.2
Mile III	4.8 ± 1.6
Rumuokoro	4.9 ± 1.4
ANOVA (p-value)	0.1245

Fig 1 shows the bacteria isolated and identified from the blenders include *Bacillus* sp. (33%), *Staphylococcus* sp. (22%), *Streptococcus* sp. (18%), *Pseudomonas* sp. (10%), *Micrococcus* sp. (6%), *Escherichia coli* (6%) and *Enterobacter* sp. (5%).

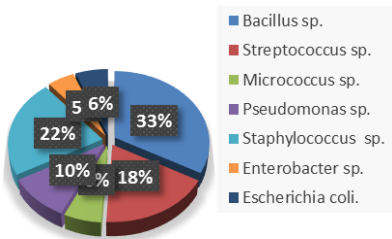
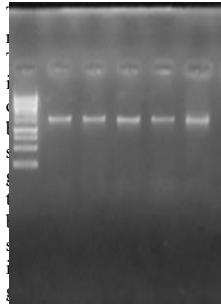


Fig 1: Frequency of Bacteria Isolates



molecular weights of 16S rRNA gene bands of the bacterial isolates. The amplified genes from the bacterial isolates are compared with the 1kb ladder (Lane M) to confirm the molecular size of 1kb (Kilobase). The amplified genes from the bacterial isolates are compared with the 1kb ladder (Lane M) to confirm the molecular size of 1kb (Kilobase). The amplified genes from the bacterial isolates are compared with the 1kb ladder (Lane M) to confirm the molecular size of 1kb (Kilobase).

Fig 2: Agarose gel electrophoresis showing 16SrRNA gene bands: Lanes 1-5 represent the amplified gene from the bacteria isolates; M represents the 1kb ladder.



M 1 2 3 4 5

Fig. 3: agarose gel electrophoresis showing ITS gene bands. Lanes 1 and 2 represent the amplified gene from the fungal isolates from the commercial manual blenders; L 3-5 represent the 1kb ladder

Figure 4 shows the plasmid profiling composition of replicons, the gel bands of agarose electrophoresis with Lanes 1 and 10 having no plasmid-7, 9 show isolates with one plasmid while lane 8 shows an isolate with two plasmids of 10 and 8kb, M represents the 10kb ladder.

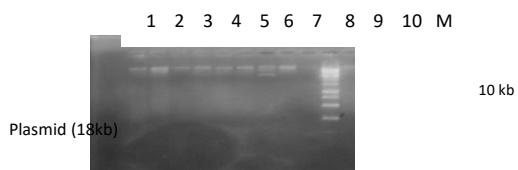


Fig 4: Agarose gel electrophoresis showing the plasmid bands

Table 2 illustrates the purity of plasmids on nanodrop technology, the absorbance from the nanodrop spectrophotometer is a cutting-edge technology used to ascertain the purity of both DNA and RNA from any isolate. The absorbance of the rRNA at 260nm and 280nm employs the absorbance of Proteins, RNA and DNA of bacterial and fungal groups isolated from medium-scale grinder. Ratio of absorbance at both 260nm and 280nm as seen is greater than 1.0nm suggest a higher value of bacterial purity. For instance the absorbance as seen in table 4.1 ranges from 1.9 -2.01nm.

Sample	ng/μg	A260nm	A280nm	260/280 nm	340 raw
B1	26.13	0.470	0.244	2.01	0.036
B2	42.13	0.523	0.261	2.03	0.036
B3	33.06	0.843	0.414	1.94	0.041
B4	41.22	0.661	0.341	1.96	0.033
F1	11.12	0.824	0.421	1.87	0.034
F2	62.86	0.222	0.119	1.90	0.057

Table 2: Shows the extract rRNA purity index from the nano-drop spectrophotometer

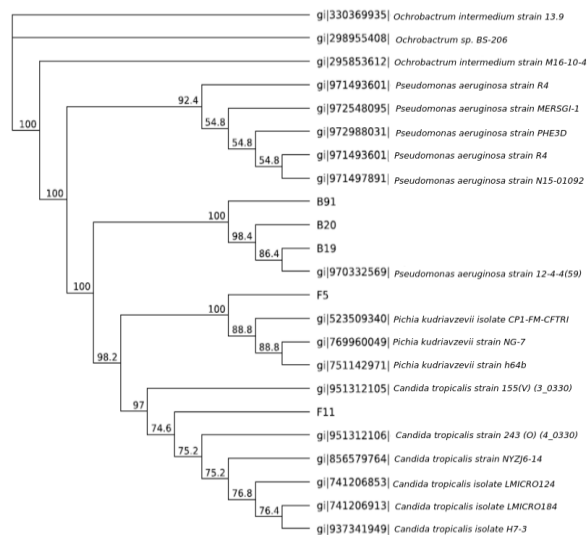


Fig. 5: Phylogenetic tree showing the relatedness of the isolates from commercial manual blenders.

DISCUSSION

Blending has gained more domestic uses than ever before, the unhygienic practices of the machines and other activities carried out with them are implicated as vehicles of food pathogens. These practices are monitored in terms of effectiveness to improve health and prevent the outbreak of diseases. Compromising safety practices always results in establishment and proliferation of pathogenic as well as detriogens on contact surfaces, processing and storage of food products. A wide array of food surfaces come under attack by surface-contaminated microorganisms, either by the farmers or retailers, who come in direct contact with most foods before they get to the end users (Xaba and Masuku, 2013).

This study observed a viable count ranging from 4.6 to 5.0 10⁵ CfU/ml. This range of values agreed with the earlier report of Bren *et al.* (2013) who reported a viable count of 7.0 log CfU/ml on blenders used in Lagos metropolis. But, similar study by Feglo and Sakyi, (2012) indicated a much lower bacterial infestation with a load of about 2.2 Log CfU/ml when they studied the microbial quality of food mixers. The materials being constantly ground with these small hand blenders are processed food substances, while some people have used them to grind some "hard leaves" which ordinarily they would have subjected to pounding. This could be attributed to the significant microbial load, probable cross-contamination from the food crops and market environment which increased the high microbial load (Argundin *et al.*, 2010; EFSA, 2012; USFDA, 2009). Blending have gained more domestic uses than ever before and have been documented as vehicles of food pathogens.

Bacillus sp., *Staphylococcus* sp. and *Pseudomonas* sp. were among the most frequent potentially pathogenic spoilage bacteria isolated from the blenders sampled that can be found in food substances. This confers with the findings of a similar study reporting a predominance of *Bacillus* sp. and *Enterobacter* sp. (Lani *et al.*, 2014). These enteric pathogens have the ability to cause infections, other organisms such as *Staphylococcus* sp. and *Micrococcus* sp. have been reported to contaminate foods from food surface-contact contamination are known to cause food intoxication with the production of staphylococcal and bacillary enterotoxins by the bacteria (Di Giannatale *et al.*, 2012; Erolini *et al.*, 2006).

PCR-amplification of the sample amplicons and the phylogenetic tree revealed a band size representative of *Ochrabacterium intermedium*, *Pseudomonas aeruginosa*, *Candida tropicalis* and *Pichia kudriavzevii* using the gel electrophoresis approach revealed band width technically similar with earlier reports (Stenfors-Arnesen *et al.*, 2008). *Ochrabacterium intermedium* are non-lactose fermenting, Gram-negative rods that are closely

related the *Brucella* sp. They are generally regarded as emerging human environmental opportunistic pathogens with mild virulence (Bharucha, *et al.*, 2017; Aujoulat *et al.*, 2014). *Pseudomonas aeruginosa* is an organism which is normally found in the soil, water and most man made environment but also thrives in normal and low-oxygen environment. The organism aggregates into enduring biofilm, capable of extensive colonization, has the ability to decompose hydrocarbon, breaks down detergent and oil from oil spillage (Itah and Essien, 2005; Hoiby *et al.*, 2010). The infections are transmitted during contact with water or soils contaminated with the organism or through contaminated hands, equipments, surfaces etc. *Candida tropicalis* is specie of yeast which belongs to the genus *Candida*, an osmotolerant, persuasive organism found in mammals, plants, temperate and humidified environment, as well as some food samples with high level of biofilm producer (Zuza-Alves *et al.*, 2017). *Pichia kudriavzevii* is a sexual reproductive form of *Candida krusei* which is found in soil, vegetables, fruit, some fermented beverages and is mainly associated with food spoilage causing surface biofilms in samples with low pH products (Kurtzman *et al.*, 2011; Chan *et al.*, 2012). The organism can be present without causing harm, an opportunistic pathogen in immunodeficient patients leading to symptoms such as fever, sepsis, high lactate and glucose level in the blood, high pulse rate and hypothermia (Nagarathnamma *et al.*, 2017).

Plasmid profiles as seen on the gel band indicate the plasmids were of the same size 10kb. The size suggests the plasmids are of high copy number and conjugative in nature but may be incompatible to themselves (Norman *et al.*, 2015). The sizes of plasmids in this indicate the surface pathogens have a vast array of resistance mechanisms. The band size of replicons suggest they may not co-exist in a vector all together, but may codes for resistance mechanisms like adaptation to harsh environmental include reduced cell wall permeability, cyst and spore formation, production of chromosomal and plasmid mediated β -lactamases, aminoglycoside-modifying enzymes and an active multidrug efflux mechanism (Oyeleke *et al.*, 2012; Samapundo *et al.*, 2011).

CONCLUSION

The findings of the study showed a significant amount of heterotrophic bacteria growth in the blenders indicating the presence of cross-contamination among the blenders. The plasmid identified is an indication of the presence of antibiotic-resistant species of *Pseudomonas*. It is imperative to improve the hygienic quality of these commercial blenders and prevent the cross-contamination of food samples by cleaning and disinfecting them after use on a food product. As well as educating the food vendors in the market using these blenders on public health awareness, proper hygiene practices to be adopted so as to avoid disease

outbreak and cross contamination that can be severely devastating and uncontrollable.

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BACTERIOLOGICAL STUDY OF DRINKING WATER FROM MUBI-NORTH, GIREI AND MAYO-BELWA LOCAL GOVERNMENT AREAS OF ADAMAWA STATE NIGERIA

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Abstract

The aim of this research is to determine some bacteriological composition in drinking water (wells and bore-holes) at Mubi-north, Girei and Mayo-Belwa local government areas of Adamawa state, Nigeria. Sample collection was done as follows; 13.0 g of nutrient broth was dissolved in 1000 ml of distilled water in conical flask. Sterilization followed by autoclaving at 121°C for 15 minutes after which the solution was allowed to cool. 9ml of the solution was then injected into already sterilized sample bottles. At the sites, 1 ml of each sample was injected and transported to the laboratory. 108 samples were collected between August-October 2018 and January-March 2019 across the study areas. For the determination of *E. coli* bacteria, EMB was used, *total coliform*, nutrient agar was used and *Salmonella species*, SS agar was used. Colony morphology were counted using microscope. Data analyzed using ANOVA SSP. Result's shows that in well water during rainy season the mean concentration of *salmonella spp.* ranged from (153– 272 CFU/ml), highest *salmonella spp.* value was recorded in Mayo-Belwa. During dry season, highest value was recorded in Mubi- North. *Total coliforms*, well water during rainy season mean concentration ranged from (418 – 624 CFU/ml), a highest mean count was recorded in Girei. During dry season, values ranged from (81 – 126 CFU/ml), highest mean counts was recorded in Mubi-North, while for *E.coli*, in well water during rainy season, the mean *E. coli* values ranged from (33 – 71 CFU/ml), highest mean concentration Counts was recorded in Girei. During dry season ranged from (97. – 131 CFU/ml), highest mean Counts were recorded in Mubi-North. Conclusively, findings indicates significant higher bacteriological composition in well water during rainy season than dry season ($p<0.05$). Hence, calls for monitoring by the concerned agencies.

Keywords: Bacteriological Composition, Salmonella ssp, Total Coliform, E.Coli and Count

Introduction

Water is essential to human life and the health of the environment. As a valuable natural resource, it has two dimensions that are closely linked, these are quantity and quality. Water quality is commonly defined by its physical, chemical, biological and aesthetic (appearance and smell) characteristics. Water quality is a tool for water quality managers to protect public health, support the economy and maintain a rich ecosystem. The population growth and development of every community requires availability of quality ground water such as rivers, streams, lakes, wells, boreholes, spring etc. Since lives depend solely on it. In most sub-Saharan African urban-rural communities, water is mostly used for domestic purposes (Dimowo, 2013).

A health environment is one in which the water quality supports a rich and varied community and protects public health. Our water resources are of major environmental, social and economic value to human and if water quality becomes degraded this resource will lose its value. Water quality is important not only to protect public health, water provides ecosystem habitats, is used for farming, fishing and mining, and contributes to recreation and tourism. If water quality is not maintained, it is not just the environment that will suffer; the commercial and recreational value of

our water resources will also diminish (EPA, 2017)

Water quality is closely linked to the surrounding environment and land use. Other than in its vapor form, water is never pure and is affected by community uses such as agriculture, urban and industrial use, and recreation. The modification of natural stream flows by dams and weirs creation can also affect water quality. Weather can also have a major impact on water quality, particularly dry countries which are periodically affected by droughts. Water quality frequently declines as rivers flow through regions where land and water use are intense and pollution from intensive agriculture, large towns, industries and recreation areas increases.

The presence of contaminants and the characteristics of water are used to indicate the quality of water. These water quality indicators can be categorized as: biological - bacteria and pathogens. Physical-temperature, turbidity and clarity, colour, salinity, suspended solids, dissolved solids, chemical - pH, dissolved oxygen (DO), biological oxygen demand (BOD), nutrients (including nitrogen and phosphorus), organic and inorganic compounds (including toxicants), aesthetic - odours, taints, colour, floating matter and radioactive - alpha, beta and gamma radiation emitters. These are the most common

standards used to assess water quality relating to health of ecosystem and safety of human contact (Nancy, 2013). Water quality depends on the local geology and ecosystems as well as human uses such as sewage dispersion, industrial pollution, use of water bodies as a heat sink, and over use (which may lower the level of the water) (Shingo, 2012).

Normally, water required for domestic consumption should be of high quality, possess high degree of purity and should be free from suspended and dissolved impurities, bacteria, pathogens, heavy metals, toxic organic compounds etc. both dug wells and boreholes water are expected to be less contaminated. However, there are possibilities of introduction of contaminants, depending upon management and the temperature gradient of the water environment (Priscilla, 2010).

Water related diseases are the largest cause of human sickness and death in the world and disproportionately affects the poor. Without water people cannot leave healthy and productive lives. Improved adequate safe water supply will lead to reduction of human suffering, improved productive capacities that at the end will reduce health care cost. This can only be realized if water is set at the border context of health objectives, it should be noted however that access to an improved source of water does not necessarily imply that it is safe to drink from that source (Bain *et al.*, 2014). Preliminary investigation revealed that majority of the rural communities in Nigeria do not have improved water supply systems such as piped water networks or boreholes, where such facilities exist are either malfunctioning or completely broken down and this forces households to rely on available sources for domestic purposes. Women and children especially spend their productive and considerable time seeking for water in far distances from their homes. They rely slowly on self-water supply sources. Households with poor access to improve water supply are vulnerable to many health problems. The role of water as a vehicle for the transmission of all manner of water related illnesses is no longer a subject for debate, even ancient histories and books contain extracts indicative of this fact (Nwabor *et al.*, 2016). The 2010 outbreak of cholera and gastroenteritis and the attendant deaths in some regions in Nigeria brought to the forefront the vulnerability of poor communities and most especially children to the infection. The outbreak was attributed to rain which washed sewage into open wells and ponds, where people obtain water for drinking and household needs. The regions ravaged by the scourge include Jigawa, Bauchi, Gombe, Yobe, Borno, Adamawa, Taraba, FCT, Cross River, Kaduna, Osun and Rivers. Even though the epidemic was recorded in these areas, epidemiological evidence indicated that the entire country was at risk, with the postulation that the outbreak was due to hyper-virulent strains of some organism (EPA, 2012).

Report from Adamawa state primary health agency showed the following as the figures representing water borne diseases from year 2008 to date. 2008: Cholera 00, Diarrhea 3221. 2009: Cholera 509, diarrhea 2, 035. 2010: cholera 106, diarrhea 2, 307 and typhoid 369. 2011: Cholera 00, Diarrhea 2, 525 and typhoid 298. 2012: cholera 00, diarrhea 3, 644 and typhoid 519. 2013: dengue 181, Diarrhea 38, 884, and dracunculiasis (guinea worm disease) 07. 2014: dengue 00, Diarrhea 30, 481, and dracunculiasis (guinea worm diseases) 74. 2015: dengue 00, diarrhea. 2016: no diseases outbreak was reported and 2017, dengue 2, diarrhea 2, 869, dracunculiasis (guinea worm diseases) 11 and diphtheria were reported. Based on these reports, this research aimed at determining the presence of some bacteria such as salmonella species, *Escherichia coli* form and total coliform in drinking water samples collected from the study areas with the view to profile possible solution to the problems caused by such organisms when present.

MATERIALS AND METHODS

Study areas: The study areas for this research covered the following local government areas in Adamawa state: Mubi-North, Girie and Mayo-Belwa located in the north eastern part of Nigeria and lies between latitude 7° and 11°, north of the equator and between longitude 11° and 14° east of the Greenwich meridian (Sanusi, 2017 cited Adebayo, 1999).

Mubi-North north local government area, well 1 is located at Shuwari Garden city (Sabon -layi) on latitude N100.16.045' and longitude E13.16.716', well 2 on latitude N100.16.591' and longitude E13.16.174', well 3 at Anguwan Qarkeje on latitude N100.16.162 and longitude E 13. 15. 667. Bore-hole point 1 is located at Shuware Qura'anic memorization school on latitude 100.16.311' and longitude E100.16 623' and longitude 130.16.311'. bore-hole 2 in Shuware primary school located on N100.16.667' and longitude E130.15, 119', bore-hole 3 at Coke-cola deport, Shagari low cost on latitude N100.16.162 and longitude E130. 15.661. In Girie local government area, well 1 is located in Bajabure, Latitude N 90 18. 340' and longitude E120 27.859', well 2 is located Latitude 90 18.278 and longitude 120 27.859, well 3 is located on latitude No90.18.161' and longitude E120 27.922', bore-water 1 is located Samunaka Bajabure on latitude No90 18. 027' and longitude E0120.28.087', bore-hole 2 is located on latitude N9018.152 and longitude E0120.27.885', bore-hole 3 is on latitude 90.18.320' and E12027.829. Mayo-belwa local government area well 1 location is Labbare on latitude N9 0.06, 566 and 120.04.617', well 2 is located at Anguwan fada on latitude N90.03.663' and E120.03.138' well 3 at Labbare 11 on latitude N90.06.639' and longitude E120 04.671, bore-hole 1 at Masagamare Sabonlayi on latitude N90.05.402' and longitude E120.04.436', bore-hole 2 at kofar fada on

latitude N90 03.738' and E120 03.249', bore-hole 3 at 03.391
Wakili Buba ward on latitude N90 03.420' and E120



Samples for bacteriological components study.

Samples were collected as follows; 13.0 g of nutrient broth was dissolved in 1000 ml of distilled water in conical flask. The solution was gently heated to

dissolve completely to a clear solution. Sterilization was carried out by autoclaving at 121°C for 15 minutes using autoclave after which the solution was allowed to cool to room temperature. 9ml of the solution was

then injected into already sterilized sample bottles and taken to the sampling sites. At the sites, 1 ml of each sample was injected into each 9ml sample bottled and properly labeled for onward transportation to the laboratory (Sulaiman et al. 2013)

Media preparation and determination of bacteria in water samples

Three different media were prepared adhering strictly to the media manufacturer's instruction. For the determination of *E. coli*, 24g of EMB was weighed, transferred into 500ml flask and dissolved in 380ml of distilled water, properly swirled until a homogenous mixture was obtained. This was boiled at a temperature of 100^oc for 15minutes. It was allowed to cool to approximately 45^oc. After cooling, 18ml of the media poured into each plate (earlier sterilized in an oven at regulated temperature) and was allowed to solidify. Samples inoculation followed and was allowed to stand for 20 -30minutes. The samples were put into an incubator and incubation was allowed to take place for 18-24 hours at 37^oc. Colonies were allowed to develop. Counting was carried out through direct examination using a microscope (Suleiman & Michael, 2013). The same method was used to determine total coliform in the samples but this time nutrient agar was weighed according to the manufacturer's instructions.

For Salmonella species SS agar was used. 60 g of the medium in one liter distilled water was mix well, heat with frequent agitation and boil for one minute. Sterilization in autoclave at 121^oC was done. The resulting media was poured into already sterilized plates i. e. after allowing the plates to warm to room temperature and the agar surface to dry, inoculation followed and samples incubation was carried out at 35-37^oC 18-24 hours. Colony morphology were then examined and counted using microscope.

RESULTS AND DISCUSSION

Bacteriological components in the drinking water are important factor that can be used to determine the quality of drinking water. According to Ekelozie et al. (2018), bacteriological components are more important than physical and chemical parameters in term of direct effect on human health. The mean scores of bacteriological components for the sampled well and bore water both during the rainy and dry seasons in the study area (Mubi-North, Girei and Mayo-Belwa local government area) are presented in the following Figures respectively.

Mean concentration of salmonella spp. in Drinking Water in the Study Area during rainy and Dry Season
The mean concentration of salmonella spp. in the

respective well and borehole water during rainy and dry seasons are presented in Figure 1 below in respect to the local government area. The results showed that in well water during rainy season the mean concentration of salmonella spp. ranged from (153–272 CFU/ml), the highest salmonella spp. value was recorded in Mayo-Belwa, while the least value was recorded in Girei. Also, the salmonella spp. value during dry season for well water across the sampled local government area ranged from (25 – 37 CFU/ml), the highest salmonella spp. value was recorded in Mubi- North local government area, while the least salmonella spp. value was recorded Girei local government area. The analysis of variance of salmonella spp. values in well water during rainy and dry season for three local government areas showed significant difference at $p<0.05$.

The mean concentration of salmonella spp. for borehole water in the study area during raining season ranged from (47 – 113 CFU/ml). The highest mean concentration of salmonella spp. was recorded in Mayo-Belwa while least value was recorded in Girei local government area. During dry season, the mean concentration of salmonella spp. recorded in borehole water ranged from (11 – 44 CFU/m). The least mean concentration of salmonella spp. was recorded in Girei local government area while the highest value was recorded in Mubi-North. The analysis of variance of salmonella spp. values in borehole water during rainy and dry season for three local government areas showed significant difference at $p<0.05$.

Studies by Levantesi et al. (2012) and Eriksson-de-Rezende et al. (2014) recorded higher salmonella spp. in water in rainy season than dry season. Also, McEgan et al. (2014) reported higher salmonella spp. in borehole water during rainy season than dry season and higher value was attributed to higher human and animal activities within their study vicinity that heighten the infection of Salmonalla spp in the water. Though, Dhino (2016) argued that not everyone who ingests Salmonella bacteria will become ill. Children, especially infants, are most likely to get sick from it. Study by Sears et al. (2014) reported that about 50, 000 cases of salmonellosis are being reported in the United States each year and about one third of those affected are kids in 4 years old or younger. Adult may not necessarily being affected when infected by salmonella spp. However, according to Rhodes and Kator (2012) a severe Salmonella infection in adult could require more tests to see which specific germ is causing the illness and which antibiotics can be used to treat it.

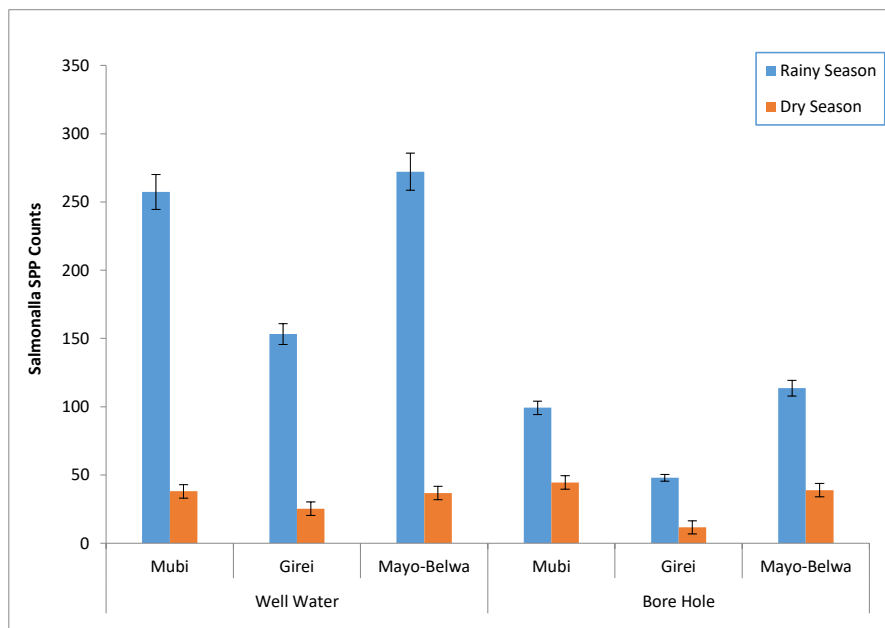


Figure 1: Mean concentration of salmonella spp. in Drinking Water in the Study Area during rainy and Dry Season

Mean concentration of total coliforms Counts in Drinking Water in the Study Area during rainy and Dry Season

The mean concentration of total coliforms counts for the respective well and borehole water during rainy and dry seasons are presented in Figure 2 below in respect to the local government area. The results showed that in well water during rainy season the mean concentration of total coliforms counts ranged from (418 – 624 CFU/ml), the highest mean concentration of total coliforms counts was recorded in Girei, while the least value was recorded in Mayo-Belwa local government area. Also, the mean concentration of total coliforms counts recorded during dry season for well water across the sampled local government areas ranged from (81 – 126 CFU/ml), the highest mean concentration of total coliforms counts was recorded in Mubi-North, while the least value was recorded in Girei local government area. The analysis of variance of total coliforms values in well water during rainy and dry season for three local government areas showed significant difference at $p < 0.05$.

The mean concentration of total coliforms counts for borehole water in the study area during raining season ranged from (288 – 435 CFU/ml), the highest mean concentration of total coliforms counts was recorded in Girei local government area while least value was recorded in Mubi- North local government area

(Figure 2). During dry season the mean concentration of total coliforms counts in borehole water ranged from (84 – 107 CFU/ml) and the least mean concentration of total coliforms counts was recorded in Mayo-Belwa local government area while the highest mean concentration of total coliforms counts was recorded in Mubi- North local government area. The analysis of variance of total coliforms values in borehole water during rainy and dry season for three local government areas showed significant difference at $p < 0.05$.

This finding agrees with that made by Adednego et al. (2013) who recorded high total coliforms counts in well water during rainy season than dry season. Also, study by Nemade et al. (2012) reported significant high total coliforms count in borehole water in rainy season than dry season ($p < 0.05$). Their study attributed high total coliform counts to the effect of erosion during rainy season which might have eroded contaminated human and animal waste into drinking water. This agrees with the view held by Olalekan et al. (2015) that the high total coliform counts are due to water pollution caused by fecal contamination. Carreira et al. (2014) expressed that one of the way a well water or borehole could be contaminated with coliform counts is when there is seepage of contaminant through well casing- cracks or holes in the well casing allow water that has not been filtered through the soil to enter the well. This seepage is

common in the wells made of concrete, clay tile, or brick. Kerr and Butterfield (2013) expressed the presence of total coliform counts in drinking water is a serious problem due to the potential for contracting diseases from pathogens. Ali et al., (2012) suggested

that whenever coliforms have been detected, boiling, repairs or modifications of the water system may be required until disinfection through re-testing confirm that contamination has been eliminated.

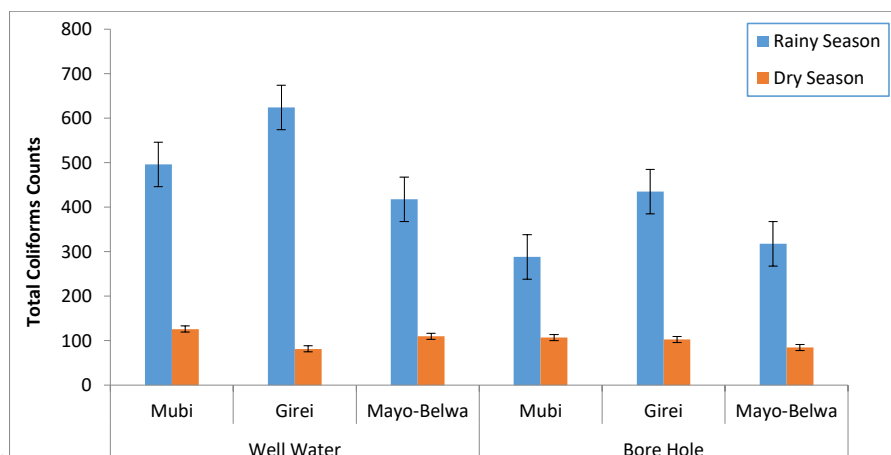


Figure 2: Mean concentration of total coliforms Counts in Drinking Water in the Study Area during rainy and Dry Season

Mean concentration of *E. coli* Counts in Drinking Water in the Study Area during rainy and Dry Season Figure 3 below presents the results on the mean concentration of total *E. coli* Counts in the respective well and borehole water during rainy and dry seasons across the Mubi-North, Girei and Mayo-Belwa local government area of Adamawa state respectively. The results showed that in well water during rainy season, the mean *E. coli* values ranged from (33 – 71 CFU/ml), the highest mean concentration of total *E. coli* Counts was recorded in Girei local government area, while the least value was recorded in Mubi-North local government area. Also, the mean concentration of total *E. coli* Counts recorded during dry season for well water across the sampled local government areas ranged from (97. – 131 CFU/ml), the highest mean concentration of *E. coli* Counts was recorded in Mubi-North, while the least value was recorded in Mayo-Belwa local government area. The analysis of variance of total *E. coli* Counts in well water during rainy and dry season for three local government areas showed significant difference at $p < 0.05$.

The mean concentration of *E. coli* counts for borehole water in the study area during raining season ranged from (9 – 94 CFU/ml), the highest mean concentration of total *E. coli* Counts value was recorded in Girei local government area while least value was recorded in Mubi- North local government area. During dry season the mean concentration of total *E. coli* Counts in borehole water ranged from

(107 – 136 CFU/ml), the least mean *E. coli* value recorded in Mubi- North local government area while the highest value was recorded in Girei local government area. The analysis of variance of *E. coli* counts in borehole water during rainy and dry season for three local government areas showed significant difference at $p < 0.05$.

The current finding concur with that made by Klein (2013) which indicated significant higher *E. coli* in well water during rainy season than dry season ($p < 0.05$). Also, studies by Rhodes and Kator (2012) and Nemade et al. (2012) recorded a significant higher *E. coli* in borehole water in different sites during rainy season than it being recorded during dry season ($p < 0.05$). The studies attributed the higher *E. coli* to higher erosion activities that eroded most of contaminated soils into drinking water. Also, studies by Brenner et al (2013) and Robin (2013) expressed that most *E. coli* bacteria are harmless and exist in the intestines of people and warm-blooded animals. This also agrees with the view held by Brenner et al. (2016) that *E. coli* is a common bacteria found in the digestive system of both humans and animals, which organically not a cause for concern. Kerr and Butterfield (2013) expressed that one of strains from *E. coli* is responsible for causing Traveler's diarrhea and the second is *E. coli* O157:H7, which contaminates meat and leafy vegetables. This strain (O157:H7) can cause serious hemorrhagic diarrhea and can have long term, if not fatal, complications. Thus, the presence of *E. coli* in a drinking water

sample usually indicates recent fecal contamination. That means there is a greater risk that pathogens are present. This agreed with the view held by Buntel (2015) that confirmation of E. coli in a water system indicates recent fecal contamination, which may pose an immediate health risk to anyone who consumes the water. Al-Abadi (2014) hinted that in most cases,

where E. coli are usually presence in drinking water boiling or disinfecting contaminated drinking water destroys all forms of E. coli, including O157:H7.

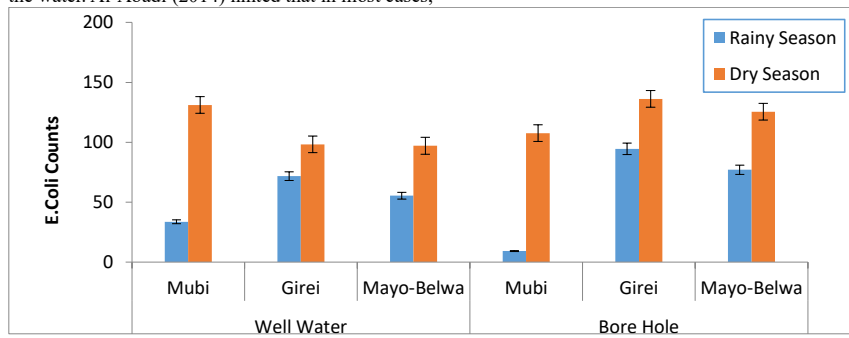


Figure 3: Mean Concentration of E. Coli Counts In Drinking Water In The Study Area During Rainy And Dry Season

Conclusion

Conclusively, the current finding indicated significant higher bacteriological composition in well water during rainy season than dry season ($p < 0.05$). Hence, calls for monitoring by the concerned agencies.

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SEASONAL VARIATIONS IN PM_{2.5} AIR POLLUTANTS AND IMPACT ON PRETERM BIRTH RATE IN KAMPALA: A RETROSPECTIVE COHORT

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Abstract

There is a growing body of evidence linking air pollution to negative births outcomes, but few studies have explored the relationship of air pollution on negative births outcomes such as preterm birth in Africa. Identifying the association between PM_{2.5} and preterm births will be a good starting point for intervention and policy changes on air pollution reduction methods. The study aimed to determine the association between seasonal variation in PM_{2.5} and preterm births in Kampala Uganda. Retrospective cohort study was carried out by collecting birth outcomes data from District Health Information System 2 (DHIS2) database, with the authority of Kampala Capital City Authority (KCCA). PM_{2.5} data were collected from US Embassy monitoring station from January 2018 to December 2019. Association between seasonal variation in PM_{2.5} and preterm births were found using STATA 13.0 version. This study showed a seasonal trend in PM_{2.5} level which was very high between the range of 56-72 µg/m³ in the two dry seasons of the year 2018 and 2019. It was found lower in both wet seasons of the year 2018 and 2019 within the range of 40-52 µg/m³. The regression analysis showed that there is a strong association between each 10 unit increase in PM_{2.5} and preterm births in second dry season of 2018 and the first dry season of 2019 (p<0.000). The results of this study support with evidence the relationship between PM_{2.5} air pollutant and preterm births, especially in dry seasons.

Keywords: Air Pollution, Preterm Births, PM_{2.5}, Dry Season, Africa

Introduction

Globally, there is an increased rate of negative births outcomes and identifying the underlying causes of negative births outcomes such as preterm birth and low birth weight are of major public health concern (Han et al., 2018). Annually, about 15 million children are born preterm globally. In Uganda, about 200,000 babies are born preterm every year (Ayebare et al., 2018; Liu, W. et al., 2018; MoH Uganda, 2019; WHO, 2018).

Annually, about one million children die due to complications of negative birth outcomes such as preterm birth and low birth-weight globally. Many who survived suffers lifetime disabilities such as learning disability, hearing impairment and vision problems (Guo et al., 2018; Ye et al., 2018; Wagura et al., 2018). Several factors can be attributed to negative births outcomes, these include; socioeconomic, maternal age, ethnic, sex of the babies and environmental factors (Ameh et al., 2016; Asiki et al., 2015). Studies have shown that there is a relationship between air pollution and negative births outcomes such as; stillbirth, low birthweight, birth defect and preterm births (Guo et al., 2018; Tan et al., 2017). Despite this growing body of knowledge, the results of the effect of exposure to pollutants like PM_{2.5} on preterm birth are inconsistent and very rare or few studies have been conducted in Africa (Tan et al.,

2017; Cheng, 2016; Choe et al., 2019). The lack of clarity can be attributed to research design, geographical location and study population. Pieces of evidence already exist on the relationship between PM_{2.5} pollutants and other negative births outcomes, but little is still known about the relationship between PM_{2.5} pollutant and preterm birth in Uganda.

Particulate matter less than 2.5 (PM_{2.5}) are fine particles which can be inhaled, with a diameter of 2.5micrometers or less. The particle is 30 times smaller than a strand of human hair which is about 70micrometers in diameter (EPA, 2018). It is generated from dust during construction activities or unpaved road and also, through complex chemical reactions from energy and industrial activities. PM_{2.5} has also been found to increase gestational weight in pregnant women (Liao et al., 2018). In many studies, PM_{2.5} exposure has been associated with preterm birth, stillbirth and low birth weight (Guo et al., 2018; Ye et al., 2018; Cheng 2016; Dastoorpoor et al., 2018; Han et al., 2018; He et al., 2018). These are mostly caused by oxidative stress in the matrix which later affects the fetal development. PM_{2.5} pollutant is capable of combining with placenta growth receptor and cause an inflammatory response of the placenta, hindering the exchange of oxygen nutrient of fetal placenta. It also alters coagulation function and hemodynamic; these mechanisms are capable of affecting the exchange of

nutrients between the placenta and fetus (Tan *et al.*, 2017).

Preterm birth (PTB) or delivery is defined as delivery of a baby before 37 weeks gestational age. It is the leading cause of neonatal mortality globally (Tan *et al.*, 2017; WHO, 2020). It accounts for 35 percent of neonatal death. Mainly because the causes of PTB are poorly understood, many local clinicians and community have failed to reduce PTB and the rates keep increasing in developing countries and even in some developed countries (Tan *et al.*, 2017). Any productive effort in reducing PTB must target any associated risk factors. Studies in African countries have reported an association between PTB and risk factors such as; socioeconomic status, antenatal attendance and genetic influence (Ayebare *et al.*, 2018). Little is still known in Africa on the association between PTB and environmental exposure like PM_{2.5}. This research focused on the seasonal relationship between PM_{2.5} and preterm births in Kampala, Uganda.

Methods
Study Area

Kampala is an urban city and the capital of Uganda. It has a population of about 2 million, with an estimate of about 781,700 males and 869,100 females (UBOS, 2020). It is a growing industrialized area, with high traffic volume and other factors that contribute to air pollution. The pollution level in Kampala is mostly found to be 5 times higher than World Health Organization limits (WHO, 2020) and has been described as unhealthy.

Data
The data includes live births that occurred in selected 21 health-facilities in Kampala district between the months of January 2018-December 2019. The facilities are less than 10 km away from the PM_{2.5} monitoring station in US embassy Kampala (Choe *et al.*, 2012), using satellite map of Kampala. The birth outcome data were assessed through the antenatal and maternal record from the district health information system (DHIS2) database through the directorate of public health and environment, Kampala Capital City Authority (KCCA) (DPHE/KCCA/201/17). The study data included a monthly record of total delivery and preterm birth delivery from the 21 selected health facilities (see figure 1 below).

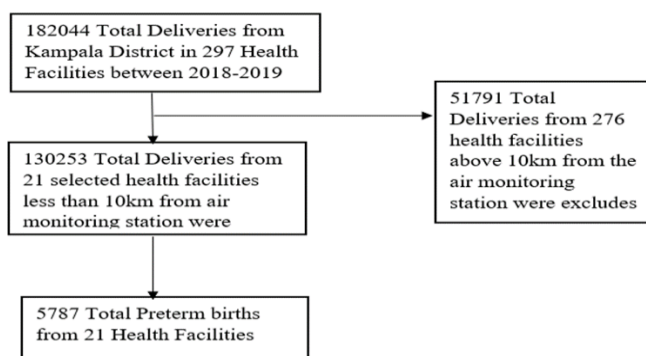


Figure 1: Flowchart of the study Population

Exposure Assessment

Daily average PM_{2.5} data for Kampala city from January 2018 to December 2019 was obtained online from the historical PM_{2.5} data made available by U.S. embassy monitoring station (US Embassy, 2019). To estimate seasonal variation in PM_{2.5}, the whole year was divided into two dry seasons (December to February as Dry1 and June to August as Dry2) and two wet seasons (March to May as Wet1 and September to November as Wet2) (Liu *et al.*, 2019). To evaluate the relationship between PM_{2.5} and preterm births, we matched monthly average PM_{2.5} exposure level to pregnancy outcomes, based on the monthly delivery report collected from DHIS2 database through KCCA. This was used to test the hypothesis that as PM_{2.5} air

pollutant increases, preterm birth delivery also increases (Smith *et al.*, 2020).

Statistical Analysis

A time series analysis was conducted using a generalized linear model (GLM). The GLM is useful in medical literature for finding correlation of data when modelling binary or count data, because of the quality of reporting that has room for improvement regarding the characteristics of the analysis, estimation method, validation and selection of the model (Casals *et al.*, 2014). The GLM was used because the outcome of interest (PTB) is a count variable and Poisson regression were performed to evaluate the association between PM_{2.5} pollutants and preterm births for each

season of 2018 to 2019. However, overdispersion was adjusted for in the model using Pearson chi-square, because the mean and SD of the outcome variable was not found equal as it should be in Poisson regression model (Dastoorpoor *et al.*, 2018; Bhaskaran *et al.*, 2013). STATA 13.0 version was used for the regression model and PM_{2.5} variable was divided by 10, to allow for proper explanation of the model estimates per 10ug/m³ increase. The confidence level was set at 95% and p<0.05 was considered to be significant (Guo *et al.*, 2018; Bhaskaran *et al.*, 2013). In this study the monthly average concentration of PM_{2.5} and the monthly number of preterm births at the 21 selected health facilities from January 2018 to December 2019 were grouped equidistant and considered as the terms of transactions (Zhu *et al.*, 2017).

Results
Prevalence of Preterm Births in the selected health facilities

The health outcome data collected which included the number of deliveries from the 21 selected health facilities in Kampala city were found to be 130253 for the study years (72495 and 57758 for 2018 and 2019 respectively). The preterm births rate was found to be 5787 for the two study years (4241 and 1546 for 2018 and 2019 respectively) (see figure 1 above), while the prevalence level was calculated to be about 5.8% and 2.7% for 2018 and 2019 respectively. Table 1 presents the description of the time series data of the seasonal records for both preterm births and PM_{2.5} air pollutant and figure 2 shows the trend in preterm births and PM_{2.5} over time.

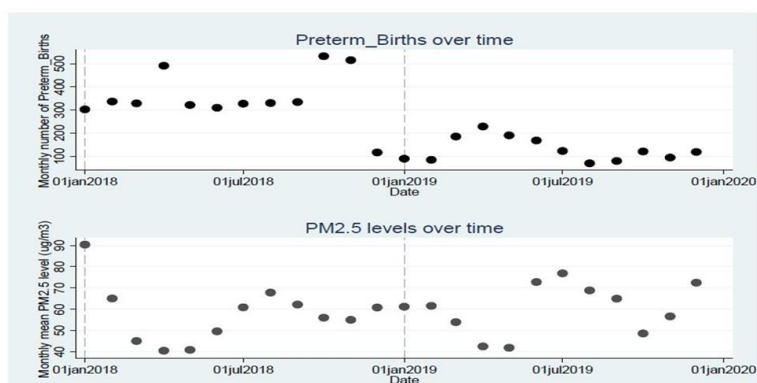


Figure2 Trend in Preterm births and PM2.5 levels in Kampala District

Table 1 Rows description of time series data from Kampala district showing monthly levels of environmental variables and monthly number of preterm births

Date	PM _{2.5} (ug/m ³)	n Preterm_Births
Jan-18	90.37062937	302
Feb-18	65.0327381	336
Mar-18	44.9992163	328
Apr-18	40.44005642	491
May-18	40.83467742	321
Jun-18	49.56732026	309
Jul-18	60.84090909	327

Seasonal Variation in PM2.5 Concentration Level

In 2018 the average concentration level of PM_{2.5} was found to be 72 µg/m, 59 µg/m, 43 µg/m and 57 µg/m for Dry1, Dry2, Wet1 and Wet2 respectively (as shown in Figure 3). The order of increase of PM_{2.5} in 2018 was found to Dry1>Dry2>Wet2>Wet1.

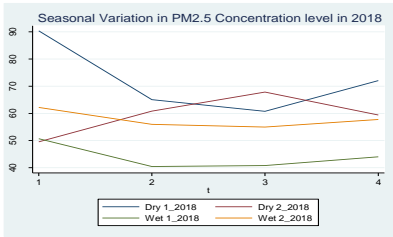


Figure 3: Seasonal variation in PM2.5 Concentration level in kampala, 2018
 In 2019 the average concentration level for PM2.5 level was found to be 65 µg/m, 72 µg/m, 46 µg/m and 56 µg/m for Dry1, Dry2, Wet1 and Wet2 respectively (See Figure 4 below). The order of increase of PM2.5 in 2019 was found to Dry2>Dry1>Wet2>Wet1.

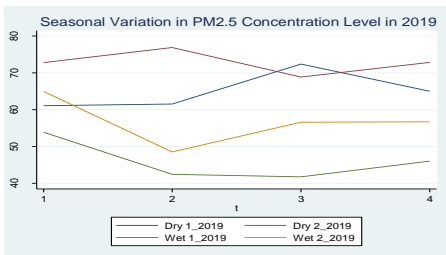


Figure 4 Seasonal Variation in PM_{2.5} Concentration level in Kampala city, 2019

Association between PM_{2.5} concentration level and Preterm Births

The association between PM_{2.5} and preterm births was found to be significant during the Dry2 of 2018, with each 10ug/m³ increase in PM_{2.5} associated with

Table 2 Odd ratio (95% confidence interval) of preterm births associated with PM_{2.5} air pollutant levels

PM _{2.5} Seasonal Association with Preterm Births	Odd Ratio (OR)	95% Confidence interval	p-value
Dry2_PM_2018/ Dry2_PB_2018	11.97	(5.89-18.06)	0.000
Dry1_PM_2019/ Dry1_PB_2019	28.19754	(17.62-38.77)	0.000

Discussion

This study further supports the evidence by WHO and other research on the increase in the prevalence of preterm births, which has been estimated to be 5-18% of every newborn (WHO, 2018; Wagura *et al.*, 2018). The prevalence of preterm births from the selected health facilities was found to be 5.8% and 2.7% for 2018 and 2019 respectively, these findings support previous work on increase in the burden of preterm birth in Uganda (Ayebare *et al.*, 2018; MoH, Uganda,

higher chances of preterm births, OR = 11.97 (95% CI 5.89-18.06, P< 0.000) and in 2019 it was found to be significant during Dry2, with each 10ug/m³ increase in PM_{2.5} is associated with OR=28.19754(95% CI 17.62-38.77, p<0.000) (see Table 2 and Figures 5 to 6 below).

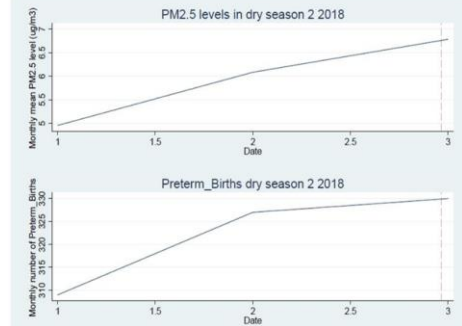


Figure 5 Association between PM_{2.5} level and Preterm Births Rate in the second Dry season of 2018

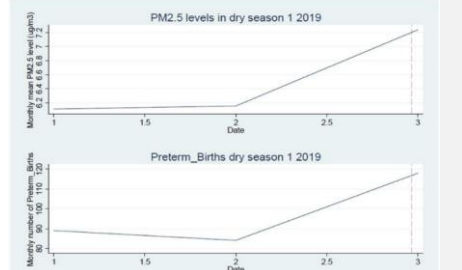


Figure 6 Association between PM_{2.5} level and Preterm Births Rate in the first Dry season of 2019

2019). This should be of serious concern because of the high neonatal mortality rate of 27.9% that has been attributed to preterm births in Uganda (UNICEF, 2015).

Similarly, the air pollution level in Uganda is Odd Ratio (OR) (95% p-value increasing Confidence interval) beyond the standard limits recommended by the World Health

Organization (WHO) and the Environmental Protection Agency (EPA). The PM_{2.5} air pollutant level was found to be between 50-72ug/m³ in the two dry seasons of the 2018 and 2019 and between 43-59ug/m³ in the wet seasons of the two years under this study as shown in figure 2. These concentration levels in both dry and wet seasons are found to be unhealthy (151-200 AQI category) for sensitivity groups and can cause everyone to begin to have health effects (AirNow, 2019). The standard limit for PM_{2.5} in a year is

25 $\mu\text{g}/\text{m}^3$, but the PM_{2.5} level in Kampala exceeds this limit (WHO, 2020).

Many studies in Africa have related preterm births with other factors, such as antenatal attendance, socio-economic status, education level, and other maternal risk factors. Few studies have considered the effects of air pollution on preterm births and to the best of our knowledge, this is the first study in Uganda to find an association between the seasonal variations in PM_{2.5} pollutant and preterm births. This study further confirms the evidence from previous studies in other nations, that increase in air pollutant might cause an increase in preterm births. Such studies include; (Ha *et al.*, 2014) in Florida, USA, (Choe *et al.*, 2019) in South Korea, (Guo *et al.*, 2018, Ye *et al.*, 2018), in China and also (Ye *et al.*, 2018) in Taizhou, China

In Figures 5-6 some levels of association were identified in the trend of PM_{2.5} air pollutant in the study area and also preterm births. Also, the concentration level for PM_{2.5} was found to be highest in the dry seasons with strong significant association with preterm births for each 10 unit increase in PM_{2.5} at $p < 0.05$ and 95% CI as shown in Table 2 (see Table 2).

In a similar study by (Enkhmaa *et al.*, 2014), a strong correlation was found between seasonal ambient air pollution and spontaneous abortion in Mongolia. The study found PM_{2.5} to correlate with spontaneous abortion with risk factor above 0.8 and p-value less than 0.001 ($r > 0.8$), ($p < 0.001$) (Enkhmaa *et al.*, 2014). A retrospective cohort study also found a strong association between PM_{2.5} and preterm births in the study area, during the entire pregnancy (HR, 1.06; 95%CI, 1.05–1.06) (Guo *et al.*, 2018).

Another study, identified the window of susceptibility to preterm birth risk from PM_{2.5} exposure (27.0 $\mu\text{g}/\text{m}^3$) to be during the 20th to 28th gestational weeks. The preterm births risk was significantly associated with the strongest effect in the 25th week (HR=1.034, 95% CI:1.010–1.059) (Wang *et al.*, 2018).

Particulate matter less than 2.5 (PM_{2.5}) are 30 times smaller in size than a strand of human hair and research has shown that they are capable of combining with placenta growth receptor and causing the inflammatory response of the placenta, hindering the exchange of oxygen nutrient of fetal placenta. It also alters coagulation function and hemodynamics; these mechanisms are capable of affecting the exchange of nutrients between placenta and fetus, which can therefore lead to preterm delivery or other negative births outcomes (Tan *et al.*, 2017; He *et al.*, 2018). As industrialization increases in urban centers the pollution level also increases. Therefore, there is need to pay attention to the health impacts of the increase in PM_{2.5} pollutant level on some sensitive groups, such as pregnant women.

Study Limitation

The study only made use of limited available data on PM_{2.5} pollutant from 2018-2019. There is need to conduct studies with larger datasets in the future that consider many years. Also, this study did not consider individual exposure level of the pregnant women and this may be necessary to validate the findings of this study.

Conclusion

This study found that there is strong association between PM_{2.5} and preterm births in the dry seasons. Pregnant women need to protect themselves from excessive exposure to ambient PM_{2.5} pollution emanating from industrial, agricultural, and environmental activities to reduce the risk of preterm births.

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We also appreciate the directorate of public health, Kampala Capital City Authority (KCCA) for releasing the health outcome data for this study.

Authors' contributions

WW, AC and AF designed the study and directed its implementation. AC collected the data, analyzed and interpreted the data. WW, AC, AF, and CA wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the DHIS2 database but restrictions apply to the availability of these data due to ethical concerns, which were used under license for the current study, and so are not publicly available.

Ethics approval and consent to participate

Approval for collecting research data was given by Mbarara University of Science and Technology, Research and Ethics committee (MUST-REC) with reference number: MUREC 1/7. The study did not require consent to be sought from participants, because it depended on secondary data.

Consent for publication: Not Applicable.

Competing interests: The authors declare that they have no competing interests.

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AN ASSESSMENT OF MICROBIAL CHARACTERISTICS OF HOSPITAL WASTE WATER
LOCATED IN ENUGU METROPOLIS

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ABSTRACT

The issue of contamination of the aquatic environment by hospital waste water has been an issue of concern to the public health of any area where these industries and hospitals are located. This study determines the levels of contamination caused by the discharge of wastewater from different wards at a Specialist Hospital Thinker's corner, Enugu, Nigeria. Wastewater samples were collected from three wastewater outlets of the hospital with pre-cleaned sterile and dried containers. The three sampling points selected for this study are: wastewater from theatre ward-(TW), maternity ward-(MW) and laboratory section-(LS). To this end, the microbial load and heavy metal analyses of the hospital wastewater were determined using established procedures. The isolated pathogenic bacteria include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus vulgaris*, *Shigella* sp., *Bacteroides* sp., *Citrobacter* sp. *Enterobacter* sp, *Pseudomonas aeruginosa* and *Serratia* sp. and *Bacillus subtilis*. The result obtained from this study showed the presence of *Aspergillus flavus*, a pathogenic fungus as the only isolated fungi specie in all wastewater samples. High level of microbial contamination was observed in wastewater effluent from the maternity ward (MW) among others especially the pathogenic ones. The heavy metals analysis showed the presence of Arsenic, Lead, Mercury, and Chromium. Most of the heavy metal concentration were found to be within the threshold of the WHO permissible limit of such metal. Therefore, it would be necessary that authorities will ensure compliances to properly treat all hospital wastewater irrespective of the ward before discharging into any receiving aquatic environment to avoid the possible environmental health risk that is associated with the discharge of such contaminated wastewater. Close monitoring of the heavy metal concentration of the discharge wastewater is also suggested to be carried out on a weekly basis in order to ensure discharge sustainability into the nearest ecosystem.

Keywords: Wastewater, Aquatic ecosystem, Heavy metals, Enugu, Pathogenic bacteria

INTRODUCTION

Wastewater is composed of inorganic and organic materials that include liquid wastes from homes, agricultural commercial sectors, pharmaceutical sectors, and hospitals (Carraro *et al.*, 2017). Wastewater is also an ideal media for a wide range of microorganisms especially bacteria, viruses and protozoa, it serves as a reservoir for resistant bacteria also (Abdel-Rouf *et al.*, 2012). It carries the resistant bacteria introduced into the sewage system that come from human excretions, liquid waste discharged from domestic home, agricultural and commercial sectors, pharmaceutical and hospitals (Amine, 2013, Fekadu *et al.*, 2015). Hospital wastewater can contain hazardous substances, such as pathogens, pharmaceutical residues, radioisotopes and chemical hazardous substances (Keen *et al.*, 2013). The discharge of wastewater or effluents directly into the environment by hospitals without treatment has been a common practice especially in developing countries (Kristell *et al.*, 2012) like Nigeria among others. The development of medical and hospital related services and products has led to practices resulting in increased generation of hospital wastewater effluents discharged directly into the environment and has received increasing attention in the recent times (Emmanuel *et*

al., 2005; Tchobanoglous *et al.*, 2004; Amouei *et al.*, 2010; Ekhaise and Omavwoya, 2008; Mahvi *et al.*, 2009; Verlicchi *et al.*, 2010; Kristell *et al.*, 2012). Untreated wastewater effluents from the hospital can have adverse effect on the environment and public health due to its components such as pathogenic organisms including antibiotic resistant organisms, hazardous chemicals, pharmaceuticals, disinfectants and radioactive isotopes among others (Baquero *et al.*, 2008, Kümmerer *et al.*, 2004, Cissé *et al.*, 2002, Matthys *et al.*, 2006). Toxicity evaluation is an important parameter in wastewater quality monitoring as it provides the complete response of test organisms to all compounds in wastewater (Mohavedian *et al.*, 2005). It becomes a great public health issue when hospital effluent water finds its way into surface waters. This poses a public health risk to individuals that make use of the water domestically, commercially and industrially due to the dangerous components found in the water. Indeed, more recently, with the development of sensitive analytical techniques, which make possible the detection of more and more active pharmaceutical compounds, it is now well established that pharmaceuticals and their metabolites are present in the environment (Kümmerer, 2004) with wastewater being the primary entry route. Sources

include households' agriculture and pharmaceutical industries (Kümmerer, 2006) and hospitals are often pointed out as a hot spot to pharmaceutical residues in influents of municipal Waste Water Treatment Plant (WWTP) (Ternes *et al.*, 2006; Hawkshead, 2008). According to the Centers for Disease Control and Prevention, about 2 million people in hospitals get infections each year, which cause 90,000 deaths. Of these, more than 70 percent of the bacteria that causes these infections are resistant to at least one common antibiotic that is typically used to treat them. Chemicals present in hospital waste water belong to different groups, such as antibiotics, X-ray contrast agents, disinfectants and pharmaceuticals (Kobayashi *et al.*, 2006). Many of these chemical compounds resist normal wastewater treatment (Mahnik *et al.*, 2007). They usually end up in different water sources where they can affect the aquatic ecosystem and interfere with the food chain. Humans are particularly at risk by drinking water, produced from these water sources. Microbial agents of special concern are of multi resistant microbial strains (Stalder *et al.*, 2014). The latter are suspected to contribute to the spread of antibiotic resistance (Pauwels *et al.*, 2006). The aim of this study is to assess and determine the microbial loads of waste water detected in hospital waste water in Enugu metropolis and to express concerns towards the suitability of the water to various uses and processes for ecosystem functioning.

MATERIALS AND METHODS

Waste water samples were collected from different units in Kenechukwu Specialist Hospital Thinker's corner, Enugu in Enugu Metropolis. Sample locations were theatre, maternity ward and the laboratory. Sterile universal containers were used to collect the waste water samples from the different wards. The wards were labeled TW, MW and LS respectively. The samples were then transported to the Godfrey Okoye University Microbiology Laboratory, for routine microbiological, chemical and biochemical analyses. Five-fold serial dilution was carried out using sterile distilled water as the diluent. Five test-tubes containing 4ml of distilled water were used for each of the samples. They were labeled and arranged in a test-tube rack. One millimeter of the sample was added to the first test-tube containing 4ml of water using a sterile syringe, each transfer was followed by a gentle agitation in order to mix the contents uniformly. The procedure was repeated for all the diluents in the same manner. The serial dilution was performed aseptically beside a lit Bunsen burner to prevent contamination (Atlas and Barta, 2004). An aliquot of 0.1 ml from each of all the serial dilutions was inoculated into already prepared media including- MacConkey broth, MacConkey agar, E.M.B agar, Nutrient agar and Salmonella-Shigella agar for bacterial growth, Potato Dextrose agar for fungal growth using spread plate method. All the media used were prepared according to manufacturer's guide.

The agar plates used which includes (nutrient agar, MacConkey agar, SSA, EMB.), were examined after 24-hrs while PDA was examined after 48 hrs of incubation. The number for bacterial colonies on the plate which had between 30-300 colonies were counted and the viable number or colony forming units were calculated while the colonies that were too numerous to count (TNTC) were discarded. The colony forming units per ml were calculated as follows.

$$\text{CFU/ mL} = \text{CFU/plate} \times \text{dilution factor} \times 1/\text{aliquot}$$

The characterization of the isolates was observed based on their pattern of growth, elevation, size, shape, colony, edge, colour, etc. after incubation on the appropriate media. The various identification tests carried out were grain staining, motility test, catalase test, oxidase test, citrate test, indole test, citrate test and indole test. A smear was made from the 18-24hr culture of the organisms on a clean grease free slide and passed over a flame to heat fix the organism. Application of a primary stain (crystal violet) to the heat-fixed smear of a bacterial culture for one minute was the first step; it was then washed off with tap. After which treatment with Lugol's iodine was done on the smear for one minute and washed off with tap water and decolorized with acetone which was also washed off immediately with water and lastly, the smear was counter stained with Safranin for one minute and washed off with tap water. The smear was allowed to dry and then viewed under the microscope with appropriate oil immersion and magnification of x100. The biochemical analyses of the various effluent samples were determined in the laboratory by standard methods. The results of the biochemical analysis of the samples and their corresponding microbiological analysis were subjected to standard statistical analysis by the use of tables and other relevant statistical tools.

RESULTS AND DISCUSSION

The dilution that yielded the colony count of 30-300 was 5³ and this was used to calculate the Colony Forming Units/ml (CFU/ml). The CFU/ml ranged from 3.13 x 10⁴ to 1.68 x 10⁵ CFU/ml for MacConkey and EMB agar. MacConkey agar yielded more colonies than its corresponding sample in EMB agar (Table 1). A similar count using Nutrient agar and Salmonella and Shigella agar yielded a CFU/ml of 8.31 x 10⁴ to 1.50 x 10⁵ CFU/ml. The SS agar yielded more colonies in theatre wastewater and Maternity waste water sample than Nutrient agar while nutrient agar yielded slightly more colonies in the laboratory waste water (Table 2).

In all the media used, the microbial load for waste water sample was consistently higher than in the other units (Tables 1 and 2).

Table 1: Total bacterial colony count of the Waste

Water Samples in MacConkey and EMB agar							
Media	Waste Water Samples (WWS)	Total Colony Count	CFU/ml				
MacConkey Agar	TW	134	1.68 x 10 ⁵	MW1	77	87 = 1.09 x 10 ⁵	
	MW	76	9.50 x 10 ⁴	MW2	97		
	LS	55	6.88 x 10 ⁴	LS1	80	68 = 8.50 x 10 ⁴	
	LS	35	3.13 x 10 ⁴	LS2	56		
EMB Agar	TW	97	1.21 x 10 ⁵	Salmonella-Shigellar agar	TW1	113	120 = 1.50 x 10 ⁵
	MW	63	7.88 x 10 ⁴		TW2	127	
	LS	35	3.13 x 10 ⁴		MW1	93	90 = 1.13 x 10 ⁵
	LS	35	3.13 x 10 ⁴		MW2	87	
				LS1	78	66.5 = 8.31 x 10 ⁴	
				LS2	55		

KEY: WWS= waste water samples, TW= theatre waste water samples, MW= maternity waste water samples, LW= laboratory waste water samples, EMB: Eosine Methylene Blue.

Table 2 below gives the total number of colonies counted on the Nutrient and Salmonella-Shigella agar plates done in duplicate, hence, TW1 and TW2.

Table 2: Total bacterial colony count of the Waste water samples for Nutrient agar and Salmonella-Shigella agar

Media used	Waste water sample	Total colony count	Average of WWS
Nutrient agar	TW1	100	93 = 1.16 x 10 ⁵
	TW2	86	

Key: TS= theatre sample, MS= maternity sample, LS= laboratory sample.

Table 2 below gives the total number of colonies counted on the Nutrient and Salmonella-Shigella agar plates done in duplicate, hence, TW1 and TW2.

A total of eleven different bacterial species were recovered from the 3 hospital units. Four different species were recovered in each of MacConkey agar, Nutrient agar and SS agar while three were recovered from EMB agar. *Bacillus subtilis*, *Staphylococcus aureus* and *Serratia* species occurred only in MacConkey agar, *Pseudomonas aeruginosa* was recovered both in M.A agar and Nutrient agar. *Escherichia coli* was recovered both in EMB and SSA

Table 3: Biochemical test results for MacConkey agar (M.A) and E.M.B agar isolates
+ = positive - = negative

Table 4: Biochemical tests results for isolates (Nutrient agar and SSA)

Media	Isolates	Catalase	Coagulase	Motility	Indole	Citrate	Oxidase	Urease	Probable organism
NA	S1	+	-	+	-	+	+	+	<i>Proteus</i> sp.
	S2	+	-	+	+	+	-	-	<i>Citrobacter</i> sp.
	S3	+	-	+	-	+	+	+	<i>Pseudomonas aeruginosa</i>
	S4	+	+	-	-	+	+	+	<i>Staphylococcus</i> sp.
SSA	S5	+	-	+	+	+	-	-	<i>Escherichia coli</i> .
	S6	+	-	+	-	+	-	-	<i>Salmonella</i> sp
	S7	+	+	+	-	-	+	-	<i>Enterobacter</i> sp.
	S8	-	-	+	-	+	-	-	<i>Shigella</i> sp

+ = positive - = negative

Table 5: Mean values of Heavy Metals in the Hospital Wastewater Samples.

Heavy Toxic Metals	TW (mg/L)	MW (mg/L)	LS (mg/L)	WHO LIMIT (mg/L)
Lead (Pb)	0.01	0.01	0.01	0.1
Chromium (Cr)	0.404	1.863	1.189	0.01
Arsenic (As)	0.003	0.012	0.002	0.1
Mercury (Hg)	0.012	0.006	0.009	0.01

recorded in Maternity ward (1.863mg/L). Also, mercury was high in Theatre ward as compared to other ward and section. With a little increase above the permissible limits of WHO.

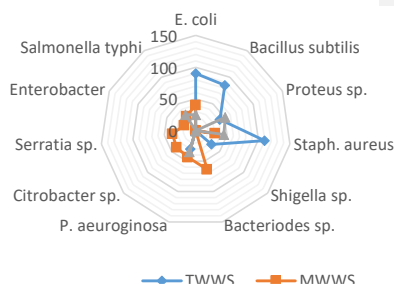


Fig. 1: Aradar analysis showing microbial counts of pathogenic bacteria present in different wastewater samples

From Table 5, mean value of wastewater sample showed that Chromium was high in all the samples and exceeded permissible limits. With higher value

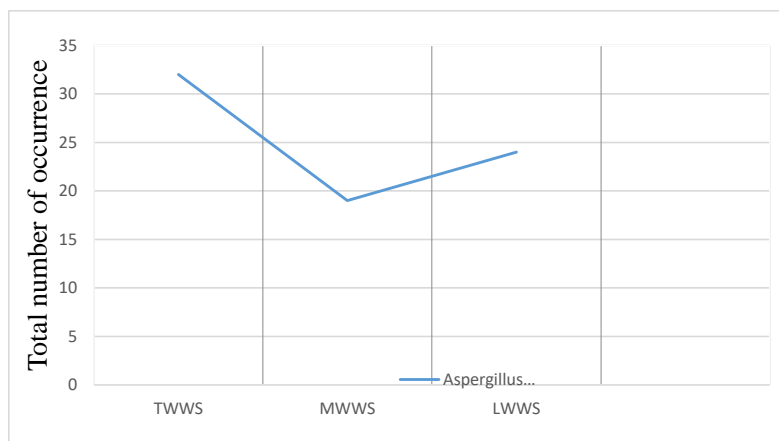


Fig. 3: A chart showing microbial counts of pathogenic fungi present in different wastewater samples. *Aspergillus flavus* was the only fungi (pathogenic), that was isolated from all three wastewater samples.

The result for the frequency of occurrences of the isolated bacterial species in the wastewater effluents showed highest total number of occurrence of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Citrobacter* sp., *Bacteriodes* sp., *Pseudomonas* sp., *Serratia* sp., *Enterobacter* sp., in wastewater effluent from the maternity ward when compared with wastewater effluents from the other wards. This suggests high level of bacterial contamination in wastewater effluents from the maternity ward as compared to other wards in line with Nwakanma and Obi (2018). Wastewater effluent from the laboratory (LS) recorded the highest total number of occurrences

of *Proteus* sp. and *Salmonella typhi*. (Fig.1). The occurrences of the pathogenic organisms obtained in this study were in agreement with the report of Giroletti and Lodola (1993). The high number of pathogenic organisms in this study proves to how toxic these effluents are and answers the question as to why they are not meant to be disposed of carelessly, due to the run-off effects it may have down-stream. Pathogenic organisms like *Escherichia coli* and *Staphylococcus aureus*, together with *Pseudomonas aeruginosa* are reported to be the only organisms present in all three wastewater samples. The biochemical characteristics of different bacteria

isolates showed both positive and negative reactions. The result also showed the presence of *Aspergillus flavus* a pathogenic fungus as the only isolated fungi specie in the hospital wastewater effluents (Fig.3). Highest total number of occurrence of *Aspergillus flavus* was found in the wastewater effluent from the theatre wastewater sample. Although the result for the test of significant difference between the varying concentrations of the identified heavy metals in the hospital wastewater effluents and WHO permissible limits showed an insignificant variation ($P > 0.05$). Conclusively, the frequency of occurrences of the microbial isolates, high microbial counts and the varying concentrations of heavy toxic metals obtained in this study suggests that there is hypothetical risk of hospital wastewater causing harm to the environment and public health especially hospital wastewater effluent from the theatre and maternity ward. It therefore calls for a proper regulatory system on the disposal of hospital wastewater effluents which are

normally discharged directly into the environment without treatment especially in the developing countries like Nigeria. Since large amounts of wastewater effluents are discharged directly into the environment without treatment on daily basis, it is therefore advised to remedy and diminish the overall impacts of these effluents on the receiving environment. Hospital wastewater is a key source of pollution, and this study affirms to it. There is need to comply with wastewater legislations and guidelines and this can be achieved through the application of appropriate treatment processes, which will help to reduce the risks to public health and the environment. There is also a need to ensure that effluents standards and limitations as set by national and international regulatory bodies are not compromised by hospital managements as this will equally ensure the sustainability of the environment and health of plants and animals.

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PREVALENCE AND OCCURRENCE OF PARASITES IN SOIL AND FAECAL DROPPINGS OF ANIMALS IN AGODI GARDEN RECREATION PARK, IBADAN, NIGERIA

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ABSTRACT

Soil transmitted helminthic infections has been recognized as an important public health problem. Agodi Garden Recreational Center receives both domestic and international tourists daily and contamination of this site may expose tourists to infection. Therefore, this study was aimed at determining the occurrence of parasite eggs, larvae and *Oocyst* in soil and faecal droppings at the site. Soil samples (40g) were collected at a distance of 20m interval from all the four sections in the recreational garden (swimming pool side, picnic area, children playground and restaurant area). Six samples of faecal droppings, voided by the animals designated for recreational activities within the garden were also obtained. Both the soil and faecal samples were examined for parasite detection using sedimentation and simple floatation methods. Overall, the prevalence of parasites was 97.5% (39/40) and 100% (6/6) in the soil and faecal samples, respectively. The frequency of parasite eggs, oocyst of protozoans and larvae that were detected in the examined soil samples were *Unsporulated coccidian oocyst* (10.4%), *Parascaris equorum* (8.3%), *Dicrocoelium spp.* (4.3%) *Cyathostomum* larvae (1.1%), *Anoplocephala spp.* (1.0%), *Trichuris spp.* (0.6%), *Strongyle* eggs (0.4%) and Mite (0.4%). From the faecal droppings of donkeys and horses, 58% (2/2) and 42% (4/4), *Strongyle* eggs were detected, respectively. The percentage parasite burden observed in the soil and the intensity at the swimming pool side, Picnic area, Playground and the restaurant area were 6.4% (10/10), 6.8% (10/10), 10.2% (10/10) and 3.0% (9/10) respectively. The present study has shown that Agodi garden environment is contaminated with parasitic eggs, Oocysts of protozoans and larvae from animals used for recreational activities in the garden. It is therefore recommended that faeces from animals should be properly disposed and animals made to undergo routine deworming. Also, provision of disinfectant at the point of exit was suggested.

Keywords: Helminth infections, Agodi garden, faecal droppings, Strongyle eggs

INTRODUCTION

Tourists can be infected with many diseases of zoonotic origins which can cause many health problems. These zoonotic diseases may be caused by many infectious agents such as parasites. There are many parasitic diseases of zoonotic origin which occur after ingestion of parasite eggs. Soil is the most common source and reservoir of parasite eggs, which may continue their lifecycle or remain and survive in the soil for months, or even years, until ingestion occurs (Zenner *et al.*, 2002). Soil is an important route of transmission of a large number of pathogens for both animals and humans. The main source of soil contamination with parasites is contamination by animals and/or humans waste, which can spread a large number of infected elements (eggs, cyst, larvae) in the environment through their faeces (Traversa *et al.*, 2012). The eggs/ larvae of many nematodes are highly resistant and they can survive in the environment for a long period of time (months, years) (Mitrea, 2011). Animals and humans are infected by accidental ingestion of the infective agents or by cutaneous contact with the contaminated soil. The concentration of pets in urban areas associated with a growing number of stray animals contributes to soil contamination in public gardens thus, spreading parasite infections (Cassenote *et al.*, 2011).

. Infected eggs can remain viable in the soil for a long time depending on several factors, such as climatic conditions, seasonal air temperatures, humidity or desiccation of soil, exposure to sunlight etc. (Storey and Phillips, 1985). Thus, contamination of soil with parasite eggs in public parks, particularly playgrounds with sandpits for children, may be an important source of infection and contributes a great risk factor for tourist infections, especially for small children aged lower than 12 years with a history of pica (O'lorcain, 1994).

Soil transmitted parasites are the large group of nematodes, protozoan, trematodes, cestodes and Arthropods that live in the soil during their development (Mandarin-Pereira *et al.*, 2010). Contamination of soil with parasite eggs, infective larvae, cysts, and oocysts constitutes a most important risk factor for zoonotic parasite infection. Zoonotic parasites such as *Toxocara spp.* and *geohelminths*, *Ascaris lumbricoides*, *Trichuris trichiura* and Hook worms) are the main parasites that could be transmitted through soil (Waenlor and Wiwanitkit, 2007). Tourist can be infected with these parasites through their direct contact with contaminated soil in public parks. The presence of animals, pets or animals without an owner, especially dogs and cats in urban public areas, is common in many cities of the world. These animals are known to harbor these parasitic diseases, which most often are found in their faecal materials dropped on the soil at the recreation centers. Therefore, contaminated soil play an important role in the spreading of parasitic diseases. The contamination of public gardens and parks with parasites has been demonstrated as a source of infection, which can cause significant health problems for tourist (Marques *et al.*, 2012; Martínez *et al.*, 2007), especially for infants. According to these authors, geohelminths were the second cause of mortality in children under

six years of age in Africa (Shumbej (2019). *Toxocariasis* is a zoonotic disease caused by the larval stage of *Toxocara cati* and *Toxocara canis* in domestic cats and dogs. Visceral larva migrant (VLM) and ocular larva migrant (OLM) are serious infections that are caused by these parasites. It has been reported that the presence of stray cats and dogs fecal droppings are the main sources of *toxocariasis* agent in the urban regions. *Toxocara* eggs require a period of four to six weeks incubation in soil to become infective (Paul *et al.*, 1988; Omitola *et al.*, 2016)). Also, intestinal canine and feline parasitic infections transmissible to humans, such as *toxocarosis*, *giardiosis* etc, have a cosmopolitan distribution and are among the ten most common infections in the world (Reference??). In most developing countries, soil-transmitted helminthes (STH) still remain a formidable public health problem of cosmopolitan importance (Anonymous, 1997, 1987).

In Nigeria, infections caused by intestinal parasites are of public health concern. Poor socioeconomic environment is a major factor facilitating the spread of the diseases (WHO, 2002).

Soil transmitted helminthes infections (STH) are common chronic tourist infections worldwide (Wirachit and Viroj, 2007; Brooker *et al.*, 2006), this has been recognized as an important health problem, particularly in developing countries (Uga *et al.*, 1997; Crompton and Savioli, 1993). The geohelminthes include *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, *Strongyloides stercoralis*, etc. tourist infections with canine intestinal worms are among the most common zoonotic infections (Pullan *et al.*, 2014) estimated worldwide in 2010 whereover 1.45 billion people were infected with at least one species of intestinal nematodes, most of the cases being concentrated in underdeveloped areas. Children in developing countries become the most important vulnerable group to these infections since they usually play within the grounds (Patel *et al.*, 2004). STH are caused by different species of parasite worms, including roundworms such as (*Toxocara spp.*; *Ascaris spp.*), hookworms (i.e. *Ancylostoma spp.*), and whipworms (i.e. *Trichuris spp.*), thread worm (*Enterobius vermicularis*) (Traversa *et al.*, 2014). The poor sanitary condition in developing countries such as Nigeria (Motarjemi *et al.*, 1993; Yusuf and Husseini, 1990) accounts for the presence of parasitic immature stages in the soil (Mahdi and Ali, 1993). The soil therefore contaminated with fecal material that may contain parasitic immature stages acts as an indicator of the potential risk of exposure of tourist to these parasitic diseases (Mizgajaska, 2001).

Agodi garden is patronized by a large number of tourists from all parts of the country, most of the time, tourists have contact with the soil in one way or another and may therefore be exposed to soil parasites.

Numerous studies conducted on soil samples in different parts of the world have demonstrated broad environmental contamination, especially in public places, such as parks and playgrounds for children (Otero *et al.*, 2014; Maraghi *et al.*, 2014; Sprenger *et al.*, 2014; Bojar and Káapeü, 2012; Stojbeviü *et al.*, 2012). The role of public places such as recreational parks, playgrounds and market as potential reservoirs of helminth parasites have been suggested as a key factor in the steady presence of (Roundworms – *Ascaris spp.*, Hookworms – *Ancylostoma spp.*, and Whipworms – *Trichiurus trichiuria*) in the tourist populations (Adekeye *et al.*, 2016; Krause *et al.*, 2015). Though the prevalence of parasitic infections among aged children in school have been detected to be high (Mafiana, 1995; Suswam *et al.*, 1992), the awareness on the infection posed by contaminated soil is still low (Maikai *et al.*, 2008). In the urban environment, a survey of soil samples collected from playgrounds, markets, motor parks, residential and recreational areas revealed that 96.3% of the samples contained ova of *Ascaris lumbricoides* (Fashuyi, 1983). These natural playgrounds have been reported as major source of infection for children (Akogun, 1989; Umeche, 1989). Thus, studying the extent of soil contamination in the ever increasing densities of tourist population within the urban square of Ibadan such as Agodi garden recreation park, will aid in the decision and suggestions on the ways to reduce or control the level of contamination of recreation centers.

Objective of the study

The main objective of this study is to investigate the occurrence of parasites in the soil and fecal droppings of animals in Agodi garden recreation park in Ibadan.

Specific objectives are to;

Determine the prevalence of soil transmitted helminth parasites in soil (STH) and animal faecal droppings in Agodi Garden Park playground.

Determine the frequency of occurrence of infective stages of the parasites (eggs/oocysts/larvae) in soil and animal faecal droppings in Agodi Garden Park.

Identify the species of STH parasites present in the soil and faecal droppings of animal and humans in Agodi Garden Park.

Materials and Methods

Study area

Agodi Garden is a top notch tourist attraction near the **Oyo State Secretariat complex** in the city of Ibadan, Oyo state with the coordinates of 7°24' 25"N 3°53' 57"E. Ibadan is characterized by three weather conditions annually. There is the rainy season which begins in April and ends in October with a temperature range of 22°C to 29°C at night. During the dry season periods, temperature can be as high as 40°C during the day and can drop to as low as 20°C at night during the harmattan period. The garden was originally created in 1967 and managed by the then Western region. The site is located in a serene environment on 150 acres of

land. However, in 1980 the infamous Ogunpa flood disaster in Ibadan destroyed the garden; the flood swept away most of the animals and left the place in a deplorable state. In 2012, the government renovated it and in 2014, the new Agodi Garden was re-opened. Since 2014, the garden has served as a tourist attraction site in Ibadan. It is usually patronized by families during festive periods and weekends. Agodi Garden has a lot of attractions such as swimming pool, children play area, rides, picnic spots, restaurant area, a lake and a mini zoo. It is very serene, lush and green; perfect for photo shoots, pre-wedding shoots, picnics, garden parties, outdoor events, concerts, etc.

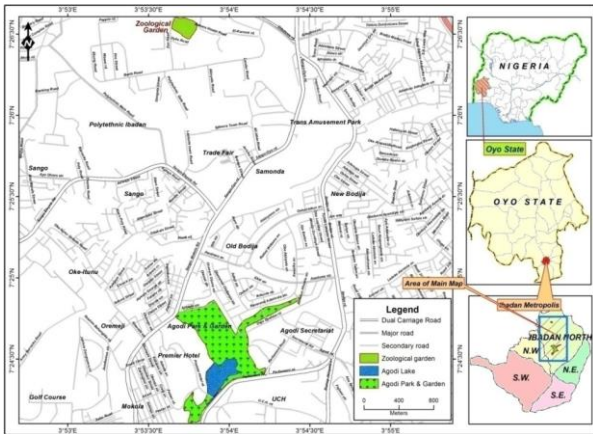


Fig. 1: Map of Ibadan North Local government showing Agodi gardens
Source: Cartography laboratory, Geography Department, University of Ibadan

METHOD OF DATA COLLECTION

Sampling procedure:

The study area was divided into four sections which are: children playground, swimming pool area, picnic spots area and restaurant area.

Sampling technique:

Soil sample collection:

Soil samples were collected at a distance of 20m interval from each section for point 1, 2, 3 ...10 within the garden from the surface layer of soil, up to the depth of approximately 5 cm using soil auger from each of the sections during raining season (July – August) using stratified random sampling method. The garden was stratified into four sections namely; children playground, swimming pool area, picnic spot area and restaurant section. Ten soil samples were taken from each section, giving a total of forty soil samples from the four sections within the recreation garden. Each soil sample collected weighed approximately 100grams from the top soil without debris and placed in a labeled polythene bags for soil analysis. All polythene bags collected from each section were placed in a larger black polythene bag and sealed. The sealed bags were stored and kept in the refrigerator at 4°C until examination.

Soil sample processing

In the laboratory, eggs/larva recovery was carried out using a modified version sample flotation method technique (John and Petri, 2006). Fifty grams (50g) of soil was weighed out of each labeled soil sample collected from each section, the soil was put in a 500ml plastic beaker each and was labeled respectively. Subsequently, 0.1%ml of Tween 80 was added into 999ml of water which was repeated thrice (3*) i.e. 0.3%ml of Tween 80 was added into 2997 approximately 3000ml of water and was stirred together with plastic rod to form a dilute solution. Thereafter, 3000ml of dilute solution was then poured into each of the weighted soil sample of each section, stirred with a glass rod and then allowed to soak overnight.

The next morning, each of the soaked soil sample was stirred thoroughly and strained through a small mesh sieve of 150 μm size. 2g of the sieve powder of each of the soil sample of each section was then poured in a 15ml centrifuge test tube and was centrifuged at 3000rpm for 10mins in order to separate the liquid from the sediment. Then the centrifuged result forms two layers comprising of the supernatant and the sediment, the supernatant was decanted/discarded. The sediment in the centrifuged test tube was then mixed with 10ml of Zinc sulphate, shaken thoroughly before it was centrifuged at 3000rpm for 10mins. The supernatant was also filled with zinc sulphate to the brim in each centrifuged test tube. Finally, the interface and the upper layer

obtained were collected and introduced into test tubes until the formation of the upper meniscus. Each test tube was covered with dry cover slip for 10mins. After 10minutes, cover slip was removed carefully and placed on dry glass slides and examined under a compound light microscope at X10 and X40 magnifications. The numbers of parasite eggs in the whole slide were counted.

Animal fecal collection:

Forty grams each of fecal droppings of animals used for recreational activities within the study site were collected from all the four horses and two donkeys available where the domestic animals are kept and around the sections within the recreational environment in a polythene bag and was transported to the laboratory for the detection of possible parasites.

Faecal sample procedure:

According to Cringoli *et al.*, (2010) and Cringoli, (2006), eggs/larva recovery was carried out in the laboratory using flotation method technique. Ten grams of the faecal sample of each animals were put in a small sieve of 150 μ m .size and immersed in a bowl containing 15ml of saturated water and washed. The sieve filtrate of each faecal sample was then poured in 15ml test tube to the brim and was covered with cover slip for 30mins. Subsequently, the cover slip was removed and placed on a dry glass slide and was examined under a compound light microscope at X10 and X40 magnifications. The numbers of parasite eggs in the whole slide were counted.

Identification of Possible Parasite Eggs/Larvae

The eggs and larvae in the samples were identified using a parasitological atlas (Cheesbrough, 2006). Eggs and larvae were counted regardless of their viability.

Data Analysis

Data obtained from the study were subjected to descriptive statistics and presented in line chart, pie chart and bar chart using statistical package (SPSS version 14) and excel.

Results

Prevalence Of Parasites In Soil In Agodi Garden Park

The overall prevalence of parasite from the various selection points within the study area showed that 97.5% (39/40) were positive for eggs and larvae of one or more parasites in the soil samples (Table 1). However, swimming pool, picnic area and children play ground had prevalence of 100% respectively while restaurant had prevalence of 90%.

Table 1: Prevalence of Parasites in Soil Sample from different Locations in Agodi Garden

Location	Number Tested	Number Positive	Prevalence (%)
Swimming Pool Area	10	10	100.0
Picnic Area	10	10	100.0
Children Playground	10	10	100.0
Restaurant	10	9	90.0
Total	40	39	97.5

Prevalence of Parasites in Animal Faecal Droppings in Agodi Garden Park

The faecal samples collected from the two animals used for recreational activities within the study area (fig. 1) showed high rate of Hookworm eggs in their faeces. The donkeys had the highest burden of hookworm eggs (58%) while the horses had (42%).

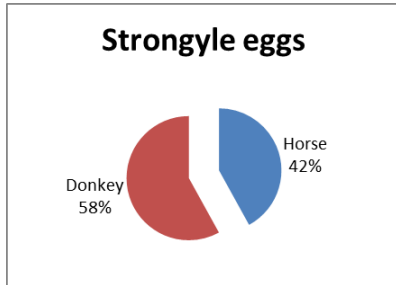


Fig 3: Percentage of *Strongyle* eggs detected from animal faecal sample

The frequency of occurrence of the different infective stages of the parasites is presented in Table 2. The result showed that rate of parasite eggs observed in children playground section was (10.2%), followed by picnic section (6.9%), swimming pool section (6.4%), while the lowest rate (0.3%) was observed in restaurant section (Table 2).

Unsporulated coccidian oocysts were the most frequently encountered in the soil samples in all the sections with a frequency rate of 10.4% (24/40) followed by *Parascaris equorum* 8.3%(30/40), *Trichuris spp.* 0.6% (5/30), *Strongyle* eggs 0.4%(4/20), *Dicrocoelium spp.* 4.3% (16/30), *Cyathostomum spp.* Larvae 1%(10/40), *Anoplocephalia spp.* 1.0%(5/20) and Mite 0.4%(3/20). Swimming pool section was the most contaminated with *Unsporulated coccidian oocysts*(3.9%), picnic section with (3.1%), followed by children playground section (3.0%). The restaurant section which was the least contaminated with *Unsporulated coccidian oocyst* had (0.4%) prevalence (Table 3).

Parascaris equorum eggs detected were (1.2%) in swimming pool, (2.5%) in picnic, (3.1%) in children playground and (1.5%) in restaurant sections. *Trichuris spp.* eggs detected were (0.3%) in swimming pool, (0.2%) in picnic, (0.1%) in children playground and none from restaurant sections respectively. *Strongyle* eggs detected were (0.3%) in swimming pool, (0.1%) in picnic, none in children playground and restaurant sections respectively. *Dicrocoelium spp.* eggs were not found in swimming pool, (0.8%) in picnic, (2.9%) in children playground and (0.6%) in restaurant sections. *Cyathostomum spp.* larvae observed were (0.4%) in swimming pool, (0.2%) in picnic, (0.2%) in children playground, (0.3%) in restaurant sections. *Anoplocephalia spp.* eggs were not detected in both swimming pool and picnic while (0.9%) was found in children playground and (0.1%) in restaurant sections. Ectoparasite (Mites)(0.3%) were observed in swimming pool and (0.1%) in restaurant sections and none was found in both picnic and children playground (Table 2).

The parasite burden observed at the swimming pool side, picnic area, playground and the restaurant area were 6.4%(10/10), 6.9%(10/10), 10.2%(10/10) and 3.0%(9/10) respectively (Table 2).

Table 2. Summary of parasites burden in soil samples at Agodi Garden Park

Section	No of sample examined	No. positive (%)	No. negative (%)
Swimming pool area	10	10(6.4%)	-
Picnic area	10	10(6.9%)	-
Children playground	10	10(10.2%)	-
Restaurant area	10	9(3.0%)	1(0.4%)
Total	40	39(26.5%)	1(0.4%)

Table 3: Frequency of Occurrence of parasites in soil samples at various sections of Agodi Garden

Section	No of samples	<i>Unsporulated coccidian oocyst</i>	<i>Parascaris equorum</i>	<i>Trichuris spp.</i>	<i>Strongyle</i> eggs	<i>Dicrocoelium spp.</i>	<i>Cyathostomum Larva</i>	<i>Anoplocephalia spp.</i>	Mite	Sample with parasite
A	10	3.9(8/10)	1.2(4/10)	0.3(3/10)	0.3(3/10)	0(0.0)	0.4(3/10)	0(0.0)	0.3(2/10)	6.4(23/60)
B	10	3.1(6/10)	2.5(10/10)	0.2(1/10)	0.1(1/10)	0.8(4/10)	0.2(2/10)	0(0.0)	0(0.0)	6.9(24/60)
C	10	3.0(8/10)	3.1(9/10)	0.1(1/10)	0(0.0)	2.9(8/10)	0.2(2/10)	0.9(4/10)	0(0.0)	10.2(32/60)
D	10	0.4(2/10)	1.5(7/10)	0(0.0)	0(0.0)	0.6(4/10)	0.3(3/10)	0.1(1/10)	0.1(1/10)	3.0(18/60)
Total	40	10.4(24/40)	8.3(30/40)	0.6(5/30)	0.4(4/20)	4.3(16/30)	1.1(10/40)	1.0(5/20)	0.4(3/20)	26.5(97/240)

Abbreviation: A=Swimming pool section, B= Picnic section, C= children playground section, D=restaurant section.

Frequency of Occurrence of parasites in faecal samples at various sections of Agodi Garden

The frequencies of detection of *Unsporulated coccidian oocyst* (10.4%), *Parascaris equorum*eggs(8.3%), *Trichuris spp.*eggs (0.6%), *Strongyle* eggs (0.4%), *Dicrocoelium spp.*eggs (4.3%), *Cyathostomum spp.*larva (1.1%), *Anoplocephalia spp.*eggs(1.0%), and Mite (0.4%) were presented in table 3. *Unsporulated coccidian oocyst*detected were (3.9%) in swimming pool, (3.1%) in picnic area, (3.0%) in children playground and (0.4%) in restaurant sections.

Parascaris equorum eggs detected were (1.2%) in swimming pool, (2.5%) in picnic, (3.1%) in children playground and (1.5%) in restaurant sections. *Trichuris spp.* eggs detected were (0.3%) in swimming pool, (0.2%) in picnic, (0.1%) in children playground and Nil from restaurant sections. *Strongyle* eggs detected were (0.3%) in swimming pool, (0.1%) in picnic, none detected in children playground and restaurant sections. *Dicrocoelium spp.*eggs detected were Nil in swimming pool, (0.8%) in picnic, (2.9%) in children playground and (0.6%) in restaurant sections. *Cyathostomum spp.*larva detected were (0.4%) in swimming pool, (0.2%) in picnic, (0.2%) in children playground, (0.3%) in restaurant sections. *Anoplocephalia spp.*eggs detected were Nil in both swimming pool and picnic, (0.9%) in children playground and (0.1%) in restaurant sections. Ectoparasite (Mite) were observed (0.3%) in swimming pool, Nil in both picnic and children playground, and (0.1%) in restaurant sections. The parasite burden detected at the swimming pool side, Picnic area, Playground and the restaurant area were 6.4%(10/10), 6.9%(10/10), 10.2%(10/10) and 3.0%(9/10) respectively.

Discussion

Soil transmitted helminthic infections are common worldwide and has been recognized as an important public health problem, particularly in developing countries. Agodi Recreational Garden receives both domestic and international tourist daily. Contamination of this garden with parasites eggs/oocyst may expose tourist to infections.

This study detected a variety of parasite oocysts that include: *coccidian oocyst*, *Parascaris equorum* eggs,*Anoplocephala spp.* eggs, *Cyathostomum larvae*, *Trichuris spp.* eggs, *Strongyle* eggs and *Dicrocoelium spp.* eggs in soil and animal faecal droppings collected from the garden. This suggests that animals in the garden are exposed to various infection sources and have not received adequate medical attention.

Furthermore, this study also determined that there was a high soil contamination rate with parasite egg/oocyst. *Unsporulated coccidian oocyst* showed highest frequency of occurrence, followed by *Parascaris equorum* eggs while *Strongyle*, and *Trichuris spp.* eggs had the lowest frequency of occurrence in the various soil sample examined. This could be due to inadequate sanitation and improper disposal of animal faeces within the environment. According to Wong *et al.* (1994), the principal source of parasitic infection in man is faecal contamination of soil. Also the results of present study showed a similar occurrence of parasites egg/oocyst to those reported previously in Ibadan and Abuja by Adekeye *et al.*, (2016); Hassan *et al.*, (2017) and Mohammed *et al.*, (2011). the fact that this garden is frequently visited by tourists from different works of life may have contributed to the high number of helminths contamination. According to Mazgajska, (1997), soles of shoes contribute to the distribution of helminth eggs in the soil. Although Uga and Kataoka, (1995) proposed fencing as a way of reducing contamination of sandpits, eggs of helminth were detected from samples collected from most recreational parks in Abuja which are fenced. In developing countries, STH infections represent a major public health problem which has constituted a universal burden. Several communities in Nigeria are widely affected by

these intestinal parasites due to inadequate sanitation and infrastructure deficit exposing the population to continuous infections (Pai and Ugwu, 2003; Odu *et al.*, 2011).

The findings in this study revealed that, the overall prevalence of the parasites eggs infection was (97.5 %) in the study area. These showed that soil transmitted heminths infection are caused by different species of parasitic worms. These parasite eggs are present in animal faeces, which contaminate the soil in areas where sanitation is poor. The soil samples from the swimming pool section maybe contaminated through the high rate at which tourist visit the section. Since it is known that eggs can be picked by sole of shoes, which means that tourists themselves could also be carriers of these parasite to swimming pool section. Some bird and reptiles faeces can also contaminate the soil due to presence of trees in such section. Parasite eggs present in the soil of this section exceeded the WHO public health standard which can infect tourists and physically impaired. Unsporulated coccidian oocyst infection can cause fever, vomiting, diarrhea, muscle pain and nervous system effect which may lead to death. Control is based on periodically deworming to eliminate infecting worms. Picnic section have a high rate of *Parascaris equorum* because horses and donkeys are used for recreation activities such as horse riding within the picnic area and the animal faeces are littered around the environment. The parasites are transmitted by eggs present in animal faeces, through runoff water or tourist shoe soles which in turn contaminate soil in area where sanitation is poor. Tourists get infected with these eggs/oocyst when ingested by having contact with contaminated soil and putting hands in mouth without washing them. The high prevalence of 97.5% of parasites eggs infections in the study site as obtained could be attributed to carelessness and unhygienic habits practiced. Lack of sanitation facilities might have also contributed to the high prevalence.

Lower prevalence in other parts of the country has also been reported (Odobu *et al.*, 2011) while reports of higher prevalence have also been observed by (Uga *et al.*, 1996; Ulukanligil *et al.*, 2001). This could be due to environmental hygiene and sanitation policies in different areas, climatic factors on the parasite ova and awareness and perception among citizens. The presence of potentially pathogenic helminth parasites in the environment highlights risk of tourist infection (Ogbolu *et al.*, 2011). Although some of the parasites: *Unsporulated coccidian oocyst*, *Parascaris equorum*, *Trichuris spp.*, Hookworm, Trematode (*Dicrocoelium spp.*), *Cyathostomum* larva, Cestode (*Anoplocephalia spp.*), ectoparasite (Mites) identified in this study are not of documented public health importance, Hookworm, *Dicrocoelium sp.*, *Trichuris* infection and mites infestations are of potential zoonotic importance which are environment highlights risk of tourist infection.

Children playground show high frequency of parasite eggs in the soil with the presence of the following parasite species *Unsporulated coccidian oocyst* which had the highest burden followed by *Parascaris equorum*, *Dicrocoelium spp.*, *Anoplocephalia spp.*, while *Cyathostomum* larvae and *Trichuris spp.* had the lowest frequency. *Strongyle* eggs and mites are not found in children playground. High prevalence of parasites eggs in children playground may be as a result of poor sanitation and high level of animal fecal materials within the environment which lead to high risk of tourist infection. The survey carried out by Dada-Adegbola (2005) in the University College Hospital, Ibadan in 2005 revealed that 5.3% of all the children (0-5 years) with diarrhea were found to have helminth parasites and according to their report, the infection can be traced to soil contamination with the parasite infective eggs and larvae, as children within that age range play on the ground and even eat the soil (geophagia). Children playground section had the highest positive samples, contributing 10.2% of the total 26.5% samples from all various sections. Children can also carry animal faeces either from their destination or within the park premises on their shoes sole to the section coupled with the poor sanitation of the area.

Trematode identified was *Dicrocoelium spp.* eggs which were also observed in sample soil of children playground. This could have been due to contamination of the environment with animal fecal droppings because *Dicrocoelium spp.* eggs are found mostly in cattle or grazing animals. This finding is similar to other studies reported in Nigeria [Dada and Belino, 1979; Ajayi and Duhlińska, 1998] and other countries of the world [Uga *et al.*, 1996]. For example, the study of Van Niekerk *et al.* (1996) found that the high prevalence of intestinal parasitic infestation in urban children in Cape Town was as a result of extensive contamination of soil in the area by parasitic eggs. The presence of potentially pathogenic helminth parasites in the environment also highlights risk of tourist infection.

The isolation of parasitic eggs from the soil has revealed the presence of different parasitic species such as *Unsporulated coccidian oocyst*, *Ascarid spp.*, *Trichuris spp.* Trematodes compared with samples collected from parks, playgrounds or sand pits in many countries such as Argentina, Zimbabwe, Japan revealed presence of *Toxocara* eggs in accordance with Alonso, 2001; Mukaratirwa, 1999; Shimizu, 1993. This showed that the soil in the countries are contaminated with pet faeces. Animal fecal dropping sample examined shows that there is high prevalence of parasites egg in the domestic animal used within the study area for recreational activities. There are two domestic animals used for recreation activities within the park which are donkeys and horses, and it was

observed that these animals are infected with parasite eggs which contaminated the soil through their fecal materials. Also,

presence of free-range animals such as rodents, birds, and pets could have also influenced the parasitic burden in the soil, which indicates that animals fecal materials play a major role as environmental contaminants with soil transmitted parasites eggs and larvae [Travalla *et al.*, 2012].

Conclusion and Recommendation

The result of this study showed relatively high prevalence and frequency of occurrence of different species of soil transmitted parasites at the study site with the implication that Agodi garden environment is highly contaminated with parasitic eggs, oocyst and larva that were from animals used for recreational activities within the garden and possibly from tourists. The study has shown the need to improve on environmental hygiene of recreational gardens and personal hygiene of the tourists in the gardens in view of the high records of helminth parasites reported in the garden and the public health implications.

It is therefore recommended that feces from animals should be properly disposed to prevent contamination of the park which could serve as a bedrock for the onset of zoonotic diseases. Animal used for recreational activities within the park should be dewormed regularly. Also, the management should provide disinfectants and hand sanitizers and encourage hand washing and dipping of feet at the gate entrance for tourist in order to avoid transmission of parasites within or outside the park.

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POSSIBLE POTENCY OF *Annona muricata* L. METHANOLIC LEAF EXTRACT IN AMELIORATING PANCREATIC β CELL FUNCTION IN ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT

The methanolic extract of *Annona muricata* leaf was investigated for its effects on β -cell dysfunction, and atherogenic dyslipidaemia in alloxan-induced diabetic mice. Thirty (30) mice of both sexes, aged between 8-10 weeks were randomly divided into normal, diabetic, diabetic + glibenclamide(5mg/kg), diabetic+250 mg/kg *A. muricata* Extract, diabetic+500 mg/kg *A. muricata* Extract, diabetic+1000 mg/kg *A. muricata* Extract, groups (n=6/group). Alloxan was administered intraperitoneally while extracts and glibenclamide were given via oral route for 21 days. The results revealed that, 250 mg/kg *A. muricata* extract elicited significant reductions in hyperglycemia, ameliorated insulinaemia as well as β -cell function and impaired weight loss in alloxan-induced diabetic mice. The extract also attenuated ($p<0.05$) atherogenic dyslipidemia and elevation in malondialdehyde and upregulated antioxidant enzymes. The effect of the extract on the test groups administered 250 mg/kg body weight (bw), 500 mg/kg bw, and 1000 mg/kg bw compared favourably with glibenclamide. The administration of methanolic extract of *A. muricata* leaves has significant ameliorative effect on alloxan-induced hypoglycemia, atherogenic dyslipidaemia and pancreatic β cell dysfunction. Our finding suggest that these positive modulatory effects are achieved via reduction in lipid peroxidation. The extract may be of immense benefits in the management of diabetes and its associated complications. Improved liver functions as well as its anti-oxidant status are beneficial in the management of chronic diseases.

Keywords: Diabetes mellitus, *A. muricata*, β -cell dysfunction, and atherogenic dyslipidaemia

INTRODUCTION

Diabetes mellitus is a chronic condition, characterized by hyperglycemia, occurring due to impaired insulin secretions. It is the most common metabolic disease worldwide (Walker and Whittlesea, 2012). Most Antidiabetic drugs available today are synthetic in origin and thus cost more and show toxicity (Diptanu *et al.*, 2016). Various studies have revealed variety of antidiabetic plants useful in the management and treatment of diabetes mellitus in various indigenous systems of medicine (Deb *et al.*, 2013). *Annona muricata* is a member of the Annonaceae family and is a fruit tree with a long history of traditional use. *Annona muricata* has shown to have both medicinal and industrial value (Mishra *et al.*, 2013; Soheil *et al.*, 2015). In ethno-medicine, *A. muricata* leaves used by local traditional healers in treatment of various ailments such as gastric discomfort, stomach ulcer, diarrhea, dysentery and skin infections (De Sousa *et al.*, 2010; Soheil *et al.*, 2015). Phytochemical analysis of *A. muricata* showed the presence of flavonoid, anthraquinone, alkaloids, saponins, steroids, terpenoids, cardiac glycoside, anthocyanin, tannins and carotenoid in both the aqueous and ethanolic extracts (Nawwar *et al.*, 2012; Yang *et al.*, 2015). Other studies have revealed that *A. muricata* extracts has antioxidant potential. Consequently, it has become imperative to investigate the plant for the management of diabetes. Presence of flavonoids and tannins in the extracts suggests they possess antidiabetic activity (Nawwar *et al.*, 2012; Jiménez *et al.*, 2014).

MATERIALS AND METHODS

Sample collection and preparation of extract

Fresh leaves of *A. muricata* used for this study were obtained from Olokoru village, Umuahia North L.G.A., Abia State in January 2017. A plant taxonomist (Dr. Mulikat A. Jimoh) in the Department of Plant Science and Biotechnology, College of Natural Sciences (COLNAS), Michael Okpara University of Agriculture Umudike, identified and authenticated the leaves as *A. muricata*. The fresh leaves of *A. muricata* were washed with tap water; room dried for one month, and macerated. The macerated samples (615 g) was soaked in 98% methanol and allowed to stand for 5days in a bell jar, shaken intermittently and filtered. The filtrate was evaporated to dryness at reduced temperature of 40°C in a water bath to yield a semisolid mass, which was stored in a refrigerator until required for use.

Experimental Animals:

Thirty (30) male mice, weighing about 24g- 30g were purchased from the animal house, Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. They had unrestricted access to standard feed and water. The animals were maintained under standard environmental conditions of temperature, relative humidity and dark/light cycle, in accordance with the guidelines of National Institute of Health Guide for the Care and Use of Laboratory Animals and the Institutional Ethical Review Board of Michael Okpara University of Agriculture Umudike. Body weight, food consumption and water intake were

monitored throughout the period of Administration. The animals were acclimatized to the condition of their new environmental prior to treatment for three weeks.

Induction of Diabetes:

The mice were fasted overnight and then injected, intra-peritoneally, with a single dose 0.24g of alloxan dissolved in 10mL of distilled water was administered to each of the mice in groups A, B, C, D, E and F based on the body weight of each mice and on a dosage of 100mg/kg. Group A served as the control. The administration of alloxan was done intraperitoneally using diabetic syringes. Stable hyperglycemia was confirmed on the 5th day using glucometer. Mice with fasting blood glucose level greater than 180mg/dl were considered hyperglycemic and used for the study.

Animal grouping and treatment

After 2 weeks of acclimatization, animals were randomly assigned to six groups (n=6/group). Group A (normal control) and B (diabetic control):received distilled water (vehicle) daily; Group C: (diabetic + glibenclamide): received 5.0 mg/kg body weight of standard drug (glibenclamide); Group D: (diabetic + 250mg/kg *A. muricata* extract): received 250mg/kg body weight of methanol *A. muricata* extract; Group E: (diabetic + 500mg/kg *A. muricata* extract): received 500mg/kg body weight of methanol *A. muricata* extract; while Group F: (diabetic + 1000mg/kg *A. muricata* extract): received 1000mg/kg body weight of the methanol *A. muricata* extract. All treatments were through oral administration and lasted for 21 days.

Blood Collection

At the end of experiment, four (4) mice, from each group, were weighed and sacrificed by cervical dislocation while under mild anesthesia and blood was collected by cardiac puncture into plain bottle. The blood was left undisturbed for 5 min and then centrifuged at 3500rpm for 10min and serum was stored frozen until needed for biochemical assays.

Biochemical assays

Insulin was determined using ELISA kit from Ray Biotech, Inc. (Georgia USA), while malon-dialdehyde (MDA), a maker of lipid peroxidation was measured by standardized method using kit obtained from Oxford Biomedical Research Ltd (Rochester Hills, MI). Estimation of total cholesterol (TC) and Triglyceride (TG) was done by standardized enzymatic colorimetric methods using assay kits obtained from Fortress Diagnostic Ltd. (Antrium, United Kingdom). High-density lipoprotein-cholesterol (HDL-C) was measured by enzymatic clearance assay (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) while low-density lipoprotein-cholesterol (LDL-C) was estimated using modified Friedewald's formular. TC/HDL-C and TG/HDL-C ratios were estimated as markers of atherogenic lipids indices

Gross necropsy and General observation

All the animals in the study were subjected to a determined gross necropsy, which included careful examination of the external surface, examination of the external surface of the body, all orifices and cranial, thoracic and abdominal cavities. The behaviour depression, salivation, diarrhea and muscular weakness were observed throughout the experimental period.

Fasting blood glucose and pancreatic β cells

Weekly fasting blood glucose was determined by glucose oxidase method of Trinder (1969) using ACCU-CHEK, Active Blood glucose monitoring system (Roche Diagnostics GmbH Sandhofer Strasse 1158305 Mannham, Germany). The pancreatic β cells function was estimated using HOMA- B formular

Statistical analysis

Data were analyzed and presented as mean ± SEM. Data were subjected to statistical analysis using one-way analysis of variance (ANOVA). Values were considered statistically significant at P<0.05.

Table 1a: Effect of methanolic extract of *A.muricata* leaves on fasting blood glucose (FBG) in alloxan-induced diabetic mice.

GROUPS	TREATMENTS	Initial FBG (mg/dl)	Final FBG (mg/dl)
A	Control	60.20±2.76 ^b	82.80±3.25 ^c
B	Diabetic Untreated	53.00±1.14 ^d	245.20±1.50 ^a
C	0.5 mg/kg Standard Drug (Daonil)	60.00±1.30 ^{bc}	95.00±0.71 ^d
D	250mg/kg <i>A. muricata</i>	55.00±1.30 ^{cd}	118.80±0.86 ^c
E	500mg/kg <i>A. muricata</i>	65.40±1.03 ^a	122.00±2.55 ^c
F	1000mg/kg <i>A. muricata</i>	59.00±1.61 ^{bc}	130.00±1.61 ^b

Values are expressed as mean± SEM of 5 mice. Values with same letters in the same column are not significantly different P<0.05. FBG- Fasting blood glucose

Table 1b: Effect of methanolic extract of *A.muricata* leaves on insulinemia in alloxan-induced diabetic mice.

GROUPS	TREATMENTS	Initial INS (mg/dl)	Final INS (mg/dl)
A	Control	60.20±2.76 ^b	82.80±3.25 ^c
B	Diabetic Untreated	53.00±1.14 ^d	245.20±1.50 ^a
C	0.5mg/kg Standard Drug (Daonil)	60.00±1.30 ^{bc}	95.00±0.71 ^d
D	250mg/kg <i>A. muricata</i>	55.00±1.30 ^{cd}	118.80±0.86 ^c
E	500mg/kg <i>A. muricata</i>	65.40±1.03 ^a	122.00±2.55 ^c
F	1000mg/kg <i>A. muricata</i>	59.00±1.61 ^{bc}	130.00±1.61 ^b

Values are expressed as mean±SEM of 5 mice. Values with same letters in the same column are not significantly different P<0.05. INS- Insulinemia

Table 2: Effect of methanolic extract of *A. muricata* leaves on body weight in alloxan-induced diabetic mice.

GROUPS	TREATMENTS	Initial BW (g)	Final BW (g)	BW Difference(g)
A	Control	22.48±1.31 ^a	45.02±1.48 ^b	22.54±1.04 ^b
B	Diabetic Untreated	23.06±0.96 ^a	18.50±1.63 ^d	-4.56±1.19 ^d
C	0.5mg/kg Standard Drug (Daonil)	22.04±0.41 ^a	54.69±1.34 ^a	32.65±1.42 ^a
D	250mg/kg <i>A. muricata</i>	25.22±0.88 ^a	33.54±1.33 ^c	8.32±2.16 ^c
E	500mg/kg <i>A. muricata</i>	24.06±0.83 ^a	29.29±1.65 ^c	5.23±1.99 ^c
F	1000mg/kg <i>A. muricata</i>	24.06±1.20 ^a	33.40±2.28 ^c	9.34±2.78 ^c

Values are expressed as mean ±SEM of 5 mice. Values with same letters in the same column are not significantly different P<0.05.

Table 3: Effect of methanolic extract of *A. muricata* leaves on lipid profile of alloxan-induced diabetic mice.

GROUPS	TREATMENTS	TAG (mg/dl)	CHOLESTEROL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
A	Control	47.11±0.55 ^b	57.71±1.48 ^b	35.34±0.07 ^a	9.42±0.11 ^b	12.95±1.39 ^{bc}
B	Diabetic Untreated	85.90±2.16 ^a	97.96±1.32 ^a	20.85±0.54 ^f	17.18±0.43 ^a	59.93±2.10 ^a
C	0.5mg/kg Standard Drug (Daonil)	45.10±1.08 ^b	60.63±5.92 ^b	32.67±0.20 ^b	9.02±0.22 ^b	18.94±5.83 ^b
D	250mg/kg <i>A. muricata</i>	48.77±4.49 ^b	60.59±6.38 ^b	31.24±0.07 ^c	9.75±0.90 ^b	19.60±6.26 ^b
E	500mg/kg <i>A. muricata</i>	44.25±2.79 ^b	44.57±2.78 ^c	28.63±0.23 ^d	8.85±0.56 ^b	7.09±3.23 ^{bc}
F	1000mg/kg <i>A. muricata</i>	46.24±2.62 ^b	41.36±1.87 ^c	27.39±0.37 ^e	9.25±0.52 ^b	4.73±1.24 ^c

Values are expressed as mean ± SEM of 5 mice. Values with same letters in the same column are not significantly different P<0.05.

Table 4: Effect of methanolic extract of *A. muricata* leaves on Antioxidant parameters of alloxan-induced diabetic mice.

GROUPS	TREATMENTS	SOD (U/mg)	CATALASE (µmol/mg)	MDA (µmol/mg)
A	Control	19.54±0.28 ^a	18.71±0.26 ^a	2.16±0.06 ^a
B	Diabetic Untreated	4.34±0.01 ^c	5.49±1.37 ^d	0.34±0.07 ^c
C	0.5mg/kg Standard Drug (Daonil)	13.24±0.01 ^b	13.27±0.30 ^b	1.04±0.01 ^b
D	250mg/kg <i>A. muricata</i>	11.17±0.08 ^c	12.99±0.02 ^b	1.00±0.01 ^b
E	500mg/kg <i>A. muricata</i>	10.94±0.11 ^c	8.22±0.05 ^c	0.84±0.02 ^c
F	1000mg/kg <i>A. muricata</i>	8.67±0.20 ^d	7.01±0.55 ^c	0.67±0.03 ^d

Values are expressed as mean ± SEM of 5 mice. Values with same letters in the same column are not significantly different P<0.05.

RESULTS AND DISCUSSION

In Africa and the world at large Diabetes mellitus is one of the leading causes of death. Currently, available synthetic drugs used by diabetic patients have a number of limitations, such as adverse effects

and high rate of secondary failure. The use of plants, plants extract, and the active compound from plants to cure diseases is a potential step in new drugs discovery (Rupeshkumar *et al.*, 2014). Different researchers suggested and proved the efficacy of plant extract and phytochemicals in diabetes treatment (Ganesh *et al.*,

2010).

This study investigated the possible potency of *Annona muricata* methanolic leaf extract in ameliorating pancreatic β cell function and dyslipidemia in type 2 diabetic mice, using alloxan-induction. Alloxan is a glucosamine-nitrosourea derived from *Streptomyces achromogenes* (gram-positive bacterium), and it is used for the treatment of pancreatic beta cell carcinoma. Alloxan inducing diabetes, hyperinsulinemia, or hyperglycemia by damaging the pancreatic beta cell.

It stimulates insulin-dependent diabetes (T2D) by inducing selective necrosis of the beta-cells of pancreatic islets, thus, destroying β -cells and reducing their function. From our findings *A.muricata* methanolic extract, alloxan-induced hyperglycemia at low dose (250 mg/kg), which compared favourably with glibenclamide.

Our result showed that alloxan treatment significantly ($P>0.05$) increased blood glucose level of mice thereby leading to diabetes (Table 1a). The increase in blood glucose level may be a result of the shrinking, destruction, ballooning, picnosis, and necrosis of the beta cells of the islet of Langerhans (Eliakim and obri, 2009; Saha *et al.*, 2016). However, treatment of diabetic mice with oral administration of 250 mg/kg, 500 mg/kg and 1000 mg/kg doses of methanolic L. extract of significantly ($P>0.05$) decreased blood glucose level of alloxan induced diabetic mice and improved pancreatic B cell function and thus, insulin production, better than the standard drug (glibenclamide) used. This may imply that, the leaf extract of *A. muricata* induced regeneration of pancreatic B cell and hence, enhanced its function. This result agrees with Sovia *et al.*, 2017 that shows *A. muricata* to have hypoglycemic effect. Hypoglycemic effect of *Annona muricata* leaves extract could be due to the presence of flavonoids in the leaves extract. Flavonoids increase insulin secretion as well as preventing beta-cell apoptosis, and modulation of proliferation (Castell *et al.*, 2008). Flavonoids can also stimulate Ca^{2+} uptake from isolated islet cells, hence it also effective even in non-insulin dependent diabetes (Sandhar *et al.*, 2011).

There was significant decrease in the final body weight and body weight difference of diabetic untreated mice when compared with control group (Table 2). The decrease in body weight observed in diabetic untreated mice is linked to the result of degradation of proteins (muscle wasting). Structural proteins are known to contribute to the body weight. In the absence of glucose and lipid sources, proteins are the next main source of energy in body. Therefore, it is clear that the decrease in body weight in diabetic mice were mainly because of degradation of structural proteins (Ananthi *et al.*, 2003; Pries *et al.*, 2006). It was observed that the body weights of mice found in three test groups treated with 250, 500, and 1000

mg/kg of *A. muricata* improved hence the extract may also help to restore body tissue proteins.

Atherogenic indices (TC/HDL-C and TG/HDL-C ratios) have been identified as successful markers for predicting individuals at the risk of atherosclerosis, which begins early in life. Therefore, our result which showed that alloxan increased TC/HDL-C and TG/HDL-C ratios and that treatment with the leaf extract of *Annona muricata* attenuated rise in the ratios are very prominent. Adam *et al.*, The result suggest that, monitoring TC/HDL-C and TG/HDL-C ratios may assist in early detection of associated atherogenic dyslipidemia in diabetics and that, leaf extract of *Annona muricata* could serve as a good therapeutic agent for early intervention against cardio-metabolic disorders.

Significant elevation of TG, TC, LDL and VLDL and decreased level of HDL in alloxan induced diabetic mice was observed. Treatment with methanolic extract of *A.muricata* leaves not only decreased serum TG, TC, LDL and VLDL but also increased HDL level significantly. The level of serum lipid profiles are usually high in diabetic mice and such elevation represents risk factor for coronary heart diseases (Maruthupandian *et al.*, 2010). The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Kanakasabapathi and Gopalakrishnan, 2015). In normal metabolism, insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic mice after *A.muricata* treatment maybe directly attributed to improvements in insulin levels (Maruthupandian *et al.*, 2010). Other research works has also reported that the flavonoids, alkaloids and tannins are responsible for hypoglycemic and hypolipidemic effect (Adeneye and Olagunju, 2009; Kanakasabapathi and Gopalakrishnan, 2015). Therefore, the presence of high amount of flavonoids in *A.muricata* (Vijayameena *et al.*, 2013) cause hypolipidemia which removes the LDL cholesterol from blood by increasing the LDL receptor densities in the liver and by binding to lipoprotein B (El-Tantaway *et al.*, 2009).

The levels of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were significantly ($p<0.05$) reduced in alloxan induced mice. These adverse changes were significantly ($P>0.05$) reversed in groups of mice treated with methanoilc extract of *A.muricata* leaves. Superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) play an important role as protective enzymes against free radical formation in tissues (Mohan *et al.*, 2010). This result suggests *A.muricata* leaves

extract to have a protective role in decreasing lipid peroxidation and by normalizing antioxidant system.

Conclusion

This study showed that methanoilic extract of *Annona muricata* leaves has the potency of ameliorating pancreatic β cell function and attenuated dyslipidemia in T2D condition by decreasing lipid peroxidation and by normalizing antioxidant system. This experiment backs the traditional usage of this plant leaves as an alternative drug source, for effective control/treatment of hypoglycemic and hypolipidemic agent and cardiovascular diseases.

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**EFFECTS OF ANTIOXIDANTS AND PROBIOTICS ON HUMORAL IMMUNITY OF
NEWCASTLE DISEASE VACCINATED BROILERS**

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Abstract

The effects of antioxidants (Vitamins A, C, and E and Selenium) and probiotics on humoral immunity were studied in 150 male Arbor Acres broiler chickens. The chickens were grouped into 5 of 30 broilers with 2 replicates in a group. The broilers were grouped as follows; group 1 (unvaccinated non supplemented control), group 2 (vaccinated non supplemented control), group 3 (antioxidants supplements), group 4 (probiotics supplements), and group 5 (antioxidants and probiotics). La Sota vaccine was administered at the age of 21 days. The feeds in groups 1 to 5 were supplemented as indicated for each group above. The unvaccinated non supplemented control group (group 1) showed very low mean HI titres which became unprotective as from week 4. The vaccinated non supplemented control group (group 2) had high mean HI titres indicating that the vaccine was immunogenic. The vaccinated and supplemented with antioxidants (group 3) had significantly ($p < 0.05$) higher mean HI titres which were protective throughout the period of study, indicating that the antioxidants engendered antibody production. The vaccinated and supplemented with probiotics (group 4) had significantly ($p < 0.05$) higher mean HI titres at week 5, the probiotics group maintained high mean HI titres till the end of the study. The vaccinated and supplemented with both antioxidants and probiotics (group 5) retained high mean HI titres which were protective throughout the study. The antioxidants and probiotic groups (3 and 4 respectively) maintained significantly ($p > 0.05$) higher mean HI titres than the control groups (1 and 2). The result of the study shows vaccination alone may not be adequate for the productive lives of chickens, therefore it will be advantageous to supplement broiler feeds with either antioxidants or probiotics. Varying levels of probiotics is recommended and a repeat of the study in laying chickens.

Keywords: Vitamin A; Vitamin C; Vitamin E; Selenium; Antioxidants; Probiotics; Broilers

Introduction

Newcastle Disease (ND) is caused by ND virus (NDV), and all strains of NDV belong to the family Paramyxoviridae, genus *Avulavirus*. They are part of one serotype, avian Paramyxovirus serotype-1 (APMV-1) which is an enveloped negative single stranded RNA virus (Alexander and Senne, 2008). Virulent ND strains may replicate in vaccinated birds, but the clinical signs will be greatly

diminished in relationship to the antibody level achieved (Allan *et al.*, 1978).

Fuller (1989) defines probiotics as live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance. Havenaar and Huisin't Veld (1992) expanded the definition to include food and non-food use and the use of mono and mixed cultures. Probiotics provide immunity resulting from gut exposure to a variety of antigens, such as pathogenic bacteria and dietary protein. It is important in the defense of young animals against enteric infection (Perdigon *et al.*, 1995).

Oxidative stress is best defined in broad terms as an alteration in the pro-oxidant–antioxidant balance in favour of the former that leads to potential damage (Halliwell and Gutteridge, 1999). According to antioxidant theory, when the concentrations of antioxidant vitamins (vitamin C and vitamin E) decrease, lipid peroxidation increases in the plasma and tissues, leading to damage of cell membranes (Klasing, 1993).

Newcastle Disease had been reported in all parts of Nigeria (Baba *et al.*, 1995). Although vaccination programmes exist that control disease among poultry, the virus continues to evolve in chickens and other species (Seal *et al.*, 1998).

Therefore, the need to investigate the effects of probiotic and antioxidant supplements on humoral immune response to Newcastle disease vaccine because it has been suggested that the inability to use vaccination alone to control Newcastle disease may be attributed to “vaccination failure” as a result of over dilution, thermal inactivation when temperature above 50⁰ C or “vaccine breaks” due to antigenic variation between infecting field and vaccine strains (Shamaki *et al.*, 1989).

The poultry industry in Nigeria is the most capitalized among the Agricultural sectors in the country and contribute the largest to the economy next to the oil industry (Adene and Oguntade, 2006). The objective of this study is to determine the effects of probiotic and antioxidant supplements on humoral immune response to Newcastle disease vaccine

Materials and Methods

The Study was carried out in Avian unit of Department of Veterinary Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAAU), which is

located between coordinate 5° 28' 33''N Latitude North of the equator and 7° 32' 56''E Longitude East of Greenwich Meridian.

The study was carried out on male Arbor Acres broiler (n = 150). One hundred and fifty (150) day-old chicks were obtained and distributed into five groups of 30 chicks; 1 (Control), 2, 3, 4, and 5 with two replicates of 15 birds in each group (a and b).

Group 1 (control): Not vaccinated not supplemented

Group 2: Vaccinated not supplemented

Group 3: Vaccinated and supplemented with antioxidants (Vitamins A, C, E, and Selenium)

Group 4: Vaccinated and supplemented with probiotics

Group 5: Vaccinated and supplemented with antioxidants (Vitamins A, C, E, and Selenium) and probiotics

Groups; 3 and 5 as indicated had the feed fortified with additional levels of vitamin A (0.375g), vitamin C (10g), vitamin E (7.5g) and Selenium (12.5g) to each 25kg of feed as recommended by US National Research Council (1994) for enhancing immune response of chickens.

Groups 4 and 5 as indicated were supplemented with Probiotic: Gro-2-Max® broiler Probiotic was manufactured by Bio-National American Institute and contains; *Bacillus subtilis* (1x10⁶cfu/g), *Lactobacillus acidophilus* (3x10⁶cfu/g), *Pedococcus acidilactici* (2x10⁶ cfu/g), *Pedococcus pentosaceus* (2x10⁶ cfu/g), and *Saccharomyces cerevisiae* (1x10⁶ cfu/g). Gro-2-Max was mixed with 25g to 45kg of feed.

Birds were housed in an intensive management system and floor covered with wood shavings. The baseline data were collected before vaccination as chicks were administered with Newcastle disease vaccine (NDV-B1 strain, intraocularly), Infectious bursal disease vaccine (IBDV in drinking water), and Newcastle disease vaccine (NDV-La Sota in drinking water) on day 2, 10, and 21, respectively. Then, second Infectious bursal disease vaccine (IBDV, in drinking water) at day 24. Chicks were fed with self-milled feed; broiler starter diet for the first 28 days of age and finisher diet from 29 to 36 days of age.

Serology was done weekly by taking about 1 ml of blood from the wing vein of ten birds per group into labeled plain bottles and sera separated (week 1, 2, 3, 4, and 5) to determine the antibody titre using Haemagglutination Inhibition (HI) test at Veterinary Medicine laboratory, University of Nigeria, Nsukka (UNN).

The Haemagglutination (HA) technique employed in the HA titration was as described by OIE Manual (2012), and 0.6% erythrocyte solution red blood cell concentration was obtained as described by Wosu (1984). The Haemagglutination Inhibition (HI) technique employed was as described by OIE Manual (2012).

Table 1: Mean Haemagglutination Inhibition (HI) Titres of Broilers Fortified with Antioxidants and Probiotics for 5 Weeks

Group	Week1	Week2	Week3	Week4	Week5
1	16.0±2.92 ^a	24.0±3.4 ^a	54.0±6.4 ^a	2.0±0.0 ^a	3.6±0.8 ^a
2	172.8±63.5 ^b	198.4±58.2 ^b	166±25.6 ^{bc}	153.0±28.9 ^b	70.4±10.5 ^a
3	259.2±52.3 ^b	153.6±17.1 ^b	128±0.0 ^b	921.6±68.3 ^d	289.4±62.7 ^c
4	243.2±51.2 ^b	128±23.4 ^{ab}	128±0.0 ^b	614.4±68.3 ^c	207.2±34.1 ^c
5	172.8±34.1 ^b	230.4±49.8 ^b	179.2±20.9 ^c	166.0±25.6 ^b	179.2±20.9 ^b

Results and Discussion

The results of the haemagglutination inhibition (HI) tests of the broilers supplemented with antioxidants and probiotics are presented in table 1.

In week 1, the HI titre of group 1 was significantly ($p > 0.05$) lower than HI titres of groups 2, 3, 4 and 5. The mean HI titres obtained in the different groups were protective though the antioxidant and probiotic groups (3 and 4 respectively) have significantly ($p < 0.05$) higher values than the control groups 1 (no vaccination) and 2 (vaccination but not supplemented).

In week 2, HI titres of groups 1 and 4 were not significantly ($p > 0.05$) different, also the titres of groups 2, 3, and 5 were not significantly ($p > 0.05$) different from one another but were significantly ($p < 0.05$) higher than the titre for group 1. The antioxidant probiotic combined group (5) had very

high HI titre though not significantly ($p > 0.05$) higher than the supplemented groups (3 and 4) and the non-supplemented group 2.

In week 3, the HI titre of group 5 was significantly ($p < 0.05$) higher than the titres in groups 1, 3 and 4. The HI titre of group 2 was not significantly ($p > 0.05$) different from those of groups 3, 4 and 5. The HI titres of groups 3 and 4 were not significantly ($p > 0.05$) different but were significantly ($p < 0.05$) higher than that of group 1. The antioxidants used in this study were vitamins A, C, E and Selenium; Kam *et al.* (2012) and Lucas *et al.* (2014) reported that vitamin A contributes to several immune - related functions, like enhancement of mucosa immunity and reduction of free radicals in chickens, Yuan *et al.* (2014) also explained that the vitamin is known for its anti-inflammatory immunostimulatory effects in chickens. El-Senousey *et al.* (2017) explained that Vitamin C is well known for both antioxidant and anti-inflammatory effects making it to be beneficial in cases of oxidative stress, inflammation and infection in chickens. Khan *et al.* (2012) reported that Vitamin E increases the number and functionality of immune system cells also stimulating antibody release in response to chicken vaccination.

In week 4, the HI titre of group 3 was significantly ($p < 0.05$) higher than those of groups 4, 2, and 1. The combined effects of Vitamins A, C, E and Selenium was able to produce such a significantly ($p < 0.05$) higher titre than both the probiotic (4) and antioxidant-probiotic (5) and the control groups (1 and 2). This was the highest mean titre obtained through out this study from the different groups. Sanda *et al.* (2015) observed and reported that high level vitamin -mineral supplementation was beneficial to enhancing immune responses of broilers that were vaccinated against Newcastle disease in their study using Vitamins A, C, E and Selenium. This agrees with the observation in this present study. Likewise, the HI titre of group 4 was significantly ($p < 0.05$) higher than those of groups 2 and 1. The HI titre of group 2 was significantly ($p < 0.05$) higher than that of group 1. The maternally derived antibody had finally waned at week 4. The mean HI titre (153.0 ± 28.9) of the vaccinated control group (2) being so high at week 4 shows the vaccine was immunogenic and vaccination procedure was adequate.

In week 5, there was no significant ($p > 0.05$) difference in the HI titres of groups 1 (3.6 ± 0.8) and 2 (70.4 ± 10.5). The HI titres of groups 3 (289.4 ± 62.7) and 4 (207.2 ± 34.1) were not significantly ($p > 0.05$) different, but were significantly ($p < 0.05$) higher than the titre of group 5 (179.2 ± 20.9) and groups 1 (3.6 ± 0.8) and 2 (70.4 ± 10.5) while the HI titre of group 5 (179.2 ± 20.9) is significantly

($p < 0.05$) higher than that of groups 1 (3.6 ± 0.8) and 2 (70.4 ± 10.5). The antioxidants and probiotic groups (3 and 4 respectively) maintained significantly ($p > 0.05$) higher mean titres than the control groups (1 and 2).

Isolauri *et al.* (1991), observed that administration of probiotics containing *Bifidobacterium bifidum* and *Streptococcus thermophiles* to children with rotaviral diarrhea resulted in faster seroconversion within IgA and IgM antibodies accompanied by the growth of cells producing IgM antibodies. This observation agrees with the findings of this study with consistently high mean HI titres of groups 4 and 5 showing probiotics alone or with antioxidants have beneficial effects in enhancing the immunity of the broilers supplemented.

Conclusion

In conclusion, Newcastle disease vaccinations were protective. The mean HI titre of the vaccinated control group continued to decline rapidly while the antioxidants group maintained a very high mean HI titre likewise, the probiotic and antioxidant-probiotic groups. It appears that vaccination alone may not be adequate for the productive lives of chickens, since poultry production is not without stress factors (like high or low ambient temperatures, overcrowding, and infections), it will therefore be advantageous to supplement broiler feeds with either antioxidants or probiotics.

Recommendations

Further studies using varying levels of probiotics is recommended. It is also recommended that this study is repeated in a flock of laying chickens.

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BIOTECHNOLOGY AND NANOTECHNOLOGY (BTN)

TISSUE CULTURE OF *Jatropha curcas*

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Abstract

There is mounting interest in *Jatropha* species as biodiesel tree to lessen the energy crises and to generally increase income in rural areas. Tissue culture have been used for propagation of many plant species to generate material for preservation. *Jatropha curcas* belongs to the genus Euphorbiaceae family which possess high amount of hydrocarbons which can be used for substitute for conventional petroleum sources. The study conducted tissue culture of *Jatropha curcas* from embryo. By this method callus responses of 60%, 50% and 70% were recorded from media supplemented by 1, 2 and 3mg/l 2- 4D respectively. The highest shoot inductions of 1.4±0.0 was observed in supplemented medium of 1mg/LBAP +2mg/L/IBA. The study revealed an efficient and reliable plant regeneration protocol that can be potentially utilized for conservation and mass production of *J. curcas* to meet the demand of energy plantation as well as for therapeutic purpose,

Keywords: *Jatropha curcas*, biodiesel, medicine and Tissue culture

Introduction

Jatropha is a large genus belonging to euphorbiaceae family comprising more than 170 species. The commonly occurring species are in India. Most of the species are ornamental, except for *J. curcas* and *J. glandulifera*, which are oily yielding species (Swarup, 2004). There are number of *Jatropha* species best among is *J. curcas*, others are *J. tanjorensis*, *J. nana*, *J. gossypifolia*, *J. integririma*, *J. multifida* and so on (Kumar, *et al.*, 2015). *J. curcas* also called physic nut is one of the best taxon for future biodiesel production. Biodiesel prepared from *J. curcas* has been successfully tested in both mobile and stationary engines without modification in any of the engine parts. Now there is a surge of interest in *J. curcas* as a biodiesel to help alleviate the energy crisis and generate income in rural areas of developing countries. Globally, *J. curcas* is promoted for large cultivation in a big way for biodiesel production (Sujatha, *et al.*, 2005). Tissue culture involves the use of small explants which are cultured in a nutrient medium under sterile condition evaluation of tissue culture propagated plants of *Jatropha curcas* revealed that they were at par with seed propagated plants in terms of yield and yield related traits multiple shoots were induced from various types of explants. shoot multiplication has been induced from leaf, petiole, stem, epicotyls, hypocotyles, peduncles and nodal segments sardan *et al.*, 1998, rajore and batra, 2005, Datta *et al.*, 2007]. But these report were not promising because multiplication rate was low. Nodal meristems are an important source tissue of micropropagation and plant raised from these are comparative more resistant to genetic variation (Pierik 1991). Also the plant regenerated are generally disease free. The important of micropropagation efficiency of *Jatropha curcas* is very important for its biodiesel production.

MATERIALS AND METHODS

Plant Material

J. curcas seeds were collected in greenhouse of Sheda science and technology complex Abuja, under optimum condition to minimize contamination in the *in vitro* culture.

Authentication of the Plants

The plants were authenticated by plant taxonomist and botanists at Shed Science and Technology Complex Abuja, Nigeria

Culture media and growth conditions.

Murashige and Skoog (MS) medium,) All the media were adjusted to pH 5.8, solidified with, 1.5g Phytigel and autoclaved at 121°C for 15–1 min. The medium used for the callus induction and multiplication contained 3% sucrose the cultures were incubated in the laboratory at 2 °C under a 16/8-h (light/dark cycle) photoperiod provided with cool white fluorescent light

Plant material and preparation of explants.

After cleaning in a solution of 5% (v/v) liquid detergent, they were washed under running tap water for 1hour. Then surface-sterilized with 75% ethanol solution for 8–20min. afterwards, they were rinsed with sterile distilled water five times. After cutting off the seeds the sterilized cotyledons were extracted and inoculated on Murashige and Skoog (MS) medium I mg l⁻¹ 2,4 Dichlorophenoxyacetic acid (2,4D) + 3% sucrose +Phytigel and kept in dark rooms

Shoot initiation.

The callus developed to plantlets and were later subculture in media supplemented with different

concentrations of Benzylaminopurine (BAP) and Indole-3-Butyric Acid (IBA).

Acclimatization.

Plantlets that were observed to have well-developed roots after four weeks were transferred to a greenhouse and kept for approximately 5–7 days. Afterwards, the plantlets were gently removed from the culture vessels and washed off the adhering medium. Subsequently, they were transplanted to plastic cups containing soil.

standard deviation

Induction rate (%) = the number of induced explants / the number of total initial explants × 100%;

Multiplication rate (%) = the total number of buds (≥0.3cm) / the number of initial buds on the subculture Explants × 100%;

Table 1: Percentage Callus formation

Media mg/l	Percentages
MS+1, 2-4D	60
MS+2, 2-4D	70
MS+3, 2-4D	50

Statistical Analysis.

All experimental values were expressed as mean

Table 2: Effects of combination of BAP and IBA on in vitro culture of *J. Curcas* at 4 weeks

BAP (mg/l)	IBA mg/l	Shoot induction	Total	% shootin g	Number of leaves > 0.5 cm	Total	Growth state of shoot
1	1	1.4±0.0	3	33	3.0±0.0	9	+++
1	2	1.5±1.0	3	33	2.0±2.0	6	+++
1	3	0.0±0.0	0	0	0.0±0.0	0	++
2	1	1.5±0.5	5	56	3.5±0.5	10	--
2	2	0.0±0.0	0	0	0.0±0.0	0	--
2	3	1.6±0.5	1	11	0.6±1.1	1	--
Control	0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0	--

RESULTS

Result from table 1 shows that combination of 1mg/L BAP and 1mg/L/BA, and 1mg/LBAP and 2mg/L/BA had the highest shoot inductions of 1.4±0.0 and 1.5±1.0

respectively. The highest number of leaves was observed in supplemented medium of 2mg/LBAP and 1mg/L/BA with 3.3±0.5

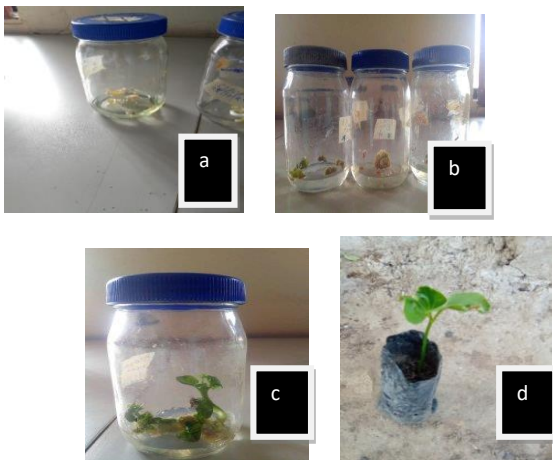


Figure 1a: Cutred embryo, 1b: callus formed from the cultured embryos. 1c: Combination of 1mg/L BAP and 1mg/L/BA plantlet, 1d: Acclimatized plantlet

Discussion

The study conducted tissue culture of *Jatropha curcas* from embryo. By this method callus responses of 60%, 50% and 70% were recorded from media supplemented by 1, 2 and 3mg/l 2- 4D respectively and no contamination(Table1).More so, theResults from table2 shows that combination of 1mg/L BAP and 1mg/L/BA, and 1mg/LBAP and 2mg/L/BA had the highest shoot inductions of 1.4±0.0 and 1.5±1.0 respectively. The highest number of leaves was observed in supplemented medium of 2mg/LBAP and 1mg/L/BA with 3.3±0.5. *J. curcas* grows well under adverse climate in low to high rainfall areas (200-1500mm) but best in 600mm-l. *J. curcas* never expressed it potential for oil productivity due to lack of moisture and nutrition. In flowering frequency and season male and female flowers ratio dependent on soil fertility, soil water content. Seed production are vulnerable to drought and waterlogging. Relative humidity and temperature in drier Agro Climatic zones of exhibits only one major flowering flush (Singh, Pathre and Rayan, 2013) Ever increasing in fuel prices and depletion of fossil reserves have ignited a worldwide search for alternative renewable energy

sources. *Jatropha* methyl esters yields biodiesel of an exceptional quality and easy adaptation. Large scale cultivation remains the single most important factor that will ultimately determine the success of *Jatropha. curcas* as source of biodiesel. Non availability of superior clones. Short of cutting, low multiplication rate, gaps in knowledge of clonal technology. (Kumar and Reddy, 2012) Study was carried out on Tissue culture with different gelling agent, different light conditions, and after care of ex vitro rooted shoots to enhance survival rate of teak. Multiple shoot formation was induced from excised seedling nodal explants on MS supplemented with BA

Conclusion

The study revealed an efficient and reliable plant regeneration protocol that can be potentially utilized for conservation and mass production of *J.curcas* to meet the demand of energy plantation as well as for therapeutic purpose.The report could also be valuable in producing more robust plants, fast growth, time saving for the growers when seeds and cutting are slow to establish.

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WIS –BTN 05

POSTHARVEST CONTROL OF YAM (*Dioscorea rotundata*) TUBER ROOT WITH LEAF EXTRACTS OF *Vernonia amygdalina* AND *Ocimum gratissimum*

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ABSTRACT

Antifungal effect of ethanolic and aqueous extract of *Vernonia amygdalina* and *Ocimum gratissimum* with four different extract concentration from each plant leaves by blending 25g, 50g, 75g and 100g in 100ml of sterile distilled water and ethanol on the growth of fungi causing rot on postharvest yam were investigated in vitro at Plant Pathology laboratory National Root Crops Research Institute, Umudike, Abia State, Nigeria. Effect of standard antibiotics (Grisovid) comparative to both plant extracts was determined. Pathogenicity test revealed that *Botryodiplodia theobromae*, *Fusarium sp.*, *Rhizopus stolonifer* and *Aspergillus flavus* induced rot in healthy yam tubers after 9 days of inoculation with *Botryodiplodia* being the most virulent (24mm). All the extracts showed varying degrees of antifungal efficacy, ethanol extract proved to be more potent. The potency of the extracts varied with the solvent of extraction, concentration of extract and test fungi. Extract of *Ocimum gratissimum* showed high rate of inhibition on all the test fungi ranging from (37.14 - 99.67%) mycelial inhibition percentage while *Vernonia amygdalina* varying from (2.5- 99.14%) inhibition percentage on all test fungi. 75% extract concentration of *Ocimum gratissimum* being the most fungitoxic showed significant ($p \leq 0.05$) inhibition on all the test fungi. This study recommends the fungitoxic potential of these plant extracts on rot inducing fungi of stored yam tubers for farmers use as an alternative to commercial synthetic fungicides. Both plant extracts are friendly, non toxic to animals and man.

Keywords: *Dioscorea rotundata*, *Ocimum gratissimum*, *Vernonia amygdalina* and Postharvest.

INTRODUCTION

Yam is a monocotyledonous angiosperm, which belongs to the order Liliiflorae, family Dioscoreaceae, and genus Dioscorea. It is considered to be among the most primitive of the angiosperms and contains over 600 species, of which about ten are edible (Brand-Miller *et al.*, 2003). Cultivated species of yam include *D. alata* (water yam), *D. cayenensis* (yellow yam), *D. rotundata* (white Guinea yam), *D. esculenta* (lesser yam), *D. bulbifera* (aerial yam), *D. opposita* (Chinese yam), *D. nummularia* (purple yam), *D. pentaphylla* (multiple-tuber yam), *D. hispida* (cluster yam), *D. trifida* (cush cush yam) and *D. dumetorum* (three-leaved yam or bitter yam) (Brand-Miller *et al.*, 2003). *Ocimum gratissimum* (L.) is a herbaceous, perennial shrub of the family Lamiaceae, often found growing around villages with its characteristic odour for seasoning dishes (Okujagu, 2008). It is a common spice with medicinal value, often known as 'scent leaf', among localities in Nigeria. It is woody at the base and has an average height of 1-3 meters. The leaves are broad and narrowly ovate, usually 5-13cm long and 3-9cm wide. *Vernonia amygdalina*, a species in the family Asteraceae, is a tropical shrub with height of 1- 3m, petiole leaf of about 6mm in diameter (Ibrahim *et al.*, 2009). *Vernonia amygdalina* commonly called bitter leaf in English, "oriwo" in Edo, "ewuro" in Yoruba, "shikawa" in Hausa, and "olubu" in Igbo (Oboh and Masodje, 2009). The leaves are consumed as vegetable and condiments, after macerating and washing thoroughly to remove the bitterness. They are known for having intense purple flowers and the genus was named after the English botanist William Vernon.

Yam rot can be a pre-harvest (occurring in the field) rot or post-harvest rot (occurring in the storage) or rot diseases carried from field to storage. Rot is a major factor limiting the post-harvest life of yams and losses can be very high. Losses due to post-harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting. The quality of yam tubers are affected by rots, which makes them unappealing to consumers. In Nigeria, over 60% of white yam varieties get rotten when stored for more than six months (Chukwu *et al.* 2008). The present work is undertaken to isolate fungi responsible spoilage of stored yam and also to ascertain the effects of ethanolic and aqueous extracts on mycelia growth on fungi causing rot on stored yam.

MATERIALS AND METHODS

(Sources of Yam tubers and plant materials)

Yam tubers { *Dioscorea rotundata* } that are rotted were collected from the Yam programme of National Root Crops Research Institute, Umudike, Abia State, Nigeria. The yam tubers were packed in sterile nylon bags and were transported to Plant Pathology Laboratory of the same Institute for pathological analysis. Clean yam tubers were equally collected for pathogenicity test. *Ocimum gratissimum* and *Vernonia amygdalina* leaves in the experiment were collected from the staff quarters of NRCRI, Umudike and were authenticated in the Germplasm Resource Unit of the Institute,

ISOLATION OF PATHOGENS

Rotted Yam tubers were washed in tap water and cut into smaller sections (3mm) with sterilized scarpel and rinsed with several changes of distilled water, the cut sections were surface sterilized with 70% ethanol for one minute, washed in sterilized water, blotted in sterile filter paper, picked with flamed sterilized forceps and inoculated on solidified potato Dextrose agar (PDA) medium in different plates. All plates inoculated were incubated at room temperature (25°C) for four days. The plates were observed daily for emergence of mycelia growth. Sub-culturing was done on the mycelia emergence to obtain pure culture. Pure isolates were kept in PDA slants and stored in a refrigerator at 4°C.

PATHOGENICITY TEST

Clean (healthy) yam tubers were washed with sterile water and later sterilized with 70% ethanol. A 5mm cork borer was used to remove cylindrical discs from the tuber, sterile 4mm cork borer was used to remove 5 discs from a 5 day pure culture and were used to plug the holes created in the tuber, the removed tuber discs was replaced, then Vaseline was applied on the point of inoculation guiding against cross contamination. The inoculated yam tubers were transferred into an improvised humidifier chamber for 7 days. Features like softening of tissues, foul smell was observed during incubation. A sharp knife was used to cut the inoculated roots into two halves and the infected parts were measured with transparent ruler and was recorded. Pathogenicity test was performed to confirm the pathogenic microorganisms responsible for the spoilage.

PREPARATION OF EXTRACTS

100g of *Ocimum gratissimum* and *Vernonia amygdalina* leaves were weighed out, washed in sterile water. The leaves were shade dried for 4 days, grinded in a laboratory Milling machine. The extract powder { 25g,50g,75g,100g }, was dispensed into 100ml of ethanol and sterile distilled water (SDW). This was vigorously stirred and left to stand for 6 hours. The solution was filtered with whatman Filter paper (NO 1) to obtain a filtrate which was used as plant extracts.

(25%,50%,75%,100%)

EFFECT OF THE EXTRACT ON FUNGAL GROWTH

The poison method of *Amadioha and Obi (1999)* was used to determine the effect of the extract on Fungal growth. This was done by drawing two perpendicular lines at the intersection indicating the Centre of the plate. The two perpendicular lines were drawn at the base of each plate before dispensing Potato Dextrose Agar into each of the plates. 2ml of the extract of the various plant material were separately introduced into the petri-dish containing the media (PDA). A disc of (5mm diameter) of the pure culture of *Aspergillus flavus*, *Rhizopus spp* *Botrydiplodia theobromae*, *fuasarinnm sp* was placed at the centre of the plate containing the media(PDA) and the extract. Plates without extract were used for experiment control. Toxicity of extract against fungi was recorded in terms of percentage colony inhibition and calculated according to the formula of Pandey et. al, (1982) Growth inhibition (%) = [(DC-DT / DC) * 100, where DC = Average diameter of control, and DT = Average diameter of Fungal colony with treatment.

DETERMINATION OF PERCENTAGE OCCURRENCE OF THE FUNGAL ISOLATES

This was done to determine the incidence of different fungal isolates. The frequency of occurrence of the pathogens from the rotten yam tubers were determined. The total number of each isolate in all isolates was obtained against the total number of all the isolates in all the samples screened. The mean value of this gives the percentage of occurrence as the following equation shows.

$$\% \text{ of occurrence} = X/N \times 100$$

Where X= total number of each isolate in all sample and
N= total number of all the isolates in all samples.

The present work is undertaken to isolate fungi responsible for spoilage of stored yam and also to ascertain the effects of ethanolic and aqueous extracts on mycelia growth on fungi causing rot on stored

RESULTS

Table 1: EFFECTS OF AQUEOUS PLANT EXTRACTS ON MYCELIA GROWTH (MM) ON RHIZOPUS SPP.

Plant extracts	<i>Vernonia amygdalina</i>		<i>Ocimum gratissimum</i>	
Concentrations%	mycelium growth (mm)	%inhibition	mycelia growth (mm)	% inhibition
100	38	5.0	20	42.86
75	39	2.5	24	31.43
50	39	2.5	25	28.57
25	39	2.5	30	14.29
Grisorid	0.3	-	0.4	-
Control	40	-	35	-
LSD(0.05)	0.44.4	-	0.2661	-

Result on the effect of aqueous plant extracts on mycelia growth of *rhizopus spp* are shown in table 1. Result revealed that there are no significant ($p \geq 0.05$) levels of inhibition of mycelia growth of *rhizopus sp* at various concentrations. However, the inhibitory effect on mycelia growth by plant extracts increased with higher concentration. The highest (42.86%) percentage

inhibition of mycelia growth by plant extracts was produced by scent leaves at the concentration of 100% while the least (2.5%) was recorded by bitter leaves of the concentration of 25%. The percentage inhibition of mycelia of 25%. The percentage inhibition of mycelia growth of both extracts at 100% did not compare well with the synthetic control (grisovid)

TABLE 2: EFFECTS OF AQUEOUS PLANT EXTRACTS ON MYCELIA GROWTH ON *FUSARIUM*

Plant extracts	<i>Vernonia amygdalina</i>		<i>Ocimum gratissimum</i>	
Concentrations%	mycelium growth (mm)	%inhibition	mycelia growth	% inhibition
100	9	94.00	5	64.28
75	9	40.00	8	42.46
50	10	33.33	10	28.57
25	10	33.33	10	28.57
Grisorid	0.1	-	0.3	-
Control	15	-	14	-
LSD (0.05)	0.46.2	-	38.963	-

The table 2 shows the effect of aqueous plant extracts on mycelia growth of *fusarium spp*. Result also showed that both plant extracts produced significant ($p \leq 0.5$) levels of inhibition of mycelia growth of *fusarium spp* at various concentrations. The highest (94%) percentage inhibition

of mycelia growth by plant extracts was produced by bitter leaves at concentration of 100% while the least (28.57%) percentage inhibition of mycelia growth was recorded by scent leaves at the concentration of 25%.

Table 3 EFFECTS OF ETHANOIC PLANT EXTRACTS ON MYCELIA GROWTH ON *RHIZOPUS. SPP*

Plant extracts	<i>Vernonia amygdalina</i>		<i>Ocimum gratissimum</i>	
Concentrations%	mycelium growth(mm)	%inhibition	mycelia growth	% inhibition
100	12	65.75	0.2	99.50
75	17	51.43	0.5	98.75
50	20	42.86	10	75.00
25	22	37.14	15	62.50
Grisorid	0.4	-	0.3	-
Control	35	-	40	-
LSD (0.05)	0.3927	-	04274	-

Result on the effect of plant extracts on mycelia growth of *rhizopus spp* is shown in table 3. The result showed significant ($p \leq 0.05$) levels of inhibition of mycelia growth of *rhizopus spp* by both extracts at various concentrations. The highest (99.50%) percentage

inhibition of mycelia growth by plant extracts was produced by scent leaves at the concentration of 100% while the least (37.14%) percentage of mycelia growth was recorded by bitter leaves at the concentration of 25%

Table 4: EFFECTS OF ETHANOIC PLANT EXTRACTS ON MYCELIA GROWTH ON *FUSARIUM SP*

Plant extracts	<i>Vernonia amygdalina</i>		<i>Ocimum gratissimum</i>	
Concentrations%	mycelium growth(mm)	%inhibition	mycelia growth	% inhibition
100	0.1	66.75	0.1	88.89
75	0.2	51.43	0.2	77.78
50	0.2	42.86	0.2	75.00
25	0.3	39.14	0.3	66.00
Grisorid	0.1	-	0.2	-
Control	35	-	0.9	-
LSD (0.05)	0.4314	-	0.378	-

Table 4 shows the effect of plant extracts on mycelia growth of *fusarium spp*. There were significant ($p \leq 0.05$) levels of inhibition of mycelia growth of *fusarium spp* by both extracts at various concentrations. The highest (88.89%) percentage inhibition of mycelia growth by plant extracts was produced by scent leaves at 100%

concentration while the least (39.14%) percentage inhibition of mycelia growth was recorded by bitter leaves at the concentration 25%. The result also showed that the percentage inhibition of mycelia growth by both plants extracts at 100% compared well with grisovid even at lower concentrations.

Table 5: EFFECTS OF ETHANOIC PLANT EXTRACTS ON MYCELIA GROWTH ON *BOTRYODIPLODIA SP*

Plant extracts	<i>Vernonia amygdalina</i>		<i>Ocimum gratissimum</i>	
Concentrations%	mycelium growth(mm)	%inhibition	mycelia growth	% inhibition
100	0.3	99.14	0.1	99.67
75	0.4	98.86	0.1	99.67
50	0.5	98.58	0.2	99.00
25	0.9	97.43	0.3	93.33
Grisorid	0.2	-	0.1	-
Control	35	-	3.0	-
LSD (0.05)	0.3308	-	0.5175	-

Table 5 shows effects of plant extracts on mycelia growth of *botryodiplodia spp*. The result revealed that both plant extracts were significant ($p \leq 0.05$) levels of inhibition on *botryodiplodia* mycelia at all the concentration levels. The highest (99.67%) percentage inhibition of mycelia growth by plant extracts was produced by scent leaves at

the concentration of 100% while the least (93.33%) percentage of mycelia growth was recorded by scent leaves at the concentration of 25%. All the percentage inhibition of mycelia growth by both plant extracts compared well with grisovid even at lower concentrations.

Table 6: EFFECTS OF ETHANOIC PLANT EXTRACTS ON MYCELIA GROWTH ON *ASPERGILLUS FLAVUS*

Plant extracts	<i>Vernonia amygdalina</i>		<i>Ocimum gratissimum</i>	
Concentrations(%)	mycelium growth (mm)	%inhibition	mycelium growth(mm)	%inhibition
100	0.2	98.00	0.1	99.33
75	0.3	97.00	0.5	96.67
50	0.5	95.00	0.5	96.67
25	0.7	93.00	0.9	94.00
Grisovid	0.2	-	0.2	-
Control	10	-	15	-
LSD(0.05)	11.39	-	0.3497	-

Table 6 shows the effect of plant extracts on mycelia growth of *Aspergillus flavus*. There were significant different ($p \leq 0.05$) levels of inhibition of mycelia growth of *Aspergillus flavus* by both extracts at various concentrations. The highest(99.33%) percentage

inhibition of mycelia growth by plant extracts was produced by Scent leaves at concentration of 100% while the least (93.00) percentage inhibition of mycelia growth was recorded by Bitter leaves at concentration of 25%. However, the percentage inhibition of mycelia growth by

both plant extracts at 100% compared very well with Grisovid.

Table 7: EFFECTS OF AQUEOUS PLANT EXTRACTS ON MYCELIA GROWTH ON *BOTRYODIPLODIA*

Plant extracts	<i>Vernonia amygdalina</i>		<i>Ocimum gratissimum</i>	
	mycelium growth (mm)	%inhibition	mycelium growth(mm)	%inhibition
Concentrations(%)				
100	0.2	7.0	0.1	50.00
75	0.3	3.5	0.5	47.00
50	0.5	2.5	0.5	35.57
25	0.7	2.5	0.9	28.57
Grisovid	0.2	-	0.2	-
Control	10	-	15	-
LSD(0.05)	0.413	-	0.3497	-

Table 6 shows the effect of plant extracts on mycelia growth of *Botryodiplodia* sp. There is no significant different ($p \leq 0.05$) levels of inhibition of mycelia growth of *Botryodiplodia* by *vernonia amygdalina* extracts at various concentrations. The highest (50.00%) percentage inhibition of mycelia growth by plant extracts was

produced by Scent leaves at concentration of 100% while the least (28.57%) percentage inhibition of mycelia growth was recorded by Scent leaves at concentration of 25%. However, the percentage inhibition of mycelia growth on Scent leaves plant extracts at 100% compared very well with Grisovid.

Table 8: EFFECTS OF AQUEOUS PLANT EXTRACTS ON MYCELIA GROWTH ON *ASPERGILLUS FLAVUS*

Plant extracts	<i>Vernonia amygdalina</i>		<i>Ocimum gratissimum</i>	
	mycelium growth(mm)	%inhibition	mycelia growth	% inhibition
Concentrations%				
100	0.3	51.43	0.1	98.75
75	0.4	42.86	0.1	95.00
50	0.5	39.14	0.2	66.00
25	0.9	35.17	0.3	62.50
Grisovid	0.2	-	0.1	-
Control	35	-	3.0	-
LSD (0.05)	0.224	-	0.331	-

Table 5 shows effects of plant extracts on mycelia growth on *Aspergillus Flavus* spp. The result revealed that both plant extracts were significant ($p \leq 0.05$) levels of inhibition on *Aspergillus flavus* mycelia at all the concentration levels. The highest (98.75%) percentage inhibition of mycelia growth by plant extracts was produced by scent leaves at the concentration of 100%

while the least (62.50%) percentage of mycelia growth was recorded by scent leaves at the concentration of 25%. All the percentage inhibition of mycelia growth by both plant extracts compared well with grisovid even at lower concentrations.

Table 9: Fungi Occurrence in rotten yam tubers.

Isolates	Occurrence Percentage	No of Occurrence
<i>Botryodiplodia theobromae</i>	28.56	8
<i>Fusarium Oxysporum</i>	10.72	3
<i>Aspergillus Niger</i>	25.00	7
<i>Penicillium spp</i>	7.15	2
<i>Rhizopus spp</i>	17.85	5
<i>Aspergillus Flavus</i>	7.15	2
<i>Geotrichum spp</i>	3.56	1

Table 10: Pathogenicity Test shows radial growth (mm) of pathogenic fungi

Isolates Fungi radial growth(mm)

<i>Botryodiplodia theobromae</i>	24mm
<i>Rhizopus sp</i>	8mm
<i>Fusarium Oxysporum</i>	12mm
<i>Aspergillus flavus</i>	10mm

DISCUSSION

All the isolated fungi organisms implicated with post harvest rot of yam in this study are not strange as they have been well reported (Okigbo and Ikediugwu, 2001 and Ogundana et al., 1970). This result is in agreement with reports of past researchers on the antimicrobial efficacy of various medicinal plant extracts against phytopathogenic fungi and equally being reported they play an important role in controlling diseases of plants caused by these fungi. (Ekwere et al, 2015, Nweke, 2015 and Parveen et al. 2014) *Ocimum gratissimum* extracts demonstrated higher antifungal properties as against that of *Vernonia amygdalina*.

This work has shown that fungitoxic compounds were present in these two plant extracts since they were able to inhibit the growth of fungi tested to some degrees. The identification of *Fusarium* and *Aspergillus flavus* as rot spoilage organisms presents a serious health concern as they are known to produce highly potent mycotoxins which are dangerous to man and animals if consumed (WHO, 1979). Results have exposed the antifungal activities of some plant extracts on rot causing fungal pathogens in post harvest storage of yam tubers. This can provide an alternative ways of reducing and controlling of yam rot diseases by farmers.

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WIS-BTN 07

PRODUCTION OF PECTINASE BY *Aspergillus niger* FOR EXTRACTION OF BEN OIL FROM *Moringa oleifera* SEEDS

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ABSTRACT

The annual cost of importing enzymes for a wide range of processes is alarming. Orange fruits can be processed into products such as juices, nectars, concentrates, jams, jelly powders and flakes amongst others. During this process, wastes are generated. The peels of oranges generated as agricultural wastes can be turned to wealth as in the extraction of pectin for pectinase production. Pectinases have important uses in oil extraction from seeds. This study was carried out to produce pectinase from *Aspergillus niger* under submerged fermentation for the extraction of Ben oil from *Moringa oleifera* seeds. Pectinase was produced using a disc with a cork borer (10mm) of a 96-h-old culture of *Aspergillus niger* inoculated into a 100ml basal medium supplemented with 1% (w/v) laboratory pectin and Experimental pectin from orange peels separately. The medium was incubated at 25°C for eight day to obtain the crude enzyme. Pectin extraction yield was 6.94% at pH 2.2, 100°C for 30min. Optimum conditions for temperature, pH and substrate concentration for pectinase activity were ascertained at 40°C, 5.0 and 1% respectively and the enzyme lost all its activity within 30min of heating at 70°C for both laboratory and experimental pectin. Extraction of Ben oil from *Moringa oleifera* with Experimental pectinase, Laboratory pectinase and Water had a percentage oil yield of 43.3%, 46.7% and 26.7% respectively. This research established the production of pectinase from *Aspergillus niger* using pectin from orange peels as substrate and its effectiveness for extraction of Ben oil from *Moringa oleifera* seeds.

Keywords: Pectinase; Pectin; *Aspergillus niger*; Enzyme activity; Enzymatic-oil-extract

Introduction

Orange fruits could be processed into products such as juices, nectar, concentrates, jams, jelly powders and flakes among others. During this process, wastes are generated. Wealth could be derived from these wastes by their utilization, as in the extraction of pectin (Pourbafrani *et al.*, 2007). Pectin are high molecular weight acid polysaccharide primarily made up of α (1→4) linked D-galacturonic acid residues (Kashyap *et al.*, 2001). It has long been used for its gel formation, thickening and stabilizing properties in a wide range of applications from food to the pharmaceutical and cosmetic industries (Enkuahone, 2018). New application opportunities have emerged and pectin is no longer just a gelling agent but also used in wide applications (Nitin *et al.*, 2017). Pectinolytic enzymes also referred to as pectinases, include a group of enzymes that are responsible for the degradation of pectic substances and have important applications in the food industry (Ivana and Mauricio, 2018; Ajayi *et al.*, 2018). Pectinases are extensively used in fruit processing, juice clarification, oil extraction, coffee and tea fermentation, paper making and in textile industries (Schwan and Wheals, 2004; Ajayi *et al.*, 2015). The main sources for the pectinolytic enzymes are yeast, bacteria, and a large variety of filamentous fungi for which the most relevant ones are *Aspergillus* strains which are generally regarded as safe (GRAS) in food industry (Hayley, 2019). *Moringa oleifera* is a native plant of the Western and Sub-Himalayan parts of Northwest India, Pakistan and Afghanistan. It is widely cultivated as a vegetable, spice, cooking or cosmetic oil and as a medicinal plant (Latif *et al.*, 2007). The seeds have attracted scientific interest as *M. oleifera* seed kernels contain a significant amount of oil (up to 40%) with a high-

quality fatty acid composition (oleic acid > 70%) and, after refining, a notable resistance to oxidative degradation (Anwar *et al.*, 2005). The oil is known commercially as “Ben oil” or “Behen oil”. Its properties make it suitable for both human consumption and commercial purposes (Latif *et al.*, 2007). *Moringa* oil could also be a good substitute for olive oil in the diet as well as for non-food applications, like biodiesel, cosmetics, and a lubricant for fine machinery. Moreover, after oil extraction, the seed cake can be used in waste water treatment as a natural coagulant or as an organic fertilizer to improve agricultural productivity (Ndabigengesere and Narasiah, 1998; Emmanuel *et al.*, 2011). Solvent extraction is not environmentally friendly and the process lessens the nature of the extraction oil, as well as denatures the protein and alters the unique nutrition of the oil (Latif *et al.*, 2011). *Aspergillus niger* a common mould and can be used in simple cultivation methods to produce pectinases using the pectin extracts from orange peels (Ezike *et al.*, 2014). The main sources of pectinolytic enzymes are yeast, bacteria, and a large variety of filamentous fungus with the most being *Aspergillus* strains which are generally regarded as safe (GRAS) in food industry (Sumantha *et al.*, 2005). Enhanced release of bioactives from plant cells by cell disruption and extraction through the cell wall can be optimized using enzyme preparations either alone or in mixtures. Enzyme assisted extraction methods are gaining more attention because of the need for eco-friendly extraction technologies (Puri *et al.*, 2012).

Methodology

Sample Collection

Orange peels were purchased from Poka market in Epe, Lagos state in sterile plastic bag. They were transported to the Microbiology Laboratory of Augustine University, Ilara-Epe, Lagos. *Moringa oleifera* seeds employed for this research were purchased from Ajah market in Lagos state. They were transported in a dry plastic container to the Microbiology laboratory of Augustine University, Ilara-Epe, Lagos state.

Preparation of Orange Peels

The orange peels were washed and cut into small bits and then washed with water and oven-dried for seven days. The dried peels were then ground to powder using an electric grinder.

Extraction of Pectin from Orange Peels

Pectin was extracted by the method of McCready (1970) whereby samples (50g) were placed in 2.5L boiling distilled water and 8 mL HCl was added to give a pH of 2.2 ± 0.1. This was followed by the addition of 20g paper pulp filter aid. The mixture was heated at 95– 100°C for 30min with constant stirring, after which the mixture was filtered and the residue was washed once with 500mL boiling distilled water. The filtrate was cooled before adding to 1.5ml volumes of 95% ethanol containing 2mL/L HCl. The mixture was slowly stirred and left to stand for 30 min. The residue was collected and dried at 60°C overnight.

Determination of Pectin Yield

Determination of pectin yield as carried out according to the method of Ernias and Teshome (2016).

$$Y_{pec} (\%) = \frac{P}{Bi} \times 100$$

Where; Y_{pec} (%) is the extracted pectin yield in percentage
 P is the amount of extracted pectin in grammes
 Bi is the initial amount of powder fruit peels

Isolation of Pectinolytic Fungi

Soil sample was dissolved in 100ml saline peptone water in a sterile conical flask. The stock solution was serially diluted to dilution 10^{-4} . Saboraud Dextrose Agar (SDA) medium was prepared, sterilized in the autoclave at 121°C for 15 min. It was allowed to cool to about 45°C. 1ml from dilution 10^{-2} and 10^{-4} each were plated out on sterile petri dishes with duplicates. Appropriate amount of medium was poured over the inoculation, stirred and allowed to solidify. On solidifying, the plates were incubated at 30°C for 5 days. Using an inoculating needle, distinct fungal colonies were inoculated onto the solid medium (SDA). The plates were incubated at 30°C for 5 days. All morphological contrasting colonies were purified by repeated streaking and sub-culturing on separate plates. This process was continued till pure fungal culture was obtained (*Aspergillus niger*). The pure fungal isolate was maintained on SDA slants as stock cultures. The SDA medium was prepared according to the manufacturer's instructions.

Macroscopic Fungal Identification

A four-day-old culture of the organism was examined and the color, texture, nature of mycelia or spores and growth patterns were observed.

Microscopic Fungal Identification

Four days old cultures were used in preparing microscopic slides. A tuft of the mycelia was dropped on the slide and a drop of lacto-phenol blue was added to it. A cover slip was placed over it and viewed under a light microscope at ×400 magnification. Identification was carried out by relating the microscopic features and the micrographs to Atlas of Mycology by Barnett and Hunter (1972).

Preparation of the Basal Medium

Submerged fermentation (SmF) was carried out using the method described by Yogeshi *et al.* (2009). Seven 250ml Erlenmeyer flasks containing 50ml of sterile cultivation medium made up of 2% NaNO₃, 1% K₂HPO₄, 5% MgSO₄, 5% KCl, 0.001% FeSO₄, and 1.5% Pectin. The initial pH of medium was adjusted to 3.8. The flask was covered with aluminium foil and autoclaved at 121°C for 20min. One disc of actively growing fungus (TIE 1) from a 4-day-old culture was added using a cork borer (10mm) in the sterile flask. The culture was incubated in a rotary shaker (150 rpm) at 100°C for 4 days. After every 24h, 10ml of the broth was separated by filtration using muslim cloth and the culture filtrate served as the crude enzyme. This was carried out for four days.

Enzyme Assay

Pectinase assay was carried out according to the method described by Miller (1959) with slight modifications on both experimental and laboratory pectinases. The activity was determined using a solution containing 100ml of 0.2M citrate-phosphate buffer (pH 5.0) containing 0.1g pectin. Crude enzyme (0.5ml) was reacted with 1ml solution and incubated for 3h at 35°C. Three millilitres of 3, 5-dinitrosalicylic acid (DNSA) reagent was added to the mixture, boiled at 100°C for 15min and allowed to cool.

Enzyme Activity

Pectinase activity was determined by estimating the reduced glucose liberated using 3,5-dinitrosalicylic acid reagent (Miller, 1959).

Protein Determination

Protein estimation of the enzyme samples was carried out according to the method described by Lowry *et al.* (1951).

Enzyme Characterization

The enzyme incubation day that showed the greatest activity was subjected to various parameters to ascertain the optimum conditions for maximum enzymatic activity

Effect of Temperature on Enzyme Activity

The effect of temperature was studied on the crude enzyme, with incubation temperature of 25°C, 30°C, 35°C, 40°C,

45°C, 50°C, 55°C, 60°C, 65°C and 70°C. Pectinase activity was measured as described under enzyme assay.

Effect of pH on Enzyme Activity

The effect of pH was studied on the crude enzyme using citrate-phosphate buffer, with pH of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0. Pectinase activity was measured as described under enzyme assay.

Effect of Heat on Enzyme Activity

The effect of heat was studied on the crude enzyme using heating temperature of 70°C for 0min, 1min, 5min, 10min, 15min, 20min, 25min, 30min, 35min and 40min. Pectinase activity was measured as described under enzyme assay.

Effect of Substrate Concentration on Enzyme Activity

Different concentrations of both crude enzyme (0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.2%, 1.4%, 1.6%, 1.8 and 2.0%) in 0.1M Citrate Phosphate buffer (pH 5.0) were prepared and employed as substrate. Pectinase activity was measured as described under enzyme assay.

Enzyme Kinetics

The initial reaction rate of pectinase was determined at different substrate concentrations as described under effect of substrate concentration above. The kinetic constant (Km) was estimated following the method of Lineweaver and Burk (1934).

Aqueous Enzymatic Extraction

The *Moringa oleifera* seeds were rinsed with clean water then allowed to air dry overnight. The dry seeds were then ground to fine particle size. Aqueous enzymatic extraction was carried out according to Abul-Hamid *et al.* (2014) whereby fifteen Grams (15g) of ground seed was weighed into 250 ml conical flask and 150 ml of distilled water was added to give a ratio of 1:10 (w/v). The samples were gently boiled for 5min and immediately cooled to room

temperature in a freezer. The suspension was then mixed with 3ml of the enzyme at a 2.0% concentration and incubated at 50°C at pH 5.0 overnight.

Oil Extraction

The miscella was collected and filtered. The crude oil was dried over anhydrous sodium sulphate. Determination of oil yield and oil recovery was carried out according to the method described by Abdulkarim *et al.* (2006).

$$\% \text{Oil Yield} = \frac{\text{Volume of oil extracted/weight (g) of seed} \times 100}{\text{seed}}$$

RESULTS

The pure culture of the fungal isolate appeared sand-like, with dark brown coloured spores, dense colony that had cream coloured boarder and the hyphae was non-septate with sporangiospores having greenish boarder (Plate 1). Pectin extraction yield was 6.94%. The extraction of pectinase within four days revealed that day 4 for laboratory pectinase and day 3 for experimental pectinase gave the highest enzyme activity (Fig. 1 & 2). There was a gradual decline of the enzyme activity at a temperature of 45°C for laboratory pectinase and at 50°C for experimental pectinase (Fig. 3 & 4). The highest pectinase activity was recorded at pH 5.0 for both experimental and laboratory pectinases (Fig. 5 & 6). There was a decline in activity when pH was increased beyond 5.0. Optimum enzyme activity was recorded before heat application at 0 min, on heating, the enzyme activity reduced (Fig. 7 & 8). The substrate concentration contributed to the activity of the enzyme. The optimum pectinase activity was observed at 2.0% (Fig. 9 & 10). The Michealis Mentens constant (Km) and the maximum rate of reaction (Vmax) for experimental pectinase was 0.27 and 44.46, and laboratory pectinase was 0.25 and 52.25 respectively.



Figure 1: Fermentation days for Laboratory Pectinase

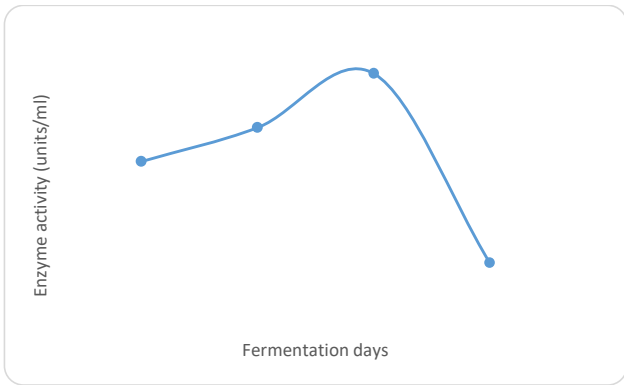


Figure 2: Fermentation days for Experimental Pectinase

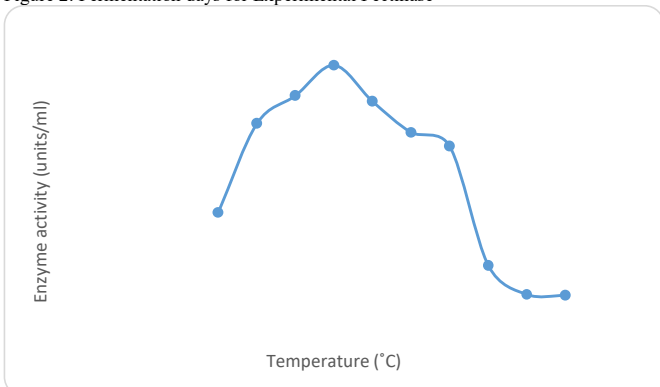


Figure 3: Effect of Temperature on the activity of Laboratory Pectinase obtained from *Aspergillus niger*

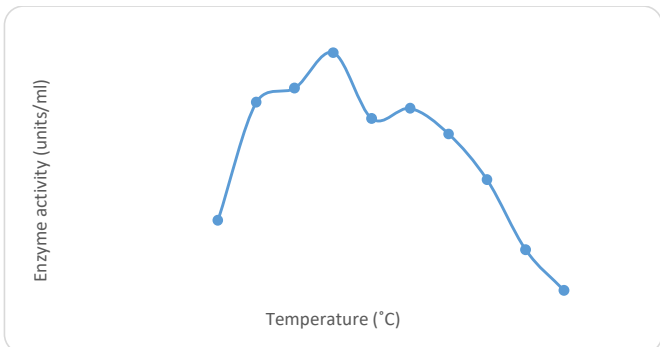


Figure 4: Effect of Temperature on the activity of Experimental Pectinase obtained from *Aspergillus niger*

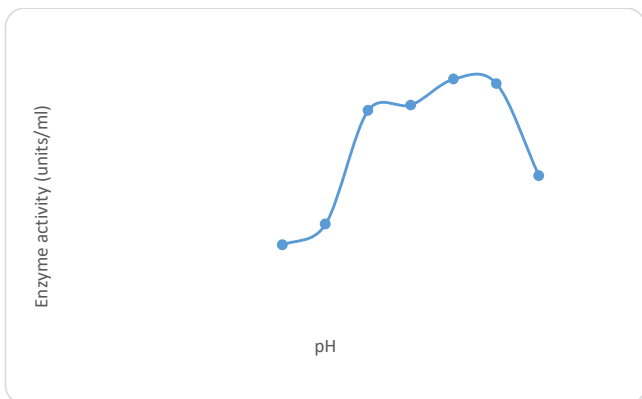


Figure 5: Effect of pH on the activity of Laboratory Pectinase obtained from *Aspergillus niger*

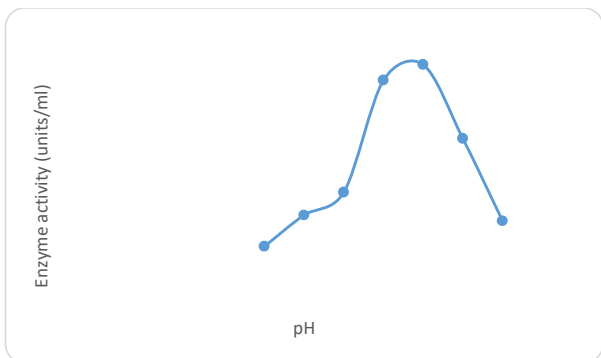


Figure 6: Effect of pH on the activity of Experimental Pectinase obtained from *Aspergillus niger*

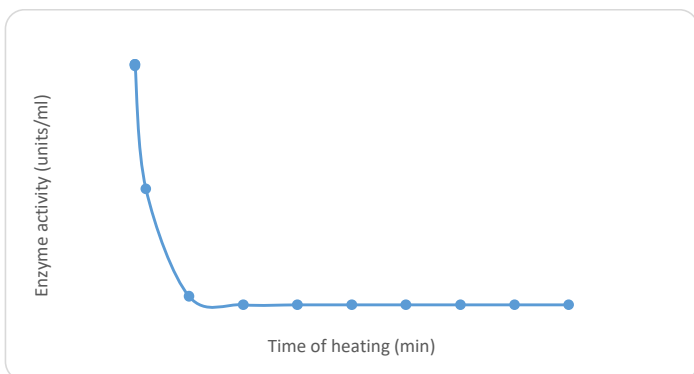


Figure 7: Effect of Heating on the activity of Laboratory Pectinase obtained from *Aspergillus niger*

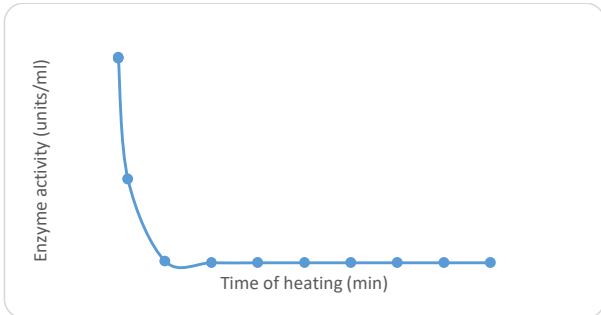


Figure 8: Effect of Heating on the activity of Experimental Pectinase obtained from *Aspergillus niger*

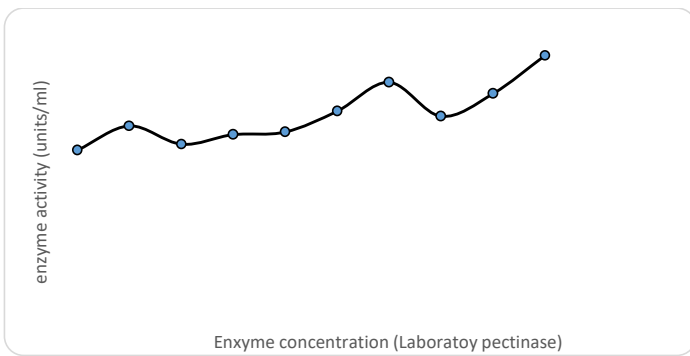


Figure 9: Effect of Substrate Concentration on the activity of Laboratory Pectinase obtained from *Aspergillus niger*

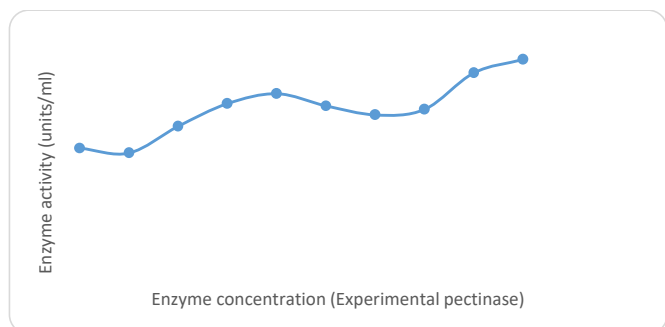


Figure 10: Effect of Substrate Concentration on the activity of Experimental Pectinase obtained from *Aspergillus niger*

Table 1: Enzyme kinetics

Enzyme	V_{max}	K_m
Experimental Pectinase	44.46	0.27
Laboratory Pectinase	52.25	0.25

Oil Extraction

Table 2: Percentage Oil Yield of Ben Oil from *Moringa oleifera* seeds

Parameters	Method of Extraction	Oil yield (% v/v)
Experimental Pectinase	Enzymatic oil extraction	43.3%
Laboratory Pectinase	Enzymatic oil extraction	46.7%
Water	Aqueous oil extraction	26.7%

DISCUSSION

The results of this study revealed a yield of 6.94% pectin from orange (*Citrus sinensis*) peels which are agricultural wastes at pH 2.2. Ezike *et al.* (2014) and Normah and Ku Hasnah (2000) obtained similar results for extraction of orange and pineapples respectively. The yield could be affected by the pH of the extraction medium and extraction time. In this research, submerged fermentation technique was applied due to the fact that change in temperature, pH and in heat would have been difficult to control under limited water availability. Submerged liquid fermentation is the cultivation of microorganisms in liquid nutrient broth. Industrial enzymes can be produced using this process. Submerged fermentation involves growing carefully selected microorganisms (bacteria and fungi) in closed vessels containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen (Grigelmo-Migeul and Martin-Belloso, 1998). *Aspergillus niger* isolated from soil sample was used for the fermentation process. The highest enzyme activity for pectinase produced using pectin extracted from Orange peel was observed on the 3rd day of fermentation and that of refined laboratory pectin was observed on the 4th day of fermentation. The increase in the time of fermentation could be attributed to the decrease in enzyme activity since *A. niger* produces extracellular enzymes and over time denatures due to effect of surrounding medium (Haq *et al.*,

2005). A lot of factors influenced the production of pectinases. These include; concentration of nutrients, pH,

temperature, moisture content and influence of extraction parameters on recovery of pectinases. The optimum temperature of an enzyme is the temperature in which the enzyme functions if optimal therefore, temperature has a complex effect on enzyme activity. A change in the temperature results in a change in the kinetic energy of enzymes. This leads to either higher or less efficiency of enzyme-substrate complex formation. It differs in each enzyme, depending on its nature and structure. At extreme temperatures above the optimum, the increased kinetic energy destroys the bonds holding the active site; the enzyme is unstable and the shape of its active site changes. This means that the enzyme is less efficient and successful at enzyme-substrate complex formation. When the enzyme is said to be denatured; it has lost its ability to catalyse reactions. 40°C was the temperature at which pectinase extracted from *Aspergillus niger* exhibited the highest activity, which falls within the range 40-45°C as reported by Favela-Torres *et al.* (2006). At a higher temperature, the activity dropped and this could be as a result of the denaturation of the enzyme (Campbell and Reece, 2002). The optimum enzyme activity as observed for the change in pH was observed at pH 5.0 for pectinase produced by *Aspergillus niger* using orange peels as substrate which is comparable to the optimum pH for most microbial pectinases in the pH range of 3.5-5.5 and optimal temperature range from 30-50°C as reported by Jayani *et al.* (2005). Also, the maximum pH level is in correlation with the optima pH range for thirty (30) fungal pectinases reported by Nitire *et al.* (2004). Extremely high or low pH

concentrations usually result in complete loss of enzyme activity due to denaturation (Helms *et al.*, 1998). Increased heating time at 70°C caused the enzyme to denature. According to Haltrich *et al.* (1996), increased heating time causes denaturation of enzyme and therefore, loss of enzyme activity. Ajayi *et al.* (2003) had earlier reported that the loss in enzyme activity due to heating beyond the optimum temperature could be attributed to the effect of temperature on the velocity of the enzyme reaction. This experiment showed optimum enzyme activity at 2.0% substrate concentration. This investigation is in correlation with the research work described by Fadel (2000) that optimum substrate concentration gave the optimum enzyme activity. The K_m and V_{max} obtained for the experimental pectinase was 0.6699 and 0.0751 and laboratory pectinase 0.0904 and 0.4683 respectively. Oliyad and Dawit (2017) recorded K_m value higher than that of the V_{max} . The results of this study showed that the oil yield for experimental and laboratory pectinases were 43.3% (v/w) and 46.7% (v/w) respectively while the conventional method (non-enzymatic process), had 26.7% (v/w) oil yield. This result agreed with the studies of Abul-Hamid *et al.* (2014) and Zhang *et al.* (2013) whereby enzyme assisted oil extraction of *Moringa oleifera* seeds resulted in greater oil yield.

Conclusion

This work suggests that pectin from orange peels can be successfully used to induce the production of pectinase under submerged fermentation system. Since orange peels utilized in this process are readily accessible as waste with little or no cost and also contain an appreciable amount of pectin, they can be regarded as a low-cost substrate for efficient and economical production of pectinases using *Aspergillus niger*. The pectinolytic enzyme obtained can be industrially used in the production of Ben oil. This study also established the fact that the purer the pectin used for the fermentation process the more enzyme yield which would further increase the rate and quantity of product formed in a production process. Also, the significance of enzymatic oil extraction method over the conventional oil extraction method was observed.

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MARKER ASSISTED SELECTION: A REVIEW OF GENETIC INTERVENTIONS FOR NATURAL RUBBER (*Hevea brasiliensis* Muell. Arg) IMPROVEMENT.

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ABSTRACTS

Natural rubber trees (*Hevea brasiliensis* Muell. Arg) are extensively cultivated in the tropical regions like Nigeria as main sources of latex production which is an important raw materials for many industries. The tree crop is faced with some production constrains. The increase rate in the loss of natural rubber trees (*Hevea brasiliensis* Muell. Arg) to pest and disease, drought tolerance and resistance, has greatly reduced the productivity of natural rubber in meeting the annual global demand. In recent time, conventional breeding methods has significantly improved rubber yield from 300-400kg/ha/yr of unselected Wickham clones to 900-1600kg/ha/yr of exotic clones and 2000-3000kg/ha/yr of Nigeria clones developed by Rubber Research Institute of Nigeria. The use of improved clones with intrinsic mechanism that are tolerant or resistant to any of these production constrains is the best and cost effective means to increase productivity in smallholder farmer's production systems under unfavorable condition. Since the advent of DNA-based markers in tackling the current challenges of conventional breeding and disease management in rubber tree, the genetic analysis and manipulation of important agronomic traits has greatly improved speed sensitivity and accuracy in crop improvement. Such markers that have been studied and found with complex traits of interest can be used to screen and select rubber trees having traits of interest before flowering. This review highlights the significant role of bio markers intervention in *Hevea brasiliensis* improvement and calls for adoption by the government of rubber producing countries.

Keywords: *Hevea brasiliensis*, crop improvement, DNA markers, Diseases tolerance and resistance, Conventional breeding

INTRODUCTION

Plant breeding is the art and science of changing traits of plants in order to produce desired characteristics. Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques. Plant breeding has been practiced for thousands of years, since the beginning of human civilization. It is practiced worldwide by individuals such as gardeners and farmers, or by professional plant breeders employed by organizations such as government Institution, Universities, crop specific industry associations or research centers. International development agencies believe that breeding new crops is important for ensuring food security by developing new varieties that are higher-yielding, resistant to pest and disease, drought-resistant or regionally adapted to different environments and growing conditions.

It is also the science, art and business of improving plants for human benefit. Combined with the developments in agricultural technology such as agrochemicals, remarkable progress has been made in increasing crop yields for a country through plant breeding. Despite this progress, plant breeders must always respond to many changes that could be seen in agricultural practices, which gives way for the development of new genotypes

with specific agronomic characteristics to meet the constant changes in consumer's preference and requirements. Plant breeders are faced with the unending task of continuous development of new crop varieties Afolayan *et al.* (2014).

Natural rubber tree (*Hevea brasiliensis* Muell. Arg) is one of the Nigerian's major agricultural export crops. It is an essential raw material for many industries (Haliru *et al.*, 2011). It is an economic tree known for its latex production/yield. Apart from latex production/yield, there are many other benefits derivable from the rubber tree especially from the seed and rubber wood. It is indigenous to the Amazon basin of South America. The cultivated clones (known as Wickham clones) which were all derived from 22 seedlings surviving from the Wickham collection in 1876, showed a very narrow genetic base. Therefore, International Survey of *Hevea* wild germplasm have been conducted in the Amazon forest. Exploitation of these resources requires information on genetic diversity of germplasm Lekwaipat *et al.* (2003).

Traditionally *Hevea* clones have been identified by using morphological and agronomic characters i.e. the leaves, branches, seeds characters and yield performance etc. Although, these characters are helpful in determining the phenotype variability among accessions, they are highly

sensitive to environmental effects. Molecular markers have been developed to detect genetic variation of *Hevea* accessions, since they are more precise than morphological markers, they are unaffected by environmental effects and capable of plant genotypic characterization at an early stage of development. With agriculture been practiced on marginal lands and with increase in demands for natural rubber as a vital agricultural commodity used in the manufacture of a wide range of products which also plays a major role in the socio-economic fabric of many developing countries, natural rubber tree (*Hevea brasiliensis*) is a perfect tree crop for a more intensive crop improvement Mesike *et al.* (2014).

Omo-Ikeroda *et al.* (2014) reported that genetic engineering/DNA marker technology and the development of methods for in-vitro culture has greatly increase the possibility of producing rubber trees with desirable traits. The heterogeneous nature, long breeding cycle of the tree crop, prolong period required for the evaluation of mature traits are strong limitation for conventional breeding and selection methods. Genetic modification involves inserting short and specific sequences of DNA into chromosomes, to incorporate new genes, fixing structural genes or to modify activity level of resident genes that are already present. These developments have helped to improve latex yield, tolerance to tapping panel dryness (TPD) syndrome, growth rate, wood quality and reduction in undesirable traits. Genomic technologies have assisted research groups working on *Hevea brasiliensis* genotypes to identify new target for breeding and/or complementing genetic transformation Omo-Ikeroda *et al.* (2014). Molecular markers provide simultaneous and sequential selection of genes/traits for important agronomic characters in *Hevea brasiliensis* breeding programs to effectively replace time consuming bioassays in early generation screens.

In conventional breeding, it is easy for breeders to select for simple and easy to measure traits such as stem height in rubber tree than selecting for more complex traits like disease resistant. However, the use of DNA markers in plant breeding known as marker assisted selection (MAS), also called marker assisted breeding (MAB) avoids the stress of selecting for such complex trait on the field by using genetic markers that are linked to the traits of interest Afolayan *et al.* (2014).

Molecular Markers Used in Crop Improvement.

Molecular markers also known as DNA markers is a form of biotechnology, which uses genetic fingerprinting techniques to assists plant breeders in matching molecular profile to the physical properties of the variety. It is the identification of DNA sequences located

near genes that can be tracked to breed for traits that are difficult to observe Angarawai *et al.*, 2006. Molecular markers can be of any kind of marker technique that is able to discriminate between two individuals at the molecular level. Thus, they are said to be polymorphic markers and they can be dominant (e.g RAPDs) or co-dominant (eg. SSRs). Co- dominant markers can differentiate between homozygotes and heterozygote genotypes (Collard *et al.*, 2005, Afolayan *et al.*, 2014). There are many types of molecular markers presently available, but no single marker technique have been used for all processes.

Importance of Marker Assisted Selection.

When compared to conventional breeding, DNA marker assisted selection increases the efficiency and value for plant breeding significantly. MAS is always simpler with selection been carried out at seedling stage and single plants may be selected with high reliability Collard and Mackill (2008).

MAS is very important when selecting disease resistant traits in rubber tree (eg. South American Leaf Blight SALB, *Corynespora* leaf fall etc.)at are difficult to manage through conventional phenotypic selection. This is so because such traits are expensive or time-consuming to measure, or have low penetrance or complex inheritance. MAS is also very crucial in selection, if the traits in question is dependent on specific environments or developmental stages that influence the expression of the target phenotype. Collard and Mackill (2008), Afolayan *et al.*, (2014) reported that selection based on molecular markers, helps to maintain recessive alleles when carrying out backcrossing and also useful in speeding backcross breeding. It is also useful when pyramiding multiples monogenic traits which includes pest and disease resistances or quality traits or when introgression several QTL for a single quantitatively inherited trait like drought tolerance or other adaptive traits (Xu and Crouch, 2008, Afolayan *et al.*, 2014).

DNA markers can be employed to assist a wide range of components of modern plant breeding. The use of DNA markers for indirect selection offers the greatest benefits for quantitative traits with low heritable as these are the most difficult characters to assess in field experiments. The application of DNA markers in the plant breeding process can create substantially more added value than just improving the quality or cost of existing selection programs. Although, molecular marker-assisted selection is an effective tool but it cannot be a substitute for conventional plant breeding, but a means to improve the efficiency of plant breeding. The most important value of marker-assisted selection is in accelerating the rate of genetic gain from selection for best genotypes and in the manipulation of QTLs influencing complex traits

of agronomic and economic importance. In *Hevea brasiliensis*, a number of microsatellite markers have been used to identify rubber clones and construct a linkage map in Thailand (Lespinasse *et al.*, 2000, Low *et al.*, 1995). Lekawipat *et al.*, (2006) worked on the evaluation of genetic diversity of 108 *Hevea brasiliensis* accessions, including 40 Wickham clones and 68 wild accession collected from Amazon forest using application of microsatellite markers.

Table 1: Advantages and Disadvantages of different types of molecular markers used in natural rubber tree crops.

Types of markers	Advantages	Disadvantages
Restriction Fragment Length Polymorphism (RFLP)	<ul style="list-style-type: none"> -High genomic abundance -Co-dominant markers -highly reproducible -Can use filters many times -Good genome coverage -Can be used across species -No sequence information -Can be used in plants reliably (well tested) -Needed for map based cloning. 	<ul style="list-style-type: none"> -Need large amount of good quality DNA -Laborious (compared to RAPD) -Need radioactive labelling -Cloning and characterization of probe are required
Randomly Amplified Polymorphic DNA.	<ul style="list-style-type: none"> -Highly genomic abundance -Good genome coverage -No sequence information -Ideal for automation -Less amount of DNA (poor DNA acceptable) -No radioactive labeling -Relatively faster 	<ul style="list-style-type: none"> -No probe or primer information -Dominant markers -Cannot be used across species -Not very well-tested
Simple Sequence Repeat (SSR)	<ul style="list-style-type: none"> -Highly genomic abundance -Highly reproducible -Fairly good genome coverage -Highly polymorphism -Easy to automate -Multiple alleles 	<ul style="list-style-type: none"> -Cannot be used across species -Need sequence information -Not well-tested
Amplified Fragment Length Polymorphism (AFLP)	<ul style="list-style-type: none"> -High genomic abundance -High polymorphism -No need for sequence information -Can be used across species -Work with smaller RFLP fragments -Useful in preparing coting maps 	<ul style="list-style-type: none"> -Very tricky due to change in patterns with respect to materials used -Cannot get consistent map (not reproducible) -Need to have very good primers
Single Nucleotide Polymorphism (SNP)	<ul style="list-style-type: none"> -PCR products can be very small: markers will work with extremely degrade DNA samples -More common in genome -May possibly multiplex hundreds of thousands on one chip -Sample processing may be completely automated -No stutter products 	<ul style="list-style-type: none"> -Less alleles -Each marker is less informative. Therefore have to genotype many more SNPs to get same level of information about DNA sample -Mixture interpretation is more difficult -Multiplexes don't actually work yet -Currently uses more of the DNA sample than STRs use.

Latex yield improvement started in Rubber Research Institute of Nigeria in the year 1960s using the method of hand pollination. Initially it was in collaboration with some rubber estates and later in Rubber Research Institute of Nigeria headquarters when the imported exotic clones attained flowering maturity. The progeny obtained from hand pollination carried out in the year 1960s were all labeled C-clones and they were all evaluated on-station. Latex yield potential of the C-clones were also evaluated in batches (Omokhafa *et al.*, 2014). The first round of selection produced fourteen C-clones which were evaluated in four location in Nigeria. These clones yield 2000-300kg/ha/yr and they have been registered with the National Committee on Naming, Registration and Release of Crop Varieties, Livestock Breeds and Fisher- NCVR (Table 2) NACGRAB, 2013. Cultivation of these clones is being promoted through activities such as field days, stakeholder meetings, workshops, conferences, publication etc. Rubber Research Institute of Nigeria is involves in multiplication and distribution of these clones to farmers based on request. Farmers are often advised to make their request in the first quarter of the year.

Development of centers for multiplication and distribution of the RRIN developed clones is also encouraged. In this regards, RRIN offers technical assistance to cooperative groups who wish to establish multiplication centers. The second round of selection among the C-clones produced the NIG 900 series clones currently evaluated in three rubber producing States in Nigeria, which are Edo, Ondo and Akwa Ibom States. The NIG 900 series clones yield 2000-3500kg/ha/yr Table 2 (Omokhafa and Nasiru 2005, Omokhafa *et al.*, 2014). The current evaluation of the NIG 900 series clones in multiplication trials will form the bases of presentation for registration with the NCVR. Pollination continued after the phase of C-clones to develop new hybrid seedlings labeled K-clones which are currently evaluated in the seedling nursery (Omokhafa 1998, Omokhafa *et al.*, 2014). This will be followed by field evaluation and selection on-station. Selected genotypes will be multiplied for inter-locational trials before presentation for registration.

Genetic Diversity works done in *Hevea brasiliensis* Muell Arg. using Microsatellite Markers.

Isozymes electrophoresis was successfully used to demonstrate the investigation of genetic diversity and clonal identification in rubber trees. Mini-satellite, Restriction Fragment Length Polymorphism (RFLP) Besse *et al.*, (1994) Mitochondria DNA RFLP analysis Lou and Boutry (1995), Random Amplified Polymorphic DNA (RAPD), DNA Amplification Finger-Printing (DAF) Low *et al.*, (1995) and Amplified Fragment Length Polymorphism (ALFP) were developed and used

in *Hevea* variability detection in order to improve the number of available molecular markers Lekawipat *et al.*, (2003). Microsatellites also known as Simple Sequence Repeats SSR are based on tandem repeat of short (2bp-6bp) DNA sequence. Microsatellite are very attractive to plant geneticists as they combine several features of utility markers, typically co-dominant, highly polymorphic and thus allow precise discrimination of even closely related individuals. Also microsatellites are abundant and uniformly dispersed in plant genome. In *Hevea brasiliensis*, a number of microsatellites markers have been used to identify rubber clones and construct a genetic linkage map.

Conclusion:

The gains in crop production through research advances in genetic improvement will help to achieve sustainable food security, poverty alleviation and environment protection in the tropics (Angarawai *et al.*, 2016). The use of molecular tools to study the genetic basis of crop traits for indirect selection and plant breeding in natural rubber trees had offered an alternative help to traditional transgenic. These technologies helps to alleviate some of the constraints surrounding intellectual property rights and biosafety that limit the dissemination of GMOs in developing countries. Latex yield improvement has increased from 300-400kg/ha/yr in exotic clones and 2000-3000kg/ha/yr for registered RRIN developed clones (Table 2). These clones are available for evaluation and further improvement, especially by Institutions that enjoyed research academic freedom. Prospect of high yield are being explored in RRIN using marker assisted selection.

Table 2: Latex yield of some exotic clones of *Hevea brasiliensis* in Nigeria

Exotic clones	Origin	Parentage	Mean yield /kg /ha /yr.
RRIM 501	Malaysia	Pil A44 x Lun A44	1627
RRIM 600	Malaysia	Tjir1 x PB86	1651
RRIM 605	Malaysia	Tjir1 x PB49	1300
RRIM 614	Malaysia	Tjir1 x RRIM 509	1370
RRIM 628	Malaysia	Tjir1 x RRIM527	1454
PB 5/51	Malaysia	PB56 x PB 24	1064
PB 28/63	Malaysia	PB56 x PB 24	1463
PB 28/59	Malaysia	Primary clone	1554
PR 107	Indonesia	Primary clone	954
GT1	Indonesia	Primary clone	1300

Table 3: Twenty Four Clones of *Hevea brasiliensis* selected in Rubber Research Institute of Nigeria

Class 1 clones				Class 2 clones			
National code	RRIN Code	Parentage	Mean Yield	National code	RRIN Code	Parentage	Mean Yield
NIG 800	RRIN C 76	RRIM 501 x Har 1	2679	NIG 901	RRIN C 289	PB5/51 x PR 107	3528
NIG 801	RRIN C 83	RRIM600 x PR107	2229	NIG 902	RRINC 292	PB5/51 x PR 107	3351
NIG 802	RRIN C 114	RRIM501 x RRIM 628	2014	NIG 903	RRIN C 291	PB5/51 x PR 107	3237
NIG 803	RRIN C 48	RRIM600x PR 107	2765	NIG 904	RRIN C 367	PB5/51x RRIM600	3233
NIG 804	RRIN C 1	RRIM 600 x Tjir1	3207	NIG 905	RRIN C 227	RRIM501 x RRIM 628	3152
NIG 805	RRIN C 15	RRIM 628 x RRIM 501	1944	NIG 906	RRIN C 380	PB5/51x RRIM 600	3069
NIG 806	RRIN C 163	RRIM 501 x RRIM 628	2723	NIG 907	RRIN C 321	RRIM 501x Har 1	3043
NIG 807	RRIN C 145	RRIM 501 x RRIM 628	2699	NIG 908	RRIN C 366	PB5/51xRRIM 600	3033
NIG 808	RRIN C 143	RRIM 501 x RRIM 628	2411	NIG 909	RRIN C 369	PB5/51x RRIM 600	3009
NIG 809	RRIN C 150	RRIM 501 x RRIM 628	2388	NIG 910	RRIN C 368	PB5/51x RRIM 600	3000
NIG 810	RRIN C 159	RRIM 501 x RRIM 628	2383				
NIG 811	RRIN C 154	RRIM 501 x RRIM 628	2334				
NIG 812	RRIN C 162	RRIM 501 x RRIM 628	2312				
NIG 813	RRIN C 202	RRIM600 x PR 107	2090				

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ENVIRONMENT AND CLIMATE CHANGE (ECC)

WIS-ECC 03

EFFECT OF THE APPLICATION OF DIFFERENT ANIMAL COMPOST ON THE PROPERTIES OF AN ALFISOL IN A FOREST ECOLOGY

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Abstract

Application of compost to agricultural fields is a widely used method of increasing soil organic matter and fertility. Cowdung and poultry droppings were separately collected and composted for three months. The composts were applied singly and in-combination to a typical Alfisol soil, maize was the test crop and the research aimed at determining the effect of these compost applications on soil properties. The field experiment was carried out at the Teaching and Research Farm of the Obafemi Awolowo University (OAU), Ile-Ife, Nigeria in early and late cropping season of 2014. The experiment consisted of seven treatments; namely, 100% cowdung compost (CD), 100% poultry compost (PT), 50% CD + 50% PT, each at 3 and 6 t ha⁻¹ and zero compost application which, served as control. The experiment was a randomized complete block design and each treatment plots (3.0 m x 2.5 m) was replicated three times to give a total of 21 plots. Plots treated with equal combination of 50% cowdung and 50% poultry at 6 tonnes per hectare (50% CD + 50% PT) has the highest organic carbon, nitrogen content with high cation exchangeable capacity after late cropping season. All the plots treated with compost had high concentration of exchangeable micronutrients except for Fe, As and B. We concluded that application of animal composts to an Alfisol soil enhanced soil properties, particularly the soil organic matter, a major key in crop husbandry. Also, the application of compost application showed positive residual effect on soil properties after cultivation.

Keywords— Climate Change; Compost; Cowdung; Forest Ecology; Poultry Droppings; Waste Management

Introduction

The impacts of torrential pattern of rainfall, high temperature, frequent flooding and drought from climate change can include increased infestation of crop by pests and diseases, depletion of household assets, increased rural-urban migration, increased biodiversity loss, depletion of wildlife, changes in the vegetation type, soil moisture and nutrients depletion, increased health risks and the spread of infectious diseases [1]. Drought-tolerant maize [2] and organic farming [3] are some of the mitigating mechanisms adopted biologically in climate changing scenario, globally. Sustainability of soil fertility is very important in achieving and maintaining high crop yields over a period of time, but due to the decline in soil fertility which could result from biotic and abiotic factors, and posing a major production constraint in Africa particularly Nigeria, to ensure lasting soil production is becoming vital [4]. Cowdung and poultry dropping are mostly used by farmers who engage in mixed farming such that there is no or little waste generated [5]. Nevertheless, the direct application of fresh cowdung and poultry droppings to soil can be difficult to handle due to its high moisture content and odour. Excessive application of fresh manure can also result in the accumulation of ammonia in soils causing hazard to soil, seedlings and roots. Thus, poor management of this resource can also lead to surface and groundwater pollution. Therefore, environmental-friendly technology such as; compost could take advantage of turning these waste resource into organic

amendments as nutrients contained are released slowly and stored for long period. This study assessed the effects of cowdung and poultry compost on the physical and chemical properties of an Alfisol soil in a forest agro-ecology.

Materials and Methods

Cowdung and poultry droppings were collected from the Beef and Poultry Unit of the Teaching and Research Farm, Obafemi Awolowo University (OAU), Ile-Ife. The two animal wastes were separately and aerobically composted for three months. Fresh cowdung and poultry droppings were separately heaped under a shed. The heaps were manually stirred once in two weeks with a strong wood to enhance the rate of curing. Water was occasionally added stirring to facilitate decomposition and aeration respectively. The temperature of the compost at fully-cured stage was recorded (32 °C) with a hand-held thermometer. The field experiment was carried out at the Teaching and Research Farm, OAU, Ile-Ife, Nigeria (Lat. 7.55° N and Long. 4.55° E) during the early cropping seasons (April – July) and late (September – December) maize cropping season of year 2014. Viable seeds of maize variety DT-SR-WC₂ were obtained from the Institute of Agricultural Research and Training, Ibadan. The experimental plot was cleared twice. The experiment was laid out in a randomized complete block design with seven treatments: 100% cowdung (CD), 100% poultry manure (PM), 50% cowdung + 50% poultry manure and each at the rate of

three and six tonnes per hectare and zero manure application to serve as control. Each treatment plots (3.0 m x 2.5 m) was replicated three times to give a total of 21 plots in the experimental site. Composts were applied at 2 weeks after sowing. The maize seedlings were later thinned to two stands per hole at two weeks after sowing (WAS) to give a population of 53,333 plants per hectare. Collection of data on growth parameter (cm) of maize commenced at 2 WAS and continued fortnightly till maturity. At full maturity, maize ears were harvested per plot, manually shelled and grains were weighed. The treatment plots used in the early season were cleared again for maize cultivation in the late season without new treatments addition.

Composite surface soil samples (0-15 cm) were taken for each plot before and after the sowing. Samples of the composts were also taken. The samples were air-dried

for analysis. Soil pH was determined in a 1:1 soil to water suspension using the Dwyer model WPH1 waterproof pH tester. Pre- and post-cropping soil and compost samples were analyzed using standard methods (Page et al., 1982). The soil pH was determined in 1:1 soil-water suspension using a glass electrode pH meter. The organic carbon in soil and compost were determined using Walkley- Black wet oxidation method [6]. Total nitrogen of the soil and compost were determined by the macro-Kjeldahl method [7]. Available phosphorus in the soil and compost were extracted using Bray P1 method and P in the extractants was determined by colorimeter [8]. Exchangeable acidity was determined by titration method [9]. Particle size distribution was determined using the hydrometer method [10]. Data obtained were subjected to analysis of variance and their treatment means were separated using Bonferroni Multiple Comparison Tests at $p > 0.05$.

Table 1: Properties of soil, composted cowdung and poultry manure used for the experiment.

Property	Soil	Cowdung	Poultry manure
pH 1:1 soil	7.86	nd	nd
Organic Carbon (g kg ⁻¹)	22.51	135.37	160.21
Total Nitrogen (g kg ⁻¹)	2.12	11.60	15.10
Available P (mg kg ⁻¹)	1.63	3.50	4.50
Sand (g kg ⁻¹)	792.00	nd	nd
Silt (g kg ⁻¹)	94.00	nd	nd
Clay (g kg ⁻¹)	114.00	nd	nd
Cation exchangeable capacity (cmol kg ⁻¹)	21.01	3.41	3.22
Exchangeable acidity (cmol kg ⁻¹)	0.40	nd	nd
Exchangeable micronutrients			
Mn (mg kg ⁻¹)	308.00	385.00	490.00
Fe (mg kg ⁻¹)	141.45	353.00	420.00
Zn	1.39	500.00	700.00
As	3.00	1.30	1.00
B	1.00	0.80	0.60
Textural class	Sandy loam	nd	nd

* Nd: Not determined

Results

The pre-cropping soil properties and chemical composition of cowdung and poultry compost are shown in Table 1. The chemical compositions of poultry compost were: total N 15.1 g kg⁻¹, total P 4.50 mg kg⁻¹, organic carbon 148.67 g kg⁻¹ and C/N ratio 9.85; while cowdung compost contained total N 11.6 g kg⁻¹, total P 3.50 mg kg⁻¹, organic carbon 145.88 g kg⁻¹, and C/N ratio 12.58. The compost C/N ration measures the ease with which the compost mineralizes in the soil [4]. Cowdung compost reduced by 23.2% for total N, 22.2% for total P and 1.9% for organic carbon when compared with

poultry compost. The pre-cropping soil was slightly alkaline with pH 7.86. Other results included: 2.12 g N kg⁻¹, 22.51 g OC kg⁻¹, 1.63 mg P kg⁻¹ and cations exchangeable capacity 21.01 cmol kg⁻¹. The sandy loam soil texture of the study area may be attributed to the parent material from which the soil was formed and the climate of the area. Enujoke *et al.* [11] earlier reported that high sand content of a soil could be attributed to high content of quartz in the parent material.

Soil properties of the experimental site after the early and late cropping seasons are shown in Table 2 and 3. The

properties of the soil reduced after the early cropping harvest (N 1.35-1.72 g kg⁻¹, P 0.82-2.00 mg kg⁻¹ and OC 14.84-19.61 g kg⁻¹ early season) but increased after the repeat experiment (N 0.40- 0.52 g kg⁻¹ P 2.39-7.95 mg kg⁻¹ and OC 25.70-30.41 g kg⁻¹ late season). Plots treated with equal combination of 50% cowdung and 50% poultry at 6 tonnes per hectare (50% CD + 50% PT) has the highest organic carbon, nitrogen content with high cation exchangeable capacity after late cropping season.

All the plots treated with compost had high concentration of exchangeable micronutrients except for Fe, As and B. This could be as a result of the release of the long stored nutrient-contained in the organic manure thus, ensuring a long term effect. The slow-decomposition rate of compost could be attributed to this [12]. Hoover *et al.* [13] had found that application of poultry manure to soil increased soil N and P and aggregate stability.

Table 2: Post-cropping soil properties of the experimental site for the two seasons

Treatments	pH	OC g/ kg	TN g/kg	P mg/kg	K cmol/kg	Ca cmol/kg	Mg cmol/kg	Na cmol/kg
Early Season								
CD3	7.29 ^b	15.52 ^{ab}	1.37 ^a	0.82 ^a	0.16 ^b	12.89 ^a	0.36 ^c	0.21 ^b
CD6	7.29 ^b	15.65 ^{ab}	1.46 ^a	1.60 ^a	0.18 ^{ab}	3.34 ^a	0.41 ^c	0.83 ^a
PLT3	7.55 ^a	14.84 ^b	1.35 ^a	1.67 ^a	0.17 ^{ab}	3.00 ^a	0.32 ^d	0.35 ^{bc}
PLT6	7.34 ^{ab}	17.22 ^{ab}	1.54 ^a	1.76 ^a	0.18 ^{ab}	7.63 ^a	0.39 ^c	0.23 ^{bc}
CD/PLT3	7.28 ^b	15.35 ^{ab}	1.41 ^a	0.92 ^a	0.17 ^{ab}	3.71 ^a	0.44 ^c	0.46 ^b
CD/PLT6	7.48 ^{ab}	16.54 ^{ab}	1.43 ^a	1.11 ^a	0.16 ^c	15.06 ^a	0.45 ^b	0.42 ^{bc}
CT	7.45 ^{ab}	19.61 ^a	1.73 ^a	2.00 ^a	0.21 ^a	17.52 ^a	0.48 ^a	0.18 ^c
Late Season								
CD3	6.08 ^d	27.05 ^f	2.62 ^f	2.39 ^f	0.42 ^c	3.70 ^f	0.27 ^c	0.33 ^g
CD6	6.24 ^a	29.20 ^b	2.95 ^c	3.71 ^c	0.45 ^b	3.40 ^g	0.23 ^d	0.44 ^b
PLT3	6.00 ^c	28.62 ^c	2.95 ^c	7.95 ^a	0.40 ^c	4.08 ^d	0.33 ^a	0.51 ^a
PLT6	6.21 ^b	28.26 ^d	2.99 ^b	5.50 ^b	0.40 ^c	6.43 ^b	0.27 ^c	0.42 ^d
CD/PLT3	6.24 ^a	27.34 ^c	2.64 ^c	4.16 ^c	0.42 ^c	5.45 ^c	0.33 ^a	0.43 ^c
CD/PLT6	6.24 ^a	30.41 ^a	3.16 ^a	2.78 ^d	0.52 ^a	6.46 ^a	0.29 ^b	0.37 ^f
CT	6.11 ^c	25.70 ^g	2.73 ^d	2.50 ^c	0.41 ^d	4.03 ^e	0.29 ^b	0.39 ^e

Mean with the same letter(s) in each column are not significantly different by Bonferroni Multiple Comparison Tests at p > 0.05.

Legend: CD3= Cowdung 3t/ha, CD6= Cowdung 6t/ha, PLT3= Poultry manure 3t/ha, PLT6= Poultry manure 6t/ha, CD/PLT3= Cowdung + Poultry manure 3t/ha, CD/PLT6= Cowdung + Poultry manure 6t/ha, CT= Control.

Table 3: Effect of composted cowdung and poultry manure on the concentration of exchangeable micronutrients in the soil after harvest of maize

Treatments	E.A. cmol/kg	Zn cmg/kg	Fe mg/kg	Cu mg/kg	Mn mg/kg	As mg/kg	B mg/kg
Early Season							
CD3	0.25 ^c	0.96 ^a	90.99 ^{ab}	12.76 ^a	240.50 ^b	3.75 ^a	1.05 ^a
CD6	0.50 ^b	0.87 ^{ab}	113.50 ^a	12.70 ^{ab}	361.00 ^a	2.40 ^a	1.20 ^a
PLT3	0.40 ^{ab}	0.84 ^b	88.60 ^b	11.76 ^a	261.00 ^b	2.40 ^a	1.15 ^a
PLT6	0.60 ^a	0.90 ^{ab}	104.15 ^{ab}	12.55 ^a	258.50 ^b	3.10 ^a	1.10 ^a
CD/PLT3	0.60 ^a	0.93 ^{ab}	115.65 ^a	12.31 ^a	225.00 ^b	2.85 ^a	1.05 ^a
CD/PLT6	0.50 ^b	0.98 ^a	99.30 ^{ab}	7.85 ^b	196.50 ^b	3.15 ^a	1.20 ^a
CT	0.50 ^b	0.91 ^{ab}	94.55 ^{ab}	14.00 ^a	239.50 ^b	2.80 ^a	0.95 ^a
Late Season							
CD3	0.83 ^f	1.12 ^c	8.18 ^a	0.93 ^c	18.53 ^f	1.44 ^c	0.80 ^b
CD6	1.47 ^b	1.04 ^c	7.17 ^c	0.91 ^f	19.76 ^d	1.55 ^a	0.91 ^a
PLT3	1.55 ^a	1.06 ^d	6.01 ^g	0.94 ^d	20.99 ^c	1.31 ^c	0.72 ^d
PLT6	1.01 ^d	1.18 ^a	6.59 ^e	0.96 ^b	22.91 ^a	1.52 ^b	0.75 ^c
CD/PLT3	0.99 ^c	1.16 ^b	6.81 ^d	0.85 ^g	21.15 ^b	1.09 ^f	0.72 ^d
CD/PLT6	1.15 ^c	1.03 ^f	6.27 ^f	0.95 ^g	18.51 ^g	1.33 ^d	0.61 ^e
CT	0.77 ^g	0.95 ^g	7.31 ^b	1.04 ^a	19.25 ^c	1.52 ^b	0.61 ^e

Mean with the same letter(s) in each column are not significantly different by Bonferroni Multiple Comparison Tests at p > 0.05.

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Legend: CD3= Cowdung 3t/ha, CD6= Cowdung 6t/ha, PLT3= Poultry manure 3t/ha, PLT6= Poultry manure 6t/ha, CD/PLT3= Cowdung + Poultry manure 3t/ha, CD/PLT6= Cowdung + Poultry manure 6t/ha, CT= Control

Conclusion

We concluded that application of animal composts to an *alfisol* soil enhanced soil properties, particularly the soil

organic matter, a major key in crop husbandry. In addition, positive residual effect of compost addition were noticed in the soil properties.

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Appendix



(d)
* a: Cowdung undergoing compost Aerobically b: Poultry dropping undergoing compost Aerobically, c:Compost application on filed and d: Maize plant at Maturity

WIS-ECC 09

BACTERIAL DETERIORATION OF SELECTED LUBRICATING OIL FROM PETROL GENERATOR SETS.

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ABSTRACT

Lubricating oils are hydrocarbon-derivatives that reduce friction between machine components in contact. Lubricating oils can be susceptible to bacterial deteriorogens. Hence, this study assessed the bacterial deterioration of some selected lubricating oils used in petrol generators used in Umuahia, Abia State. The biodeteriogens isolated from selected used lubricating oils were incubated at different temperatures to determine the temperature growth ranges. The bacteria isolates accessed for the lubricating oil utilization screen test. These isolates identified were *Bacillus* sp., *Corynebacterium* sp., *Actinomyces* sp., *Citrobacter* sp., *Micrococcus* sp., and *Pseudomonas* sp. The total heterotrophic counts from the used and unused oil samples, ranged from 3.8×10^7 cfu/ml to 9.3×10^7 cfu/ml; and 0.6×10^3 cfu/ml respectively. Hydrocarbon utilizing bacterial counts ranged from 2.3×10^7 cfu/ml to 9.0×10^7 cfu/ml; and 0.2×10^2 cfu/ml respectively. Incubation temperature effect showed that all isolates grew at 30°C, four grew at 45°C, and 60°C, three grew at 50°C; only *Bacillus* sp. grew at 70°C. No organism grew at 80°C. The screen test showed that all isolates utilized the lubricating oil as their carbon source at varied degrees. The utilization of lubricating oil indicates that it can serve as the carbon source of the bacterial isolates. The used oil served as a better substrate for the organisms than the unused one. The lubricating oil can be susceptible to biodeterioration before use if not properly preserved. There is a need for lubricating oils in petrol generator sets to be changed regularly to ensure the good working condition of the engines.

Keywords: Bacterial deteriorogens, Biodeterioration, Generator sets, Lubricating Oil, Petrol

INTRODUCTION

Lubricating oil not only reduces friction in petrol generator set engines but also cleans them. It does this by removing the wear particles around metallic moving surfaces. The pistons are cooled. The presence and proliferation of microbes can lead to the contamination of these lubricating oils thus, impairing their functionalities (Montagnoli *et al.*, 2009). Many microorganisms can cause the deterioration of oil in service (Billett, 1979). Gram-negative bacteria such as the *Pseudomonas* are the most common (Hill, 1977) and the anaerobic sulphate-reducing bacteria (Khan and Rizvi, 2011). The growth of fungi in lubricating oil can be problematic. The metabolic activities of microorganisms in lubricating oil in-use can lead to acidic pH, emulsion instability, increase rusting, malfunctioning of vital components, slime and dirt accumulation, and other organoleptic changes (Koma *et al.*, 2003). There may be no significant losses noticed in industrial water-soluble lubricating oils with high microbial counts. Initial laboratory efforts to correlate microbial activity with adverse lubricant performance have met with no appreciable success (Okpokwasili and Okorie, 1988).

Lubricating oils and additives used in generator engines, are majorly made of long-chain saturated hydrocarbons (Osadebe *et al.*, 2018). Recently, the global usage of

petroleum-based lubricants has increased with increments of 13,726 million tons of oil equivalent per annum (Jeevan and Jayaram, 2018). The cyclic alkanes (c-alkanes) make up the main components. Lubricants made up of C₁₆-C₃₆ hydrocarbons are more than 75% C-alkanes. These base oil constituents with long alkyl side chains are recalcitrant, thus resistant to microbial attack (Koma *et al.*, 2003). The biodegradation of long-chain hydrocarbons is inhibited by not being bioavailable. At temperatures less than 10°C, the hydrocarbons are in solid-state; thus, microorganisms find it difficult to degrade the waste oil (Ijaha *et al.*, 2003). Besides, the recalcitrance of hydrocarbons and the inhibition of microorganisms by minor ingredients in waste oil, these ingredients also act as hindrance to the deterioration of the waste oil (Leke *et al.*, 2011). Microbial degradation of hydrocarbons depends on the type and size of the hydrocarbon molecule. Intermediate chain length alkanes (C₁₀-C₂₄) are faster to degrade, while very-long-chain alkanes are not susceptible to microbial deterioration (Ijaha *et al.*, 2003; Mbachu *et al.*, 2016).

Biodeterioration is any change that is undesirable in the properties of a material caused by essential activities of an organism (Sanyaolu *et al.*, 2012). The change is often termed a programmatically negative process. Of course, desirable changes occur, such as the breakdown of detergents and toxic chemicals in the soil. This process

is often referred to as biodegradation and covers such areas as pesticide breakdown in the environment and the treatment of agriculture and domestic waste (Appenroth, 2010). The quality of lubricating oils can be negatively altered by the metabolic activities of microorganisms (Hill, 1977). The agents which cause undesirable changes do this as part of their normal activities. The microorganisms of importance in a biodeterioration process are the bacteria, fungi, actinomycetes, and algae (Mbachu *et al.*, 2016). These groups have been reported widely as biodeterioration agents in all parts of the world and under many environmental conditions (Appenroth, 2010). Studies of biodeteriorative problems draw on basic information regarding the organism, such as nutritional, ecological, and physiological preferences (Eze and Okpokwasili, 2010).

Complex hydrocarbons, especially pollutants constituting various compounds, need a consortium of microorganisms to be deteriorated. Single species can metabolize only a limited range of hydrocarbons, while the mixed cultures possess broad-spectrum enzymatic capacities required in petroleum biodeterioration (Olajire and Essien, 2014). Several microbial populations have been detected in petroleum-contaminated soils or water. This exhibits a microbial role in hydrocarbon transformation processes. The deterioration capacity of any microbial culture is not just a result of combined single strain capacities (Ghazali *et al.*, 2004). The environmental factors which affect the biodeterioration of lubricants and other materials include temperature, pH, water activity (Wa), water, redox potential, and material interactions. The hydrocarbon-degradative capability to use petroleum products as growth substrates by hydrocarbon utilizers portrays their application and importance (Appenroth, 2010).

Studies and reports on the bacterial deterioration of lubricating oils in Nigeria are few. Car users, among others, have little or no knowledge of why oil in the motor and other engines should be constantly changed (Ghazali *et al.*, 2004). The problem of power supply in Nigeria has led the populace to seek alternatives. The practical option being the use of different types of generator sets. Lubricants are prone to spoilage when they are exposed to microbial activities, especially when they are in use. Microbial growths can serve as proxies for deterioration in lubricating oils (Stanley *et al.*, 2018).

Used lubricating oil samples from different petrol generator sets, therefore, were assessed in this study under the same conditions to isolate, identify and characterize bacteria associated with the biodeterioration of the lubricating oil, and also their ability to utilize the lubricating oil as a sole carbon source.

Material And Methods

Collection of Samples

Used lubricating oil samples from different petrol generator sets were evaluated. The used lubricating oil samples were collected from the engines of five different generator sets (Sumen Generator set 3800v, Parson 1900v, Sumen Generator set 2700v; Yamaha 1900v and Elepac Ec 1800CS) which had been used between 2 to 3months. They were poured into sterile containers, labelled, and transported to the laboratory for analyses. The unused oil sample, AGIP super motor oil (SAE 20W-50) which served as the control was purchased in a sealed container from retailers.

2.2 Isolation, Enumeration, Characterization, and Identification of Total Heterotrophic Bacterial Count (THBC) and Hydrocarbon Utilizing Bacterial Count (HUBC).

Ten-fold serial dilution was carried out to obtain various dilutions. An aliquot of 1ml of the serially diluted lubricating oil samples was plated out in duplicates on Nutrient agar plates using the pour plate method. The plates were incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 to 48hours. At the end of the incubation, the total heterotrophic counts were determined.

To isolate and enumerate the hydrocarbon utilizing bacteria (HUBC), five drops of each oil sample were added to 10ml of nutrient broth. Ten-fold serial dilution was carried out to obtain various dilutions. The oil samples were plated out using the pour plate method. This was done by adding 10-15ml of molten Nutrient agar which had been previously been sterilized and left to cool to 45°C . The plates were gently swirled for adequate mixing to occur. Upon solidification, the plates were inverted and incubated at $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 48hours. The bacterial colonies which grew on the plates were enumerated and purified by subculture onto fresh nutrient agar using streak plate technique for identification and further analyses as described by Okpokwasili and Okorie, (1988).

The bacterial isolates were examined for colonial morphology as well as for cell micro-morphological and biochemical characteristics. Confirmatory identities of the bacteria were made using Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Growth of Bacterial Isolates at Different Temperature

The ability of the bacterial isolates from the test oil samples to grow under different temperatures (30, 45, 60, 70, and 80°C) was examined. This is to determine the temperatures the organisms tolerated and grew and those at which they were inhibited (Okpokwasili and Okorie, 1988). About 10ml of nutrient broth was dispensed into

six test tubes (for each sample and the control) for each isolate. They were sterilized by autoclaving and subsequently inoculated with the isolates. The tubes were labeled and inoculated at varying temperatures for 48hours. The test tubes were examined for turbidity as a sign of growth. Results were recorded as positive or negative.

Utilization of Lubricating Oil Samples by Bacterial Isolates

Bacterial isolates from the lubricating oil samples were tested for their ability to utilize the lubricating oil as their sole carbon source. The modified mineral salt medium as described by Okpokwasili and Okorie (1988) was employed. To the 9.9 ml of mineral salt broth in each test tube, 0.1 ml of the relevant test oil was added before autoclaving at 121°C and 15 psi for 15 minutes. On cooling, each test tube was inoculated with the corresponding bacterial isolate (the bacteria initially

isolated from the test oil in the test tube). A test tube containing only media and the test oil without any inoculum served as control. The test tubes were incubated for seven days at 23-25°C. Lubricating oil utilizing bacteria turned the broth turbid as against a clear control.

Results And Discussion

The mean total heterotrophic bacterial counts (THBC) from the selected lubricating oil varied from 3.8×10^7 cfu/ml to 9.3×10^7 cfu/ml; while the mean hydrocarbon utilizing bacterial counts (HUBC) were from 2.3×10^7 cfu/ml to 9.0×10^7 cfu/ml. The unused lubricating oil gave 0.6×10^3 cfu/ml for total heterotrophic bacterial counts and 0.2×10^2 cfu/ml for hydrocarbon utilizing bacterial counts. The bacterial isolates from used and unused oil were identified as follows: *Corynebacterium* sp., *Bacillus* sp., *Citrobacter* sp., *Micrococcus* sp., *Actinomyces* sp., and *Pseudomonas* sp.

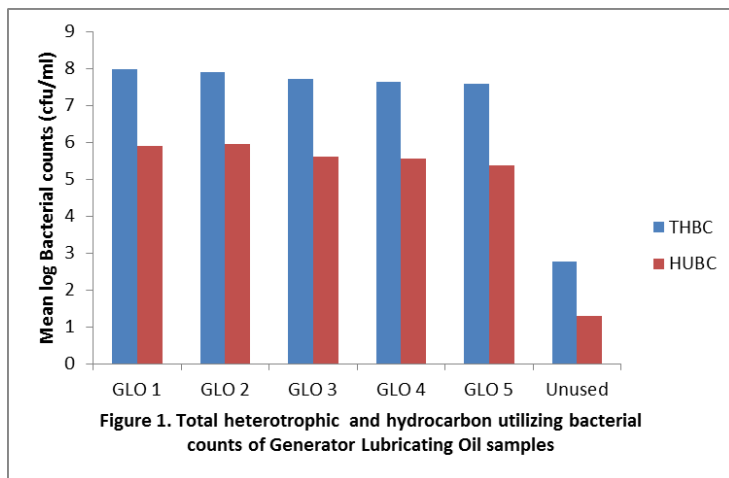


Table 1: Growth of bacterial isolates at different temperatures

Isolates	Temperature °C				
	30	45	60	70	80
<i>Bacillus</i>	+	+	+	+	-
<i>Actinomyces</i>	+	+	+	-	-
<i>Citrobacter</i>	-	-	-	-	-
<i>Corynebacterium</i>	+	+	+	-	-
<i>Micrococcus</i>	+	-	-	-	-
<i>Pseudomonas</i>	+	+	+	-	-

Key: += Growth; - = No Growth

Incubation of the bacteria at different temperatures for 48hours showed *Bacillus* sp. growing at all temperature ranges except at 80°C, *Actinomyces* sp., *Corynebacterium* sp., *Pseudomonas* sp. grew at all temperature ranges except 70 and 80°C. *Citrobacter* sp. and *Micrococcus* sp. grew only at 30°C as shown in Table 1.

Table 2: Screen test for the utilization of used and unused lubricating oil by the bacterial isolates.

Isolates	Used Oil	Unused Oil
<i>Corynebacterium</i> sp.	+++	-
<i>Actinomyces</i> sp.	++	-
<i>Pseudomonas</i> sp.	+++	+
<i>Citrobacter</i> sp.	++	-
<i>Bacillus</i> sp.	+++	+
<i>Micrococcus</i> sp.	+	-

Key: - =No growth; + = Little growth, ++ =Moderate growth; +++ = Heavy growth

The screen test for the utilization of used and unused lubricating oils by bacteria isolates showed that the organisms grew at different rates with *Corynebacterium* sp., *Pseudomonas* sp. and *Bacillus* sp. having heavy growth (+++), *Actinomyces* sp. and *Citrobacter* sp. had

moderate growth rate (++) , followed by *Micrococcus* sp. that had little growth rates on used oil. *Pseudomonas* sp. and *Bacillus* sp. had little growth (+) on unused oil, while the other organisms had no growth at all (Table 2).

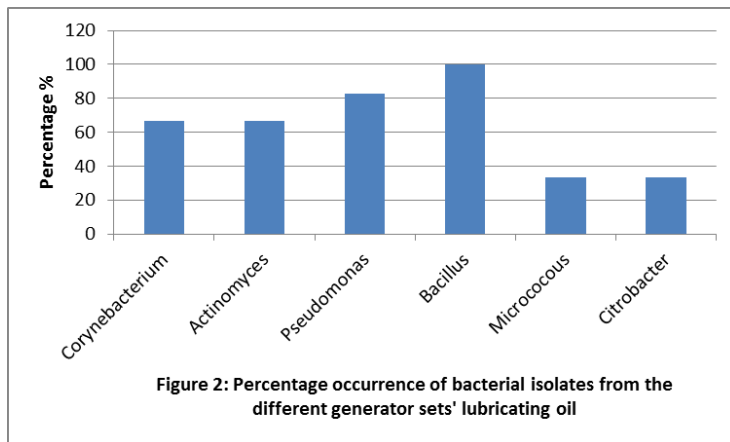


Figure 2: Percentage occurrence of bacterial isolates from the different generator sets' lubricating oil

The prevalence of isolates in the different oil is shown in Figure 2 with *Bacillus* sp. having the highest (100 %)

prevalence and *Micrococcus* sp. and *Citrobacter* sp. having the least (33.3%).

Solid and liquid lubricants made of petroleum Hydrocarbons are readily affected by bacteria during operation and storage in unprotected containers (Eisentraeger *et al.*, 2002). Madanchi *et al.*, (2017) reported that microbial growth and oil spoilage occur mainly when the petroleum products come in contact with water. This indicates a variety of organic substrates are present. Some or all of which are potential sources of food and energy to invading microorganisms (Yemashova *et al.*, 2007).

The mean counts of total heterotrophic bacterial counts from the lubricating oil from different petrol generator sets ranged from 3.8 to 9.3 x 10⁷ cfu/ml, and when compared with the (control) unused lubricating oil that gave 0.6 x 10³ cfu/ml showed the proliferation of bacteria in used oil. It was also deduced that the lubricating oil from petrol generator sets that have been used for three months gave higher bacterial counts than those that have been used for two months. The same was noticed in the mean hydrocarbon utilizing bacterial counts for used oil that ranged from 2.3 x 10⁷ cfu/ml to 9.0 x 10⁷ cfu/ml when compared with the control that gave 0.2x 10² cfu/ml hydrocarbon utilizing bacterial counts. Yemashova *et al.*, (2007) reported that concentration of bacteria isolated from the bottom of fuel tank varied from 1x10² to 8.8x10⁷ cells/ml and 85% of samples contained high concentrations of these bacteria, typically from 10⁴ to 10⁷ cells/ml.

The bacteria isolated from used and unused oil were identified as follows: *Corynebacterium* sp., *Bacillus* sp., *Citrobacter* sp., *Micrococcus* sp., *Actinomyces* sp., and *Pseudomonas* sp. Studies by Okpokwasili and Okorie, (1988), Ismail *et al.*,(2014) and Osadebe *et al.*, (2018) have reported the isolation of bacteria such as *Serratia*, *Actinomyces*, *Bacillus*, *Edwardsiella*, *Corynebacterium*, *Micrococcus*, *Citrobacter*, *Nocardia*. They further stated that different microorganisms play dominant roles in the spoilage of generator engine lubricating oil at different times.

Hydrocarbon utilizing bacteria that use oil and oil products as a sole source of carbon and energy can be found virtually in the air, water, and soil. *Corynebacterium* sp., *Pseudomonas* sp., and *Bacillus* sp. expressed the highest turbidity in test tubes. Initial oil degradation could be carried out by these organisms. All

the isolated bacteria grew with the used lubricating oil as the sole source of carbon and energy. This could be an indication that used oil is a better substrate for the growth of the infecting bacteria than the unused oil. This result is in concordance with the study of Stanley *et al.*, (2018) who reported that the highest turbidity was shown in the medium containing used lubricating oil. This result also suggests that microbial activities might have affected some of the lubricating oil constituents; leading to their more rapid utilization than the unused oil. *Pseudomonas* sp. and *Bacillus* sp. were able to grow slightly on unused oil.

The effects of incubation temperature on the organisms were also studied. It showed that all isolates grew at 30°C and a few at temperatures of 45°C and 60°C but *Bacillus* spp., exhibited growth at 60°C and 70°C. However, there was no growth at 80°C. This could be as a result of spore-forming nature of *Bacillus* species. *Bacillus* spores can resist adverse temperature conditions. The inhibition temperature rate of organisms changes depending on the organisms and oil type (Hill and Genner, 1980).

Overtime bacteria in used lubricating oil produce metabolites and enzymes necessary for oil deterioration. Unused oil, which appears pristine is not susceptible to bacterial deterioration. For organisms to grow on unused oil, adequate time is needed for the requisite enzyme systems to be established for further actions under optimum conditions. Furthermore, it seems plausible that bacteria are the principal biodeteriogens in oil when the pH is near neutral.

Conclusion

The physicochemical changes that result when the lubricating oil is in service may correlate to the selection of different dominant organisms present at different times. The presence of oil-utilizing species in used oil suggests that bacterial deterioration of the oil can take place whether the engine is in use or not. This establishes the occurrence of both mesophilic and thermophilic organisms. When the engine becomes very hot, *Bacillus* species infecting the oil can be isolated. Various microorganisms play specific roles in the deterioration of generator set engine lubricating oils at different times. Used oil is more prone to biodeterioration than unused oil.

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ASSESSMENT OF ABATTOIR WASTEWATER ON THE QUALITY OF BOREHOLE WATER SOURCE IN ABA, ABIA STATE

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ABSTRACT

The assessment of abattoir wastewater discharge on the quality of borehole water in old express Aba, Abia state was carried out. Water samples were collected from three points within abattoir discharge point, point 1 was 30 meters away, and point 2 was 50 meters away while point 3 was 200 meters away from the point of the abattoir wastewater discharge which was used as the control. Wastewater from the abattoir is channeled into a burrow pit as the means of waste discharge. The borehole water samples and the abattoir waste water were analyzed to see if there is any effect of the abattoir waste discharge on the physicochemical, heavy metal and bacteriological quality of the borehole waters. The result of the physico-chemical properties of the waste water analyzed shows that the pH (6.45) was below WHO standard, the TDS (795.00mg/l), EC (795.00u/cm), Turbidity (68.50NTU), BOD (296.25mg/l), Total Hardness (127.85mg/l) was higher than the acceptable standard stated by WHO. The acidity (13.61mg/l), Chloride (57.98mg/l), Nitrate (8.92mg/l), Phosphate (5.68mg/l), Sulphate (23.93mg/l), Dissolved Oxygen (0.30mg/l) of the abattoir waste water fell under WHO Standard. The result of the heavy metals shows that Zinc (12.55mg/l), Iron (7.78mg/l), Lead (0.76mg/l), Arsenic (0.48mg/l) were all higher than the WHO Standard. The result of the Bacteriological quality of the waste water shows that the abattoir waste had a total Coliform load count of 7.8×10^7 , E. Coli and Salmonella load count of 2.0×10^7 and 2.5×10^7 respectively. While the result of the physicochemical properties of the borehole water samples shows that the pH of the samples (4.23 – 5.83) were below acceptable standard stated by WHO, while the TDS (54.50 – 77.0mg/l), EC (109.00 – 157.50u/cm), turbidity (0.67 – 0.85NTU), acidity (30.20 – 50.00mg/l), chloride (19.19 – 22.27mg/l), nitrate (ND - 0.03mg/l), phosphate (0.72 – 1.45mg/l), Sulphate (4.25 – 6.81mg/l), total hardness (25.66 – 25.93mg/l), dissolved oxygen (4.80 – 8.93mg/l) and biological oxygen demand (0.70 – 0.95mg/l) of the borehole water samples were within the stipulated acceptable limit of WHO. The result of the heavy metal concentration shows that the zinc (0.68 – 1.25mg/l) and iron (0.08 – 0.20mg/l) concentration of the borehole water samples also fell within acceptable limit. Arsenic and lead were not detected in the borehole water samples. The result of the bacteriological quality shows that the borehole water samples had total viable load count that was higher than the stipulated standard. Coliform was only detected in point 1 borehole water which indicated presences of water borne disease.

Keyword: Abattoir, wastewater, heavy metal concentration, bacteriological quality

INTRODUCTION

Water is a universal resource and because of its free nature, it is often subjected to abuse, especially in the third world nations where information is not disseminated to society. Water can be gotten everywhere but safe and clean water are hard to come by in almost all parts of the world (Omole *et al*; 2008). Water performs three roles of transporting body nutrients to other vital organs, regulating the body temperature, as well as carrying waste out of our internal body organs and is second only to air in its importance. The highest rate that water is potentially available for human use and its management is often considered the best measure of the total water resources in any given region. With the advent of increasing population and industrialization, the range of requirements for water has increased, together with greater demand for higher water quality (Turdukulov, *et al*. 2003). The physical hazards presents are the volatile organic compounds (VOCs), dissolved solids and

suspended solids etc. The chemicals hazards are the Iron, Mercury, Copper, Manganese, Lead, Cadmium, Phosphate, Nitrate etc. (Omole *et al*; 2008).

Borehole water requires ever increasing need for management. Borehole water is the most readily available, yet the most polluted as a result of anthropogenic activities, but notably, controllable activities. In developing countries, especially the rural areas where potable water supply is a near rarity, most of the dwellers rely mainly on water in streams, borehole, brooks, rivers, ponds and lakes, well, tube wells. Borehole water is characterized with adequate aquifer protection, groundwater has excellent microbial and chemical quality and it therefore requires minimal or no treatment. Also, the capital cost of groundwater development when compared to surface water development is modest and groundwater lends itself to flexible development making it attractive as a source of

potable water supply. The wells terminate in the weathered crystalline rocks and if deep enough could sustain the dry season. The major sources of borehole water pollution is indiscriminate discharge of untreated. The principal sources and causes of groundwater pollution are municipal, industrial, agricultural and others.

Abattoir activities is another source of pollution since human activities such as animal production and meat processing have been reported to impact negatively on

soil and natural water composition leading to pollution of the soil, natural water resources and the entire environment (Adesemoye, 2006). It has also been reported that animals which graze on contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues and milk if lactating. When such animals are killed, these metals are released in the soil as natural sink but subsequently leached out into nearby streams or water bodies. (Verheijen *et al.*, 2006).

Study Area

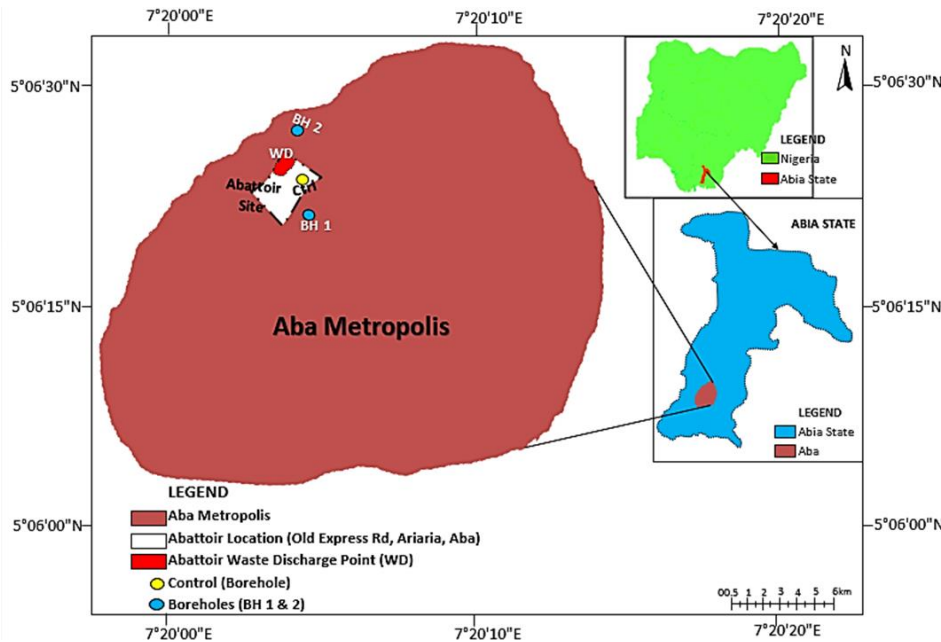


Fig. 1: Map of Abattoir site.
Source: (Field work, 2018)

This study was carried out at old express road, a sub-city found in the mega city of Aba in Abia State which is located at latitude 5° 06' 23¹¹ N, and longitude of 7° 22' 00¹¹ E with the elevation of 64m = 209ft above the sea level.

The main occupation of the dwellers in this area are mostly farming and trading.

Abattoir waste water – N5°6'17.1756. E7209.7224, Elevation: 39.0m

Borehole 1 – N5° 6'15.984; E7°20'11.418; Elevation.33.0m – distance from wastewater = 30.78m

Borehole 2 – N5°6'19.6228; E7°20'12.4728 Elevation. 40m, distance from wastewater = 50.96m.

Borehole 3 = N5°6'27 2952¹¹ E7208.466°⁰, Elevation. 39.0m, distance from wastewater = 200.27m.

MATERIALS AND METHODS

Water samples were collected randomly in 4 different locations of Old Express abattoir. All the samples were collected on the same day in the morning for various Physical, Chemical, and Micro-biological analysis. Water samples were collected into 4 (2 liters) clean

sterilized plastic bottles. Sample bottles were rinsed twice with the water obtained from designated boreholes and waste water prior to collecting samples for testing and the sample bottles were filled completely to prevent any loss of dissolved gases from the water samples. The sample containers were labeled appropriately using a marker with the information on collection point which will be cross-checked with the field notebook and the sample collection sheet and transported to the laboratory.

The Water quality parameters measured/analyzed includes; pH , TDS, EC, Turbidity, Acidity, Chloride, Nitrate, Phosphate, Sulphate, Total hardness, Dissolved oxygen, Biological oxygen demand, Zinc, Iron, Arsenic, Lead, Total viable load count, Coliform, E coli. Descriptive statistics (means, ranges, minimum and maximum, standard error), was carried out with the aid of statistical packages of SPSS v.22.0 and MS Excel 2007

RESULTS

Table 3.1 Physicochemical properties of Borehole water and Abattoir waste water

Parameters	Abattoir waste water	Point 1 Borehole water	Point 2 Borehole water	Control Borehole water	SE	WHO Standard
Colour	Dark brown	Colourless	Colourless	Colourless		
Taste	-	Tasteless	Tasteless	Tasteless		
Odour	Offensive	Odourless	Odourless	Odourless		
pH	6.45 ^a	5.83 ^b	4.23 ^d	4.87 ^c	0.18	6.5-8.5
TDS	795.00 ^a	55.50 ^c	77.00 ^b	54.50 ^c	0.50	1000
EC	795.00 ^a	109 ^c	157.50 ^b	110 ^c	0.01	500
Turbidity (NTU)	68.50 ^a	0.67 ^d	0.76 ^c	0.84 ^b	0.01	5
Acidity(mg/l)	13.61 ^a	30.20 ^d	50.60 ^b	41.93 ^c	0.18	100
Cl (mg/l)	57.98 ^a	22.27 ^b	19.19 ^d	14.98 ^c	0.48	250
NO ³ (mg/l)	8.92 ^a	0.03 ^b	ND	ND	0.23	10
PO ⁴ (mg/l)	5.68 ^a	1.15 ^b	0.72 ^b	1.45 ^b	0.12	50
SO ⁴ (mg/l)	23.93 ^a	5.41 ^c	4.25 ^d	6.81 ^b	0.39	250
TH (mg/l)	127.85 ^a	25.22 ^b	22.66 ^c	25.93 ^b	7.91	100
DO (mg/l)	0.30 ^c	4.80 ^b	4.85 ^b	8.93 ^a	0.08	2.0
BOD (mg/l)	296.25 ^a	0.95 ^b	0.80 ^b	0.70 ^b	0.17	50

Source: Field Work 2018.

Table 3.1 presents the result of the physicochemical properties of the water samples. The result obtained shows that the pH of the water sample ranged from 4.23 – 6.45. The values obtained shows that the water samples were acidic. However, the abattoir waste water was less acidic than the borehole water samples. The result revealed that the pH of the borehole waters decreased as the distance away from the abattoir site increased. Value obtained were below the stipulated standard of 6.5 – 8.5 stated by WHO. The low pH values of the river may be an indication of high CO₂ content of the water (Coker *et al.*, 2001).

The total dissolved oxygen (TDS) of the water samples ranged from 54.50 – 795.00mg/l. The TDS value of the

abattoir waste water was significantly higher than that of the borehole water samples. The higher value obtained in the point 2 borehole compared to that of point 1 and the control shows that the abattoir waste water does not have any effect on the TDS of ground water within. The values obtained for the borehole water samples were below the maximum limit stated by WHO.

The conductivity value of the abattoir waste (1563.00us/cm) was significantly higher than that of the borehole water samples (109 – 157.50us/cm). The higher value obtained in the water sample from point 2 and the control compared to that of point 1 is a clear indication that the abattoir has no effect on the electrical conductivity of ground water within. The result shows

that the value obtained for the borehole water samples was within the stipulated limit (500us/cm) stated by WHO. Similar observation was reported by Hassan *et al.* (2014) on the effect of abattoir on ground water quality in Ikotun, Lagos state.

The turbidity value of the abattoir waste water (68.50 NTU) was higher than that of the borehole water samples (0.67 – 0.84 NTU). The result shows that the abattoir waste did not affect the conductivity of borehole water, as the water collected from the borehole closest to the discharge point (point 1) had lower values compared to the others. The values obtained for the borehole water samples were within WHO acceptable limit (5 NTU).

The acidity of the samples ranged from 13.61 – 50.00mg/l. values obtained were significantly different ($p < 0.05$). The result shows that point 1 borehole water sample had acidity value closer to that of the abattoir waste water than the others, thus showing likeliness that the abattoir waste might have influenced its acidity. However, the acidity of the point 1 borehole water sample could probably be as a result of anthropogenic activities of people and not necessarily the abattoir waste. Since industrial activities is at minimal at the study area, the major source of acidity could be attributed to vehicular activities, use of power generating sets of varying sizes by the residents, indiscriminate refuse burning and old practice of bush burning by few hunters and farmers in the study area The World Health Organization (WHO, 2001),

The chloride content of the water samples ranged from 19.19 – 57.98mg/l. The result shows that the chloride value in the borehole water sample decreased as the distance away from the abattoir discharge point increased. This shows that the abattoir waste had significant effect on the borehole water chloride content. This is in line with the report of Olaiya *et al.* (2016) on the effect of abattoir waste on ground water in Edo state. The chloride level obtained in the borehole water sample was within acceptable limit (250mg/l) by WHO.

Nitrate was not detected in point 2 borehole water sample and the control, while seemingly low concentration was obtained in point 1 borehole water sample (0.03mg/l) compared to that of the abattoir waste water (8.92mg/l). The levels of nitrate obtained in point 1 borehole water sample could suggest the presence of higher amount of nucleic acids from dead organisms and nitrogenous animal wastes such as urea and uric acid that leach deep in to the ground water level. The result shows that nitrate could have been carried in to the ground water, however, the level obtained in point 1 borehole water sample was below the maximum limit (10mg/l) stated by WHO.

Phosphate content obtained ranged from 0.72 – 5.68mg/l. the result shows that the high phosphate content of the abattoir waste water did not affect the level in the borehole water, as the value obtained in the control was significantly higher than that of point 1 borehole water sample. Hassan, *et al.* (2000), reported that abattoir waste did not affect ground water phosphate content in Ikotun, Lagos state. The value obtained in this study fall within the acceptable limit (0.1 – 50mg/l) stated by WHO. Phosphorus is an essential component of ATP, DNA and phospholipids (Nelson *et al.*, 2000). However, phosphorous levels obtained may be due to high degree of organic pollution, eutrophication, agricultural activities, and use of detergents very close to the water sources which could be washed into groundwater by erosion or leaching.

Sulphate level obtained ranged from 4.25 – 23.03mg/l. values obtained were significantly different ($p < 0.05$). The higher value obtained in the control compared to that of point 1 borehole water sample suggests that the abattoir waste did not affect the Sulphate level of groundwater. Ezemonye *et al.* (2016) reported that abattoir increased the Sulphate level of Anwai River in Asaba, Delta state. This could be as a result of runoff from the discharge point into the River. Value obtained in this study for the borehole water samples was within the acceptable limit (250mg/l) stated by WHO.

The total hardness of the abattoir water sample (127.85mg/l) was significantly higher than that of the borehole water sample (22.66 – 25.93mg/l). There was no significant difference in the value obtained for point 1 borehole water sample and the control. Thus, suggesting that the abattoir waste didn't affect the CaCO₃ level of groundwater within its point of discharge. The value obtained for the borehole water samples was within acceptable limit (100mg/l).

The dissolved oxygen of the abattoir waste water (0.30mg/l) was significantly lower ($p < 0.05$) than that of the borehole water samples (4.80 – 8.93mg/l). The dissolved oxygen content of point 1 and point 2 borehole water sample was lower than that of the control. The lower value obtained could be as a result higher biofilm present in the underground pipes. Some of these organisms are carried in the overhead tanks where they multiply and use up part of the dissolved oxygen present in the tank. However, values obtained for the borehole water sample was within the acceptable limit of (2.0mg/l) as stated by WHO, (2003).

The BOD of the abattoir waste water 296.25mg/l was higher than that of the borehole water samples. The value obtained for the borehole water sample was significantly lower than that of the control and falls within the acceptable limit of 50mg/l by WHO.

Table 3.2 metal concentration properties of borehole water and Abattoir waste water

Samples	Zn (mg/l)	Fe (mg/l)	Pb (mg/l)	Ar (mg/l)
Abattoir waste water	12.55 ^a	7.78 ^a	0.76 ^a	0.48
Point 1	0.86 ^b	0.08 ^b	0.02 ^c	ND
Point 2	1.25 ^b	0.20 ^b	0.04 ^b	ND
control	0.68 ^b	0.05 ^b	ND	ND
SE	0.13	0.27	0.05	0.01
WHO Standard	5.0	0.3	0.1	0.01

Source: Field Work, 2018.

Table 3.2 presents the result of the heavy metal concentration of the water sample. The result shows that the zinc level in the abattoir waste water was 12.55mg/l. this value was significantly higher than the range of 0.68 – 1.25mg/l obtained for the borehole water samples. Point 2 borehole water sample had higher zinc level compared to the control and point 1 borehole water samples. The low zinc concentration of point 1 and 2 borehole water samples shows that the abattoir waste discharge did not affect the zinc level of the groundwater. Hassan *et al.*, (2014) reported low zinc level in groundwater obtain with abattoir waste site in Ikotun, Lagos state. While Unamba *et al.*, (2016) did detect zinc in borehole water in the Federal Capital Territory, Nigeria. However, values obtained in this study fell within the maximum permissible limit (5.00mg/l) stipulated by WHO.

The iron concentration of the abattoir waste water was 7.78mg/l, while that of the borehole water samples ranged from 0.08 – 0.20mg/l. the lower concentration obtained in the control sample compared to that of point 1 and 2 borehole water sample could be attributed to the type of soil in which the borehole was dug. The high value obtained in this point 1 borehole water samples could be due to rusty irons used in drilling the borehole that could have left huge mass of its part underground (Nkansah *et al.*, 2010). Values obtained for the borehole water samples were lower than the acceptable limit of 0.30mg/l stipulated by WHO.

The lead and arsenic concentration of the abattoir waste water was 0.76mg/l and 0.48mg/l respectively. Lead concentration obtained in the borehole samples ranged from ND - 0.04mg/l. level obtained in the borehole

samples was within the acceptable limit (0.05mg/l) stipulated by WHO. The result shows that the abattoir did not affect the lead and arsenic level in borehole 1 and 2 as Arsenic was not detected in the borehole samples.

Table 3.3: Bacteriological quality of Borehole water and Abattoir waste water

Samples	TVC (cfu/ml)	TCC (cfu/ml)	TEC (cfu/ml)	TSal (cfu/ml)
Abattoir	3.5 X 10 ⁷	7.0 X 10 ⁷	2.0 x 10 ⁷	2.5 x 10 ⁷
Point 1	1.5 X 10 ³	3.0 X 10 ³	NG	NG
Point 2	5.0 X 10 ⁷	NG	NG	NG
Control	3.0 X 10 ³	NG	NG	NG

Source: Field Work 2018. NG = No Growth

Table 3.3 shows the result of the bacteriological quality of the water samples. The result shows that the total viable load count of the abattoir waste water was 3.5 X 10⁷, while that of the borehole water samples ranged from 1.5 X 10³ – 5.0 X 10³. The result shows that point 1 borehole water sample had the least load count followed by the control, the point 2 borehole water samples had the highest. The trend shows that the total viable load count of the groundwater was not affected by the abattoir waste discharge. Load count obtained were beyond the acceptable level (1.0 X 10² cfu/100ml) stated by WHO. The result shows that the abattoir waste water and point 1 borehole water sample had a total coliform load count of 7.8 X 10⁷ and 3.0 X 10³ respectively. Whereas, no coliform was not detected in point 2 borehole water samples and the control. However, the result also shows that the abattoir waste water had an E. coli and salmonella load count of 2.0 X 10⁷ and 2.5 X 10⁷ respectively, while in all the borehole water samples, they were not detected. The presence of coliform in point 1 borehole water samples is an indication of fecal contamination and pose a threat to human life. If the source of this contamination is not checked and possible remedy applied, the coliform load could increase over time. This value is low compared to the range of 2.45 – 3.45 X 10⁶ reported by Unamba *et al.* (2016) for some borehole waters in the Federal Capital Territory, Nigeria. Coliform count obtained in point 1 borehole sample is higher than the stipulated standard (<10cfu.100l) for drinking water by WHO. The source of the coliform might not only be from the abattoir waste through leaching and ground water movement, but could also be from the storage overhead tank, since there is no strict legislation mandating people to wash their tank

regularly. To reduce the incidence of water borne disease that could emanate from the presence of abattoir in an area, it is advisable not to site water boreholes at close proximity to abattoir wastewater discharge point especially when the discharge is done on a permanent borrow pit.

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SPATIAL CHARACTERIZATION OF AMBIENT AIR QUALITY AROUND ABATTOIR OPERATION SITE IN WATERSIDE ABA, ABIA STATE, NIGERIA.

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ABSTRACT

Ambient air pollution has become a major problem in most towns and cities in Nigeria. The study aimed at assessing the effects of abattoir operation on ambient air quality at waterside Aba, Abia State. Monitoring of gases was done in two weeks at the abattoir site. The samples were collected from four points at the study area: Point 1 measured from 0 - 50m (the point the burning of animals took place with the aid of tyres), point 2 measured from 50 - 100m, point 3 measured from 100 - 150m, while point 4 measured from 150 - 200m distance. Air quality sampled includes particulate matter (PM_{2.5}) and carbon monoxide (CO), temperature and relative humidity. Measurements were taken in the morning (8 - 11.30 am) in three replicates per sampling point using Aeroqual handheld monitor (series 500) with interchangeable and replaceable gas sensor heads. Wind mates (WM 350) for temperature and relative humidity. All equipment was all properly pre-calibrated before usage for quality assurance. Results of air parameter were above and within WHO (24hours) and NAAQS permissible limit. The mean concentration of air quality and meteorological parameter in waterside Aba ranged as followed 13-115.333 µg/m³ (PM_{2.5}), 20.780-70.000 mg/m³ (CO), 25.428-33.886 °C (Temperature) and 25.428- 70.300 % (Relative Humidity) across the sample location. The level of gaseous and particulate pollutant differed significantly (p<0.001) across the sampled location. The AQI rated the monitored air pollutants as “moderate to hazardous” in the location. Conclusively, continuous operation of this abattoir within the residential area can constitute a great environmental menace to the residents of the area and can result in complication to those with an existing health challenge.

Keywords: Abattoir, air quality, particulate matter (PM_{2.5}), CO, AQI

INTRODUCTION

Air pollution is considered one of the main environmental problems bedeviling developing countries today. Its adverse effects on human health and on the environment are enormous (Schraufnagel *et al.* 2019). Air is said to be polluted when it is contaminated as a result of alteration of its natural composition either by natural occurrence or anthropogenic activities (Umunnakwe and Njoku, 2017). When air is polluted either by the release of thick smoke emanating from burning or foul smell from heaps of waste materials, waste disposal/collection spots, it does not only cause interference to the comfort of human beings but also adversely affect the lives of properties, plants and animals within the vicinity (Oguntoke *et al.*, 2019). Adoki, (2012) reported that air quality around a particular location are influenced by activities such as burning of fossil fuel, from waste disposal/collection spots, gas flaring from oil production facilities, burning of fuel in the operation of high capacity power generators over long period of time, and emissions from vehicles among others.

Abattoirs are globally recognized as a major source of air pollutants (Osibanjo and Adibe 2007; Adelegan 2002). In a typical Aba abattoir, Animals are roasted with kerosene and condemned tyres in the course of

processing the meat for marketing leading to the emission of carbon monoxide into the atmosphere (Umunnakwe and Njoku, 2017). The blood from the slaughtered animals is left flowing on the ground with offensive odour causing pollution in the environment. Meat processing activities are mostly carried out in unsanitary environment and by untrained personnel or butchers who are mostly unaware of sanitary principles (Olanike, 2002). Cattle dung are dumped on the surface without evacuation, animal blood is channeled to a soakaway which is open and left flowing into the environment constituting nuisances and also drains into the river (Magaji and Hassan, 2015, Fearon, 2014). The area is rampant with filth and scattered rubbish, which is left uncollected, apart from the blood draining trenches through which the filth is scattered rather than eliminated causing pollution to the atmospheric environment (RMAA, 2009). The waste from the burnt tyre also contaminates the soil colouring it black which can also pollute surface and groundwater. In an abattoir operational system where incinerators are used to burn wastes and carcasses, a range of air pollutants are released into the atmosphere (Ubuoh *et al.* 2017) Such pollutants include sulphur dioxide (SO₂); oxides of nitrogen (NO_x), such as nitrogen oxide, (NO) and nitrogen dioxide (NO₂); carbon monoxide (CO); volatile organic compounds (VOCs); ozone (O₃); suspended

particulate matter (SPM) also called particulates. These harmful gases constitute a serious nuisance to the atmosphere. The variable gases of the atmosphere can be altered by human activities, thereby having effects on the functions of the atmosphere which house the air we breathe. Abattoirs also consume fuel for energy production thereby emitting CO₂, NO_x, SO₂, and particulates. Abattoir emission also contributes to global problem caused by greenhouse gas emission (Adesemoye *et al.*, 2006). This study will provide information on the magnitude of air pollution from abattoir and the need for reduction of concentration of the air pollutants to residential areas.

MATERIALS AND METHODS

Water side, abattoir is situated in the commercial nerve center of Aba in Aba North Local Government Area of Abia state. Aba is located in South Eastern region of Nigeria, between latitude 5.05°N 7.22°SE and longitude 5.30°N 7.15°E. The town is a major economic hub in commercial enterprises such as pharmaceuticals, abattoir operations, manufacturing, plastics among others, which generate enormous waste. The components of these wastes include metals, organic materials, papers, plastics, among others. The residents are mainly traders, artisans, including professionals in different fields and civil servants. The climate involves two distinct seasons,

dry and wet season with the former starting from November to February while wet season ranges from March to October though variations may occur. The area lies within the sub equatorial, sub humid region. However a period characterized by dusty winds, cold and dry conditions known as harmattan normally starts from December and February. Annual average rainfall is about 2200mm.

Air quality sampling

The samples were collected from four points in the study area. They include;

- Point 1: 0 - 50m (The point the burning of animals took place with the aid of tyres,)
- Point 2: 50 - 100m
- Point 3: 100 - 150m
- Point 4: 150 - 200m

The coordinates were taken at each of the stations with Global Positioning System (GPS). The measured parameters include PM_{2.5}, Carbon monoxide (CO), temperature and relative humidity. Measurements were taken in the morning (8-11.30am) in three replicates at waterside Aba, Abia State. These samples were recorded along the direction of the wind within the measurement times for one week. Measurement was carried out using Aeroqual handheld monitor (series 500) with interchangeable and replaceable gas sensor heads.

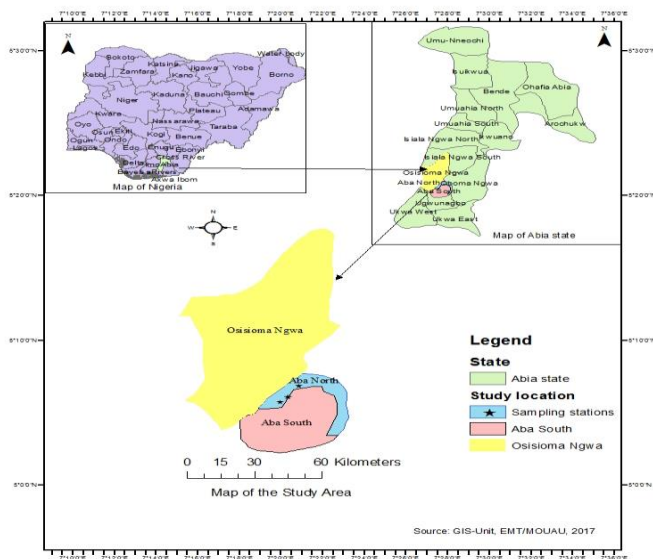


Fig 1: Map of study area

The readings were displayed on the screen of the instruments after three minutes when the green light becomes steady.

Air Quality Index (AQI) of the Pollutant

AQI is an index for reporting air quality in a location. It tells how clean or polluted the air around us is. AQI of the pollutants will be determined to evaluate the health risk which the public is exposed to due to atmospheric pollution. The indices for each of the pollutants derived using the mathematical formula below:

$$AQI\ Pollutant = \frac{Pollutant\ data\ reading \times 100}{Standard}$$

Table 1 Air quality index values and the levels of health concerns

AQI Values	Levels of Health concern	Colour codes
0 to 50	Good	Green
51 to 100	Moderate	Yellow
101 to 150	Unhealthy for sensitive groups	Orange
151 to 200	Unhealthy	Red
201 to 300	Very Unhealthy	Purple
301 to 500	Hazardous	Brown

Source: (USEPA, 2006)

Statistical Analysis

After the data was gathered from the survey, the responses were inputted into Statistical Package for Social Sciences (SPSS) version 20.0 for analysis. Data generated were analyzed using both descriptive and inferential statistics such as frequency, ANOVA,

correlation and regression analysis.

RESULTS

The range of PM_{2.5} was between 63.34-115.33 µg/m³. The amount of particulate matter (PM_{2.5}) in the ambient air differed significantly (F=1.651, p=0.001). Higher values of PM_{2.5} were observed at P1 (0-50m) when compared with other distance. The mean concentration of PM_{2.5} is higher than the 24 h NAAQS (65 µg/m³) and 25 µg/m³ WHO (2018) permissible limits. Mean concentration of CO did not varies significantly (F=1.243, p = 0.357) higher at P1 (0-50m) and least at P4 (150-200m) in sampled location.

DISCUSSION

The result of this study revealed that the monitored gases were higher at P1 (0-50m) and lower in P4 (150-200m). As observed in the study area, the recorded highest concentration of PM_{2.5} was at P1 which could be attributed to carcass combustion and vehicular emission. The concentration level of PM_{2.5} exceeded 24 hours National Ambient Quality Standard (NAAQS) limit (65ug/m³) and WHO (25 ug/m³) permissible limit. The mean concentrations of the pollutants recorded in this study were higher than the result reported by Magaji and Hassan (2017). Increased levels of fine particles in the air are linked to health hazards such as heart disease, asthma and lung cancer (WHO, 2018., Molles, 2005).The concentration particulate matters emanating from the roasting of skins of animals with tyres clog and travel from this point to other areas (Okhimamhe and Okelola, 2013) .

Table 2: Mean concentration of air quality and meteorological parameters at Abattoir site Aba

Distance	PM2.5 µg/m ³	CO mg/m ³	Temperature(°C)	Relative humidity(%)
0-50m	115.333±52.386 ^a	78.000±28.213 ^a	31.000±5.000 ^a	60.000±6.557 ^a
50-100m	87.333±29.670 ^a	50.333±26.083 ^b	27.467±5.353 ^a	67.333±19.604 ^a
100-150m	26.100±20.843 ^a	35.000±10.066 ^a	29.333±1.527 ^a	60.333±10.115 ^a
150-200m	13.933±9.550 ^a	20.750±13.528 ^a	28.000±2.645 ^a	56.667±11.455 ^b
NAAQS(24 hrs)	65 µg/m ³	43.2 mg/m ³	-	-
WHO (2018)	25 µg/m ³	30.8 mg/m ³	35°C	-
Anova; p value	1.651,0.001	1.243,0.357	0.473,0.001	0.382,0.769

Values are means ± standard deviation; Different superscripts in the same column indicate significant differences at p <0.05 according to Duncan Multiple Range Test(DMRT). WHO: World Health Organisation. NAAQS: National Ambient Air Quality Standard.

The mean concentration of carbon monoxide (CO) ranged from 20.750 – 78.000 mg/m³ across the location, respectively. Generally, higher mean CO values were observed in P1 and least at P4. The result shows that CO exceeded NAAQS and WHO (2018) permissible limit. This result is in agreement with Umunnakwe (2017) on

Assessment of the abattoir activities on air qualities of Ogbor Hill, Aba and Environs. This signifies that continuous emission of CO in waterside abattoir can cause harmful health effects by reducing oxygen delivery to the body's organs and tissues, as well as adverse effects on the cardiovascular and central nervous

systems. CO displaces oxygen in the blood, forming carboxyhaemoglobin with the human haemoglobin depriving the blood of oxygen and resulting in severe damages of the human cells causing headaches, weakness, dizziness, nausea, coma, respiratory failure and even death in very severe cases (US-DLOSHA, 2002, Hays *et al.*, 2005). CO emanates from combustion processes from fossil fuels and the decomposition of organic wastes in municipal solid wastes and the raising of livestock (Nwachukwu *et al.*, 2012).

Ayoola *et al.*, 2012 reported that pollutant such as animal dung, refuse, domestic waste were not properly dumped leading to drainages blockages and causing hazardous effect to human existence and also to the health of the inhabitants of the area.

pollutants on human health have been widely reported by many researchers. However, the overall assessment of the ambient air quality in the study area indicates a result that would be described as unhealthy by various health standards.

For the reduction of risk of air pollution-related illness, agricultural industries should utilize the waste generated from abattoir operation. The river side abattoir should be relocated to a place with a minimum distance of 500m from residential areas. A good means of disposal facilities should be provided and constant monitoring and inspection of the activities carried out in the abattoir.

Table 3. Air Quality Index of the analyzed pollutants

Pollutants AQI rating	PM _{2.5}	CO
0-50m	Unhealthy	Hazardous
50-100m	Unhealthy	Hazardous
100-150m	Moderate	Very healthy
150-200m	Moderate	Unhealthy to sensitive people

The AQI of PM_{2.5} ranged from moderate to unhealthy across the sampled location. The polluted air quality may pose a threat to sensitive people with respiratory symptoms. This finding is in accordance with the report of [Magaji and Hassan \(2017\)](#) at Ile-Ife, Ondo State.

The AQI of CO ranged from unhealthy to sensitive group to Hazardous across the sampled location. This implies that air quality around the can pose serious health risks to sensitive groups such as asthmatics, children and elderly, people with heart or lung diseases.

CONCLUSION AND RECOMMENDATION

Assessment of ambient air quality is an essential tool in evaluating the quality of air present around abattoirs and the effects of the pollutants present in the abattoir on man and the environment. Results of this study revealed that some of the ambient air pollutants studied have high concentration levels which exceeded permissible limits. PM_{2.5} and CO were mostly predominate as they recorded the highest level during the monitoring period. The source of the observed level of the ambient air pollutants at the location may be attributed to indiscriminate disposal of animal waste, meat processing, dehairing, burning of tyres and carcass. The criteria pollutants are responsible for the health risk of the public around the study area. The level of pollutants in all the locations varied. The adverse health effects of the various air

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ENGINEERING AND INNOVATIONS (EIN)

WIS-EIN 02

AN ONLINE FOOD ORDERING AND DELIVERY SYSTEM USING SHORTEST PATH ALGORITHM

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Abstract:

It has become a culture for most people to order food online. However, the delivery aspect still suffers hiccups, as sometimes, these perishable orders are not delivered to customers on time. This study improved on the delivery aspect of food ordering systems by applying the Floyd-Warshall Algorithm to obtain the shortest path between restaurants and customers' locations. The System Development Methodology (SDM) framework was adopted in developing the system. Java programming language was used to develop the frontend of the system and MySQL database system served as the backend of the system. The communities under Obio/Akpor Local Government Area of Rivers State, Nigeria, were used as the graphical dataset. For the shortest path, a bi-directional weighted graph holding various locations was used to get shortest paths between the nodes. The developed system recorded success in identifying the most economic paths between every community pair within the target local government area.

Keywords: Floyd-Warshall Algorithm, Shortest Path Algorithm, Nodes, Vertices, Weights.

Introduction

In a digitally evolving world, consumers tend to get everything they need with just some few clicks on their devices; this also includes food of all kinds. The online food ordering systems are being developed for this purpose and it avails restaurants the opportunity to increase sales and expand their businesses exponentially, by providing customers with the facility to order varieties of food online (Suryadev & Mahik, 2018). The adoption of technology in food industry has not only simplified the lives of consumers but has also positioned the catering domain at a good edge in the very competitive economic environment of today. Although only about 13% of restaurants in the world offer online ordering and delivery services (Restolabs, 2019) due to several reasons such as; funding, unwillingness to embrace a different approach to food business, misinformation on new trends amongst others. Online ordering of food, go side by side with the delivery aspect. Food delivery is defined as a courier service in which a restaurant, store or independent food delivery company takes food to customers. An order is made through the restaurant, store or company's website, and then the delivery team transports the order to the desired location of customers. The ordering of food so far, has not been a challenge, however, the aspect of the delivery of these booked meals still suffers some setbacks. Nevertheless, due to the importance of food delivery, there is still a growing increase in the practise of online ordering and delivering of food as seen in figure 1. The Online Food Delivery Statistics (OFDS) recorded a steady growth in percentage of consumers that patronize restaurants via online ordering and delivering (Gloriafood, 2020). It is depicted from figure 1 that 8% of the total food delivery orders in the year 2011 were made online, and this percentage can be seen to have experienced a constant surge, even till

the year 2020 where the percentage is presently on a 60% mark and is still set to go in an upward direction with the current state of the pandemic, COVID-19, that has hit the world in an unprecedented manner.

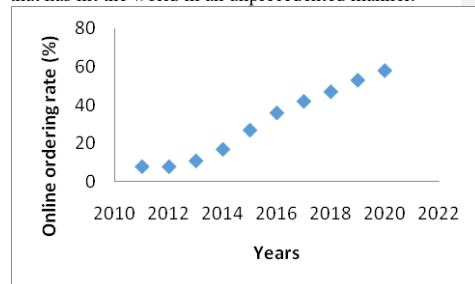


Figure 1: Graphical representation of percentage increase of online ordering rate per year (Gloriafood, 2020).

There is a dire need to improve on the intelligence of delivery systems just as technology advances, so as to ensure that every trip made is time efficient and cost effective. The Market Research Report, in their annual report, gave a base year estimate in online delivery for the year 2018 as USD23,539.4 million, and this sum is expected to register a compound annual growth rate (CAGR) of 15.4% in coming years of 2019-2025 (Grandviewresearch, 2019). And in same light, the growing use of smartphones and greater awareness of the internet has also contributed to market growth of online food ordering and delivery systems (Ghajargar et al., 2016). These systems have eliminated a lot of problems that existed in the traditional way of offline ordering and delivering of food. Some of these problems included; long queues, delay in service, non-conducive eating area, and untimely deliveries, amongst many (Yanbo et al., 2018). With the steady

growth of the adoption of the food delivery culture, it is paramount to develop intelligent algorithms that will be implemented to create robust systems, which would in turn sustain and service the huge number of prospective customers coming on board.

This study applied a shortest path algorithm in the bid to improve the intelligence of the delivery system. The shortest path, otherwise known as economic path, can be said to be the least number of edges or a path with the shortest length within a graph area, where the nodes, otherwise known as vertices, represent locations on the graph and edges represent the distance between these locations. The act of finding the shortest path in a graph from one vertex to another can be termed as the shortest path problem and this problem is addressed by applying shortest path algorithms, which are being developed within computer science domain.

Related Literature

Existing works in literature were reviewed and the contributions, strengths and weaknesses of each have been highlighted.

Ricky (2014) developed an application with design workflow for customer side, restaurant side, courier side, and administrator side using the storyboard design; the user experience design; the Unified Modelling Language (UML), consisted of use case, class diagram, sequence diagram, and activity diagram; and database structure design. MySQL and Java programming was used for customer and courier side application, and Java Server Page for restaurant side and administrator side websites. The application was named "Pick the Food" and it helped customers place order easily, it also gave information needed to book an order. However, the application was more focused on the ordering aspect of food rather than delivering.

Choudhari et al., (2015) implemented an e-food ordering system whereby customers placed orders via the mobile application and once the transaction is completed, the processed order was transferred to the database through the server where the database generates a token which was sent to the user's device on one hand, and on the other hand, send the order data to the kitchen's display, where the chefs can access it and start working on the order. The system architecture has three main components: server, application and kitchen display. In the system the server links all the component of the system which ensures that the system works effectively. The application developed was convenient as it permitted customers to place orders from their phones from whatever location; however, delivery was not factored into the system.

Patel (2015) worked on an Online Food Order System, which was made of three sub units: web ordering system, menu management and order retrieval system.

The web ordering module provided functionality for customers to place orders and supply necessary details. The menu management module provided functionality for the power user-administrator only, while the order retrieval module is designed to only be used by the restaurant employees. Hence, the work focused on only onsite services.

Nandhini et al., (2016) worked on an automated food ordering system that has features involving wireless communication and feedback. They compared the major automation tools in restaurant sector which are: the PDA based System, QORDER system and Android based system. Their system was able to perform a lot of features which include: combining of wireless technology to automate food ordering process, allowing the restaurant to operate faster, reduction of error by employee(s), thereby increasing customer happiness, provision of a mechanism for obtaining feed-back from the customers and provide the restaurant a means of review of their service, and minimizing of flaws in conventional system by atomizing the working of a restaurant.

Tobing (2016) developed a food ordering system on two main applications: Web-based and Android-based application. The Web-based platform was designed for customers to order the products after a one-time registration, while the Android-based platform was installed on the smart phones used by the Delivery Staff. Their work applied the heuristic algorithm for routing optimization and simple web-based application was developed to test the heuristic algorithm for routing optimization using Google Maps API. The system worked intelligently in terms of time efficiency but was not cost effective.

Several other works were reviewed and most works did not factor in the shortest distance problems, rather, attention was given to developing user friendly interface, ordering processes and connectivity of sub systems.

The Floyd-Warshall Algorithm, which was published in its current form by Robert Floyd in 1962, was adopted in the work to address the problem of shortest distance in food delivery, the choice of algorithm used, was as a result of the nature of the algorithm and the success it has recorded in literature when applied to other domains such as; rail networks (Esuabana et al., 2015), navigation app for the blind (Sohrawordi et al., 2015), accessibility to health care (Risald et al., 2017), cable wiring (Amoako, 2019) amongst others. Shortest paths can also be applied in robot navigation, urban traffic planning, maps, routing of telecommunications messages and even network routing protocols.

Shortest path problem is a problem towards finding the most economical and immediate path from a starting point to a last destination (Akinwol et al., 2017). Three main types of shortest path problems include: Single

Source Shortest Path (SSSP), Single Destination Shortest Path (SDSP) and All-Pairs Shortest Path (APSP). The shortest path linking two nodes does not have to be a direct edge involving both nodes, but instead involve a diversion through some other nodes (Amoako, 2019). All-pairs shortest path problems require that shortest path distances between every pair of nodes in a network is determined.

Shortest path algorithms are a family of algorithms designed to solve the shortest path problem. They are algorithms designed essentially to find a path of minimum length between two specified vertices of a connected weighted graph. Some applications of shortest path algorithm include: Geographic Information System (GIS), remote sensing, economics, critical path analysis, military mission planning systems, shipping/distribution problem and even automated traveling advisory system.

The Floyd-Warshall Algorithm which is also referred to as Floyd's algorithm, WFI algorithm, Roy-Floyd algorithm or the Roy-Warshall algorithm refers to an algorithm for discovering paths in a weighted graph with negative or positive edge weights, and no negative cycles. The algorithm finds the total sum of the weights of shortest paths between all pairs of vertices. It does comparison on paths by incrementally improving an estimate on the shortest path between two vertices, until the estimate is optimal.

System Implementation

The system prototyping was adopted as the suitable system development methodology for the work, considering main features of the system, such as less complexity and few analysts. The proposed system architecture as seen in figure 2 was designed to show the process flow, whereby the front end interface, labelled as the web ordering system, hold features which permits several customers to place their food orders by indicating their preferred meal and destination, this information goes into the database that is at a centralized position on the architecture, which was developed using the MySQL database system; the administrative staff also accesses the menu management system, where maintenance and modification can be done on menus and rates and these changes impact on the database. The restaurant employees retrieve the placed order and attend to it. After which, the delivery team, depends on the routing system, which is the intelligent system on which the Floyd-Warshall algorithm was developed, to suggest the shortest path, amongst several options, for the delivery.

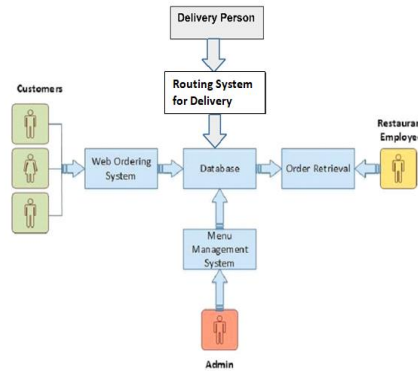


Figure 2: Proposed System Architecture

The Floyd-Warshall Algorithm was implemented using the java programming language. The algorithm worked with pairs of vertices within the weighted graph. The implementation was carried out on the algorithm as thus:

Construct the initial distance matrix D^0 of dimension $k \times k$, where k is the number of vertices.

Initialize $d_{ij}^{(0)} = w_{ij}$ for all edges (i,j) . The rows are indexed as i and the columns as j . If $i=j$, assign $d_{ij}^{(0)} = 0$. Where there is no path going from i to j , $d_{ij}^{(0)} = \infty$.

Initiate for loop for i,j .

Generate subsequent distance matrix D^1 .

Increment w_{ij} counter by 1; fix all elements in target row and column.

For the shortest path from i to j such that any transitional vertices on the path are selected from the set $\{1,2,\dots,k\}$; if k is not a vertex on the desired path, then the shortest path has length $d_{ij}^{(k-1)}$ else if k is a vertex on the path, then the shortest path has length $d_{ik}^{(k-1)} + d_{kj}^{(k-1)}$.

Set the iterations counter $k=1$. Where $i,j \neq k$, use the relation, $d_{ij}^{(k)} = \min \{d_{ij}^{(k-1)}, d_{ik}^{(k-1)} + d_{kj}^{(k-1)}\}$ to find the value of D^k .

A bi-directional weighted graph of Obio/Akpor Local Government Area (LGA), Port Harcourt Rivers State, Nigeria, was used for this study. The communities of the LGA that are located within the restaurants range can be located inside the red boarder, as in figure 3, while the areas outside the red boarder were not labelled or considered in the study. The weights between the nodes were summed based on the length or distance between them. The map shows 26 nodes which are; A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z together with paths that connects them.

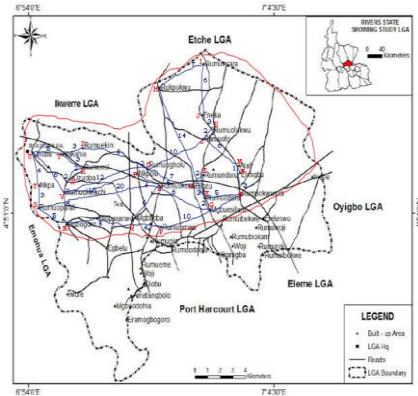


Figure 3: Map of Obio/Akpor Local Government Area.

Table 1 shows the labelling of the communities within the LGA. They serve as the nodes of the graph, while the distances between the nodes are termed edges or links. The developed graph is bi-directional as movements can go either way of the edges.

Table 1: Labelling of Obio/Akpor Graph Nodes

Label	Name of Community
A	Rumuokoro
B	Nkpolu
C	Rumuogbolu
D	Eliozu
E	Rumuduru
F	Rumudara
G	Mgbuesilaru
H	Rukpokwu
I	Rumuesara
J	Encka
K	Rumuolukwu
L	Rumuofu
M	Atali
N	Elimgbu
O	Rumuokwurushi
P	Rumuomasi
Q	Mgboba
R	Ogbogoro
S	Rumuoparali
T	Nkpa
U	Choba
V	Rumuokwachi
W	Ozuba
X	Rumuosi
Y	Alakahia

Z	Rumuekini
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Result Discussion

With the aid of the weighted map, the edges and nodes where fed into the software at development and Floyd-Warshall Algorithm calculated the shortest path between all pairs of node in the map as shown in table 2. The labelled communities in table 1 were represented as row and column heads in table 2, in order to ascertain the shortest distance between every pair. Table 2 shows the resultant matrix containing the shortest path between every community pair within the Obio/Akpor Local Government Area of Rivers State. The resultant matrix was arrived at after 25 iterations of the Floyd-Warshall Algorithm on the graphical dataset. From the matrix, the algorithm ran iteratively to populate the common cells in hundreds of meters (X100meters), which are the points where the locations on the horizontal and corresponding vertical intercept. Locations that are traced to themselves always resulted to a zero weight, as no movement is expected to be done. However, those locations that intercept with a different location have a positive weight registered at the intercepting cell. Given that the graph used in the study is bi-directional, hence, the weight of the edge EF given as 300meters will also be same for the edge FE, as delivery can be made both ways.

Conclusion and Future Work

The Floyd-Warshall algorithm has been applied as a novel approach to build a food ordering and delivery system. The system examined the data points on the Obio/Akpor local government area of Port Harcourt, Rivers State, Nigeria, and intelligently calculated the shortest distance between every two location. This system is set to provide objective information for food delivery businesses in order to guide their decision on routes to be adopted to ensure food deliveries that are time efficient and cost effective. The route optimization offered by the work, is of great benefit to the restaurant as well as the customers.

The study could be extended to mobile device as this present system was built with the screen size of a computer only; and adjustment to various phones screen sizes was not factored in.

Table 2: Calculation of shortest paths between all nodes in the map using Floyd-Warshall Algorithm.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
A	0	4	6	5	7	8	6	20	27	21	18	16	17	16	13	10	6	14	19	22	20	14	16	18	17	14
B	4	0	2	9	11	12	10	24	23	17	14	12	18	19	17	14	10	15	20	20	16	10	12	14	13	10
C	6	2	0	7	9	12	12	26	21	15	12	10	16	17	17	16	12	17	22	20	16	12	14	14	11	8
D	5	9	7	0	2	5	17	21	28	22	19	17	14	13	11	15	11	19	24	27	23	19	21	21	18	15
E	7	11	9	2	0	3	5	19	26	23	20	18	12	11	8	17	13	21	26	29	25	21	23	23	20	17
F	8	12	12	5	3	0	2	16	23	20	17	15	9	8	5	15	14	22	27	30	28	22	24	26	23	20
G	6	10	12	7	5	2	0	14	21	22	19	17	11	10	7	16	12	20	25	28	26	20	22	24	23	20
H	20	24	26	21	19	16	14	0	7	13	16	18	24	24	21	30	26	34	39	42	40	33	36	38	37	34
I	27	23	21	28	26	23	21	7	0	6	9	11	17	18	21	31	33	38	43	41	37	34	35	35	32	29
J	21	17	15	22	23	20	22	13	6	0	3	5	11	12	15	25	27	32	37	35	31	27	29	29	26	23
K	18	14	12	19	20	17	19	16	9	3	0	2	8	9	12	22	24	29	34	32	28	24	26	26	23	20
L	16	12	10	17	18	15	17	18	11	5	2	0	6	7	10	20	22	27	32	30	26	22	24	24	21	18
M	17	18	16	14	12	9	11	24	17	11	8	6	0	1	4	14	18	26	31	34	32	28	30	30	27	24
N	16	19	17	13	11	8	10	24	18	12	9	7	1	0	3	13	17	25	30	33	33	29	31	31	28	25
O	13	17	17	10	8	5	7	21	21	15	12	10	4	3	0	10	14	22	27	30	33	27	29	31	28	25
P	10	14	16	15	17	15	16	30	31	25	22	20	14	13	10	0	4	12	17	20	23	17	19	21	24	24
Q	6	10	12	11	13	14	12	26	33	27	24	22	18	17	14	4	0	8	13	16	19	13	15	17	20	20
R	14	15	17	19	21	22	20	34	38	32	29	27	26	25	22	12	8	0	5	8	11	5	7	9	12	15
S	19	20	22	24	26	27	25	39	43	37	34	32	31	30	27	17	13	5	0	3	7	10	12	14	12	15
T	22	20	20	27	29	30	28	42	41	35	32	30	34	33	30	20	16	8	3	0	4	10	12	12	9	12
U	20	16	16	23	25	28	26	40	37	31	28	26	32	33	33	23	19	11	7	4	0	6	8	8	5	8
V	14	10	12	19	21	22	20	34	33	27	24	22	28	29	27	17	13	5	10	10	6	0	2	4	7	10
W	16	12	14	21	23	24	22	36	35	29	26	24	30	31	29	19	15	7	12	12	8	2	0	2	5	8
X	18	14	14	21	23	26	24	38	35	29	26	24	30	31	31	21	17	9	14	12	8	4	2	0	3	6
Y	17	13	11	18	20	23	23	37	32	26	23	21	27	28	28	24	20	12	12	9	5	7	5	3	0	3
Z	14	10	8	15	17	20	20	34	29	23	20	18	24	25	25	24	20	15	15	12	8	10	8	6	3	0

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FOOD SAFETY AND VALUE CHAIN (FSV)

WIS-FSV 01

EFFECT OF COMMERCIAL YOGHURT STARTERS ON THE SHELF LIFE SOY-COCONUT MILK

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ABSTRACT

Soy-coconut milk (soy beverage) is a nutritive and healthy drink made from soya beans and coconut. Soy-coconut milk is however limited in availability due to its low shelf life. This study aimed at evaluating Soy-coconut milk shelf life using commercial yoghurt starters (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). The Soy-coconut milk was prepared, pasteurized and divided into two equal parts. One part was inoculated with commercial yoghurt starter and incubated for 12 hours at 45°C to obtain fermented Soy-coconut milk, while the other part was without starter (control). Result of sensory evaluation analysed using one way Analysis of Variance (ANOVA), revealed that there was significant difference between the sensory attributes of the fermented and unfermented Soy-coconut milk at $p < 0.05$. However proximate composition of the fermented showed that there was a large increase in crude protein (8.08%) and ash content (0.9%) of the fermented compared to unfermented Soy-coconut milk which was 6.5% and 0.76% respectively at $p < 0.05$ confident level. The study on the shelf life of fermented Soy-coconut milk showed that no spoilage was detected beyond 4 days of storage at room temperature but after 2 days of storage of the unfermented, logarithmic increase of $8.02 \log_{10}$ cfu/ml in microbial cell number was observed and at day 8, log of cell numbers was $8.41 \log_{10}$ cfu/ml, an indication that yoghurt starter was able to preserve Soy-coconut milk over a reasonable period (61 days) of time.

Keywords: Soy-coconut milk, preservation, preparation, food safety, shelf life

Introduction

The importance of fermented milk in human diet is well known and documented. For a long while, these products have been a useful component of nutritional diet (Bibek, 2018). However, soymilk (also known as soy juice) is a beverage made from soybeans is an aqueous extract of whole soybeans which closely resembles dairy milk in physical appearance and composition (Patil and Jha, 2008). It is a nutritious beverage rich in high quality protein with no cholesterol or lactose, inexpensive, highly digestible, and rich in water soluble proteins, polyunsaturated fatty acids, linoleic acid carbohydrate and oil nutrient (Deshpande *et al.*, 2008).

Coconut on the other hand is classified as a "functional food" due to provision of many health benefits beyond its nutritional content such as its fibre and oil contents (Rita, 2009). The oil improves insulin secretion and utilization of blood glucose; reduces symptoms associated with mal-absorption syndrome and cystic fibrosis, relieves symptoms due to crohn's disease; ulcerative colitis and stomach ulcers; improves essential fatty acids utilization as well as its protection from oxidation (Chee and Choon, 1997). The fortification of soymilk with coconut to produce soy-coconut milk will be a good alternative to dairy milk for adults and children. However, soy milk being of high protein content predisposes it to spoilage as well as long term availability on the shelf. Chemical preservatives though if added could reasonably prolong its shelf life, but many of these chemicals have been reported to be carcinogenic; therefore, there is the need for a replacement. Lactic acid bacteria being

generally regarded as safe (GRAS) have been widely used as food preservatives. Their ability to ferment substrates homo-lactically (lactic acid only) and or hetero-lactically (many products) to produce metabolites that are lethal to spoilage organisms have brought about the uniqueness of these bacteria. Lactic acid bacteria used as starters for production of cultured dairy foods are divided into two types, based on the optimum temperature ranges at which they grow. The lactic acid bacteria incubated at temperatures above 35°C are known as thermophilic starters and those incubated at 20–30°C are called mesophilic starters (Ramesh and Arun, 2013). Thus, incorporating yoghurt starter into symbiotic drinks that contain prebiotics (fibers) and probiotics (lactic acid bacteria) would not only offer added nutritional benefits that can help boost overall health and well-being but also improve the shelf life of the product. Therefore, the objective of this study is to preserve soymilk enriched with coconut and to determine sensory qualities.

Sample collection

The soybeans and coconut samples used were purchased from Towobowo Market in Igboora, Ibarapa local government of Oyo State and taken to Oyo State College of Agriculture and Technology Laboratory for further analysis.

Production of fermented soy-coconut milk

Soy-coconut milk was produced locally using the method of Abiodun and Adelodun (2008). Three hundred grammes of the soybean seeds were sorted (to remove cracked, damaged and discoloured seeds) and

winnowed. This was then soaked in three litres of clean water for 10 hours. After soaking it was dehulled and the shells of the coconuts were removed using kitchen knife and washed with clean water and the flesh was cut into smaller pieces. Ninety grammes (90 g) of the cut coconut were added and were ground with 900 ml of water to paste using an electric blender. The paste obtained was sieved using a clean muslin cloth to separate the milk (filtrate) from the chaff. The extracted milk was transferred into a covered plastic container and 24 g of sugar was added and it was pasteurized and heated to 70°C for 30mins and allowed to cool gradually to a temperature of about 45°C. One hundred and fifty grams (150 g) of skimmed milk was dissolved in 1litre of sterile water and was pasteurized at 70°C for 30 minutes, cooled and inoculated with 4% freeze-dried commercial yoghurt starters (*Lactobacillus bulgaricus* and *Streptococcus lactis*). The inoculated soy-coconut was incubated at 45°C for 18 hrs.

Sensory Evaluation

Sensory evaluation of fermented and unfermented soy-coconut milk was carried out according to the method of Pithong *et al.*, (2010). All the soy-coconut milk samples were evaluated for organoleptic characteristics and overall acceptability by 20 panellists that comprise Oyo State College of Agriculture and Technology students. Evaluation was based on appearance, flavour, taste, mouth feel and overall acceptability. Scores were based on a 9-point hedonic scale of 1-9 (1=like extremely and 9=dislike extremely).

Proximate composition of unfermented and yoghurt starter fermented soy-coconut milk

Samples of fermented and unfermented soy-coconut milk were analysed for the following parameters; protein, crude fibres, ether extract, moisture and ash content using the methods of the Association of Official Analytical Chemists (2009).

Ash content determination

Ash content as determined by heating 5 g of soy-coconut milk sample in a clear dry crucible. This was charred over a Bunsen flame in a fume cupboard to destroy most of the organic matter. The heated soy-coconut milk sample was further heated in a muffle furnace at about 500°C for about 3 hours until white ash remains. Heated soy-coconut milk sample was cooled in desiccators reweighed. Ash was calculated as follows;

$$\% \text{ Ash (dry basis)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Protein content determination

Burette method as outlined by AOAC (2009) was used in the determination of the protein content. A standard calibration curve was plotted using Bovine serum albumin (BSA) as standard protein and absorbance read at a wavelength of 540 nm

Moisture content determination

Dry matter was determined by oven drying at 105°C to a constant weight. Five grammes (5 g) of the sample was weighed in to pre-weighed drying dishes and kept in oven adjusted at 105°C. After 6 hours, the sample was withdrawn, cooled in desiccators and reweighed. Moisture content of each sample was calculated as follows;

$$\% \text{ Moisture content (M)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

W1 = Initial weight of crucible

W2 = Weight of crucible + sample before drying

W3 = weight of crucible + sample after drying

Determination of Shelf life of soy-coconut milk

The starter inoculated soy milk produced in the laboratory under aseptic condition was placed in sterile Zip ploc bag; it was labelled and stored on the shelf at room temperature of 25°C. The control (soy-coconut milk without starter) was stored along with test samples at room temperature. The soy-coconut milk was observed daily along with control to determine when spoilage would start. The total microbial load was determined at the beginning of spoilage by serially diluting the samples and plating them out on Plate count agar. The plates were incubated at 35°C for 48 hrs and the colony-forming units per millimetre of samples were determined (Ogunbanwo *et al.*, 2004)

Results and Discussion

Table 1 showed the result of sensory evaluation of fermented and unfermented soy-coconut milk. Organoleptic parameters such as colour, taste, flavour, mouth feel and over all acceptability revealed that the colour (2.2) taste (2.4) and flavour (2.2) of the unfermented soy-coconut milk were more acceptable than that of the fermented which were 2.6, 2.5, and 2.5 respectively. The mouth feel of the fermented (2.5) was more acceptable than unfermented (2.7) this may be due to the addition of yoghurt starter that was added to the fermented. In overall acceptability, the unfermented (1.9) was more acceptable than the fermented (2.9). However statistical analysis of the fermented and unfermented showed that there was significant difference in the acceptability of both fermented and unfermented soy milk at P<0.05. The result of sensory evaluation of soy-coconut milk has shown that the unfermented was more acceptable than the fermented soy-coconut milk this may be due in part to the quantity of coconut milk added and genetic variation in individual response to organoleptic parameters used. However, according to Abiodun and Adelodun, (2008); acceptability of soy-coconut by 'western palate' depends on the amount of coconut premix before fermentation.

The proximate composition of fermented and unfermented soy-coconut milk is shown in table 2.

Observation revealed that crude fibre of the fermented soy-coconut milk had least concentration of 0.08% and slight decrease when compared with that of the unfermented whose concentration was 0.12%. The ash content of the fermented soy-coconut milk (0.90%) was more than the unfermented (0.76%). In all, moisture content of the unfermented soy-coconut had highest concentration of (52.36%) which was more than the fermented (49.21%), followed by crude protein of the fermented (8.08%) which was also more than the unfermented (6.5%). The ether extract concentration of the fermented soy-coconut milk (3.5%) was lesser than that of the unfermented (3.81%). The significant increase (p<0.05) in the crude protein of the fermented over the unfermented may be due to the additive. This means that soy-coconut milk

could be taken as supplement to meet the required daily nutrients. Also soy-coconut milk nutritional composition have been improved upon and therefore could serve to be more promising source of nutrients for both old and young people, particularly the lactose intolerant patients. In this research, there was significant increase (p<0.05) in the ash content of the fermented soy-coconut milk compared to the unfermented this might be due to the loss of moisture content during lactic acid bacteria fermentation (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) since microorganisms requires certain amount of water for growth, which is a measure of the amount of water available in the milk for microbial growth (water activity).

Table 1: Sensory of Fermented and Unfermented Soy-coconut milk drink

Test Samples	Colour	Taste	Flavour	Mouth feel	Overall acceptability
Fermented Soy Coconut Milk	2.6 ^a	2.5 ^c	2.5 ^c	2.5 ^e	2.9 ⁱ
Unfermented Soy Coconut Milk	2.2 ^b	2.4 ^d	2.2 ^f	2.7 ^h	1.9 ^j

Mean values within the column with different superscripts are significantly different at p<0.05

Table 2: Proximate composition of fermented and unfermented Soy-coconut milk drink

Test Samples	Crude Protein	Ether Extract	Crude Fibre	Ash Content	Moisture Content
Fermented Soy Coconut Milk	8.08 ^a	3.5 ^c	0.08 ^e	0.9 ^e	49.21 ⁱ
Unfermented Soy Coconut Milk	6.5 ^b	3.81 ^d	0.12 ^f	0.76 ^h	52.36 ^j

Mean values within the column with different superscripts are highly significantly different at p<0.05

The shelf life profile of the fermented and unfermented soy-coconut drink is shown in table 3. The result showed no spoilage in the fermented soy-coconut milk till 61 days of storage at room temperature while that of unfermented spoilt on the third day of storage. There was logarithmic increase in the microbial cell daily until cells were too numerous to count. On day 3 the microbial cell number for the unfermented soy-coconut milk was 8.02

(log₁₀cfu/ml) while day 7 was 8.41(log₁₀cfu/ml) this logarithm increase in cell numbers might be due to high moisture content of the unfermented soy-coconut milk when compared with the unfermented soy-coconut milk. According to Yang *et al.* (2018), high moisture content supports enzyme and

microbial activities; an implication on shelf life elongation of the milk. The lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) used as preservative, prolonged the shelf life of the fermented soy-coconut milk beyond that of the unfermented (without starter) this might be due to the fact that lactic acid bacteria grew, multiplied and dominated the other microorganisms which must have escaped into the milk during processing. In addition, lactic acid bacteria reportedly, produce certain metabolites such as Lactic acid, propionic acids and acetic acid that could render the fermenting medium (milk) unfavourable for the growth of spoilage and pathogenic microorganisms; there by extending the shelf life of the soy-coconut milk (Rattanachaikunsopon and Phumkhachorn, 2010).

TNT means Too numerous to count

Keys: SND means spoilage not detected

Table 3: Shelf life of soy-coconut milk (log10 cfu/ml)

Days of storage at room temperature	Fermented soy-coconut milk	Unfermented soy-coconut milk
1	SND	SND
2	SND	SND
3	SND	8.02
4	SND	8.17
5	SND	8.22
6	SND	8.24
7	SND	8.30
8	SND	8.41
9	SND	TNT
10	SND	TNT

Conclusion and Recommendation

The result of this analysis showed that fermented Soy-coconut milk contained desirable proximate composition than the unfermented and the yoghurt starter culture extended the shelf life of the produced milk effectively. Hence, fermented soy-coconut milk is good for human health as it contains high protein content than the unfermented and could therefore act as supplement to complement the diet of young and adult people. Also the presence of probiotics organisms in the milk could act as additional source of nutrients which when present and taken in adequate amount, confers health benefits on the host.

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WIS –FSV 04

EFFECT OF DIFFERENT PROCESSING METHODS ON THE CHEMICAL COMPOSITION OF PURPLE JOY LEAF (*Alternanthera brasiliana*).

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ABSTRACT

Effect of different processing methods on the chemical composition of purple joy (*Alternanthera brasiliana*) leaves was studied. The leaves were subjected to different processing methods such as boiling for 5 minutes, shade drying at a temperature of 30 °C for 96 hours and sun drying for 72 hours. The fresh leaves served as the control. The mineral and phytochemical contents of these samples were determined. The boiled sample had the least values for calcium, zinc, magnesium and sodium while shade dried sample and sun dried sample had the least value for potassium. The control sample had the highest values for all the minerals analysed. Calcium contents ranged from 16.677 -18.133 mg/g, zinc contents ranged from 0.287-0.343 mg/g, magnesium contents ranged from 8.653-9.193 mg/g, sodium contents ranged from 0.810-1.217 mg/g and potassium contents ranged from 21.03-21.23 mg/g. The control sample recorded highest for all the phytochemical contents analysed while the boiled sample recorded least. Alkaloid contents ranged from 507.92-513.92 mg/kg, anthocyanin contents ranged from 1543.25-1571.22 mg/kg, phenol contents ranged from 14689.97 mg/kg-14801.41 mg/kg, tannin contents ranged from 139.83-143.57 mg/kg and saponin contents ranged from 151.02-168.75 mg/kg. This study has shown that the different processing methods adopted had significant effects on the nutrient contents of *Alternanthera brasiliana* leaf.

Keywords: Processing methods, Chemical composition, Purple joy leaf.

INTRODUCTION

Purple joy leaf (*Alternanthera brasiliana*) is a neotropical native species that grows on poor and deforested soil. It is an ornamental as well as a medicinal plant found growing wild in bushes and along the road sides (Ogedegbe *et al.*, 2013). The chemical composition of purple joy leaf has shown that it contains various bioactive compounds that are beneficial to humans like vitamins, nutrients, anticarcinogens and many other compounds with medicinal value (Novak and Haslberger, 2000). The extract of purple joy leaf exhibited antinociceptive (inhibition of pain) effect in mice, anti-microbial effect due to presence of important chemical constituents including alkaloid, phenols, flavonoids, saponins, glycosides etc (Baura *et al.*, 2009).

Purple joy leaf plays significant role in human nutrition especially as a source of vitamins (C, A, B and E), dietary fibre, minerals such as calcium, phosphorus and iron (Aletor and Adeogun, 1995; Anonymous, 2005). Few researchers have investigated the nutritional and phytochemical properties of this leaf. However, there is no information on the effect of pretreatment (boiling, shade drying and sun drying) on the nutritional and phytochemical properties. This study is aimed at evaluating the effect of processing on the chemical properties of purple joy leaf.

MATERIALS AND METHODS

SAMPLE COLLECTION

Purple joy leaves used for this work were harvested from Michael Okpara University of Agriculture,

Umudike. The sample does not have any voucher specimen number.

SAMPLE PREPARATION

The sample was subjected to different pre – treatments. The sample was washed and drained. A 100 g portion of the sample was boiled in 200 ml of water for 5 minutes after which it was drained and ground. Another sample was sun dried for 72 hours while the last sample was shade dried at room temperature (30 °C) for 96 hours. The fresh sample served as the control. The various pre – treated samples were ground, packaged in different air tight plastic containers and stored at ambient temperature (30 °C) until needed for analysis.

CHEMICAL ANALYSIS

Determination of mineral composition of *A brasiliana* samples

The mineral content of samples was determined by the dry ash extraction method described by Carpenter and Hendricks (2003). A quantity of each sample was burned to ashes (as in ash determination) in a muffle furnace at 550°C. The resulting ash was dissolved in 10 ml of 2M HCL solution and diluted to 100 ml in a volumetric flask using distilled water and filtered. The filtrate was used for the mineral analysis. The minerals analyzed are Calcium, Magnesium, Potassium, Sodium and Zinc.

Determination of Calcium and Magnesium

Calcium and Magnesium contents of the sample extract was carried out using Versanate EDTA complexometric titration described by Carpenter and

Hendricks (2003). A quantity of 20 ml of each extract was dispersed into a conical flask pinch doses of the masking agents (Potassium cyanide, Potassium ferrocyanide, Hydroxylamine hydrochloride) were added to it. Then 20 ml of Ammonia buffer was added to adjust the pH to 10.0. A pinch of the indicator Eriochrome black T was added and the mixture was shaken very well. Then it was titrated against 0.02N EDTA solution, until the colour changed from mauve to a permanent deep blue colour. This is a result of Ca²⁺ and Mg²⁺ forming complexes at pH 10.0 with EDTA. A second titration was conducted to determine Calcium alone. This was a repeat of the previous one with slight change, in that 10% NaOH solution was used to raise the pH of the digest to 12.0 and then titrated with 0.02N EDTA using seochrome dark blue as indicator in place of Eriochrome black T. At pH 12.0 Ca²⁺ complexes with EDTA. A reagent blank was titrated to serve as control. The experiment was repeated two more times. The Calcium and Magnesium contents were calculated separately using the formula;

$$\frac{\% \text{ calcium or magnesium}}{100} = \frac{EW \times N \times V_f}{V_a \times T - B}$$

Where:

W = weight of sample analyzed

EW = equivalent weight

N = normality of EDTA

V_f = total volume of extract

V_a = volume of extract titrated

T = titre value of sample

B = titre value of blank.

Determination of Zinc

Zinc content was determined using Atomic Absorption spectrophotometer (AAS) following dry ash acid extraction described by carpenter and Hendricks (2003). A quantity of the sample was weighed in a crucible and was incinerated to ashes. The ash was dissolved in 10 ml of 2 M HCL solution, diluted to 100 ml of distilled water in a volumetric flask and filtered through whatman NO 42 filter paper. The filtrate was used for the analysis. The instrument (AAS) was set up according to the manufacturer's instructions. It was switched on and allowed to equilibrate for 10 to 15 minutes. There appropriate hollow cathode lamp (for Zn) was put in place and the monochromator was set at the appropriate wavelength 212 nm for Zn. Then the instrument was flushed by aspirating distilled deionized water into the system followed by serially diluted standard solutions of the test element (in turns) and their respective absorbance were recorded and plotted into a curve in the system. Carefully the test sample extracts were aspirated in turns into systems and their absorbance were recorded with reference to the standard curve earlier plotted, the concentration of the test element in the sample was extrapolated. The formula below was used to calculate the concentration in the sample.

$$E \text{ (mg/100g)} = 100 \times X \times D$$

$$\bar{W} = 1000$$

Where:

W= weight of sample analyzed

X=concentration of the element extrapolated from the standard curve

D= dilution factor as applicable.

Determination of Potassium and Sodium

Potassium and Sodium contents were determined by flame photometry method as described below by James (1995). The instrument (photometer) was set up according to the manufacturer's instruction. One ml of prepared potassium and sodium standard solutions were aspirated into the machine and sprayed over the non -luminous butane gas flame. The sodium and potassium emission (having been appropriately filtered) from the different concentrations were recorded and made into standard curve subsequently, the optical density emission recorded from each of the sample were against those in the curve to extrapolate the quality of each (potassium and sodium) in the sample.

DETERMINATION OF PHYTOCHEMICAL COMPOSITION OF LEAF SAMPLES

Determination of alkaloid contents of the leaf samples

This was done by the alkaline precipitation gravimetric method of Harborne (1973) as described by Onyeka and Nwambekwe (2007). One (1) mg of the sample was dispersed in 10 ml of 10 % acetic acid solution in ethanol to form a ratio of 1:10 (10 %). The mixture was allowed to stand for 4 hours at 28 °C. It was later filtered with a Whatman No 42 grade filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH₄OH until the alkaloid was precipitated. The alkaloid precipitated was filtered, received in a weighed filter paper, washed with 1 % ammonia solution dried in the oven at 80 °C for 30 minutes. Alkaloid content was calculated as:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W} \times 100$$

100

W = weight of sample

W1 = weight of empty filter paper

W2 = weight of paper + alkaloid precipitate

2.4.2 Determination of flavonoid contents of the leaf samples

This was determined according to the method of Harborne (1973) as described by Onyeka and Nwambekwe (2007). A quantity of 5g weight of each sample was boiled in 50 ml of 2M HCl solution for 30 minutes under reflux. It was allowed to cool and then filtered using Whatman No 42 filter paper. A known volume (5 ml) of the extract was treated drop wise with equal volume of ethyl acetate. The flavonoid

precipitated was recovered by filtration using weighed filter paper. The weight difference gave the weight of flavonoid in the sample.

% Flavonoids =

$$\frac{W_2 - W_1}{W} \times \frac{100}{1}$$

W = weight of sample

W1 = weight of empty crucible

W2 = weight of paper + flavonoid precipitate

2.4.3 Determination of tannin contents of the leaf samples

Tannin content was determined by the Folin-Denis colorimetric method described by Kirk and Sawyer (1991). A quantity of 5g weight of each sample was dispersed in 50 ml of distilled water, shaken and allowed to stand for 30 minutes at 28 °C before it was filtered through whatman filter paper (No. 42). A quantity of 2 ml volumetric flask and mixed with 2 ml standard reagent and 2.5 ml of

saturated Na₂CO₃ solution. After mixing, the content of each flask was made up to 50 ml with distilled water and allowed to incubate at 28 °C for 90 minutes. The respective absorbance was measured in a spectrophotometer at wave length of 260 nm. The reagent blank was used to calibrate the instrument while the absorbance values of the samples was plotted to determine tannin content against the weight of the sample. Tannin content was calculated as:

$$\% \text{ Tannin} = \frac{100}{w} \times AU \times \frac{C}{AS} \times C \times \frac{Vf}{1000} \times D/Va$$

W = weight of sample analyzed

AU = Absorbance of the test sample

AS = Absorbance of the standard solution in mg/ml

C = Concentration of standard solution in mg/ml

Vf = Total volume of extract

Va = Volume of extract analyzed

D = Dilution factor

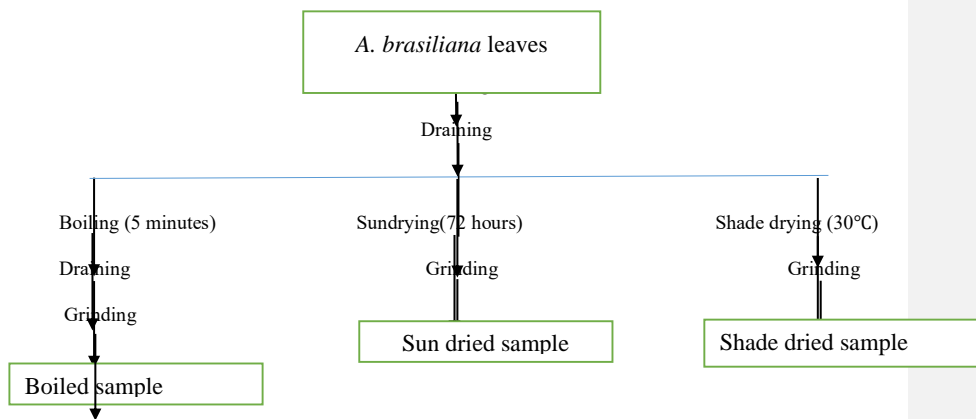


Figure 2.1: Flow chart for the processing of samples of purple joy leaf

Determination of Anthocyanins (glycosides) contents of the leaf samples

This was done gravimetrically by the method of Harborne (1973) as described by Onyeka and Nwambekwe (2007). Five (5) grams of each test sample was hydrolyzed by boiling in 10 0ml of 2M HCl solution for 30 minutes. The hydrolysate was filtered using whatman No 42 filter paper. The filtrate was transferred into a separation funnel, mixed with equal volume of ethyl acetate and allowed to separate into two layers. The ethyl acetate layer (extract) was discarded while the aqueous layer was recovered, transferred into a crucible and evaporated to dryness on a steam bath. The dried extract was rehydrated with concentrated amyl alcohol in an evaporating dish of known weight and evaporated to dryness. It was further dried in the oven at 30 °C for 30 minutes and cooled in a desiccator. The weight of anthocyanin was determined and expressed as percentage of the original

sample as:

$$\text{Glycoside (HCN)} mg/kg = \frac{100}{W} \times \frac{AU}{AS} \times C$$

W = weight of sample

AU = Absorbance of the test sample

AS = Absorbance of the standard solution

C = Concentration of standard solution

Determination of saponins contents of the leaf samples

Saponin content of the samples was determined by the double solvent extraction gravimetric method as described of Harborne (1973). Five (5) grams of the powdered sample was weighed and mixed with 50 ml of 20 % aqueous ethanol solution. The mixture was heated with periodic agitation on a water bath for 90 minutes at 55°C. It was filtered through Whatman No. 42 filter paper and the residue re-extracted with 50 ml of 20 % ethanol, both extracts were combined

together. The combined extracts were reduced to 40 ml over a water bath at 90 °C and the concentration transferred into 250 ml separating funnel and 40 ml of diethyl ether added and shaken vigorously. Separation was by partition during which the aqueous layer recovered and the ether layer discarded. Re-extraction by partition was done repeatedly until the aqueous layer became clear in colour. The saponins was extracted with 60 ml of normal butanol and the combined n-butanol extract washed with 5 % aqueous NaCl (sodium chloride) solution and evaporated to dryness in a pre-weighed evaporating dish. It was further dried at 60°C in the oven and weighed. The saponin content was determined and expressed as percentage of the weight analyzed using the formula:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

W = weight of sample

W1 = weight of empty evaporating dish

W2 = weight of dish + saponin extract

Determination of phenol contents of the leaf samples

The phenol content was determined by the method of the Association of Official Analytical Chemists (AOAC, 1990). A quantity of 0.2 g of the sample was treated with 10 ml of concentrated methanol to extract the phenol and filtrate. This was mixed for 30 minutes at room temperature. The mixture was centrifuged at 500 rpm for 15 minutes and the supernatant was used for the analysis. One ml portion of the extract from each sample was treated with equal volume of folin-ciocalteu's reagent followed by the addition of 2 ml of 2 % Na₂CO₃ solution. The standard phenol solution was prepared and diluted to a desired concentration. One ml of the standard solution was also treated with F-D reagent and Na₂CO₃ solution. The intensity of the resulting blue coloration was in a spectrophotometer at 560 nm wavelength. Measurements were made with a reagent blank at zero.

Statistical Analysis

All experimental analysis in this study were done in triplicates. One way analysis of variance of a completely randomized design using statistical package for social sciences (SPSS) version 20.0 software was used while mean separation was done using least significant difference (LSD) at 95% confidence level (p<0.05).

RESULTS AND DISCUSSION

MINERAL COMPOSITION OF PURPLE JOY LEAF SAMPLES

Mineral composition of purple joy leaf samples are shown in Table 3.1. The calcium contents of the four samples ranged from 15.677 - 18.133 mg/g. Boiled sample had the least value (15.677 mg/g) while control had the highest value (18.133 mg/g). The different processing methods affected the calcium content of the samples. Calcium is necessary for the strong bones and

teeth. It is relatively high in cereals, nuts and vegetable (James, 1996). Calcium is main component in bone and helpful for regulating skeletal and cardiac muscles contractions (Toyoshima *et al.*, 2000).

The zinc contents of the four samples ranged from 0.287 - 0.343 mg/g. Boiled sample had the least value (0.287 mg/g) while control had the highest value (0.343 mg/g). There was no significant (p>0.05) differences between control and shade dried sample. The zinc contents of the samples vary from 0.28727 mg/g reported by Pratap (2017). According to the Chikwendu and Nmalubuibe (2014) variation in minerals could be due to changes in environmental factors or the laboratory used for the analysis.

The magnesium contents of the four samples ranged from 8.653 - 9.193 mg/g. Boiled sample had the least value (8.653 mg/g) while control had the highest value (9.193 mg/g). There were significant (p<0.05) differences among the samples. The sodium contents of the samples are slightly different from 0.943 mg/g reported by Pratap (2017). This could be as a result of environmental factors. According to the Chikwendu and Nmalubuibe (2014). Sodium is essential and it plays crucial role in the cellular homeostasis (Pohl *et al.*, 2013).

The potassium contents of the four samples ranged from 21.03 - 21.23 mg/g. Shade and sun dried samples had the least values (21.03 mg/g) while control and boiled sample had the highest values (21.23 mg/g). There were no significant (p>0.05) differences between control and boiled sample and also shade and sun dried samples.

PHYTOCHEMICAL CONTENTS OF PURPLE JOY LEAF

Phytochemical contents of purple joy leaf are shown in Table 3.2. The alkaloid contents of the four samples ranged from 507.92 - 513.92 mg/kg. Boiled sample had the least value (507.92 mg/kg) while control had the highest value (513.92 mg/kg). There were no significant (p>0.05) differences between shade and sun dried samples. These results are in line with the findings of Onyeka and Nwambekwe (2007) who reported that boiling drastically reduced the alkaloid content of vegetables.

The flavonoid contents of the four samples ranged from 459.92 - 471.20 mg/kg. Boiled sample had the least value (459.92 mg/kg) while control had the highest value (471.20 mg/kg). There was no significant (p>0.05) difference between shade and sun dried samples. The flavonoid contents of purple joy leaf is very close to the value (462.00 mg/kg) reported by Attaugwu and Uvere (2017). According to Müller and Heindl (2006), post-harvest processes like drying take authoritative impact on the quality of the product influencing its value which is mostly shown on its

appearance. The losses observed in flavonoids were very high when the vegetables were subjected to boiling. This is in line with the findings of Okwu (2004) that flavonoids are water-soluble and are easily destroyed by boiling.

The phenol contents of the four samples ranged from 14689.97 - 14801.41 mg/kg. Boiled sample had the least value (14801.41 mg/kg) while control had the highest value (14689.97 mg/kg). There were significant ($p < 0.05$) differences among the samples. The phenol contents of *A. brasiliensis* vary from 14702.40 mg/kg content reported by Attaugwu and Uvere (2017).

The tannin contents of the four samples ranged from 139.83 - 143.57 mg/kg. Boiled sample had the least value (139.83 mg/kg) while control had the highest value (143.57 mg/kg). There were significant ($p < 0.05$) differences among the samples. Chinma and Igyor (2007) reported a range of 0.01g/100g to 0.25g/100g for raw vegetables in south East Nigeria. The differences in the tannin contents could be attributed to genetic variation and topography of the soil. The saponin contents of the four samples ranged from 151.02 - 168.75 mg/kg. Boiled sample had the least value (151.02 mg/kg) while control had the highest value (168.75 mg/kg).

CONCLUSION

This study has shown that the different methods adopted had significant effect on the quality of the vegetable. Findings from this research showed that the values obtained vary with different processing techniques adopted. The unprocessed sample which served as the control was the best in terms of vitamins and mineral retention. Boiling method was found to reduce the nutrients and the anti-nutrients present in the sample more than the other methods adopted.

Table 3.1: Changes in mineral contents of *Alternanthera brasiliana*

Sample	Calcium (mg/g)	Zinc (mg/g)	Magnesium (mg/g)	Sodium (mg/g)	Potassium (mg/g)
Control	18.133 ^a ±0.07	0.343 ^a ±0.00	9.193 ^a ±0.02	1.217 ^a ±0.00	21.23 ^a ±0.06
Shade drying	18.010 ^b ±0.09	0.340 ^a ±0.00	9.083 ^b ±0.06	1.130 ^a ±0.13	21.03 ^b ±0.06
Boiling	15.677 ^c ±0.02	0.287 ^c ±0.00	8.653 ^d ±0.01	0.810 ^b ±0.02	21.23 ^a ±0.06
Sun drying	17.997 ^b ±0.06	0.323 ^b ±0.01	9.007 ^c ±0.01	1.127 ^a ±0.13	21.03 ^b ±0.12

Values show the mean of triplicate analysis and ± standard deviation. Mean values with different superscript in the same column are significantly different (p<0.05)

Table 3.2: Changes in phytochemical contents of *Alternanthera brasiliana*

Sample	ALKALOIDS (mg/kg)	FLAVONOIDS (mg/kg)	ANTHOCYANIN (mg/kg)	PHENOL (mg/kg)	TANNIN (mg/kg)	SAPONIN (mg/kg)
Control	513.92 ^a ±0.07	471.20 ^a ±0.19	1571.22 ^a ±0.01	14801.41 ^a ±0.01	143.57 ^a ±0.01	168.75 ^a ±0.02
Shade drying	511.26 ^b ±0.77	464.17 ^b ±0.30	1549.93 ^b ±0.07	14710.61 ^b ±0.55	143.23 ^b ±0.01	168.32 ^b ±0.02
Boiling	507.92 ^c ±0.09	459.92 ^c ±0.08	1543.25 ^d ±0.07	14689.97 ^d ±0.06	139.84 ^d ±0.02	151.02 ^c ±0.02
Sun drying	510.84 ^b ±0.28	463.97 ^b ±0.06	1549.51 ^c ±0.02	14708.96 ^c ±1.16	143.02 ^c ±0.02	168.30 ^b ±0.01

Values show the mean of triplicate analysis and ± standard deviation. Mean values with different superscript in the same column are significantly different (p<0.05)The anthocyanin contents of the four samples ranged from 1543.25 - 1571.22 mg/kg. Boiled sample had the least value (1543.25 mg/kg) while control had the highest value (1571.22 mg/kg). There were significant (p<0.05) differences among the samples.

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WIS-FSV 05

ASSESSMENTS OF HOME-MADE DRINK (ZOBO AND TIGER-NUT) PRODUCTION IN OBIO-AKPOR LGA, RIVERS STATE, NIGERIA

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ABSTRACT

Zobo and tiger nuts drinks are widely acceptable local beverages and its processing is gaining wide acceptance from locals and city dwellers. The study assessed homemade drink (zobo Hibiscus roselle and tiger nut *Cyperus esculentus*) production Obio/Akpor Local Government Area of Rivers State, Nigeria. Specifically, the study examined socioeconomic variables of the processors and estimated the cost and returns in zobo and tiger nuts drink processing in the study area. A total sample size of 100 home-made drinks (zobo and tigernut) processors were randomly selected from 10 communities in the study area. Descriptive statistics, multiple regression and gross margin techniques were the analytical tools used. The result indicated that 86% of the respondents were females and had spent at least 3 years in the fruit drink processing. A weekly gross margin (₦17,290.00) was realized in zobo processing while ₦61,443.00 was realized as the weekly margin from tiger nut processing. Linear model was used as the lead equation because it had the highest R² of 80%. The coefficient gender was positive and significant at 5% level. However, cost and quantity of zobo and tiger nuts showed negative signs and were significant at 1% level each. This implies that increasing the cost and quantity of zobo and tiger nuts would reduce processors revenue earning. Inadequate energy power supply, poor storage facilities, unfavourable weather condition were identified as the major challenges facing the industry. It is recommended that homemade drink processors should maintain good hygiene and form association of processors to increase their revenue.

Keywords: Assessment, home-made, drink, zobo and tiger-nut

INTRODUCTION

Homemade drink is becoming very popular among households in Nigeria. This is evidence as the products are sold and served as drinks at homes and parties. The drive to eradicate poverty and enhance self-reliance has led individuals both male and females to engage in income making ventures such as processing of fruits and other plant parts into refreshing drinks and beverages that are sold commercially. Zobo and Tiger-nut drink processing are some of such ventures. Zobo as is popularly known in Nigeria, as a drink obtained from *sorrel* plant is found in many other countries in the world. Knowledge of the benefits of a food material enhances its use and acceptance among populations. *Hibiscus sabdariffa* plant (Zobo) has been reported to be of high nutritive and medicinal properties. According to Ezearigo, Adeniji & Ayoade (2014), the flowers contain carbohydrate, sucrose, mannose, proteins, fats and certain vitamins. The seeds are rich in starch, cellulose, oleic acid, formic acid and alcohol. The calyx contains alcohol, malic acid, fibre and ash. The fruits are rich in formic acid, acetic acid, alcohol, pectin and minerals, while the roots possess tartaric acid and saponin (Shivali & Kamboj, 2009). *H. sabdariffa* is used in traditional medicine due to its phytochemical contents (Ghazala & Rajni, 2018).

Tiger-nut (*Cyperus esculenta*) is an underutilized crop that is rich in nourishment. In Nigeria, tiger nut is available in fresh, semi-dried and dried forms in local markets. The nuts are consumed raw. The under exploitation of tiger nut is attributed to lack of knowledge of its nutritional and health benefits (Bamishaiye & Bamishaiye, 2011). A number of

products can be derived from tiger nut with which income can be generated. It has been reported to be rich in energy giving nutrients such as carbohydrate and fat and protein. It is rich in phosphorus, potassium and magnesium as well as fibre and vitamin C (Suleiman *et al.*, 2018). Tiger nut contains moderate amounts of calcium which is needed for teeth and bone health, blood clotting and regulation of muscle contraction. It is low in sodium so helps to reduce high blood pressure when consumed (Suleiman *et al.*, 2018). The shelf life of local drinks depends on a number of factors among which include: packaging material, contamination during preparation and refrigeration (Ezearigo *et al.*, 2014). The zobo drink has an average shelf life of 24 to 48 hours after which spoilage begins to set in (Nwafor and Ikenebomeh, 2009).

Profitability is a measurement of efficiency, and a guide to further improvement. It is critical in determining a firm's overall health, in terms of revenues and profits. It can be defined as the capacity to generate profit from all the aspects of a business. Profitability measures how well the farm business uses the resources available to generate income and profit (Kahan, 2010). There is an enormous demand for various types of drinks and beverages in Nigeria. Many homes and indeed many individuals in Nigeria today consume drinks and beverages on a fairly regular basis Federal Institute of Industrial Research, Oshodi (FIRO, 2018). For the elite homes, they constitute part of the basic items on the dining table. In other cases, drinks and beverages come in handy to serve guests or as a readily affordable drink for

quenching thirst. In hotels and restaurants, they are served with almost every meal ordered. A big contributing factor to increasing demand for drinks and beverages is the fact that Nigerians are lovers of social functions. Major social engagements at weekends and sometimes during week days are ceremonies such as: wedding, burial, birthday party, housewarming, thanksgiving, etc., drinks and beverage are served in all these occasions. Among the drinks are zobo and tiger-nut drink popularly known across the nation.

Zobo drink is the aqueous extract from the flower of sorrel *Hibiscus sabdariffa* by cold or hot extraction methods. The drink is consumed as a beverage for its nutritional and medicinal value. The major raw material for the drink sorrel calyx is found in commercial quantities in many markets in Nigeria but more especially in northern part of Nigeria. Drink and beverage manufacturers face serious competition from producers who buy imported flavour concentrates and dilute them to make drinks that are much cheaper. Consequently, the production and marketing of drinks should focus on the fact that they are made from available local materials with no additives. Furthermore, the cost of equipment to form cartons and sealant is too high for small scale producers and they are only sold under license. The problem of price fluctuations of Nigeria's traditional export such as minerals, cocoa and timber has called for diversification in agricultural production to expand her export product base. Fruits are ubiquitous in most temperate and tropical zones.

Poor packaging of zobo and tiger-nut drinks as stated earlier may predispose the drink to contamination and spoilage, which can limit its acceptability. However, it has been demonstrated by nutritionist that the major nutritional problems especially among the poor in developing countries could be solved through exploitation of the nutritious and economic potentials of the local food resources (Ndubuisi, 2009). Tiger-nut and sorrel are good examples of underutilized crops with great potentials for domestic and commercial purposes. Sad to note that, not much research has been carried out on cost and returns of zobo and tiger-nuts drink processing in Nigeria. With the proliferation of markets with processors of these drinks, it is imperative to assess the profitability of home-made zobo and tiger nut drink production. Therefore, the broad objective of this study is to assess the profitability of home-made drink (zobo and tiger-nut) production. Specifically, the study; examined the socio-economic characteristics of zobo and tiger nut drink producers; estimated the cost and returns *in zobo* and tiger nuts drink processing in the study area; determined the effects of socioeconomic variables on the drink gross margin of the processors. Identified challenges faced by home-made drink processors in the study area.

METHODOLOGY

The study was carried out in Obio-Akpor Local Government Area (LGA) in Rivers State, Nigeria. It is located at 4°45'N and 4°60'N and longitudes 6°50'E and 8°00'E. The LGA has a total land mass of approximately 311.7sqkm with a population of 464, 789 (National Population Commission, 2006). It is one of the major centres of economic activities in Nigeria, and one of the major cities of Niger Delta Nigeria.

A sample size of 100 home-made drinks (zobo and tiger-nut) processors were selected randomly from ten communities in Obio-Akpor L.G.A. for the study. These communities are known for their high population and been hubs of business activities in the L.G.A. They include Rumuokoro, Rumuigbo, Rumueme, Eneka, Rumuodomaya, Ozuoba, Choba, Alakahia, Rumuekini and Rumuosi. Ten (10) drink/beverage processors (5 zobo and 5 tiger nut processors each) were purposively selected from each of the communities based on their scale of production, to make up the sample size for the study. Both primary and secondary data were used in this study. Primary data collection was by a structured questionnaire and one on one interview which elicited information on the socioeconomic characteristics of the respondent, purchase and sales records, production process and challenges faced by the fruit drink processors. While nutritional and health information of the drinks were obtained from secondary sources. Data was analyzed using frequency, mean, percentages and multiple regressions.

Production process of Zobo and Tiger nut drink
Flow chart for Tiger nut (*Cyperus esculenta*) drinks production

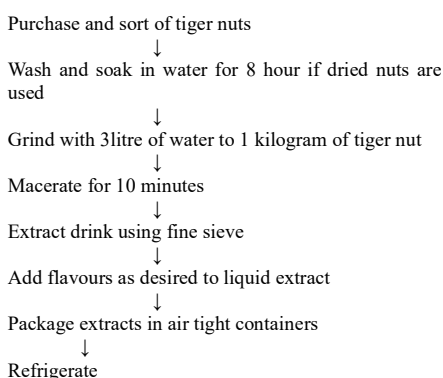


Fig1. Production process of tiger nut drink adapted

from Bamishaiye & Bamishaiye, (2011)

Flow chart for zobo (*Hibiscus sabdariffa*) drinks production

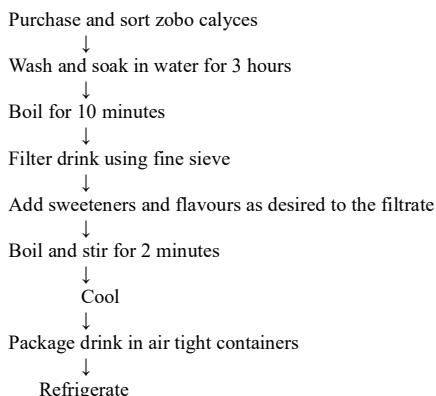


Fig 2. Production process of zobo drink adapted from Okereke et al.,(2015)

Model Specification

$$Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9) \quad \text{eq 1}$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \beta_9 X_9 + e \quad \text{eq 2}$$

Functional Forms of Regression Models

Linear Form

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \beta_9 X_9 + e \quad \text{eq 3}$$

Semi Log Form

$$\text{Log } Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \beta_9 X_9 + e \quad \text{eq 4}$$

Double Log Form

$$\text{Log } Y = \beta_0 + \beta_1 X_1 + \log \beta_2 X_2 + \log \beta_3 X_3 + \log \beta_4 X_4 + \log \beta_5 X_5 + \log \beta_6 X_6 + \log \beta_7 X_7 + \log \beta_8 X_8 + \log \beta_9 X_9 + e \quad \text{eq 5}$$

Where:

Y = Gross Margin in ₦

β_0 = intercept

$\beta_1 - \beta_9$ = slope

X_1 = age

X_2 = sex

X_3 = marital status

X_4 = educational Level

X_5 = experience

X_6 = cost of zobo leaves

X_7 = cost of tiger nuts

X_8 = cost of labour

X_9 = cost of transport

e = error term

Gross Margin Model Specification

$$GM = \sum TR - \sum TVC \quad \text{eq 6}$$

Where

$\sum TVC$ = Cost of zobo leaf + cost of tiger nut + cost of labour + cost of transport eq 7

$$TR = Y \times P \quad \text{eq 8}$$

GM = Gross Margin

TR = Total Revenue

TVC = Total Variable Cost

Y = Output

P = Price

Results and Discussion

The result in Table 1 shows the socioeconomic characteristics of drink processors. It indicated that 86% of the respondents are female and 14% are males. The result indicates that women are dominating in the home made drink processing business. This is not far from research outcome of researchers in food processing in Africa countries. This agrees with the finding of Ndubueze-Ogaraku and Edema (2015) which reported that that about 78.6 percent of processors were female because processing of agricultural products is generally seen as a female job. Majority of respondents (28%) were between the ages of 31-40 years. This result shows that majority of zobo and tiger nut drinks processors are in their youthful ages thus would be active in the processing activities. Majority of respondents (81%) had one form of education or the other. This indicates that a high percent of individuals involved in the production of zobo and tiger nut drink are informed and would understand the need for best production/ processing practices in the course of preparing the drinks. It is obvious that most of the respondents (48%) had a household size of 1-5 persons while 11% of them had household size above 11 individuals. This is interesting because large family size is an advantage to farm families because it is a common practice in Africa that members of household provide labour in doing their economic activities.

The results show that the respondents have experiences in drink production, with most of them having either 2 (24%) and 3 (27%) year's experiences each in drink production while 19% had more than 5 years' experience in drink production. This result showed that drink processing is relatively new among processors in the study area.

Table 1: Distribution of respondents according to socio-economic variables

Variables	Frequency	Percent
Sex		
Male	14	14.0
Female	86	86.0
Age in years		
20 and below	13	13.0
21-30	27	27.0
31-40	28	28.0
41-50	16	16.0
51 years and above	16	16.0
Marital status		
Single	43	43.0

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Married	49	49.0
Separated	7	7.0
Divorced	1	1.0
Educational level		
No formal education	19	19.0
Primary education	22	22.0
Secondary education	39	39.0
Tertiary education	20	20.0
Household size in persons		
1-5	48	48.0
6-10	33	33.0
11 persons and above	19	19.0
Drink processing experience in years		
1	14	14.0
2	24	24.0
3	27	27.0
4	16	16.0
5	19	19.0
Total	100	100.0

Survey: Field survey 2019

Table 2 shows the weekly gross margin analysis of zobo drink production. The gross income realized by the processors was (₦135,450.00) per week. Total variable cost was (₦118,160) with gross margin is ₦17,290. A weekly gross margin of seven thousand two hundred and ninety naira (₦17,290.00). The gross margin is a good indicator which shows that if the scale of production is increase, processors revenue earning will be increase which is expected to increase the margin.

Table 2: Weekly Cost and Returns of Zobo Drink Processing

Variables	Items	Amount in Naira (₦)
Revenue	Sales from zobo drink	135,450.00

TR	Total Sales	135,450.00
Variable Costs	Cost of zobo leaves	47,800.00
	Cost of flavor	17,860.00
	Cost of ginger	10,580.00
	Cost of pineapple	15,900.00
	Cost of ice-block	8,200.00
	Cost of bottles	2,750.00
	Cost of water	1,870.00
	Cost of cloves	8,250.00
	Cost of transportation	4,950.00
	Total Variable Costs	TVC
Profit	Gross Margin= TR-TVC	17,290

Source: Field survey, 2019

The result in Table 3 shows the profitability estimates of the gross margin analysis. The result showed that gross revenue of (₦105,000.00) was realised by the respondents. Total variable cost was (₦43,557.00) while gross margin was ₦61,443.00. This shows that the business of tiger nut processing was profitable. This could account for the multitude of processors involved in this business as the ingredients needed are relatively cheap, locally and readily available.

Table 3: Weekly cost and returns of tiger nut drink processing

Variables	Items	Amount in Naira (₦)	
Revenue	Sales from tiger-nut drink	105,000.00	
TR	Total Sales	105,000.00	
Variable Costs	Cost of tiger-nut	23,350.00	
	Cost of coconut	7,500.00	
	Cost of sugar dates	4,400.00	
	Cost of ice-block	3,300.00	
	Cost of bottles	2,275.00	
	Cost of water	732.00	
	Cost of transportation	2,000.00	
	Total Variable Costs	TVC	43,557.00
	Profit	Gross Margin= TR-TVC	61,443.00

Source: Field Survey, 2019

The result of multiple regression analysis as showed in Table 4, showed the four functional forms of the model specification used, which include linear, semi log, exponential and double log. From the results, the linear functional form was selected as the lead

equation of the regression analysis, this was based on the value of R² (coefficient of determination) which considers the model with the highest value and the number of significant variables. Based on the result of the analysis, the coefficient of determination (R²) was 0.803. This shows that 80% of the variation in the revenue of the processors was accounted for by the independent variables in the model, while 19.9% of the variation in the revenue carried could be due to some other important variables that were not captured

in the regression model. The coefficient gender was positive signed and significant at 5% level. This implies that increasing female processors participation in fruit drink making, would increase the revenue realized by the processors. However, the coefficients cost of tiger nut and quantity used in production showed negative signs and were significant at 1% level each. This implies that an increase in the in cost and quantity of *zobo* and tiger nuts would result to reduction in the gross margin.

Table 4: Result of multiple regression analysis

Variables	Linear	Semi-log	Double log	Exponential
Constant	1053.08*** (2.263926)	7.357190*** (42.76668)	2.586772*** (6.283238)	14750.22*** (-11.44415)
Age	122.3071 (1.542986)	0.048245 (1.641696)	0.125170 (1.688215)	-223.3029 (0.962016)
Edu. Level	-7.177102 (-0.099539)	0.016149 (0.604128)	-0.083826 (-1.350536)	-403.7303** (-2.077672)
Gender	-437.9665** (-1.996017)	-0.164827** (-2.026182)	-0.191612 (-1.597107)	-384.2487 (-1.023018)
Household size	-95.13344 (-0.785313)	-0.043010 (-0.957639)	-0.77444 (-1.040072)	-165.4816 (-0.709881)
Processing Exp	-3.884010 (-0.076220)	-0.006635 (-0.309749)	-0.051375 (-0.906304)	-98.23169 (-0.553520)
Cost of Processing	-	0.000394*** (12.82792)	0.722637*** (13.08964)	2422.527*** (14.01638)
Quantity of tiger nut/ zobo leaves	36.09359*** (8.069594)	0.011331*** (6.833114)	0.152294 (6.907719)	485.2643*** (7.030556)
R Squared	0.803724 (3117.000)	0.711658 (7.937784)	0.702231 (7.937784)	0.726935 (3117.000)
F value	53.81829 (1.809802)	32.43799 (1.421031)	30.99496 (1.674338)	34.98805 (2.156128)

Source: Field survey, 2019

The result in Table 5 showed the challenges faced by respondent in processing zobo and tiger nut drinks for sale. Power failure (2.93), preservation problems (2.70) and poor weather conditions (2.63) were the major challenges faced by the respondent in processing these drinks as their mean scores were higher than the decision rule of 2.5. This challenge is to be expected as the country has never boasted of constant power supply to any of its states. Irregular and epileptic power supply will likely hinder scaling up processing operations in the business especially for small scale entrepreneurs. So the drinks which are prepared without artificial preservatives cannot be kept fresh beyond their production day if not sold, thereby leading to spoilage and loss of revenue. The shelf life of zobo drink depends on various factors among which include: packaging material,

contamination during preparation and refrigeration (Ezearigo *et al.*, 2014), The drink has an average shelf life of 24 to 48 hours after which spoilage begins to set in (Nwafor and Ikenebomeh, 2009). The extremely hot weather condition experienced in the country as a result of climate change makes it impossible for the drinks to remain fresh as the ice cubes used for cooling the drinks melt faster, thereby increasing the cost of production as more ice cubes will be required to prevent early fermentation of the drink as a result of activities of microorganisms, (especially tiger nut drink) which makes the drink go sour. Sourness of the drink is a sign of spoilage and also reduces patronage by buyers. The sweetness of the drinks is better savoured when served chilled hence the need to overcome the identified major challenges.

Table 5: Challenges faced by *zobo* and tiger nut drink processors in the study area

Challenges	SD	D	A	SA	Mean	Rank	Remark
Power failure	13 (13)	21 (21)	26 (26)	40 (40)	2.93	1 st	Major Constraint
Problem of preservation	20(20)	17(17)	42 (42)	21 (21)	2.70	2 nd	Major Constraint
Problems of unfavorable weather condition	20 (20)	17(17)	42 (42)	21 (21)	2.64	3 rd	Major Constraint
High cost of tiger nut	34 (34)	20 (20)	26(26)	20(20)	2.32	4 th	Minor constraint
Low returns from sales	29 (29)	40(40)	23 (23)	8 (8)	2.10	5 th	Minor constraint
Time consuming	38(38)	28(28)	23 (23)	11 (11)	2.07	6 th	Minor constraint
High of cost of <i>zobo</i> leaves	52 (52)	18 (18)	18 (18)	12 (12)	1.90	7 th	Minor constraint
High cost of processing cost	51 (51)	43(43)	4(4)	2 (2)	1.57	8 th	Minor constraint

Source: Field survey, 2019; frequency (percentage); SD-strongly disagree, D-disagree, A-agree, SA-strongly agree; mean < 2.5=major constraint while mean >2.5= minor constraint.

Conclusion

The study assessed homemade drink *zobo* (*Hibiscus sabdariffa*) and tiger nut (*Cyperus esculentus*) production Obio/Akpor Local Government Area of Rivers State, Nigeria. Females dominated the drink processing sector and were mainly youths. A weekly gross margin of (₦17,290.00) was realized in *zobo* processing while ₦61,443.00 was realized as the weekly margin from tiger nut processing. The coefficient gender showed positive and significant level. However, cost and quantity of *zobo* and *tiger nuts* showed negative signs and were significant at 1% level each. Poor electricity power supply, poor preservation and unfavourable weather condition were the major identified challenges facing the industry. It

is recommended that:

Government should improve in the infrastructure especially provision of electricity power supply that will support survival and growth of small businesses. Processors should form cooperative society and pull their resources together to make bulk purchase of the inputs and equipments used in processing.

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WIS-FSV 07

SENSORY AND PROXIMATE COMPOSITION OF CHIN- CHIN PRODUCED FROM WHEAT AND CASSAVA GENOTYPE CULTIVATED IN NATIONAL ROOT CROPS RESEARCH INSTITUTE UMUDIKE, NIGERIA

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ABSTRACT

Cassava *Manihot esculenta* is a tuberous crop with carbohydrate as its main nutrient. It can be used for the production of gari, fufu, cassava chips, and high quality cassava flour. High quality cassava flour (HQCF) can serve as an alternative to wheat flour for the production of confectioneries. Four cassava breeding lines NR/12/PD/0024, NR/12/PD/0035, NR/12/PD/0036, NR/12/PD/0038 and TME 419 (which served as a check) were all bred in the National Root Crop Research Institute Umudike. The cassava roots were processed into HQCF and were used with wheat flour, sourced from Umuahia main market for the production of chin chin. The products were evaluated for sensory attributes and proximate composition. The sensory parameters investigated were appearance, taste, texture, crispiness and general acceptability. The result of the sensory attributes showed that the appearance, taste, texture, crispiness and general acceptability of the entire samples ranged from 5.67–7.33, 5.37–7.20, 5.47–6.97, 5.53–6.77 and 6.33–7.37 respectively. The result of the proximate composition for the whole samples ranged from 2.70 - 4.90%, 1.50 -2.40%, 29.50 - 44.60%, 0.80 -1.75%, 3.48 - 8.03% and 46.20 - 58.32% for moisture, ash, fat, crude fiber, crude protein and carbohydrate

Keywords: proximate, cassava, sensory genotype

INTRODUCTION

Snacks are light food eaten in between the main meal to assuage hunger. They are usually used for pleasure rather than the nutrient attached to them. Currently in Nigeria the demand in snacks is on the increase. Chin chin is a fried snack popular in West Africa. It is a sweet, cookie-like product made from wheat flour, fat, sugar and egg [1].

Wheat has the capacity to form viscoelastic dough as a result of the gluten protein it contains, which is essential for the leavening of bread [2]. Consumption of wheat-based snacks is associated with celiac disease which is an immunological disease of the upper intestine due to the ingestion of gluten, which can lead to severe malnutrition [3, 4]. The dependency on the use of wheat flour and high cost of importation is a major constraint in cookies production and the economy of Nigeria [5]. The substitution or the use of cassava flour with improved varieties as an alternative to complement wheat flour is a welcome initiative. Cassava (*Manihot esculenta crantz*) supplies about 70% of the daily calorie to Nigeria populace as a major food crop. Cassava has nutritional value relative to high yield flour hence, can be used wholly or in combination to prepare snacks [6]. It can also be used for the production of fufu, gari, cassava chips, starch, aligbo, atiekke and high-quality cassava flour (HQCF) which is a local alternative to wheat flour. Breeding of cassava varieties with improved qualities will boost utilization and farmer acceptability of cassava.

The objective of this research work is to compare the sensory and the nutritional properties of Chin-chin produced from wheat flour and HQCF from newly bred cassava varieties.

MATERIALS AND METHODS

Four cassava genotypes NR/12/PD/0024, NR/12/PD/0035, NR/12/PD/0036, NR/12/PD/0038 and TME 419 (which serves as check were cultivated at National Root Crops Research Institute Umudike. The cassava samples were peeled, thoroughly washed (manually), and grated into a mash with a grater. The mash was dewatered by manual pressing in clean jute bags, the lumps in the dewatered mash were disintegrated, and finally the mash was oven dried at 60°C for 5 hours. Cassava Hammer Mill (Large Handling Capacity Cassava Grinding Hammer Mill, JH, Henan, China) was used to mill the dried flour to a particle size of about 2 mm, and the resulting flour was sieved with muslin cloth to obtain HQCF. The 100% wheat flour and 100% HQCF were used separately for Chin -chin production without sample substitutions.

This was achieved by mixing 30g of flour, 0.5 g of splenda, 5g of butter, 30ml of egg, 1g of baking powder, 25ml of water, and 20g of milk in a bowl. The resultant dough was placed on a working table and kneaded until smooth and elastic. The kneaded dough was rolled out and cut into desired shapes. This was later fried using deep frying method at 160°C for 6mins. The Chin-chin was later spread out on a paper towel to drain excess oil, this was later stored in an air tight container and kept outside for further use. Proximate composition: moisture, ash, crude fiber, protein and carbohydrate were determined as described by Association of Official Analytical Chemist [7].

The sensory analysis was done by the 9-point hedonic scale assessment as described by Iwe [8]. Students from the Department of Food Science and Technology Michael Okpara University of Agriculture were selected based on their familiarity with Chin-chin. The

panelists scored the coded snacks in terms of degree of likeness for appearance, taste, texture, crispness and general acceptability.

STATISTICAL ANALYSIS

The statistical package IBM SPSS Programme version

20 was used to analyze data. Results were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) with Duncan post hoc test were used to evaluate the statistical difference between the different groups, the results were considered significance at (P <0.05).

RESULTS AND DISCUSSION

Table 1: Sensory attributes of chin- chin produced from wheat flour and cassava genotype

Samples	Appearance	Taste	Texture	Crispness	GA
NR/12/PD/0024	7.33 ^{ab} ±1.45	6.23 ^{bc} ±1.30	5.97 ^{bc} ±1.75	5.70 ^{bc} ±1.73	6.63 ^{bc} ±1.00
NR/12/PD/0035	7.70 ^a ±1.06	7.20 ^a ±1.30	6.97 ^a ±1.16	6.63 ^{ab} ±1.35	7.60 ^a ±1.04
NR/12/PD/0036	7.13 ^{ab} ±1.36	5.37 ^c ±1.88	5.47 ^c ±1.96	5.53 ^c ±2.15	6.33 ^c ±1.52
NR/12/PD/0038	6.53 ^b ±1.91	6.93 ^{ab} ±1.89	6.73 ^{ab} ±1.80	6.67 ^{ab} ±2.19	7.37 ^{ab} ±1.65
TME 419	7.03 ^{ab} ±1.83	6.50 ^{ab} ±2.05	6.63 ^{ab} ±1.5	6.77 ^a ±2.10	7.10 ^{ab} ±1.69
Wheat	5.67 ^a ±1.37	6.10 ^{bc} ±1.75	6.03 ^{bc} ±1.83	6.20 ^{bc} ±1.58	6.67 ^{bc} ±1.45

Values are mean± SD of 3 replications. Means within rows with the same superscripts were not significant difference (P>0.05). Key: 100% NR/12/PD/0024, 100% NR/12/PD/0035, 100% NR/12/PD/0036, 100% NR/12/PD/0038, 100% TME 41 and 100% wheat

Sensory evaluation is a scientific method used for measuring, analyzing and interpreting those responses to products perceived through senses of sight, smell, touch, taste and hearing [9]. The results of the sensory attribute of Chin- chin produced from wheat flour and HQCF of new bred cassava genotype is presented in Table 1. The result was evaluated on 9-point hedonic scale. There were significant differences (P<0.05) in all the sensory parameters. The panelists rated NR/12/PD/0035, high in the scoring for all the sensory parameters evaluated except in crispness. NR/12/PD/0038 compared favorably with the check in

terms of taste, texture and general acceptability. Chin- chin produced from wheat flour was rated low by the panelist in appearance and taste. All the Chin -chin produced with HQCF scored the highest in term of appearance this is in accordance with the findings of Eke Ejiofor *et al.*, [10], who reported that 100% high quality cassava flour had the highest score in appearance. This can be attributed to the white colour of the HQCF when compared to wheat flour. Among the Chin -chin produced from cassava genotypes NR/12/PD/0036 had the least score in all the sensory parameters evaluated except for appearance

Table2: Proximate composition of Chin- chin produced from wheat flour and cassava genotype

Samples	MC	ASH	FAT	CF	CP	CHO
NR/12/PD/0024	4.90 ^a ±0.14	1.50 ^c ±0.00	37.45 ^c ±1.63	1.75 ^a ±0.07	4.90 ^b ±0.00	49.50 ^{bc} ±1.84
NR/12/PD/0035	4.50 ^a ±0.14	1.50 ^c ±0.14	38.40 ^c ±0.28	0.80 ^c ±0.00	3.48 ^d ±0.04	51.33 ^b ±0.60
NR/12/PD/0036	4.70 ^a ±0.14	1.50 ^c ±0.14	30.70 ^d ±0.99	1.10 ^b ±0.14	3.68 ^c ±0.00	58.32 ^a ±0.85
NR/12/PD/0038	3.20 ^b ±0.28	2.40 ^a ±0.14	41.00 ^b ±0.28	0.90 ^{bc} ±0.14	5.00 ^b ±0.07	47.50 ^{cd} ±0.07
TME419	2.70 ^c ±0.14	1.92 ^b ±0.11	44.60 ^a ±0.28	1.15 ^b ±0.07	3.43 ^d ±0.00	46.20 ^d ±0.18
WHEAT	3.10 ^{bc} ±0.14	2.00 ^b ±0.00	29.50 ^d ±0.14	0.90 ^{bc} ±0.14	8.03 ^a ±0.11	56.48 ^a ±0.25

Values are mean± SD of 3 replications. Means within rows with the same superscripts were not significant difference (P<0.05) Key: 100% NR/12/PD/0024, 100% NR/12/PD/0035, 100% NR/12/PD/0036, 100% NR/12/PD/0038, 100% TME 41 and 100% wheat

The moisture content of the Chin- chi produced from high quality cassava flour and wheat flour ranged from 2.70 % to 4.90 %, with NR/12/PD/0024 being significantly higher than all the samples while TME 419 recorded the lowest value. The significant differences (P<0.005) in the moisture content of the Chin -chin may be due to differences of the cassava varieties. The moisture content of all the samples are within the acceptable limit which will not encourage microbial proliferation and thus, have a longer shelf life [11]. The ash content is a rough estimate of the

mineral contents of the samples [2]. The value obtained from this study ranged from 1.50 to 2.40%. Highest ash content was observed for NR/12/PD/0038 while the lowest was observed for NR/12/PD/0024, NR/12/PD/0035 and NR/12/PD/0038 with no significant difference (P>0.005). The ash content of the Chin- chin produced from the cassava breeding lines were higher than the value obtained by Eke-Ejiofor and Allen [10]. The fat contents of normal Chin- chin ranged from 29.50 to 44.60% while the fat content of the Chin -chin obtained in this study was

high. The fiber content ranged from 0.80 to 1.15% while the fiber content of TME 419 gave the highest value when compared to other cassava. This is in line with the finding of Iwe *et al.*, [12] which stated that TME419 has higher fiber content when compared with other cassava varieties in their work where they evaluated TME 419 and other cassava varieties.

The protein content of the Chin- chin ranged from 3.43 to 8.03% and Chin-chin produced with wheat flour recorded the highest, cassava is known to be low in protein content and high in carbohydrate. This can be supported by the work of Olatunde *et al.*, [13] that wheat flour has the highest score in protein compared to the other sample produced with HQCF since HQCF has carbohydrate as its bulk nutrient. The carbohydrate ranged from 46.20 to 58.32% with the Chin -chin produced from NR/12/PD/00 38 flour recording the highest value followed by the wheat flour and the least is TME419 (46.20 %). All the samples have high carbohydrate content which suggests that they can be good source of energy.

CONCLUSION

This study showed that differences in cassava genotype have implications on the sensory and proximate composition of Chin -chin. Chin -chin produced from NR/12/PD/0035 recorded the highest level of acceptance by the panelists in terms of appearance, taste, texture and general acceptability. The panelist rated NR/12/PD/0036 lowest in all the sensory parameters evaluated except in appearance. All the samples have low moisture and, high fat contents. Amongst all other samples. TME 419 has the highest crude protein content

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WIS-FSV 08

IN-VITRO STARCH DIGESTIBILITY AND GLYCEMIC INDICES OF DIFFERENT ACCESSIONS OF COCOYAM FLOUR

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ABSTRACT

Hyperglycaemia, a hallmark of diabetes mellitus, is exacerbated by the consumption of foods with high glycaemic indices. This study evaluated the *in vitro* starch digestibility and glycaemic indices of six accessions from two species of cocoyam: *Colocasia esculenta* (NC_s001, NC_s012, NC_s011) and *Xanthosoma maffafa* (NX_s001, NX_s002, NX_s003). The samples were cleaned, oven-dried at 50 °C and processed into powdered form. Amylose and total starch determination as well as *in vitro* starch digestion were carried out on each of the accessions. Total starch content was significantly ($p < 0.05$) lower in all the accessions: NX_s001 (77.64±3.38^f %), NX_s002 (99.65±0.07^d %), NC_s012 (210.14±1.81^b %), NC_s011 (200.14±1.81^c %), NX_s003 (206.05±6.73^{bc} %) and NC_s001 (85.55±2.22^e %) relative to white bread (217.39±1.88^a %) which served as control. The highest amylose content (19.01±0.15^a %) was found in NX_s001 which was significantly ($p < 0.05$) higher than other accessions. The results of *in vitro* hydrolysis showed significantly ($P < 0.05$) lower levels in the accessions relative to the control while the equilibrium constant ranged from 0.03 (NX_s003) to 0.30 (NX_s001). A low glycaemic index relative to the control was observed in all the accessions. These findings suggest that the cocoyam accessions studied may not increase postprandial blood glucose level and so could be recommended for the management of diabetes mellitus.

Keywords: Diabetes mellitus, Cocoyam accessions, *Colocasia esculenta*, *Xanthosoma maffafa*.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is currently a significant public health burden that has remained persistent (Tuomilehto *et al.*, 2001). The number of people living with diabetes mellitus worldwide rose astronomically from 108 million in the year 1980 to 422 million in 2014 suggesting a corresponding increase in the health and financial burden arising from its complications (WHO, 2016). The World Health Organization (WHO) predicted that by 2030, diabetes will be the seventh leading cause of death (WHO, 2011). Diabetes prevention studies have revealed that adhering to a healthy dietary pattern together with lifestyle modifications are as effective as, or even better than the use of synthetic medicine in the prevention of Type 2 diabetes mellitus. It is known that carbohydrates have the greatest effect on blood glucose concentration relative to other components of a diet (Bhupathiraju *et al.*, 2014). The notion of glycemic index (GI) was presented in the early 1980s due to the difference in the abilities of carbohydrates to increase blood glucose (Jenkins *et al.*, 1981). The GI is a ranking of carbohydrates according to their effect on postprandial glycemia when compared to a reference food, usually glucose (Brand-Miller *et al.*, 2003a). Although the GI can effectively rank foods on the basis of their blood glucose response, it does not account for the amount of carbohydrate in a typical serving. The glycemic load (GL) was therefore developed as the product of the GI and the amount of carbohydrate in a serving. Several beneficial effects of low-GI such as lowering of postprandial glucose and possible improvement of insulin sensitivity have been shown (Jenkins *et al.*, 1981). Previous epidemiologic studies have provided sufficient evidence that a diet

based on carbohydrate-rich foods with a low-GI, high-fiber content may protect against diabetes in non-diabetic patients (ADA, 2010). Low glycaemic index foods have been shown to improve glucose and fat tolerance in diabetes mellitus patients and improve insulin resistance. In addition they can also help in control of appetite by slowing the emergence of hunger to help in weight control. (Regina, 2012)

Cocoyams (*Colocasia esculenta* (L.) Schott and *Xanthosoma sagittifolium* (L.) Schott) are the important species of edible aroids, grown in tropical and sub-tropical countries and known mainly for their corms. Cocoyams are used as subsistence staples in many parts of the tropics and sub-tropics. Cocoyams are grown mainly for their edible starchy corms, cormels, and their leaves that could serve as vegetables (Aregheore and Perera, 2003). The corms could be processed into many products including poi (fresh or fermented paste, canned, and canned-acidified), flour, cereal base, beverage powders, chips, sun-dried slices, grits, and drum-dried flakes (Owuamanam *et al.* 2010).

There is paucity of literature on the phytonutrient profile on cocoyam accessions in Nigeria despite studies on functional and proximate properties of cocoyam flours thus making cocoyam an under-utilized crop (Osisiogwu *et al.*, 1974; Lawal, 2004). The need to evaluate the glycaemic indices of these cocoyam accessions studied is to enable individuals living with diabetes make informed choices is one of the major reasons for this study. This study was aimed at determining the *in-vitro* starch digestibility and glycaemic indices of different accessions of cocoyam flour.

MATERIALS AND METHODS

Collection and Preparation of Sample

Six (6) different accessions (varieties) of cocoyam both (*Colocasia esculenta*) Nce001, Nce012, Nce011 and (*Xanthosoma maffafa*) (Nxs001, Nx002, Nxs003) were harvested from the experimental field of cocoyam programme, National Root Crops Research Institute, Umudike. The samples were processed (sorted, washed, peeled and chopped into particle sizes) and oven dried at 50°C. Thereafter, they were ground into flour and kept in an airtight container for further analysis.



Figure 1. Corms of *Colocasia sagittifolium*; NX_s002, NX_s003 and *Xanthosoma Maffafa* NX_s 002 respectively.

Biochemical assays

Amylose determination

Amylose Content was determined by the modified Iodine Binding methods of McCready *et al.*, (1950) and Onwuka (2005). A portion (0.1 g) of each sample was weighed into a 100 ml conical flask and dissolved in 1 ml of 95% ethanol. A quantity (9 ml) of 1N NaoH was used to hydrolyze the starch in the flask, the content of the flask was transferred and made up to 10 ml with distilled water. Some (5ml) of the filtrate was transferred from the 100 ml into another conical flask and 1ml of concentrated acetic acid was pipetted into each sample along with 2 ml iodine solution each for a colour change. Distilled water was added again to make up to 100 ml while the absorbance was read at 620 nm using the spectrophotometer. The result of the blank was subtracted from the absorbance of the sample. Amylose Content was calculated using the formular
 Amylose content (%) = 3.06 x A x 20
 Where A = Absorbance value

Amylopectin content (%)

The amylopectin content was determined by subtracting amylose content from one hundred (Williams *et al.*, 1970)

100 - % amylose content

Total starch

A portion (0.05g) of cocoyam flour was weighed and dispersed each in 6ml KOH solution. The solution was allowed to stand in a vortex until starch completely dissolves. A quantity of (10ml) amyloglucosidase was pipetted into 48ml of sodium acetate buffer to form an enzyme solution. Four milliter of the enzyme solution was added to the dissolved starch solution and kept in water bath for 45 minutes at 60°C with constant shaking. Thereafter 1ml of the obtained solution was measured and diluted to 10ml using distilled water (to get glucose concentration of 10ng/ml). Glucose concentration and absorbance was determined using a spectrophotometer at 500nm, Starch concentration was obtained by multiplying the absorbance value by 0.9 (AOAC, 2007)

In vitro starch digestibility

Each cocoyam flor sample (0.3g) was weighed out into 50 ml beakers, then 25 ml of 0.2M phosphate buffer solution at pH 6.9 was added to each of the beakers. Exactly 5 ml of $\alpha\alpha\alpha$ -amylase was added to the beaker (starch hydrolysis). The mixture was incubated at 37 °C for 3 hours with constant shaking. An aliquot of 0.2ml was taken from the mixture at intervals of 30 minutes over 3 hours and α -amylase was inactivated immediately by placing the beaker in a boiling water bath for 5 minutes. Amyloglucosidase (7.2ml) is added into 10ml of 0.4ml/L sodium acetate to get an enzyme solution then 0.6ml each of the enzyme solution was added to the beaker. The sample was incubated at 60°C for 45mins; the volume was adjusted to 20ml using distilled water. Glucose concentration was measured at a range of 25-100mg/ml using a spectrophotometer
 In vitro starch is calculated using the formula for first order exponential studies developed by Goni *et al.*, (1997)

$$\Delta t = \frac{(0.9 \times C_G \times (\frac{1}{100}) \times V)}{W \times \left[\frac{T_s(\%)}{100} \right]}$$

C_G = glucose concentration (mg/ml)
 0.9 = stoichiometric constant of glucose converted to starch
 $\frac{1}{1000}$ = conversion of Ng to Mg.
 V = Volume of digesta (ml)
 W_s = weight of sample
 T_s (%) = total starch expressed as dry matter.

Determination of Glycaemic Index

The glycaemic indices of the samples were estimated using glucose as the reference food according to the equation of Goni *et al.*, (1997).

The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding AUC of a reference sample (fresh white bread). According to Goni *et al.*, (1997) the HI is good predictor of glycaemic response.
 The glycaemic and hydrolysis indices were calculated

using the area under curve by integration between zero time and highest.

Statistical analysis

Data obtained were analyzed by using SPSS Version 20.0 software. All values were expressed as the Mean value \pm Standard deviation (SD) and the level of significance was calculated by one-way Analysis of Variance (ANOVA). The Duncan Multiple Range test (complemented with Student's T test) post- hoc test were used for comparison of the means of the various doses and fractions. A probability level of less than 5% ($p < 0.05$) was considered statistically significant difference between the test and control groups as well as among test groups for measured values

RESULTS AND DISCUSSION

The amylose, amylopectin and total starch contents of the six (6) different accessions (varieties) of cocoyam both (*Colocasia esculenta*) Nce001, Nce012, Nce011 and (*Xanthosomamaffafa*) (Nxs001, Nx002, Nxs003) are shown in table 1.

From Table 1, NXs 001 ($19.01 \pm 0.15\%$) had the highest amylose content while Nce 012 recorded the highest amylopectin content ($90.41 \pm 0.01\%$). Nce 012 therefore had the highest starch content ($210.14 \pm 1.81\%$).

Table 4 shows the starch hydrolysis of the accessions from (0 -180 min). The values ranged from (2.00- 42.00). The control gave the highest value. (42.00) at 30mins.

Table 5 shows the equilibrium constant, hydrolysis and glycaemic index. The control gave the highest value on both the hydrolysis(100%) and glycaemic index(26.96%). The highest value for GI and Km^{-1} was on Nce 001 (9.44%) and (0.12%).

HI-Hydrolysis index, GI-Glycemic Index, Km^{-1} – Kinetic constant

The *In vitro* method for measuring the rate of hydrolysis of starch has been suggested as an inexpensive and time-saving method compared to *in vivo* starch digestion (Jenkins *et al.*, 1987). From the values observed on the hydrolysis constant; the control (white bread) gave the highest value compared to the accessions used. The slow hydrolysis observed in the test accessions could be due to factors other than the amylose content owing to the fact that the results obtained do not agree with the findings of Riley *et al.*, (2014) which stated that food sample with low amylose contents are more digestible than food samples with high amylose content but may be as a result of the particle size of the starch. Recent research studies have shown that larger particles are more slowly digested than smaller particles because of the less amount of starch released due to enzymatic breakdown of starch during preparation. The equilibrium constant demonstrates the extent of starch hydrolysis curve. The highest equilibrium constant values was found in NX,001 (0.30).

The kinetic constant of the six (6) cocoyam accessions was low and hence a slower hydrolysis. The highest kinetic constant was observed in Nce 001 (0.12m) and this corresponded with the results of starch hydrolysis index (HI). This high value of kinetic constant could be as a result of its susceptibility to enzymatic hydrolysis. Low kinetic constant suggest higher resistance to enzymatic hydrolysis and is supported by Jaisut *et al* (2009), implying the accessions studied possibly produce a decrease in glycemic response.

The Glycemic Index (GI), is a dietary measuring system (Brand-Miller *et al.*, 2003b) that grades carbohydrate containing foods and compares the rate at which the blood sugar is raised after two hours or more of consuming the food (post-prandial glycemia) to a reference food, usually glucose, though white bread may be used instead (Wolever *et al.*, 1993; Anon, 2006). GI is expressed as percentages on an absolute scale. Carbohydrate-containing foods are graded as either having a high, medium (intermediate) or low GI depending on the rate at which blood sugar level rises (Mendosa, 2000), which in turn is compared to the rate of digestion and absorption of sugars and starches available in that food. The estimated glycemic index (eGI) of the six accessions of cocoyam studied (NXs001, Nce001, NXs003, Nce011, Nce012 and NXs002), were 55.63%, 58.93%, 45.75%, 50.14%, 49.04%, 58.38% respectively while bread had 94.61%. Therefore, it can be readily summarized using standard criteria (Brand-Miller *et al.*, 2003a) that accessions NXs003 and Nce012 are low glycemic foods, while the other accessions are intermediate glycemic foods.

Conclusion:

The *in vitro* starch digestion of all accession depicts that the accessions studied are good sources of nutrients which will make a plethora of low glycemic diets and thus may be beneficial in the fight against diabetes.

Table 1: Amylose Content and Total Starch Analysis

Samples	Amylose Content (%)	Amylopectin (%)	Total Starch (%)
NX _s 001	19.01 ± 0.15 ^a	80.99 ± 0.15 ^d	77.64 ± 3.38 ^f
NCe001	12.53 ± 0.19 ^c	87.48 ± 0.19 ^b	85.55 ± 2.22 ^e
NX _s 003	14.48 ± 0.20 ^b	88.49 ± 2.67 ^{a, b}	206.05 ± 6.73 ^{bc}
NCe011	10.32 ± 0.13 ^d	89.68 ± 0.13 ^a	200.14 ± 6.81 ^c
NCe 012	9.96 ± 0.09 ^e	90.41 ± 0.01 ^a	210.14 ± 1.81 ^b
NX _s 002	14.63 ± 0.11 ^b	85.37 ± 0.11 ^c	99.65 ± 0.07 ^d
White Bread (control)	-	-	217.39 ± 1.88 ^a

Values with different superscript across columns are significantly different (P<0.05) while values with the same superscript across columns are not significantly different (p>0.05).

Table 2: Starch hydrolysis table with time 0min-.180 mins

TIME (Min)	NX _s 001	NCE 001	NX _s 003	NCE 011	NCE 012	NX _s 002	CONTROL
0	6.00±0.00 ^d	6.00±0.00 ^e	2.00±0.00 ^a	3.00±0.00 ^b	4.00±0.00 ^b	7.00±0.00 ^f	27.00± ^a
30	6.00±0.01 ^c	6.00±0.00 ^c	3.00±0.00 ^a	6.00±0.00 ^b	3.00±0.00 ^a	9.00±0.00 ^d	42.00± ^c
60	7.00±0.00 ^d	6.00±0.00 ^d	6.00±0.00 ^c	4.00±0.00 ^b	4.00±0.00 ^c	10.00±0.00 ^a	26.00± ^f
90	7.00±0.00 ^d	6.00±0.00 ^c	3.00±0.00 ^c	6.00±0.00 ^c	5.00±0.00 ^b	10.00±0.00 ^a	12.00 ^e
120	10.00±0.00 ^f	13.00±0.00 ^d	4.00±0.00 ^c	6.00±0.00 ^b	6.00±0.00 ^a	11.00±0.00 ^a	25.00 ^e
150	7.00±0.00 ^c	9.00±0.00 ^d	3.00±0.00 ^c	5.00±0.00 ^b	5.00±0.00 ^b	8.00±0.00 ^a	32.00 ^e
180	14±0.00 ^e	6.00±0.00 ^c	2.00±0.00 ^d	4.00±0.00 ^b	4.00±0.00 ^b	8.00±0.00 ^a	30.00 ^f

Values with different superscript across rows are significantly different (P<0.05) while values with the same superscript across rows are not significantly different (p>0.05).

Table 5: Equilibrium constant, hydrolysis and estimated glycemic index table

SAMPLE	HI (%)	GI (%)	Km ⁻¹
NX _s 001	29.00	55.63	0.01
NCe001	35.00	58.93	0.12
NX _s 003	11.00	45.75	0.06
NCe011	19.00	50.14	0.01
NCe012	17.00	49.04	0.03
NX _s 002	34.00	58.38	0.04
White bread (control)	100	94.61	0.01

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WIS-FSV 09

EFFECT OF DRYING METHODS AND SLICE THICKNESSES ON BETA CAROTENE CONTENT ON ORANGE-FLESHED SWEET POTATO FLOUR

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ABSTRACT

Agricultural products of high moisture contents require appropriate drying method for nutrient component retention, particularly the bio-fortified crops, such as orange-fleshed sweet potato (OFSP). Effect of five different drying methods (oven, open sun, freeze drier, direct solar and indirect solar dryings) and slice thickness (2mm and 4mm), on carotene retention of fresh OFSP was researched. The study determined stability of β -carotene ($\mu\text{g/g}$) dry weight (DW) in OFSP by different drying methods and slice thickness. The beta carotene content was highest in indirect solar dried samples, having values of 504.08 and 432.85 $\mu\text{g/g}$ for 2mm and 4mm thickness, respectively, while other results followed in decreasing order: freeze dried (2mm thickness) (423.49 $\mu\text{g/g}$); 379.21 $\mu\text{g/g}$ (4mm) direct solar dried 371.80 $\mu\text{g/g}$ (2mm thickness) 337.58 $\mu\text{g/g}$ (4mm thickness); oven dried 246.78 $\mu\text{g/g}$ (2mm); 233.54 $\mu\text{g/g}$ (4mm) and open dried 115.72 (2mm thickness) and 104.48 $\mu\text{g/g}$ (4mm). One could therefore recommend indirect solar drying method and 2mm slice thickness as most appropriate for OFSP flour processing.

Keywords: Drying; β -carotene; OFSP; Thickness; Flour.

INTRODUCTION

Drying is one of the most widely used methods for preserving agricultural products or food. The main purpose of drying agricultural products is to reduce their water content so as to minimize microbial and other sources of spoilage during storage (Sim'eon *et al.*; 2016). Drying is important because, quality deterioration during storage virtually depends on the level of moisture in products, since moisture content reduction reduces degradation and chemical enzymatic reactions (Lee, 2013). Sweetpotato is cultivated throughout the tropics and warm temperate regions of the world for its starchy roots as a source of energy and other nutrients (Woolfe, 1992). The concentration of β -carotene in orange-fleshed sweet potato (OFSP) variety was responsible for the orange flesh colour (Simon *et al.*, 1993), hence, it has been recognized as a readily available vitamin A source (Nzamwita *et al.*, 2017). Appropriate drying method is needed for β -carotene (nutrient) retention (Fana *et al.*; 2015 and Ibrahim, 2012).

Though, OFSP is gaining importance as a cheap source of antioxidant (high in beta carotene) to combat VAD, however, its high moisture content and high nutrients makes it highly perishable and hardly store for long after harvest (Gouado *et al.*; (2011); Xiao *et al.*, 2009). Puree making for OFSP intermediate product has been in use, dried form (flour) require less facility to process and stored (Juliana *et al.*;2017). Hence, processing OFSP to flour by adequate drying methods may translate to its increased utilization.

Therefore, the objective of this study was to determine and compare the stability of β -carotene in OFSP by different drying methods and slice thickness. Application of the study will be to identify appropriate drying method(s), that could preserve beta carotene

and to provide information on optimal reconstitution quality of dried OFSP flour.

MATERIALS AND METHODS

Materials:

Fresh OFSP purchased from Super market (Woolwhorths) Hath field Pretoria, South Africa was used for this experiment.

Drying/Flour Preparation:

The following unit operations: sorting for uniform sizes, washing under clean running tap water to remove sand and other extraneous material and then air dried away from direct light. The fresh procured OFSP samples were cut into 2mm and 4mm using semi-automated slicer. Each slice thickness was further sorted for uniformity, divided into five portions and dried under five drying methods at approximate durations: oven (60°C 24 hours); open sun 3days; freeze 12hours, indirect solar 2days and 2days direct solar.

Well dried chips were milled (MF 10. IKA WERKE 3000-6500 1/Min. and sieved (500 μm) to obtained uniform particle size flour, vacuum packed and stored under -20°C freezer for further analysis to prevent oxidation reaction.

β -carotene content analysis

The following protocol was used in the extraction of the samples: Two (2g) of each sample was weighed into 50ml beaker, 20ml Tetra Hydro Furan (THF) was added, stirred on magnetic stirrer for 30minutes under dark environment and the upper clear layer was carefully filtered to rotary evaporator flask covered with aluminium foil and the residue was re-extracted until it became colourless (Nzamwit *et al.*, 2017). The filtrate was then evaporated to dryness at 40 °C on

rotary evaporator (IKA HB 10, IKA RV8 SO99, and Germany) to separate the extraction solvent from the sample. Then, 1 ml Toluene was used to dissolve the dried residue and 4ml HPLC mobile phase (Acetonitrile-58%, methanol-35% and THF-7%) was used to quantitatively rinsed the solution into 10ml test tube, well labelled and wrapped in aluminium foil and kept in -20°C condition. The β -carotene content of the dried OFSP samples were evaluated by following the procedures of Kelvin et al., and Munzuroglu et al. (2003) using a prominence Ultra-Fast Liquid Chromatography (UFLC), SHIMADU Tokyo, Japan, equipped with a SIL 20A Prominence auto sampler, DGU-20A Prominence degasser, a CTO-10 as VP Shimadzu column oven and a SPD-M 20A Prominence diode array detector. The β -carotene

production was quantified using a standard calibration curve which was obtained by authentic β -carotene from Sigma Chemical Co. (Cat. no. 7235407) from the slope of five concentrations (10pp to 200ppm). About 3ml of each dried sample extract was filtered (0.45 μ m) into Eppendorf tube. The filtrate was then transferred to the insert vial already packed in the 2ml brown vial before injecting. The 20 μ l of the extract was injected to the UFLC. Separations were carried out on the Waters Nova Pak-C18 4 μ m 3.9X300mm Column, serial No: 11913700613812, Part number: WAT011695. The flow rate was 1 ml/min. The chromatograms (peaks width, retention time and resolution) were calculated using Microsoft excel 2016.

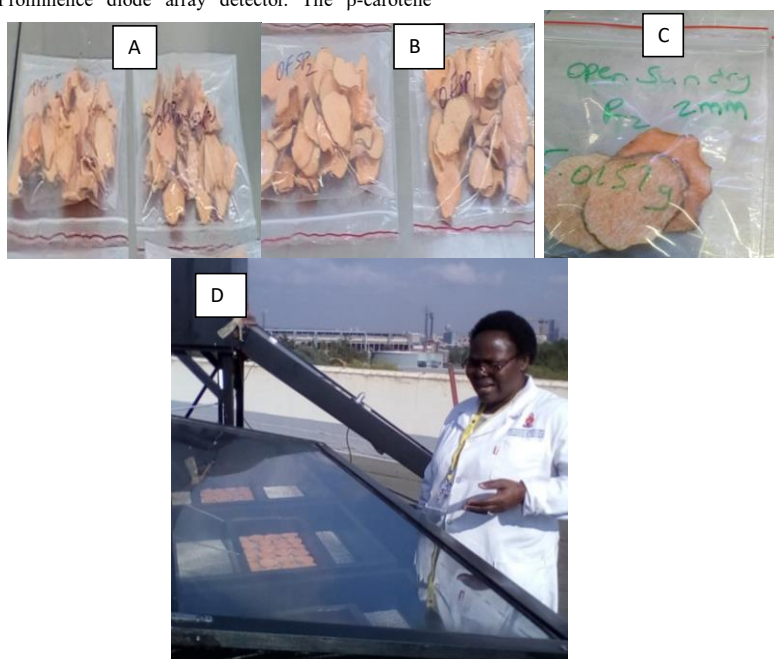


Plate 1: Photograph of A & B = Oven and Freeze dried OFSP samples; C = Open sun dried OFSP 2mm sample and D = Research scientist Examining Indirect solar drying

Statistical Analysis

The experiments were performed with two replicates for each five drying methods and two thicknesses. Descriptive statistics were performed for all variables by XLSTAT (2014) and SPSS Version 20. Two factorial analysis of variance (ANOVA) was performed to test the differences among the drying methods and two thicknesses for rehydration ratio and total carotenoids contents by ultra-fast high-performance liquid chromatography. The 5% level of least significance was used to determine any differences in the mean values between different drying methods and two thicknesses.

RESULT AND DISCUSSIONS

The beta carotene contents of the experimental OFSP are presented in Table 1. There was variance in percentage (Figure 1) of orange colour in the dried chips by five methods of drying and different thickness as shown in Plate 1. Orange-fleshed sweet potatoes contain carotenoids in different amounts (Fana et al., 2015; Fesco et al., 2002). The beta carotene contents on dry weight, ranged from 115.32 \pm 0.56 to 504.62 \pm 0.78 μ g/g for 2mm slice thickness, while 4mm slice thickness had a range of 104.48 \pm 0.66 to 432.85 \pm 0.5 μ g/g, for open sun drying and indirect solar drying respectively in all cases. There was general decrease in the beta

carotene contents in the flours compared to the fresh value (942.731±0.04 µg/g) , probably due to adverse effect of heat on stability of beta carotene content. This agrees with the report of Laura *et al.*, (2013) of reduction in β-carotene content of heat treated β-carotene rich experimental samples. Thickness 2mm had significant (p<0.05) higher amount of beta carotene than values observed in 4mm thickness across the five drying methods (oven, open sun, freeze, direct solar and indirect solar dryings). This may be caused by the exposure of samples to oxygen or/and heat for a longer period during drying for thicker slices (4mm) than the 2mm thick slices. Also, exposure of the samples, in each unit operation could be responsible for the general reduction. Indirect solar drying had highest beta carotene values of 504.62±0.78 µg/g followed by freeze drying 423.40±0.96 µg/g, direct solar drying 379.29±0.00 µg/g, conversely, direct solar drying had higher significant (p<0.05) value 371.67±1.02 µg/g than freeze drying 337.58±1.39µg/g in 4mm thickness samples, followed by open drying 246.77±0.05 and 233.53±0.41µg/g. The other drying method (open sun drying) had significantly (p<0.05) lowest amount (115.32±0.56 and 104.48±0.66 µg/g of beta carotene contents among all the drying methods.

Table 1: Beta carotene of OFSP flour as influenced by drying method and slice thickness

DRYING METHOD	BETA CAROTENE (µg/g)DW	
	2mm	4mm
Oven Dry	246.78 ^g ±0.05	233.54 ^h ±0.41
Open Sun Dry	115.72 ⁱ ±0.56	104.48 ^j ±0.66
Freeze Dry	423.49 ^c ±0.96 ^c	337.58 ^f ±1.39
Direct Solar Dry	379.21 ^d ±0.00	371.80 ^e ±1.02
Indirect Solar Dry	504.08 ^a ±0.78	432.85 ^b ±0.51

Key: -Values are means ± standard deviation of duplicate runs of beta carotene Means in vertical with different superscripts are significantly (p<0.05) different among the drying methods and thicknesses. While, FR* = fresh sample, OD = Oven drying, OS = open sun drying, FD = freeze drying, DS = direct solar drying, and ID = indirect solar drying.

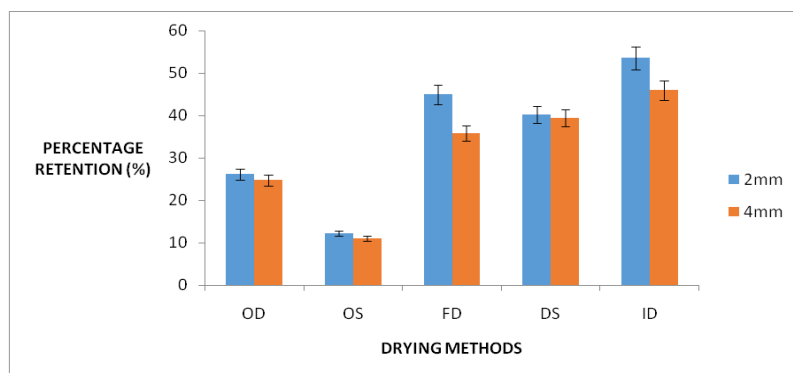


Figure 1: Percentage Retention of drying methods and slice thicknesses on Beta Carotene content of Orange-fleshed Sweet potato
Key: FR* = fresh sample, OD = Oven drying, OS = open sun drying, FD = freeze drying, DS = direct solar drying, and ID = indirect solar drying.

CONCLUSION

We conclude that indirect solar drying method is most appropriate for OFSP flour processing. Hence, for high percentage of beta carotene retention in OFSP flour indirect solar drying is recommended. This study provides information for food stake holders on appropriate drying method, for outstrip beta carotene content retention in dried OFSP flour, which could translate to postharvest preservation of the crop during season against off season.

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PASTING PROFILE DURING SHORT TERM STORAGE OF FLOURS PROCESSED FROM TWO CASSAVA VARIETIES

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Abstract

High quality cassava flour processed from two cassava varieties (TME 419 and UMUCASS 36) were evaluated for their pasting properties to ascertain their suitability in the food industry. Results showed significant ($p > 0.05$) varietal differences in their viscosities. Peak viscosity was higher in TME 419 (266.20 ± 4.35 RVU) compared to UMUCASS 36 (228.57 ± 0.46 RVU). Breakdown viscosities of the flour from both varieties reduced at the end of storage from 142.53 to 129.43 RVU in TME 419 and from 121.10 to 113.20 in UMUCASS 36. A lower final viscosity (160.90 ± 2.59 RVU) was observed in the flour from 'UMUCASS 36'. The peak temperature of the cassava varieties ranged from 85.07 °C to 88.80 °C during storage. However, storage duration had no significant ($p > 0.05$) difference on the pasting properties of flour from both cassava varieties. The observed differences in the pasting properties shows that cassava flour can be used for various purposes in the food industries such as for confectionery production, weaning food, salad dressing and thickeners.

Introduction

The use of high quality (unfermented) cassava flour as composite for bread making has become a very known practice across West African countries. Apart from using cassava flour as composite flour in bread making, it can be utilized for other purpose in the food industry, like in production of baby foods, noodles, as soup thickener (Nwabueze and Anoruh, 2011), confectionery and in the textile industries. These industrial applications of cassava flour are accredited to its desirable functional properties which are very distinct with certain varieties. Pasting profile of cassava flour is essential for characterizing its usefulness in the food industries (Etudaiye et al., 2009). For instance, high viscous flour is useful in the production of jelly, food thickeners and binders, while flours with low viscosity could be suitable for weaning food and in composite flour because their starch molecules are highly resistant to breakdown during high temperature cooking (Tsakama et al., 2010). Pasting profile can also enable the food processors to identify how gelation processes affect the texture and stability of the food products during cooking.

Despite the detailed reports on the functional and pasting properties of cassava flour, there is little information on the impact of storage on the pasting properties of cassava flour stored at ambient temperature ($23 \pm 2^\circ\text{C}$). Hence, for a short term storage period, the pasting profile of flour from two cassava varieties were studied. The findings could influence its utilization: large scale production in the food industry, market value, and consumers' acceptability.

Materials and Methods

Source of Materials

Two cassava varieties 'TME 419' and 'UMUCASS 36' were used for this study. Both varieties were obtained from experimental field of National Root Crops Research Institute (NRCRI) Umudike, at 12 months after planting. The freshly harvested cassava roots were separately processed into high quality flour. Thereafter, the flour samples were packaged in Low Density Polyethylene (LDPE) bags, and stored for 12 weeks at $23 \pm 2^\circ\text{C}$ and 50-55% relative humidity (RH).

Determination of pasting properties

Pasting properties of the different flour samples during storage were determined at four-week intervals using a Rapid Visco Analyzer (RVA) (Newport Scientific Pty. Ltd., Australia). Parameters determined were peak viscosity, trough, breakdown, final viscosity, setback, peak time and pasting temperature, using thermocline software (Newport Scientific 1998). The results were expressed in Rapid Visco Unit (RVU).

Statistical analysis

Analysis of variance (ANOVA) was conducted on all data using Statistical software, (Version 12.0, Statistical, Statsoft, USA) Significant differences were established at $p \leq 0.05$ according to Fisher LSD test, and data obtained were reported as mean and \pm standard deviations.

Results and Discussions

The result showed that the pasting properties of the flour are basically a factor of the varieties and their inherent components. Flour from TME 419 had higher peak viscosity (266.20 ± 4.35 RVU) compared to UMUCASS

36 (228.57 ± 0.46 RVU) (Table 1). Similar significant changes were observed in previous study on the effect of storage on physicochemical quality of cassava flour (Uchechukwu-Agua et al., 2015). Trough which is the hot paste viscosity ranged from 123.67 to 107.47 RVU as TME 419 had higher value after storage (Figure 1). Trough has been reported to be influenced by high swelling power allowing the granules to reach its highest peak faster thus leading to decrease in shear resistance force at temperature above 80 °C (Marta et al., 2019). During storage, the peak viscosity and trough increased significantly in both varieties. Highest values at 12 weeks were observed in flour of TME 419 (Table 1). There was no significant ($p < 0.05$) difference in storage duration between both varieties. The mean breakdown viscosity of flour was significantly ($p > 0.05$) higher for 'TME 419' before and during storage than 'UMUCASS 36'. Although, the breakdown viscosity reduced at the end of storage, low breakdown viscosity has been reported to show better resistance to shear during heating where the formed paste will be stable under hot or heating condition (Abiodun et al., 2009). Therefore, it could be expected that flour obtained from 'UMUCASS 36' will show greater resistance to shear during heating compared to flour from 'TME 419'.

The initial values before storage of the cassava flour for the setback viscosity were 51.00 RVU and 47.80 RVU for 'TME 419' and 'UMUCASS 36', respectively. These values closely correspond to the values reported by Ikegwu et al. (2009) who evaluated the pasting properties of starch isolated from some improved cassava cultivars. Low setback values imply that the flour would have high paste stability and resistance to retrogradation on cooling (Etudaiye et al., 2009). A decrease was observed in both flour varieties after storage.

Final viscosity has been reported as the ability of flour to form gel after cooking and cooling, giving an indication of flour paste stability. Both varieties used for this study differed significantly ($p > 0.05$) in their final viscosities (Figure 1). Iwe and Agiriga (2014) also reported significant ($p > 0.05$) difference in the final viscosity of

cassava flour cultivars. Lower final viscosity in 'UMUCASS 36' flour could be attributed to the amylose content in the flour. Higher amylose starches are known to reorganize more rapidly because the linear chain allows the molecules to tightly arrange and held together by hydrogen bond. Hence, the 'TME 419' flour with higher final viscosity could be more preferred in the food industries for the production of jelly formation, salad dressings, confectionery and in other products where high viscosity is required. The final viscosity increased significantly ($p < 0.05$) at 12- weeks storage with values ranging from 160.90 to 181.00 RVU.

No significant ($p < 0.05$) difference was noticed in the pasting time during the storage. However, cassava flour with shorter pasting time could be expected to use lesser energy during processing thus reducing the production time and cost. Tsakama et al., (2010) reported that samples with short peak time would swell quickly which will lead to fast disassociation of the granules and thus shorter cooking time.

The peak temperature ranged from 85.07 °C to 88.80 °C during storage. Peak temperature indicates the minimum temperature required to cook a product as well as the energy cost involved (Ikegwu et al., 2009). 'UMUCASS 36' was higher (88.60 ± 0.35 °C) than the TME (85.43 ± 0.15). Therefore, it clearly shows that flour from 'TME 419' would require less energy during cooking compare with 'UMUCASS 36'.

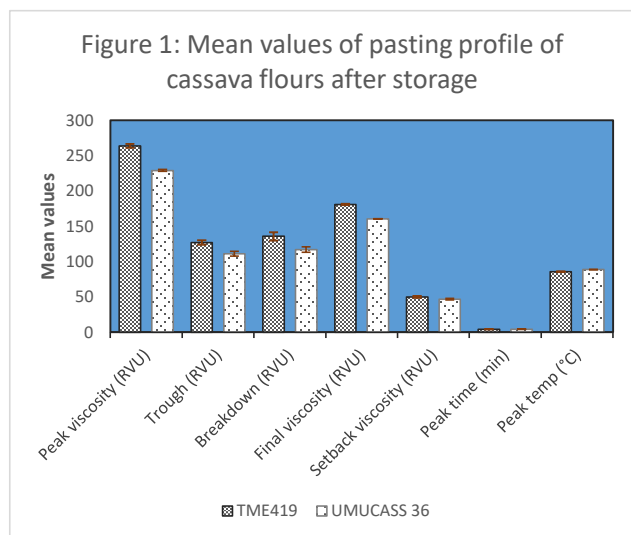
Conclusion

Varietal differences clearly influenced the pasting properties of the flour. Cassava flour from UMUCASS 36 would have the most resistance to gelatinization as indicated by the high temperature as well as its lower final viscosity. The varietal differences allow flour samples from different cassava varieties to be used for various industrial purposes like baking, production of noodles, weaning food, thickeners, sugar syrup and many others.

Table 1. Pasting Profile of flours from two cassava varieties during storage

Varieties	Storage duration (weeks)	Peak viscosity (RVU)	Trough (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback viscosity (RVU)	Peak time (min)	Peak temp (°C)
TME 419	0	260.23 ± 0.51	123.67 ± 1.06	142.53 ± 3.35	181.00 ± 1.92	51.00 ± 0.95	4.00 ± 0.00	85.43 ± 0.15
	4	263.20 ± 3.16	125.07 ± 2.13	138.41 ± 3.22	181.00 ± 1.72	50.70 ± 0.65	4.00 ± 0.00	85.13 ± 0.10
	8	265.50 ± 2.35	128.75 ± 1.49	132.53 ± 1.65	180.60 ± 0.92	49.50 ± 0.48	4.05 ± 0.00	85.98 ± 0.13
	12	266.20 ± 4.35	130.80 ± 2.46	129.43 ± 1.96	179.20 ± 1.71	48.40 ± 0.75	4.07 ± 0.06	86.10 ± 0.00
UMUCASS 36	0	228.57 ± 0.46	107.47 ± 2.01	121.10 ± 1.57	160.35 ± 2.60	47.80 ± 2.19	4.20 ± 0.00	88.60 ± 0.35
	4	227.55 ± 1.14	109.15 ± 1.09	119.16 ± 1.14	160.31 ± 2.10	47.32 ± 2.75	4.20 ± 0.00	88.60 ± 0.26
	8	229.59 ± 0.53	112.64 ± 1.51	114.10 ± 0.83	160.55 ± 2.45	46.12 ± 1.89	4.20 ± 0.00	88.61 ± 0.35
	12	230.57 ± 2.40	115.27 ± 0.85	113.20 ± 0.53	160.90 ± 2.59	45.63 ± 2.95	4.00 ± 0.00	88.57 ± 0.15

Values are given as means of triplicate determinations ± standard deviation



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MAINSTREAMING GENDER IN SCIENTIFIC RESEARCH (MGR)

WIS-MGR 03FL

**GENDER PARTICIPATION IN BEE HONEY FARMING ACTIVITIES AMONG BEE FARMERS IN
ISIALA NGWA SOUTH LGA OF ABIA STATE, NIGERIA**

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Abstract

The study analyzed bee honey production by gender in Isiala Ngwa North local government area of Abia state. A purposive sampling technique was used to collect data from sixty males and sixty female respondents. Data were collected from primary and secondary sources. The primary data were collected by the use of a structured questionnaire administered to the respondents and were analyzed using descriptive statistics such as frequency, means and rank order. The result showed that the mean, household size and farming experience of the male respondent were 49, 7 and 9 respectively while that of the female were 50, 8 and 8 respectively. 100% of the female respondents partook in site selection, hive installation, baiting and processing of honey. All the male respondents (100%) participated in honey harvesting, colony transfer and processing. Also greater percentage of male and female farmers 93.3% and 97% respectively had information through fellow farmers. Majority of the farmers both male and female reported decrease in output, deforestation, weather condition, shortage of bee forage and pest/predators as their major constraints. The study concluded that males are more involved in bee farming than their female counterparts. The female is encouraged to go into bee farming to maximize the benefits involved in bee farming.

Keyword: Bee honey, Gender and Isiala Ngwa

Introduction

Agriculture is an important component of a national economy which serves as a vehicle for diversifying the economics and enabling economic growth. Agriculture still remains the main employer of over seventy percent of the country's labor force and account for about thirty one percent of the nation's gross domestic product (World Bank, 2008). Consequently Nigeria still manifest the typical symptoms of peasant Agriculture resulting to low productivity in virtually all the sub-section of agriculture. Gender is a term often associated with roles and responsibility of males and females in the society as a social classification of sex. It is the socio-cultural differences between males and females as against the biological differences (Sinkaiye & Jibowo, 2005). Many rural women who are faced with the challenges of low income and gender inequality improve their role in the farming system based on participation in the agricultural value chain, thereby enhancing agricultural productivity, household income and sustainability development. A previous study showed that 53% of the labor force in the agricultural sector of a developing countries is made of women who play an indispensable role in agricultural production (Apata, 2013). Women engage on a continuous basis in home related and income generating activities and often spend most of their time engaged in both productive and domestic activities (Azubike, 2005), therefore the gender gap is closing and this may be because women participation in economic activities is more active than before.

Beekeeping is a form of agriculture that involves management of wild or domesticated bee colonies for production of honey and other bee products. Honey production has been identified as having the potential to provide employment and reduce poverty among rural household in Nigeria. Beekeeping has been practiced since time immemorial and has often been regarded as male-dominated enterprise, many cultural taboos and the method of beekeeping used has been prohibitive to the

involvement of women to beekeeping activities (Mujuni et al., 2012; Vlek et al., 2003; Shackleton, 2011).

This has contributed to the slow development of the beekeeping industry considering that women contribute about 80% to the households' food in most African families (Karunde, 2011), but beekeeping generally now has become important economic activity for both men and women in the country. As observed from literature, gender roles along the beekeeping value chain vary from one country to another. In the advent of modern beekeeping technology and involvement of development agencies, apiculture roles performed by women are increasingly changing to resemble those of men in the previously male dominated enterprise (Presser and Sen, 2000). Beekeeping is an endeavor that provides for time flexibility and can be incorporated in other farming activities. It is also suitable for women because it can be operated from the ground level without necessarily climbing trees.

The study was designed to examine gender participation in bee honey farming activities among bee farmers in Isiala Ngwa North LGA of Abia State, Nigeria.

Methodology

The study was purposively carried out in Isiala Ngwa South LGA. Ngwa people are a group of Igbo speaking clan who reside in Abia state, Nigeria. They are located between latitude 50.30°N and longitude 90° and 70.30°E. Its land mass is about 1312.768 square kilometers and has a population above 400,000. The temperature is about 21°C, the climate is humid tropical and is characterized by wet and dry seasons (Chigbu, 2011). The agronomic practices attach solely on annual rain fall which has a mean value of 200cm and relative humidity of 80%. The predominant food crops of the people are yam, maize, cassava, vegetables, palms and fruits. It was chosen because of the predominant of

wood lands and tree species which provide excellent forage that is suitable for beekeeping. Multistage sampling technique was used in selecting 6 communities namely Omoba, Alaoma, Ovu-kwu, Isieketa, Nncise and Ovu-Ngwu. In the second stage 20 bee farmers comprising of 10 male and 10 female were randomly selected from each community, making it 60 male and 60 female respondents, giving a total of 120 respondents for the study. Structured questionnaire was used to collect data from the respondents. Data analyses were done using descriptive statistics such as frequency, percentage, mean and ranking.

Result and Discussion

The result in Table 1 showed that majority (40%) and (30%) of the male and female respondents respectively were within the age range of 41-50 years, with the mean of 49 and 50 years respectively. This indicates that the population was made up of adults in their active age. The study conformed with the findings of Agada and Ejembi(2010) who reported that female farmers were younger than their male counterparts. This agrees with Rahman (2002) who found that the age bracket is the economically active age and as such will respond positively to any intervention aimed at improving their productive capacity. The result also revealed that (65%) of the male and(50%) of the female respondents are married. This may be attributed to the fact that married people who have families have more responsibilities than the unmarried ones and therefore will engage in honey production to improve their living standard. Table 1 also showed that majority(83%) male and (68%) of the female respondents has a household size ranging between 1 - 9 persons with the mean of 8 and 7 persons for male and female respectively, this finding is in agreement with Nwobiala *et al.*, (2009) who reported

Table 1: Distribution of Respondent According to their Socio-Economic Characteristics

Variables Gender	Male (n=60)		Female (n=60)	
	Frequency	%	Frequency	%
Age				
21 - 30	10	17	9	10
31 - 40	19	32	20	29
41 - 50	24	40	15	30
51 - 60	4	6	11	19
61 - 70	3	5	5	12
Marital status				
Single	4	6.67	5	8
Married	39	65	30	50
Widow	10	16.67	12	20
Widower	7	11.67	13	21.67
Household size				

1 - 9	50	83	41	68
10 - 15	-	-	16	27
16 - 21	10	17	3	5
Mean	7		8	
Major occupation				
Farming	7	12	16	27
Civil servant	31	52	27	45
Business	22	36	17	28
Mean	14		10	
Level of education				
Non-formal edu	-	-	10	17
Primary education	3	5	6	10
Secondary edu	4	7	22	37
O N D	14	23	21	35
B S C	29	48	1	1
Post-Graduate	10	17	-	-
Year of experience				
1 - 4	14	23	16	27
5 - 9	37	62	23	38
10 - 14	6	10	13	22
15 - 19	-	-	8	13
20 - 24	3	5	-	-
Mean	9		8	
Mode of honey production				
Full-time	10	17	21	35
Part-time	50	83	39	65
Membership of association				
Yes	17	28.3	26	43.3
No	43	71.7	34	56.7
Total			60	100

Source:-Field Survey data, 2019.

that large household size is a cheaper means of providing farm labor and reducing labor cost.

Beekeepers rely so much on household labor for beekeeping activities. This is in line with finding of Effiong (2005) who reported that large household size enhances the availability of labor. The result also revealed that (52% and45%) of the male and female respondents respectively are civil servants. This implies that they use savings from salary to develop their bee farms with the intension for commercial production in preparation for retirement. Majority of the male (48%) and the female (37%) respondents had post primary education, this means adoption of innovation by the farmers in the study area could be more effective. This result agrees with the findings of Eze (2002) who reported that acquisition of education by famers positively influence the use and acceptance of improved practices. The distribution of the respondents by year of experience showed that(62%)of the male and(38%) of

the female respondents had 5 - 9 years' experience in farming with the mean of 9 and 8 for male and female respondents, this indicates that male bee farmers are more experienced than their female counterparts. Farming experience is used as a measure of management ability, this conformed with the findings of Nwaru *et al.*, (2004) who reported that the higher the farming experience the more the farmer would have gain more knowledge and technical ideas on how to tackle farm production problems .Table 1 result also revealed that majority of the male (83%)and the female (65%) respondents are into part-time bee farming. About (71.7%) of the male and (56.7%) of the female respondent do not belong to any farmers' association, 28.3% and 43.3% of male and female respondents are members of association/organization. This indicates low level of social participation and hence resulting to low innovativeness among the respondents due to lack of group dynamic effect.

Table 2:Distribution of male and female farmers according to participation in Bee Farming activities

Farming Operation	Male (n=60) Frequency	Percentage	Female (n=60) frequency	Percentage
Site selection	58	96.7	60	100
Hive installation	59	98.3	60	100
Honey harvesting	57	95	60	100
Colony transfer	60	100	47	78.3
T o t a l	60	100	60	100

Source:Field survey, data 2019;

*Multiple responses recorded

Table: 2 show the results of participation of male and female farmers in bee farming activities. It indicated that (96.7%) of the male respondent partook in site selection, hive installation(98.3%), honey harvesting (95%).In contrast,(100%) of the female respondents partook in site selection, hive installation(100%)and honey harvesting (100%).In addition (78.3%) of the female respondents were involve in colony transfer. The findings showed that although both male and female bee farmers are involved in honey production the males are more involved in colony transfer than their female counterparts. This could be due to the energy demanding nature of the activities.

Table3:Distribution of respondents according to sources of information on honey production

Source	Male(n=60) Frequency	percentage	Female(n=60) frequency	Percentage
Extension agents	20	33	30	50
Fellow farmers	56	93.3	58	97
Internet	50	83.3	52	87
R a d i o	40	67	47	78.3
Television	42	70	56	93.3
B o o k s	51	85	49	82
Seminar/workshop	39	65	38	63.3

Source: field survey data, 2019.

Multiple responds recorded

Table 3: shows the distribution of the respondents according to their sources of information on honey production. The result indicated that majority of the respondents male(93.3%) and female(97%) rely mainly on fellow farmers as their source of information, this agrees with the Adereti *et al.*, (2006) who reported that majority of the farmers rely on group discussion with fellow farmers as their major source of technical information, similarly (83.3%)of the male and (87%) of the female source came from internet, the table shows that(85%) of the male and (82%) of the female also sourced information from books, this could be as a result of high level of literacy among the bee farmers. This conforms to the findings of Agbamu(2006)who reported that farmers sources of information is mostly influence by the level of education or available source of innovation and extent of modernization in the locality.

Table 4:Gender distribution of respondents according to constraints encountered in honey production

constraints	Male(n=60) Frequency	Percent	Female (n=60) Frequency	Percent	Rank
Decrease in output	60	100	60	100	1
Deforestation	60	100	60	100	1
Weather condition	60	100	60	100	1
Shortage of bee	60	100	60	100	1
Pest and predator	60	100	60	100	1
Inadequate skill	40	67	38	63	2
Lack of information	33	55	30	50	3

Poor access to credit	30	5	0	4	2	0	3	3	4
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Source:field survey,2019;

*multiple responses recorded

Table 4 shows the constraints that limited honey production in the study area. The major constraints faced by both male and female respondents in the study area were ranked in descending order and they are as followed ; decrease in output, deforestation, weather condition, shortage of bee forage and pest/predators was ranked first followed by inadequate skill which was ranked second. the result conformed with Yirga *et al.*,(2012) who reported shortage of forage as a major constraint affecting bee farming sector thereby causing them to relocate to the area where resources are available for their survival. According to the respondents ants is a predators of honey bees that limited honey production in

the area . Lack of information was also another constraint that limited honey production in the area.

Conclusion

The study concluded that both male and female respondents were in their productive age. Male respondents were more experienced than their female counterparts, probably because of the belief of the people that beekeeping activities are male's job due to physical and cultural reasons which might not be true . Both genders rely more on their fellow farmers as source of information. Male and female farmers face similar constraints in bee farming in the study area. However, honey production is yet to be maximized since several constraints still limits its production. The study recommended that women should be encouraged in honey bee farming activities to maximize the benefits therein.

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WIS-MGR 05

CHALLENGES OF WOMEN IN ENGINEERING: A NIGERIAN PERSPECTIVE

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ABSTRACT

Engineering as a discipline is greatly dominated by the masculine gender. This scenario seems to be the bedrock for relegating women to rear position when it comes to engineering research, innovation, entrepreneurship and management. There is need to build confidence to restore the fact that female engineers can excel in all aspects of engineering profession. This paper therefore addresses: 1, the challenges of women pursuing a career in male dominated profession. 2, the difficulties of female Engineers attaining leadership/managerial position in a male dominated work force. 3, Proactive measures that will enable female Engineers gain work experience, develop entrepreneurial skills and increase their relevance and 4, Suggestions on narrowing down the skewed gender ratio in the Engineering profession. The methodology includes quantitative data collection across the sampled region. The results were collated as primary data source and summarized in graphical representation. The result shows that only 10% of engineers employed in industries and the academia are females. Since more than half of the Nigerian population are females, this however should serve as a great asset to her economy through engineering development. Engineering therefore should be made gender friendly to ensure full women participation and ensure equal opportunities.

Keywords: gender disparity, female entrepreneurial engineers, equal opportunities, skewed work force.

INTRODUCTION

Women in engineering practice, is as old as the profession itself. Prior to the formal recognition of the profession, women had made significant impact to register their presence in the profession. Hypatia of Alexandria was given the honor for inventing the hydrometer between 370 – 415AD. The positive journey continued with the emergence of women like Elizabeth Brags, Julie Morgan as graduate Engineers but their achievement still had infinitesimal impact (All-together, 2019). From the late 19th century to the early 20th century, there exists an unwritten perception and emotional persuasion where woman's ability to acquire university education is limited especially in sciences. This however reduced the number of women who gained international awards and recognition.

Some prestigious universities in the early days allowed women to study engineering but were not honored with degrees. The likes of Eller Henrietta Swallow, Nora Stanton Blatch and Elsie Gregory Machell broke the barrier. These female engineers worked really hard to achieve their professional engineering status. Fortunately, by the half of the last century, women were regarded as oddities defying gender norms in engineering. The revolution seems to draw a positive effect as the shortage of male engineers after the World War II created an opportunity to train female engineers who took up job in companies. Presently the statistics and the records by American society for engineering shows that 19% of women obtain bachelor's degrees in

United States of America. Despite the surge in the number of women that obtain PhD in engineering, the gender disparity still persists. To address this anomaly, there must be a better understanding and a concerted approach by both industrial organizations and educational institutions to meet the demands occasioned by the sudden change that the society is experiencing. The challenges facing women in STEM (Science, Technology, Engineering and Mathematics) emanate from the primary through the secondary, tertiary institutions and then to area of employment. Each of these stages presents a visible hoax that discourages women in professional discipline. In Nigeria, the story is not different; the competitive environment both in industry and educational institution has not allowed women to prove their capabilities, explore their potentials and talent. Generally, women in engineering are faced with less hazard allowances since they do not have the opportunity to be called upon for such task, very slow promotion rate and less leadership appointment (Byko, 2005). Despite the advantage of the large female population size in Nigeria, infinitesimal numbers of women are involved in STEM. Approximately 49.5% of Nigeria populations are women while 22% of the total population of female graduates in Nigeria are graduate of engineering and technology. There is no doubt that Science, Technology, Engineering and Mathematics are the back bone of any country's technological advancement, but the socio- economic development of any country hinges on one important aspect of human emancipation which rallies on equitable distribution of

resources. Women have been disadvantaged in this aspect since equal opportunity has not been given to them when compared to their male counterpart.

There is still the discrepancy and assumption that in engineering, only men can accomplish a task successfully. This leads to a scenario where women are devalued, leaving them with low self-esteem. Their efforts are biasedly assessed leading to unequal remuneration and promotion. The women most times need to perform exceptionally better than their male counterparts to gain professional recognition. Women at times do not have practical experience in their field and as such, feel inferior to their male counterpart. They realize that there is a wide gap between the concept of being an engineer and the real practice of engineering. Female engineering lecturers, encounter some challenge teaching certain courses effectively due to lack of exposure and real time industry experience. In certain circumstances, married women are not even considered for employment in engineering firms. The concept is that female engineers cannot work beyond official working hours or at weekends and their employment literally regarded as an economic burden.

It is often seen that Women cannot embark on far distance trip and engage in marine or other tasking engineering field works. Motherhood and family inclinations bar them from exhibiting their best talents. Considering these constraints, women are still yet to find an equitable position in engineering profession or STEM when compared to the men still leaving engineering as a male-dominated profession. The fear of being exposed to hazardous environment or getting involved in off-site work, places women at disadvantaged position of being selected in technical team in the industry. Women at times show unnecessary level of incompetence by asking too many questions when given a task. This creates mistrust in their ability to deliver on the job requirement, contrary to their male counterparts (Lombarch, 2017). The scenario leads to men's insistence not to allow women lead in engineering field (Niemeier and Gonzalez, 2014). This apathy towards their capabilities affects the job distribution from the role of a graduate engineer to managerial position in the organization. This however affects the salary distribution due to differences in the degree of responsibility (Faulkner, 2016). There is lack of confidence in the employers due to the work/life balance conflicts in women. Women are faced with child bearing, child care, and other family responsibility which definitely impede their developmental growth and prevents maximum level of job satisfaction (Subri, 2018). This scenario places the leadership of women engineers in industry, organizational management and academia to the rear until at an advanced stage of their lives.

Challenges facing women in Nigeria is multifaceted. There is no enabling environment that encourages women to embrace STEM in their academic pursuit. Strategic policy makers do not consider women in curriculum development for STEM. This poses a lot of obstacles in creating a positive emancipation for women in a male dominated field. According to Chitra (2007) "don't give up, don't doubt yourself and don't think about what people say about you but be strong and embrace the engineering profession". Despite the noticeable gender gap in woman's involvement in higher levels of engineering, industry and academic emancipation, there are still societal, personal and occupational factors that exclude women from attaining the highest echelon in academic, industrial and research institutions. Accumulation of these bottlenecks contribute to the unforeseen barrier facing women; stampeding their participation in engineering and affects the attitude of females in STEM subjects (Sarkar, Tyther and Palmer, 2014). The discouraging factors facing women as a challenge to participating in engineering emanates from the early stages of education.

METHODOLOGY

Quantitative data collection was used in this study to obtain the Numerical strength of women who are involved in STEM and those in engineering. A stratified random sampling was used in choosing the primary and secondary schools for the studies in the geographical region where the data were collected. This was done to ensure holistic representation of the variables both male and female in the STEM. The tertiary institutions were randomly selected from the geographical region and it covers both the students and lecturers in the STEM departments. The industries employing STEM professionals were considered for data collection for the study. The data was collected from south-south and south-East demographic region of Nigeria. Correlational data were also collected from selected private and public secondary schools across states from the southern and south-east region of Nigeria; of males and female students offering mathematics and sciences. The study also x-rayed and showed the ratio of women in engineering employed in different departments in the industry (construction companies, oil companies etc.) as compared to the number of their male counterparts. Quantitative data of the numerical strength of women in academia as compared to that of men were collected from selected Universities from the southern part of Nigeria.

RESULTS and DISCUSSION

Attitude of Females Towards Stem in Secondary Education

It is obvious that STEM is the cornerstone of technology and industrial development in any society. Provision of good health, good environment, food for the entire populace and fostering technological advancement rallies on STEM. The approach and attitude of females to STEM in primary and secondary school are not different from that of the boys. Globally, there is gender parity in achievement, enrolment and completion for both boys and girls at primary school level which gives a visible achievement in recent times. In West African countries especially in Nigeria, gender gap is significant and socio-economic status of communities contribute to

the gender gap between boys and girls. During primary education, there is no significant gender difference in STEM.

Data collected from both public and private primary schools across the specified region in Nigeria shows that there is no significant difference in the performance of girls and boys in STEM subjects. Same trend was found in private and public secondary school. In some significant cases, girls perform better than boys at the secondary school levels.

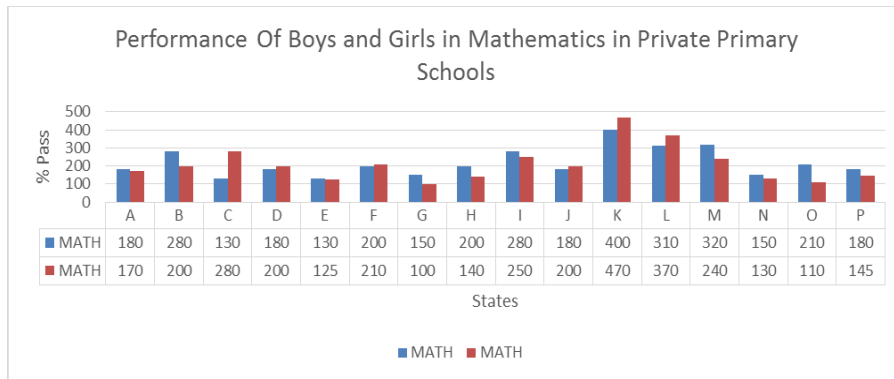


Figure 1: Shows the Performance of Girls and Boys in Mathematics in Private Primary School

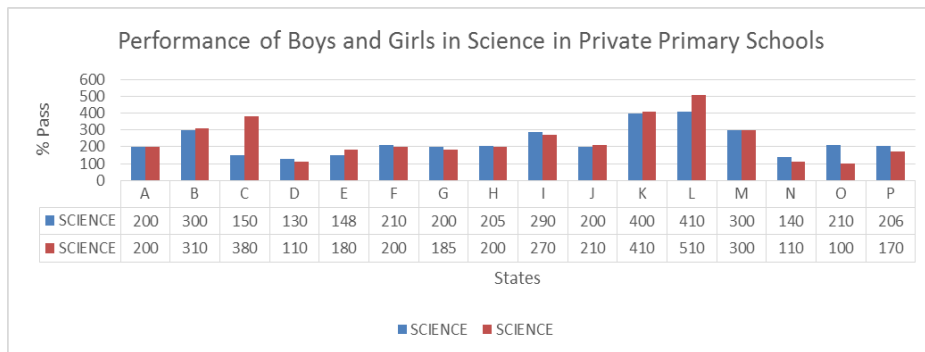


Figure 2: Shows the Performance of Girls and Boys in Science in Private Primary School

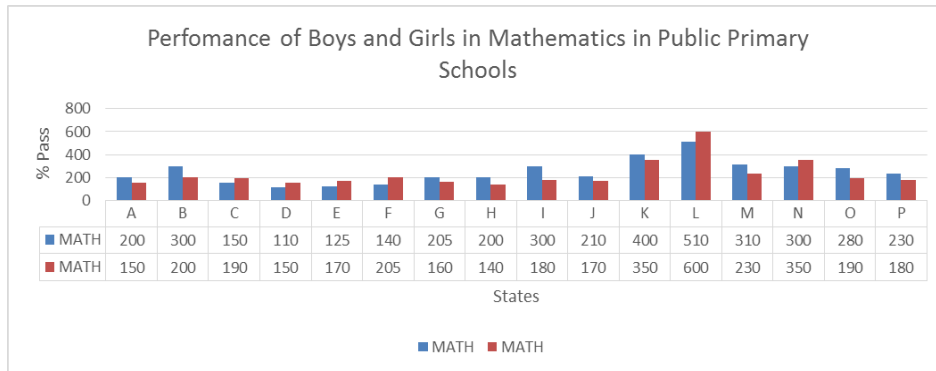


Figure 3: Shows the Performance of Girls and Boys in Mathematics in Public Primary School

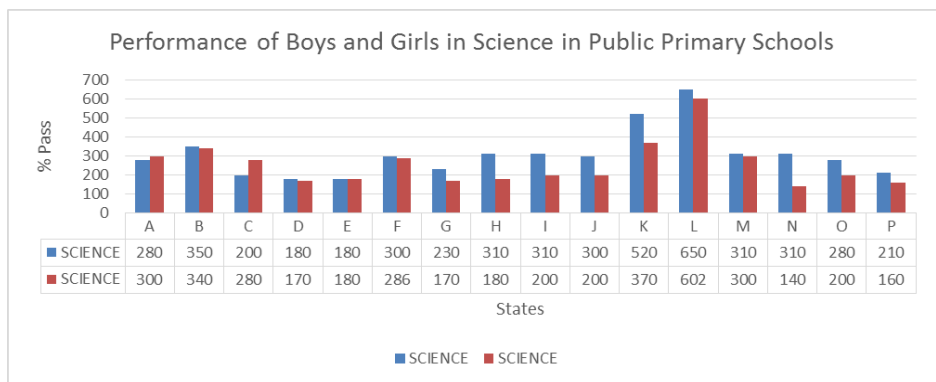


Fig 4: Shows the Performance of Girls and Boys in Science in Public Primary School

A similar trend was obtained at secondary school level but the numerical strength of boys and girls differ. There is a greater number of boys offering mathematics and sciences than the girls. There is decrease in the number of girls enrolled into sciences and mathematics than boys. This might not be unconnected to culture, beliefs and socioeconomic status of the families. In Nigeria and other African countries peer influence, effects of social media, early marriage, and parent’s opinion on the girl child status in the family are responsible for poor enrolment (Veronica and Amaka, 2019).

The customary conception that women were destined for domestic and child bearing responsibilities; worsen the scenario in Nigeria. There is lower overall rate of enrollment and completion of secondary education by girls but the performance between girls and boys at this level remains the same. This is statistically shown from

data collated from five private secondary schools in the south east region.

CAREER ACCEPTANCES OF FEMALE STUDENTS IN STEM AT TERTIARY LEVEL OF EDUCATION

Gender gap for STEM in primary and secondary school is not statistically significant except on the numerical strength of the boys which is occasioned by cultural and socioeconomic factors. The participation rate of females in tertiary education in engineering or STEM is relatively low with regard to the population demographics (Jannis, Deilla and Diane, 2013). Females are still racially skewed without any balance in the short term. The female students in Nigeria might be very intelligent, hardworking and interested in engineering but this genuine zeal is stunted by socio-cultural belief and practices, family, economic status, fear of

unemployment, gender description in terms of job placements and more importantly the professional bias created by the age-long belief that engineering is meant for men.

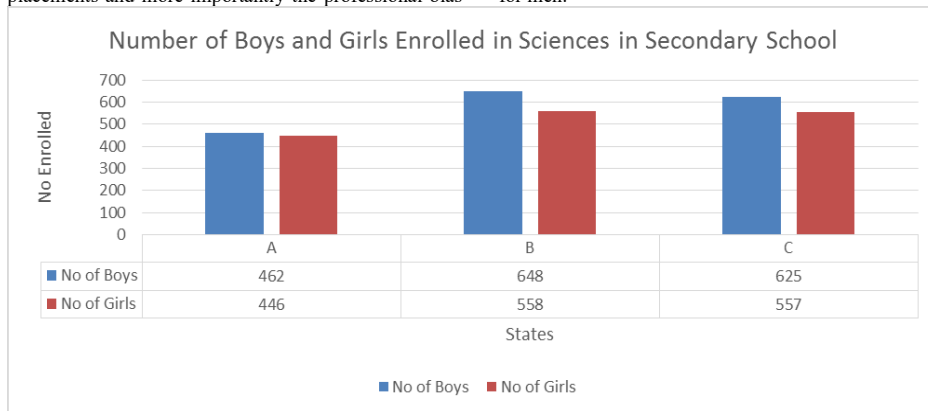


Fig 5: Statistics of Number of Boys and Girls Enrolled in Sciences in Secondary Schools

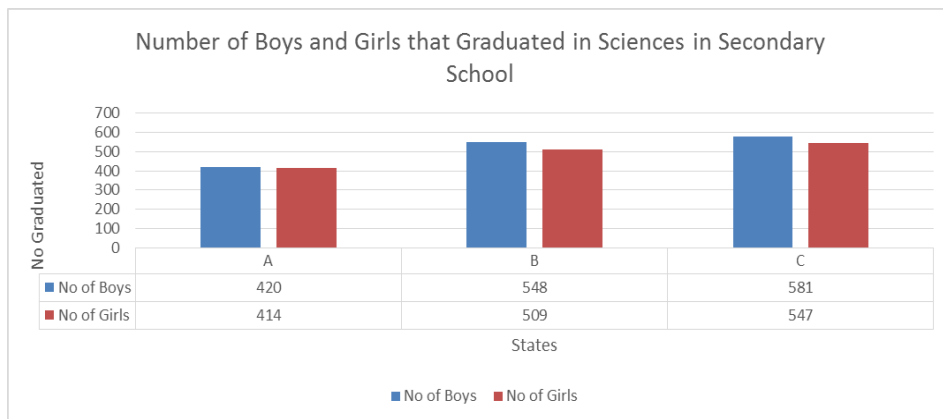


Fig 6: Statistics of Number of Boys and Girls that Graduated in Sciences in Secondary Schools

Despite these challenges, women have been encouraged to study and excel in STEM more than before (Corbett and Hill, 2015). The excellent performance of female in STEM in tertiary institutions has not been matched by the total representation of females in these fields. In Nigeria, 70% of university students are females but less than 20% of their student graduate with STEM degree. Admission quota of Universities in the STEM faculties

does not encourage women to fill the spotlight to produce engineering graduates who will contribute to the predicated million STEM jobs anticipated due to shift in global technology and engineering.

Analysis carried out in 4 universities in the South-East demographic region shows that in a STEM faculty that has 100% students, 25% average of them are females.

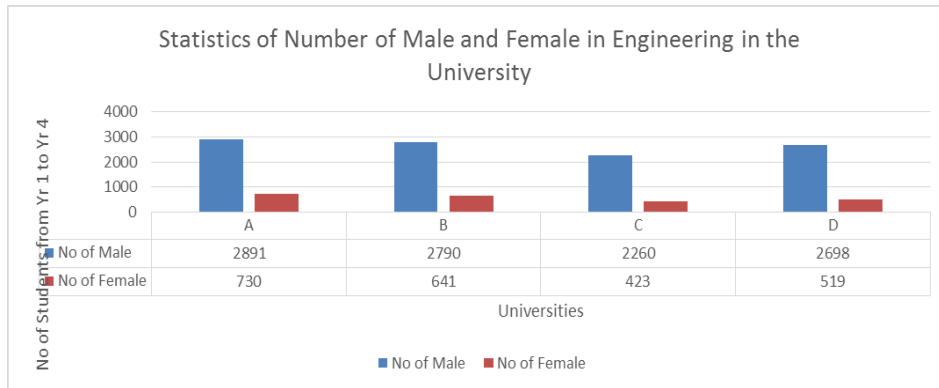


Figure 7: Statistics of Number of Male and Female in Engineering in Nigerian University.

The unhealthy data occasioned by the unseen reason for such statistical imbalance shows that the trajectory of learning that saw these students from secondary school to University education has unattended flaws. It shows that the initiatives carried out in primary and secondary school levels to encourage female students in STEM have little noticeable impact and gendered patterns of participation in engineering courses still persists (Smith, 2010). Due to the excellent performance of women in STEM, a variety of causes has contributed to failed admission and recruitment endeavors in Nigeria. These include social factors (Cho *et al.*, 2019), institutional structures as x-rayed by Bottia *et al.* (2015), Bad or poor guidance and counseling (Lee, 2008), Early classroom and educational environment also contribute to this scenario (Han, 2016).

If Nigerian girls are encouraged to develop interest linked to STEM from young age (Bieri, Busclor *et al.*, 2014), and mentored all the way, University intake will definitely be increased. Having supporting family (Burge, 2013) is an added advantage. Lee (2016) expressed confidently that family has a role to play. Active and quality counseling is very important (Byars – Winston 2014). These approaches can play a part in guiding the female child in the choice of career in engineering discipline before seeking for admission into the University. It is very important to understand that early exposure to STEM can be a precursor to pursuing engineering discipline (Bishop, 2015). There should be massive advocacy in increasing gender diversity which should focus in the field among primary and secondary students (Valla and Williams, 2012). Secondary school background plays a vital role in determining the percentage of females that seek admission in engineering and STEM courses (Redmond–Sanogo, Angle and Davis 2016). Exposing the girl child in secondary school to

interact with influential women in engineering and mathematics will help to motivate them in choosing STEM courses as a career (Conklin, 2015).

Encouraging the women to attend STEM focusing secondary schools (Science school) will enhance their interest in science and engineering careers (McKnight, 2016). This will positively motivate the girl child to view science and engineering as a career before seeking admission in the University (Heilbronner, 2011). There must be a holistic approach to disabuse the mind of the elites in academia and industries of the purported assumption that engineering is a masculine profession.

JOB OPPORTUNITIES AFTER UNIVERSITY EDUCATION

This discrimination spans through the job market and this is more particular in Nigeria and other African countries. America and European countries motivate the women to take up STEM as a career. Their industry leaders emphasize on high level skilled employees in engineering (Bottia *et al.* 2015). A current study in the United States shows that the motivation to pursue STEM as a career was the greatest platform in the recruitment of employees especially women and this makes stake holders to increase the intake of female students to study STEM as a career. (Franchetti, Ravn and Kuntz 2010).

According to the National Bureau of statistics, women make up on average about 22% of total number of engineering graduates; just 8% of this number becomes gainfully employed. This scenario indicates that women are passive users of engineering technology. Research has shown that more useful tools are created when developers fully represent the diversity of the societies, we live in. In the near future, 90% of jobs will require STEM skills and if women are under-represented as

engineering & Technology developer and sophisticated users, Nigeria and Africa will be left behind in the tomorrow's world.

We lack accurate statistical data, but available data shows that infinitesimal number of women engineers are employed in industries and academic institutions. Research has shown that 9% of the total engineering workforces in multinational companies are women. This shows the stark reality of gender discrimination of women in engineering. The inter-sectionalism of ethnicity, socioeconomic background and gender makes it extremely difficult for women to secure good jobs in engineering and technology firms. This situation is worsened by the complexities and nuances that intersect with social class, sexual orientation, ability and religion. The worst and biggest barrier to women in engineering is the perception of engineering as a male dominated profession (Lee, 2008). This encourages knowingly or unknowingly the reservation of engineering and technology job vacancies for men. This contributes to lack of interest in engineering by women and purely analyzed, occupational segregation and afflicted stereotypes are strong career index which make it one of the biggest barriers for women to go into STEM courses and eventually get employed.

In the academic environment women engineers who scaled the hurdle to secure a job as lecturers and administrative staff are trapped with social experience in the academic environment. They are faced with the challenges of making themselves relevant in the acclaimed male dominated profession. They must do something extraordinary in particular to have sense of belonging in the STEM fields (Seaton, 2011). They will feel more pressure to conform to the masculine norms, spanning through high level of gender competition; working extremely hard to prove their claims of scientific authority. Inadvertently, having to exert additional effort than the male counterparts to belong and succeed at the workplace can discourage women to pursue and excel in the work place. Those who are not aware of the challenges at the workplace may be discouraged to go into engineering or STEM courses simply because job placement advertisement in these courses are not targeting women (Glass and Minnoth, 2010).

From a positive perspective, if there is assurance of job placements, supportive graduate experience program; there will be successful career trajectories in the end, both in academic, industries and private sector. (Clark *et al.*, 2016). The gender discrimination and the obnoxious environment which women are subjected to in terms of

job placement after their graduate program makes them unlikely to view academics as paying career (Morrison, 2013).

ENTREPRENEURIAL SKILLS

Female engineers need to be encouraged to develop entrepreneurial skills and be supported once such skill is ready to be harnessed. This can be achieved by providing increased awareness on the hardware, software and digital resources the women can access, to successfully float and scale their companies. Also, Grants and loans can be given by the government and institutions to support programs that help women become entrepreneurs. Incentives can be given to organizations that engage female owned engineering firms for their support and patronage. Women must always seek to learn more skills to make themselves very relevant among their contemporaries. However, the need to build self-confidence, have a role model/mentor and be dedicated in hard work is highly encouraged.

Below are statistical data calculated from higher institution of learning and industries in South-South and South-East Nigeria.

EXECUTIVE GENDER BIAS IN THE INDUSTRY AND ACADEMIA.

Women experience gender bias despite the infinitesimal number employed by industries and academic institutions. In reality, the ability of women to persist and work in engineering field is linked to their internal motivation and commitment. The domineering attitude of men and the persistent perception of engineering profession as a male dominated profession contribute to this situation. This scenario contributes to lack of interest and discouragement for women to excel. These complexities bring to the fore the difficulties women pass through as STEM professionals.

Definitely, challenge abounds for women pursuing a career in male dominated field like engineering. It is very intimidating since the former are the minority and this can lead to a lack of confidence. Despite the achievement of the present-day women in engineering, they are still under represented in leadership positions. In Nigeria, approximately, 10% of women are deans and departmental heads. According to McCullough (2011), data in United States show that one quarter of deans and heads of department in engineering are woman. Following data from national science foundation (2011), women have almost approached parity in several field of STEM, yet the gap in executive cadre of industrial work force and academia still persists.

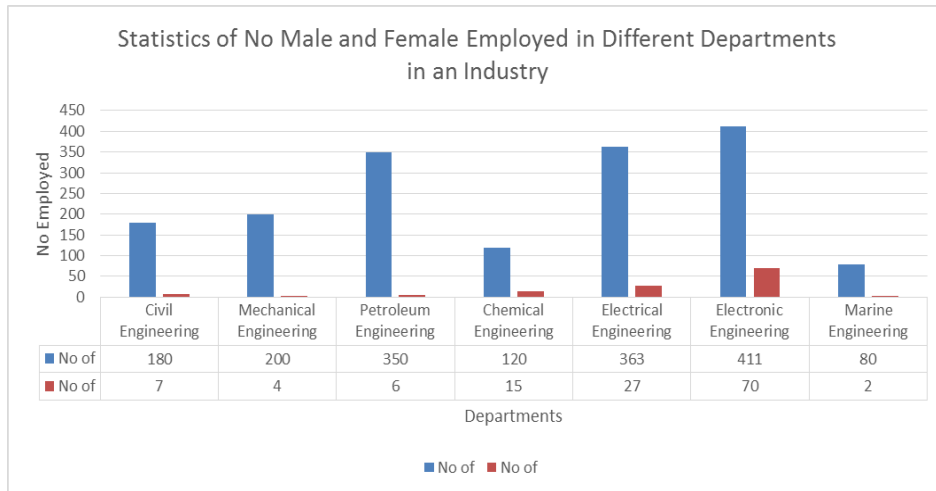


Figure 8: Statistics of Number of Male and Female Employed in Different Departments in an Industry

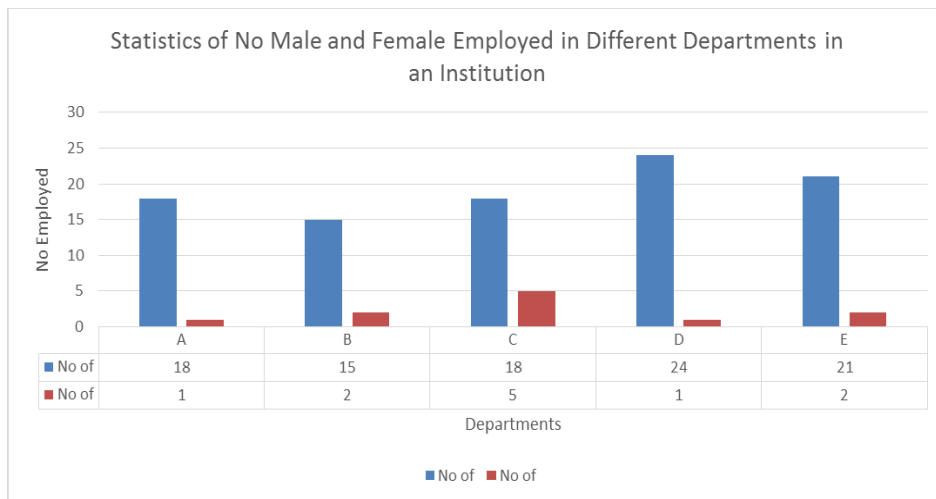


Figure 9: Statistics of Number of Male and Female Employed in Different Departments in Tertiary Institution; A-Chemical, B-Civil, C-Electrical/Electronic, D-Mechanical and E-Petroleum Engineering.

Even at the executive level, overt sexism is declining but covert sexism and discrimination are still major factors that prevent women from getting to the high executive position. This raises the issue of prejudice in engineering leadership positions.

A correlational study that compares women salaries against men in industries shows that even if the background factors are the same, women progress is slower. Using experimental case study, where biases are examined, and the clinical trial shows that both men and

women exhibit biases against women as leader (Eagly and Carli, 2007). Considering executive and leadership styles in existence, women are not accustomed to transactional leadership style which is the base of male historical leadership style. This leadership style includes keeping people in line, given direction praising and punishing. Presently women are more likely to exhibit transformational leadership style which entails inspiring workers, promoting innovation and serving as a role model and building a community. In engineering, the leadership of the woman can be problematic when she does not follow gender norms. Resistance to women leadership style or attaining the highest level of organizational leadership in engineering is strongest in highly masculine domain. These women engineers need to behave extra-ordinarily different from other women leaders on other professions. These scenarios determine the level a woman can attain in a male-dominated profession.

PROACTIVE MEASURES TO REMEDY THE SKEWED GENDER RATIO

Women in engineering seem to be at the receiving end in the industries, academia and management. They are still in the minority and needs to be encouraged. This can be achieved by implementing the following:
There should be a proactive approach to increase the number of women admitted in the Universities to pursue career in STEM.

Concerted awareness program should be designed to enlighten women in engineering of their potentials and right as an engineer.

Distinguished women engineers should be celebrated as mentors to encourage other women to aspire high. Women should present themselves as fellow contributors to engineering process measured on competences and excellent results. When they exercise empathy and diplomacy either in the industry, academic environment or management position, it will repose confidence on women engineers.

Government must legislate laws that will ensure equity in terms of opportunity given to women engineers. Private and government industries must be made to uphold policies and reinforced elites that will ensure zero discrimination.

Consideration must be given to women engineers of child bearing age. There should be flexibility in assigning jobs to them.

There should be proper advocacy and awareness program to ensure equity and justice for both genders. There should be re-orientation of the general society and some parochial cultures that conceal women's future to

perpetual housekeepers and for child bearing purposes. Families of women engineers must help to encourage women career engineers.

Women engineers (e.g. Women in Engineering WIE, Association of professional women engineers of Nigeria APWEN etc.) should continue as a body to chart an undivided course for the progress and growth of women engineers. These bodies can intensify the advocacy program from the grass root, through the formative stages of primary and secondary education.

CONCLUSION

From the research conducted, it can be seen that there is limited number of female engineers in public and private owned establishment, industries and the academia. The root cause seems to be the very low participation of females in STEM. Many girls struggle to be associated with celebrities than engineers. Engineering job require resilience, determination and personal belief to succeed. These attributes are still lacking in women (Mrityunjaya, K. 2013) thus, Female engineering graduates find it very difficult to get a job (Parikh and Sukhatme, 2004). In many

Countries, participation in engineering by women is negligible and the work force is about 6.1% of the general engineering work force population. In academic environment, about 25% of the total faculty strength is women. This seems to be the preferred areas for employment by women (Parik and Sukhatane, 2004).

To remain relevant in the engineering industry, women must balance the pressure that is associated with field, office and industrial work in relation to family responsibilities to reduce the family/work conflict and achieve a fulfilled career life.

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SCIENCE EDUCATION (SED)

WIS –SED 07

INVOLVING THE GIRL-CHILD IN THE LEARNING OF SCIENCE, TECHNOLOGY, ENGINEERING AND MATHEMATICS (STEM): Panacea to National Development and Social Equality.

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Abstract

The role of Science and Technology as the fulcrum of National Development and Social Transformation, in the development of any human Society at this digital age cannot be overemphasized. Involvement in Science, Technology and Mathematics (STEM) among Citizens should thus be well embraced by all, irrespective of Gender in order to develop the Nation and promote Social equity among the people. However, this is not so in most African Societies. It is in unequal basis, with the female Gender at the receiving end. This paper looked into the enrolments of Students by Gender into basic Schools being the foundation on which Children intellectual capability is believed to be formed. It examined the societal biases hindering the Girl-child involvement into Science and Technology. The paper discussed the low representation of women in Science, Technology and Mathematics fields and concluded by suggesting ways of enhancing the learning of Science, Technology and Mathematics in the Girl-child as panacea to National Development and Social equality in the Society.

Keywords: Girl-child, STEM, National development, Social equality.

Introduction

The importance of Science and Technology in the development of any human Society especially in recent times cannot be overstated. It is the fulcrum for National Development and Social Transformation. Hence there is the constant need for the development of Science and Technology in every Sector, and the involvements of both gender in order to achieve the required science education. Despite this, a limited participation of the Girl-child in Science, Mathematics and Technology in many Countries in Africa, Nigeria inclusive. is an issue that calls for attention; same has also been recorded around the World (Kishore, 2008; McCarthy, 2003; Ellis, 2003 and Andrew, 2007). This resulted from many factors noted to affect girls' learning. Key among the factors are 'Cultural Biases'. These biases are inculcated into Science, Mathematics and Technology instruction and assessment, thus influencing the attitudes of both girls and boys towards learning Science. The culture learned directly or indirectly to a large extent determines how a person thinks and feels, directs his or her actions, and defines his or her outlook in life. Surprisingly, parents and teachers are also influenced with these cultural biases which are developed in an unequal basis in many African Societies, with the female gender at the losing end. The plethora of Science sub-Disciplines often share a male-dominant culture, which has been inherited from institutional environment that created them. Many believed that Science is the domain of males and that it is not necessary for girls' future lives, as girls will ultimately end in the kitchen after all their Education. Also, male Children are believed to be more capable when it comes to Science learning. The gender inequality of the Science Community as well as lack of interest

from girls in Science is a well-known challenge among Scientists. The wrong notion that science is for males need to be corrected.

The importance of Science Education for all

Science Education is a field of study concerned with producing a scientifically literate Society. It acquaints learners with certain basic knowledge, skills and attitudes needed for future work in Science and Science-related fields (Omorogbe and Ewansiha, 2013). Science plays a central role in the contemporary Society, having the potentials of improving lives in diverse ways to promote National Development. Science being a part of everyday life helps us develop a growing body of ideas which is essential for understanding the World. The scientific skills and knowledge acquired by the citizenry increase their access to information and give jobs opportunities. It is of importance to know that any Nation that disregard the provision of good quality science education may become a dumping ground of other Countries innovations, and lack the necessary human resources for development and competence in the global economy, since Science can be consider as the central of industrial development and the link between Technology and Socioeconomic Development. A Country's competence in securing good health, combating diseases, reducing pollution of environment, producing good food for its people, creating job opportunities and developing new Industries and Technologies is all dependent on the Scientific knowledge and skills of its people. It was reported by Omosewo (2009) that many developed Nations of the World were able to achieve much in Science and Technology through Science Education.

Declining interest and negative attitude towards Science Education among youths in general and girls specifically.

Recently, discussions on Education have moved from focusing primarily on School access to placing greater emphasis on the learning that takes place in classrooms. It is of importance that Science is a critical part of the learning that all children need to acquire. It is noted that although many children enroll for Primary Education, they still lack adequate basic knowledge and numeracy skills, with their Science competency not systematically assessed. Science, Mathematics and Technology are prominent among the foundational learning of every child. Any child denied this, may find it hard to progress in learning at higher levels. While looking at enrollment and gender in basic classes, it is noticed that girl-child maintained a reduced number compared to boys as children grows to higher learning. This is evidenced below

Table 1.1: Distribution of Enrolment in Primary Education by Year and Sex

Year	Male	Female	%F	%M
2014	13,255,789	12,545,408	48.6	51.4
2015	13,393,310	12,049,225	47.4	52.6
2016	13,435,940	12,155,241	47.5	52.5

Source: Nigeria Digest of Education Statistics, Federal Ministry of Education

Nigeria has in recent years sought to ensure equitable access to Education for all. In this context, girls' access to Education has received attention at the policy level, and progress has been made toward gender parity in Primary School enrollments. However, far less attention has been paid to girls' participation in different areas of learning, including Science and Technology. Junior Secondary Education

Table 1.2: Distribution of Enrolment in Junior Secondary School by Year and Sex

Year	Male	Female	%F	%M
2014	3,311,470	2,891,624	46.6	53.4
2015	3,260,109	2,920,182	47.2	52.8
2016	3,181,810	2,786,332	46.7	53.3

Source: Nigeria Digest of Education Statistics, Federal Ministry of Education

Percentage Distribution of Enrolment in Junior Secondary Schools by Sex, 2014 - 2016 Table 1.2 reveals the percentage Distribution of Enrollment in Junior Secondary Schools by Sex for the period of 2014 to 2016. The table shows that Enrolment into junior secondary schools on the average was about 47 per cent

for girls and 53 per cent for boys in the period under reference.

Nigerian Government in recent years, have been attentive to improving the National capacity in Science and Technology, a key vision for her 2020 Plan for Economic Transformation but yet to fully consider the relevance of gender equality in Science Education and initiate National Policies toward this goal. Although, female participation in Science receives mention in Nigeria's contemporary Science and Technology Policy, but has no specific objectives developed (Federal Ministry of Science and Technology, 2012). This could be due to the unconsciousness of the Science gender gap and the relevance of females' participation in Science. Some clues have been recommended by Ekine, (2013) to be of importance in promoting gender equity in Science by improving Science Education for girls and boys in Nigeria these include; improving data quality and accessibility; incorporating gender in Science classrooms; building on Science, Technology, Engineering and Mathematics (STEM) initiatives in Nigeria; women helping women in Science; and enhancing partnerships to promote girls' participation in Science.

Low Representation of Women in STEM Fields and Science Research Globally

Despite the efforts to increase women's involvement in many diverse fields of study, UNESCO revealed that Women constitute a minority of the World's Science Researchers. Throughout the World, women is noticeably underrepresented in Science, Technology, Engineering and Mathematics (STEM), Men are more numerous than women as Students, Educators, Researchers, and Workers in these fields. Though there has been an increase in number of Women enrolling in higher education, Men significantly outnumber Women in Science and Engineering Disciplines. Ekpo (2004) and Ithen (2002) observed that though there has been considerable progress in facilitating Women access in Education lately, there is still gender disparity in performance and completion of Science, and Technology based programmes. It has been speculated that Women shy away from Science, Mathematics and Technology related courses. Means of closing the gender gap in Science, Mathematics and Technology is of critical importance for all countries to promote gender equality and Women empowerment in a bid to fight poverty and diseases, also to arouse sustainable development (UNGA, 2000). It will also help check the loss of vast human resources that could contribute to National Development and could further entrench social inequality of gender in Society.

The major determinant of a Country's socioeconomic

development and National competitiveness is her ability to create, apply and diffuse Scientific and Technological knowledge which should involve both genders. Many African Countries are deficit in generating the human Technological capacity for their economic development because of ascribed gender ethics, cultures and belief. Women should not be limited to be being passive users of Science and Technology but should be active participants in Scientific development, applications and decision making, Nations need to be proactive, ensuring that Science and Technology initiatives are implemented to address the needs and preferences of both gender (Rathgeber, 2009). The exclusion of Women from participation and high achievement in Science Education may result to limited access to jobs in these fields, which are among the fastest growing and highest paying. Studies have shown that a Student's performance in Science and Mathematics is a strong indicator of later earnings (Crawford and Cribb 2013). Furthermore United Nations Department of Economic and Social Affairs (2010) opined that the exclusion of females from generation and application of Scientific knowledge represents a tremendous waste of human potentials.

Nigeria and Science Education

Education is believed all over the World to be a catalyst for National Development. The Nigerian Government has also shown their recognition of this fact in Nigeria's National Policy on Education. Education was set as the greatest investment that the Nation can make to address the needs of the Nation and to support the Government Development goals. Nigeria has identified Education as a fundamental human right and shown her full involvement in being a signatory to major conventions for the protection of the rights of children and women. Education is believed to be for all, irrespective of gender. In order to achieve the Education For All (EFA) Goals, Universal Basic Education was enacted in 2004. This consists of six years of Primary Education and three years of Junior Secondary Education, which amount to nine (9) years continuous Education. If only more girls could be persuaded to take up Science and Technology subjects in Schools, and could be persuaded to do better in them, there will be more benefit of an increased Technological output with little inputs. Full benefits of Education can be derived only when there is no bias in the gender concept a Society holds. For Science to largely remain the domain of men is a sure means to perpetuate existing inequalities on the basis of gender in Society. The need for gender complementarity goes beyond mere lending of helping hands for effective contribution to National Development.

The nature of the concept of gender in any Society determines the existence and way of life of the members

in that Society For effective Social transformation therefore there is need for both formal and informal Education to address the gender imbalance in Society that prevents females from being at their optimum best in the development race. There are 69 million women and girls in Nigeria UNESCO (2012) and their exclusion from the generation and application of Scientific knowledge represents a tremendous waste of human potential. Women undertake 60 to 90 percent of agricultural production activities in the developing World, and they carry the primary responsibility for providing for the water, energy, sanitation and health care needs of their family and communities (UNESCO, 2012) Moreover, women exclusion from participation and high achievement in Science Education offers then a limited access to jobs in these fields, which are among the fastest growing and highest paying. Studies have shown that a Student's performance in Science and Mathematics is a strong indicator of later earnings (Crawford and Cribb, 2013). This is the case even within the Education system itself, where teachers and lecturers in Science are often paid more or have a competitive advantage over their colleagues in other fields (Crawford and Cribb, 2013).

It was also recorded by Marshall and Horton (2011) that it is in Science and Mathematics subjects, that many of the cognitive, affective and psychomotor skills necessary for individual and National Development, such as higher order thinking and problem solving, are expected to be learned. The study which is on the Relationship of Teacher-Facilitated, Inquiry-Based Instruction to Student Higher-Order Thinking on School Science and Mathematics further revealed that cognitive ability has no effect on participation of females in SMT Education and in support Viadero (2006) discovered that difference in brain structure and maturation rates may account for differential performance in School-related tasks but Erinosh (2008) poised that ability is not a determining factor in performance because males and females are found to perform equally if instructional context is fair and conducive. Thus, cognitive ability though a vital tool for retention is not a determinant for participation in SMT education but strategies for increasing female participation could be incentives like scholarship, and the use of pragmatic approach in the teaching/learning process to help increase female participation in SMT.

Results from studies revealed that after decades of Science and Technology interventions for development, women's overall position are seen to be declined in relative to men's, and women have become disproportionately poor in comparison with men in their communities (Ekine, 2013). Rathgeber, (2006) recorded that the gender gap in Science and Technology must be more effectively addressed to benefit both Society and

the individual. As women increasingly participate in Science and Technology, their Communities and Nations will also reap the benefits. However, Educationalists must reexamine the pedagogy and practice of Science, given the ongoing underrepresentation of girls and women in Science. Rathgeber, (2009) thus recommend that women should not be limited to being passive users of Science and Technology but should be active participants in Scientific Development, in application and decision making and ensuring that Science and Technology initiatives are implemented to address the needs and preferences of both sexes. Closing the gender gap in Science is thus essential for ensuring that women as much as men benefit as citizens and contributors to their Societies.

Reasons for the Science Gender Gap

There are various factors identified as contributing to female's on-going low rate of involvement and low-performance in Science. This could be in relation to nature or nurture.

One argument by Gray (1981) for example, is that because girls' brains develop differently from boys', biological differences explain the gender gap in Science. Some have posited that the developments in boys' brains result in better developed visual spatial ability than that possessed by girls, and that this could explain differences in abilities and interest in Mathematics and some Science Subjects (Johnson, 2007). Others like IEA, TIMSS (2011) find the evidence inconclusive and disputed the argument that biological factors cause gender differences in performance or interest in Science. Moreover, the fact that in some instances girls perform as well as or even better than boys in Science Popoola, (2002) would seem to contradict the view that gender disparities are due to innate, biologically determined characteristics. Furthermore, counter evidence to the view that boys are "naturally" better at Science than girls is recorded in the most recent TIMSS, conducted in 2011, where data were gathered from 63 Countries regarding Student performance at the fourth and eighth grade levels. Although only two African countries (Botswana and South Africa) were included, the Study revealed that in 10 countries, girls outperformed boys in Science in the eighth grade. In 9 countries, gender disparities in performance favoring boys persisted, but in 12 countries that had previously shown such a disparity, gender parity has now been achieved. This provides counterevidence to the view that girls are not as capable as boys in learning Science.

It is also important to note that social positions ascribed to a child in the society, to some extent determine their areas of interest, even in terms of Education. Home orientations have major effect on a child's choice of Subject. In majority of African homes, anything that

requires repair or need to be mended is known to be for the male child, while those ones that require cleaning or caring attention are seen as females' responsibilities. The girl child grows with the formation of Societal expectations, which continue to dictate her way of life, nature of work she could do, areas she could be seen, what she could say in the public to reflect her decency, etc. In Nigeria it is ethical for a good woman not be inquisitive or curious about anything. Inquisitiveness is for men and this is one of the requirements of Science. Therefore for effective participation, there is the need to refocus home orientations to current requirements for Development to meet the challenges of the future.

It is not surprising, then, that research gathered in multiple cultural contexts draws attention to extrinsic factors that contribute to the persistence of the gender gap in Science participation, interest, and, in some contexts, achievement. The most influential of these factors are sociocultural beliefs that favor males and classroom practices that discourage girls in their pursuit of Science. These include societal beliefs about females' innate abilities and social role biases in the curriculum, teacher-student and peer interactions, and the methods of pedagogy and assessment. It has also been observed that teachers treat Students differently depending on their gender. It is of the researcher's opinion that teachers' assumptions and expectations have remarkable impact on Students' achievement in Science. Thus, Udeani (2012) recorded that schools play important role in the girl-child involvement in science by the way and manner of which the curriculum is implemented.

Sociocultural Beliefs and Practices

In many African countries, girls' exclusion from Science, according to Kitetu, (2008) can be attributed largely to the construction of feminine identities, ideologies of domesticity and gender stereotypes. Formal and informal sociocultural norms and expectations about the role of females in Society have tremendous effects on girls' Educational opportunities, learning outcomes and decisions about Study and work. Gender discrimination is a critical factor facing females' effective participation in every field of Science. The discrimination results from combination of built in biases that make them less likely to participate in Mathematical, Critical and Technical profession.

At the most basic level, obstacles to School access and retention remain fundamental barriers to girls' participation in Science, both as children and adults. Girls outnumber boys among children out of school, and they are more likely to begin schooling at a later age. In Nigeria, late school entry is a particular problem among poor children and girls. Less than 50 percent of the poorest girls are enrolled in school at age six (Kitetu, 2008). The Girl-child, according to Lewis and Lockheed

(2006) also face greater constraints on pursuing their studies due to household demands on their labor, threats to their physical safety, a lack of necessary sanitation facilities at school and societal beliefs that privilege investments in boys' Education.

SUGGESTED WAYS OF EARLY INVOLVEMENT OF GIRL-CHILD INTO SCIENCE LEARNING:

Some ways of involving girl-child in the learning of Science in Nigeria has been encouraged. Learning should starts early in children, so the 'Catch them Young' idea is necessary as Primary School Basic Science learning is vital to the girl-child involvement in Science.

The way and manner of inculcating ideas to learners should be active participation, such that catches children's interest and build positive attitude towards Science learning. These can be easily done through

Parents encouragement

Active Learning:

Demonstration teaching method (using real objects)

Use of role-play and storytelling

Use of collaborative pedagogy

Pictorial teaching models

Outdoor Science class when required

Use of audio visual instructional materials

Including native dialect in pedagogy:

Use of local examples in teaching science.

Gender Sensitivity:

Women mentoring Women in Science

Building on STEM Initiatives in Nigeria

Data Quality and Accessibility

bias cultural norms that affect classroom practices and Students' learning, and Policy responses are also needed in order to involve girls in Science from an early age. Science Education and Social Equity

Educational equity is the achievement of fairness, justice and impartiality in Education. This arises from the fact that some Students have different need than others because they may come from a disadvantaged environment. Essentially, power relations, discrimination and the guarantee of equality are defined through Education. According to Liliانا (2015) and Women in Parliament (2015) it can be used as a tool to form prejudices that lead to discrimination, or we can teach students to accept a multicultural and diverse Society in which men and women are equal. An equitable Education system helps all Students to develop the knowledge and skills they need to be engaged and become productive members of the Society. More importantly the act of giving all children an equitable start, would lead to better economic and social outcome for individuals, regions and for the country . This paper emphasize the importance of targeting girls at the primary school level, when fundamental knowledge and skills are being acquired, when interest can be most easily sparked, and when the greatest numbers of both boys and girls are in school. The Study also recommends that the Society should be enlightened on the need to change attitude to gender roles, and the need to initiate affirmative actions for females in SMT education. In the same view, females are encouraged to be assertive, standing for their right to avoid unnecessary marginalization, and the School and the curriculum should be gender responsive.

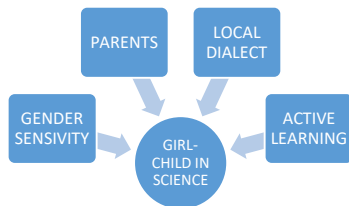


Fig: Involving the Girl-child in science Learning

The quality of what these children learn is of great consequence and serves as a pull factor that will bring children to School and keep them in School. In particular, learning in Science builds cognitive and non-cognitive skills in Students that are invaluable for both their individual lives and the Development of Society as a whole. It is now thought necessary that the girl child should be engaged in Science, Mathematics and Technology at their early lives, that is, in Primary School level where their interests and attitudes about learning are formed. There is need for consciousness to gender

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WIS-SED 08

CHARACTERISTICS OF RESEARCH IN SCIENCE TEACHING AND LEARNING LITERATURE IN NIGERIA FOR NATIONAL DEVELOPMENT

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Abstract

Academic research has been identified as the basis for the development of national policies in Nigeria. This is because evidence-based planning and implementation of policies rest mainly on data that inform decision making in an empirical fact findings. The study was therefore conducted on the characteristics of research on science teaching and learning literature in Nigeria. The design of the study was the single case design. Data collection used, geographical dispersion of citation, core journal in use, form of authorship and categories of authors. Percentages were used to analyse the data collected. The study revealed that the preferred format by authors in research on science teaching and learning is the monograph, followed by journals. It also revealed that most academics preferred single authorship and that the literature was dominated by male authors. It was concluded that researchers in science teaching and learning paid more attention to relevance of citation in literature such as journals, theses, conference proceedings, newspaper and magazines. The study recommends that there should be provision for foreign literature of various format in our libraries and that libraries should be provided with recent journals among others.

Keywords: Bibliometrics, Literature, Research in Science Teaching and Learning

INTRODUCTION

Science education can be seen as an integral part of study, putting into consideration both the subject matter of science disciplines such as traditional sciences (The Biology, Chemistry, Physics) as well as the processes involved in the teaching and learning of the subject. This implies that the learner should be exposed to the nature of science and scientific processes. Going by the current low level of our national development, there is the need to reposition the stance of the academic in the area of science teaching and learning. This can adequately be carried out by reviewing the related literature in science teaching and learning through research.

Research in its broad sense is the formal systematic application of the scientific method to the study of problems. It is a means of providing solution and answer to identified problems. Research can therefore be seen as building on existing foundations (Ayeni, 2007). Research can also be seen as the careful diligent search. It is a studious inquiry or examination or investigation or experimentation aimed at the discovery and interpretation of facts; revision of accepted theories or laws in the light of new facts or practical application of such new or revised theories or laws (Apeji, Omole and Ubani, 2015).

Research in science teaching and learning is the fulcrum of technology and national development. Changes in economic competitiveness are creating demand for science and technology education. This can adequately be achieved through proper research in science teaching and learning by having access to related research report where they may exist and by

being conscious of the pattern of publication within a given field or body of literature in respect of the various teaching methods and their effects on students' academic performance.

According to Apeji and Akinsola (2015), the work of Cole and Eales (1917) on statistical analysis of the growth of the literature of comparative anatomy from 1550 to 1856 opened a new direction in intellectualism which consequently led to new sphere in research, known as bibliometrics. Bibliometrics is a type of research method used in information science and which utilizes quantitative analysis and statistics to describe pattern of publication within a given field or body of literature (Apeji and Akinsola, 2015). A major area of bibliometrics research is citation analysis which helps to establish links or relationship between authors or their works. When one author cites another, a relationship is established. Many links such as links between journals, between field or even between countries etc. can be ascertained. Citation both from and to certain documents may also be studied. A combination of all or some of the aforementioned characteristics could also be a basics for study.

The studies of Earle and Vickey (1969) and Aiyepoku (1973) on the characteristics of subject literature have established a basis for critical examination of subject literature. In their various studies, Adewole and Ajala (2007) Nnadozie (2008), and Apeji and Akinsola (2015), revealed that journals were the major medium of publication in Nigeria. However Apeji and Akinsola (2015), pointed out that monographs were found to be most preferred medium in Nigeria. In another development, Adewole and Ajala (2007), Apeji and

Akinsola (2015) revealed in their various studies that the literature of brucenosis in Nigeria is dominated by single authorship.

Available literature show that efforts have so far not been directed at the characteristics of science teaching and literature. It is on this basis the present study is being carried out.

Problem of the Study

Authors in science teaching and learning need to generate and expand the literature in the field for findings and publications of new facts. The characteristics of the literature are supposed to be subjected to scientific examination in the interest of scholarship, but they are not. This is the basis for this study which intends to analyse the characteristics of the literature of science teaching and learning in Nigeria and thereby making it more accessible for effective use.

Research Questions

The study seeks to answer the following research questions:

- What are the formats of the use for the literature of science teaching and learning?
- What are the geographical dispersion of the citations in the literature of science teaching and learning?
- What are the forms of authorship in science teaching and learning literature?
- What are the gender categories of authors in science teaching and learning literature?

Purpose of the Study

The main purpose of this study is to determine the characteristics of the literature of science teaching and learning in Nigeria. Specifically, the study sought to find out the
 Format of use
 Geographical dispersion of citations
 Form of authorship
 Gender categories.

Method of Study

The study is a single case design, which assures the form of an experimental design when an existing case is observed for sometime so as to study or evaluate it. The target population for the study is all the journals relating to science teaching and learning in Federal College of Education Library Kontagora, Niger State.

To select a journal in the area of science teaching and learning and to generate the data base for this study, the following criteria were used:
 the journal must be Nigeria base
 it must be published by a professional association of science education
 articles published in the journal largely make original contributions to empirical studies, therefore contribute to knowledge

high academics and professional standing of authors published.

The journal that satisfactorily meets these criteria in my view, is the journal of Science Teachers Association of Nigeria (STAN). The Journal is a publication of Nigeria Science Teacher Association. The journals were then subjected to examination and evaluation on the basis of distribution of article by volume, issue and year; distribution of citation by format and geographical location; distribution by forms of authorship, distribution of authors by forms of gender.

At the end of the selection exercise, a total of 167 articles from 12 Journal of Science Teachers Association of Nigeria were used for this study. This distribution of the articles selected by volume, issue and year is presented in the table below.

Table 1: Distribution of Article by Volume, Issue and Year

S/N	Year	Volume and Issue No	No of Articles
1.	1998	33 (1 & 2)	15
2.	1999	34 (1 & 2)	17
3.	2000	35 (1 & 2)	11
4.	2001	36 (1 & 2)	11
5.	2002	37 (1 & 2)	11
6.	2003	38 (1 & 2)	14
7.	2004	39 (1 & 2)	16
8.	2005	40 (1 & 2)	13
9.	2006	41 (1 & 2)	12
10.	2007	42 (1 & 2)	11
11.	2011	46 (1)	16
12.	2015	50 (1)	20
Total			167

Result and Discussions

The results are presented based on research questions

Research Question 1

What are the formats of use for the literature of science teaching and learning?

Table 2: Distribution of Cited Literature by Format

Categories

Format categories	Cited	Literature
	No	Percentage
Monographs	924	39.5
Journals	942	40.5
Theses	224	9.6
Conference proceedings	121	5.2
Newspaper magazines	11	0.47
Others	113	4.8
Total	2341	100

The result of Table 2 reveals that a total number of 2,341 citations were consulted by the authors of teaching and learning. Table 2 also shows that monographs have 924 (39.5%) citations, Journals 948 (40.5%) citations, Thesis 224 (9.6%) citations, conference proceedings 121 (5.2%), Newspapers, magazines 11 (0.47%) and other types of format lumped together 113 (4.8%) citations.

The results presented in Table 2 on the distribution of cited literature this indicates that Journal was the most useful format for disseminating research findings in science teaching and learning in Nigeria. The result is in agreement with the earlier study by Apeji and Akinsola (2015).

Research Question Two

What are the geographical dispersion of the citations in the literature of science teaching and learning.

Table 3: Distribution of Citation by Format and by Geographical Location

Format	No of citation	Citation from Nigeria		Citation from outside Nigeria	
		N	%	N	%
Monographs	924	497	53.8	427	46.2
Journals	948	746	78.6	202	21.3
Theses	224	198	88.4	26	11.6
Conference proceeding	121	106	87.6	15	12.4
Newspapers /magazines	11	09	81.8	02	18.2
Others	113	88	77.9	2.5	22.1
Total	2,341	1644	70.4	6.97	29.6

Table 3 revealed a total of 2,341 citations out of which 1,644 (70.4%) citations were from Nigeria while 697 (29.6%) were from outside Nigeria. Monograph accounted for 924 citations out of which 497 (53.8%) were from Nigeria while 427 (46.2%) were from outside Nigeria. Journals have a total of 948 citations out of which 746 (78.6%) were from Nigeria while 202

(21.3%) were from outside Nigeria. The theses consulted by researchers of science teaching and learning were 224 out of which 198 (88.4%) were from Nigeria while 26(11.6%) were from outside Nigeria. Conference proceedings has 121 citations out of which 106 (87.6%) were from Nigeria while 15 (12.4%) were from outside Nigeria. Newspapers and magazine recorded 11 citations out which 09 (81.8%) citations were from Nigeria while 02 (18.2%) citations were from outside Nigeria. Table 3 revealed that other format categories had 113 citations out of which 88 (77.9%) were from Nigeria while 25 (22.1%) were from outside Nigeria.

Research Question 3

What are the forms of authorships in research in science teaching and learning literature?

Table 4: Number of Percentage Distribution of Articles by Forms of Authorship

Forms of Authorship	Authors		Article produced	
	N	%	N	%
Single authorship	97	39.3	97	58.1
Joint authorship	150	60.7	70	41.9
Total	247	100	167	100

Table 4 indicates that two forms of authorship (i.e. single and joint authorships) were involved in the production of articles for research in teaching and learning. The table revealed that a total of 247 authors produced 167 articles. Single authors produced 97 (39.3%) articles while 150 (60.7%) joint authors produced 70 (41.9%) articles. The total number of 247 authors contributed to the production of the entire 167 articles for research in science teaching and learning.

In spite of the fact that joint or collaborative research is encouraged in scholarship, table 4 however revealed that the trend of authorship in research in science teaching and learning was dominated by single authors. These findings corroborated the findings of Apeji and Akinsolar (2015).

Research Question 4

What are the gender categories of authors in research in science teaching and learning?

Table 5: Number and Percentages Distribution of STAN Journal Authors by Gender

Authors Gender	Number	Percentages
Male	201	81.4
Female	46	18.6
Total	247	100

From table 5, it could be seen that out of 247 authors, 201 (81.4%) were male while 46 (18.6%) were female. This shows that literature of research in science teaching and learning was dominated by male authors.

Conclusion

Researchers in science teaching and learning recognized the use of citations in literature, such as monograph, journals, theses, conference proceedings, newspapers and magazines. The analysis of the data collected revealed that journal is the dominant format for the dissemination of findings of research in science teaching and learning in Nigeria.

Recommendations

Based on the findings of this study, the following recommendations are made:

Female educators should be encouraged to contribute to the development of research in Nigeria with emphasis on girls education. Their findings and recommendations will become a challenge to stakeholders in education

It was discovered that most of the journals and monographs that a mainly used by researchers in science teaching and learning are Nigeria base. This is likely because of poor access or unavailability of relevant foreign literature in our libraries. The Nigeria libraries should therefore help to address the gap by acquiring foreign literature of various formats for researchers.

Joint or collaborative research should be encouraged in scholarship. This will enable researchers to improve their knowledge since they have the chance to share ideas with one another.

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WIS-SED-09

ASSESSMENT OF SKILLS THROUGH MAINSTREAMING GENDER IN RESEARCH IN TARABA STATE UNIVERSITY

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Abstract

The study seeks to identify the practical skills through mainstreaming in scientific research in, Taraba State University, Jalingo, Nigeria. A survey research design was adopted for the study. Three research questions and three null hypotheses were formulated to guide the study. The population for the study was 57 made up of 42 students and 15 work based lecturers. The study employ proportionate stratified random sampling techniques to sample students. While the work based lecturers were used for the study, 57 structured questionnaires titled; Assessment Skills through Mainstreaming Gender in Scientific Research (ASMGSR) was used for collecting data from the respondent. The instrument was validated by three experts. Cronbach Alpha was used to determine the reliability of the instrument, which yielded overall reliability index of 0.91. The data of the study was analyzed using Mean to answer the research questions and T- test was used to test the three null hypotheses at 0.05, level of significance. The findings showed that Mainstreaming Gender in scientific research assessment was by lecturer efforts in teaching and Gender adheres pertinently. Considering the result of the study, the researcher recommended that science is very importance in everyday life activities. Mainstreaming gender in scientific research should be strictly introduced and monitor from primary/post primary through tertiary institution because it is a strategy for women as well as men concerns and experiences an integral dimension for any planned action programme in the society at large.

Keywords: Assessment, Skills, Mainstreaming, Gender, Science.

INTRODUCTION

In our today generation, the word 'science' can be defined as a means to satisfy interest, for producing solutions to everyday problems and improving the quality of life. It is also to understand how things work and arouse the economy. The wealth and quality of life of the women and men in Taraba State University is mostly dependent, to a significant extent, upon the successful development of lecturer assessment of skills through mainstreaming gender in scientific research. The word, 'Mainstreaming' is the systematic integration of equal opportunities for women and men into the organisation and its culture and into all programmes, policies and practices into ways of seeing and doing thing. However, this global picture is disfigured by the fact that a person's gender plays unequal role in the likelihood of their being able to enter, remain in and succeed within the scientific research. Hence the presence of women in science has been increasing, extremely few have an equal chance to make a contribution and enjoy the benefits of a scientific research. This is both unjust and inefficient that women play a very negligible role in decision-making about scientific policies and priorities and relatively few follow scientific research. This is not withstanding the facts that they (women) represent over half of the population of this country, they also make an extensive contribution to the scientific development of science and technology and they are on the receiving end of outcomes derived from science research. Thus, women should have the same right to enjoy the advantages that a scientific research can offer and to be involved in decision- making on it. Indeed, their contribution is fundamental to the future

outlook of development of scientific research as today they are striving to see their effectiveness to the better development as mainstreaming in Taraba State University in order to develop science and its claim to the highest standards, we need the best human resources at our disposal, both those of women and men. Therefore, gender mainstreaming has been embraced worldwide as a strategy towards realising gender equality. It involves the integration of a gender outlook into the preparation, design, implementation, monitoring and evaluation of policies, regulatory measures and spending programmes, with a view to promoting equality between women and men, and fighting inequality. The concept of bringing gender issues into the mainstreaming in scientific research was clearly established as a universal strategy for promoting gender equality as contained in the Platform for Action adopted at the United Nations Fourth World Conference on Women, which held in Beijing in China, 1995 respectively. In that conference it highlighted the necessity that gender equality is a primary goal in all areas of social and economic development. In nutshell July, 1997, the United Nations Economic and Social Council (ECOSOC) defined the concept of gender mainstreaming as follows "Mainstreaming is a gender perspective in the process of assessing the implications for women and men of any planned action, including legislation, policies or programmes, in any area and at all levels. It is a strategy for making the concerns and experiences of women as well as of men an integral part of the design, implementation, monitoring and evaluation of policies and programmes

in all political, economic and societal spheres, so that women and men benefit equally, and inequality is not achieved. The vital goal of mainstreaming is to achieve gender equality”.

Mainstreaming includes gender-specific activities and positive action, whenever women or men are in a particularly detrimental position. Gender-specific interventions can target women exclusively, men and women together, or only men, to enable them to participate in and benefit equally from scientific activities. These are necessary temporary measures designed to fight against the direct and indirect punishment of the past discrimination. Mainstreaming is not about adding a "woman's component" or even a "gender equality component" into an existing activity. It goes beyond increasing women's participation. It means bringing the experience, knowledge, and interests of women and men to bear on the development agenda.

It may entail identifying the need for changes in that agenda. It requires changes in goals, strategies, and actions so that both women and men can influence, participate in, and benefit from scientific research. The goal of mainstreaming gender equality is thus the alteration process of unequal social and institutional structures into equal and just structures for both men and women. In this regard, therefore what is the assessment of skills through mainstreaming gender in scientific research in Taraba State University, Nigeria? However, this research work seeks to.

Statement of the Problem

From the foregoing facts on the assessment skills through mainstreaming gender in scientific research, the problems arise through the kind of assessment skills to identify the strength and weaknesses of women and men in scientific research. Skills assessment has various methods to apply, in each level, when and how such skills are available to ascertain the mainstreaming gender in scientific research. By dealing with this kind of assessment there are lack of resource personnel or trained personnel to understand the basis of using the appropriate skills to diagnose the efficiency and reliability measure of mainstreaming gender in scientific research. However, the assessment skills through mainstreaming gender in scientific research in any organised institution should be based in the effectiveness of available resources, methods, and expertise, in handling it in efficiency by the formalised assessment skills of mainstreaming gender in scientific research. With the above buttressing problems involved in mainstreaming gender in scientific research, the researcher is zealous to create a balance in mainstreaming gender of both women and men particularly in Taraba State University, Nigeria.

Purpose of the study

The purpose of the study is to expose both women and men into assessment of skills through mainstreaming gender in scientific research in Taraba State University. Specifically, the study sought to;

1. Identify the assessment skills through mainstreaming gender in scientific research in Taraba State University.
2. Verify the measurable methods that can be put in place while dealing with mainstreaming gender in scientific research in Taraba State University.
3. Recognize roles of different lecturers and students in the University and their ways of improving the mainstreaming gender in scientific research in Taraba State University.

Research questions

The following research questions guided the study. These are;

1. What are the assessment skills through mainstreaming gender in scientific research in Taraba State University?
2. What are the measurable methods that can be put in place while dealing with mainstreaming gender in scientific research in Taraba State University?
3. What are roles of different lecturers and students in the University and their ways of improving the mainstreaming gender in scientific research in Taraba State University?

Hypotheses

The following hypotheses were tested at 0.05 level of significance.

HO₁. There is null all through significant difference in the likert scale response of respondent on the assessment skills through mainstreaming gender in scientific research in Taraba State University.

HO₂. There is an alternate significant difference in the likert scale response of respondents on the assessment skills through mainstreaming gender in scientific research in Taraba State University

HO₃. There is null all through significant difference in the likert scale response of respondent on the roles of different lecturers and students in the university and their ways of improving the mainstreaming gender in scientific research in Taraba State University

Methodology

A survey research design was adopted for the study. According to Osuala (2001) survey research design are studies that both large and small population are chosen from the population to discover the relative incidence, distribution and interrelation of sociological and psychological variables. This method was chosen to help the researchers on assessment skills through mainstreaming gender in scientific research in Taraba State University, Jalingo. The population for the study was 57 people made up of 42 students and 15 work based lecturers. The study employed proportionate stratified random sampling techniques and the

structured questionnaire titled: Assessment Skills through Mainstreaming Gender in Scientific Research (ASMGR) was used for data collection. The instrument was validated by three experts trial tested. Cronbach Alpha reliability technique was used in determining the internal-consistency of the instrument which yielded reliability index of 0.91. Three research questions and two null hypotheses were formulated while T-test was used to test the two null hypotheses at 0.05 level of significant. The data collected from the respondents were generated from the study contained 57 items on a five point Likert scale with response options of Strongly Agree (AS), Agree (A), Under Disagree (UD) - Disagree (D) Strongly Disagree (SD) and the corresponding values of 5, 4, 3, 2, and 1, respectively.

Results:

The results of the analysis are presented as follows;

Research Question 1: What are the assessment skills through mainstreaming gender in scientific research in Taraba State University?

Research Hypothesis 1: There is no significance difference in the Likert scale response of respondent on the assessment skills through mainstreaming gender in scientific research in Taraba State University.

The result in table 1 shows that ten items have mean value less than 2.5 and 14 items have mean value range from 2.5 and above. This shows that the respondents agree with the facts that, the skills assessment through mainstreaming gender in scientific research in Taraba State University are very important

Research Question 2: What are the measurable methods that can be put in place while dealing with mainstreaming gender in scientific research in Taraba State University?

Hypothesis 2: There is significant difference in the Likert scale response of respondents on the assessment skills through mainstreaming gender in scientific research in Taraba State University

The result presented in table 2 indicated that almost all the results have their mean scores greater than 2.5 which show that, there is no significance different in the likert scale response of respondents for the measurable methods that can be put in place while dealing with mainstreaming gender in scientific in Taraba State University.

Research Question 3: What are roles of different lecturers and students in the University and their ways of improving the mainstreaming gender in scientific research in Taraba State University?

Hypothesis 3: there is no significant difference in the likert scale response of respondents in the roles of different lecturers and students in the university and ways of improving the mainstreaming gender in scientific research in Taraba State University.

Table 3: shows that 12 items had their mean values range from 2.50 and above are in cut off point. This indicates that the respondents strongly agreed that the role of lecturers and students and their ways of improving the mainstreaming gender in scientific research is very pertinent in Taraba State University. Therefore, result from the hypothesis showed that no much significant difference exists between the mean ratings of the items. Thus, the hypothesis is accepted.

Summary of the Findings

1. There were strongly agreed facts by the respondents that there was need in the assessment of skills through mainstreaming gender in scientific research in Taraba State University
2. The result also showed that the mean scores of respondents were greater than 2.5 and above the cut-off point. This indicates that there were enough facts by the respondents in the measurable methods to be put in place while dealing with mainstreaming gender in scientific research in an established organisation.
3. Based on the last table, the results showed that the respondents strongly agreed that there was important need in the roles of different lecturers and students and their ways of improving the mainstreaming gender in scientific research because without the lecturers' roles in mainstreaming gender, the assessment of skills will not be important in verifying the strength and weaknesses of both women and men in times of any programme planning to take action, either in form of economic, policy making and decision taking or implementation of such programme in any given country.

Conclusion

The study concluded that, assessment of skills through mainstreaming gender in scientific research is almost very important in the established organisation. However, the integration of both women and men in the society carry value and should be recognised to have equal opportunity in order to eliminate the assumption of discrimination that women are in equal, because without women in the society the world is unfinished and imperfect.

Recommendations

The following recommendations were made based on the findings.

1. Mainstreaming gender in scientific research should be recognised and encouraged in all spheres of established institutions.

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2. Government should put more emphasis in the integration of women and men in the local, state and federal characters in times of plan action like, economic, politics, policy making and implementation of any programme like Non-governmental organisation (NGO) as a national building, development and policies.
3. Government should recognise the effort of both lecturers and students involved in mainstreaming gender in scientific research by providing funds, adequate structures by building laboratories for research and facilities to facilitate research works or activities.

Table 1: Mean rating of respondents on the assessment skills through mainstreaming gender in scientific research

S/N	Items	N	X	Std	T test	Remarks
1	Skill in mainstreaming gender in scientific research is an ability to change one's behaviour.	26	2.8846	.95192	-1.351	NS
2	Assessment in scientific research is to ascertain the extent of one's knowledge in science.	31	2.5161	1.17958	-1.377	NS
3	Skill is an ability to be persistently in pursuing goals despite an obstacle in mainstreaming gender in sciences.	26	2.3462	.89184	.807	S
4	Assessment of skills through mainstreaming gender in scientific research involves an interest.	31	2.7097	1.10132	.806	NS
5	Mainstreaming gender in scientific research exposes women and men to innovation in science and technology.	26	2.7692	1.03180	-1.213	NS
6	Gender is the female sex only that strives to create awareness in scientific research.	31	2.5484	1.02758	-1.224	NS
7	Mainstreaming gender in scientific research is the integration of women and men in societal development.	26	2.2692	1.00231	.492	S
8	Mainstreaming in scientific research helps one's talent.	31	2.6129	1.11587	.493	NS
9	Assessment of skills helps one to take rational decision in achieving its goals.	26	2.5000	1.10454	.731	NS
10	Skills are fundamental of readiness in scientific research.	31	2.3548	1.11201	.742	S
11	Mainstreaming gender in scientific research helps impromptu in decision-making.	26	2.6923	.97033	-.148	NS
12	Assessment of skill through mainstreaming gender in scientific research enables individual to rightly adjust well in the science and technology.	31	2.4839	1.15097	-.149	S
13	The Research on mainstreaming gender has increased knowledge in scientific research in Taraba State University?	26	2.3462	1.01754	.676	NS
14	Gender equality helps in purposeful goals in the tertiary institution.	31	2.3871	1.05443	.675	S
15	Intentional motive in mainstreaming gender in scientific research initiate wickedness.	26	2.5000	1.17473	-.674	NS
16	Mainstreaming gender in scientific research is useful in career choice.	31	2.2903	1.16027	-.689	S
17	Mainstreaming gender in scientific research improve effectiveness and efficiency of research in science.	26	2.3846	.94136	1.245	S
18	Assessment of skills through mainstreaming gender in scientific research decreases one's maladaptive behaviour.	31	2.5806	1.20483	1.260	NS
19	Assessment of skills in mainstreaming gender in scientific research increases one's adaptive behaviour to function well in the society.	26	2.6538	1.01754	.366	NS
20	Assessments in mainstreaming gender make girls have access to science and technological studies and career.	31	2.2903	1.16027	.366	S
21	Attracting Women in science poses challenges in education in Taraba State University.	26	2.5000	1.17473	.352	NS
22	Mainstreaming gender discrimination is a violation of human rights.	31	2.3871	1.14535	.350	S
23	The under representation of women threatens excellence in Taraba State University.	26	2.6154	1.09825	-1.212	NS
24	Assessment of skills through mainstreaming gender improves quality of life in women.	31	2.5161	1.02862	-1.227	NS

Table 2: mean ratings of respondents on the measurable methods that can be put in place while dealing with mainstreaming gender in scientific research

S/N	Items	N	X	Std	T test	Remarks
25	Mainstreaming gender in scientific research helps individual's knowledge to advance in policy orientation and counselling of the youths.	26	2.5769	.94543	-.130	
26	Mainstreaming gender in scientific research help to place an individual to a rightful position in the working establishment.	31	2.6129	1.11587	-.132	
27	Assessment of skills through mainstreaming gender in scientific research enjoin individual to discharge their knowledge in the developmental organization.	26	2.5769	1.17211	.324	
28	Assessment of skill in mainstreaming gender in scientific research helps the students to adjust well in chosen a better career that matches their interest.	31	2.4839	.99569	.320	
29	The Taraba State University gender strategy has been implementing.	26	2.8846	.95192	.894	
30	Mainstreaming gender in scientific research builds individual to associate with internal and external bodies of sciences.	31	2.6452	1.05035	.902	
31	Skills in mainstreaming gender clarify doubt of inequality in the organisational sphere	26	2.3077	1.19228	- 1.213	
32	Assessment of skills in mainstreaming gender in scientific research increase self-understanding and help in carry out self-job effectively.	31	2.6774	1.10716	- 1.205	
33	Mainstreaming gender in scientific research provided immediate feedback of learning input and output.	26	2.7692	1.14220	.880	
34	Mainstreaming gender in scientific research enable an individual to be independent in life.	31	2.5161	1.02862	.872	
35	The methods, actions and technologies can be used to increase women's participation in research and decision making.	26	2.8462	1.04661	1.162	
36	The Research on mainstreaming gender has increased knowledge in scientific research in Taraba State University?	31	2.5484	.88840	1.145	
37	Institution that admits women in science helps them to add more value in the society.	26	2.8462	1.08415	1.197	
38	There is benefit of stronger gender equality in Taraba State University.	31	2.5161	.99569	1.188	
39	Women involved in planning, design and evaluation work within the Taraba State University.	26	2.3846	1.09825	-.900	

Table 3; Mean ratings of respondents on the role of different lecturers and students in the university and their ways of improving the mainstreaming gender in scientific research

S/N	Items	N	X	Std	T test	Remarks
40	Mainstreaming gender in scientific research make both lecturers and students to become aware of the need to be well rooted in science and technology.	26	2.4615	1.10384	-1.041	
41	Mainstreaming gender in scientific research enable both lecturer and student to update their knowledge.	31	2.7419	.92979	-1.026	
42	Mainstreaming gender in scientific research increased the motivation of student and lecturer to be science-oriented.	26	2.3846	1.02282	-.234	
43	Mainstreaming gender in scientific research helps to share responsibilities in the programmed implementation.	31	2.4516	1.12068	-.236	
44	There is equal opportunity of both women and men in Taraba State University.	26	2.6923	1.15825	.505	
45	Assessment of skill through mainstreaming gender in scientific research increase individual's awareness to act in appropriate standard of self-direction.	31	2.5484	.99461	.498	
46	Taraba State University developed a gender equality policy and strategy for programme implementation.	26	2.4615	1.02882	-1.127	
47	The research results have been communicated or reach women in the area of mainstreaming gender in scientific research.	31	2.7742	1.05545	-1.129	
48	Does the Taraba State University have a gender equality policy and strategy based on mainstreaming gender in scientific research?	26	2.6923	1.08699	.161	
49	Does the Taraba State University reflect upon why a mainstreaming gender in scientific research is important?	31	2.6452	1.11201	.161	
50	The ageing population makes it essential to target both genders in the wrinkle pool of young scientists.	26	2.5000	.94868	-.841	
51	General view of men says, it is wasteful to educate and train young women in sciences but then they think they (women) cannot make use of their skill to get employment.	31	2.7419	1.18231	-.857	
52	Mainstreaming gender in scientific research improve information of women's in science and technology.	26	2.2308	.99228	-1.332	
53	Mainstreaming gender in scientific research achieves greater equality in the relation between women and men throughout in Taraba State	31	2.6129	1.14535	-1.349	

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	University.					
54	School and media encourage girls to involve in scientific research.	26	2.7692	1.17670	.554	
55	Mainstreaming gender in scientific research improves gender equality.	31	2.6129	.95490	.544	
56	Mainstreaming gender in scientific research improves competitiveness and development of stimulus.	26	2.5385	1.13950	.513	
57	Mainstreaming gender in scientific research makes both lecturers and students to become aware of the need to be well rooted in science and technology.	26	2.4615	1.10384	.510	

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WIS-SED 10

**EXTENT OF IMPLEMENTATION OF SAFETY PRACTICES IN BIOLOGY LABORATORY
IN PUBLIC AND PRIVATE SECONDARY SCHOOLS IN ABIA STATE.**

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ABSTRACT

This study determined the extent of implementation of safety practices in Biology laboratory in public and private secondary schools in Umuahia North Local Government Area of Abia state. Four research questions and two hypotheses were used in the study. A survey research design was utilized. A total of 285 SS2 students and 45 Biology teachers in both public and private secondary schools, served as sample for the study. The instrument for data collection was questionnaire with a reliability coefficient of 0.71 for students and 0.76 for teachers. Mean and Standard Deviation were used to answer the research questions. Results also show that fittings like storage facilities, position of the demonstration table are appropriate to a great extent in public and private schools; Results show that safety practices such as students always working under the supervision of the teachers, eating and drinking forbidden in the laboratory, are implemented to a very great extent by the Biology teachers in both public and private schools. However the safety practice of having first aid supplies approved by a consulting physician is implemented to very low extent in public and private schools by Biology teachers. Safety practices such as washing of hands and face with water after biology practical, knowing the location of safety equipment in the laboratory are implemented by Biology students to a great extent in public and private schools. However, students' knowledge of how to operate safety gadgets is still at a very low extent in public schools while this is of moderate extent in private schools. Recommendations were made among which is need for curriculum experts to introduce laboratory safety courses into the curriculum of Science Education in Nigeria and Biology teachers and students to be well tutored on safety practices in the laboratory.

KeyWords: *Implementation, Safety practices, Biology, laboratory, Teachers, Students*

Background

Biology is the systematic study of living forms. It is the study of life and its evolution of organism and their structures, function, processes, and interaction with each other and the environment (Hofstein&Tunetta, 2004). Biology is an incredibly fascinating field of study, because it is so fundamental to the world. It plays a role in everyone's lives and touches almost every aspect of human existence in some way. People keep pet, nurture house plants, and invite avian visitors with backyard bird houses and visit zoos and natural preserves. Biology is the scientific extension of this humans' tendency to feel connected to and curious about all forms of life. It takes us, personally or vicariously, into jungles, deserts, sea and other environment, where a variety of living forms and their physical surroundings are interwoven into complex webs called ecosystems. Studying lead humans into laboratories to examine more closely how living things called organisms, work. Biology draws us into microscopic world of the fundamental unit of life known as cell and into the sub-microscopic realm of the molecules that make up those cells (Campbell & Reece, 2002).

Modern Biology is as important as it is inspiring; Genetics and cell Biology are revolutionizing medicine and agriculture. Molecular Biology is providing new tools for anthropology; help us trace the origin and the dispersal of early humans. Ecology is helping to evaluate environmental issue such as the causes and consequences of global warming.

Neuroscience and evolutionary Biology are reshaping physiology and sociology. These are just a few examples of how biology is waving into the fabric of our culture as never before (Campbell & Reece, 2002).Biology education, in general is included in the school curriculum for the same reason as any other subject, but in addition biology inculcates certain special values peculiar to it and which no other subject can provide. But before satisfying the usual needs for its inclusion as a subject in the curriculum such as intellectual, cultural, moral, aesthetic, utilitarian and vocational values-learning of Biology provides training in scientific method and also help to develop a scientific attitude of mind in the learner. The qualities imbibed by the learner through learning Biology are of great value to a citizen living in the modern society (Digumarti &Pushpa, 2017). Biology brings behavioural changes in the learner and enriches his character and personality. It gives opportunity for creative thinking and constructive imagination. Furthermore, it is a subject where ideas can be experimented upon and verified.

Biological science laboratory enriches the quality of life in numerous ways by providing new solutions to problems in health and materials and energy usage. Thus, the role of the laboratory in learning becomes a key component in understanding biology. Laboratory activities and inquiry provides student with opportunity to question, sample, experience and experiment with scientific phenomena in their quest for knowledge with living things. The most effective

vehicle by which the process of inquiry can be learned appears to be a laboratory where the student experiences, first-hand, the inquiry process.

According to Omiko (2015), a laboratory is a room, or building equipped and set apart for practical or experimental studies to take place. He sees the laboratory as the heart of a good Biological program which allows students in the school to have experience which are consistent with the goals of Biological literacy. This implies that Biology teaching and learning cannot be completely done in a Secondary School where there is no equipped laboratory. Ufondu (2009) observed that the laboratory is an indispensable organ of the school if effective teaching and learning of Biology subject is to be achieved. Omiko (2015) observed that the use of laboratory has the following benefits:

Laboratory teaching makes the student to learn the nature of Biology in order to foster the knowledge of Human enterprises of Biology and thus enhance the authentic and intellectual understanding of the child. Learning Biological inquiry skills that can be transferred to other spheres of problem solving (that is, acquisition of problem solving skills). One of the basic goals of Biology education is to help student learn skills that can be applied to other life situations in future.

Students learning to appreciate and emulate the role of Biologists through acquisition of manipulative skills. Developing interests, attitudes and values by considering what Biology entails. It is clear that a laboratory experience has the best potential for stimulating a lifetime experience in Biology in the student when accorded the chance for personal experience by handling the real things. Students interest in Biology increases as they yearn to investigate and explore more about their environment.

According to Omiko (2015), eight aspect of Biological attitude exists all of which can be nurtured in the Biology laboratory in the school. They are: Curiosity, Open mindedness, Objectivity, Intellectual honesty, Rationality, Willingness to suspend judgement, Humanity and Reverence for life. Queens (2008), an internet website on good practice (laboratory-based learning) stated that Biology teachers believe that the laboratory is an important means of instruction in Biology since late 19th century. Laboratory instruction is considered essential because it provides training in observation, supply detailed information and arouse students' interest.

Based on the roles of the Biology laboratory in Biology teaching and learning, it implies that schools without laboratory where student can carry out Biological practical could end up producing or graduating students who have no knowledge of Biology practical required by the West African

Examination Council (WAEC) and the National Examination Council (NECO) to pass the Senior School Certificate Examination. Consequently, those students will lack requisite requirement qualification for courses like Medicine, Microbiology and any of the Biology related career.

Practical and safety practices should run concurrently in laboratories. Safety practice can be explained to mean the process of averting danger (Adam & Salome, 2014). Learner – centred or activity –based learning is one of the recognised methods of teaching Biology. This method teaching ensures a high degree of student participation. Safety in school laboratory is everyone's concern and the aim is to ensure that no one gets hurt or become ill. Some of these safety practices to be maintained in a Biology laboratory, especially at the secondary school level, could be outlined as follows:

Do not enter the laboratory or use equipment without permission.

Do not eat or drink in the laboratory.

Be aware of safety signs and adhere to them.

In the event of an accident, inform the teacher immediately.

Be aware of the location of fire extinguisher or fire blankets; first aid box and eye wash station.

Wear eye protection when instructed.

Hands should be washed after laboratory practical.

Do not run; pay attention to where you are going to etc.

Laboratory safety is often a major concern for many schools that offer experiences to the students in scientific investigation and experiments. This is because the Biology laboratory which usually is an active site for learning also possess potential risks for the students and even teachers themselves. Since accidents are unpredictable by nature, threats due to the use of tools, devices, or chemicals while conducting experiments are highly possible in the school Biology laboratory. It is therefore indispensable to take the necessary precautions and minimise possibilities of threats and hazards. For the Biology laboratory to achieve its goals it should be organised, well equipped with facilities and safety measures, precautions should be taken and most importantly it should be managed well.

The people involved in the management, organization and the establishing of safety practices of a Biology laboratory could include the teacher, the laboratory assistants and the students (Dumcho & Sonam, 2016). Equipment and facilities in most laboratories have been lacking as well as implementation of safety practices such as safety equipment and precautions in most secondary school laboratories both in public and private secondary schools. Blames have been on the inability of the school authorities to provide materials and safety equipment in the laboratories; teachers fail to recognise the importance of safety precautions during practical session. Since Biology involves practical activities and accidents could happen, it is

important to include safety measures in the laboratory in teaching biology theoretically before practical takes place. Practical work in Biology may involve exposure to something that may have the potential to cause harm or injury that is, a hazard.

It is important to know the hazards, to understand the risks (the likelihood of causing harm and the severity of that harm if it occurs) and apply control or manage the risks, for example by wearing eye protection in a laboratory in which corrosive chemical are used. It could be that if safety measures are practiced judiciously during practicals, hazards could be prevented and if Biology is taught with practical, the student achievement could be better yearly. However, reports show that students' performance keeps declining every year.

According to Nnamonu (2003), students perform poorly in Biology practical leading to their failure in Biology examination. A close look at the WAEC result records confirm that students' performance has been very poor generally and particularly in Biology practical. Poor performance of Biology could be as a result of the fact that senior secondary school students are not sufficiently exposed to Biology practical classes before going into external examination or laboratory equipment are not being used to teach Biology during Biology practical classes.

Gimba (2011), carried out a survey on the availability and use of laboratories in secondary school, it was discovered that the laboratories in most schools had the multipurpose type of laboratory where all science practical were conducted using the same laboratory at different times. This probably led to students carrying out practical only once in a while. The study also revealed that there was insufficient number of trained staff, equipment and chemicals and inadequate safety equipments.

It has been observed that little attention is placed on taking precautionary measures in biology laboratory in Nigeria which could be dangerous to both the teacher and student (Adedayo &Owolabi, 2014). Gimba (2011), observed the need for government to employ laboratory assistants and technicians and also to make adequate provision for safety for staff and students in the laboratory to enhance effective teaching and learning of Practical Biology in the secondary schools. It is also imperative to check how far schools are equipped with safety equipment and awareness and implementation of safety practices during laboratory sessions in schools. This study therefore tends to find out the extent of implementation of safety practices in Biology laboratory, by teachers and students in both private and public secondary schools.

Specifically, the study intended to find out:
Extent of general design and fittings of Biology laboratories relevant to safety in public and private

secondary schools

The extent to which Biology teachers implement safety practices in public and private secondary schools

The extent to which Biology students implement safety practices in public and private secondary schools

The following research questions were posed:

To what extent are the type of design and fittings in the Biology laboratory appropriate to ensure safety in public and private secondary schools?

What is the extent of implementation of safety practices during practical sessions by Biology teachers in public and private schools?

To what extent do students implement safety practices during practical sessions in public and private schools?

Method

The study adopted a survey research design. The area of the study was Umuahia North Local Government Area of Abia state. The population comprised of all the senior secondary school two (SSII) Biology students and teachers in Umuahia North Local Government Area secondary schools, which was made up of forty-five (45) Biology teachers and one thousand one hundred and thirty (1130) Biology students in public and private schools based on the statistical data obtained from Secondary Education Management Board (SEMB of Abia state, 2019).

Simple random sampling techniques was used to select four (4) secondary schools; one private and three public. From the four schools selected for the study, simple random sampling was used to select two hundred and eight-five Biology students. All the senior secondary school Biology teachers which were forty-five (45) in number were all used for the study.

Questionnaire instrument developed by the researchers was used for the data collection. The instrument was Biology students' implementation of safety practices questionnaire (BSISPQ) and Biology teachers' implementation of safety practices questionnaire (BTISPQ). The response mode was a modified Likert-type 4-point scale of : Very great extent (VGE), Great extent (GE), Moderate extent (ME) and Very low extent (VLE) with ratings of 4, 3, 2, 1 respectively. Mean of 3.00 and above were rated as VGE, 2.50-2.99 GE, 2.40-2.49 ME and less than 2.40 as VLE. The instruments (BSISPQ and BTISPQ) were face validated and establishment of the reliability was carried out using test re-test method, and analyzed using Pearson product moment correlation coefficient. This yielded correlation coefficient of 0.71 for BSISPQ and 0.76 for BTISPQ.

The questionnaire was administered to the Biology teachers and students and collected the same day. Data was collated and analyzed in line with the research questions and hypotheses. The research questions

were analyzed using , mean and standard deviation. design and fittings in the biology laboratory
 Results appropriate to ensure safety in public and private
 Research Question 1: To what extent are the type of secondary schools?

Table 1: Extent to which the Following Fittings and General Designs are Appropriate in Ensuring safety in Public and private Schools

S/N	ITEM	PUBLIC SCHOOLS			PRIVATE SCHOOLS		
		MEAN	SD	REMARK	MEAN	SD	REMARK
1	storage facilities	2.64	0.93	GE	3.20	0.78	VGE
2	displaying shelves	2.43	1.16	ME	2.73	1.03	GE
3	position of the demonstration table	2.64	1.08	GE	3.07	0.80	VGE
4	fitting of the laboratory window	2.21	1.31	VLE	2.93	0.96	GE
5	quantity and fitting of laboratory doors	2.14	1.10	VLE	2.93	0.80	GE
6	electrical wiring and fitting	2.29	1.33	VLE	3.40	0.63	VGE
7	water piping	2.21	1.12	VLE	3.13	0.74	VGE
8	position of the sinks	2.43	1.22	ME	2.73	0.96	GE
9	gas fitting	2.29	1.07	VLE	2.67	0.82	GE
10	position of the gas cylinder	2.36	1.15	VLE	3.07	0.70	VGE
11	laboratory flooring	1.93	1.14	VLE	3.07	1.03	VGE

Key: Very great extent (VGE), Great extent (GE), Moderate extent (ME), Very low extent (VLE).

The results in Table 1, indicate that only storage facilities and position of demonstration table are appropriate to a great extent in ensuring safety in public schools, while these facilities are appropriate to a very great extent in ensuring safety in Biology laboratory in private schools. Apart from displaying shelves and position of sinks, which are appropriate to a moderate extent, all other listed fittings and designs are appropriate to a very low extent in ensuring safety in public schools. For Private schools majority of the

listed facilities are to a very great extent appropriate in ensuring safety in private schools.

Research question 2: What is the extent of implementation of safety practices during practical sessions by biology teachers in public and private schools?

Table 2: Extent of Implementation of Safety Practices during Practical Sessions by Biology Teachers in Public and Private Schools

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S/N	ITEM	PUBLIC SCHOOLS			PRIVATE SCHOOLS		
		MEAN	SD	REMARK	MEAN	SD	REMARK
1	Students always work under the supervision of the teacher	3.29	0.61	VGE	3.60	0.50	VGE
2	Eating and drinking are forbidden in the laboratory	3.64	0.63	VGE	3.60	0.91	VGE
3	All bottles and containers are sealed with lids	3.57	0.65	VGE	3.67	0.48	VGE
4	Bare foot is not allowed in the laboratory	3.93	0.27	VGE	3.47	0.64	VGE
5	Gas cylinders are capped, supported to prevent rolling	3.36	0.84	VGE	3.20	1.14	VGE
6	Gas cylinders are placed away from heat sources and open flame	3.57	0.94	VGE	3.87	0.35	VGE
7	Chairs and stools where available do not obstruct movement	3.43	0.76	VGE	3.07	0.79	VGE
8	Waste are carefully segregated into labelled containers	2.00	1.04	VLE	3.00	0.65	VGE
9	Separate disposal containers are available for broken glass wares	1.71	1.07	VLE	2.93	1.03	GE
10	Goggles are worn when working in the laboratories	1.93	0.73	VLE	2.40	0.98	ME
11	Personnel knows the location and use of all safety equipment	3.43	0.85	VGE	3.40	0.63	VGE
13	Suitable protective gloves are worn when transferring or handling hazardous chemicals	2.57	1.16	GE	3.27	0.79	VGE
14	Mechanical pipette is used instead of mouth	2.36	1.39	VLE	3.40	0.73	ME
15	Laboratory coat are always worn when working in the laboratory	3.43	0.85	VGE	3.67	0.61	VGE
16	Teachers are trained in resuscitation	2.36	1.15	VLE	2.87	0.74	ME
17	First aid supplies are richly available	2.43	1.09	ME	3.00	1.13	VGE
18	First aid supplies have been approved by a consulting physician	2.21	1.12	VLE	3.33	0.90	VGE
18	Any injured student is treated promptly	3.07	1.21	VGE	2.93	1.03	GE
20	First aid is taught by the Biology teachers	3.43	0.85	VGE	3.13	0.74	VGE
21	There is periodic inspection of the laboratory by the biology teacher	3.07	0.83	VGE	3.00	0.92	VGE
22	Publication on laboratory safety is readily available to the personnel	2.86	1.09	VLE	3.07	1.03	VGE
23	There is a laboratory safety information notice board	3.50	0.94	VGE	3.47	0.74	VGE
24	Stock record is kept for all chemical/materials	3.14	0.86	VGE	2.80	0.77	GE
25	Storage cabinets inspected periodically by the teacher for rust and chemical/material decomposition	3.29	0.91	VGE	2.93	0.70	GE
26	Special cabinets for flammables and combustibles	2.64	1.15	GE	2.87	1.06	GE
27	Chemical/materials stored are classified	3.43	0.85	VGE	3.60	0.82	GE
28	Large bottles especially acids are stored on low shelves or in acid cabinets	3.36	0.63	VGE	3.53	0.83	VGE
29	All containers are clearly labeled	3.64	0.63	VGE	3.33	0.72	VGE
30	Labels are firmly attached to containers	3.64	0.49	VGE	3.67	0.61	VGE
31	Labels are readable and free of blockage	3.36	0.75	VGE	3.27	1.03	VGE
32	Fume hood is available	1.71	0.99	VLE	2.60	1.12	GE
33	Fire extinguishers inspected, with dates	2.43	0.94	LE	2.53	0.99	GE
	Pooled Mean	2.96		GE	3.19		VGE

Table 2 shows that all the listed safe practices are implemented averagely to a great extent (pooled mean 2.96) in public schools. All the listed safe practices are implemented averagely to a very great extent (pooled mean 3.19) in private schools. Comparatively, this result shows that Biology teachers in private school

implement the listed safety practices better than those in public schools (pooled mean 2.96).
 Research Question 3: To what extent do students implement safety practices during practical sessions in public and private schools?

Table 3: Extent to which Students Implement Safety Practices during Practical Sessions in Public and Private Schools

S/N	ITEM	PUBLIC SCHOOLS			PRIVATE SCHOOLS		
		MEAN	SD	REMARK	MEAN	SD	REMARK
1	Always work under the supervision of the teacher	3.22	1.12	VGE	3.86	0.55	VGE
2	Know how to operate safety gadgets. E.g. fire extinguisher	2.33	1.27	VLE	2.05	1.07	VLE
3	Questioning in times of difficulties in the laboratory	2.88	1.22	GE	3.36	0.89	VGE
4	Wear hand gloves during practical	2.69	1.33	GE	1.92	1.18	VLE
5	Wash hands and face with water after biology practical	2.92	1.25	GE	2.66	1.21	GE
6	Wear laboratory coat before entering the biology laboratory	2.49	1.27	ME	2.71	1.20	GE
7	Know the location of safety equipment in the laboratory	2.66	1.17	GE	2.69	1.14	GE
8	Knowledge on the disconnection of electric gas after use	2.62	1.22	GE	2.40	1.26	ME
9	Know the precautions to take in the case of spills and splashes of chemicals	2.42	1.19	ME	3.13	1.11	VGE
10	Wear gloves when working with hazardous chemicals in the laboratory	2.42	1.26	ME	2.13	1.21	VLE
11	Use of chemical pipette when working with hazardous chemicals	2.48	1.27	ME	3.33	1.05	VGE
Pooled Mean		2.64			2.78		

Key: Very great extent (VGE), Great extent (GE), Moderate extent (ME), Very low extent (VLE).

Table 3 shows that all the listed safe practices are implemented averagely to a great extent (pooled mean 2.64) in public schools. All the listed safe practices are implemented averagely to a great extent (pooled mean 2.78) in private schools. Comparatively, this result shows that Biology students in private school implement the listed safety practices better than those in of public schools, considering the higher pooled mean of students in private schools.

Discussion

The findings on Table 1 for research question 1, indicated that majority of the fittings and general designs are appropriate to ensure safety in the biology laboratory only to a moderate extent in public schools while they are appropriate to a very great extent in private schools. This finding is in agreement with the finding of Aniodo (2008) who observed that despite the importance of safety laboratory equipment and materials, they are not adequately available in many secondary school laboratories. This may be due huge sum of money required to provide them. Moreover the issue of vandalisation of facilities is more rampant in public schools than in private schools. Moreover many private schools take the issue of standardization, especially of facilities, more seriously than public schools where many people believe that government

owned property are owned by nobody. However, there is an indication from the study that some ensure safety. This in line with Maduka (2018) findings that the respondents agreed that the designs and fittings ensure safety.

Results in Table 2 and 3 for Research question 2 and 3 show that the extent to which Biology teachers and students implement safety practices in private schools is better than those in public schools. This could be as of the fact that teachers and students in private schools are more strictly monitored than those in public schools. This is in further corroborated by Adams and Salome (2014), who indicated that most laboratory hazards can be reduced by good value judgement, careful manipulation, adequate supervision and most of all the knowledge of how to use the safety materials

or device present in the laboratories.

Conclusion

The findings of the study indicate a major flaw in our educational system as it relates to the use of safety materials in the laboratories and implementation of safety practices in the lab. In both public and private schools there are still fittings and designs not ensuring adequate safety in biology laboratory and these need to be addressed. It was also revealed in this study that there are still many safety practices not being practiced by biology teachers and students.

Recommendations

The following recommendations have been made based on the findings:

1. Basic safety equipment like first aid kit, hand

gloves, eye goggle and fire extinguishers must be provided in the laboratories by the government.

2. Trained and experienced laboratory attendants and technicians should work in the laboratory.

3. The tertiary institutions should also review the nature of their programs to include enough laboratory instructions so as to prepare adequately the student, teachers so as to enable them acquire safety practices and be able to transfer what they have learnt to their students.

4. Curriculum experts should introduce a laboratory safety course into the curriculum of Nigeria education.

5. Safety rules and regulations should be displayed at entrances of the laboratory and other places by laboratory staff or class teacher.

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ILLITERACY AS AN IMPEDIMENT TO MAINTAINING PUBLIC HEALTH IN NIGERIAN COMMUNITIES: THE ROLE OF ADULT EDUCATION

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Abstract

The study examined illiteracy as an impediment to maintaining public health in Nigerian communities and the role of adult education as a remedy. Illiteracy can chain one in ignorance, poor health status, hopelessness, slavery, inaction, intimidation and exploitation in a number of ways. Illiteracy is not only an individual problem, it is also a social problem not peculiar to Nigeria but Africa and the world. A number of health programmes have been established in Nigeria to improve the public health status of the people but the health workers still maintain that lack of education (illiteracy) among the people and the health workers has continued to be a challenge in maintaining public health. Poor education, poor communication and lack of frequent enlightenment leads to repeated hospitalizations and this is costing the health care industry billions of currencies. In response, the paper reveals the status of public health in Nigeria, how illiteracy affects public health, causes of illiteracy and steps aimed at improving healthcare systems through adult education. Among others, the researcher recommended that adult educators should be given the chance to show their expertise in reducing the level of illiteracy especially on health through developing learning packages relating to public health. There should also be constant training workshops for the health workers on the need for effective teaching of patients in various ailments for proper understanding and effective service delivery which invariably tackles the problem of lack of knowledge in health matters and poverty of mental power.

Keywords: illiteracy, health literacy, public health, adult education.

Introduction.

Illiteracy includes ignorance, lack of enlightenment on a particular issue or area, holding on the false belief, cuddling a wrong decision, not being objective and rational in thinking, having superstitious belief on a scientifically proven issue, and sticking in conformity with the uninformed/uneducated persons to show unity in a wrong opinion. Globally, illiteracy has manifested as ignorance and wrong beliefs, with all the professionals multiple degrees and skills, still the perception towards issues such as the time of "COVID-19" cannot be easily forgotten. Some people were of the view that it was a divine punishment, some saw it as a disease, some saw it as signs of end time, many believed it was not actually existing which gave rise to mixed reactions that helped in spreading the disease as wide as it went.

In other words, illiterates deserve preferential treatment (serial provision of information and education) in order to maintain the desired public health standard. Since the development goals are part of a determined effort to alleviate poverty, reduce mortality rate and increase life expectancy, then literacy as a part of basic services and meeting of basic human needs is itself a major contributor. It is important to recognize also that the role of literacy in improving public health is not tied to formal education only. In previous studies, Obiozor (2018) indicated that adult education has widened to include a variety of learning opportunities outside the school system. These include non-formal and informal education. Non-formal education include organized adult literacy

programmes, instructions in family planning methods, instructions on oral dehydration therapy, proper hygiene, instruction on how to maintain good health status; check-ups, keeping up appointments with the doctors, accepting counselling, needs for referrals and compliance to doctor's prescription while informal education captures lifelong process by which every person acquires, aids and accumulates knowledge, skills, attitudes from daily living experiences at home, workplaces, social interactions, markets, motor parks, churches, mosques and other meeting places through sensitization, enlightenment programmes, workshops and other forms.

Illiteracy, a silent barrier to health is a problem facing many Nigerian communities and affecting the health of the public as a whole. Health literacy is the degree to which individuals have the capacity to obtain, process and understand basic health information and services needed to make an appropriate decisions concerning their health. It is one of the links by which the health status of individual and the health indices and statistics of our country can be improved upon. Factors influencing health literacy in Nigeria include the culture and belief system, poor and ineffective communication, lack of education, low educational level, low socioeconomic status and lack of patients' knowledge of their health rights (Ekwueme & Ochonma 2011). Illiteracy predisposes one to poorer health status, poor disease management and outcome and lack of understanding in the use of preventive services, frequent hospital visitations amongst others.

Factors influencing literacy are identified and modified in this paper and ways to help improve the literacy level and health of the populace. However, it is a well-established fact that there are health programmes targeting to improve the status of public health in Nigeria which include national healthcare Resources, National health systems and management, partners of Health development, Health research and National Health Care Laws (Faisal, Jamil & Chowdhury 2017). With this known fact, are the citizens satisfied, fulfilled and reached? This is a call for the Government and Health Care Professionals to acknowledge illiteracy as a problem to maintenance of public health and therefore, be willing to play their roles in tackling this problem to achieve a healthy Nation by frequently updating the healthcare workers with new trends of knowledge and regularly educating the people not minding their level of education or extent of exposure to information which may not tally with the desired knowledge.

Public Health in Nigeria.

Public health is the science of protecting and improving the health status of people and their communities. According to the Centre for Disease Control (CDC) (2018), public health can be seen as the science and art of preventing disease, prolonging life and improving quality of life through organized efforts and informed choices of society, organizations, public and private, communities and individuals. Overall, public health is concerned with protecting the health of entire populations. These populations can be as small as a local neighborhood, or as big as an entire country or region of the world. Public health is about helping people to stay healthy and protecting them from threats to their health. Public health activities involve helping individuals, with the knowledge of health provision of health facilities, making available health workers at appropriate locations, organizing programmes that focus on health matters and healthy living (CDC 2018).

Public health in Nigerian communities is a call for concern. Illiteracy being a silent barrier to public health, a major issue and an impediment to the above defined. Besides that, the Nigerian health care system is poorly developed and has suffered several backdrops, especially at the local level. No adequate and functional surveillance systems are developed and hence no tracking system to monitor the outbreak of communicable diseases, bioterrorism and chemical poisoning (CDC 2018). To achieve success in public health care in this modern era, a system well-grounded in medical intelligence and literacy as the backbone of the health sector is necessary, besides adequate management coupled with strong leadership principles, proper sensitization and education of Nigerians especially the senior citizens (adults) on the need to take measures effective to maintenance of their health as a whole, there would not be compliance still.

According to Faisal, Jamil and Chowdhury (2017), the major public health challenges Nigeria faces are infectious diseases, maternal mortality, infant mortality, poor sanitation and hygiene, disease surveillance, non-communicable diseases and traffic injuries. The researchers maintain that these are prevalent despite the collaborative efforts of the government and non-governmental organizations to provide efficient and effective healthcare delivery in Nigeria. The researchers noted that inadequate programmes designed to address the numerous health problems in Nigeria have led to the little improvement in our health problems in Nigeria. Global overall life expectancy at birth is 54 years; infant mortality rate is 86 per 1000 live birth while maternal mortality ratio is 840 per 100,000 live births (WHO 2011). The researchers also discovered that the primary health care on which the Nigerian health care system is based has not helped in effectively solving the numerous health problems in Nigeria. As WHO (2011) noted, Nigeria has one of the largest stocks of human resources for health in Africa but these health workers are still in small number of health workers to effectively deliver essential health services. Not only that, migration to foreign countries has given rise to inadequate production and inequitable distribution of health workers. On this note, National health system remains weak while its management is ineffective and inefficient because every year, Nigeria records an outbreak of diseases which kill innocent people in their thousands such as lassa fever, meningitis, diarrhea, measles, malaria, tuberculosis, etc. Life expectancy at birth in Nigeria stands at 46.8 years for men and 48.4 years for women and great shortage of health workers as a result of social brain drain (Bolaji, 2012).

Concept of Illiteracy

Alvin Toffler, a great educationist saw it that the illiterates of the 21st century will not be those who cannot read and write but those who cannot learn, unlearn and relearn. The researcher emphasized on the adult illiterates in this study because they are the immediate nation builders, family owners, responsible for all affairs in the communities and take decision of what is to be or not. They take decision on the health issues of themselves and that of their family members.

Illiteracy as perceived in the context of this study goes beyond the lack of knowledge of how to read and write and compute. Illiteracy may also be perceived as an illness, social blindness, underdevelopment, backwardness, and failure. Illiterates do not have access to written cultures and modern trends of knowledge and therefore ignorant of the least values and must be given adequate education. Illiteracy is defined as the quality or state of being unable to read or write and the lack of knowledge in common sense. The Oxford University press (2020) defines

Illiteracy as the inability to read or write, or the actual or perceived state of being uneducated or insufficiently educated. Formerly, the term illiterate was used to describe someone without book learning, even though such a person could read in a vernacular language or handle accounts and correspondence. However, the word also carries the connotation of unpolished, ignorant, or inferior, as in the disadvantage of an illiterate education. It is estimated that about one billion adults in the world (one in five of the world's population) are unable to read.

Nigeria is often referred to as the giant of Africa, owing to its large population and economy. Nigeria is the most populous country in Africa with more than 170million people, it is a nation made up of more than 250 ethnic groups, 380 languages and a diverse range of cultural and religious beliefs and practices (Faisal, Damil & Chowdhury 2017). *According to the Global Monitoring Report (2008), the most recent data for Nigeria shows an adult literacy rate of 69 % (78 % for men and 60 % for women). More than 22 million people are illiterate, 65 % of whom are women (adults). This shows that the literacy efforts attempted in Nigeria so far have not produced the desired results and millions of people are still being denied access to literacy because of lack of effective education policies.* This shows that in Nigeria, majority of the illiterates are adults and this has in one way or the other posed a harm to the health of the general public as this blurs their knowledge about health. Though not peculiar to Nigeria, Nordquist (2019) discovered that worldwide, 880 million adults have been labelled as illiterates and also maintained that in the United States, it is estimated that almost 90 million adults are functionally illiterate, in other words, they do not have the minimal skills need to function in society. This shows that the subculture of illiteracy is larger than anyone on the outside would ever believe.

Causes of illiteracy in Nigeria

It is of no doubt that there are major reasons attributed to illiteracy nationally and globally. The different causes of illiteracy especially in Nigeria and all over the globe are explained as follows:

Poverty/Economic Condition: Economic condition or in layman's term poverty is the major root cause of illiteracy in the world. How can someone study if he needs to fill his stomach first. This is the irony situation of most of the people who are in the very edge of poverty line, but have no choice, but to continue living, by working in order to live in a day or two. It's the same reason why the pattern is being passed on because when one is not properly educated, they are the ones who are vulnerable to commit crimes, contribute to early pregnancy and engage in atrocities to make a living. These are all correlated, but the root cause of it is lack of education or illiteracy. As it has

been stated and elucidated in one of the adages, if you want to save the world, you have to educate the minds first. (Beranda,2012).

Population growth: Population growth in Nigeria is one of the derivative factors that affect not just the quality education, but also the budget allocation for each family. In fact, in most families who have more than the standard number of members of the family would be forced to stop, and let other family members to continue studying with the promise that they can continue after their brothers or sisters have finished their schooling. Hence, they have no options left, but to assist the family in all expenses by working early. There are policies in Nigeria with regards to extending free education, but because of the growing population, the budget for this cannot cover the whole and extensive need for education. This is especially true in developing African communities, Nigeria especially.

Ignorance and Mindset about education: In Nigeria today, many have seen education as a scam for lack of proper job employment for all levels of education. There are people who do not seem to feel the value of education in their lives because of contentment in family business and trades. We cannot force people to accept the importance of early education, and completing a course, since it lies on what the person believes, and stands for especially when it is obvious. There is a ratio of people who feel that they can live a normal life if they would just work harder, but to some, it is less significant for them to study if they are not properly fed. Others still practice the notion that women still have to stay at home, and only men could take advantage of what education can give and offers. Despite of the contemporary changes that the world undertakes, there are still people who are still living in traditional scope, and cannot shun away that principle from their minds as a result of religious backgrounds.

Lack of funds: Emphasis should be placed on funding education in Nigeria. This has to capture all the levels of education and precisely gives opportunity for the adults to further their education at all levels. Adults are socially responsible people and do not like whatever that militate against the family upkeep. The proper and honest utilization of funds provided is another factor to be considered in the education system. At time the mismanagement of funds can be an internal problem within the system which must be monitored to achieve expected goals (Obiozor 2018).

Preference to work over education no matter how little the pay is: It happens in any country especially if one has experience the feeling of earning something for him out of his hard work would just forego or forget the need of completing an education. There are various reasons why some would work at an early age, but majority of them is because of lack of financial

resources. Hence, some would just work temporarily to save some money, but when one becomes use to the scope of the job, the desire to continue studying leaves their hearts. Others would rather choose working than to face piles of books. Hence, it's a common knowledge that work does not guarantee stable financial income, this is the reason that one needs to avail and complete a course in order to progress not just on the kind of job that he or she would choose in the future. (Beranda 2012)

Poor Educational Implementation Policies: This is the common problem of some school institutions and government agencies that actually assigned to oversee and supervise the major educational programmes in their countries. It is a typical scenario and complains that there is a high ratio of unsupervised programmes in most of the schools. Again, it is financially related to funds allocation, and of course to people who would be dedicated and committed to religiously observe that every policy will be followed. Moreover, there are also existing problems that have been the cause of educational disintegration, but have not been discussed, wherein possible solutions are still in the shadow of a hopeless case. If all situations will be listed down, there is a unified and balanced effort regardless of the weight of neglect and poor educational implementation policies, there are no problems that cannot be broken down for the benefit of all (Beranda2012).

Causes of illiteracy are summarized by Fasokun and Pwol (2008):

Parents with little schooling.

Difficult living conditions.

Learning disabilities such as dyslexia, dysorthographia etc.

Problems in primary education (low enrollment rates, high dropout rates, inadequate facilities, poor teaching/learning materials, irrelevant curriculum).

Poor enrollment rates in adult literacy programmes;

High dropout rates linked both to economic problems which force adult learners to abandon classes in favour of income-generating activities and to problems relating directly to the adult literacy programme such as lack of relevance, funding issues and low morals among adult literacy instructors;

Literacy instructors not properly trained in facilitation skills

The exclusion of women from adult education programmes;

Higher dropout rates among women due to irrelevant curricula and competing demands;

Poor access to adult education for "hard" to reach communities, such as nomads, fishermen and pastoralists.

Failure to sustain literacy rates due to poor resources, including equipment, materials and teachers, donor dependency and a negative value of education

A poor literate environment that means that literacy

skills are not mentioned in the long term. (Fasokun & Pwol 2008).

Effects of illiteracy in relation to public health in Nigerian communities.

Over the past decade, many studies have reported linkages between illiteracy and health outcomes, such as health status, chronic illnesses, and hospitalization. Nigerian remote communities over the past years have been battling with the health of the public as a result of illiteracy, experts suggest that the effects of illiteracy on health status and utilization may be indirect, possibly through conditions such as disease knowledge, health behavior, patients' knowledge of health rights and use of preventive care. The effects of illiteracy are highlighted as follows:

Inadequate Disease Knowledge: Individuals who are illiterate not only in Nigeria but all over the world are less knowledgeable about diseases and less-capable of taking proper care of themselves. Many of them are usually ignorant of certain symptoms of different ailments which may be persistent and therefore make themselves vulnerable to bad health. This lack of disease knowledge affects the health of the general public as a whole. The areas of medical knowledge, management and self-care documents to be made auxiliary in health literacy programme should include the knowledge of tobacco effects, diabetes, hypertension, chronic heart failure, asthma, HIV/AIDS, and the knowledge of prostate cancer screening and management, mammography screening, and cervical cancer prevention and lots that have been troubling the Nigerian Communities (Research Gate 2008).

Unhealthy health behavior and habits: Research evidence linking health behavior specifically to health literacy is limited. Several studies, however, have reported associations between general literacy level and substance use. It is conceivable that individuals with lower health literacy are more likely to engage in negative health behaviors, such as smoking, drug abuse, drinking, abuse of illegal substances, and living a sedentary lifestyle. This maybe in part because of their limited access to and ability to understand health and medical information. (Arnold, Davis, Berkel, Jackson, Nandy & London 2001)

Bad preventive measures and poor hygiene:

Limited ability to comprehend information about the importance and methods of early disease detection and treatment may lead to a lower rate of preventive care utilization among people with illiteracy. Having problems following physician instructions and understanding information on an appointment slip or referral form from a hospital (Davis, Arnold, Berkel, Jackson, Nandy, Glass, 1996) also may limit access to preventive care and routine physician visits. Hygiene is a factor that affects public health and can lead to a terrible state of public health. Taking a look at the

outbreak of the corona virus (COVID-19), the WHO(2020) has given measures to prevent the further spread of the virus; but very few people who are literate understand the need to comply with the given measures like regular washing of hands and avoiding touching the orifices of the body. These illiterate adults see people who put on face masks as aliens and people are scared out of ignorance, hereby leading to spread of diseases and bad public health.

Issues with compliance, poor communication skills: Lorenc and Braithwaite (1993) conducted a study to understand factors leading to better medication compliance. Five of seven factors were potentially related to patients' health literacy level: accurate knowledge of regimen, belief in taking tablets exactly as prescribed, less fear of illness, ability to read the label on the bottle, contraindications and understanding what the doctor had said. These factors, in turn, cause delay in seeking timely and appropriate care, leading to worse health status and increased use of emergency and hospital services.

Unknown health status and low self-esteem: Illiterates find it very hard to know their health status as a result of bad health knowledge and lack of proper exposure to health issues. Some relate whatever symptoms they have to spiritual roots and prayers. Some do not believe that science and technology would be able to solve their problems hence they keep quiet and watch themselves go worse daily. The health of the public is endangered by the aforementioned.

Unemployment, myopic view of life, settling for less and poverty: Research has it that unemployment rate in Nigeria is 2-4 times higher in people with little or no education than with people with degrees. It has been observed that adults who have failed to attain western education usually see themselves as people whose time is past. Most of the illiterate Nigerian adults usually resort to petty jobs and less paying jobs not minding if the pay is commensurable to the work they do and the time they spend doing it. This limits their reasoning and makes them see their life span as being cut short, therefore they put less attention to their health and the state of their body and just live for the moment. (Literacy Foundation 2008). The above mentioned factors lead to death, bad public health, and poverty, precarious financial condition, less income, lesser-quality jobs and inability to foot hospital bills and cater for day to day bills. Hence the need for proper sensitization and education of the illiterate masses especially the adults on steps to maintain public health in the Nigerian communities is very important.

The Role of Adult Education

Adult literacy is a global problem. UNESCO Institute for Statistics (UIS) (2015) reported that 85% of the world's adults age 15 and older lack basic reading and

writing skills equivalent to 757 million adults, and two-thirds of them are women. *Everyone has to work together to defeat illiteracy and its impact on public health. Without the participation of individuals, community organizations, business and government, the situation will never be turned around. Adult education can function and resolve this global issues through the following ways:*

Funding: Perhaps the greatest challenge facing education in Nigeria is inadequate funding by federal, state and local governments. At all levels, adult and non-formal education suffers seriously from under-funding. If Nigeria is to attain even part of the EFA Millennium Development Goals, there is a real need to consult widely and come up with a reliable and workable funding mechanism. The Federal Government must support workforce literacy by developing tax incentives, infrastructure development and support, public awareness campaigns and supportive policies to ensure accessibility of literacy and numeracy skills training to the masses. State and local governments need to take greater responsibility and treat adult education with the seriousness it deserves. To help reach that goal, governments must make literacy a policy and funding priority. (UNESCO 2015).

Training of the adults and implementations of adult literacy programmes: There is a need to recruit and train competent adult education personnel who will be motivated to support policies and programmes on adult education throughout the country. Without an adequate number of good facilitators and administrators, no amount of funding will yield the desired results. The NNCAE should be supported by government and non-government agencies to map out effective training strategies (Aliwa, 2018). Furthermore, literacy is often related to health matters, especially such issues like preventive health and maintaining health, pre-natal and post-natal health (infant care), public health, and so on. In essence, the quality of literacy services available will in turn improve the quality of life. Illiterate citizens deserve serious attention so that they cannot only contribute to the socio economic and political growth of the country, but also to improve their welfare and life expectancy. In addition, most adults are parents; their level of education undoubtedly will influence positively or negatively the quality and level of education their children would attain. Finally, adult literacy could help its recipients to be gainfully employed and earn higher income and higher status. In fact, it will help its beneficiaries to make use of the computer, internet and e-mail for more information, connections on their health issues.

Mass literacy campaign: The present UBE programme in Nigeria is moving in the right direction to combat illiteracy in the country (Fasokun 2008). In

order to address the poor literacy rates and low enrolment, the Government, in collaboration with NGOs and local communities, should ensure there is improved access to quality non-formal education for adults and out-of-school young people, especially girls and women, mobile communities and the disabled. The Federal Government should as a matter of urgency set the machinery in motion to institute another mass literacy campaign that will be effectively carried out. Fliers, posters and handouts can be shared to the masses giving them information about health and the needs to maintain good health practices not only in the time of emergencies (Obiozor 2016).

Self-education and private tutorship: It can be very embarrassing for an adult to admit that he cannot read or work simple calculations. If the thought of attending adult education classes freaks someone out, private tutors are always available. Some adults due to low self-esteem and fear of shame; also inability to express oneself find it hard sitting in classes. Hiring a private teacher who would teach them would be a good way to reduce illiteracy in some places. This would improve ability to read, write and communicate hereby enabling compliance with doctors' report, lesser chances of drug and substance abuse and healthy living promoted.

Preventive healthcare education and disease knowledge:

Illiterate citizens educated in healthcare literacy tend to adopt healthier lifestyles, enjoy better mental health, and engage in fewer types of risky behaviour. When faced by illness, they tend to be patients or carers who react in the earlier stages of the disease, mitigating severe situations and intervening in the disease when it is easiest to do so, thereby resulting not only in lower levels of healthcare expenditure, but also better recoveries, both faster and with less suffering. The Sustainable Development Goals (SDG) that specifically targets health contains a total of thirteen objectives to be attained by 2030. Of these, only two do not entail a direct relationship (substantially increase health financing and the recruitment; support the research and development of vaccines and medicines). In three instances, healthcare literacy is absolutely critical to attaining the set objectives: end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases, and combat hepatitis,

water-borne diseases and other communicable diseases; reduce by one-third premature mortality from non-communicable diseases through prevention and treatment; promote mental health and well-being; reduction and management of national and global health risks. (WHO 2016).

Adult education is concerned with mental excellence reflecting in performance and behavior. A person is at peace with himself when he is literate, informed, bold and virtuous and regarded as having attained what is the ideal and achievable in our material world. Adult education helps to conquer fears and empowers people of less privileged, the physically sick, the healthy challenged, professionals, drop-outs, stark illiterates and their like with knowledge, attitudes, behaviours and skills to live effectively in their communities. Health is wealth, one who is not healthy does not struggle for progress, adult education through its flexible nature has the capacity to design programmes for every person both young and old in all geographical zones. The experts in adult education (educators, facilitators) have been trained to handle all issues bothering man including health issues., with emphasis on methodology, programme content, mode of delivery, the participants, or beneficiaries and the need on ground. Therefore adult education according to the researchers is perceived to be the solution of illiteracy as it affects public health in Nigeria.

Recommendations

Adult educators should be given the chance to show their expertise in reducing the level of health illiteracy through developing learning packages relating to public health.

The government should create a platform that would give the adult masses the opportunity to have access to education without spending their meagre income.

There should be frequent health enlightenment programmes by the adult educators sponsored by the government focusing on the rural Nigerian communities through the radio, market, churches, town unions, teaching and learning about health matters.

Constant workshops are recommended for the health workers on the need for effective teaching of patients in various ailments as the matter arise not minding their level of professionalism.

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SENIOR SECONDARY STUDENTS' LEVEL OF KNOWLEDGE, ATTITUDE AND AWARENESS OF ENVIRONMENTAL POLLUTION IN LAGOS STATE

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Abstract

This study investigated Senior Secondary School Students' Level of Knowledge, Attitude and Awareness of Environmental Pollution in Lagos State. The descriptive survey research design was adopted for the study. Simple random sampling technique was used to select twelve schools and 540 biology students from Senior Secondary Schools. The instrument comprised of 30 modified Likert type questions on air, water and land pollution, including attitude and awareness scales ($r=0.645$). Nine research questions were raised for the study. Descriptive statistics of mean and standard deviation and inferential statistics of independent t-test, tested at 0.05 level of significance were used. Male and female students had an equal level of knowledge. Urban senior secondary students possessed more knowledge than their rural senior secondary school counterparts. Students from private owned senior secondary schools possessed more knowledge than their government-owned counterparts. Gender, school location and type of school had no influence on attitude and awareness towards environmental pollution. It was hence recommended among others that the government through the Federal Ministry of Education should introduce Environmental Education as a subject in the senior secondary school curriculum as against environmental pollution appearing as a topic across school subject areas.

Keywords: environment, pollution, knowledge, attitude, awareness

INTRODUCTION

Human societies have continued to change from traditionalism to modernism with rapid technological advancement. There is increasing industrial production to satisfy growing human needs and comforts to improve civilisation. New lifestyles and increased production activities have created unexpected industrial pollution, and population explosion has also worsened the situation.

The term environment is defined by the Oxford Advanced Learner's English Dictionary of Current English as conditions and circumstances affecting people's lives. According to Pandey (2014), the environment is the surrounding around us; that is, life-sustaining support of all living beings. It is the complex physical, chemical and biotic factors that act on an organism or an ecological community and therefore determine its form and survival (Encyclopedia Britannica, Vol. 4).

According to Banergee (2018), pollution derived from the Latin word "Pollutionem" meaning to defile or make dirty. Hence, pollutants are substances which defile or make the environment dirty. Air pollution, water pollution and land pollution are the three major classes of pollution. Air pollution is the contamination of the air with unwanted gas, smokes, particles and other substances. Wastes remaining from the ways we produce goods and generates energy to heat our environment also pollute the air (Ferronato, 2019). Some sources of air pollutants include thermal power stations, burning fossil fuel and moving vehicles which emit harmful pollutants like sulphur dioxide, nitrogen oxide and carbon monoxide. These can cause acid rain, global warming, reduced sunlight and visibility, increased corrosion, asthma, cancer, and other lung diseases.

Water pollution is primarily caused by water run-off, which carries fertilising chemicals such as phosphates and nitrates from agricultural fields into lakes, streams, and rivers. These

chemicals combine with the phosphates and nitrates from sewage to speed the growth of algae, a type of plant-like organism. The water body may then become choked with decaying algae, which severely depletes the oxygen supply. This process is called eutrophication; it can cause the death of fish and other aquatic life. Erosion which is the wearing away of topsoil by wind and rain, also contributes to water pollution. Soil and silt, fine sediments washed from logged hillsides, ploughed fields, or construction sites, can clog waterways and kill aquatic vegetation.

In land pollution, the earth's surface is degraded or destroyed directly or indirectly as a result of human activities. Each year, households produce tons of garbage and garbage like aluminium, plastic, paper, cloth and wood are collected and sent to local recycling units. Items that cannot be recycled become a part of the landfills that hampers the beauty of the city and causes land pollution. In developing countries of the world like Nigeria, household wastes are often dumped by the street sides constituting danger because they become breeding grounds for pathogenic vectors. The resultant effects of environmental pollution are serious adverse socio-economic and ecological implications such as flood disasters, desertification, respiratory disorders, allergies, malignancies, cardiovascular disorders, increase in stress and various other harmful effects (Ferronato, 2019).

Nigerian cities such as Lagos are witnessing high rate of environmental degradation and is rated among urban areas with the lowest livability index in the world (Ohwo and Abotutu, 2015). Lagos State is the smallest state in Nigeria with an area of 356,861 hectares of which 75,755 hectares are wetlands, yet the rate of population growth is about 600,000 per annum with a population density of about 4,193 persons per sq. Km. The implication is that the population is growing ten times faster than New York and Los Angeles in the U.S. with a grave impact for urban sustainability. Lagos

state has the largest population of industries of the four most industrialised states in Nigeria (Lagos, Rivers, Kano and Kaduna) (Oketola & Osibanjo, 2011). These industries generate and introduce wastes of varying types such as SO₂, NO₂ and CO, into the environment. This is the appropriate time to action and control the pollution otherwise, the waste products from consumption, heating, agriculture, mining, manufacturing, transportation, and other human activities will further degrade the environment to the utmost harm of man.

Humans make these problems; hence, different kinds of interventions should be taken into account, and the most effective solution is education. Environmental knowledge needs to be a part of societal knowledge; in this way, environmental problems become public worries. The aim of environmental instruction and consequently, environmental literacy is training citizens who, both theoretically and practically, has supportive behaviour toward the environment (Goulgout, Plakitsi and Stylos, 2019).

STATEMENT OF THE PROBLEM

The environment is highly polluted and threatened, and there is an urgent need to create environmental awareness as to conserve, protect and nurture our environmental resources. Environmental education has been included in the Nigerian senior secondary school biology curriculum to acquaint the younger generation with appropriate knowledge, skills and attitudes relating to the environment. Environmental pollution yet continues to be on the increase with little or no observable positive behavioural change towards preserving the environment in Lagos State. Previous studies focusing on students' environmental knowledge, awareness and attitude were carried out in other countries; Sharma (2014); Olufemi, Mji and Mukhola (2016a); Olufemi, Mji and Mukhola (2016b); Mangat (2016); Banga (2016); Sultana, Hossen and Khatun (2017) and Alam (2017). There is a scarcity of research designed to compare male and female, urban and rural and public and private secondary school students' level of knowledge, attitude and awareness of environmental pollution in Biology in Nigeria. This study was, therefore carried out to fill the gap.

PURPOSE OF THE STUDY

The purpose of the study was to compare the knowledge, attitude and awareness of environmental pollution in biology among secondary male and female, urban and rural, government-owned and private senior secondary schools' students in Lagos State.

RESEARCH QUESTIONS

What is the difference in knowledge about environmental pollution between male and female senior secondary school students?

What is the difference in knowledge about environmental pollution between urban senior secondary school students and rural senior secondary school students?

What is the difference in knowledge about environmental pollution between senior secondary students of government-owned and private schools?

What is the difference in attitude between male and female

students towards environmental pollution?

What is the difference in attitude between urban senior secondary students and rural senior secondary school students towards environmental pollution?

What is the difference in attitude between senior secondary students of government-owned and private schools towards environmental pollution?

What is the difference in the level of awareness of environmental pollution between male and female senior secondary students?

What is the difference in the level of awareness of environmental pollution between urban senior secondary students and rural senior secondary students?

What is the difference in the level of awareness of environmental pollution between senior secondary students of government-owned and private schools?

RESEARCH HYPOTHESES

There is no significant difference in the mean scores of male and female students on knowledge about environmental pollution.

There is no significant difference in the mean scores of students of urban senior secondary schools and rural senior secondary schools on knowledge about environmental pollution.

There is no significant difference in the mean scores of senior secondary students of government-owned and private schools on knowledge about environmental pollution.

There is no significant difference in attitude between male and female students towards environmental pollution.

There is no significant difference in attitude between urban senior secondary students and rural senior secondary students towards environmental pollution.

There is no significant difference in attitude between senior secondary students of government-owned and private schools towards environmental pollution.

There is no significant difference in the level of awareness between male and female senior secondary students.

There is no significant difference in the level of awareness between urban senior secondary students and rural senior secondary students.

There is no significant difference in the level of awareness between senior secondary students of government-owned and private schools toward environmental pollution.

METHODOLOGY

The study adopted a descriptive survey research design. The population of the study was the Senior Secondary II students in Epe and Apapa local government areas of Lagos State. The study sample consisted of 540 biology students selected from 12 senior secondary schools from the local government areas under study. The schools in each of the two local government areas were selected by simple random sampling technique. The instrument used for data collection was adapted from the Children Environmental Attitude, and Social Knowledge Scale (CHEAKS) developed by Lemming and Dwyer (2008), The ROSE questionnaire developed by The ROSE project in 1962 and Gallup Poll (1992).

Data obtained from the instrument were analysed using descriptive statistics of mean and standard deviation and inferential statistics of independent t-test at a significance

level of 0.05. The analysis was done using SPSS version 17.0.

RESULTS

Hypothesis One: There is no significant difference in the mean scores of male and female students on knowledge about environmental pollution.

Table 1: Difference in the mean scores of male and female students on knowledge about environmental pollution

Variable	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
Gender								
Male	48.22	273	6.30	0.24	538	1.96	.658	Not significant
Female	48.46	267	6.33					

Significant at $p \leq 0.05$

Table 1 reveals that there was no significant mean difference between male and female students with regards to their knowledge of environmental pollution ($t=1.96$, $df: 538$, $p>0.05$). Thus, the null hypothesis, which states that there is no significant difference in the mean scores of male and female students on knowledge about environmental

pollution, was accepted.

Hypothesis Two: There is no significant difference in the mean scores of students of urban senior secondary schools and rural senior secondary schools on knowledge about environmental pollution.

Table 2: Difference in the mean scores of students of urban senior secondary schools and rural senior secondary schools on knowledge about environmental pollution.

Variable	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
School Location								
Urban	48.96	284	6.30	1.05	538	1.97	.049	Significant
Rural	47.91	256	6.33					

Significant at $p \leq 0.05$

Table 2 reveals that there was a significant difference in the mean scores of students of urban and rural senior secondary schools with regards to their knowledge of environmental pollution ($t=1.97$, $df: 538$, $p<0.05$). Thus, the null hypothesis, which states that there is no significant difference in the mean scores of students of urban senior secondary schools and rural senior secondary schools on knowledge about environmental pollution, was rejected. Hence, there was a significant difference in the mean scores

of students of urban senior secondary schools and rural senior secondary schools on knowledge about environmental pollution.

Hypothesis Three: There is no significant difference in the mean scores of students of government-owned senior secondary schools and private-owned senior secondary schools on knowledge about environmental pollution.

Table 3: Difference in the mean scores of students of government-owned senior secondary schools and private-owned senior secondary schools on knowledge about environmental pollution.

Variable	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
School Type								
Government	47.71	300	6.02	1.48	538	2.75	.006	Significant
Private	49.19	240	6.31					

Significant at $p \leq 0.05$

In the table above, there was a significant difference in the mean scores of senior secondary students of government-

owned and private-owned schools regarding their knowledge of environmental pollution ($t= 2.75$, $df: 538$,

$p < 0.05$). Based on this result, the null hypothesis which states that there is no significant difference in the mean scores of students of government-owned senior secondary schools and private-owned senior secondary schools on knowledge about environmental pollution was rejected. Thus, there is a significant difference in the mean scores of students from government-owned senior secondary schools

and private senior secondary schools on knowledge about environmental pollution.

Hypothesis Four: There is no significant difference in attitude between male and female students towards environmental pollution.

Table 4: Difference in attitude between male and female students towards environmental pollution.

Variable Attitude	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
Male	13.57	273	2.92	0.26	538	1.00	.317	Not Significant
Female	13.31	267	3.13					

Significant at $p \leq 0.05$

Table 4 above shows that there was no significant difference in the mean scores of male and female students concerning their attitude towards environmental pollution ($t=1.00$, $df: 538$, $p > 0.05$). Thus, the null hypothesis, which states that there is no significant difference in attitude between male and female students towards environmental pollution, was

accepted.

Hypothesis Five: There is no significant difference in attitude between urban senior secondary school students and rural senior secondary school students towards environmental pollution.

Table 5: Difference in attitude between urban senior secondary school students and rural senior secondary school students towards environmental pollution.

Variable School Location	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
Urban	13.48	284	2.75	0.14	538	0.56	.573	Not Significant
Rural	13.34	256	3.15					

Significant at $p \leq 0.05$

Table 5 above shows that there was no significant difference in the mean scores of urban senior secondary students and rural senior secondary students regarding their attitude towards environmental pollution ($t=0.56$, $df: 538$, $p > 0.05$). Based on this result, the null hypothesis, which states that there is no significant difference in the attitude between students of urban senior secondary schools and rural senior

secondary schools towards environmental pollution, was accepted.

Hypothesis Six: There is no significant difference in attitude between senior secondary students of government-owned and private schools towards environmental pollution.

Variable School Type	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
Government	13.35	300	2.94	0.16	538	0.63	.853	Not Significant
Private	13.51	240	3.03					

Table 6: Difference

in attitude between senior secondary students of government-owned and private senior secondary schools towards environmental pollution.

Significant at $p \leq 0.05$

Table 6 shows that there was no significant difference in the mean scores of senior secondary students of government-owned and private schools regarding their attitude towards environmental pollution ($t= 0.63$, $df: 538$, $p>0.05$). The null hypothesis which states that there is no significant difference in attitude between senior secondary students of government-owned and private schools towards

environmental pollution was therefore accepted based on this result.

Hypothesis Seven: There is no significant difference in the level of awareness about environmental pollution between male and female senior secondary school students

Table 7: Difference in the level of awareness about environmental pollution between male and female senior secondary school students.

Variable	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
Male	16.05	273	3.01	0.22	538	0.87	.384	Not Significant
Female	15.83	267	2.95					

Significant at $p \leq 0.05$

Table 7 above shows that there was no significant difference in the mean scores of male senior secondary students and female senior secondary school students regarding their awareness level about environmental pollution ($t= 0.87$, $df: 538$, $p>0.05$). Based on this result, the null hypothesis which states that there is no significant difference in the level of awareness about environmental pollution between male and

female senior secondary students was accepted.

Hypothesis Eight: There is no significant difference in the level of awareness about environmental pollution between urban senior secondary school students and rural senior secondary school students.

Table 8: Difference in the level of awareness about environmental pollution between urban senior secondary school students and rural senior secondary school students.

Significant at $p \leq 0.05$

Variable	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
Urban	16.12	284	2.80	0.30	538	1.18	.238	Not Significant
Rural	15.82	256	3.08					

The table above reveals that there is no significant difference in the mean scores of students of urban senior secondary school students and rural senior secondary school students regarding their awareness level about environmental pollution ($t=1.18$, $df: 538$, $p>0.05$). The null hypothesis which states that there is no significant difference in the level of awareness about environmental pollution between

urban senior secondary students and rural senior secondary students was accepted.

Hypothesis Nine: There is no significant difference in the level of awareness between senior secondary students of government-owned and private-owned schools towards environmental pollution.

Variable	Mean	N	SD	Mean Difference	Df	t-value	Sig. of P	Remarks
Government	15.89	300	2.75	0.08	538	0.34	.734	Not Significant
Private	15.97	240	2.99					

Table 9: Difference in

the level of awareness between senior secondary students of government-owned and private schools towards environmental pollution

Significant at $p \leq 0.05$

Table 9 above shows that there was no significant difference in the mean scores of senior secondary students of government-owned and private senior secondary schools with respect to their level of awareness about environmental pollution ($t= 0.34$, $df: 538$, $p>0.05$). Based on this result, the null hypothesis, which states that there is no significant difference in the level of awareness between senior secondary students of private and public schools towards environmental pollution, was accepted.

SUMMARY OF FINDINGS

In the analyses of hypotheses one, four, five, six, seven, eight and nine, it was observed that there were no significant differences in the mean scores of the variables under study. Hence, the stated null hypotheses were accepted. Analysis of hypothesis two showed that there was a significant difference in the mean scores of students of urban senior secondary schools and rural senior secondary schools on knowledge about environmental pollution; hence the null hypothesis was rejected. Analysis of hypothesis three also showed that there was a significant difference in the mean scores of students of government-owned senior secondary schools and their private school counterparts on knowledge about environmental pollution. The null hypothesis was, therefore rejected.

It was observed from the research findings with regards to knowledge that male and female students of senior secondary schools have an equal level of knowledge about environmental pollution. Students of urban senior secondary schools had more knowledge of environmental pollution than their counterparts in rural schools while private senior secondary school students had more knowledge than students of government-owned schools. The study also revealed that gender, school type and school location had no influence on the attitude and awareness of senior secondary school students to environmental pollution.

DISCUSSION

The study revealed that there was an equal level of knowledge of environmental pollution among male and female students. The finding that gender was not a factor in the knowledge of environmental pollution aligns with the finding of Olufemi et al. (2016a) in a study assessing secondary school students' awareness, knowledge and attitude to environmental pollution issues in the mining regions of South Africa: implications for instruction and learning. They discovered that students' knowledge level was not statistically different according to gender. Similarly, the finding is also in agreement with that of Sultana et al. (2017) that there was no significant difference between male and female students on the average level of environmental knowledge, in a related study of the assessment of environmental knowledge and attitude of secondary level students of Tangail, Bangladesh.

This study also revealed that students of urban senior

secondary schools had more knowledge of environmental pollution than their counterparts in the rural senior secondary schools. This disparity may be the result of the influence of other factors as, the exposure of urban students to information about environmental issues from the mass media such as televisions and radios and the social media (Sultana, 2017). This is pleasing to note and especially significant since the urban centres are unarguably highly polluted. Embracing a positive behaviour towards the environment begins with knowledge of environmental issues like pollution. However, this finding is not in agreement with that of Sultana et al. (2017) who reported that there was no significant difference between urban and rural students on the average level of environmental knowledge.

The study revealed that senior secondary students of private schools were more knowledgeable about environmental pollution than their counterparts in government-owned senior secondary schools. This finding supports that of Olufemi (2016a), which showed that the mean score for knowledge of private school learners was higher than that of public-school learners. This could be as a result of factors like the quality of educational resources in the private schools; engagement in E-learning and high engagement in extracurricular activities geared towards environmental issues (Edsand and Broich, 2019). This result is at variance with that of Sultana et al. (2017), which stated that no significant difference was found in knowledge level between students from government school students and private school students.

This study further revealed that gender, school location and school type had no influence on the attitude of students towards environmental pollution. This result is in agreement with the findings of Olufemi et al. (2016b). They discovered that gender did not affect students' attitude of environmental pollution. However, the result is not in agreement with the research finding of Sultana et al. (2017) who found out that there was a small difference between male and female students' attitude towards environmental issues, with the female students having a higher environmental attitude than the males. In terms of school type, the finding of this study differs from that of Olufemi et al. (2016a) who reported that private school students had significantly higher scores in attitude towards the environment compared to the students from government-owned schools. It also differed from the finding of Sultana et al. (2017), which indicated that there was a significant difference in the level of attitude between government and private school students. Their result showed that government schools' students had a high positive attitude towards environmental issues as compared to their private schools' counterparts. The finding of this study does not agree with that of Olufemi et al. (2016a) regarding school location. Their result indicated that there was a significant difference in school location (Coal mining region and a non-coal mining region) in the attitude of students towards environmental pollution. They found

out that the students in the coal mining region had a better attitude towards environmental pollution than their counterparts living in the non-coal mining region. The result of this study does not also support that of Sultana et al. (2017), who found out that rural students had higher environmental attitude level than their urban counterparts.

Furthermore, this study revealed that gender, school type and school location do not influence senior secondary school students' level of awareness of environmental pollution. This finding is in agreement with Sharma (2014) in a study on environmental awareness of college students in relation to sex, rural-urban background and academic streamwise. He reported that male and female students possessed an equal level of environmental awareness ability. Olufemi et al. (2016b) also found out that there was no gender difference in awareness of environmental pollution. The finding of this study is however not in agreement with that of Alam (2017), who reported that the female students were more environmentally aware than the male students in a study of environmental awareness among senior secondary school students.

About school location, the finding of this study is in support of Mangat (2016), who reported that there was no significant difference in environmental awareness level between rural and urban senior secondary school students. The finding is also at par with that of Alam (2017), he found out that there was no significant difference in the level of awareness of rural and urban students. However, this result is not in agreement with the finding of Banga (2016). He discovered that rural graduate students had fairly good awareness than their urban counterparts in a study of the environmental awareness among rural and urban government college students of Ludhiana District of Punjab, India. In terms of school type, the result of this study is not in agreement with the finding of Alam (2017), who found out that private

school students were more environmentally aware than their government school counterparts.

CONCLUSION

It was concluded from the study that; the senior secondary students of urban schools and private schools possess more knowledge of environmental pollution than their rural and government-owned schools' counterparts. There is an anticipation that this knowledge be competent enough in influencing environmentally friendly behaviour in the students. The reverse is observed as the case, as the impact of environmental pollution on life in Lagos urban centres continue to rise exponentially. For there to be a change, biology educators should focus on nurturing positive environmental attitudes in learners through a thorough environmental education via curricular and co-curricular activities.

RECOMMENDATIONS

The Federal Ministry of Education should introduce environmental education as a subject in the school curriculum as against environmental pollution topic appearing across school subject areas. It would allow for enrichment of the curriculum and adequate presentation of the relationship between human activity and the quality of the environment in the presentation of content.

Teachers should be regularly trained on environmental education to help them develop and improve students' environmental suitable behaviours. It is especially crucial for the teachers in schools located in the rural areas. Adequacy of curricular materials which shed light on current social, environmental pollution problems should be ensured, as to evoke the emotions of the students. It would lead to the desire to want things to be done differently, develop attitudes of concern for the environment and the motivation to improve or maintain environmental quality.

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ASSESSMENT OF INFORMATION COMMUNICATION TECHNOLOGY (ICT) KNOWLEDGE AND COMPETENCE OF PRE-SERVICE PHYSICS TEACHERS IN MICHAEL OKPARA UNIVERSITY OF AGRICULTURE, UMUDIKE, NIGERIA

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Abstract:

This study adopted the survey design to investigate the knowledge and competence of Information and Communication Technology among pre-service physics education teachers in Michael Okpara University of Agriculture, Umudike. By the use of simple random sampling 55(23males, 32females) out of a population of 104 Physics Education Undergraduates served as sample. Two research questions and one null hypothesis guided the study. Questionnaire of the four point scale Likert type was the instrument used for data collection. The instrument was validated and its reliability coefficient was obtained as 0.86. Mean was used to answer the research questions while t-test statistics was used to test the hypothesis. Results showed that the students are only able to chat online, use the E-mail, make use of Microsoft Word and download materials from the internet. Result also showed no significant difference in the male and female students' knowledge and competence of ICT. Recommendations were made to include the need for workshops and training on ICTs and computer operations to be organized for pre-service physics education teachers by the administration of Michael Okpara University of Agriculture, Umudike. It was also recommended that the Physics Education curriculum should be revised in a way that it incorporates relevant courses and training in ICT.

Keywords: Science Education, Physics Education, Information and Communication

Introduction

Physics is a science that studies matter, energy and their interactions. It is an international enterprise which plays a key role in the future progress of humankind. Physics is one of the prerequisite subject for the study of medicine, engineering and other applied science courses in the university. Physics is sometimes refers to as the science of measurement and its knowledge has contributed greatly to the production of instruments and devices that are of tremendous benefits to human race (Omosewo, 2013). Physics according to Adeyemo (2010) is seen as a discipline that has an abstract nature but despite this abstract nature, its teaching is to bring about scientific thinking in students; a mindset that require students to test out theoretical concepts through experimentation. According to Erinoshio (2013), Physics is basic for understanding the complexities of modern technology, and essential for technological advancement of a nation. Not only this, many of the tools on which the advances of science and technology depend are direct product of physics. Physics education is very important to the technological development of any nation and should be taught properly. Unfortunately the learning of physics has been observed to have some flaws like using the lecture method which is teacher centred to teach physics; concentrating on the use of textbooks and not exploring other teaching materials; laying more emphasis on the mathematical computations than the physics concepts; learning process is confined to reading and memorization – critical and creative skills are rarely developed.

These flaws have unfortunately affected the academic

achievement of students in physics over the years. Achievement of students in physics has been poor (WAEC Chief Examiners report, 2016, 2017 and 2018). The reason for this poor achievement cannot be separated from the fact that there is deficiency in the teacher's knowledge and students' interaction especially in the area of knowledge and competence in Information and communication Technologies (ICT) which is now a global phenomenon (Kola, 2013). The global market has become highly digitalised and there is barely any employer of labour that will not require reasonable competency from its employees. There is therefore a great need to improve academic achievement of students in physics and prepare physics graduates adequately for the global labour market. This can be done through changing the teacher's methods of teaching physics by using appropriate computer tools relevant in developing essentials ICT skills that are central to a successful learning in physics (Wanbugu and Changeiro 2008). Physics teachers can effectively deliver their physics lessons by using active learning strategies such as ICT tools in the teaching and learning of physics. Such ICT skills will get the students involved in learning activities that will enable them develop the needed knowledge and competence in ICT related issues. According to Shedd (2014), physics teachers must incorporate technology into their physics classes since teaching has gone beyond traditional method of talk and chalk. This implies that the integration of Information and Communication Technology (ICT) in physics classes is very imperative (Aina, 2012). Explaining further, Aina (2012) stated that such methods will create excitement in students and thus

induce their critical thinking and conceptual understanding of the subject. According to Kola (2013) Information and communication technology (ICT) is defined by UNESCO as forms of technology used for creating, displaying, storing, manipulating, and exchanging information (UNESCO, 2011). ICTs, in general, consist of computers, hardware and software, networks, learning management systems, e-mail, internet, telephone, television, radio and so forth. Information and communication technologies (ICTs) is an umbrella term which includes communication devices or applications like radio, cellular phones, computers, network hardware and software, television, satellite systems, internet and other electronic delivery systems as well as the various services and applications associated with them which include e-learning, distance learning and videoconferencing. According to Prasad and Prasad (2019), ICT is defined as computer based tools used by people to work with the information and communication processing needs of an organisation. It encompasses the computer hardware and software, the network and several other devices (video, audio, photography camera, etc.) that convert information (text), images, sound, and motion, and so on into common digital form (Milken Exchange on Education Technology, 1999). According to European Union (2005), Information and communication technology refers to the applications found on most thin client computers, internet, radios, digital televisions and projectors among others that secondary school teachers can use as pedagogical tools. ICTs included not only learning resources but also tools to facilitate interaction and collaboration (European Union, 2005). It is obvious from the above that for pre service physics teachers to ensure the acquisition of appropriate levels of ICT literacy and life skills needed for life-long learning by the physics students, such pre-service teachers need to have innovative strategies to reinforce their capabilities. No wonder Ndirika (2015) stressed that pre-service science teachers must have the knowledge of information and communication technology to adequately communicate scientific knowledge to students in this twenty first century. The concept of National Economic Empowerment and Development Strategy (NEEDS) is a programme that emphasizes creation of employment opportunities, which is to say that its emphasis is on self-employment and self-reliant for economic growth and development. This is the main thrust of entrepreneurship education. It focuses on empowering citizens and if properly harnessed will address the societal needs into the much talked about economic, social-political, industrial and technological development. It is also believed that well harnessed NEEDS can bring about the needed socio-economic development of a nation if properly managed. According to Iyamu and Ojeaga (2015), the adoption of National Economic Empowerment and Development Strategy (NEEDS) in 2004 became

necessary due to the global changes in the social and economic context coupled with the economic situation of the country. NEEDS, have the major targets of value reorientation, poverty reduction, job creation, and wealth generation. According to Obioma and Ajagun (2006) there was a need to re-define the country's education system, as exemplified in the reforms which are meant to move Nigerian education from the theoretical orientation to practical and knowledge orientation. Bello (2007) highlighted some of the reasons for educational reforms to include the need to: have education relevant to the need of the country, make education accessible to more people, and pay more attention to science and technology, equip students with the relevant knowledge to change their private and professional lives and prepare the citizens of the country to face the challenges of globalization.

According to Dappa and Thom—Otuya (2010), the effects of globalization in the education system include; the adoption of information and communication technology for increased access to education and also for quality in education, the decentralization and privatization which are considered strategic for ensuring quality and flexibility in a global economy, as exemplified in global market and global skills, changes in both the labour market and the education systems due to the increasing demand for skilled labour, the demand for additional resources for education in a policy environment that is not receptive to the expansion of the role of the public sector in education and the increase in cross-national measurement of education system.

Due to the highly digitalised global market which has resulted in all employers requiring reasonable competency with the computer from its employees, there is therefore need for pre-service teachers to have adequate knowledge and competence of ICT related issues. The implication is that ICT has thus become a necessity to be included in the training of undergraduates irrespective of their disciplines. There has also been growing interest in the use of ICT to support whole class teaching and learning to complement ICT based activities for individual students. Ton and Trinh (2019) explained that teaching Physics education using ICTs has a lot of benefits which include: learners motivation, students engagement in activities, increased interest, access to a huge range of resources that are relevant to scientific learning, visualization and manipulation of complex models to enhance understanding of scientific ideas, increased variety of ways that material can be used for whole class and individual learning, access to more up to date resources and the possibility of repetitive tasks to be carried out to allow for more student time on thinking about the scientific data that has been generated.

The first holistic attempt at introducing ICT in all facets of the country's life was the approval by the Federal Government of a national policy on ICT. The Nigerian national policy for information technology (FRN, 2004), recognised the need for ICT to be used for education. This policy has several objectives which include the need to: integrate ICT into the mainstream of education and training, and establishment of multifaceted ICT institutions as centres of excellence on ICT and empower youths with ICT skills to prepare them for competitiveness in a global environment. In order to achieve these objectives, nine major strategies were outlined. These objectives are using ICT in distance education, and ICT companies' investment in education; giving study grant and scholarship on ICT, training the trainers' scheme for Youth Corp members on ICT, ICT capacity building at the zonal, state, and local government levels; establishing private and public dedicated ICT institutions, and working with international and domestic initiatives to transfer ICT knowledge and making the use of ICT compulsory at all educational institutions, developing of ICT curricular for all levels of education (Yusuf & Yusuf, 2009).

It is very pertinent at this point to find out whether Physics pre-service teachers in Michael Okpara University of Agriculture, Umudike have adequate knowledge and competence of ICT skills in order to compete effectively in the global labour market. The present study thus set with the purpose of assessing ICT knowledge and competence of pre-service physics education teachers in Michael Okpara University of

Agriculture, Umudike.

Research Questions:

The following research questions are to guide this study

What is the mean knowledge and competency in ICT possessed by pre-service physics teachers?

Do male and female pre-service physics teachers differ significantly in their ICT knowledge and competencies?

Hypothesis:

The following Null hypothesis is set for analysis at $p \leq 0.05$:

There is no significant difference in the ICT knowledge and competency of male and female pre-service physics teachers

Method

This study adopted the survey design. By the use of simple random sampling, 55 (23 males, 32 females) out of a population of 104 Physics Education Undergraduates served as sample for the study to investigate the knowledge and competence of Information and Communication Technology among pre-service physics education teachers in Michael Okpara University of Agriculture, Umudike. Two research questions and one null hypothesis guided the study. Questionnaire of the four-point scale Likert type was the instrument used for data collection. The instrument was validated and its reliability coefficient was obtained as 0.86. Mean was used to answer the research questions while chi-square statistics was used to test the hypothesis.

Results

Table 1: Mean Responses of Students on Level of Knowledge and Competencies in ICT

Knowledge and Competence in:	SA	A	D	SD	Mean	Remarks
Microsoft word	16	34	3	2	3.16	Agree
Microsoft Excel	2	8	11	34	1.60	Disagree
Microsoft PowerPoint	-	11	14	30	1.65	Disagree
Microsoft Access	-	5	18	32	1.51	Disagree
Online Communication-chatting	18	32	5	-	3.24	Agree
E-mail	15	38	2	-	3.24	Agree
Downloading from internet	28	19	2	6	3.26	Agree
SPSS	8	4	13	30	1.89	Disagree
Desktop Publishing	4	4	15	32	1.64	Disagree
Computer-aided AutoCAD, Arch CAD	2	9	13	31	1.67	Disagree
Computer-aided Manufacturing CAM	-	4	13	38	1.38	Disagree
Write a Programme using a simple programming language	2	8	19	26	1.60	Disagree
Operate and teach with the Interactive Whiteboard	-	12	30	13	1.98	Disagree
Design educational websites	2	8	11	34	1.60	Disagree
Design educational software	1	3	12	39	1.38	Disagree
Design ICT rich learning environment	2	2	16	35	1.47	Disagree
Critically select appropriate technology to support the learning process	7	5	15	28	1.84	Disagree

Results on Table 1 showed that all the items except items 1, 5, 6 and 7 have mean responses of students below 2.50 which is the mean value of the scale used. This further implies that the students disagreed on having adequate and sufficient knowledge of ICT in thirteen out of the seventeen aspects of ICT listed above.

Research Questions 2: Do male and female pre-service physics teachers differ significantly in their ICT knowledge and competencies?

Table 2: Mean Responses of Male and Female Students on level of Knowledge and Competence in ICT components.

I have a high level of Knowledge and Competence in:	Male Mean(X)	Remarks	Female Mean(X)	Remarks
Microsoft word	3.23	Agree	3.10	Agree
Microsoft Excel	1.57	Disagree	1.63	Disagree
Microsoft PowerPoint	1.73	Disagree	1.60	Disagree
Microsoft Access	1.57	Disagree	1.47	Disagree
Online Communication-chatting	3.21	Agree	3.26	Agree
E-mail	3.23	Agree	3.25	Agree
Downloading from internet	3.25	Agree	3.26	Agree
SPSS	2.15	Disagree	1.71	Disagree
Desktop Publishing	1.69	Disagree	1.60	Disagree
Computer-aided AutoCAD, Computer-aided Manufacturing CAM	1.65	Disagree	1.68	Disagree
Write a Programme using a simple programming language	1.41	Disagree	1.36	Disagree
Operate and teach with the Interactive Whiteboard	1.59	Disagree	1.62	Disagree
Design educational websites	2.01	Disagree	1.95	Disagree
Design educational software	1.63	Disagree	1.58	Disagree
Design ICT rich learning environment	1.37	Disagree	1.38	Disagree
Critically select appropriate	1.43	Disagree	1.50	Disagree
	2.01	Disagree	1.69	Disagree

technology to support the learning process

Results on Table 2 clearly showed that all the items except items 1, 5, 6 and 7 have mean responses of both male and female students below 2.50 which is the mean value of the scale used. The implication of this is that both male and female Physics students do not agree to having adequate and sufficient knowledge of ICT in thirteen out of the seventeen aspects of ICT listed above.

Table 3: Chi-square Analysis of Male and Female Responses to ICT Knowledge and Competence.

	Value	df	Significant (2-Sided)
Pearson Chi-Square	22.627 ^a	27	.568
Likelihood Ratio	28.683	26	.386
Linear-by-Linear	1.245	1	.132
No of Valid Cases	55		

Table 3 shows a non-significant difference ($P \geq 0.05$) in the ICT knowledge and competence of male and female pre-service Physics education students.

Discussion

Results on Table 1 show that out of the 17 items listed, the students only have knowledge and competencies in only 4 items which are Microsoft Word, Online communication-chatting, using E-mail and Downloading from the internet. This means that the students lack knowledge and competence on the use of Microsoft Power point, Microsoft Excel, Microsoft Access, SPSS, Desktop Publishing, Computer-aided AutoCAD and Computer-aided Manufacturing CAM. More so, the students cannot write a programme using a simple programming language, operate and teach with the interactive whiteboard, design educational websites, design educational soft-wares or even design ICT rich learning environment. The result of this study is contrary to observation of Middleton (2000) who stated that digital video, audio, software simulation, synchronous and asynchronous chats and interactive software, among others, bring dynamism in describing a method or reporting result. The result of this study indicates that physics students are not exposed to courses that could equip and prepare them with useful ICT skills in their course and students on their own part have not made any personal efforts to acquire these skills. This result is in line with the observation of Kola (2013), that there is a great deficiency in the teacher’s knowledge and students’ interaction especially in the area of knowledge and competence in Information and communication Technologies (ICT).

A further observation in this result is that none of the objectives stated in the ICT Policy is being achieved. This includes empowering youths with ICT skills to prepare them for competitiveness in a global environment and integrate ICT into the mainstream of education and training, and establishment of multifaceted ICT institutions as centres of excellence on ICT.

The result of Chi-square analysis shows a non-

significant difference between male and female students’ ICT knowledge and competence. The implication is that gender differences does not exist between male and female physics pre-service teachers in ICT related issues which is a good development. This result is in line with the findings of Ndirika (2015) who reported a non-significant difference in the male and female biology pre-service teachers’ ICT knowledge and competence. This result contradicts the findings of Akanwa (2011) who reported a significant difference in the usage of the internet by male and female science teachers.

Conclusion

The importance of Information and Communication Technologies in the Physics Education curriculum cannot be over emphasized. Information and communication technologies are very important and invaluable tools necessary for the success of the educational reform programmes of the Federal Government of Nigeria. Information and Communication Technologies are very pertinent and vital in curbing the unemployment menace among Nigerian youths. Unfortunately, there is a gap in the training of undergraduate’s students who are not studying computer in Michael Okpara University of Agriculture, Umudike. Supporting this, Aniebonam (2007) reported a similar gap in ICT skills between average Nigeria student and students of comparable economies around the world. It is therefore very necessary that the government and National Universities Commission (NUC) should address this gap for Nigerian graduates to be able to compete with others globally.

Recommendations

Based on the findings of this study, the following recommendations are made:

Physics Education Undergraduate Curriculum should be revised to incorporate relevant courses and training

in ICT in line with one of the nine strategies for achieving the National ICT objectives of the nation. Physics Education teachers should as a matter of great importance be effectively trained in ICT and this training should be linked to ICT usage. Physics Education lecturers in the universities should be encouraged to utilise available ICTs in lecture delivery as a way of encouraging students to use them.

Government should encourage science teachers by providing computers and other ICT infrastructures to create an enabling environment for ICT training and usage especially in secondary schools. University administration should organize trainings and workshops on useful ICTs and basic computer operations for both physics education lecturers and teachers

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WIS-SED 16FL

IMPACT OF TEACHER CHARACTERISTICS ON STUDENTS' ACADEMIC PERFORMANCE IN FEDERAL COLLEGES OF FORESTRY, NIGERIA

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Abstract

This paper was carried out to explore the technical knowledge and vocational skills of students and the influence of teaching staff factors on students' performance. This was initiated as a result of the importance of technical and vocational education as an integral part of national development. The study was a survey study. A sample of three hundred and eighty-two (382) National Diploma students and eighty (80) teaching staff of Federal Colleges of Forestry were used for this study. Three instruments were used to collect data for this study: Technical Knowledge Test (TKT), Vocational Skill Test (VST) and Teaching staff performance Observation Scale (TSPOS). Data collected were analysed using descriptive analysis and multiple regression. The result of study revealed that 68.3% of the students scored 50% and above in technical knowledge while 55.5% of the students scored 50% and above in vocational skills. The result also revealed that the four teachers' characteristics (evaluation approach, course mastery, delivery approach and attitude) contributed significantly to students' technical knowledge and vocational skills. It is recommended that evaluation approach, course mastery, course delivery approach and attitude of teaching staff should be taken into consideration as factors that affect students' achievement in Technical/Vocational Education. In addition, training of teaching staff should be encouraged especially in the area of teaching pedagogy for proper service delivery.

Keywords: Technical Knowledge, Vocational Skills National Diploma, National Development,

Introduction

The role of education in enhancing social and economic progress of a nation has long been recognized as the foundation for the development of any nation. Education is not just an instrument of enhancing efficiency but it is also an effective tool useful for widening and upgrading the overall quality of individual and societal life and for sustainable development. Educational systems vary from one country to another, depending on the needs, aspiration, goal and philosophy of education of the country in question. The Nigerian government is of the view that majority of tertiary education graduates should acquire sufficient technical skills and knowledge to be self employed with wealth creation (Umunadi, 2012); this is also why entrepreneurial and skill acquisition has been incorporated into the National Youth Service Corp (NYSC) scheme lately, for self-employment and self-reliance after the one-year compulsory service. Technical education becomes important because it facilitates the acquisition of practical and applied skills as well as basic scientific knowledge. Technical and vocational education and training has been an integral part of national development this corroborates with Marope et al. (2015), that technical and vocational education and training (TVET) is steadily gaining popularity at the global debates and government priorities for education and national development agendas. This will help in promoting economic development, expand employment size and improve its quality. Technical and vocational education and training aim at developing human abilities in terms of knowledge, skills and understanding, this also make

students efficient in carrying out the activities in the vocational pursuits of his or her choice. This is in line with Nwogu and Nwanoruo (2011) that technical and vocational education as an educational training has been designed technically and systematically to accommodate both the trainer and the trainee. Most importantly, this is in order to enable the trainee acquire (the) basic knowledge, skill abilities, understanding and attitude needed for efficient performance in a chosen occupational career for self-reliance and national development. The specific goals of Technical and vocational education known as technical education at the tertiary level as stipulated by National Policy of Education (FRN, 2013) are as follows:

- (a. Provide courses of instruction and training in engineering, other technologies, applied science, business and management, leading to the production of trained manpower.
- (b. Provide the technical knowledge and skills necessary for agricultural, industrial, commercial, and economic development of Nigeria.
- (c. Give training that imparts the necessary skills for the production of technicians, technologists and other skilled personnel who shall be enterprising and self-reliant
- (d. Train people who can apply scientific knowledge to solve environmental problems for the convenience of man
- e. Give exposure on professional studies in the technologies

A critical analysis of the aims and objectives of Technology Education stated above indicate that such education or training leads to acquisition of practical skills which empowers the trainee to also become self-reliant. Technical and vocational training is among the vital tools needed by an individual to be developed. This implies that technical and vocational education and training are living experiences meant to be imparted to an individual systematically in order to get him/her adequately equipped for a good employment in a recognized occupation. Hence, it is a training for useful employment in trade, industries, agriculture, forestry, business and home making etc (Obioma 2011) Despite the importance of technical and vocational education and training, Nigeria seems not to accord it adequate attention it deserves and students' achievement have not been encouraging either. This appears to be one of the reasons for the rising rate of unemployment and poverty in the society. To realise its potential to impact development, however TVET systems need sustained transformation and revitalization. This is reflected in Shanghai consensus (UNESCO, 2012).

Forestry education is a unique and dynamic field of study, it is a vital instrument for sustainable development and societal transformation in the field of forestry, agriculture and natural resources. This education, therefore, should help to provide sufficient number of individuals that will contribute to adapting and identifying solutions to new problems, in a wider field than forestry. Federal College of Forestry is one of the tertiary institutions accredited by the National Board for Technical Education (NBTE) to offer technical and vocational education in the field of forestry and agriculture. The objectives of technical and vocational education offered in Federal College of Forestry include among others, to provide technical knowledge and vocational skills requisite for Agricultural and Forestry development (National Policy of Education (2013), Federal College of Forestry (2008)). To make sure that the above stated objectives of technical and vocational education offered in Federal College of Forestry are achieved, there is need for constant assessment of the quality of the output and also to explore the factors that affect the quality of technical and vocational education offered in Federal College of Forestry. Federal College of Forestry is characterized by students' knowledge and skill acquisition in the field of forestry and its related fields. Hence, the role of the teacher/lecturer is essential as in any other institution of learning, as the end implementer of educational curriculum. This explains why teacher quality has been of great interest to many researchers.

There is no doubt that students taught by experienced teachers in terms of knowledge of subject matter perform better than those taught by teachers that do not

have full knowledge of the subject matter. This view is supported by Fakeye (2012), who stated that ability to teach effectively depends on the teacher's knowledge of the subject matter. Since, teachers who do not have full mastery of the courses could get confused and mix up the content of the topic taught thereby causing the students to receive wrong information which could ultimately lead to poor achievement among the students. Competency and subject mastery are positive characteristics that must be possessed by any good teacher, because a teacher does not give out what he or she does not have. The teachers become handicapped when they are not familiar with the body of knowledge taught. Hence, lecturers and instructors must be grounded in the courses they teach and must be convincing enough so that the students will not doubt their competency. This is so because every educational system requires highly skilled teaching personnel to move the system forward and if students are left in the hands of untrained and unskilled lecturers and instructors, it will be difficult to accomplish the objectives of a school.

There is also the likelihood that if students' learning is adequately evaluated regularly, and prompt feedback and remediation provided, the level of students' performance will improve. Bardwell (1981) as cited by Ajogbeje and Alonge (2012), is of the opinion that when students are evaluated and feedback provided to them, the affected students are prone to have a better understanding of their capabilities and might begin to have different perception of themselves. This is because the major purpose of evaluating learners is to assess the strength and weakness of the learner and for evaluation to be effective, students should benefit from it. Students who undergo feedback and remediation usually accomplish a greater number of objectives than students who participate in an instructional programme that does not include remediation. This is attributed to the fact that, when a teacher provides feedback and remediation to learners, the teacher has gone a long way in leading the learners to be aware of their errors, in addition to providing them with the corrective measures.

The way and manner students perceived some courses, could be attributed to the fact that instructional delivery strategies adopted by Teachers and Teachers' attitude to teaching in classroom and on the field during the learning process are not impressive. This fact is supported by Egun (2007), who stated that most teachers of Agricultural science in our secondary schools today are still known to have difficulty in teaching some Agricultural science concepts thus leading to students' poor performance in the subject. Some still believe in using only lecture method whereas there are many other methods which can be used to make teaching and learning very interesting and effective. This is because lecture method is not the most effective method for diverse learners in today

school (Sleeve 2001). Teaching staff are therefore, required to use different and effective pedagogy such as demonstration, discussion and other methods for easy assimilation of technical and vocational courses. This is important because the use of wrong methodologies can affect students' performance. In addition, no matter how good a course content is, if there are no qualified and experienced teachers to use appropriate teaching methodology to deliver or teach the course, the desired goals of that course may not be achieved (Seng, 2007).

Okoro (2002) also reported that attitudinal factors have great influence on learning and students' achievement. The attitude which the teacher exhibit may likely affect the students either positively or negatively. Science teachers generally are required to employ various teaching approaches which will bring about effective teaching of the subject. Students level of achievement in technical knowledge and vocational skills may be a function of teachers' factors. This study assessed students' performance in technical knowledge and vocational skills and the extent to which teachers' factors affect students' performance. To achieve this aim, the following research questions were stated:

What is the level of performance of ND students in technical knowledge?

What is the level of performance of ND students in vocational skills?

What are the composite and relative influence of teaching staff on performance of the students (i.e. teaching styles, course mastery exhibition evaluation approach and attitude to teaching job) on the technical knowledge of the student?

Vocational skills?

What are the composite and relative influence of teaching staff on performance of the students (i.e. teaching styles, course mastery exhibition evaluation approach and attitude to teaching job) on the vocational skills of the student?

Methodology

The population for this study comprised of all the students of federal colleges of forestry.

Multi- stage sampling techniques were employed in this study. Stage one; two colleges namely Federal College of Forestry, Ibadan and Federal College of Forestry, Jos, out of the four colleges under the supervision of Forestry Research Institute of Nigeria (FRIN) were purposively selected. The reason for this is that the two colleges have the same programme and therefore share the same programme objectives. Stage two; census approach was used to select all the year two National Diploma students from the two colleges considering the size of population of the students. Simple random sampling was adopted to select eighty (80) teaching staff, forty (40) from each college.

Three instruments were used to collect data for this study: Technical Knowledge Test (TKT), Vocational Skill Test (VST) and Teaching staff performance Observation Scale (TSPOS). Simple random sampling technique was used to select eighty (80) teaching staff.

Technical Knowledge Test (TKT)

Total of sixty (60) multiple choice items with four options A to D were developed out of which forty (40) questions were selected after the validation of the questions. The content validity of this test items was established using the National Board for Technical Education curriculum and course specification for National Diploma to develop the items on technical knowledge in forestry and agriculture general questions. This test items covered the following course contents: Silviculture/Farm Management, Forest Management/Utilisation, Forest Extension, Horticulture, Technology of Wood Product/Timber Seasoning, Agriculture Extension and Forest Engineering

The instrument was pilot tested on fifty (50) ND II students of Federal College of Agriculture Moore plantation, Ibadan. Kudar Richardson Formula 20 (KR- 20) was used to establish the internal consistency of the instrument which gave 0.83 as the reliability coefficient and the items with difficult level ranging from 0.4- 0.6 were selected. The students were given one hour to respond to the items. The students' correct response to an item was scored 1 while wrong response was scored 0.

Table 1. TABLE OF SPECIFICATION

TOPIC	Knowledge	Comprehe nsion	Thinking	Total
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Silviculture/Farm management	18, 2, 5, 6, 9, 10, (6)	11, 12, 13 (3)	3,4,7,8 (4)	13 (32.5%)
Forest management/utilization	16 (1)	14 (1)	15 (1)	3 (7.5%)
Forest extension	18, 19 (2)	17 (1)		3 (7.5%)
Horticulture	20, 21 (2)	22f (1)	23 (1)	4 (10%)
Technology of Wood product/Timber seasoning	26, 28 (2)	27 (1)	24, 25 (2)	5 (12.5%)
Crop production	32 (1)	30, (1)	29,31 (2)	4 (10%)
Agriculture extension	33, 34 (2)	36 (1)	35 (1)	4 (10%)
Forest engineering	40 (1)	37 (1)	38,39 (2)	4 (10%)
Total	17 (42.5%)	10 (25%)	13 (32.5%)	40 (100%)

Vocational Skill Test (VST)

This instrument consists of sections A and B. Section A, deals with the Bio-data of the students, comprising the name of school, gender and age. Section B consists of the instruction and the time allowed for the test and twelve (12) questions (having forty-six (46) items) which are alternatives to practical questions. The items were generated using National Board for technical Education National Diploma curriculum and course specification Practical guide. The students were given

one hour to respond to the items. The students' correct response to an item was scored 1 while wrong response was scored 0. This instrument was validated by the researcher on a similar sample of fifty (50) students not included in the study. Kudar Richardson Formula 20 (KR-20) was used to establish the internal consistency of the instrument which gave 0.79 as the reliability coefficient and the items with difficult level ranging from 0.4- 0.6 were selected.

Table 2. TABLE OF SPECIFICATION

TOPIC	Application	Analysis	Synthesis	Total
Silviculture I/Farm management	2.(a, b, c, d, e, f, g, h)(8)		1. (a, b, c, d,e, f, g) (7)	15 (32.6%)
Crop production		3. (a, b, c) (3)		3 (6.5%)
Silviculture ii			4. (a, b, c,) 5. (a, b, c) (6)	6 (13.1%)
Forest measurement		7. (a, b, c, d, e, f) (6)	6. (1)	7 (15.2%)
Farm wood and land management	9. (a, b, c, d, e) (5)		8, 10. (a, b, c) 11. (a, b, c) (7)	12 (26.1%)
Agriculture extension	12. (a, b, c) (3)			3 (6.5%)
Total	16 (34.8%)	9 (19.6%)	21 (45.6%)	46 (100%)

Teaching Staff Performance Observation Scale (TSPOS)

This instrument tagged teaching staff performance

observation scale (TSPOS) was adapted from Ojo (2013) to measure teacher effectiveness. The instrument consists of thirty-six (36) items and the

index has three (3) dimensions which included instructional delivery of the teaching staff, teaching staff exhibition of course mastery and their evaluation approach. The observation scale was subjected to face and content validity by the researchers' supervisor and other experts in the field of observation and instrument construction and development. After the corrections have been effected the instruments was validated on

30 teaching staff that are not included in the study. Inter-rater reliability was established using scot pie formula and the reliability was 0.83. To administer this instrument, each teaching staff selected for this study was observed and rated using the instrument. The data collected were analyses using descriptive analysis and multiple regression.

Research question 1: What is the level of performance of ND students in technical knowledge?

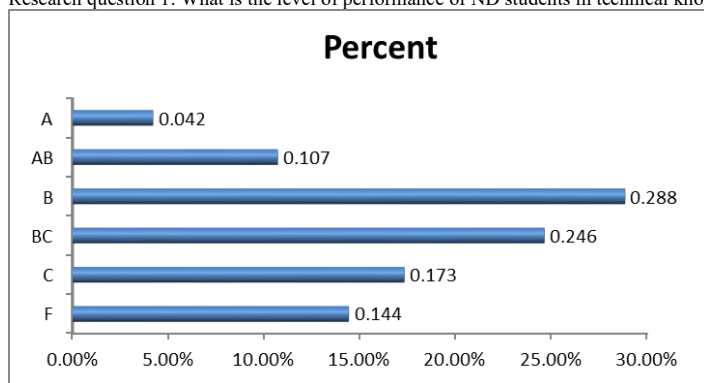


Fig 1: Performance of ND students of Federal Colleges in Technical knowledge

Fig. 1 show the status of ND students of Federal Colleges of Forestry in Technical knowledge. Out of three hundred and eighty-two (382) students sampled in this study, 110 (28.8%) scored Grade B (i.e. between

60 and 69 marks), 94(24.6%) obtained Grade BC (i.e. 50 - 59 marks), 66(17.3%) recorded weak pass (i.e.40-49 marks), while 55(14.4%) failed (i. e 0-39 marks). Meanwhile 57(14.9%) recorded marks between 70-

Table 3: Stanine norm on Technical Knowledge Test

Stanine	1	2	3	4	5	6	7	8	9
Resulting ranking	4%	7%	12%	17%	20%	17%	12%	7%	4%
Cumulative frequency	15	42	88	153	229	294	340	361	382
Scores	0-13	13-24	24-40	40-53	53-62	62-71	71-80	80-87	87-98

Table 3 shows the stanine scores range from a low of 1 to a high of 9 to determine the relative standing of individual students on vocational skill test. The table revealed that out of three hundred and eighty-two (382) students that sat for this test, 15 (4%) of them scored below 13% while 27(7%) of them scored between 13% and 24%. 46(12%) of the students scored between 24% and 40% and 65(17%) of them scored between 40% and 53%. The table also shows

that 76(20%) of the students scored between 53% and 62%. Another 65(17%) of the students scored between 62% and 71%, 46(12%) of them equally scored between 71% and 80%, 27(7%) scored between 80% and 87% while another 15(4%) of them scored above 87%

Research question 2: What is the level of performance of ND students in vocational skills?

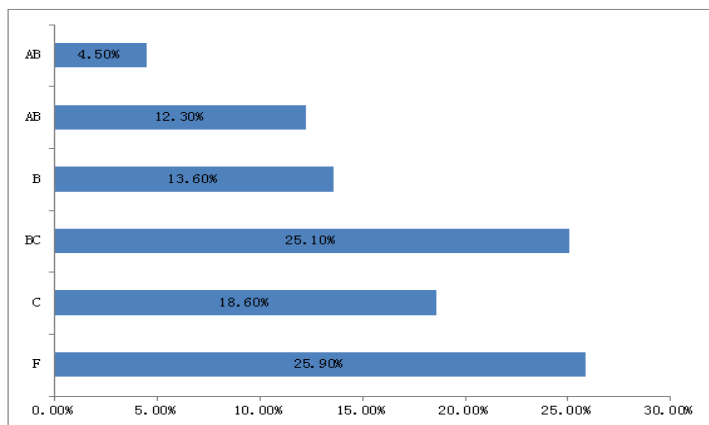


Fig 2: Status of students of Federal Colleges in Vocational skill

Fig. 2 show the status of students of Federal Colleges of Forestry in vocational knowledge. Out of three hundred and eighty-two (382) students sampled in this study, 99 (25.9%) scored F (0-39%); 71(18.6%) obtained weak pass grade C (40-49%); 96(25.1%)

recorded average score grade BC (50 - 59%); while 52(13.6%) are within grade B (60- 69%). However, only 47(12.3%) and 17(4.5%) scored grade AB (70-79%) and A (80-100%) respectively.

Table 4 Stanine norm on vocational skill test

Stanine	1	2	3	4	5	6	7	8	9
Resulting ranking	4%	7%	12%	17%	20%	17%	12%	7%	4%
Cumulative frequency	15	42	88	153	229	294	340	361	382
Scores	0-13	13-24	24-40	40-53	53-62	62-71	71-80	80-87	87-98

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62% and 71%, 46(12%) of them equally scored between 71% and 80%, 27(7%) scored between 80% and 87% while another 15(4%) of them scored above 87%

Research Question 3: What are the composite and relative influence of teaching staff on performance of the students (i.e. teaching styles, course mastery exhibition evaluation approach and attitude to teaching job) on the technical knowledge of the student?

Table 5: Correlation Matrix showing relationship between independent variables and dependent variable.

Variables	Technical Knowledge	Evaluation approach	Course mastery	Course delivery method	Attitude
Technical Knowledge	1				
Evaluation approach	.053	1			
Course mastery	.439**	-.020	1		
Course delivery method	.396**	-.296*	.293*	1	
Attitude	-.152	-.125	.237	.045	1

** Sig at 0.01 Level *Sig at 0.05 Level

The result from table 5 shows the correlation coefficients among independent variables (evaluation

approach, course mastery, delivery approach and attitude of the teaching staff) and dependent variable (Students' technical knowledge). The table showed that there was weak relationship between each of the teachers' variable, this indicated that there was no multi-collinearity between teacher staff variable. As shown in the table, there was a positive significant

relationship between students' technical knowledge ($r = .439$) and teaching staff course mastery and course delivery method ($r = .396$). positive relationship existed between evaluation approach and technical knowledge of the student ($r = .053$), and there was also negative relation between attitude and students' technical knowledge ($r = -.152$).

Table 6: Summary of regression analysis showing composite Contribution of combined independent variable to dependent variable.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	752.232	4	188.058	9.593	.000 ^b
Residual	1058.649	54	19.605		
Total	1810.881	58			

$R = .645$ $R^2 = .475$ Adjusted $R^2 = .420$

The joint effect of the four teachers' variable (evaluation approach, course mastery, delivery approach and attitude of the teaching staff) on students' technical knowledge was significant ($F(4,54) = 9.593, P < 0.05$). The multiple correlation coefficient of all the combined predictor variable with students'

technical knowledge is .645. The adjusted R^2 is .475 and the adjusted R^2 is .420. this means that the predictor variables (evaluation approach, course mastery, delivery approach and attitude of the teaching staff) contributed to 42% of the variation in students' technical knowledge.

Table 7: Multiple Regression Analysis showing Contribution of each independent variables to the dependent variable.

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-13.914	8.397		-1.657	.103
Teaching staff evaluation approach	.168	.078	.239	2.163	.035
Teaching staff Course mastery	.498	.122	.456	4.072	.000
Teaching staff Course delivery method	.324	.105	.352	3.086	.003
Teaching staff Attitude to teaching	-.294	.104	-.306	-2.829	.007

Table 7 revealed the relative contribution of each the independent variables to the dependent variable as follows: evaluation approach ($\beta = .239, P < 0.05$), course mastery ($\beta = .456, P < 0.05$), course delivery

approach ($\beta = .352, P < 0.05$), and attitude ($\beta = -.306, P < 0.05$) on students' technical knowledge. All the variables were significant.

Table 8: Summary of regression analysis showing composite Contribution of combined independent variable to dependent variable.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	2407.770	4	601.942	10.830	.000
Residual	3057.080	55	55.583		
Total	5464.850	59			

$R = .664$, $R^2 = .441$, Adjusted $R^2 = .400$

The joint effect of the four teachers' variable (evaluation approach, course mastery, delivery approach and attitude of the teaching staff) on students' vocational skill was significant ($F(4,55) = 10.830, P < 0.05$). The multiple correlation coefficient of all the combined predictor variable with students'

technical knowledge is .664. The adjusted R^2 is .441 and the adjusted R^2 is .400. this means that the predictor variables (evaluation approach, course mastery, delivery approach and attitude of the teaching staff) contributed to 40% of the variation in students' technical knowledge.

Table 9: Multiple Regression Analysis showing Contribution of each independent variables to the dependent variable.

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	8.993	13.327		.675	.503
Teaching staff Evaluation Approach	1.197	.198	.652	6.030	.000
Teaching staff Course Mastery	-.846	.231	-.391	-3.658	.001
Teaching staff Course Delivery Method	-.445	.218	-.213	-2.047	.045
Teaching staff Attitude to teaching	.243	.111	.226	2.197	.032

Table 9 revealed the relative contribution of each the independent variables to the dependent variable as follows: evaluation approach ($\beta = .652, P < 0.05$), course mastery ($\beta = -.391, P < 0.05$), course delivery approach ($\beta = -.213, P < 0.05$), and attitude ($\beta = .226, P < 0.05$) on students' technical knowledge. All the variables were significant.

Discussion

The findings of the student' performance in technical knowledge test revealed that 57(14.9%) scored 70% and above while 55(14.4%) failed the test, this is shown in figure 1. The findings of the students' performance in vocational skill tests also revealed that only 64 (16.8%) of the students scored 70% and above, while 99 (25.9%) of them failed the test, this is shown in and figure 2. Apart from that 44.5% of the students scored below 50% in vocational skill test. The implication of this is that majority of the students did not get up to average in the test. This could be as a result of inadequate background knowledge of some of the courses. Indeed, some of the courses presently being offered require prerequisite knowledge of mathematics as foundation for enhanced performance by the students. This finding correlates with that of Adeleke and Appah (2011) who reported that Mathematics serves as a strong reinforcement to most courses offered in Federal College of Forestry and Federal College of Animal Health and Production technology. However, the programme presently being run by the colleges do not contain some relevant mathematics courses. Hence for students of Federal Colleges of Forestry to perform better in their courses they need to offer some prerequisite courses that will help them understand other courses.

Findings from tables 6 and 7 revealed that there was a composite significant contribution of the four variables (highest qualification, teaching experience, course mastery, evaluation approach and attitude) on ND students' of FCF technical knowledge. This is an indication that the characteristics of the teaching staff of FCF have positive impact on the learning process of the students. This finding corroborates the report of Adepaju (2002) that a significant relationship exists between teachers' variables and the learning outcomes of students. It is also in agreement with Odinko (2002)

that teacher factor is an important input in teaching/learning situation. This finding also complements that of Koedel and Betts (2007) who asserted that teacher quality is an important factor in predicting students' achievement.

Tables 8 and 9 revealed that four predictor variables (evaluation approach, course mastery, course delivery and attitude) contributed meaningfully to the regression model. Evaluation approach of the teaching staff contributed significantly to technical knowledge and vocational skills of the students this can be attributed to the fact that evaluation gives the lecturers and the instructors useful information about how to improve their teaching method and make them go extra mile to see that their students' performance improves regularly. However, this finding is supported by Baku (2008) assertion that assessment help to improve learning if it is well carried out as expected. Since, teachers constitute a significant variable in the learning process; quality teaching depends on the teacher's characteristics such as academic qualification, teaching experience and instructional delivery approach. As revealed from table 4.19 that four predictor variables (highest qualification, teaching experience, course mastery and evaluation) contributed meaningfully to the regression model. The reason for the significant contribution of teaching experience to vocational skill of the students might be that lecturers and the instructors who have stayed long in the colleges have mastered the course content and fully acquired skills and strategies in handling the students. This is in consonance with earlier report of Gibbons, Kimmel and O'shea (1997) that there was a significant relationship between teachers' experience and students' academic achievement. Since students taught by more experienced teachers achieved higher levels due to the simple reason that the experienced teachers have mastered the subject contents. Also they

had acquired classroom management skills and strategies to handle and tackle different classroom and practical problems.

The findings from tables 6 and 8 revealed that there was composite contribution of all the four variables (evaluation approach, course mastery, delivery approach and attitude of the teaching staff) on students' technical knowledge and vocational skill. This an indication that teaching staff constitute significant variable in learning process. This finding agrees with that of Hattier (2009) and Seng (2007) who affirmed that teachers' quality is an important moderating factor responsible for differences found in students' performance. In addition, no matter how good a course content is, if there are no qualified and experienced teachers to teach the course well, the desired goals of that course may not be achieved (Seng, 2007).

Conclusion

Based on the findings, it was observed that teaching staff evaluation approach, course mastery, delivery approach and attitude influence the students' performance in technical knowledge and vocational training in Federal Colleges of Forestry. Since technical and vocational education involves the acquisition of skills, knowledge, attitude and abilities necessary for entry into advancement in forestry and its related fields, students should be properly integrated into it to enable them acquire the technical knowledge and vocational skills needed.

Recommendation.

Based on this study, the following recommendations were made.

Evaluation approach, course mastery, course delivery approach and attitude of teaching staff should be taken into consideration as factors that affect students' achievement in Federal College of Forestry.

Teaching staff should be undergoing regular training, workshops, seminar and lectures on teaching methods, students need assessment for proper service delivery. Federal College of Forestry should have adequate teaching personnel who are well equipped with variety of pedagogy in order to impart technical knowledge and vocational skills thus improve students' achievement.

It is important that all teaching staff should be equipped with requisite tools and inputs to ensure that the objectives of technical and vocational training as stated by National Policy on Education are sustainably achieved.

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**SOCIOECONOMIC
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WIS-SSD 01

**SOCIOECONOMIC DETERMINANTS OF BEHAVIORAL RESPONSES TO CLIMATE CHANGE
COPING STRATEGIES AMONG WOMEN RICE FARMERS IN SOUTH-EAST, NIGERIA:
IMPLICATIONS FOR ENVIRONMENTAL MANAGEMENT**

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Abstract

This study examined the socioeconomic determinants of behavioral responses to climate change coping strategies among women rice farmers in South-East, Nigeria and their implications for environmental management. Multistage sampling technique was employed in the selection of 180 respondents from three out of the five States in Southeast Nigeria. Structured questionnaire were used to obtain relevant data for the study which was analyzed using simple descriptive statistics such as frequency counts, percentages and means as well as ANOVA and OLS regression model. Results revealed that major responses exhibited by the women towards the use of the adaptation measures were “seek for information based on observed changes ($\bar{X} = 3.8$), consult fellow farmers for advice ($\bar{X} = 3.3$) and opt for Value addition (processing of remaining crops into other products to preserve them) ($\bar{X} = 3.1$) indicating that there was a very proactive response to the adaptation measures. Education (2.501**), farm size (1.907)*, farming pattern (3.570***) and income (3.570***) influences the women’s behavioural response to the adaptation measures. Gender mainstreaming in access to resources, including credit, extension services, training, information and technology, must be taken into account in developing activities designed to curb climate change. Therefore, ICT driven virtual platform that provides weather advisory services for small-scale farmers should be made available through extension and Rural Advisory Services (RAS). This platform will enable farmers get prompt alerts about impending weather conditions and recommendations on what they should plant and adaptation strategies to apply according to their location.

Keywords: Behavioral responses, women farmers, rice production, coping strategies

Introduction

The focus on gender and recognition of how it influences climate change impacts has led to its inclusion in international plans, programmatic interventions and policies (United States Aid for International Development, 2018). The inclusion and study of gender as a component of development studies has gone through several revolutions as it became evident that men and women experience life in different ways based on socially constructed rules and expectations. The United Nations Framework Convention on Climate Change, (2018) has justified its focus on gender by noting that “Impacts of climate change, such as drought, floods, extreme weather events and reduced food and water security, affect women and men differently with the poorest being the most vulnerable. 70 per cent of the world’s poor are women. Even though women are therefore disproportionately affected, at the same time they play a crucial role in climate change adaptation actions. It is increasingly evident that involving women and men in all decision-making processes on climate action is a significant factor in meeting the climate challenge and achieving the long-term objectives of the Convention” (Ayesha, 2016).

Behavioural response is the range of actions and mannerisms made by individuals, organisms, systems, or artificial entities in conjunction with themselves or their environment, which includes the other systems or

organisms around as well as the (inanimate) physical environment (Intergovernmental Panel on Climate Change, 2010). Farmers have a long record in adapting to changes in rainfall and temperature over time. Future changes in the climate could have significant impacts on agriculture that will challenge farmers to adapt to changes in land use, commodity production, and its location. Moreover, agriculture is a major source of global greenhouse emissions, representing 10-12% of total global anthropogenic emissions of greenhouse gases (GHGs) (Wreford and Adger, 2010). Agriculture will be expected to reduce its own greenhouse gas emissions, and offset emissions from other sectors through carbon storage. All of these actions are closely related to farmers’ management practices. It is therefore important to understand the cultural and social (education, information, traditional local practices) factors and the incentives in place that can facilitate or hinder the implementation of adaptation actions.

Agricultural activities depend on and have a very large impact on natural resources which will affect a farmer’s behaviour. For example, when farmers are aware of how their own practices contributes to natural resource management and to their role within the local community, the effect of this awareness on uptake of coping strategies enhances sustainability in livelihood processes, environmental management and ecosystem balance Chukwu and Atukpawu, 2014)

Incorporating gender perspectives and involving women as agents of change is a critical building block in to climate change response action. Adaptation efforts should systematically and effectively address gender-specific impacts of climate change in the areas of food security, agriculture and fisheries; biodiversity; water; health; human rights, environmental management; and peace and security (United Nation Women Watch, 2013). Technological developments related to climate change should take into account women's specific priorities, needs and roles, and make full use of their knowledge and expertise, including indigenous knowledge and traditional practices. Women's involvement in the development of new technologies can ensure that they are user-friendly, affordable, effective and sustainable (Clot, 2015). The study therefore examined the socioeconomic determinants of behavioral responses to climate change coping strategies among women rice farmers in South-East, Nigeria and their implications for environmental management. Specifically, the study described the socio-economic characteristics of the women rice farmers, assessed perceived benefits of the use of climate change adaptation measures and their behavioural responses to the practice of adaptation measures to climate change in the study area.

Hypothesis

Ho= There is no significant relationship between behavioural response to adaptation measures to climate change and selected socioeconomic characteristics of the women rice farmers.

Methodology

Study area

The study was conducted in South-East agricultural zone of Nigeria. The South-East zone of Nigeria lies between latitude 4°20' and 7°25'N and longitudes 5°25'E and 8°51'E covering a land area of about 109,524 sq km, which represents about 11.86% of the total area of Nigeria (Federal Republic of Nigeria Official Gazette 2007.). South-East Nigeria comprises of five states namely; Abia, Anambra, Imo, Enugu and Ebonyi state. Nigeria as a nation lies wholly within the tropical zone with wide variability in climate across different parts of the country. The study followed a survey approach.

Sampling procedure

Multi-stage sampling procedure was used in selecting 180 respondents for the study. The research adopted Agricultural Development Programme (ADP) zoning pattern. In the first stage, three states Ebonyi, Anambra and Enugu States were purposively selected out of five states in the zone because they are the major producers of rice in the zone (Okeke and Oluka, 2014). In the second stage, one agricultural zone was purposively selected from each of the sampled State making it three (3) agricultural zones for proximity and good coverage by the researcher. The third stage was

random selection of two blocks from each of the agricultural zones making it six (6) blocks, (i.e. two blocks from each state). Furthermore, two circles were randomly selected from each block making it twelve (12) circles. Finally in the last stage, fifteen (15) female rice farmers were randomly selected from each circle making it 180 respondents for the study.

Data for this study were generated from primary sources which are the farmers. A structured questionnaire and interview schedule were used to elicit responses from the respondents. Tours for direct observation of field activities in the study areas to observe the adaptation/mitigation practices adopted by the farmers in coping with these challenges were organized. Focal group discussions with male and female rice farmers were conducted in definite locations among the states in the study area.

Data analysis

Objective i, was realized using descriptive statistics such as frequency tables, percentages and mean. Objective ii and iii were realized using mean count while the hypothesis was tested using Ordinary Least Square multiple regression analysis at 5% alpha level.

Results and discussion

Socioeconomic characteristics of the farmers

Results on socio-economic profile (Table 1) shows that the mean age of the women is 35.5 years indicating that the women are young, within the working age and vibrant, they are very active in the field and therefore able to recall and detect notable changes in climate, avoid and or tackle these challenges they may encounter in their rice farming activities given favourable working conditions. This result is an improvement on the study of Ajewole, Ojehomon, Ayinde, Agboh-Noameshie and Diagne (2016) which reported 49 years as the mean age of female rice farmers. Younger women are also going into rice production. Majority (90%) of them are also married with household size of 6 persons. there is appreciable level of literacy among the women as 53.3% have attained secondary school and mean rice farming experience of 6 years. Level of literacy could have significant influence on their ability to understand and apply adaptation coping strategies based on the situation in their rice farm as well as identify their challenges in the process. Umeh and Ekwengene (2017) agreed that education helps the farmers to make better, faster and informed decision while Ndamani, & Watanabe, (2016) reported that farmers with higher levels of education are more likely to use improved technologies in order to adapt to climate change. The result also indicates that the women have mean farm size of 1.5ha mean monthly income of N30,000. This indicates the women are smallholder low income earning farmers, therefore they need to protect the available farm land against vagaries of weather and other adverse working

conditions in order to maintain their farming production and sustain themselves and family. Farming experience is also listed as one of the factors which enable farmers to accept new ideas and practices and also enhances proper utilization of innovation and helps farmers to learn from their past mistakes. 48.8 % of the women have contact with extension agents fortnightly while most (68.2%) of them belong to cooperative societies. Access to extension and membership to cooperatives enhance their access and exchange of information on production innovations, weather forecasts, adaptation/mitigation strategies and other important updates concerning their farming operations. Table 1 also shows that most (52.2%) of the women do not have access to government credit facilities while 57.8% do not own their farm land as they on lease implying that they are self sponsored farmers, 58.8% still work with local level of technology operations in their farm while 74.4% of the women have accessed extension trainings on climate change. Similarly, Eden (2012) added it is important to identify gender-sensitive strategies for responding to environmental and humanitarian crises caused by climate change. These efforts should focus on: reducing women’s vulnerability, in tandem with men’s susceptibilities; promoting gender sensitive emergency responses; and enlisting women as key environmental actors in natural disaster management decision-making processes, alongside men, tapping on women’s skills, resourcefulness and leadership in mitigation and adaptation efforts.

Level	(SSCE/WAEC/GCE)	
Household Size	61.1	6
Income (N)	72.2	30,000.00
Rice farming pattern (Swamp rice production)	72.2	
Farming Experience (Years)	29.0	8
Farm Size (HA)	47.8	1.5
contact with extension	48.8 (Fortnightly)	
Membership of cooperative societies (yes)	62.2	
Access to government credit	52.2 (none)	
Access to land level of technology operations in the farm (local)	57.8 (lease) 58.8	
Access to extension trainings on climate change (yes)	74.4	

Field survey 2018

Perceived benefits of use of climate change adaptation measures

Results from Table 2 show that improved livelihood outcome ($\bar{X} = 4.0$), higher technical efficiency ($\bar{X} = 3.9$), higher income ($\bar{X} = 3.8$) and improved quality of rice produce ($\bar{X} = 3.8$) were identified as major benefits by the women. From the findings, the women agree that implementation of climate change adaptation coping strategies is very beneficial in their rice production enterprise and these benefits influence their responses towards implementation of adaptation measures. Jackson et al, (2010) agreed that although farmers manage at multiple scales, their adaptation decisions are primarily driven by immediate private benefits reaped.

Table 1: percentage distribution of the socio-economic characteristics of the women farmers engaged in climate adaptation measures in Abia State

Socio-economic variables	Percentage (pooled)	Mean
Age	36.8	35.5
Marital Status	90.0 (married)	
Educational	53.30	

Table 2: Distribution of Respondents based on Perceived Benefits of use of Climate Change Adaptation Measures to Rice Producing Households (n=180).

s/n	Variables	Anambra	Enugu	Ebonyi	(pooled mean) Southeast zone
1	Increased yield of rice produce	3.6	3.4	3.3	3.4
2	Higher income from rice farms	3.9	3.9	3.5	3.8
3	Improved quality of rice produce	3.9	4.0	3.5	3.8
4	Availability of food for farmers	3.1	4.1	4.0	3.7
5	Sustainable agricultural practices	3.5	3.9	3.5	3.6
6	Value addition to rice produce	3.8	3.0	3.5	3.4
7	Reclamation of land	3.9	3.8	3.5	3.7
8	Higher technical efficiency in rice production	4.7	4.0	2.9	3.9
9	Improved livelihood outcome	4.4	3.9	3.7	4.0
	Grand mean	3.9	3.8	3.5	3.7

Source: Field survey, 2018.

Farmers’ behavioural responses to adaptation measures of climate change

To effectively address the challenges of communicating climate change adaptations, there is need to understanding local-level perceptions around climate change risks and adaptation responses. Results show that the women responded proactively to the practice of adaptation measures. Major responses exhibited were change of cropping time ($\bar{x} = 3.4$) seek for information on expected method of farming based on observed changes ($\bar{x} = 3.0$), consulting fellow farmers for advice ($\bar{x} = 2.6$), reverting to indigenous methods or self help methods ($\bar{x} = 2.5$) and value addition to the remaining produce ($\bar{x} = 2.5$).

Similarly, Arbuckle, Morton and Hobbs (2015) also reported that farmers face pressures to adjust agricultural systems to make them more resilient in the face of increasingly variable weather (adaptation) and reduce GHG production (mitigation). These proactive responses indicate that farmers show close awareness of the role of climate in farming enterprise performance and risk, illustrating the importance of improving climate information dissemination channels. There is need for effective means of communication to extend priority location and gender based specific information to the farmers. This will address their individual vulnerability and increase their resilience towards weather variability as they voluntarily respond positively to implementation of these adaptation measures.

Table 3: Distribution of Respondents Based on Farmers Behavioural Responses to Adaptation Measures of Climate Change (n=180)

Source: Field survey 2018.

s/n	Behavioural responses	BAP	END	IOI	MFW	CR	LR	MST	CPT	PRV	ICH	AIT	Pooled mean
1	Cs/t	4.1	3.2	3.5	3.3	3.0	2.8	3.3	3.0	3.5	3.4	2.7	3.3
2	Info	3.8	2.8	2.9	3.0	2.6	2.2	2.8	3.0	3.3	3.0	2.5	3.0
3	Indg	2.5	2.4	2.6	2.6	2.6	2.2	2.6	2.4	2.8	2.5	2.2	2.5
4	Cff	3.3	2.5	2.7	2.6	2.5	2.4	2.6	2.5	2.9	2.9	2.2	2.6
5	Va	3.1	2.3	2.2	2.5	2.2	2.3	2.4	2.3	2.3	2.6	2.0	2.5
6	Sto	2.6	2.5	2.6	2.5	2.3	2.3	2.3	2.4	2.7	2.5	2.1	2.4
7	Pig	1.7	1.6	1.7	1.5	1.6	1.6	1.6	1.7	1.9	1.6	1.7	1.7
8	Aban	1.4	1.6	1.6	1.5	1.6	1.6	1.5	1.6	1.7	1.6	1.6	1.6
	Grand mean	2.8	2.4	2.5	2.4	2.3	2.2	2.4	2.4	2.6	2.5	2.1	2.1

Keyfor Behavioural responses = Cs/t=Change of cropping style and time,Info=Seek for information on expected method of farming based on observed changes,Indg=Revert to indigenous methods or self help methods, Cff=Consulting fellow farmers for advice, Va=Value addition, Sto=Storage of remaining crops, Pig=Play ignorant of the situation at hand, Aban=Abandonment of farming enterprise due to loss of crop to adverse climate condition. Keys for adaptation measures = BAP=Use of best agronomic practices of rice, END=Enterprise diversification, IOI=Increase in use of organic and inorganic manures, MFW=More frequent weeding, CR=Crop rotation,

LR=Land rotation, MST=Minimum soil tillage (zero or minimum), CPT=Change of planting time, PRV=Planting of resistant varieties, ICH=Increase use of chemicals like herbicide, insecticide to combat weed or insect outbreak, AIT=Adoption of irrigation techniques)

Table 4: Ordinary Least Square regression estimate of the influence of socioeconomic characteristics on the behavioural response to adaptation measures to climate change in the study area

Variables	Parameter	+Cobb Douglas
(Constant)	B_0	8.980 (9.566)***
Age	X_2	0.007 (0.681)
Marital status	X_3	-0.303 (-2.794)**
Education	X_4	0.064 (2.501)**
Household size	X_5	0.005 (0.172)
Farming experience	X_6	0.010 (0.832)
Farm size	X_7	0.009 (1.907)*
Farming pattern	X_8	0.002 (3.570)***
Monthly income	X_{10}	0.002 (3.570)***
Member of social organizations	X_{11}	0.014 (0.390)
	R ²	0.735
	R	0.715
	Adjusted F - Ratio	32.813***

Source: STATA, RA Result from

Key: * Significance at 10%, ** Significance at 5%, *** Significance at 1% ***, + = Lead Equation and the values in bracket are the t-values

The Cobb-Douglas functional form was chosen as the lead equation based on a high R² value for the women. The R² value of 0.735 implies that 73.5% variability in behavioural responses of the women was explained by the independent factors. Furthermore, the F-value of (32.81) was highly significant at 1% α -level indicating a regression of best fit. Furthermore, the result indicates as follows:

Education: The coefficient for education (2.501) was positive and significant at 5% α -level for the women. This implies that education has great influence on the behavioural response of the women rice farmers towards practice of adaptation measures. The more educated the women rice farmers are, the more they will respond more positively to the practice of adaptation measures of climate change. Similarly, Umar, Musa, Olayemi, and Suleiman, (2015) also identified literacy as a serious factor for utilization of innovations among extension service workers and farmers.

Farming pattern: The coefficient for farming pattern was positive and highly significant at 1% level of probability (3.57). This implies that the farming pattern the farmers adopted encouraged and influenced their practice of adaptation measures. Earlier results in the socioeconomic characteristics indicate that majority of women practiced swamp rice production pattern. Accurate and constant practice of adaptation measures which include good cultural practices are needed to combat adverse weather vagaries and achieve maximum yield. Langtau (2013) likewise asserted that excessive flooding, iron toxicity and lack of water control structures have been the bane of low land and swamp rice production in Nigeria, therefore effective action need to be taken against the climate menace to encourage rice production among the women.

Monthly income: The coefficient for monthly income (3.57) was positive and highly significant at 1% α -level. This implies that the higher the income, the higher their ability to respond positively to adaptation intervention measures in their rice farms. Again, this calls for timely assistance from government and non-governmental bodies in the form of access to credit, grants and subsidies to the women to sustain their fight against climate change menace in rice production in Southeast, Nigeria. This result collaborate Osondu1, Obike and Ogbonna (2014) who inferred that limited financial resources at the disposal of farmers act as barriers to entry into investments in their farms. Therefore, farmers need to adopt adaptation measures to combat climate change menace in order to increase their income level through their farming enterprise.

However, the hypothesis which stated that there is no significant relationship between farmers' behavioural response to adaptation measures to climate change and selected socioeconomic characteristics of the farmers in the study area was rejected.

Conclusion and recommendations

To ensure full participation of the women rice farmers in climate change initiatives, socioeconomic variables such as education in form of better access to training, credit and skills-development programmes in good cultural practices need to be enhanced. Gender mainstreaming in access to resources, including credit, extension services, information and technology, must be taken into account in developing activities designed to curb climate change. Against this backdrop, the study recommend as follows:

ICT driven virtual platform that provides weather advisory services for small-scale farmers should be

made available through extension and Rural Advisory Services (RAS). This will help farmers handle climate risks situation promptly, This platform will enable farmers get prompt alerts about impending weather conditions and recommendations on what they should plant and adaptation strategies to apply according to their location.

Government should, therefore, boost the capacity of research scientists and agricultural staff to develop and promote more appropriate and effective technologies

(e.g. drought-tolerant and early maturing crop varieties) to help the women adapt to extreme weather events.

Location and gender specific menace coping capacity building should be provided to the women farmers to absorb their socioeconomic peculiarities in the management of their environment and rice production activities.

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WIS-SSD 13

GENDER DIMENSION OF POVERTY AMONG RICE FARMERS IN KOGI STATE, NIGERIA

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ABSTRACT

This study analyzed the gender dimension of poverty among rice farmers in Lokoja Local Government area of Kogi State, Nigeria. It had specific objectives of describing the socioeconomic characteristics of farming households, examining input access among the respondents, assessing gender dimension of poverty in the study area and analyzing the determinants of poverty among the respondents. Primary data was collected through structured questionnaire from 120 farmers using multistage random sampling technique. Analysis of data was carried out using simple descriptive statistics, Foster Greer and Thorbecke poverty model (FGT) and Logit regression analysis. Results of the analysis revealed that 61.67% of the respondents are male while 38.33% are female. It is evident that the percentage of male farmers that had access to credit is 37.5% while that of female farmers is 23.3%. The result of the poverty status of male headed rice farming households shows that 43.2% were income poor while 56.8% are non-income poor. Among the female headed rice farming households, 58.7 % are income poor while 41.3% are non-poor.

The significant determinants of poverty among the male rice farmers in the study area are the years of farming experience and farm size while that of female rice farmers in the study area is farm size. The contribution made by farmers to rice production and to rural agricultural development in Nigeria is grossly underappreciated in spite of the role farmers' play in Nigeria rice sector. It is therefore recommended that farmers in this area should be granted access to more land and other inputs, to help them improve their rice production.

Keywords: Gender, Poverty, Rice Production

Introduction

Gender analysis focuses on the different role and responsibilities of women and men and how these affect society, culture, the economy and politics. For example, there is an important difference between women and men in their quality of life, in the amount, kind and recognition of work, health, literacy levels and in their economic, political and social standing. According to Spielhoch (2007), women comprise the majority of the world's population in both the urban and rural sectors and the majority of those working in the informal sector.

Across sub-Sahara Africa, agriculture is considered to be a major answer to the issue of extreme poverty among both male and female gender. To achieve this, gender inequality that inhibits the productivity of female farmers must be addressed. There are 450 million women and men working as agricultural laborers worldwide who do not own or rent the land on which they do work nor the tools and equipment they use. These workers comprise over 40% of the world's agricultural labor force often living below the poverty line and forming part of the majority of the rural population in many parts of the world (FAO/ILU 2005).

According to the United Nations (2006), women are responsible for over half of the world's rice production. In developing countries, rural women produce between 60-80% of the rice. The Food and Agricultural Organization (FAO) indicates that women produce as much as 80% of the basic

foodstuffs for household consumption and sale in Nigeria. These agricultural activities employs both gender with women playing vital roles in food production, processing and marketing in Nigeria; producing about 60-80 percent of total output (Rahman,2004) and contributing about 60-80 percent of agricultural labor force (Mgbada,2000).They also contribute more than two-thirds of their produce towards household subsistence (Ayoola, 1999; Rahman, 2004).

Poverty deals with the welfare of individuals. Poverty is a reflection of economic development and at a time when Nigeria is experiencing bad economy it is essential to discuss the concept of poverty. In Nigeria today, most people subsist on a mere ₦306 (slightly more than \$1) a day (World Bank, 2019). A large proportion of Nigerians lack adequate health care, shelter and remunerative jobs. Nigerian women are the most affected being marginalized in decision-making process, employment, economic opportunities and access to credit. Most of them suffer from illiteracy, high maternal mortality; low income and poverty (CBN/World Bank, 2019). According to Nigeria Poverty Profile (NPP) in 2010 released by NBS (2012), food poverty in Kogi State was 50.1%, absolute poverty 67.1%, dollar per day 67.3% and per capita expenditure was 73.5%. While based on derived subjective poverty measure, 58.7% were core poor, 38.0 moderate poor, and 3.3% non-poor.

Rice is a major crop for poverty reduction: a staple food which many families cannot do without in

Nigeria today. Rice has become a strategic commodity in the Nigeria economy. Since the mid-1970s, rice consumption in Nigeria has risen tremendously, at about 10% per annum. According to Akande (2003), increasing population growth, increase income level, changing consumer preference and associated change in family occupational structures as factors responsible for the growing of rice. However, the cultivation of rice is not a practice confined to a sex category. Fakoya *et al.* (2010) reported that poor rural women play important roles in rice based farming systems as unpaid family workers, hired laborers, income earners and major caretakers of family health and nutrition, the role which has been overshadowed by gender insensitivity by policy makers (Kandiwa, 2013). There is unequal division of labor and other numerous marginalization and subordination of women; the policy making body has consistently turned blind eye with insensitive and oblivious behavior on such gendering issues in rice production in Nigeria. If gender issues bordering on such imbalance in rice production is not addressed, Nigeria cannot achieve its intended aim of poverty eradication among rural rice farming households. It is upon this background that this study seeks to examine the socio-economics characteristics of male headed and female headed rice farming household, the disaggregated poverty profile of rice farming households and the determinants of poverty of these households.

Despite the some poverty eradication programs initiated and implemented by successive administration in Nigeria (for example, Better Life Program for rural women, National Poverty Eradication Program (NAPEP), Family Economic Advancement (FEAP), among others) about 86.9 million Nigerians are living in extreme poverty, which implies that nearly 50% of it estimated 180 million population are very poor (Damilare, 2019).

Poverty analysis and studies in Nigeria reveals that men, women, boys and girls experience poverty in similar yet different ways (Ajani, 2008). A gender perspective of poverty needs to be integrated in agriculture as a strategy pathway towards sustainable and effective rice production implementation program in Kogi State, Nigeria. Previous efforts at measuring poverty in Kogi State have always focused on assessment of poverty alleviation programs, multidimensional study of poverty among others. Measurement of poverty in Kogi State has rarely focused on the gender dimension indicators as the objective of policy programme. For example of previous efforts in analyzing poverty in Kogi State include, Adebayo (2004), Salifu (2010), Mohammed and, Awoyemi (2017), Ejima *et al.* (2019). Hence, this study seeks to examine the gender dimension of poverty among rice farmers in Lokoja local government area of Kogi State.

The broad objective of this study is to examine gender dimension of poverty among rice farmers in Lokoja, Kogi state. Specific objectives are to:

- i. describe the socio-economic characteristics of the rice farmers in the study area,
- ii. identify if rice farmers have access to the same kind of inputs,
- iii. examine the gender dimension of poverty status of rice farmers in the study area; and
- iv. identify determinants of poverty among the respondents in the study area.

Methodology

Area of study

This study was carried out in Lokoja Local Government Area of Kogi state, Nigeria. It lies at the confluence of the Niger and Benue rivers and is the capital of Kogi State. It is a confluence town and is contiguous to a lot of water bodies and wetland areas. The rainy season lasts from April to October. The dry season, which lasts from November to March, is very dusty of cold as a result of the northeasterly winds, which brings in the harmattan. The main vegetation type in Lokoja is Guinea savannah or parkland savannah belt with tall grasses and some trees. These are green in the rainy season with fresh leaves and tall grasses, but the land is open during the dry season, showing charred trees and the remains of burnt grasses.

Lokoja has a population of about 77,516 in 1991 which has increased to 195,261 in 2006, with 100,573 males and 94,688 female (National Population Commission, 2006). Agriculture serves as the main occupation of the people. Wetland areas have great advantages for farmers because they provide opportunities for planting different crops such as, rice, sugar cane, corn, vegetables, among others throughout the year. There are 10 wards in Lokoja local government area of Kogi State, they include: Eggan, Kakanda, Kupa north east, Kupa south west, Lokoja A, Lokoja B, Lokoja C, Lokoja D, Lokoja E, Oworo.

Data collection and sampling methods

In carrying out this study, primary data was used. The data were obtained from a field survey through the use of questionnaires, administered through personal interview. The target population was the male and female rice farmers. A Multistage sampling technique was used in the selection of the respondents. First stage involves a purposive selection of five wards from the 10 wards in Lokoja Local government Area that are known for rice production. In the Second stage, two communities were selected from the five wards and finally 12 respondents were randomly selected from each of the communities. Thus, a total of 120 respondents were selected for this study.

Analytical Framework

Simple frequency tables and percentages were used to analyze the socio-economic characteristics and

examine rice farmers' access to inputs.

Foster, Greer and Thorbecke poverty measure (Income approach)

Foster, Greer and Thorbecke poverty measure (Income approach) was used to determine the gender dimension of poverty status of rice farmers

$$FGTa = \sum_{k=1}^K ak FGTa,k$$

where $FGTa,k$ is the poverty measure for the k th group (Foster, Greer and Thorbecke, 1984). This implies that total poverty is a weighted average of poverty levels in all groups—the weights being proportional to the groups share of the population

Logit Regression Model

Logit Regression Model was used to identify determinants of poverty among the respondents.

The respondents were classified into poor and non-poor using the poverty line. The relative poverty line of 2/3 of mean per capita income was used. Farmers that have per capital income below the poverty line will be classified as poor and non-poor otherwise. The response variable will be binary taking values of one if the farmer is poor and zero otherwise.

The general logit regression model is mathematically expressed as

$$Logit\left(\frac{p}{1-p}\right) = \alpha + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \dots + \beta_nx_n + e$$

Where;

P == probability that a farmer will fall below the poverty line or not given as Xi; (0 = Non-poor; 1 = poor)

- x_1 = education level in number of years spent in school
- x_2 = farming experience` 3`
- x_3 = farm size
- x_4 = household size
- x_5 = access to credit
- α = constant
- β_1 - β_5 = regression coefficients
- e = error term

Results and Discussion

Socio-economic Characteristics of the Respondents

The socioeconomic characteristics of rice farmers is presented in Table 1. The gender distribution of the respondents as presented in Table 1 revealed that 61.67% were male and 38.33% were female implying a male dominance in rice farming in Lokoja though the roles of females cannot be underestimated in rice production. Females are majorly involved in the processing of rice. The result shows a male dominance in farming activities in the study area is in consonant with the result of a study carried out in 2006 by (National Bureau of Statistics (NBS) which reported that majority of farmers are male in most parts of

Nigeria.

Most of the male rice farmers (90.54%) were in the age range of 25 and 60 years, 6.76% were within the age bracket of 25 and less than 25 years. Majority (90.00%) of the women rice farmers were in the age bracket of 25 and 60 years. The mean age of the respondents was 46 years. This shows that most farmers involved in rice production were in their active age. According to Sofoluwe *et. al.*, (2011) younger famers have been found to be more knowledgeable about better practice and maybe more willing to bear risk. The age factors are very important for resilience purpose. It facilitates farmer's susceptibility to innovation. The younger farmers have the ability to adopt innovation and to retain changes more than the old. On the other hand, older farmers are more experienced and can easily predict and reduce risks associated with rice production.

Majority of the male rice farmers (33.78%) had primary school education while only 25.68% and 21.62% of men farmers had secondary and tertiary education respectively. For the women farmers, majority (47.83%) had primary school education while 23.91% and 6.52% respectively had secondary and tertiary education. The result also shows that 18.92% of the men farmers had no formal education while 21.74% of the women had no formal education. It is obvious that the level of educational of the respondents was low when compared to other region in the south eastern and northern part of Nigeria (Akinsanmi and Doppler, 2005). This relatively low level of educational may not encourage acceptance of innovation which may increase rice production.

Table 1. Distribution of Respondents According to their Socioeconomics Characteristic

Socio-economic characteristics	Men (%)	Women (%)	All (%)	Mean
Sex				
Male	74(100)	-	74(61.67)	
Female	-	46(100)	46(38.33)	

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Age (years)				
<25	5(6.76)	3(6.52)	8(6.67)	
25-60	67(90.54)	41(89.13)	108(90.00)	
>60	2(2.70)	2(4.35)	4(3.33)	
Educational Level				
No formal Education	14(18.92)	10(21.74)	24(20.00)	
Primary education	25(33.78)	22(47.83)	47(39.17)	
Secondary education	19(25.68)	11(23.91)	30(25.00)	
Tertiary education	16(21.62)	3(6.52)	19(15.83)	
Household size				
1-5	23(31.08)	17(36.96)	40(33.33)	
6-10	20(27.03)	17(36.96)	37(30.83)	8
11-15	16(21.62)	11(23.91)	27(22.50)	
>16	15(20.27)	1(2.17)	16(13.33)	
Farm size (Hectare)				
≤1	24(32.43)	3(6.52)	27(22.5)	
1.1-2.0	27(36.49)	32(69.57)	49(49.17)	
2.1-3.0	15(20.27)	7(15.21)	22(18.33)	
>3.0	8(10.81)	4(8.70)	12(10.00)	
Farming Experience				
1-10	26(35.14)	23(50.00)	49(40.83)	
11-20	19(25.68)	11(23.91)	30(25.00)	19.52
21-30	11(14.86)	5(10.87)	16(13.33)	
>30	18(24.32)	7(15.22)	25(20.84)	
Annual income (N)				
≤100,000	0(0.00)	2(4.35)	2(1.67)	
₦100,001- N200,000	34(45.95)	15(32.61)	49(40.83)	
₦200,001- N300,000	16(21.62)	11(23.91)	27(22.50)	₦464,462.50
₦300,001- N400,000	8(10.81)	4(8.70)	12(10.00)	
>N400,000	16(21.42)	14(30.43)	30(25.00)	
Membership of Association.				
Yes	58(78.38)	12(26.01)	70(58.33)	
No	16(21.62)	34(73.91)	50(41.66)	

Field Survey, 2019

About 31.08% of the male headed households had a household size of between 1 and 5 persons, while 27.03% had the range 6 and 10 persons as their household size. Also about 20.62% had above 16 persons as their household size. About 36.96% of the female headed households had between 1 and 5 persons, and 36.96% had between 6 and 10 persons. Only 2.17% had persons more than 16 persons as their household size. The overall respondents show that a greater proportion (33.33%) of the respondents had household size of between 1 and 5 persons. About 30.83%, 22.50% and 13.33% affirmed that they had 6 – 10 persons, 11-15 persons and above 16 persons in

their households. The mean number of persons per household was 8 persons.

About 36.49% of male headed household had farm size of between 1.1 and 2.0 hectares, while 32.43% had farm size less than equal 1 hectares. 20.27% had a farm size between 2.1 and 3.0 hectare. Only 10.81% of the male headed household head had greater than 3 hectares. Majority of the female headed households (69.57%) had between 1.1 and 2.0 hectares, while only 8.70% had farm size more than 3 hectares. The overall result shows that a greater proportion (49.17%) of the respondents had farm size of between 1.1 and 2.0 hectares.

Greater proportion of men (35.14%) had between 1 and 10 years farming experience. 25.68% had 11-20 years, while 14.86% and 24.32% had 21-30 years and greater than 30 years respectively. 50.00% of women farmers had between 1-10 years of farming experience. Of the total respondents, 40.83% of them had between 1-10 years of farming experience. Those that had between 11-20 years of farming experience were 25.00%. Another 13.33% of the respondents noted that they had between 21 – 30 years of farming experience while the remaining 20.84% had above 30 years of farming experience. The mean years of farming experience was 19.52 years. This implies that a great number of the respondents have been practicing agriculture long enough and could therefore have noticed significant changes in the weather as it affects their farming activities.

About (45.95%) of the men farmers earned between ₦ 100,001 and N200,000, 21.62% earned between N200,001 and N300,000 annual income while only 10.81% earned between N300,001 and ₦ 400, 000. Majority of the women farmers (32.61) earned between ₦ 100,001 - ₦ 200,000 annual income while 8.70% earned between 300000-400000 and 30.43% of the respondents earned above ₦ 400,000.

Most of the male respondents (78.38%) and 26.01% of women farmers were members of different social organizations. The overall result indicates that most (41.66%) do not belong to any association. While about 58.33% of the respondents belong to at least one association or society.

Respondents Access to Farm Inputs

The respondents' access to farm inputs is presented in Table 2. As shown in Table 2, most of the male respondents 37.5% had access to credit while only 23.3% of the women had access. Also, 55.0% of the male farmers had access to agro-chemicals while 35.8% women had access. 60.0% of the male rice farmers had access to improved varieties while the result shows 35.8% of the female rice farmers had access improved varieties. 45.8% of the male farmers claimed ownership of farming equipment, while the

result indicates that 28.3% of the female farmers claimed ownership of farming equipment. 26.7% of the male farmers had access to mechanized farm implement, while the result indicates that 9.2% of the female farmers had access to mechanized farm implement. Only 29.2% have farmland of their own, while only 18.3% of the female farmers had land of their own. This result obviously shows that men had more access to all the inputs than women. This unequal access to some farm inputs can promote their productive and income generating capacities, promote unequal access to social services which may excluded them from the social, economic and political processes that affect their lives.

Table 2: Respondents access to farm inputs

INPUT TYPE	Male %	Female %
Access to Credit	37.5	23.3
Access to Agro-chemicals	55.0	35.8
Access to improved variety of seeds	60.0	35.8
Ownership of farming equipment	45.8	28.3
Access to mechanized farm implement	26.7	9.2
Ownership of farmland	29.2	18.3

Source: Field Survey, 2019

Poverty Profile and Average Annual Income of the Respondents

The income level of moderate poor rice farmers fall below the poverty line of ₦25, 803.40. Similarly, for core poor, their income was below the poverty line of ₦12,901.70. The result suggest if the moderately poor were assisted to meet whatever is their shortfall from the threshold of ₦25,803.40, they will become non-poor. This means that a core – poor rice farmers needed addition to their income in order to be moderately poor or to become non-poor. The result furthers shows the average per capita income of the respondents is ₦234.22. Furthermore, the result showed 59 of the rice farmers were moderately poor representing 49.17% while 61 of the farmers were non-poor representing 50.83%. The income level of moderate poor rice farmers fall below the poverty line of ₦25,803.40 by 17 % amounting to ₦52,638.94 annually in addition to their income in order to be non-poor as it is shown in Table 3.1. That amount is needed annually in addition to a rice farmer average annual income to move one from the level of moderate poverty status to non-poor. The headcount ratio shows that only 49 % of the individuals in the study area were poor and that 51% were non-poor. The implication of this shows that targeted effort aimed at increasing the income of farmers in an attempt to alleviating poverty

among rural farming household would help reduce income poverty considerably. This position is in consonance with the report of the World Bank that the poverty profile of Nigeria dropped by 2% in 2013 according to World Bank 2013 as reported by Ajewole *et al.*, (2016).

Table 3.1: Poverty Profile and Average Annual Income of the Respondents

Distribution of Responses	Amount of income ₦
2/ 3 of the mean income	25,803:40
1/ 3 of the mean income	12,901:70
Average per capita income	234:22
Head Count Ratio	0.49
Moderately Poor	59
Moderately Non-poor	61
Moderately poverty gap index	0.17
Moderately poverty severity index	0.03
Chronic poverty	13

Source: field survey, 2019

FGT Gender distribution of Poverty Status among the Rice farming Households

Table 3.2 shows the poverty status of male headed and female headed rice farming households in the study area; Among the male, 43.2% were income poor while 56.8% are non-income poor. Among the female category, 58.7 % are income poor while 41.3% are non-poor

Table 3.2: Gender distribution of Poverty Status among the Rice farming Households

Poverty Status	Male		Female	
	Freq.	Percentage	Freq.	Percentage
Non Poor	42	56.8	19	41.3
Poor	32	43.2	27	58.7
Total	74	100	46	100

Source: Field Survey, 2013

Determinants of Poverty among male and female rice farming households

Table 4 present the disaggregated poverty determinants of male and female rice farming households, The positive values of the coefficient implies that increasing the independent variables by one unit will increase the poverty level by the value of the coefficient while negative values of the coefficient implies that increasing the independent variable by one unit will reduce the poverty level by the value of the coefficient. In Table 4, the disaggregated model for the rice farmers in the study is significant at one per cent. The pseudo R-square shows that 42.5 per cent of the variable affect their poverty level for male while that of the female shows that the pseudo R-square

indicates that 56.2 per cent of the variable affect their poverty level. This indicates that 42.5 per cent variation in poverty severity is explained by variations in the specified explanatory variables, suggesting that the model has fairly good explanatory power on the changes in poverty status among the respondents with 95% level of confidence.

The significant determinants of poverty among the male rice farmers in the study area are the years of farming experience and farm size. The probability of a male rice farmer being poor decreases with increase in the number of years of farming experience as well as with increase in their farm size. Both statistically significant at 5 per cent and 1 per cent respectively.

The coefficient of household farm size had significant and positive relationship with poverty status among

the male respondents. This means that a unit increases in the size of farm holding would lead to a decrease in the probability of household poverty and vice versa. Households with larger farm sizes were on average, poor than those that cultivated smaller farm sizes. The finding was in agreement with previous studies. The significant determinant of poverty among the female rice farmers in the study area is the years of farm size. The probability of a female rice farmer being poor decreases with increase in their farm size. The variable was statistically significant at 1 per cent. The coefficient of household farm size also had significant and positive relationship with poverty status among the female respondents. This means that a unit increases in the size of farm holding would lead to an increase in the probability of household poverty and vice versa.

Table 4: Disaggregated Logistic Regression Analysis showing the Determinants of poverty status

Logistic regression Variables	Male			Female		
	Coefficient	Std. Err.	T-values	Coefficient	Std. Err.	T-values
Educational level	-0.0750804	0.053937	-1.39	0.43489	0.365022	-0.99
Farm Experience	-0.0086645**	0.003963	-2.19	0.922514	0.050589	-1.47
Household size	-0.007593	0.009558	-0.08	0.994014	0.145111	-0.04
Credit Access	0.1598583	0.1446767	-1.10	1.349222	1.878766	0.22
Farm size	0.02038778***	0.0411642	4.95	0.037014***	0.041553	-2.94
-const.	1.191897	0.1621571	7.35	0.459783	1.48272	0.01
No. of Observation	74			46		
Prob >F	0.0000			0.0000		
R-Squared	0.4247			0.5616		

Source: Computed from Survey Data, 2019 ***Significant at the 1 per cent level, ** Significant at the 5 per cent level

CONCLUSION

This study assesses the gender dimension of poverty among rice farmers in Lokoja LGA of Kogi State. The study reveals that there is a high level of poverty in the study area among the rice farmers in the area. The study contribute to knowledge by showing that the poverty situation or rice farming households in the study area across the gender lines. It has been able to show that efforts targeted at reducing poverty among rice farming household in Nigeria should be targeted at both gender. Gender consideration in policy making in rice production should be a priority among stakeholders with focus on both male and female

headed households

Recommendations

1. The study recommends that poverty reduction strategies in the study area should be gender specific and should focus mainly on variables that influence the poverty status of each category of household.
2. The contribution made by farmers to rice production and to rural agricultural development in Nigeria is grossly underappreciated in spite of the role farmers play in Nigeria sector It is recommended that farmers in this area should be granted access to loan in cash and kind to help them improve their rice production.

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WIS SSD 19

ECONOMIC ANALYSIS OF VALUE ADDED CASSAVA PRODUCTION AMONG CASSAVA PROCESSING HOUSEHOLDS IN SOUTH EAST, NIGERIA

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Abstract

This study examined the economics of value added cassava production in Southeast, Nigeria. Descriptive statistics, farm budget analysis, ordinary least square and factor analysis were the major analytical tools employed for the study. Results of the analysis showed that the processors were young with basic formal education and highly experienced in cassava processing. The study further showed that the gross margin was ₦326814.53, ₦353978.00 and ₦267791.69 for *garri*, *fufu* and *abacha* enterprises respectively while the BCR was 2.17:1.00, 1.77:1.00 and 2.44:1.00 respectively for the enterprises implying that value added cassava production in the study area is profitable. The coefficients for age were negative and significant at 1% for *garri* and *fufu* enterprises and 10% level for *abacha*. Sex and household size were positively correlated with profit at different levels of significance for all the enterprises. The result also showed that high cost of labour, input prices, processing and weather conditions were some of the constraints militating against value added cassava production in the area. The study therefore recommends that, accessible, affordable processing inputs and storage facilities should be provided to improve processors technical efficiencies. More so, the emphasis for public investments should be on improving general knowledge, transport and market infrastructures.

Keywords: Cassava processing, Value added, Farm budget analysis, factor analysis

INTRODUCTION

Nigeria is faced with the challenge of ensuring adequate food supply for her teeming population with current population estimated at more than one hundred and forty million individuals (FGN, 2006). Milcah *et al.*, (2013) observed that among the strategies being pursued to achieve food security is agribusiness development, through strengthening smallholder farmers' entrepreneurial capacity and promotion of traditional crops production and utilization.

Cassava production is characterized by small scale producers who use old varieties and traditional production technologies which largely accounts for low yield. Oyebanji and Akwashilik (2003) noted that these small holders accounts for over 80 percent of cassava production in Nigeria. Meanwhile, over 90 percent of cassava production is consumed locally with less than 10 percent utilized for industrial purposes (Ayodele *et al.*, 2011). This may result from the fact that cassava is majorly produced by smallholder farmers cultivating 1-5 hectares of land intercropped with maize, yam or legumes in the rainforest and savannah agro-ecologies of Southern, Central and lately Northern Nigeria (National Bureau of Statistics, 2007). Agwu and Anyaechie (2007) opined that cassava value added products are the major source for food for many households in Nigeria. However, Ikeorgu and Iloka (1994) observed that cassava is one of the most dominant and main crop components in crop mixtures in South Eastern Nigeria.

The South Eastern region accounts for about 20 percent of the total cassava output in the country with

Abia, Enugu and Imo states producing a total of 634 metric tonnes, 2599 metric tonnes and 2315 metric tonnes respectively of the crop annually (NBS, 2007). The crop is produced in many forms both in the fresh and processed forms, starting from the producers through myriads of processors to the end users. Hence, processing and diversification of uses transfer cassava into wide range of food forms in order to increase its consumption, increase shelf life of the products, improve product palatability, enhance market value, reduce post harvest losses, facilitate transportation and provide raw materials to cassava based agro industries (IITA, 1990; Achems, 2011). Nevertheless, producers face many challenges in maintaining and developing cassava in a sustainable way. Currently, they cannot meet demand. At the farm level, production costs are high relative to those in other countries. If producers could harvest cassava and process them into staple commodities that could be stored for several months, they would earn high profits during the periods of scarcity (Hillocks, 2002).

More so, the processing of cassava is labour intensive and almost exclusively non-mechanized in Nigeria and in south east in particular. Peeling represents the most labour intensive unit operation of the cassava value chain, non-mechanized and traditionally done by women and sometimes, children (Fresco, 1993). Improvement of cassava processing techniques would also increase labour efficiency, incomes and living standards of cassava producers (Westby, 2002).

Though, remarkable progress has been made by agricultural research and development organizations on increasing agricultural productivity and promoting

sustainable intensification of major food crops for small scale farmers (Obisesan, 2012), sustaining success in productivity based agricultural growth however, depends on expansion of market opportunities (Diao and Hazell, 2004) through value additions. This also requires a holistic view beyond productivity to incorporating profitability and competitiveness (Kaplinsky, 2000). This study therefore summarized the descriptive statistics of the variables used in the multiple regression model analysis. The study also estimated the level of profit of cassava value added product and its determinants in the study area; and identified the constraints to households investment cassava processing.

METHODOLOGY

Study Location

The study was conducted in South Eastern Nigeria. The South East agro-ecological zone is made up of five states namely Abia, Anambra, Ebonyi, Enugu and Imo and has a rural population density of 173 persons per square kilometre (Iloka and Anuebunwa, 1995). The zone has a total land mass of 10,952,400ha with over 16 million people (NPC, 2006). Farming is the predominant occupation of the people in the zone majority of who are the small-holder farmers. Major food crops cultivated include cassava, maize, rice, sweet potatoes, yam, plantain, banana and vegetables (Onyeukwu, 2012).

Sampling Procedure

A multi stage sampling technique was used to select the respondents. Three out of the five states in the south east agro ecological zone were randomly selected for the study as shown in Table 1 below. A list of 270 cassava processors was compiled from the 24 communities. Out of these, 60 processors were purposively selected for a particular product (20 processors from each state), depending on the level of cassava processing activities going on in those communities. This gave a sample size of 180 cassava processors for the study Data for the study were collected from primary sources using structured questionnaire sets which were distributed to the processors.

Table 1: Multi Stage Sampling of Cassava Processors in the Study

State	Agricultural Zone	Local Government Area	Communities
Abia	Umuahia	Umuahia North	Ubani Ibeku and Okwoyi
		Ikwuano	AhabaUkwu Oloko and Ugwu-Ibere
	Ohafia	Bende	Bende and Uzuakoli
Enugu	Enugu East	Ohafia	Nkporo and Aro
		Isi-Uzor	Ehamufu and Ikem
	Enugu West	Enugu East	Ugwogo Nike and Agbogazi
Imo	Okigwe	Oji River	Akpugoeze and Akwuachi
		Aninri	Nenwe and Okpanku
	Owerri	Onuimo	Okwe and Umuna
		Okigwe	Agbobu and Umualumoke
		Ngor-Okpuala	Obikwe and Ntu
		Owerri West	Iheagwa and Umuguma

Source: Ahameful (2017)

Analytical Technique

Data from this study were analyzed using different tools and technique. Quantitative analytical techniques were employed in order to achieve the objectives. Specifically, the descriptive statistics were analyzed using statistics tools such as means and standard deviation. The level of profit and its determinants were analyzed using the farm budgeting technique employed by Ibekwe *et al.* (2012) and the ordinary least square multiple regression technique. The constraints militating against cassava processing was analyzed using factor analysis based on Ashley *et al.*, (2006) and Kessler (2006). The constraint factors were extracted based on the response of the processors. Only variables with loadings of 0.30 and above were used in naming the factors.

The Gross Margin technique was given thus:

$$GM = GR - TVC \dots\dots\dots(1)$$

Where; GM = Gross Margin in naira per kg

GR = Gross Revenue in naira

TVC = Total Variable Cost in naira

$$NI = TR - TC \dots\dots\dots(2)$$

The result of the budgetary analysis was used to obtain the following ratios;

$$RRI = \text{Rate of Return on Investment} = \frac{NI}{TC} \times \frac{100}{1} \dots\dots\dots(3)$$

Where; NI = Net Income

TR = Total Revenue

TC = Total Cost

TFC = Total Fixed cost

The profit function was fitted into the data and estimated using the multiple regression technique. The various forms of regression model were used to examine the influence of socio economic characteristics on profit level. The model is implicitly expressed as:

$$Y = f(x_1, x_2, x_3, x_4, x_5, x_6, e) \dots\dots\dots (4)$$

Explicitly the model is stated thus:

$$Y(garri) = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 + e \dots\dots\dots (5)$$

$$Y(fufu) = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 + e \dots\dots\dots (6)$$

$$Y(abacha) = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 + e \dots\dots\dots (7)$$

Where;

Y = profit (naira)

X₁ = age (years)

X₂ = marital status (dummy, married=1; single=0)

X₃ = gender (dummy, male=1; female=0)

X₄ = level of education (years)

X₅ = household size (no of persons)

X₆ = experience (years)

e = error term

RESULTS AND DISCUSSION

The summary statistics of the socio-economic characteristics of the cassava processors in South East, Nigeria is presented in Table 2. They include the sample mean and the standard deviation for each of the variables.

variables used in the multiple regression model in South East, Nigeria

Variables	Mean	Standard deviation	Minimum	Maximum
Age(years)	52.282	10.103	29	75
Marital	1.846	0.393	1	3
Status	6.079	2.989	1	18
Household size	0.618	0.487	0	1
Sex	1.634	0.955	0	3
Education (years)	17.901	11.589	3	48
Experience				

Source: Field Survey

Findings from Table 1 showed that the processors were young with basic formal education and highly experienced in cassava processing. The average age of the processors was 52years. This suggests that they belong to the economically active population category which is between 25-59 years (Muhammad-Lawal, *et al.*, 2013) and can therefore increase their output by increasing their capital investment and putting more effort into cassava processing. Majority of the processors in the study area were married with a mean household size of 6 persons. The contribution of marital status on agricultural production can be explained in terms of the supply of agricultural family labour. More so, both males and females engaged in cassava processing and were quite experienced with an average processing experience of 17.90 years. The years of experience is likely to impact positively on cassava processing as experience coupled with acceptance and adoption of improved processing technology will probably have direct relationship with value addition (Bassey and Okon, 2008; Muhammad-Lawal *et al.*, 2013).

Profitability Analysis of Cassava Value Addition

This section discusses the profitability of the major products from cassava processing and it's determinants in the study area. The budgetary technique was used while Benefit Cost Ratio was calculated. The result of the analysis is presented in Table 3 below.

Table 2: Summary of the descriptive statistics of the

Table 3: Cost and Returns Analysis for Cassava processing in the study Area

Variable		Value		
		Garri	Fufu	Abacha
A	Revenue/ton	544490.94	750450.00	431770.05
B	Variable Cost			
	items(₦/ton)			
	Cassava root	63487.15	99199.61	61997.06
	Other raw material cost	71818.28	236426.14	70306.96
	Labour cost	82370.98	60846.25	31674.34
	Total Variable Cost(TVC)	217676.41	396472.00	163978.36
C	Total Fixed Cost (TFC)	163946.69	208956.68	113314.00
	Depreciation	32789.34	27754.18	13062.80
	Total Cost	250465.75	424226.18	177041.16
	Gross Margin(Revenue-TVC)	326814.53	353978.00	267791.69
	Net Income (NI)=TR-TC	294025.19	326223.82	254728.89
	Benefit Cost Ratio(Revenue/Total Cost	2.17:1.00	1.77:1.00	2.44:1.00
	Rate of return on Investment(%)=NI/TC ×100/1	117.39	76.90	143.88

Source: Field Survey

Table 3 showed the result of the profitability analysis for value added products in cassava processing enterprises. The cost incurred in cassava processing included cost of raw materials (cassava tubers, firewood, fuel, water, bags, palm oil etc), labour (peeling, washing, grating (soaking, drying, sieving, frying etc), and cost of depreciated assets.

From Table 3, an average of 2730.63kg of cassava was processed into *garri*. An average of 4266.65kg fresh cassava root was processed into *fufu*. The average quantity of cassava tuber that was processed into *abacha* was 2666.54kg. The estimated total cost was ₦250,465 for *garri*, ₦424,226.18 for *fufu* enterprise and ₦177,041.16 for *abacha* enterprise respectively. The estimated total revenue was ₦544,490.94/ton for *garri*, ₦750,450.00/ton for *fufu* and ₦431,770.05/ton for *abacha* enterprises respectively. Furthermore, the result showed a gross margin of ₦326,814.53/tonne, ₦353,978.00/tonne and ₦267,791.69 /tonne for *garri*, *fufu* and *abacha* enterprises respectively.

The rate of return on investment (ROI) was calculated as a percentage ratio of the Net Income to the Total Cost (TC). ROI ratio in this study was used to estimate earnings per ₦1.00 investment. The estimated ROI for *garri*, *fufu* and *abacha* enterprises were 117.39%, 76.90% and 143.88% respectively. This implies that the *garri* enterprise made more than double the amount on every ₦1.00 invested. The result is the same for

abacha while the *fufu* enterprise made 77 percent profit on every ₦1.00 invested in the venture. This could be seen in the estimated Benefit-Cost ratio for the enterprises. For every N1.00 spent, there was a return of N1.17 (2.17:1.0), 77 kobos (1.77:1.0) and N1.44 (2.44:1.0) for *garri*, *fufu* and *abacha* enterprises respectively. This confirms the findings of Ehinmowo *et al.*, (2015) that the processing of cassava is profitable, returning a BCR of 1.75 to 2.24 in South Western, Nigeria.

Determinants of Level of Profit of the Cassava Value Addition

The determinants of the profitability of the value added cassava production were analyzed using multiple regressions. The results are presented in Table 4.5 below.

Table 4: Summary of the Socio economic Determinants of the Level of Profit of Cassava Value Addition

Variable	Garri Exponential	Fufu Exponential	Abacha Linear (L)
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	(L)	(L)	
Constant	9.0990 (10.00)***	8.7304 (10.32)***	12383.36 (1.42)
Age	-0.0635 (-4.08)***	-0.0673 (-4.47)***	-402.2984 (-2.30)*
Marital Status	-0.1881 (-0.38)	0.0564 (0.15)	3168.511 (0.84)
Sex	2.9969 (24.22)***	2.9874 (24.34)***	6919.96 (5.62)***
Education	0.0687 (0.24)	0.1754 (0.64)	3060.642 (1.13)
Household size	0.2358 (1.88)*	0.1955 (1.73)*	-464.6324 (-0.41)
Experience	0.0206 (0.33)	0.0275 (0.45)	906.1707 (1.50)
R-squared	0.9387	0.9391	0.5399
Adj R-square	0.9308	0.9313	0.4811
F-ratio	119.86***	120.69***	9.19***

Source: field survey 2015

L = lead equation

, * and * is significant at 10%, 5% and 1% level

Figures in parenthesis are t – values

The results of the regression analysis to estimate the socio-economic determinants of the level of profit for cassava processing are shown in Table 4. Six independent variables were included in the multiple regression model. Four functional forms of the multiple regression analysis were estimated for the different products and they are linear, double log, semi log and exponential functional forms. The exponential functional form was chosen as the lead equation for garri and fufu processing while the linear functional form was the lead equation for abacha enterprise. The lead equations were chosen based on the high R², number of significant variables and F-ratios. Age, gender and household size were significant at different levels for garri and fufu enterprises while only age and gender were significant for abacha enterprise. The coefficients for age were negative and significant at 1% level for garri and fufu enterprises and also negative, but at 10% level for abacha. This indicates that an increase in age will lead to a decrease in the profit accruing from processing cassava. This may be attributed to the fact that, as one gets older, strength and vigour tend to decrease thereby affecting the outcome of any endeavours as well as its profit. This conforms to the study of Iheanacho (1997) and Ibekwe *et al.*, (2012) that age was significantly related to profitability of cassava processing at the 1% level of significance.

Sex was positively correlated with profit and is significant at 1% for all the enterprises. This showed that an increase in the variable will increase the profit level of cassava processing. The a priori expectation of the sign of gender coefficient should be negative.

The positive sign could be attributed to the fact that more number of males are now involved in profit oriented activities. Household size also affected the profitability of garri and fufu processing positively at the 10% level of significance. This implies that an increase in household size would definitely lead to an increase in the returns from garri and fufu enterprises. Bamine *et al.*, (2002) asserted that large family size was associated with greater labour force for timely execution of agricultural activities. Other studies such as Bassey and Okon (2008) and Nandi *et al.*,(2011) reported that household size impacted positively on cassava production. The R² values were 0.9387, 0.9391 and 0.5399, which implies that 94%, 94% and 54% of the variations in the dependent variables were caused by variations in the independent variables included in the model for garri, fufu and abacha enterprises respectively. The F-values were highly significant at 1% level indicating that the regression lines fitted.

Constraints to Cassava Processing in the Study Area

The results in Table 5 showed the responses of the processors on possible factors/constraints militating against the value added cassava production in the area. Exploratory factor Analysis was used to group the variables into possible constraint factors for policy relevance. The constraint factors were extracted based on the response of the processors. Only variables with loadings of 0.30 and above were used in naming the factors following Ashley *et al.*, (2006). According to Kessler (2006), each factor is given a denomination based on the set of variables or characteristics it is composed of. Therefore actors 1, 2 and 3 were named economic/institutional factors, socio - financial factors and Techno-infrastructure factors.

The result from Table 5 shows that Economic /institutional factor was dominated by high cost of labour (-0.9871), weather conditions (0.9871), inconsistent government policies (0.6037). Under socio-financial factor, the major constraints militating against value added cassava production were high cost of transportation (0.9475), high input prices (0.9522), high cost of processing (-0.5164) and lack of extension services (-0.3924). This is consistent with the findings of Amusa *et al.*, (2011) who stated that access to extension service was a significant constraint militating against agricultural production in Nigeria. Hence, extension agents should be empowered to take agricultural innovations to the rural areas of Nigeria in general and the South East in particular. The techno-infrastructure factor affecting cassava value addition was lack of basic amenities (0.4880) which includes health services, water supply, road networks etc. The techno-infrastructure factor was a huge constraint affecting the processors in the study area. Consequently, the shortage of infrastructural facilities, like good roads hamper the effective movement of

agricultural products from production sites to the final consumers.

Source: field survey, 2015

Table 5: Constraints Militating against Value Added Cassava Production.

Variable	Economic /Institutional Factor	Socio - Financial Factor	Techno - Infrastructural factor
Inadequate Capital	0.6360	0.7549	0.1603
High cost of labour	-0.9871*	0.1511	-0.0535
Lack of market	-0.9066	0.3852	0.1724
Weather condition	0.9871*	-0.1511	0.0535
Higher cost of transportation	-0.1450	0.9475*	-0.2851
Lack of basic amenities	-0.2029	0.1422	0.4880*
Lack of processing equipment	0.5630	0.7005	0.4387
Unfriendly bank policies	0.6360	0.7549	0.1603
Inconsistent government regulations	0.6037	0.0140	0.7971
High interest rate	0.3228	-0.9396	-0.1139
High input prices	0.2443	-	0.0362
High cost of processing		0.9522*	
		-0.5164*	0.1339
	0.2410		
Inadequate extension services	0.2888	-0.3924*	-0.1921

CONCLUSION

Based on the findings, the enterprises were profitable at varying levels with *garri* and *abacha* enterprises returning more than double the amount invested in them, though the *fufu* enterprise has the highest gross margin. The study therefore concludes that several socio-economic characteristics of cassava processors in the study area such as age, household size, gender and experience, among others affect cassava processing while problems such as high cost of labour, lack of basic amenities, inconsistent government policies and high cost of processing were among the major problems faced by the processors. Therefore, it is necessary to provide accessible, affordable key processing inputs and storage facilities for processing cassava so as to reduce the drudgery involved in cassava processing which will ultimately enhance processors technical efficiencies. Government should ensure better management of extension services in the study area through more training (workshops and seminars), provision of mobility such as motor cycles and motivation. The emphasis for public investments should be on improving general knowledge, transport, healthcare services, communication, power supply and market infrastructures.

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WIS-SSD 20

ANALYSIS OF THE PROFITABILITY OF COCOA MARKETING IN IKWUANO LOCAL GOVERNMENT AREA OF ABIA STATE, NIGERIA

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Abstract

This study investigated the analysis of the Profitability of Cocoa marketing in Ikwuano Local Government Area of Abia State, Nigeria. The specific objectives include: describing the socio-economic characteristics of cocoa marketers in the study area, examining marketing functions performed by the respondents, estimating the cost, returns and efficiency associated with cocoa marketing in the study area, estimating the determinants of cocoa marketing efficiency and identifying the challenges faced by cocoa marketers in their marketing activities. Multi-stage sampling technique was used to select 160 cocoa marketers. Descriptive statistics, marketing efficiency model, budgetary and multiple regression analysis were employed to analyze data. Results of analysis showed that 69% of the respondents were men, 44% of the farmers (majority) were between ages 35 and 44 years with mean age of 42 years. 92.00% of them were married with average household size of 6 and 88.00% were literate. Average age was 46.49 years while respondents claimed 16.89 years (mean) of marketing experience. The marketers transacted an average of 771763.26kg per annum. Budgetary analysis showed that Cocoa marketing is profitable in the study area with net return of ₦15596.88 per bag sold and marketing efficiency of 51%. Respondents were found to perform marketing activities efficiently. Age, quantity sold and marketing experience were positively related to profit at 5%, 10% and 1% significant levels respectively while household size, distance to market, transportation costs and storage cost affected cocoa marketing profitability negatively at 5%, 10%, 10% and 1% significant levels respectively. Lack of capital, high cost of the commodity poor road network were the major constraints to cocoa marketing in the study area. The study concluded that cocoa marketing is a profitable enterprise in the study area and therefore recommends that the cocoa marketers should initiate cooperative society through which financial assistance could be provided to members.

Keywords: Cocoa marketing, profitability, marketing efficiency, budgetary analysis, marketing functions

INTRODUCTION

Agriculture is the most important sector in terms of contributions to domestic production, employment and foreign exchange earnings in Nigeria. Before the discovery of crude oil in Nigeria, major agricultural products such as palm oil, rubber, cocoa, groundnut and cotton played prominent roles in the growth, development and stability of the nation's economy. Agriculture is the mainstay of the Nigerian economy, providing 65% of Gross Domestic Product (Oluyole, 2010).

Cocoa (*Theobroma Cacao*) is an important export crop in Nigeria producing about 5% of the total world production (FAO, 2011). It is an important source of raw materials, as well as source of revenue to governments of cocoa producing States (Olowolaju, 2014). Nigeria was once the second leading producer of cocoa in West Africa and palm oil globally. During this period, cocoa was ranked as the first Nigerian foreign exchange earning commodity. The situation remained sustained until the discovery, exploration and production of oil in commercial quantity. The contribution of cocoa to Nigerian economy cannot be over emphasized owing to its contribution to the foreign exchange and economic development of the nation (Folayan *et al.*, 2006; Fadipe *et al.*, 2012). In terms of foreign exchange earnings, cocoa has

remained the largest non- oil export earner. Nigeria produces about 250,000 metric tonnes of cocoa (Adesina, 2012). Although its contribution to the total natural exports earning during the past two decades dropped considerably due to the enormity of foreign exchange earning of crude petroleum. Cocoa is grown in fourteen states on Nigeria which includes Abia, Akwa Ibom, Cross River, Delta, Edo, Ekiti, Ogun, Ondo, Osun, Oyo, Kogi, Adamawa and Taraba. Cocoa is used for cosmetics, drinks, candles and pharmaceuticals.

Marketing involves all the legal, physical and economic activities necessary in moving a product from the producer to the consumer. Marketing of cocoa ensures the movement of cocoa from the producer to the final consumer in the form, time and place of need (Ejike R. D. and Chidiebere N. M, 2019). Despite the significant role of marketing in agricultural development, it is still faced with several problems that lead to low productivity. This has resulted to a fall in percentage share of cocoa output. As Amos (2007) noted, two reasons are said to be responsible for the fall in percentage share of cocoa output. Firstly, is the negligence of the agricultural sector by the past administration due to the discovery of the petroleum resources that now accounts for the bulk of foreign exchange earnings. Evidence has

however shown that the growth rate of cocoa production has been declining, which has given rise to a fall in the fortunes of the sub-sector among other reasons. Secondly is the endemic problem in the cocoa industry. Due to low productivity, cocoa has declined in economic importance at the aggregate national level. Such uncertainties in productivity of a very important export crop like cocoa, calls for concern and gives threatening signals to realizing the sustainability potentials of the sub-sector. Besides, information on how the different management systems affect efficiency and profitability has scarcely been documented (Nkang *et al.*, 2007). In Abia State, Cocoa farmers produce cocoa using any of these management systems. Their choice is hardly based on value appreciation of the management system relevant to the environment. Most importantly, researches carried out so far are yet to establish, which of the management systems of cocoa production best suit the cocoa farmers in his/her immediate environment and conditions.

However, evidence has shown that the return performance from cocoa production in Abia state recently has been declining. This may be due to export capacity reduction in the industry and a number of other factors. According to Villalobos (2009), low yield, inconsistent crop production pattern, and use of simple tools have dampened returns from the venture. The implication of these is cocoa production reduction at a time when there are high cocoa demands as a result of increase growth in consumption of chocolate in the world (Kamla, 2010). It has been stated that Nigeria was the first to liberalize cocoa export in terms of trade in West Africa, but it is discouraging to state that less is known about the nations 'cocoa production performance (Wilcox and Abbot, 2014). However, Dandi (2011) reported that most post-harvest activities that generate better returns are still handled by multinationals participating in cocoa industry in Nigeria. Also in Abia State, illegalities in the marketing system, poor implementation and inconsistency in government policies is a problem which affects the performance of cocoa in the study area. Efficiency in agricultural industry is the most frequently used to measure market performance and marketing efficiency is a common objective of farmers, food marketing firms, consumers and the society at large (Olukosi and Isitor, 2004). Given this background, it is expected that a lot of research efforts should be directed towards finding solutions to problem of poor marketing in cocoa business in Abia state. This study is therefore a response to filling this knowledge gap as well as providing some policy impetus to stakeholders in Abia State, especially the cocoa industry in solving the challenges to cocoa marketing activities in Nigeria. The broad objective of this study is to analyze the profitability of cocoa marketing in Ikwuano Local Government Areas of Abia State. The specific objectives include: describe

socio-economic characteristics of cocoa marketers in the study area; examine marketing functions/activities performed by the respondents; estimate the profitability and marketing efficiency of cocoa marketing; identify the challenges to marketing activities of respondents in the study area

MATERIALS AND METHOD

The study was conducted in Ikwuano L.G.A. of Abia State Nigeria. Ikwuano L.G.A. has a land mass of about 60059km as well as population of about 61,214 (National Population Commission, 2006). It is made up of about 52 villages and communities and is bounded by Ini LGA of Akwa Ibom State by the West and Umuahia South to the north. It lies between the latitudes 5 24N and 5 30N and between the longitudes of 7 32E and 7 37E. The main occupation of Ikwuano people is farming. The soil of the L.G.A. is fertile though slightly acidic. The major food crops grown are cassava, yam, vegetable, maize etc while tree crops grown mainly are cocoa, rubber and oil palm. Various kinds of livestock and poultry are also raised by Ikwuano people. It is known for her agricultural activities (farming) with much concentration on palm oil/carnal, cocoa cassava, yam, broom, basket, etc.

The sample of the study comprised of all cocoa marketers in Ikwuano L.G.A. of Abia State. Two stage sampling procedures were adopted to select the sample size. The first stage was purposive sampling of four autonomous communities from the Local Government Area where cocoa producers were also the marketers. The second stage involved the random selection of twenty cocoa marketers from each autonomous community who were also the producers making a total of eighty marketers. In addition, forty marketers were randomly selected from two markets (Ndiru and Ariam) to give a total of eighty cocoa marketers. In all, the sample size was made up one hundred and sixty respondents. The data were obtained using a well-structured questionnaire administered through oral interview and personal observation. The socio-economic characteristics of the respondents and the constraints militating against cocoa marketing in the study area were analyzed using simple descriptive tools like tables and frequencies while the profitability of cocoa marketing and marketing efficiency were realized using the Budgetary analysis and marketing efficiency index. Multiple regression technique was used to analyze the determinants of the profitability of cocoa marketing in the study area.

Model specification

Marketing margin (mm) model is given as:

$$MM: \frac{\text{Selling price} - \text{Purchase price}}{\text{Selling price}} \times \frac{100}{1}$$

Marketing Efficiency (M.E.) model is given as:

$$M.E. = \frac{\text{Net return}}{\text{Total marketing cost}} \times 100$$

Costs and returns model is given as:

Profit (π) = TR - TC
 Where,
 TR = Total Return
 TC = Total Cost
 N/B: TC = TFC + TVC
 Where,
 TFC = Total Fixed Cost
 TVC = Total Variable Cost

Multiple regression analysis

The implicit form of the model is specified as follows:

$$Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9)$$

Where

- Y = Marketing Efficiency (%)
- X₁ = Age of marketers (Years)
- X₂ = Household size (number)
- X₃ = Level of education (years)
- X₄ = Distance to the nearest market (Km)
- X₅ = Transportation cost (N)
- X₆ = Storage cost (N)
- X₇ = Quantity of cocoa sold (kg)
- X₈ = Cooperative membership (1=member, 0=otherwise)
- X₉ = Marketing experience (Years)
- e_i = Error term.

The model specified was subjected to four functional forms and the lead equation was selected based on the economic, econometric and statistical criteria. The four functional forms fitted were linear, semi-log, Cobb-Douglas and exponential. Explicitly, the four functional forms are shown thus:

Linear; $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + \dots + b_9X_9 + e_i$

Semi-log:

$$Y = \log b_0 + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 + b_4 \log X_4 + \dots + b_9 \log X_9 + e_i$$

Cobb-Douglas;

$$\log Y = \log b_0 + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 + b_4 \log X_4 + \dots + b_9 \log X_9 + e_i$$

Exponential;

$$\log Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + \dots + b_9X_9 + e_i$$

Where;

b₀ is the intercept
 b₁ – b₉ are the coefficients to be examined and
 X₁ – X₉ are the explanatory variables defined in equations 5 to 8 above

RESULTS AND DISCUSSION

The result presented in Table 1 shows some selected socio-economic characteristics of the cocoa marketers. The information drew from the survey shows that 69% of the respondents were male cocoa marketers. This indicates that more men participated in cocoa marketing than females. Since most of the marketers were cocoa producers, the stress associated with cocoa farming would have been the reason for the result.

About 44% of the respondents were between ages 35 and 44years, which formed the majority indicating most of the respondents were young and still in their economic active age. Age relates to strength, innovativeness, activeness and efficiency according to Onu and Echebiri (2019). The marital status of the respondents shows that majority of the respondents accounting for about 92% were married and only 8%of them were single. Marital status is therefore no barrier to being involved in the enterprise. The dominance of the married could indicate a large household and may be a source of labour thereby reducing expenses on hired labour for the marketing operations. Marketers with secondary education accounted for the highest proportion of the entire respondents (63%). This implies that the marketers could read, write, make better resource allocation and efficiently too. About 56% of the respondents had a household size of 1-5 family members, which is followed by 6-10 family members with the percentage of 31%. Household size has been known to affect labour supply, thus, labour was more available among larger households and expenses on hired labour consequently low and the reverse on the other hand. The distribution of the respondents by cooperative membership shows that 51% of the marketers are cooperative members while 49% are non-members. This implies that 51% of the marketers would have access to loans and innovative ideas while the remaining may not (Okoye *et al.*, 2008). This is because cooperative membership is a major requirement for receiving certain loans especially from agricultural banks.

Table 1: Frequency Distribution of Cocoa Marketers According to their Socio-economic Characteristics (n=160)

Variable	Frequency	Percentage
Gender		
Male	110	69
Female	50	31
Age Range (Years)		
25-34	30	19
35-44	70	44
45-54	40	25
55-64	20	12
Mean: 42		
Marital Status		
Single	12	8
Married	148	92
Level of Education		
No formal education	20	12
Primary education	24	15
Secondary education	100	63

Tertiary education	16	10
Household size		
1-5	90	56
6-10	50	31
11-15	20	13
Mean: 6		
Cooperative Membership		
Yes	82	51
No	78	49

Source: Field Survey Data, 2019

Table 2 shows the Marketing Activities performed by the Cocoa Marketers. Results implies that cocoa marketing is practiced at both small and large scale levels in the study area. Average sales volume per annum was 1,225.02 bags. According to the findings a bag of cocoa weighs 63 kilograms, thus respondents transact 771763.26kg per annum. The results also show that the average marketing experience stood at 16.89years. This implies that on the average, the respondents are experienced cocoa marketers, which should have a positive effect on marketing efficiency. The result indicates that the marketers were well experienced and as such were expected to understand the operations of the system and thus make good management decisions, which will enhance the efficiency of the marketing process. Analysis further revealed that 35% of the respondents got their cocoa supplies directly from the marketers, 50% got theirs from the licensed buying agents (LBA), 7.5% claimed to obtain cocoa supplies from both marketers and licensed buying agent, 2.5% obtain their supplies from brokers, 3.8% claimed to obtain supplies from brokers and licensed buying agents while the remaining 1.3% claimed to do business with marketers and cooperative groups. This result revealed that a large percentage of the respondents obtain supplies from LBAs, and it looks like what Fatchamps and Mintenn (2001) found among developed economies, that liberalization has resulted in concentration and vertical integration with a small number of large corporations purchasing directly from marketers and selling to distributors.

Table 2 further depicts that 25.0% of the respondents do not store their cocoa while the remaining 75.0% store their cocoa. This result implies that most of them store their cocoa because an increase in storage would increase care given to cocoa which will have a positive effect on the quality and profit incurred. Concerning labour use, 10% of the respondents do not make use of hired labor while 90.0% of the respondents make use of hired labor, which may be an indication that cocoa marketing is labor intensive. It was found that 28.8% of the respondents do not belong to any marketer's association, while 71.3% belong to one marketer's association or another.

Table 2: Marketing Activities Performed by Cocoa Marketers, n=160

Variable	Frequency	Percentage
Quantity transacted per annum (bags)		
≤2000	114	71.3
2001-4000	26	16.3
4001-6000	16	10.0
6001-8000	4	2.5
Mean: 1,225.02		
Years of Experience		
≤10	40	25.0
11-20	62	38.8
21-30	38	23.8
31-40	20	12.5
Mean: 16.89		
Source(s) of supply		
Marketers	56	35.0
LBA	80	50.0
Marketers and LBA	12	7.5
Brokers	4	2.5
Brokers and LBA	6	3.8
Marketers and Marketing Cooperatives	2	1.3
Storage functions		
No	40	25.0
Yes	120	75.0
Utilization of hired labour		
No	16	10.0
Yes	144	90.0
Membership of Marketer Association		
No	46	28.8
Yes	114	71.3

Source: Field Survey, 2019

Table 3 shows the Budgetary Analysis. According to the table, net returns for marketers indicate that cocoa marketing is profitable as shown by the positive profit received. The marketers incurred an average variable and fixed cost of ₦26403.12/bag and ₦4000/bag respectively. The profitability index/ratio for cocoa marketing is 0.3390. This means that for every ₦1.00 invested by the marketers, a return of 5kobo was realized. This conforms to the findings of Ahamefula et al. (2017) that cocoa marketing is a profitable venture.

Table 3 Cost, Returns and Efficiency Estimates of the Cocoa Marketers

Market variables	Value (₦)
Variable Cost items	
Average Cost of transportation	853.00
Average cost of cocoa/bag	25050.12
Average cost of electricity	400.00
Average cost of other items (govt. charges etc.)	100.00
Average Variable Cost (AVC)	26403.12
Fixed Cost items	
Rent on transaction land	500.00
Rent on warehouse	1000.00
depreciated cost on marketing tools and equipment depreciation	2000.00
basins/trays/scales	500.00
Average Fixed Cost	4000.00
Total Cost	30403.12
Average Gross Returns from sales (Total Revenue, TR)	46000.00
Net Returns (NR)	15596.88
Profitability index : 0.3390	

Source: Field Survey Data, 2019

MARKETING EFFICIENCY AND MARKETING MARGIN ANALYSIS OF THE COCOA MARKETERS

The result of the marketing efficiency analysis in Table 4 showed that cocoa marketing in the study area is operationally inefficient for the traders. According to Scarborough and Kydd (1992), marketing efficiency ranges from zero (0) to infinity. 100% indicates perfectly efficient market, and less than 100% indicates market inefficiency and above 100% indicates excess profit. However, Folayan *et al.*, (2007) stated that efficiency ratio greater than one indicates an efficient cocoa marketing. Given this, the efficiency value of 0.51 (51%) as shown in Table 4 suggests that cocoa marketing is inefficient in the study area

Table 4: Marketing Efficiency and Marketing Margin Analysis of the Cocoa Marketers

Items	Value (₦)
Selling price	30000.00
Purchase price	25050.12
Gross marketing margin	20949.88
Net Return	15596.88
Marketing cost	30403.12
Marketing efficiency (%)	51.3
Marketing Margin (%)	16.5

Source: Field Survey Data, 2019

DETERMINANTS OF COCOA MARKETING EFFICIENCY IN IKWUANO LOCAL GOVERNMENT AREA OF ABIA STATE

The regression estimates of the factors influencing the

marketing efficiency of cocoa in the study area are presented in Table 5

The multiple regression model was employed to examine the factors affecting the marketing efficiency of the cocoa marketers in the study area. Four (4) functional forms of the model were tested and the lead equation chosen based on a number of statistical, economic and econometric considerations such as number of significant variables conforming to *a priori* expectations, the R² value, F-ratio etc. Considering the above, the semi-log was chosen as the lead equation. The model R² showed that 74.6% of changes in profits from cocoa marketing were accounted for by changes in the explanatory variables included in the model and the remaining 25.4% due to error. The F-ratio was significant at 1% implying a good test of fit. The coefficient of age of marketers was positive and significantly related to marketing efficiency at the 5 percent levels. This showed that an increase in age would increase profit of marketers possibly because age relates to experience, thus, the more aged marketing gets, the more profitable and efficient they become. This finding contradicts such previous findings as Obasi *et al.* (2012) and Bassey *et al.* (2015) which hold that aged marketers are not innovative and lack the vigor and energy to withstand the rigor of marketing. Household size showed a negative relationship with cocoa marketing efficiency meaning that efficiency reduced as household size increased. The reason is that with a large household, more expenses are made in terms of feeding, shelter, healthcare, education and therefore reduce the profits available at the marketers' disposal having made other house expenses. Distance to the nearest market also had a negative influence on efficiency at 10%. This implies that as distance increased, efficiency decreased. This may be as a result of the high transportation cost experienced. Transportation cost also impacted negatively on the profit and efficiency of marketers at the 10 percent significance level. Its coefficient (0.222) showed that increasing transportation cost would decrease cocoa profit by 2.22 percent. Bassey *et al.*, (2015) also agreed to this position. The coefficient of storage cost was negative and significant at the 1 percent level. Since storage facilities are grossly inadequate in the study area, most marketers resort to smoking and home storage which is not only costly but damage prone, thereby increasing the marketing cost. Though this result agrees with Bassey *et al.*, (2015), it is at variance with (Obasi *et al.*, 2012). Quantity sold was positively related to the efficiency/profitability of cocoa marketing at the 10 percent level of significance. This shows that increasing the quantity of cocoa sold yielded more profit for the marketers in the study area. The coefficient for marketing experience was positive and significant at the 5 percent level. Experienced marketers are perceived to have learnt from the other marketer's experiences due to their prolonged fraternity with them. They have also accumulated

enough marketing knowledge through several years of marketing trials and errors (Bassey *et al.*, 2013). This finding lends credence (Obasi *et al.*, 2012).

Table 5 Regression Estimates of Factors Affecting Cocoa Marketing Efficiency in Ikwuano Local Government Area of Abia State

Variables	Linear	Exponential	Semi-Log (+)	Double-Log
Constant	0.234 (-1.200)	0.361 (4.383)***	0.146 (-1.468)	0.738 (0.336)
X ₁ (Marketers' age in years)	0.188 (1.330)	0.347 (0.947)	0.194 (2.310)**	0.358 (0.925)
X ₂ (Household size)	0.780 (-0.281)	0.903 (2.122)*	0.695 (-2.394)**	0.832 (-2.213)*
X ₃ (Educational qualification, yrs)	0.173 (1.769)*	0.876 (0.156)	0.177 (0.632)	0.873 (0.160)
X ₄ (Distance to market, Km)	0.536 (-2.009)*	0.635 (-453)	0.756 (-1.998)*	0.423 (1.002)
X ₅ (Transportation cost, ₦)	0.142 (-1.485)	0.937 (-1.797)*	0.222 (-1.831)*	0.872 (0.161)
X ₆ (Storage cost, ₦)	0.154 (1.542)*	0.428 (0.797)	0.164 (-2.907)***	0.459 (0.745)
X ₇ (Quantity sold, Kg)	0.081 (1.770)*	0.650 (2.456)**	0.088 (1.730)*	0.683 (2.092)*
X ₈ (Cooperative membership)	0.002 (1.342)	0.342 (1.324)	0.452 (0.922)	0.984 (1.552)
X ₉ (Marketing experience, Years)	0.023 (2.348)**	0.054 (2.012)*	0.445 (2.774)***	0.007 (2.326)**
R ²	0.553	0.533	0.746	0.435
Adj. R	0.473	0.366	0.546	0.372
F-Ratio	5.890***	5.602***	7.558***	2.503**

Source: Field Survey, 2019.

+ Lead equation

PROBLEMS ASSOCIATED WITH COCOA MARKETING IN THE STUDY AREA

Some of the major problems associated with cocoa marketing in Ikwuano Local Government Area are presented in Table 6

The result of the problems militating against cocoa marketing in the study area showed that all the problems considered had responses on them. The result further showed that lack of capital and funds was the major problem faced by cocoa marketers in the study area as opined by all the cocoa marketers in the study area. Other problems like cost of the commodity (93.8%), poor road networks (90.0%), high transportation cost (77.5%) and poor processing/storage facilities (50.0%) were identified constraints militating against cocoa marketing in Ikwuano Local Government Area of Abia State. The result further showed that lack of capital and funds was the major problem facing cocoa marketing in the study area. It has been discovered that cost is the major hindrance faced by several business outlets. This had about 34% respondents agreeing to this.

Table 6. Constraints Militating against Cocoa Marketing in the study area.

S/N	Problems	Frequency*	Percentage	Rank
1	Lack of capital /funds	80	100.0	1st
2	High cost of the commodity	75	93.8	2nd
3	Poor road networks	72	90.0	3rd
4	High cost of transportation	62	77.5	4th
5	Poor storage/processing facilities	40	50.0	5th

*Multiple Responses

Source: Field Survey Data, 2019

CONCLUSION AND RECOMMENDATION

The study concluded that cocoa marketing is a profitable enterprise and marketing activities are efficiently performed in the study area. Variables influencing revenue generated include quantity of cocoa transacted, storage cost, rent on transaction land

/ warehouse, marketing experience, years of formal schooling. Pressing challenges to cocoa marketing enterprise in the study area include financial constraint, unstable market price, high transportation cost, irregular supply and high taxation.

Based on the finding that cocoa marketing was found to be profitable, which indicates that it has a potential of improving the standard of living of marketers, this

study recommends that our unemployed youths and young school leavers should be encouraged through awareness campaigns to venture into cocoa marketing. Provision of credit support to cocoa marketers not only as agricultural loans but as part of small and medium scale enterprises (SMEs) development grant, to expand their capacities and income is recommended.

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PERFORMANCE ANALYSIS OF SEASON AND OFF-SEASON RETAIL YAM MARKETING IN UMUAHIA A SOUTH LOCAL GOVERNMENT AREA OF ABIA STATE, NIGERIA

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ABSTRACT

The study analyzed net returns and its determinants from season and off-season yam marketing in Umuahia South Local Government Area of Abia state, Nigeria. Specifically, it analyzed socio-economic characteristics of yam marketers, cost and return from season and off-season yam marketing, determinants of net return and the constraints that militated against yam marketing in the study area. A multistage sampling procedure was used to select respondents. Stage one involved purposive selection of three markets where yam marketing is carried out in large quantity. Stage two involved the proportionate selection of retailers from the selected markets. Twelve yam marketers were randomly selected from Apumuri- Ubakala due to high level of yam marketing activity as the head quarter of the LGA. Ten respondents were selected from Ahia- ukwu Olokoro and eight marketers were selected from Abam- Nsirimo market. These gave a total of thirty yam retailers for the study. Data were analyzed using descriptive and inferential statistical tools. Objective one and four on socio- economics characteristic and constraints were realized using mean and percentages. Objective two was accomplished using net-return formulae and the fourth objective was achieved using regression model. The result on the socio –economic characteristics shows that male dominated yam retailing, marketers had mean age of 37 years. The business was viable, variables that influenced the net return of yam marketing were education and marketing experience. Constraints identified by marketers in the study area were inadequate finance and high cost of transportation. The study recommends, that marketers should form yam marketers’ cooperative to mobilized funds to boost their business.

Keywords: yam, season and off-season, marketing

INTRODUCTION

Yam is a major food crop widely grown and massively consumed in Nigeria and is popularly regarded as the “King” of all arable crops in Nigeria because it earns more money per unit weight than other food crops, National Root Crops Research Institute (NRCRI), (2008) in Ogbonna, Onyenweaku, and Nwaru, (2012). Nigeria is a tropical country; one of the highest yam producers in the world. Recent world data showed that Nigeria accounts for 65% of the total world production; about 38 million metric tons are cultivated on 2.9 million-ha cultivated area of land in 2012 and valued at \$7.75 billion (Odigbo, Ogbidi, and Ewa, (2015). It is major source of energy for large population of Africa. Its tubers can be eaten in different forms, ranging from roasting, boiling, frying, pounding into paste as well as processing into yam flour which can be eaten with soup. Its peels can also be processed into livestock feed. Hence it is considered an important staple to combat food insecurity in areas where it is cultivated (Offor, Nse – Nelson and Asokome 2016).

Marketing of agricultural produce is as important as its production. Marketing is essential in the distribution of produce from point of production (rural area) to consumption centers in (urban areas) where there is high demand for the produces. Marketing plays enormous roles in providing employment opportunity for individual marketers at the local and national levels. In the course of marketing certain function are performed to provide utility to the consumers. The

utility of time is actualized through the function of storage that ensures the availability of products all year round. The marketing of yam at season and off-season involved storage of yam from the peak of harvest to off –season when the produce will be limited in supply. This however, involves a cost, the cost of storage is the opportunity cost of holding inventory. More so, economic theory stipulates that, the cost of products at off season should increase by the cost of storage, but an increase in price higher than the storage cost provides the traders opportunity to make abnormal profit Offor *et al.*, (2016). The marketing of off-season yam is constrained with problems such as high cost of produce, high rate of damage in terms of perishability of the yam tubers, unavailability of produce hence the study intends to investigate in the performance of season and off-season yam marketing in Umuahia South Local Government Area of Abia State, Nigeria. Specifically, the study examined the socio –economic characteristics of retail yam marketers in the study area, estimated the net returns of yam marketers at season and off-season; marketing margin, marketing efficiency, return on investment for season and off-season respectively. The study also estimated the determinants of net returns from season and off-season yam marketing and finally identified the constrain that militated against yam marketing in the study area.

METHODOLOGY

The study was conducted in Umuahia South Local Government Area (LGA) of Abia State. Umuahia

South LGA has its headquarters at Apumiri Ubakala, it lies on latitude 5^o27' and 44.42' N and longitude .7^o 26' and 23.89'E of the green wick - meridian. It is bounded in the south with Umuahia North LGA, in the north with Isiala- Ngwa North LGA, in the east with Ikwuano LGA. It has a land mass of 140km² and a projected population of 182,200 people, National Bureau of Statistics (NBS, 2016). Umuahia South LGA is made of nine (9) communities. They include Apumiri Ubakala, Olokoru, Nsirimo, Amakama, Omaegwu, Ezeleke, Amankwo, Ekenobizi and Ngodo. A multistage sampling technique was used to sample for respondents. Stage one involved purposive selection of three (3) markets where yam marketing is carried out in large quantity. The selected markets were; Apumiri, Ubakala, Ahia- ukwu Olokoru and Abam- Nsirimo . Stage two involved the proportionate selection of yam marketers from the selected markets. Twelve (12) yam marketers were selected from Apumiri Ubakala due to the high activity of yam marketing in the market as the head quarter of the LGA. Ten (10) yam marketers were randomly selected from Ahia- ukwu Olokoru and eight (8) yam marketers were randomly selected from Abam- Nsirimo market. These gave a total of thirty retail yam marketers for the study. The study made use of primary data source, data were collected through the use of structured questionnaire. Objective one and four on socio-economic characteristics and constrain respectively were achieved through the use of descriptive statistics such as mean and percentage. Objective two on marketing margin, marketing efficiency and net-return were realized using marketing margin, marketing efficiency and net -return formulae adopted from Olukosi Isitor, and Ode.(2005).

$$\text{Marketing margin} = (MM) \frac{\text{Selling Price} - \text{Purchasing Price}}{\text{Selling Price}} \dots (1)$$

$$\text{Marketing efficiency}(ME) = \frac{\text{Value addition by marketing (net return)}}{\text{Total marketing}} \dots (2)$$

$$\text{Net return} = \text{Total Return from sales (TR)} - \text{Total marketing cost (TMC)} \dots (3)$$

Objective three which estimated the determinants of net return was analyzed using multiple regression model stated in its implicit form. The model is specified as follows:

$$Y = F(X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9) \dots (4)$$

Y = Net return (naira)
 X₁ = Age (years)
 X₂ = Households size (persons)
 X₃ = Education (years)
 X₄ = Marketing experience (years)
 X₅ = Capital investment (naira)
 X₆ = Quantity of yam sold (kg)
 X₇ = purchase price (naira)
 X₈ = Distance (km)
 e = error term

RESULT AND DISCUSSION

Table: 1 shows the socio –economics characteristics of yam marketers in the study area. The result on sex shows that male counterparts dominated the business of yam retailing in the study area. Male represents 63.3% of the total marketers sampled for the study. The study is similar the findings of Okwuokenye and Onemolease (2011) who reported that yam marketing was majorly dominated by males representing (78%) of the marketers. On age of the respondents, the marketers had a mean age of 37 years. This implies that, the marketers were still in their active age and could withstand the stress involved in yam marketing. The work is in agreement with the findings of Ikpeazu, and Moguluwa (2014) who reported that the mean age of 39 years for yam marketers. On marital status, the result shows that single dominated the business representing about 50% of the respondents. On education, the result shows that all respondents had one form of education or the other. Although majority had secondary education. This is a good development; because education can influence an individual business positive through knowledge acquisition. Okwuokenye and Onemolease (2011) also reported a good level of education that majority of the marketers had secondary education rep representing (73.8%) of the marketers. On household size, a mean household size of 5 persons was obtained. This implies that yam marketers in the study area had a relatively small household. The result is in agreement with the work of Kassali, Girei, and Sanu, (2018) who reported a mean household size of 5 persons in the study area. In terms of marketing experience, the marketer had a mean marketing experience of 9 years. This implies that yam marketers were well experience and as such can handle some challenges in yam marketing. Kassali *et al.*, (2018) also reported a marketing experience of 11 years of yam marketing by marketers.

Table 1: Socio-Economic Characteristics of Retail Yam Marketers in the Study Area

Variables	Frequency	Percentage
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Sex		
Male	19	63.3
Female	11	36.7

Total	30	100
Age		
20-29	5	16.7
30-39	14	46.7
40-49	9	30.0
50-59	2	6.6
Total	30	100
Mean	37.2	
Marital status		
Married	13	43.3
Single	15	50.0
Others	2	6.7
Total	30	100
Educational level		
Primary	5	16.7
Secondary	18	60.0
Tertiary	7	23.3
Total	30	100
Household size		
1-3	8	26.7
4-6	17	56.7
7-9	5	16.6
Total	30	100
Mean	5	
Marketing experience		
1-5	5	16.7
6-10	15	50.0
11-15	10	33.3
Total	30	100
Mean	9	

Source: Field survey 2015

Table 2: shows season and off- season net returns of yam marketers. The result shows that at season the yam marketers purchased an average quantity of 364.8 tubers of yam, with a total average purchase price of ₦ 182.48. The marketers incurred an average total marketing cost of ₦ 81,568.704. More so the marketers at season sell a tuber of yam on the average for ₦ 263.33. The yam marketers made an average monthly net returns of ₦14,494.08. Furthermore, the result shows that at off- season the yam marketers purchased an average quantity of 283.33 tubers of yam, with a total average purchase price of ₦334.99. The marketers incurred an average total marketing cost of ₦ 109,912.72. More so the marketers at off-season sell a tuber of yam on the average for ₦ 424.99. The yam marketers made an average monthly net returns of ₦10,499.7.

Table 2: Net return, marketing margin and marketing efficiency analysis of yam marketing in the study area

ITEM	MINIMUM	MAXIMUM
A: Season		
Quantity bought (kg)	1344	9600
Average quantity (AQ)	364.8	
Purchase price (₦)		
Small size	40	91
Medium	100	183
Big	315	366
Average purchase	151	213.3
Total average purchase price (TAPP)	182.48	
STAPC= (TAPP) × (AQ)	66568.704	
B: SEASON RETURN		
Selling Price (₦)		
Small size	120	170
Medium size	160	250
Big size	280	600
Average selling price	186.66	360
Total average selling price (TASP)	263.33	
Season total average Revenue (STAR) = (TASP) × (AQ)	96062.784	
C: OFF SEASON		
Quantity bought (tubers)	1000	7500
Average quantity (AQ)	283.33	
Small size	150	200
Medium size	250	370
Big size	300	740
Average purchase price (APP)	233.33	436.66
Total average purchase price (TAPP)	334.99	
OTAPC= (TAPP) × (AQ)	94912.72	
D: OFF SEASON RETURNS		
Selling price (₦)		
Small size	200	250
Medium size	350	500
Big size	450	800
Average selling price	333.33	516.66
Total average selling price (TASP)	424.99	
Off- season Total average Revenue (OTAR)= (TASP) × (AQ)	120,412.42	
E: Other cost		
Transportation	7,000	
Storage cost	3,000	
Market charges	500	
Loading and off -loading	4,500	
Total other cost (TOC)	15,000	
F: STMC = (STAPC) + (TOC)	81568.704	
G: Net Return for Season= (STAR) - (STMC)	14,494.08	
H: OTMC= (OTAPC) + (TOC)	109,912.72	
I: Net Return for off- Season= (OTAR)- (OTMC)	10,499.7	
Marketing Margin for Season (MMS)	30.7%	
Marketing Margin for Off- season (MMO)	21.2%	
Marketing efficiency for season (MES)	17.8%	
Marketing efficiency for off season (MEO)	9.6%	
Return on investment for season (RIS)	1.2	
Return on investment for off- season (RIO)	1.1	

Source: Field survey 2015

Note: Sample size for the study was 30.

STMC: Season total marketing cost

OTMC: Off- season total marketing cost

This implies that yam marketing even at the retail level is profitable at both season and off- season in the study area. Although the business is more profitable at

season. This could be due to the fact that, at season marketers with smaller amount of money can purchase more tubers and sell more quantity of yam hence make higher net turn from the business than off-season when the price of yam had increased due to scarcity of the product. The result of the study further shows that marketers had a marketing margin of 30% and 21% for season and off-season respectively. This is an

acceptable margin for storable goods. On marketing efficiency, at season the marketers had an efficiency value of 17% while at off- season the efficiency value was 9.6%. All represents inefficient marketing system; but it could be said that the efficiency at season is better than off-season. On return on investment, the result shows that the business is viable since 1.2 and 1.1 were estimated.

Table 3: Determinants of net returns of yam marketers in the study area.

Variables	Linear	Exponential	+ Double log	Semi log
Constant	171122.204 (1.585)	12.333 (28.193)***	10.11 (6.416)***	-1969338.989 (-0.851)
X ₁ Age	-2208.841 (-1.28)	-0.005 (-1.050)	-0.292 (-0.921)	0.020 (0.245)
X ₂ Household size	-935.940 (-0.133)	-0.026 (-1.252)	0.087 (-0.74)	-0.065 (-0.759)
X ₃ Education	-22448.765 (-1.474)	-0.074 (-1.623)	0.274 (1.745)*	-0.050 (-0.603)
X ₄ Marketing Experience	-831.451 (0.249)	-0.005 (-0.523)	0.290 (2.522)**	0.152 (1.798)*
X ₅ Capital investment	0.096 (-0.607)	-5.931E-007 (-1.248)	0.011 (0.180)	0.78 (0.750)
X ₆ Quantity of yam sold	65.144 (2.243)**	0.001 (9.904)***	0.709 (6.736)***	0.524 (3.082)***
X ₇ Purchase price	(-42.352) (-1.974)**	0.000 (-3.59)***	-0.768 (-4.381)***	-0.581 (-4.697)***
X ₈ Distance	1.111 (0.935)	-4.073E-007 (-1.601)**	-0.323 (-3.198)***	0.563 (3.779)***
R ²	0.994	0.939	0.855	0.729
R ⁻²	0.983	0.929	0.832	0.686
F- statistics	39.604	38.705	36.737	32.356

Source: Field survey 2015. Note: figures in parentheses are t- ratio, ***, **, * represents 1, 5, and 10 percent level of significance respectively; + is lead equation.

The result of the multiple regression presented in Table: 3 shows that the double log functional form was chosen as the lead equation based on the number of significant variable and other *a priori* signs. The coefficient of multiple regression R² value was 0.855. This implies that about 85.5 % of the total variation in the dependent variable was accounted for by the independent variables included in the model. The F- statistic was also significant at 1 % level of significance indicating the goodness of fit of the model. The variables that influenced the net return of yam marketers were education, marketing experience, quantity of yam sold, purchase price and distance from source of yam. The coefficient of education had a positive sign and was significant at 10 % level of significance. This implies that as the level of education of yam marketers increase, the net return from yam marketing also increase. The coefficient of marketing experience was positively related to net return, and also significant at 5% level of significance. This

implies that as the marketing experience of marketers increases the net return from yam marketing also increase. This is because, the marketers would have gotten some knowledge to handle the challenges associated with yam marketing. The variable of quantity of yam sold had a positive relationship with net return and was significant at 1% level of significance. This is in line with *a priori* expectation. The higher the quantity of yam a marketer sells the higher the net return of the marketer. The coefficient of purchase price was negatively related to net- return but also significant at 1% level of significance This implies that as the purchase price of yam increases the net return from yam would decrease. This could be true because as the purchase prices increases, the quantity of yam that would be bought and sold by marketers would be reduced and such the net return from the business will be reduced. This work is contrary to the study of Ugwumba Omojola, and Chidebelu (2014) who reported that per unit price of

yam had positive and significant relationship with profit; that is as the unit price of yam increases the profit from yam production also increases. The variable of distance had negative sign but was significant at 1% level of significance. This implies that the farther away a marketer from the source of yam the lesser the net returns he realizes from yam marketing. This is because, the longer the distance from source, the higher the cost of transportation that would influence the net return of the marketers negatively.

CONSTRAINTS MILITATING AGAINST YAM MARKETING IN THE STUDY AREA

Table 4: Constraints to season and off-season marketing in the study area

Constraints	Frequency	Percentage
Finance	30	100
Transportation cost	30	100
Damage	25	83.3
Seasonality of product	23	76.7
High price of product	25	83.3

Source: Field survey, 2015

Note: Multiple responses taken*

Table: 4 shows the constraints that militated against

yam marketing in the study area. The marketers identified finance and high cost of transportation as the most important constrain that hindered yam marketing in the study area. These were followed by damage of product and high price of product due to seasonality of the product. The study found a similar result to the work of Kassali *et al.*, (2018) who reported high cost of transportation and high cost of yam as major challenges in the business.

CONCLUSION, SUMMARY AND RECOMMENDATIONS

The study investigated into performance of season and off-season yam marketing in Umahia South Local Government Area of Abia State Nigeria. The study found out that male dominated the business, the business was viable; yam marketers made higher net returns at seasoning marketing. Factors that influenced net returns in the study were marketing experience and level of education. More so, the factors that hindered the marketing of yam in the study area are high transportation, inadequate finance and high rate of perishability of tubers of yam especially in the off season period. The study therefore recommends that marketer should form yam marketers’ cooperatives that could aid them to mobilize their finances, develop an improve storage facility that could reduce spoilage and hence increase in their net return.

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FACTORS AFFECTING THE PRO-VITAMIN A CASSAVA PRODUCTION AMONG SMALLHOLDER FARMERS IN IKWUANO LGA, ABIA STATE, NIGERIA

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Abstract

The study determined the factors affecting pro vitamin A cassava production in Ikwuano, Abia State. A structured questionnaire and interview schedule were used to collect relevant information. Random sampling method was used to collect the data. Ikwuano local government area (LGA) was selected for the study and in it sixty (60) farmers were randomly selected. Frequency, percentages and mean scores were employed to summarize the data collected, while regression analysis were used to determine the factors affecting the production of pro vitamin A cassava. Majority of the farmers were females (61.7%) while the remaining (38.3%) were males. The study revealed that farmers within the age of 45-54 years had the highest percentage (51.7%). The study also revealed that 86.7% of the farmers were married, 8.3% were single while 5.0% were divorce. The mean value of household composition of the farmers in the study area was 8 while the mean value of farmers' level of education was 6.4. Majority of the farmers in the study area were into commercial farming, hence cultivating farm size ranging from 4.6-5.5 hectares. The result of regression showed that cost of labour and cost of herbicide were negatively significant at 1% and 10% level respectively, while cost of fertilizer, farm size, and F-ratio were positively significant at 1%. Lack of access to credit and poor road network were the most pronounced constraint facing the farmers in the area. Policy recommendation such as, establishment of infrastructure (road network, and market facility) in the area should help in boosting up pro vitamin A cassava.

Keywords: Determinants, Pro-vitamin A Cassava, Constraints.

INTRODUCTION

Background of the Study

Cassava (*Manihot esculenta*) is a perennial shrub in the family *Euphorbiaceae* grown primarily for its storage roots which are eaten as a vegetable (FAO, 2003). The plant is woody with erect stems and spirally arranged in simple lobed leaves with petioles up to 30 cm in length. The crop is produced by smallholders who produce a wide range of crops often as intercrop and the mean field area produced by each household is 0.6 ha (Nweke *et al.*, 2002). It is more widely grown in the humid zone where it occupies about 35% of cultivated arable fields than in the sub-humid zone where it occupies about 15% and outside the humid zone where it occupies less than 5% of the cultivated arable fields (Nweke *et al.*, 2002). In 2002, cassava gained more prominence in Nigeria following the pronouncement of presidential initiative on the crop which was aimed at using cassava production and processing as the engine of growth in the economy (Fakayode *et al.* 2008). Following the initiative, there has been a significant increase in industrial demand for cassava product primarily as substitution for imported raw materials and semi-finished products in bread flour, noodles, adhesive and animal feeds industries (Adenisa, 2013).

Harvest plus a global leader in developing crop to have higher levels of essential micro-nutrients such as

vitamin A, iron and zinc has currently bred and promoted Vitamin A bio-fortified cassava (yellow cassava) in Nigeria. Currently, six varieties of vitamin A cassava (UMUCASS; 35, 36, 37, 44, 45, and 46) have been released by National Root Crops Research Institute (NRCRI) Umudike in collaboration with International Institute for Tropical Agriculture (IITA) Ibadan (NRCRI News Report, 2015) to cushion the effect of vitamin A deficiency. Studies have shown that cassava especially pro-vitamin A cassava has the potential to industrialize Nigeria more than any other crop if it is well utilized (FAO, 2008). Based on this the research focused on the following objectives:

Specific objectives are:

To describe the socio-economic characteristics of the respondents.

To analyze the factors affecting the production of pro vitamin A cassava among smallholder farmers in the study area.

To examine the constraints challenging the production of pro- vitamin A cassava farmers in the study area.

MATERIALS AND METHODS

Study Area

This study was conducted in Abia State, Nigeria. Abia State has a land mass of about 6,320 square kilometers,

a population of over two million people and a Gross Domestic Product (GDP) of about \$8.69 billion and per capita of \$3.003 (C-GIDD, 2008). Abia State is made up of 17 Local Government Areas (LGAs) and 3 agricultural zones. The zones are Ohafia, Umuahia, and Aba; with 5, 5 and 7 LGAs respectively.

The State has a tropical climate with rainy season starting from March to October and it ranges from 200 mm to 250 mm, while dry season occurring from November to February. Temperature ranges between 31°C maximum to 22°C minimum (FAO, 2000).

The majority of the farmers in the study area are small scale farmers with an average farm size of less than five hectares. The prominent food crops grown in this area are cassava, yam, maize, plantain, banana, sweet potato, beans, and vegetables. The ecology of the state favors the growing of root and tuber crops, cereals and vegetable crops and the rearing of livestock.

Method of Data Collection

Data were collected using well-structured questionnaire which was administered to cassava farmers in the area. Data such as socio economic characteristics, factors affecting the production of Pro-A cassava and constraints against the production were collected.

Sample Procedure

A multistage random sampling procedure was used to select samples for the study. In the first stage, Ikwuano LGA was purposively selected for the study because of its nearness to National Root Crops Research Institute (NRCRI), Umudike from where the pro-vitamin A cassava originated, has access and familiarity to Pro-vitamin A cassava. Stage two involved the selection of villages and 12 villages were randomly selected from the LGA. Furthermore, stage 4 involved the selection of farmers (Pro-vitamin A cassava farmers). Hence, 5 farmers were randomly selected from each of the villages. Thus a total of (60) farmers were selected for the study.

Analytical Techniques

Descriptive statistics such as mean, frequency and percentage were used to analyze the objectives one and three while objective two was analyzed using linear regression analysis. The explicit form of the equation is as follow:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + B_5X_5 + e$$

Where

Y = output in ton

B₀ = constant

B₁ = coefficient of regression

X₁ = cost of fertilizer

X₂ = cost of herbicide

X₃ = farm size

X₄ = cost of labour

X₅ = cost of planting material

RESULTS AND DISCUSSIONS

Table 1: Summary of The Socio Economic Characteristics of Respondents

Gender	Frequency	Percentage	
Female	37	61.7	
Male	23	38.3	
Total	60	100.00	
Age			
25-34	7	11.7	
35-44	17	28.3	
45-54	31	51.7	
55-64	05	8.3	
Total	60	100.00	
Marital status			
Married	52	86.7	
Single	05	8.3	
Divorce	03	5.0	
Total	60	100.00	
Household Size			Mean
1-6	6	10.0	8
7-12	23	38.3	
13-18	09	15.0	
Total	60	100.00	
Level of Education			Mean
No Formal Education	09	15.0	6.4
Primary education	37	61.7	
Secondary school	10	16.7	
Tertiary education	04	6.7	
Farm Size			
0.1-1.5	5	8.3	
1.6-2.5	4	6.7	
2.6-3.5	7	11.7	
3.6-4.5	12	20.0	
4.6-5.5	21	35.0	
5.6-6.5	11	18.3	
Total	60	100	
Access to credit			
No	53	88.3	
Yes	7	11.7	
Total	60	100.00	

Table 1 shows the distribution of the respondent according to socio economics characteristics. Majority (61.7%) of the respondents were females while the remaining 38.3% were males. This result confirms that women participation in Pro-vitamin-A cassava variety (like other cassava varieties) is predominant than their male counterparts (Adeyemo *et al.*,2010). Also, a research in Busia indicated that women participation in yellow root cassava production is more predominant than men Esuma et al

(2019). Furthermore, 51.7% were within the age range of 45-54 years. This implies that majority of the cassava farmers were within their active age and are capable of taking decision. This could be as a result of the labor intensity of cassava production in the study area. This work agrees with the findings of Onyenucheya and ukoha,(2007) that youth have the ability to withstand the rigors, strain and stress involved in cassava production. The result revealed that 86.7% of the respondents were married with a mean household size of 8persons. This is an indication of large household sizes, implying relative high food demand.

The result in Table 1 shows that 15% of the respondents have no formal education, 61.7% have primary education, and 16.7% have secondary education while 6.7% have tertiary education. This implies that most of the respondents had low level of education but could have rudimentary knowledge of production since they would be able to read and write. Furthermore, because of farmers' level of education, they would be able to take and avert risks associated to Pro-vitamin A cassava production and make proper decision to adopt new technologies. Education is strong tool that helps farmers in using modern technologies in agriculture. The result is agreed with Bolarinwa, (2000), who found that majority of farmers with higher education level in rural areas migrated to cities to search for white-collar jobs.

The result showed that 8.3% of the respondents had farm sizes of about 0.1-1.5 hectares, 6.7% of the respondents had farm sizes of 1.6-2.5 ha and 11.7% had 2.6-3.5ha of farm, 20.0% had farm size of about 3.6-4.5ha, 35% had farm sizes of 4.6-5.5ha and 18.3% had farm sizes of about 5.6-6.5ha. This indicates that farmers are smallholders, cultivating less than three hectares of cassava farm. This is in consistent with Onyebinama, (2004) who stated that farmers with less than 5 ha of farm land are small holder farmers. This is also in confirmation with Okunmadewa, (2003) who asserted that most of Nigeria farmers are of small farm holdings. The result showed that majority (88.3%) of the respondents had no access to credit while 11.7% had access to credit. the availability of credit helps to finance the procurement of material inputs which have a positive effect on cassava production

Table 2: Summary of The Factors Affecting the Pro-vitamin A cassava Production in the Study Area

Variables	B	Std. Error	Beta	t-ratio
(Constant)	-8.493	.734		-11.564***
Cost of fertilizer	.677	.088	.527	7.693***

Cost of herbicide	-.104	.064	-.078	-1.627*
Farm size	.935	.160	.416	5.855***
Cost of labour	-.093	.027	-.169	-3.499***
Cost of planting materials	-.036	.041	-.041	-.884
R ²			.905	
F-ratio				83.860***

Source: Field Survey, 2017

The factors affecting the production of pro vitamin A cassava in the study area is presented on the Table 2. The R square was .905 which indicated that about 90.5% of the variation in the dependent variable was explained by the explanatory variable, the f-ratio was significant at 1% indicating the overall significance of the model. The result showed that cost of fertilizer, cost of herbicide, farm size and cost of labour were the significant variables and factors affecting the production of pro vitamin A cassava in the study area.

Cost of fertilizer was significant at 1% level and was directly related to the quantity of cassava produced. This implies that the higher the cost of fertilizer, higher the quantity of pro vitamin A cassava variety produced. This could be as a result of the importance of fertilizer to farmers during production seasons. Most farmers cannot do without fertilizer because of the poor fertility of the soil. Fertilizer helps to boost yield and thereby encouraging production; most farmers cannot produce without fertilizer no matter the cost. In this case, it has become necessary for subsidy to be given for fertilizer to support and encourage farmers in their production seasons.

Cost of herbicide and cost of labour were significant at 10% and at 1% respectively and were negatively related to quantity of cassava produced. This implies that the higher the cost of herbicide, the lower the quantity of cassava produced and vice versa. It also implies that the higher the cost of labour, the lower the quantity of pro vitamin A produced and vice versa. The results imply that high cost of herbicide and labour will perhaps discourage farmers from engaging into the enterprise.

Constraints to the production of pro-vitamin A cassava variety

The constraints to the production of pro vitamin A cassava is presented on the table 3.

Table 3: Summary of the Result on The Constraints to the Production of Provitamin A cassava

Constraints	Frequency	Percentage
Poor road network	56	93.33
Lack of access to credit	55	91.67
Inadequate		

information on pro-vitamin A cassava variety	46	76.67
High infestation of pest and diseases	39	65.0
Government policies	37	61.67
High cost of planting material	34	56.67
High cost of chemical	28	46.67
Land tenure system	20	33.33

Source: Field Survey, 2017

The result showed that 93.33% of the respondents were constraint by poor road network which prevented them from accessing pro vitamin A cassava variety, lack of access to credit (91.67%), inadequate information about the cassava variety (76.67%), high cost of planting material (56.67%), and high cost of chemical (46.67%), high infestation of pest and diseases (65%) and government policies (61.67%).

Conclusion and Recommendation

Conclusion

From the study carried out, gender, age, marital status, household size, occupation, educational level and farm

size pictured out the socio-economic characteristics of the pro vitamin A cassava farmer in the area.

The result of the factors affecting the production of pro vitamin A cassava showed that cost of fertilizer, cost of herbicides, farm size, cost of labor and cost of planting material were the major factors affecting the production of Pro-vitamin A cassava in the study area. Cost of fertilizer and farm size were positive and significant at 1%, while cost of herbicides and cost of labor, were negative but significant at 10% and 1%, levels respectively. Furthermore, from the result of constraint against the production of pro vitamin A cassava variety, poor road net work with 93.33% showed the highest constraint, followed by lack of access to credit which showed 91.67%. land tenure system did not contribute much on the constraint against the production of Pro-vitamin A cassava in the area.

Recommendations

Based on findings, the following policy recommendations were made and they include:

Effort of the extension agents are highly needed to organize training programs, workshop, agric show and seminar. These would undoubtedly increase the skills, knowledge and techniques in pro vitamin A cassava production and hence improve the health and living standard of the farmers in the area

There is also need to provide infrastructure such as road network and market facilities in order to reduce transportation cost.

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SOCIO-INSTITUTIONAL FACTORS MILITATING AGAINST COMMERCIALIZATION OF CASSAVA AMONG COOPERATIVE FARMERS IN ABIA STATE, NIGERIA

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ABSTRACT

The study analyzed the socio-institutional barriers militating against commercialization of cassava among cooperative farmers in Abia State, Nigeria. Structured questionnaire was used to collect data from one hundred and twenty (120) Cassava cooperative members using multi-stage random technique. The data collected were analyzed using descriptive statistic, commercialization index and multiple regression models. The result shows that majority of the respondents were males and married, literates with large household size and had many years of farming experience. The regression result estimated that the level of education (1%), household size (10%), income (1%) and access to credit (5%) are positive and significantly related to cassava commercialization, while input costs was significant and negatively related to cassava commercialization. However, the result identified insufficient capital, unfavourable government policies, lack of patronage, pest and diseases, poor road infrastructure, late delivery of inputs, high cost of living, climate change and community unrest as constraints militating against cassava commercialization in the study area. The study calls for policy implementation, which will lead to increase commercialization of cassava. It also showed that farmers sensitization on important of marketing on their surplus products to reduce wastage and empowering farmers through provision of accessible credit at reduced interest cost access road, and provision of farm input at affordable rate, will lead to increase in commercialization and also leads to economic development.

Keywords: Cassava, Commercialization index, Cooperatives and Market

Introduction

Agriculture has continued to play important role in the Nigerian economy. It is the second largest sector after oil despite falling from 48% in terms of GDP in 1970 to 20.6% in 1980 and was only 23.3% of GDP in 2005. However, this sector has continued to make contribution to employment, food production, foreign exchange earnings and industrial inputs. It is estimated that 60% of Nigerians are employed in agriculture and are predominantly smallholders (CBN, 2002; Daramola, 2007). This means that a large majority of the farmers operate at the subsistence, smallholder level, with intensive agriculture being uncommon. A characteristic feature of the agricultural production system in Nigeria that is a disproportionately large fraction of the agricultural output is in the hands of these smallholder farmers whose average holding is about 1.0-3.0 hectares (CTA, 1999). This may be as a result of limited access to modern improved technologies and their general circumstance does not always merit tangible investments in capital, inputs and labour (Yemisi *et al.*, 2009) thus; there is urgent need to increase the level of operation through commercialization.

Commercialization refers to the process of increasing the proportion of production that is sold by farmers (Pradhan *et al.*, 2010). Commercialization of cassava production can take many different forms by either occurring on the output side of production with increased marketed surplus or occur on the input side with increased use of purchased inputs. Commercialization is the outcome of a simultaneous

decision-making behaviour of farm households in production and marketing (von Braun *et al.*, 1994). International cooperative Alliance, ICA(2015), defined cooperative as an autonomous association of persons unified voluntarily to meet their common economic, social and cultural needs through a jointly owned and dramatically controlled enterprise. It is a business voluntarily owned and controlled by its members patrons and operates for them and by them on a non- profit basis. Furthermore, the use of agricultural cooperatives on appropriate technology dissemination through extension services to increase their agricultural sustainability, development of rural poverty alleviation and commercialization has been recorded. Ibe, (2002) reported that the introduction of formal cooperatives in Nigeria, over seven years ago has helped to improve agricultural production and farmers welfare. Improving commercialization among the cooperative group is essential to increase the bargaining power of such group. The primary objective of forming group farming cooperatives is to increase the agricultural outputs (Zarafshani *et al.*, 2010). Although agricultural cooperatives are constrained by some factors and require supports from the government to enhance the effectiveness of their production and marketing of items, with the hope of increasing output and benefit to members economically. Some of these supports needed by the cooperatives were outlined by Ogieva, (2003) include: access to loans, farm inputs such as fertilizers, agricultural extension services, farm equipment, mobilization of savings, management of credit and attraction of government's support.

However, as it is recognized that agricultural commercialization is the key strategies for promoting accelerated modernization, sustainable growth and development and, hence, poverty reduction in the sector. Investment in agriculture should gear towards commercialization especially in cassava sector. It is imperative that those constraints inhibiting the performance of this sector are first identified with a view to unlock them and create a conducive environment for smooth operation. Identifying challenges to commercialization will unveil appropriate strategies for promoting accelerated commercialization and investment in the sector such that, in the final analysis, agriculture will become one of the most important growth points in the economy. Moreover, there are still gaps in the literature particularly in comprehensively conceptualizing the level of cassava commercialization, modelling and estimating the factors influencing commercialization. This study therefore, bridge the lacuna by analyzing factors affecting commercialization, level and determinants of cassava commercialization among cooperative farmers in Abia State, Nigeria.

METHODOLOGY

The study area was conducted in Abia state, Nigeria. The state is located in the South Eastern Nigeria. The State has a population density of 580 persons per square kilometre and a population of 2,833,999 persons (NPC 2007).The state have 17 Local Government Area. Agriculturally, the state is divided into three agricultural zones namely; Umuahia, Ohiafia and Aba zones. There are also various cooperatives societies in the study area.

Primary data were used for this study. Structured questionnaire were used to collect data from the respondents with the assistance of extension agents from ADP.

Multi-stage random sampling technique was adopted in sample selection. In the first stage the three agricultural zones were selected. In the second stage, two (2) LGAs were randomly selected from each of the three zones, giving a total six (6). And in the third stage Four (4) communities were purposively selected giving total of 24. Finally a random selection of five (5) cassava cooperative farmers, each from the communities, bringing a total of one hundred and twenty respondent used for the study.

Data collected were analyzed using descriptive statistical tools such as frequencies and percentages, factors influencing cassava commercialization and level of commercialization were analyzed using multiple regression and commercialization index respectively.

The models are shown thus:

$$Commercialization\ Index = \frac{Value\ of\ Cassava\ sold}{Total\ Value\ of\ Cassava\ production} \times 100$$

The implicit form of the regression is stated below as follows:

$$Y = f(x_1x_2x_3x_4x_5x_6x_7x_8)$$

Where;

- Y = Commercialization index
- X₁ = Education (Years)
- X₂ = Household (Number of people living under same roof)
- X₃ = Family experience (Years)
- X₄ = Farm Size (Hectares)
- X₅ = Input cost (Naira)
- X₆ = Access to Credit (Access to credit = 1, non access to credit = 0)
- X₇ = Income (Measured in Naira)X₈ = Farmer asset (Naira)
- X₉ = welfare

RESULTS AND DISCUSSION

Socio-economic characteristics of the respondents in the cooperative groups

The Socio-economic characteristics of the respondents was summarized and presented in table 1. The result shows 66.6 percent of the respondents were predominately male. The marital status result revealed that 58.3percent were married with large household size of about 9-12 person each. Majority of the respondent were literate since 49.1 percent represented those that attained either secondary or tertiary education. Over half (58.3%) of the respondent had farming experience of between 10-29 years. The result also shows that majority (79.1%) of the respondent had access to credit. This indicates that majority of the respondents had access to credit. This could be attributed to the fact that access to credit is one of the benefit derived by being members of farmers cooperative. Majority of the respondents had a relative low input costs since 75.0 % represented those who had input costs ranging between ₦1000- ₦49, 999. This could be due to their involvement in cooperative, cooperative societies assist their members in subsidizing inputs when purchased through them. And lastly majority of the respondents had low welfare level since 66.6% of the respondents had income generated from cassava production between ₦1000 – ₦99, 999,

Table 1: Summary of the Socio-economic Characteristics of the Respondents

Variables	Frequency	Percentage
Observation	120	100
Sex		
Female	40	33.3
Male	80	66.6
Marital Status		
Single	50	41.6
Married	70	58.3
Educational level		
Primary	36	30.0
Secondary	59	49.1
Tertiary	25	20.8
Household size		
1-4	27	22.5
5-8	83	69.1
9-12	10	8.33
Farming experience		
3-9	12	10.0
10-19	70	58.3
20-29	25	20.8
30-39	13	10.8
Access to credit		
Yes	95	79.1
No	25	20.8
Farm size		
0.1-1.49	20	16.6
1.5-2.89	75	62.5
2.90-4.29	20	16.6
4.30-5.69	5	4.2
Input cost		
1,000-49,999	90	75.0
50,000-96,999	15	12.5
99,000-147,999	-	-
148,000-196,999	15	12.5
Welfare		
1000-99,999	80	66.6
300,000-499,999	22	18.3
>500,000	18	15

Source: Field survey, 2019

Table 2: Level of Cassava Commercialization

Commercialization index	Frequency	Percentage
0.1-0.39	15	12.5
0.4-0.69	10	8.33
0.7-0.99	87	72.5
1.0-1.29	8	6.66
Total	120	100

Survey: Field survey, 2019

Cassava Farmers in Abia State.

Variables	Linear	Exponential	Semi-log	Double Log+
Constant	375.161 (2.208) ^{xx}	5.674 (6.737) ^{xxx}	-455.240 (-0.972)	0.011 (0.007)
Education	4.091 (0.757)	-0.001 (-0.024)	34.740 (0.711)	0.530 (3.011) ^{xxx}
Household size	31.052 (1.883) ^{xx}	0.167 (2.045) ^{xx}	-103.258 (-1.040)	0.586 (1.635) ^x
Experience	-0.766 (-0.199)	0.013 (0.678)	-23.094 (-0.393)	0.181 (0.852)
Farm size	0.179 (1.042)	0.001 (1.041)	21.885 (0.868)	0.054 (0.589)
Input Cost	-3.647 (-1.818) ^{xx}	-0.012 (-1.211)	-71.575 (-1.827) ^{xx}	-0.278 (-1.968) ^{xx}
Access to credit	157.142 (1.952) ^{xx}	0.696 (1.743) ^x	-164.173 (-1.977) ^{xx}	0.580 (1.935) ^{xx}
Income	0.001 (4.197) ^{xxx}	3.78E-006 (4.195) ^{xxx}	86.763 (3.337) ^{xxx}	0.615 (6.546) ^{xxx}
Farmer asset	0.119 (0.718)	-0.001 (-1.045)	3.488 (0.072)	0.165 (0.942)
R ²	0.563	0.507	0.482	0.690
R ²	0.424	0.350	0.317	0.591
F-ratio	4.031 ^{xxx}	3.218 ^{xxx}	2.911 ^{xxx}	6.954 ^{xxx}

Source: Field Survey, 2019.

Note : *significant at 10%, ** significant at 5%, *** significant 1%

Table 4: Constraints Faced by Cassava Farmers in Cooperative Society on Commercialization of Cassava

Constraints	Frequency	Percentage (%)	Ranking
Insufficient	85	100	1 st
Lack of patronage	11	24.4	7 th
Unfavorable Government	39	86.7	4 th
Late delivery of inputs	14	31.1	6 th
Poor road infrastructure	44	97	2 nd
Pest and Diseases	42	93	3 rd
Community unrest	4	8.9	8 th
High cost of living	44	97.8	2 nd
Climate change	27	60	5 th

Source: Field survey, 2019.

Commercialization index

The Table 2 shows the commercialization index of the cooperative in the study area. The result shows the commercialization index range of 0.7 - 0.99 had 72.5%. This implies that majority of the farmers involved in cassava farming are still tilting towards commercialization or becoming market oriented.

Factors influencing cassava commercialization among cooperative farmers

Table 3: Factors Influencing Commercialization of

The factors influencing cassava commercialization among cooperative farmers is shown in Table 3. Form the result of the regression model, double log was chosen as the lead equation based on the value of R² coefficient of multiple determinations, F ratio and the conformity of the sign of regression coefficients with prior expectations. The result shows a value of R² which was 0.690 meaning that 69% of the total variations observed in the dependent variable were accounted for by the independent variables. F-ratio was significant at 1% indicating the fitness of the model. The result shows that seven variables namely: level of education, household size, family experience, farm size, input cost, access to credit, income, farmer asset significantly influenced the commercialization among cassava cooperative farmers.

Coefficient for level of education attained by farmers was significant at 1% Level and positively related to cassava commercialization. The implication is that the higher the level of education attained by the farmer the greater the market orientation of cassava. This is in conformity with a prior expectation that the level of education of farmers enhances productivity.

Coefficients for household size and income were highly significant at 1% level and positively related to cassava commercialization. This implies that as household size and income of the farmers increased, commercialization of cassava increased. Household size is a ready and cheap source of family lab our. The greater the ready and cheap source of family lab our, the greater the optimum utilization, of scarce resources especially land, capital and time consequently, leading to commercialization of cassava. Commercialization of cassava has contributed to the increased in the income level of farmers through increased in their production, resulting in higher proportion of marketed surplus (Ufomadu, 2005).

Coefficient for access to credit was positively related to cassava commercialization at 5%. Access to credit has been positively linked to agricultural productivity in several studies in Nigeria (Abu, *et al.*, 2010 and Ugbaja, 2011). Access to credit could influence the

purchase of productive assets for cassava production. Coefficient of input cost was negatively related to commercialization and significant at 5% level of probability. The inverse relationship implies that as the input cost of the farmers' increases, their commercialization decreases. This is expected and accordance with a prior expectation.

The result in Table 4 shows the distribution of cooperatives according to constraints militating against cassava commercialization. The result shows that insufficient capital (100%), poor road infrastructure. (97.8%) high cost of living (97.8), pest and diseases (93.3%) unfavourable government policies (86.7%), climate change (60%), late delivery of inputs (31.1%), lack of patronage (24.%) and community unrest (8.9%) were the problems identified by cassava farmer in cooperative society on commercialization. However, insufficient capital, poor road infrastructure, high cost of living, pest and diseases were the major problems faced by farmers on commercialization

Conclusion and Recommendation

The study identify constraints militating against cassava commercialization as Insufficient capital, poor road infrastructure, high cost of living, pest and diseases, unfavourable government policies, climate change, late delivery of inputs, lack of patronage, and community unrest. However, factors influencing commercialization of cassava among cooperative members in the study area were level of education, household size access to credit, income and input cost. The study also shows cassava farming is tilting towards commercialization and calls for policy if attended to, might increase commercialization among farmers through adequate sensitization on the benefits of cassava farmers joining cooperatives. Government should empower the farmers through available credit access at reduced interest cost, access road, and provision of farm input at affordable rate. Orientation and encouragement should be given to farmers on need to increase their marketable surplus and reduce waste at the peak period to increase commercialization.

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SOCIO-ECONOMIC CHARACTERISTICS OF PALM OIL MARKETERS AND CONSTRAINTS IN PALM OIL MARKETING IN EDO STATE, NIGERIA

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ABSTRACT

The study identified the socio-economic characteristics of palm oil marketers as well as the constraints to palm oil marketing in Edo State, Nigeria. A multistage sampling technique was used in selecting 180 respondents for the study. Data were analysed using simple descriptive statistics such as means, frequency counts and percentages. The results showed that young married women constituted 93% of the respondents, while 66% of the respondents, had more than 4 years of marketing experience in palm oil marketing. Also, it was observed that 71% of them were married; an indication that they supported their families with proceeds from the business. The respondents were fairly literate, as 66% of the respondents had secondary school education as their highest educational qualification. Transportation cost and lack of credit facilities were the major constraints faced by the marketers in the study area. It was recommended that the government should provide an effective transport system / good road network besides provision of loans/credit facilities at low interest rates to assist the palm oil marketers.

Keywords: Marketing, Constraints, Socio-economic

INTRODUCTION

Palm oil marketing remains a major business in Nigeria, especially in the rain forest zones of the country, especially in Edo State (Omoti, 2003). Palm oil and kernel oil accounts for an average of 70% of the Nigerian oil market, even though the annual demand for palm oil in the country is above one million tonnes.

In the nineteenth century, palm oil marketing began with international communities, while that of palm kernel began after 1832 (Omoti, 2003). Palm oil became the principal cargo for slave ships (Ojuigo, 1984). Locally, the uses of palm oil are varied and many. It is used for cooking, soap making and lamp oil. It is also used in metal plating. Other uses include a source of glycerine, for manufacturing of margarine, cooking fats and for making pomade (Biochemistry Division, NIFOR; Adegbola *et al.*, 1979). The industry is an important sub-sector of the agricultural sector of the Nigerian economy. 4 million Nigerians have been directly employed in about 20 oil palm growing States in Nigeria and indirectly to other numerous people involved in its processing and marketing. Its production on a large industrial scale has directly provided or catalysed the provision of rural infrastructure and other facilities such as hospitals, dispensaries, schools, roads, electricity and water in some parts of Nigeria (Abubakar, 2001). In the early 1870s, exports of palm oil from the Niger Delta were between 25,000 to 30,000 tonnes per annum and by 1911, the British West African Territories exported 87,000 tonnes of palm oil (Omoti, 1999).

Hartley (1988) reported that throughout the first half of the 20th century, the world production and export of palm oil was led by Nigeria and Zaire, now known as the Democratic Republic of Congo, Abubakar (2001).

Hartley (1988) also reported that before the Nigerian civil war, and the period before the oil boom, the palm oil and palm kernel oil exports were a significant source of foreign exchange to the country. He reported that Nigeria, as at that time, was the highest producer and exporter of palm oil and palm kernel oil. Total world production of palm oil reached 73.9 million metric tonnes (MMT) in the 2018/2019 production season representing a 31% increase from the 56.38MMT total world production in the 2012/2013 production season (www.statista.com). The increase of 17.52 million (or 31% increase) is explained largely by the expanded production from Asia. The global production is expected to grow up to around 75.7MMT by the end of the year 2020. Indonesia and Malaysia have been the major producers of palm oil, followed by Thailand, Colombia and Nigeria.

Presently, domestic production stands at 1,015 metric tonnes (USDA, 2019). Abubakar (2001), Amaechi *et al.* (2003), Corley and Tinker (2003) and Omoti (1999) all attributed the increasing demand for palm oil in the country to increase in population growth, increased income levels and rapid urbanization. Consumption of palm oil in Nigeria has been increasing at an average annual rate of 11% with 3% of the increase attributed to population growth (Omoti, 1999).

Like every other business venture, palm oil marketing is not without problems or challenges. Problems associated with palm oil marketing can negatively impact on the living standards, income of the investor, and the palm oil industry at large. It becomes expedient therefore, to examine these factors in order to proffer solutions.

The objective of the Study

The objective of this study was to profile the socio-

economic characteristics of palm oil marketers in Edo State Nigeria, identify the respondents source of palm oil, examine the prices of palm oil across the selected markets in the State and examine the constraints faced by the palm oil markers in the study area .

Statement of the Problem

Low income from palm oil marketing as a result of the problems associated with marketing, can affect income and the living standards of the investors.

METHODOLOGY

Area and Scope of the Study

The study was conducted in Edo State, an inland state in southern Nigeria with its capital in Benin City. The State is known for oil palm production because of the weather condition that favours the growth of the crop. Edo State has a landmass of 17,802km² (6,873 square miles) and a population of 3,497,502 (NPC, 2006). It is made up of eighteen (18) Local Government Areas (LGAs). It lies roughly between longitude 06.04E and 06.43E and latitude 05.44N and 07.34N. The State has boundaries with Delta State to the South, Ondo State to the West, Kogi State to the North and Anambra State on the East. Benin City has a landmass of 249km² and a population of 374,671 (NPC, 2006).

The State is divided into three agro-ecological zones namely, Edo South, Edo Central and Edo North. Edo Central is divided into five blocks as follows: Esan Central, Esan West, Esan North-East, Esan South-East and Igueben Local Government Areas (LGAs). Edo North Comprises 6 blocks, namely: Owan West, Akoko-Edo, Etsako West, Etsako East, Owan East and Etsako Central LGAs. Edo South consists of seven (7) blocks namely, Oredo, Ovia South West, Ovia North East, Ikpoba-Okha, Egor, Uhumwode and Orhionwon LGAs.

Sampling Technique

A multistage sampling technique was used to select 180 palm oil marketers across Edo State. Stage 1, involved the purposive selection of 5 Local Government Areas (Oredo, Ovia South-West, Ovia North-East, Ikpoba-Okha and Igueben). This was done as a result of the high concentration of palm oil producers in these LGAs. Stage 2, involved the random selection of 9 markets from the LGAs selected which were Iguobazuwa, Udo, Ugbogiobo, Ekiadolor, Aduwawa, Oka, New Benin, Ekiosa and Igueben markets. The last stage was a random selection of 20 respondents across the 9 markets, giving a total of 180 respondents. Information was elicited from the marketers using structured interview schedule, on socio-economic characteristics of the marketers, sources and trading point of palm oil and problems inhibiting the effective flow of palm oil from marketers to consumers in the State.

Method of Data Analysis

Data were analyzed using simple descriptive statistics such as means, frequency counts, and percentages

Data Sources

Both primary and secondary data were used for the study. Primary data used for this study were collected from the respondents through the use of a structured questionnaire while the secondary data used were from relevant sources to support the discussion.

RESULTS AND DISCUSSION

Socio-economic Characteristics of the Respondents

Table 1, shows the distribution of respondents according to their socio-economic characteristics. The result showed that 93% of the total respondents were women who were below the age of 41. This observation aligns with those of Ilo (2002); Anzankuet *al.*, (2006) and AWARD (2010) where young female adults form the major workforce in agricultural activities were found to have contributed significantly (60%-80%) to household income from marketing activities. Also, it was observed that 71% of them were married; an indication that they supported their families with proceeds from the business. Furthermore, 66% of the respondents have secondary school education as the highest educational qualification. This indicates that palm oil marketers in the study area were fairly literate. This agrees with the findings of Edeoghon, C.O, and Oria-Arebutun (2011) where the bulk of the respondents of women in urban agriculture in Ovia North East Local Government Area of Edo State, Nigeria, had secondary school education. It was also observed that 65% of the respondents were members of a cooperative society which they use in supporting the business with 66% of them having over 4 years of marketing experience.

Table 1: Distribution of Respondents According to their Socio-economic Characteristics

Variable	Frequency	Percentage (%)
<i>Age (Years)</i>		
<20	7	4
21-30	60	33
31-40	82	46
41-50	29	16
>50	2	1
<i>Sex</i>		
Male	13	7
Female	167	93
<i>Education</i>		
No formal education	17	9
Primary	51	28
Secondary	109	61
Tertiary	3	2
<i>Marital status</i>		
Single	52	29

Married	128	71
Cooperative membership		
Member	117	65
Non-member	63	35
Years of experience		
<2	21	12
2-3	39	22
4-5	62	34
>5	58	32

Source: Field survey, 2019.

2. The supply channel for palm oil in Edo State was found to be complex with little or no specialization with regards to the roles played by middlemen in the process. This is because, at each channel level, the middlemen sold palm oil to all market intermediaries at the lower levels including to the final consumer. The study, however, showed that 92% of the marketers got their palm oil supply from companies within Edo State, such as Presco, Okomu, e.t.c. 75% of them got their supplies from other wholesalers usually from nearby villages and towns, while 16% got palm oil from individual farmers/processors. Find details in Table 2, below.

Marketers' Source of Palm oil

The marketers' source of palm oil is presented in Table

Table 2: Marketers' Source of Palm oil

L.G.A.	Market	Farmer /Producer	Owner Plantation	Wholesalers	Companies
Ovia West	South Iguobazuwa	3	-	18	19
	Udo	12	2	10	16
Ovia East	North Ugbogiobo	5	-	15	17
	Ekiadolor	5	-	8	20
IkpobaOkha	Aduwawa	3	-	17	20
	Oka	-	-	6	20
Oredo	New Benin	-	-	13	17
	Ekiosa	-	3	18	19
Igueben	Igueben	2	4	17	17
	Total	30*	9*	135*	165*
	%	16	5	75	92

*= multiple responses

Source: Field survey, 2019.

Palm Oil Prices (Retail)

Table 3, presents the retail palm oil prices at various markets during the peak and lean production season of the year. Price differences in palm oil trade were observed in all the markets studied as prices generally tend to be higher during the lean season (Jul.-Aug. & Nov.-Feb.) when the commodity is scarce and drops in the peak season (Mar.-Jun. & Sept.-Oct.) when the product is in abundance. Details are presented in Table 3, below.

Palm oil prices (wholesale)

Table 4, presents the wholesale palm oil prices at various markets during the peak and lean production season of the year. Just as it was observed for the retail prices, the wholesale prices of palm oil generally tend to increase during the lean season (Jul.-Aug. & Nov.-Feb.) ,i.e when production is low or when the commodity is scarce and drops in the peak season (Mar.-Jun. & Sept.-Oct.) when the production is high. Find details in Table 4, below.

Table 3: Retail Palm Oil Prices (₦) at Various Markets during the Peak and Lean Production Season of the year.

Peak Season (Mar.-Jun. & Sept.-) Lean Season (Jul.-Aug. & Nov.-)

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L.G.A.	Market	Oct.) Cost price(₦)	Selling price(₦)	Feb.) Cost price(₦)	Selling price(₦)	Mean cost price(₦)	Mean selling price(₦)
Ovia South West	Iguobazuwa Udo	2480.31	2904.19	3301.56	3801.02	2890.94	3352.61
		2480.00	2902.03	3310.85	3801.05	2895.43	3351.54
Ovia North East	Ugbogiobo Ekiadolor	2528.36	2900.16	3349.29	3805.10	2938.83	3352.63
		2541.69	3107.13	3394.75	3812.01	2968.22	3459.57
IpkobaOkha	Aduwawa Oka	2589.12	3159.25	3373.91	3826.02	2981.52	2492.64
		2509.76	3127.09	3353.58	3819.01	2931.67	3473.05
Oredo	New Benin Ekiosa	2619.73	3156.15	3317.31	3817.90	2968.52	3487.03
		2598.75	2986.10	3339.01	3800.03	2968.88	3393.07
Igueben	Igueben	2585.33	2981.37	3384.58	3818.16	2984.96	3399.77
Total		22933.05	27223.47	30124.84	34300.3 0	26528.97	30761.91
Mean Standard Deviation		2548.12 3.42	3024.83 3.02	3347.20 2.05	3811.14 1.98	2947.66	3417.99
Coeff. of Var. (V)		0.0013	0.0010	0.00061	0.00052		

Source: Field survey, 2019.

Table 4: Wholesale Palm Oil Prices (₦) at Various Markets during the Peak and Lean Production Season of the year.

L.G.A	Market	Peak Season (Mar.-Jun. & Sept.-Oct.)		Lean Season (Jul.-Aug. & Nov.-Feb.)		Mean cost price	Mean selling price
		Cost price	Selling price	Cost price	Selling price		
Ovia South West	Iguobazuwa Udo	2000.31	2480.31	2532.38	3301.56	2266.35	2890.94
		2015.00	2480.00	2525.22	3310.85	2270.11	2895.43
Ovia North East	Ugbogiobo Ekiadolor	2029.39	2528.36	2537.53	3349.29	2283.46	2938.83
		2201.09	2541.69	2598.73	3394.75	2399.91	2968.22
IpkobaOkha	Aduwawa Oka	2216.95	2589.12	2596.32	3373.91	2406.64	2981.52
		2212.32	2509.76	2587.65	3353.58	2399.99	2931.67
Oredo	New Benin Ekiosa	2276.09	2619.73	2599.33	3317.31	2437.71	2968.52
		2248.08	2598.75	2596.15	3339.01	2422.12	2968.88
Igueben	Igueben	2232.53	2585.33	2583.43	3384.58	2407.98	2984.96
Total		19431.75	22933.05	23156.74	30124.84	21294.27	26528.9 7
Mean Standard Deviation		2159.08 1.43	2548.12 3.42	2572.97 2.11	3347.20 2.05	2366.03	2947.66
Coeff. of Var. (V)		0.00066	0.0013	0.00082	0.00061		

Source: Field survey, 2019.

Problems Faced by Oil Palm Marketers

Table 5, presents the constraints faced by the respondents. The marketers were interviewed on five problem areas they were likely to have difficulties in

the course of their trade activities. The problem areas focused on, include incidences of theft, taxation, transportation expenses, access to credit facilities and access to information on prevailing market prices of

palm oil (Table 5). The result obtained from the study showed that theft was not a problem at all to both wholesalers and retailers. About 30% of the wholesalers and retailers indicated that payment of tax was a problem, while less than 35% of them had no access to information on the prevailing market price for palm oil. However, all the marketers identified transportation to be a major problem. Transportation is one very important factor in agricultural marketing. The results from the study agreed with Impey's (2000) findings that the cost of transportation has an important influence on the market price of commodities, and income received by farmers. That is, the higher the cost of transportation, the higher the marketing cost of the marketers, and therefore, the lower their income. This observation is applicable to palm oil marketing since the price received per unit

quantity of the commodity is more or less relatively the same in all the markets due to the competitive structure of the market. Poor access to credit facilities was also identified as one of the bottlenecks faced by all the wholesalers and retailers in palm oil trade in Edo State. This could have constrained the marketers from expanding their business frontiers as well as afford efficient/effective storage facilities for storing their palm oil long enough to bridge the effect of seasonal price variation. In concordance with the above assumption, Clive (2001) revealed that lack of capital (plants or money) is a serious problem to business expansion and growth which can lead to the death of industries or even cause entrepreneurs to dispose (sell) off their goods in order to remain in the market.

Table 5: Problems Faced by Oil Palm Marketers

L.G.A	Market	Wholesalers					Retailers				
		Theft	Tax	Transport	Poor credit facilities	Lack of information	Theft	Tax	Transport	Poor credit facilities	Lack of information
Ovia West	South Uguobazua	-	6	10	10	4	-	6	10	10	3
	Udo	-	5	10	10	1	-	5	10	10	4
Ovia East	North Ugbogiobo	-	10	10	10	5	-	-	10	10	1
	Ekiadolor	-	3	10	10	2	-	3	8	10	2
IkpobaOkha	Aduwawa	-	1	10	10	2	-	1	9	10	4
	Oka	-	1	10	10	3	-	1	10	10	2
Oredo	New Benin	-	5	10	10	1	-	5	7	10	3
	Ekiosa	-	-	10	10	5	-	-	8	10	2
Igueben	Igueben	-	6	10	10	6	-	6	10	10	1
Total		-	28	90	90	29	-	27	82	90	22
%		-	31	100	100	32	-	30	91	100	24

Source: Field survey, 2019.

CONCLUSION

Statistics of the socio-economic characteristics of the marketers showed that young married women with more than 4 years of marketing experience dominated the palm oil market in Edo State. Their marketing system was complex and their major source of palm oil was mainly from companies. The respondents carried out such marketing functions as business financing and transportation of palm oil. Palm oil storage in the State was not yet efficient and/or effective as seasonal price variations were observed at both wholesale and retail market levels in all the markets studied. The major problems confronting the palm oil marketers in the study area were found to be

high transportation cost and inadequate finance to manage the business. Steps to overcome these constraints in palm oil marketing in the State would include, provision of loans or credit facilities at single-digit interest rates to the marketers, provision of effective storage facilities by government or service providers specialized in bulk storage to reduce or help eliminate the fluctuation of palm oil prices as well as ensure its all-year supply and availability. Lastly, there is the need for government to develop an efficient transport system as well as improve on the road network system in the State to provide easy access to marketers and consumers of palm oil alike and reduce the marketers marketing cost.

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WIS –SSD 26FL

SUB THEME H: SOCIO ECONOMIC RESEARCH, SUSTAINABLE DEVELOPMENT AND
COMMUNITY-RELATED ISSUES

TITLE: INCOME BENEFITS OF NON-TIMBER FOREST PRODUCTS (NTFPs) TO RURAL HOUSEHOLDS AND THE CHALLENGES OF HUMAN ACTIVITIES IN OHAFIA ABIA STATE NIGERIA

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Abstract

Demand for NTFPs has enabled the expansion of rural domestic markets. The study was carried out in Ohafia local government area of, Abia State with the following objectives; to describe the socio economic characteristics of the respondents, determine the knowledge level on the impact of livelihood activities in the study area, estimated the income share contribution of NTFPs to their economy and its significance. A simple random technique was used in the selection of 216 respondents from 9 villages using a structured questionnaire. Data obtained were analysed using both descriptive and inferential statistics. About 26% of the respondents were between the ages of 25- 34 years. Their occupations are mainly farming and harvesting of NTFPs (19.4% and 45.8%). Their sources of income are crop production (27.8%) and NTFPs trading (20.4%). High importance and priority placed on NTFPs reflected in households' income share with an average of (11.3%) from NTFPs, next to crop production (43.5%), off-farm income (22.2%) and livestock production (21.4%). Income share from NTFPs harvest had a positive relationship with the total household income and was statistically significant at ($P \leq 0.05$). Respondents' knowledge on the challenges of various human activities especially on agriculture is (42.1%), but they lack capacity for a sustainable adaptation. Their rigorous human activities worsen the environment and may jeopardize non timber forest production and distress the peoples' livelihood. It is imperative for local policy makers to formulate policies and strategies aimed at sustaining the future availability of NTFPs through a consolidated community-based forest management using modern approaches and techniques that would ensure more domestication of NTFPs.

Keywords: Environment, Income, Livelihood, NTFPs, Ohafia.

Introduction

Non-Timber Forest Products (NTFPs) support rural livelihoods and food security in many developing countries by providing vital supplies of food, medicine, shelter, building materials, fuels, and cash income. Mulenga *et al.* (2012) noted that the increasing demand for NTFPs has enhanced rural livelihoods and enabled the expansion of domestic markets. Therefore, non-timber forest products may offer sources of income and opportunities for poverty alleviation in both rural and urban areas.

In Ohafia L.G.A, most rural households residing near the forest zones extract a range of forest products for both direct consumption and trade (including food products and wood for cooking and charcoal production), and forest products are among the top sources of household income in some rural areas.

Mulenga *et al.* (2012), reported that households engage in the trade of non-timber forest products (NTFPs) because of low capital requirements and they relatively gain easy access to markets. Overtime, NTFPs have helped to bridge seasonal gaps in income for many farmers, and they provide a safety net for many rural households during years with low crop yields.

Human activities have worsened the environment which indicates high temperature, irregular rainfall

pattern, flooding, erosion etc. and this can jeopardize non timber forest production by leading to low or unproductive yield in NTFPs, which will ultimately distress the peoples' livelihood. Many forest products are over-exploited, unfortunately anthropogenic activities, such as: hunting, farming, bush burning, tree felling etc has also negatively impacted on NTFPs in the rural communities. Many of these NTFPs are threatened and/or endangered while some have totally gone extinct due to over-exploitation pressure and resultant impacts even though the NTFPs play a significant role in the rural dwellers' economy, poverty as well as food security.

This study therefore seeks to establish the income benefits of non-timber forest products (NTFPs) to rural households and the challenges of human activities in Ohafia Abia State Nigeria mostly resulting from intensive agriculture. The objectives were to ascertain the socio economic characteristics of the rural households, determine the knowledge level on the impact of livelihood activities in the study area, and estimate the income share contribution of NTFPs to their economy and its significance. The results from this study will be integrated into local policies and stakeholders' forum.

Materials and Methods

Study Area

The study was carried out in Ohafia Local Government

Area of Abia State, Nigeria. Ohafia is one of the 17 local government areas in Abia State, which comprises 26 villages. It is an Igbo speaking region and it is located in the South-eastern region of Nigeria. Ohafia is predominantly known for having a lot of NTFPs, but then, they experience a series of harsh weather conditions too. Its geographical coordinates in degrees and decimal minutes are Latitude 5° 36" and 5° 48" N and Longitude 7° 48" and 7° 52" E with an altitude of 124m above sea level in the moist rainforest zone. It has an average rainfall of 2177mm yearly with a relative humidity of about 72% and monthly ambient temperature ranges from 17°C to 36°C Meteorological Station of NRCRI (Umudike, 2004 and 2005). The vegetation is of tropical rainforest (NEST, 2011). There are two distinct seasons in a year – the rainy season which is experienced between early March and October. November to February is the dry period, and then the harmattan comes between December and January.

Data Collection, Sampling Procedure, Sample size and Data Analyses

Data were collected on the socioeconomic characteristics of the rural households, the knowledge

level on the impact of livelihood activities in the study area, the income share contribution of NTFPs to rural households' economy and the NTFPs' income shares total household economy from 216 respondents.

The study adopted a simple random sampling technique which was used to select 9 villages out of the 26 villages in Ohafia Local Government Area (Bernard, 2005). A systematic sampling method was then used to select every 6th house in each village, so as to attain every corner of the households. A total of 216 household heads were interviewed (table 1). The respondents selected were 20% of the total population from each of the nine villages. (Odobode, 1999). The qualitative tools used to elicit information from the households were participatory tools such as Focus Group Discussions (FGDs), In-depth Interviews (IDIs) with key informants (village leaders) as well as field notes and direct observations. The FGDs were two groups from each of the sampled locations made up of male groups comprised 5 elderly and 5 youths, female groups comprised 5 elderly and 5 youths. The IDIs comprised 3 key informants from each village, these included (Ezeogo, Queen mother and Chief Farmer). This is summarised below.

Table 1: Distribution Showing the Sampling frame

S/n	No. of Villages	Villages stratified random technique	Selected by simple sampling	Stratified locations	No. of Households in Each village	No. of Households selected in each village (20%)
1.	Ohafia = 26	Akanu			220	44
		Asaga		Urban	110	22
		Ebem			80	16
		Eziafor			90	18
		Ndi lbe		Semi urban	108	20
		Amuke			118	22
		Ndi Orieke			120	24
		Nkwoebi		Rural	135	26
		Amuma			120	24
Grand Total					1101	216

Source: Field survey data, 2017.

Based on social norms and values in African culture, data was disintegrated based on gender needs, livelihood and their capabilities. This is because in the rural areas, there are certain responsibilities of men and different responsibilities of women. Women tend to pick NTFPs in groups from the forest edges, while the men are more interested in deep forest cultivation and processing of NTFPs. Therefore, data was disseminated by gender to recognize the different experiences of male and female using the method (Sen, 1999). All data obtained, were analysed using a number of analytical methods like: descriptive statistical methods, regression analysis, means and percentages. The method used to fit the regression line was the linear equation, which tried to calculate the best fitting line for the observed data by minimizing the sum of the squares of the vertical deviations from each data point to the line. Linear equation was used to calculate the relationship between NTFPs and total

household economy by fitting a linear equation to the observed data. $Y = a + bX$; where 'Y' is the dependent variable, 'a' is the slope, 'b' is the intercept and 'X' is the explanatory variable. Data on socioeconomic characteristics were analyzed using the percentage frequency calculation method.

Results and Discussion

Socio-economic characteristics of the sampled households

Table 2 reveal that (26%) of the respondents were between the ages of 25- 34 years. This shows that the household heads were still in their productive working age. The implication of this is that these household heads were young, energetic and able-bodied and should be able to pursue their livelihood activities with some ease and effectiveness such that they are able to provide for their household needs adequately. It also implies that the respondent will have a lot of experiences in regard to their environment. This agrees

with the findings of (Msalilwa *et al.*, 2013; Akponikpe *et al.*, 2010) which affirm that age has an influence in the accumulation of knowledge as regards to environmental challenges and variability in a particular locality. Majority of the household heads (66.2%) were male, while (33.8%) were female. It was observed that married women only gather NTFPs close or around fallow lands and farmlands while men and young ladies cultivate and harvest NTFPs deep in the forest areas. The households' occupations were majorly on farming and harvesting of NTFPs (19.4% and 45.8%) respectively. Their sources of income were mainly crop production (27.8%) and NTFPs trading (20.4%). The highest educational qualification of Secondary School Certificate was 44%, this shows that most of the rural dwellers are not tertiary graduates; this has affected their decisions on the exploitation of NTFPs and the resultant effect on their livelihood. This agrees with findings of Msalilwa *et al.* (2013) in a study they conducted in Tanzania, which affirms that educational level, age and residence duration influence the people's perception on their environment. The occupations of household heads were grouped into thirteen occupation- mix as shown in Table 2 Crop/ livestock/collection of NTFPs produce was the primary occupation of majority of the household heads with (45.8 and 19.4) % respondents engaged in it. This agrees with the finding of Blench (2003) which state that mix cropping helps farmers to cope and adjust to prevailing environmental conditions.

Married	121	56.0
Widowed	19	8.8
Separated	1	0.5
Total	216	100.0
Gender		
Male	143	66.2
Female	73	33.8
Total	216	100.0
Educational qualification		
None	18	8.3
Primary	32	14.8
JSS	35	16.2
SSS	95	44.0
OND/HND	28	13.0
B.Sc	7	3.2
PhD	1	0.5
Total	216	100.0
Household head occupation		
Crop/livestock	42	19.4
Crop/livestock/NTFPs	99	45.8
Civil servant	12	5.6
Health worker	7	3.2
Hair dresser	14	6.5
Fishing	3	1.4
Hunting	5	2.3
Technician/Artisan	5	2.3
Construction worker	7	3.2
Wage labourer	15	6.9
Housewife	7	3.2
Total	216	100.0

Source: Field survey data, 2017

The large household size (6-10) 61.1% may be explained by the high level of polyandrous and polygamous lifestyle among the respondents and this is invariably supported by their culture which encourages polygamy activity. This is consistent with the findings of Feto (2009), Acquah (2011) who also reported large household size in Ethiopia and Bawku Municipality in Upper East Region of Ghana which also as result polygamous activities. The study showed that the household type most common among the respondents was the Nuclear/polygamous household type as noted by 75% of the respondents. 19.4% and 5.6% of the respondents practice Nuclear/monogamous and extended household types

Table 2: Socio-economic characteristics of the sampled households

Socioeconomic characteristics	Frequency	Percentage
Age of respondents		
18-24	45	20.8
25-34	56	26.0
35-44	54	25.0
45-54	39	18.1
55-64	22	10.2
Total	216	100.0
Marital status		
Single	75	34.7

respectively. Majority of respondents (60.6%) were born and still live in the study area while the remaining 39.4% of the respondent migrated from neighbouring Local Government Areas (LGA) for trade or as migrant labourers. 21.8% of these migrants have lived in the study area for at most 5years, 8.8% of them have lived in the study area between 5-10years, and 5.1% of them have lived in the study area between 11-20years, while 3.7% of them have lived in the study area for over 20years. This is consistent with Udeagha (2014) who noted that most of the respondents in most rural areas are mostly indigenes of the area. A large proportion (60.2%) of the households own farmland while the remaining 39.8% of the respondents never owned any farmland. Among the households that own farmland, a large proportion (69.2%) of the households acquired the farmland by inheritance while the least proportion (6.2%) of the households acquired the farmland by gift. This result conforms to the finding of Banta *et al.* (2012) who posited that agricultural production is mostly performed on inherited land in most areas. This may make the control of production resources to be under the control of the male farmers rather than their female counterparts and thus may discourage the desire of female involvement in farming as expected. Also land

tenure system influence the farmer's productive behaviour and land improvement practices embarked upon (Nwosu, 2000) and decides which crop to plant and how long they will remain on the land. The farm size of large proportion (65.7%) of the households in the study was in plots. This was followed by 16.7% of households whose farmland in size are in hectares. This implies that most of the farmers are small scale farmers. The small farm size of the respondent might be due to the land tenure system or ownership which is characterized by fragmentation of farm lands. Nzeakor, (2013) averred that land ownership through heritage is the sole reason for the series of farm land fragmentation in the rural area. This will show their commitment towards increasing their production. A large proportion (27.8%) of the households engaged in crop farming as their major source of income in the study area, this was followed by 20.4% of the respondents that engaged in NTFPs production and trading as their main source of income. The least proportion of the respondents (4.6%) depends on remittances from relatives as their major source of income. The mass engagement of the respondents in crop production and non- timber forest production/trading in the study area may be due to the high level of income generated from these activities which supports household needs. According to Nnamerenwa *et al* (2017), rural farmers engage in those activities that best gives them high revenue as a coping strategy to mitigate against low household income and to meet up with family responsibility. Of the sampled respondent, (76.8%) harvested NTFPs in 2016, while (23.2%) did not harvest any NTFPs in last year 2016. The dominance of those that collected NTFPs last year (2016) shows the importance of NTFPs collection as a means of income diversification in the study area. According to Udeagha (2014), dependency of rural households in NTFPs collection and trading as a means of income diversification grows with time. More people got involved in NTFPs collection and trading in Akwa Ibom state of Nigeria in the current year of his study than in the preceding year as indication that harvesting of NTFPs increased with time in the study area.

Table 2: Socio-economic characteristics of the sampled households Continued.

Socioeconomic characteristics	Frequency	Percentage
Household size		
1-5	66	30.6
6-10	132	61.1
11-15	18	8.3
Total	216	100.0
Household type		
Nuclear/monogamous	42	19.4
Nuclear/polygamous	162	75.0
Extended	12	5.6
Total	216	100.0
Duration of living in		

the community		
Born here	131	60.6
<5yrs	47	21.8
5-10yrs	19	8.8
11- 20yrs	11	5.1
>20yrs	8	3.7
Total	216	100.0
Own farm		
Yes	130	60.2
No	86	39.8
Total	216	100.0
How land was acquired		
Outright Purchased	14	10.8
Inheritance	90	69.2
By gift	8	6.2
By lease	18	13.8
Total	130	100.0
Farm size		
Acre	34	15.7
Hectare	36	16.7
Plot	142	65.7
Others	4	1.9
Total	216	100.0
Household main source of income		
Crop production	60	27.8
Livestock production	13	6.0
Non-timber forest production/trading	44	20.4
Full-time wage/salary	42	19.4
Part-time wage/salary	31	14.4
Remittance from relatives	10	4.6
Others	16	7.4
Total	216	100.0
Household harvest of any NTFP in 2016		
Yes	166	76.8
No	50	23.2
Total	216	100.0

Source: Field survey data, 2017

Knowledge level on the impact of livelihood activities in the study area

Considering the mean score of the people, the result on table 3 showed that (39.4%) of the respondents accepted that bush burning emits CO₂ into the atmosphere and causes "extreme" irregular rainfall pattern; (46.3%) agreed that the use of chemicals, such as: herbicides, pesticides, insecticides, negatively affect their environment in a "very high" manner; (42.1%) affirmed that farming near water bodies causes depletion of the water bank which encourages "very high" flooding; (39.8%) said that deforestation and bush slashing causes the emission of CO₂ gases into the atmosphere, which in turn "highly" reduces the abundance of O₂ needed by man for survival

likewise, (36.1%) said that deforestation and bush slashing encourages excessive heat that leads to different kinds of diseases like meningitis, measles, chickenpox, heat-rashes in a “high” manner. (41.2%) believed that disposal of sewage in streams are harmful to sea bodies like fish and makes the water unsafe for drinking, “very highly” while (50.0%) affirmed that continuous cropping leads to loss of soil fertility as well as encourages easy leaching of soil nutrients, which leads to erosion and flooding in a “very high” manner. The overall mean score of 3.67 was higher than the decision cut-point of 3.00, implying that there was an acceptance that the aforementioned perception assessment statements were all accepted by the respondent. This result implies that the respondents have a good knowledge of the cause of environmental challenges from the various human activities especially on agriculture but lack the capacity for a sustainable adaptation. This agrees with the work of Enete *et al.* (2011) which states that households in Imo and Enugu states were also fully aware of the effect of their activities on agriculture and the environment.

Estimation of the income share contribution of NTFPs to rural household’s economy

The result of this study reveals that the entire sampled household engaged in the collection of NTFPs because of their income return and their overall contribution to their household economy. This high importance and priority placed on NTFPs reflected in households’ income share (Table 4). With an average income share of (11.3%) from NTFPs accounting for the fourth largest income share next to crop production (43.5%), off-farm income (22.2%) and livestock production (21.4%) with Ugbaga (Oil Beans) (3.1%) followed by Anuofia (Bushmeat) (2.6%) having the highest income share among the NTFPs. Cassava production commanded the largest portion of the household income with about 16.4% income share. The findings of this study is supported by the result of the following studies. In the Northern Ethiopia, Babulo *et al.* (2009), reported 27% and in southeast Ethiopia 35% by (Feto, 2009) of total income. In southwest Ethiopia, (Ermiyas *et al.*, 2014), NTFPs accounted for 47% of annual household income. In Zambia, NTFPs contribute 34% to household income on average (Mulenga *et al.*, 2011). In the northern savannah region of Benin, Heubach *et al.* (2012) reported that NTFPs form 39% of the total income accounting for the second largest income share next to crop production. Kamanga *et al.* (2009) reported 15% from Malawi and in Nigeria

(Udeagha, 2014) reported a 20% contribution to income share from the selling of *Irvingia spp.* The implication of the result is that improvement in income of households is mostly possible through active participation in NTFPs harvesting and trading. The z-test used to the income share of NTFPs and other income sources of the household presented in table 4 shows that the income share of NTFPs to the total household income was significantly lower than the income share of Crop production (-2.689 at P≤ 0.05), Livestock/fish (-2.946 at P≤ 0.05), and Non-farm income (-2.778 at P≤ 0.05), but was significantly higher than that of remittance (2.311 at P≤ 0.05). This goes a long way to show that apart from other income contribution, NTFPs also contributes its income shares to rural households in the study area. But its contribution is very minimal, this can be attributed to excessive human activities like bush burning, massive deforestation, excessive overgrazing, continuous cropping etc which have some negative impacts on the forest resources.

Significance of NTFPs income share to the total household economy

The double log model was selected as the model of best fit (the lead model) on the basis of its possession of the highest number of significant variables with appropriate theoretically expected signs and a high value of the coefficient of the multiple determinations (R²). The coefficient of multiple determination (R²) value was 0.926. This indicates that all the regressors included in the model explained about 92.6% of the variation in the total household economy in the study area. The F-Statistic was highly significant at (p ≤ 0.01) which indicates that the entire regressors included in the model significantly influenced the explained variable (total household Income) and that the model is well specified.

The result of the regression analysis showed that income from Crop production, Livestock/fish production, NTFPs harvesting and Off-farm activities were the significant variables that influenced the total household economy in the study area. Income share from NTFPs harvest had a positive relationship with the total household income and was statistically significant at 5 percent probability level. This indicates an increase in income diversity of the household through NTFPs harvest leads to an increase in the total household income in the study area.

Table 3: Impact of livelihood activities in the study area

S/N	Livelihood activities	E	VH	H	M	L	\bar{X}	STD
1.	Bush burning emits CO ₂ into the atmosphere that causes irregular rainfall pattern in your area.	64 (29.6)	85 (39.4)	37 (17.1)	25 (11.6)	5 (2.3)	3.82	0.72
2.	The use of chemicals, such as: herbicides, pesticides, insecticides etc.	35 (16.2)	100 (46.3)	52 (24.1)	25 (11.6)	4 (1.9)	3.63	0.71

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	negatively affects the environment in your area.							
3.	Farming near water bodies causes depletion of the water bank which encourages flooding.	29 (13.4)	91 (42.1)	54 (25.0)	39 (18.1)	3 (1.4)	3.48	0.62
4.	Deforestation and bush slashing causes the emission of CO ₂ gases into the atmosphere, which in turn reduces the abundance of O ₂ needed by man for survival.	29 (13.4)	67 (31.0)	86 (39.8)	32 (14.8)	2 (0.9)	3.41	0.54
5.	Deforestation and bush slashing encourages excessive heat that leads to different kinds of diseases like meningitis, measles, chickenpox, heat-rashes etc.	41 (19.0)	64 (29.6)	78 (36.1)	32 (14.8)	1 (0.5)	3.52	0.52
6.	Disposal of sewage in streams are harmful to sea bodies like fish and makes the water unsafe for drinking.	36 (16.7)	89 (41.2)	62 (28.7)	25 (11.6)	4 (1.9)	3.59	0.64
7.	Continuous cropping leads to loss of soil fertility as well as encourages easy leaching of soil nutrients, which leads to erosion and flooding.	64 (29.6)	108 (50.0)	39 (18.1)	3 (1.4)	2 (0.9)	4.06	0.89
8.	Overgrazing leads to loss of vegetation and forest cover which protects the soil surface and as such reduces the nutrient capacity of the soil.	42 (19.4)	127 (58.8)	39 (18.1)	6 (2.8)	1 (0.5)	3.94	0.96
9.	Burning of fuel wood releases the emission of CO ₂ gases and other harmful pollutants into the atmosphere.	30 (13.9)	89 (41.2)	73 (33.8)	24 (11.1)	-	3.58	0.65
10.	Littering of household waste creates offensive odour in the environment and as such makes the environment un-conducive for living.	81 (37.5)	102 (47.2)	25 (11.6)	7 (3.2)	1 (0.5)	4.18	0.96
11.	Burying of wastes leads to ground water contamination and can also release chemicals into the soil that will make the plants unfit for consumption and blockage of O ₂ aeration in the soil.	18 (8.3)	43 (19.9)	109 (50.5)	45 (20.8)	1 (0.5)	3.15	0.57
Overall mean score							3.67	0.71
Number of respondents							216	
Decision mean cut-point							3.00	

Source: Field Survey Data, 2017.

E-Extreme; VH-Very High; H-High; M-Moderate; L-Low; X-Mean;

STD- Standard Deviation

Table 4: Household livelihood strategies total and mean income (in Naira) and income share.

S/N	Income Source	Total Income by source(₦)	Mean Income per Household (₦)	Standard Error	Income Shares (%)
A	Crop Production	14,643,880.04	95,089.93	36,893.98	43.5
	Yam	2,237,200.00	25,372.00	4,300.58	6.6
	Maize	1,135,000	5,675.00	1,166.81	3.4
	Cassava	5,524,000	27,620.00	19,085.45	16.4
	Melon	1,691,750.02	10,458.75	2312.83	5.0
	Cowpea	1,444,780	7,223.90	2705.13	4.3

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	Plantain/banana	2,074,400	10,372.00	5,279.77	6.2
	Cocoyam	182,750.02	4427.5	982.83	0.5
	Cucumber	354,000.00	3940.78	1060.58	1.1
B	Livestock/fish Production	7,200,666.87	87,393.25	29,747.69	21.4
	Goat	2963333.36	10,800.56	3,162.09	8.8
	Sheep	646166.67	7,315.54	2796.08	1.9
	Poultry	1755833.33	42,675.39	12678.56	5.2
	Rabbit	302000.00	2,060.77	548.3	0.9
	Fishery	1533333.51	24,540.99	10562.66	4.6
C	NTFPs	3812560	44809.89	14710.04	11.3
	Ugbaga(Oil Beans)	1060000	7737.23	2680.75	3.1
	Uda(Guinea Pepper)	33700	362.37	141.53	0.1
	Ukazi (Gnetum)	582600	3664.15	641.11	1.7
	Ochiogochio (Tetraptera)	24900	315.19	88.82	0.1
	Ikolo (Snail)	265800	2631.68	1005.62	0.8
	Nmanu anu (honey)	275560	4239.38	1174.16	0.8
	Mmimmi (Pepper fruit)	19600	332.2	173.43	0.1
	Mgborogwu (roots)	579500	10536.36	4344.79	1.7
	Anuofia (Bushmeat)	859500	13223.08	4019.65	2.6
	Osu,Erue (mushrooms)	111400	1768.25	440.18	0.3
D	Off-farm Income	7,474,526.00	86,745.60	22,344.66	22.2
	Pub. Servant	3489400.00	36,571.46	7,804.76	10.4
	Private Servant	1607636.67	18,760.23	5712.89	4.8
	Hunting	488059.33	3660.45	1585.07	1.5
	Trading	710696.67	10,660.45	2585.07	2.1
	Other self-employment	1178733.33	17,093.01	4656.87	3.5
E	Remittance	520,034.00	5,208.87	2,555.83	1.5
	Children	443,378.00	4,433.78	2188.32	1.3
	Relatives	76,656.00	775.09	367.51	0.2
	Grand Total(a,b,c,d,e)	33,651,666.91	319,247.54	106,252.20	100.0

Z-test: NTFPs-Crop = -2.689**; NTFPs-Livestock/fish = -2.946**; NTFPs- Off farm = -2.778**; NTFPs- Remittance = 2.311**

Source: Computed by the author from field survey data, 2017 ; NB: ** represent 5% significance level.

This result strengthened the result in Table 5 below to indicate that diversification of household income through NTFPs is highly important. This finding is

supported by the findings of Owoh (2015) who reported that household income diversification through agroforestry harvesting is imperative in the

sustenance of rural household economy with the income may not be available as expected. supposition that other major sources of household

Table 5: Linear Equation regression result of the contribution of NTFPs to total household economy in the study area

Variable	Linear	Exponential	Double-log+	Semi-log
Constant	170526.3 (9.679)***	12.561 (47.544)***	12.449 (6.105)***	390622.1 (2.983)***
Crop income share	3533.457 (2.325)**	0.826 (3.831)***	1.347 (3.096)***	3443.888 (0.902)
Livestock/fish income share	1800.197 (3.431)***	1.26E-05 (4.927)***	0.929 (3.423)***	1522.958 (2.131)**
NTFPs income share	3533.457 (5.325)***	0.106 (1.509)	0.308 (2.416)**	3317.76 (2.979)***
Off-farm income share	0.152 (0.602)	0.199 (3.419)***	0.896 (2.978)***	2193.703 (0.596)
Remittance income share	0.075 (1.028)	0.022 (1.281)	1.054 (1.210)	424.073 (1.557)
R ²	0.741	0.782	0.926	0.591
Adj. R ²	0.718	0.767	0.909	0.577
F-statistic	52.846***	56.392***	74.204***	48.476***

Source:
Field
Survey

Data, 2017

***significant at 1%; and ** significant at 5%. + = represents lead equation. Values in parenthesis are t- ratio.

Conclusion and Recommendations

This study focused on the income benefits of non-timber forest products (NTFPs) to rural households and the challenges of human activities in Ohafia, Abia State, Nigeria. NTFPs contributes its income shares to rural households in the study area. But its contribution is very minimal, and this can be attributed to excessive human activities like bush burning, massive deforestation, excessive overgrazing, continuous cropping etc which have some negative impacts on the forest resources.

Based on the findings of this study, the following recommendations were proffered;

It is very pertinent for local policymakers to formulate policies and design strategies aimed at sustaining the future availability of NTFPs through a consolidated community-based forest management and other conservation measures using modern approaches and techniques that would ensure more domestication of NTFPs in public, government and private lands. This

would define more access to NTFPs gathering and extraction for cultural and economic uses while increasing the resilience of the rural dwellers to adjust to their livelihoods. Additionally, this would enhance the genetic diversity of NTFPs and conserve their innate resilience to excessive exploitation.

The study proposed an integrated assessment exercise to evaluate NTFPs in the neighbouring communities and states in Nigeria as very little has been done on investigating their income importance in Nigeria and its varying effects on the livelihood of the people.

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IMPACT OF MILLENNIUM DEVELOPMENT GOALS (MDGS) ON SWEET POTATO PRODUCTION IN NIGERIA: TREND ANALYSIS AND POLICY IMPLICATIONS

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Abstract

The study examined the impact of Millennium Development Goals (MDGs) on sweet potato area cultivated, production and productivity in Nigeria. The study sourced data from FAOSTAT from 1980-1999 as pre- MDGs and 2000-2018 as post MDGs intervention periods. A graphical presentation of area, production and productivity were projected, trend and growth rate functions estimated and confirmation of acceleration, deceleration and stagnation function. The coefficients for area and production showed positive significant trends and negative for productivity at pre and post MDGs period at 1% level each. The result also showed positive growth rate for area and production at pre-MDGs period (24.43% and 19.62% respectively) and post- MDGs (4.40% and 2.71%) but negative for productivity (3.86% and 1.61% respectively). The result confirmed acceleration in area and production at pre MDGs periods and stagnation at post-MDGs. The result also confirms stagnation in productivity within the two periods. The calculated chow's F-statistic shows significant values for in area, production and productivity of sweet potato within the two periods implying impact of policy (MDG) intervention. The study therefore calls for policies aimed at sustainable increase in the productivity of sweet potato in Nigeria by initiating programmes/projects that will result in higher priority to broad based research for development hence initiation of Sustainable Development Goals (SDGs) in 2006.

Keywords: Area, Intervention, Pre- and Post- MDGs, and Productivity

Introduction

Despite the tremendous increase in human population and resources, food supply has continued to decrease below demand globally (FAO (2013). To cushion this effect, the world leaders of 191 UN members in 2000 signed commits to combat poverty, hunger, disease, illiteracy, environmental degradation, and discrimination against women through Millennium Development Goals (MDGs) by the year 2015. The MDGs are derived from this declaration, and all have specific targets and indicators (MDG Report, 2015). There was therefore, clear series of efforts at the various levels of the food production chain in other to achieve this goal. Increase in production and productivity of crops (like sweetpotato) with high yielding potentials was one of them. A number of initiatives (RAINBOW, RAC and AGRA) in Nigeria during this period of MDGs encouraged increase in production and consumption of sweet potatoes (Okoye *et al.*, 2017). Rainbow and RAC focused on production and consumption of sweetpotato with particular interest on Orange fleshed sweetpotato (OFSP), which contain beta-carotene and help fight vitamin A deficiency-precursor for night blindness, good for children (0-5years), pregnant and lactating mothers. These interventions also focused on the breeding of new varieties, improved agronomic practices, and supply of planting materials (vines) to farmers (Andrade *et al.*, 2009).

These series of interventions and programs and others were targeted to employ over 60 percent of the labour force into agricultural sector in the country, provide food for the poor and hungry masses and improve their

livelihoods. However, with the wind-up of MDGs, the world still seems to have problems with development and poverty eradication especially amongst the developing countries (MDG Report, 2015). These categories of country also encompass Nigeria. There is need therefore, for precise knowledge of where the economy is, where we are going and the gap therein, by evaluating the performance of the various sections of the Agricultural sector before and after the interventions with special emphasis on sweetpotato and ways to improve them.

Sweet potato (*Ipomoea batatas L*), a root crop emerged as an attractive crop of choice for resource-constrained households in Nigeria because of its ability to tolerate drought, low demand for inputs, multiple uses, and income-generating potential in marginal areas that are not suited for production of traditional cash crops (Okoye, *et al.*, 2020). Sweet potato had the tenth highest production level of any single food crop in Nigeria (after cassava, yam, oil palm fruit, maize, sorghum, millet, paddy rice, and plantains) (FAO, 2013). It is the 3rd most important root and tuber crop after cassava and yam. Sweet potatoes offer a significant potential for increased food security and income of many households in Nigeria. It presents diverse home, industrial and economic uses and potentially highly profitable (Adewumi and Adebayo, 2008). Production of this crop is one of the drivers necessary for economic growth and reduction in poverty (Reardon and Timmer, 2014).

Nigeria being the highest producer of sweetpotato in the world after China and Uganda with about 4.03mmt

(FAO, 2018) must have contributed towards achieving the MDGs. In 2016, another initiation Sustainable Development Goals (SDG) was targeted to zero hunger in the world and by implication, Nigeria in 2030. There is, therefore, a clear need for series of efforts at the various level of the food production chain in other to make this possible. This study estimated the impact of MDGs by estimating trend in sweetpotato production, area cultivated and productivity before and after the MDGs and also estimated the impact of agricultural policies on sweetpotato productivity between the two periods.

Methodology

The study made use of secondary data sourced from FAO statistics, Central Bank of Nigeria (CBN) and National Bureau of Statistics (NBS). The data on annual output, area and productivity of sweetpotato were obtained for the period 1980-1999(Pre MDGs) and 2000-2018 (post MDGs). Annual compound growth rates of sweetpotato production, area and productivity were computed by fitting an exponential equation in time to the data as follows:

$$Y = a_0 b_1 t \dots \dots \dots (1)$$

Which when linearized in logarithm becomes:

$$\ln Y = a + b_1 t + Ut \dots \dots \dots (2)$$

Where Y is output in metric tonnes, area in hectares and productivity (t/ha) each, t is the time trend (variable), while a and b are estimated regression parameters.

The annual compound growth rate (r) in sweetpotato output, area and productivity is given as follows:

$$r = (e^b - 1) \times 100 \dots \dots \dots (3)$$

Where e is Euler's exponential constant (2.71828)

In order to confirm the existence of acceleration, deceleration or stagnation in sweet potato output, area and productivity in Nigeria, quadratic equations in time series were fitted to the data as follows:

$$\ln Y = a + b_1 t_1 + b_2 t_2^2 \dots \dots \dots (4)$$

The impact of MDGs on sweetpotato production was analyzed using Chow's test following Onyenweaku (1997) as follows:

$$F^* = \frac{[\sum e_3^2 - (\sum e_1^2 + \sum e_2^2)/k_3 - (k_1 - k_2)]}{\sum e_1^2 + \sum e_2^2 / (k_1 + k_2)} \dots \dots \dots 6$$

If the calculated F (f cal) exceeds the tabulated F (f tab), it implies that MDGs had impact on the estimated variables.

Test for Homogeneity of slope: The F- statistics was

estimated as

$$F^* = \frac{[\sum e_3^2 - (\sum e_1^2 + \sum e_2^2)/k_3 - (k_1 - k_2)]}{\sum e_1^2 + \sum e_2^2 / (k_1 + k_2)} \dots \dots \dots 6$$

If the calculated F (f cal) exceeds the tabulated F (f tab), it implies that MDGs brought about a structural change or shift in estimated parameter.

To test for the stability of the intercepts

$$F^* = \frac{[\sum e_3^2 (\sum e_4^2) / k_3 - k_4]}{\sum e_4^2 / k_4} \dots \dots \dots 7$$

If the calculated F (f cal) exceeds the tabulated F (f tab), it implies that the post-MDGs period differ from pre-MDGs period.

Where;

- $k_1 = n_1 - m$
- $k_2 = n_2 - m$
- $k_3 = n_3 - mn$
- $k_4 = n_4 - mn$
- n_1 = Number of observations for the first regression (pre-MDGs)
- n_2 = Number of observations for the second regression (post-MDGs)
- n_3 = Number of observations for the third regression (pooled sample)
- n_4 = Number of observations for the fourth regression (with dummy)
- mn = Number of regression estimates including b_0
- $\sum e_1^2$ = Residual sum of squares (unexplained variation) for pre MDGs
- $\sum e_2^2$ = Residual sum of squares (unexplained variation) for post MDGs
- $\sum e_3^2$ = Residual sum of squares for the pooled data samples
- $\sum e_4^2$ = Residual sum of squares (unexplained variation) for the fourth regression
- F^* = chow's F-statistic

Result and Discussion

Trends in the area cultivated, production and productivity of Sweetpotato pre and post MDGs

The result in Figure 1 shows area (*000 ha) of sweetpotato cultivated in Nigeria pre MDGs (1980-1999) and post MDGs (2000-2018) periods. The result shows an average area cultivated with sweet potato (pre-MDGs) as 121.03 thousand hectares compared to 1,214 thousand hectares (MDGs period). The result also indicated a sudden rise in slope in the area cultivated from 282 thousand hectares in 1998 to 800 thousand hectares in 1999 before the commencement of MDGs. The result recorded fluctuations in area cultivated with sweet potato within the pre MDGs period, while there was continuous increase in area cultivated over the years in the post MDGs period as

indicated in Fig. 1.

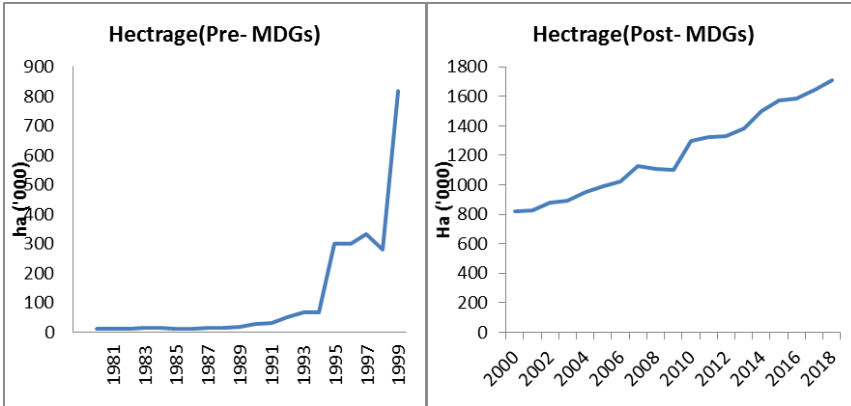


Fig. 1: Area ('000ha) cultivated with Sweetpotato for Pre-MDGs (1980-1999) and Post MDGs (2000-2018)
Source: Basic data from FAO 2018 (FAOSTAT, June 2018, accessed April, 2020)

The result in Fig. 2 shows production (million tons) of sweet potato for pre-MDGs (1980-1999) and post-MDGs (2000-2018) period. The result shows that there was an increase in production in pre MDGs period, although slightly from 1980 (0.1mt) to 1993(0.605mt) till sharp increase observed in 1995 (1.168mt) to 1999 (2.451mt), while there was

fluctuation in production in post MDGs period. The fluctuation was obvious in year 2007 from 3.462mt (2006) to 2.43mt (2007) and then a sudden rise (3.31mt) in 2008. The result also shows an average production of 0.5173mt and 3.30mt for pre and Post MDGs indicating an increase in production in post MDGs period over the pre-MDGs period.

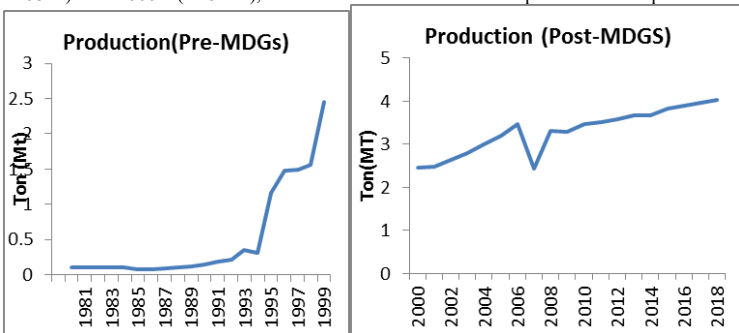


Fig. 1: Production (Million tons) of Sweet potato for Pre-MDGs (1980-1999) and Post MDGs (2000-2018)
Source: Basic data from FAO 2018 (FAOSTAT, June 2018, accessed April, 2020)

The result in Fig. 3 shows the productivity (t/ha) of Sweet potato for Pre-MDGs (1980-1999) and Post MDGs (2000-2018) periods. The result shows that there was a decline and fluctuation in yield of sweet potato over the years studied both for pre- and post-MDGs years. Findings have shown that the poor yield of root and tuber crops are often associated with nutrient- poor soil, lack of irrigation, and weak infrastructure (Spencer and Badiane, 1995). In sweet potato production, decrease in productivity has been attributed to quality of seed use, poor management and inadequate technical know-how etc (Okoye *et al.*, 2020). To harness the current potentials for sweet

potato production, its productivity must be improved and challenges eliminated. Agricultural productivity is one of the key determinants of impact of programme on livelihood of farmers. Low yield or productivity can discourage farmers towards commercialization and programme participation. Low yield results in low farm incomes, higher food prices, curbing inflation and low farm growths. Gollin *et al.*, (2002) noted that rising agricultural productivity leads to enhanced livelihood of farmers, hence structural transformation needed for developing economies to catch up with more advanced economies.

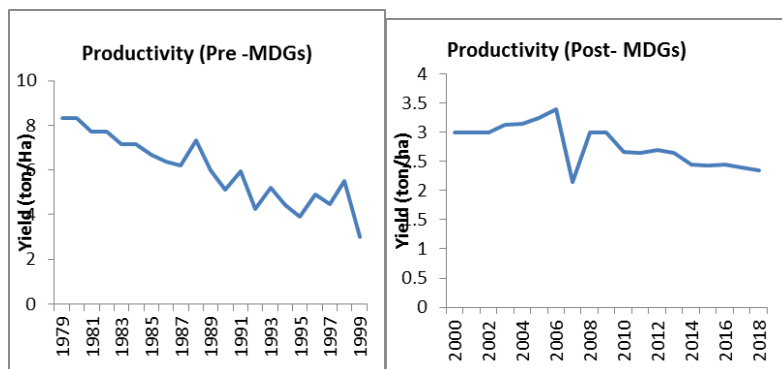


Fig. 3: productivity (t/ha) of Sweet potato for Pre-MDGs (1980-1999) and Post MDGs (2000-2018)
 Source: Basic data from FAO 2018 (FAOSTAT, June 2018, accessed April, 2020)

Growth in Area Cultivated, Production and Productivity of Sweet potato in Nigeria: Pre MDGs (1980-1999) and Post MDGs (2000-2018)

The result in Table 1 shows the estimated log linear functions in time variables for sweet potato area cultivated, production and productivity in Nigeria for pre- and post-MDGs periods. The finding indicates that the coefficients for trend variables of sweet potato area for pre- and post-MDGs periods were positive and highly significant at 1% level of probability each. This is an indication of increase in area cultivated in both periods over time. The coefficients for trend

variables of production also show a positive trend for both periods which were highly significant at 1% level each, indicating increase in production with time probably because of increase in area cultivated. The coefficients of productivity showed negative trends at pre- and post-MDGs periods and highly significant at 1% level each. This is an indication that there was a decline in yield over the two periods sampled. FAO (2013) noted that in terms of area and production in Nigeria, sweet potato production increased with time while yield decreased with time. This may probably be because increase in area cropped with sweet potato was accelerating while production decelerated.

Table 1: Estimated function for area, production and productivity of Sweet Potato in Nigeria: Pre (1980-1999) and post MDGs (2000-2018)

Variables	Constant (b ₀)	Time (b ₁)	R ²	F-value
Area				
Pre-MDGs	8.3663 (31.73)***	0.2186 (9.93)***	0.8457	98.66***
Post-MDGs	13.5513 (965.99)***	0.0431 (35.01)***	0.9863	1225.37***
Production				
Pre MDGs	10.5179 (41.29)***	0.1792 (8.43)***	0.7978	71.03***
Post MDGs	14.7229 (402.99)***	0.0267 (8.34)***	0.7920	69.52***
Productivity				
Pre- MDGs	2.1516 (35.40)***	-0.0394 (-7.77)***	0.7702	60.34***
Post-MDGs	1.1780 (27.24)***	-0.0163 (-4.31)***	0.5220	18.57***

Source: Basic data from FAO 2018 (FAOSTAT, June 2018, accessed April, 2020)

*** is statistically significant at 1%

The result in Table 2 showed computed growth rates of area, production and productivity for sweet potato in Nigeria, Pre- and Post-MDGs periods. The result showed a positive growth rate for area cultivated and production within the pre-MDGs period (24.43% and 19.62% respectively) and post-MDGs (4.40% and 2.71%). This implied that the area cultivated and production increased by 24.43% and 19.63% at the pre-MDGs period compared to 4.40% and 2.71% at the post-MDGs period respectively. This implies that

there is an increase in growth for area cultivated and production. However, a continuous rise in area and production is a sustainable source of growth on the long run. Increasing production and globalization could increase the productivity and may affect the agricultural growth adversely following Kalarlapudi (2010). Both periods recorded drops in growth rate of productivity by 3.86% within the pre-MDGs and 1.61% post-MDGs periods.

Table 2: Computed Growth Rate (%) of Area, Production and Productivity of Sweet Potato in Nigeria (Pre and Post MDGs Periods)

Period	Area	Production	Productivity
Pre-MDGs (1980-1999)	24.4333	19.6259	-3.8654
Post-MDGs (200-2018)	4.4042	2.7059	-1.6198

Source: Basic data from FAO 2018 (FAOSTAT, June 2018, accessed April, 2020)

Confirmation of Acceleration, Deceleration and Stagnation in Sweet Potato Area, Production and Productivity in Nigeria: Pre and Post-MDGs Periods

The estimated quadratic trend function equation for confirmation of acceleration, deceleration and stagnation in sweet potato area, production and productivity in Nigeria for pre- (1980-1999) and post-MDGs (2000-2018) periods are shown in Table 3. The

result shows a positive (0.0158) trend in growth within the pre MDGs period and significant at 1% level of probability, and a non significant but negative (-0.0007) trend within the post-MDGs for sweet potato area cultivated. This indicates that within the pre MDGs period, the trend was accelerating over time. At the post-MDGs period, the result shows a stagnation in growth for area cultivated.

The result also confirms a significant and positive (0.0157) growth trend for production (pre-MDGs period) and significant at 1% level and negative but not significant (-0.0007) trend for the post-MDGs period. This implies that the growth was accelerating over the pre-MDG period and stagnated within post-MDGs period. The coefficients for productivity trend within the pre- and post-MDGs periods were negative and not significant confirming stagnation in productivity over these periods.

Table 3: Estimated Trend Function Equation for Confirmation of Acceleration, Deceleration and Stagnation in Sweet Potato Area, Production and Productivity in Nigeria: Pre (1980-1999) and Post-MDGs (2000-2018) Periods

Variables	Constant (Bo)	B ₁	Time (b ₂)	R ²	F-value
Area					
Pre-MDGs	9.5857 (44.94)***	-0.1139 (-2.44)*	0.0158 (7.32)***	0.9628	220.19***
Post-MDGs	13.5493 (583.17)***	0.0437 (8.16)***	-0.00003 (-0.12)	0.9863	577.13***
Production					
Pre MDGs	11.7252 (62.04)***	-0.1500 (-3.62)**	0.0157(8.18)* **	0.9590	198.90***
Post MDGs	14.6841 (249.69)***	0.0397 (2.93)*	-0.0007 (-0.98)	0.8147	35.18***
Productivity					
Pre- MDGs	2.1395 (21.37)***	-0.0361 (-1.65)	-0.0002 (-0.15)	0.7706	28.55***
Post-MDGs	1.1349 (16.14)***	-0.0040 (-0.25)	-0.0006 (-0.78)	0.5397	9.38***

Source: Basic data from FAO 2018 (FAOSTAT, June 2018, accessed April, 2020)

*, ** and *** are statistically significant at 10%, 5% and 1% respectively

Impact of MDGs on Area, Production and Productivity of Sweet Potato in Nigeria

The result of the statistical tests for impact of MDGs on area, production and productivity of sweetpotato in Nigeria is shown in Table 4. The calculated chow's F statistic for impact of MDGs on area, production and productivity of sweetpotato were significant at 1% each. The result confirms presence of significant difference in area cultivated, production and productivity of sweetpotato, within the pre- and post-MDGs periods. The results on the test for homogeneity of slopes for pre- and post-MDGs periods show that the calculated Chow's F statistic was significant at 1% for area, production and productivity. The result confirms that MDGs intervention brought about

structural changes and shift in area, production and productivity of sweetpotato in Nigeria within the period. It also indicated that the production functions are factor-biased. The calculated chow's F statistic for the test of differences in intercept was significant at 5% for area cultivated and 1% for productivity. This confirmed heterogeneity of intercepts for area cultivated and productivity during pre-and post MDGs periods. The significant coefficients indicate impact of MDGs intervention in area and productivity within the period. Tables 1, 2 and 3 indicated negative trend, negative growth rate and stagnation for productivity for the two periods but with a significant impact (Table 4). This might be because the rate of productivity decreased within the two periods was not the same.

Table 4: Tests for Impact of MDGs on Area, Production and Productivity of Sweet Potato in Nigeria

	Classification	Error sum of square	d.f	F-cal
Area	Pre MDGs	5.7994	19	
	Post MDGs	0.0147	18	
	Pooled data	16.2619	38	
	Pooled data with dummy	15.2742	37	
	Test of impact			66.4881***
	Test for homogeneity of slope			60.2026***
	Test for difference in intercept			2.3926**
Production	Pre MDGs	5.4127	19	
	Post MDGs	0.0996	18	
	Pooled data	13.1978	38	
	Pooled data with dummy	12.6492	37	
	Test for impact			51.5871***
	Test for homogeneity of slope			47.9047***
	Test for difference in intercept			1.6047
Productivity	Pre MDGs	0.3082	19	
	Post MDGs	0.1394	18	
	Pooled data	0.6751	38	
	Pooled data with dummy	0.6109	37	
	Test for impact			18.8059***
	test for homogeneity of slope			13.4989***
	Test for difference in intercept			3.8884***

Source: Basic data from FAO 2018 (FAOSTAT, June 2018, accessed April, 2020)

*** and ** are statistically significant at 1% and 5% respectively

Conclusion

This study analyzed the impact of MDGs intervention on area, production and productivity of sweetpotato in Nigeria. The study estimated positive growth rate in area and production of sweetpotato in pre- and post-MDGs period and negative for productivity in both periods. The study result found that the trend variable of growth for area and production accelerated over timewithin the pre-MDGs period, and stagnant at post MDGs period for productivity. This indicates that rate of change in area was higher than the rate of change in

production and productivity over the period. Although there was a positive impact of MDGs in area, production and productivity over the periods studied, the study needs further research to determine the level and rate of impact for future projections. The study therefore calls for policy interventions to increase the productivity of sweetpotato in Nigeria by initiating programmes/projects that will give higher priority to broad based research for development especially if Sustainable Development Goals (SDGs) will be attainable

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ECONOMIC PERFORMANCE OF SWEET POTATO SEED PRODUCTION UNDER DIFFERENT SOIL FERTILITY MANAGEMENT TECHNIQUES IN UMUDIKE, NIGERIA

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Abstract

The study provides empirical analysis on economics of sweet potato seed production under different soil fertility management techniques in Western Farm of National Root Crop Research Institute (NRCRI), Umudike, Nigeria. The study employed real time costing procedures for data collection and cost and returns analytical model to determine the cost, revenue, profit and revenue-cost ratio for each of the soil fertility management techniques. Treatment 5 (T5) (N1864.1) had the highest total cost of production, followed by T7 (N1623.2) and T3 (N1559.7), while T4 (N4414.5), T2 (N4053.7) and T7 (N2288.37) generated the highest profit. The study estimated positive profit and revenue for all treatments in the experiment and returned N4.45, N4.23 and 2.67 for every N1.00 invested for treatment T4, T2 and T0 respectively. The result of the study showed positive and high margins (above 50%) for all the treatments. T4 (77%), T2 (76.3%) and T0 (62.48) had the highest net margin indicating percentage margins they were able to return. The study therefore recommends the use of treatment T4 (100% PM and opt. Zn) where Zinc can be accessible, otherwise T2 (100% PM) with second highest return on investment.

Key words: Treatments, Organic, Inorganic, Cost and Return analysis

Introduction

Several studies have been conducted to assess the effects of different soil fertility managements on soil properties and crop yields, and different agronomic and environmental outcomes have been observed depending on the specific agro ecosystem (Son, *et al.*, 2004; Havlin, *et al.*, 2005; Saha, *et al.*, 2008) with no economic impact of these technologies on farmers. With a target of improving the quality and quantity of seeds (planting materials), hence improving food shortage, maximum efforts have been directed towards crop yield. Under subsistence and even commercial farming, the yield of many root crops especially sweet potato is very low, while their genetic potential for increased yields has not yet been fully expressed. This has been traced to the use of virus infected planting material usually caused by unavailability of planting material at the onset of raining season (Okoye *et al.*, 2017) and poor soil management (Ogwuet *et al.*, 2019).

NOAN (2012) and FAO (2017) observed that soil management practices influence the farmer's level of output and indicated the practices used such as: agrochemicals, tillage system, soil nutrient mining, removal or loss of vegetative cover, continuous cropping and use of soil fertility materials. The study noted that when intensely practiced these practices may adversely affect crop production with level of the crop output decreasing. The use of these unfavorable soil management practices are referred as unsustainable. Scholars have identified many soil management practices for sustainable measures for different crops and little or none on its seeds. Ullah, *et*

al., (2008) identified that the highest yield was obtained for maize from the combined application of organic and inorganic sources of soil nutrients (ISFM). The study further explained that the highest vegetative growth was recorded from the use of the ISFM and the lowest vegetative growth with the use of only organic fertilizer (cow dung). Ogwo *et al.*, (2019) indicated that the application of organic and inorganic fertilizers solely or combined are found to have great influence on the vegetative growth and yield of the crops such as sweet potato seed.

Sweet potato seed refers to the vegetative part of the plant known as the vines. Presently, there is high demand for sweet potato (especially the Orange fleshed varieties) seed in Nigeria due to increased demand for human consumption (varieties rich in beta carotene for combating vitamin A deficiency), animal feed and industrial uses (Okoye *et al.*, 2017). For farmers to meet up with the increasing demand there is a need for availability, affordability and accessibility of sweet potato seed. A lot of investors, farmers and cooperative groups have been trained by relevant institutions (e.g. NRCRI) on the process of conservation, multiplication and of sweet potato seed as a business to meet the increasing demand. For farmers to remain and sustain in the business, there is a need to develop technologies for soil fertility managements for maximum profit at least cost, accessible and sustainable. Therefore, this paper analyzed the economics of sweet potato seed production using different soil fertility management techniques and made recommendations to improve sweet potato seed production.

Methodology

Location: The experiment was carried out at the Western Farm of National Root Crops Research Institute, Umudike, Abia State Nigeria. Umudike is a humid rainforest region located at 05°29'09.1"N, 007°32'29.5"E, with temperature of 27.08°C and mean rainfall of 2524mm.

Materials: UMUSPO4 (Sologold-Orange fleshed sweetpotato) was purposively used for the study because it is the most recent released variety with high demand from the farmers. Net tunnel was constructed and used for seed production.

Plot Size: 1 x 1.5m replicated 3 times for each treatment giving a total of 24 plots.

Treatments: the following treatments were adopted for the study

- T0= No fertilization (absolute control)
- T1= 100% N from inorganic source (Urea) applied alone
- T2= 100% N from organic source (poultry manure) applied alone
- T3= 100% N from inorganic source + optimum level of Zn
- T4= 100% N from organic source + optimum level of Zn
- T5= 50% N from inorganic source and 50% N from organic source
- T6= 75% N from inorganic source + 25% N from organic source+ opt level of Zn
- T7= 75% N from organic source + 25% N from inorganic source + opt. level of Zn

Data Collection: Cost Route Approach was used to collect real time data for all the activities carried out during the experiment. Data collected include:

- (a) Labour cost: time taken to do each activity e.g. time it takes to plant, apply fertilizer etc.
- (b) Consumable cost: real cost of items used within the four month production period e.g. cost of fertilizer, poultry manure, planting materials etc.
- (c) Fixed cost: depreciated cost of items used for more than 4 months e.g. cost of net tunnel constructed.
- (d) Output: the yield of the vine (bundles)

Analytical Technique: Cost and Return Analysis (CRA) was used to estimate the cost and returns of production of different soil fertility techniques.

Labour Cost (LC)= time spent per activity(MD) x Amount/MD

Consumable Cost (CC) =quantity used x amount bought

Fixed Cost (FC) = (Amount purchased fixed item/Salvage value)/ four Months

Profit= Total Cost – Revenue

Total Cost = LC+CC+FC

Revenue = Output (bundle)x amount sold (Naira)

Revenue-Cost Ratio (RCR) = Revenue/Total Cost

Results and Discussion

Labour cost

The result in Table 1 shows that labour cost associated with production of sweetpotato seed under different soil treatments. The result shows that treatments T5 (N1146.2) had the highest labour cost per plot, followed by T7 (N912.82) and T3 (N821.14), while T1 (N446.15) had the least cost of labour, followed by T0 (N5570.9) and T2 (N557.09). Against expectation, the results show that the labour cost for T0 was higher than T1 probably due to cost associated with harvesting and packing of vines as shown in the Table. However, the high cost labour found in the study was in agreement with the previous studies that labour cost contributes up to 60% of total cost of production following Okoye *et al.*, (2017).

Table 1: Labour Cost Associated with Production of Sweetpotato (Solo Gold) Vines under Different Soil Treatments

ITEMS	T0	T1	T2	T3	T4	T5	T6	T7
A= LABOUR (N)								
Construction of Net tunnel	208	208	208	208	208	208	208	208
Land preparation	52.08	52.08	52.08	52.08	52.08	52.08	52.08	52.08
Herbicides application	0.651	0.651	0.651	0.651	0.651	0.651	0.651	0.651
Manure application	0	0	41.67	0	41.67	41.67	41.67	41.67
Preparation of vine	39.06	39.06	39.06	39.06	39.06	39.06	39.06	39.06
Planting	15.63	15.63	15.63	15.63	15.63	15.63	15.63	15.63
N and Zn application	0	39.06	0	39.06	39.06	39.06	39.06	39.06
Harvesting/packing	241.67	91.67	200	466.67	183.33	750	333.33	516.67
Total Labour	557.09	446.15	557.09	821.14	579.48	1146.2	729.48	912.82
Rank	6 th	7 th	6 th	3 rd	5 th	1 st	4 th	2 nd

Field experiment, 2019 Plot size: 1.5m²

Consumable cost

The result in Table 2 shows the consumable cost associated with production of sweetpotato seed under different soil treatments per plot. The result shows that treatments T3 (N408.82) had the highest cost of consumable items, followed by T1 (N399.44) and T6

(N399.44). Other consumable cost for treatments include: T5 (N388.25), T7 (N380.7), T4 (N371.32), T2 (369.5) and T0 (354.50). The lowest cost recorded for T0 is expected as it was an absolute control with no treatment application.

Table 2: Consumable Cost associated with Production of Sweetpotato Seed under different Soil Treatments using Net Tunnel

B =Consumables	T0	T1	T2	T3	T4	T5	T6	T7
Vine (cuttings)	225	225	225	225	225	225	225	225
Premextra +Round up (ml)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Manure (kg)	0	0	15	0	15	7.5	3.75	11.25
Urea(kg)	0	52.5	0	52.5	0	26.25	39.37	13.13
Zinc(kg)	0	0	0	1.82	1.82	0	1.82	1.82
Twine(roll)	75	75	75	75	75	75	75	75
Labels(pcs)	50	50	50	50	50	50	50	50
Total Consumable	354.5	407	369.5	408.82	371.32	388.25	399.44	380.7
Ranking	8 th	2 nd	7 th	1 st	6 th	4 th	3 rd	5 th

Field experiment, 2019 Plot size: 1.5m²

Fixed cost

The result in Table 3 shows the fixed cost associated with production of Sweetpotato seed using Net Tunnel per plot of 1.5m². The result shows a fixed price for all

the treatment at N329.71. The equipment (fixed cost items) used were depreciated within four months production cycle for sweetpotato vine production.

Table 3: Fixed Cost Associated with Production of Sweetpotato (Solo Gold) Vine under Different Soil Treatments

C=Fixed Cost	T0	T1	T2	T3	T4	T5	T6	T7
Weighing scale	13.889	13.889	13.889	13.889	13.889	13.889	13.889	13.889
Scissors	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Pliers	13.88	13.88	13.88	13.88	13.88	13.88	13.88	13.88
Binding wire	2.77	2.77	2.77	2.77	2.77	2.77	2.77	2.77
Net tunnel	286.67	286.67	286.67	286.67	286.67	286.67	286.67	286.67
Total FC	329.71	329.71	329.71	329.71	329.71	329.71	329.71	329.71

Field experiment, 2019 Note: all cost was depreciated for 4months Plot size: 1.5m²

Total cost

The result in Table 4 shows the total cost associated with production of sweetpotato seed under different soil fertility management techniques. The result shows that T5, T7, T3, T6, T4, T2, T0 and T1 had total production cost of N1864.1, N1623.2, N1559.7,

N1458.6, N1280.5, N1256.29, N1241.29 and N1182.85 and ranked 1st, 2nd, 2nd, 3rd, 4th, 5th, 6th, 7th, and 8th respectively. The highest cost from T5 is mainly associated with the high cost of labour (from cost of harvesting). The result also shows that total cost of production for T0 was higher than that of T1.

Table 4: Total Cost associated with Production Sweetpotato (Solo Gold) Vine under Different Soil Treatments using Net Tunnel

Items	T0	T1	T2	T3	T4	T5	T6	T7
Labour cost	557.09	446.15	557.09	821.14	579.48	1146.2	729.48	912.82
Consumables	354.5	407	369.5	408.82	371.32	388.25	399.44	380.70
Fixed cost	329.71	329.71	329.71	329.71	329.71	329.71	329.71	329.71
Total Cost	1241.29	1182.85	1256.29	1559.7	1280.5	1864.1	1458.6	1623.2
Ranking	7 th	8 th	6 th	3 rd	5 th	1 st	4 th	2 nd

Field Experiment, 2019 Plot size: 1.5m²

The result in Figure 1 shows the distribution according to Total Cost of production. The result shows that about 50.43% of the total cost were accounted as labour cost, while 26.43% and 23.14% were consumable and fixed costs respectively. Results show that labour had the highest cost incurred for all the different soil fertility management systems. This is in

accordance with Olayide and Heady (1999) who noted that labour accounted for the highest cost item in small scale agricultural production. The high percentage of fixed cost (26.43%) unlike other production enterprises was as a result of short production cycle of the sweet potato system of 4 months.

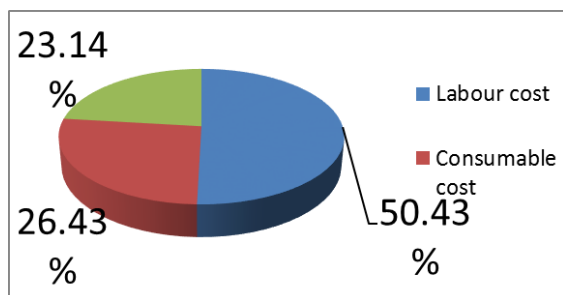


Figure1: Percentage Distribution according to Cost Incurred in Sweet potato Seed production under Net tunnel

The result in Table 5 shows the economic analysis of sweetpotato seed production under different soil treatments. The result shows an average output/plot generated from each treatment as shown in the Table 5. Treatment T4 had the highest revenue (N5695) and profit (N4414.5) compared to other treatments in the experiment. Treatment T2 and T7 estimated profit of N4053.71 and N2301.58 respectively, while T6, T0, T3, T5 and T1 had profit of N2114.7, N2067.04, N1901.16, N1857.56 and N1702.15 and ranked 4th, 5th, 6th, 7th and 8th respectively. Hence, in line with the findings, Ogwo *et al.*, (2019) indicated that the application of organic and inorganic fertilizers solely

or combined are found to have great influence on the vegetative growth and yield of the crops such as sweet potato seed.

The result also shows that for any N1 invested in sweet potato seed production, N4.45 and N4.23 revenue was generated using T4 (100% + opt. Zn) and T2 (100% PM only) respectively (also shown in Fig. 2). T0 ranked 3rd by returning N2.67 for every N1.00 invested. This indicates that any treatment above T0 (no application) was viable and can be recommended while treatments below T0 are not viable, and thus be regarded as economic waste.

Table 5: Economic analysis of production of Sweetpotato (Solo Gold) Vine under Different Soil Treatments using Net Tunnel

Items	T0	T1	T2	T3	T4	T5	T6	T7
Total Cost (TC)	1241.29	1182.85	1256.29	1507.2	1280.5	1864.1	1458.6	1610.0
Output (O)	6.62	5.77	10.62	6.82	11.39	7.45	7.15	7.82
Amount Sold	500	500	500	500	500	500	500	500
Revenue	3308.33	2885	5310	3408.33	5695	3721.67	3573.33	3911.67
PROFIT = R-TC	2067.04	1702.15	4053.71	1901.16	4414.5	1857.56	2114.71	2301.58
Revenue-Cost Ratio	2.67:1	2.44:1	4.23:1	2.26:1	4.45:1	1.99:1	2.45:1	2.429:1
Rank	3 rd	5 th	2 nd	7 th	1 st	8 th	4 th	6 th

Field Experiment, 2019 Plot size: 1.5m²

Conclusion

The study provides empirical analysis on economics of sweetpotato seed production by the use of different soil fertility management techniques in NRCRI, Umudike, Nigeria. Although all the treatments had positive profit and revenue, the study therefore

recommends the use of treatment T4 (100% PM and opt. Zn) and T2 (100% PM) with highest return on investment and discourage the use of any treatment below RCR of T0. Any treatment with RCR less than that of T0 is unviable, and thus regarded as economic waste.

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STRUCTURAL EVALUATION AND PERFORMANCE OF SEED YAM MARKET IN NIGERIA.

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Abstract

The structure and conduct of the seed yam market was analysed in three states, which were the major markets where seed yams are sold in Nigeria. A multi stage sampling procedure was used to select 150 marketers (30 wholesalers and 20 retailers) from the states. The data collected were analysed using descriptive statistics, market structure and conduct approach. The result of analysis on the market structure shows that the market was not perfectly competitive with a Gini coefficient of 0.41 and 0.48 for seed yam wholesalers and retailers respectively. Analysis of barrier to entry into seed yam market indicates that union registration and payment of toll were the major entry barrier into seed yam market for most traders. As regards to the conduct of seed yam market, the study finds that seed yams are transported from point of purchase to place where it will be sold by a commercial transporters. The major information source used by the marketers are obtained from their fellow traders and about 88% of the traders finance their own business. This implies that the seed yam traders have limited access to credit to support their business. However, there is need to increase market concentration among seed yam retailers as well as wholesaler. Access to credit for seed yam traders could be enhanced and easy entry into the market is needed. This can be done through reduction in toll payment and other unnecessary levies.

Keywords: Market structure, conduct, seed yam and Gini coefficient

Introduction

Yam production is an important agricultural activity in sub Saharan Africa as it supplies more than 95% of the world production (FAO, 2019). In Nigeria, yam is among the main staple food crops that is widely consumed with high in nutrient value. It has significant quantity of protein that is much more than the commonly grown cassava (Asiedu and Sartie, 2010). Hence, yam tremendously contributes to the food security and income generation for millions of producers, distributor, processors and consumers in the regions where it is produced. More so yam plays an important role in the sociocultural life of most tribes in Nigeria.

The production of seed yam as an integral part of ware yam production is widely practiced throughout the yam belts of Nigeria. There are several approaches through which seed yams are produced, which can be group into traditional and non-traditional methods (Kalu *et al* 2019). In traditional method, farmers do set aside about 25 to 30 percent of the harvested tubers as seeds for the next planting season or at the end of first six months of planting, yams are milked and left in the soil to produce sets which would be used for planting. The low rate of multiplication and the use of the edible tubers for propagation makes seed yam very expensive that about 50 - 70% of production costs is spent on purchase of seed yam (Asumugha, 2007). Expansion and increasing intensification of yam cultivation have raised the need for ensuring a sustainable availability of high quality seed yam on a commercially viable basis in yam growing areas.

Although seed yam is produced in every part of the

country, the major seed yam markets can be found in north central and south east part of Nigeria. The country depends greatly on these regions to move surplus planting materials to other parts where seed yam may be insufficient. A well-established marketing system will argument farmers' production efforts and assist them to achieve their cherished goals by making available time, place, form and possession utilities (Offor *et al.*, 2016).

However, the production of seed yam tubers in Nigeria are in the hands of small-scale farmers who operates in a highly competitive market. It is therefore imperative for countries such as Nigeria to put in place appropriate policies to boost production and distribution of planting material especially, traditional crops which have long been advertised as food security crops such as yam through institutionalizing formal market structure. Anuebunwa, 2002 defined market structure as the number and size distribution of buyers and sellers, product differentiation and presence or absence of barriers to entry into the market. It is the characteristics of markets that influence the behaviour and performance of firms/farms that sell. The use of perfect competition market model is widely acknowledged as a tool for evaluating the structure and conduct of a market. The presence of large numbers of buyers and sellers, product uniformity, low barrier to entry and a better understanding of alternative choices available to producer and consumer characterize the competitive market model. The information gained on market structure can give insight about market competitiveness. Market structure can also be analysed using the degree of market concentration,

mode of entry into the market, the extent of product differentiation and vertical and horizontal integration.

Individuals in the market are required to conduct themselves in line with the conditions guiding where they buy or sale their product. These choice of conduct is known as market behaviour. There are different approaches that participants who operate in the market use, and these approaches depend on the structure of the market and the individual power of the traders in the market. Chogou et al (2019) opined that components of market behaviour include: transportation, storage, information on purchases and sales conditions and financing.

Most studies on seed yam marketing focus on supply and constraints experienced by farmers (Asumugha, 2007). This study analysed the structure and conduct of some selected seed yam markets in Nigeria, with a view to analyse the socio economic characterises of the respondents, analyse the mode of entrance into the market, identify the product differentiation and analyse the market concentration among seed yam traders.

Methodology

A multi stage sampling procedure comprising of purposive and random techniques were used for the study. The study was purposively carried out in major seed yam markets located at Edo, Delta and Anambra state, Nigeria. One seed yam market was selected from each state, these three markets surveyed were known for selling of seed yams. In the second stage fifty marketers were randomly selected from each market comprising of 30 wholesalers and 20 retailers, making a total of 150 respondents. Data were collected with the use of structured questionnaire. About 149 questionnaire were retrieved and used for result analysis.

Analytical procedure

Data collected were analysed using descriptive statistics such as percentages, frequencies, means, tables and Gini coefficient.

The Gini coefficient (G) was used to determine the inequality in market share among the seed yam marketers.

The model is expressed following Anueunwa, 2002 as:

$$G = \sum_k (X_k - 1 - Y_k - X_{k,yk-1}) * 1/100$$

Where,

X₁ = percentage of seed yam traders in the *i_{th}* class of traders

Y₁ = cumulative percentage of seed yam traders in the *i_{th}* class of traders

K = number of class

The Gini coefficient varies from 0 to 1, where 0 implies perfect equality in the distribution (perfect market) and 1 implies perfect inequality (imperfect market, high level of seller concentration)

Result and Discussion

Socio –Economic Characteristics of the Respondents

The result of socio-economic characteristics of the seed yam marketers in the study area is presented in table 1. The result shows that 50.34% and 49.66% constitute the female and male marketers in the study area respectively. This implies that both gender are well represented in the seed yam business. We further deduced from the result that about 45.64% of the marketers are within the age bracket of between 35-50 years, while about 42.28% are below 60 years. This implies that many of the respondents are within their productive age. Respondents of within this age bracket are risk averse and can initiate good business ideas, and will also have strength to move from one location to the other. More so, greater percentage of the marketers are married (90.60) with about 55% having a household size of about 5-7 persons. The size of household of seed yam marketer can reflect availability of family labour which could promote seed yam business.

The result from the table also shows that about 82 % of the traders were into full time trading as either wholesalers or retailers. Majority (71.82%) of the respondents have between 10-29 years of marketing experience in seed yam marketing while those between 40-50 years of experience are only 2.01%. One could infer from the result that more experienced seed yam marketers will better manage their business and similar result was gotten by Okwuokenye and Onemolease, (2011).

Market Structure

In analysing market structure four major indicators of competition were used namely; mode of entrance into the market, barrier to entry, product differentiations, market concentration and market share.

Mode of entrance into the market

The result in Table 2 shows that, about 54.44% and 33.33% -of the wholesalers and retailers respectively of the respondents gives positive response to free entrance into the market while about 45.56% of the wholesalers and 66.67% of retailers indicated that there were barriers to the entry into the market. This implies that the market was assumed not to be perfect since the entrance into the market was not free.

The result from table 2 also shows that about 44.94% and 36.67% of wholesalers and retailers respectively indicated that payment for toll act as a barrier to entry into the market. The greatest barrier observed by both the wholesalers and retailers was registration with the union, about 55% of wholesaler and 63% of retailers' indicated not registering with the union can prevent one from selling in the market. It was also observed

that seed yam was the only good sold in the market and as such only those that trades on seed yam are registered in the union.

Product Differentiation

The seed yams sold at the markets surveyed were mainly landraces, most of the improved varieties were not observed. The seed yams in the market under study were differentiated based on their varieties and grade (size) of the seed yam. The heap of 400 seed yam are kept according to their varieties. The varieties identified in their local names include Ekpe, Adaka, Abii, Alumaco, and Obiaoturugo. Ekpe was identified as the variety that has highest demand because it matures early. The seed yams that fall under grade one weighs more than 1Kg while the once under grade two and three are below 1Kg. The information gotten from the sellers confirms that their customers could identify the various seed yam types in the market, the size and buyers often based their choices on these factors. Product differentiation do a times result in price differential for some product that are less preferred. This is a problem because it is a deviation from norms of a perfect market.

Market concentration

Market concentration explains the size distribution of sales or income by different categories of traders in the market. This usually analysed using market share, Gini Coefficient and Lorenz curve.

Market Share

This is stated in terms of sales or income generated, the market share for the wholesalers and retailers are shown in Table 3. Equal shares among distribution of income/sales among the market operator means that the market is perfectly competitive and no particular sector dominates the sales of a particular product, but if is otherwise it means that the market is imperfect following Olukosi and Isitor, (2005).

Variable	Frequency	percentage
Gender		
Female	75	50.34
Male	74	49.66
Age		
< 40	18	12.08
41-50	50	33.56
51-60	63	42.28
61-70	14	9.40
71-80	3	2.01
81-90	1	0.67
Marital status		
Married	135	90.60
Single	5	3.36
Divorced	4	2.68
widowed	5	3.36
Household size		
2-4	14	9.40
5-7	86	57.72
8-10	43	28.86
>11	6	4.03
Mode of operation		
Full time	123	82.55
Part time	26	17.45
Marketing experience		
<10	12	8.03
10-19	58	38.93
20-29	49	32.89
30-39	27	18.12
40-49	3	2.01
Total	149	100
Source of capital		
Personal savings		88
Borrowed capital		12

Source: Field survey 2020.

Table 1: Distribution of respondents on socio economic characteristics of seed yam Marketers (N=149)

Table 2 Distribution of markets according to mode of entrance in the market and Barriers to Entry into the Market.

.Mode of entrance	Wholesalers'		retailers	
	Frequency	Percentage	Frequency	Percentage
Free	49	54.44	20	33.33
Not free	41	45.56	40	66.67
Total	90	100	60	100
Barriers				
	Frequency	Percentages	Frequency	Percentage
Toll payment	40	44.94	22	36.67
registration	49	55.06	38	63.33
Total	89	100	60	100

Source Field Survey Data 2020.

Table 3 market share for wholesalers and retailers				Wholesalers	234,792	0.56	59.29
Institution	Income (N)	Market share	percentage	Retailers	161,258	0.41	40.71
				Grand total	396,055		

Source Field Survey Data 2020.

The result in Table 3 shows that the wholesalers' value of sales (income) is greater than the retailers' income. This implies that the wholesalers control the market with a 56% of sale proportion while 41 % is being controlled by the retailers. More so, this also indicates that the seed yam market under study was imperfect and this could probably be as a result of the financial limitations faced by the retailers which may affect the expansion of their business.

The market share of seed yam wholesalers and retailers as computed with Gini coefficient is shown in Tables 4 and 5 respectively. The result from the tables show that Gini coefficient of 0.41 and 0.47 were gotten from seed yam wholesalers and retailers respectively. This implies that the concentration of sales among them. It could also be deduced that that wholesalers or retailers of seed yam does not have control over market price, which reflects a competitive market structure. Thus, the market was competitive but not perfectly competitive since the coefficients were far from zero.

The market share with Gini Coefficient

Table4: percentage distribution of seed yam wholesalers by average size and total value of monthly sales

Range of sales	Frequency	% of wholesalers (x _i)	Cum% of wholesalers	Total value of monthly sales	% of total sales	Cum. % (y _s) sales	X _i Y _i
100,000	25	28.09	28.09	1790000	8.57	8.57	0.02
101,000-200,000	22	24.72	52.81	3491000	16.71	25.28	0.06
201,000-300,000	33	37.08	89.89	8851000	42.36	67.64	0.25
301,000-400,000	7	7.87	97.76	2565000	12.27	79.91	0.06
501,000-600,000							
701,000-800,000							
801,000-900,000							
901,000-1000,000	1	1.12	98.88	1000000	4.79	84.7	0.009
1,000,0000	1	1.12	100	3200000	15.31	100.01	0.011
Total	89	100					0.410
Mean	234797.75			20897000			

Source Field Survey Data 2020.

Table 5: percentage distribution of seed yam retailers by average size and total value of monthly sales

Range of sales	Frequency	% of Retailers (x _i)	Cum% of Retailers	Total value of Retailers	% of total sales	Cum. % (y _s) sales	X _i Y _i
1-100,000	26	43.33	43.33	1,799,500	18.60	18.60	0.081
101,000-200,000	30	50	93.33	4,796,000	49.57	68.17	0.341
201,000-300,000	1	1.67	95	400,000	4.13	72.30	0.012
301,000-400,000	1	1.67	96.67	480,000	4.96	77.26	0.013
501,000-600,000							
701,000-800,000							
801,000-900,000							
901,000-1000,000	1	1.67	98.34	1,000,000	10.34	87.6	0.015
1,000,0000	1	1.67	100	1,200,000	12.40	100	0,017
Total	60			9,675,500			0.479

Source Field Survey Data 2020.

Market behaviour

The study observed that most traders engage the services of commercial drivers to move Seed yams from place of purchase to the point of sale. The majority (81.00%) of traders complained of high cost of transport which weighs heavily on their transaction cost and this in line with the findings of (Offor *et al.*, 2016) who identified high transportation cost as one of the constraints experienced by yam traders in Abia state.

allowed to store their commodities in the rented shops while non-members display their seed yams under the shades outside the stalls. Lack of storage space reduces the bargaining power for non-member who are compelled to sale the goods as they brought them to market or take them back home, thus increasing competitiveness in seed yam business (Asumugha, 2007; Anyaegbulam, 2012). Several information sources were identified, majority of the markets about 80 % sources information from follow traders. This implies that since common interest was shared among the trader, there will be greater reliability on

In the markets, the traders registered with the union are

information exchanged among them. The result from table 1 shows that most traders (88%) finance their own business. This implies that the seed yam traders has limited access to credit to support their business. The complicated process required to access credit from microfinance structures and high interest rate were among the major constraints to credit accessibility

Conclusion and Recommendation

The degree of competition in seed yam market was evaluated by gaining better knowledge of how the market is being organized. The result from this study shows that the seed yam markets under study were not perfect market. Some degrees of seller concentration exist in the seed yam market with greater concentration in the wholesale market. Policy should be focused on reducing the illegal levies required from the marketers.

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ANALYSIS OF WATERMELON MARKETING IN ABIA STATE, NIGERIA

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ABSTRACT

This study analyzed watermelon marketing in Abia state Nigeria. Its specific objectives were to: ascertain the viability of watermelon marketing and its marketing efficiency in the study area; analyze determinants of marketing efficiency and constraints to watermelon marketing in the State. Multi stage sampling techniques were adopted in selecting zones, local government areas, communities and marketers. The sample size for the study was seventy-two watermelon marketers. The primary data was used and information was collected with the aid of a well-structured questionnaire administered on the respondents by the researcher and the trained enumerators from the study area. Descriptive and inferential statistical tools were used in analyzing data. Results showed that watermelon marketing was a viable business in the area with a gross margin of ₦722.327 and marketing efficiency of 60.92%. Gender (5.0%) and age (1.0%) were the negative determinants of water melon marking efficiency, while marital status (5.0%), educational qualification (1.0%) and access to credit (1.0%) were the positive determinants. Lack of storage facilities (x =4.74), perishability of the produce (x =4.78), irregular supply of watermelon (x= 4.36), among others posed serious constraint to the marketing. Thus, it was recommended that the marketers association in the study area should device means of ameliorating the numerous marketing constraints to the business in order to encourage new entrants and its consequent expansion.

Keywords: Watermelon, Marketing, Watermelon Marketing

INTRODUCTION

Fruit vegetables are edible plant products that are good for health. They are nutritionally important to man as they provide the much needed vitamin, minerals and fibre which are the important components of diet in human nutrition as sources of adequate energy, nutrients and vitamins where they play key roles in neutralizing the acids produced during food digestion and helps in reducing constipation (Ebiwoei, 2013)). In Nigeria, the production and consumption level of these exotic fruit vegetables namely: water melon, sweet melon, cucumber cabbage and pumpkin is gradually increasing annually owing to greater appreciation of their food values and crops that bring in the needed income for improved livelihood (Oguntola, 2006; Adamu *et al.*, 2013).

Watermelon (*Citrullus lanatus*) is a member of the cucurbit family (Cucurbitaceae) and is one of the most important and the most widely cultivated fruits crops grown in the tropics and in the world at large (Odinwa *et al.*, 2015; Oladele, 2015). It is consumed worldwide because of its numerous nutritional benefits (Onyemauwa, 2010). Watermelon is mainly eaten raw when it is fresh after being washed and sliced into bits. It is highly relished as a fresh fruit because of its thirst-quenching attribute in addition to many other identified nutritional values and advantages such as its preferable nutritional values to its consumers. It supplies the body with low calories, lycopene which is an antioxidant that prevents cancer and other diseases; vitamin A, vitamin C, protein, carbohydrate, fibre, potassium, calcium, iron, fats and up to 92 mills of

water (Adamu *et al.*, 2013). Its consumption in the recent times has witnessed remarkable development and this has boasted the business.

The potentials of watermelon as a cash generating crop is significant especially for small scale entrepreneurs in Abia State. The commodity is mainly distributed to Abia state in bulk mostly by Hausa traders from the northern part of Nigeria who sale to the wholesalers in the state, who then resale to the retailers and the small scale entrepreneurs who includes mainly traders from the northern part of Nigeria as well as some Abians that sale either in balls or in slices to consumers. There has been increased trade and commerce activities surrounding the commodity in Abia State, thus, Ekerete (2014) stated that efficient marketing of watermelon is important to ensure its all-year round availability due to its increasing demand by its consumers.

Despite the nutritional, commercial values and increasing demand of water melon, its production is low in Nigeria (Adamu *et al.*, 2013) and is a recent phenomenon in Abia state as the crop was believed to thrive well only in the northern part of the country (Oladele, 2015). Since the edaphic and climatic factors in the state are not favorable for the growth of the fruit crop, this has adverse impact on its marketing. Incidentally, fruit crops marketing in Nigeria is ineffective and inefficient due to inadequate infrastructures and social amenities such as transportation facilities, communication system, good storage facilities and good pricing systems (FAO,

2003). Ibok (2012) and Ekerete (2014) further attested that the marketing system of watermelon in Uyo Metropolis of Akwa Ibom State is very inefficient due to certain constraints such as losses resulting from fruits spoilage, lack of preservation facilities, high cost of transportation of fruits and lack of credit facilities, to mention but a few.

Furthermore, the fruit is bulky and perishable if not timely stored or consumed. It is likely to attract more cost of transportation and storage since it is to be imported from outside Abia state.

Objectives of the Study

The broad objective of the study was to analyze watermelon marketing in Abia State, Nigeria. The specific objectives are to:

- ascertain the viability of watermelon marketing in the study area,
- determine marketing efficiency of watermelon,
- analyze determinants of marketing efficiency of watermelon in the state, and
- describe constraints to watermelon marketing in the Abia state.

Hypotheses Tested

The following hypotheses in their null forms were tested for the study:

- Ho₁: Watermelon marketing is not viable
- Ho₂: Watermelon marketing is not efficient
- Ho₃: Gender, age, marital status, household size, educational qualification, Years of business, access to credit and selling price do not determine watermelon marketing efficiency.

METHODOLOGY

The study was conducted in Abia state Nigeria. Abia is in south eastern Nigeria. It is located within latitudes 4° 45' and 6° 07' North, and longitudes 7° 10' and 8° 10' East. It is bounded on the north and north east by the states of Anambra, Enugu and Ebonyi. To the west is Imo State, to the east and south east are Cross River and Akwa Ibom States, and to the south is Rivers State (Independent National Electoral Commission, 2008). The State has three agricultural zones and seventeen local government areas. Abians are predominantly farmers and traders as well. Both Abia indigenes and non-indigenes are seen participating in watermelon marketing in the State, and the goods are transported mostly from the northern parts of Nigeria where they are produced to urban areas and markets as well as the local markets.

Multi stage sampling technique was adopted for this study. In the first stage, two agricultural zones, namely: Aba Zone, Umuahia Zone, in Abia state were selected out of the three agricultural zones. In the second stage, purposive selection of one local government from each of the two selected agricultural zones was made. The essence of the purposive selection was to ensure the selection of areas where

watermelon business is predominant. The third stage involved the random selection of two communities from each of the two local governments making a total of four communities. The fourth stage involved the random selection of three villages from each of the four communities, while the last stage entailed the random selection of six watermelon retailers from each villages to give a sample size of seventy-two watermelon marketers for the study.

One source of data was used for this study which is the primary data. The primary information was collected with the aid of well-structured questionnaire administered on the 72 respondents by the researcher and the trained enumerators from the study area.

Descriptive and inferential statistical tools were used in data analysis. To ascertain the economic viability of watermelon marketing in the study area, gross margin analysis was used. Efficiency index was used to determine marketing efficiency of watermelon. Multiple regression analysis was employed in analyzing determinants of marketing efficiency of watermelon, while mean score of 5 points likert scale was adopted in describing constraints to watermelon marketing

Models Specifications

Gross margin

Gross margin analysis used was as follows:

$$GM = TR - TVC$$

Where:

GM = Gross margin

TR = Total Revenue

TVC = Total Variable Cost

Efficiency Index

$$\text{Marketing Efficiency, ME} = \frac{\text{Gross Marketing Margin}}{\text{Marketing Costs}} \times 100$$

Adopted from the works of Yusuf *et al.* (2016).

Multiple Regression Model

The model is explicitly specified thus:

$$\text{Linear, } Y = a + b_1X_1 + b_2X_2 + b_3X_3 + \dots + b_nX_n + e$$

$$\text{Double-log, } L_n Y = L_n a + L_n b_1 X_1 + L_n b_2 X_2 + L_n b_3 X_3 + \dots + L_n b_n X_n + L_n e$$

$$\text{Semi-Log, } L_n Y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + \dots + b_n X_n + e$$

$$\text{Exponential, } Y = L_n a + L_n b_1 X_1 + L_n b_2 X_2 + L_n b_3 X_3 + \dots + L_n b_n X_n + L_n e$$

Where :

Y = Watermelon marketing efficiency (%)

X₁ = Gender (female =0, male =1)

X₂ = Age (years)

X₃ = Marital Status (married =1, others =0)

X₄ = Household size (Number of persons)

X₅ = Educational qualification (years)
 X₆ = Years of business (years)
 X₇ = Access to credit (amount accessed in Naira)
 X₈ = Selling price (Naira)
 e = stochastic variable.

RESULTS AND DISCUSSION

Viability of Watermelon Marketing in Abia State, Nigeria

Table 1 showed the result of gross margin analysis on the viability of watermelon marketing in Abia State. The result showed a total variable cost of ₦1,185,773 and a total revenue ₦1,908,100.00. The gross margin was ₦722,327.00 indicating that the business was a viable one. This finding is in tandem with Oladejo (2015) that marketing of fruits was a viable business Nigeria. Adinya *et al.* (2018) equally discovered that fruits marketing was viable.

Table 1. Gross Margin Analysis on Viability of Watermelon Marketing in the Study Area

Variables	Average		Total Amount (Naira)
	Average Quantity	Unit Price (Naira)	
Variable Costs			
Transportation			144,080
Loading			193,603
Off-loading			168,490
Market Charges		100	17,200
Cost of Fruits	3312	200	662,400
Total Variable Costs			1,185,773
Marketing Revenue			
Sales of Whole Fruit	2006	500	1,003,000
Sales of Sliced Fruits	1293	(100x7)	905,100
Total Marketing Revenue			1,908,100
Gross Margin			722,327

Source: Field Survey; 2018.

Marketing Efficiency of Watermelon in the Study Area

Table 2 showed the marketing efficiency of watermelon in the study area. The result revealed that total variable costs were ₦1,185,773 while the total

revenues were ₦1,908,100. The marketing efficiency was 60.92%, confirming that the business of watermelon was efficient. The finding agreed with Oladejo (2015) who suggested that fruit crops marketing was profitable and efficient.

Table 2. Presentation Marketing Efficiency of Watermelon in the Study Area

Items	Amount (Naira)
Total Revenues	1,908,100
Gross Margin	722,327
Total Variable Cost	1,185,773
Marketing Efficiency	(722,327/1,185,773) X 100/1 = 60.92%

Source: Field Survey; 2018.

Determinants of Marketing Efficiency of Watermelon in the Study Area

Table 3 showed the multiple regression estimate on the determinants of marketing efficiency of watermelon in the study area. The semi-log functional form appeared to be the lead equation based on the values of F-ratio (12.365), R² (0.743) and R⁻² (0.641). The coefficient of the multiple determination (R²) indicated that about 74.3% of the variations in the dependent variable (Y) were accounted for by the variables included in the model. The F-ratio was highly statistically significant at 1.0% which indicated the goodness of fit of the model used for the analysis. Gender, age, marital status, educational qualification and selling price were the determinants of marketing efficiency. The coefficient of gender (-0.511) was statistically significant at 5.0% risk level and negatively related to marketing efficiency, indicating that decreasing number of male marketers increased the efficiency of the business. The coefficient of age (-31.009) was statistically significant at 1.0% level of significance and negatively related marketing efficiency. This implies that any decrease in age of the respondents will lead to a corresponding increase in their marketing efficiency. Marital status was statistically significance at 5.0% level of significance and positively related to marketing efficiency of water melon. This implied that the more the marketers got married, the more their water melon business flourished. Educational qualification was statistically significant at 1.0% risk level and positively related to the marketing efficiency, implying that as the marketers got more educational qualification, it lead to a corresponding increase in their marketing efficiency of water melon. Access to credit was statistically significant at 1.0% of significance and positively related, implying that

increase in the marketers' access to credit lead to a corresponding increase in their marketing efficiency.

Table 3. Multiple Regression on Determinants of Marketing Efficiency of Watermelon in the Study Area

Parameters	Linear	Semi-Log +	Exponential	Double-Log
(Constant)	11.478 (4.331)***	8.15597 (8.913)***	2051.318 (2.888)**	1.324 -1.545
Gender	-1.138 (-2.730)**	-0.511 (-2.44)**	9664.462 (1.192)*	-0.743 (-1.851)*
Age	-0.086 (-0.346)	-31.009 (2.907)***	-26541.942 (1.911)*	-0.37 (-0.972)
Marital Status	0.167 -0.649	0.004 (2.132)**	43781.366 -0.94	0.071 (1.830)*
Household Size	-0.042 (-0.272)	-0.077 -0.9	12144.834 (2.586)**	-0.117 (-0.685)
Educational Qualification	0.233 -1.159	0.002 (3.570)***	1401.411 -0.987	0.003 -0.033
Years of Business	-0.057 (-0.400)	-0.014 -0.39	1290.794 (1.744)*	0.045 -0.255
Credit Access	-0.728 (-1.868)*	0.008 (-0.044)	-0.114 (-0.739)	0.107 (1.716)*
Selling Price	170.124 (-0.587)	2.30E-07 (6.988)***	29844.052 (2.269)**	0.006 (2.505)**
R ²	0.662	0.745	0.78	0.85
R ⁻²	0.618	0.641	0.76	0.83
F-ratio	9.519***	12.367***	11.813***	7.942***

Source: Field Survey; 2018.

Note: * = statistically significant at 10.0%, ** = statistically significant at 5.0%, and *** = statistically significant at 1.0%.

Constraints to Watermelon Marketing in Abia State, Nigeria

The result of the constraints encountered by watermelon marketers was presented in Table 4 with a benchmark of 3.0. According to the result on the Table, the grand mean was 4.33 which confirmed that watermelon marketers faced serious challenges in the business. All the variables considered as constraints were all above the benchmark mean of 3.0 indicating that they were all serious constraints to water melon marketing. This consolidated with the findings of Oseni *et al.* (2015) who stated that watermelon marketing is constrained by so many factors such as armed robbers on Nigeria's highways, bad roads, among others. This result may be plausible in that watermelon marketing have higher risks and

uncertainty associated with it.

CONCLUSION AND RECOMMENDATIONS

Based on the findings of the research, it could be deduced that watermelon marketing in Abia State is viable and efficient, but has a whole lot of constraining factors. Its efficiency is positively determined by marital status, educational qualification and access to credits, and negatively determined by gender and age. Thus, it was recommended that the marketers association in the state should device means of ameliorating the numerous marketing constraints to the business in order to encourage new entrants and its consequent expansion.

Table 4. Presentation of Watermelon Marketing Constraints in Abia State

	5	4	3	2	1	$\sum fx$	\bar{x}
CONSTRAINTS	SA	A	UN	D	SD	Total	Mean
Lack of storage facility	54(270)	17(68)	1(3)	0(0)	0(0)	341	4.74
Lack of reliable marketing channel	30(150)	40(160)	2(6)	0(0)	0(0)	316	4.38
Inadequate transportation facilities	31(155)	29(116)	8(24)	4(8)	0(0)	303	4.21
Perishability of the product	56(280)	13(52)	4(12)	0(0)	0(0)	344	4.78
Marketing policy	42(210)	30(120)	0(0)	0(0)	0(0)	330	4.58
Seasonality	26(130)	45(180)	0(0)	1(2)	0(0)	312	4.33
Influence of marketing association	27(135)	42(168)	3(9)	0(0)	0(0)	312	4.33
Poor access to market	33(165)	26(104)	9(27)	4(8)	0(0)	304	4.22
Poor access to credit	19(95)	40(160)	9(27)	4(8)	0(0)	290	4.03
Poor marketing information	23(115)	29(116)	16(48)	4(8)	0(0)	287	3.99
Poor handling and packaging	35(175)	25(100)	8(24)	4(8)	0(0)	307	4.26
Irregular supply	37(185)	28(112)	3(9)	4(8)	0(0)	314	4.36
Bulking nature of fruit	31(155)	27(108)	5(15)	4(8)	5(5)	291	4.04
Price instability	46(230)	15(60)	4(12)	3(6)	4(4)	312	4.33
Grand mean							4.33
Benchmark mean							3

Source: Field Survey; 2018.

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FACTORS THAT AFFECT THE PROFITABILITY OF OIL PALM PROCESSING IN IMO STATE, NIGERIA

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ABSTRACT

Palm fruits processing is an important economic activity in South East zone of Nigeria. The study ascertained the factors that affect the profitability of palm oil processing in Imo State, Nigeria. Specifically, the study examined the various technologies utilized by the processors; determine the profitability of oil palm processing and ascertain factors that affect the profitability of palm oil production in the study area. Multistage and random sampling procedure were used in the selection of 60 palm fruits' processors for the study. Data were collected through the use of a well structured questionnaire. Data collected were analyzed using descriptive and inferential statistical tools such as mean, frequency, profitability technique and regression analyses. Result show that age, education, household size, processing experience, quantity of palm fruits processed and technology –use were significant variables the affect the profitability of palm fruits processing in the study area. It was recommended that the processors in the study area form a cooperative society in order to acquire modern equipment for efficient and profitable processing.

INTRODUCTION

Palm fruits processing is an important economic activity in South East zone of Nigeria. This is because the oil palm tree is widely grown in the area as an economic tree providing source of income for families and communities (FAO, 2010). Most agricultural produce, require processing to the forms in which can be consumed. In other words, create utility functions needed to satisfy consumers.

Three varieties of oil palm are available in Nigeria; namely Dura, Pisifera and Tenera. The preferred variety among palm oil farmers in Nigeria is the hybrid Tenera which is a crossbreed of the Dura (female) and the Pisifera (male). Tenera seedlings are produced by the Nigeria Institute for Oil Palm Research (NIFOR) and commonly referred to as the extension work seeds. In terms of comparison, the fruit of the Tenera variety contains 25% oil, by weight, and the Dura variety 18%, so the same amount of Tenera can yield 30% more oil than the equivalent fruit of the Dura (NIFOR, 2009).

In Nigeria, the oil palm tree is a useful crop that is relevant in all aspects of live with socioeconomic and socio-cultural values. According to Ibitoye *et al.*, (2011) reported that oil palm is a versatile tree crop with almost all parts having economic value and useful for everyday livelihood. The different parts of oil palm include: the fronds, leaves, trunk and roots. These parts give a wide range of products which are of benefit to mankind.

Generally, food crop processing adds value to the produce and increase the shelf life, as well as the availability of the product for consumption (ITDG, 2003). The most important product of oil palm is the palm fruit, which is processed to obtain three

commercial products. These include palm oil, palm kernel oil and palm kernel cake. Processing palm fruits could either be carried out either manually or mechanically with the use of machineries for each process. The traditional method of oil extraction consists of pounding cooked/soaked fruits in large wooden or concrete mortars with a wooden pestle and foot trampling the cooked but cold fruits in canoes or specially constructed wooden troughs (FAO, 2002). The modern or mechanical methods are further grouped into categories according to the degree of complexity of the unit operational machinery. These are the small-scale mechanical units, medium-scale mills and large industrial mills.

Furthermore, the importance of oil palm to the national economy of Nigeria cannot be over emphasized. It ranges from production of food for human consumption, employment, income to farmers and nation and raw materials for industries. Oil palm which is a production of palm oil has been a major source of foreign exchange to Nigeria as well as source of revenue to major segment of the rural population of South East Nigeria (Onoh and Peter-Onoh, 2012). The palm oil is rich in carotene and contains vitamin A. It is also used in the manufacture of soaps and other detergents (Agwu, 2006).

Oil palm is among the most productive and profitable of tropical crops for bio-fuel production. High-yielding oil palm varieties developed by breeding programmes can produce over 20 tonnes of fresh fruit bunches/ha/yr under ideal management, which is equivalent to 5 tonnes oil/ha/year (excluding the palm kernel oil) (FAO 2002). World demand for vegetable oils is rising sharply, from 100 million tons in 2005 to an estimated 150 million tons in 2020, as the world population continues to grow and the standards of living increase in many developing countries. The role

of oil palm as a supply of relatively inexpensive and versatile edible oil is, therefore, expected to become ever more prominent.

From the aforementioned, the research was intended to study the factors that affect the profitability of oil palm processing in Imo state, Nigeria. The profitability approach to the study was to ascertain whether the palm oil processing is no longer lucrative that brought about its short of supply in the recent times. Consequently, the study addressed the following objectives;

examine the various technologies utilized by the processors;
determine the profitability of oil palm processing;
ascertain factors that affect the profitability of palm oil production and Hypothesis formulated was that there is no significant different between the income of the palm fruits processors from modern and traditional technologies utilized by the processors in the study area.

Methodology

The study was carried out in Imo State, Nigeria. The state is located in the South-East of Nigeria and share boundaries with Anambra, Abia, Delta and Rivers states. The state has a land area of about 19,000 square kilometers and with a population of over 4.8 million people and the population density varies from 230 to 1,400 people per square kilometer. Imo state is geographically situated within the latitudes of 4°45'N to 7°15'N and longitudes 6°50'E to 7°25'E. There are three (3) agricultural zones in the state namely; Okigwe, Orlu and Owerri zones. The three (3) zones are made up of twenty seven (27) Local Government Areas. The occupational distributions of the people of the area are civil service, agricultural production and processing which include palm fruits' processing.

Multistage and random sampling procedure were used in the selection of the respondents for the study. Firstly locations were purposively selected from the three agricultural zones. This was based on the areas high population of oil palm plantations and areas predominantly into oil palm processing. Secondly, two (2) Local Government Areas were purposively selected from each agricultural zone. In Orlu zone, Nwangele and Nkwere LGAs, Owerri zone; Ikeduru and Mbaitoli LGAs and Okigwe zone; Oguta Ohaji Egbema LGAs were selected. Thirdly, two (2) communities were selected from each of the six (6) LGAs giving a total of twelve communities. From the communities, five (5) palm fruits processors were randomly selected from the each of the communities giving a total sixty (60) respondents. Primary data were used for the study. Data were collected through the use of a well structured questionnaire. Data collected were analyzed using descriptive and inferential statistical tools such as mean, frequency, budgetary and regression analyses.

Profit (π) of the oil palm production was determined by finding the difference between the Total Revenue (TR) and Total Cost (TC) of processing. This was expressed as;

$$\pi = GM - TFC$$

$$GM = TR - TVC$$

Where: π = profit, TFC =Total Fixed Cost, TVC = Total Variable Cost and GM = Gross Margin

Total Revenue in Naira = sales of palm oil + sales of cracked palm Kernel + sales of cracked kernel shell.

Total Variable Cost = cost of labour + cost of FFB + transportation + fuel + water.

Fixed Cost = depreciated value of fixed cost items.

Gross margin = Total sales - Total variable cost (TVC).

Profitability Ratio (PR) of oil palm production was calculated using;

$$PR = \frac{TR - TC}{TC}$$

Where; TR = Total Revenue and TC = Total Cost

Regression analysis was employed to ascertain the factors that affect the profitability of oil palm production. It is explicitly expressed as: $Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8)$

Where;

Y = Profitability ratio

X₁ = Gender (male=1, female =0)

X₂ = Age (in years)

X₃ = Marital status (single = 0, married = 1)

X₄ = Education (in years)

X₅ = Household size (number)

X₆ = Processing experience (in years)

X₇ = Quantity of palm fruits processed (in Kg)

X₈ = Technology used (traditional = 0, modern = 1)

Hypothesis was tested using the Z-test. The test of significant between the means of income of modern and traditional palm fruits processors.

The z- test is given as:

$$Z = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

Where;

\bar{X}_1 = Mean income of respondents that utilize modern method

\bar{X}_2 = Mean income of respondents that utilize traditional method

σ_1^2 = Variance of mean income of modern palm fruits processors

σ_2^2 = Variance of mean income of traditional palm fruit processors

n_1 = Number of respondents engaged in modern method

n_2 = Number of respondents engaged in traditional method

Decision rule

If Z calculated exceeds Z tabulated we reject the null hypothesis and accept the alternative hypothesis and if Z calculated is less than Z tabulated we accept the null

hypothesis and reject the alternative hypothesis.
 Results and Discussion
 Results from the study were discussed according to stated objectives.
 Technologies used by the oil palm processors

Total
 Source: Field survey, 2017

The distribution of respondents according to palm fruits' processing methods show that majority (65%) utilized traditional processing technology while 35% utilized modern technologies in the study area. The implication was that palm fruits' processing was dominated by the use of traditional method of processing. Gourichon, (2013), asserted that palm oil is the main vegetable oil produced in Nigeria and that its production is characterized by low yields due to traditional methods of processing.

Table 1: Distribution of respondents according to the processing method used.

Technology-used	Frequency	Percentages (%)
Mechanized	21	35
Traditional	39	65
	60	100

Factors affecting oil palm production

The estimates of the various factors affecting processing are presented as follows:

Table 2: Regression estimates of factors that affect oil palm processing in the study area

Variables	Linear	Exponential	Semi – Log	+Double Log
Constant	-2338.142 (-0.032)	8.980 (9.566)***	103387.027 (4.714)***	11.173 (4.507)***
Gender	39594.605 (0.651)	0.659 (0.473)	29725.679 (1.373)	0.637 (0.588)
Age	-194.886 (-0.2255)	0.007 (0.681)	-37351.323 (-0.972)	-0.773 (-1.779)*
Marital Status	-26405.1 (-3.097)***	-0.303 (-2.794)**	-28333.8.511 (1.236)	-0.417 (1.361)
Education	3244.229 (1.805)*	0.064 (2.501)**	-3.4888.386 (1.151)	1.149 (3.355)***
Household size	-302.356 (-0.122)	0.005 (0.172)	1376.132 (0.106)	0.068 (3.461)***
Processing experience	1950.902 (1.983)**	0.010 (0.823)	14972.501 (4.160)***	0.89 (3.767)***
Quantity processed	0.422 (2.071)	1.823E-6 (0.705)	474.305 (2.037)***	0.113 (5.768)***
Technology	0.054 (0.247)	1.766E-6 (0.633)	8394.982 (0.767)	0.048 (3.390)***
R – Square	0.685	0.655	0.616	0.765
R adjusted	0.618	0.609	0.597	0.733
F- ratio	14.710***	11.711***	12.27***	16.144***

Source: Field data 2017

*Significant at 10%, ** significant at 5%, ***significant at 1%, + Lead equation, values in bracket are t-value.

The result in Table 2 shows the estimates of the factors influencing palm fruit processing in the study area. Double-log functional form was chosen as the lead equation based on the magnitude of the R² value (0.765), number of significant variables and the F- ratio. From the model, seven explanatory variables were statistically significant. Age, education, processing experience, quantity processed and technology used were all significant and affect the profitability of processing oil palm in the study area.

The coefficient of age was significant at 10% and negatively related to processors' profitability. The inverse relationship implies that as the processors advance in age, their profit from palm fruits' processing decreases. This may be that as the processors get older the doggedness to process palm fruits diminishes

following possible reduction in quantity processed.

Education was positive and significant at 1% percentage. The result show that an increase in the level of education of the respondents, it leads to a corresponding increase in the profit from the processing business. Attainment of any level of education must have exposed the processors to ways to cost minimization. Ani *et al.*, (2008), opined that increase in education of farmers positively influenced access, participation and adoption of improved agricultural practices.

Household size was positively related and significant at 1% level of probability. Increase in household size suggests that families with relatively high number of people are likely to process more bunches of oil palm

using family labour. This can result in increased output/profit. Processing experience had a positive coefficient of 3.767, this means that the more the increase in the processing experienced in years, it makes the processors' more knowledgeable over time in the art of processing corresponding in increased profit.

It means increase in the use of modern technology will increase the profitability of the processors by 3.390 percent. Although manual processing dominated the study area but, from the result the use of mechanical/modern method was more profitable. The modern method increased the efficiency in processing compared to the manual method.

Quantity of oil palm processed has a positive coefficient (5.768) and statistically significant at 1%. This means that a unit increase in the quantity of oil palm processed will lead to 5.768 increase in profit. Technology used had positive coefficient (3.390) and significant at 1%.

Result of hypothesis tested using Z-test. The test of significant between the means of income of modern and traditional palm fruits processors is presented in Table 3.

Table 3: Comparative analysis of the difference in the income from modern and traditional palm fruits' processors

Technology used	Frequency	Mean income	Variance	df	Z-cal	Z-tab
Modern method	21	198, 920	150, 975.0			
Traditional method	39	153,600	95, 889.8	58	6.214***	3.58

Source: field survey, 2017

The hypothesis formulated was rejected since the Z-calculated (6.214) was greater than the Z- tabulated (3.58). The difference between the mean income of modern palm fruits processors from traditional processors tested was significant at 5%. This shows that the processors that utilized modern processing method made more income more than the processors into traditional method practices and this also translated into more profit to the former. This is attributed to ability of the modern method influencing the output processed unlike the manual method characterized by drudgery with lesser output.

Conclusion

The study shows that oil palm processing is profitable in the study area. Although manual processing dominated the study area but, from the result use of mechanical/ modern method was more profitable. Its recommended that the processors in the study area form a cooperative society. This will enable them acquire the necessary equipment through the cooperation or external support from Non Governmental Agencies that will facilitate the use of improved/modern technology in oil palm processing. Expansion in capacity of the quantity of palm fruits processed by the processors will be achieved.

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WIS-SSD 42

IMPACT OF THE ADOPTION OF IMPROVED CASSAVA VARIETIES BY THE WOMEN FARMERS IN AKWA IBOM STATE, NIGERIA

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Abstract

The study investigated the impact of selected improved cassava varieties on the socioeconomic characteristics of women farmers in Akwa Ibom state, Nigeria. The data for the study were collected from 120 women farmers. A well-structured questionnaire was used for the study. The data were analyzed by descriptive statistics such as tables, frequencies percentages, mean and likert type scale. The result revealed high awareness and adoption of all the five selected improved cassava varieties studied, TMS 89/0505, TME 419, TMS 89/0851, NR 8082 and pro vitamin A among the farmers. The result also revealed that the mean annual farm income of women farmers in Akwa Ibom state after adopting improved cassava varieties was ₦345, 215.64. The study concluded that most of the women farmers in Akwa Ibom state adopted the improved cassava varieties and it has increased the farm income levels and livelihood of the women. It is highly recommended that, since the adoption of improved cassava varieties had significant impact on farm income and livelihood of the women, the government of Akwa Ibom should encourage and empower women farmers through the provision of incentives (such as farm credit, planting materials and subsidies) to enable them improve their level of adoption and greatly enhance their livelihood.

Keywords: Impact, Adoption, Cassava, Women Farmers

Introduction

Cassava (*Manihot esculenta*) is one of the important root crops grown in Nigeria (Amadi *et al.*, 2019). Notably, assessing the impact of some selected improved cassava varieties for their expected performance on the socio-economic status of farmers particularly women farmers is a pre-requisite and basis for the suitability of new varieties in Nigeria (Amadi *et al.*, 2018; Ironkwe *et al.*, 2012). These improved varieties have high yielding capacity per unit land area. They tolerate major diseases and pests of cassava. They also have low cyanide content, high starch, high garri index and are early maturing and bulking, stay green, drought tolerant, yellow root, protein and vitamin A content (ADP Akwalbom, 2001; Nwakor, 2010; NRCRI, 2014 and IITA, 2017).

However, most of the women farmers in the rural areas still depend on local cassava varieties for their planting materials which in turn result to very poor yield at harvest. Previous research studies have shown that cassava yield in farmers field stood at 5-10 tonnes per hectare (Nwosu *et al.*, 2014). Furthermore, Nigerian cassava yield of 9.98 tonnes per hectare is still lower than the world average yield of 10.76 tonnes/ha irrespective of her being ranked highest cassava producer in the world. The main solution to this problem is adoption of improved cassava varieties and production technologies by the farmers (Imo, 2006; Nwakor, 2010). In order to relieve farmers from this problem of poor yield in cassava, IITA, Ibadan and NRCRI, Umudike collaborated to developed more than 46 hybrid cassava varieties through selective breeding, capable of adapting to a wider range of ecological conditions and farming systems (IITA, 2017). Among these varieties are TME419, NR8082,

Pro vitamin A cassava, TMS89/0505, TMS89/0851 among many others, and have been disseminated to the farmers through the extension services programme of NRCRI Umudike (NRCRI, 2014). Notably, it was observed that many of the farmers have adopted the varieties. An understanding of women contributions to food output in rural households in Nigeria is essential. Women play a central role in cassava production, processing and marketing (Onyemauwa, 2012). They are entirely responsible for virtually all activities like making mounds, weeding, harvesting, transporting, processing, marketing and domestic chores provides them with additional income-earning opportunity percentages to measure the strength of agreement or and enhances their ability to contribute to household food security (Oladejo *et al.*, 2011). Therefore, there is need to find out the extent the improved cassava varieties has positively impacted on the socio-economic status of the women farmers in Akwalbom State, been one of the most important food crop cultivated and consumed widely in the state.

Methodology

Study Area

The study was conducted in Akwalbom State, Nigeria. Akwalbom is located in the coastal south-southern part of the country, lying between latitudes 4° 32' and 5° 33' north, and longitude 7° 25' and 8° 25' east. The state is credited with a resident population of about 5 million (projected from NPC, 2006). The state is divided into six agricultural zones namely: Abak, Uyo, Etinan, Eket, Ikot ekpene, and Oron. The State has comparative proximity advantage to National root crops research institute, umudike, for production of improved cassava varieties than most of the South-southern states.

Sample Selection

A multistage sampling procedure was used in selecting the sample for this study. In the first stage two agricultural zones (Uyo and Ikot-Ekpene) were purposively selected. This is because cassava is massively cultivated in those areas. The second stage involved the selection of three (3) blocks from each of the two agricultural zones, making up a total of six (6) blocks. The third stage involved the random sampling of two circles from each of the blocks totaling twelve (12) circles. Ten (10) rural women cassava farmers were randomly selected from the list cassava farmers in of the area, making it a total number of 120 women farmers for the study.

Data Collection

Preliminary visits were carried out to the two agricultural zones before the commencement of the actual data collection. The visit was an aid for the familiarization of the researchers with the study locations, resident agricultural extension workers, key informants, women group and field guides. Data were collected using structured questionnaires and interview schedule. Data collection covered level of awareness and adoption of improved cassava varieties, extent the selected improved cassava positively impacted on their socio-economic status and tangible achievement made with the income from the improved cassava varieties.

Data Analysis

Descriptive statistical tools (such as frequencies, percentages and mean) and 5 points likert-type scale rating with a bench mark of 3 such as very much (5), much (4), moderate (3), low (2) and very low (1). Not that any technologies rated 4.0 and above is regarded as very much, from 3.5 to 3.99 is much, from 3.0 to 3.49 is moderate and below 3.0 is regarded as having low impact on the livelihood of women farmers.

Results and Discussion

Level of Adoption of selected Improved Cassava Varieties

The results in Table 1 revealed high awareness of all the five selected improved cassava varieties among the women farmers studied as over 91% of the respondents were aware of the improved cassava varieties. The result also shows that the improved cassava varieties introduced to the farmers in the study area were adopted in varying degrees. Greater proportion of the women adopted the five improved cassava varieties with pro vitamin A cassava having the highest adoption rate (94.2 %), NR 8082 (93.4%), TMS 419 (85.1%), TMS 89/0505 (83.5) and TMS

89/0851 (74.4%).The implication of the results of the adoption level in the study implied that adopters adopted the technology because of the impact on their socio-economic status. This finding is in accordance with the report of Abdoulaye *et al*, (2014) who stated that several factors could drive the adoption process. Amadi *et al* (2019) reported that high adoption of pro vitamin A cassava would bring about improvement in the health and economic condition of poor farmers and reduce the level of vitamin A deficiency in the study area. This is in agreement with the findings of Nwakor, (2010) which reported high adoption of TME419 and NR 8082 by farmers. In a review, Oparinde *et al*. (2016) identified the adoption of the improved cassava varieties to be dependent on political changes and policies abounding in Nigeria.

Table 1: Distribution of respondents according to the level of awareness and adoption of improved cassava varieties

Improved Cassava Varieties	Unaware	Aware	Not-adopted	Adopted
TMS 89/0851	19.7	80.3	25.6	74.4
TME 419	11.6	88.4	14.9	85.1
NR 8082	2.1	97.9	6.6	93.4
Pro vitamin A	0.0	100.0	5.8	94.2
TMS 89/0505	13.2	86.8	16.5	83.5

Source: field survey, 2019

Extent the selected improved cassava positively impacted on the livelihood of the women farmers

Table 2 shows that majority of the selected improved cassava varieties had positive impact on the livelihood of the farmers in the study area. Improved cassava varieties like pro vitamin A cassava (4.5), NR 8082 (4.1) and TMS 419 (3.6) had high impact particularly on increased availability of food, good health and nutrition, wedging of poverty, income generation and employment for household members, while TMS 89/0505 (3.3) recorded moderate impact and TMS 89/0851 (2.9) had low impact. The grand mean (3.2) indicated that most of the technologies adopted by the farmers had relative impact on the socio-economic status of the women farmers in the study area. This finding agrees with the report of Ironkwe *et al.*, (2012) that farmers adopt those technologies that have high impact on their socio-economic status

Table 2: Distribution of the women according to the extent the improved cassava varieties positively impacted on

the livelihood of the women

Improved Cassava Varieties	Good health and nutrition	Wedging of poverty	Income Generation(money)	Employment for household members	Acquired new properties	Increased availability of food	Mean	Remark
TMS 89/0851	3.1	2.9	3.0	2.9	2.8	3.0	2.9	low
TME 419	4.0	3.9	3.4	3.2	3.0	4.2	3.6	much
NR 8082	4.0	4.6	4.3	4.0	3.1	4.8	4.1	very much
Pro -vitamin A	4.8	4.5	4.7	4.6	3.7	4.5	4.5	very much
TMS 89/0505	3.8	3.1	3.3	3.1	3.0	3.2	3.3	moderate
Grand mean							3.7	

Source: field survey, 2019 5=Very much, 4 = Much, 3 = Moderate, 2 = Low, 1 = Very low

Impact of Improved Cassava Varieties on Farm Income of women farmers before and after adoption in Akwa Ibom States

Table 3 shows that 44.17% of women farmers had annual farm income less than ₦100, 000 before adoption of improved cassava varieties while 34.17% of them had annual farm income of between ₦100,000 and ₦199,000. Also, 33.33% of women farmers after adoption of improved cassava varieties had annual farm income of ₦400, 000 and above while 25.00% of them had farm income of ₦200, 000 and ₦ 299,000 and 18.33 others had annual farm income of between ₦300,000 and ₦399,000. The mean annual farm income of women farmers in Akwa Ibom state after

adopting improved cassava varieties was ₦345, 215.64. This implied that adoption of improved cassava varieties by women farmers has increased the farm income levels of the women. Income is a major indicator of the farmer’s livelihood. This collaborated with the findings of Owuor *et al.* (2004) that adoption of improved crop varieties increased farmers income which in turn improves the capacity to adopt other agricultural innovations as they have the necessary capital to start the innovation. Also, adoption of improved crop varieties apparently offers opportunity to increase production and income substantially (Nweke and Akorhe, 2002) and reduce food insecurity (Nata *et al.*, 2014).

Table 3: Distribution of women farmers according to Impact of Improved Cassava Varieties on farm income before and after adoption in Akwa Ibom state

Farm Income (₦)	Before		After	
	Frequency	Percentage	Frequency	Percentage
100,000>	53	44.17	14	11.67
100,000 – 199,000	41	34.17	14	11.67
200,000 – 299,000	11	9.16	30	25.00
300,000 - 399,000	10	8.33	22	18.33
400,000 – and above	5	4.17	40	33.33
Total	120	100.00	120	100.00
Mean (#)	123,076,73		345,215,64	

Source: Field survey, 2019

Conclusion

Considering the role women play in agricultural production in Nigeria, it is quite obvious that adoption of improved cassava varieties and benefit from the cassava business in a sustained and effective manner will impact positively to the livelihood of women. The study concluded that most of the women farmers in Akwa Ibom state adopted the improved cassava varieties and it has increased the farm income levels and livelihood of the women. It is highly recommended that, since the adoption of improved cassava varieties had significant impact on farm income and livelihood of the women, the government of Akwa Ibom should encourage and empower women farmers through the provision of incentives (such as farm credit, planting materials and subsidies) to enable them improve their level of adoption and greatly enhance their livelihood.

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ANALYSIS OF CONSUMERS' PREFERENCE AND WILLINGNESS TO PAY FOR PROCESSED TURMERIC POWDER IN ABIA STATE NIGERIA.

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Abstract

The study examined consumers' preference and willingness to pay for processed turmeric powder in Abia State Nigeria. Through focus group discussions, shop observation and personal interview information were gathered from 80 respondents. The data obtained was analysed using descriptive statistics and 5 point Likert rating scale model. The consumers' preferred attribute identified from the study include colour, size, appearance etc. It was observed that all the identified attributes were preferred by the consumers with a mean value of above 3.0. Turmeric powder with yellow colour and big in size were highly preferred with a mean score of 4.06 and 4.25 respectively. It was recommended that variety of turmeric used in processing should be considered during processing fresh turmeric into powder as this will help increase consumption of turmeric in the study area.

Keywords: Turmeric, Consumer, and attributes.

Introduction

Turmeric is a perennial crop from the family of 'Zingiberaceae' and member of *Curcuma Longa*, has been identified for its various uses. Its broad usage covers the area of food, medicine and as raw material for the industries (Roshan and Gaur 2017). Turmeric production has been promoted over the years as it is being identified among crops that can generate income to resource poor farmers in Nigeria (Idowu-Adebayo *et al* 2020). It can be grown under different tropical conditions, but optimal productivity could be gotten from drained sandy or clay loam soils between the temperature range of 20-30⁰C (Akinpelu *et al* 2012). Its production requires less use of fertilizer and has low pest/disease infestation. Turmeric qualifies as a good cash crop because it has great potential in attracting foreign exchange earnings. Although turmeric production receives less attention among Nigerian farmers, National Root Crops Research Institute in Umudike Nigeria has promoted and popularised the production among farmers by spear heading the research on turmeric accessions.

Lack of awareness on the potentials and uses of turmeric is among reasons why most people do not go into its production, processing and consumption of this root crop. Turmeric production is left in the hands of a few farmers in Nigeria, most of who use crude implements in their farm operations.

The farmers have been experiencing among others: low volume of production, low access to market and market information, lack of information and availability of seeds, dispersed production, lack of knowledge on proper cultivation and post-harvest handling and no specific programmes on turmeric. (Olojede and Nwokocho 2011)

A yellow tasty powder could be gotten when the

rhizomes of turmeric is dried and grinded, and could be commercially processed and packaged, while some locally processed non branded ones also exist. However, for easy acceptability of processed turmeric product by consumers, it is therefore pertinent that consumers' preferences must be ascertained. (Kalu *et al* 2020). The poor acceptance of new product has been attributed to the insufficient priority given to consumer preferred traits by processors. Information on consumer preference is needed more than ever before, in order to enhance the adoption of new product. Studying the utility that consumers derive from consuming the products at different stages of processing will give more insight into the solution of low acceptance among consumers.

On this note, the broad objective of this study is to analyze consumers' preference and willingness to pay for processed turmeric in umuahia Abia State. With the following specific objectives; identify processed turmeric power product attributes, to determine consumer preferences for processed turmeric, and to ascertain consumers' willingness to pay for the preferred attributes.

Methodology

Study area

The study was carried out in Abia state, Nigeria. Abia State is located in the South East geopolitical zone of Nigeria. It has three senatorial zones namely: Abia North, Abia South and Abia Central with seventeen local Government Areas. The state is housing about three research institutes, among them is National Root Crops research institute whose mandate among other things is to promote and popularize the cultivation of turmeric. The climate of the state is tropical one, having rainy and dry seasons. Farming and trading are major occupations of the people.

Sampling technique

The Study adopted a mixed sampling techniques using focus group discussions, key informant and consumer intercept. The first stage involved the identification of participants and questionnaire formulation. Purposive selection of 20 participants for two consumer focus group discussion made of male, females, old and younger people. Shop observation and visit to canteen and restaurants was done. Personal interviews were carried out with farmers, retailer, processor and consumers. About 80 persons participated in the study.

Data collection

The study adopted an interactive process of data collection over a 4 months period in Abia state Nigeria, from November 2018 to February 2019. The consumer value data was collected in two stages. The first was a focus group discussion which was to determine the range of attributes that consumers consider when purchasing processed turmeric powder. The participants in the group discussion were randomly selected making the group heterogeneous, comprising of males, females, younger persons as well as older ones. A total of 20 persons participated, which focus was on finding out the awareness, preference and attribute they are willing to pay for in both fresh and processed turmeric. Consumers’ insight was also gotten as we visited chefs in four major eatery in the city that makes use of turmeric in cooking. The qualitative insights from the focus group were then enhanced by a quantitative survey of shoppers with the aid of an interview guide a survey was conducted with 60 respondents.

Analytical Techniques

Objective 1 and 2 were analysed using 5 point Likert rating scale model., while objective 3 was analysed with the aid of descriptive statistics such as mean, frequency and percentage The study ascertained the consumer preferred attribute of processed turmeric powder using Strongly Agree SA (5), Agree A (4), Undecided UD (3), Disagree DA (2) and Strongly Disagree SD (1). Respondents with mean score of 3.0 and above imply they are in agreement that the said attribute was preferred while respondents with mean score of less than 3.0 were not in agreement. To determine the mean Likert level = $X_s = \Sigma X$. X_s of each item was computed by multiplying the frequency of each response pattern with its appropriate nominal value and dividing the sum with the number of respondent to the items.

Model specification

The Likert model can be specified
This can be expressed with equation below.

$$X_s = \frac{\Sigma fn}{N} \text{----- (1)}$$

Where X_s =mean score
 Σ = summation
 f = frequency
 n = likert nominal value
 N = number of the respondents
 $X_s = 1+2+3 +4 +5/5 = 15/5 = 3.0$

Result and Discussion

Consumer’s Preference on processed Turmeric powder
 The distribution of consumers according to the preferred their preferred attributes was presented in table 1. The results from table 1 shows that all the identified attributes were preferred by the consumers with a mean value of above 3.0.

Table 1. Distribution of Respondent according to consumer preferred attributes for processed turmeric powder.

Attribute	Strongly agree (5)	Agree (4)	Moderate (3)	Disagree (2)	Strongly disagree	Total respond	Total point	Ave point
Freshness	25	15	40			80	305	3.81
Appearance	35	15	10	10	10	80	295	3.68
Size	40	20	20			80	340	4.25
Color	45	15	10		10	80	325	4.06
Taste	30	15	20		15	80	280	3.5
Expiry dates	45	15	10	5	5	80	330	4.13
Product information	30	20	20	10		80	310	3.88
Processing information	20	30	10	10	10	80	280	3.5
Product certification	20	30	10	5	15	80	275	3.44

Source: field survey 2019

It was shown from the result on table 1 that in processed turmeric powder colour and size of the product were highly preferred with a mean score of 4.06 and 4.25 respectively. This was followed by turmeric powder

that has date of expiration and product information having a mean score of 4.21 and 4.02 respectively. Similar result was observed in an earlier study by Kalu 2018.

The distribution of respondents according to their willingness to pay for the preferred trait was presented on table 2. The results show that about 43% of consumers were willing to pay two more times extra if the processed turmeric powder possessed any of the preferred traits.

Table 2. Distribution of Respondent according to Consumers' Willingness to Pay for the Preferred Trait.

How many times more	frequency	percentage
0	15	18.75
2	35	43.75
3	20	25.00
5	10	12.50
	80	100

Source: field survey 2019

The result further shows that 25.00% and 12.50% of the respondents indicated interest in paying three and five more extra respectively if a processed turmeric powder have their desired attribute. This implies that these identified attributes were so important to turmeric consumers in the study area.

Conclusion and Recommendation

The study identity processed turmeric power product attributes, ascertained the consumer preferred attribute for processed turmeric powder and determined their willingness to pay for these preferred attributes. It was observed that size, colour and dates were among the attribute that consumers will like to see on processed turmeric powder and will be willing to pay for these preferred traits. We therefore recommend that more attention should be paid to this attribute during processing fresh turmeric into powder as this will help increase consumption of turmeric in the study area.

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WIS-SSD 44FL

ECONOMIC ANALYSIS OF TOMATO MARKETING IN LOKOJA LOCAL GOVERNMENT AREA OF KOGI STATE, NIGERIA.

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Abstract

This study investigated the economic analysis of tomato marketing in Lokoja local Government Area of Kogi State. The objective of the study was to determine the socio-economic characteristics of tomato marketers, examine the performance of marketers, determine the profitability of tomato marketing and identify the constraints in tomato marketing in the study area. A total of 75 respondents were selected through purposive sampling technique from different market. Data were collected through structured questionnaire and analyzed using descriptive statistics, Marketing margin, Gross margin and likert scale. The result revealed that majority of the respondents (83%) were females with a mean age of 48 years. Majorities (64%) of the respondents were married and 24% were single. The average family size was 7 persons per household. Most of the respondents had no formal education. The mean marketing experience was 26 years. The result also showed that the average gross margin was ₦430 and the Benefit Cost Ratio was 1.33 indicating the profitability of tomato marketing. Constraints to tomato reported were inadequate capital, Perishability, Poor storage facilities, High transportation cost, Low output price, Irregular supply. The study therefore recommended that Provisions of inputs such as storage facilities should be made available by the Government to enable farmers store their products for a relatively period so as to reduce perishability of the harvested produce. Also resource should be made available by the government to tomato marketers in form of loan with low interest rate as this will help them expand their scale of marketing.

Keywords: tomato, marketing, profitability

INTRODUCTION

Tomato (*Lycopersicon esculentum* mill) belongs to the family *solanaceae*. It is the world most widely grown vegetable, other than potato. Tomato rank first (1st) in the world for vegetable and also account for 14% of world vegetable production (over 100 million metric tons/year and 1.6 billion market)(FAO 2010).

Tomato is a rich source of vitamins, minerals, carbohydrates, proteins and dietary fibres which are important to the human diet. A balanced diet should contain 250-325g of tomatoes and the recommended average human requirement for tomato is 285g/person/day (Nwachukwu and Onyenweaku, 2007).

The production of tomato has been on the increase in most areas, both in acreage and yield. The food and agricultural organization (FAO, 1993a) revealed that the mean annual fruit yield world over has increased from 52.597 million metric tons to 77.07 million metric tons. In Nigeria, FAO (1993b) quarterly statistical report shows that tomato production rose from 403,000 metric tons to 670,000 metric tons.

Tomatoes have tremendous potentials to address poverty alleviation and nutritional security because they are affordable and easily available, easy to grow, require minimum production inputs, rich in vitamins and minerals and are loaded with photochemical and antioxidants properties (Eusebio,2009).

Marketing is the activity, set of institutions, and processes for creating, communicating, delivering, and exchanging offerings that have value for customers, clients, partners, and society at large ([American Marketing Association](#) (AMA), 2007)

Women actively participate in tomato marketing activities .Usually; women are involved in this for self-reliance. In terms of income and other aspect of agricultural production. However, women are confronted with heavy losses due to wastages, large quantity of tomato littered around the place during the season in market places in the study and this has led to heavy enormous loss of tomato produce and consequently financial loss in Lokoja Local Government Area.

The marketing of tomatoes in Lokoja metropolis has grown rapidly over the years and is surrounded by a myriad of problems ranging from high exploitation by middlemen, high marketing costs, instability of government policies in assisting marketers and lack of good infrastructure to ease movement of products. Tomato marketing is highly risky due to wide yield and price variation which need quick disposal because of the perishable nature of tomatoes .It is against this backdrop the study tend to examine the economic analysis of tomato marketing in Lokoja Local Government Area of Kogi State, Nigeria.

The specific objectives are:-
 describe the socioeconomic characteristics of tomato marketers in the study area
 examine the performance of tomato marketers
 determine the profitability of tomato marketing in the study area
 identify the constraints in tomato marketing in the study area

STUDY AREA

The study was conducted in Lokoja, the capital city of Kogi State. Lokoja is located in the south-central Nigeria on the west bank of the River Niger opposite side of the Benue River. Formerly the capital of Kabba province, Lokoja was part of Kwara from 1967 to 1991, when it became the capital of the newly formed state of Kogi. The State lies between longitudes 7°8'17"N and 6°7'50"E East and latitude 7°49'N and 6°45'E with an area of 3180Km² and a population of 195,261 at 2006 census. Lokoja serves as the gateway to the North and south of the country. It shares common boundaries with Koton-karfe (Kogi L.G.A) Kabba/Bunu, Bassa, Ajaokuta and Adavi Local Government Area (*Encyclopaedia Britannica*, 2014).The area has an agro based economy with numerous agricultural potentials .Vegetables produced and marketed include: Tomato, Spinach ,Sorrel and Pepper while food crops produced are Maize ,Rice and Guinea corn .Cattle rearing and fishing also predominate. Lokoja zone experiences two major climatic conditions as harmattan .The dry season starts from November-March, rain fall occurs from late March to the end of October. With an annual rainfall of 20cm³ and temperature is about 27.9°c.The relative humidity varies from 30 in the dry season and 90 in wet seasons.

METHOD OF DATA COLLECTION

Tomato marketers in the state constituted the population for the study. A purposive sampling technique was used to select respondents. . Fifteen (15) tomato marketers from five (5) different markets in the study area were randomly sampled. This gave a sample size of 75

Data for the study were collected through a well-structured questionnaire and personal interview which was administered to tomato marketers in the study area. In order to characterize the respondents on their socio-economic status, marketing characteristics, variables related to costs and returns in tomato marketing and major problems encountered in tomato marketing were also gathered

ANALYTICAL PROCEDURE

Objective one: Socio-economic characteristics of the respondents was analyzed using descriptive statistics such as mean, frequency and percentage

Objective two: Performance of tomato marketers ' was analyzed using marketing margin

Objective three: Profitability of tomato marketers

was analyzed using Gross Margin
 Objective four: Constraints in tomato marketing was identified using mean score from a four point likert scale.

Model Specification

Marketing Margin (Olukosi and Isitor, 2005).The formula is given as:

$$\text{Marketing Margin} = \frac{\text{Total selling price} - \text{Total cost price}}{\text{Total selling price}} \times 100\%$$

Gross Margin Analysis

The Gross Margin Analysis was used to determine the costs and return associated with tomato marketing .Gross Margin is the difference between total revenue and the total variable cost of tomatoes (Oluosi and Isitor, 2005).The formula is given as:

$$GM = TR - TVC$$

Where:

GM =Gross Margin

TR =Total revenue (Gross income or total income)

TVC =Total variable cost

Mean Score

Problems militating against tomato marketing in the study area were analyzed with the use of mean score after marketers responses were obtained using a four-point Likert type of scale. Likert scale was developed by RensisLikert in the 1930s to measure the mean scores of variables. The four point Likert type of scale was used as specified below

Opinion	Point
Very serious	4
Serious	3
Not serious	2
Not a problem	1

The mean response to each item as calculated using the following formula:

$$Y = \frac{\sum FX}{N}$$

Where Y =Mean response, \sum = summation , F = number of respondents choosing a particular scale point, X =numerical value of the scale point and N =total number of respondents to the item.

The mean response to each item was interpreted using the concept of real limits of numbers .The numerical value of the scale points (Response modes) and their respective real limits are as follows:

Not a Problem (NP) =1 Point with real limits of 1.50 -2.49

Not Serious (NS) =2 Points with real limits of 2.50 -3.49

Serious (S) =3 Points with real limits of 3.50 -4.49

Very Serious (VS) =4 Points with real limits of 4.50 -5.49

Decision Rule: score equal to and above is considered a problem

RESULTS AND DISSCUSSION

Majority of the farmers (82%) were between the age group of (31-60 years) as shown in table 1. This implies that majority of tomato marketers in the study area were still in their active and productive age. The frequency according to gender as presented in Table 1 indicates that most (82.7%) of tomato marketers in the study area were female and the male constituted 17.3%. This implies that women are more involved in the marketing of tomato than male. This agrees with Bakari and Usman (2013) who reported high percentage of women involved in tomato marketing in Adamawa State. However, this is in contrast with Haruna et al., (2012) who confirmed low percentage of women participation in tomato marketing in Bauchi Metropolis and attributed it to religion and culture of the people in the area, which emphasized more on domestic economic activities of women than outdoor home ventures. The result of Marital Status of the respondents as presented in Table 1 shows that majority (65.4%) were married, 24.0% were single, 9.3% were widowed, 1.3% were divorced. This is an indication that tomato marketers in the area were married women. Income from tomato marketing can supplement household income and increase the living standard of individuals in the household. Educational status of the respondents revealed in Table 1 showed that majority (54.7%) of the marketers had no formal education, 38.7% only had primary education, and 5.3% had secondary education while few number of the respondents (1.3%) attended tertiary institutions. Educational status can affect marketing skills and its multiplier effect on increased return. Usman *et al* (2006) pointed out that educational level of a trader does not only raise its productivity but also increase its ability to understand and evaluate new techniques and processes for better marketing of his goods. The result in Table 1 showed that majority (65.4%) of the sampled tomato marketers were into full time tomato marketing, 28% combined farming activities with tomato marketing, 5.3% combined civil service with tomato marketing while 1.3% of the marketers combined artisan activities with tomato marketing in the area.

Socio-economic characteristics	Frequency	Percentage	Mean
Age			
20-30	04	5.3	
31-40	06	8.0	
41-50	45	60.0	
51-60	18	24.0	
Above 60	02	2.7	
Total Sex	75	100	48 years
Male	13	17.3	
Female	62	82.7	
Total	75	100	
Marital status			
Single	18	24.0	
Married	49	65.4	
Widowed	07	9.3	
Divorced	01	1.3	
Total	75	100	7 persons
Family size			
1-5	11	14.7	
6-10	57	76.0	
Above 10	07	9.3	
Total	75	100	
Educational status			
Non-formal education	41	54.7	
Primary education	29	38.7	
Secondary education	04	5.3	
Tertiary education	01	1.3	
Total	75	100	
Major occupation			
Tomato marketing	49	65.4	
Farming	21	28.0	
Civil service	04	5.3	
Artisan	01	1.3	
Total	75	100	
Marketing experience			
1-10	02	2.6	
11-20	14	18.7	
Above 20	59	78.7	
Total	75	100	26 years

Table 1: Socio-economic Characteristics of the Respondents. The information regarding socioeconomic characteristics of tomato marketers is presented in table 1

Source; Field Survey, 2018
The frequency distribution according to the Family

size of the respondents as presented in Table 1 also indicated that majority (76%) Of tomato marketers in the study area had family size of between 6-10 persons,14.7%had household size of 1-5 persons ,while 9.3% of the respondents had above 10 persons. The mean household size was 7 persons. Members of the family can serve as source of labor in carrying out marketing activities. Results in Table 1 also revealed that majority of the marketers (78.7%) had marketing experience of above 20 years, 18.7 % had experience of between 11-20 years while only 2.6% had marketing experience of 1-10 years. The mean marketing experience was 26 years. The marketing experience is also an indication that tomato marketing in the area is profitable because it is believed no one will remain in an unprofitable venture for a long time.

Marketing Performance of Tomato Marketers in the Study Area

Marketing Margin = $\frac{\text{Total selling price} - \text{Total cost}}{\text{price}} \times 100\%$

$$= \frac{\text{Total selling price} - \text{Total cost}}{\text{price}} \times 100\%$$

$$= \frac{1600 - 1195}{1600} \times 100\%$$

$$= 25.3\%$$

The marketing margin of tomato marketers in the study area was 25.3%.This implies a fair performance by tomato marketers in the study area .This result agrees with Obayelu *et al* (2014) that the higher the value, the higher the performance and the more efficient is the marketing system.

Cost items	Amount(#)
Variable cost	
Acquisition cost	850(72.65)
Transportation cost	120 (10.26)
Cost of empty basket	80 (6.84)
Cost of loading and offloading	40 (3.42)
Taxes	30(2.56)
Sanitation cost	50(4.27)
Total variable cost (TVC)	1170 (97.9)
Fixed cost	25 (2.1)
Depreciation on rent	25
Total fixed cost	1195
Total cost	
Returns	
Total revenue	1600
Gross Margin (TR –TVC)	430
Return per Naira invested (TR/TC)	1.33

Source: Field survey, 2018

The costs and return analysis of tomato marketing was indicated in table 3. The table showed variable cost (97.7%) and fixed cost (2.1%) of the total cost of tomato marketing in the study area. The results further indicated that the cost of acquisition (72.65%),cost of transportation (10.26%),cost of empty basket (6.84%) and sanitation cost (4.27%) were major variable costs incurred in tomato marketing .Based on the computation per basket, the average price per basket was #1600,total cost of marketing was #1,195 while the total revenue of # 1,600 was realized. The gross margin from tomato marketing was #430 per basket. In review of this costs and return results, tomato marketing in the study area was highly profitable since the return on naira invested was 1.33.This implies that for every one naira invested on tomato marketing, 1.33k will be realized. This finding is in agreement with Sani and Haruna (2010) who stated that costs and return analysis of tomato crop production was economically viable and sustainable based on the applied planning model implying that supply response was not a problem but rather value addition and marketing components of the produce which needed to be planned and sustained.

Table 3 Cost and return analysis in tomato marketing

Table 4: Distribution of respondents according to constraints in tomato marketing

Constraints	VS (4)	S (3)	NS (2)	NP (1)	Total	Total sum of square	Mean score	Rank
Inadequate capital	47	28	0	0	75	272	3.63	1
Perishability	39	33	03	0	75	261	3.48	2
Poor storage facilities	37	36	02	0	75	260	3.47	3
High transport cost	25	37	13	0	75	237	3.16	4
Low output price	16	55	01	03	75	234	3.12	5
Irregular supply	12	49	09	05	75	218	2.91	6
Unavailability of the product	04	11	43	17	75	152	2.03	7
Low demand for tomato	0	06	28	41	75	115	1.53	8

Source: Field Survey, 2018

From the result obtained in table 4, the mean score was used to ascertain the level of seriousness of the

problems. Inadequate capital was ranked 1st, had a mean score of 3.63, Perishability of tomato ranked 2nd with a mean score of 3.48, Poor storage facilities was ranked 3rd with a mean score of 3.47, Transportation cost was ranked 4th with a mean score of 3.16, Low output price was ranked 5th with a mean score of 3.12 and irregular supply of tomato was ranked 6th with a mean score of 2.91. The problems indicated are all considered a serious problem (with a mean score above average) affecting tomato marketing.

CONCLUSION AND RECOMMENDATIONS

The study is on economic analysis of tomato marketing in Lokoja local government area of Kogi State, Nigeria. It can be concluded that tomato marketing in the study area is profitable as revealed by the study. This is because it has generated ₦1.33k as

returns per naira invested. This is an indication that the performance of tomato marketing based on profit is good and equally viable. Based on the research findings, the following recommendations are made:

Provision of input such as storage facilities should be made available by the government to enable farmers store their products for a relative period so as to reduce perishability of the harvested produce

Capital should be made available to tomato marketers in form of loan with low interest rate as this will help them expand their scale of marketing.

There is the need for the facilities to be in place in order to help perishability

Tomato marketers in the study area should be organized to act under an umbrella of a marketing union in order to stabilize the price of tomato

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WIS-SSD 45

ASSESSMENT OF GROUP AGRICULTURAL ACTIVITIES AMONG CASSAVA WOMEN FARMERS

IN KABBA/BUNU LOCAL GOVERNMENT AREA OF KOGI STATE

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Abstract

The study investigated group agricultural activities among cassava women farmers in Kabba/bunu Local Government Area of Kogi State. The specific objectives were to describe the socioeconomic characteristics of cassava women farmers, identify various cassava products produced by the women farmers, examine the status of participation in group agricultural activities by them, determine the effect of socioeconomic characteristics on their participation in group agricultural activities and identify the constraints faced by cassava women farmers in their group agricultural activities in the study area. A total of 160 respondents were selected and administered questionnaire using a combination of both purposive and random sampling techniques. Data obtained was analyzed using descriptive statistics and Binary Logit Regression. Result showed that Majority (53.1%) of the respondent were married with average household size of 5 persons per household. The mean age of respondents was 43 years. The result also showed that election of groups executives, attend regular meetings, frying, moving of cassava to grater, peeling of cassava, planting and harvesting were the major group agricultural activities engaged by cassava women in the study area. The study shows that corruption ($\beta=2.07$), old age of farmers ($\beta=2.01$), poor management and leadership ($\beta=2.18$) and health status of farmers ($\beta=2.21$) were the major constraint faced by cassava women farmers in their participation in group agricultural activities. The study recommends that, women farmers should be encouraged to form farmers' cooperatives or join existing ones to pool their resources together in order to take advantage of development assistance.

Keywords: cassava, participation, group activities, women

Introduction

Cassava (*manihot esculanta*) is a popular crop in the countries of sub-Saharan Africa. It is an important food source in the tropics and provides the third-highest carbohydrate yield among the crop plants. Cassava grows well in poor soils and low rainfall areas and tolerates a wide variety of growth conditions including soils with pH ranging from acidic to alkaline, annual rainfalls from 50 mm to 5 m, elevation between sea-level and 6,600 feet, and even equatorial temperatures (Worldatlas, 2017).

Cassava is one most important food crops in Nigeria and in most developing countries; it is one of the most important carbohydrate sources. Moreover, the country is the largest producer of cassava in the world, with about 50 million metric tons annually from a cultivated area of about 3.7 million ha. Nigeria accounts for cassava production of up to 20 per cent of the world, about 34 per cent of Africa's and about 46 per cent of West Africa's. The national average yield of cassava is estimated at about 13.63 MT per ha, as against potential yield of up to 40 metric tons per ha. Close to two-thirds (66 per cent) of total production is in the southern part of the country, while about 30 per cent is in the north-central, and 4 per cent in other parts of the north (FAO, 2018). It is a basic staple food to more than 70% of Nigerian population and consume at least once every day (Eke-okoro and Njoku, 2012). Cassava has been described as a miracle crop because of the numerous products and by-products which add value to it. Every part of the tuber is useful for one purpose or the other. While the peel can be used as feed

for livestock, the rest of the root can be processed into various products such as gari, fufu, tapioca, starch, chips, flour, fried balls, among others chin-chin, salad cream, bread, etc) (Agbarevo and Okeke, 2014). The main traditional cassava products in Nigeria are *gari*, *fufu*, and *lafun*. *Gari* is roasted cereal with a slightly fermented taste. It is made from yellow-fleshed varieties or from white roots fortified with red palm oil to make it yellow in color; otherwise, it is creamy white. *Gari* processing in Nigeria is on the rise because it is seen as a convenience food; it is quick and easy to cook and can be stored. In both urban and rural markets, *gari* competes with rice and cereals due to its low price and high convenience. *Fufu*, a fermented paste, is similar to *gari* in its importance in a household's diet. Instant *fufu* is gaining popularity because it is easy to prepare, has a long shelf life, and is packaged compactly. *Lafun* is fermented, dried cassava that is turned into flour and then into a stiff paste to be eaten with sauce (Emily and Adewale, 2015)

Women play a central role in cassava production, harvesting, processing and marketing, contributing about 58 percent of the total agricultural labour in the southwest, 67 percent in the southeast and 58 percent in the central zones (FAO, 2004, Onyemauwa, 2012). According to Cadena (2017), women oversee 70% of the activities of cassava production and processing. In Nigeria, women are involved in almost all phases of food production. These women are found in various women groups in the rural areas and they engage in exchange of labour, production, processing and marketing of farm produce. Others include: social

groups and consumer cooperatives (Ajani and Mgbenka, 2013). The women groups assist members to increase their farm output and farm income, increase their opportunity for capital formation, provide services to the members at low cost, ensure sales of the farmer's produce and other agricultural commodities to their best possible advantage and ensure equitable distribution of bonuses to the members from annual profits (Lakwo 2008).

Rahman, (2004) reported that women carry the major responsibility for both farm production and domestic works which negatively affect their labour productivity in farm production. The absence of qualitative and quantitative data on gender -labour productivity has contributed to the inadequate recognition of women's effort and insensitivity to their needs in the farming communities despite the contribution of women farmer in agriculture they are faced with problems (such as difficulties in gaining access to resources as land, credit, productivity – enhancing inputs and other services) Which affect their productivity in agricultural sector compared to men who have more access to productive resources. According to Ajani and Mgbenka,(2013) there exist some problems encountered by majority of rural women groups, who wish to fully participate and contribute more to agricultural production. Some of these problems are due to lack of recognition of women's contribution to food production by government through its established agencies. This study is an assessment of group agricultural activities among cassava women farmers in the study area.

The specific objectives are to:
 describe the socio-economic characteristics of cassava women in the study area.
 identify various cassava products produced by the women farmers in the area.
 examine the status of participation in agricultural activities women in the study area.
 determine the effect of socio-economic characteristics on their participation in group activities.
 identify the challenges faced by cassava women farmers in their group agricultural activities.

Methodology

The Study Area

The study area is Kabba-Bunu Local Government Area of Kogi State, Nigeria. Kabba-Bunu local Government Area is one of the 21 Local Government Areas in Kogi State. It is located in the western senatorial district of Kogi State. The Local Government was created in 1991. It is bounded in the North by Lokoja Local Government and by Ijumu Local Government to the South, Yagba- East and Mopamuro Local Government share boundary with the Local Government to the west and to the East by Okehi Local Government Area. According to the National Population Census (2006), Kabba-Bunu

Local Government Area has a population of 145,446 people which is made up of 74,289 males and 71,157 females. It has land area of about 2,706 km². The local government usually experience 2 distinct seasons, the wet and dry seasons. The wet season usually spans from the middle of March to October while the dry season cover the period between November and early March. The vegetation of the area comprise of derived savannah and rain forest in some areas. There are vast available lands for farming. Agriculture is the most important economic activities in the Local Government as majority of the population derive their livelihood from it. Agricultural practice in the area is still at subsistence level, which invariably makes the farmers vulnerable to poverty. The soil is viable for growing crops such as yam, maize, cassava, sorghum, cashew, cocoa, oil palm and coffee. Kogi State Economic Empowerment and Development Strategy, KOSEEDS, (2004).

Sampling Procedure

Multiple stage random sampling technique was used in selecting the sample for the study. At the first stage, the two district of Kabba-Bunu local government area was selected. The second stage involved purposive selection of two villages from each district; Otu and Okedayo were selected in Kabba district, Odoape and Aiyegunle were selected in Bunu district and the third stage involves the random selection of forty (40) respondents from each villages giving a total of 160 respondents for the study.

Method of Data Analysis

Data were analyzed using descriptive statistics. This involves the use of frequency distribution, mean score from likert scale, percentages, and regression analysis. Objective I, II, III, and IV were realized using descriptive statistics such as frequency distribution, mean score from likert type of scale, percentage. Objective V was achieved using binary Logit regression

Model Specification

3 point likert scale was used to analyse the level of participation in group activities.

Low 0 point
 Medium 1 point
 High 2 point

Social economic characteristics that determines farmers level of participation in the group agricultural activities was analysed using binary logit regression model, and the model is specialized as follows;

$$\ln y = \ln (P/1 - P) \dots\dots\dots \text{equation 1}$$

$$\ln (P/1 - P) = b_0 + b_1 X_1 + b_2 X_2 \dots\dots b_{10} X_{10} + e \dots\dots\dots \text{equation 2}$$

Where;
 y = farmers participation in group agricultural activities (1 = yes, 0 = otherwise).
 P = probability of the farmers participation in the

group activities
 Ln = natural logarithm function
 b₀ = constant
 b₁ – b₁₀ = Logit Regression Coefficient
 X₁ = Age of farmers (in years)
 X₂ = Marital status (married = 1, unmarried = 0)
 X₃ = Household size (number)
 X₄ = Farming experience (years of cassava farming)
 X₅ = Educational level (years in formal school)
 X₆ = Extension visit
 X₇ = Income Level (monthly = 1, otherwise = 0)

RESULTS AND DISCUSSION

Household size

Result in Table 1 also shows that 52.5% of the cassava women had household size of 5-10 persons while 47.5% had household size of less than 5 persons. The mean household size was 5 persons. It is expected that members of the household will serve as source of labour in cassava processing and production. This is in line with Anyiro and Onyemachi (2016) who studied the socio-economic characteristics of the rural women that benefited from training on value additive on to cassava crop organized by government and private organizations in their study area revealed the mean household size of 5.3 persons. Large household size indicates availability of more persons that will serve as source labour thereby reducing cost of production.

Marital Status

Majority (53.1%) of the respondents were married individuals, while 10.6% divorced. Marriage is a sign of responsibility. It could also be seen as readiness to settle for a particular kind of profession/activity. This finding corroborates with that of Okebiurun and Jatto (2017) who carried out study on Value Addition in Cassava Processing: Evidence from Women in Ilesa West Local Government Area of Osun State reported that majority of the cassava farmers are married.

Farming Experience

Table 1 also shows that 67.5% of the respondents had farming experience of less than 10 years while 1.3% had above 30 years. The mean farming experience was 9 years. It was gathered that years of farming experience had a positive impact on production system and household income among women farmers in Nigeria. Enete *et al.*, (2002) observed that farmers would count more on their farming experience for improved productivity rather than their educational attainment, this is because the number of years a farmer has spent in farming business may give an indication of the practical knowledge she has acquired on how to cope with the inherent farm

Age				
21-40	78	48.8		
41-60	62	38.7	43 years	
61-80	20	12.5		
Total	160	100.0		
House hold size				
Less than 5	76	47.5		
5-10	84	52.5	5 Persons	
10 and above	0	0		
Total	160	100.0		
Marital status				
Single	37	23.1		
Married	85	53.1	Married	
Divorced	17	10.6		
Widowed	21	13.1		
Total	160	100.0		
Major Occupation				
Farming	98	61.3		
Trading	62	38.7	Farming	
Total	160	100.0		
Farming Experience (years)				
Less than 10	108	67.5		
11-20	41	25.6		
21-30	9	5.6	9 years	
Above 30	2	1.3		
Total	160	100.0		
Educational Level				
No formal education	49	30.6		
Primary education	46	28.8	No formal education	
Secondary education	36	22.5		
Tertiary education	29	18.1		
Total	160	100.0		
Access to Extension Service				
No	99	61.9		
Yes	61	38.1		
Total	160	100.0		

Source: Field Survey 2018

production, processing, and marketing problems leading to higher levels of production efficiency.

Educational level

The socioeconomic characteristics also showed that 30.6% of the respondents had no formal education while 18.1% had tertiary education as their highest level of education. The study shows that about 70% of the respondents had one form of formal education or the other. The level of education of a farmer not only

Table 1 Distribution of Respondents According to Socioeconomic Characteristics

Socioeconomics Characteristic	Frequency	Percentage	Mean /Mode
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increases productivity but also enhances his ability to understand and evaluate new production techniques. This agrees with the findings of Oyesola and Oladeji (2008) that education is very important in farming.

Access to Extension Service

Results on Table 1 finally shows that majority (61.9%) of respondents had no contact with extension agent in the last cropping seasons while only 38.1% had contact with extension agents. This implies that extension services in the study area are poor and inadequate. This agrees with the findings of Shaibu (2016) who reported low extension service among small scale farmers in Kogi state. Increased extension contacts would lead to more knowledge on improved cassava technologies which are expected to lead to increased productivity and profitability.

Table 2 Distribution of Respondents According to types of Cassava Product Produced

Cassava Products	Frequency	Percentage
Garri	155	96.9
Cassava bread	18	11.3
Cassava vegetables	58	36.3
Starch	126	78.8
Fufu	155	96.9
Cassava flour	118	73.8
Cassava chips	22	13.8

Source: Field Survey, 2018

Result in Table 4.2 shows that *garri* (96.9%), *fufu* (96.9%), starch (78.8%) and cassava flour (73.8%) were the major cassava products produced by respondents in the study area. This result is in line with that of Anyiro and Onyemachi(2014) who jointly revealed that cassava fufu, flour, garri and high quality cassava flour were the most cassava value added products disseminated to the rural women.

Table 3.1: Distribution of Respondents According to Participation in Group Agricultural Activities

Variables	Frequency	Percentage
Participants in group agricultural activities	131	81.9
Non Participants in group agricultural activities	29	18.1
Total	160	100.0

Source: Field Survey, 2018

Result in Table.3.1 shows that majority (81.9%) of the

respondents participate in group agricultural activities while only 18.1% did not participate in group agricultural activities. This implies that majority of the women farmers participate in group agricultural activities base on the knowledge and understanding of the benefit they derived in working together as a body than individual.

Table 3.2: Distribution of Respondents According to Level of Participation in group Agricultural activities N= 131

Agricultural Activities	Low (1)	Medium (2)	High (3)	Total	Mean
Land clearing	62	47	22	222	1.69
Planting	16	53	62	308	2.35
Cultivation	58	56	17	221	1.68
Fertilization	66	47	18	214	1.63
Harvesting	19	55	57	300	2.30
Peeling of Cassava	17	47	67	312	2.38
Moving of cassava to grater	13	41	76	323	2.47
Frying	12	44	75	325	2.48
Attend regular meetings	9	40	82	335	2.56
Participation in Election of groups executives	11	31	89	340	2.60

Source: Field Survey, 2018

Result in Table 4.3.2 shows that majority of the respondents participate in election of groups executives (mean score = 2.60), attend regular meetings (mean score = 2.56), frying (mean score = 2.48), moving of cassava to grater (mean score = 2.47), peeling of cassava (mean score = 2.38), planting (mean score = 2.35)and harvesting (mean score = 2.30).This may be because majority of the respondents in the study area have the cultural belief that most of these activities are feminine in nature and as such are mostly attributed to the women. Women play a central role in cassava production, processing and marketing, contributing about 58 percent of the total agriculture labour in the southwest, 67 percent in the southeast and 58 percent in the central zones (FAO, 2004, Onyemauwa, 2012). Cassava women farmers in the study carry out agricultural activities like tilling of land, making ridges, planting, weeding, harvesting, processing, marketing, storing and other activities involved in cassava production in group as these will help reduce labour cost and also help small scale cassava women farmer increase their productivity This finding agrees with the notion that women provide

some 60-80% of agricultural labour and are responsible for 80% of food production (Rahman, 2004).

Participation in group activities

Estimates of the binary regression model on effect of socioeconomics characteristics on their participation in group activities are presented in Table 4.4.

Effect of Socioeconomic characteristics on their

Table 4.4: Estimates of the Binary Logistic Regression showing the Effect of Socioeconomic characteristics on their participation in group activities

Variable	Coefficient	Standard error	Z	P> z
Age (years)	0.0376998	0.03025070	1.25	0.213
Educational level (years)	0.0721537	0.20742	0.35	0.728
Marital status (dummy)	1.560072	0.4863576	3.21	0.001
Income status (dummy)	-0.5967932	0.6743894	-0.88	0.376
Farming experience(years)	-0.0256304	0.0419406	-0.61	0.541
Household size (No.)	-0.1164647	0.12815	-0.91	0.363
Extension visit(dummy)	0.319258	0.4451413	0.72	0.473
Constant	0.1736508	1.177137	0.15	0.883
Number of observations =160				
Log-likelihood	= 13.71			
Prob>Chi ²	= 0.0567			
Pseudo R ²	= 0.0905			

Source: Field Survey, 2018.

***, ** and * = significant at 1%, 5% and 10% respectively.

Table 4.4 shows the effect of socioeconomic characteristics on their Participation in group activities. Among all the variables only marital status is statistically significant with coefficient of 1.056 implying that marital status has a positive effect on cassava women participation in group activities and this means that marriage increases the probability of cassava women participation in group agricultural activities in the study area. Oluwatayo et al.,[2008]

posited that most farmers usually have more than one wife with a large family for having access to farm labour.

Constraints Faced by Cassava Women in their Group Agricultural Activities

The major constraints faced by cassava women farmers in their group agricultural activities is presented in Table 4.5

Table 4.5: Distribution of Respondents According to Constraint faced by Cassava Women

Constraints	Not serious	Serious	Very serious	Total	Mean
Lack of Govt. Support	72(45.00)	47(58.75)	41(76.88)	289	1.80
Level of Education	85(53.13)	41(51.25)	34(63.75)	269	1.68
Corruption	39(24.40)	71(44.40)	50(31.30)	331	2.07
Poverty Level	70(43.75)	50(62.50)	40(75.00)	290	1.81
Old age of Farmers	45(28.13)	69(43.12)	46(28.75)	321	2.01
Financial Problems	33(20.63)	49(30.63)	78(48.75)	365	2.28
Health Status of Farmers	32(20.00)	62(38.75)	66(41.25)	354	2.21
Management and Leadership Problems	41(25.63)	49(30.63)	70(43.75)	349	2.18

Source: Field Survey, 2018

Table 4.5 presented the constraints faced by cassava women farmers in their group agricultural activities in the study area. Corruption, Old age of Farmers, Financial Problems, Health Status of Farmers, Management and Leadership Problems with the mean scores of 2.07, 2.01, 2.28, 2.21 and 2.18 respectively were seen as the major constraints. This implies that cassava women farmers are faced with a wide range of challenges that deterred their participation in group agricultural activities. These include corruption, old age, financial problems, health status and management

and leadership problems.

CONCLUSIONS AND RECOMMENDATION

Majority of cassava women farmers involve in group agricultural activities are in their active and productive ages. The major cassava products produced by women in the study area are gari, fufu, and starch and cassava flour. Planting, harvesting, peeling of cassava, moving of cassava to grater frying, attending meetings are the agricultural activities cassava women farmers in the study area participate in group . The major constraints

faced by the respondents were financial problems, corruption, health challenge, old age and management and leadership problem.

It is highly recommended that cassava women farmers should be encouraged to form farmers' cooperatives or join existing ones to pool their resources together and to take advantage of development assistance, and they should be Strict supervision to ensure that farmers do not divert development assistance to non-farm uses. To enable women cassava processors realize higher benefit from their activities, relevant authority should liaise with researchers to develop appropriate processing technologies to suit local conditions and to satisfy the literacy levels of the respondents

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FARMERS' PERCEPTION OF CLIMATE SMART TECHNOLOGIES IN MITIGATING THE EFFECTS OF CLIMATE CHANGE ON COCOYAM PRODUCTION IN IHI-ALA LGA OF IN ANAMBRA STATE, NIGERIA

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ABSTRACT

The study investigated Perception Of Climate Smart Technologies In Mitigating The Effects Of Climate Change On Cocoyam Production In Ihi-ALA Local Government area Of Anambra State, Nigeria. Multistage sampling procedure was used to select 80 cocoyam farmers. Data was collected through a well-structured questionnaire and analyzed with descriptive statistics and inferential statistics such as regression analysis. Results showed that weather smart technology (81.3%), water smart technology (62.5%), carbon smart technology (37.8) and nitrogen smart technology (18.8%) were the various levels awareness on climate smart technology. The study showed that agronomic practices ($x=3.40$), water management ($x=2.90$) and tillage and residue management ($x=2.85$) were the perception farmers had on climate smart technology. The study further showed increase production output ($x=2.8$), reduced pest infestation ($x=2.78$), controlled disease ($x=2.98$) and increase adaptation/resilience ($x=2.5$) were the various perceived farmers had on climate smart agriculture. The logit regression showed F-value of 19.792 which was significant at 1% and two out of the six coefficient of the explanatory variables were significant at 5% level of probability. From the findings, it concluded that climate smart technologies mitigated effect of climate change on cocoyam production. The study recommend that more efforts must be taken to develop policies on awareness creation, intensify the dissemination process on climate smart technologies in the study area.

Keywords: Climate Change, Climate Smart, Women Farmers, Cocoyam Production

Introduction

Climate Smart Agricultural (CSA) practices has been identified as a key element in the successful response to the threats of climate change to sustainable agricultural production and food security in Nigeria (FAO, 2018). Climate change is becoming a threat to sustainable agricultural production and food security in Nigeria. Farmers need to be more resilient to climate change and produce more food through adoption of Climate Smart Agricultural Practices (Saliu, Uduma, Timothy , Rahman, Sanusi, Enitan and Adebayo ,2017). Climate change and agriculture are interrelated processes, both of which take place on global scale (Climate Education Modules (CEM), 2010). Global warming is projected to have significant impact on conditions affecting agriculture, including temperature, carbon dioxide, glacial run-off, precipitation and the interaction of these elements. There are innumerable potential effects climate change could have on agriculture. It could affect crop growth, quality,

pest control farming practices and the varieties of crops that could be grown in particular climatic areas. These could in turn, affect the availability and price of agriculture products as well as the costs of farming. Climate-Smart Agriculture (CSA) is an approach to help the people who manage agricultural systems respond effectively to climate (2010, 2013). The CSA approach pursues the triple objectives of sustainably increasing productivity and incomes, adapting to climate change and reducing greenhouse gas emissions where possible. Climate-Smart Technology is an explicit consideration of climatic risks that are happening more rapidly and with greater intensity than in the past.

Cocoyam production is commonly described as the women crop in some states in southeast of Nigeria. Chukwu *et al.* (2014) revealed that cocoyam is a generic name for *Colocasia esculenta* (Tara) and *Xanthosomamafafa* (Tennia) which is cultivated for its corms and cormels which are used as edible aroids.

Cocoyam has more food value than yam and cassava in terms of percentage crude protein and essential minerals. Cocoyam corms and cormels are recommended as edible starch for diabetic patients due to its high glycemic property. Cocoyam as food can be eaten for control, prevention and reduction of some incidence and prevalence of health risks associated with high blood pressure, cardiac problems, prostate and breast cancers (Chukwu *et al.*, 2014).

Women farmers are the principal labour force on small holder farms and perform the largest labour in land preparation, weeding, transporting, processing and marketing of agricultural products (Ugboaja, 2013; Odebode, 2012). This is found to be true in the case of cocoyam where women play an active role in cocoyam production, processing and marketing (Onyenobi *et al.*, 2010).

Climate-smart agricultural (CSA) approach was developed to provide this assistance, Ogbonna and Orji (2013) pointed out that cocoyam production has suffered serious neglect due to low yield per hectare and low economic return. Among the reasons given for the decline in cocoyam production as the inability of cocoyam industry to increase output include generally climate variability's which escalate insect vectors causing diseases and pests. Draught associated with climate change causes poor growth and cocoyam development, increase seedling mortality and affect the root size. All these deteriorate quality of produce, reduce quantity produced per year. Inability to combat these anomalies may be due to poor technological approach against climate change variables on cocoyam production in Anambra State. Climate-smart agriculture involves farming practices that improve farm productivity and profitability, help farmers adapt to the negative effects of climate change and mitigate climate change effects, e.g. by soil carbon sequestration or reductions in greenhouse gas emissions (FAO,2013). Climate-smart practices, such as the locally practiced conservation agriculture, aim at conserving soil moisture, retaining crop residues for soil fertility, disturbing the soil as minimally as possible and diversifying through rotation or intercropping (IPCC, 2014;Saliu , Uduma , Jude And Musediku , 2018). As Nigeria population continues to grow and is expected to double by 2050 (reaching the 1 billion mark),the continent will be challenged to meet the food security and nutritional requirements of its people, while also ensuring continued economic growth and sustainable livelihoods on a continent where agriculture is the backbone of many African countries' economies (Clare,2019).

Consequently, Anambra State being part of Nigeria is highly sensitive to variations in climatic factors most especially rainfall, temperature, sunshine, cloud cover,

precipitation, etc. Several views have been given about the impacts of irregularity of climate on crop production; some assert that rural and poor farmers are more affected (*Ekweanya, Iyang and Okiringbo*2017) For these reasons, the study seek to investigate farmers' perception of climate-smart technologies impacts in offsetting these negative effects on cocoyam production in the study area.

The specific objectives were to;
ascertain the level of farmers' awareness of climate smart technology
ascertain the perception of the farmers on climate smart technologies
ascertain the perceived effect of climate change on cocoyam production
determine factors influencing the adoption of climate smart technologies in the study area

Materials and Methods

The study was conducted in Ihiala , a city in [Nigeria](#), located in the southern part of [Anambra State](#), and has long served as the local administrative capital of the Local Government Area in which its located. The Local Government Area has a population of about 87,796 persons with a Coordinates: [5°51'14"N](#)6°51'36"E. Ihiala Local Government Area consists of several town, such as [Amorka](#), [Azia](#), [Lilu](#), [Okija](#), [Mbosi](#), [Iseke](#), [Orsumoghu](#), [Ubuluisuzor](#) and [Uli](#). It lies in the Agricultural belt of the state. Anambra State, which is located in the south-east geopolitical zone of Nigeria. It consist of twenty-one Local Government Areas grouped under four agricultural zones of Anambra State Agricultural Development Programme, these are Awka, Aguata, Anambra and Onitsha zones *Ekweanya, et al (2017)*. It has an approximated land area of 4,416 km²and lies between longitude 6°20N' and 7°00E' and latitudes of 7°16N' and 7° 00E'. Its boundaries are formed by Delta State to the West, Imo State to theSouth, Enugu State to the East and Kogi State to the North (NBS, 2007).

Multi-stage sampling technique was used in the selection of the sample from the population. The first stage involved the purposive selection of four (4) communities out of the total communities in the selected Local Government Area. This was because; cocoyam production is the major agricultural activity in the communities since most of them use the product to prepare soup for traditional marriages and burial ceremony. The second stage involved simple random selection of one (1) village from the number of villages that make up each of the four communities because; most household farmers that produce cocoyam generate income from it. That is they produce not only for household consumption. Data for the study were collected through the use of a questionnaire. Data were

analyzed using mean score and multiple regression analysis.

Decision rule:

A 4-point rating scale of $4+3+2+1=10/4=2.5$

Model specifications

$$Y = \alpha_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + U$$

Where:

Y = Adoption of climate-smart technologies (1= Adoption and 0 = Non adoption)

X₁ = Age (years)

X₂ = Household size (number of people)

X₃ = Level of education (years)

X₄ = Farming experience (years)

X₅ = Extension visits (number of times)

X₆ = Farm size (hectares)

U = Error term

α_0 = Constant term

β_1 - β_6 = Regression coefficients.

RESULT AND DISCUSSIONS
THE LEVEL FARMERS' AWARENESS OF CLIMATE SMART TECHNOLOGY

Table 1: Distribution of respondents according to their level of awareness of climate smart technology in the study area (n = 80)

Climate Smart Technology	Aware (%)	Not Aware (%)
Weather Smart Technology	65 (81.3)	15 (18.8)
Water Smart Technology	50 (62.5)	30 (37.5)
Carbon Smart Technology	5 (37.5)	75 (93.8)
Nitrogen Smart Technology	15 (18.8)	65 (81.3)

Source: Field Survey Data, 2019.

* Multiple respondents were recorded

Table 1 revealed that majority the farmers (81.3%) and (62.5%) were aware of weather and water smart technology respectively while 93.8% and 81.3% claimed no awareness of carbon and nitrogen smart technology respectively in the study. The result shows progressive awareness of some of the climate smart technologies in the study area.

Hassan and Nhemachena (2008) opined awareness is an important determinant of adaptation to climate change effects. Similarly, Maddison (2006) argued that farmers' awareness of climate smart agriculture is important to adaptation decision making. This was further confirmed by Araya and Adjaye (2001) which reported that farmers' awareness and perceptions of climate smart agriculture as a result of changes in climate showed positive effect on crop production.

PERCEPTION OF THE FARMERS ON CLIMATE SMART TECHNOLOGIES

Table 2: Distribution of respondents according to perception of climate smart technologies (n = 80)

Variables	Agreed (4)	Disagreed (3)	Strongly Agreed (2)	Strongly Disagreed (1)	Mean score	Rank *
Agronomic practices (Improved seed varieties, crop rotation, intercropping, cover crop)	51(63.8)	15(18.8)	10(12.5)	4(5.0)	3.41	1 st
Water Management (Irrigation, bunds, terracing, Contouring, water harvesting);	21(26.3)	42(52.5)	9(11.3)	8(10.0)	2.95	2 nd
Integrated Soil Fertility Management (Organic fertilizer, efficient use of inorganic fertilizer)	15(18.8)	47(58.8)	13 (16.3)	5(6.3)	2.90	3 rd
Integrated Pest Management (blend of cultural, biological and chemical control)	10(12.5)	14(17.5)	18(22.5)	38(47.5)	1.95	5 th
Tillage and residue Management (Conservation tillage, incorporation of crop residues)	19(23.8)	42(52.5)	7(8.8)	12(15.0)	2.85	4 th

Source: Field Survey, 2019

Decision Rule 2.0 and above = Perceived

Decision Rule <2.0 = Not Perceived

Values in Parenthesis () are Percentages

The result in Table 2 shows the perception of farmers on climate smart technology. The result showed that agronomic practices ranked 1st with a mean of 3.41 on a four point rating scale, integrated soil fertility management with a mean of 2.90 and tillage and residue management with a mean of 2.85 ranked 2nd and 3rd respectively. This implies that farmers had strong perception on climate smart technology because the means responses were great than the bench mark of 2.5. still, the reason for high perception of improved seed

varieties could be the favourable policy environment for development and distribution of improved seed varieties to farmers. There is a specialized body- National Seed Certification Council of Nigeria - which was established specifically to administered seed release and production in the country. Research and extension activities were also widely focused on breeding of improved varieties which has led to the release of some rice varieties to farmers (NCRI, 2014).

THE PERCEIVED EFFECT OF CLIMATE SMART TECHNOLOGIES ON COCOYAM PRODUCTION

Table 3: Distribution of respondents according to perceived effects of climate smart technology on cocoyam production (N = 80)

Perceived Effects	HI	MI	I	NI	ΣFX	Mean	Rank *
Increased production output	30	20	18	12	228	2.85	2 nd
Reduced pest infestation	28	16	26	10	222	2.78	3 rd
Early maturity	42	18	12	8	146	1.83	6 th
Controlled Disease	30	28	12	10	238	2.98	1 st
Increased livelihood	16	24	6	34	218	2.73	4 th
Increased adaptation/resilience	36	9	22	13	228	2.85	2 nd
Increased soil fertility	26	16	12	26	202	2.53	5 th

Source: Field Survey, 2019. HI = highly intense; MI = moderately intense; I = intense; NI = not intense

Decision Rule: 2.0 and above = Perceived

Decision Rule: <2.0 = Not Perceived

Table 3 shows the result of perceived effects of climate smart technology on cocoyam production. The result showed that controlled disease was ranked 1st with a mean of 2.98, increased production output ranked 2nd with a mean of 2.85, reduced pest infestation ranked 3rd with a mean 2.78 and increased livelihood ranked 4th with a mean of 2.73 respectively. This result implies that Climate-smart agriculture (CSA) is increasingly gaining ground as a valuable tool in tempering the negative

effects of weather-based changes on agricultural output. Vernoooy *et al.* (2018) revealed that through the practice of smart technologies, farmers had good bio-fertilizer for their crops and vegetables. The result also confirmed decrease soil erosion, increase soil fertility, increased productivity, less soil erosion as the effects of practicing climate smart technologies in 2015 by household farmers. These results were observed and/or measured through visual observations mostly.

FACTORS INFLUENCING ADOPTION OF CLIMATE SMART TECHNOLOGIES

Table 4: Regression estimation of factors influencing adoption of climate smart technologies

Variables	Coefficient	T-value	Significance
Constant	-3.027	-0.475***	S
Age(X ₂)	- 0.050	-1.287*	S
Household size(X ₄)	2.904	0.055**	S
Education (X ₅)	0.311	1.436*	S
Extension visit (X ₇)	0.250	1.486*	S
Farm Income (X ₈)	0.450	3.956***	S

Farming Experience (X ₉)	0.466	0.369	NS
R ²	0.667		
Adj.R ²	0.633		
F. value	19.792***		

Source: Field Survey, 2019 *** = Significant at 1% ** = Significant at 5% * = Significant at 10%

Table 4 shows that the coefficient of age, household size, education, extension visit and income were significant at different levels of probability. The coefficients of multiple determinations was 0.667% of the variations in cocoa production level or output and were accounted for the variables included in the model. The F- ratio was significant at 1% indicating the goodness of fit of the model. The regression coefficients of Age, household size, education, extension visit, income, experience were positive and statistically significant at 5%, 10%, and 1% respectively, this implies that the independent variables were positively related to the likelihood of adopting the various options of adaptation of climate smart technology. Increase in farmers’ income enables farmers to adopt recommended adaptative strategies to adjust to challenges of climate change; this is because farmers with high income will be able to produce more cocoyam than the farmers with low income.

This result corroborates with the findings of; Saliuet *al.* (2017) who have noted in their respective studies, that there was a positive relationship between the selected socio-economic characteristics of farmers and their adoption of improved agricultural practices or technology.

Conclusion

The study concluded that the cocoyam farmers were aware of some climate smart technologies and that climate smart technologies were able to mitigate effect of climate change on cocoyam production

Recommendations

the following recommendations were made:
To ensure successful adaptation to climate change,

concerted efforts are needed to design and promote planned adaptation measures that fit into the local context and also educate farmers on climate changes and climate smart technologies which can reduce vulnerability and escalate cocoyam production level per annum.

More efforts must be taking to develop policies on awareness creation; intensify the dissemination process on climate smart technologies.

Policy formulation that will enhance socio-economic conditions of the farmers should be uphold to enable them adopt climate smart technologies and consequently, adapt to the adverse effects of climate change in various agricultural activities.

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REVENUE ANALYSIS OF UNRIPE PLANTAIN BUSINESS IN RIVERS STATE, NIGERIA

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ABSTRACT

This study analysed revenue of unripe plantain business in Rivers state, Nigeria. The specific objectives were to: examine marketing activities of unripe plantain traders in the study area, determine their net returns, Rate of Return on Investment, ascertain determinants of unripe plantain net revenue, describe unripe plantain marketing channels in the study area and examine constraints to the business. A multi stage sampling technique was adopted for the study. Primary data which were collected with a well-structured questionnaire were used. Simple descriptive statistics, mean score, rate of returns and net returns as well as exponential regression function formed the analytical tools used to analyse data collected. The result of the analysis showed that unripe plantain traders in the study area make a profit of ₦1.59 per every ₦1.00 investment in the business. Purchase price was the most important cost variable in the business. It was recommended that roads linking the rural communities where unripe plantain is being produced should be made more motor-able by association members and the government to enhance prompt movement of goods from the farms to the market.

Keywords: Revenue Analysis, Unripe Plantain, Plantain business

INTRODUCTION

Plantain (*Musa paradisiaca*) is one of the major food crops in the humid and sub-humid parts of Africa and a major source of energy for millions of people in these regions. Plantain belongs to the family of banana and is popularly called cooking banana, since it is seldom eaten raw (Adeoye and Oni, 2014). Banana plants are monocotyledonous perennial and important crops in the tropical and subtropical world regions (Cauthen *et al.*, 2013). Plantain is very similar to unripe dessert banana in exterior appearance, although often larger; the main difference being that the flesh of the plantain is starchy rather than sweet as in desert banana and also requires cooking while dessert banana is consumed usually as ripe fruit.

Plantain has become a staple food in Nigeria, just like garri, yam and cassava, and a source of income for subsistence farm families, as the main source of energy and important source of carbohydrate, and play a key role in providing food security in food scarce months when most other starchy staples are difficult to harvest (Akinoyemi *et al.*, 2010). It is an excellent source of substantial amount of recommended nutrients when eaten as food (Baruwa *et al.*, 2011).

Unripe plantain is an economic crop which has a relatively high value in common with most other horticultural crops (Aina *et al.*, 2012). It has the potential to serve as a vehicle for poverty reduction and source of livelihood for a majority of smallholder farmers and traders in Rivers state. Unripe plantain has shown great adaptation to both urban and rural consumption for both humans and animals as feed. The demand and consumption of unripe plantain has risen tremendously in Rivers state in recent years because of the rapidly increasing urbanization and the

great demand for easy and convenient foods by the non-farming urban populations, and besides being the staple for many people, unripe plantain is a delicacy and favoured snack for the people.

Despite the nutritional values of unripe plantain, her role in world plantain economy is relatively minor and does not project a promising outlook. Nigeria does not feature among plantain exporting nations (Akinoyemi *et al.*, 2010). Presently, plantains are of less importance than banana in terms of world trade. The relative attention given to plantain is focused on the technical and productive viability of plantain while little is done on its marketing (Ekboir *et al.*, 2002; Owusu-Benoah *et al.*, 2007).

The plantain business is faced with a lot of marketing problems which determine whether production can be expanded or not (Adetunji and Adesiyani, 2008). Low farm-gate pricing with traders determining prices has been a major hindrance to production and marketing. The marketing of plantain is very difficult because of the dispersal of the production zones, the lack or poor conditions of the lines of communication with urban consumption centers and the irregular supplying in the market by wholesalers and middlemen who set the prices. In addition, perishable produce like plantain suffers from continuous deterioration resulting from poor post-harvest management. This aggravates loss of quality and quantity and thus affects the final price. Furthermore, plantain is a seasonal crop with relative short shelf life hence, it is available for a limited period and postharvest losses are very high. These situations necessitate a scientific survey of the course under study.

Objectives of the Study

The broad objective of this study is to analyse revenue of unripe plantain business in Rivers state, Nigeria, and the specific objectives are to: examine the marketing activities of unripe plantain traders in the study area, determine the net returns of the respondents, determine rate of returns of unripe plantain Investment, ascertain determinants of unripe plantain net revenue in the study area, describe unripe plantain marketing channels in the area, and examine constraints to unripe plantain business in the study area.

RESEARCH METHODOLOGY

The study was carried out in Rivers State, Nigeria. Rivers State is one of the 36 states of Nigeria. Its capital is Port Harcourt. About two thirds of Rivers state lies in the Niger Delta geographical terrain of Nigeria and the state is bounded in the south by the Atlantic Ocean which has a great influence on the its climate. To the North, the state is bounded by Anambra, Imo and Abia States, to the East by Akwa Ibom State and to the West by the Bayelsa and Delta States (NPC, 2006). The inland part of Rivers state consists of tropical rainforest; towards the coast the typical river delta environment features many mangrove swamps (River State Ministry of Information, 2008). Agriculture is the main occupation of the people of Rivers State and the agricultural policy of the state government is anchored on food production. Major crops cultivated in the state include yam, cassava, maize, oil palm, banana and plantain. Plantain is produced in nearly all the local government areas of Rivers State, and the business is also predominant.

Multi stage sampling technique was adopted for this study. In the first stage, purposive selection of two local government areas from the State was made. The essence of the purposive selection is to ensure that the areas were unripe plantain business is predominant was selected. The second stage involved the random selection of two communities from each of the two local governments making it a total of four communities. The third stage involved the random selection of three villages from each of the four communities making it a total of twelve villages, while the last stage entailed the random selection of five unripe plantain marketers in each village making a total of sixty respondents for the study. Unfortunately, out of this 60 distributed questionnaires only forty-four of it were returned by the respondents, as a result, the sample size became forty-four unripe plantain marketers.

Only one sources of data was used for this study which was the primary data. The data was collected by the researcher and some trained enumerators on data collection from the study area with the aid of well-structured questionnaire distributed to the respondents.

Descriptive and inferential statistical tools was used in analysing the objectives of the study. To describe unripe plantain marketing channels in the study area and examine constraints to unripe plantain business in the study area descriptive statistics such as tables, frequency distributions and means were used. Means score of five points likert scale was used in examining marketing activities of unripe plantain traders in the study area. Net returns model was used in determine net returns earned by unripe plantain traders; Rate of Return on Investment formula was used to rate of returns on unripe plantain business, while exponential regression formula helped in ascertaining determinants of unripe plantain net revenue in the study area.

Models Specifications

Mean Score (Likert scale)

Five points likert scale was used in examining marketing activities of unripe plantain traders in the study area (i.e. where activities were graded thus: very high = 5, high = 4, moderate = 3, low = 2 and very low = 1).

Unripe plantain marketers with activity score of 3.0 and above were regarded as haven reached average score to be considered as a marketing activity, while those with activity score of less than 3.0 have no activity or hindered activity.

To determine the mean of marketing activity level = $\bar{X} = \sum X/N$

\bar{X} s of each item was computed by multiplying the frequency of each response pattern with its appropriate nominal value and dividing the sum with the number of respondent to the items. This can be summarized with the equation:

$$\bar{X} = \sum fn/N$$

Where: \bar{X} s = mean score

\sum = summation

F = frequency

N = number of respondents

n = likert nominal value.

Net returns model

Net returns model was used in determining net returns earned by the traders. This model was used by Osuji *et al.* (2012) in determining the net returns earned by the small holder irrigation vegetable farmers in Imo state of Nigeria

The net revenue model is specified as follows:

$$NR = TR - TC,$$

$$TC = TVC + TFC.$$

$$NR = TR - (TVC + TFC)$$

Where:

NR = Net Revenue

TR = Total Revenue

TC = Total Cost (TVC + TFC)

TVC = Total Variable Cost

TFC = Total Fixed Cost.

Rate of Return on Investment

Rate of Return on Investment (ROI) is stated as follows:

$$ROR = TR/TC$$

Where ROR = Rate of Return on unripe plantain business

TR = Total Revenue

TC = Total Cost (TVC + TFC)

TVC = Total Variable Cost

TFC = Total Fixed Cost.

Exponential regression

The model is specified explicitly thus:

$$Y = L_n a + L_n b_1 X_1 + L_n b_2 X_2 + L_n b_3 X_3 + L_n b_4 X_4 + L_n b_5 X_5 + L_n b_6 X_6 + L_n b_7 X_7 + L_n e$$

Where Y = net income of unripe plantain marketers (Naira)

X1 = Sex (1= male, 0= female)

X2 = Marital status (1 married, 0 single)

X3 = Age of the marketers (years)

X4 = Household size (no. of person)

X5 = Marketing experience (years)

X6 = Level of education (no. of years spent in school)

X7 = Social organization membership (1=membership, 0= non membership).

RESULTS AND DISCUSSION

A total number of 60 copies of questionnaire was distributed to the unripe plantain traders in Rivers State. Unfortunately, 44 questionnaire were successfully retrieved by the researcher, thus, the study made use of 44 respondents.

Marketing Activities of Unripe Plantain Traders in Rivers State.

Table 1 shows the marketing activities of unripe plantain traders in Rivers State. The findings revealed that a high proportion of the unripe plantain traders (72.7%) made use of regular marketing activities in terms of supply. The result further revealed that 45.5% and 29.5%, of the traders made use of home market, and home and urban markets respectively as their marketing activities. This implies that the study area was dominated by unripe plantain traders whose marketing activities were mostly regular at homes and urban market. This finding is in agreement with the findings of Ogazi (2006) that regular supply rate was the marketing activity of traders pointing out that it brings about steady supply of product and yield more profit to the trader.

Table 1: Distribution of respondents according to Marketing Activities

Marketing Activities	Frequency	Percentage
Supply Rate:		
Irregular	12	27.3

Regular	32	72.7
Total	44	100
Point of sales:		
Home market	4	9.1
Home and urban market	20	45.5
Street hawking	7	15.9
Urban market	13	29.5
Total	44	100

Source: Field Survey, 2018

Net Returns of Unripe Plantain Traders in Rivers State.

Table 2 shows the estimated net revenue of unripe plantain business in the study area. The table shows that transportation, damage during transit, rent and purchase price were the major costs undertaken by the traders. The purchase price was the most important cost variable in the business, and an average unripe plantain marketers realized a net revenue of ₦83,674. This result implies that unripe plantain business in the study area was profitable and generated a good revenue. This finding is in line with the findings of Cramer and Hense (2013) that plantain business is a profitable business.

Table 2: Distribution of respondents according to the Net Returns earned in the study area (n = 44)

Variable	Quantity (bundles)	Unit Price	Total Amount (₦)
Revenue			
Capital			49,000
Sales	132	1360	179,520
Total Revenue			223,520
Cost			
Purchase price	132	825	109,296
Rent	44 stores	200	8,800
Transportation			6,900
Damage during transit	18	852	14,850
Total Cost			139,846
Net Revenue			83,674

Source: Field Survey, 2018

$$\text{Net Revenue (NR)} = \text{Total Revenue (TR)} - \text{Total Cost (TC)}$$

$$\text{Thus Net Revenue (NR)} = \text{₦}223520 - \text{₦}139,846 = \text{₦}83,674$$

Rate of Return on Unripe Plantain Investment

Table 3 revealed the rate of return on unripe plantain investment as ₦1.59. This implies that the traders made profit of ₦1.59 per every ₦1.00 investment in the business. The finding further revealed that plantain business in the area yielded returns to the traders. This is in line with the findings of Jeng (2000) who opined

that the rate of return in plantain business was approximately ₦2.5 which accounted to lots of traders to in the business.

Table 3: Distribution of respondents according to rate of return on unripe plantain investment in the study area.

Variable	Amount (₦)
Revenue	223520
Cost	139,846
Rate of Return (ROR)	1.59

Source: Field Survey, 2018.

Determinants of Unripe Plantain Net Revenue in the Study Area.

Table 4 showed the exponential regression results of the multiple regression analysis on the determinants of unripe plantain net revenue. The results revealed the F-ratio of 6.702 which is highly statistically significant at 1.0% level of significance. This implied that the variables included in the model had line of best fit. The result also revealed a correlation coefficient (R) of 0.755 as well as coefficient of determination (R²) 0.626. The coefficient of multiple determinations (R²) of 0.755, indicates that 75.5% of the variation in net revenue was explained by the dependent variables in the model while the remaining 24.5% was due to error of estimates.

Sex (10.0%), age (10.0%) and education level (1.0%) negatively determined revenue of unripe plantain in the study area, while association membership (5.0%) positively determined the revenue of the traders. The implication is that as the number of male traders, their age and the more their educational levels increase, the lesser their revenues in unripe plantain business, whereas the more their membership associations to groups, the more their revenues. This conformed to a priori expectation, because union involvement increases participation on every trade (Ugwumba, 2009), and traders who are association members are better placed to participate fully in trades than non members (Nze, 2016). Ogazi (2006) opined that there is a relationship between net revenue and determinant factors such sex, age, membership of cooperatives and educational qualification.

Unripe Plantain Marketing Channels in the Study Area.

Table 5 revealed that majority of the unripe plantain marketers (56.8%) were retailers, 38.6% were wholesalers and 4.5% were farm gate collectors. This implies that unripe plantain marketers in the study area were mostly retailers. This shows why the business is attracting lots of easy entrants. This finding is line with that of Nze (2016) that retailers are mostly involved in the marketing of agricultural product.

Table 4: Exponential Regression Analysis on the Determinants of Unripe Plantain Net Revenue in the Study Area.

Explanatory variables	Linear	T value
Constant (β_0)	1914.436	(2.650)***
Sex (x_1)	-152.913	(1.892)*
Marital Status (x_2)	-12.640	(-0.062)
Age (x_3)	-8.093	(-1.938)*
Household size (x_4)	-26.519	(-0.342)
Years of experience (x_5)	5.897	(0.169)
Education level (x_6)	-28.329	(-2.769)***
Association membership (x_7)	264.487	(2.261)**
R ²	0.755	
R ⁻²	0.626	
F-ratio	6.702***	

Source: Field survey data, 2018

Note: values in parenthesis, (), are the respective t-test ratio.

***, ** and * implies statistical significance at 0.01, 0.05 and 0.10 probability levels respectively.

Table 5: Distribution of respondents according to Marketing Channels in the Study Area.

Marketing channels	Frequency	Percentage
Farm gate collector	2	4.5
Retailer	25	56.8
Wholesaler	17	38.6
Total	44	100

Source: Field Survey, 2018

Constraints to Unripe Plantain Business in the Study area

Table 6 showed that majority (75.0%) of the respondents attested that consumer behaviour is among the constraints faced in the unripe plantain business. This finding is in line with that of Adetunji and Adesiyani (2008) that consumer behaviour affects plantain business. Most of the respondents, 93.2%, were constrained by purchase issues. According to Cauthen *et al.* (2013) purchase issues affects plantain business. Some of the traders, 63.6%, 68.2% and 56.8% of the unripe plantain traders attested that inadequate capital, transportation cost and storage problem were their major problems. It was also seen that majority (93.2%) of the respondents reported they were faced with the problem of bulky nature of the produce. 84.1%, 72.7% and 68.2% of the respondents said that they faced the problem of theft, perishability and seasonality respectively in their unripe plantain business. This findings is in tandem with Aina *et al.*

(2012).

CONCLUSION AND RECOMMENDATIONS

Haven analysed unripe plantain business in Rivers state, Nigeria, it was concluded that the business is profitable in Rivers State, with diverse marketing channels, determining factors such as age, sex and association membership, and constraints such as Consumer behaviour, purchase constraints, inadequate capital, transportation cost, bulky nature of produce, storage problem, theft, perishability, competition and seasonality, among others. It was recommended that members of the associations should assist in ensuring good transportation system in the area in order to linking the rural communities where unripe plantain is being produced to urban markets. Special vehicle that could help in curbing quantitative and qualitative losses should be made available for the business. Programmes that will improve plantain marketing should be organized for the marketers by relevant government or non-government agencies, and researchers should assist in devising means of unripe plantain storage to minimize spoilage.

Yes	37	84.1
No	7	15.9
Perishability		
Yes	32	72.7
No	12	27.3
Competition		
Yes	28	63.6
No	16	36.4
Seasonality		
Yes	30	68.2
No	14	31.8
Total	44	100

Source: Field Survey, 2018

Table 6: Distribution of respondents according to constraints facing unripe plantain business in the study area

Constraints	Frequency	Percentage
Consumer behaviour		
Yes	33	75.0
No	11	25.0
Purchase constraints		
Yes	41	93.2
No	3	6.8
Inadequate capital		
Yes	28	63.6
No	16	36.4
Transportation cost		
Yes	30	68.2
No	14	31.8
Bulky nature of produce		
Yes	41	93.2
No	3	6.8
Storage problem		
Yes	25	56.8
No	19	43.2
Theft		

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EMPIRICAL EVIDENCE ON CREDIT RISK ATTITUDE OF MALE AND FEMALE AGRIBUSINESS
ENTREPRENEURS TOWARDS FORMAL AGRICULTURAL LOANS IN SOUTH EAST NIGERIA

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Abstract

This study focused on empirical evidence of credit risk attitude of male and female agribusiness entrepreneurs towards agricultural loan or credit from formal financial sources in south east Nigeria. Multi stage random sampling method was used in sampling for respondents. Data were collected from primary source. A total of 360 agribusiness entrepreneurs respondent were interviewed using a well structured questionnaire. To analyze their credit risk attitude towards formal sources, multinomial logit model was employed. Results showed that the model was well fitted. The variables: age, income level, experience, education, number of loan applications, length of lending relationship and cooperative membership all showed significant relationship at different levels indicating the effect these variables have on the agribusiness entrepreneurs attitude towards credit risk from formal sources. Agribusiness entrepreneurs are encouraged to improve their educational level as this will help enlighten them about business management, credit and financial risk and also join cooperative. Cooperative membership enhances access to credit and foster a risk seeking attitude among the cooperator

Keywords: Credit risk attitude, Banks, agribusiness entrepreneurs.

Introduction

Ojo (1998) defined agricultural credit as all financial assistance in form of loans and advances given to farmers or other beneficiaries of agricultural reform to finance and service product activities relating to agriculture. The role of bank loan in financial development has been accepted and productive as credits are obtained by the various economic agents to ensure investment operating expenses and at the long run bring about economic development. Ademu (2006) said that availability of credit to a reasonable extent with adequate involvement of the price system is one of the ways to generate self employment opportunities. This is because credit helps to generate and maintain a reasonable business size, expand the business and at the long run bring about efficiency. In Nigeria, governments had recognized the importance of credit to agribusiness entrepreneurs and have formulated and organize credit policies, programmes that favours agriculture and also created institutions which ensures that agribusiness entrepreneurs acquire credit. The Agricultural credit insurance Fund is among the policy interventions for agricultural financing in the country. They are created especially for agricultural support for farmers and agribusiness entrepreneurs in Nigeria. (Akpan, Obot, and Ubon 2012).

According to Appa (1996), Risk management is the human activity which integrates recognition of risk, risk

assessment, developing strategies to manage it, and mitigation of risk using managerial resources. In terms of gender assessment, female agribusiness entrepreneurs are risk-averse and take minimum risk especially in the area of funding for their businesses more than the male (Wanger, 2001). Existing empirical research on gender and credit risk suggests females are more risk-averse than male entrepreneurs. This finding is particularly strong in studies that measure risk through financial resource allocation experiments (Eckel and Grossman, 2003). Existing literature exploring the causes of females risk aversion indicates that “females appear more fearful of losses” and more “pessimistic” than males financial scenario experiments about allocating money suggest females may weigh risk disproportionately relative to reward. The more money allocated to options with higher possible returns and lower probability outcomes, the “riskier” the behavior (Harrant and Vailant, 2008).

Irrespective of the numerous benefits associated with agricultural loans in terms of employment generation, maintaining a reasonable business size and general promotion of agricultural sector in the economy, agribusiness entrepreneurs are not fully utilizing these opportunities because of the risk associated with loans. Formal agricultural credit fail to cater for the credit needs of the poor agribusiness entrepreneurs for some reasons like high interest rates attached to the loans and more administrative cost in processing the loans. Nweze

(1995) is of the view that banks require collateral, which the entrepreneurs find difficult to provide. Again, their procedure of filling the loan request forms and other formalities for accessing loans are too complex for the illiterate small scale and resource poor farmers. They also prefer handling large loans rather than the petty loans that these Agric entrepreneurs need and most times their loans are not granted. These entrepreneurs term these experience as risky and time wasting since they hardly achieve their aim of accessing loans. However, the general experience of the small farmers credit programmes, which has long been popular with governments in most developing countries such as Nigeria, has had limited progress reaching the target group. The small scale farmers however, have access to the informal source of credit. These informal sources include money lenders, Isusu, friends, traders, relatives etc (Nosike 1996). Watson and Robinson (2003) opined that female entrepreneurs show higher display greater credit risk avoidance than male entrepreneurs. Therefore, the former have a lesser propensity to seek indebtedness, or they are less fascinated by firm increase but more interested in control (Carter and Shaw, 2006). Female entrepreneurs would possibly use less funding from external sources than male ones due to the fact that they are less inclined to lose total control over their firms than male entrepreneurs (Constantinidis and Comet, 2005; Verheul and Thurik, 2000).

The main objective of the study was to examine male and female entrepreneur's attitude towards credit risk from formal finance sources of loan to agribusiness firms. Specifically, the study determined the respondents' attitude to credit risk from formal finance sources and estimates the factors determining agribusiness entrepreneur attitude towards credit risk from formal sources.

Methodology

The study was carried out in South Eastern Geo-Political Zone of Nigeria. The Zone lies between latitude 6°E and 9°N and 4°E and 7°N longitude, has a total land mass of 10,952,400ha. The zone has over 16 million resident populations (NPC, 2016) and made up of five states viz: Abia, Anambra, Ebonyi, Enugu and Imo States. Agriculture is a major occupation of the people in the zone. Most agribusiness ventures are found in urban areas like Aba and Umuahia in Abia state, Owerri and Okigwe in Imo state, Enugu in Enugu state, Onitsha in Anambra state and Abakaliki in Ebonyi state (Onwumere, 2010).

The study adopted a multi stage sampling procedure in sampling for respondents. In the first stage, three out of the 5 states (Abia, Imo, and Anambra) in the South-east geo-political zone was randomly sampled for the study.

In the second stage, two Agricultural Zones from each state were randomly selected giving a total of six agricultural zones. In the third stage, two Local government areas were randomly selected from each zone giving a sample of 12 LGAs. In the fourth stage, three communities were selected randomly from each local government areas giving a sample of 36 communities. At the last stage about 10 agribusiness entrepreneurs were randomly selected from each of the community giving a total of 360 respondents. Data for the study were collected from primary sources. Primary data were collected by the use of structured questionnaire, which were administered on sampled agribusiness entrepreneurs. The instrument was designed to collect a range of information, which entails information about gender of the entrepreneur, age, educational background, type of company, location of firm, staff capacity, sales and profit made in business and loan applications procedures from the selected Banks. Data collection was done by the researcher and some enumerators selected from the study area and trained to assist in the administration of data collection instruments.

Method of data analysis: To examine Agribusiness Entrepreneurs' attitude towards credit risk from formal finance sources, was realized in line with their responses to three different contextualized business-related statements. Contextualized methods used to measure an individual's risk attitude as well as to investigate patterns in decision-making processes was as applied, in Weber *et al.* (2002), Fausti and Gillespie (2006) and Mann *et al.* (1997). Participants selected from the following business-related, contextualized statements:

- 1 'I am willing to borrow money from formal financial sources on reducing the entrepreneurial risk of success because too high entrepreneurial risks cause me a lot of worry'.
- 2 'I am not willing to borrow money from formal financial sources on changing the entrepreneurial risk of success because I do not care about the risk'.
- 3 'I am willing to borrow money from formal financial sources on increasing the entrepreneurial risk of success because I like to take on entrepreneurial risks in general'.

The formulation 'entrepreneurial risk of success' which is included in each of the three statements above, establishes a concrete contextual reference. If the participant agrees with statement (1), it can be concluded that he/she is risk-averse. An agreement with statement (2) implies risk-neutrality, while statement (3) stands for risk-seeking behaviour. To examine the factors affecting agribusiness entrepreneurs' attitude towards credit risk from formal finance sources, the multinomial logit model was employed.

Multinomial logit regression for risk attitude analysis

The generalized multinomial model is expressed as (Babcock *et al.*, 1995):

$$P_{ij} = \frac{e^{B_j X_i}}{1 + \sum_{k=0}^j e^{B_k X_i}} \dots\dots\dots (1)$$

The probability that the *i*th entrepreneur belongs to the *j*th risk behavior group reduces to:

$$P_{ij} = \frac{e^{B_j X_i}}{1 + \sum_{k=j}^j e^{B_k X_i}} \dots\dots\dots (2)$$

While the probability of being in the base outcome group or group 0 is

$$P_{ij} = \frac{1}{1 + \sum_{k=0}^j e^{B_k X_i}} \dots\dots\dots (3)$$

Where;
i = 1, 2n variables;

- k = 0, 1, 2 groups;
- B_j = a vector of parameters that relates X_i's (independent variables) to the probability of being in group j where there are j+1 groups.
- X₁ = Age of entrepreneur (years)
- X₂ = Education (years spent in school)
- X₃ = Income level (₦)
- X₄ = Firm size (Value of sales)
- X₅ = Business type (1=Sole proprietorship, 0 = partnership)
- X₆ = Business experience (years)
- X₇ = Number of loan applications
- X₈ = Length of lending relationship (Years)
- X₉ = Cooperative membership (Yes=1, No=0)

RESULTS AND DISCUSSION

Figure 1 Sector of Activities

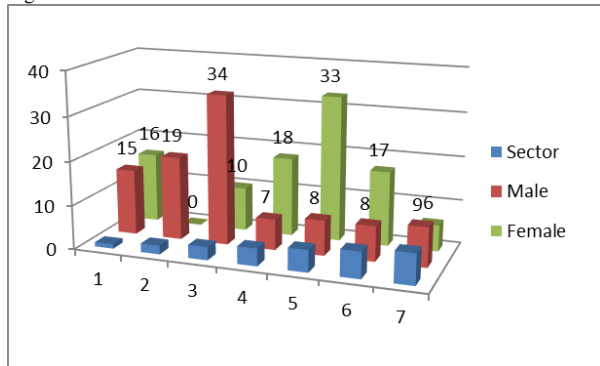


Figure 1 Sector of Activities

Figure 1 explained the different sectors of activities of the agribusiness entrepreneurs used as sample for the study. These sectors were grouped into seven (7). Group (1) are livestock entrepreneurs, group (2) forestry entrepreneurs, group (3) crop production entrepreneurs, group (4) fishery entrepreneurs, group (5) food services (bakery and fast food), group (6) poultry entrepreneurs and finally group (7) agro-production (fertilizers and feeds). Result showed in term of the distribution of the firms by area of specialization that majority of the male-owned firms were into crop production (34%), followed by forestry (19%) and livestock (15%). For the female-owned agribusiness entrepreneurs firms, majority were into food services (33%), fish-farming (18%) and poultry (17%). This finding re-validates the age-long assertion that males are the dominant force in agriculture. Summarily, the study shows that the firms are broadly categorized into farm and non-farm for the male and female-owned agribusiness firms respectively.

Determinants of Entrepreneurs credit risk attitude

In this section, borrower-lender related factors that show agribusiness entrepreneurs' attitude towards credit risk is examined in line with their response to three different contextualized business-related statements. Contextualized methods to measure an individual's risk attitude as well as to investigate patterns in decision-making processes had applied, for example, in Weber *et al.* (2002), Fausti and Gillespie (2006) and Mann *et al.* (1997). The multinomial logit model was employed after the respondents were categorized into risk-averse, risk-seeking and risk-neutral based on gender differences.

The risk-neutral was chosen as the base/reference category. The result of the diagnostic statistics shows that the model is well fit. The log-likelihood ratio (LR) statistic (-556.66, -278.36 and -379.4) was significant at 1% for the male-owned, female-owned and pooled firms

respectively, meaning that the explanatory variables included in the model jointly explained the probability of accessing loans on collateral basis. Similarly, the pseudo R² of 0.679, 0.635 and 0.812 implies that 67.9%, 63.5% and 82.1% changes in the likelihood event of accessing

loans on collateral basis by the male-owned, female-owned and pooled firms respectively was accounted for by changes in the independent variables included in the model and the result presented in Table 1

Table 1: Multinomial logistic regression estimates of agribusiness entrepreneurs' determinants of credit risk attitude

	Male Risk averse	Risk- seeking	Female Risk averse	Risk- seeking	Pooled Risk averse	Risk- seeking
Entrepreneurs' age	-2.059**	-1.57	4.25***	-0.60	-4.29***	-4.66***
Education (Years)	-2.21**	0.22	-2.56**	1.63	-0.45	0.03
Income level (N)	-2.14**	0.37	2.93**	-3.66***	-2.67**	-0.83
Firm size (sole proprietorship =1 others =0)	-2.63	1.17	0.04	-1.78*	0.26	-1.21
Experience (Yrs)	-2.73**	0.96	-2.51**	1.06	-2.04**	1.62
No. of Loan apps.	0.59	-2.08**	1.16	-3.47***	1.26	-2.82**
Length of lending	-3.71***	-1.58	0.30	0.27	-4.36***	-1.94*
Coop. membership	-0.39	2.39**	0.65	3.20***	-2.51**	2.26**
Constant	3.39	0.79	-4.63	-0.26	5.49	3.89
Observations	360.000		180.000		180.000	
LR Chi ² (10)	-556.66		-278.36		-379.4	
Prob> Chi ² (10)	0.000		0.000		0.000	
Pseudo R ²	0.6794		0.635		0.812	
Log likelihood	-131.333		-80.057		-43.948	

Source: Field survey, 2018.

***, ** and * significant at 1, 5 and 10% levels of significance respectively.

The result showed that age of entrepreneur was negative for the male-owned agribusiness firms and pooled sample risk averse category but positive for the female-owned firms while negative for the pooled risk-seeking category. The negative coefficient implies that advancing in age breeds courage and reduces the fear of business failure; thus, as age increases, male entrepreneurs get more involved in riskier investments irrespective of the possibility of business failure and consequent inability to repay loans. The positive coefficient for risk-averseness implies that female entrepreneurs will become more risk averse as they grow older.

Sepúlveda and Bonilla (2014) observed that age affects fear of failing quadratically. When young, a person experiences an increase in the probability of feeling a fear of failing for each year that his age increases. However, their study was not conducted on gender basis and as such the positive pool effect of the female entrepreneurs would have exceeded the negative effect of the male entrepreneurs. The negative pooled effect in this study suggests that males had a stronger pool on the age-risk relationship. This negative coefficient agrees with the findings of Haneishi, Maruyama, Takagaki, and Kikuchi (2014); Dadzie and Acquah (2012); Aye and Oji (2007); Nzenwa (2015); Shahabuddin, Mestelman, and

Feeny (1986) and Binswanger (1980) who observed that older people having dealt much more in risky economic games at high stakes might be more willing to take risks at high levels than young people. Age may also be indexing for the wealth status of the household and accumulation of social capital as older entrepreneurs are more likely to have accumulated more wealth than younger farmers. Moreover, as posited by Aye and Oji (2007), older entrepreneurs are more likely to have greater wealth, social capital and networks which serve as some form of traditional insurance or fall-back strategies in the process of decision making. Yesuf and Bluffstone (2007) and Lucas and Pubuayon (2012) were of contrasting thoughts, arguing that older entrepreneurs are less willing to take risks than their younger counterparts.

The coefficient of education showed a negative relationship with risk averseness for all categories except that it was not significant for the pooled sample. This result implies that as entrepreneurs acquire more education, they tend to be more risk seeking and in relation to this study, they could make risky investments despite the possibility of business failure and inability to repay loans. This buttresses the findings that at low game levels, education variable had little influence on risk

aversion, but at higher game levels, it generally reduced the level of risk aversion while increasing risk preference simultaneously (Binswanger, 1980; Dazie and Aquah, 2012). As indicated by Nmadu, Eze, and Jirgi (2012), more educated agribusiness entrepreneurs would have acquired the knowledge base that is necessary to understand the nature of risk and the various technologies available to fight it which will of course encourage risk seeking. This result is also consistent with Nzenwa (2005) and conforms to the *a priori* expectation that the more educated respondents will be more willing to bear risk than the less educated ones relative to risk-neutrality.

The income coefficient was significant and negative for the risk-averse male-owned and pooled sample categories respectively while positive for female-owned risk averse. The negative coefficient implies that risk-averseness decreases with increase in income and vice-versa that is, as male entrepreneurs acquire more income, they tend to become more risk-seeking as evidenced by the positive coefficient. On the other hand, female entrepreneurs tend to become risk averse as they acquire more income. This stands to reason that the lower enterprise's gross income, the more risk averse it will be. Hence, all other things being equal, enterprises whose incomes fall would be less willing to take risk than those whose incomes are higher because such entrepreneurs will be afraid of making risky investments in order to avoid business failure. The study result is consistent with the findings that weak income entrepreneurs are more risk averse than wealthy ones and as such avoid prospects in which the probability of failure looms large (Mosley and Verschoor, 2003; Lamb, 2003 and Dazie and Aquah, 2012).

The result further showed that firm size had a negative relationship with the risk-averse male agribusiness entrepreneurs and risk-seeking female agribusiness entrepreneurs. The negative relationship implies that increase in firm size is an indication of risk-seeking attitude of entrepreneurs and vice-versa. From the foregoing, male entrepreneurs have been shown to be more risk-loving than the female entrepreneurs. It is expected that increasing firm size will lead to an increased output and consequently, increased market share and profit. On the other hand, female-entrepreneurs showed a different outcome.

The result is in line with the findings of Ajetomobi and Binuomote (2006) that having more areas to explore will increase the vulnerability to bear higher risks. According to Sepúlveda and Bonilla (2014), the adverse effects caused by increased attitude toward risky undertakings by large scale operators are mitigated by the ability of operators to implement thorough risk analysis. However,

the effects towards risky undertakings can have serious implications among financial institutions that cater for the entrepreneurs because default is likely to occur at higher rounds of loaning, which normally involves larger amounts than the initial loan.

Firm type was positively related to the risk-seeking attitude of male entrepreneurs. Firm type was dummied 1 for sole proprietorship and 0 for others. The positive relationship means that male sole proprietors are risk-seeking while female sole-proprietors are risk-averse. The majority of the female-owned agribusiness firms are self-owned and since they are the decision makers and considering their risk attitude, they are less likely to take risky investments to avoid losses of investment capital.

Years of business experience negatively impacted on the risk-averseness of all the sample categories (male, female-owned and pooled) implying that experience entrepreneurs irrespective of their gender are willing to take risks. Experienced entrepreneurs have proper knowledge of input-markets, resource allocation and market intricacies. These factors form strong backgrounds to involve in risky investments. It is expected that with growing experience in business, the entrepreneur is able to better understand the production technology and its associated challenges. This finding agrees with Adeyinka *et al* (2015) and Ogoke (2009) who reported that the longer the years of entrepreneurship, the more efficient the entrepreneur becomes because the number of years an entrepreneur has spent in business may clearly give an indication of the practical knowledge he has acquired. This is an advantage to boost production in any pre-determined period in business through increased investment, expansion of firm size, use of improved technologies, etc.

Number of loan applications had a negative relationship with risk-seeking attitude of all the sample categories implying that entrepreneurs who have several numbers of applications are less likely to be risk-seeking. This is because multiple loan application could be an indication of previous application failures and such entrepreneurs would not be willing to invest in risky businesses due to fear of failure and possibility of loan default. The consequence is that they may not be able to access loans from such financial institutions. On the other hand, years of lending relationship had a negative effect on the risk-averse attitude of male and pooled sample entrepreneurs implying that male-entrepreneurs who have long lending relationship with financial institutions are less risk-averse as they have been able to manage previous lending activities and repaid when due, thus, building a healthy lender-borrower link.

Cooperative membership variable was significant and positively related to the risk-seeking attitude of all the entrepreneur categories implying that membership encourages risk-seeking behaviour. According to Nmaduet *et al.* (2012), it is thought that when entrepreneurs associate with members in similar social and economic status, the knowledge base for dealing with risks associated with economic production environment is enhanced thus boosting risk seeking abilities. According to Nzenwa (2005), cooperative membership enhances access to credit and other production inputs and this is expected to foster a risk-seeking attitude among the co-operators. There is also evidence that females tend to be more risk averse than men when confronting financial decisions (Jianakoplos and Bernasek, 1998). Previous researches have also documented that females are less willing to sort into relatively risky schemes (Niederle and Vesterlund, 2007). According to Dohman *et al.* (2014), gender difference in willingness to take risks could be part of the explanation for this important difference in behaviour. In fact, another study also found females prefer less risky compensation over risky ones (Dohmen and Falk, 2006). They found that lower willingness to take risks among females explains a substantial part of the gender difference in sorting decisions.

Conclusion

From the result is concluded that older male entrepreneurs are more involved in risky investments irrespective of possibility of business failure and consequent inability to repay loans than the female entrepreneurs. More educated agribusiness entrepreneurs are more risk takers. They are willing to make more risky investment despite the possibility of business failure. The level of income of the entrepreneurs also determines the ability and credit risk attitude of the entrepreneur in considering applying for agric loan. Other variables like firm size, firm type, years of business experience, number of loan applications, and cooperative membership were significant and affected the entrepreneur credit risk attitude from formal financial sources.

Recommendations

Based on the findings it is recommended that young entrepreneurs should enroll in financial risk management programs that will help enlighten them on the consequences of lack of financial risk and proper management. Education is very important the more educated entrepreneurs are willing to make risky investments because they understand how to limit the level of uncertainty that comes with credit risk from formal financial sources. Finally, agribusiness entrepreneurs are encouraged to join cooperatives

according to their sector of activities. Cooperative membership encourages risk seeking behavior. Through cooperative they are enlightened on basic knowledge for dealing with loans and credit from financial institutions. It enhances access to credit and other production inputs. This encourages risk seeking attitude among cooperators.

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