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FOREWORD

I am pleased to put into the hands of readers Volume-3; Issue-5: Sept-Oct 2018 of **"International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)",** an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from CrossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

I thank all the authors of the research papers for contributing their scholarly articles. Despite many challenges, the entire editorial board has worked tirelessly and helped me to bring out this issue of the journal well in time. They all deserve my heartfelt thanks.

Finally, I hope the readers will make good use of this valuable research material and continue to contribute their research finding for publication in this journal. Constructive comments and suggestions from our readers are welcome for further improvement of the quality and usefulness of the journal.

With warm regards.

Editor-in-Chief
Date: Nov, 2018

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Molecular characterization and DNA fingerprinting of some local eggplant genotypes and its wild relatives

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Abstract**—** *Collection and characterization of local genotypes and landraces are prerequisite for any crop improvement program. Molecular diversity and DNA profiling shown exact genetic blue print of any crop. Hence, the experiment was design to establish the molecular diversity and polymorphism among some local eggplant genotypes and its wild relatives for future breeding program. The experiment was carried out at the Biotechnology Laboratory, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, with twenty-five local and two wild relatives (Solanum sisymbriifolium and S. villosum) of eggplant to study molecular diversity and DNA fingerprinting at those genotypes. Five well-known SSR primers (EPSSR82, smSSR01, EM114, EM120 and smSSR04) were used for the molecular characterization of the genotypes. Quality DNA was isolated with 27 genotypes and PCR amplification was carried out with these primer. The amplified DNA fragment was visualized by 2% agarose gel and data were analyzed by POWERMAKER (version 3.25) and NTSYS-PC (version 2.2). Some total at 10 different alleles were generated with a range of 1 to 3 alleles per locus and an average of 2.0 alleles. The highest number (2) of polymorphic bands was observed in the primers EPSSR82 and smSSR01. The Polymorphism Information Content (PIC) of SSR markers ranged from 0.37 to 0.67 with an average value of PIC = 0.54. Gene diversity ranges from 0.49 (smSSR01) to 0.72 (EPSSR82), with an average value of 0.61. UPGMA method separated the of 27 genotypes into two major clusters (I and II). From the clusters, wild species Solanum villosum belonged to the sub-cluster (IIb), that revealed its distinct variation from the others. On the other hand, wild species Solanum sisymbriifolium showed a close relatedness by forming the same cluster together with thirteen local eggplant genotypes. Molecular diversity and DNA profiling was identified among 25 local eggplant germplasm and its wild relatives. The finding of the experiment could be used for selection of diverse parent for eggplant improvement.*

Keywords**— eggplant, molecular diversity, SSR marker, wild relatives.**

I. INTRODUCTION

Eggplant (*Solanum melongena L.* 2n = 2x = 24) belongs to the plant family of Solanaceae. It is the sixth most important vegetable after tomato, watermelon, onion, cabbage, and cucumber and the most important *Solanum* crop native to Asia [1]. Eggplants have a remarkable demand and are considered as the second important vegetable crop after potato in Bangladesh [2]. As eggplant is a native plant of Indian sub-continent which surely can define its abundance in this region. Though it is cultivated almost all over the country its production is not as good as expected for being an ancient plant of this region. In the year 2014-15, total area devoted to eggplant cultivation was 1,22,014 acres with annual production of 4,50,146 metric tons [2]. Eggplant has a number of health benefits. It is an important source of fiber, potassium, manganese, as well as vitamins C, K, and B6. Phenolic compounds in eggplant contain significant amounts of chlorogenic acid, one of the most powerful free radical scavengers found in plants. Chlorogenic acid has been shown to decrease low-density lipid (LDL) levels, and also serves as an antimicrobial, antiviral, and anti-carcinogenic agent. Despite eggplant's economic importance, its improvement and molecular study of different land races, local genotypes and germplasm characterization was not well studied. The development of new eggplant varieties addressing old and new breeding objectives requires of genetic diversity [3, 4, 5]. Collection and characterization of genetic resources and local cultivars are required for the improvement of new varieties. SSR markers for eggplant have been developed in the recent years and are being mainly used for assessing the genetic diversity and genome

similarity in the related species [6,7]. Co-dominant markers such as simple sequence repeat (SSRs) could generate more information and has high repeatability than other dominant markers like RAPD or AFLP [8,9]. SSRs have proved as a more powerful marker than AFLPs to study the relationships amongst closely related eggplant materials [10]. SSR markers are multi-allelic, highly abundant, well distributed in the genome and are suitable for high throughput PCR which makes them ideal for molecular diversity studies [11]. Genetic diversity assessment is very important to identify groups with similar genotypes and to conserve, evaluate and utilize the genetic resources. The diversity of the germplasm can be used as a potential basis of genes that lead to improved performance of the superior cultivars and can also be used to determine distinctness and uniqueness of the phenotypes and the genotypes. Wild species remain largely unexploited for eggplant breeding. *S. villosum* and *S. sisymbriifolium* are two wild relatives of Solanum *melangena* which showed considerable resistance to bacterial wilt. So, the study was focused on genetic diversity of some local eggplant germplasm through SSR marker to generate more information and to assess relatedness among local landraces and also with their wild relatives.

II. MATERIALS AND METHOD

Collection of material

A sum total of 27 materials were used in the study and among those 25 were local eggplant genotypes and 2 were wild relatives viz. *Solanum villosum and S. sisymbriifolium*). Germplasms were collected from different districts of Bangladesh. A list of local germplasm and their collected area was given in Table 1. Wild relatives were collected from the Gene Bank of Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The experiments were carried out at the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh.

Seedling raising

Good quality, disease free, healthy seed were sown in plastic pots and kept in nets house. All management practices were done for raising quality seedlings from those materials. Fresh leaves were collected at 3-4 leaf stage of plant for isolation of DNA.

Extraction and quantification of DNA

Total genomic DNA from each genotypes was isolated by CTAB method with slight modification [12]. The extracted DNA was purified by propanol and treated with 10µg/ml RNase A for 20-25 minutes at 37°C to remove the RNA. The purified DNA was dissolved in TE buffer and quantification of DNA was done through electrophoresis on 1% agarose gel staining by ethidium bromide. The sample DNA were stored at -20°C freezer for further use.

Primer selection and PCR amplification

Five SSR primers were selected on the basis of previous works to evaluate the molecular polymorphism study the eggplant local genotypes and wild relatives. PCR reaction was performed using BIONEER KIT (Korea). The PCR reaction having 20.0 µl mixture containing with 3.0 µl sterile de-ionized water, 4.0 µl 10X PCR buffer, 4.0 µl enzyme dilution buffer, 3.0 µl 20 mM $MgCl_2$, 1.0 µl dNTPs (10mM), 0.5 µl top DNA polymerase, 2.5 µl primer (forward and reversed) and 2.0 µl sample DNA (approx. 40-50 ng). The reaction mixture was subjected to the following thermal profile for amplification in a thermocycler: 5.00 min at 95°C for initial denaturation, followed by 33 cycle of 1.00 minutes denaturation at 94°C, 1.00 minutes at annealing with various temperature according to primer melting point and 1.30 minutes at 72°C for extention. A final extension step was done at 72°C for 7 minutes. Electrophoresis was done to visualize the PCR amplified product. It was carried out on 2.0% agarose gel and amplified fragments were visualized by staining with ethidium bromide.

Documentation of PCR amplified DNA products and SSR data analysis

The gel was taken out carefully from the gel chamber and was placed on high performance ultra-violet light box (UV trans-illuminator) of gel documentation for checking the DNA band and photographed by a Gel Cam Polaroid camera. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were determined using POWERMARKER (version 3.25) [13]. Molecular weight for each microsatellite products, in basepairs were estimated with AlphaEaseFC (Alpha Innotech Corporation) version 4.0 software. The individual fragments were assigned as alleles of the appropriate microsatellite loci.The allele frequency data from POWERMARKER was used to export the data in binary format (presence of allele as 1 and absence of allele as 0) for analysis with NTSYS-PC (Numerical Taxonomy and Multiware Analysis System) version 2.2 software [14,15]. Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram was constrcured using a computer programme, POPGENE (Version 1.31) based on Nei"s [16] genetic distance.

III. RESULTS

Eggplant (*Solanam melongena*) is an import vegetable in our country which has wild relatives as well as primitive cultivars and landraces. The molecular genetic maps developed in eggplant have been used both for the tagging of simply inherited traits and the localization of the loci underlying complex morphological characters. The assessment of genetic diversity or relatedness is not only

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important for eggplant improvement but also for the conservation and maintenance of germplasm. Highly polymorphic and repeatable PCR based microsatellite markers or Simple Sequence Repeat (SSRs) markers were used here to assess the polymorphism, diversity and similarity within those local and wild relatives.

DNA amplification by SSR markers and its polymorphism

Five SSR primers *viz*., EM114, EM120, smSSR01, amSSR04 and EPSSR82 produced different banding pattern separately with 25 eggplant genotypes and two wild relative. The amplifications of each SSR primers are presented in Table 2 and Fig. 1 to 4.

The SSR primer EM114 produced only one DNA fragment among all the genotypes under study. The approximate fragment size was 225 bp. It was a monomorphic DNA band which was common in all the genotypes. The amplification product is presented in Fig. 1.

Two fragments of DNA amplification were noticed by the SSR primer EM120. The size of amplification ranged from 50 to 180 bp. All the genotypes produced 180 bp fragment which indicated a monomorphic band. Whereas, the genotypes Salta begun, Ashary, Lalmoni local-1, Cricket, Nilphamari local and Dinajpur local were able to produce 80 bp polymorphic band (Fig. 2). The SSR primer smSSR01 was able to amplify three fragments of DNA among all the individuals. The DNA product ranged from 200 to 350 bp. Among them 300 bp fragment was common in all genotypes. The germplasm Khotkhotia, Thakurgaon local, Bogra local and Khulna local-1 showed second amplification of DNA band. It's indicated that the second fragment at 320 bp is polymorphic in nature. Kurigram local, wild species *Solanum sisymbriifolium* and *Solanum villosum* produced third amplification at 250 bp, which was polymorphic (Fig. 3). The SSR primer EPSSR82 has the ability to amplify three fragment of DNA among all the experimental materials. The band size ranged from 50 to 180 bp. It was noticed that 180 bp fragment was common in all the genotypes and was monomorphic for all. The genotypes Salta begun, Ashary, Lalmoni local-1, Kurigram local, Cricket, Rangpur local, Thakurgaon local, Bogra local, Iswardi and Jessore local-3 were able to regenerate two additional DNA bands between the size ranging from 50 to 70 bp. The above finding indicated that, two polymorphic DNA were regenerated by the primer EPSSR82. A 50 bp DNA fragment was amplified by the primer smSSR04 and it was monomorphic for all the genotypes under study (Fig. 04). On an average, five SSR primers were able to generate some total of 10 DNA amplification (10 bands) with an average amplification for each primer was 2.0. Out of them, five DNA fragment were polymorphic among the genotypes under studied.

Allelic frequency, gene diversity and Polymorphism Information Content (PIC)

Allelic frequency, gene diversity and Polymorphism Information Content (PIC) value of experimental genotypes are presented in Table 3. PCR products of five SSR markers were characterized. Some total 10 alleles were detected for the five polymorphic SSR loci, with an average number of alleles/locus is 2.0. The frequency of the major allele ranged between 0.33 to 0.56 with an average value of 0.49. Polymorphic Information Content (PIC) value for the 5 markers ranged from 0.37 (smSSR01) to 0.67 (EPSSR 82) and the average PIC value was 0.54. Highest PIC value (0.67) was observed in the primer EPSSR82 and it was lowest (0.37) in the primer smSSR01. The primer EPSSR82 was considered as the best marker for diversity analysis in eggplant germplasm followed by EM114 and EM120, respectively. The marker smSSR04 was considered as the least powerful marker. Gene diversity ranged between 0.49 (smSSR 01) to 0.72 (EPSSR 82) with an average of 0.61.The results indicate that the 25 local eggplant landraces present a high degree of homozygosity and are closely related to the wild variety *Solanum villosum* and *Solanum sysimbriifolium*, and also considerable intra-varietal group diversity, and a certain degree of genetic differentiation and polymorphism really do exist.

Nei's Genetic Distance and Genetic Identity

The value of pair-wise comparisons of Nei"s (1972) genetic distance (D) among twenty-five local and two wild relatives of eggplant were computed from combined data of the five primers and it was ranged from 0.200 to 1.000 with an average of 0.600.Comparatively higher genetic distance (1.000) was observed between a number of genotypes. Among them Ashary showed highest genetic dissimilarity with maximum number (14) of genotypes *viz*., Bogra local, Comilla local, Dohazari, Jamalpur local, Jessore local-1, Jessore local-2, Jessore local-3, Jessore local-4, Khulna local, Narsingdi local, Sada Khulna, Thakurgaon local and two wild species. The wild species *Solanum villosum* showed highest genetic distance among twelve eggplant genotypes. The highest genetic distance between them indicated that genetically they are diversed. Genotypes pair with higher value (1.000) of genetic distance is more dissimilar than a pair with a lower value. The lowest genetic distance (0.200) was found in a variety of pairs indicating that they are genetically much closer among them. The highest Nei's genetic identity was observed in various genotype pairs. Among them Bogra local showed maximum genetic similarities with maximum number (10) of genotypes viz., Iswardi, Jamalpur local, Jessore local-1, Jessore local-2, Jessore local-4, Khulna local-1, Narsingdi local, Sada Khulna, Thakurgaon local. From Nei's genetic distance and identity value it was

clearly revealed that the 25 eggplant genotypes and 2 wild species had distinct genetic diversity.

UPGMA dendrogram

A dendrogram was constructed based on the Nei"s genetic distance calculated from 25 eggplant genotypes and two wild species. Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated the segregation of 27 genotypes into two main clusters I & II (Fig.. 5). Ashary, Khotkhotia, Kurigram local, Lalmoni local-1, Lalmoni local-2, Salta begun, Cricket, Dinajpur local, Nilphamari local, Rangpur-1, Rangpur-2, Rangpur-3 were formed cluster-I. On the other hand, Bogra local, Comilla local, Dohazari, Iswardi, Jamalpur local, Jessore local-1, Jessore local-2, Jessore local-3, Jessore local-4, Khulna local-1, Narsingdi local, Sada Khulna, Thakurgaon local, wild species (*Solanum sisymbriifolium*) were fallen in the cluster II(a) and only one wild species (*Solanum villosum*) formed cluster-II (b). The genotypes – Ashary, Khotkhotia, Kurigram local, Lalmoni local-1, Lalmoni local-2, Salta begun were formed cluster I(a) and the germplsm Cricket , Dinajpur local, Nilphamari local,Rangpur-1, Rangpur-2, Rangpur-3 formed cluster-I(b). Based on above result, it may be concluded that, the close relatives of the eggplant germplasm are grouped in the same cluster due to lower genetic distance and the genetically dissimilar germplasms were placed in another cluster due to higher genetic distance. It was clearly observed that wild species (*Solanum villosum*) was very much different from all the genotypes. The result indicates that the low or high level genetic distance exits within the genotypes.

IV. DISCUSSION

Eggplant is an important vegetable crop in Bangladesh. Different local genotypes and wild relatives were found in Indian sub-continent. Morphologically those genotypes showed huge variation. Diversity study through molecular marker expressed the actual genetic make-up of eggplant genotypes. The present observation noticed the polymorphism at DNA level among the twenty-five local and two wild relatives. This finding also supported by various scientist. Some of them were discussed below.

The 22 amplified DNA products using nine SSR primers with an average amplification for each primer of 2.2 were noticed in six eggplant genotypes showed 70% monomorphic and 30% polymorphic band through using 9 primers in eggplant genotypes [17]. Nineteen SSRs markers for the molecular characterization of 30 eggplant accessions were studied. The polymorphism information content (PIC) of SSR markers ranged from 0.07 to 0.77, with an average value of PIC=0.50[18]. The mean observed heterozygosity (Ho) presented a very low value Ho=0.01, while the mean expected heterozygosity (He) had a value of He=0.57. Genomic SSRs that previously proved to be highly polymorphic in eggplant have been found to be of great value for evaluating the genetic diversity and relationships in a collection of eggplants from different cultivar groups[18, 19]. It possesses a number of desirable horticultural traits such as disease resistance [20] and has medicinal uses [21]. This result clearly indicated that different levels of genetic identity and distance present within the eggplant germplasm and shown in the UPMGA dendrogram (Fig. 5). Different levels of cluster analysis was reviewed which was performed by several scientists. Constructed a dendrogram with scale from 0.16 to 0.97 based on Jaccard's similarity coefficient. Separated the 32 accessions into 4 main clusters (*S. Melongena* and 3 small CWR clusters) and 8 sub-clusters (I-VIII) when a line was drawn at similarity coefficient of 0.42[22]. An experiment with 19 SSR markers to analysis genetic diversity among 30 Spanish eggplant genotypes revealed a considerable diversity exists within each of the cultivar groups. Germplasm from different regions shows a wide range of genetic diversity as well as phenotypic diversity indeed.

V. CONCLUSION

Bangladesh has wide range of diverse eggplant landraces. This experiment was carried out to investigate the diversity and relatedness among twenty-five local and two wild species found in Bangladesh using five highly polymorphic Simple Sequence Repeats (SSR) markers. Total ten DNA bands were generated from the five SSR primers *viz.* EM114, EM120, EPSSR82, smSSR01 and smSSR04. Amplified alleles ranged from 1 to 3 per locus with an average 2.0 alleles/locus were detected. SSR primer EPSSR82 and smSSR04 produced two polymorphic bands whereas, primer EM120 produced single polymorphic band. But, rest of two SSR primers such as EM114 and smSSR04 were not able to generate any polymorphic band. The Polymorphism Information Content (PIC) for all the markers ranged from 0.37 to 0.67 with an average value of PIC = 0.54. Gene diversity ranges from 0.49 to 0.72, with an average value of 0.61. SSR markers showed an average gene diversity of 0.61 for all the genotypes. Dendrogram figure revealed that, the 25 local and two wild relatives of eggplant into two major clusters. It is concluded that SSR markers have been proved to be a powerful tool for molecular genetic analysis of eggplant germplasm for plant breeding programs to assess genetic diversity for the improvement of cultivars. Molecular characterization of local eggplant data might be helpful to select the diverse parents for development of a new variety.

SIGNIFICANT STATEMENT

This work able to identify polymorphism among local genotypes through SSR markers. Molecular diversity and genetic distance also estabilshed between cultivated eggplant and its wild relatives viz. *Solanum villosum* and *Solanum sisymbrifullium*. The result may utilized as a source of diverse parent for any hybridization program.

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Diversity at DNA level information will be used to conserved the local germplasm for future use.

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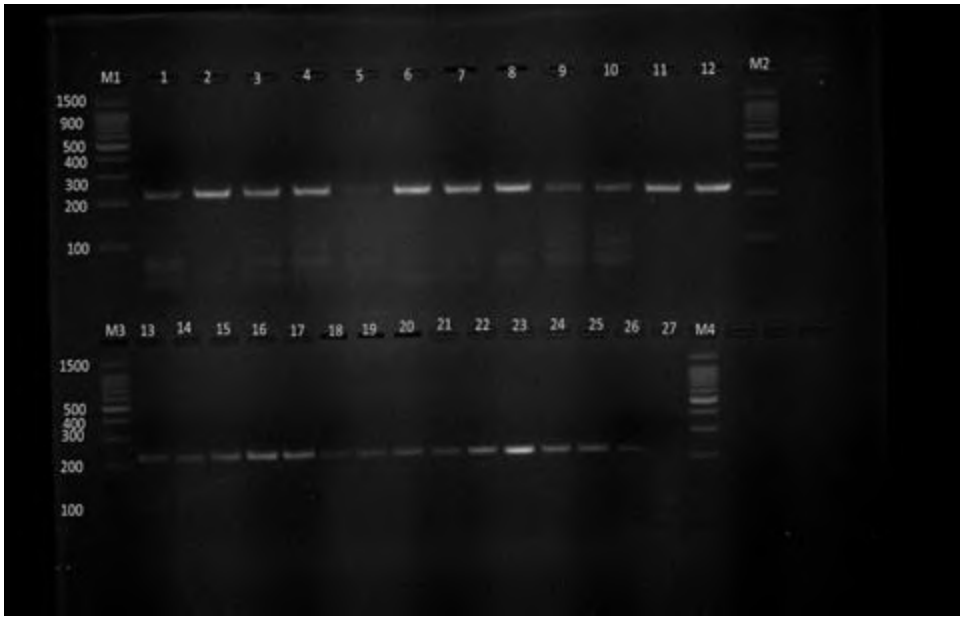

Fig. 1: SSR profile of 27 local and wild eggplant germplasm using primer EM114.

(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpurlocal-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local- 2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla Local; Lane 25: Dohazari; Lane 26 : Wild species Solanum sisymbriifolium; Lane 27: Wild species Solanum villosum and M1=M2=M3=M4=100 bp DNA ladder).

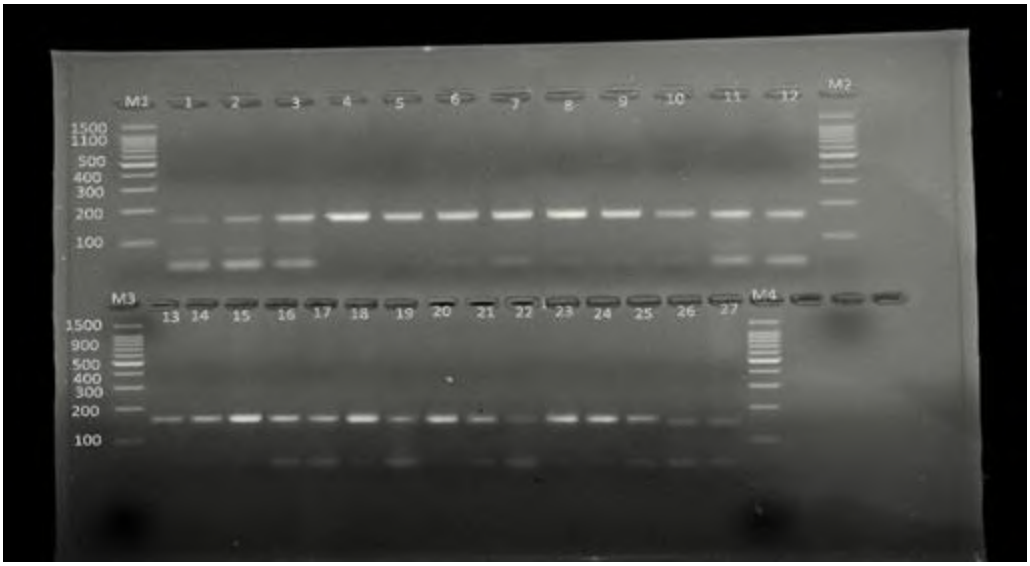

Fig. 2: SSR profile of 27 local and wild eggplant germplasm using primer EM120.

(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local- 2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla Local; Lane 25: Dohazari; Lane 26 : Wild species Solanum sisymbriifolium; Lane 27: Wild species Solanum villosum and M1=M2=M3=M4=100 bp DNA ladder).

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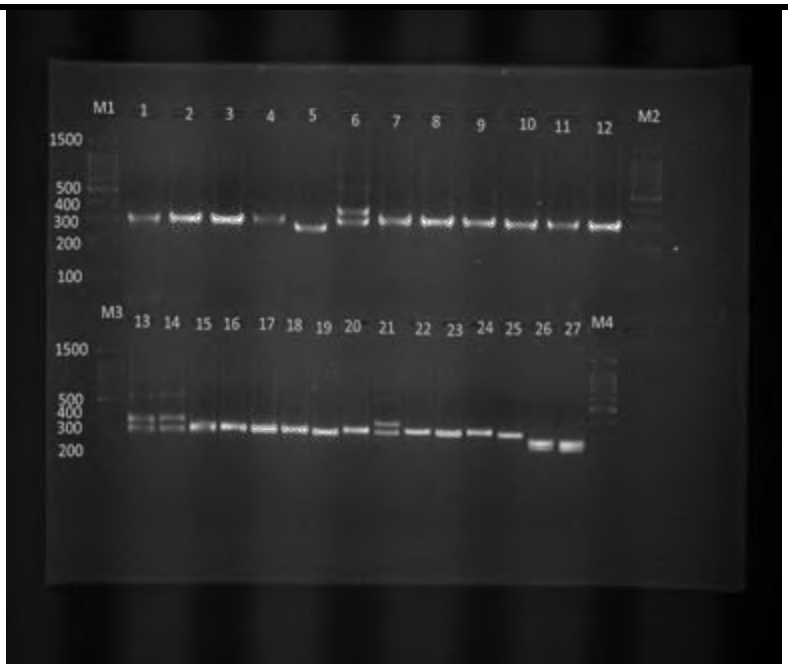

Fig. 3: SSR profile of 27 local and wild eggplant germplasm using primer smSSR01.
(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8: Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local- 2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla local; Lane 25: Dohazari; Lane 26 : Wild species Solanum sisymbriifolium; Lane 27: Wild species Solanum villosum and M1=M2=M3=M4=100 bp DNA ladder.

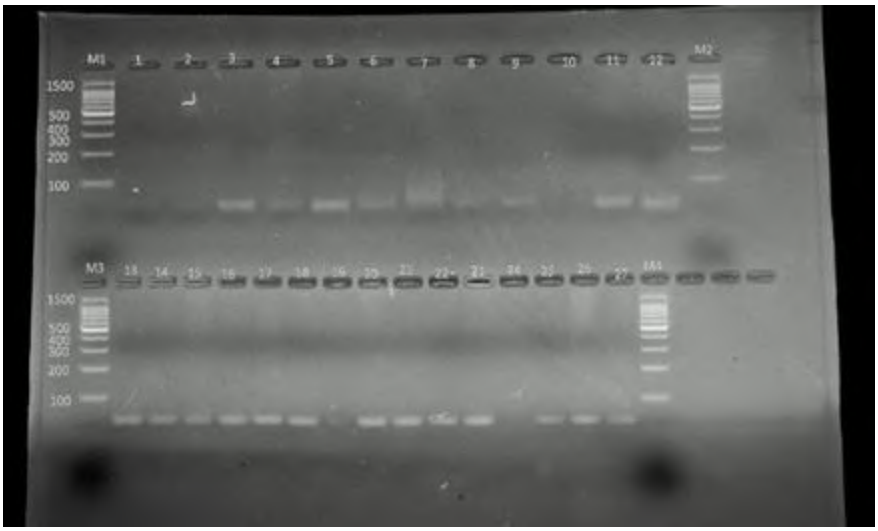

Fig. 4: SSR profile of 27 local and wild eggplant germplasm using primer smSSR04.
(Lane 1: Salta begun; Lane 2: Ashary; Lane 3: Lalmoni local-1; Lane 4: Lalmoni local-2; Lane 5: Kurigram local; Lane 6: Khotkhotia; Lane 7: Cricket; Lane-8:Rangpur local-1; Lane 9: Rangpur local-2; Lane 10: Rangpur local-3; Lane11: Nilphamari local; Lane 12: Dinajpur local ; Lane 13: Thakurgaon local; Lane 14: Bogra local; Lane 15: Iswardi local; Lane 16: Jessore local-1; Lane 17: Jessore local- 2; Lane 18: Jessore local-3; Lane 19: Jessore local-4; Lane 20: Sada khulna; lane 21: Khulna local-1; Lane 22: Jamalpur local; Lane 23: Narsingdi local; Lane 24:Comilla Local; Lane 25: Dohazari; Lane 26 : Wild species Solanum sisymbriifolium; Lane 27: Wild species Solanum villosum and M1=M2=M3=M4=100 bp DNA ladder.

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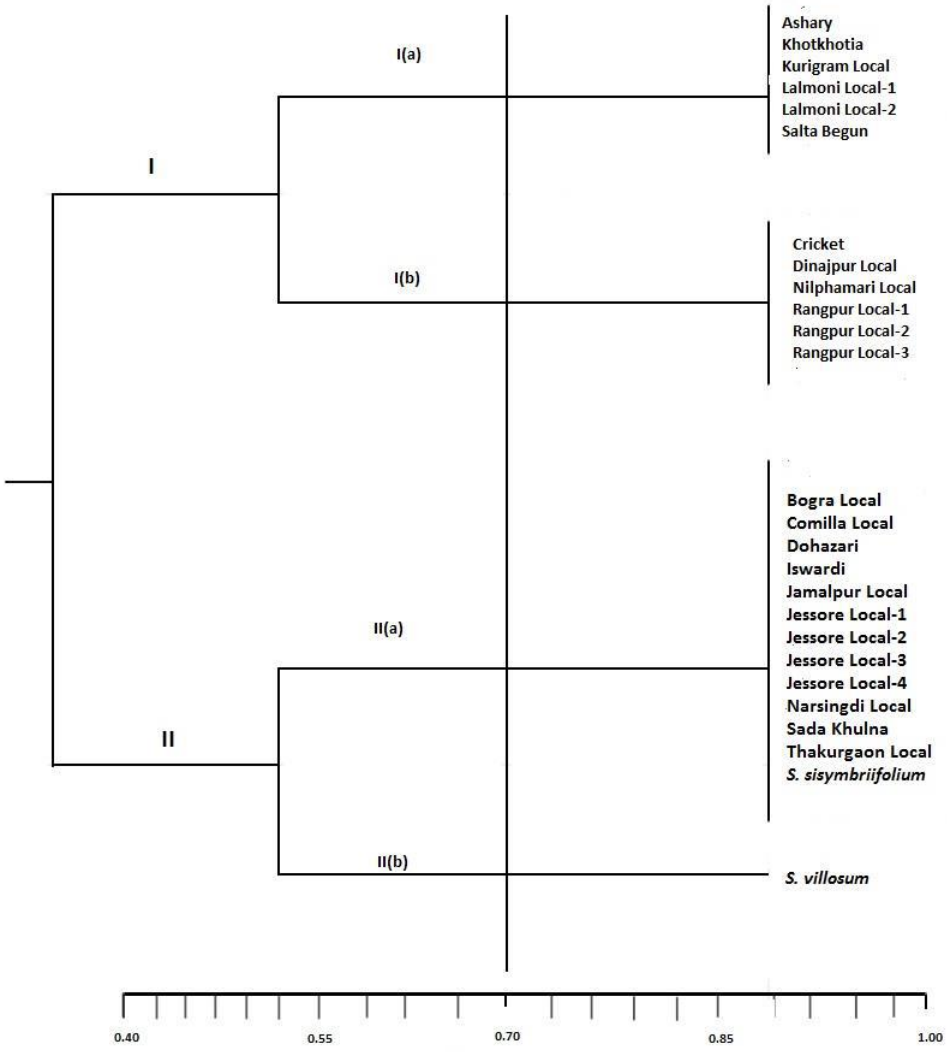

Fig.5: Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei"s (1972) genetic distance, summarizing data on differentiation for twenty-five local and two wild relatives of eggplant.

Table 1: Name of the local genotypes and their collected area in Bangladesh.

SL. No.	Entry Name	Collected Area
1	Salta Begun	Lalmonirhat District,
2	Ashary	Lalmonirhat District
3	Lalmoni Local-1	Lalmonirhat District
4	Lalmoni Local-2	Lalmonirhat District
5	Kurigram Local	Kurigram District
6	Khotkhotia	Rangpur District
7	Cricket	Rangpur District
8	Rangpur Local-1	Rangpur District
9	Rangpur Local-2	Rangpur District
10	Rangpur Local-3	Rangpur District
11	Nilphamari Local	Nilphamari District
12	Dinajpur Local	Dinajpur District
13	Thakurgaon local	Thakurgaon District
14	Bogra Local	Bogra District

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15	Iswardi	Local	Pabna District
16	Jessore	Local-1	Jessore District
17	Jessore	Local-2	Jessore District
18	Jessore	Local-3	Jessore District
19	Jessore	Local-4	Jessore District
20	Sada Khulna		Khulna District
21	Khulna	Local-1	Khulna District
22	Jamalpur	Local	Jamalpur District
23	Narsingdi	Local	Narsingdi District
24	Comilla	Local	Comilla District
25	Dohazari		Comilla District
26	Wild species (*Solanum sisymbriifolium*)		*BARI, Gazipur
27	Wild species (*Solanum villosum*)		*BARI, Gazipur

*BARI= Bangladesh Agricultural Research Institute

Table.2: PCR amplified DNA fragment size and number of polymorphic band with 27 genotypes

Primer no.	Primers' Name	Primer sequences (5'-3')	(G+C) %	No.of DNA band(s)	No.of polymorphic band(s)	Band size range (bp)
1	EM114	For. AGCCTAAACTTGGTTGGTTTTTGC Rev.GAAGCTTTAAGAGCCTTCTATGCAG	43	1	0	225
2	EM120	For. GGATCAACTGAAGAGCTGGTGGTT Rev.CAGAGCTTCAATGTTCCATTTCAC A	44	2	1	50-180
3	EPSSR82	For. ACATGCCACTCATGTTGGTG Rev. CTTCAGCCATGGACCACATT	50	3	2	50-180
4	smSSR01	For. GTGACTACGGTTTCACTGGT Rev. GATGACGACGACGATAATAGA	46	3	2	200 - 400
5	smSSR04	For. AATGAGTCAGAAACCACGCC Rev. CGTTTAACCTTTGGCTCGGAA	49	1	0	50-80
Total	-	-	-	10	5	-
Mean	-	-	-	2.0	1.0	-

Table.3: Major allelic frequency, gene diversity and PIC value of different eggplant genotypes

Markers	Obs. No.	Availability	Allele no.	Major allele frequency	Gene diversity	PIC value
EM114	27	1.00	1.0	0.52	0.63	0.57
EM120	27	1.00	2.0	0.56	0.61	0.55
EPSSR 82	27	1.00	3.0	0.33	0.72	0.67
smSSR 01	27	1.00	3.0	0.56	0.49	0.37
smSSR 04	27	1.00	1.0	0.48	0.61	0.53
Mean	27	1.00	2.0	0.49	0.61	0.54

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Evaluation of structure and natural regeneration status of woody plant species in sudanian domain : Case of eastern part of National Park of Sena Oura, Chad

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***Abstract*—** *Many scientific studies confirmed that plants have an important ecological role maintaining the productivity of the environment and regulating the global climate. In order to valorize the wild phytogenetic resources for the efficient in situconservation and sustainable use in sudano-zambezian region, a study was carried out in sudanian domainprovidingquantitative informations on the community structure and natural regeneration status of woody plant species.The study site is the eastern part of National Park of Sena Oura in Chad. Adults plants (trees and shrubs) were systématically collected in 10 linear transects (20 m x 1000 m). Juvenile plants (saplings and seedlings) weresystematically collected within 40 plots (20 m × 20 m). These plots were randomly stablished in the transects, at a rate of four plots per transect.In total, 84 adults plants species grouped in 58 genera and 29 families and 66 juvenile plants species grouped in 45 genera and 27 families were inventoried. Bell andreverse J-shaped patterns of selected woody species were identified.The stand regeneration status wasgood.The stand regeneration rate were SRR = 52.29% and Hymenocardia acida (SIR = 17.95%), Combretum collinum (SIR = 14.12%), Annona senegalensis (SIR = 6.67%) and Isoberlinia doka (SIR = 6.22%) had the most important specific index of regeneration. The specific structures showed that the structure of the total stand is the result of the dynamics of all species and their interactions.The global stand regeneration status was good.The obtained results provided quantitative informations on the community structure and natural regeneration status of woody plant species for the efficient conservation and sustainable use.*

***Keywords*— Structure, regeneration status, wild phytogenetic resources,in situ conservation,sudano-zambezian region, forest dynamic.**

I. INTRODUCTION

Sudanian domain is the phytogeograpical area loacted between the sahelian domain (drier) and the guineo-congolese domain (semi-deciduous moist dense forest).The National Park of Sena Oura (NPSO) is the third National Park in Chad and it covered 73520 haarea. It was created by Law N° 011/PR/2010 of 10 June 2010 on the initiation of the local communities of the cantons Dari and Goumadji and one of the main objectives of which was to propagate, protect and conserve wild animal and plant species.The vegetation of easthern part of this Park is rich in plant species (85 species) that *Isoberlinia doka*,*Burkea africana*, *Daniellia oliveri*, *Terminalia laxiflora*, *Datarium microcarpum*, *Monotes kerstingii* and *Anogeissus leocarpus* are the most abundant (Todou et al. 2017a). Many scientific studies confirmed that tropical ecosystems are the most rich in biodiversity, particulary in plant biodiversity. These plants have an important ecological role maintaining the productivity of the environment and regulating the global climate(Quijas et al. 2012). They provide food for humans and animals, serve as medicine basis and construction materials as well as combustible. Somes wild fruits are edible. They are sold locally or sold after being processed locally (Todou et al. 2017b).They also protect the land from wind and water erosion, stabilizing the water cycle, facilitate the process of evaporation. They serve to absorb carbon dioxide to reduce global warming, give off oxygen, and renew the atmosphere.The main mission of protected areas is to conserve plant populations, specific diversity or genetic diversity. They also act as buffers against anthropogenic or natural uncertainties, including natural disasters and climate change.

Despite their richness in biodiversity and their usefulness for humanity, tropical ecosystemsare facing a lot of pressure from human as well as natural phenomenon to ensure sustainable improvement of the resources

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(Aubertin and Vivien 1998).Humans are still dependent for a large part of his natural environment. They get all that is necessary for their daily survival. The impacts of anthropogenic activities are negative on biodiversity (Akpagana and Bouchet 1995). On the other hand, in almost all sub-saharan african countries, *in situ* conservation policies lack rigor in their design and implementation and have failed. For example, protected areas are generally abandoned and are transformed into plantations, feeding areas and zones of anarchic woodcutting (Akpagana and Bouchet 1994). Currently,few studies on plant biodiversity and its natural regeneration are carried out in sudano-zambezian region.It is known that studies on floristic composition, structure and natural regeneration in forests are instrumental in the sustainability of forests since they play a major role in the conservation of plant species, and the management of forest ecosystems as a whole (Ssegawa and Nkuutu 2006). These studies are imperative to identify and develop the potential for innovations derived from plant richness, particularly those of the developing countries and these studies becomes more imperative in the face of the ever increasing threat to the forest ecosystem. They are also help to understand the dynamics of woody vegetation (Adane 2011).

In easthern part of NPSO, Todou et al. (2017a) had study floristic composition, diversity and ecological importance of woody plants. The resultsgave a general idea of the diversity of ligneous plants in the Sudanian area considering all its phytogeographical sectors.The present study enables to fill some of the information gaps especially of structure and natural regeneration. The idea is to provide quantitative informations on the forest restoration and specific information for conservation measureswhich may be applicable during forest management in all soudano-zambezian region. The main objective is tocontribute to efficient conservation and sustainable use of the sudano-zambezian wild plant resources.The specific objectives are: (1) to evaluate stand structure and individual structure of most abundant species as well as (2) to evaluate the natural regeneration status.

II. MATERIALS AND METHODS

Study area

National Park of Sena Oura is located in the Department of Mayo-Dallah, Mayo-Kebbi West Region. It is located between 8°25'43" and 9°13' 06" North latitude and 13°58'47" and 15°30'09" East longitude. It is located at an altitude of between 350 and 671 m and it is cross-border with the Bouba-Ndjidda National Park in Cameroon (fig. 1).

This study was carried out in the eastern part of the Park, limited to the western part by the river 'mayo sena oura. The NPSO is located in the Sudanian domain (sudano-zambezian region) according to the phytogeographic subdivision (Letouzey 1985). The climate is of the tropical sudano-guinean type with a dry season which extends from October to April and a rainy season from May to September. Annual cumulative rainfall is about 900 to 1200 mm per year. The hydrographic network consists of rivers flowing between July and September (Bemadjim 2014). The vegetation is a wooded savanna identical to that of the National Park of Bouba Ndjidda but with the particularity of sheltering in the zone of confluence of the streams, vegetation of guineo-sudanian type and forests gallery along the rivers.

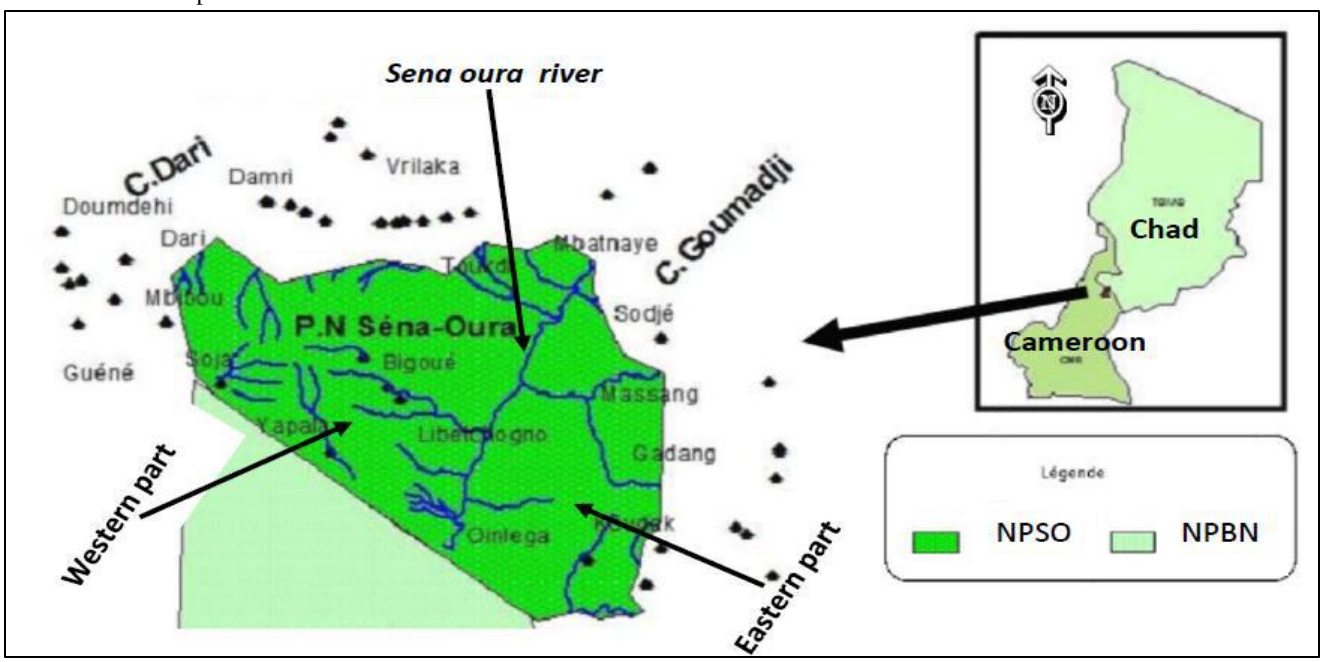

Fig.1 : Location map of study site.

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Data collection

The inventory was done during four months (April-May-June-July 2016).Data collection for adult plants (adult trees and shrubs) was done in 10 linear transects (20 m x 1000 m).These transects were established about more than 500 m one away from each other in order to cover the eastern part of the Park and to represent the maximum of species. In total, 20 ha were surveyed. Juvenile plants (saplings and seedlings) collection was done within 40 plots (20 m × 20 m)randomly stablished in the transects,at a rate of four plots per transect. Within each plot, all juvenile plants were systematically counted. Scientific identification of the most common species was done directly in the field whenever possible. Some specimens were collected in order to authentify scientific names in laboratory of Agriculture and Development Research Institute (IRAD) in Maroua and by botanists of University of Maroua (Cameroon).

Data analysis

The stand structure and specific structure of the eight most abundant adult plant species were assessed through histograms constructed by using the density of individuals (Y-axis) categorized into height and diameters classes (Peters 1996).

All recorded data of plots were pooled and the total number of species were tallied. Using the pooled data, number of species, of genera and of families were calculated in order to evaluate their richness.

The number of juvenile plants was compared with those of adult plants. According to Dhaulkhandi et al. (2008) and Tiwari et al. (2010), there is good regeneration if number of juvenile plants> number of adult plants;there is fair regeneration if number of juvenile plants ≤ number of adults plants; there is poor regeneration if the species survives only in juvenile stage (number of juvenile plants may be <, > or = number of adult plants). If a species is present only in an adult form it is considered as no regenerating. Species is considered as new in the stand if the species has no adult plants but onlyjuvenile plants.

The Shannon Weaver index was calculated according to formula:

$$H = -\sum_{S=1}^{S} (Pi * \ln(Pi))$$

The diversity is low if H < 3; the diversity is moderate if 3 ≥ H > 4 and the diversity is high if H ≥ 4 (Yédomonhan 2009).

The equitability index of Pielou was calculated using the formula:

$$J = \frac{H}{Hmax} = \frac{H}{\ln(S)}$$

J ranges between 0 and 1. One species is present in the site if J = 0 and all species have same probability if J = 1. This index means that the degree of diversity reaches the possible maximum ratio.

The stand regeneration rate(SRR) was calculated accordingPoupon (1980) formula:

$$SRR = \frac{JUV}{ADT + JUV} \text{ x } 100$$

JUV = number of juvenile plants of stand ; ADT = number of adult plants of stand.

The specific index of regeneration (SIR)was calculated accordingAkpoand Grouzis (1996) formula:

$$SIRi = \frac{JUVi}{JUV} \text{ x } 100$$

SIRi = specific index of regeneration of species i; JUVi = number of juvenile plants belonging to species i.

The *Statistical Package for Social Sciences software* version 20.0 (SPSS, Inc., Chicago, IL, USA) and Excel (Microsoft Office 2013) were used for data processing and presentation of the results.

III. RESULTS AND DISCUSSION

Stand structure of adult plants based on height class

The height class frequency distribution of the stand exhibited a tendency towards a reverse J-shaped distribution (fig. 2).The adjustment of the distribution of all adult plants to the mathematical model gave an exponential function ($y = 4350.6e^{-0.74x}$), highly significant ($p < 0.001$, $R^2 = 0.94$), with y = numberof species and x = the center of diameter class.About 44.02% (1670 individuals)were the highest between 6 and 11 m height class. However,23.29%(800 individuals) were inferior to6 m height. This class was represented by shrubs and also juvenile trees. It can therefore be deduced that up to 76.71% of plants were trees more than 11 m height.In this case where all plants species were grouped, reverse J-shaped distribution indicate that there were less and less species possessing greater individuals than 11 m height.This structure which decreased exponentially from small height classes to great height classes testifies to the stability of the total stand, characterized by regular natural regeneration.

Structure of most abundant species based on diameter class

According Todou et al. (2017), 3792 adult plants grouped in 84 species, 58 genera and 29 families, were identified in eastern part of NPSO. The study of structure was based on diameter class distribution of the eight most abundant species. They were *Isoberlinia doka*,*Burkea africana*, *Daniellia oliveri*, *Terminalia laxiflora*, *Datarium microcarpum*, *Monotes kerstingii*, *Anogeissus leocarpus* and *Combretum glutinosum*.

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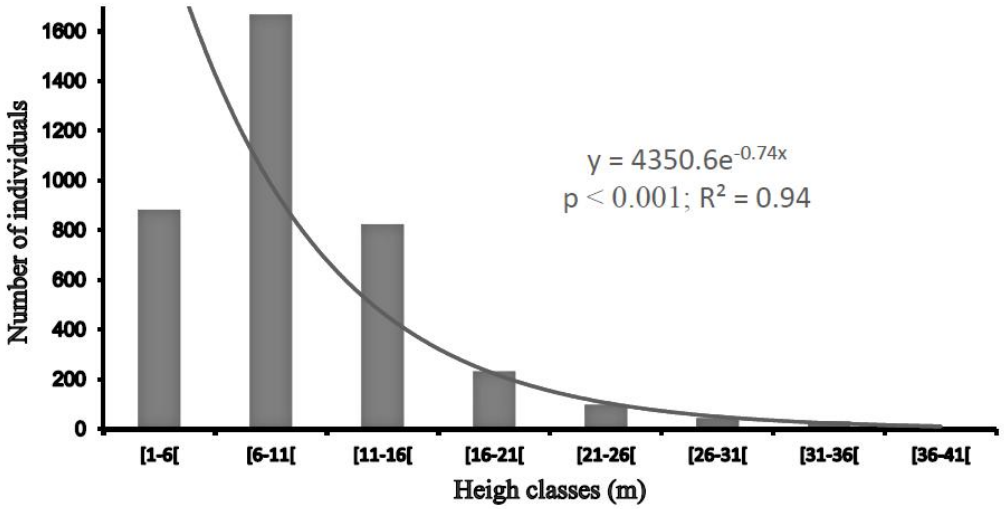

Fig.2 : Vegetation structure of the stand on height class.

By studying species' structure differently, it has been demonstrated that two types of patterns of population structure. In *C. glutinosum* and *B. africana*, diameter class frequency distribution exhibited a tendency towards a bell-shaped pattern (figs 3 and 4). Diameter classes < 10 cm anddiametr class]20, 30]had the least individuals number and diameter class]10,20] had highest individuals number. For the other six species, diameter classe frequency distribution exhibited a tendency towards a reverse J-shaped pattern (figs 5-10). There were*A. leiocarpus* (peack in]10, 20]), *D. oliveri* (peack in]20, 30]), *D. microcarpum* (peack in]10, 20]),*I. doka* (peack in]20, 30]), *M. kerstingii* (peack at diameter < 10 m) and *P. lucens* (peack in]10, 20], but always with individuals of smaller diameter class than these peaks. These specific structures, different from each other, show that the structure based on the height of the total stand was the result of the dynamics of all species and their interactions(Guedjé2002). In eastern part of NPSO, the potential natural regeneration were not negligeable, according the results of specific structures, beacause there was a large number of individuals fell in the smaller diameter classes than the peacks of some species.

In bell-shaped pattern the distribution of individuals of a species in the middle diameter classes is high and low in lower and higher diameter classes. According to Feyera et al. (2007), bell-shaped pattern indicates a poor reproduction and recruitment of species which may be associated with intense competition from the surrounding trees. This similar situation was observed by Kuma and Shibru (2015) in Oda Forest of Humbo Carbon Project (Ethiopia). Inverted J-shaped pattern has shown high distribution of individuals of a species in the lower diameter classes and a gradual decrease towards the higher classes. It has shownalso good reproduction and recruitment potential of the species.Analysis structures for adult plant species could provide more realistic and specific information for conservation measures.

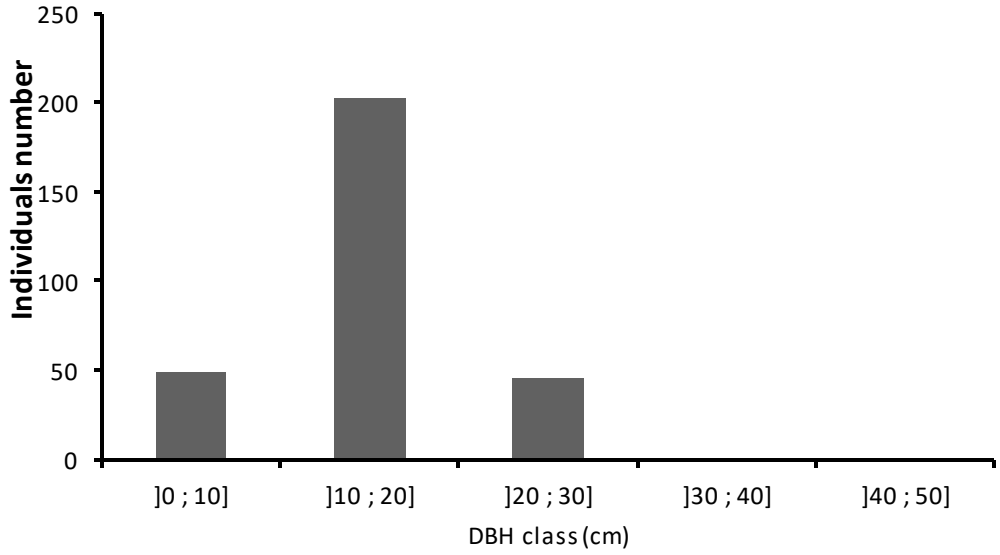

Fig.3 : Structure of C. glutinosum based on diameter class.

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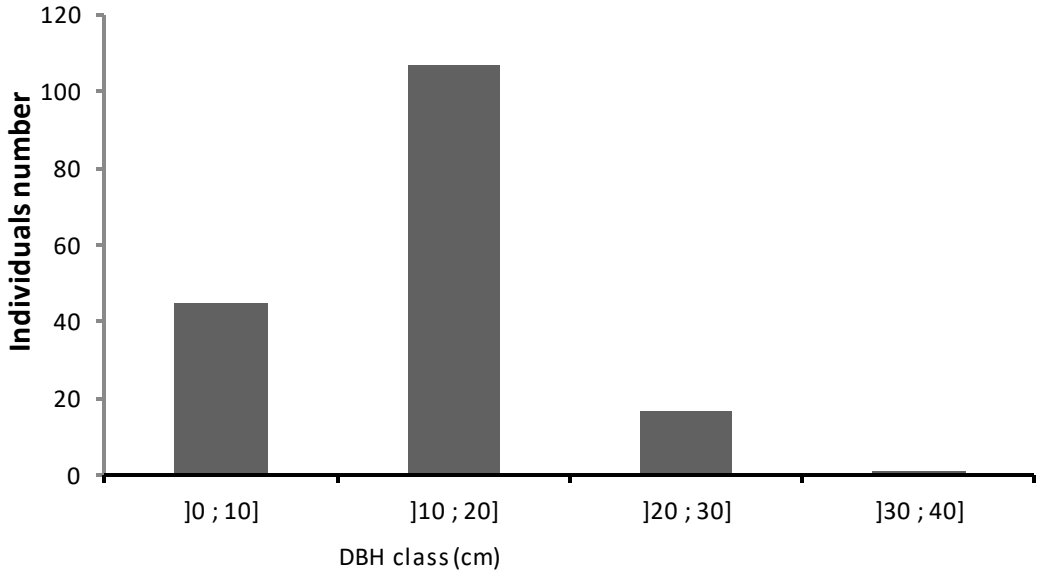

Fig.4: Structure ofB. africana based on diameter class.

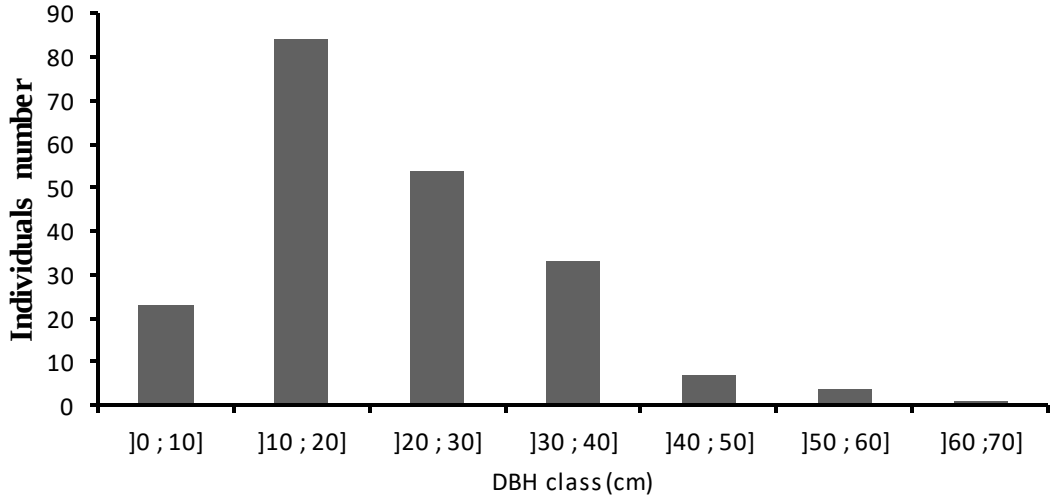

Fig.5: Structure of A. leiocarpus based on diameter class.

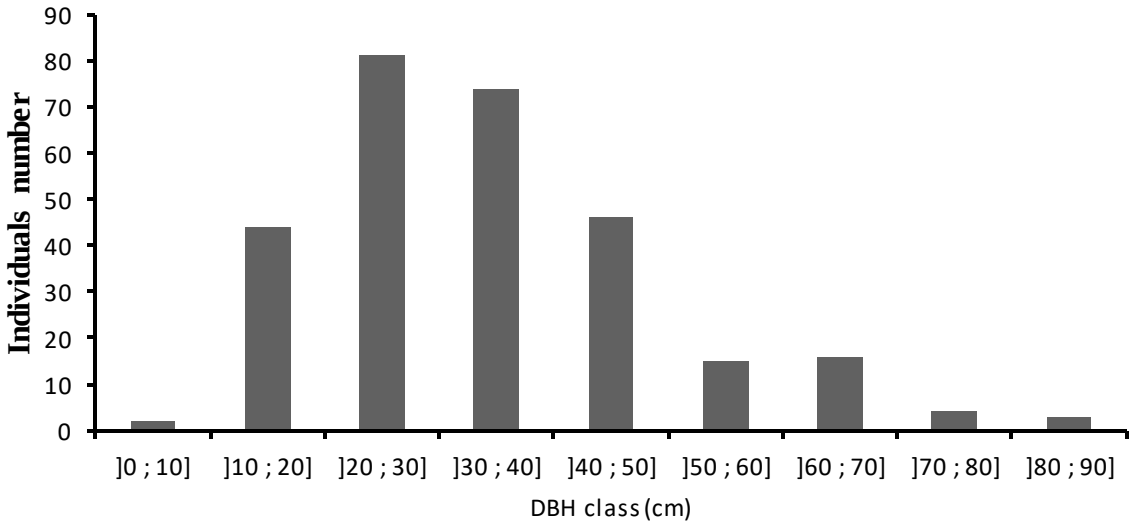

Fig.6: Structure of D. oliveri based on diameter class.

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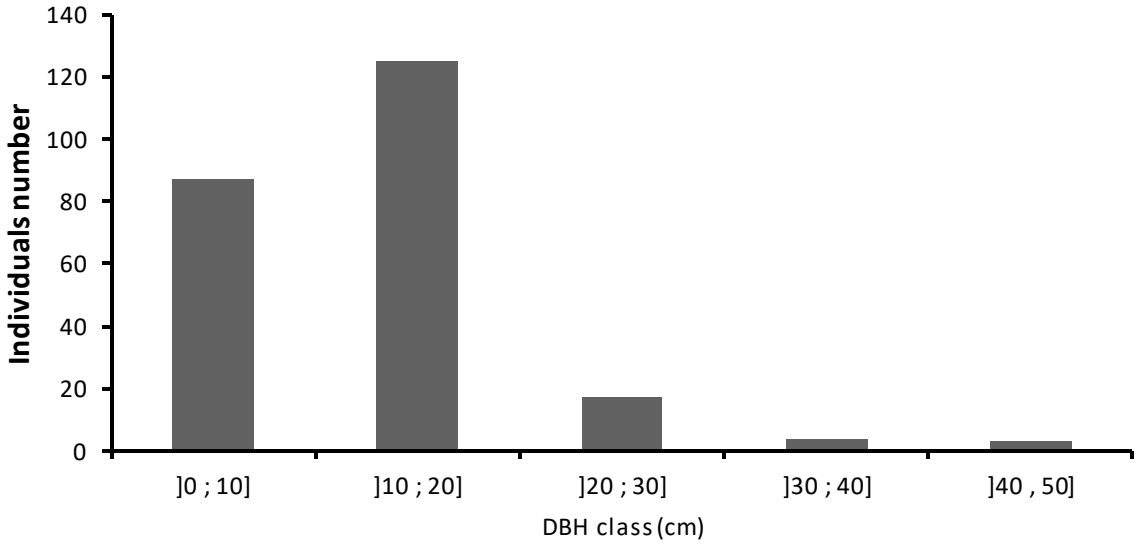

Fig.7: Structure of D. microcarpum based on diameter class.

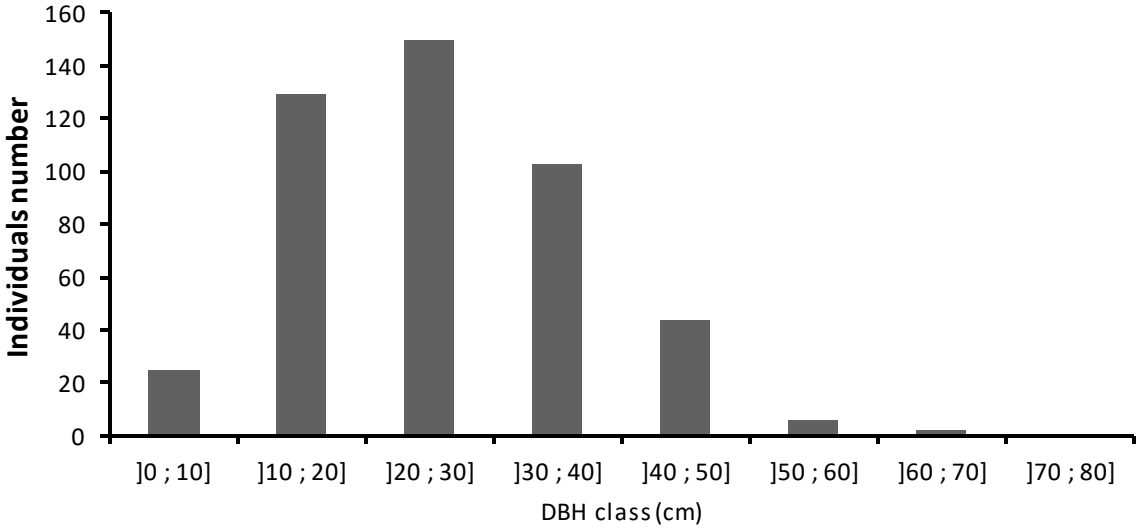

Fig.8: Structure ofI. doka based on diameter class.

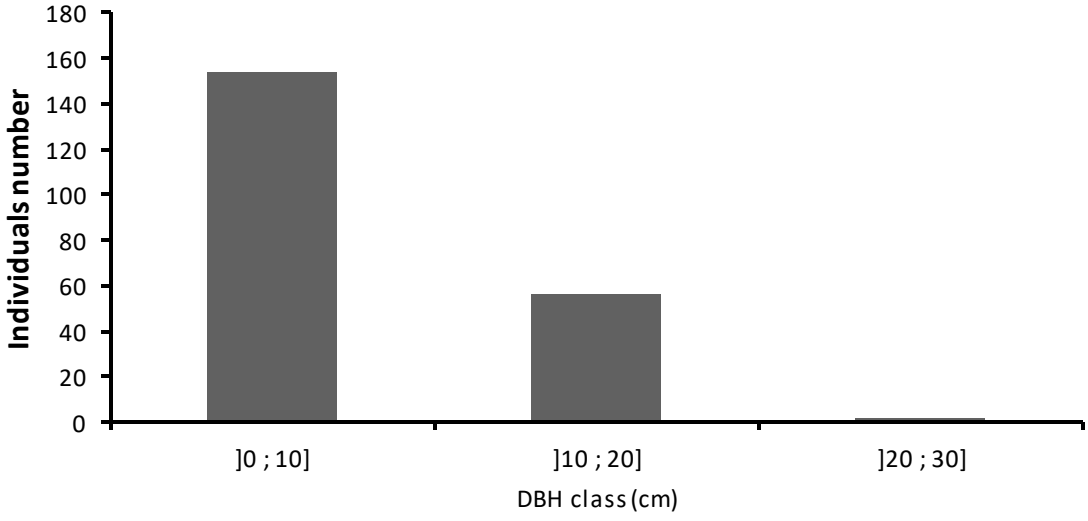

Fig.9: Structure of M. kerstingii based on diameter class.

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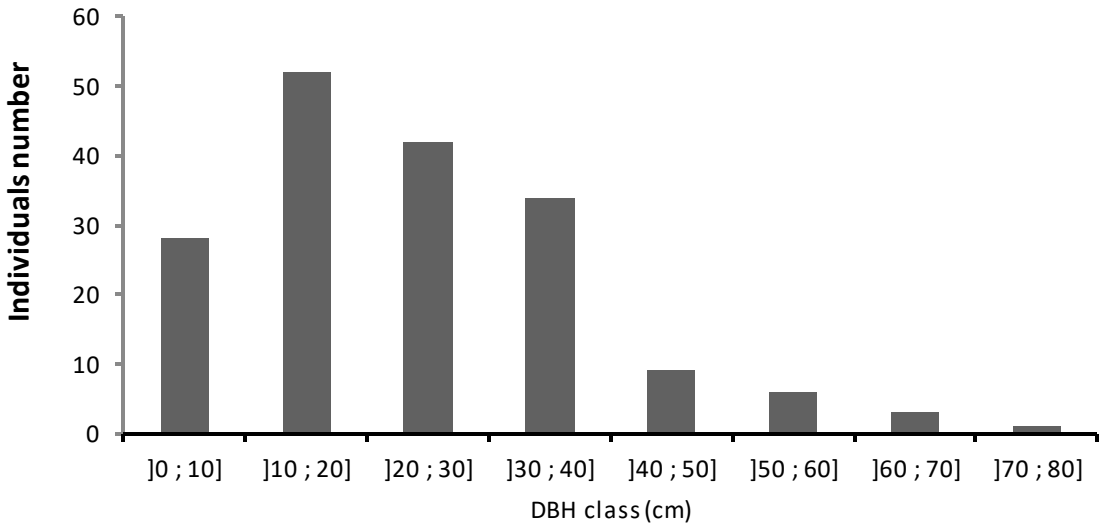

Fig.10: Structure of P. lucens based on diameter class.

Stand diversity of juvinile plants and natural regeneration status

In the stand, there were 66 species belonging to the natural generation flora (number of juvenile plants species). They belonged to 45 genera and 27 families. The number of these juvenile plants species, genera and familes were inferior to those of adult plants. However, according to Dhaulkhandi et al. (2008) and Tiwari et al. (2010), the stand regeneration status was good because the juvinile individuals (Nt = 4146 individuals and D = 2657.67 individuals per ha) was more represented than adult indivuduals (Nt = 3792 invidividuals and D = 189.60 individuals per ha). Juvinile stand diversity (Shannon index = 3.20 and Pielou equitability = 0.76) was comparable to those of adult plants (Table 1).Thisstand diversity was moderated with fairly good equitablility according Yédomonhan (2009) analysis. The Shannon index was usually found to fall between 1.5 and 3.5 and is rarely above 5.0 (Magurran 2004). The found value (Shannon index = 3.20)in this inventory fall within the expected range, but sufficient for sudanian landscapeaccording Todou et al.(2017a).

There were 53 species with good regeneration (densities of juvenile plants were superior to those adult individuals), two species with fair regeneration (*B. africana* and *T. macroptera*) with densities of juvenile plants were inferior to densities of adults individuals and 29 species with no regeneration (no juvenile plants were recorded). On the other hand, 11 species were represented by juvenile plants only. There were *Acacia erythrocalyx*, *Acacia sieberiana*, *Bridelia scleroneura*, *Flueggea virosa*, *Grewia barteri*, *Grewia venusta*, *Grewia vilosa*, *Maytenus senegalensis*, *Psorospermum senegalensis*, *Vitex simplicifolia*, *Ziziphus mucronata*(Table 2).They were considered as new species in the stand according Dhaulkhandi et al.(2008). In Oda Forest of Humbo Carbon Project in Ethiopia, 23.7% represented juvenile plants and 74% represented adults trees (Kuma and Shibru 2015). Combretaceae (13 species), Caesalpinaceae (9 species) and Mimosaceae (8 species) specifically dominated in the studied area (Table 2). It was same to adult plants that Combretaceae (16 species), Caesalpinaceae (10 species) and Mimosaceae (10 species) were specifically dominated in the same site (Todou et al. 2017a).The so called new species could be present in the forest, more or less far from the study site, but dispersed by frugivorous animals. This could justify the important ecological role of animals in a forest ecosystem.

Natural Regeneration is the establishment of trees from seeds that fall and germinate *in situ* (Harmer 2001). It is the basis for understanding the dynamics of woody vegetation. It involves recruitment, juvenile mortality and different stages of development, and survival (Traoré 1997). It can be vegetative or by natural seedling, but in easthern part of NPSO, natural regeneration was assessed by the importance of sapling. The general regeneration important, stand regeneration rate were SRR = 52.29%.This value is not negligible because it is higher than average. It indicates a stability of the total stand assumed in the structural study. Stand regeneration rate of easthern part of NPSO was slightly lower than that of Ngom et al. (2013) in Ferlo Biosphere Reserve (northern Senegal). That was 72%.The importance of regeneration according different species was represented by Specific Index of Regeneration (SIR). Species with the greatest potential for regeneration were*Hymenocardia acida* (SIR = 17.95%) *Combretum collinum* (SIR = 14.12%), *Annona senegalensis* (SIR = 6.67%) and *Isoberlinia doka* (SIR = 6.22%). These species alone accounted for up to 45% of regeneration. Species with low regeneration potential

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were*Grewia barteri*, *Maytenus senegalensis*, *Psorospermum seneglensis*, *Pterocarpus erinaceus*, *Sterculia setigera*, *Terminalia macroptera* and *Ziziphus mauritiana* whith SIR = 0.02% each (Table 2).According Ngom et al. (2013), species with the highest specific index of regeneration are species which regenerate readily by stump in the absence of bushfires. This is the case of Hymenocardiaceae and Combretaceae.

Table.1: Diversity characteristics of each stage of stand development in the study site

Parameters	Adult plants*	Juvenile plants
Number of individuals	3792	4146
Number of species	84	66
Number of genera	58	45
Number of families	29	27
Density (stems.ha^{-1})	189.60	2657.67
Shannon index	3.41	3.20
Simpson index	0.95	-
Pielou equitability index	0.76	0.76

* Results published in Todou et al (2017a)

Table.2 : List of species and their density and their generation status

Families	Species	Adult plants*	Juvenile plants		Status
		D	D	SIR	
Anacardiaceae	*Lannea acida*	0.35	-	-	No
	Lannea barteri	0.1	-	-	No
	Lannea velutina	3.7	9.62	0.36	Go
	Lannea schimperi	0.5	-	-	No
Annonaceae	*Annona senegalensis*	0.7	177.56	6.67	Go
	Hexalobus monopetalus	0.2	-	-	No
Bignoniaceae	*Stereospermum kunthianum*	0.5	37.82	1.42	Go
Bombacaceae	*Bombax costatum*	0.25	1.28	0.05	Go
Burseraceae	*Commiphora pedonculata*	0.55	-	-	No
	Boswellia dalzielii	0.05	1.92	0.07	Go
Caesalpiniaceae	*Afzelia africana*	0.95	10.9	0.41	Go
	Burkea africana	15	10.9	3.08	Fa
	Piliostigma reticulatum	3.95	21.79	0.82	Go
	Piliostigma thonningii	2.6	12.18	0.46	Go
	Tamarindus indica	2.25	30.77	1.16	Go
	Cassia sieberiana	0.35	-	-	No
	Daniellia oliveri	14.1	16.03	0.6	Go
	Detarium microcarpum	11.8	28.21	1.06	Go
	Isoberlinia doka	23	165.38	6.22	Go
	Swartzia madagascariensis	2.25	20.51	0.77	Go
Capparaceae	*Crateva adansonii*	0.1	-	-	No
	Maytenus senegalensis	-	0.64	0.02	Ne
	Maerua angolensis	0.05	-	-	No
Chrysobalanaceae	*Parinari curatellifolia*	0.5	3.85	0.14	Go
Combretaceae	*Combretum collinum*	5.35	375.64	14.12	Go
	Combretum micranthum	0.25	3.85	0.14	Go
	Combretummolle	2.65	3.85	3.42	Go
	Combretum paniculatum	1.1	3.21	0.12	Go
	Anogeissus leiocarpus	10.3	56.41	2.12	Go
	Combretum adenogonium	4.05	13.46	0.51	Go
	Combretum glutinosum	8.75	41.03	1.54	Go
	Terminalia albida	5.15	7.69	0.29	Go
	Terminalia avicennioides	2.95	68.59	2.58	Go

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	Terminalia brownii	0.45	6.41	0.24	Go
	Terminalia catappa	1.75	30.77	1.16	Go
	Terminalia laxiflora	12.4	101.28	3.81	Go
	Terminalia macroptera	1.9	0.64	0.02	Fa
	Terminalia mantaly	0.15	-	-	No
	Terminalia mollis	0.45	-	-	No
	Terminalia schimperiana	0.6	-	-	No
Dipterocarpaceae	*Monotes kerstingii*	10.6	24.36	0.92	Go
Ebenaceae	*Diospyros mespiliformis*	0.3	3.21	0.12	Go
Euphorbiaceae	*Croton macrostachyus*	0.35	-	-	No
	Antidesma venosum	0.1	-	-	No
	Bridelia scleroneura	-	27.56	1.04	Ne
	Flueggea virosa	-	1.28	0.05	Ne
	Bridelia ferruginea	0.8	29.49	1.11	Go
Fabaceae	*Erythrina sigmoidea*	0.1	-	-	No
	Pericopsis laxiflora	1.7	-	-	No
	Pterocarpus lucens	8.75	102.56	3.86	Go
	Lonchocarpus laxiflorus	1.1	20.51	0.77	Go
	Pterocarpus erinaceus	0.35	0.64	0.02	Go
Guttiferae	*Psorospermum senegalensis*	-	0.64	0.02	Ne
Hymenocardiaceae	*Hymenocardia acida*	2	477.56	17.95	Go
Loganiaceae	*Strychnos innocua*	2.4	44.87	1.69	Go
	Strychnos spinosa	1.35	55.77	2.1	Go
Meliaceae	*Ekebergia senegalensis*	0.05	-	-	No
	Khaya senegalensis	0.25	1.28	0.05	Go
	Pseudocedrala kotschyi	2.05	26.28	0.99	Go
Mimosaceae	Acacia ataxacantha	0.05	7.05	0.27	Go
	Acacia erythrocalyx	-	7.05	0.27	Ne
	Acacia gerrardii	0.1	-	-	No
	Acacia macrostachya	1.1	3.21	0.12	Go
	Acacia sieberiana	-	1.28	0.05	Ne
	Acacia polyacantha	0.3	2.56	0.1	Go
	Acacia tortilis	0.15	-	-	No
	Albizia zygia	0.05	-	-	No
	Parkia biglobosa	0.05	-	-	No
	Prosopis africana	1.65	21.15	0.8	Go
	Dichrostachys cinerea	0.05	62.18	2.34	Go
	Entanda africana	0.45	2.56	0.1	Go
Moraceae	*Ficus sur*	0.05	-	-	No
	Ficus thonningii	0.05	-	-	No
	Ficus ingens	0.15	-	-	No
Myrtaceae	*Syzygium guineense* var. *macr.*	0.15	-	-	No
Olacaceae	*Ximenia americana* L.	2.9	19.87	0.75	Go
Opiliaceae	*Opilia celtidifolia*	0.3	20.51	0.77	Go
Polygalaceae	*Securidaca longepedunculata*	0.05	17.95	0.67	Go
Rhamnaceae	*Ziziphus mauritiana*	0.05	0.64	0.02	Go
	Ziziphus mucronata	-	3.85	0.14	Ne
Rubiaceae	*Crossopteryx febrifuga*	3.95	24.36	0.92	Go
	Gardenia aqualla	0.6	59.62	2.24	Go
	Gardenia ternifolia	0.55	48.08	1.81	Go
	Morelia senegalensis	0.05	-	-	No
	Sarcocephalus latifolius	1.4	-	-	No
	Feretia apodanthera	0.15	14.74	0.55	Go
Sapotaceae	*Vitellaria paradoxa*	0.2	1.28	0.05	Go

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Sterculiaceae	*Sterculia segitera*	0.35	0.64	0.02	Go
Tilliaceae	*Grewia barteri*	-	0.64	0.02	Ne
	Grewia lasiodiscus	0.1	-	-	No
	Grewia venusta	-	96.79	3.64	Ne
	Grewia vilosa	-	3.21	0.12	Ne
Ulmaceae	*Celtis integrifolia*	0.25	-	-	No
Verbenaceae	*Vitex doniana*	0.05	-	-	No
	Vitex simplicifolia	-	1.92	0.07	Ne

D = density (stems/ha); SIR = specific index of regeneration; Go = Good regeneration, Ne = New species, Fa = Fair regeneration and No = No regeneration; * = results published in Todou et al (2017a).

IV. CONCLUSION

At the end of this study, it was demonstrated the structure of all plants species grouped together was reverse J-shaped distribution characterized by regular natural regeneration. Structure of eight selected species demonstrated two types of patterns of population structure: bell-shaped pattern for two species and reverse J-shaped pattern for six species. These specific structures showed that the structure based on the height of the total stand is the result of the dynamics of all species and their interactions.The diversity of juvenile plants stand was moderated with fairly good equitablilitysimilar to adult plants diversity. The global stand regeneration was good that species with the greatest potential for regeneration were*Hymenocardia acida*, *Combretum collinum*, *Annona senegalensis* and *Isoberlinia doka*. But some species with no regeneraion were recorded too and 11 species were represented by only juvenile plantsproving the ecological role of frugivorous animals.These results of structure and natural regeneration status provided quantitative informations on the community structure and natural regeneration status of woody plant species for the efficient conservation and sustainable use.They may be applicable during forest management.

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Prevalence of Microbial Loads on Betel Leaf with Special emphasis on Multidrug Resistance Salmonella spp and its Public Health Implications

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Abstract**—** *Presence of pathogen especially Salmonella spp in the Betel leaves suspended export of Betel leaf in Europe. Bangladesh has a subtropical monsoon so the present study was undertaken to determine Microbial loads of Betel leaf on the basis of seasonal variation (rainy and winter season). A total of 50 Betel leaf samples were collected from five sources (betel field, transport, whole seller, local shop, betel leaf washing water used in local shop Highest TVC (total viable count) were counted from local shop sample (5.3X 10^5CFU/ml) and the lowest TVC was found from field sample (2.5X 10^3 CFU/ml). This study results showed that during rainy season (July-October) TVC count was higher than winter season (November-February). From this study 10 genera of bacteria, were isolated from betel leaf such as E.coli, Vibrio spp, Bacillus spp ,Pseudomonas spp, Klebsiella spp, Salmonella spp, Shigella spp, Staphylococcus spp, Enterococcus spp and Proteus spp) and 5 genera of fungus (e.g. Aspergillus spp , Fusarium spp, Rhizopus spp, Zygosaccharomyces spp and Rhizoctonia spp)were isolated. Out of 184 isolates we found the following percentage of isolated microorganisms: 17.9% in betel leaf field, 19.5% in Transport, 19.5% in wholesaler, 28.8% in local shop and 14.3% in betel leaf washing water from local shop. Antibiotic sensitivity test showed that all of the isolates were resistant to Bacitracin, Penicillin, Vancomycin, Erythromycin and against other 5 antibiotics (Azithromycin, Gentamycin, Cephalexin, Ciprofloxacin and Chloramphenicol) isolates showed Resistant, Moderate and Sensitive Results.Data of this study suggest that Betel leaves from different source could harbor multidrug resistant bacteria specially Salmonella spp which underscore the need of implementation of hygienic practices during production, harvesting, transportation, storage, selling and preparation of Betel leaves to safeguard public health.*

Keywords**— Antibiotic Sensitivity, Betel leaf, Drug Resistance, Seasonal Variation, TVC.**

I. INTRODUCTION

Botanical name of betel vine is *Piper betel*. In Bangladesh, it is known as 'paan'. It is available in many Asian countries including Bangladesh. The betel plant originated from the South and South East Asia. The betel leaf is cultivated in most of South and Southeast Asia. Betel leaves has good export potential and Bangladesh exports betel leaves to the countries like Pakistan, India, Indonesia, Malaysia, Burma and Thailand. The harvested leaves are consumed locally or exported to other parts of Asia, the Middle East, Europe, and the United States.

In Bangladesh, farmer prepares a garden called a barouj in which they grow betel. The barouj is fenced with bamboo sticks and coconut leaves. The soil is plowed into furrows of 10 to 15 meters length, 75 centimeters in width and 75 centimeters' depth. Oil cakes, manure, and wood ash are thoroughly incorporated with the topsoil of the furrows. The creeper cuttings are planted at the beginning of the monsoon season. The harvest lasts for 15 days to one month. Betel plays an important role in the economy of rural Bangladesh. In some regions betel leaf cultivation is the main source of income for farmers. A total of 2,825 hectares of land is dedicated to betel vine farming. The average production cost for these betel farms in Bangladesh are about Tk. 300,000 per hectare, and the farm owners can earn a profit of over Tk. 100,000 per hectare. Betel vine is an important medicinal and recreational plant in Southeast Asia.

Betel leaf (Paan) export to the European and Middle Eastern countries stood at over US $ 31 million in 2012.

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Detection of *Salmonella* bacteria in betel leaf from Bangladesh in the UK prompted the European Union to suspend imports. Expatriates from Bangladesh and India are the primary customers of betel leaf in European countries. Saudi Arabia and the USA are other big markets for betel leaf [1]. The government has taken an initiative to produce bacteria-free betel leaf in order to resume its export.

The surface of Betel leave can be contaminated with microbial pathogens by polluted air, water and soil, during pre-harvest stage. Packaging materials used for carry and storage at Betel leaf, moisture content and water used for washing of Betel leaf are important sources of contamination during post-harvest stage [2].

1.1 Rationality of the Study

According to the Food Standards Agency, UK- Since October 2011 there have been several food safety notifications concerning the presence of a range of pathogenic *Salmonella* strains found in foodstuffs containing or consisting of betel leaves originating in or consigned from Bangladesh. There is a temporary suspension of imports of betel leaves from Bangladesh until 30 June 2018 [3]. So it's high time to develop methods for controlling *Salmonella spp*. in the Betel leaf for local consumption.

Detection of *Salmonella* bacteria in betel leaf from Bangladesh in the UK prompted the European Union to suspend imports. Expatriates from Bangladesh and India are the primary customers of betel leaf in European countries. Saudi Arabia and the USA are other big markets for betel leaf [4].

The surface of Betel leaves can be contaminated with microbial pathogens by polluted air, water and soil, during pre-harvest stage. Packaging materials used to carry and storage Betel leaf, moisture content and water used for washing are important sources of contamination during post-harvest stage [5].

1.2 Prevalence of microorganism in food (leaf) products

Post- harvesting the spoilage of betel leaves accounts for the post-harvest loss in the range of 35%–75% respectively [6][7].

A comprehensive microbiological investigation of pathogen causing leaf diseases has been conducted to isolate, classify and characterize micro flora of Betel leaf. *Xanthomonas compestris* pv. *Betticola* bacteria have been identified previously from damaged Betel leaves [8].

Across Trinidad the prevalence and microbial load of *Listeria spp*. *Escherichia coli* O157 and *Salmonella spp*. was determined in the products of supermarkets. The microbial load was assessed using the total aerobic plate count (TAPC) per g/ml of foods and prevalence of *Escherichia coli* O157 and *Salmonella spp*. were determined using conventional methods. For *Listeria monocytogenes*, immune magnetic separation (IMS), TECRA (enzyme-linked immune sorbent assay, ELISA) and conventional methods were used. The $\log_{10}$ mean ± SD TAPC per g or ml was highest for vegetables (11.0±11.6), and lowest for seafood (5.2±5.7) ($p < 0.05$). The prevalence of *L. monocytogenes* was 1.7%. Sixteen (4.5%) of 153 samples yielded *E. coli* but all samples were negative for *Salmonella spp*. and *E. coli* O157 [9]

A significant bacterial count (CFU g) was detected in jhal-muri (1.66x10 CFU g), betel-leaf (1.49x10 CFU g), hog-plum (1.87x10 CFU g), sweet (3.39x10 CFU g) and bun (3.11x10 CFUg). Sola (6.24x10 CFU g), cup-cake (6.19x10 CFU g), peaju (4.96x10 CFU g), sheek-kabab (2.63x10 CFU g) an vhel-puri (1.96x10 CFU g) found to be contaminated with moderate bacterial count whereas, in singara (8.93x10 CFU g) and somosa (4.11x10 CFUg) load was found. Jhal-muri, hog-plum, betel-leaf, peaju, sheek-kabab, singara and vhel-puri were found to be 100 % contaminated with coliforms with an unacceptable range, as compared to somosa (75 %), sola (50 %) and bun (25 %). But cup-cake and sweet were free from contamination with coliforms (0 %). So among the 48 RTE food samples, 29.16 % of them did not contain coliforms. It was found that, sola (6596 CFU g), hog-plum (6197 CFU g), betel-leaf (3856 CFU g) and jhal-muri (2312 CFU g) were hazardously contaminated with fungi [10], when evaluated bacterial loads in salad vegetables using spread plate agar dilution method was done. Bacterial loads ranged from 1.6 x 10^6 to 2.9 x 10^8 CFU/g .*Escherichia coli*, *Klebsiella* and *Enterobacter* were amongst the Coliforms (lactose fermenters), while *Proteus*, *Pseudomonas aeroginosa*, *Salmonella* and *Shigella* were non-lactose fermenters associated with the samples.

Salmonella spp is an important zoonotic pathogen that cause an estimated 1.4 million illness, 16000 hospitalization and between 400 to 600 deaths annually in the united states alone [11][12]. *Salmonella* can produce invasive infections that lead to sepsis and death. Young children, the elderly and those with compromised immune systems are especially susceptible to severe disease.

The prevalence of multidrug resistant among *Salmonella* strain has increased over the past two decades [13][14][15], making treatment failures more common among those with serious disease. In addition, infections with resistant strains of Salmonella tend to be more severe and lead to higher rates of hospitalization than those caused by susceptible strains [16][17][18][19].And multidrug - resistant strains of zoonotic *Salmonella spp* present on ready-to-eat Paan is a public health concern. It may be one of the factors responsible for the hyper endemic status of salmonellosis [20]. People generally acquire salmonellosis through foodborne exposure,

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although direct contact with infected animals is another possible route [21][22]. The outcome of different experiments showed that the best season for longer storage of betel leaves in any of the form which may be petiolated or depetiolated is winter seasons i.e. December-January [23] .

Fungus is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds as well as the more familiar mushrooms. These organisms are classified as a kingdom, Fungi which are separate from the other eukaryotic life kingdoms of plants and animals. Fungi spoiling organisms are silently invading, acidifying, fermenting, discolouring, and disintegrating microbes that render corn such as maize, wheat, barley etc. Fungi spoilage is caused by two factors, (biotic) living which includes insects, birds, rodents and microorganisms and (non-biotic) non-living which includes temperature, humidity and time. The world is concerned with food safety that has enhanced interest in fungal and subsequent food spoilage. Contamination with mould causes deterioration of product which affects human and animal health. [24]. Fungal spoilage of corn reduces the nutritional value and palatability of the feed, thereby increasing its allergic potential and may result in mycotoxic contamination [25].

II. RESULTS

2.1Collection and transportation of samples

A total of 50 betel leaf samples were collected from Different sources (betel field, transport, whole seller, local shop, betel leaf washing water used in local shop) on the basis of seasonal variation (rainy and winter season). Individual sample placed in the sterile container. The samples were transported carefully to the Bacteriology laboratory for bacteriological analysis.

2.2 Processing of betel leaf samples

The betel leaf samples in polythene-bag were washed with sterile PBS (phosphate buffered saline). One Betel leaf was washed with 20ml of sterile PBS. A 5 fold serial dilution of the washed samples was prepared in nutrient broth.

2.3 Determination of Total Viable count (TVC) of betel leaf

A total of 0.1 ml 10 fold diluted sample (10^{-1} to10^{-6}) was transferred and spreaded onto nutrient agar (NA) and incubated at 37ºC for 24-48 hours. TVC was determined by using the following formula

CFU/ml=Number of colonies/ml x dilution factor

Table.1: Total Viable count of betel leaf

Betel leaf source	Sample	Seasonal variation		TVC, CFU/ml
Route level(field)	1,6,11,16,21	Winter	1.68 X 10^4	1.82 X 10^4
	26,31,36,41,46	Rainy	1.96 X 10^4	
Transport	2,7,12,17,22	Winter	1.32 X 10^5	1.41 X 10^6
	27,32,37,42,47	Rainy	1.55 X 10^5	
Whole seller	3,8,13,18,23	Winter	2.7 X 10^5	2.64 X 10^6
	28,33,38,43,48	Rainy	2.58 X 10^5	
Local shop	4,9,14,19,24	Winter	4.42 10^4	2.31 X 10^6
	29,34,39,44,49	Rainy	4.18 X 10^5	
L.S.W.W	5,10,15,20,25	Winter	1.51x 10^5	1.98 X 10^6
	30,35,40,45,50	Rainy	2.46 X 10^5	

* L.S.W.W= Local Shop Washing Water

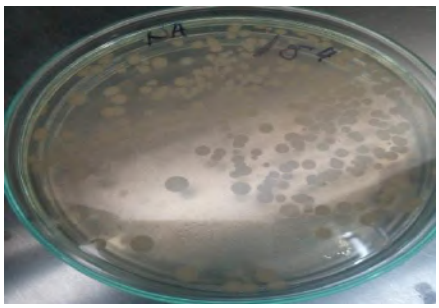

Fig1: Growth of microorganism on Nutrient Agar medium (TVC)

2.4 Statistical Data Analysis for significance level

Test for two independent Samples/Two –tailed test was performed to show statistical significance (Table-2).

A one way ANOVA (Table-3, 4) followed by Analysis of the differences between the categories Fisher (LSD) test (Table-5) were also used. We also conducted t Paired test, ANOVA test followed by Fisher (LSD) to find out whether our calculated value had any significance.

Table2: Test for two independent Samples/Two-tailed test, 95% confident interval on the difference between the means of different collection site

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Variable	Rainy	Winter
Observations	25	25
Obs. with missing data	0	0
Obs. without missing data	25	25
Minimum	10000	10000
Maximum	630000	300000
Mean	219440	122960
Std. deviation	187260.754	117572.488
Difference		96480
t (Observed value)		2.182
|t| (Critical value)		2.011
DF		48
p-value (Two-tailed)		0.034
Alpha		0.05

t-Paired test interpretation

H0: The difference between the means is equal to 0.

Ha: The difference between the means is different from 0.

As the computed p-value is lower than the significance level alpha=0.05, we can reject the null hypothesis H0, and accept the alternative hypothesis Ha

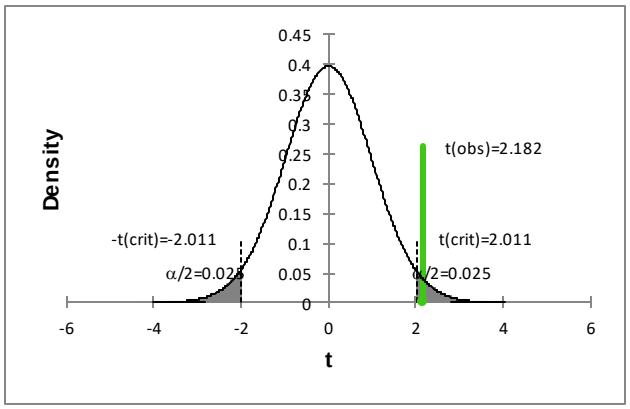

Fig2: t-test for two independent samples / Two-tailed test

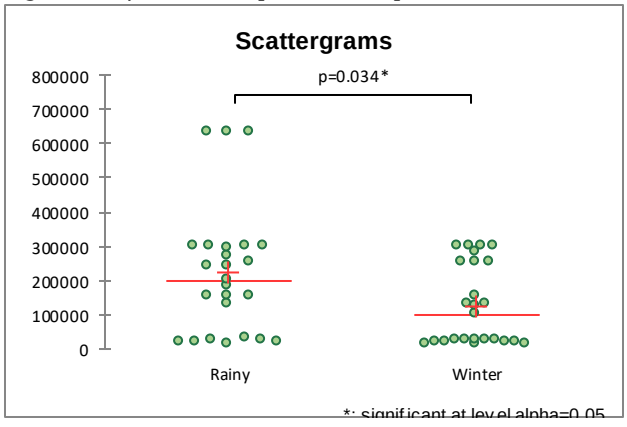

Fig 3: Scatter grams of TVC Count on the basis of Rainy and Winter Season.

Table 3: Summary statistics (Quantitative data): One way ANOVA test

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
TVC	50	0	50	10000	630000	171200	162236.411

Table 4: Analysis of variance, ANOVA (TVC)

Source	DF	Sum of Square	Mean square	F	Pr>F	F Crit
Between Groups	4	370940200000.000	92735050000.000	4.542	**0.004**	**2.45**
Within Groups	45	918771799999.999	20417151111.111			
Total	49	1289712000000.000				

Our Calculated value, F (4, 45) = 4.452, P=0.004 is higher than F Critical Value 2.45 so there is a significant difference among the TVC count of different collection Site.

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Table 5: Summary of all pair wise comparisons for C.S (Fisher LSD)

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Wholeseller	264000	45185.342	172992.050	355007.950	A	
Local shop	231100	45185.342	140092.050	322107.950	A	
L.S.W.W	198600	45185.342	107592.050	289607.950	A	
Transport	144100	45185.342	53092.050	235107.950	A	B
Field	18200	45185.342	72807.950	109207.950		B

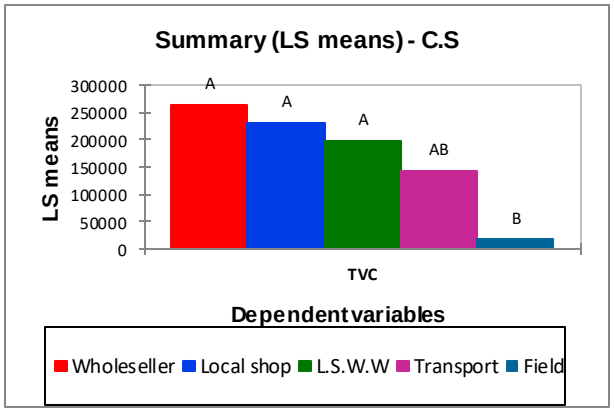

Fig 4: Bar diagram of TVC Count (LS means) on the basis of Collection Site.

2.5 Isolation and Identification of Microorganisms

After Microscopic observation followed by cultural and biochemical test results observation , 10 genera of bacteria (e.g. *E.coli*-21.73%, *Vibrio* spp - 7.6% ,*Bacillus spp* -2.7%, *Pseudomonas* spp-3.84%, *Klebsiella* spp - *7.06%* , *Salmonella* spp -19.5%, *Shigella* spp - 5.43%,*Staphylococcus* spp-5.43%, *Enterococcus* spp-*4.89%* and *Proteus* spp-*1.63%*) and 5 genera of fungus (e.g. *Aspergillus* spp-*5.43%*, *Fusarium* spp- *4.89%*. *Rhizopus* spp-*3.84%*, *Zygosaccharomyces* spp- *3.26%*, *Rhizoctonia* spp-*2.71%*) were isolated [26] [27].

Out of 184 isolates we found the following percentage of isolated microorganisms 17.9% in betel leaf field, 19.5% in Transport, 19.5% in wholesaler, 28.8% in local shop and 14.3% in betel leaf washing water from local shop.

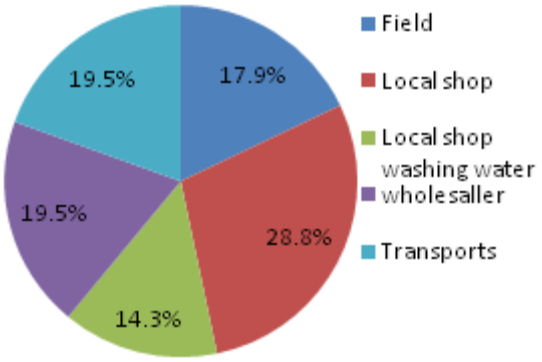

Fig.5: Percentage of isolated pathogen from betel leaf

Antibiotic sensitivity test showed that all of the isolates were resistant to Bacitracin , Penicillin, Vancomycin, Erythromycin and against other 5 antibiotics (Azithromycin, Gentamycin, Cephalexin , Ciprofloxacin and Chloramphenicol) isolates showed Resistant, Moderate and Sensitive Results. [28]

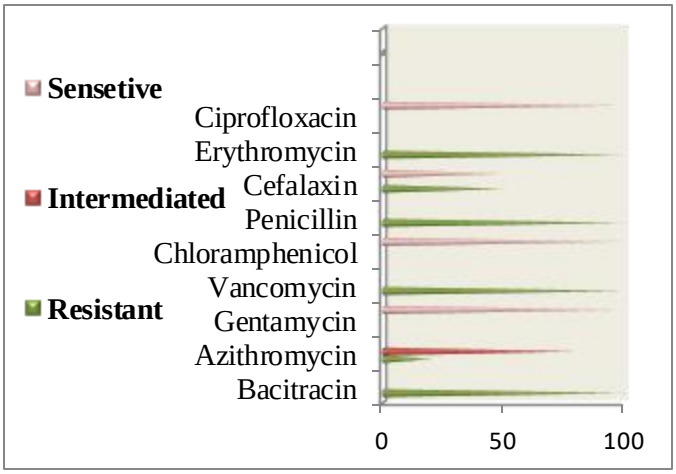

Fig.6: Antibiotic Sensitivity of Bacteria isolated (total 125) from betel leaf

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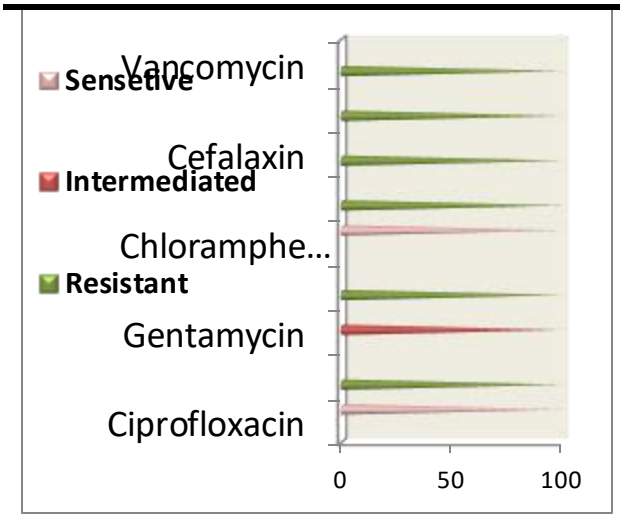

Fig.7: Summary of antibiogram profile of Salmonella spp. against 9 antibiotics.

III. DISCUSSION

Highest TVC counted from Whole seller sample (2.64 X 10^6) and the lowest TVC counted from field sample(1.82 X 10^4).TVC count from different source vary might be due to unsanitary environment, use of polluted water to wash Betel leaf and unclean utensil used to storage Betel leaf.

This study results showed that during rainy season (July-October) TVC count was higher than winter season (November-February).

We also conducted t Paired test, ANOVA test followed by Fisher (LSD) to find out whether our calculated value had any significance. From the statistical data analysis we have found that our observed data were significant at 95% confidence level.

In the present study, selective media (EMB, TCBS. SS. Macconkey, SDA, MSA) were used for isolation of *E. coli, Vibrio* spp. *Salmonella* spp.,and *Klebsiella spp Bacillus spp, Staphylococcus spp, Enterococcus spp* .

In this study, the colony characteristics of *Vibrio* spp. on TCBS agar plate were similar to the findings of Tankeshwar Acharya. In Gram's staining bacteria exhibited curved rod shaped appearance which was also observed by other researchers [29][30][31].

The colonies of *Salmonella* spp. on agar SS plate were opaque, translucent with black centers which were similar to the findings of Cheesbrough.[32].In Gram's staining *Salmonella* spp. exhibited short rods, Gram negative, single or paired in arrangement. Similar findings were also reported by Buxton and Frase [33][34].

From this study 10 genera of bacteria, were isolated from betel leaf such as *E.coli*-21.73%, *Vibrio spp -7.6%, Bacillus* spp -2.7%, *Pseudomonas* spp-3.84%, *Klebsiella* spp-*7.06%* , *Salmonella* spp-19.5%, *Shigella* spp-5.43%,*Staphylococcus* spp-5.43%, *Enterococcus* spp-*4.89%* and *Proteus* spp-*1.63%*) .

A study conducted in Bangladesh found that the prevalence of *E. coli* was 17.34% (17 of 98), *Salmonella* spp. was 25.51% (25 of 98), *Vibrio* spp. was 19.39% (19 of 98), *Bacillus* spp. was 18.37% (18 of 98), and *Staphylococcus* spp. was 19.39 (19 of 98) [35].

A total of 5 genera of fungus (e.g. *Aspergillus spp-5.43%, Fusarium spp- 4.89%. Rhizopus spp-3.84%, Zygosaccharomyces spp- 3.26%, Rhizoctonia spp-2.71%*) were isolated

A study conducted in India isolated from Betel leaves isolated *Xanthomonas compestris* PV. Betticola fungi from diseased Betel leaves. **[2]**

From our observation out of 184 isolates we found the following percentage of isolated microorganisms: 17.9% in betel leaf field, 19.5% in Transport, 19.5% in wholesaler, 28.8% in local shop and 14.3% in betel leaf washing water from local shop.

In case of *Salmonella* we have found that 38% of betel leaf sample was contaminated with *Salmonella* spp. Among them 7, 6 and 5 no of *Salmonella spp* were isolated from Transport, Whole seller and Local Shop Betel leaf Samples respectively. Our study showed that Transport is the major source of *Salmonella* spp contamination in Betel leaf consumed in Bangladesh.

A study conducted in Bangladesh found that77% betel leaf sample collected from different markets of Dhaka city was found to be contaminated with Salmonella spp. [36].

Antibiotic sensitivity test show most of the isolates were resistant to bacitracin, penicillin. More shocking report is, most of the people in Bangladesh use Erythromycin and Azithromycin antibiotic vigorously but this study show erythromycin were resistant against four isolates and azithromycin show both moderate and resistant result and Ciprofloxacin was sensitive to all tested isolates. On the other hand gentamicin shows sensitive against the isolates. Cephalexin show both sensitive and resistant result [37].

Our isolated 18 isolates of *Salmonella spp* showed completely resistance to Bacitracin, Penicillin, Vancomycin, Erythromycin, Azithromycin, Amoxicillin and sensitive against other 2 antibiotics, Ciprofloxacin and Chloramphenicol. They are intermediately sensitive to Amoxicillin. Indiscriminate use of antibiotic is responsible for emergence of multidrug resistant *Salmonella spp*. [19] [38].

Data of this study suggest that Betel leaves from different source harbor multidrug resistant [39] bacteria which underscore the need of implementation of hygienic practices during production, harvesting, transportation, storage, selling and preparation of Betel leave to safeguard public health. From this study we could suggest that Betel leaves might be contaminated with bacteria not

only due to use of potable water for washing, handling of Betel leave with unclean hands but also use of unclean utensil or cutting board when preparing ready to eat Betel leaves.

IV. CONCLUSION

Data of this study suggest that Betel leaves from different source harbor multidrug resistant bacteria which underscore the need of implementation of hygienic practices during production, harvesting, transportation, storage, selling and preparation of Betel leaves to safeguard public health.

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Status and Management of Cashew Disease in Tanzania

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Abstract**—** *Cashew (Anacardium occidentale L.) is one of the most important export crops and the main source of cash income in the southern part of Tanzania. However it is challenged by a number of factors such as drought, declining soil fertility, un-improved low yielding cashew genotypes, insect pests and diseases. Of these factors, diseases have been cited to result in high production costs, poor nut quality and low market price. The most devastating diseases that attack cashew are powdery mildew, cashew leaf and nut blight, dieback and fusarium wilt. Other minor diseases include anthracnose, damping off and leaf spots. Despite the negative role that these diseases possess to cashew growers, there is limited or no critical updated information on their current infection status and management in Tanzania. Thus, this review article discusses the status of the most important cashew diseases and their management options in the country. Such information will be vital to cashew farmers and other stakeholders in making appropriate improvements in cashew production in Tanzania.*

Keywords**— Cashew, dieback, fusarium wilt, cashew Leaf and nut blight, powdery mildew.**

I. INTRODUCTION

Cashew *(Anacardium occidentale* L.) is a perennial nut crop, native to Brazil that belongs to the Anacardiaceae family (Ohler, 1979; Masawe, 2006; Zhongrun and Masawe, 2014). It was introduced to East Africa by the Portuguese in the 16th century and it is now widely cultivated, especially in Tanzania. (Masawe, 1994; Topper and Boma, 1997). The most important product derived from the plant is cashew nuts that are processed into kernels. The crop also produces other products such as juices, jam, alcohol and non-alcoholic beverages; all of which are produced from the cashew apples (Sobhana *et al*., 2010). In Tanzania cashew is the main cash crop and the leading source of income for over 300,000 households in South-Eastern Tanzania (Kasuga, 2013). It is estimated that more than 80% of the national cashew production comes from Mtwara, Lindi and Ruvuma (Tunduru District) regions (CBT, 2015). The area under cashew is estimated to be more than 400,000hectares in mono or mixed crop production systems. An average cashew farmer owns 1-2 hectares of cashew trees (Topper *et al.*, 1997). The average yield in farmers' fields ranges from 500kg/ha to 800kg/ha (Masawe, 2006).

Cashew has been one of the most important export crops since independence in Tanzania. The cashew production increased rapidly in 1960s towards mid-1970s, recording as high as 145,000 MT (metric ton). Thereafter there was drastic decline in production up to 16,400 MT in 1973/74. The reasons for the decline in cashew nut production were drought, declining soil fertility, unimproved low yielding genotypes, insect pests and diseases (Ellias, 1980; Brown *et al*., 1984). Of the factors, diseases (Table 1) have been a major challenge in cashew since the 1970s. Of the diseases cashew powdery mildew disease (PMD) has been cited to be among important constraints to cashew production causing crop losses if not controlled ranging from 70 to 100% (Castellani and Casulli, 1981; Sijaona, 1984; Sijaona and Shomari, 1987; Intini, 1987; Shomari, 1988). The historical timeline for cashew diseases in Tanzania is shown in Table 1. In 2003, a second deadly disease known as 'cashew leaf and nut blight' caused by *Cryptosporiopsis* spp was reported for the first time, attacking cashew at all growth stages (Sijaona *et al.*, 2005; Sijaona *et al*., 2006). The disease causes crop losses of up to 48.4% annually if not controlled (ACRR, 2006). The third major disease was reported in 2012 by Tibuhwa and Shomari (2012), as cashew fusarium wilt caused by *Fusarium oxysporum*. This disease affects cashew trees leading to yield losses of up to 100% if not controlled (Tibuhwa and Shomari, 2016).

The current status of these major and minor diseases such as dieback, anthracnose, pestalotia leaf spot and damping off is discussed in this article. Such disease status information and how they are managed is vital for different cashew stakeholders in Tanzania. It can alert policy makers on how to collectively address cashew disease problems and plant breeders and or pathologists on how to develop resistant crop varieties and or integrated pest management options, respectively, against

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the diseases. A chronological history of occurrence of major cashew diseases in Tanzania is as shown in Table 1.

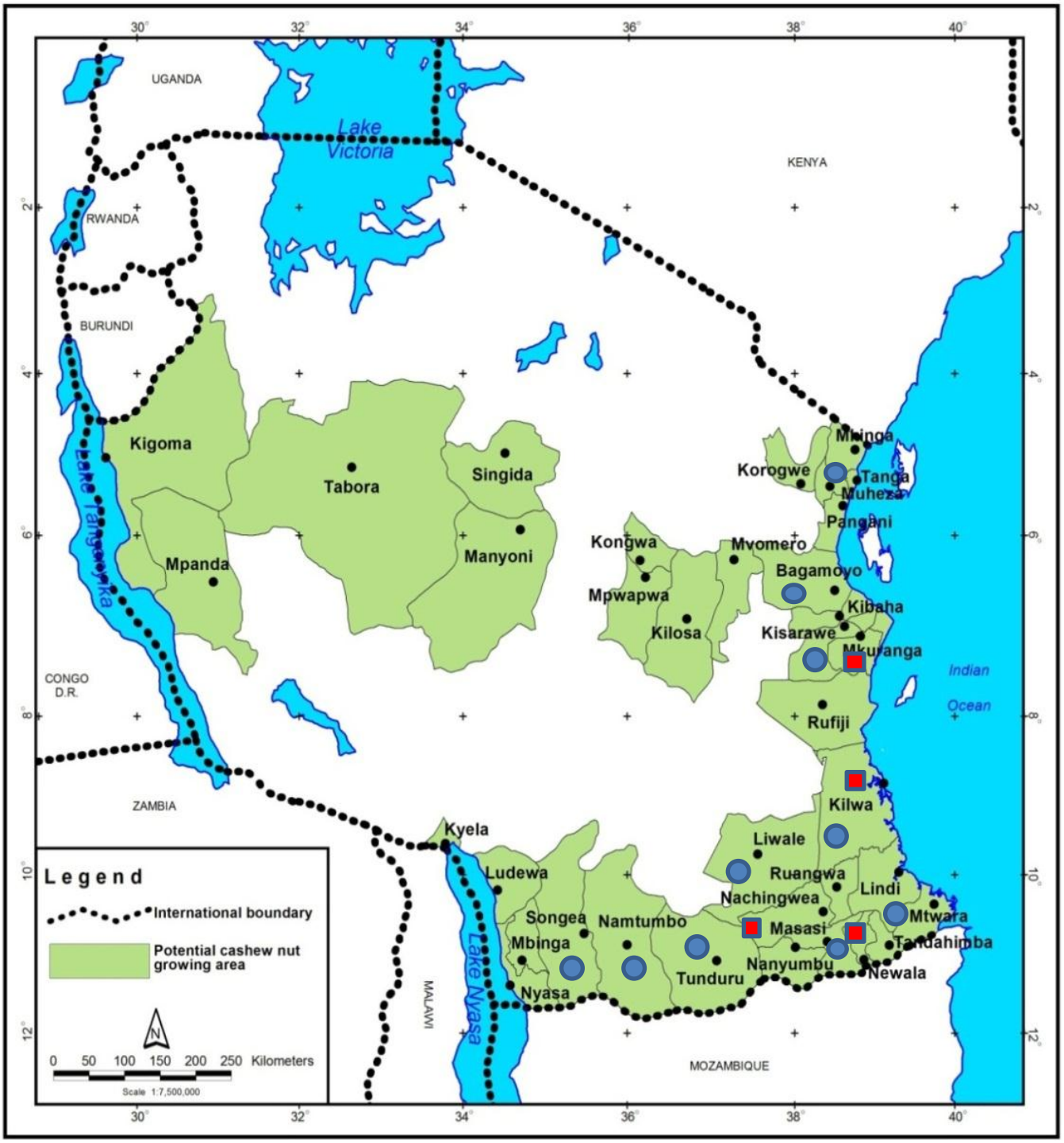

Fig.1: Distribution of cashew growing areas and major diseases affecting the crop in Tanzania (Source: ARI-Naliendele).Green coloured regions represents all cashew growing areas in Tanzania but affected by powdery mildew, dieback, and anthracnose; deep blue coloured circles represent cashew producing areas affected by cashew leaf and nut blight disease and the red coloured blocks represent locations affected by cashew fusarium wilt disease.

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Table.1: Historical time line of cashew diseases occurrence in Tanzania.

S/n	Disease	Causal agent	Year reported	References
1	Cashew powdery mildew disease	*Oidium anacardii* Noack	1970	Casulli, (1979) Sijaona, (1984) Shomari, (1988)
2	Anthracnose disease	*Colletotrichum gloeosporoides* Penz	1978	Casulli, (1981)
3	Dieback disease	*Phomopsis anacardii*	1980	Intini and Sijaona (1983)
4	Pestalotia Leaf Spot disease	*Pestalotia* spp.	1980	Intini and Sijaona (1983)
5	Damping off disease	*Fusarium* spp., *Pythium* spp., *Phytophthora palmivora* Butler,	1980	Intini and Sijaona (1983)
6	Cashew leaf and nut blight disease	*Cryptosporiopsis* spp	2003	Sijaona *et al*, (2005) Sijaona *et al*, (2006)
7	Cashew fusarium wilt disease	*Fusarium oxysporum*	2012	Tibuhwa and Shomari, (2016)

II. CASHEW PRODUCTION TREND AND DISTRIBUTION OF MAJOR DISEASES IN TANZANIA

Tanzania is the world's eighth and Africa's third largest cashew nut producer after Mozambique and Ivory Coast (CBT, 2011). The main producing areas and distribution of major cashew diseases in Tanzania is as shown in Figure 1 and the production trend from 1945s shows a zigzag production style in Figure 2, nevertheless the production is currently increasing possible due to increased acreage of production.

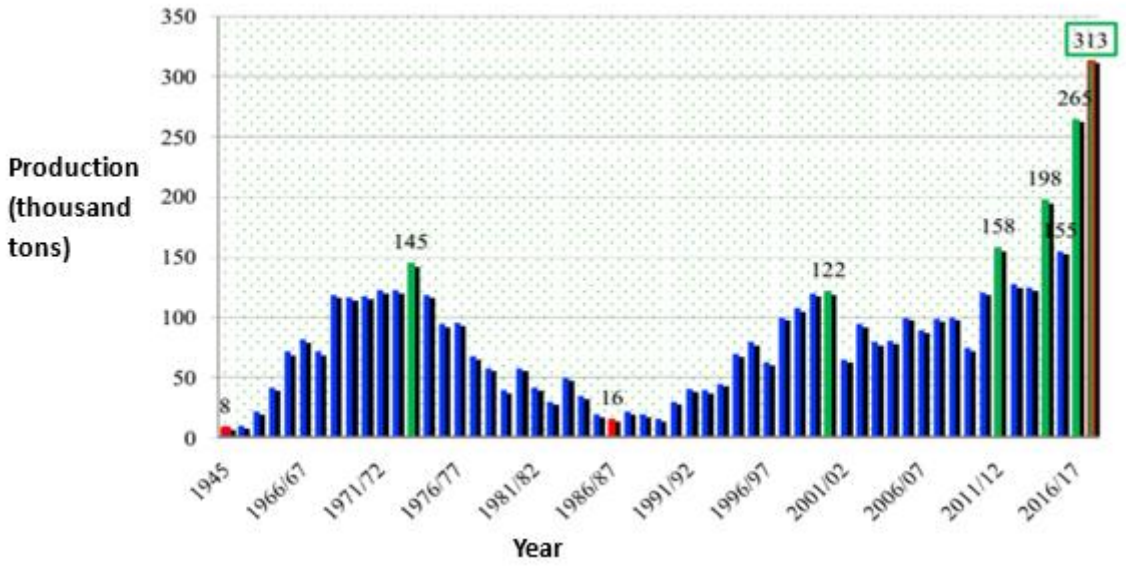

Fig.2: Raw cashew nut production trend in Tanzania since year 1945 to year 2017(Source: Cashewnut Board of Tanzania).

III. HISTORY, EFFECT, CONDITIONS FAVOURING CASHEW DISEASE AND THEIR MANAGEMENT IN TANZANIA

Cashew Powdery mildew disease

The history of powdery mildew goes back to the 1950s; however it was not economically important in Tanzania until mid-1970s (Casulli, 1979; Castellani and Casulli, 1981; Sijaona, 1984; Sijaona and Shomari, 1987; Shomari, 1988). In 1975 the rate of powdery mildew disease infection on cashew started to increase (Sijaona and Shomari, 1987) which resulted into decline in cashew production from 145,000 MT in 1973/74 to 16,4000 MT in 1986/87 (CRP Report, 2006). The decline in cashew production was consistent in all cashew-growing areas in the country, which resulted into huge losses of revenue for both growers and the government (Topper *et al.*, 1997). The increased outbreak of the powdery mildew disease was then associated with change of environmental conditions and poor cashew management resulting from villagilization program which took place in 1974/1975

(Ellias, 1980; Brown *et al*., 1984). To date, powdery mildew disease is the main constraint of cashew in Tanzania. The disease is caused by *Oidium anacardii* Noack, a fungus of genus Odium of the Deuteromycotina (Fungi Imperfecti) (Shomari, 1996; Sijaona, 1997). Powdery mildew disease infests all tender tissues of the cashew trees, mainly the tender leaf and inflorescence including the part not well unfolded. The disease seldom attacks old and mature leaves (Sijaona *et al.,* 2006). A white powdery growth is formed on the infested fruit bearing branches and inflorescence. The lesions of the infected parts turns to brown and after 2-3 weeks they shrink gradually and become dry and shed, leading to drying out and drop of numerous flowers and tender fruits (Sijaona and Shomari, 1987). Infected apples turn dull and their skin becomes much coarser. The apples when heavily infected show deep cracks on the surface and gradually shrivel and dry up (Sijaona *et al*., 2005). The tender nuts when infected are deformed on the shell. The lesions turn grey on infected tender apples and nuts. Infected nuts deteriorate in quality during storage, decays easily and produce poor quality kernels when processed (Shomari 1996; Waller *et al.,* 1997; Sijaona, 1997).

All cashew varieties are susceptible to the powdery mildew disease but at different levels. Most unimproved cashew varieties succumb more to the disease compared to improved varieties, which have a certain level of resistance or tolerance (Masawe 2006; Sijaona, 2013). The conducive environment for PMD are cold nights which are followed by warm daytimes leading to mist and fog conditions in the early mornings. An optimum temperature ranges between 25-28^{o}C with optimum at 26^{o}C. Relative humidity that is conducive to the environment ranges between 80-100% with optimum at 95% (Noak 1898; Ohler 1979; Casulli, 1979; Chacko et al. 1990; Sijaona, 2013). The PMD spores are mainly dispersed by wind as rainfall inhibits its development. However, perennation and survival of the pathogen from one season to another takes place in fallen infested leaves, water shoots and off season flowers (Sijaona *et al.,* 2006). Powdery mildew disease is not dormant and can occur on the tree canopy all the year around by wind dispersal (Shomari, 1996; Sijaona, 1997). Undertaking sanitation, which is basically the removal of water shoots underneath tree canopies, pruning branches to allow aeration, clearing of dropped branches and leaves to remove the source of the inoculum can reduce and delay occurrence of this disease for weeks (Casulli, 1979). Overlapping branches and twigs under the crown without penetration of sunlight and lack of rains are optimum condition for the powdery mildew fungus to survive (Shomari, 1996; Zhongrun and Masawe, 2014). Powdery mildew is currently controlled mainly using fungicides (chemical control). Several fungicides including sulphur dusts, wettable powders (Cassulli, 1981; Intini and Sijaona, 1984) and water based organic fungicides (Topper *et al.,* 1997; Sijaona *et al*., 2001) have been recommended for control of the PMD in Tanzania but more efforts are still going on to have more fungicides. Cashew varieties partially resistant to PMD have been developed in Tanzania (Masawe, 2006). These varieties have been commercialized and made available to farmers as grafted seedlings through Cashew Development Centres (CDCs) located in the main cashew growing areas in Tanzania. Polyclonal Seed Orchards (PSG) was established using these varieties for production of planting materials in form of seeds. Still there is a need of developing other new resistant materials for controlling powdery mildew disease to increase cashew production.

Cashew Anthracnose disease

The history of Anthracnose in Tanzania goes back to 1978 (Casulli, 1981). This fungal disease caused by *Colletotrichum gloeosporoides* Penzis not only a problem in cashew but also infects other tropical fruits trees including mango, citrus, avocado and papaya (Sijaona, 2013). The disease attacks all young and tender vegetative organs together with nuts and pseudo fruits/apples. The disease is favoured by relative humidity of 95% - 100% and temperature ranging between 22^{o}C - 28^{o}C during flowering and fruiting period (Sijaona, 2013; Zhongrun and Masawe, 2014). Early symptoms are reddish brown shinny water-soaked lesions and resin exudation on the affected parts. Infected shoots appear as “hanging nuts” Hanging nuts may act as a source of disease infection during the next season (Zhongrun and Masawe, 2014). Cashew varieties resistant or partially resistant to anthracnose were developed in Tanzania (Masawe, 2006). These varieties have been commercialized and made available to farmers as grafted seedlings. Other approaches of controlling anthracnose disease have been discussed in table 2.

Cashew Dieback disease

Cashew Dieback disease was reported in Tanzania in 1980 according to Intini and Sijaona (1983). This fungal disease caused by *phomopsis anacardii* Early and Punithalingam is believed to be facilitated by damage caused by mirid (*Helopeltis* spp) or coconut bug (*Pseudotheraptus wayii*) on cashew plant (Zhongrun and Masawe, 2014). The symptoms of the disease include withering of the panicles, followed by a progressive dieback of small flower stalks. This starts from the tips then advances downward to the main floral shoots (Intini and Sijaona, 1983). The normal greenish colour of the health shoots progressively turns brown resulting in loss

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of flowers. Infected young nuts and apples become black and fluffy and remains attached to the floral stalks. Heavy infection appears similar to fire damage (Sijaona, 2013). Damage caused by insect attack (*Helopeltis* spp *or Pseudotheraptus wayii*) are considered as predisposing factors to dieback infection. The fungus attack young and tender shoots and flowers followed by dieback infection starting at tips and spreading downwards. The dieback disease of cashew is found in every cashew growing areas. The different methods used to control the infections are well explained in Table 2.

Cashew Pestalotia leaf spot disease

Pestalotia leaf spot is a disease caused by a fungus known as *Pestalotia heterocornis* Gubaand it was reported in 1980 (Intini and Sijaona, 1983). The fungus attacks mature leaves, forming angular to irregular leaf lesions, reddish brown on upper surface and pale gray to whitish on underside of leaves. Later on lesions become thinner, papery and necrotic. Severe infection may cause defoliation (Intini and Sijaona, 1983).The infected leaves show regular or irregular polyclonal lesions or round lesions. These lesions mostly appear on the leaf tip and they enlarge gradually and coalesce, expanding from the leaf tip downwards to more than half of the leaf within masses of conidia appearing on both lower and upper sides of the leaves (Zhongrun and Masawe, 2014). The pathogen develops at an optimum temperature and relative humidity range between 26^{o}C-28^{o}C and 80-100% respectively (Sijaona, 2013). The dispersal mechanism of the pathogen is mainly wind and free running water. The disease can best controlled by application of copper based fungicides (Zhongrun and Masawe, 2014), such as Kocide at the rate of 3-5 gm per litre of water at two week intervals. Other management methods are described in Table 2.

Cashew Damping off disease

Damping off cashew disease is caused by number of fungal organisms including Fusarium spp, Pythium spp, Phytophthora palmivora Butler, Cylindrocladium scoparium Morgan, Selerotium rolfsii Sacc and Pythium ultimum Trow; most of which occurs mainly at nursery (Intini and Sijaona, 1983). It was reported in Tanzania in 1980's and mainly infects young cashew seedlings in the nursery with poor drainage or container-raised young plants (Zhongrum and Masawe, 2014). The infected plantlets cease to grow and wither gradually and show circular water soaked stripes on the root collar (Sijaona, 2013). The roots may rot, leading to lodging of the plants (Zhongrum and Masawe, 2014). The nursery or the land for container raised young plants should be well drained to stop waterlogging. Seed beds can be leached by spraying with carbendazim 50% WP, Chlorothalonil 75% WP, carbendazim thiram zineb 80% WP, or cymoxanil manacozeb 72% WP. Further details on disease management are as described in Table 2.

Cashew Leaf and nut blight disease

The cashew leaf and nut blight disease was reported for the first in Tanzania in August 2002, (Sijaona *et al.*, 2005). The disease was reported in Nanyanga, Mtopwa sub-station and Chiwindi in Newala District. It was also observed in a neighbouring country at Itoculo farm in Monopo District, Nampula Province, Mozambique in 2005 (Sijaona, 2005 and Sijaona *et al.*, 2006). The disease has been reported to be more active during wet weather especially during off-season rains, where severe infections affect the young flushing material (Sijaona *et al.*, 2006). Infected cashew leaves develop silver/grey lesions with a dark reddish brown margin that enlarge and coalesce causing defoliation (Sijaona *et al.*, 2006). Infected young nuts blacken while older nuts results in characteristic dark lesions that under favourable conditions form white spore masses of the fungus within the nut lesions (Menge, 2013 and Menge, 2014). Cultural methods are done by removing, gathering, burning and burying all diseased fruits and branches and twigs left in the cashew plantation to reduce pathogen source in the field (Zhang and Masawe, 2014). Chemical methods are done by spraying fungicides such as Trifloxystrobin 10% SC, Difenaconazole WG, Picoxystrobin and Trifloxistrobin + tebuconazole (Table 2). Disease control commences when first symptoms occurs particularly during fruiting season. Two varieties which are tolerant or resistant to cashew leaf and nut blight disease have been developed and these are AZA 2 and AZA 17 (Masawe, 2006). Farmers are advised to use resistant varieties to control this disease. These varieties have been commercialized and made available to farmers as grafted seedlings (Masawe, 2006; Zhang and Masawe, 2014).

Cashew Fusarium wilt disease

The cashew fusarium wilt disease caused by *Fusarium oxysporum* was first reported in Tanzania in 2012 at Magawa village in Mkuranga Districtin the Coast region (Tibuhwa and Shomari, 2012). There after it was reported in Masasi District (Nanganga), Tandahimba District (Lindumbe) and Mtwara District (Mnongodi). The cashew fusarium wilt can cause the entire cashew plant to wilt within three to four weeks after first symptoms. The disease can attack the next nearby cashew trees until trees in the entire field are all wilted (Tibuhwa and Shomari, 2016). Infected cashew plant is characterised by gradual loss of natural green colour of leaves of some branches and then turns yellow (chlorosis) (Tibuhwa and Shomari,

2016). Looking from a distance, affected trees appear yellow and green and later within three to four weeks the entire tree(s) wilt (Tibuhwa and Shomari, 2016). Different methods including field sanitation and destruction of infected plant parts have been proposed controlling fusarium wilt disease. No other fusarium wilt managerial option has been proposed to the moment. Some fungicides have shown positive response in controlling the disease at laboratory level and field trials are in progress (Tibuhwa and Shomari, 2016). The room is open for scientists to work on in order to have positive control for this new devastating cashew disease.

Table.2: Current management options for cashew diseases in Tanzania

Disease	Causal agent	Management and description option
Cashew powdery mildew	*Oidium anacardii* Noack	**Cultural methods**:- Include sanitation by removing suckers, clearing of dropped branches and leaves also thinning and pruning unwanted branches (Casulli, 1981; Shomari, 1996) **Chemical methods**: - Sulphur dusts and Wettable powder at 250gm per tree at 14 days interval. Water based organic fungicides e.g Triadimenol, Hexaconazole and Penconazole at the rate of 10-15ml per litre per tree at an interval of 21 days (Intini and Sijaona, 1983; Sijaona, 1884 Topper *et al*., 1997; Sijaona *et al*., 2001)
Cashew anthracnose	*Colletotrichum gloeosporoides* Penz	**Cultural methods**:- Clearing off and burning of diseased and dead shoots, leaves, fruits on trees and fallen dry twigs, fruits and leaves after harvest (Sijaona, 2006). **Chemical methods**:- Chlorothalonil 75% WP 1ml per 0.6-0.8 litre, Prochloraz 25% EC 1ml per 0.8-1.5 litres, copper oxychloride, copper hydroxide, captafol, benomyl, Anilazine, Triadimenol and Dithianon (Sijaona, 2013; Zhongrun and Masawe, 2014)
Cashew dieback	*Phomopsis anacardii*	**Cultural methods**:- Remove all infected leaves on the entire trees (Intini and Sijaona, 1983). **Biological methods**:- Use weaver ants to control sucking pests (Sijaona *et al*., 2001) **Chemical method**: - use Lambda cyhaothrin EC 5 mls per litre, Beta-cypermethrin 4.5% EC 1ml per 2.5-3 litres, Dimethoate 40% EC 1ml per 1-1.5 litre and Trichlorfos 90% crystal 1g per 1 litre Zhongrun and Masawe, 2014).
Cashew pestalotia leaf Spot	*Pestalotia sp*.	**Chemical methods**: - Metalaxl Mancozeb 58% WP (1g per 0.8-1litre), Chlorothalonil 75% WP (1g per 0.8-1litre) and propamocarb 72.29% (1g per 0.8-1 litre) (Zhongrun and Massawe, 2014).
Damping off disease	*Fusarium* spp., *Pythium* spp., *Phytophthora palmivora* Butler,	**Cultural methods:-**The nursery and the land container raised young plants should be well drained to stop waterlogging (Sijaona, 2013; Zhongrun and Massawe, 2014). **Chemical methods**: - Carbendazin 50% WP (1g/0.5-1 litre), chlorothalonil 75% WP (1g/0.8/1 litre) and cymoxanil manacozeb 72% WP (1g/0.5/1 litre) (Zhongrun and Massawe, 2014).
Cashew leaf and nut blight	*Cryptosporiopsis* spp	**-Cultural methods: -**Clear off, gather, burn and bury all the diseased fruits and leaves infected in the field (Sijaona, 2006). **-Chemical method:-** Fungicides are used such as difenaconizole 14g/litre, Pecoxystrobin 10 mls per litre, Chlorothalonil 14 g/l Trifloxistrobin+Tebuconazole 14mls per litre (Sijaona, 2006) **-Resistant varieties:-** Such as AZA2 and AZA 17 (Masawe, 2006)
Cashew fusarium wilt	*Fusarium oxysporum*	**-Cultural methods:-** Clean the equipment after use and plant satisfied materials from authorised institutes (Tibuhwa and Shomari, 2016)

IV. CONCLUSION

This document highlights the status of the most important cashew diseases and their management options in the country. The information provided here is vital to cashew farmers and other stakeholders in making appropriate improvements in cashew production in Tanzania. However, there is need to conduct in-depth research especially on characterizing pathogen strains and

developing appropriate management options that will discourage use of chemical pesticides for environmental safety and improved cashew production in Tanzania.

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Yields gap evaluation of wheat grown in Piedmont plain and Floodplain soils of Bangladesh through compositional nutrient diagnosis (CND) norm

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***Abstract*—** *Mineral nutrient stress is one of the major yield gap factors, especially in floodplain and piedmont plain soil. The compositional nutrient diagnosis (CND) provides a plant nutrient imbalance index in statistical distribution patterns, which is important for adjusting the soil-plant systems specific fertilization for maintaining sustainable soil fertility. This study calculated the CND norms of wheat (Triticum aestivum L.) and identified optimum wheat yield target of high-yielding subpopulation in farmers' fields. It also categorized the most yield limiting nutrient(s) for wheat grown. Popular high-yielding wheat was grown in 62 farmers' fields, maintaining farmers' nutrient management plan (FP) and improved nutrient management plan (INM). Nutrient composition analysis was done from 62 young foliar composite samples, collected at 7th leaves stage (vegetative stage). The CND generic model gave 3.47 Mg ha^{-1} as minimum cutoff yield of the high-yield subpopulation. Nitrogen was identified as the core yield limiting nutrient for wheat in piedmont and floodplain soils. However, the yield limiting nutrients for wheat grown in the studied are were established the following series: N > S > K, Mg > P, Ca and Mn >Fe >B >Zn respectively. The CND generic model, allowed us to suggest that N, P, K, Mn, B were the factors discriminating high- from low–yielding subpopulation in piedmont plain and floodplain soils of Bangladesh.*

***Keywords*— Compositional nutrient diagnosis (CND), wheat, piedmont plain, floodplain.**

I. INTRODUCTION

Bangladesh is an agrarian country having three dominant physiographic soil types includes floodplain, terrace and hilly area. Among these land types, floodplain and piedmont plain soil have a greater intensification of agriculture. Yield potential of currently cultivated cultivars in a farmer's field decreased due to rigorous cultivation. Thus, mineral nutrient constraint might be one of the major yield limiting factors for farmer's field. Although evaluations of local scale yield limiting factors are essential for ensuring food security but little attempt was taken. Moreover, tropical climatic situation and multiple geomorphic features of Indo-Gangetic region favor higher nitrogen loss and high P and K fixation admits larger nutrient deficient soil, thus more fertilizer inputs are required for intensive cropping system (Timsina and Connor, 2001; Ali *et al.*, 1997). Several evidence also

showed that available K concentration of floodplain and piedmont plain soil store < 0.1 meq/100 g soil and mean annual balance of P was found -1 to -9 kg ha^{-1} (Saleque *et al*., 2006; Panaullah *et al*., 2006). Besides, less conspicuous deficiency symptoms of P and K in wheat compared to the symptoms of N and S retain farmers from applying these fertilizers. Therefore, understanding of multi-environmental soil nutrient dynamics and nutrient absorption, transport accumulation in plant tissue is essential, to improve the nutritional value of the plant and reducing the yield gap (Mattos *et al*., 2003; Vargas *et al*., 2013).

Leaf analysis is a good tool to monitor, evaluate and adjust agricultural fertilization programs to reduce the yield gap (Tomio et al., 2015; Cunha et al., 2016). Because, the leaf is the prime portion of plant that reflect any stresses. Foliar nutrient status can be diagnosed by mineral composition analysis and several mathematical approaches like- Critical Value Approach (CVA) (Bates, 1971), Diagnosis and Recommendation Integrated System (DRIS) (Walworth and Sumner, 1987), and Compositional Nutrient Diagnosis (CND) (Parent *et al*., 1994). Among these methods, CND approaches, calculate nutrient balance considering all foliar nutrient elements and their interactions and dry mass of plants, provide greater accuracy of diagnosis (Cunha et al., 2013). For selecting suitable nutrient norms, an arbitrarily yield cutoff value is needed for defining a high yield subpopulation (Khiari *et al*., 2001). Parent and Dafir (1992) and Parent *et al*., (1994) proposed the X^2 distribution function to define a CND threshold value for nutrient imbalance when relating yield and the cumulative variance ratio function for each nutrient. The CND approach has a robust mathematical basis to define a minimum yield target useful for discriminating between high and low yield subpopulations for identifying specific element related yield gap. Thus, the CND approach is applicable for solving nutrient imbalance problems in specific physiographic unit soil (Khiari et al., 2001).

In Bangladesh, wheat (*Triticum aestivum* L.) is commonly grown in rice-wheat cropping patterns during the "rabi" season from October to March. It is the region's second most important food security crop after rice (Debnath *et al*., 2011; Krupnik *et al*., 2015). The consumption of wheat is increasing due to increase in food diversity in the country. Currently, per capita wheat demand is a 17.3 kg $year^{-1}$, which is approximately 20% of rice consumption. With 3% more protein than rice, wheat makes an important contribution to per capita protein intake at 4.3 g day^{-1} (FAOSTAT, 2014). Production of wheat is increasing day by day, although the country still imports significant quantities of wheat to meet the rapidly growing domestic demand. Nutrient constraints present in Bangladesh soil become prime yield limiting problem in wheat growing areas especially piedmont and floodplain soils. However, a big knowledge gap is detected in the area of demarcating nutrient based yield gap in the farmer's field of Bangladesh. Although several nutrient diagnosis approaches were identified for nutrient balance in relation to yield of conifer seedling, onion, garlic, pepper, potato and fruits (Parent *et al*., 1995; Cunha *et al*., 2016). Among the different methods, the CND approach was identified as an effective multivariate for distinguishing yield gap by considering the leaf nutritional disorder (Cunha *et al*., 2016). At present it is fact that there is no information about the nutrient diagnosis approach for wheat in farmers' fields in floodplain and piedmont plain soil.

Considering the facts, the study was intended the

compositional nutrient diagnosis (CND) norms of wheat (*Triticum aestivum* L.) and identifies optimum wheat yield target of high-yielding subpopulation in farmer's fields and nutritional interaction between high and low yielding subpopulation. Moreover, it also categorizes the most limiting nutrient(s) that should be applied to reduce the yield gap of wheat in the region.

II. MATERIAL AND METHODS

Experimental data

This study was conducted based on the data acquired from dry season irrigated wheat plant grown in 62 farmers' fields, in three different districts (Rangpur, Dinajpur and Nilphamari) of northern part of Bangladesh. This area is located between 25°50′N to 89°00′E which incorporate three agro-ecological zones of Bangladesh i.e., Old Himalayan piedmont plain, Active Tista floodplain and Tista meander floodplain. Two nutrient-management practices were tested. The plan-included farmer's practice (FP), which constituted farmer's traditional nutrient management program and improved nutrient management plan (INM). Sixty-two farmer's practices field was randomly selected within the study area. The nutrient doses in farmer's practice field were varied from place to place. For FP, doses of N, P and K varied from 48-114, 8-25 and 0-19 kg ha^{-1} respectively. Twelve experimental field was managed according to soil test based improved nutrient management system (INM). The doses of N, P and K in INM followed field varied from 81-160, 23-39 and 55-97 kg/ha respectively.

At 7^{th} leaves stage 30 young leaves of each experimental plot was collected to prepare foliar composite sample. A total of 62 foliar composite samples were collected from randomly chosen healthy plants at 45-50 days after sowing (DAS). For determining nutrient concentration, each sample was taken from the most recent expanded leaf (immediately before the flag leaf), collected from the standing crops of farmer's field. The leaf sample were dried at 69°C for 72 hours and grinned by Wiley mill. The total N content was determined by micro Kjeldahl method (Yoshida *et al.*, 1976). The concentration of K, Ca, Mg, S, Na, Zn, Fe, Mn and B were analyzed by digesting 0.5 g of the leaf sample with 10ml 5:2 HNO_3: $HClO_4$ (Yoshida *et al.*, 1976). P was estimated colorimetrically by the phospho-molybdate blue complex method (Chapman and Parker,1961). For calculating the yield $1m^2$ area of each plot was harvested after complete maturity and separated the unfilled grain. Then the nutrient data set were matched with the yield of the same field. Descriptive statistics were determined for leaf nutrient concentration and nutrient ratio expression data. Compositional nutrient diagnosis norms were calculated using Microsoft Excel 2000 Software (Microsoft Corp., 2000).

Theory of the CND approach

To calculate the preliminary compositional nutrient diagnosis norms, we used the CND approach, which has been described in Khiari *et al.*, (2001a). The approach is based on the plant tissue composition, which forms a *d*-dimensional nutrient arrangement, i.e., simplex (*Sd*) made of $d + 1$ nutrient proportions including d nutrients and a filling value defined as R (Parent and Dafir, 1992). The theory is applied as follows:

$$S^d = [(N, P, K, \text{-------} -Rd): N > 0, P > 0, K > 0, Rd > 0, N + P + K + \cdots + Rd = 100] \quad (1)$$

Where S^d is simplex made of d nutrient, 100 is the dry matter concentration (%); N, P, K... are nutrient proportions and R_d is the filling value between 100% and the sum of d nutrient proportion computed as follows;

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$Rd = 100 - (N + P + K + \cdots)$. (2)

The nutrient proportions become scale invariant after they have been divided by the geometric mean (G) of the $d + 1$ components, including Rd (Aitchison, 1986), as follows:

$G = [N \cdot P \cdot K \cdots Rd]\ 1/d+1$. (3)

Row-centered log rations are computed as follows:

$$V_N = 1n\left(\frac{N}{G}\right),\ V_p = \left(\frac{P}{G}\right),\ V_K = \left(\frac{K}{G}\right)\ldots,\ V_{Rd} = \left(\frac{Rd}{G}\right)$$

(4)

and

$$V_N + V_p + V_K + \ldots + V_{Rd} = 0 \quad (5)$$

Where, V_X is the CND row-centered log ratio expression for nutrient X. The sum of tissue components is 100%, as in equation (1), and the sum of their row-centered log ratios including the filling value must be zero, as in equation (5).

Thereafter, the database is partitioned between two subpopulations using the Cate–Nelson procedure, once the observations have been ranked in a decreasing yield order (Khiari *et al.*, 2001). At each iteration, the group A comprises n_1 observations, and the group B comprises n_2 observations for a total of n observations ($n = n_1 + n_2$) in the whole database. For the two subpopulations, the variance of the CND V_X value must be computed. The variance ratio for component X can be estimated as:

$$f_1(V_x) = \frac{Variance of\ V_x\ n_1\ observations}{Variance of\ V_x\ n_2\ obervations} \quad \text{--------(6)}$$

Where $f_1(V_x)$ is the ratio function between two subpopulations, for nutrient X at the ith iteration (i=n_i-1) and the V_x is the CND row-centered log ratio expression for nutrient X.

The cumulative variance ratio function is the sum of variance ratios at the ith iteration from top. The cumulative variance ratio function $F^C i$ (VX) can then be computed (Khiari *et al.*, 2001) as:

$$F^c_i(V_x) = \left[\frac{\sum_{i=1}^{n_1-1} fi(Vx)}{\sum_{i=1}^{n_1-1} fi(Vx)}\right] [100] \ldots\ldots\ldots \quad (7)$$

is partition number and n is total number of observations (n_1+n_2). The denomination is the sum of variance ratios across all iterations and thus is a constant for nutrient X.

The cumulative function F^c_1 (V_x) related to yield (Y) shows a cubic pattern:

$F^c i(V_x) = aY^3 + bY^2 + cY + d$ ……(8)

The inflection point is the point where the model shows a change in concavity. It is obtained by delving equation [8] twice:

$$\frac{\partial F^C i(Vx)}{\partial Y} = 3aY^2 + 2bY + C \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

$$\frac{\partial^2 F^C i(Vx)}{\partial Y} = 6aY + 2b \ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

The infection point is then obtained by equating the second derivative of equation (10) to zero. Thus the solution for the yield cutoff value is -b/3a. The highest yield cutoff values across nutrient expressions (N, P, K and S) were selected to ascertain the minimum yield target for a high yield subpopulation. CND norms were computed using means and standard deviations corresponding to the row-centered log ratios V_X of d nutrients for high-yield specimens.

III. RESULTS

The compositional nutrient diagnosis norms comprised the eleven nutrients and the filling value R. Nutrient concentrations were transformed into CND row-centered log ratios V_N, V_P, V_K, V_{Ca}, V_{Mg}, V_S, V_{Mo}, V_{Zn}, V_{Mn} V_{Fe}, V_B and VRd through equations (1–4). Equation (7) was

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used to calculate the cumulative variance ratio functions [$F^c i$ (VN)] values.

Table 1. Grain Yield of wheat at inflection points of the cumulative variance functions for row-centered log ratios in the survey population (n=62)

Components	$F_i^c(V_x) = aY^3 + bY^2 + cY + d$	R^2 Value	Yield at inflection point = -b/3a (Mg ha^{-1})
N	$-63.18Y^3 + 657.18Y^2 - 2253.8Y + 2549.9$	0.90	3.47
P	$-84.406Y^3 + 832.71Y^2 - 2683.6Y + 2823.5$	0.75	3.29
K	$-71.818Y^3 + 729.82Y^2 - 2442.3Y + 2696.5$	0.93	3.39
S	$-74.523Y^3 + 771.32Y^2 - 2628.6Y + 2949.8$	0.85	3.45
Ca	$-74.712Y^3 + 738.49Y^2 - 2388.2Y + 2527.9$	0.76	3.29
Mg	$-79.062Y^3 + 738.49Y^2 - 2388.2Y + 2527.9$	0.92	3.39
Zn	$-86.33Y^3 + 870.62Y^2 - 2881.5Y + 3130.3$	0.92	3.36
Mn	$-53.847Y^3 + 8553.67Y^2 - 1883.6Y + 2129.1$	0.92	3.43
Fe	$-78.109Y^3 + 800.77Y^2 - 2700.8Y + 2997.7$	0.89	3.42
B	$-82.82Y^3 + 840.46Y^2 - 2800.7Y + 3064.5$	0.92	3.38
Mo	$-67.078Y^3 + 595.28Y^2 - 2048.3Y + 2327.9$	0.77	2.96
Rd	$-61.20Y^3 + 603.59Y^2 - 1946.2Y + 2052.1$	0.61	3.28

The cutoff yield between the low and high-yield subpopulations were determined after examining the eleven cumulative variance ratio functions [$F^c i(V_N)$, $F^c i(V_P)$, $F^c i(V_K)$, $F^c i(V_S)$, $F^c i(V_{Ca})$, $F^c i(V_{Mg})$, $F^c i(V_{Mo})$, $F^c i(V_{Zn})$, $F^c i(V_{Mn})$, $F^c i(V_{Fe})$ and $F^c i(V_B)$] related to yield. (Table 1 and Fig. 1, Fig. 2 and Fig. 3).

The cutoff yield between the low and high yielding subpopulations obtained fromcumulative variance ratio functions of nitrogen, phosphorus, potassium and sulfur ranged from 3.29 to 3.47 Mg ha^{-1} (Fig. 1 and Table 1). These nutrients are usually deficient in the study area and fertilizer application for these nutrients is recommended. The yields (Mg ha^{-1}) at inflection points of the cubic functions, computed by setting the second derivative of $F^c i(V_x)$ to zero were 3.47 Mg ha^{-1} for $F^c i(V_N)$, 3.29 Mg ha^{-1} for $F^c i(V_P)$, 3.39 Mg ha^{-1} for $F^c i(V_K)$, 3.45 Mg ha^{-1} for $F^c i(V_S)$, 3.29 Mg ha^{-1} for $Fi^c(V_{Ca})$, 3.39 Mg ha^{-1} for $Fi^c(V_{Mg})$, 3.48 Mg ha^{-1} for $F^c i(V_{Mo})$,, 3.36 Mg ha^{-1} for $F^c i(V_{Zn})$, 3.43 Mg ha^{-1} for $F^c i(V_{Mn})$, 3.42 Mg ha^{-1} for $F^c i(V_{Fe})$ and 3.38 for $F^c i(V_B)$ respectively. The highest cutoff yield was obtained with $F^c i(V_N)$ and $F^c i(V_{Mo})$. At $F^c i(V_N)$ yield cutoff, 5 to 42 observations had yield of 3.47Mg ha^{-1} or more.

Summary statistics for high and low yielding subpopulations of wheat yield and leaf nutrient concentration are given in

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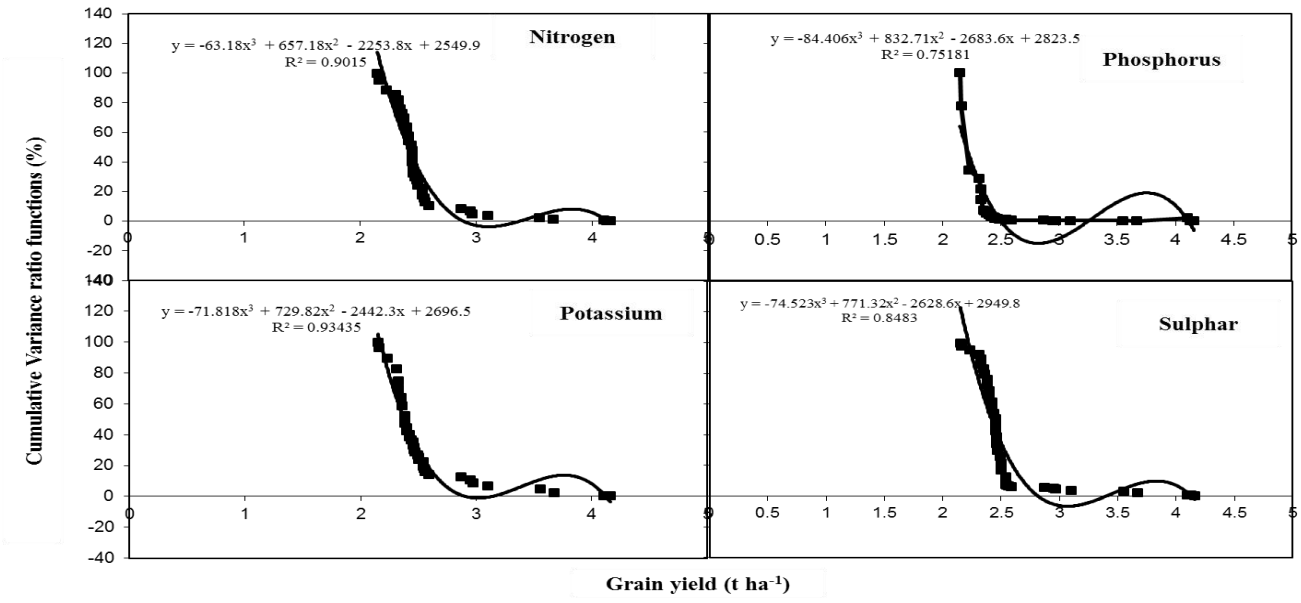

Fig. 1 Relationship between grain yield and cumulative variance ratio function percentage in N, P, K and S for wheat in farmer's fields

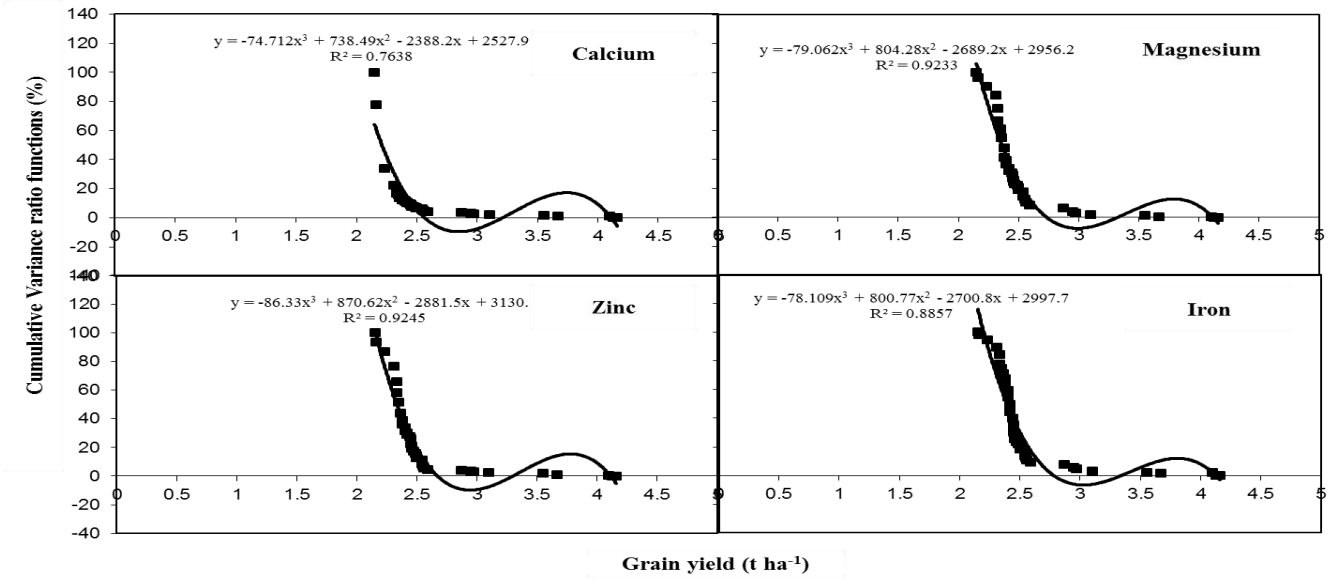

Fig. 2 Relationship between grain yield and cumulative variance ratio function percentage Ca, Mg, Zn and Fe for wheat in farmer's fields

Table 2. The mean concentration of N, P, K, S, Ca, Mg, Mn and Fe was slightly higher in high yielding subpopulations, however, the differences was greater in case of N. Mean N concentration in high yielding subpopulation was 32.22 g kg^{-1} compared to 18.04 g kg^{-1} in low yielding subpopulation. Mean concentration of K, both high and low-yielding subpopulations were only 5.42 and 4.48 g kg^{-1} respectively. The nutrient concentration in both high and low-yielding subpopulation showed good symmetry. Skewness in the high-yielding subpopulation varied from –0.82 in case of S to 2.20 in Mn. In low-yielding subpopulation, varied from –0.12 in case of K to 1.34 in Mg.

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Table 3 summarizes the significant nutrient inter-correlations identified in previous section but expressed as nutrient ratios. With the aim of elucidating if these expressions are important to differentiate between the subpopulations, an F-test was performed for each of them.

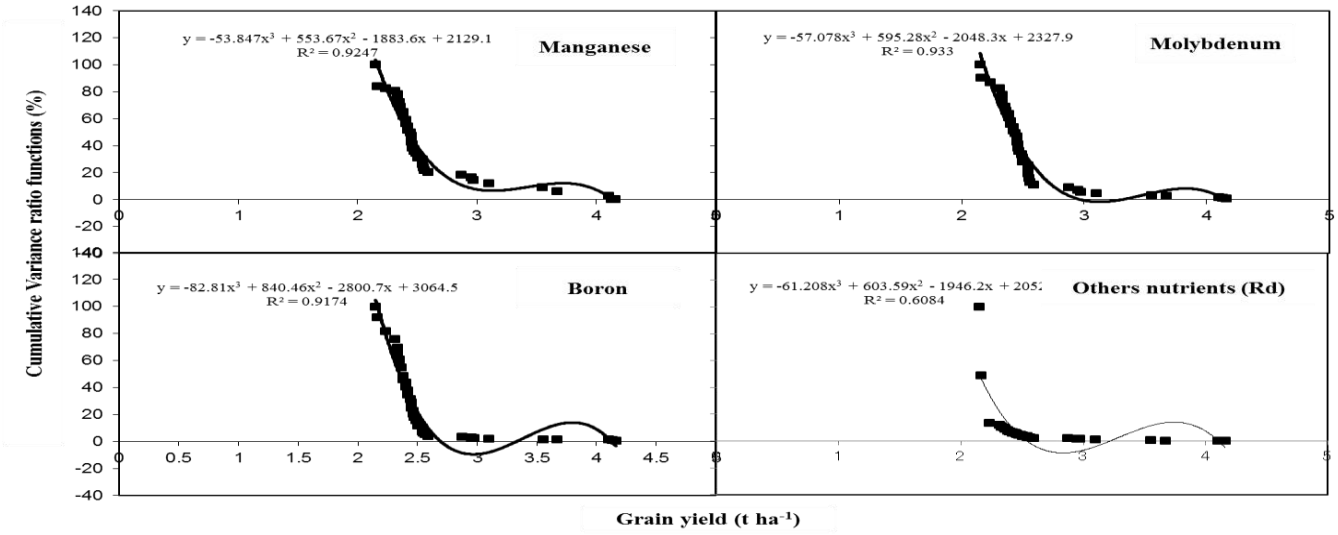

Fig. 3 Relationship between grain yield and cumulative variance ratio function percentage in Mn, Mo, B, and Rd for wheat in farmer's fields

Molar ratio of nutrient showed a big difference between high yielding and low-yielding subpopulation.

Molar ratio of nutrient (Table 3) showed that, the N/K, N/Ca, N/S,

N/Mg, P/S, K/S K/Ca, K/Mg, S/Ca, S/Mg and Ca/Mg ratios were lower than the DRIS norms for rice proposed by Bell and Kovar (2000). N/Mg ratio was very close to the DRIS norm for rice. The observed N/P ratio was 40.06% lower in high-yielding subpopulation and 53.21% lower in low-yielding subpopulation than the DRIS norm for rice. Coefficient of variation of the N/P ratio was 26.34% in

Table 2. Summary statistics for wheat grain yield and leaf nutrient concentration data for high-yielding (n = 12) and low-yielding (n = 50) subpopulations

Parameters	High yielding sub-population (n=12)					Low yielding sub-population (n=50)				
	Mean	Median	Minimum	Maximum	Skewness	Mean	Median	Minimum	Maximum	Skewness
Yield (t ha^{-1})	3.92	4.10	3.55	4.16	-0.68	2.45	2.45	1.95	3.10	0.78
N (g kg^{-1})	32.22	31.10	29.70	38.00	2.00	18.04	17.70	9.80	29.70	0.50
P (g kg^{-1})	5.72	5.90	3.70	6.70	-1.42	4.29	4.20	2.00	6.80	0.22
K (g kg^{-1})	5.42	5.40	4.50	6.50	0.28	4.48	4.40	0.90	7.70	-0.12
S (g kg^{-1})	5.24	6.20	2.50	6.90	-0.82	3.27	2.90	0.78	8.60	1.32
Ca (g kg^{-1})	1.85	1.94	1.40	2.03	-2.09	1.48	1.40	0.34	3.50	0.79
Mg (g kg^{-1})	0.24	0.20	0.20	0.30	0.61	0.38	0.30	0.10	1.10	1.34
Zn (g kg^{-1})	0.03	0.03	0.02	0.04	-1.92	0.03	0.03	0.01	0.04	0.58
Mn (g kg^{-1})	0.06	0.04	0.03	0.15	2.20	0.09	0.07	0.02	0.19	0.20
Fe (g kg^{-1})	0.24	0.20	0.20	0.35	1.89	0.32	0.28	0.20	0.49	0.50
B (g kg^{-1})	0.05	0.05	0.05	0.06	0.61	0.07	0.07	0.05	0.10	0.86
Mo (g kg^{-1})	0.01	0.01	0.01	0.01	1.07	0.01	0.01	0.01	0.01	-0.32

P/K, P/Ca, P/Mg and Fe/Mn ratios were greater whereas, N/P, high-yielding subpopulation and 43.88% in low-yielding

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subpopulation. Skewness of N/P ratio was 1.68 in high-yielding and 0.96 in low-yielding subpopulation. Observed N/K ratio was 90.75% higher in high-yielding and 61.34% lower in low-yielding subpopulation than the DRIS norm for rice, which signifies greater imbalance of N and K nutrition in the observed wheat plant. Higher N/K ratio in the high-yielding subpopulation than the low-yielding subpopulation further confirmed the role of imbalanced N/K ratio in lowering wheat yield. The N/S ratio was 65.27% lower in high-yielding and 69.73 % lower in low-yielding subpopulation than the DRIS norm for rice. N/Ca ratio was 4.43% higher in high-yielding and 6.01% higher low-yielding subpopulation than the norm of 6.77. The N/Mg ratio was very close to the DRIS norm of 19.72, only 10.5% lower in high-yielding and 21.90% lower in low-yielding subpopulation. Due to low K concentration and optimum P concentration in wheat plant tissue, P/K ratio appeared 233.3% in high-yielding and 175% higher in low-yielding subpopulation than the DRIS norm for rice. The P/S ratio was 40.55% lower in high-yielding and 33.33% lower in low-yielding subpopulation than the DRIS norm of 1.8 for rice. Higher S concentration in the plant tissue caused this imbalance of P/S ratio. In both high and low-yielding subpopulations, P/Ca ratio was higher over 65.28% and 136.11% to the DRIS norm of 0.72. The P/Mg ratio showed 44.34% higher in high yielding and 64.62% higher in low-yielding subpopulation than the DRIS norm of 2.12 for rice. The K/S ratio was another important nutrient imbalance in wheat plant. Compared to the DRIS norm for rice of 16.06, the K/S ratio was 2.66 in high yielding and 3.81 in low yielding subpopulation. Lower K concentration decreased K/Mg ratio by 61.11% in high yielding and 44.41% in low yielding subpopulation compared to DRIS norm of 20.06 for rice. Compared to the DRIS ratio of 0.15 for rice, the observed Fe/Mn ratio in high yielding subpopulation was 5.06 and in low yielding subpopulation it was 6.17. However, the higher Fe/Mn ratio in high yielding subpopulation than the low yielding subpopulation signifies that the imbalance due to Fe and Mn did not contribute much

Table 3. Mean values of nutrient molar and or dual ratios for high and low-yielding subpopulations together with their respective coefficients of variance (CV's), standard deviation and skewness

Molar ratio	High yielding sub-population (n=12)				Low yielding sub-population (n=50)				F-ratio	Mean Reference
	Mean	SD	CV (%)	Skewness	Mean	SD	CV (%)	Skewness		Molar ratio
N/P	5.88	1.55	26.34	1.68	4.59	2.01	43.88	0.96	1.73	8.24
N/K	2.27	0.23	10.27	1.6	1.39	0.46	33.15	0.48	16.84	0.21
N/S	6.00	0.58	9.62	0.49	5.23	3.74	71.65	1.90	0.16	9.92
N/Ca	7.07	3.31	46.8	1.61	7.16	5.37	75.06	2.42	0.01	6.76
N/Mg	17.65	3.01	17.07	1.39	15.40	11.67	75.77	2.39	0.16	19.72
P/K	0.40	0.08	20.75	-1.32	0.33	0.10	30.21	0.15	2.29	0.93
P/S	1.07	0.27	25.18	-0.39	1.20	0.76	63.78	1.74	0.13	1.80
P/Ca	1.19	0.39	32.33	0.74	1.70	1.18	96.48	2.43	6.12	0.72
P/Mg	3.06	0.34	11	0.16	3.49	2.21	63.26	2.62	0.18	2.12
K/S	2.66	0.35	13.15	-0.13	3.81	2.49	65.26	1.83	1.01	16.06
K/Ca	3.14	1.49	47.49	1.44	5.17	3.18	61.65	2.35	1.87	6.23
K/Mg	7.80	1.21	15.45	2.09	11.15	7.55	67.72	2.60	0.94	20.06
S/Ca	1.19	0.57	48.31	1.7	1.77	1.48	83.49	3.03	0.72	0.68
S/Mg	2.97	0.61	20.5	0.6	3.65	2.22	60.86	0.74	0.44	1.99
Ca/Mg	2.76	0.79	28.58	-0.58	2.70	1.90	71.34	1.72	0.01	2.92
Fe/Mn	5.06	1.49	29.42	-2.17	6.17	5.97	96.75	2.18	0.16	0.15

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to the wheat yield. Compositional nutrient diagnosis (CND) difference in the mean row centered log ratios for the high

Table 4. Compositional nutrient diagnosis (CND) row-centered log ratio of nutrients with their standard deviation and coefficient of variation (CVs)

Row-centered log ratio	High yielding sub-population (n=12)			Low yielding sub-population (n=50)		
	Mean	SD	CV(%)	Mean	SD	CV (%)
V_N	2.43	0.34	14.04	2.02	0.16	8.07
V_P	0.97	0.3	31.36	0.78	0.13	17.22
V_K	2.15	0.17	7.97	2.31	0.13	5.56
V_{Ca}	0.64	0.48	75.18	0.12	0.37	306.03
V_{Mg}	-0.12	0.39	-28.87	-0.19	0.06	-32.88
V_S	0.97	0.48	49.43	0.5	0.2	39.92
V_{Zn}	-4.14	0.32	-7.76	-3.97	0.24	-6.02
V_{Mn}	-3.08	0.66	-21.30	-2.42	0.55	-22.78
V_{Fe}	-1.60	0.3	-18.5	-1.2	0.19	-15.63
V_B	-3.09	0.27	-8.74	-3.1	0.1	-3.31
V_{Mo}	-1.58	0.6	38	-1.42	0.25	-17.54
V_{Rs}	6.45	0.18	2.71	6.59	0.14	2.17

row-centered log ratio (V_X) for N, P, K, Ca, Mg, S, Zn, Fe and Mn are presented in Table 4. The high and low-yielding subpopulation had V_N 2.43 and 2.02, V_P 0.97 and 0.78, V_K 2.15 and 2.31, V_{Ca} 0.64 and 0.12, V_{Mg} – 0.12 and – 0.19, V_S 0.97 and 0.50, V_{Zn}– 4.14 and – 3.97, V_{Mn} – 3.08 and – 2.42, V_{Fe} – 1.60 and – 1.20 and V_B -3.09 and -3.10. Difference in V_X was not large for any of the tested nutrient between high and low-yielding subpopulation.

IV. DISCUSSION

The CND norms of nutrients

The CND norms were derived from high yielding sub-population and low-yielding sub-population farmer's field yield of wheat. Nutrient concentrations that were transformed into row-centered log ratios were used for the derivation of CND norms. There was however a significant and low-yielding sub populations, suggesting that the yield difference is due to nutritional disorder (Nkengafac and Ejolle, 2014). These obtained nutrient norms helps to nutrient assessment in wheat grown in Piedmont and Floodplain soil. Yield depended database shown that for nitrogen the cutoff yield was 3.47 Mg ha^{-1} indicates commensurate to a reasonable good yield for wheat (Table 1). Thus, it is most likely that N was the most limiting nutrient of yield, as this was evidenced by a significant negative correlation between N and yield (data not shown) when considering low performance observations. However, the cutoff yield for F^ci (V_S), F^ci (V_K) were 3.45 and 3.39 Mg ha^{-1} respectively also matching to a reasonable good yield for wheat (Table 1). This trends suggests that K and S also limited the yield of wheat considered as experimental unit, which can be interpreted as insufficiency of this nutrient,

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especially in the subpopulation of low yields. The acute K deficiency was indicated by highly negative average CND, K indices and the low average leaf K concentrations. The results of CND analyses suggest that inadequacy in K was largely responsible for the underperformance of wheat in piedmont and floodplain soils of Bangladesh. Nutrient concentration and CND dual and or molar ratio involving K also agreed well that K was the main limiting plant nutrient for wheat yield. Continual cultivation of wheat- rice cropping and removal of straw for either fuel or fodder purpose and application lesser K fertilizer than crop removal are the primary factors of K deficiency in the piedmont soils. Soil test based fertilizer application 55-97 kg ha^{-1} K was under dose for piedmont and floodplain soils of Bangladesh. Under dose of K fertilizer application create a negative K balance in rice – wheat cropping (Timsina et al., 2006). Depletion of soil nutrients, particularly K, is a possible cause of yield decline in long-term experiments in northwest India (Bhandari *et al.*, 2003). Saleque et al. (1998b) reported an economic optimum dose of K fertilizer of about 80 kg ha^{-1} in Barind soil of Bangladesh. Potassium play a key role in N uptake and translocation of (Cushnahan et al., 1995), and therefore both N and K need to be present in quite specific proportions if N accumulation and subsequent assimilation into protein is to take place at optimal rates (Ramakrishna et al., 2009). Moreover, the studied area contained high concentration of P but low amount of Mg indicates non-calcareous alluvium soil in nature (García -Hernández, et al., 2007).

Nutrient molar and or dual ratio

The molar ratios of different nutrients are used as a simple indicator of nutrient bioavailability (Zheng et al., 2010). This molar ratio of different nutrients indicates apparent antagonistic and synergetic effects of a particular nutrient on other nutrient in wheat plants (Cunha et al., 2016). However, these study identified that some of molar nutrient ratio become more important for wheat production in Piedmont and Floodplain soil in Bangladesh.

Like this study shown that, the most consistent negative skewness was observed in the Ca/Mg ratio. Several reporters reported that commonly Ca^{2+} is strongly competitive with Mg^{2+}in substrates and often results in increased leaf-Ca along with a marked reduction in leaf Mg (Ruiz *et al.*, 1997; Grattan and Grieve, 1999). Another explanation for leaf Mg deficiency might be absent of Ca-Mg synergism (García -Hernández, *et al.*, 2007; Hernández, *et al.*, 2008). However, this interaction is not important to discriminate between high- and low-yield subpopulations as proved by the F test (Table 3).

However, the N/Ca molar ratio had shown the most consistent positive skewness in this studied area. This finding is strongly disagrees the previous findings by Marschner (1986) who indicated that $NH_4^+$and Ca^{2+} ions are strongly competitive with each other for substrate. But, this interaction was not important in the discrimination between high and low-yield subpopulations as indicated by the F-test (Table 3).

A symmetric skewness was observed in P/Ca molar ratio (Table 4) and with a significant level of the F value (Table 3). This negative relationship may result from higher activity of P in the soil solution due to forming higher solubility of P minerals, especially on soils having lower exchangeable Ca2+, and thus increase P uptake by plants (Barł óg, 2014). There is no robust physiological explanation for the antagonism between N and Mg. This negative interaction has been found in corn leaves by Dara *et al.*, (1992). The

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ratio between these two nutrients was not prominent to differentiate high- and low-yield datasets using the F-test (Table 3).

In contrast, the symmetric skewness between Ca and P was found to discriminate between high- and low-yield subpopulations as shown by the F test (Table 3). This finding is disagrees with report of Parent *et al*. (1994) who had reported the antagonistic effects of these two nutrients. These trends may be happened due to the sandy and or silty soil type of these areas with low cation exchange capacity. Another important molar ratio was the K/Ca ratio (Table 4). This positive interaction was also useful to differentiate high from low-yield subpopulations (Table 3).

The P/K ratio appeared significant to discriminate high and low-yield subpopulations (Table 3). Sumner and Farina (1986) found that the K–P interaction was important in the forage sorghum production, indicating that the balance between K and P is important.

The N/P ratio, as evidenced by a symmetric skewness between N and P (Table 4) and a significant level of the F-value (Table 3), was important for discriminating the ratio between the low and high- yielding subpopulations. Moreover, it should be pointed out that N–P interactions are probably the most economically important of all interactions involving P (Sumner and Farina, 1986; García -Hernández, *et al*., 2007).

A symmetric skewness was observed in N/K molar ratio (Table 4) and with a significant level of the F value (Table 3), was the most discriminating ratio between the low- and high- yielding subpopulations. These trends may be happened due to continual cultivation of wheat- rice cropping, application of lesser K fertilizer than crop removal in the piedmont soils. Under dose of K fertilizer application create a negative K balance in rice – wheat cropping (Timsina *et al*., 2006). These results agreed with findings of Saleque *et al*., (2008) indicating that soils of the study area had low (0.06 – 0.11 cmol kg^{-1}) soil exchangeable K. Moreover, Timsina *et al*., (2006) reported that with continual cropping and low application of K fertilizer create a negative K balance in rice – wheat cropping piedmont and floodplain soils of Bangladesh. However, the interpretation of interactions identified by diagnostic techniques, as the multivariate CND approach could help in overcoming some of the drawbacks of the classical approaches.

V. CONCLUSION

Generic approach to select a minimum yield target for the high yield subpopulation was found effective for a small database of wheat. The corresponding optimum ranges of nutrients for wheat gave 3.47 Mg ha^{-1} as minimum cutoff yield of the high-yield subpopulation. According to the model, macro nutrients (N, K and S) and micro nutrients (Mn, B) inadequacy were the major limiting nutrient factor for wheat yield in piedmont and floodplain soils of Bangladesh. Moreover, five interactions were strongly evident for wheat N-K, N-P, P-K, P-Ca, K-Ca, and K-S. Nitrogen, sulphur and potassium fertilizer including some micronutrient i.e., Mn and B dose for wheat should be increased to improve wheat yield in piedmont plain and floodplain soils of Bangladesh.

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CONFLICT OF INTEREST

There is no conflict of interest.

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Local Knowledge and the Adoption of Science Knowledge in Cocoa Cultivation Community in East Kolaka Regency

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***Abstract*—** *This study aims to analyze the existence of local farming knowledge by cacao farmers community and their integration with science knowledge from outside.The results showed that local knowledge in cacao cultivation is based on customs and traditions as well as the insistence of family life which has been the custom of farmers and then processes through repetitions which then form a farming experience. Science knowledge in cacao cultivation is formed based on the response to the decline in productions and user preferences and the inovation in farming technology which is introduced to users through technology transfer by researchers, extension agents and plantation assistants. The acceleration of technological innovation by the government was not followed by the speed and utilization of science knowledge by cacao farmers, and the weaknesses are in the delivery subsystem and the recipient subsystem. It takes a continuous bridge between research institutions as suppliers of science technology/ knowledge with their users, so that the resulting science knowledge is guaranteed to be followed by users on an ongoing basis.*

Keywords*— *local knowledge, science knowledge, cultivation, cocoa.

I. INTRODUCTION

Science starts from human curiosity, from this curiosity makes humans always observe the existing natural symptoms and try to understand them. The word *science* means knowledge which is consists of the science of social science (social science) and natural science (natural science) (Syukri., Et al. 2015). Knowledge is the information that has been combined with understanding and the potential to act; which then sticks to someone's mind. Knowledge has meaning only after it has been placed in a particular social network (Collins, 1990; Collins and Pinch, 1998; Jasanoff, 1990) in (Carolan, 2006).

Drucker (1998) defined knowledge as information that changes something or someone, so that knowledge is considered as a power to master others. In line with Drucker's opinion, Sveiby (1997) defines knowledge as the capacity to act. Foucault (2012) explained that the power to create knowledge and power and knowledge influence each other directly. Wisdom is the fruit of knowledge and knowledge generated from human perception of the world through their senses or intuition (Geertz, 1983). Furthermore, Kenickie and Mphahlele (2002) stated that indigenous knowledge is an accumulation of knowledge that has been created for decades, reflecting creative thinking and action of various generations in individual communities, in a permanent ecosystem of residence in an effort to deal with an ever-changing agroecological and socio-economic environment.

Warren (1993) stated that local knowledge is unique in a culture or society. The end result of indigenous psychology is knowledge that describes local wisdom, which is a picture of attitudes or behavior that reflects the original culture (Ridwan, 2006). Ali (2000) pointed out that knowledge owned by farmers is named by experts with different names. According to Forsyth (2004) local meaning in terms of local knowledge refers to knowledge that is limited by space in a particular area, or may also be based on certain cultural and ethnic aspects. This means that local knowledge is something that is specifically tied to a particular person or place. According to Chamber (1987) local knowledge is often also referred to as folk science, ethnoscience, rural science, and there are also those who use the term indigenous technical knowledge.

II. RESEARCH METHODS

Paradigm, Type and Research Approach

This research paradigm uses the post positivism paradigm, where discourse and knowledge are seen as social reality. This type of research is descriptive. The

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approach used is qualitative research (qualitative research) which is a method to explore and understand the meaning by a number of individuals or groups of people considered come from social or humanitarian problems, aims to reveal the process, interpretation of meaning and lead to the disclosure of individual circumstances or behavior who are holistically obsessed.

Location and Time of Research

This research was conducted in the location of the program of the National Movement for Production Improvement, Cacao Productivity and Quality (*Gernas Kakao*), specifically in Penanggosi Village, Lambandia District, East Kolaka Regency for 3 months.

Data Collection and Analysis Techniques

The data collection of this research is done by: interviews, observation, documentation and archival records. The techniques in exploring these problems are described as follows: (1) Inventorying local knowledge information and science knowledge (2) Documenting it in the form of unitary statements. (3) Accumaliting the statements into connected pattern to draw a model of local knowledge and science knowledge in cocoa cultivation. (4) Making discourses in acquiring knowledge, disseminating knowledge, and utilizing local knowledge and scientific knowledge in cocoa cultivation. (5) Interpreting the discourse

Results and Discussion

Cocoa plants have long been known by the people of Southeast Sulawesi, the development of cocoa was launched since the Gersamata program in 1980 through the Makmur Merata Village Movement, abbreviated as Gersamata. The program's approach at that time was reforestation of regions and the environment grew in Kolaka Regency, Southeast Sulawesi Province. At that time, cocoa was distributed to the farming community in the form of fresh fruit which is then nursed and planted by farmers in their gardens individually.

Local Knowledge About Cocoa Cultivation

Various local knowledge in cocoa cultivation which is conventionally practiced by farmers described as follows:

Land Preparation

Land preparation for cocoa plantation development and settlement by farmers was done by exploring and clearing the forest which is locally termed as " *pammulanna majjama dare or mabbele* ". In the beginning, as the preparation to open a new location for planting cocoa, because the majority of farmers were buginese community (transmigrants), the steps begined with the traditional habits/rituals which is aimed to prevent disturbances from gost (local believe) when opening a new land for cocoa cultivation and also asking for successfull farming to God.

Local knowledge of land clearing that they understand both individually and in groups is by cutting off all large and small trees gradually by using machetes and "*banci-banci*" (axes) which is brought from their native land (South Sulawesi). A few days after it is estimated that they have dried slowly then they burn it completely. This kind of practice is traditionally gained from the experiences and hereditary knowledge from their ancestors. Hence, it can be concluded that the land preparation knowledge is an original knowledge which is based on daily experiences. However, this kind of practice does not concern the environmental damage due to the burning of the land in opening a new farming location and this has been a great discussion in postmodernity.

Nursery and Planting Materials

One of the ways to determine the planting materials is done by visual selection, farmers usually see ripe cocoa fruits which have large fruits and stem as well as considering its physical growth and helath, regardless the type and the origin of the cocoa. This way of selection is purposively done because it is more practical, free and can be done by the farmers themselves, this is also because there is no outside access or other alternatives to obtain planting material.

Cocoa beans are spread on burlap sacks that have been prepared to germinate for 5 to 7 days in an empty room at farmers homes. At the same time, while waiting for cocoa seeds to germinate/the root out, they have prepared a nursery medium in the form of white plastic that has been cut into various sizes, i.e. 10 cm x 10 cm or 12 cm x 12 cm.

Shade Planting

Shade planting or known as cocoa crop protectors. Farmers have never thought about giving shade between cocoa plants, coincidentally at that time, various types of plants grew around the cocoa plants by themselves at farmers' gardens after cocoa cultivation.

Pruning

Local term in the practice of cocoa pruning is known as "Mapparoning" which means cleaning. Method used in pruning cocoa plants is by cutting branches and twigs that grow thickly at random, the pattern and timing of pruning is done at any time, and pruning is done when the plants bear fruit or do not bear fruit. Pruning is done because the branches of the cacao plant are very tight to one another and pruning starts when the plants are at least 3 years old. The equipment that is used is a machete.

Planting Space Determination and Planting Holes

Determination of planting space and planting holes is done by farmers after the land has been cleared from woods and shrubs. Usually the planting process begin by determining the planting distance by using equipment such as ropes from banana stems and bark,

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then making *pengajiran* or "Mabecci". Before making the planting holes, the farmers make sure the place where the cocoa is planted first, by pulling the rope perpendicularly with 4 meters long for each, the rope is marked with a tie knot, and each 4 meters distance is marked with a wooden mark as the sign of the planting hole spot. This is done to make sure that the lines of cocoa plants are neat / straight and at the same spacing line. The equipment for making plant holes are hoe and *Pattiba*. *Pattiba* is a traditional tool made by farmers which is designed to make planting holes. According to the farmers, the use of *Pattiba* is preferred because it works fast and can break large wood's roots in the ground, while the hoes sometimes break and slower when used in making planting holes. The size of the planting hole made by the farmer is 20 cm x 15 cm or locally termed as 1 hoe eye. As for the spacing that is made varies which is 4 m x 4 m. Farmers knowledge in sizing the hole and distance obtained from neighbor village farmers who had previously planted cocoa.

planting

Cocoa planting by farmers in Penanggosi village is known as "*mattaneng*". Usually planting is done when cocoa is 3 - 7 months old seeded or conditioned with available time. Planting activities are carried out individually by family groups and planting is carried out at the beginning of the rainy season. This is done so that young cocoa / new seeds can grow immediately and no longer need to be watered. Planting time is adjusted to farmers belief which is should be performed in a good day (according to Islamic Calendar) and it is done in the early morning before the sun rises or before the time passes trough 9:00 o'clock a.m.

Fertilization

The efforts to increase cocoa production have been carried out through fertilization to regain soil fertility. In the early years of plainting (1-2 years), farmers do not use fertilizer because they think that the land is still fertile and loose. Fertilizers begin to be given when the plants are 3 years old or more, this is based on the reason that young plants before producting fruits do not need to be fertilized because the soil is still fertile and loose. This is also done because according to them there is already capital from cocoa plants that have already produced fruits and need to maintain soil fertility. Fertilizers used for cocoa plants in the location of this study are Urea, ZA, TSP and KCL and NPK. The general method of fertilization is the fertilizer is scattered under the cacao trees around the stem by cleaning the leaves and dried stems around the trunk first.

Sanitation

In farmers native language, sanitation refers to term "*mapparakai*" or cleaning the grass that grows wild around the plants. Therefore, farmers knowledge about sanitation in this study location is limited to cleaning all types of grass / weeds and cutting off branches that grow into other plants. Cleaning is carried out at any time manually by using equipment such as hoe and *subbe*.

Pest and Diseases Control

Pests and diseases that are concerned by farmers are cocoa fruit borer (PBK) and VSD and cocoa stem borer. These pests and diseases make cocoa fruit hard so it is very difficult to separate between the pod and cocoa beans, another effect is the decrease in cocoa productivity resulted by the attacks from pests and diseases, even it can cause death in cocoa plants if the plans experience the severe attack.

Basically the knowledge of farmers about pests and diseases in cocoa plants gained after the phenomenon where farmers found their cocoa fruit hardens when it split and some fruits are rotten. This symptom resulted in actions to overcome the pests and deseases by smoking the cocoa plants with burning twigs and leaves around the garden and maintaining red ants around the garden. This way is done in order to make adult pests are not free to move and develop normally through smoke and ants as their natural enemies. Some farmers also used chemical substance by spraying pesticides to pests such as pest and disease control practices on rice fields. Pesticides are obtained from cocoa brokers / buyers who buy cocoa from farmers as well as offering these drugs.

Harvest

The application of cocoa harvesting practices is the determination of harvesting which is done visually, in slecting fruits to be harvested farmers can do it simply by looking at the color of the fruits that are yellow or orange. The fruit picking equipment using tools made by themselves namely machete and *pakekadang* / stacker which is a knowledge that has been developed from generation to generation by the cocoa farming community.

The descriptions of cocoa community development based on local knowledge as previously described can be extracted as presented in the following table:

Table.1: Local Knowledge In Cocoa Cultivation Community at Penanggosi Village

No.	Cocoa Cultivation Aspects	Local Knowledge
1.	Land Preparation	Traditional (burning land)
2.	Nursery and Planting Materials	- Seed from the garden itself and not certified

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		- Nurseries use soil on plastic media, nursery time 3-7 months.
3.	Shade Planting	Productive plants for daily consumption are bananas, tomatoes, chilies and lamtoro trees
4.	Determination of Distance and Planting Holes	Size of spacing: 4 m x 4 m Hole size according to the size of the nursery media
5.	Planting	Early rainy season
6.	Fertilization	Slope in the ground
7.	Pruning	Irregular
8.	Sanitation	Cleaning weeds using machetes and *subbe*
9.	Pest and Disease Control	Manual and chemical
10	Harvest	Visually, when cocoa fruit is yellow or orange. Harvesting equipment: homemade machetes and *pakkadang* / sticks.

Science Knowledge About Cocoa Cultivation

Science knowledge in this case is the knowledge of cocoa cultivation carried out by the government. Various programs have been promoted in developing cocoa community, such as the Integrated Pest Management School Field and the National Movement for Cocoa Production and Productivity Improvement (Gernas Cocoa). This activity aims to increase cocoa production, productivity and quality on an ongoing basis through rejuvenation, rehabilitation and intensification of cocoa (Pedum Gernas Kakao, 2012).

Land Preparation

Knowledge about land preparation which is recomended by government is very difficult to be accepted by farmers, because farmers are prefer to use their traditional way which is opening a new farming land by cutting off (wood and shrubs) and immediately burned when it is dry. Farmers usually open a new farming land in dry season. This habit is difficult to be changed although this way can cause environmental damage, but because of time, energy, limited costs, wild animal treat such as snakes and limited modern farming equipments availability, farmers incline to maintain their traditional way of land preparation which has been inherited in their family farming habits

Planting and Nursery Materials

Nursery and planting materials are received from a cocoa rejuvenation program carried out by the government. The program that offered for certified planting material are in the form of Somatic Embryogenesis (SE) cocoa seed assistance comes from Clones: Sulawesi-1, Sulawesi-2, ICCRI-3, ICCRI-4 and Scavina-6, and 2) Assistance for cocoa Entres from cocoa Entres gardens which is recommended by the Southeast Sulawesi Province Plantation and Horticulture Agency. It is expected that with this program, farmers' habits of preparing planting materials from their own gardens can be abandoned.

The use of planting material in the form of SE cocoa seeds recomended by the government is not well received by farmers, because the fruit is small and it is susceptible to pest and disease attacks. Farmers prefer homemade seeds because they are better suited to the local climate. The nursery delivered by the government through researchers and extension workers is also difficult to implement by farmers because the costs, energy and time are quite large, while farmers prefer practical ones but the results are also maximum. However, the selection of planting media in the form of black polybags has been followed by farmers, as well as the government assistance programs programmed by the government have been well received, but the government grafting knife is not well received because it is easily damaged and take longer time while used.

Shade Planting

Shading plants for cacao plants are *gamal* and banana plants which has generally been well received by cocoa farmers. The concept of providing banana as shading plant is the local knowledge of the farmers followed up by the government support with the provision of plant spacing, where the banana plant as a life support during cocoa yields. Temporary shading plant space (banana) is 3m x 6m and a permanent shade range (*gamal*) is 6m x 6m. Research results by Agung A and Shahabuddin (2014), that Percentage of beans damaged in themonoculture shade was not significantly different from the polyculture shade

Determination of Planting Space and Planting Hole

Knowledge of technology introduction offered by the government is the determination of distance and planting holes in cocoa plants delivered by extension workers with the size of planting holes 60cm x 60cm x 60cm and the size of the planting distance of 3m x 3m and the making of planting holes 2-3 months before planting and manure on planting hole. Science knowledge is still difficult to be accepted by farmers, because it requires a lot of money, energy and time. On the other hand farmers want more practical and fast farming.

Planting

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Planting can be done if cocoa seeds and planting holes are ready in the field in accordance with the provisions. Technique and planting method is by using polybag which is slashed first, the plant is inserted into the planting hole, after that the soil is compacted so that it does not fall easily and provides security poles and fence in order to keep the plants thrive and productive as expected. The provision of organic fertilizer in the form of cow / goat manure as the nutrient reserves for young plants has not been followed by farmers because organic fertilizer is not freely available. Therefore, to provide organic fertilizer, a livestock assistance program is needed for farmers who are integrated with the cocoa plantation. This program will create a symbiosis mutualism where animal manure becomes fertilizer for cocoa plants and shading plants, then cocoa skin can be processed as animals' feed.

Fertilization

The efforts to maintain the sustainability of cocoa are carried out by increasing the effectiveness of fertilizing the cocoa plants through the Gernas Cocoa approach by the use of NPK Special Formula based on soil analysis results namely N: 19%, P2O5: 8%, K2O: 10% MgO: 3% and K2O : 2%. Fertilization has an impact on increasing soil fertility and causing more stability level of crop production so that maximum crop production can be achieved. The provision of manure and organic fertilizer is significantly different from farmers fertilizing practices (control), but the best treatment is found in the combination of inorganic fertilizer (P3) which shows growth and the highest production number of fruits (Azri, 2015).

Pruning

There are 3 ways of pruning offered by the government, those are; trimming, production pruning and maintenance pruning. Trimming is carried out on cocoa plants aged 8-24 months, while production pruning is at the beginning of the dry season and the end of the rainy season, maintenance pruning is done on the sidelines of production cuts that are 2-3 months. Intensive pruning is carried out in a precise time and appropriate manner that aims to keep the branches of the plant organized, and more importantly to increase production and to control pests and diseases (Firdaus AB et al, 2008).

Sanitation

The concept of sanitation as a whole is in the form of weed control by using manual hoe and sickle equipment and the chemical methods are the actions by spraying with various types of herbicide with active contact / systemic ingredients according to the recommended dosage. The garden sanitation is in the form of making holes / *rorak* with a length of 1.5 m, width and depth is 50 cm, this is useful to accommodate the results of pruning in the form of leaves, twigs and branches of cocoa / shading plants as a source of organic fertilizer for plants. Besides it can be an organic fertilizer, this also a way to avoid pests and deseases which can cause a decrease in production and poductivity. Another way of garden sanitation is done by making drainage around the cocoa garden to avoid flooding and high humidity around the garden during the rainy season.

Pest and Disease Control

Various pest and disease control programs on cocoa plants have been carried out through SL-PHT Cocoa and Gernas Cocoa. The method used is mechanical, chemical and biological control that has been conveyed to cocoa farmers both formally and non-formally in their gardens. However, this offered concept , although it can reduce pest and disease attacks and can increase production, but farmers still maintain their traditional ways of controlling pests and diseases, farmers are maintaining the use of chemical control by spraying using various types of insecticides and fungicides offered by traders /drugs sellers.

Harvest

The determination of cocoa fruit harvesting age is marked by changes in the color of cocoa pods which are yellow or orange or 5-6 months since flowering. Introducing the use of harvest equipment in the form of pruning shears and pole scissors, solely to avoid damage to the fruit bearing /next shelter and harvesting can be faster than using machetes as farmers traditionally do. Pruning shears and giant scissors have been received by farmers, but giant scissors are used to cut branches and not to harvest / to pick fruits because it will damage other fruits. The cocoa fruit breaking equipment which is recommended made from wood has not been received by farmers because of its slow working, especially while breaking the fruits attacked by CPB, so that the farmers still use their own method which is using machetes.

Table.2: Science Knowledge In Cocoa Cultivation Community at Penanggosi Village.

No.	Cocoa cultivation aspects	Science knowledge
1.	Land Preparation	Modern (without burning land)
2.	Nursery and Planting Materials	- Planting material from certified seeds Nurseries use a mixture of soil + sand + organic fertilizer (1: 1: 1), 4-6 months of nursery.
3.	Shade Planting	Gamal plants

4.	Determination of Distance and Planting Holes	Size of spacing: 3 m x 3 m Planting hole size: L: 60cm x inside: 60cm
5.	Planting	Early rainy season
6.	Fertilization	Immersed in the ground
7.	Pruning	Intensive (Trimming, trimming production, trimming maintenance)
8.	Sanitation	Weed control, making *rorak* and drainage
9.	Pest and Disease Control	Integrated Pest Management (PHT), namely mechanical, chemical and biological
10	Harvest	Age 5-6 months since flowering, harvesting tools and pruning shears

III. CONCLUSION

Local knowledge in cocoa cultivation is rooted in customs and traditions as well as the insistence of family life which is the habit of farmers and then processes through repetitions which then form a farming experience. Science knowledge in cocoa cultivation is formed based on the response to decreased production and user preferences. Sustainable development of cocoa plantations is carried out jointly by the government, farmers / smallholders and private communities. The government issued research and gave birth to a technology that was introduced to users through technology transfer by researchers, extension agents and plantation assistants.

The acceleration of technological innovation by the government was not followed by the speed and utilization of science knowledge by cocoa farmers, and the weakness of the delivery subsystem and the recipient subsystem. A follow-up plan and valid evaluation are needed between research institutions as suppliers of science technology / knowledge with their users, so that the resulting science knowledge is guaranteed to be followed by users on an ongoing basis.

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Kinetic Modelling of Vitamin C Degradation in Selected Fruits under Market Storage Conditions

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***Abstract*— *The degradation kinetics of vitamin C in three fruits namely; orange, banana and apple under different market storage conditions prevalent in Nigeria were investigated. Orange and banana samples were stored in sacks and open air at ambient conditions, while open air and refrigerator were used for apple samples. Storage was for 6 days duration. .Iodometric titration was used to determine vitamin C content of the fruits on a daily basis. Regression analysis was employed to fit the variations in vitamin C concentration in the different samples with time, to three kinetic models, to determine which model best describes the degradation trend. Results showed that vitamin C concentration in all the fruit samples reduced over time following zero order kinetics. Kinetic studies obtained the following degradation rate constants: orange 0.44g.L^{-1}.s^{-1} and 0.29 g.L^{-1}.s^{-1}, for sack and open air storage respectively, banana 0.316 g.L^{-1}.s^{-1} and 0.264 g.L^{-1}.s^{-1}, for sack and open air, and apple 0.122 g.L^{-1}.s^{-1} and 0.188 g.L^{-1}.s^{-1}, for refrigerated and open air respectively. The study indicated that with respect to vitamin C retention, open air storage is preferable to sack for oranges and bananas while apples are preferably stored in refrigerator.*

Keywords*— *Diet, samples, rate constant, open air, biological activity, anti-oxidant.

I. INTRODUCTION

Fruits such as orange, banana, mango and apple are a major component of a healthy diet. They are low in fat and provide significant levels of some micronutrients. Most fruits when ripe have a short durability, which is further reduced when the fruits are exposed to unfavourable conditions during transportation, processing and storage. Fruits are important sources of vitamin C. (Giannakouurou *et al*., 2003).

Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of L-ascorbic acid (AA) (Lee *et al*., 2000). Vitamin C or ascorbic acid is a nutrient that is valuable for its antioxidant effect (Giannakouurou *et al*., 2003). It is a water soluble vitamin, and very essential to human beings (Uddin *et al*., 2002). Most plants and animals have the ability to synthesize vitamin C. The only mammals that are unable to synthesize vitamin C are primates, including man, and guinea pigs. Therefore, humans depend on exogenous sources of the vitamin which include fruits and vegetables as well as food supplements and pharmaceutical preparations (Parviainen, 1995). Vitamin C is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments and bone. It is also important in the synthesis of the neurotransmitter norepinephrine, which is critical to brain function and can affect mood.

Vitamin C content of fruits vary depending on fruit size and species. Oranges contain about 50mg/100g, while banana and apple contain 9mg/100g and 6mg/100g respectively (Bellow and Krebs, 2007). Vitamin C is easily destroyed and various studies have been carried out on the rate of vitamin C degradation in foods and fruits alike, and various kinetic models adopted. However, the vitamin C degradation mechanism is specific to a particular system, as it depends on several factors (Tannenbaum, 1976), such as temperature, water activity, pH and metal ions (Masamba *et al*., 2013). Vitamin C is most sensitive to destruction when the commodity is subjected to adverse handling and storage conditions. Losses are enhanced by extended storage, higher temperatures, low relative humidity, physical damage, and chilling injury (Lee and Kaddar, 2000). Ascorbic acid is recognized as one of the most heat sensitive nutrients in foods, therefore, it is a marker of the loss of other nutrients (Esteve *et al*., 1999). Numerous analytical techniques are available for the determination of the vitamin C content in various fruits and vegetables, amongst them are iodometric titration, chromatographic methods, enzymatic methods, and electrochemical methods (Abraha *et al*., 2014; Gunjun and Mangla, 2012; Iwase, 2000).

This study was carried out to (i) determine the rate of degradation of vitamin C in orange, banana and apple under the market storage methods prevalent in Nigeria namely;

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ambient storage in open air, sack storage, and refrigeration, so as to recommend the best option; (ii) develop kinetic models for predicting vitamin C degradation in the selected fruits under the studied conditions.

II. MATERIALS AND METHOD

Source and preparation of samples

Ripe and fresh fruits were purchased from Relief market in Owerri, Nigeria. The fruit samples were divided into two groups and stored using the different market storage methods. The first group of oranges, apples and bananas were kept on a tray and exposed to open air under ambient conditions, the second group of oranges and bananas were packed in two separate jute bags and kept on concrete floor, while the apples were stored in a refrigerator. The fruits were stored for a period of 6 days. The fruit juice from the orange and banana samples was prepared by blending 100g of the fruit sample with 50ml of distilled water and the mixture strained. Distilled water was then added to make a final solution of 100ml in a volumetric flask. The apple samples were squeezed to extract the juice.

Preparation of standard solution

Starch solution was prepared by dissolving 0.5g soluble starch in 50ml near boiling distilled water, while vitamin C solution was prepared by dissolving 0.25g of standard vitamin C in 100ml of distilled water, and the solution was diluted to 250ml with distilled water. 5g of potassium iodide (KI) and 0.268g of potassium iodate (KIO_3) were dissolved in 200ml of distilled water, and 300ml of 3M sulphuric acid (H_2SO_4) was added to obtain standard iodine solution.

Sample analysis

The iodine solution was titrated with 25ml of the standard vitamin C solution, and then with 25ml of prepared fruit juice samples using starch indicator. Triplicate samples were analyzed and the average titre used for calculation.

a) *Calculation of the mass of vitamin C present in fruit sample* - The mass of vitamin C contained in each of the samples was calculated from the relationship:

$$\frac{\text{volume of iodine solution required to react with standard vitamin C sample}}{\text{mass of standard vitamin C used}} = \frac{\text{volume of iodine solution required to react with vitamin C in fruit sample}}{\text{mass of vitamin C in fruit sample}} \quad (1)$$

b) *Calculation of the Concentration of vitamin C present in fruit sample* – This was carried out using the relationship:

$$\text{Concentration of vitamin C} = \frac{\text{mass of vitamin C in fruit sample}}{\text{volume of fruit juice sample used}} \quad (2)$$

Kinetic modelling

The degradation of vitamin C was modeled using the integral rate law. Different models were fitted using the integral method of analysis. The integral law equation is stated as;

$$\frac{dC}{dt} = -k[C]^n \quad (3)$$

where k = rate constant

C = concentration of vitamin C in sample at time t

n = order of reaction

This equation was used to develop three models based on concentration (for order of reactions n = 0, 1 and 2) and their associated half lives ($t_{1/2}$).

Zero order model (n = 0) is given as:

$$C = C_0 - kt \quad (4a)$$

$$t_{1/2} = {C_0}/{2k} \quad (4b)$$

First order model (n = 1):

$$\ln(C) = \ln(C_0) - kt \quad (5a)$$

$$t_{1/2} = {\ln(2)}/{k} \quad (5b)$$

Second order model (n = 2):

$$\frac{1}{C} = \frac{1}{C_0} + kt \quad (6a)$$

$$t_{1/2} = {1}/{kC_0} \quad (6b)$$

where C_0 = initial concentration of vitamin C in sample

$t_{1/2}$ = half-life of vitamin C in sample

Concentration or a function of concentration was plotted against time for each model and regression analysis was used to determine the 'Goodness of fit' employing Matlab software (Version 8.2, MathWorks Inc., USA). Goodness of fit is characterized by Coefficient of Determination (R^2),

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Sum of Squared Errors (SSE), and Root Mean Sum of Errors (RMSE). The model with maximum R^2 and minimum RMSE is adjudged the best (Silva *et al*., 2011; Mitra *et al*., 2011)

III. RESULTS AND DISCUSSION

The variations in vitamin C concentration of fruit samples during storage are presented in Tables 1 - 3. Tables 4- 6 summarize the results of kinetic model regression analysis, while Table 7 compares vitamin C zero order degradation kinetic parameters for the samples under different storage methods. As can be observed in Tables 1-3, the concentration of vitamin C decreased steadily with time during storage in all the samples. This confirms the fact that vitamin C degrades during storage in fruits. The vitamin C concentration of the fruit samples decreased during storage, but in different degrees, depending on the method of storage. This is in agreement with the report of earlier workers on citrus and strawberry fruit juices (Burdurlu *et al*., 2006; Derossi *et al*., 2010,) and in accordance with the degradation kinetics of ascorbic acid in model systems as put forth by Liao and Seib (1988). Besides, it is also evident that the ascorbic acid content in a given mass of orange is greater than that in an equal mass of banana and apple.

A visual inspection of the kinetic plots of models (4a), (5a) and (6a) respectively, presented in Figures 1-3 for orange, Figures 4-6 for banana, and Figures 7-9 for apple, shows that the zero order, that is, model (4a) fitted the kinetic data best in all fruit samples. This is confirmed by the goodness of fit data in Tables 3, 4, and 5. The zero order kinetics exhibited R^2 values; 0.9335, 0.9822 and RMSE values; 0.1621, 0.1229 for orange under open air and sack storage respectively, R^2 values; 0.9312, 0.9558 and RMSE values; 0.1505, 0.1424 for open air and sack storage respectively for banana, and R^2 values; 0.9850, 0.9574 and RMSE values; 0.0486, 0.05395 for apple under open air and refrigerated storage respectively.

The zero order model generally exhibited the highest R^2 values and the lowest RMSE values. Thus, the vitamin C degradation kinetics in orange, banana and apple can be best described by zero order kinetics. . Furthermore, the degradation rate constants of fruit samples stored in sacks were generally higher than those kept in open air and refrigeration. Refrigerated samples exhibited lower rate constants relative to open air for apple. Since the magnitude of the rate constant is a reflection of the rate of reaction, the inference is that degradation of vitamin C occurred faster in samples stored in sacks than in those exposed to the air, and slower in refrigerated samples for apple. This trend manifested in the half life of the samples. The time at which the concentration of vitamin C in the samples reduces to half of its original amount (half life) was shorter in sack stored samples for orange and banana, and longer in refrigerated samples for apple. This implies that the air exposed and refrigerated samples will be expected to have longer shelf life than the sacked ones. Refrigerated apple samples recorded the longest half life of 10.79days, with a proposed zero order model $C = C_0 - 0.1223t$, while sacked banana exhibited the shortest half life of 2.40days with the proposed model $C = C_0 - 0.3166t$. These models and others presented in Table 7 are proposed for monitoring vitamin C degradation for the respective fruits under the indicated market storage conditions.

Table.1: Vitamin C concentration in orange during storage

Time (day)	Concentration (g/l)	
	Open air	Sack
1	3.76	4.24
2	3.68	3.96
3	3.52	3.64
4	3.20	3.20
5	2.80	2.64
6	2.32	2.04

Table.2: Vitamin C concentration in banana during storage

Time (day)	Concentration (g/l)	
	Open air	Sack
1	1.32	1.52
2	1.16	1.32
3	0.92	0.88
4	0.30	0.36
5	0.28	0.20
6	0.12	0.10

Table.3: Vitamin C concentration in apple during storage

Time (day)	Concentration (g/l)	
	Open air	Refrigerated
1	1.78	2.01
2	1.44	1.63
3	1.17	1.33
4	0.98	1.10
5	0.83	0.98
6	0.68	0.72

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Table.4: Results of kinetic model regression analysis for orange

Storage Method	Kinetic order (n)	R^2	Adjusted R^2	SSE	RMSE
Open air	0	0.9335	0.9169	0.00256	0.16210
Sack	0	0.9822	0.9778	0.06046	0.12290
Open air	1	0.9034	0.8792	0.01692	0.06505
Sack	1	0.9390	0.9238	0.02322	0.07619
Open air	2	0.8626	0.8282	0.02826	0.02658
Sack	2	0.8841	0.8551	0.00533	0.03652

Table.5: Results of kinetic model regression analysis for banana

Storage Method	Kinetic order (n)	R^2	Adjusted R^2	SSE	RMSE
Open air	0	0.9312	0.9139	0.09057	0.15050
Sack	0	0.9558	0.9447	0.08113	0.14240
Open air	1	0.9264	0.9080	0.34240	0.29260
Sack	1	0.9548	0.9435	0.30590	0.27650
Open air	2	0.7893	0.7367	8.67800	1.49000
Sack	2	0.7408	0.6760	27.0600	2.60100

Table.6: Results of kinetic model regression analysis for apple

Storage Method	Kinetic order (n)	R^2	Adjusted R^2	SSE	RMSE
Open air	0	0.9850	0.9813	0.00944	0.04860
Sack	0	0.9574	0.9468	0.01164	0.05395
Open air	1	0.9639	0.9549	0.04399	0.10480
Sack	1	0.9505	0.9381	0.00244	0.02479
Open air	2	0.7408	0.6760	27.0600	2.60100
Sack	2	0.90450	0.8806	0.28960	0.69120

Table.7: Comparison of zero order kinetic parameters and proposed model

Fruit samples (Storage method)	Rate constant k g(lday)$^{-1}$	Half life $t_{1/2}$ (day)	Proposed model
Orange			
Open air	0.2903	6.47	$C = C_0 - 0.2903t$
Sack	0.4371	4.85	$C = C_0 - 0.4371t$
Banana			
Open air	0.2646	2.49	$C = C_0 - 0.2646t$
Sack	0.3166	2.40	$C = C_0 - 0.3166t$
Apple			
Open air	0.1886	3.28	$C = C_0 - 0.1886t$
Refrigerated	0.1223	10.79	$C = C_0 - 0.1223t$

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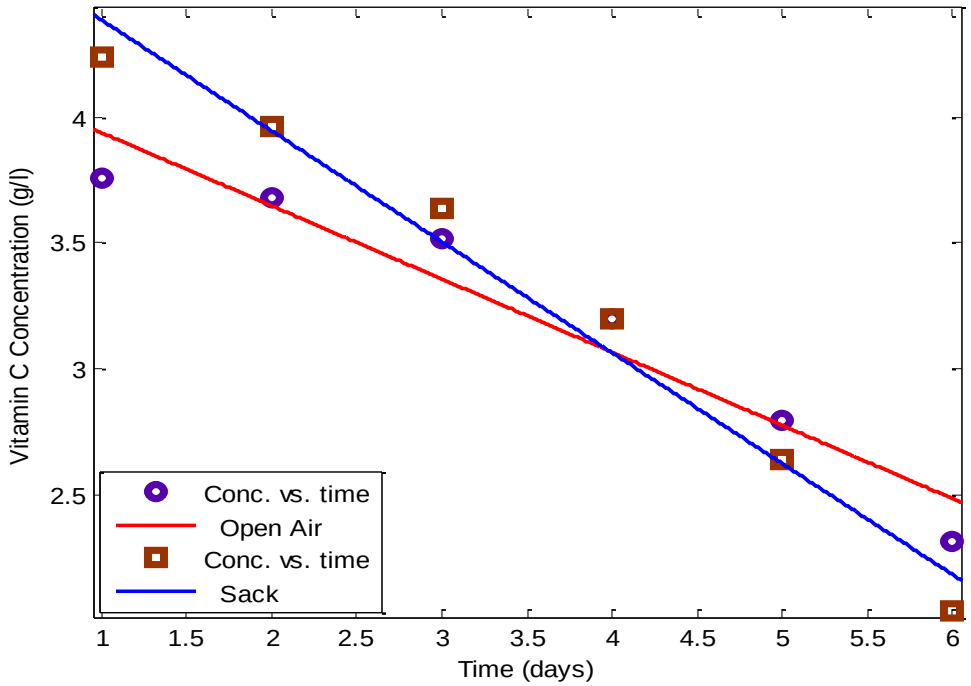

Fig.1: A plot of zero order kinetics for orange

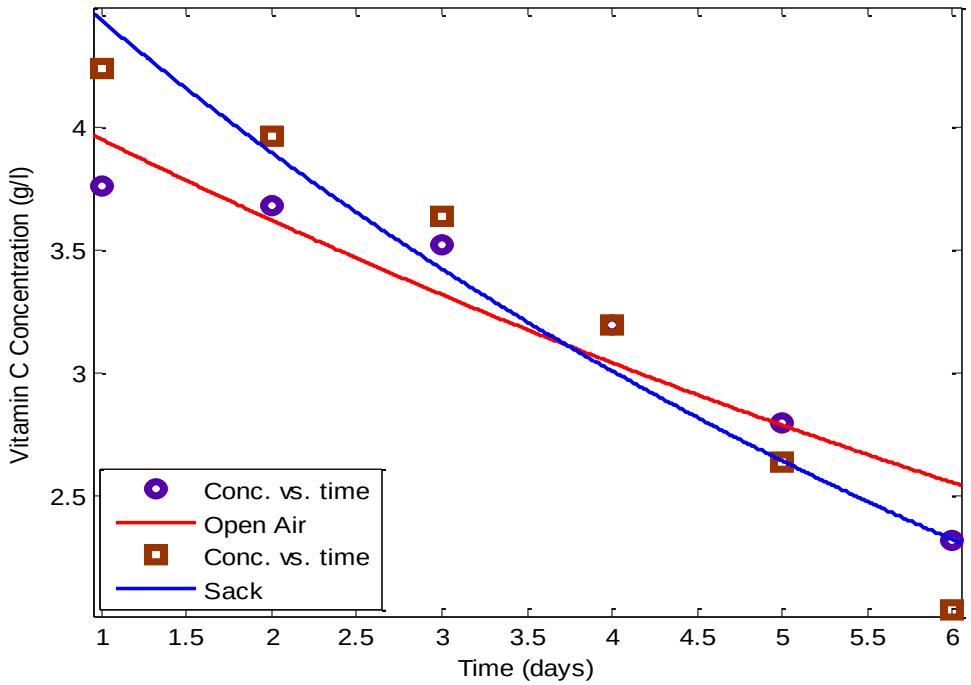

Fig.2: A plot of first order kinetics for orange

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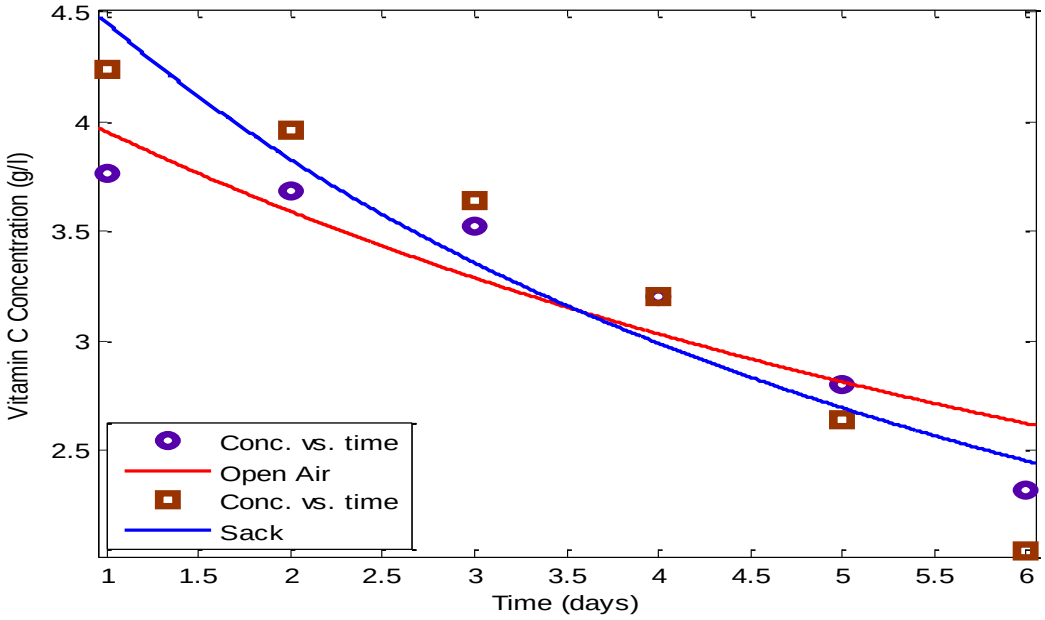

Fig.3: A plot of second order kinetics for orange

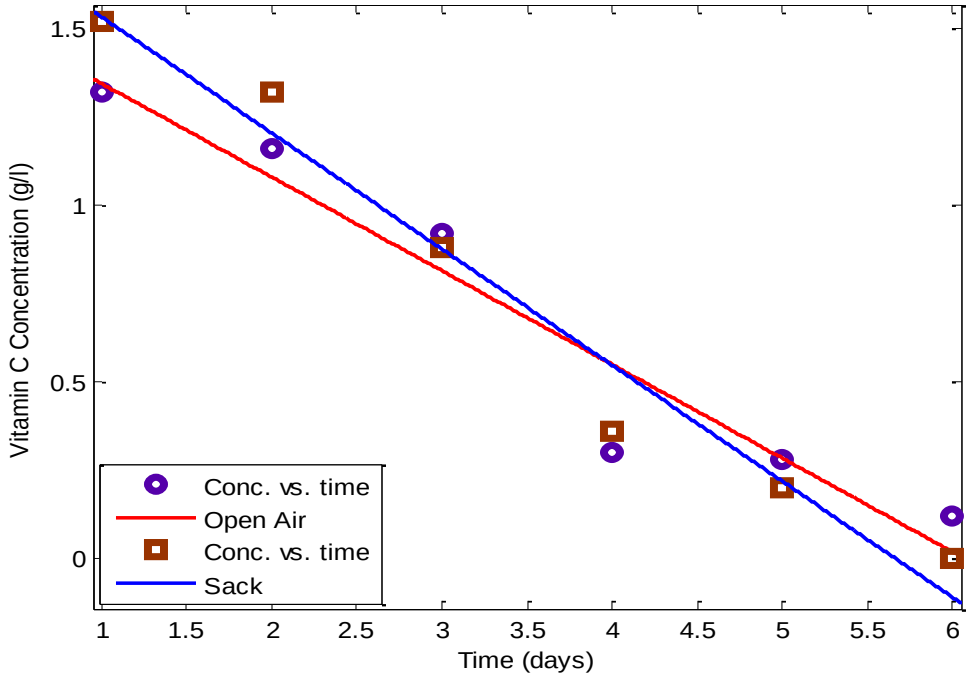

Fig.2: A plot of zero order kinetics for banana

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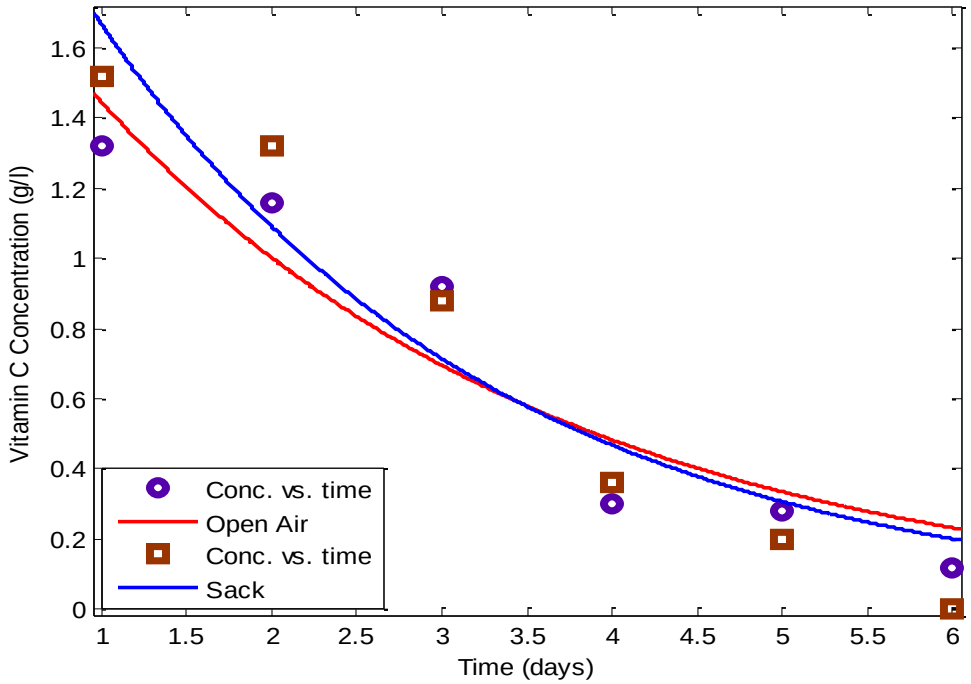

Fig.5: A plot of first order kinetics for banana

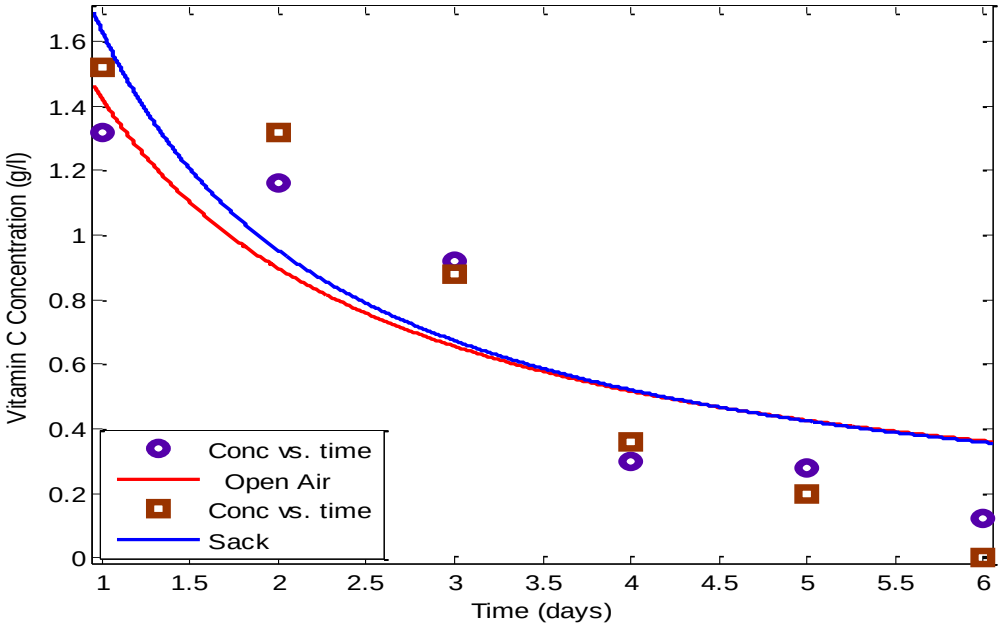

Fig.6: A plot of second order kinetics for banana

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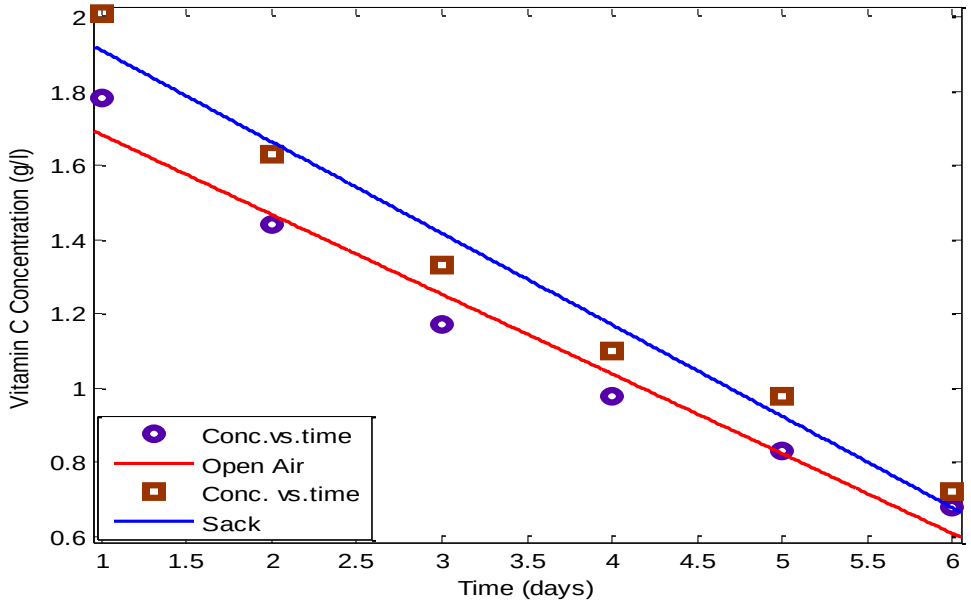

Fig.7: A plot of zero order kinetics for apple

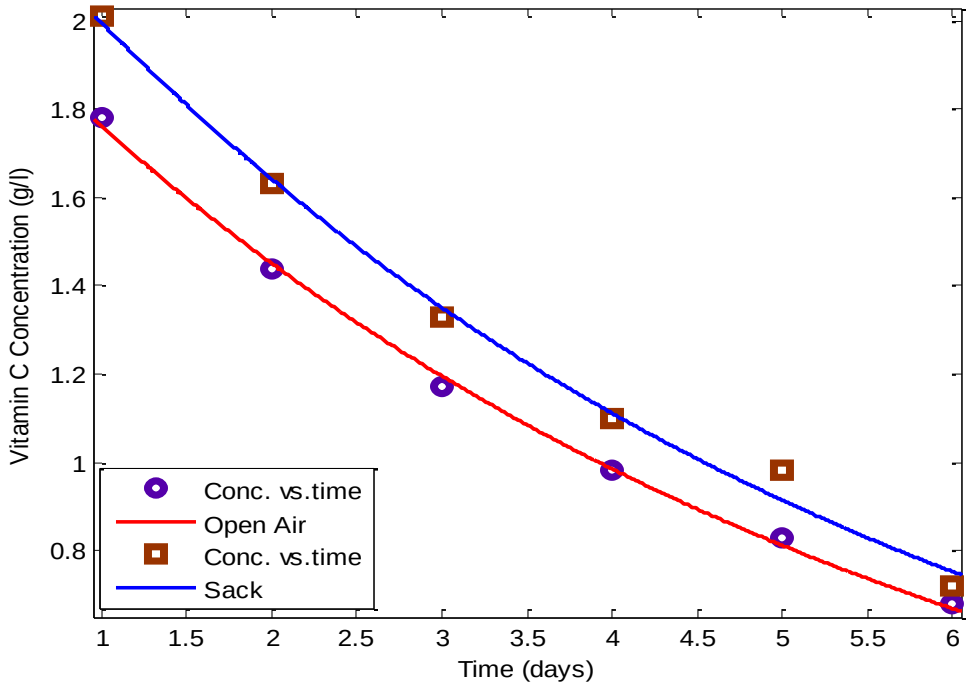

Fig.8: A plot of first order kinetics for apple

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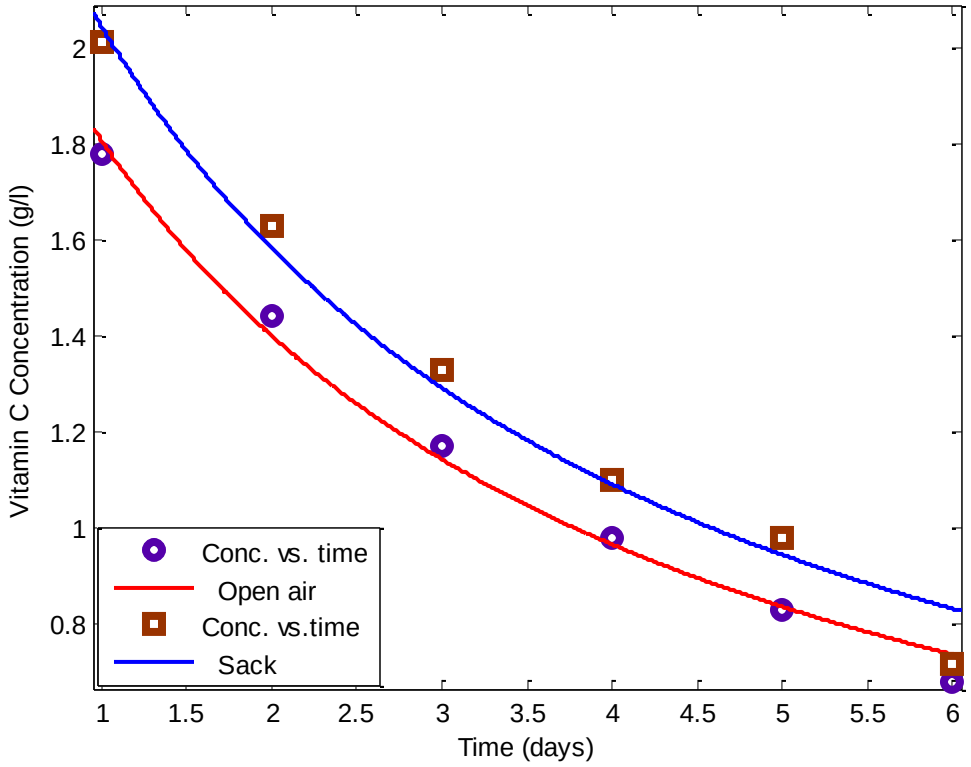

Fig.9: A plot of second order kinetics for apple

IV. CONCLUSION

The influence of market storage conditions on vitamin C degradation in orange, banana and apple samples was evaluated in this study. Vitamin C content of these fruits decreased with time following zero order kinetics. It was found that open air storage and refrigeration helped to significantly reduce the rate of degradation when compared to storage in sacks as shown by the degradation rate constants and half lives. Thus, refrigeration for apples and open air storage for oranges and bananas are preferable in terms of vitamin C retention to sack storage, and hence, recommended for the storage of the indicated fruits in Nigeria local markets. However, in situations where refrigeration facilities are not available, properly managed open air storage can go a long way to retain vitamin C in apples for extended periods.

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Coping Behaviours of Frontline Extension Workers in Akwa Ibom State of Nigeria

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***Abstract*—** *The study determined the coping behaviours exhibited by Akwa Ibom State Agricultural Development Programme (ADP) extension workers in the bid to ease their job-related tension.*

The study also highlighted the relationship between selected personal characteristic and coping behaviours of the extension personnel. Data for the study were obtained from 88 respondents who were randomly selected. Mean score, and correlation statistic were the statistical tools employed in analyzing the data. The findings show that the extension agents (BESs) in Akwa Ibom State ADP dealt rarely with the job-related tension by using many of the coping behavioural variables such as talking with friends, spouse and relatives, involvement in religious activities, seeking professional help among others respectively. Level of formal education, household size, and extension experience of EAs were strong predicators of coping behaviours. Age and extension experience of BEAs and BESs were significantly associated with coping behaviours. It was concluded that the socio-economic characteristics of the extension agents influenced their coping behavior. Necessary recommendations such as improving extension agents' education and working experience as well as constant training for capacity enhancement were made among others.

Keywords*— *Coping Behaviour, Frontline Extension Workers, Akwa Ibom State, Nigeria.

I. INTRODUCTION

Field extension workers deals with wide varieties of constituencies and programme requirements. Dealing with organizational and public demands will make these extension educators to feel tense up. Extension must be address the issue of job-related tension to attract and retain leading professionals, if it intends to continue as a principal provider of non formal educational programmes (Goering, 1991, Place and Jacob, 2001). Subsequently, determining the behaviors exhibited by frontline extension staff to cope with job-related tension will provide policy makers, programme administrators, and development practitioners at all levels with relevant information they need for improvement and future planning.

Tension is part and parcel of work. However, the worker's ability to cope with it is what makes the difference between fulfilling and unfulfilled job. Coping is an individual action that is learned from one's reference group, such as one's colleagues. Each individual has to manage his own coping; it is not something that can be done by others, no matter how supportive they may be. Ability to cope with tension is important in extension work. Coping well with tension plays a large part in achieving a sense of well-being at all levels (Long, 1988; Goering , 1991 Place and Jacob,2001). Every worker has to discover for himself effective methods or ways of coping with job-relate tension that work for him and put these into practice. This study will help to identify coping strategies used by extension personnel to ease job-related tension. This will form an information pool which extension workers will use to cope rationally with tension arising from their work. Extension administrator will also use the information to help their subordinates to cope well with work-related tension.

Human beings continually assess what is happening in their environment because different behavioural situations demand different coping strategies (Bernard and Bandler, 1998, Oranye, 2002). In coping with tension with tension arising from extension work, some personal characteristics having positive or negative influences may come into play. There is need to identify the personal characteristics of extension staff that effect (Favour or disfavour) their abilities in coping with job-related tension. Herein lies the importance of this study.

The study investigated the behaviours exhibited by frontline extension workers in the bid to deal with their job-related tension. The relationship between selection personal characteristics and coping behaviours of these extension workers was explored.

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II. MATERIALS AND METHODS

Akwa Ibom State of Nigeria was the study area. The population of the study was made up of frontline extension workers that included the block extension supervisors (BESs), block extension agents (BEAs), and extension agents (EAs) in Akwa Ibom state ADP (AKADEP). AKADEP has 6 agricultural zones, viz, Abak, Eket, Etinan, Ikot Ekpene, Oron and Uyo consisting of 9, 7, 4, 8, 4 and 8 blocks, respectively. The blocks are made up of 59, 40, 27, 63, 23 and 62 circles, respectively. Altogether, AKADEP has 6 agricultural zones, 40 blocks and 274 circles (Okpongete, 2000; Umoh, 2003).

Multi-stage random sampling procedure was used in the selection of the agricultural zones, blocks and circles. The first stage involved simple random selection of two agricultural zones. Ikot Ekpene and Uyo zones were selected. The second stage involved simple random sampling of seven blocks from each of the agricultural zones. The BESs and BEAs whose blocks were selected served as respondents. The third stage involved simple random selection of five circles from each of the blocks. The EAs whose circles were selected served as respondents. This gave a total of 14 BESs, 14 BEAs and 70 EAs. On the whole, a total of 98 respondents made up the sample size for the study. However, 88 questionnaires (14, 12 and 62 questionnaires from BESs, BEAs and EAs, respectively) where found suitable for the study.

Three sets of questionnaires were used to elicit information from EAs, BESs and BESs.

Questionnaires for EAs, BEAs and BESs designed to elicit information on their personal characteristics and job-related tension coping behaviors.

The coping behaviours of the frontline extension workers were measured by asking each of them to indicate the degree of their agreement with each of the 28 different coping behavioral/attitude statements or items on a five point likert-type scale. The five point on the scale were weighted according to the degree of agreement as follows: 1=Not at all, 2=Rarely, 3=Sometimes 4=Often, and 5=Always. The mean degree of agreement for each of the 28 different coping behavioral/attitude score of the group by the member of the respondents. The coping behavioral/attitude level for each group of respondents was computed by dividing the grand mean coping behaviourial/attitude score of the group by the number of the different coping behaviourial/attitude statements (28).

Mean scores and correlation statistic were utilized for data analysis. The correlation statistic was used to test the relationship between selected personal characteristics and the job related tension coping behaviourial variable of the frontline extension workers. The level of probability that was accepted as indication of a statistically significant relationship for correlation analysis was at 0.05

Coping behaviours of extension agents

Data in Table 1 indicate the means of the job-related tension coping behaviours for the EAs. Involvement in religious activities was the first most frequently used coping behavioural variable in dealing with job-related tension by EAs. Prioritizing work and sticking to priorities was the second most frequently exhibited coping behaviours by EAs. The table reveals that talking with friends was the third most frequently used coping behavioural variable in dealing with job-related tension by EAs. Prioritizing tasks and cutting out unnecessary work are actions that can be taken by management to help workers cope with job-related tension thereby promoting better working conditions in the organization (Mullins, 2007).

The grand mean coping behavioural score for EAs was 68.32. The coping behavioural level for EAs was 2.00 implying that majority of the EAs dealt rarely with job-related tension by using many of the coping behavioural variables.

Coping behavioural of block extension agents

Table 1 shows the means of the job-related tension coping behaviours for the BEAs.

Talking with friends was the first most frequently exhibited coping behavior by BEAs. Involvement in religious activities was the second most frequently exhibited coping behavior by BEAs. Involvement in organized groups and talking with relative were the third most frequently used coping behavioural variables by BEAs. The ability to hold good-quality conversation is becoming a core individual and organizational skill. Conversations are intrinsically creative and roam freely across corporate gossip, work projects and personal issues. Conversations are a defence against stress/tension and other mental health problems. Employees with good social relationship at work are much less likely to be anxious or stressed or tensed up (Reeves, 2003). A growing number of organizations are introducing an email- free day to encourage workers to use the telephone or walk across the corridor to talk more with one another (Mullins, 2007).

Date in Table 1 show that the grand mean coping behavioural score for BEAs was 65.67.

The coping behavioural level for BEAs was 2.00 implying that majority of the BEAs dealt rarely with Job-related tension by using many of the coping behavioural variables.

Coping behaviours of block extension supervisors

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Setting realistic goal for my work was the first most frequently used coping behavioural variable by BESs (Table 1). Date in Table 1 reveal that involvement in religious activities, and prioritizing work and sticking to priorities were the second and the third most frequently exhibited coping behaviours by BESs, respectively.

The grand mean coping behavioural score for BESs was 68.64. The coping behavioural level for the BESs was 2.45 approximately 2.00 implying that majority of the BESs dealt rarely with job-related tension by using many of the coping behavioural variables.

Table.1: coping behaviours exhibited by frontline extension workers

Coping Behaviour	**X**		
	EAs	BEAs	BES
Talking with spouse	2.82	2.83	3.43
Talking with Friends	3.53	3.83	3.43
Involvement in religion activities	3.61	3.75	3.79
Involvement in organized groups	3.40	3.42	3.21
Smoking	1.26	1.00	1.14
Going out to have a drink	1.66	1.17	1.43
Working around the house	2.16	1.67	2.00
Finding activities that will take mind off	2.44	2.17	2.50
Going for shopping	2.40	2.25	2.21
Seeking professional help	2.87	2.75	3.07
Going on tranquilizers	1.74	2.08	1.14
Changing of eating habits	1.58	1.92	1.57
Going on other drugs	1.44	1.17	1.50
Just get away from everybody	1.66	1.25	1.57
Gambling	1.31	1.00	1.57
Talking with relatives	2.81	3.42	3.07
Tight with money	2.16	2.17	2.21
Over-spending	2.10	1.67	2.00
Visiting favourite places	2.57	2.25	2.71
Savouring special relationships	2.48	2.00	1.79
Leaving problem at work	2.42	3.00	2.36
Searching out relevant information to deepen understanding	2.92	2.67	3.36
Watching videos/film	2.77	2.92	2.71
Listening to music	2.97	2.75	3.14
Setting realistic goals for my work	3.50	3.33	3.86
Prioritizing work and sticking to priorities	3.57	3.33	3.50
Engaging in other income generating activities e.g motor cycle taxing.	1.36	1.33	1.57
Refusing to become over stretched by another person's unrealistic expectations	2.82	2.58	2.79

Grand Mean (X) Coping Behaviour Score: (a) EAs= 68.32 (b)BEAs= 65.67 (c) BESs=68.64
Coping behavior level: (a) EAs=2.44≈2.00 (b) BEAs=2.35≈2.00 (c) BESs=2.45≈2.00

Relationship between selected personal characteristics and job-related tension coping behaviours of frontline extension workers

Information on the relationship between selected personal characteristics and job-related tension coping behaviours of frontline extension workers are presented under hypothesis 1 to 3. Age, level of formal education, household size and extension experience of frontline extension workers were tested for their relationship with job-related tension coping behaviours using Pearson correlation.

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Relationship between selected personal characteristics and job-related tension coping behaviours of extension agents

The first hypothesis states that there is no significant relationship between selected personal characteristics and job-related tension coping behaviours of extension agents. Data in Table 2 indicate the relationship between selected personal characteristics and job-related tension coping behaviours of EAs.

The table shows that the level of formal education of EAs was negatively and significantly correlated with their going out to have a drink (r=0.42), going on other drugs e.g. anti-depressants sleeping tablets and so on, and engaging in other income generating activities e.g motor cycle taxing (r=-0.27), respectively. The implication of this finding is that the higher the level of formal education received by the EAs, the less they dealt frequently with job related tension by going out to have a drink, going on others e.g. anti-depressants, and sleeping tablets among others, and engaging in other income generating activities e.g. motor cycle taxing and thus, the less they coped effectively with job-related tension.

The table further reveals that household size of EAs was positively and significantly correlated with talking with spouse (r=0.25), implying that the larger the household size of the EAs, the more they dealt frequently with job-related tension by talking with their respective spouses and hence, the more they coped well with job-related tension. Extension experience of EAs was positively and significantly correlated with being tight with money (r=0.27), meaning that the more experience the EAs acquired in extension work, the more they dealt frequently with job-related tension by being tight with money and thus, the more they coped better with job-related .

Relationship between selected personal characteristics and job –related tension coping behaviours of block extension agents in Akwa Ibom State ADP.

Table 2 reveals that extension experience of BEAS was positively and significantly correlated with seeking professional help (r=0.71), going on tranquilizers (r=0.65), setting realistic goals for my work (r=0.61), and prioritizing work and sticking to priorities (r=0.61), respectively. The implication of this finding is that the more experience the BEAs acquired in extension work, the less they dealt frequently with job-related tension by being tight with money and thus, the less the coped well with the job-related tension.

Age of BEAs was positively and significantly correlated with talking with spouse (r=0.67), Implying that the older the BEAs, the more they dealt frequently with job-related tension by talking with their spouses and hence, the more the coped effectively with job-related tension. Age of BEAs was negatively and significantly correlated with seeking professional help (r=0.66), and changing of eating habits (r=0.61) this findings implies that the older the BEAs, the less they dealt frequently with job-related tension by seeking professional help, and changing of eating habits and thus, the less they coped well with job-related tension.

The table further indicates that household size of BEAs was negatively and significantly correlated with being tight with money (r=0.64). The implication of this finding is that the larger the household size of the BEAs, the less they dealt frequently with job-related tension by being tight with money and thus, the less they coped effectively with job-related tension.

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Table 2: Relationship between selected personal characteristics and job-related tension coping behaviours of front-line extension workers

Personal characteristics	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12	Y13	Y14	Y15	Y16	Y17	Y18	Y19	Y20	Y21	Y22	Y23	Y24	Y25	Y26	Y27	Y28
Age (a)	.23	.02	.02	-07	-01a	-0.20	-08	-12	.03	.09	-10	.16	.15	-14	-20a	-04	-.16	.12	.11	.07	.07	-02	-05	-08	.11	-08	.10	.17
(b)	.67*	-18	-000	.07	.02	-o.9	.13	.24	-25	.66*	-23	-61*	36	-11	.13	-09	-14	.07	-15	.05	-27	.58	.19	.10	.04	-07	-20	-34	.14
(c)	.41	-02	-09	-15	.02	13	.11	.02	10	-00	-10	.12	-24	-37	.13	.54*	-14	-09	-35	.04	-11	.25	.18	.11	10	-20	46	-14	
Level of (a) Formal (b) Education (c)	.01 .45 21	-16 -43 -23	.09 .42 .05	.23 -57 -06	-01a .06	-42* -47 .25	-15 -09 .07	.16 .03 .46	-06 .24 .09	.01 -19 .09	-11 -18 .06	-07 -42 .45	-27* -44 .41	.24 -23 11	-24a .11	-01 -35 -07	-06 -43 -05	.11 -11 .16	-11 .00 -07	-04 .49 -34	-11 -17 .12	.17 .24 -24	-11 -24 -44	.09 .00 .13	.03 .07 -19	-03 .07 .07	-27 .11 -17	-11 .41 .24	
Household Size (a) (b) (c)	.25* .51 .10	-09 .22 .03	.14 .04 -12	-09 .03 -36	.17a -11	.21 -42 -31	.05 .21 -05	.12 .39 -13	.15 .22 -06	.13 .21 -03	.08 .27 -29	.18 .07 -o3	.13 -33 -30	.05 -23 .30	.06a -07	-06* .03 .29	-17 -64* .17	-02 .03 -20	.03 .33 -.09	.08 .35 .00	-04 -15 -06	.08 .35 .43	.02 -05 .23	.08 -18 -.06	.14 .07 .07	.15 .01 -.25	.12 -.35 .26	.10 .40 -.02	
Extension Experience (a) (b)	.13 .12 .10	-.09 .2	-.09 .3	-.05 -.04	-.01a	.01 -.52 -	.10 -.1	.13 .00	.00 .17	.11 .71* -	.05 .65 -.30	-.03 .06 .47	.12 -.26 -	-.03 -.33	.12a .32	.15 .05 .85*	.27* -63	-.05 -.31	.06 -.29 -	.09 -.19 -	-.10 .33 .04	.00 .00 .10	-.00 -.48	.14 -.20 -	.16 .67* -	-.00 .61*	.18 .15 .24	.20 .04 -.28	

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(c)		4.17	8-41	-.65*	.20	.42	3.28	.25	.18	.09			.49	.00			*-.15	.02	.12	.45			-.15	.13	.25	-.29		

*significant at p<0.05(a) EAs (b) BEAs (c) BESs

A cannot be computed because at least one of the variables is constant

Key

Y_1 = Talking with spouse
Y_2 = Talking with friends
Y_3 = Involvement in religious activities
Y_4 = Involvement in organized groups
Y_5 = Smoking
Y_6 = Going out to have a drink
Y_7 = Working around the house
Y_8 = Finding alternative things to think about
Y_9 = Going for shopping
Y_{10} = Seeking professional
Y_{11}= Going tranquilizers
Y_{12} = Changing of eating habits
Y_{13} = Going on others drugs e.g. anti-depressant, sleeping tablets, etc
Y_{14} = Just get away from everybody
Y_{15} = Gambling
Y_{16} = Talking with relative
Y_{16} = Tight with moneys
Y_{17} =Over-spending
Y_{18} = Visiting favorite places
Y_{19} = Savouring special relationship
Y_{20} = Leaving problems at work
Y_{21} = Searching out relevant information to deepen understanding
Y_{22} = Watching video/films
Y_{23} = Listening to music
Y_{24} = Setting realistic goals for my work
Y_{25} = Prioritizing work and sticking to priorities
Y_{27} = Engaging in other income generating activities e.g motor cycle taxing
Y_{28}= Refusing to become over-stretched by another person's unrealistic expectations

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Relationship between selected personal characteristics and job-related tension coping behaviours of block extension supervisors in Akwa Ibom State ADP.

The third hypothesis states that there is no significant relationship between selected personal characteristics and job-related tension coping behaviors of block extension supervisors in Akwa Ibom state ADP. Data in Table 2 show the relationship between selected personal characteristics and job-related tension coping behaviours of BEASs in Akwa Ibom state ADP.

Extension experience of BESs was positively and significantly correlated with talking with relatives (r=0.85), This finding implies that the more experience the BESs acquired in extension work, the more they dealt frequently with tension arising from their job by talking with their relatives and hence, the more they coped effectively with job-related tension. Age of BESs was positively and significantly correlated with talking with relatives (r=0.54), meaning that the older the BES, the more they dealt frequently with job-related tension by talking with their relatives and thus, the more they coped well with job-related tension.

III. CONCLUSION AND RECOMMENDATIONS

The paper concludes that the socio-economic characteristics of extension agents such as their level of formal education, household size, age and extension experience were strong predictors of coping behavior and should be enhanced. Necessary recommendations such as employing those with higher level of formal education; recruiting extension workers based on their practical experience in agriculture constant in-service trainings to enhance coping behavior and job related tension were made.

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Cocoa Pod Husk Biochar Reduce Watering Frequency and Increase Cocoa Seedlings Growth

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Abstract**—** *Biochar amount applied in the growing soil medium may decrease water use of cocoa seedling during dry season and hence may increase water use efficiency, thus a polybag experiment was carried out in the Glasshouse Agricultural Faculty, Halu Oleo University, Kendari, Southeast Sulawesi Indonesia in 2016to evaluate the effect of cocoa pod husk (CPH) biochar and watering frequencies on growth of cocoa seedlings.The experiment was arranged in a randomized block design with seven cacao pod husk (CPH) biochar levels (without CPH biochar, 3 g CPH biochar kg^{-1} soil, 6 g CPH biochar kg^{-1} soil, 9 g CPH biochar kg^{-1} soil, 12 g CPH biochar kg^{-1} soil, 15 g CPH biochar kg^{-1} soil dan 18 g CPH biochar kg^{-1} soil) and three watering frequencies (every two days, every four days and every six days) in three replications. Results showed that CPH biochar and watering frequency significantly influenced soil moisture. The rate of CPH biochar amendment determined watering frequency and cocoa seedling growth rate. CPH biochar improved cocoa seedling growth and reduced watering frequency. Cocoa seedlings treated with 9 g CPH biochar kg^{-1} soil and 60 g CPH biochar kg^{-1} soil with every six days of WF increased WUE by 208.8% and 262.22%, respectively, compared to no biochar application.*

Keywords**— biochar, cacao, growth, rate, soil, water.**

I. INTRODUCTION

Increasing cocoa production and improving quality of cocoa seedling is a major target to meet the domestic and export demand since Southeast Sulawesi as a national area center of cocoa development in Indonesia. Cocoa (*Theobroma cacao* L) seedlings require a significant amount of nutrient and water for their proper growth and development. It has been reported that water being essential in growth and development of plants, a specific amount of water is needed for optimum growth and very large amount of water required to sustain seedlings growth is obtained primarily from the soil. [1] regular watering for tree nurseries is necessary to produce good quality seedlings at economic rate.

The amount of water in the soil varies widely over time and almost never is ideal for maximum absorption by roots. Usually there is too much or too little water in the soil, and mostly the latter. The major sources of water for seedlings in the nursery phase are irrigation water. However, a portion of this water is lost by surface run off and some evaporates before it can percolate into the soil. The amount that is lost by evaporation varies with atmospheric conditions and with soil texture, color, and porosity [2]. The big challenges for obtaining high quality of seedling in the nursery growing period during dry season with the current trend of changed weather are limited water resources, therefore, seedling frequently exposed to drought conditions.

Drought is one of the main limiting factors affecting seedling growth in Indonesia. Drought decreases soil moisture content by evaporation and limits water availability to seedling root systems [3]. Seedlings close their stomata during drought and photosynthesis is stopped, resulting in seedling mortality due to“carbon starvation” [4]. This conditions has in a long way discouraged the farmer leading to fall in the level of production of high quality of seedling to be transplated in the field. The possible approach to addressing those constraints to obtain the high quality of seedlings is the application of biochar and regulating water applied. Biochar amendment may be a viable means of mitigating current water shortages on drought-prone soils and future water shortages accompanying climate change [5].

Biochar has been proposed as a beneficial amendment concerning various agricultural and environmental aspects such as increasing soil fertility, retaining water in the soil and enhancing plant growth [6]. Most biochars

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made from plant materials have a high porosity and surface area [7] and thus a large capacity to hold water at field capacity [8]. Biochar application improves nutrient availability and water holding capacity for supporting plant growth [9,10]. Biochar from cocoa pod husk influenced soil temperature, soil moisture and seedling growth [11]. Research reports have been shown that biochar addition significantly increased the available water contents of the soils by both increasing the amount of water held at field capacity, allowing plants to draw the soil to a lower water content before wilting and increase produktivity in drought-prone regions or reduce the frequency of irrigation [5]. This indicates that biochar applied into the growing soil medium may decrease water irrigation for cocoa seedlings during dry season and may, therefore, increase water use efficiency. Thus, CPH biochar has the potential to be used as a material for improving growth and water use efficiency of cocoa seedlings. It would therefore be important to also study growth of cocoa seedling under different rate of biochar and watering frequencies. The aim of the current study was to investigate the effects of CPH biochar and watering frequencies on growth and water use efficiency of cocoa seedlings.

II. MATERIAL AND METHODS

A polybag experiment was conducted in the Glasshouse of Agricultural Faculty, Halu Oleo University, Kendari, Southeast Sulawesi Indonesia, 2016. The experiment was arranged in a randomized block design with seven levels of cacao pod husk (CPH) biochar (i.e. without CPH biochar, 3 g CPH biochar kg^{-1} soil, 6 g CPH biochar kg^{-1} soil, 9 g CPH biochar kg^{-1} soil, 12 g CPH biochar kg^{-1} soil, 15 g CPH biochar kg^{-1} soil dan 18 g CPH biochar kg^{-1} soil) and three levels of watering frequencies (i.e. every two days (V0), every four days (V1) and every six days (V2)) in three replications. The mean daily temperatures in the glasshouse varied from 22°C to 30°C, and the relative humidity ranged from 68% to 88%.

Biochar was produced from cocoa pod husk (CPH) by using a drum kiln, in which carbonization was done within 4-6 h [12]. The hot biochar produced after pyrolysis was quenched with distilled water, collected, air-dried, crushed and sieved through a 2 mm sieve before being used. The soil for trial was collected from the sandy loam (76% sand, 21% silt and 11% clay) of the experimental farm of Agricultural Faculty, Halu Oleo University.

Cacao seedlings were raised on germination media for 14 days and each seedling was then transplanted into a polybag (25 cm X 30 cm size) seedling madia which have been filled with 5 kg dry soilmixed witha treatment-based rate of biochar from cacao podhusks (0.5 mm particle size) at a planting space 20 cmx 20 cm [11]. The amount of water applied was 200 ml per plant for the three months under glasshouse conditions with a frequency depending on the given treatment. Seedling growth and soil moisture were monitored for three consecutive months. The data collected include: seedling height, number of leaves, leaf area, root dry weight,shoot dry weight and water use efficiency. The soil moisture was monitored with asoil moisture meter (model: PMS-714), while soil temperature with soil thermometer at the depth of 12 cm below the surface every two days at 17.00 pm (before being irrigated). Seedling height, number of leaves and leaf area were measured 90 days after planting. Thereafter, seedlings were removed from the nursery and sent to the laboratory, in order to obtain their dry weight of root and shoot. Dry weight was obtained after drying the material at 85 °C for 48 hours. The WUEwas determined by using the formula: WUE= shoot dry weight (g)/total water use (L) [13]. Data were analyzed by using anova followed by Duncan's multiple test at an error rate of 5% ($P < 0.05$).

III. RESULTS AND DISCUSSIONS

As shown in Figure 1, biochar from cocoa pod husk (CPH) and watering frequencies (WF) significantly influenced soil moisture. Soil moisture increased with an increase in CPH biochar rate at different WF. The rate of CPH biochar application determined soil moisture at all WF treatments. Our results showed that CPH biochar maintained soil moisture even at every six days of WF. This indicates that CPH biochar could retain moisture and minimize WF, therefore, less water was required for cacao seedling growth. The application of biochar improves soil structure [8] and improves the soil's ability to retain moisture [14,15]. Further, CPH biochar increases pore aeration and water availability [10]. The significant increase in soil moisture under treatment of CPH biochar was in conformity with the findings that biochar amendment in soils increased the plant available water content [16,17].

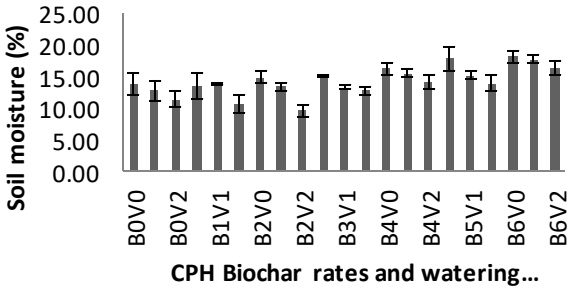

Fig.1: Effects of cocoa pod husk (CPH) biochar rates and watering frequencies on soil moisture. Error bars indicate standard deviations.

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Cocoa seedlings grown on soils treated with 6 g CPH biochar kg^{-1} soil with every four days of watering frequency were higher in dry root weight but insignificantly different from those treated with 3 g CPH biochar kg^{-1} soil with every four and six days of WF and other rates of CPH biochar at different WF (Figure 3a). This indicates that small amount of CPH biochar improved root growth due to the capacity of CPH biochar in increasing water holding capacity. Dose of CPH biochar exceeding 6 g CPH biochar kg^{-1} soil significantly decreased root dry weight due to high soil moisture (Figure 1). This means that CPH biochar greatly influences root growth and root growth may be determined by soil water conditions. Root development is largely influenced by the soil moisture. This strengthens the previous findings that soil water content was a crucial component that influenced root growth [18], with possible affects on leaf growth and cocoa seedling growth as a whole. A positive influence on plant growth and development due to the plant available water content is increased [19].

As shown in Figure 2 and 3, CPH biochar and WF significantly affected seedling height, number of leaves, leaf area, root dry weight and shoot dry weight of cocoa seedling. Cocoa seedlings grown on soils treated with 9 g CPH biochar kg^{-1} soil with every four days of WF were higher in plant height, number of leaves, leaf area and dry shoot weight of cocoa seedling, but insignificantly different from cocoa seedlings grown on soils without CPH biochar with every two days of WF and 9 g CPH biochar kg^{-1} soil with every six days of WF. This clearly indicates that adding CPH biochar can significantly affect cacao seedling growth and reduce WF. It means that CPH biochar increased both cocoa seedlings biomass and water use efficiency (Figure 3 and 4). The application of 9 g CPH biochar kg^{-1} soil increased seedling height, number of leaves, leaf area, and shoot dry weight of cocoa seedling. Similar results were reported by a number of researchers [20,21,22,23] that biochar significantly increased plant growth and biomass. Our results also followed similar trends established in different studies involving application of biochar in increasing WUE. The greatest WUE (1.63 g L^{-1}) was found in the soils treated with 12 g CPH biochar kg^{-1} soil with every six days of WF and the lowest was found in the soils without CPH biochar (0.45 L^{-1}) application. Such a greatest WUE found in the former treatment was, however, not significantly different from cocoa seedlings treated with 9 g CPH biochar kg^{-1} soil with every six days of WF. Cocoa seedlings treated with 9 g CPH biochar kg^{-1} soil and 12 g CPH biochar kg^{-1} soil with every six days of WF increased WUE by 208.8% and 262.22%, respectively, compared to no biochar application. On the other hand, as CPH biochar rates exceeded 12 g, the WUE tended to decrease at different WF. Our study showed that biochar was effective in increasing the WUE of cacao seedlings, however, the rates of biochar application should be considered.

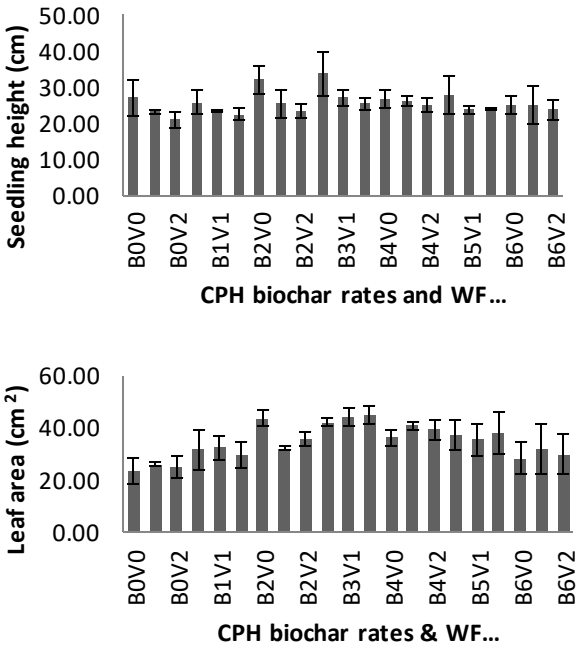

Fig.2: Effects of CPH biochar rates and watering frequencies on seedling height (a) and leaf area (b). Error bars indicate standard deviations.

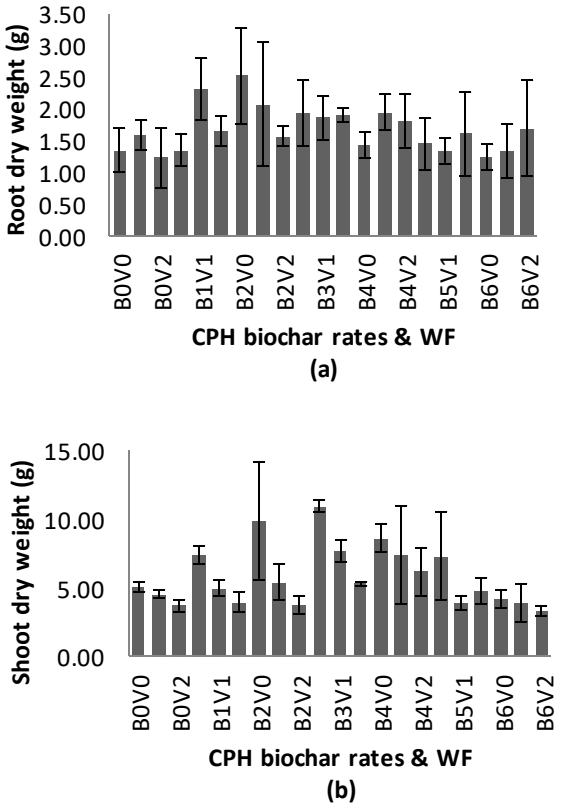

Fig.3: Effects of CPH biochar rates and watering frequencies on root dry weight (a) and shoot dry weight (b). Error bars indicate standard deviations.

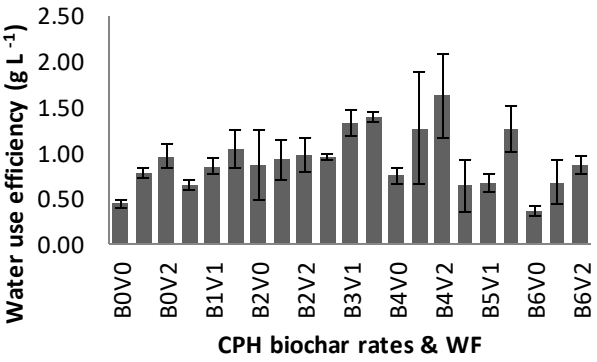

Fig.4: Effects of CPH biochar rates and watering frequencies on water use efficiency. Error bars indicate standard deviations.

In general, the results also showed that the biochar treatment exceeding 9 g CPH biochar kg^{-1} soil significantly decreased seedling growth. The above result has been reported by [11]. However, such a decrease in cocoa seedling growth rate also depended on WF. Interestingly, mean of cocoa seedling growth at a rate of 15 g CPH biochar kg^{-1} soil and 18 g CPH biochar kg^{-1} soil with every six days of WF was higher than those grown on soils treated with CPH biochar and every two days of WF. This is presumably a consequence of the big changes in soil moisture (Figure 1). The decrease in seedling growth may be due to great changes in soil bulk density, restricted aeration or higher soil moisture at a rate of 18 g CPH biochar kg^{-1} soil compared to without biochar and the other treatments (Figure 1). This indicates that roots were exposed to limited oxygen and high water content conditions [11]. Lack of oxygen content in the soils may be damaged of root development[24]. This is similar to findings by [25] who found reduced permeability to water due to poor aeration may cause decreased tree growth.

IV. CONCLUSION

Results showed that CPH biochar and watering frequency significantly influenced soil moisture. The rate of CPH biochar amendment determined watering frequency and cacao seedling growth rate. CPH biochar improved cacao seedling growth and reduced watering frequency. Cacao seedlings treated with 9 g CPH biochar kg^{-1} soil and 12 g CPH biochar kg^{-1} soil with every six days of WF increased WUE by 208.8% and 262.22%, respectively, compared to no biochar application. Application of 9 g CPH biochar kg^{-1} soil and 12 g CPH biochar kg^{-1} soil are recommended for increasing growth and water use efficiency of cocoa seedling.

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Crude glycerol in the diets of the juveniles ofAmazon catfish(female *Pseudoplatystoma punctifer x* male *Leiarius marmoratus*)

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Abstract*—This research aimed to determine the best inclusion level of crude glycerol in the diet of the Amazon catfish (Pintado), through zootechnical performance, body composition, metabolic profile and histopathology. The experiment was conducted at the Laboratory of Morphophysiology and Biochemistry of Neotropical Fishes of the Federal University of Tocantins. There was used 150 juvenilles of pintado, these with initial weight of 6,83 ± 1,11 (g) and 10,06 ± 0,57 (cm) lenght in a completely randomised design, with 3 replications (10 animals in each one). They were fed with five diets containing increasing levels of glycerol (0 g kg^{-1}, 50 g kg^{-1}, 75 g kg^{-1}, 100 g kg^{-1}, and 125 g kg^{-1}) during 90 days (30 days of adaption and 60 experimental days). The indexes were evaluated and they did not present statistical difference between each other, except for the specific growth rate, which showed a moderate linear behavior and muscular glycogen that at the level of 125 g kg^{-1} presented a lower concentration compared with the control diet (0 g kg^{-1}). Regarding histology, the crude glycerin did not cause significant hepatic and renal changes in the referred specie, since the alterations found in the two tissues were considered lesions that did not compromise the functioning of the organ or that are reversible. Finally, it was indicated that the juveniles of Amazon Pintado are able to metabolize the crude glycerin up to 100 g kg^{-1} level.*

***Keywords*—biodiesel, fishfarming, hematology, metabolism, performance.**

I. INTRODUCTION

The accentuatepopulation growthentailssome implications such as the increasingdemand for basic inputs inherent to survival. This is why the scientific community has focused its attention on issues that promote the perpetuation of humanity and the sustainability of the planet, such as the development of renewable energy sources and the increase of food production.

Thus, the production of biodiesel has been highlighted as an alternative to fossil fuels, which are considered the main responsible for the greenhouse gases emissions as carbon dioxide, for example. In a national scenario biodiesel started to stand out with greater intensity starting in 2010, after the ratification of the mandatory use of biodiesel together with diesel from fossil fuels, according to current national legislation.

However, biodiesel production generates a significant amount of byproduct known as crude glycerol and it has a high polluting potential when not disposed of correctly. A possible solution to this problem is the use of this glycerol as an alternative food for farm animals, because besides the advantages linked to its bioavailability, glycerol has a low cost.

The fish farming has been one of the research areas that have tested this byproduct as an alternative food. Even though it is a sector that contributes to the world food production, it faces some obstacles that make it difficult to expand, such as a the need for dietary ingredients, which are available in small quantities. As a result the production costs increases, since food represents between 70 and 90% of the production costs of captive fish.

Studies that have investigated the use of crude glycerin in fish diets are still in the beginning. Hence, in view of the current scenario of growth in aquaculture and biodiesel production, the search for information in association with animal experimentation on the use of this b-product as an energy ingredient in the fish diet is essential to evaluate the substitution of conventionally used dietary energy sources and for the foundation of later studies with glycerol in fish nutrition.

The present work was conducted with the aim of determining the best level of the inclusion of glycerol in the diet of the hybrid Amazon catfish "Pintado" by the evaluation of zootechnical performances, body composition, metabolic profile and histopathology.

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II. MATERIAL AND METHODS

2.1 Experimental Design

The experiment was conducted in the School of Veterinary Medicine and Animal Sciende of the Federal University of Tocantins - UFT, Araguaína Campus - TO, at the Laboratory of Morphophysiology and Biochemistry of Neotropical Fishes, from january to april 2017. Following the standards written in the Law of Procedures for Scientific use of Animals of the Federal University of Tocantins, the process number is 23101.005896/2016-56. There was used 150 juvenilles of pintado, these with initial weight of 6,44 ± 0,89 (g) and 10,06 ± 0,57 cm lenght, they were displaced in fiber boxes with 1000 liters capacity and constant water flow. Five treatments were tested with three repetitions (fiber boxes) and 10 animals per experimental unity. The experimental design used was completely randomised design (DIC). The diets were created to be isoproteic and isoenergetic and all the nutritional requirements were met, according to Almeida (2014). The treatments consisted of five experimentais diets and four of them with inclusion level (50 g kg^{-1}, 75 g kg^{-1}, 100 g kg^{-1}, and 125 g kg^{-1}) of crude glyrecol in partial substitution of maize and a control treatment as reference (no inclusion of glycerol) (Table1).

Table.1: Percentage and chemical composition of the experimentais diets with different glycerol levels for the pintado juveniles.

	Inclusion Level				
INGREDIENTS	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**
Viscera flour	55,00	55,00	55,00	55,00	55,00
Soy flour	21,43	22,45	22,96	23,47	23,98
Feather meal	4,00	4,00	4,00	4,00	4,00
Farelo de milho	18,56	12,54	9,53	6,52	3,51
Crude glycerol	0,00	5,00	7,50	10,00	12,50
Nucleus[1]	1,00	1,00	1,00	1,00	1,00
TOTAL	100,00	100,00	100,00	100,00	100,00
	Requirements				
Calcium (%)	2,69	2,69	2,69	2,69	2,69
Gross energy (Kcal/kg)	4630	4626	4623	4621	4619
Metabolizable energy (Kcal/kg)	1,36	1,38	1,39	1,40	1,41
Ethereal extract (%)	9,02	8,82	8,72	8,62	8,52
Crude protein (%)	42,00	42,00	42,00	42,00	42,00
Crude fiber (%)	2,02	1,96	1,93	1,90	1,87
Total phosphorus (%)	1,63	1,62	1,62	1,61	1,61
Total lysine (%)	2,59	2,61	2,62	2,62	2,63
Total methionine (%)	0,80	0,79	0,79	0,79	0,79

[1]Micronutrient levels per kilogram of product: folic acid 20,25mg; antioxidant 66,15mg; cobalt 33,75mg; copper 337,50mg; iron 337,50mg; iodine 50,62mg; manganese 1350,00mg; methionine 1,20mg; calcium pantothenate 315,56mg; selenium 10,12mg; sodium 55,58mg; tyrosine 810,00mg; vit. A 216. 000U.I/kg; vit. B1 45.56mg; vit. B2135,00mg; vit. B6 67,50 mg; vit. D3 50.625,00UI/kg; vit. E 506.25UI/kg; vit. H 2.70mg; vit. K3 50,62mg; vit. B12 675.00mcg; zinc 3375,00mg.

The ingredients were pelleted in a meat grinder and dried in a greenhouse with circulation and air renewal at 55ºC. The fishes were fed twice a day (around 8h and 17h) to apparent satiety for a period of 90 days of experiment, being 30 days of adaptation. The siphoning of the boxes was performed every day (15h) and the water quality parameters such as pH, oxygen, temperature and ammonia were measured weekly.After the experimental period, fish were fasted for 24 hours to empty the gastrointestinal tract. Five animals from each experimental unit were selected in order to execute biometrics, length (through pachymeter) and weight (high precision scale), for zootechnical performance data as proposed by Fracalossi and Cyrino (2013). After this, the animals were desensitized on ice and then the body composition analysis was performed. The indices were used to verify if the tested food interferes negatively or positively in the performance and health conditions of the animals.

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2.2 Zootechnical performance

The survival (SOB%) - was calculated through the equation:

$$SOB\ (\%) = nf / ni \times 100 \quad (1)$$

In which: nf = Final number of animals
ni = Initial number of animals

Specific Growth Rate (SGR) - it shows the daily growth of the animals, obtained in percentage. For this calculation, the following expression was used:

$$SGR\ (\%\ days) = (lnpf - lnpi) / t \times 100 \quad (2)$$

ln which: ln pf = Logarithm of final weight
ln pi = Logarithm of initial weigh
t = time

Hepatosomatic (HI) Index - it is the ratio between the total of the liver weight and the total of the fish weight. This index is obtained according to the following formula:

$$HI = \text{liver weight (g)} / \text{fish weight (g)} \times 100 \quad (3)$$

Weight gain (WG) - it is the final weight of the animal subtracted from the initial weight. This calculation is obtained by the following formula:

$$WG = \text{final weight (g)} - \text{initial weight (g)} \quad (4)$$

Condition factor (CF) - The condition factor is the parameter that indirectly measures the physiological state of the animal, in relation to stored energy, such as hepatic glycogen and body fat. For its determination, there was used the following formula:

$$CF = \text{weight (g)} / \text{total length (cm)} \quad (5)$$

Apparent feed conversion (AFD) - it is equal to the amount of food needed for the animal to gain 1 kg of live weight:

$$AFD = \text{consumed diet (g)} / (\text{final weight} - \text{initial weight}) \quad (6)$$

Food Efficiency (FE) - this is the average weight gain per fish in the group, divided by the average fish feed intake. Thus, this measures the efficiency that the animal had to convert the consumed diet in live weight:

$$FE = \text{mass gain (g)} / \text{amount of the diet that were ingested(g)} \times 100 \quad (7)$$

2.3 Body composition

Analysis of fish body composition was performed according to the standard methodology described by INCT/ Detmann et al. (2012). They were made with the five treatments tested (0g $kg^{-1,}$ 50 g kg^{-1}, 75 g kg^{-1}, 100 g kg^{-1}, and 125 g kg^{-1}) of crude glycerin with 5 replicates (for each fish used).

2.4 Hematologic parameters

For the hematological analyzes, fifteen individuals from each treatment were randomly selected. The blood collection was performed by caudal puncture using syringes and needles bathed in Ethylenediamine Tetra-Acetic Acid - EDTA and then they were desensitized on ice.

Subsidiary blood samples were used immediately for the determination of hematocrit - Htc (microhematocrit technique, according to Wintrobe (1929), hemoglobin-Hb (cyanometahemoglobin method, according to Drabkin (1948) and red cell count - RBC in Neubauer's chamber using as the citrate formaldehyde as diluent.

The hematimetric indexes MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) were also evaluated.

For the hematimetric indexes the following formulas were used:

$$MCV\ (\mu m^3) = (Hct \times 10) / RBC$$

$$MCH\ (pg) = (Hb \times 10) / RBC$$

$$MCHC\ (d/L^{-1}) = (HCM \times 100 / VCM)$$

Blood distension slides were made for total and differential leukocyte (Lc) counts and total thrombocyte counts (Tr) and stained with PANOTE kit, according to the methodology recommended by Ranzani-Paiva et al. (2013).

2.5 Analyzes of biochemical parameters

The blood was centrifuged at 3000 rpm for 5 minutes to obtain blood plasma. The total of proteins, cholesterol, triacylglycerol, Aspartate Amino Transferase enzyme (AST) and Alanine Amino Transferase enzyme (ALT) were analyzed through the Labtest Kit and the readings in the spectrophotometry apparatus.

For the quantification of the blood glucose concentration, the One Touch Ultra2 portable reading device (reading between 20 and 600 mg/dL) was used with disposable tapes that were suitable for the apparatus. About 1 to 5 µl of blood was placed on the tape, after the monitor switched on automatically and then it was waited 5 seconds for quantification of the blood glucose concentration in mg/dL.

For the determination of hepatic and muscular glycogen this researche used the technique described by Bidinotto et al. (1997). In addition to the the referred methodology, hepatic glycogen was also analyzed using Image J software to quantify the total area occupied by it.

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2.6 Histopathology of the liver and the kidney

There were selected 9 animals (randomly) from each treatment and they were desensitized on ice to remove hepatic and renal tissue samples

The samples were washed with 0.9% saline solution and fixed for 24 hours in Bouin solution. Subsequently, the material was washed for 24 hours in running water and stored in containers containing 70% alcohol. The samples were then dehydrated in successive alcohol baths (70%, 80%, 90%, 95%, and 100%) and clarified in xylol FERNANDEZ et.al., 2011).

After the dehydration and clarification processes were completed, the samples were embedded in paraffin for histological sections of 3 µm thick using a manual microtome, which were stained with Hematoxylin and Eosin (HE) for later analysis under the light microscopy. All sections were analyzed using images obtained on a LEICA DM500 microscope connected to a computer by the LAZ 2.0 program.

To identify alteration in the liver or the kidney alterations it was observed 5 fields of each slide in 100x increase. The histopathological analyzes of both tissues were evaluated by two semi-quantitative methods: Mean Value of Changes (MVC) and Histological Alterations Index (HAI).

The calculation of (MVC) results in the incidence of lesions, according to Schwaiger et al. (1997). Thus, a numerical value was assigned for each animal according to the scale: grade 1 (absence of histopathological alteration), grade 2 (occurrence of localized lesions) and grade 3 (lesions widely distributed by the organ).

To evaluate the degree of liver and kidney alterations, the Histological Alteration Index (HAI) was used according to Poleksic and Mitrovic-Tutundžic (1994), which each alteration was classified in progressive degrees related to tissue function impairment: stage I, for changes that do not compromise the functioning of the body; stage II for more severe alterations that compromise organ functioning, but are reversible; and stage III, for the most serious alterations that irreversibly compromise the functioning of the organ.

HAI value was calculated for each animal according to the formula: HAI = (1x ΣI) + (10 x ΣII) + (100 x ΣIII), in which ΣI, II and III correspond to the diferent stages numbers I, II and III respectively.

The HAI values between 0 and 10 indicate normal tissue functioning; between 11 and 20 indicate mild damage to the organ; between 21 and 50 indicate moderate damage; from 51 to 99, severe damage and greater than 100 indicates irreversible tissue damage.

2.7 Statistical analyzes

The data were submitted to analysis of variance (ANOVA) and the averages were compared by Tukey's test ($p > 0.05$) and those without normal distribution were submitted to non-parametric analysis (Kruskall Wallis) using the Instat Program v 3.0 for Windows, also, the results were expressed as average ± standard deviation. The parameters of performance and body composition were also submitted to linear regression analysis.

III. RESULTS

3.1 Water analysis

Regarding the environmental variables quantified during the experiment, differences between treatments were not identified ($p > 0.05$). The average values for water quality parameters that were recorded during the experimental period are displayed in (Table 2).

Table.2: Water quality parameters during the experimental period.

	CRUDE GLYCEROL (%)					
WATER QUALITY PARAMETERS	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**	**CV%**
pH	6,2 ± 0,08	6,2 ± 0,09	6,2 ± 0,09	6,2 ± 0,11	6,2 ± 0,01	0,25
O_2 dissolved (mg/L)	8,0 ± 1,03	8,0 ± 0,99	8,0 ± 0,78	8,0 ± 0,69	8,0 ± 0,69	1,96
Temperature (°C)	27,1 ± 0,74	27,0 ± 0,63	27,1 ± 0,67	27,2 ± 0,61	26,8 ± 0,59	0,22
Ammonia (mg/L)	0	0	0	0	0	-

The values are followed by the calcultation of the averages and standard deviation

3.2 Zootechnical performance

In the comparison of the zootechnical performance indexes, it was observed that there was no statistical difference between the treatments for the analyzes of the final length, final weight, survival, hepatosomatic index, condition factor, weight gain, conversion or diet efficiency.However, the animals specific growth rate was influencedby the levels of crude glycerin presenting a moderate and an increasing linear behavior compared to the control diet (Fig. 1). The zootechnical parameters of juveniles "Pintado" were analyzed in the present study and are demonstrated in (Table3).

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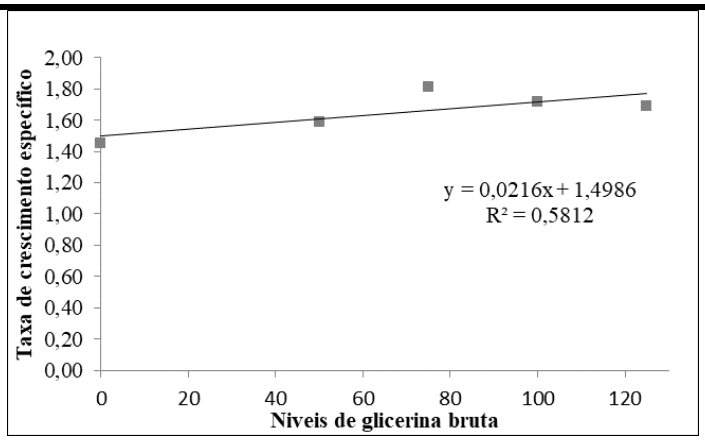

Figure. 1: linear regression of specific growth of juveniles of pintado

Table.3: Zootechnical parameters of the hybrid Amazon catfish "Pintado", that were feed with different levels of crude glycerin in the diet.

	CRUDE GLYCEROL (%)					
ZOOTECHNICAL PARAMETERS	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**	**CV%**
FL (cm)	16,66 ± 1,35	17,36 ± 1,59	18,23 ± 0,51	15,86 ± 1,41	16,83 ± 1,25	2,46
FW (g)	27,66 ± 7,00	26,66 ± 5,10	35,62 ± 3,26	28,06 ±7,06	28,73 ± 5,34	4,21
SOB (%)	96,66 ± 5,77	93,33 ± 11,54	83,33 ± 28,86	96,66 ± 5,77	96,66 ± 5,77	10,71
SGR (%) [1]	1,45 ± 0,39	1,59 ± 0,16	1,81 ± 0,39	1,71 ± 0,40	1,68 ± 0,37	6,31
HI (%)	0,01± 0,00	0,01± 0,01	0,01± 0,02	0,01± 0,03	0,01± 0,04	13,06
CF (g/cm)	1,63 ± 0,32	1,62 ± 0,13	2,22 ± 0,08	1,75 ± 0,28	1,69 ± 0,21	5,64
WG (%)	20,20 ± 7,44	21,46 ± 2,21	33,86 ± 2,38	22,06 ± 7,70	22,33 ± 6,42	11,36
AFD	2,58 ± 0,63	3,01 ± 1,26	1,72 ± 0,19	3,50 ± 1,23	2,68 ± 0,53	16,54
EA (%)	4,06 ± 1,13	3,90 ± 2,13	5,84 ± 0,72	3,13 ± 1,24	4,00 ± 1,25	12,29

The values are followed by the calcultation of the averages and standard deviations. Fl = final length; FW = final weight; SOB = survival; SGR = specific growth rate; HI = hepatosomatic index; ICF = initial condition factor; FCF = final condition factor; GP = weight gain; AFD = apparent feed conversion; FE = food efficiency. [1] Linear effect y = 0.0216x + 1.4986 R^2 = 0.5812.

3.3 Body composition

The body composition of the pintado juveniles was not influenced by the partial replacement of maize by crude glycerin. Since no significant differences ($p > 0.05$) were found between the treatments in any of the indices analyzed.

The results of the analysis of body composition of juveniles of pintado (moisture content, ashes and crude protein) are presented in (Table 4).

Table .4: Body composition of the Amazon catfish "Pintado", that were feed with different levels of crude glycerin in the diet

	CRUDE GLYCEROL (%)					
COMPOSITION	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**	**CV%**
Moisture (%)	77,39 ± 3, 02	73,53 ± 6,5	73,91 ± 4,79	80,68 ± 4,27	73,71 ± 4,10	1,68
Ashes (% MN)	2,88 ± 0,51	3,85 ± 0,96	3,17 ± 0,78	2,72 ± 0,84	3,48 ± 0,50	6,28
Crude Protein (% MN)	14,29 ± 2,33	15,30 ± 4,47	14,74 ± 3,10	12,46 ± 1,68	16,06 ± 2,20	7,44

The values are followed by the calcultation of the averages and standard deviations .

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3.4 Evaluation of the red trial

The increasing levels of crude glycerin in the diet of pintados did not result in changes in the erythrocyte variables in relation to the control (P> 0.05): hematocrit, hemoglobin, erythrocyte cell count and hematimetric indexes (mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) as presented in (Table 5).

Table.5: hematimetric parameters of juveniles of pintado, that were fed with different levels of crude glycerin.

	CRUDE GLYCEROL (%)						
PARAMETERS	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**	**EFFECT**	**CV%**
RBC (milions/mm3)	0.67 ± 0.25	0.54 ± 0.21	0.48 ± 0.15	0.69 ± 0.28	0.67 ± 0.16	NS	8,96
Hb(g/dL)	7.23 ± 1.19	7.30± 1.84	7.32 ± 1.35	7.32 ± 1.48	7.51 ± 1.14	NS	3,8
Hct (%)	23.66± 2.82	25.26 ± 3.26	24.93 ±3.12	24.60 ± 3.52	25.86 ± 3.44	NS	1,12
MCV (fL)	333.58 ±142.65	389.54 ± 178.59	458.16 ± 101.74	335.73 ± 113.62	355.74 ± 121.80	NS	8,05
MCH (pg)	130.07. ± 57.50	138.59 ± 57.72	140.56 ± 36.67	118.96 ± 57.62	136.09 ± 46.35	NS	7,12
MCHC (%)	28.35 ± 5.03	26.31 ±4.85	28.35 ± 6.26	25.13 ± 3.75	28.50 ± 4.16	NS	3,51

Values expressed as mean ± standard deviation. RBC = number of erythrocytes; Hb = hemoglobin; Hct = hematocrit; MCV = Mean corpuscular volume; MCH = mean corpuscular hemoglobin; Mchc = Mean corpuscular hemoglobin concentration.

3.5 Evaluation of the white trial

The total and differential counting of leukocyte were not altered by diet, (P> 0.05). The averages followed by the standard deviations of this count are demonstrated in (Table 6).

Table.6: Total and differential leukocyte counts of the juveniles of pintado that were fed with different levels of crude glycerin.

	CRUDE GLYCEROL (%)						
PARAMETERS	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**	**EFEITO**	**CV%**
Thrombocytes (uL)	22.38 ± 29.98	25.19 ± 33.32	24.96 ± 32.57	23.78 ± 28.92	23.04 ± 27.50	NS	10,25
Leukocytes (uL)	122.06 ± 35.94	139.03 ± 73.49	117.78 ± 73.25	120.92 ± 51.05	126.76 ± 54.33	NS	12,75
Lymphocytes (%)	97.30 ± 3.02	97.40 ± 2.83	96.60 ± 2.41	97.90 ± 1.66	98.30 ± 1.41	NS	0,72
Neutrophils (%)	2.20 ± 2.61	2.10 ± 2.68	3.00 ± 2.16	1.50 ± 1.71	1.30 ± 1.25	NS	11,03
Monocytes (%)	0.10 ± 0.31	0.20 ± 0.42	0.10 ± 0.31	0.20 ± 0.42	0.20 ± 0.42	NS	36,08
*LG-PAS (%)	0.20 ± 0.42	0.10 ± 0.31	0.20 ± 0.42	0.20 ± 0.42	0.10 ± 0.31	NS	36,08
Eosinophils (%)	0,20 ± 0,42	0,20 ± 0,42	0,10 ± 0,31	0,20 ± 0,63	0,10 ± 0,31	NS	80,69

The values were expressed as average ± standard deviation. NS = not significant for the Turkey Test (P> 0.05). * LG-PAS-granular leucocyte-PAS positive.

3.6 Biochemical analysis

The analysis of the hepatic glycogen did not present any statistical difference in comparison with the treatments with inclusion of glycerol in relation to the control as shown in (Table 7 and 8). The muscle glycogen analyzed in this study had a higher concentration in the control diet (0% glycerol) when compared to the treatment with 12.5% glycerol as presented in (Table 7).

Table.7: Hepatic and muscle glycogen of the juveniles of pintado, according to the methodology o Bidinotto et al. (1997).

	CRUDE GLYCEROL (%)						
PARAMETERS	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**	**EFFECT**	**CV%**
HEPATIC GLYCOGEN (umoles)	33,76 ± 10,32a	33,29 ± 11,00a	32,44 ± 3,28a	41,42 ± 5,44a	35, 66 ± 5,82a	NS	9,44

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MUSCLE GLYCOGEN (umoles)	31.88 ± 4.86a	35.45 ±7.24a	24.71 ± 7.00a	25. 09 ± 6.78a	18.03 ± 2.73b	S	7,11

Averages on the same line, followed by distinct letters, differ (P> 0.05) by the Turkey Test. The values are expressed as average ± standard deviation. NS = not significant; S = significant.

Table.8: Hepatic glycogen of juveniles of pintado, analyzed with the Image J program.

	CRUDE GLYCEROL (%)						
PARAMETERS	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**	**EFFECT**	**CV%**
HEPATIC GLYCOGEN (%)	33,76 ± 10,32	33,29 ± 11,00	32,44 ± 3,28	41,42 ± 5,44	35, 66 ± 5, 82	NS	9,44

The values are expressed as average ± standard deviation. NS = not significant (P> 0.05) by the Turkey Test.

3.7 Histopathological analysis

3.7.1 Liver

The alterations observed in the liver of the pintado that were feed with the control diet and in those fed with increasing levels of crude glycerin were mostly stage I lesions, considered to be alterations that did not compromise the functioning of the organ and a stage II alteration considered more severe, but also reversible. The alterations found in hepatocytes were: nucleus at the periphery of the cell, nuclear hypertrophy, cytoplasmic vacuolization, sinusoidal dilatation and biliary stagnation. The frequency of the hepatic changes and the classification of the severity and the impairment of hepatic function found in the treatments are presented in (Table 9). The most frequent alterations found in the liver of the pintados are presented in the (Fig. 2).

Table.9: *Frequency of histopathological changes in the liver of pintado juveniles that were feed with different levels of crude glycerin in the diet.*

		DIETS				
ALTERATIONS	**STAGES**	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**
nucleus at the periphery of the cell	I	+	+	0+	0+	0+
nuclear hypertrophy	I	0	0	0+	0+	0+
cytoplasmic vacuolization	I	0	0	0+	0+	0+
sinusoidal dilatation	I	0	0+	0+	0	0
biliary stagnation	II	0+	0+	0+	0+	0+

0 = nonexistent; 0+ = alterations of rare occurrence; + = alterations occurring; ++ = fairly frequent alterations; +++ = alterations of intense occurrence

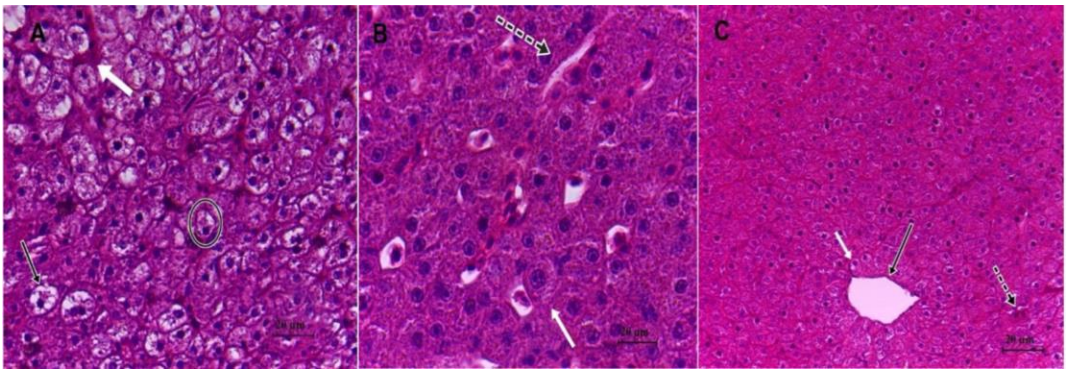

Fig.2: histopathological alterations in the liver of pintado. (A) biliary stagnation (white arrow), cytoplasmic vacuolization in hepatocytes (black arrow) and nucleus in the periphery of the cell (circular area). (B) sinusoidal dilatation (dashed black arrow) and nuclear hypertrophy (white arrow). (C) normal hepatic tissue - hepatocytes (white arrow), central vein (thin black arrow) and sinusoids (dashed black arrow).

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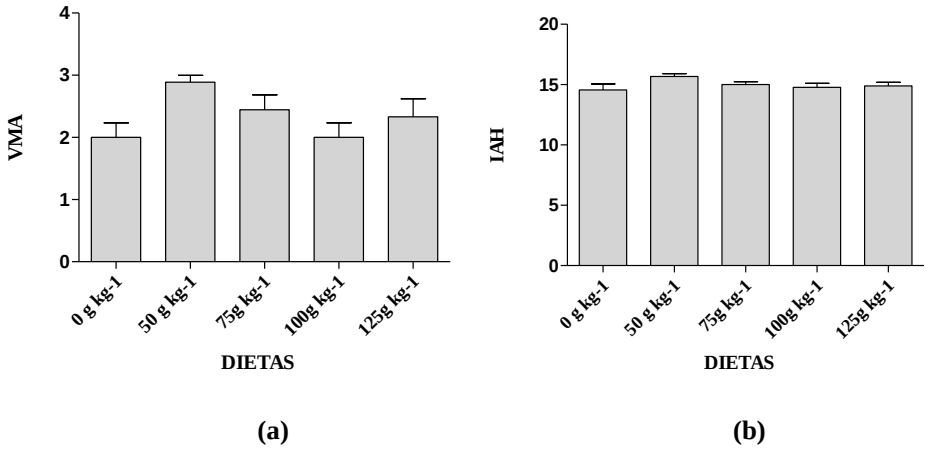
The results of the average value of alterations and the indexes of histopathologival changes are displayed in (Fig. 3).The Mean Value of Histological Alteration (VMA), that is obtained considering the hepatic alterations did not present significant differences ($p > 0.05$) between the experimental diets (0 g kg^{-1}, 50 g kg^{-1}, 75 g kg^{-1}, 100 g kg^{1}, and 125 g kg^{-1}) and the control (fig.3a) group. The alteration that were observed in the liver, were punctually distributed in the organ and they did not exceed grade 2. The Histological Alteration Index (HAI) also showed no significant difference (fig.3b), which demonstrates that changes in the hepatic tissue did not compromise liver functioning.



(a) **(b)**

Fig.3: Average values of VMA and HAI in the liver of pintado. Data are the averages ± standard deviation ($p > 0,05$).

3.7.2 Kidney

The renal histopathological analysis that was performed in this study showed that the alterations observed in the kidney of the juvenilles of pintado were limited to the renal tubules and were mostly stage I lesions (alterations that do not compromise the functioning of the organ) and stage II (considered more severe, but still possible to reverte).

The alteration were: nuclear hypertrophy, tubular light dilation and tubular light occlusion and are displayed in (Fig.4).

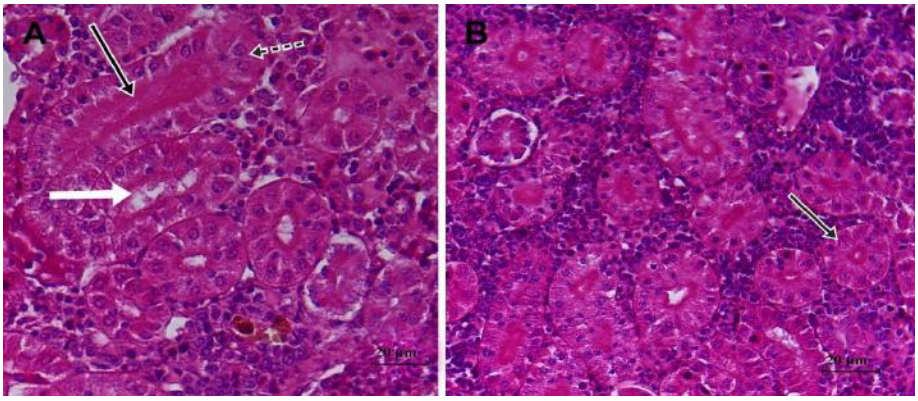


Fig.4: Histopathological changes in the pintado kidney. (A) tubular light occlusion (black arrow) , tubular light dilatation (white arrow) and nuclear hypertrophy (dashed black arrow). (B) Renal tubule with normal morphology (black arrow).

The frequency of renal changes and the degree of severity and impairment of renal function found in the treatments are showed in (Table 10).

The results of the the average value of alterations and the index of histopathological changes are presented in (Fig.5).

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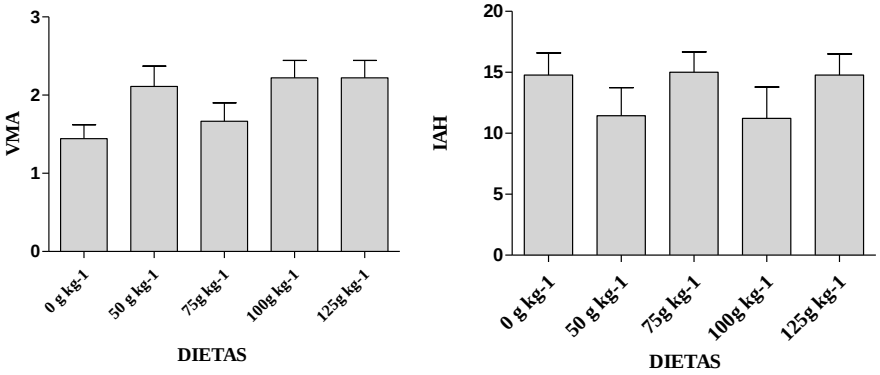
Table10: Occurrence of alterations in the renal tissue of pintado juveniles that were feed with different levels of crude glycerin in the diet.

		DIETS				
ALTERATIONS	**STAGE**	**0 g kg^{-1}**	**50 g kg^{-1}**	**75 g kg^{-1}**	**100 g kg^{-1}**	**125 g kg^{-1}**
nuclear hypertrophy	I	0+	0+	0+	+	+
tubular light dilatation	I	0	0	0	0+	0
tubular light occlusion	II	+	+	0+	0+	0+

0 = nonexistent; 0+ = alterations of rare occurrence; + = alterations occurring; ++ = fairly frequent alterations; +++ = alterations of intense occurrence.



(a) **(b)**

Fig.5: Average values of VMA and HAI in the pintado kidney. Data are the average ± standard deviation (p >0,05).

Hence, as observed in the liver analysis, the Mean Value of Histological Change (VMA) obtained for renal alterations did not present significant differences (p> 0.05) between the experimentais diets (50 g kg^{-1}, 75 g kg^{-1}, 100 g kg^{1}, and 125 g kg^{-1} of crude glycerin) and the control (Figure 5a) one. The alterations were distributed punctually in the renal area, because they did not exceed stage 2.

The Histological Alteration Index (HAI) also showed no significant difference (figure 5b), wich demonstrates that the renal alterations were not serious in order to compromise the functioning of the organ.

IV. DISCUSSION

The water parameters analyzed during the experiment were not influenced by the diets tested. All the analyzed parameters were kept within the standards that are recommended for the cultivation of fish of tropical climate (CAMPOS, 2010; RODRIGUES, 2013), this result is probably linked to the constant renewal of the water.

In the comparison of the zootechnical indexes only the specific rate of growth of the animals was influenced by the levels of crude glycerin, it presented a moderate increasing of linear behavior in comparison to the control diet.

Balen et al. (2017) also observed that the SGR of Curimbatá juveniles (*Prochilodus lineatus*) was influeced by the crude glycerin (0, 40, 80,120, 160 e 200 g kg^{-1}). In this referred study, the 40 g kg^{-1} diet promoted the highest values whilist in the diet of 200 g kg^{-1} there was the lowest values of inclusion.

Neu et al. (2013) reported that there was no difference in the growth performance of Nile tilapia juveniles that were feed different levels (0, 25, 50, 75 and 100g kg^{-1}) of dietary glycerol was observed. Li et al. (2010) showed that the use of up to 100 g kg $^{-1}$dietary glycerol for channel catfish (Ictalurus punctatus) did not cause changes in weight gain, feed efficiency, hepatosomatic index and survival. However, the inclusion of 150 and 200 g kg^{-1} of glycerol in the diet adversely affected its growth performance.

The length of the animals was relatively close, ranging from 15.86 cm to 18.23 cm in treatments with 100g kg^{-1} inclusion of glycerol and with 75g kg^{-1} addition of the product, respectively. One factor that may have influenced the non- statistical differentiation of the values of length and final weight is that the fish of the current

study received, even with the increasing levels of crude glycerin in the diets, isoproteic and isoenergetic diets.

In the present study the survival rate in all diets tested was greater than 80%. These results were close to the values described by Gonçalves et al. (2015) and Moesch et al. (2016) for juveniles of Nile tilapia fed with increasing levels of glycerol.

However, in contrast to this result Neu et al. (2012) found that the survival of Nile tilapia (*Oreochromis niloticus*) fingerlings was higher in treatment that did not contain glycerol. The highest mortality rates according to the author were at the two lowest levels tested, the 75 g kg^{1}followed by the 25 g kg^{-1}. Therefore, differences species and developmental phases may influence the response of the animal metabolism to adapt to glycerol in the diet.

According to the referred author, the development phase of the animals used in the research may have been preponderant for a high mortality rate, however, other factors may have contributed to this occurrence, among them, the temperature and the tested food source itself.

Hepatosomatic index can be altered given the great importance of energetic metabolism in fish liver, since hepatic deposition of lipids as well as glycogen is common. However, in the present study, this index did not present any alterations in the metabolic functions of the liver, even in comparison to the differents levels of crude glycerin. Moesch et al. (2016) when testing the influence and the best level of replacement of maize by crude glycerol (0, 200, 400, 600, 800 and 1000 g kg^{-1}) in diets for Nile tilapia fingerlings, observed a significant difference for the hepatosomatic index.

The condition factor consists in a important indicator of the degree of hygiene of an individual, where its value reflects the recent nutritional conditions and/or the expenses of the reserves in cyclical activities, making it possible to relate it to the environmental conditions and to the behavioral aspects of the species (GOMIERO et al., 2010).

This parameter was not influenced by the diet tested in this study, considering that there was no significant difference between the treatments. Neu et al. (2013) also did not identify a significant difference in the condition factor of juveniles of Nile tilapia (*Oreochromis niloticus*) fed with different levels of glycerol.

In relation to the weight gain, it did not show any variation between the treatments ($p > 0.05$). This result corroborates with what was described by Matos et al. (2016) and Neu et al. (2012) who also did not verify change in this variable for Tambaqui and Nile tilapia, respectively. However, Balen et al. (2017) observed an influence of dietary glycerin levels on the weight gain of juveniles of *P. lineatus*, these authors reported that as the inclusion levels of crude glycerin increased, weight gain decreased for the referred species.

Thus, as the results found by Matos (2016), the conversion and feed efficiency analyzed in this work were not influenced by the diets analyzed. Neu et al. (2012), although they did not verified statistical differences for AFE, they had observed that these values were considerably higher for Tilapia in the larval phase, which means that depending on the environmental conditions or the physiological phase in which the species is, this parameter can be influenced.

Crude glycerin has also been studied in other monogastric species and the results found in these studies are in agreement with those found in the present research. Berenchtein et al. (2010) concluded that glycerol can be used as an energy ingredient in the diet for growing and finishing pigs at levels of up to 90 g kg^{-1}, as it did not influence performance, carcass characteristics and meat quality.

Freitas et al. (2017), when analyzing the glycerin in the broiler diet, observed that the glycerin can be added up to the 50 g kg^{-1} level without causing effects on the performance, litter humidity and carcass yield.

The body composition of the juveniles of pintado was not influenced by the partial replacement of maize by crude glycerin. Thus, as in the study of Neu et al. (2013), the study in the body evaluation of juveniles of Nile tilapia fed with crude glycerol did not verify statistical differences ($p > 0.05$) for moisture, ashes and crude protein. However, lipid deposition was influenced by the diet containing glycerol, where they presented the highest values in fish fed with 50 g kg^{-1} of glycerol and the lowests in fishes that were fed with 100 g kg^{-1}.

Moesch et al. (2016) observed in Nile tilapia fingerlings fed with crude glycerol an increase in protein deposition and moisture of the whole body and the decrease of mineral matter and ethereal extract, which presented a minimum value in 720 g kg^{-1} substitution.

The mentioned authors point out that the higher deposition of protein and the lower deposition of lipids confirms the effects of glycerol on energy metabolism. Because the use of carbohydrates and lipids is intensified as a source of energy resulting in the saving of dietary protein, which can be deposited in the body tissues of the fish. However, the decrease in the mineral matter content would be due to the fact that the carcass analyzes were carried out using the whole fish, influencing the mineral matter levels of the body composition. The humidity in turn, even though it presented an increase in its content, remained within the range observed by other authors.

In other monogastrics animals such as finishing pigs, Melo et al. (2014) found that crude glycerin (50, 100, 150 and 200g/kg^{-1}) did not influence moisture, ash, protein. In

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ruminants, Lage et al. (2009) observed that despite the different levels of crude glycerin (0, 30, 60, 90 and 120 g kg $^{-1}$) in the finishing lamb diet it did not influence moisture and ash contents and crude protein levels were reduced.

Analysis of the hematimetric indexes is usually used in the control of pathologies and stress, in addition to being able to demonstrate the physiological state of the animal (SILVA et al., 2012).

The concentration of erythrocytes (red blood cells) found in the present study ranged from 0.48 to 0.69 (million/mm^3) between treatments. These values were higher than those found by Lundstedt (2003) when testing different levels of protein and energy in the diets of juveniles of pintado (*Pseudoplatystoma corruscans*) measuring 21.8 ± 0.7 cm, where erythrocytes ranged from 0.27-0, 30 (million/mm^3). However, it must be considered that individuals with different sizes release energy in different amounts according to their length, and this may interfere in their hematological characteristics (RANZANI-PAIVA; TAVARES-DIAS, 2002).

The average values of hemoglobin-Hb that were found (7.23 to 7.51 g/dL) were within the range established by Weiss et al. (2010) which is 5 to 10 g/dL, they are lower when compared to the concentration of Hb in mammals. Tavares-Dias et al. when studying hybrid fishes of the genus Pseudoplatystoma (*P. fasciatum x P. coruscans*) that were from 568.0 to 1350 g of weight, found a range between 5.2 and 6.1 g/dL of hemoglobin. While, Fagundes and Urbinati et al. (2008), when evaluating the hemoglobin concentration of *P. coruscans* with average weight of 14.04, 24.94 and 44.78 g, had observed average values of 10.50; 8.01 and 10.10 g/dL respectively.

Hematocrit (globular volume) corresponds to the volume occupied by erythrocytes contained in a certain amount of the whole blood, and low values may indicate anemia. The percentage of hematocrit analyzed in this study ranged from 23.66 to 25.86% and remained within the range predicted by Weiss et al. (2010) for fish, which is 20 to 45%. The values referenced by Razani-Paiva et al. (2000a, 2000b) for captive *Pseudoplatystoma fasciatum* (23.0-32.5%) and for wild-type *Pseudoplatystoma corruscans* (26.0%) remained close to the results described in this study.

The mean corpuscular volume (MCV) is related to cardiac dynamics and blood flow (FRIES et al., 2013). The range of MCV for fish predicted by Weiss et al. (2010), ranges from 150 to 350 fL. Although this parameter in the present study distinguished from the range of the mentioned author, it did not present a significant difference between the treatments. Tavares-Dias et al. (2009), cites a range of (159.2-180.3fL) for hybrids of the genus Pseudoplatystoma (*P. fasciatum x P. coruscans*). While Razani-Paiva et al. (2000a) and (2000c) cite the values of (129.1-189.0 fL) and (165.2 fL); respectively

MCH varies considerably between species, ranging from 30 to 100 pg (picograms) due to differences in the size of circulating globules (WEISS et al., 2010). In this study the identified MCH (mean corpuscular hemoglobin) was higher than those referenced by Weiss et al. (2010), however there was no statistical difference between treatments.

The distinction between the values found for VCM and MCH results is due to the peculiarities of fish erythrograms, since hematological parameters can be influenced by numerous factors, such as age, species, stress, temperature, photoperiod, nutritional status and the methodology used for (PEDRO et al., 2004), and they may present different values even for animals belonging to the same genus (TAVARES-DIAS, MORAES, 2004).

Moreover, according to McCarthy et al. (1973), the values of VCM and MCH of fish require caution in their interpretation, since they are calculated from the total erythrocyte count, in which it may present a certain range of variation. However, MCHC is considered more accurate since it is calculated from the percentage of hematocrit and hemoglobin.

The average values of MCHC (25.13 a 28.50 g/dL) were in the range estabilished by Weiss et al. (2010) ehich is from 18.0 to 30.0g/dL. According to Tavares-Dias et al., (2009) for hybrid fishes (*P. fasciatum x P. coruscans*), weighing between 568 and 1350g range of 16.8-18.8g/dL to MCHC.

Total and differential counting of leukocyte were not altered by diet. Leukocytes are cells that play an important role in nonspecific immunity and their indices are determinant in the evaluation of fish health status (ISHIKAWA et al., 2008).

The average values of the number of leukocytes and thrombocytes from pintado juvenilles were different in comparison to the values reported for healthy hybrid Surubins (*Pseudoplatystoma reticulatum x P. corruscans*) (PÁDUA et al., 2009), however, lymphocytes and neutrophils were the most frequent leukocytes in both species.

Tomaz and Campos (2011), studying the defense cells of *Pseudoplatystoma reticulatum* males and females during their reproductive period in pisciculture, reported averages for lymphocytes (74.2 ± 12.7-males and 76.1 ± 9.91- female) lower than those found in the present study. The average values for eosinophils (3.63 ± 3.09 - male and 2.80 ± 3.09 - female), monocytes (1.75 ± 3.46 - male and 1.30 ± 0.86 - female), leukocyte-PAS-positive/LG-PAS (0.68 ± 0.25 0.82 - male ± 0.52-female) and

neutrophils (14.6 ± 8.30 - male and 11.8 ± 8.22- female) were larger than those reported for the Pintado.

Knowledge about the origin and development of thrombocytes and leukocytes in fish is considered scarce, although some ideas have been proposed since the beginning of the last century. However, the data acquired through the studies of hematology and/or hematopoietic organs are still inconclusive. However, organic defense blood cells present interspecific variation (TAVARES-DIAS et al., 2002), which may explain the variation in the values compared above.

In addition, differential leukocyte count has some barriers that make it difficult to compare results among different authors, even for studies that use the same species. Among the problems faced are the divergence of terminology, mainly involving granulocytes and the diversity of techniques for quantification and identification of leukocytes (TAVARES-DIAS && MORAES, 2004). However, leukogram is considered an important tool in the understanding of infections and other processes of homeostatic imbalance (SILVA et al., 2012).

The biochemical parameters analyzed in this study were not altered by the different levels of crude glycerin. The biochemical composition of the blood reliably portrays the constancy between ingress, egress, and metabolization of nutrients in animal tissue. This balance is termed homeostasis, in which complex metabolic-hormonal mechanisms are involved (BOCKOR, 2010).

The concentrations of total cholesterol were not affected by the inclusion of crude glycerin in the diet, corroborating with Balen (2017) results for juveniles of Curimbatá (*Prochilodus lineatus*), that were feed diets containing crude glycerin (0, 40, 80, 120, 160 and 200 g kg $^{-1}$) and Neu et al. (2013) for juveniles of Nile tilapia with diets containing (0,0, 25, 50, 75 and 100 g kg $^{-1}$) glycerol.

Enzymes ALT (alanine aminotransferase) and AST (aspartate aminotransferase) also had not presented a significant difference between the treatments, demonstrating that the diet did not alter their activities. These enzymes are considered important in the diagnosis of hepatic lesions, since the increase of their serum activity may be related to hepatocyte rupture, resulting from processes such as cellular necrosis or aggression by toxic agents (HARZER et al., 2015).

Total protein values found in this work did not differ much from those found by Costa et al. (2015) for Nile Tilapia, that remained between 3.93 to 4.13 and that also did not present significant difference. According to this same author, the diet containing glycerol protects protein catabolism for energy purposes in Nile tilapia. Corroborating with this study, Menton et al. (1986) also found no significant difference in plasma protein concentration of trout that were fed diets containing 60 and 120 g kg $^{-1}$ glycerol.

Glucose acts as an energetic substrate stored in the form of hepatic and muscular glycogen (through glycogenesis) and can be mobilized to provide energetic support to fish. Plasma glucose is highly variable not only among species, but also interspecifically, at different stages of life or under certain feeding diets (HEMRE et al., 2002).

The results for glucose found in this research were not altered with the inclusion levels of crude glycerol in the diet. Distinguishing from the results found in this work for glucose concentration, Li et al. (2010) observed that the level of glucose in the blood of the catfish of the channel (*Ictalurus punctatus*) was influenced by the different levels of glycerol (0, 50, 100, 150 and 200g kg $^{-1}$). The values were significantly higher in fish fed with 0 g kg $^{-1}$and 50 g kg $^{-1}$of glycerol than in fish fed with the other diets. Also, at levels containing 100, 150 and 200 g kg $^{-1}$ of glycerol, glucose generally decreased

For Nile tilapia in the growth/fattening phase Moesch et al. (2016) found that glycerol-containing treatments (200, 400, 600, 800 and 1000 g kg $^{-1}$) had the lowest blood glucose levels, a fact that the author associated with the reduction of starch (corn) in the diet.

Carnivorous fishes use lipids efficiently as a source of energy, due to the restricted ability of their metabolism to regulate glycemia (CASERAS et al., 2002; HEMRE et al., 2002), where triacylglycerols are the main form of storage of body energy in these animals

The results for the concentration of triglycerides observed in this study are similar to those obtained by Costa et al. (2015) and Neu et al. (2013). Both found that dietary glycerol levels did not influence the plasma triglyceride concentration in juvenile Nile tilapia. Costa et al. (2015) stated that there was no change in plasma triglyceride levels because there was no energy deficiency in the animal and therefore, there was no need for the species to mobilize energy and consequently to perform lipolysis on adipocytes.

Crude glycerin has been tested in a diet of other monogastric species. Romano et al. (2014) studied the effects of glycerol on the metabolism of broilers. Similarly to the present study, there was no significant difference in total cholesterol concentrations between the control group and the groups that received diets with different glycerin inclusion levels (25, 50, 75 and 100 g kg $^{-1}$),

Gallego et al. (2014), when testing levels (0.0, 35, 70, 105 and 140 g kg $^{-1}$) of semipurified glycerin neutralized in the diet, observed that the plasma glucose, cholesterol and triglycerides levels had also no effect of the inclusion of glycerin.

In ruminant animals, Maciel et al. (2016) analyzed the performance and carcass characteristics of dairy cows that were fed diets containing crude glycerin and observed that it did not alter serum glucose concentrations, triacylglycerol, total cholesterol, high density lipoprotein cholesterol and creatinine.

Ribeiro (2015) when studying crude glycerin (0, 70, 140, and 210g kg $^{-1}$) in the diet of confined lamb, observed no significant difference for albumin, globulin, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltransferase. However, serum concentrations of urea and glucose decreased linearly with the increasing of inclusion and cholesterol presented increasing linear behavior, therefore, they were influenced by glycerol.

Glycogen levels present in the hepatic tissue are adaptable to diet (SHEMU, 1997; HEMRE et al., 2002). Hepatic glycogen did not present statistical difference with the treatments with inclusion of glycerol in relation to the control. Menton et al. (1986), when examining glycerol (ranging from 10 to 120 g kg $^{-1}$) in the diet of rainbow trout (Oncorhynchus mykiss) replacing part of wheat bran, observed that in diets containing 60 and 120 g kg $^{-1}$ of glycerol there was an increase in the level of plasma glucose, but the hepatic glycogen concentration was not altered.

The concentration of muscle glycogen was higher in the control diet (0 g kg $^{-1}$glycerol) when compared to the treatment with 125 g kg $^{-1}$glycerol

It is well known that muscle glycogen is essential only for muscle and that its metabolization provides energy for muscle contraction, whereas hepatic glycogen regulates glycemia and provides energetic substrate for other tissues.

Silva et al. (2012), when testing two levels of glycerol (0 and 50 g kg $^{-1}$) supplementation, as a way of replacing the muscular glycogen reserves of gilthead (*Sparus aurata*), observed that fish that were feed the inclusion of 50g kg ^{1} crude glycerol showed a significantly higher glycogen deposition than the control (0g kg $^{-1}$) group.

In previous studies involving glycerol, it has been shown that glycerol may also influence lipogenic activity. Lin (1977), when studying the addition of 200g kg $^{-1}$glycerol in the diet of rats for three weeks, reports that it caused an increase in liver weight. On the other hand, when the same author studied 200 g kg $^{-1}$ of glycerol in the diet of broilers fed for three weeks, he did not observe alteration in liver weight

Lin (1977), when studying the feeding of non-ruminant animals, observed that glycerol causes species-specific and organ-specific responses. Based on this hypothesis, in the literature, glycogen deposition and lipogenic activity can be influenced by dietary glycerol. It suggests that more studies have to be done with this food in order to uncover the metabolic effects of glycerol on energy reserves in fish.

Crude glycerin may have methanol concentrations in its constitution and that an acute intoxication by this residue can lead to the accumulation of formic acid, resulting in a process of metabolic acidosis (LAMMERS et al., 2008). In this way it is of great importance studies that investigate pathological alterations linked to the glycerol.

The liver is an important organ in the digestion and absorption of nutrients from food and, therefore, the monitoring of this organ is considered essential (RAŠKOVIÉ et al., 2011). Morphological changes in the liver can be triggered by chemicals, drugs and even by unbalanced nutrition, which can result in adaptations, injury and even cell death. This organ is highly susceptible to changes in the nutritional status of fish, where diet quality interferes directly with functional histo-morph structure (HONORATO et al., 2014).

The alterations observed in the liver of the pintados that were feed on the control diet and those that were feed with increasing levels of crude glycerin were considered to be mostly alterations that did not compromise organ function.

Research on the histopathology of animals that were feed with crude glycerin are still incipient. However, even there is just a few studies performed the results corroborate with those found in the present study. Moesch et al. (2016) when studying the replacement of corn bran by crude glycerol in diets for *O. niloticus* fingerlings at concentrations of (0, 200, 400, 600, 800 and 1000 g kg $^{-1}$) concluded that there were no differences in the area of hepatocytes and that the possible toxic compounds present in the crude glycerol composition did not affect the area of hepatocytes.

According to Lammers et al. (2008) growing pigs fed with the addition of up to 100 g kg $^{-1}$ glycerol in the diet had not suffered hepatic damage, since the frequency of histological lesions was not influenced by dietary treatment.

Also, in studies with ruminants, Leão et al. (2012) did not identify hepatic alterations in samples from cull cows and heifers that were feed with up to 240 g kg $^{-1}$inclusion of glycerol. As in the liver histopathological analysis, the renal lesions performed in this study, renal, were not serious in order to compromise the functioning of the organ.

Which also happened in the liver histopathological analysis of this study, the renal lesions performed were not serious in order to compromise the functioning of the organ.

There was no studies that associate the renal histology of fish with the diet containing crude glycerin. However,

there are some reports for other monogastric species such as swine, for example, in which there was no frequency of histological lesions in the kidney (LAMMERS, et al., 2008). In ruminants, Leão et al. (2012) also had not observed pathological alterations in the renal samples of cattle fed with crude glycerin.

V. CONCLUSION

The juveniles of Amazon catfish "Pintado" are able to metabolize crude glycerin up to the level of 100 g kg $^{-1}$. Considering that, up to this level, no significant alterations were observed in the zootechnical parameters, body composition, hematological, biochemical and histological parameters of the animal.

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Genetic diversity and population structure of *Peronosclerospora sorghi* isolates of Sorghum in Uganda

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***Abstract*—** *Sorghum is the third most important staple cereal crop in Uganda after maize and millet. Downy mildew disease is one of the most devastating fungal diseases which limits the production and productivity of the crop. The disease is caused by an obligate fungus, Peronosclerospora sorghi (Weston & Uppal) with varying symptoms. Information on the genetic diversity and population structure of P.sorghi in sorghum is imperative for the screening and selection for resistant genotypes and further monitoring possible mutant(s) of the pathogen. Isolates of P. sorghi infecting sorghum are difficult to discriminate when morphological descriptors are used. The use of molecular markers is efficient, and reliably precise for characterizing P. sorghi isolates. This study was undertaken to assess the level of genetic diversity and population structure that exist in P. sorghi isolates in Uganda. A total of 195 P. sorghi isolates, sampled from 13 different geographic populations from 10 different regions (agro-ecological zones) was used. Eleven (11) molecular markers, comprising of four Random amplified microsatellite (*RAM) and seven (7) Inter-Simple Sequence Repeat (ISSR) markers were used in this study. *The analysis of molecular variation (AMOVA) based on 11 microsatellite markers showed significant (P < 0.001) intra-population (88.9 %, PhiPT = 0.111) and inter-population (8.4 %, PhiPR = 0.083) genetic variation, while the genetic variation among regions (2.7 %, PhiRT = 0.022) was not significant. The highest genetic similarity value (0.987 = 98.7 %) was recorded between Pader and Lira populations and the lowest genetic similarity (0.913 = 91.3 %) was observed between Namutumba and Arua populations. The mean Nei's genetic diversity index (H) and Shannon Information Index (I) were 0.308 and 0.471 respectively. Seven distinct cluster groups were formed from the 195 P. sorghi isolates based on their genetic similarity. Mantel test revealed no association between genetic differentiation and geographical distance (R^2 = 0.0026, p = 0.02) within the 13 geographic populations.*

***Keywords*— AMOVA, Genetic diversity Index, ISSR, Mantel test, RAM, Shannon Information Index.**

I. INTRODUCTION

Downy mildew disease of sorghum, caused by an obligate soil-borne fungus *Peronosclerospora sorghi* (*P. sorghi*) [Weston and Uppal (Shaw)] (Frederiksen, 1980) poses a serious biotic stress to sorghum production and productivity worldwide (Williams, 1984). The prevalence and distribution of the disease is favoured by factors such as high relative humidity (Wang *et al*., 2000), low temperature (Bock *et al*., 1998) and rainfall which favours conidia production and subsequent development of the disease. When the disease is not managed at the early stages of infection, losses can reach 100 %. Infected plants show localized and systematic symptoms varying from lesions on leaf lamina to chlorosis (Jeger *et al*., 1998). Previous reports suggest the prevalence and distribution of Sorghum downy mildew disease (SMD) in Uganda at varying incidence and severity levels (Bigirwa *et al*., 1998; Kumi *et al*., 2018), but no study has been

done to unravel the state of genetic diversity of *P. sorghi* isolates in Uganda.

Morphological and molecular characterizations are two methods commonly used to assess genetic variability of *P. sorghi* (Bock, 1995). Unlike molecular characterization, morphological characterization is exclusively dependant on morphological traits of *P. sorghi* which come with several shortcomings ranging from; influence by environmental factors, time consuming and lack of efficient resolution power required to discriminate between genetically related isolates. More so, the use of morphological descriptors to assess genetic variation among isolates of *P. sorghi* is reported to be limited (Bock, 1995).

The advent of molecular markers has made it easier to study genetic diversity and population structure of *P. sorghi* with much precision and better accuracy (Perumal *et al*., 2006). Random Amplified Polymorphic DNA (RAPD) primers, Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphism (AFLP) have been extensively used to explore the variability among *P. sorghi* isolates (Bock *et al*., 2000) and different pathotypes of *P. sorghi* in sorghum (Perumal *et al*., 2008). Compared to other parts of the world, pathogenic and molecular variability among the *P. sorghi* isolates of sorghum have been well documented (Bock *et al*. 2000; Mathiyazhagan *et al*., 2008). Outcome of such research confirmed the existence of genetic diversity in *P. sorghi* and led to identification of new pathotypes (Perumal *et al*., 2006).

Perumal *et al*. (2006) analyzed the genetic variability among the 14 isolates of *P. sorghi* including metaxyl resistant isolates and reported that approximately 25% of the bands were polymorphic across the isolates in the tested populations. Mathiyazhagan *et al*. (2006) also reported similar genetic variability between isolates from sorghum and corn in India, using restriction fragment length polymorphism analysis of the polymerase chain reaction (PCR). Sequence characterized amplified region (SCAR) marker have also been used to assess genetic variability of *P. sorghi* (Ladhalakshmi *et al*, 2009).

Knowledge regarding the extent of genetic diversity and genetic relationships of *P. sorghi* in Uganda will be valuable for designing a comprehensive breeding strategy for identifying resistant sorghum genotypes that will be resilient to SDM disease. Thus, studying and quantifying genetic diversity of *P. sorghi* from different agro-ecologies will offer a valuable marker assisted selection and breeding strategy. The objective of this study was to characterize the isolates of *P. sorghi* in Uganda by assessing the population structure, genetic diversity and/or relatedness using ISSR and RAMS markers.

II. METHODOLOGY

Collection of samples

Hierarchical sampling technique was used to collect a total of 195 SDM samples from 13 districts (Table 1 and Fig 1) covering all the ten agro-ecologies in Uganda. These samples were taken from sorghum plants with leaves showing systemic and/or localized characteristic lesions of mildew with conspicuous conidia growth. Leaves samples were carefully covered with aluminum foil, labeled and stored in a cooler.

DNA extraction and purification

DNA extraction was done following the protocol described by McDermott *et al*. (1994). Conidia found on leaves were collected using a camel hair brush and transferred to a 1.5-ml microcentrifuge tube containing 500 µl of extraction buffer (50 mM Tris–HCl, pH 8.0; 0.7 M NaCl and 1% SDS) (1 × 108 spores/ml), vortexed for 30 s and incubated at 60 ° C for 1 h. After incubation, the mixture was centrifuged at 12,000 × g for 10 min, and the aqueous phase was collected and extracted twice with an equal volume of phenol: chloroform: isoamylalcohol (25:24:1). The aqueous phase was transferred to a 1.5-ml microcentrifuge tube and the DNA was precipitated by addition of an equal volume of cold isopropanol and incubation at –20°C for 1 h.

Table.1: Geographical and climatic data of P. sorghi populations in Uganda.

Population	Latitude	Longitude	Altitude (m)	Ave. Temp (° C)	Annual Rainfall (mm)	Agro-ecological zone
Iganga	N00°37.402'	E033°29.657'	1022	22.3	1313	Lake Victoria Crescent
Namutumba	N00°48.309'	E033°39.273'	1091	23.4	1011	Lake Victoria Crescent
Pallisa	N00°48.309'	E033°39.273'	1081	23.2	1353	Lake Kyoga Basin
Kumi	N01°16.556'	E033°52.238'	1125	23.2	1238	Lake Kyoga Basin
Serere	N01°31.167'	E033°33.089'	1104	23.8	1362	Eastern Highlands
Lira	N00°18.478'	E032°34.292'	1153	23.6	1219	Northern Grassland

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Pader	N02°48.549'	E033°06.440'	979	23.7	1239	Northern Grassland
Hoima	N01°26.270'	E031°21.541'	1113	22.6	1382	Western Mid-Altitude
Masindi	N01°40.888'	E031°43.540'	1194	22.9	1355	Lake Albert Crescent
Arua	N03°01.303'	E030°54.684'	1109	22.9	1404	West Nile
Nebbi	N02°32.943'	E031°06.064'	1061	23.2	1098	Northwestern Grassland
Kabarole	N00°36.511'	E030°15.428'	1106	19.3	1459	Western Mid-High Altitude
Kabale	S01°15.660'	E030°01.444'	1934	17.2	1018	Southwestern Highlands

Source: Field data, 2016.

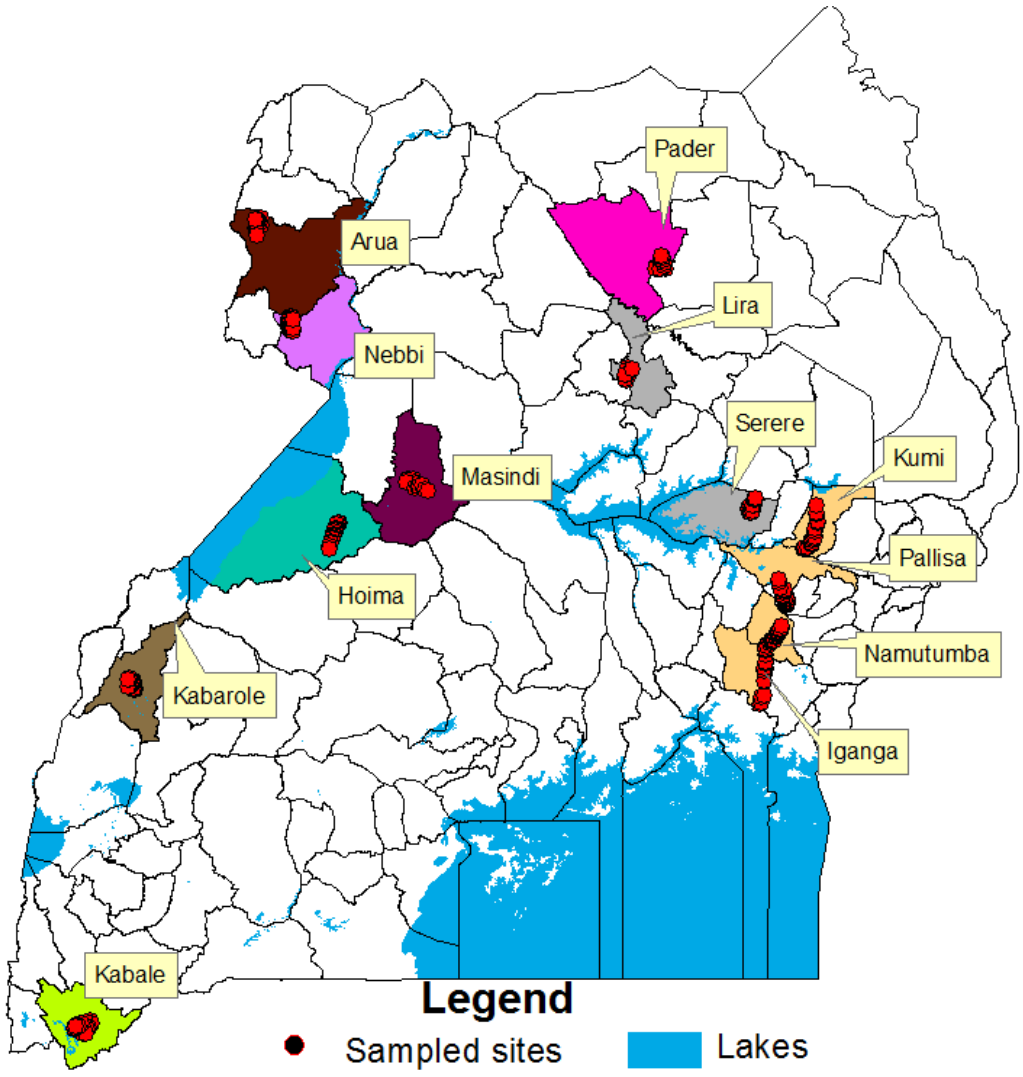

Fig. 1: Sampling sites of Peronosclerospora sorghi for the study. The small circled marks in each population (District) are the precised sampling points.

The DNA was pelleted by centrifugation at 12,000 × g at 4°C for 10 min. The pellet was washed twice with cold 70% ethanol, air-dried and re-suspended in 50 µl of Tris–EDTA buffer (10 mM Tris– HCl and 1 mM EDTA, pH 8.0). The genomic DNA was checked by agarose gel electrophoresis and the concentrations of the DNA were determined using a NanoDrop spectrometer nm (Thermo Scientific, Waltham, MA) by measuring the absorbance at 260.

Amplification and electrophoresis

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A total of twenty (20) primers, consisting of nine (9) Random Amplified Microsatellites (RAMS) and eleven (11) Inter Simple Single Repeats (ISSRs) (Table 2) were used to screen the 195 *P. sorghi* isolates, but eleven (11) primers yielded clear visible PCR products and those were used in this study.

Table.2: List of Primers used in this study

Primer	Primer sequence (5'-3')	Length	AT (°C)
UBC809[a]	AGAGAGAGAGAGAGAGG	17	50
UBC824[a]	TCTCTCTCTCTCTCTCG	17	47
UBC825[a]	ACACACACACACACACT	17	50
UBC826	ACACACACACACACACC	17	47
UBC836[a]	AGAGAGAGAGAGAGAGYA	18	50
UBC841[a]	GAGAGAGAGAGAGAGATC	18	47
UBC842[a]	GAGAGAGAGAGAGAGA	16	45
UBC847	CACACACACACACACAAC	18	47
UBC848	CACACACACACACACACG	18	45
UBC849	CTCTCTCTCTCTCTCTCA	18	47
UBC880[a]	GGAGAGGAGAGGAGA	15	47
RAMS1[a]	CACACAACAACAACAACA	18	45
RAMS2[a]	GACCACCACCACCACCA	17	55
RAMS3 [a]	ATCCGACGACGACGACGA	18	50
RAMS4	AGGGTGTGTGTGTG	14	45
RAMS5	AACACACACACA	12	35
RAMS6[a]	ACCAGAGAGAGAG	13	40
RAMS7	GCATATATATATGT	14	35
RAMS8	CTAGAGAGAGCTCTG	15	45
RAMS9	GATCGCGCGCGCGTC	15	55

a = Primers which yielded visible polymorphic bands.

These primers have the ability to differentiate among isolates of *P. sorghi*. Each PCR reaction (20 µl) contained 50 ng of genomic DNA as template, 5 mM each dNTPs, 10 pmol of primer and 0.5 µl of Taq DNA polymerase. DNA Amplification was performed using the following thermal cycle parameters; 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 37 °C for 1 min, and 72°C for 1 min and a final extension at 72 °C for 10 min at the end of amplification. The PCR products were analyzed by electrophoresis on a 1.5 % (w/v) agarose gel containing 0.5 µg/ml of ethidium bromide in Tris-acetate-EDTA (TAE) buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0) and subsequently visualized under UV light. Selected ISSRs and RAMs amplifications were further repeated to ensure reproducibility. Polymorphic bands were subsequently screened with a 100 base pair (bp) marker.

Data collection and analyses

Data collected from the amplified multiloci bands which were clearly visible were scored manually for each isolate using a binary format according to presence (1) or absence (0) (Santacruz-Varela et al., 2013) from the image of ethidium bromide stained gels (Fig. 2) of the target isolates.

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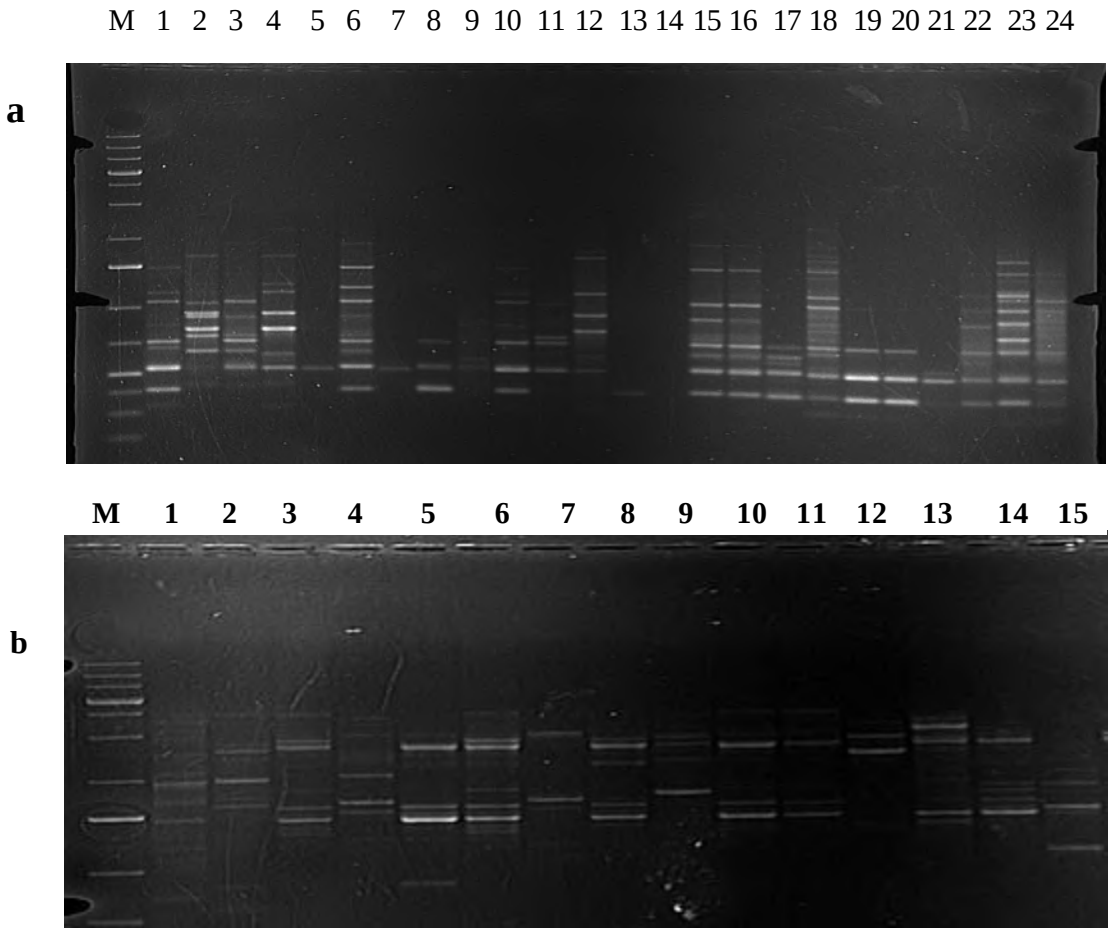

Fig. 2 Agarose gel electrophoresis of PCR amplified products from genomic DNA of P. sorghi using UBC 880 primer (ISSR primer) (a) Lane 1 (M) in both figure are 100 bp DNA ladder; Lane 2 – 24 (P. sorghi Isolates, Lane 14 is an empty well) and RAMS 3 (RAMS primer) (b) Lane 2 – 16 (P. sorghi Isolates).

The binary data were subjected to analysis of molecular variance (AMOVA) (Reyes-Valdés *et al*., 2013; Excoffier *et al*., 1992) with 999 permutations using GenAlEx6.5 (Peakall & Smouse, 2006; 2012). The total genetic variation among 195 isolates was generated using phi-statistic through AMOVA. The genetic variations were partitioned into three; variation among regions (PhiRT), variation among population (PhiPR) and variation within population (PhiPT).

PhiPT coefficient values denote the proportion of estimate variance among population relative to the total variance and the pairwise between populations expressed as PhiPT = (V_{AP} + V_{AR}/ (V_{WP} + V_{AP}), where V_{AP} is the estimate of variance among populations, V_{AR} is the estimate of variance between the geographical regions, and V_{WP} is the estimate of variance within the studied population. PhiPT was used to determine the genetic differentiation between the population, it is a measure which allows intra-individual variation suppression when comparing binary and codominant data (Teixeira *et al*., 2014).

The genetic variability of the populations (districts) was analyzed using the Hardy-Weinberg equilibrium (HWE) (Crow *et al*., 2008) assumption in GenAlEx 6.5 (Peakall & Smouse, 2006; 2012). Pairwise Nei's genetic distances (Nei, 1972) and Pairwise Nei's genetic identity between geographical populations of *P. sorghi* were obtained based on 999 permutations. The percentage of polymorphic loci (PPL), number of bands (No. Bands), number of different alleles (Na), number of effective alleles (Ne), Shannon Information Index (*I*), expected heterozygosity/ Genetic Diversity Index (H) and unbiased expected heterozygosity (uHe). The Shannon diversity index (*I*) is an index that is commonly used to characterize species diversity in a giving population. Shannon's index (*H*) accounts for both abundance and evenness of the species present and expressed as a proportion of species *i* relative to the total number of species (p_i), and then multiplied by the natural logarithm of this proportion (lnp_i). The resulting product is summed across species, and multiplied by -1.

$H = \sum_{i=1}^{s} - (pi * lnpi)$ (Spellerberg & Fedor, 2003)

Data on latitudinal and longitudinal coordinate for each isolate was converted into decimal degrees to

estimate geographical distance matrix. Similarly, genetic distance was obtained by transforming the binary binary data (0 for absence and 1 for presence of amplicon) from the ISSR and RAMS amplified product. Mantel Test with 999 permutation was computed in GenAlEx to examine whether populations (districts) that are geographically close are also genetically similar.

Mantel test in GenAlEx (Peakall & Smouse, 2006; 2012) was performed to examine whether genetic isolation was associated with the geographic distance among *P. sorghi* populations. The pairwise Nei's population genetic distances were calculated based on gene frequency differences between populations, and these distances were then compared to geographic distances between populations and a correlation was run for these two parameters. The genetic distance matrix generated from 195 isolates in GenAlEx was used to perform a hierarchical cluster analysis based on Ward's hierarchical agglomerative clustering method (Ward, 1963) using R statistical package (R Core Team, 2013). A cluster dendogram was generated for the isolates (Maechler *et al*., 2016). In other to determine the optimal number of clusters for the dendogram, the Bayesian Inference Criterion (BIC) (Akogul, 2017) for k means method was used. This method deploys expectation-maximization, initialized by hierarchical clustering for parameterized Gaussian mixture models (Reynolds, 1992).

Following the cluster analysis, Discriminant Analysis of Principal Components (DAPC) (Jombart *et al*., 2010) was carried out in R statistical package to assess the relationships between the different clusters, using a method that focus on variability between-group, while neglecting variability within-group variation, which is precisely the rationale of Discriminant Analysis (DA) (Lachenbruch & Goldstein, 1979) to achieve the best discrimination of isolates into pre-defined groups. DAPC scatter-plot was generated which allows for a graphical assessment of the genetic structures between clusters.

III. RESULTS

Genetic variation and relationship in *P. sorghi* at varied population levels

Statistical analyses which were performed on the total of 120 amplified polymorphic loci from PCR reaction for *P. sorghi* isolates using ISSR and RAMS revealed a wide genetic diversity and structure in the population. The overall genetic differentiation was examined for the 195 *P. sorghi* isolates from 13 geographic populations (Districts) covering 10 regions (AEZ) in Uganda (Table 3). The AMOVA results are presented below.

Table.3: Analysis of molecular variance (AMOVA) showing the partitioning of genetic variation within and among populations of Peronosclerospora sorghi

Sources of variation	df	MS	Est. variance	% Total variation	Phi Statistic	Value	*P* value
Among regions	9	63.519	1.102	2.7	PhiRT	0.022^{NS}	0.487
Among population	3	44.100	3.368	8.4	PhiPR	0.083***	0.001
Within population	182	22.468	35.876	88.9	PhiPT	0.111***	0.001
Total	194		40.346	100			

Df= degree of freedom, SS= sum of square, Phi statistic, *P* value is based 999 permutation. *** = Significant at P < 0.001

Partitioning of genetic differentiation at three levels (among population, within population and among regions) contributed varying degrees of genetic variation to the total variation observed. Genetic variation among and within population of the 195 isolates examined by AMOVA were significant (P <0.001), while genetic variation of isolates among regions where not significant. Variation within population accounted for 88.9 % of the total genetic variance observed in *P. sorghi* isolates, this means the highest genetic diversity of these isolates occurred within population level. Variation among population and regions contributed 8.4 % and 2.7 % genetic variation respectively to the total diversity of *P. sorghi*. Additionally, significant (P <0.001) Phi values for genetic diversity were recorded among population (PhiPR = 0.083) and within population (PhiPT = 0.111) while the Phi value recorded among regions (PhiRT = 0.022) was not significant.

The results from the analysis of genetic diversity among 195 isolates of *P. sorghi* from13 different populations are presented in Table 4.

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Table.4: Estimated Heterozygosity, number of bands and percentage polymorphism loci by Population

Population	NPL	PPL (%)	Na	Ne	*I*	H	uHe
Kabarole	107	89.17%	1.783 (0.057)	1.462	0.436	0.285	0.294
Arua	117	96.67%	1.942 (0.030)	1.564	0.493	0.329	0.341
Nebbi	105	87.50%	1.750 (0.061)	1.378	0.381	0.243	0.251
Iganga	109	90.83%	1.817 (0.053)	1.444	0.437	0.282	0.292
Namutumba	105	87.50%	1.750 (0.061)	1.559	0.488	0.329	0.340
Pallisa	113	94.17%	1.883 (0.043)	1.433	0.436	0.279	0.289
Kumi	116	96.67%	1.933 (0.033)	1.502	0.485	0.316	0.327
Kabale	112	93.33%	1.867 (0.046)	1.470	0.464	0.300	0.311
Hoima	112	93.33%	1.867 (0.046)	1.599	0.525	0.354	0.366
Masindi	116	96.67%	1.933 (0.033)	1.481	0.476	0.308	0.319
Lira	119	99.17%	1.983 (0.017)	1.490	0.483	0.312	0.323
Pader	119	99.17%	1.983 (0.017)	1.518	0.503	0.328	0.339
Serere	118	98.33%	1.967 (0.023)	1.543	0.515	0.339	0.350
Mean	113	94.04%	1.881	1.496	0.471	0.308	0.319
Mean SE		1.17%	0.012	0.007	0.005	0.003	0.004

PPL= Percentage of Polymorphic loci, NPL= Number of polymorphic loci, Na = Number of different Alleles, Ne = Number of Effective Alleles, *I*= Shannon's Information Index, H = Expected Heterozygosity/ Genetic diversity index, uHe = Unbiased Expected Heterozygosity. Values in parenthesis are standard errors.

The mean number of different polymorphic loci, percentage of polymorphic loci, number of different Alleles, number of effective alleles, Shannon's Information Index, Nei's genetic diversity index/expected heterozygosity (measure the number of alleles and their abundance) and unbiased expected heterozygosity were 113, 94.04 %, 1.881, 1.496, 0.471, 0.308 and 0.319, respectively. The results from the analyses revealed high levels of genetic variations within population.

The percentage of polymorphic bands was high in all the 13 distinct populations, with values ranging from 87.50 % for both Nebbi and Namutumba to 99.17 % also for both Lira and Pader. The number of different alleles ranged from a minimum of 1.750 recorded for population in Nebbi to a maximum of 1.983 for both Lira and Pader. The number of effective alleles ranged from a minimum of 1.378 for Nebbi population to a maximum of 1.599 for Hoima. Shannon Index was highest for Serere population (0.515) and lowest at Nebbi (0.381). The results show a low genetic diversity index (He) ranging from 0.243 for Nebbi population and 0.345 for Hoima population with an overall mean of 0.308.

Relationship for genetic distance and geographical distribution

The results of pairwise Nei's genetic distance between geographical population of *P. sorghi* (above diagonal) and pairwise Nei's genetic similarity between geographical populations of *P. sorghi* (below diagonal) are presented in Table 5. The general Nei's genetic distance (genetic difference) values recorded between the tested populations of *P. sorghi* were very low, ranging from a minimum of 0.014 to maximum of 0.091. The smallest genetic distance was observed between Lira and Pader population (0.14), both of which are in the same agro-ecological zone (Northern grassland), while the largest genetic distance was observed between Arua and Namutumba population in which fall within West Nile and Lake Victoria Crescent ecological zones respectively. The entire population of *P. sorghi* isolates recorded high genetic similarity values ranging from 0.913 (91.3 %) to 0.987 (98.7 %). The minimum genetic similarity value of 91.3 % was recorded between Namutumba and Arua populations while the highest genetic similarity value of 98.7 % was recorded between Pader and Lira population.

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Table.5: Pairwise Nei's genetic distances between geographical population of P. sorghi (above diagonal). Pairwise Nei's genetic similarity between geographical population of P. sorghi (below diagonal)

Population	Kabarole	Arua	Nebbi	Iganga	Namutumba	Pallisa	Kumi	Kabale	Hoima	Masindi	Lira	Pader	Serere
Kabarole	-	0.074	0.045	0.035	0.065	0.045	0.039	0.030	0.033	0.032	0.036	0.038	0.039
Arua	0.929	-	0.088	0.071	0.091	0.086	0.065	0.068	0.059	0.072	0.074	0.067	0.069
Nebbi	0.956	0.916	-	0.036	0.062	0.027	0.033	0.034	0.064	0.033	0.035	0.040	0.036
Iganga	0.965	0.931	0.965	-	0.057	0.040	0.030	0.031	0.048	0.030	0.031	0.030	0.031
Namutumba	0.937	0.913	0.940	0.945	-	0.077	0.072	0.072	0.074	0.072	0.082	0.069	0.069
Pallisa	0.956	0.918	0.974	0.961	0.926	-	0.030	0.031	0.050	0.027	0.022	0.026	0.024
Kumi	0.962	0.937	0.967	0.971	0.931	0.970	-	0.016	0.045	0.017	0.022	0.022	0.019
Kabale	0.970	0.935	0.967	0.969	0.930	0.970	0.985	-	0.047	0.016	0.021	0.019	0.022
Hoima	0.967	0.943	0.938	0.953	0.929	0.952	0.956	0.954	-	0.040	0.042	0.041	0.037
Masindi	0.968	0.930	0.967	0.970	0.930	0.974	0.984	0.984	0.961	-	0.018	0.019	0.018
Lira	0.964	0.929	0.966	0.970	0.921	0.978	0.978	0.979	0.959	0.982	-	0.014	0.015
Pader	0.963	0.935	0.960	0.970	0.934	0.974	0.979	0.981	0.960	0.981	0.987	-	0.016
Serere	0.962	0.933	0.965	0.970	0.933	0.976	0.981	0.978	0.963	0.982	0.985	0.984	-

The results from Mantel test to examine whether genetic distance was associated with geographic distance among *P. sorghi* populations revealed no association (Fig 4. $R^2 = 0.0026$, $p = 0.02$).

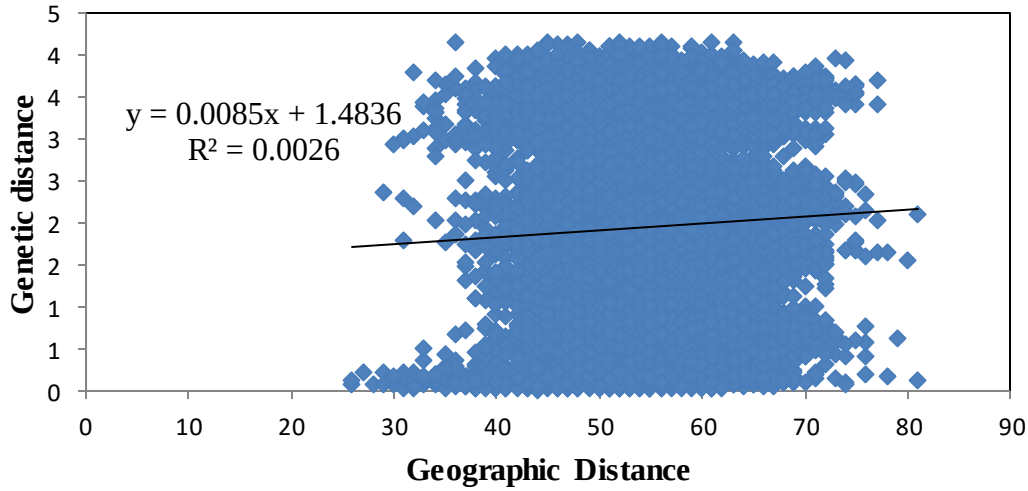

*Fig. 4: Mantel test between Nei's genetic distance and geographic distance for 13 populations of P. sorghi (**r = 0.014**.)*

Cluster analysis

The 195 *P. sorghi* isolates from 13 different geographic populations clustered into seven distinct cluster groups (Fig. 4) which indicates genetic diversity within the population. Each cluster was mutually exclusive of isolates identity but not in geographical

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population of the isolates. The largest cluster was cluster 2 with a total of 64 isolates from 10 different geographic populations namely; Kabarole, Nebbi, Iganga, Pallisa, Kumi, Kabale, Masindi, Lira, Pader and Serere. The least weighted cluster was cluster 3 with 10 isolates from two geographic populations namely, Kabarole and Hoima. The remaining clusters, cluster 1, 4, 5, 6, and 7 constituted a total of 15, 25, 28, 31 and 22 isolates respectively from varied geographic populations.

Cluster 1 constituted isolates from Kabarole and Namutumba while cluster 4 constituted population of Arua, Iganga, Kumi, Masindi and Pader. Furthermore, clusters 5 and 6 shared nine (9) geographical populations namely; Arua, Pallisa, Kumi, Kabale, Hoima, Masindi, Lira Pader and Serere. But in addition to the aforementioned populations, cluster 6 also Iganga. Lastly, cluster 7 consisted of populations namely; Nebbi, Iganga and Pader.

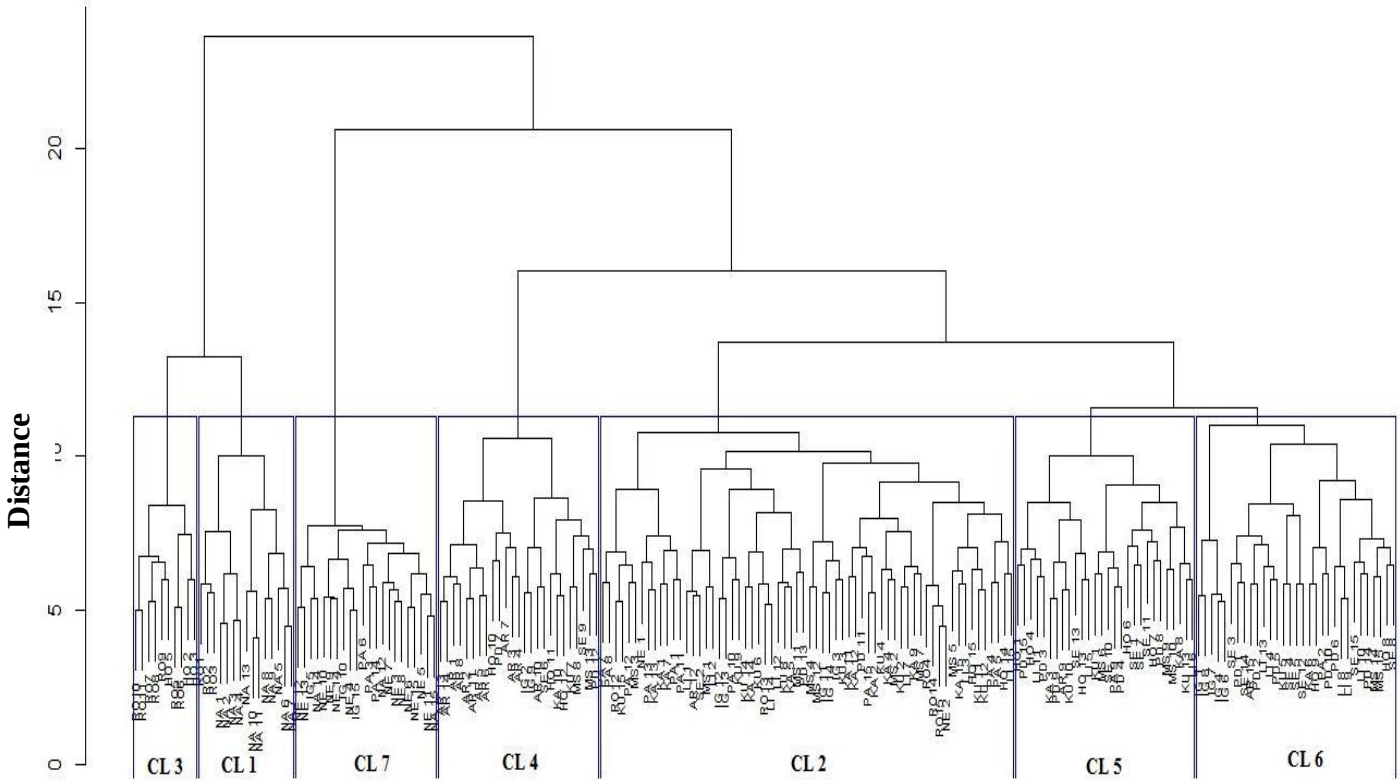

Fig 4: Ward's cluster dendogram of 195 isolates of P. sorghi from 13 different populations in Uganda.

Following cluster analysis and mantel test, Discriminant analysis was carried using the detected number of clusters from the dendogram computed from the Bayesian Information Criterion (BIC, which employs *k* means) Model to determine whether or not population structure exist within the *P. sorghi* isolates. DAPC results (Fig. 5) showed genetic variability among the isolates but there was no clear separation pattern among the study population. The results from the DAPC analysis showed no clearly defined population structure and a total of seven discriminant eigenvalues for principal components (PCs) were retained. The proportion of variance conserved by the PCA principal components accounted for 36.9 % variance. Discriminant analysis eigenvalues for PC1, PC2 and PC3 were 40.436, 17.523 and 9.166 respectively.

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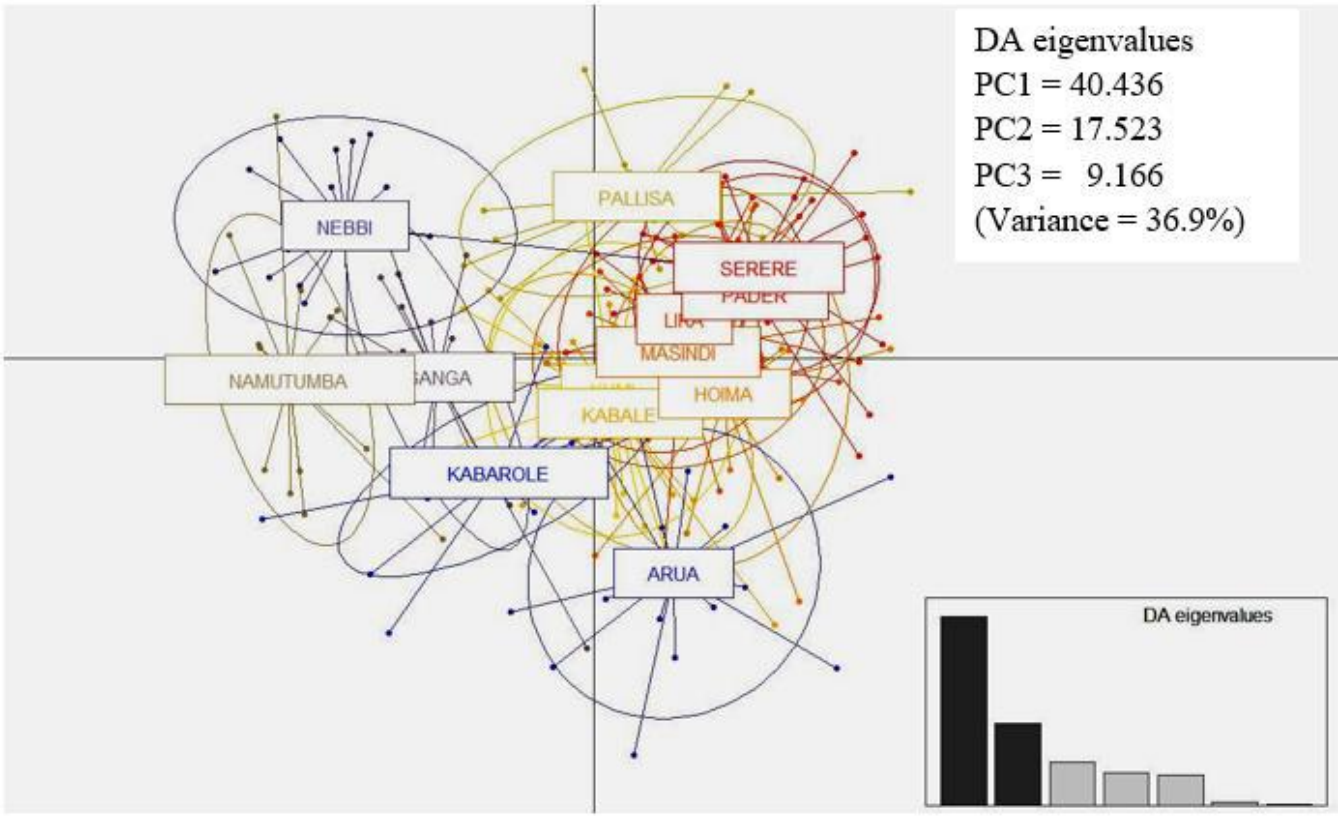

Fig.5: Discriminant analysis of principal components (DAPC) for 195 P. sorghi isolates from Uganda. Each circle represents a cluster and each dot represents an isolate.

IV. DISCUSSION

Genetic variability is significant to plant breeders for purposes of screening, selection and developing improved crop varieties that confers resistance to biotic stresses and are highly adaptable/ resilient to varied abiotic conditions while offering high quality yield and products for industrial purposes. In this present study, two different primer sets, Random Amplified Microsatellites (RAMs) and Inter-Simple Sequence Repeats (ISSRs) were used to assess genetic diversity and population structure of 195 *P. sorghi* isolates from 13 different populations in Uganda. The combination of these two molecular markers was effective in evaluating the genetic diversity, population structure and estimating genetic variations.

The observed genetic variation in this study was partitioned within population rather than among population or among regions (Table 3). These results indicate that majority of the genetic differentiation (88.9 %) existed within the defined population of *P. sorghi* isolates and among populations (8.7 %). However, the insignificant genetic differentiation value of *P. sorghi* isolates among regions (2.7 %) in this study showed that genetic differentiation was independent of the geographic regions (agro-ecological zone) of the isolate. These result agreed with other findings on genetic diversity in *P. sorghi* reported from other parts of the world such as India (Mathiyazhagan *et al*. 2008; Ladhalakshmi *et al*., 2009), Indonesia (Lukman *et al*., 2013) and the United States (Perumal *et al*., 2008) and Africa (Bock *et al*., 2000).

Results from this study also showed high percentage of genetic polymorphism (94.04 %) (Table 4) which explained the high genetic differentiation observed within the *P. sorghi* population in the AMOVA results. Similar results were reported by Sireesha & Velazhahan (2015) and Perumal *et al*., (2008) who reported high genetic polymorphism in *P. sorghi* isolates from sorghum. In addition, Sireesha & Velazhahan (2015) reported high percentage polymorphic values for *P. sorghi* in sorghum which confirmed the high genetic variations observed in this study. High polymorphic percentage values recorded in this study (Table 4) further confirmed similar findings by Perumal *et al*., (2008) and (Mathiyazhagan *et al*., 2008) who also reported high percentage polymorphism (33 %- 100%) in *P. sorghi* isolates.

Low genetic diversity index (0.304) (Table 4) and high genetic similarity (98.7 %) (Table 5) values recorded in this study was not surprising because *P. sorghi* is reported to exhibit high sexual recombination (Heffer-Link *et al*., 2002) and therefore explained the high level of inbreeding. Another contributing factor

could be exchange of *P. sorghi* infected sorghum seeds among farmers for cultivation and thereby perpetuating the spread and development of the pathogen (seed-borne). These results were similar to findings of Sireesha & Velazhahan (2015) and Mathiyazhagan *et al*., (2008) who reported high genetic simmilarity values of 93 % and 90 % respectively within *P. sorghi* isolates. Their results further reported low genetic diversity.

Mantel test results between geographic distance and genetic differentiation among the 13 geographic populations of *P. sorghum* revealed no significant correlation (Fig. 3). This result showed that, the observed genetic variability of *P. sorghi* isolates (within population) from the 13 different populations was not structured in geographic space (there is no spatial structure). Cluster analysis result (Fig. 4) revealed that, *P. sorghi* isolates in Uganda could cluster into seven (7) genetically distinct groups according to genetic similarity/identity of the populations. These findings suggested that geographical origin of *P. sorghi* isolates has no influence on the clusters formation.

Discriminate analysis result (Fig. 5) showed no clear well-defined pattern of clusters of genetic structure among the study populations. DAPC analysis therefore confirmed the Mantel test results, that the observed genetic variability of *P. sorghi* isolates within the study populations was not spatially structured.

V. CONCLUSION

The study revealed a high genetic variation of *P. sorghi* within populations of Sorghi in Uganda. A weak association of genetic isolation by geographic distance was established for *P. sorghi* populations, which suggested that the observed genetic differences of *P. sorghi* was unaffected by the geographical populations. Seven genetically distinct clusters groups were formed from *P. sorghi* isolates according to the genetic similarities.

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Causes and Measures for Controlling Loan Default among Agricultural Cooperatives in Bune State, Nigeria

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Abstract— *The studies analyzed the causes and measures for controlling loan default among agricultural co-operatives in Benue State of Nigeria. Data were collected from 130 respondents using structured questionnaire, and was analyzed using descriptive statistics. The result shows that factors responsible for loan default were classified into Institutional, Client-related, Geo-economical, and Market related factors. Late disbursement of (92.31%), lack of loan monitoring (76.92%), inadequate loan appraisals (69.23%), and lack of clear cut policy on lending (69.23) were the institutional factors responsible for loan default. Lack of integrity (80.77%), poor business practice (78.46%), and loan diversion (50.0%) were client-related factors. Death of client (70.77%), economic downturn (55.38%), and natural calamity (52.31%) were geo-economic factors, while, lack of market information (81.54%), market location (49.23%) and wrong economic decision (40.77) were market related factors. The result also showed that obtaining information on borrowers' integrity (92.23%), and training of borrowers on the terms and conditions (82.54%) were main measures to control loan default in the study area. Information on client integrity, and training of borrowers on terms and condition of loan before loan are granted were found to be best measures to control loan default. It was recommended that sound and flexible lending policies measures which must be reviewed frequently by the Central Bank of Nigeria (CBN) and Bank for Agriculture and Agricultural Co-operatives (BAAC) be put in place in order to curtail bureaucracies involved in the management approvals and disbursement of agricultural loan should be formulated. This will ensure early disbursement of funds to co-operative members.*

Keywords— Loan, default, Agricultural Cooperatives, Benue State of Nigeria.

I. INTRODUCTION

The importance of agricultural cooperative organizations in Nigeria is a long-standing one. The cardinal objective of introducing agricultural cooperative was to increase crop production and credit facilities to cultivators. They have been deeply involved in activities that have impacted on the livelihood of members in particular and rural people in general (International Labour Organization (ILO, 2002).

Agricultural cooperatives pool production and resources of farmers and rural entrepreneurs in order to maximize the benefits for its members. Unlike corporations they are focused on services rather than profit. Although members receive a payment for their capital contributions, it is not linked to the profitability of the cooperative; rather it is usually held at a fixed interest rate that can be tied by law to a maximum permitted rate (Yebisi, 2014).

Agricultural cooperatives can be classified into service cooperatives or production cooperatives (Lerman, 2013). Production cooperatives involve farmers who operate the cooperative on jointly owned agricultural plots (Chambo, 2009). The service cooperative members carry out their activities independently, and the cooperative provides them with a range of services which may include machinery, processing, transport, packaging, distribution, marketing and information (Lerman, 2013). A credit cooperative is also a service cooperative, and allows members to jointly finance their investments or working capital. Through credit unions, farmers' pool funds to be loaned to members and at the same time loans can be raised at better interest rates than those offered by commercial banks.

One problem faced by formal and informal lending institutions in Nigeria is loan default among farmers (Okorie and Iheanacho, 1992). Several efforts have been made by lenders and policy makers to deal with the situation. For instance, government at the three tiers of governance have been involved directly or indirectly in providing financial assistance to farmers as a major policy strategy for increased agricultural output in an attempt to cover up the negative effect of nonperforming loans. The

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establishment of the Agricultural Credit Guarantee Scheme Fund by the Federal Government in 1988 was one of such efforts (Nwosu *et al*., 2010). Despite strategies evolved by formal and informal lenders to prevent loan defaults, it is observed that default rates are generally higher among those who borrow from government sponsored sources than those who borrow from moneylenders and other informal lenders (Eyo *et al*., 2013, Ameh and Iheanacho, 2017)

Afolabi (2010) opined that loan recovery is one of the critical determinants of profitability and viability of a cooperative institution. Poor recovery hampers the cooperatives' capability to recycle funds and adversely affect the effective management of its resources and ultimately its profitability. The incidence of over dues in the agricultural credit system has been increasing over the years and has turned out to be the single most important factor responsible for steady erosion of the financial soundness and fitness of the cooperative institutions.

Unless the over dues are substantially brought down, the impact of various measures to improve viability of banks would not be visible. Strategic defaulting of loan is quite widespread among the opportunist farmers who consider government sponsored loans more as gift than as debt that have to be repaid. It is against this backdrop that this study is designed to analyze the causes of loan default and default prevention strategies of agricultural cooperatives in Benue State of Nigeria.

A numbers of studies have been carried out on causes of loan default (Udoh 2008, Aseyo 2013, Kuye 2015, and Owusu *et al*., 2015). These studies focused mainly on formal financial institutions. No study has been carried out on causes of loan default and default preventive measures among agricultural co-operatives. This study fills the gap in the literature by analyzing the causes and measures for controlling loan default among agricultural cooperatives in the study area. The study was designed to provide answers to the following questions:

i. What are the causes of loan default among agricultural cooperatives?
ii. What are the measures for controlling loan default among agricultural cooperatives?

II. METHODOLOGY

The study was carried out in Benue State of Nigeria. The state was created from the former Benue Plateau State on February 3rd, 1976 when the country was further split from 12 to 19 states (Ajaero (2007)). It lies at the middle-belt region of Nigeria and has a population of 4,219,244 people (National Population Census (NPC, 2006). Benue State occupies a land mass of 30, 955 square kilometers and is bounded by Nasarawa State to the North, Taraba State to the North East, Cross River State to the South, Enugu State to the southwest and Kogi State to the west. It also shares a small section of the national boundary with Republic of Cameroun to the South East of the State (BNARDA, 1986)

The State lies between latitudes 6°C 25' and 8°C 8' North of the equator, and Longitudes 7°C and 10' East (Ajaero, 2007). The State is endowed with a tropical climate. The rainy season starts from April and lasts until October, while the dry season begins from November and ends in March. The annual rainfall is between 150mm – 180mm. The temperature fluctuates between 23°C to 30°C most of the year. The main occupation of the people of Benue State is farming. Benue State has a vast area of land and produce part of the food which feeds the whole nation. The present Benue State is blessed with agricultural produce, such as yam, cassava, potatoes, rice, millet, guinea corn, groundnut, maize, sesame, soya beans, and a vast range of fruits and vegetable. This earned the State the slogan "Food Basket of the Nation". The State has 23 Local Government Areas (LGAs) and 275 council wards (Ajaero, 2007). For administrative and operational purposes, Benue State is divided into three agricultural zones by Benue State Agricultural and Rural Development Authority (BNARDA, 1986). The three zones are as follows: - North Eastern zone (Zone A):- Katsina-Ala, Kwande, Konshisha, Logo, Ukum, Ushongo and Vandeikya LGAs, North Western Zone (Zone B): - Buruku, Gboko, Guma, Gwer East, Gwer West, Makurdi and Tarka LGAs, Southern Zone (Zone C): - Ado, Agatu, Apa, Obi, Ogbadibo, Ohimini, Oju, Otukpo, Okpokwu LGA. It adopted multistage sampling techniques.

The first stage involved purposive selection of one Local Government Area (LGA) each from the three agricultural zones based on the high concentration of Co-operatives, namely Ukum, Makurdi and Otukpo. In the second stage, a sample proportion of 25% across board was used to obtain a sample of 130 respondents from the selected co-operatives.

Data were collected using structured questionnaire. Information were collected on the causes of loan default, and measures to control default among agricultural cooperatives in the study area. Data collected were analyzed using descriptive statistics.

III. RESULTS AND DISCUSSION

Causes of loan default among agricultural cooperatives

Causes of loan default were categorized into institutional, client related, geo-environmental and market related factors, and presented on table 1.

Institutional factors

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The result showed that late disbursement of fund to borrowers was the major (92.31%) cause of loan default. This could be attributed to bureaucratic practices of most lending institutions in the study area. Clients' requests pass from table to table and this prolongs the management appraisals and results to late disbursement of fund. Most affected are those involved in the agricultural sectors because their activities are usually tied to the prevailing weather conditions. Late receipt of loan delays the planting season, and results to reduction in yield. This may result to loan default. This finding concurs with Sheila (2011) that early disbursement of fund is critical to minimize default.

Client-related factors

Lack of integrity of client (80.77%) causes loan default, as farmers no longer adhere to the terms and conditions of loan set up by Agricultural Cooperatives. They diverted funds to unproductive business other than their original request, thereby resulting to their inability to repay loan. This finding agrees with Neininger *at.al* (2011) that lack of integrity in the business environment is a major contributor of organizational cynicism. Organizational cynicism can cost company money in productivity, waste, injuries, sick calls and inability to repay loan by an organization.

Geo-environmental factor.

Death of client (70.77%) causes loan default. This is an external factor which becomes very difficult for Agricultural Cooperatives to have control over, hence natural phenomenon. Agricultural Cooperatives that take borrowers who did not take life insurance cover stand a chance of losing their investment via loan default. This agrees with Dorfman (2004) that insurance should be part of risk management when starting an agricultural business.

Market-related factor

Lack of market information (81.54%) is related to lack of information about production. For instance, clients keep their produce for a very long period of time because they have limited information on who to buy, where to sale, when to sale to maximize profit and the buyer in other hand have a limited information on whom to buy, where to buy and when to buy to minimize loss thereby resulting to delays in loan repayment and eventually default. This finding agrees with the finding of Chadnra (2017) that one of the major problems confronting agricultural marketing in India if lack of market information.

Table.1: Cause of loan default (n = 130)

Causes	frequency	percentage*
Institutional		
Late disbursement of fund to borrowers	120	92.31
Lack of loan monitoring	100	76.92
Inadequate loan appraisal	90	69.23
Lack of clear cut policy on lending	90	69.23
High interest rate on lending	36	27.69
Conflict of interest by loan administrators	33	25.38
Monopoly of power	18	13.85
Others (Gift to client during festivities)	3	2.31
Client related factors		
Lack of integrity	105	80.77
Poor business practice	102	78.46
Loan diversion	65	50.0
Lack of technical skills to keep record	34	26.15
Conflict of interest among administrators family	22	16.92
Geo-environmental factors		
Death of client	92	70.77
Economic downturn	72	55.38
Natural calamity	68	52.31
Market related factors		
Lack of market information	106	81.54
Market location	64	49.23
Wrong economic decision	53	40.77
Proximity of market	36	27.69

Source: Field survey data, 2017. *Multiple responses existed, hence >100%

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Measures to control loan default

The result of measures control loan default is shown in table 2, obtaining information on client integrity (92.23%) is the best measure to control loan default. This is an appraisal issue. Customer integrity is a key factor in appraisal process. Lending officers consider borrowing proportion and subsequent repayment in isolation from security. The borrowers are screened based on the future and the past. Lending is based on integrity, liquidity, purpose, profitability, spread, and suitability. This result agrees with Bigambah (1997) that the loan default in Uganda was attributed to loan appraisal as the key factor. Sometimes, information received is not verified, while in some cases the information received is doctored or falsified.

The result further revealed that training of borrowers on terms and condition of loan was indicated by 81.54% of the lenders. Prevention is better than cure. Borrowers are made aware of the condition of loan in the offer letter presented to them by the lending institutions and this plays an important role in loan default prevention. This concurs with the finding of Teskiewicz (2007) that it is important to stress during the client-education stage, both the benefits received due to punctual payment as well as the cost incurred by the client for late payments.

The result also shows granting loan amount proportionate to business expenses (28.46%) is a measure that is capable of preventing default. This is because if the amount is granted proportionately, there will be no room for wastage and the business will not be under funded. This is in consonance with the finding of Peter (1995) that accurate estimates are the best outcome. Underestimation can impact dependencies and the overall quality of the project.

Overestimation may be wasteful for the resources on a particular task, but it is less likely to impact other tasks or overall quality. Others are monitoring of borrowers business activities, granting loan amount above business expenses and below business expenses.

Table.2: Measure to control loan default among the agricultural cooperatives (n=130)

Measures	Frequency	Percentage*
Obtaining available information on borrowers integrity	120	92.23
Training borrowers on the terms & condition of loan	106	81.54
Granting loan amount proportionate to expenses	37	28.46
Monitoring of borrowers business activities	15	11.54
Granting loan amount above business expenses	14	10.77
Granting loan amount below business expenses.	8	6.15

Source: Field survey data, 2017

*Multiple responses existed, hence >100%

IV. CONCLUSION AND RECOMMENDATIONS

Evidence from the study has shown that late disbursement of fund, lack of monitoring, lack of integrity, poor business practice, death of client, economic downturn, lack of market information, and market location were major factors that cause loan default while, obtaining available information on client integrity, training of borrowers on terms and condition of loan before loan are granted were found to be best measures to control loan default.

It is recommended that Sound and flexible lending flexible policy measures must be reviewed frequently by Central Bank of Nigeria (CBN) and Bank for Agriculture and Agricultural Co-operative (BAAC) in order to curtail bureaucracies involved in the management approvals and disbursement of agricultural facilities. This will ensure early disbursement of funds, and in line with prevailing weather condition. Professional appraisal officers should handle and properly assess the repayment capacity and/or willingness of the client to repay loan, while life insurance cover on every borrower must be in place by all lending institutions to cushion the effect of loan defaults as a result of client death. Market information is essential for producers, traders, consumers as well as the government. Marketing Boards should be set in all agricultural zones to disseminate the character and the volume of supply of commodity, the present and expected level of consumers demand, current price quotation and future price trend for different type of products and their probable impacts on price market information to the public.

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Climate change and dynamic of lands occupation at the hippopotamus pond biosphere reserve in Burkina Faso

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Abstract— *In Africa, forest cover and timber resources experienced a sharp decline, especially in the last thirty years. Burkina Faso has experienced major droughts, especially from1965-1966to 1991-1992 and 1994 – 1995 with serious impact on agriculture, water resources and natural vegetation as well as the indirect consequences on health, economy and institutions.*

The forests are located in areas dominated by subsistence production agricultural and many herds of cattle. The growing needs for firewood leads to anarchic cuts causing the deterioration of forest genetic resources. The Biosphere Reserve of "Mare aux hippopotames (RBMH)" although its International statute knows these phenomena. The study aims generally to contribute to an assessment of the impacts of climate change on the lands occupation of the Biosphere reserve. The specific objectives are: i) understand the perception of the people on climate change and its effects; (ii) study the dynamics of lands occupation in the RBMH and iii) Identify the causes of decline of ecosystems. The approach consisted in a diachronic analysis to assess the dynamics of lands occupation and semi structured interviews to collect the effects and manifestations of climate change with60 men and 40 women from 10 villages of the RBMH.A list of 29 climate resiliencehas beencited by all the villages. The incidence of disease is the largest followed by the lack of drinking water and drought; floods, overload work and drought are the most severe.

Our results could contribute to take actions coping with climate change and variability.

Keywords— Climate shock, Impact, Land exploitation, Vulnerability, Severity.

I. INTRODUCTION

In Africa, forest cover and timber resources experienced a sharp decline, especially in the last thirty years. According to [17], studies on the evolution of the climatic zones in Burkina Faso show that over 40 years, the Sahelian zone extends while the Sudanian zone is reduced. Indeed, Burkina Faso had major droughts, especially in 1965-1966, 1972, 1974, 1981 to 1984, in 1986-1987, 1991-1992 and 1994 - 1995 [6]. The result is a severe direct impact on agriculture, water resources and natural vegetation as well as the indirect consequences on health, economy and institutions.

Accordingly, population, migration and climate pressures resulted in an intense desertification and over-exploitation of natural resources, threatening protected areas. In the classified areas hunting activities farming and agriculture and other human activities are prohibited or regulated, and the country conducted strategy of conservation of biodiversity, from 1926 to 1937., In general, this classification has been done without the participation of the populations, causing frustrations and various hostilities such (poaching, bush fires, agricultural clearings, pastures). Thus, the classified forest of the Hippopotamus pond was created in 1937 by the Decree No. 836 SE of March 26, 1937, like various other forests in Burkina Faso. This forest got the statute of Biosphere Reserve by decision of the MAB International coordination in 1987, then as Ramsar site in 1990.

According to [10] the forests are located in areas dominated by a subsistence agricultural production and by many herds of cattle. The growing needs for fires wood of populations and particularly in large urban areas, lead to anarchic cuts which are one of the main causes of the deterioration of forest genetic resources.

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The Hippopotamus pond Biosphere Reserve is not in secure of certain phenomena which may bring into question its status as International Biosphere reserve. Biosphere reserves have objectives to serve research, education and training in the field of ecology [20].

The overall objective of our study is to contribute to an assessment of the impacts of climate change on vegetation formations of the Hippopotamus pond Biosphere Reserve (RBMH)of Burkina Faso..

.The specific objectives are to i) understand the perception of local communities on climate change, ii) to study the dynamics of the occupation of the lands of the RBMH and the impact on ecosystems and iii) to identify with local people the causes of regression of some vegetation formations.

II. MATERIALS AND METHOD

2.1. Presentation of study site

2.1.1 Localization of the study site

Hippopotamus pond Biosphere Reserve is located between 11°30' and 11°45' N and 04°05'and 04°12'O at South-west of Burkina Faso.It covers 19 200 hawith a permanent river of 660 ha.

This forest is surrounded by a dozen of villages including Bala, Sokourani, Tierako. "Fig 1". The name of the forest derives from the pond it hosts and where Hippopotamus live permanently[18]. The RBMH is located in south-soudanian phytogeographic sector and in the south-soudanian climatic domain according to [11]. The strictosensu Biosphere Reserve is concerning 16354 ha according to [1]

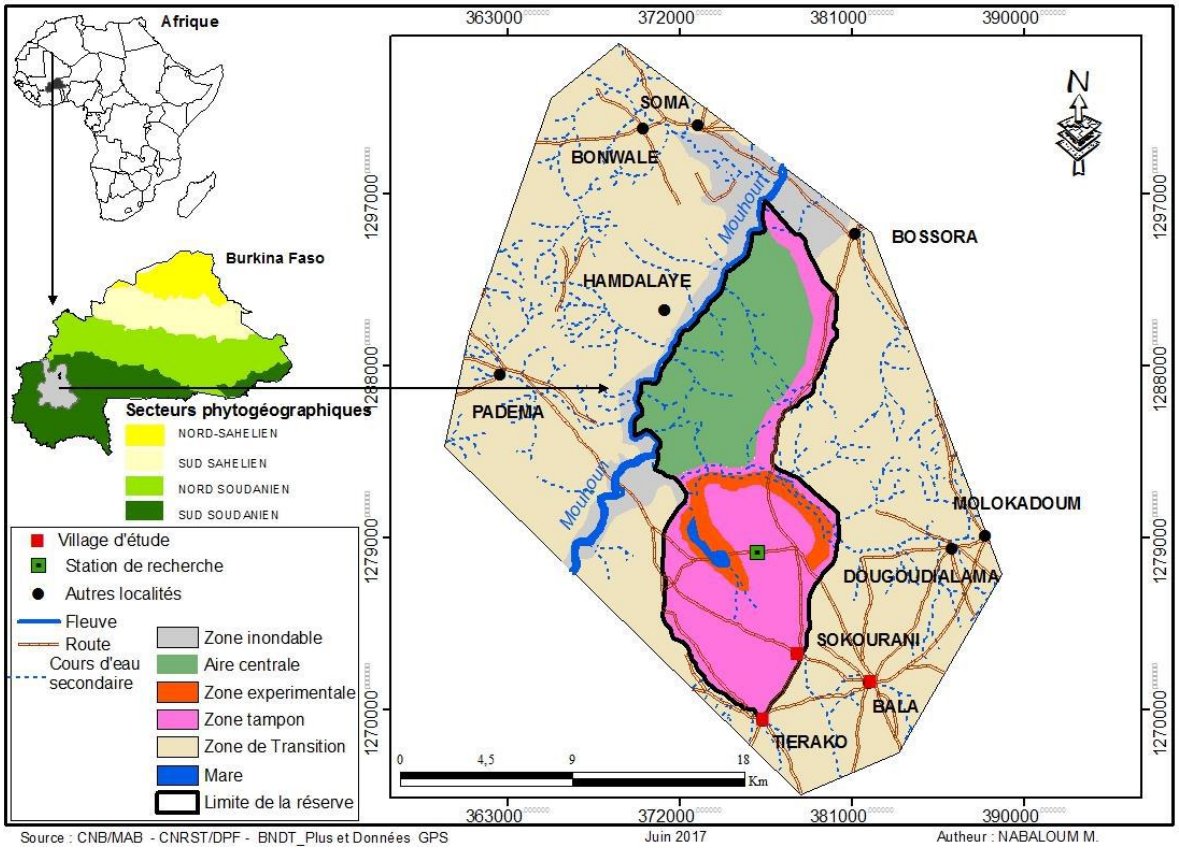

Fig: n°1: Localization of Hippopotamus pond Biosphere Reserve

2.1.2. Climate of RBMH area

The south-sudanian climate of RBMH is characterized by a dry season which runs from November to April and a rainy season or winter from May to October. The average rainfall over the period 1986 to 2015 is about1080 mm/year. The evolution of rainfall during this period indicates a very strong inter annual variability with an upward trend. The monthly mean temperatures are relatively weak between 25 and 31°C. "Fig2".Some droughts, particularly severe have been registered as those of 1972-73 and then from 1983-84, which have had dramatic consequences for the agro-forest-pastoral productions[6]. One of the direct consequences of the reduction of rains and their variability is the increase in the frequency of dried years, with an acceleration of desertification and silting of waterways, accentuated by the decline in density of the vegetation, the ageing of stands and the threat of extinction of some species.

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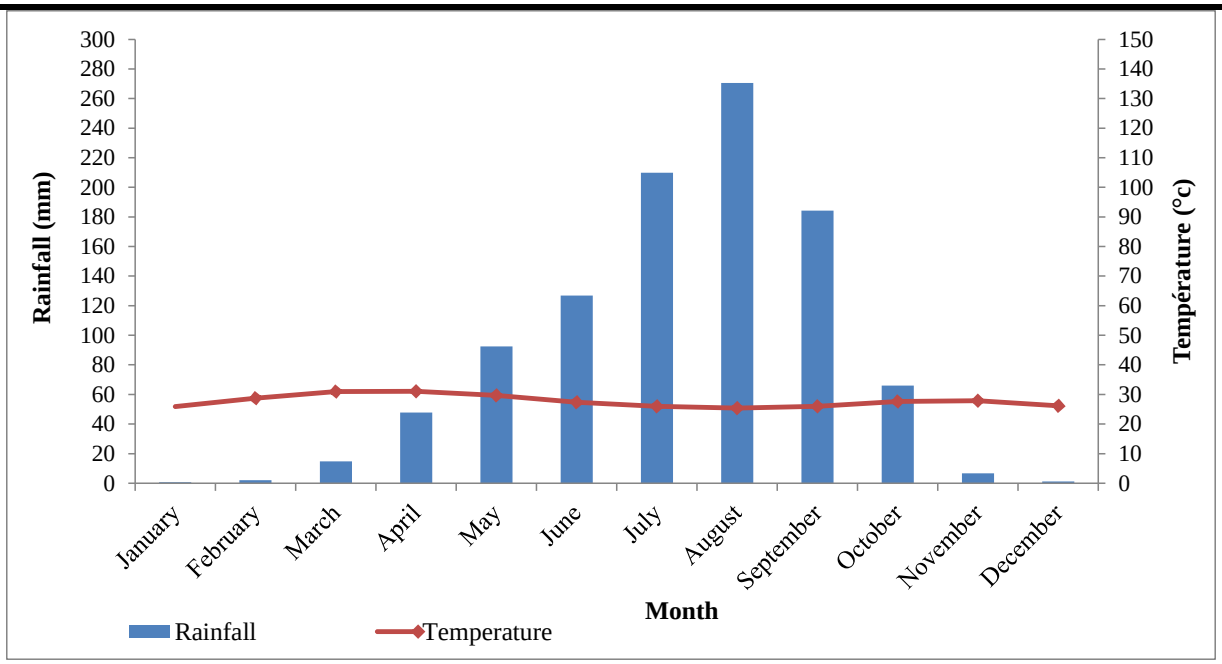

Fig.n°2 :Ombro-thermic diagram of the station of BoboDioulasso (1986-2015)

The annual height of rainfall reported in "Fig. 3" do not meet certain species of Combretaceae rainfall needs such *Combretumglutinosum, Pteleopsissuberosa, Terminaliamacroptera*, etc. [12].

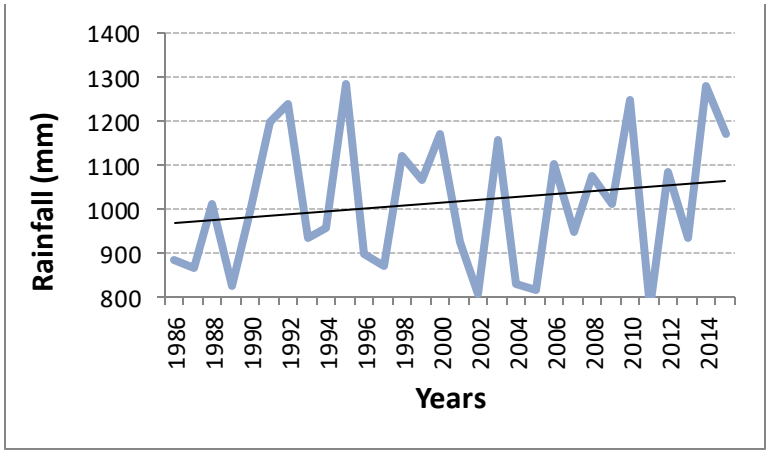

Fig. n° 3 : Rainfall High at the station of BoboDioulasso from 1986 to 2014

2.1.2. Relief and Soils

The relief of the Biosphere Reserve and the surrounding villages is relatively flat with an average altitude ranging between 280 and 320 meters.

Four types of soils are distinguished at the level of the reserve area according to [1] :

- soils on battleship made up of ferruginous soils truncated;
-tropical ferruginous soil leached;
-alluvial soils including mineral soils to "Gleyoxydated" along the temporary streams and on the alluvial strip of Mouhoun.
-hydromorphic soils at the level of the flood plains.

2.1.3.Hydrographyof RBMH area

The Mouhounriver, main watercourse with permanent flow, with its tributaries as the Wolo, the Tinamou and the Leyessa, irrigate the reserve and the villages in the South and central parts [1]. The pond is a lake lying N/NW-S/SE of about 2.6 km long and 700 m wide. In times of flood, water runs and floods the East Coast and West, favouring thus the displacement of the hippopotames from the flooded area to any backwater or pocket of water[18], [20].Floodings of the pond are highly dependent on those of the Mouhoun.

2.2. Study method

2.2.1. The mapping

The method of production of the maps of the lands occupation at the RBMH in 1992, 2002 and 2013 is

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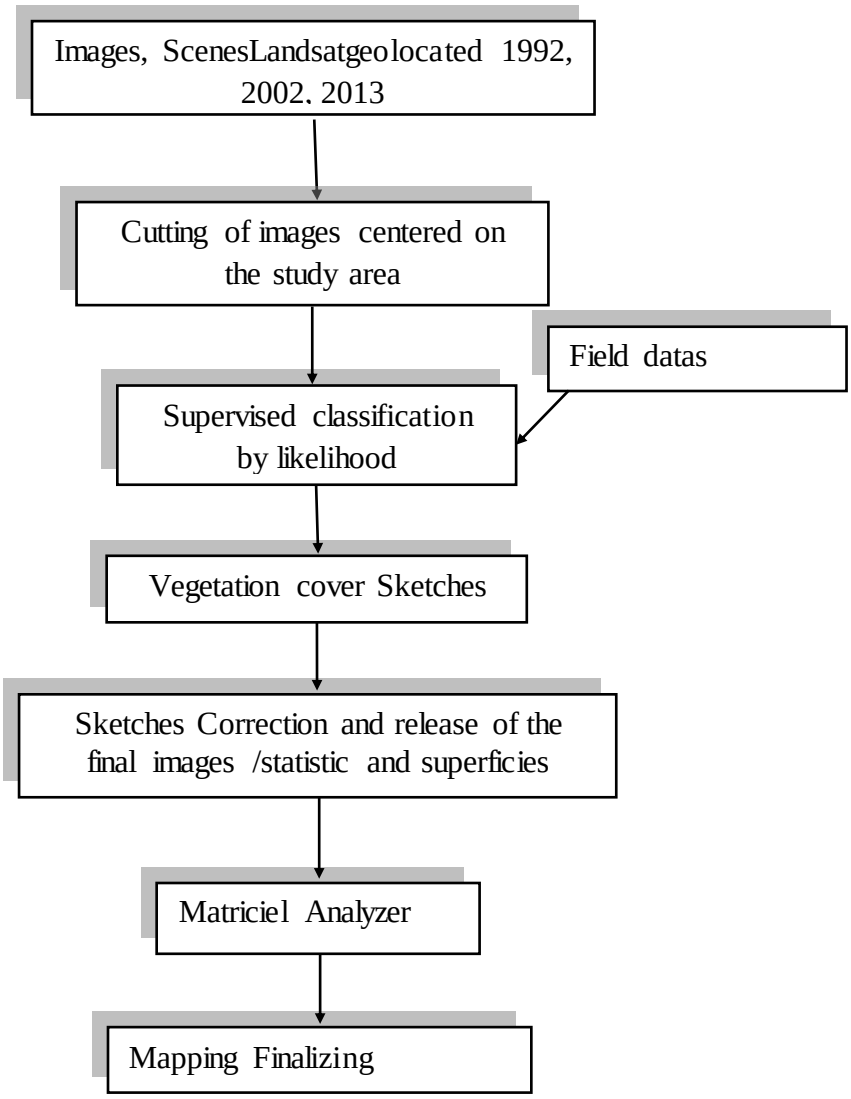
summarized in "Fig.4" below. It required the exploitation of data and their treatment. The images used are type Landsat imagesTM, ETM, ETM+ for the years 1992, (L4B10_19921016196050S), 2002 (L7B50_20021028196052), 2013 (LC8_1960522013 3 07LGN00_8bit). The vector datas used are those of the national topographic database (BNDT) of IGB. ENVI 4.5 for the treatment and classification of satellite images (phase 1).



Source: the authors
Fig.n°4: Methodological Scheme of Maps production

2.2.3.The inquiries

Investigations consisting to collect the perception of each group on climate change effects, feelings and coping strategies. A questionnaire guide used helped identifying all felt shock and adaptation strategies. Once the shocks are listed in each group, we did a harmonization of the terms. Then, the populations have themselves hierarchized the shocks according to their importance. Adaptation strategies developed by people to deal with the multifaceted climate shocks have been recorded with a focus on adaptation in relation to the vegetation according to the perception of men and women.

Analyzing the severity of the climate gave a resulting list of shocks. This list was used then for the calculation of the impact, of the severity and Risk Index (Ij). From the impact and the severity, the severity map of a village, the group of men or women can be established. In the analysis of the map, a strong value translated a significant impact of the shock. However, great severity is associated with a low value.

Risk indices were calculated in a participatory manner according to the method cited by [17] and [19].Thus, the equations below have been applied:

: Index of severity Sj (1-2) :1 + (r-1) /(n-1)

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r = rank of the threat (by order of importance according to the participant)
n = total number of threats listed by the participant;
Then we calculate the average for all participants who have listed a certain threat to get the average severity**, Sj :**
Impact Ij= listed type of threat x-time / number of participants
Risk Index (0-1) :Rj :Ij/Sj

III. RESULTS AND DISCUSSION

3.1. Description of vegetation of RBMH

This description indicates that the reserve is the clear,field of woodlands, treed and shrubby savannas and forest galleries.

3.1.1. Forests Gallery and clear Forests

Forests Gallery are found on the banks of the Black Volta River and its tributaries, and are characterized by*Berliniagrandiflora*, *Vitexdoniana*, *Cola cordifolia*, *Khayasenegalensis*, *Erythrophleumguineensis* and *Diospyrosmespiliformis*.

The clear forests of RBMH are composed of *Prosopisafricana*, *Danielliaoliveri*, *Ostryoderrisstuhlmannii* and treed savannas constituted by a treed strata with *Pterocarpuserinaceus*, *Prosopisafricana*, *Danielliaoliveri*, *Xeroderrisstuhlmannii* and *Anogeissusleiocarpus*. The big characteristic of these two vegetation kinds is the existence of herbaceous stratum made up of perennial grasses dominated by *Andropogontectorum*[1], [8].

3.1.2.The treed savannas

In the RBMH, the treed savannas have a medium coverage. The floristic composition is composed of*Isoberliniadoka*, *Terminaliaspp*, *Danielliaoliveri* and *Vitellariaparadoxa*[1],[9],[20].

3.1.3.The shrubby Savannah:

It covers large areas in the biosphere reserve. The shrub stratum is composed mainly of *Detariummicrocarpum*, *Combretumcollinum* and *Crossopteryxfebrifuga*. The herbaceous stratum is made up of perennial grasses [1], [8].

3.1.4. The bushes:

They thrive on the micro reliefs such areas attacking in the Biosphere Reserve. This type of vegetation contributes to the maintenance of these micro reliefs by contributions of plant debris and by promoting the installation of the mounds [20]. Thicket vegetation is composed of shrubs of *Combretumglutinosum*, *Combretumghazalense*, *Capparissepiaria*, *Leptadeniaangolensis*, *Gardenia sokotensis*, *Acaciamacrostachya*. andcreepers as *Saba senegalensis*, *Baisseamultiflora*,and *Acacia penneta*.

3.1.5. Wetland Vegetation

It is found in the Biosphere Reserve because of the permanent presence of water in the pond and the Mouhoun River. The aquatic and/or semi-aquatic vegetation are developed around the pond as well as the floodplains of Mouhoun[1],[9],[20].Thus, from the pond to the outside, there isprogressively : i) a floating vegetation or water meadow with a belt around the pond consisting of species likes *Pistiastratiotes*, *Trapanatans*, *Azollaafricana*a,ii) a dense thicket hardly penetrable composed of *Ficustrichopoda*, iii)a treedsavannah with *Mitragynainermis* and *Vetiverianigritana*.

3.1.6. The parklands

They occupy more than 80% of the Burkina national territory. The trees of the field or parklands have been so preserved because of the products and services they provide to local communities [5], [22].The fields of the RBMHRiverside villages are dominated by woody species with in majority *Vitellariaparadoxa*, *Parkiabiglobosa*, *Tamarindusindica*, with some companions species such as *Terminaliamacroptera*, *Ficusgnapalocarpa*, *Lanneamicrocarpa*. *Sclerocaryabirrea*, *Bombaxcostatum* and *Khayasenegalensis*.

3.1.7. The sacred wood

The sacred woods are islands of vegetation located near villages, often presented as relics of natural forest, preserved of human action in respect to traditions and to fear the criminals minds that they host. They are forest fragments associated with a spirit, a divinity or a temple. These are traditional protected areas, directly managed by the local population for cultural purposes[21]. The component speciesof these areas in the RBMH include *Afzeliaafricana*, *Annonasenegalensis*, *Cordiamyxa*, *Diospyrosmespiliformis*, *Gardenia erubescens* and *Securidacalondepedunculata*.

3.1.8. The fields

In houses fields are cultivated cereals like millet, sorghum and maize which are the main food of the population. A little further away from the houses are villages' fields where speculations like peanuts, cotton and tobacco are grown in addition to grain. Several kilometers of the course, households cultivate in Bush's. fields[22]. .

3.1.9. Bare areas

They correspond to two facies, one hydromorphic and the other gravillonnaire. The hydromorphicfacies appears

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onthe hollower, with a compact slab on the microtopographies. The gravillonnaire layer, although very little deep (about 10 cm), is not flush; the 'termite mushrooms' are particularly numerous. The vegetation, which is very poor, is composed of some annual grasses like *Loudetiatogoensis* and *Andropogonpseudapricus*, with a perennial feature at very low recovery *And ropogonascinodis*. The gravillonnairefacies is an intermediate microtopography little affected by the hydromorphy.

3.1.10. Water, aquatic areas and flood plans

These facies of vegetation are observed around the pond itself as well as in the areas of overflow of Mouhounriver. According to [12],from the pond to the outskirts, there are several landscape units whose the aquatic grassland. Woody riparian vegetation or Gallery of the pond and treed savannah with *Mitragynainermis* and *Vetivernianigritana*.

The aquatic grassland of the flooded area includes two strata. The first, submerged and the water or floating, includes species such as *Ceratophyllumdemersum, Trapanatans, Pistiastratiotes, Azollaafricana* and *Utriculariainfexa var. infexa*. [3]. The second, about a meter high contains the following set *Apodostigmapallens, Ipomoea rubens, Neptuniaoleracea, Oxycaryumcubense, Vossiacuspidata, Echinochloastagnina, Pycreusmundtii, Ludwigiaadscendens, Ludwigiastenoraphe, Cyclosorusstriatus, Leersiahexandra and* diverses Polygalaceae.. Woody riparian Vegetation encircling the water meadow has two levels: a stratum of shrub with*Ficuscongensis, Canthiumcornelia, Alchorneahirtella, Trichiliaemetica, Phyllanthusreticulatus* and *Mimosa pigra*a treed stratum with *Morelia senegalensis, Syzygiumguineense* covered with *Ipomoea rubens* and *Pterocarpussantalinoides*[4]. [5].

3.2. Dynamic of lands occupation at RBMH from 1992 to 2013

The "Table1"defines each unit and its evolution in the time and in the space.

Table.I: Dynamic of lands occupation at RBMH

lands occupation	1992 (ha)	% 1992	2002 (ha)	% 2002	2013 (ha)	% 2013	Tendancy (1992-2013)
Galleryforest	1179,79	6,9	1179,79	6,9	1424,67	8,4	progression
Clear Forest claire	11640,37	68,6	9265,93	54,6	9636,92	56,8	regression
ShrubbySavannah	1864,13	11	4129,28	24,33	4520,25	26,6	progression
*Parklands*territories	0,0022	0,13	0,0022	0,13	252,04	1,5	progression
Field	339,74	2,13	449,31	2,73	826,79	4.9	progression
Water	1039,11	6,12	1039,11	6,12	55,33	0,32	regression
Aquatic grassland	683,88	4,03	683,59	4,02	447,60	2,6	regression
Bare grounds	200,22	1,18	200,22	1,18	58,11	0,34	regression

In the RBMH, the clear forest being the largest land unit went from 68.6% in 1992 to 56.8% in 2013. The second unit is the shrubby Savannah who went from 11% to 26.6% between 1992 and 2013. The gallery forest grew from 1179,79 ha to 1424,67 ha from 1992 until 2013, causing a reduction of 245 ha. It remains the vegetation having the lowest area. The fields and parklands territories are on the rise in the reserve. They move from 2 to 4.9% and 0.13 to 1.5 during the same period. These have increased at the expense of the natural wooded formations. The increase in very close crops of the RBMH vegetation formations against natural one is another cause of the destruction of the vegetation according to [13]. The following map shows the land occupation at RBMH from 1992 to 2013

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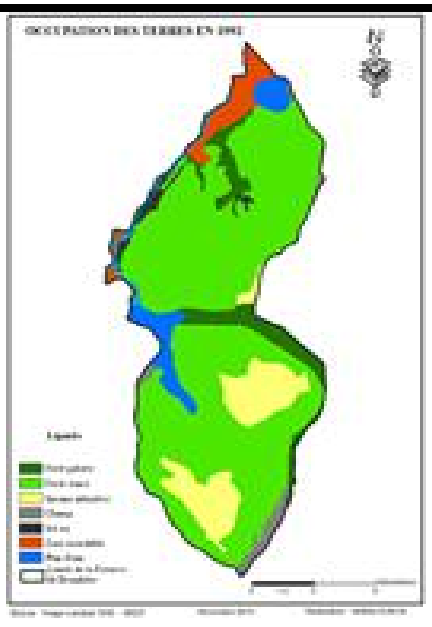
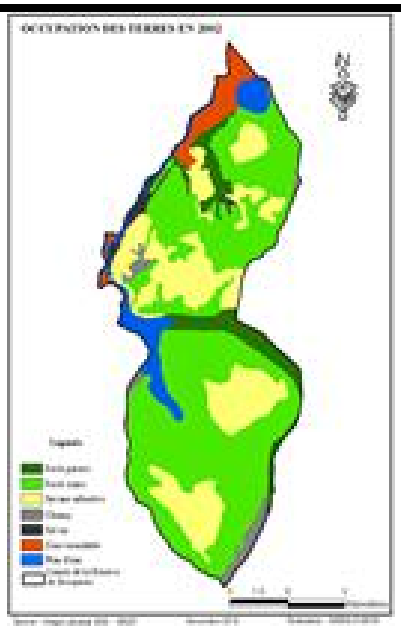
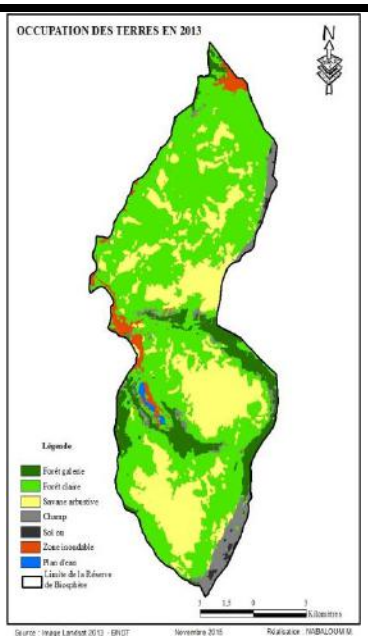

Land occupation in1992


Land occupation in2002



Land occupation in2013

Fig. n°5: Land occupation Map of RBMH from 1992 to 2013

After seven years survey, [1] has clearly shown that there is also a tendency to the disappearance of many species within the forest galleries of the RBMH. However, this study showed no evidence that the decline is directly linked to the actions of local residents (removal, Bush fires). Indeed, the causes of this degradation are also at the level of climate change (precipitation down) but also in facilities designed around these environments (dams, track,...)[11]..

A general observation of the environment of Burkina Faso shows a degradation of natural resources (soil, water, biomass and biodiversity resources) because of multiple factors[7], [10].These factors are, among others, the pressure on natural resources due to the increase of the population, access to bit secure land, low productivity of farming systems and livestock, a weak implementation of the legislative framework. Low ownership of the management of natural resources and a lack of development of biodiversity.

Of course, it was impossible for [3]to definitively identify the specific reasons for the decline in quality of these areas of great ecological interest "Fig 5". But this study may confirm that certain practices can be modified to slow down, or even to counter the impoverishment of middle in terms of diversity of woody according to [15],[16],

3.3. Climate Change and people vulnerability according to gender

3.3.1. Climate Change impact according to gender

The observation of the population indicates a significant increase in temperatures and a decline in rainfall. "Table 2" shows 29 shocks listed by all of the surveyed population. Among these shocks, diseases are the most important with an incidence of 0.6, followed by the lack of drinking water and the drought with respectively 0.5. Low impact shocks include deforestation, fruit reduction, and poverty, with a value of 0.4. population growth, degradation of soils, the disappearance of wildlife, the disappearance of fishing, the Hunger (0;3); the disappearance of medicinal plants, high temperatures (0.25); unemployment, Exodus, lack of wood, agricultural mechanization, human mortality, the land problem, work overload and the drying up of River (0.2). Eight very low impact shocks were given: the lack of fodder, Guinea worm, the winds, the livestock mortality, floods. the disappearance of market gardening, the abandonment of customs.

Table n°2 :Impact values in the whole villages according to gender

N°	**Shocks of all men and women**	**Impact**		
		W, M	**W**	**M**
1	Abandonment of customs	0,1		0,1
2	Unemployment	0,2	0,3	0,1
3	Demographicgrouth	0,3		0,3

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4	Deforestation	0,4	0,4	0,3
5	SoilsDegradation	0,3	0,3	0,3
6	Diminution of fruits plants	0,4	0,4	0,3
7	Disappearance of medicinal plants	0,25	0,25	
8	Disappearance ofwildlife	0,3	0,3	0,2
9	Disappearance of market gardening	0,1	0,1	0,1
10	Disappearanceof fishering	0,3	0,25	0,2
11	Animal divagation	0,1		0,1
12	Exodus	0,2		0,2
13	Hunger	0,3	0,2	0,2
14	High temperatures	0,25	0,25	
15	Floodings	0,1		0,1
16	**Diseases**	**0,6**	0,6	0,5
17	Lack of wood	0,2	0,3	0,1
18	**Lack of water**	**0,5**	0,5	0,4
19	Lack of Fodder	0,1	0,1	
20	agricultural mechanization	0,2	0,2	0,2
21	Lifestockmortality	0,1	0,1	
22	Humanmortality	0,2	0,2	0,1
23	Poverty	0,4	0,6	0,2
24	Bussiness land problem	0,2	0,2	0,2
25	**Drought**	**0,5**	0,3	0,7
26	Overloadwork	0,2	0,2	
27	River drying	0,2		0,2
28	Violent winds	0,1	0,1	0,1
29	Guinea worm	0,1		0,1
			22	24

3.3.1.1. According to women

The data of "table 2" show that 24 shocks have been identified by the women. The highest incidence (0.6) is given by poverty, disease and lack of water. Seventeen shocks have a low impact and are as follows: 2 with impact = 0, 4 (fruit reduction, deforestation); for 6 shocks impact is 0.3 (drought, degradation of land, unemployment, hunger, endangered wildlife and lack of wood);. We noted 3 shockswith impact = 0.25 (high temperatures, endangered medicinal plants, and endangered fishing); and 6 shocks with impact = 0.2 (land problem, famine, lack of wood, lack of agricultural equipment, human mortality and work overload). Four shocks have a very low incidence (0.1): lack of fodder, disappearance gardening, winds and livestock mortality.

3.3.1.2. According to the men

On a total of 22 given shocks, drought emerges as the first shock with high incidence (0.7). Diseases have an incidence of 0.5. Low impact shocks are the lack of water (0.4); degradation of soils, deforestation, fruit reduction, population growth (0,3); poverty, land problem, agricultural mechanization, hunger, endangered wildlife, endangered fishing and exodus (0.2); floods, abandonment of customs, Guinea worm, straying of animals, human mortality, violent winds disappearance gardening and unemployment (0.1).

3.3.2. Severity in all the study villages according to gender

Concerning the severity of shocks (ranging from 1 to 2), a low value means great severity. For this purpose, the floods, overload work and drought are the most severe with respectively 1, 1 and 1.1. Five shocks have a severity equal to 1.4: abandonment of customs, unemployment, population growth, lack of water and drying up of rivers. A single shock has a medium severity (1.5) the hunger. Low severity impacts include respectively the hunger (2), exodus (1.9), and six shocks with severity = 1.8 as: high temperatures, lack of wood, soil degradation, lack of fodder, human mortality and straying of animals. Three shocks with severity = 1.7 are: gardening, agricultural mechanization, and cattle mortality. Seven shocks with severity = 1.6 are: diseases, fruit drop, loss of medicinal plants, poverty, land problem, violent winds and Guinea

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worm. From all, the drought seems to be the most indicative shock of the climate change.

Table n°3: Severity values in all the study villages according to gender

N°	Shocks of all men and women	Sévérité		
		W,M	W	M
1	Abandonment of custums	1,4		1,4
2	Unemployment	1,4	1,4	1,5
3	Dmographicgrougth	1,4		1,4
4	Deforestation	1,3	1,5	1,2
5	SoilDegradation	1,8	1,6	1,9
6	Diminution of fruits trees	1,6	1,6	1,7
7	Disapearanceof medicinal plants s	1,6	1,6	
8	Disapearanceof wildlife	2	1,9	2,1
9	Disapearanceof gardenmarket	1,7	1,5	2,0
10	Fisheringdisapearance	1,5	1,5	1,4
11	Animalsstraying	1,8		1,8
12	Exodus	1,9		1,9
13	Hunger	1,5	1,4	1,4
14	Hightemperatures	1,8	1,8	
15	Floodings	1		1,0
16	**Deseases**	1,6	1,4	1,8
17	Lack of wood	1,8	1,6	1,6
18	**Lack of water**	1,4		1,7
19	Lack of fodder	1,8	1,8	
20	Agricultural Mechanization	1,7	1,8	1,7
21	Cattlemortality	1,7	1,7	
22	Humanmortality	1,8	1,8	2,0
23	Poverty	1,6	1,5	1,7
24	Land problems	1,6	1,9	1,3
25	**Drought**	1,1	1,1	1,2
26	Overloadwork	1	1,0	
27	Riverdrying	1,4		1,4
28	Violent winds	1,6	1,2	2,0
29	Guineaworm	1,6		1,6

3.3.2.1. According to women

In terms of the severity of each of these 24 shocks, overwork has maximum severity (1). Then successively follow the drought (1,1), lack of water, winds (1,2), disease, unemployment, and hunger (1,4). 5 shocks have a medium severity = 1, 5 (poverty, deforestation, gardening disappearance, hunger and fishing disappearance). The less severe shocks are represented by the degradation of the soil, fruit reduction, lack of wood, endangered medicinal plants (1.6); cattle mortality (1.7); lack of agricultural equipment, lack of fodder, human mortality and high temperatures, (1.8); then the land problem, endangered wildlife and lack of wood (1.9). Women severity S map "Fig. 6" shows that water scarcity and drought stand out among the shocks with to strong impact and average severity.

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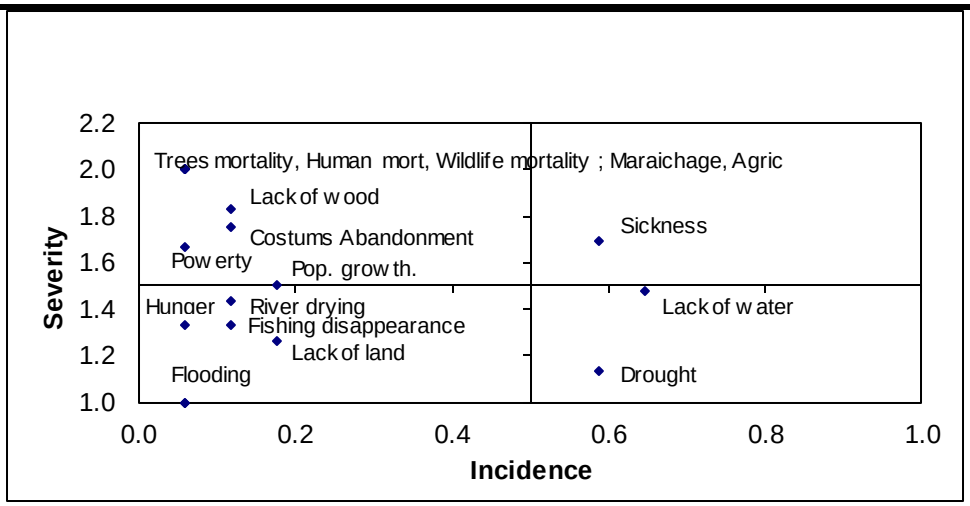

Fig n°6: Women severity S map

The surveyed women are unanimous in recognizing that the flooding, lack of land, the Hunger and the drying up of the pond are the main causes of degradation of natural resources.

3.3.2.2. According to men

Respectively the severity of shocks is dominated by the floods with a maximum severity (1), drought and deforestation (1,2), the property issue (1,3), hunger, endangered fishing, population growth, dry river,. customs abandonment (1,4). Unemployment has amedium severity. Then follow in last position, Guinea worm, lack of wood (1.6), poverty, lack of water, agricultural mechanization, fruit decrease (1.7), diseases, river drying (1.8) exodus, degradation of soils (1.9). 3 shocks have zero severity (winds, human mortality, and disappearance gardening).

3.3.2.3.Men Severity Card

Map of severity of shocks "Fig 7" listed by men let see shocks groups emerging: shock with impact and high severity (drought), shock with impact and medium severity (lack of water), shock with high incidence and low severity (diseases). Shock with low impact and maximum severity (flood), the rest of the shocks are divided among groups of shock with impact and low severity and shocks with low impact and high severity.

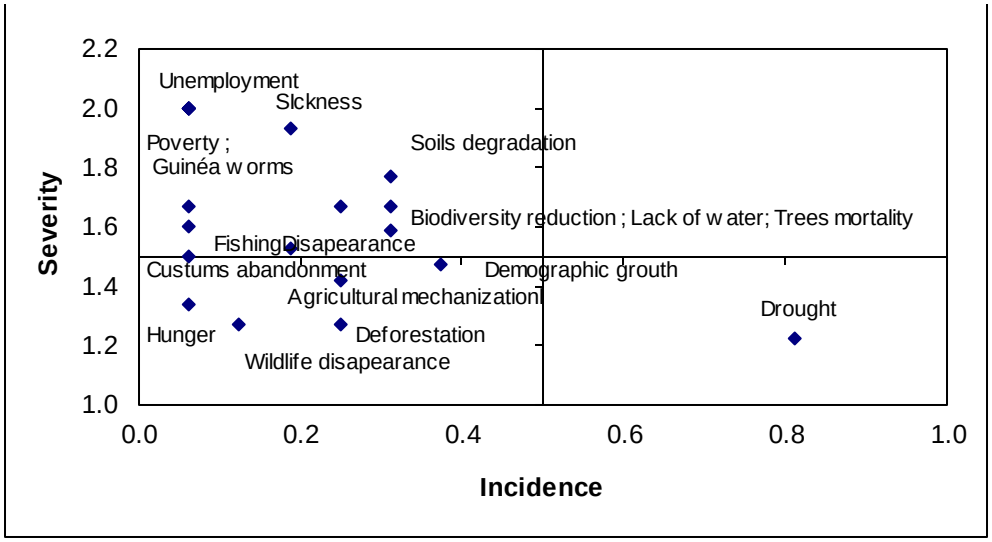

Fig. n°7 : Severity Map S of Men

For men, deforestation is the first cause of natural resources degradation in all the villages. Then come the hunger, agricultural mechanization and population growth which have greatly contributed to a deep and accelerated degradation of natural resources in the region

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3.4. Adaptation practices of populations to climate change

Studies of vulnerability to climate change in Burkina Faso [6] have shown the difficulties to separate the effects of the variability of the anthropogenic factors. Despite the efforts to combat desertification, recurrent droughts are only exacerbating environmental imbalances [17]. Shocks listed in this study as the disappearance of wildlife, soil degradation, the rural exodus, the lack of forage are more important than those cited in the southwest of the Burkina [17], [14] showed that the lack of rain has the value of the highest incidence while the poor quality of the seeds and the straying of cattle present as much severity. In the face of climate change, populations have initiated a set of adjustments or adaptation in natural resource systems: extension of the area under cultivation, crop intensification; introduction of animal culture, the culture of cotton (annuity), techniques of conservation of water and defense and restoration; operation of the shallows and the lowlands, etc. In terms of our study area, mapping noted a regression of the natural formations in favor of fields and agroforestry parks. This was confirmed by investigations which also showed dynamism in the formation of farmer organizations in the area. These often mixed professional organizations specialized in specific production sectors such as cotton and oilseeds and for the monitoring of forest or pasture, etc. The adaptation of people living along the RBMH to climate change is reflected on the different sectors of activity. At the farm level, it was noted among other things, the increase in area planted; diversification of operating systems (culture attached, irrigation techniques to techniques of conservation of water and defense and restoration) the intensification of crops (seeds, fertilizer and manure); cash crops (cotton, Cowpea) and market gardening.

IV. CONCLUSION

The populations of the Biosphere Reserve of the Hippopotamus pond perceive climate change and variability. Awareness of the effects of climate change by the people has occurred especially in the elderly. The youngest, because of their short existence in time, less perceive the effects of climate change. Awareness would have occurred from the years 1975-1980, following a significant decline in rainfall over several agricultural seasons. This study showed that climate change has a negative impact on 4 sectors as agriculture, livestock, forests, and water resources. In agriculture, cultivated areas are increased; pastures are more degraded by livestock; water resources present an irregularity in their distribution of a pool to the other. This is noticeable as well by the mapping study by local people.... Also, local people are developing various adaptation strategies to mitigate or even curb the impacts and effects of climate change. It is apparent that the increase in the cultivated areas at the expense of the natural formations is a form of adaptation to climate change.

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Tnyafar: Women, Livelihoods Strategy in Selaru Island, West Southeast Maluku District

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Abstract**—** *Tnyafar as local wisdom is closely related to women as a regulator of family income strategy. Through tnyafar women express alternatives to meet the needs of families in a variety of conditions, both social, economic, and physical environment. One of the strategies undertaken in tnyafar concept is to do farming activities with a variety of food crops, plantation crops, even developed a special food crops currently facing food emergencies also utilization of coastal areas for seaweed commodities. Thus, women have demonstrated the role of implementing farming activities had even surpassed conception of agroforestry is known so far. Therefore, at this time the role of women not only dominant in the domestic area, but be extended to cover public areas.*

Keywords**— local knowledge, women, livelihood strategies, small island, dusun, and arin.***

I. INTRODUCTION

Background

Women role was recognizable since a long ago. Communal tradition divided work categories into light and heavy. Such work division has been attended by Tanimbar community in Maluku Tenggara Barat. Men were charged with heavy work, including opening field for a new planting land, cutting trees, clearing spot for business location, and others. Women only continued what men did after heavy work finished. It means that after field was opened, then activities of preparing seeds, planting, managing against pest and disease, weeding, harvesting and marketing, were works that must be done by women, and it became their legacy throughout generations. All these processes were given a label of "*Tnyafar*" where households left their village and settled temporarily on their farmland. Indeed, *Tnyafar* process was a local wisdom that placed women in an important position for fulfilling household necessities. Early description of *Tnyafar* was explained as following:

1. Women role was aligning with *Tnyafar* implementation, where women role was extensive and severe. It impacted on the continuity of *Tnyafar* which also influenced the sustainability of fulfilling household necessities because *Tnyafar* was also considered as a strategy of household livelihood.
2. Women activities were compatible to *Tnyafar* implementation because the displacement of women role dominated public sector, meaning that women was previously occupying domestic sector, but their role shifted from domestic to public realm, with a possible domination in public sector in the future.

The community of Selaru Island was the occupant of coastal part of the island. Their local economic was dominated by farming and fishery sectors. In farming sector, the community cultivated crops including tubers, legumes, corns, vegetables, and dry-land rices. Farming and fishery sectors were managed on Tnyafar. Land-open method for shifted-farming or gardening was using slash-and-burn system. It involved traditional technology and local technique, and it was still influenced by customs that regulated the relationship between human and soil/land. In some villages, the opening of new garden must apply procedures in the customs although few changes had recently been made (Atsea, 2011). Pattiselanno et al (2015) explained that in recent days, fallow period was shorter because people tended to look for the land nearby their residence to be cultivated as garden. It was evident possibly because primary forest was too far from the village. The opening of new road could help shortening the journey the farmers must take to go to their gardens because it provided clean path free from bushes that could be past over by farmers by riding on rented-motorcyclist.

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As shown by Cabuy et al (2012) in Papua and Ratnawati et al (2014) in Jogjakarta, local wisdom was important element in community life. Local plants were cultivated on immediate environment in order to produce farming work. Tnyafar attempted to create the relationship between all elements in the livelihood system of the households. Tnyafar helped satisfying the living standard of the households in sustainable ways. Tnyafar gave a consequence of fixed-work which at least prevented the possibility of shifted-farming and also reduced the unfavorable impact of environmental degradation. All of them allowed the households to have livelihood sustainability. This reality was the manifestation of local wisdom. From this matter, problem of research could be made: Why Tnyafar as main economic process of the households could be said as local wisdom in the environmental sustainability context and also be useful in creating livelihood satisfation sustainability?

Objective

The objective of research was to analyze the existence of Tnyafar as local wisdom of the community.

II. METHOD OF RESEARCH

Qualitative Approach

This research used qualitative approach. Main strategy to operate this approach was case study as suggested by Yin (1996), Stake (1995), Bogdan, R.C and Biklen, S.K., (1982), and Creswell (1994). Case study was used because it was relevant with the articulation of Tnyafar which until today it was still continually professed by Selaru Island community. Case study also allowed the author and respondents to develop interaction and dialog, as explained by Yin (1996) and Stake (1995).

Location of Research, Population and Informants of Research

Selaru Island belonged to the admnistration area of Selaru Island District, West Southeast Maluku Regency (MTB Regency). Selaru Island was one of few islands in MTB Regency occupied by Tanimbar Ethnic as the native inhabitant. The administrative area in Selaru Island District comprised of five villages. The distinctive marker of the inhabitant was high capacity of migration among Tanimbar Ethnic in Selaru Island. This ethnic could settle on few islands with few inhabitants, and also assimilate easily with community in few villages in the Selaru Island or beyond the island.

Research population was Selaru Island community who conducted Tnyafar. Informants included men and women who occupied Tnyafar. There were 25 informants, and their status were leader, former-leader and member of Tnyafar. The leadership structure of Tnyafar consisted of Chair, Secretary, and Treasurer. Mostly, treasurer was a woman, but in some Tnyafar, woman worked as secretary. Other Tnyafar even selected woman as the Chair. The Head of Customs was key informant who understood well the working structure and the activity of Tnyafar.

Case Selection, Deep Interview, and Participative Observation

For obtaining the information, the author asked some questions to the key informant, precisely the Head of Customs as the community figure in Adaut Village in Selaru Island, who regularly conducted Tnyafar local wisdom. Snowball technique as explained by Moleong (1989) was used to obtain other informants. Deep interview was performed to obtain more relevant informations. Focused Group Discussion was implemented to strengthen information previously obtained. Participative Observation (Moleong, 1989:138) was also conducted because it allowed the author to see, to feel and to sense the world, events, and social symptoms in the way the actor did. It was also helpful to establish mutual understanding between the author and the actor (inter-subjectivity).

Data Analysis

Miles and Huberman (1992:15-21) mentioned about three paths in analyzing qualitative data. It started with continuous data reduction that involved some activities such as selecting, concentrating attention, making simplification and abstraction, and transforming data given by informants. It was followed by data presentation which displayed important information about Tnyafar. The presentation helped the author in preparing conclusion and next action. Conclusion remark included a verification on previous conclusion about Tnyafar as local wisdom.

III. RESULTS AND DISCUSSION

The Development of Women Role

The problem was related with the displacement in how to preserve the culture and how to utilize natural resource. It caused a role gap between men and women. Women existence also displaced threatening the traditional role of rural women. The impact of social change and also of intensive exploitation of natural resource forced women to have narrower engagement. When the nature was explored to be human settlement, forest must be the subject of utilization. Women were forced to support the fulfillment of household necessities, meaning that they must seek for additional income, including being the laborer in various activities. Such double roles keep the gap between two genders becoming wider. As a result, it marginalized the function of women in the household.

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According to Andriani and Euis Sunarti (2008), traditional role of women concerned with taking care of domestic affair, while men did subsistence work. But, it is not a rare to find women in livelihood employment. Therefore, women indeed incur double charges. In household affair, women must manage household resource to produce welfare. On agriculture, double livelihood pattern was a strategy for survival because relying only on farming output was not adequate to fulfill household necessities. All members of household must enter employment market outside farmland. Dharmawan (2007) said that livelihood strategy was a tactic and an action developed by individual or group to maintain their existence, social infrastructure, social structure, and cultural value system. Livelihood strategy used by each man and woman may differ to each other (principally, it was gender-oriented).

Scoones (1998) admitted that in applying livelihood strategy, farmer household utilizes various resources to maintain their life. Livelihood strategy was classified into three categories such as: farming livelihood engineering, which utilizing the farming sector in efficient and effective ways by considering various external inputs such as technology and workers (intensification), or by enhancing the cultivated land (extensification); double livelihood pattern (diversification), which looking for jobs out of farming land to increase household income or to deploy household-based workers (father, mother and kids) for additional income; and finally, spatial engineering (migration), which represents a mobility to other region out of the village, either permanently or circularly to obtain additional income.

Puspitawati (2012) insisted that gender was reflecting the difference on role, function, status and responsibility between men and women, as a consequence of socio-cultural construction internalized through a socialization process from a generation to another generation. Instruction of Indonesia Republic President No.9/2000 about Gender Equality had stated that men and women must be given equal standing to ensure their usage of opportunity and rights as human with equal role and participation into political, economical, socio-cultural, national defense and security sectors, and also into the enjoyment of the development result. Gender Equality was important in the household because it kept men and women to have equal standing.

Tnyafar : The Implementation of Functions of Production and Consumption, Settlement and Value Inheritance

The existence of Tnyafar in Selaru Island was proved a fact that the community of the islad professed local wisdom. It involved some activities. Livelihood satisfaction method was adaptable to the surrounding nature. The crops were planted by adjusting them with immediate weather and planting culture the immediate community had used. General farming, including fishery, was performed on Tnyafar. The community might exploit land and sea sides in accordance to weather. In dry season, the harvest of the crops might be not good. Therefore, the activity was oriented toward the sea, and it involved cultivating sea grass, collecting sea cucumber and lola, and catching fish with rod. During rain season, farming was dominant. The longevity plant, such as coconut, was main crop to consider because it also produced copra. Review of Jiri, Mafongoya, Mubaya, and Mafongoya (2016) gave evidence that scientific estimation on the condition had failed to help smallholding farmers because of their incapacity to access this information. Sometimes, there was a dispute between scientific method and customs-based prediction. The prediction of customs was without challenge, and therefore, it was directly used by community as the reference in selecting adaptive strategy although it was difficult by farmers to integrate customs with scientific knowledge. A similar reality was reported by Chanamuto and Hall (2015). Farmer-women were quite susceptible to climate change that affected greatly their farming activity, and therefore, breeding the livestock was becoming the other option. Indeed, every household did radical action when their food security was threatened. This reality was understood by farmers in Tnyafar, and even considered as reasonable habit that preceded the maturity of local wisdom in Tnyafar. Besides relying on the commodities such as tubers and corns, the community also planted certain commodity that would only be stored and even never be consumed when main food was secured. This commodity (including sago with harsh fiber and red color - mawere) was cultivated in Tnyafar because it could gave food reserve during food crisis.

Local wisdom was applied in Tnyafar to regulate the harvesting of crops and sea products. At certain times, coconut was subjected to Sasi (the prohibition of harvest on certain rules) in order to maintain the productivity of the plant. However in the case of immediate need, such as school tuition, the owner of coconut might ask for allowance *(Exceptional Sasi)* for meeting such need. The procedure of this Exceptional Sasi was varying across each plant owner. It did not comply anymore with the customs but it was submissive to the Church Organization. Such procedure was strongly effective because the deviation of this sasi would be considered as violating God's Rule. The penalty was more heavier than monetary fine.

Sea products were also subjected to Sasi, especially for sea cucumber and lola. The ownership of

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sea area was given to the Village, and it was the Village that would rent the area to any individuals based on rent agreement. Result of Focused Group Discussion could be explained as following. The harvest from the shallows was subjected to Sasi. The contract of a week would require payment of IDR 3 millions tothe Village. The depth of the rented sea area was measured by the border of the ebb and also by the capacity of the renter to dive into the sea. The sea could be set to Sasi of 2-3 years period, and this period was considered as long enough to maintain the lola. Opening and closing the period of Sasi would require local government to give report to the Church for blessing. Reverend went to the sea wearing Toga (Church Cloth) and did praying by expecting that there was no stealing. The government was equivalent to men, and Church Organization was equivalent to women.

The explanation above indicated that Sasi was the effort by the community to maintain the sustainability of the harvest. Therefore, village government would take Sasi as good initiative in order to reduce the possibility of stealing and also to eliminate the possibility of conflict among citizens who fought for certain sea area. Sasi could also be conducted by individuals for rare crops or certain species of plants, at least for the purpose of conservation. Commodity that was usually subjected to Sasi was orange to conserve the utility of fruit and leaf. The commodity that would be ready for harvest could be subjected to Sasi by the request of the owner after giving note to the Church, and it was done to prevent stealing. Therefore, Sasi had two main principles. One was to maintain the continuous production of certain commodity, where the second was to reduce the possibility of social clash due to stealing and individual conflict.

As explained by Keraf (2010), Marfai (2012), and Juniarta (2013), the implementation of Tnyafar had three important functions, as shown in the following table 1. As indicated by the table, Tnyafar had important functions in the household, respectively being the shelter, being the source of production and consumption, and being the value inheritance system. All these three aspects were related and supporting to each other. All of them underlined the existence of Tnyafar and produced the unity of local wisdom. All activities, from the starting of work until the gratitude during harvest, were done in Tnyafar, and the result was enjoyed by all members of Tnyafar, and even shared with neighboor Tnyafar. The selection of activity to be done in Tnyafar was determined by two things, precisely the determinancy in doing the work and the accessibility of the work. When determinancy and accessibility was hard, fixed-farming in the garden (orchard) was the only option. The expression of Tnyafar aslom was still prevailed onwards, and it possibly developed further when the accessibility to work became easier.

This finding was aligned with Kardooni et al (2014). Their research revealed some indications. Some variables were representing traditional knowledge. Men had higher participation than women. There was a close relation between age and knowledge. Old generation tended to have much traditional knowledge than the young. Result of research by Swarnam et al (2015) had shown that the knowledge of the people about how to process post-harvest coconut into main food had facilitated their livelihood satisfaction activity. This knowledge was passed on the next generation to be the important stock for their living. Even, this knowledge was still relevant in recent days.

Table.1: The Functions of Tnyafar

No	Function	Explanation
1	Shelter	Parental time was mostly spent in Tnyafar from Monday to Saturday. The living day of the household was also occuring in Tnyafar, including resting and gathering with household members (besides the parent, there was also unschooled children).
2	Production and Consumption	The satisfaction of livelihood was done in Tnyafar.
	Food Security	Garden was planted with crops such as tubers, corn, and rice as main food, and the excess was sold.
		Coastal area was used for fishing to satisfy the need of protein. The excess was sold or exchanged with other commodity the household did not possess.
	Sustainable Life Security	Non-food necessity, such as school tuition, was satisfied through longevity plant. The coconut was one plant that was usually exchanged for fast money. The copra could be directly sold and becoming the source of household income for non-food need.
	Strengthening the Household Economic	Coastal area was used for sea-grass cultivation to produce additional income, and it succesfully gave significant contribution to the household.
		The shallow side of the coastal area was providing sea products with quite high economical value, including sea cucumber and lola.

3	Value Inheritance	Through Tnyafar, the values of livelihood satisfaction could be inherited to the descendants. During school holiday, children were taken to Tnyafar at least to help their parent. All activities in Tnyafar were indirectly learnt and becoming a part of the sustainable process of knowledge acquisition among the children. Indeed, local wisdom values in Tnyafar, including Sasi, could also be understood by children as the knowledge that must be inherited to the next generation.

Source: Sopamena, 2018

IV. CONCLUSIONS

The burden of women in Selaru Island was getting heavier due to efforts to maintain *Tnyafar* local wisdom on agroforestry. Such agroforestry would be a strategy of farmer household livelihood which may impact the position of women in community life. In general, women position faces two contrasting alternatives:

1. They should have better position than before in the context of socio-economic life of Tanimbar communities, recalling that women played important role, or possibly dominant, in fulfilling household necessities, especially when they must choose proper livelihood strategy. This role means that women in Tanimbar incur heavier burden and therefore, their role in the context of socio-economic life of Tanimbar communities must be recovered to what should be the place. The excess of work should be relieved to reduce the burden of Tanimbar women into a harmony, and be manifested as women in their household life.
2. They may suffer from lower position because the domination of men forces women under pressure in fulfilling household necessities. There is no change of women position in the context of socio-economic life of Tanimbar communities. Fulfilling household necessities and contributing to household income were mandates that must be met by women. In one side, role of women was the articulation of *Tnyafar* local wisdom in relative with the utilization of natural resources for fulfilling household necessities. In other side, role of women was the manifestation of local wisdom, but it displaced from domestic realm to public realm. It is possible if this role remains in the realm of public sector.

The question includes that whether this local wisdom is compatible with expectation and self-interest of women, or whether women accept their excess role as the manifestation of *Tnyafar* local wisdom in relative with household livelihood strategy or just accepting the role with sense of perforce and pressure? Further research must understand Tanimbar women in the context of role linkage, livelihood strategy and local wisdom.

The separation of rights and obligations between men and women also occurred in spatial aspect. For instance, the access and control of men and women were separated between public and private spaces. It means that it not only separates the access to natural resources, but also separates their rights to obtain and to manage natural resources. The rights of ownership and the rights of usage of men were associated on *de jure* perspective, meaning that it was valid based on jurisprudence. Women were associated more with the rights of natural resources on *de facto* perspective, precisely only based on a habitual practice.

The rooms separating rights and obligations were mediated by activities crossing space limits. Agroforestry concept was developed through *Tnyafar* showing that women had activities on land and sea. Besides cultivating annual plants and crops, women in *Tnyafar* also manage seaweed works. Across the rooms, there will be a path for women to across domestic and public realms. The position of women is the part of *Tnyafar* activities, on which women do their domestic and public activities, recalling the fact that they must embed themselves into land and sea works.

Tnyafar describes a kind of intact agroforestry through the utilization of long-age plant (annual plant, including coconuts) and short-age plant (corns and tubers), and both plants are arranged together in similar planting area based on the agreement of household. Besides, in *Tnyafar* processes, seaweed commodities are the supplementary contribution to household income. The utilization of land and sea regions in *Tnyafar* had shown that agroforestry system was unique and different from the usual. Indirectly, women activities had strengthened the presence of *TnyafarAgroforestry* not only crossing the borders of land and sea, but also exceeding agroforestry concept itself.

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Pesticidal Effects of Extracts from *Hyptis suaveolens and Hyptis spicigera* on Cowpea Weevils

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***Abstract*—** *This experimental research was conducted with the view of determining the effectiveness of Hyptis suaveolens and Hyptis spicigera extracts on cowpea seeds in deterring the feeding habit and life span of cowpea weevils. Masses of 400 and 500 grammes of pulverized samples of the two plants, Hyptis suaveolens and Hyptis spicigera were separately extracted by percolation with 2200 millilitres of 95% ethanol respectively. The percolates were evaporated to dryness at room temperature to give crude extracts of both plants which were each subjected to a partition process. Soluble solvent extracts of the two plants were applied on cowpea seeds and subjected to cowpea weevils by two-choice feeding deterrent method. The chloroform soluble extracts were found to be most active extracts of both plants with the least percentage consumption indices and higher percentage mortalities. This was a clear indication that these chloroform soluble extracts contained most of the toxic component(s) of the plants which can be used to protect cowpea seeds to some period of time. The study tasks the Ministry of Food and Agriculture to consider providing funding and further research into the use of the pesticides from these plant extracts to maintain the buoyancy of biodiversity as well as the environment in Ghana.*

Keywords*— *Pesticidal Effects, Plant Extracts, Hyptis suaveolens, Hyptis spicigera, Environmental Conservation.

I. INTRODUCTION

A pesticide is any substance used to kill, repel or control certain forms of plant or animal life that are considered to be pests. Elemental sulphur dusting against lice in 2000 B.C. is the first recorded use of pesticides (Fishel, 2013). Pesticides include herbicides for destroying weeds, and other unwanted vegetation, fungicides used to prevent the growth of molds and mildew, disinfectants for preventing the spread of bacteria, and compounds used to control mice and rats (Leroy *et al*, 2009). Bio-pesticide such as pyrethrum and rotenone, from the roots of tropical vegetables, was introduced in the 19th century (Miller, 2002).

The behavior of a pesticide in the environment depends on its stability, physico-chemical properties, the nature of the medium in which it is applied, the organisms present in the soil, and the prevailing climatic conditions (Singh & Walker, 2006). Because of the wide spread use of agricultural chemicals in food production, people are exposed to low levels of pesticide residues through their diets. Scientists do not yet have a clear understanding of health effects of these pesticide residues. Results from agricultural study, an on-going study of pesticide exposures in farms, show that farmers who used agricultural pesticides experienced an increase in headaches, fatigue, insomnia, dizziness, hand tremors, and other neurological symptoms (Hipkins *et al*., 1996).

It has been established that pesticides could become a nuisance if they are misused or continuously applied. Some of the negative effects of pesticide misuse include undesirable residue accumulation in food crops (Owens *et al*., 2010). Evidence suggests that children are particularly susceptible to adverse effects from exposure to pesticides, including neuro-developmental effects. People may also be

exposed to pesticides used in a variety of settings including homes, schools, hospitals and workplaces. (Knutson, 2009). Pesticides can be classified as synthetic pesticides or biological pesticides (bio-pesticides), although the distinction can sometimes be blurred (Miller, 2004). Pesticides are used to control harmful organisms such as mosquitoes that transmit parasites which cause potentially deadly diseases like yellow fever and malaria. They can kill invasive weeds in parks and wilderness, and also kill organisms that destroy storage products which may cause environmental damage (Eurekalert, 2009). Pesticides are used in grocery stores and food storage facilities to manage rodents and insects that infest food such as grains (Kellogg *et al*., 2000).

Each use pesticide carries some associated risks. Proper pesticide use decreases these associated risks to a level deemed acceptable by pesticide regulatory agencies such as the United States Environmental Protection Agency and Pest Management Regulatory Agency of Canada. Strict pesticide regulation and enforcement mechanism are put in place to ensure its safe use and proper handling. The control schemes further ensure that approval for the sale and use of pesticide is based on scientific data that support its effectiveness against target pests and that it is not unduly hazardous to human health and the environment (Glover-Amengor & Tetteh, 2007).

The application of pesticides on crops can save farmers some money by preventing crop losses to insects and other pests. In the United States, farmers get an estimated fourfold return of money they spend on pesticides (Kiniuki *et al*., 2001). One study found that not using pesticides reduced crop yields by about 10% and another study, conducted found that a ban on pesticides in the United States may result in a rise of food prices, loss of jobs, and an increase in world hunger (Knutson, 1999).

The 19th century saw the introduction of two more natural pesticides, pyrethrum which is derived from chrysanthemums and rotenone which is derived from the roots of tropical vegetables (Miller, 2002). In 1939, Paul Muller discovered that diphenyl dichloro trichloroethane (DDT) and other synthetic compounds were very effective pesticides. His discovery prompted many manufacturers in the 1940's to produce synthetic pesticides in large amounts because they were widely used (Daly *et al*., 1998). The use of DDT and other synthetic chemicals as pesticides began to pose serious threats to human health and the environment. In the 1960's, it was discovered that DDT was preventing many birds from reproducing, which was a serious threat to biodiversity (Lobe *et al*., 2005). The agricultural use of DDT, lindane, and karate, is now banned under the Stockholm Convention on Persistent Organic Pollutants, but these pesticides are still used in some developing countries to store grains and prevent tropical diseases by spraying on interior walls and fields to kill or repel insects (Lobe, 2006). Surveys conducted in vegetable growing areas in Ghana identified lindane, karate, unden and dithane as the most used pesticides by farmers. However, the agricultural use of these compounds has been banned and the pharmaceutical use of lindane is prohibited in some countries because it causes damage to the central nervous system and weakens the immune system (Glover-Amengor & Tetteh, 2007).

Residues of pesticide can be found in most consumable items such as, vegetables, fruits, fish and some processed foods made from them making them unsafe (Amoako, 2010). A study to assess the residue levels of selected pesticides on tomato crops in Ghana revealed that pesticide residues were indeed present (Essumang *et al*., 2008). Another research was carried out to determine and compare the levels of vulnerability of the Ghanaian population to pesticides and faecal coliform contamination through the consumption of fresh vegetables produced in intensive urban and peri-urban waste water irrigation which revealed that the public health of Ghanaians are threatened from pesticide because many vegetables are consumed in their fresh forms (Amoah *et al*., 2006)

These cause serious health effect and environmental problems. The cumulative effects resulting from the use of these synthetic chemical compounds had diverted the attention of most chemists and environmentalists to the search of compounds from natural sources which are directly or indirectly non-toxic to humans, wildlife and the ecosystem. Leaves, seeds, barks and roots of plants have been investigated by many researchers by isolating, identifying and screening for chemical compounds that deter and inhibit growth of pest (Oparaeke, 2007). The objective of the research is to deduce the efficacy of *Hyptis suaveolens* and *Hyptis spicigera* extracts on cowpea weevil (*Callosobruchus maculatus*) in terms of anti-feedant property and mortality rate. It is the aim of the work to identify the most active extracts from the two plants which could lead to the isolation of active compounds that could serve as pesticides. The scope of this research involves the testing of components obtained from *Hyptis suaveolens* and *Hyptis spicigera* on cowpea weevils using cowpeas in a two-choice feeding deterrent bioassay.

1.1 Hyptis suaveolens Lam (Lamiaceae)

Hyptis suaveolens is a dicotyledonous plant and belongs to the family *Lamiaceae* (Leroy *et al*, 1979). It is an annual shrub or an erect branched herb with white pilose, strongly aromatic stems, up to 120 centimeters high, the leaves are ovate with the base rounded to 5.5 centimeters long and 40

millimeters broad. The flowers are blue to purple with the corolla 5.5 millimeters long and 6 millimeters across in a dense raceme of cymules. It has an inflorescence leaves with lateral cymules and a few flowers. The petals are up to 6 millimeters long while the calyx-teeth are equal in size (Chopra *et al*., 1956).The plant is usually found growing around abandoned construction sites and refuse dumps in tropical countries (Yoganarasimhan, 2000).

The uses of *Hyptis suaveolens* are categorized into medicinal and other uses. Hyptis suaveolens (L.) Poit; [Lamiaceae] can cause infertility; it is anti-inflammatory and also has anti-plasmodial properties (Sharma *et al*, 2013). Almost all parts of this plant are being used in traditional medicine. Its leaves are utilised as a stimulant and as a cure for parasitic cutaneous diseases in India (Mandal *et al*., 2007). A decoction prepared from the leaves of the plant is drunk to combat indigestion of food in the stomach in some parts of Senegal, whereas in Ivory Coast, the same part of the plant is pounded, mixed with water and administered to children as enema for the treatment of gastrointestinal troubles (Bouquet, 1969). The crude leaf extract of this plant is used as relief to colic and stomachache, while fumes of its dried leaves are used to control insect pests of stored grains and as a mosquito repellent (Kirtikar *et al*., 1991). In Mali, the liquid obtained from the leaves by squeezing with the fingers is applied on the nose against headaches and catarrh (Chopra, 1956) whereas in northern parts of Nigeria, Kenya and Gabon, a concoction of the leaves and stem is drunk to cure piles and as a remedy to abortion in pregnant women (Dalziel, 1937). In Mali, Nigeria and Senegal, the leaves and stems are used to bath dead bodies so as to prevent decomposition (Chopra, 1956). The same parts of the plant are used as anti-inflammatory and anti-fertility agents in India. In Ivory Coast, the stem is chewed and the extract from it is swallowed as treatment for stomach upsets (Kerharo & Bouquet, 1950).

A decoction prepared from the roots is used as an appetizer and is reported to contain urosolic acid, a natural HIV-integrase inhibitor. The shoot-tips are eaten in India, and in Thailand they are added to food as flavouring (Chatterjee *et al*., 1997). Biological activities of the leaves of *Hyptis suaveolens* have been reported in literature. The steam distillation and petroleum ether extracts of the leaves have displayed antifungal activity on *Aspergillus niger, Fusarium oxysporum* and *Helminthosporium oryzae*. These same extracts have also exhibited a broad spectrum of antibacterial activity on *Bacillus subtilis, Pseudomonas aeruginosa* and *Micrococcus luteus*. *Hyptis suaveolens* is found to contain some essential oils, alkaloids, flavonoids, phenols, saponins, terpenes, and sterols, for example diterpenes: suaveolic acid, suaveolol, methyl suaveolate, steroids: β-sitosterol, Oleanolic acid, ursolic acid, 3β-hydroxy-lup-12-en- 28-oic acid (Chukwujekwu *et al*., 2005).Essential oils from the leaves have presented higher antimicrobial effects on *Mucor species* when compared to ketoconazole (Malele *et al*., 2003). Works have been published on the isolation of terpenoids and steroids from the plant. Investigations on the aerial parts of this plant have led to the characterisation of a new pentacyclic triterpene (Murkherjee *et al*., 1984). Essential oils from the leaves have indicated the existence of compounds such as β-caryophyllene, β-elemene, 1,8-cineole, sabinene, trans- α-beryamotene, spathulenol and bicyclogermacrene (Malele *et al*., 2003; Mandal *et al*., 2007; Corey *et al*., 1964).

1.2 Hyptis spicigera Lam (Lamiaceae)

Hyptis spicigera is also dicotyledonous and belongs to the same family as *Hyptis suaveolens*. The plant is an erect shrub of 120 to 150 centimeters tall, having a stem of 6 to 8 centimeters long with a diameter of 2 to 3 centimeters. It has a terminal inflorescence with either a dense cylindrical or ovoid cylindrical spike which consists of very small white/mauve flowers (Birkill, 1985).*Hyptis spicigera* can be found growing on roadsides, waste places and cultivated land, but mostly on dump sites (Parson *et al*, 1992). The plant is commonly seen growing across Senegal to West Cameroun (Kerharo & Bouquet, 1950). The uses of *Hyptis spicigera* can be categorized into nutritional, medicinal, social and others. The leaves and seeds are used in Gabon to prepare sauces and these parts of the plant serve as condiments and flavours in foods. The leaves, flowers and seeds are orally consumed in Senegal and Nigeria for the treatment of diarrhoea, dysentery, nasopharyngeal and cutaneous infections (Birkill, 1985). The leaves are squeezed with the fingers and drops of the liquid obtained are poured in the nose to treat catarrh and severe headaches. In Brazil, the leaves are dried and pounded and applied on the skin as treatment for eczema (Chopra, 1956). In Northern Ghana, the leaves are placed in guinea corn barns to prevent weevils from perforating grains (Dalziel, 1937), while in Senegal, the entire plant is known as dead plant because it is used to preserve dead bodies from decomposition (Kerharo & Bouquet, 1950).The plant has been used to control *Striga hermonthica* infestation which predominates on maize farms. This finding has improved the yield of crops in *Striga*-prone environment (Orthira *et al*., 2008). Extracts from the plants have shown some biological activities. Ethanol extracts from the leaves of the plant have been tested on *Callosobruchus maculatus F*, a pest of stored cowpea and have proved active (Sanon *et al*., 2006).Leaf extracts of *Hyptis spicigera* and *Parkia*

biglobosa have shown activity against root knot-nematodes (Yussuf *et al*., 2006).Structural elucidation had also shown the presence of some compounds in the plant. Essential oils extracted from the stem and leaves of the plant have indicated the presence of β-caryophyllene, C_{16} and C_{18} fatty acid methyl esters which could serve as flavours (Onayade *et al*., 2003; Leonti *et al* 2008).

Research works have provided understanding of the chemistry of some plants of the *lamiaceae* family of which *Hyptis suaveolens* and *Hyptis spicigera* are among. The mode of action for these plants were analysed and has shown that these plants can be used reliably and safely to treat grains and legumes when stored in small quantities. In a Rural Participatory Appraisal (RPA) survey conducted in semi-arid regions, *Hyptis suaveolens* and *Hyptis spicigera* were among sixteen plants identified as grain protectant (Brice *et al*., 1996).

1.3 Weevils

Weevils are generally divided into two major divisions, the *Orthoceri* or primitive weevils, and the *Gonatoceri* or true weevils. E. C. Zimmerman proposed a third division, the Heteromorphi, for several intermediate forms. Primitive weevils are identified by their straight antennae, while true weevils have elbowed (geniculate) antennae. The elbow occurs at the end of the scape (first antennal segment) in true weevils, and the scape is usually much longer than the other antennal segments. Some exceptions occur. Nanophyini are primitive weevils (with very long trochanters) but have long scapes and geniculate antennae (Zimmerman, 1994). Weevils are often found in dry foods including nuts and seeds, cereal and grain products. In the domestic setting, they are most likely to be observed when a bag of flour is opened, although they will happily infest most types of grain including wheat and barley. Their presence is often indicated by the granules of the infested item sticking together in strings, as if caught in a cobweb. If ingested, *Escherichia coli* infection and other diseases can be contracted (Drees & Jackman, 1999).

1.4 Callosobruchus maculatus (Fibricius)

They are commonly called cowpea weevils and their adults are $\frac{1}{8}$inch long and slightly elongated when compared to the typical rounded appearance of other members of the *bruchids* family. Although weevil-like, they are not true weevils and do not have heads prolonged into a long "snout." Wing covers (elytra) are marked with black and gray and there are two black spots near the middle of their wing covers. The elytra are short, leaving the last segment of the abdomen exposed. This last abdominal segment also has two visible black spots. The larva is whitish and somewhat C-shaped with a small head (Philips *et al*, 1996). There are other bruchids which include stored product pests and species that attack plants in the wild. The bruchids that feed on stored products include: the pea weevil, *Bruchus pisorum* (Linnaeus), which feeds primarily on green peas; the broad bean weevil, *B. rufimanus* (Boheman), which prefers kidney beans and lima beans; and the common beanweevil, *Acantoscelides obtectus* (Say). Adult *bruchids* sometimes can be quite common on flowers in the spring time. Eggs laid by females hatch in 5 to 20 days. Larvae typically feed inside the cowpea, taking from 2 weeks to 6 months to develop before pupating there. The adults may live for seventeen days and six or seven generations may occur per year. Mouthparts are for chewing. They prefer dried cowpeas but will attack other beans and peas in storage. Adults move about readily and can infest seeds in the field, but can also breed continuously in stored dry cowpeas. Larvae typically develop inside the dried peas. Larvae chew near the surface and leave a thin covering uneaten which appears as a window (Nojima *et al*, 2007).

II. METHODOLOGY

The study utilized the experimental research. The equipments used in the research include: digital chemical balance, separating funnels, beakers (600 and 300ml), retort stand, funnels, Whatmanns filter paper, vials, micro litre syringes, cowpea seeds, cowpea weevils *(Callosobruchusmaculatus F.)*, screen cages demarcated into compartments, Winchester bottles, Aluminum foil. The reagents included 95% ethanol, methanol, chloroform, petroleum ether and distilled water. The reagents used were analytical grades bought from Timster Laboratory Suppliers Limited. Two plants, *Hyptis suaveolens Lam* and *Hyptis spicigera Lam* were used for the experiment due to their local uses in some African countries and Northern Ghana in particular. Both plants were randomly harvested behind the Microbiology Laboratory of University for Development Studies, Navrongo Campus in November, 2008. The entire plants were separately air dried for twenty one days and later pulverized with the aid of a clean mortar and a pestle. The pulverized samples were stored in cleaned air-tight containers and kept in a cool dry place.

2.1 Extraction Procedure

Masses of 400 and 500 grammes of pulverized samples of the two plants, *Hyptis suaveolens* and *Hyptis spicigera* were separately extracted by percolation with 2200 millilitres of 95% ethanol respectively for two weeks. The percolates were evaporated to dryness at room temperature to give crude extracts of both plants which were each subjected to a partition process.

2.2 Partition Procedure

Crude extracts obtained as described above were partitioned between chloroform and distilled water (100, 1:1) using separatory funnels. The chloroform soluble fractions and the distilled water soluble fractions for each plant sample were separately evaporated to dryness at room temperature. The chloroform soluble residues were later partitioned between methanol and petroleum ether (100, 1:1). The methanol soluble fraction and the petroleum ether soluble fractions were also separated and concentrated. The residues derived in the whole process were transferred into vials and used in a two- choice cowpea weevil bioassay (Fatope *et al*., 1995).

2.3 Two-Choice Cowpea Weevil Bioassay

10 mg of test extracts were weighed and dissolved in 1ml of methanol. 250µl of the solution formed was poured into a vial and kept overnight to evaporate to dryness. 5 ml of methanol was later added to the vial to re-dissolve the residue obtained and 40 cowpea seeds were immersed in the solution for a few seconds. The vial and its contents were allowed to dry at room temperature. Control cowpea seeds were introduced into 5 ml of methanol in a similar manner as the treated seeds and the treated seeds were prepared in duplicates. Both the treated and control cowpea seeds were separately placed in compartments demarcated by cardboards at the bottom of a screen cage and the seeds were infested with weevils. At the end of two weeks interval, the perforated and unperforated cowpea seeds, and dead weevils were counted (Fatope *et al*, 1995).

The percentage consumption index (% C.I) and mortality were calculated using the following formulae:

$$\% \text{ C.I} = \frac{\% \text{ treated cowpea seeds perforated}}{\% \text{treated cowpea seeds perforated}} \times 100$$

$$\% \text{ Mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

III. RESULTS AND DISCUSSIONS

Table.1: Feeding deterrent activity of Hyptis suaveolens extracts on cowpea weevils

SOLUBLE SOLVENT EXTRACT/ FRACTION	CONCENTRATION (µg/ml)	NUMBER OF PERFORATED SEEDS		% TREATED SEEDS PERFORATED	% CONTROL SEEDS PERFORATED	% CONSUMPTION INDEX (% C.I)	MEAN % CONSUMPTION INDEX
		TREATED	CONTROL				
ETHANOL	500	2	9	10	45	18.18	26.29
	250	3	9	15	45	25.00	
	125	5	9	25	45	35.71	
CHLOROFORM	500	0	11	0	55	0.00	7.14
	250	0	11	0	55	0.00	
	125	3	11	15	55	21.42	
DISTILLED WATER	500	1	10	5	50	9.09	21.83
	250	3	10	15	50	23.08	
	125	5	10	25	50	33.33	
PETROLEUM ETHER	500	1	9	5	45	10.00	21.92
	250	3	9	15	45	25.00	
	125	4	9	20	45	30.76	
METHANOL	500	1	8	5	40	11.11	21.48
	250	2	8	10	40	20.00	
	125	4	8	20	40	33.33	

From table 1, there were zero % consumption indices for both the 250 and 500µg/ml concentrations of the chloroform soluble fraction of *Hyptis suaveolens*. This implies that the solvent soluble fraction protected the cowpeas from being infested by the weevils. The 125µg/ml concentration of the chloroform soluble fraction recorded the least consumption index of 21.42% when compared to all the other tested extracts of the same concentration. This clearly gives a clue that the chloroform soluble fraction of *Hyptis suaveolens* contains the most active components which prevent the weevils from feeding on the cowpeas. From the same table, the ethanol soluble extract at 125 and

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500µg/ml concentrations recorded the highest consumption indices of 35.71 and 18.18% respectively when compared to all the other tested soluble fractions at these concentrations. This is an indication that the ethanol soluble extract is the least active in terms of deterring the weevils from feeding on the cowpeas. A contributing factor to the least activity of the ethanol soluble extract is the presence of components or impurities in its content which mask its active ingredients, thus preventing it from exhibiting its complete anti-feeding property.

Table.2: Feeding deterrent activity of Hyptis spicigera extracts on cowpea weevils

SOLUBLE SOLVENT EXTRACT/ FRACTION	CONCENTRATION (µg/ml)	NUMBER OF PERFORATED SEEDS		% TREATED SEEDS PERFORATED	% CONTROL SEEDS PERFORATED	% CONSUMPTION INDEX (% C.I)	MEAN % CONSUMPTION INDEX
		TREATED	CONTROL				
ETHANOL	500	0	8	0	40	0.00	
	250	3	8	15	40	27.27	20.20
	125	4	8	20	40	33.33	
CHLOROFORM	500	0	9	0	45	0.00	
	250	1	9	5	45	10.00	11.67
	125	3	9	15	45	25.00	
DISTILLED WATER	500	1	11	5	55	8.33	
	250	2	11	10	55	15.34	19.65
	125	6	11	30	55	35.29	
PETROLEUM ETHER	500	1	8	5	40	11.11	
	250	3	8	15	40	27.27	23.90
	125	4	8	20	40	33.33	
METHANOL	500	1	10	5	50	9.09	
	250	3	10	15	50	23.07	20.24
	125	4	10	20	50	28.57	

From table 2, the chloroform soluble fraction of *Hyptis spicigera* presented the least consumption indices at the tested concentrations when compared to all the other soluble extracts or fractions. Hence, it is a clear indication that the chloroform soluble fraction contains the most active component(s) of the plant and it is capable of inhibiting the feeding habit of the weevils.

On the contrary, the petroleum ether soluble fraction of *Hyptis spicigera* gave the highest average consumption index of 23.90% when compared to those of all the other tested extracts or fractions. Therefore, it can be explained that the petroleum ether soluble fraction contains the least active component(s) of the plant which deter the weevils from feeding on the cowpeas.

Results from table 3 present the chloroform soluble fraction of *Hyptis suaveolens* with the highest average mortality of 40.74% and the ethanol soluble extract with the least average mortality of 29.16%. These two values reveal that the chloroform soluble fraction contains the most active component(s), while the ethanol soluble extract contains the least active component(s) of the plant which caused the dead of the weevils.

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Table.3: Effects of Hyptis suaveolens extracts on the mortality rates of cowpea weevils

SOLUBLE SOLVENT EXTRACT/ FRACTION	CONCENTRATION (µg/ml)	TEST MORTALITY	CONTROL MORTALITY	% TEST MORTALITY	% CONTROL MORTALITY	% MORTALITY	MEAN %MORTALITY
ETHANOL	500	6	2	60	20	50.00	29.16
	250	4	2	40	20	25.00	
	125	3	2	30	20	12.50	
CHLOROFORM	500	6	1	60	10	55.56	40.74
	250	6	1	60	10	55.56	
	125	2	1	20	10	11.11	
DISTILLED WATER	500	6	1	60	10	55.56	29.63
	250	3	1	30	10	22.22	
	125	2	1	20	10	11.11	
PETROLEUM ETHER	500	6	1	60	10	55.56	29.63
	250	3	1	30	10	22.22	
	125	2	1	20	10	11.11	
METHANOL	500	5	2	50	20	37.50	25.00
	250	4	2	40	20	25.00	
	125	3	2	30	20	12.50	

For the values given in table 4, the chloroform soluble fraction of *Hyptis spicigera* recorded the highest average mortality of 33.333% and was closely followed by the distilled water soluble fraction with an average mortality of 33.33%. The petroleum ether soluble fraction of the plant presented the least average mortality of 28.56%. From the stated values, it could be explained that the chloroform soluble fraction contains the most active component(s) of the plant, whereas the petroleum ether soluble fraction contains the least. However, the chloroform soluble fractions of both plants were found to be the most active fractions, while the ethanol soluble extract and the petroleum ether soluble fraction were the least active for *Hyptis suaveolens* and *Hyptis spicigera* respectively. Since the chloroform soluble fraction is identified to be responsible for protecting the cowpea seeds from being infested, and for causing the dead of the cowpea weevils, then there is a correlation between the feeding deterrent activity and toxicity to cowpea weevils

Table.4: Effects of Hyptis spicigera extracts on the mortality rates of cowpea weevils

SOLUBLE SOLVENT EXTRACT/ FRACTION	CONCENTRATION (µg/ml)	TEST MORTALITY	CONTROL MORTALITY	% TEST MORTALITY	% CONTROL MORTALITY	% MORTALITY	MEAN % MORTALITY
ETHANOL	500	5	1	50	10	44.44	29.627
	250	4	1	40	10	33.33	
	125	2	1	20	10	11.11	
CHLOROFORM	500	6	1	60	10	55.56	33.333
	250	4	1	40	10	33.33	
	125	2	1	20	10	11.11	
DISTILLED WATER	500	5	1	50	10	44.44	33.330
	250	4	1	40	10	33.33	
	125	3	1	30	10	22.22	
PETROLEUM ETHER	500	6	3	60	30	42.85	28.567
	250	5	3	50	30	28.57	
	125	4	3	40	30	14.28	
METHANOL	500	5	2	50	20	37.50	25.000
	250	4	2	40	20	25.00	
	125	3	2	30	20	12.50	

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IV. CONCLUSION

From the results obtained in the anti-feeding and mortality tests, it can be generally concluded that the soluble solvent extracts of the two plants were toxic to the cowpea weevils because these soluble extracts were able to kill the weevils or prevent them from feeding on the cowpeas seeds.

In all, the most toxic extracts to the cowpea weevils can be assigned to the chloroform soluble fractions of the two plants because the cowpea seeds treated with the chloroform soluble fractions recorded the least percentage consumption indices and the highest percentage mortalities. This is an indication that extracts of these plants could be used to protect cowpea seeds from damage.

V. RECOMMENDATION

It is therefore recommended that further research should be conducted on these two plants to help isolate the active compounds which could serve as pesticides for keeping the desirable qualities of cowpea seeds. This will provide a more environmentally-friendly pesticides to avoid the use of synthetic pesticides that leave residues on plants and in the soil that may be harmful to both humans and the environment at large. The Ministry of Food and Agriculture of Ghana must provide the needed funding and logistics in conducting further research into these two plant extracts used as replacements of the environmentally hazardous synthetic pesticides often used in Ghana. This would maintain environmental quality while protecting the rich biological diversities in the environment.

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Assessment of Productive Potential of Peanut Varieties (*Arachis hypogaea L.*) from the Bulgarian Breeding Program and Opportunities for Genetical Improvement

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Abstract*—The study was conducted in the experimental field of IPGR Sadovo in the period 2016-2017. Three peanut varieties type Valencia from the Bulgarian breeding program: Kalina, Kremena and Tsvetelina, are morphologically assessed. The aim of the study is to establish the possibility of genetic control over indicators directly related to productivity. The influence of the variety, the impact of the climate and the growing conditions, as well as the effect of the two factors on gynophores number, the fruit number and their weight were investigated. The relations between the studied signs are clarified. The components of the variation, phenotypic and genotypic variance are evaluated. The genetic progress and the genetic progress as a percentage of the mean are defined. The results show that the conditions of the environment are the strongest sources of variation for the studied signs. The gynophores number and the fruit number per plant are in direct positive relation to the fruit weight per plant as an element of the yield. In the studied components of the yield there is no possibility for genetic control. Their manifestation depends on applied agro-technology and the meteorological conditions. The future breeding work for obtaining high-yield peanut varieties requires finding out signs indirectly related to increasing the fruit weight per plant and possessing genetic control.*

Keywords*—*elements of productivity, environmental conditions, genetic control, peanuts.

I. INTRODUCTION

The yield of peanuts is of a complex polygene characteristic and its components are in strong relations between them (Stamatov, 2015; Stamatov and Deshev, 2015; Stamatov and Deshev, 2015a). The peanut yield depends strongly on agro-technology and environmental conditions (Giayetto et al., 2013; Gulluoglu et al., 2016). Stamatov and Deshev (2015) proved that there is a direct positive relationship between the fruit weight per plant and the yield, which confirms the results of Chifchijan and Stamatov (2007).

The nature and dimension of the genetic variability is essential for any program aimed at the crop breeding improvement. Conclusions, depending on the nature and dimension of genetic variability, are of vital importance for the planning of an effective breeding program for increasing the potential of the sign in new genotypes. Establishing of adequate variances due to phenotype, genotype, and environment allows targeted breeding activities and hybridization capabilities. The genetic advance explains the degree of progress in the indicator achieved in a variety through a certain breeding pressure. The high genetic advancement offers the most appropriate breeding. It also shows the presence of gene interactions in the expression of the indicator, suggesting reliable crop improvement by selecting such signs. Assessments of genetic advancement are more reliable and meaningful than individual parameter evaluation (Nyquist and Baker, 1991). According to Teklu et al. (2014), the higher phenotypic variance (PCV%) versus the genotype variance (GCV%) indicates that significant impact on the expression of the indicator have the growing conditions and the environment.

The aim of the study is to establish the possibility of genetic control over indicators directly related to productivity of Bulgarian peanut varieties.

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II. MATERIAL AND METHODS

1. *Place of the experiment*

The study was conducted in the experimental field of the Institute of Plant Genetic Resources – Sadovo, located in the Southern Bulgaria. The area of Sadovo is characterized by a transient continental climate, with its typical frequent and prolonged droughts. The average temperature for peanut vegetation recorded by a 120 year period is 3165.2°C with the maximum daily average temperatures of 23.7°C in August. The amount rainfall in the area is with a non-permanent character and it is equal to 247.3 l/m^2 for the peanut growing period. The droughts during the active vegetation in July-August are typical.

2. *Plant material*

The experiment was conducted with three peanut varieties type Valencia from the Bulgarian breeding achievements. The Kalina variety was created in 1987, Kremena – in 2005, and Tzvetelina – in 2008.

3. *Staging of the experiment*

The plants of all tested varieties are sowed at 70 cm between row distance and 6 cm within the row. Thereby, 166 666 plants per hectare were harvested from each variety.

4. *Data collecting and studied parameters*

The data is collected from randomized plants in the ripening phase of the fruit in 2016 and 2017. The following morphological parameters were studied: gynophores number, fruit number and the fruit weight per plant.

5. *Statistical methods*

The analysis was performed using the statistical package SPSS 19.0.By using a two-factor dispersion analysis the influence of the variety, the impact of climate and growing conditions, as well as the effect of the two factors on the gynophores number, the fruit number and the fruit weight per plant were evaluated.

The correlation analysis showed the relations between the gynophores number and the fruit number on one side and the fruit weight per plant on the other.

The evaluation of variation components, phenotypic and genotypic variance was performed using the method proposed by Burton and Devane (1953) as follows:

Environmental variance: $(\sigma^2 e) = Mse$

Phenotypic variance: $(\sigma^2 p) = (\sigma^2 g + \sigma^2 e)$

$$\text{Genotypic variance } (\sigma^2 g) = \frac{Mse - Mst}{r}$$

Where:

Mse Mean square error
Mst Mean square treatment
r Replication

$$\text{Phenotypic coefficients of variation (PCV)} = \frac{\sqrt{\sigma^2 px}}{x} \times 100$$

$$\text{Genotypic coefficients of variation (GCV)} = \frac{\sqrt{\sigma^2 gx}}{x} \times 100$$

Where:

$\sigma^2 p$ Phenotypic variance
$\sigma^2 g$ Genotypic variance
x Grand mean of a character

According Johnson et al. (1955) the genetic advancement (GA) and genetic advancement as a percentage of the mean (GAM) are identified:

$$GA = \frac{K \times \sqrt{\sigma^2 p} \times \sigma^2 g}{\sigma^2 p}$$

Where:

GA Expected genetic advance
Standardized selection differential at 5% selection intensity (K = 2.063)
$\sigma^2 p$ Phenotypic variance
$\sigma^2 g$ Genotypic variance

$$GAM(\%) = \frac{GA}{x} \times 100$$

Where:

GAM Genetic advance as percentage of mean
GA Expected genetic advance
x Grand mean of a character

III. RESULTS AND DISCUSSION

The analysis of the results presented in (Table 1) shows that the gynophores number are proved influenced by the growing year and the interaction between year-genotype. However, with a much higher variance is the growing year. The impact on the fruit number per plant has been proven for the growing year, the variety and the interaction between them. The strongest source of variation exists again the growing year and the weakest shows the variety. The fruit weight per plant repeats the effects and influences by the fruit number.

Table .1: Sources of variation in the studied elements of productivity

Variation	Source	Indi-cators	MS	Sig.	η %
Between variants	Year	GN	16236.150***	0.000	95.75
		FN	7958.017***	0.000	92.05
		FWP, g	17988.553***	0.000	87.43

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	Variety	GN	32.467	0.663	0.38
		FN	109.117*	0.037	2.52
		FWP, g	553.805**	0.005	5.38
	Inter-action	GN	328.200*	0.020	3.87
		FN	234.617***	0.001	5.43
		FWP, g	739.008***	0.001	7.18
	Error	GN	78.472		24.99
		FN	31.161		19.46
		FWP, g	92.791		24.35

***Significance for α=0.001, ** significance α=0.01, *significance α=0.05

Gynophores number (GN), Fruit number (FN), Fruit weight per plant (FWP, g)

Table.2. Relationship between the studied elements of productivity

Elements of productivity	GN	FN	FWP (g)
GN	1	0.916**	0.901**
FN		1	0.953**
FWP, g			1

***Significance for α=0.001, ** significance α=0.01, * significance α=0.05

Gynophores number (GN), Fruit number (FN), Fruit weight per plant (FWP, g)

The data in Table 2 suggests an existing direct positive relationship between the gynophores number and the fruit number per plant on one side and the fruit weight per plant on the other. Increasing the gynophores number and the fruit number directly leads to increasing of fruit yield, measured by the fruit weight per plant.

For the purpose of the crop breeding improvement on agricultural crop we need to establish the possibility of genetic control over the studied elements of productivity. From the results presented in the Table 3 it is visible that the phonotypical coefficient of variation by the indicator gynophores number per plant is higher than 10 and significantly exceeds the genotype variation coefficient.

Table.3. Estimation of phonotypical and genotypic coefficient of variation, genetic progress and genetic progress of the mean in the studied indicators

Indicators	*PCV %*	*GCV %*	*GA*	*GAM %*
GN	*17.89*	*3.12*	*0.36*	*1.73*
FN	*48.79*	*9.18*	*0.78*	*3.68*
FWP, g	*73.71*	*9.50*	*0.81*	*2.53*

Gynophores number (GN), Fruit number (FN), Fruit weight per plant (FWP, g), Phenotypic coefficient of variation (PCV %), Genotypic coefficient of variation (GCV %), Genetic advance (GA), Genetic advance of mean (GAM %)

According to the classification of Deshmukh et al. (1986), PCV and GCV values more than 20% are considered as high, values less than 10% are considered as low and values between 10 and 20% are medium. Regarding this argument, medium influence over gynophores number per plant shows the environment and negligible influence provokes the genotype. Thus, the genetic advance and the advance of the mean by this indicator are weak (Johnson et al., 1955).

By the number of fruit per plant the phonotypical coefficient of variation has a significant value, indicating the great influence on the environment in formation of this indicator. The opportunities for genetic control are weak, because the genetic variation coefficient is less than 10%. For that reason, the genetic progress and the genetic progress of the mean are with low values.

The phenotypic coefficient of variation has a significant value and it is the maximum by the three indicators. The genetic control is again weak with a genetic coefficient of variation of 9.50. This is the reason for the low genetic progress and also low progress of the mean.

For increasing the variability of the studied signs, genetically distant parental mature forms or the possibilities of mutagenesis should be used (Tiwari et al., 2011). The genetic variance of the fruit number per plant using mutagenesis methods was achieved by Nadaf et al. (2009).

IV. CONCLUSION

The environment, as a function of applied agro-technology and the meteorological conditions, is the major source of variation of the studied parameters.

The gynophores number and the number of fruit per plant are in direct positive relation to the fruit weight per plant as an element of yield.

There is no possibility for genetic control of the studied yield components. Their manifestation depends on the applied agro-technology and the conditions of the environment.

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In a future breeding program targeting high-yield peanut varieties, the signs with genetic control that are indirectly associated with increasing the fruit weight per plant should be revealed.

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Pesticide evaluation in water, sediment and in oyster shells (*Crassostrea rhizophorae*) in the Manzanillo- Niquero coastal area, Cuba

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***Abstract*—** *Occasionally pesticides have been used excessively for the pest control in agriculture, and many of these compounds become toxic for living organisms, including man. Adjacent lands to Guacanayabo gulf, Cuba, show an important use in agriculture, mainly in rice due to the contribution of fluvial waters from several hydrographical basins. The coastal area from Manzanillo to Niquero, Granma province, is the habitat of commercial shrimps, oysters and fish. The objective of the study was to determine in that region the presence of chemical residues from pesticides in the waters, sediments and shells of the mangrove oyster Crassostrea rhizophorae, Guilding (1828), as indicators of contamination. Samplings were carried out in maximum rainfall months. Besides samples for pesticide determination by gas chromatography, hydrology was studied, and there were carried out surveys (85 farmers) to know about pesticide types, dose and application frequency per crop, for conceptual analysis of the study objective. Results indicate a non-affectation by chemical residues of pesticides in any of the evaluated matrixes; and satisfactory quality of the waters for fishing use according to the used hydrological indicators. Ignorance exists on the farmer part on the application and dose of these chemical products, and recommendations are offered on pesticide use to prevent future impacts on the ecosystems.*

***Keywords*— Crassostrea rhizophorae, Guacanayabo gulf, pesticides, marine sediments, Cuba.**

I. INTRODUCTION

In the southern area of the Guacanayabo gulf, it is carried out the commercial capture of *Penaeus* sp. shrimps, fish and crustaceans as the common lobster of the Caribbean *Panulirus argus* (Latreille, 1804), and the mangrove oyster (*C. rhizophorae*).

In Cuba, the capture of these resources has diminished in the last 20 years, due to different natural factors and of human origin, such as the damming of rivers and its consequent reduction in the contribution of nutritious inorganic to the coastal area (Baisre and Arboleya, 2006). Contamination has also been pointed out by heavy metals and chemical pesticides coming from agriculture (Arencibia-Carballo *et al*., 2014). Due to the fishing environmental and biological importance of the Manzanillo-Niquero coastal region, the irrational use of pesticides constitutes a serious problem, due to the adverse effects that it causes in the health of the aquatic and marine organisms, as well as in coastal ecosystems and the environment (Arencibia, 2005).

Although one knows that in the last years it has happened a decrease in Cuba of the inorganic sources of contamination (Montalvo *et al*., 2010) and less toxic pesticides to the environment and man are used, as the buprofezin insecticide, used for the control of insectpests in rice and potato (Orta-Arrazcaeta, 2002), it is necessary to diminish risks associated to indiscriminate use of the same ones (Concepción-Villanueva *et al*., 2016).

The study of pesticide residues facilitates the evaluation of the level of contamination (Zhang *et al*., 2012). The Fishery Research Center (CIP) of Cuba carries out an environmental monitoring in a periodic way, in waters, sediments and organisms of fishing interest, where pesticide determination is a complementary analysis. Being the objective of the present paper the determination of residues of chemical pesticides in water and superficial marine sediment, as well as in shells of mangrove oyster (*Crassostrea rhizophorae*), of the Manzanillo-Niquero coastal area, in the Guacanayabo gulf.

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II. MATERIALS AND METHODS

The study area is located in the southeastern region of Cuba, Granma province, and it covered the coastal area, from 20°21.204'N and 077°08.156'W (Manzanillo city) up to 20°03.276'N, 077°36.266'W (Niquero municipality), it has a total surface of 8 969 km^2 and a current population of 47 475 inhabitants, (Fig. 1).

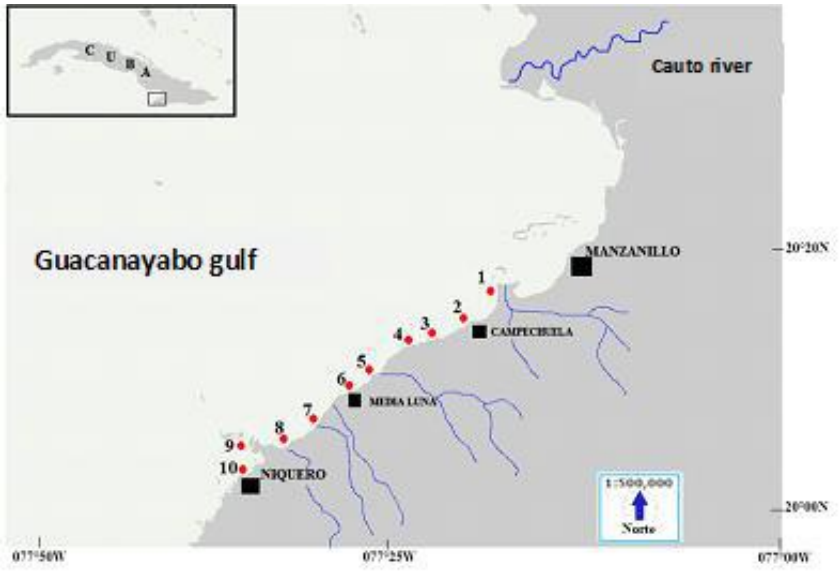

Fig.1: Sampling network, main populations and hydrographical basins in the study area.

The sampling network included 10 coastal seasons (Fig. 1) that were sampled in May and June, 2016, months of high temperature and rainfalls, since high temperatures increase the incidence of pests and the use of chemical pesticides can increase, and significant rainfalls induce a bigger terrigenous runoff with a bigger probability of contamination of coastal marine ecosystems.

For pesticide residues there were taken sediment samples in all seasons, water samples in four seasons and oyster shells in the region of Campechuela, forming a sample made up of 10 shell kg.

Water samples were collected with a Van Dorn bottle at 30 cm depth and stored in wide mouth amber glass bottles while sediment samples were collected with a dredger and they were stored in black nylon bags, being conserved frozen until their transfer to the destination laboratories.

Oyster shell samples went to the Research Center for the Mining and Metallurgical Industry (CIPIMM) to be grinded and to homogenize in a ball mill, next, they were sifted to 0.1 mm and they were sent joined to the samples of the different matrixes to the Chemistry laboratory of the National Institute for Vegetable Health Research (INISAV) for the analysis of pesticide residues in a gas chromatograph, M- DANI brand, whose detection limit for quantification is 0.2 µg/L and an uncertainty based on the standard deviation of0.4 (Ricardo-Mariño, 2009).

In each season, there were carried out *in situ* recordings of water temperature (°C), pH, salinity (ups), oxygen saturation (%), concentration of dissolved oxygen (mg L^{-1}) and total dissolved solids (mg/L), at 0.50 m depth, with a HANNA HI 9828 multi-parametric probe with an error (precision) of ± 0.1 units. Data averages are shown with their standard deviation (± DS). In parallel it was carried out a survey to 85 farmers that inhabit near towns to the coastal area, to obtain information about the pesticide type used, dose and application frequency by crop types, for conceptual analysis of the outlined study object (I annex 1).

III. RESULTS AND DISCUSSION

The results of hydrological sampling are shown in Table 1. They indicate that the evaluated parameters didn't show indicative values of contamination, with concentrations in salinity (<34.7), of oxygen over 4 mg/L and dissolved solids (<100 mg/L), so it is considered of Good and Fair quality according to NC-25 the Cuban standard for fishing use (1999).

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Table.1: Summary of standard hydrological parameters according to sampling seasons.

No.	Season	Temp.°C	Salinity (UPS)	pH	Dissolved Oxygen (mg/L)	Oxygen Saturation %	SD (mg/L)
1	Punta Gua	28.13	34.20	7.82	4.75	71.4	44.08
2	Campechuela	28.53	34.57	8.33	4.41	67.8	54.32
3	CeibaHueca	28.84	34.69	8.13	4.25	65.1	55.37
4	San Ramón	28.73	34.63	8.08	4.86	77.4	51.31
5	Media Luna	28.67	34.33	8.11	5.19	80.2	53.11
6	Punta Monacal	28.96	34.34	8.29	5.54	85.1	-
7	Tanariver	28.88	33.58	8.19	5.34	82.3	-
8	Sevilla river	29.03	33.90	8.39	4.92	76.3	-
9	PasaBalandra	28.56	34.02	8.38	5.16	79.9	53.82
10	Niquero	28.86	29.58	7.98	5.42	80.5	63.55
Mean	The whole region	28.71	33.78	8.17	4.98	76.6	52.73
SD (±)	The whole region	0.26	1.52	0.18	0.43	6.50	5.30
Max.	The whole region	29.03	34.69	8.39	5.54	85.1	63.55
Min.	The whole region	28.13	29.58	7.82	4.25	65.1	44.08

The region showed a water temperature of 28.71 ± 0.26 °C, the mean salinity of 33.78 ±1.52 ups, relatively low although characteristic of the period, and very similar to the mean (33.46 ± 1.12 ups) obtained for the region by Betanzos *et al*., (2012) for the rainy period 2008 and 2009; although annual values means of this variable are located between 36.5 and 37 ups for that region (Fernández-Vila *et al*., 2010).

The pH showed values characteristic of marine waters, and the average concentration and saturation of oxygen are acceptable, keeping in mind that the region shows a high degradation of organic matter, and bigger oxygen consumption than production of the same one (Montalvo and Perigó, 1999); The results of pesticide residues demonstrate that contamination doesn't exist in the coastal area below 0.2 µg/L, which could be a consequence that in the last years they have been applied rationally, or due to low concentrations, and when being highly biodegradable compounds in the marine ecosystem, added to the high temperatures of the region, they´re not detectable with the techniques being used.

Pesticide analyses didn't detect chemical residues in water, sediment, or accumulations in the oyster shells (Table 2), (quotation).

The results of the surveys carried out for the same period of study in the population of the coastal area are shown.

Table.2: Result of the 85 surveys carried out.

Total of surveys	**No. (%)**		
Reject	34 (40 %)		
Accepted	51 (60 %)		
Men	43 (84.3 %)		
Women	8 (15.6 %)		
Response	Yes	No	I don't know
Question 1	15 (29.41 %)	25 (49.01 %)	11 (21.56 %)
Question 2	10 (19.60 %)	41 (80.39 %)	
Question 3	5 (9.80 %)	46 (90.19 %)	
Question 4	Only 9 comments		

Statistically one can say that it exists 99% that the substance is not present in the matrixes, with 1% probability that the substance detected in the MDL be considered present when the real concentration is 0 (Type I Error).

These results are more representative in sediment than in water, since chemical residues are retained in the surface of the same one during one year, and in more depth during several years, according to the sedimentation rate. However,

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a bigger representativeness in the sampling of superficial water requires several samplings at different times and with more frequency, because when there are marine currents, it is constantly flowing and chemical residues are not retained because they´re beingdrawn by the same ones. It also could be sampled the sediment of the intertidal area as the high tide and low tide processes can contribute to their deposition in the coastal coast area.

Absence of chemical pesticides in the study area can be used as baseline to evaluate future events that can involve these compounds, which would allow a precise analysis of their persistence, bioaccumulation and toxic action in the ecosystems or marine organisms, because if there exists contamination, it can cause an unbalance in the vital cycle of some resident species, altering their reproduction (Pis*et al*., 2014).

Concepción-Villanueva *et al*. (2010) reported that during the years 2003 - 2008, in the Cauto basin, to the northeast of the Guacanayabo gulf, there have been endosulfan residues in sediment and water, which is an organochlorine chemical pesticide used for the control of pests in some crops like rice, tomato, and coffee, among other. So it becomes necessary to use more precise methods of analysis, since in the adjacent agricultural region to the coastal area agricultural cultivations exist, in state and private parcels, where the use of insecticides can be a practice in pest control.

In the last years Cuba has reduced the use of chemical pesticides in50 % (Del Puerto-Rodríguez *et al*., 2014). Insecticides and fungicides are being developed; less and less toxic to the human being, as well as less persistent in the environment, as the buprofezin and carbaryl insecticides (Official List of Authorized Pesticides, 2016).

Methods of integrated handling of pests and agri-environment politics are also developed. This has allowed the significant substitution of imported chemical pesticides, decrease of the toxic load on the ground and underground waters, improvement of the conservation of functional biodiversity, among other(Vázquez-Moreno, 2006). All which could justify the results obtained in the analyzed samples of the different matrixes.

Methods of integrated handling of pests and agri-environment politics are also developed. This has allowed the significant substitution of imported chemical pesticides, decrease of the toxic load on the ground and underground waters, improvement of the conservation of functional biodiversity, among other.

The results of applied surveys don't allow to draw conclusions, but they give a vision of the risk, although it is recognized that the type of question to formulate and the hypothesis of the problem, determine in great measure the research design that we should use (Meltzoff, 2000).

IV. CONCLUSIONS

There were not found residues of chemical pesticides in the different evaluated matrixes, for what it is considered that the evaluated coastal area doesn't present contamination due to pesticides.

Hydrological variables showed acceptable quality and in consequence with the period of sampling, of significant rainfalls.

An ignorance of the appropriate use of pesticides exists on the part of some (put the %) farmers.

V. RECOMMENDATIONS

Annually maintain a pesticide monitoring in critical points of the Manzanillo-Niquero coastal area.

Carry out pesticide analysis not only in the oyster shell but also in samples of the animal meat.

THANKING

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Annex 1: Interviews carried out to some farming of the coastal area of study.

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Phone.- 72097107
Related to this survey: malvarez@cip.alinet.cu

INTERVIEWS on aspects of pesticide use in the coastal area of Granma

No. ____ Town: _____ Province _____Date: _____

The Fishery Research Centeris carrying out a survey in the coastal area of Granma province to have preliminary information on some aspects of pesticide use in this region. This survey pursues as main objective to enlarge the level of information. We thank you for dedicating some minutes to answer these questions. Please read attentively and express freely your criterion.

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Thank you

First and Last names (optional): _____

Sex: M____ F____ Age: ____ School Level: _____

1.-Do you use pesticides or do you know any farmer that uses them?
Yes __ No __ I don't know __
2.-Do you know the application doses recommended per cultivation type?
Yes ___ No ____
3.-Do you know of mortalities of fish or other organisms in the coastal area associated to pesticide dumping or spills?
Yes ___ No ___
4.- Mention any comment on the topic that you want to express.
_____

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Assessing dynamics and productivity of tropical natural forest using permanent plots: case study of mounts kouffè and warimaro forests reserve.

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Abstract**—** *The dynamic and productivity of natural reserves in Benin were assessed in the Mounts Kouffè and Wari Maro forest reserves on the basis of sixty circular permanents plots of 1000 m^2 size each installed in 2006. The factorial correspondences analysis of the based on a matrix of 60 records and 81species. Four different plants community were discriminated:*

- *dry woodland of Diospyros mespiliformis and Anogeïssus leïocarpa*
- *woodland of Pterocarpus erinaceus and Isoberlinia doka*
- *Wooded savanna of Vitellaria paradoxa and Burkea africana*
- *Shrub savanna of Vitellaria paradoxa ans Isoberlinia doka*

The specific richness of individualized plant communities varies between 42 and 75 species. The Shannon index values calculated (dbh≥10cm) ranged between 1.25 and 4.66 bits; those of Pielou evenness ranged between 0.24 and 0.87. The diameter size classes distribution is best adjusted to Weibul three parameter function and showed a left skewed distribution.

Trees basal area decreased by 0.80 m²/ha/year for the dry woodland and 0.25 m²/ha/year for the woodland while the one of wooded and shrub savanna increased by 0.50 m²/ha/year. The density of trees decreased by 120 trees/ha for dry woodland and by 108 trees/ha for woodland over the last five years. The diameter of individuals of average basal area has increased by 2.1 cm for dry woodland. 3.95cm for woodland. 2.8cm for Wooded savanna and 3.09cm for shrub savanna. The minimum exploitable diameter (MED) of 45cm allowed for 50% of Anogeïssus leïocarpa and Isoberlinia doka basal area reconstitution while this MED allowed for 75% of Diospyros mespiliformis reconstitution.

Keywords**— Dynamic. Productivity. natural forests and plants community. Bénin.**

I. INTRODUCTION

In Benin, the sustainable management of forest ecosystems has gained awareness for a while after the adoption of a new forestry regulation that seeks to promote sustainable management approaches. Sustainable management policy requires a minimum understanding of forest dynamic which is a critical component in the designing of forests ecosystems management (Sokpon. 2006). Benin has many forest reserves which are recognized as protected areas at the national scale. Although, they provide timber wood and various non-timber forest products to the local population, these forest reserves are undergoing severe anthropogenic pressure that affects their dynamic. It is then of paramount importance to periodically lead floristic inventory in these reserves for monitoring their growth and their management. The Mounts kouffé and Wari maro forest reserves are two of the country forest reserves that have gained permanent plots-based monitoring approach over a period of five years. This monitoring approach has become usual for the surveillance and management of both ecosystems identified by Marsch in 1979 and which are of paramount resources importance to the local population compared to other regions of Benin. Although some species of high cultural, food and commercial values within the country natural forests are experiencing high anthropogenic pressure, there is still lack of information to appreciate the ecological and structural dynamic of the country forest reserves in general and in particular the one of the Mounts kouffè and Wari maro which are subjected to a restrictive mode of forest regulation application. By comparing the results of five years (2006 to 2011) dynamic study, it was possible to appreciate the dynamic and the productivity of these forest reserves. The objective of the current study is then to investigate structural and ecological dynamic of the Mounts kouffè and Wari maro forest reserves in order to provide baseline information for their sustainable management.

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II. STUDY AREA

The Mounts Kouffè and Wari maro forest reserves are shared by three Departments (Donga. Collines and Borgou) and are located between latitude 1° 40 ' and 2° 25 ' North and longitude 8° 25 ' and 9° 10 ' West. They are bordered by four municipalities including Bassila at North. Ouèssè at South. Tchaourou at East and Bantè at West (Figure 1)

The climate is of Sudano-Guinean type. It corresponds to the transition zone of Benin.

The annual average rainfall ranges between 1100 mm and 1200 mm and the annual average temperature is estimated at 27°c approximately. The annual average values of the maximum and the minimum temperature are respectively of 33.2°c and 21°c.

The relief is uniform and is dominated by plateau with an altitude that varies from 200 to 350m. The soil is of tropical ferruginous type. However hydromorphic and ferralitic soils are also met at some areas (Kakpo, 1992).

The vegetation is majorly composed of savannas. There are also community managed forests, gallery forest, dry dense forests and woodland.

The population surrounding the forest reserves is estimated at **150,618**inhabitants composed of **21,783** households with an average density of 8 inhabitants /km ^{2} (INSAE/RGPH4, 2013).

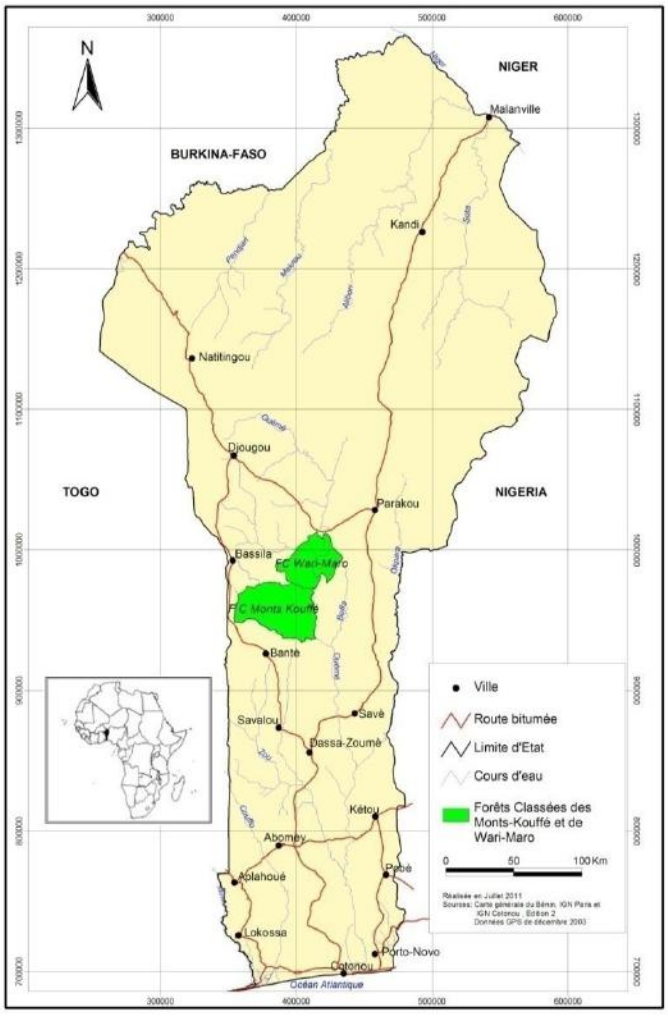

Fig.1: The localization of the Monts Kouffé et de Wari-Maro forest reserve

III. METHODS

Data collection

The data were collected in 60 permanent circular plots of 1000 m² size previously established in 2006. A number of 14 to 16 plots were established per types of vegetal formation. Table 1 shows the distribution of the inventoried plots among types of vegetal formations according to each forest reserve.

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Table.1: Distribution of the inventoried plots per types of vegetal formation according to each forest reserve

	Dry woodland	Woodland	Wooded savanna	Tree savanna	Total forest reserves
Mont kouffè forest reserve	12	13	13	12	50
Wari maro forest reserve	2	3	2	3	10
Total vegetation types	**14**	**16**	**15**	**15**	**60**

Adult trees were measured within 1000 m² size plots. To count the regeneration (dbh<10 cm), four circular subplots of 50.24m² size were established within each plots. Floristic inventory was carried out based on Braun-Blanquet (1932) phytosociological approach. This approach has been previously used by several authors (Sinsin. 1993; Sokpon. 1995). We first collected preliminary data related to plots ecological variables (type of vegetation, presence of anthropogenic pressure, type of soil, altitude and the slope). Then we measured the diameter at breast height (dbh) of all adult trees (dbh≥10cm) within the plot and numbered the regeneration (individuals' with dbh< 10cm). In order to individualize plant community groups, the phytosociological data collected were submitted to factorial correspondences analysis (AFC) using CANOCO software. Each group was after characterized in terms of structure and diversity.

Data analysis

Ecological characterization of plant communities

Three diversity metrics (species richness. Shannon and Wiener diversity index and Pielou evenness) mostly used to describe vegetation diversity were calculated to appreciate each plant community group.

the specific richness is the total number of species observed in each plant community

the Shannon and Wiener diversity index is given by the following equation (Eq1).

$$ISH = -\sum pi \log 2\ pi \quad \text{(Equation 1)}$$

where pi = Ni/N; Ni is the number of the species i within the plots; N is the total number of species within the plots and pi is the occurrence probability of a species within the plots. H varies from 0 to 5 bits and is minimal (H = 0) at plot level if all the individuals within the plot belong to one species and is maximum when all the individuals are equally distributed across all the species.

The diversity is low when H is less than 3 bits; average if H lies between 3 and 4 bits; Then high when H ≥ 4 bits.

Pielou evenness measure how individuals are distributed among species. This index is defined by the Eq.2.

$$EQ = ISH/\log 2S \quad \text{(Equation 2)}$$

Where N is the total number of species. EQ varies from 0 (community represented by one species) to 1 (all the species have the same importance). Pielou evenness is very useful to compare potential predominance between sites or dates of sampling.

Structural characterization of individualized plant communities

structural parameters including trees density (N) and the diameter of trees with average basal area (D_g) were estimated using Equations 3 and 4.

$$N = \frac{n \times 10000}{s} \quad \text{(Equation 3)}$$

n is the total number of species recorded within the plot. S is the plot size.

Diameter of trees with average basal area

$$D_g = \sqrt{1/n \sum_{i=1}^{n} d_i^2}$$

(Equation 4)

Where n is the total number of trees within all the plots and di is the diameter of a given tree.

the average basal area (m²/ha) was calculated using the equation 5.

$$G = \prod / 40000s \sum_{i=1}^{n} d_i^2$$

(Equation5)

The diameter size classes distribution was adjusted using Weibull 3 parameters distribution. This distribution has widely been used due to its great flexibility. Its function of density of probability is given by the following formula (Rondeux, 1999).

$$f(x) = c/b\left((x-a)/b\right)^{c-1} \exp\left[-\left((x-a)/b\right)^{c}\right]$$

(Equation 6)

Where x is tree diameter; a is the position parameter; a is null if all trees stages are considered; it is different from zero if the dbh ≥ a. in the present study, a equals to 10 cm for dbh structure and equals to 5 m for height structures. b is the scale parameter and c the shape parameter. Parameters b and c where estimated based on the method of maximum likelihood (Burk and Newberry, 1984).

Determination of harvesting rotation period and the MED of some high commercial value species

The rotation period and the MED were calculated based on the basal area size classes distribution (Sokpon, 2006).

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The rotation period is the laps of time taken by trees of diameter less than the MED to be shifted to a tree of diameter greater than the MED. This periodicity is a function of the speed of trees growth and the diameter size classes distribution of species population. The rotation period calculation is based on the percentage of the basal area reconstitution rate. This reconstitution rate is a function of exploitable basal area, rate of trees exploitation, the growth and mortality rates. The percentage of the basal area reconstitution (P) is given by the Equation.7

$$P = \frac{[G_o\ (1 - \Delta)]\ (1 - \alpha)^T \times 100}{G_p} \quad \text{(Equation 7)}$$

where P (%) is the percentage of the basal area reconstitution at time 0; Go = basal area of the three or four diameter classes immediately below the MED; α =rate of annual mortality (0.01); Δ = rate of damage due to the exploitation (Δ =0.1); Gp = exploitable basal area; T is the time of passage = (MED - Dbi) /AAM; with Dbi = lower limit of the diameter class below the MED; AAM = average annual diameter growth in mm/an.

IV. RESULTS

Individualization of plant communities

The factorial correspondences analysis displayed three groups of plants communities (Fig3.) including the dry woodland of *Diospyros mespiliformis* and *Anogeïssus leïocarpa* (07 plots); woodland of *Isoberlinia doka* and *Pterocarpus erinaceus* (05 plots), the wooded savanna of *Vitellaria paradoxa* and *Burkea africana* (09 plots), and shrub savanna of *Vitellaria paradoxa* and *Isoberlinia doka* (33 plots).

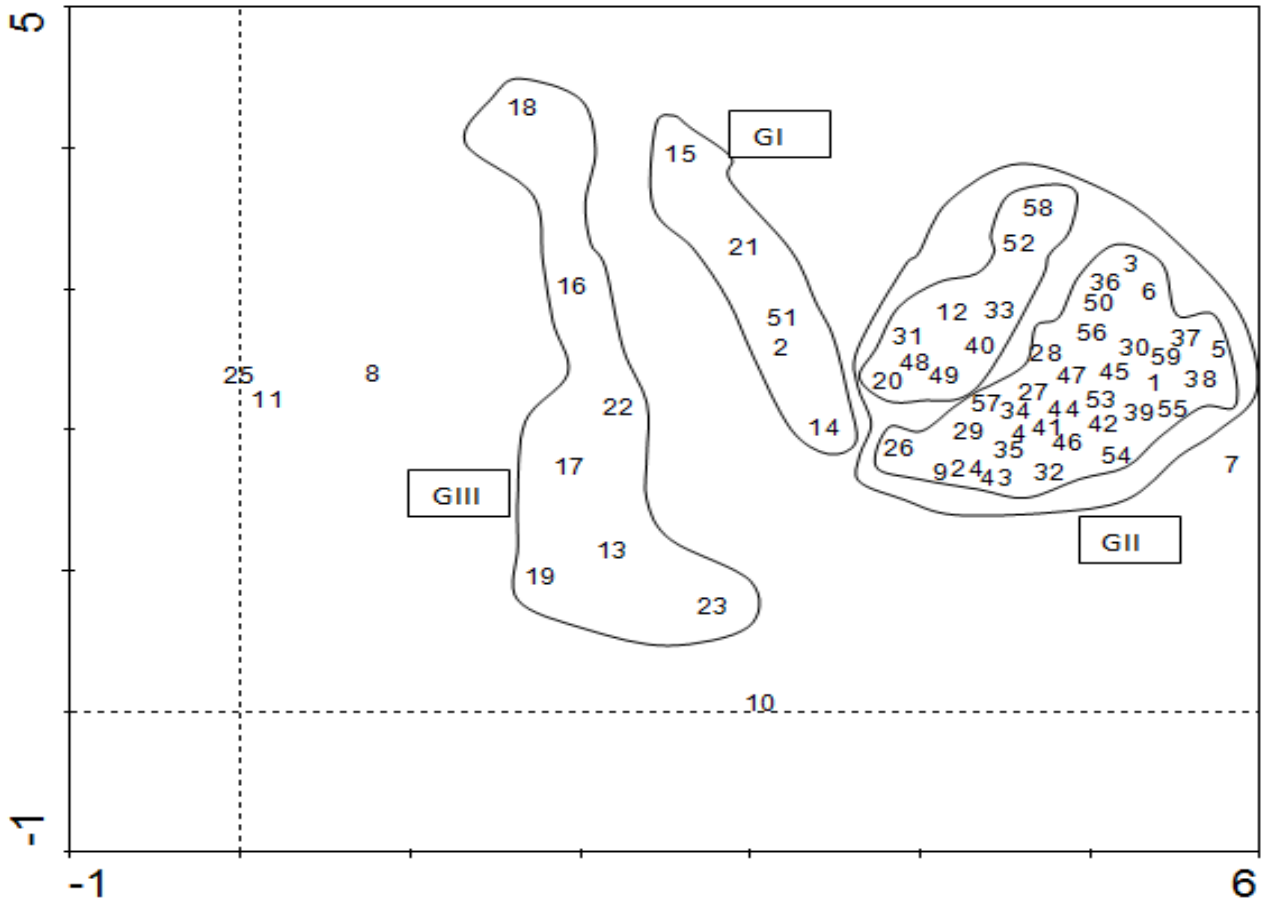

Fig.2: Individualized plant communities groups using Factorial Corespondance Analysis in CANOCO

Legend: GI=dry woodland of *Diospyros mespiliformis* and *Anogeïssus leïocarpus*. GII1= wooded savanna of *Vitellaria paradoxa* and *Burkea Africana*. GII2=Shrub savanna of *Vitellaria paradoxa* and *Isoberlinia doka*. GIII=woodland of *Isoberlinia doka* and *Pterocarpus erinaceus*.

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Table.2: structural and ecological characteristics of individualized plant communities

	Groupe I (n=05)		Groupe II_1 (n=09)		Groupe II_2 (n=33)		Groupe III (n=07)		Prob(%)
	m	se	m	se	m	se	m	se	
Structural parameters									
Shape coefficient (C. 3 Weibull parameters)	1.03	-	0.93	-	0.95	-	0.91	-	-
Dendrometric parameters									
Diameter (D. cm)	23.59ab	1.19	20.92b	0.77	21.16b	0.43	25.26a	0.99	0.000***
Density (N. trees/ha)	277a	51.57	368a	38.43	319a	20.07	287a	43.58	0.53 ns
Average diameter (Dg. cm)	27.65a	2.84	24.3a	2.12	24.59a	1.10	29.1a	2.40	0.71 ns
Basal area (m^2/ha)	15.76a	2.82	16.15a	2.10	14.36a	1.1	19.35a	2.38	0.299ns
Ecologic parameters									
Species richness (S. species)	29	-	33	-	58	-	33	-	-
Shannon index (H. bits)	4.22	-	1.25	-	4.66	-	4.18	-	-
Pielou evenness (Eq)	0.87	-	0.24	-	0.79	-	0.83	-	-
Plant community Species	Forêt claire *-Ptérocarpus erinaceus ; -Isoberlinia doka*		Savane boisée *-Vitellaria paradoxa ; -Burkea africana*		Savane arborée *- Isoberlinia doka -Vitellaria paraodoxa*		Forêt dense sèche *-Diospyros mespiliformis ; -Anogeissus leiocarpus*		-

Mean with the same letter are not significantly different (test of Tukey at the threshold of 5%). ***: significant difference at the threshold of 0.1 % ; ns= not significant. Mean (m). standard error (se). probability (Prob).

The average diameter differed significantly (Tukey) between plant communities' groups. The average diameter is of 25.26cm for trees in the dry woodland, 23.5 cm for trees in woodland. 20.9cm for trees in the wooded savannas and 21.16cm for trees in the shrubby savannas.

Diameter structure of individualized groups of plant communities

The diameter size classes distribution translates the high pressure on woodland (c=1). On the whole, all the studied plant communities showed a skewed distribution.

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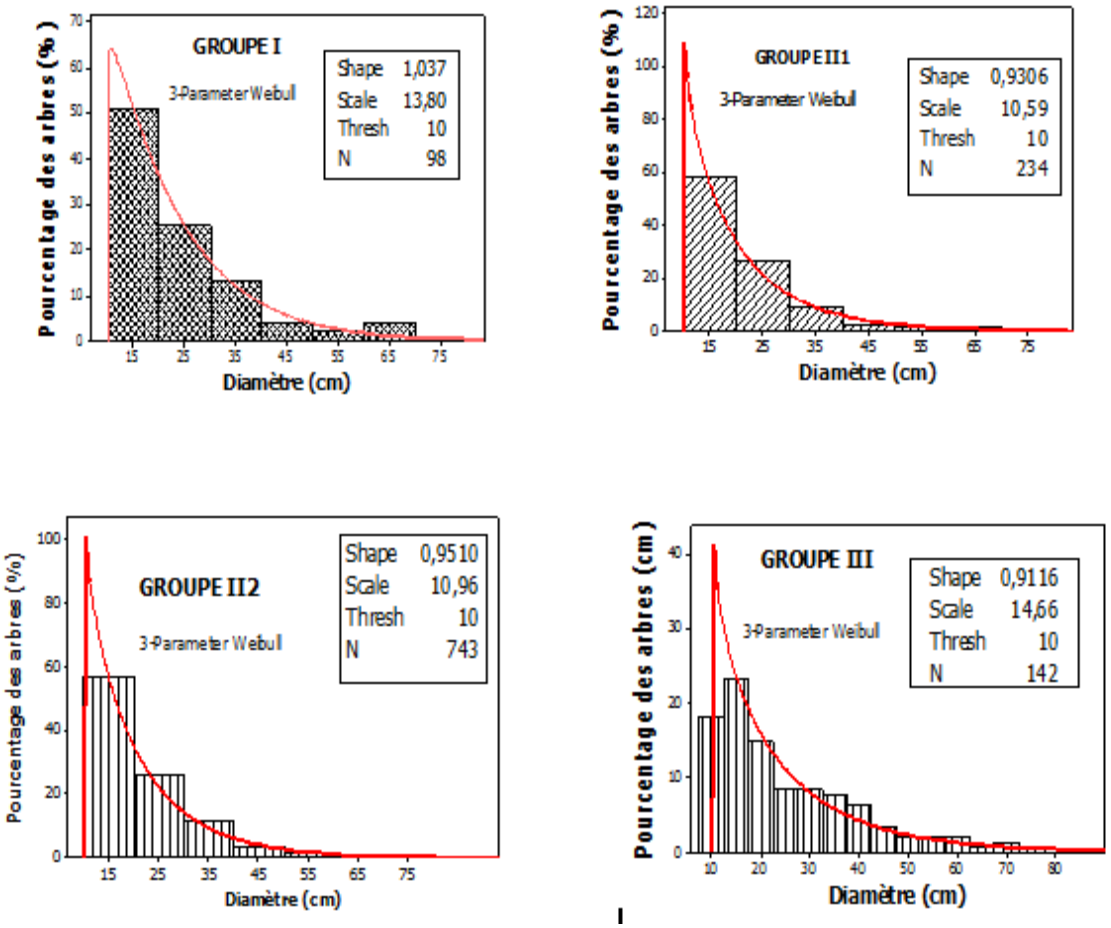

Fig.3: Diameter size classes distribution of individualized plant communities within the Monts kouffè and Wari maro forest reserves

Evolution of the structural characteristics of individualized plant communities

Over a period of five years, the density and basal area in both the dry woodland and woodland declined. The basal area decreased by 0.80 m²/ha/an in the dry woodland and by 0.25 m ² /ha/an in the woodland. Considering the trees density, it has decreased by 120 trees/ha in the dry woodland and by 108 trees/ha in the woodland. Reversely, the basal area has increased by 2.74 m²/ha and 2.64 m²/ha respectively in wooded savanna and shrub savannas. Considering both savanna types, the basal area has overall increased by 2.68m ²/ha over the period of five years. The diameter of trees of average basal area has averagely increased by 2.1cm in the dry close forests ; 3.95cm in the woodland; 2.8cm in the wooded savannas and 3.09cm in the shrub savannas.

Table.3: Evolution of structural characteristics of individualized plant communities over five years

	2006			2011			2011-2006		
Plant communities	Ni	G	Dg	Ni	G	Dg	Ni	G	Dg
Dry woodland (GI)	407	23.35	27	287	19.35	29.1	-120	-4	2.1
Woodland (GII1)	385	17.02	23.7	277	15.76	27.65	-108	-1.26	3.95
Wooded savanna (GII2)	368	13.41	21.5	368	16.15	24.3	-	2.74	2.8
Tree and shrub savanna (GIII)	323	11.72	21.5	319	14.36	24.59	-	2.64	3.09

Ni = Density (trees/ha). **G** = basal area (m²/ha)
Dg = diameter of trees of average basal area (cm).

Minimal exploitable diameter and periodicity of tree exploitation

The MED of species of high commercial values is 25 cm for *Pterocarpus erinaceus*. 45cm for *Isoberlinia doka* and *Anogeïssus leïocarpa*. 65 cm for *Diospyros*

mespiliformis and *Danielia oliveri*. The MED of 35 cm allowed 50% of species (*Anogïssus leïocarpa*, *Isoberlinia doka*) basal area reconstitution while this MED allowed 75% of species (*Diospyros mespiliformis, Pterocarpus erinaceus, Danielia oliveri*) basal area reconstitution. The average harvesting rotation period is estimated at 41 years and was species dependent. The highest exploitation was recorded for *Pterocarpus erinaceus*.

Table.4: Minimal exploitable diameter and percentage of reconstitution in the Mounts kouffè and Wari maro forest reserves

MED (cm)	*Anogeïssus leïocarpa*		*Danielia oliveri*		*Diospyros mespiliformis*		*Isoberlinia doka*		*Pterocarpus erinaceus*	
	%R	D	%R	D	%R	D	%R	D	%R	D
25	22.82	19	5.05	25	70.57	25	25.7	25	44.9	30
35	55.16	31	81.82	42	78.57	42	53.61	42	185.2	50
45	96.98	44					136.7	58		

R=reconstitution rate, D=Minimum Exploitable Diameter

V. DISCUSSION

This study has documented the structural and ecological dynamic of Mounts kouffè and Wari maro forest reserves over five years.

The study recorded low specific richness in the dry woodland (33 species), in woodland (29 species) and wooded savannas (33 species) while the richness was higher in shrub savannas (58 species). In the same Wari-Maro forest, Mensah et al. (2018) noted high species richness in 2004 compared to 2014 in dense dry forests and wooded savannas. The low number of species recorded in dry woodland and woodland is due to the degradation of these natural stands. The specific richness observed in this study is far greater than the one (39 species) observed by Sokpon and Ouinsavi (2006) in the Mounts kouffè and Wari maro forest reserves. Forest dynamics and productivity are highly dependent of plant groups and are function of time. Over the period of five years, the basal area in the dry woodland and woodland declined to 4m^2/ha and 1.26 m^2/ha respectively. Similarly, trees density declined to 120trees/ha and 108 trees/ha respectively for dry woodland and woodland. The decrease in density over time is also observed by Mensah et al. (2018). The low density and basal area values of ligneous trees in 2011 reflect the impact of human activities on natural formations. These density values are lower than the value obtained by Toko Imorou (2008) in the upper Ouémé catchment and by Mensah et al (2018) in the Wari-Maro classified forest. Values closer to those of this study were obtained by Wala (2004) in woodlands of northern Benin. These analyzes indicate that logging increased after the implementation of the management plan and especially at the end of the PAMF project. This logging is practiced mainly in dry forests, woodlands and savanna woodlands. This indicates a low level of implementation of the requirements of the management plan. The basal area declined to 2.68m^2 considering wooded and shrub savannas. The current basal area of wooded savanna and shrub savanna are respectively 16.15m ^{2}/ha and 14.36m ^{2} /ha compared to those obtained in 2006 respectively of 13.41m ^{2}/ha and 11.72m ^{2}/ha. The diameter size classes distribution of individuals showed that almost plant communities are skewed in structure. Regardless the group of plant communities, more than 50% of the individuals are of small diameter size (10–20 cm). The diameter of trees of average basal area has increased by 2.1cm in the dry woodland; 3.95cm in the woodland; 2.8cm in the wooded savannas and 3.09cm in the tree and shrubby savannas over the period of five years. In Benin like in other countries, the MED and the harvesting rotation period were often fixed in an empirical way by the forestry administration. But this empirical approach lies on the forest composition and structural dynamic majorly caused by human pressures. This is the case of the Mounts kouffè and Wari maro forest reserves which have undergone high anthropogenic pressure over the period of five years. In such context, the MED of 35 cm allowed 50% of *Anogïssus leïocarpa* and *Isoberlinia doka* basal area reconstitution while this MED allowed 75% of *Diospyros mespiliformis, Pterocarpus erinaceus. Danielia oliveri* basal area reconstitution. The average harvesting rotation period is estimated at 41ans and differed between species. The MED found in our study is not much different than that observed by Biaou (1999). Hunhyet (2000) and Sokpon et al. (2006). But the harvesting rotation period found by these authors is lower (22ans) than the one observed in the current study (41ans). This difference may result from the overexploitation of wood resources that would have increased the time of the basal area reconstitution. Trees density and the state of natural regeneration are good indicators for determining the possibilities of natural forests reconstitution (Sokpon. 1999). Also, understanding of the regeneration dynamic remains a priority for the maintenance of forest productivity (Dupuy. 1998). In the framework of this study, the regeneration has increased by 27.47% in the dry woodland, 25.71% in the woodland, 31.39% in the wooded savannas and 26.85% in the shrub savannas. This implies an improvement of the regeneration

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rate over the period of five years compared to that found by Sokpon (2006) (12.6% in the dry woodland, 17.9% in the woodland, 9.4% in the wooded savanna and 16.1% in the tree and shrubby savanna).

VI. CONCLUSION

This study has examined the dynamic and productivity of the Mounts kouffè and Wari maro forest reserves. On the whole, four types of plant communities were identified including the dry woodland of *Diospyros mespiliformis* and *Anogeïssus leïocarpus*, the woodland of *Isoberlinia doka* and *Pterocarpus erinaceus*, the wooded savannas of *Vitellaria paradoxa* and *Burkea africana* and the tree and shrubby savannas of *Vitellaria paradoxa* and *Isoberlinia doka*. We observed decline in the basal area and trees density in both dry woodland and woodland. The basal area declined to 0.80 m²/ha/an in the dry woodland and by 0.25 m ² /ha/an in the woodland. Similarly, trees density has declined to 120 trees/ha in the dry woodland and 108 trees/ha in the woodland over the past five years. Reversely, trees basal area increased by 2.74 m ² /ha and 2.64 m ² /ha respectively for the wooded savanna and tree and shrub savanna. The MED calculated allowed for more than 50% of trees basal area reconstitution and the harvesting rotation period range between 30 years and 50 years. The MED and the harvesting rotation period are baseline indicators in defining policy for the sustainable exploitation of high commercial value species.

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Forage Introduction to Support Development of Cattle in Sangkub District

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Abstract**—** *Farmers in SangkubDistrict develop cattle as a source of income, so the government seeks to give serious attention to its development.The problem is there are constrains in its development, one of them related to feed.This research has been conducted with the aim to know how far the availability of feed for cattle.The research method used is survey method, with the respondents amounted to 15 farmers determined by purposive sampling ie farmers belonging to the group, the development of science and technology for the region.Data analysis used is descriptive analysis.The results showed the ownership of cattle by each farmer ranged from 2-6 tail with a total of 43 tails.The results showed ownership by each farmers ranged from 2-6 cattle to a total of 43 cattle.Cattle have the potential to be developed in terms of available resources. However, the food consumed is the grass that grows wild and corn waste.This is due to high quality forage, not yet available continuously.Knowledge of farmers about quality feed is still low, so the introduction of feed has been done by the team. Based on results of the research can be concluded that the introduction of cattle feed has been done and responded well by farmers. Suggestions submitted, need to socialize about the development of forage with business orientation and environmentally friendly.*

Keywords**— Cattle, introduction, forage.**

I. INTRODUCTION

The government's attention to the agricultural sector is closely linked to the livestock sector. According Ikbal (2015), livestock development in this case is always associated with the reorientation of agricultural development policies. Related to the development of livestock, cattle is one of the commodities that support its development.

Farmers in Sangkub District develop cattle as a source of income, so the government seeks to give serious attention to its development. The problem is there are constraints in the development of cattle farming, one of which is related to feed. Feed problems are a problem faced by farmers in any area such as according to Alfian et al (2012), Gunawan et al (2013), Rahmansyah et al (2013), Rusdiana and Adawiyah, (2013) and Nugraha et al (2013). Based on that thought has been conducted research with the aim to know how far the availability of feed for cattle.

II. MATERIALS AND METHODS

The materials used in this study are land, cattle and agricultural waste. Land is land under coconut that is used for the development of cattle. Cattle are amounts owned by farmers. Agricultural waste is residue of corn consumed by cattle. The forages introduced are dwarf grasses. The research method used is survey method. Respondents as many as 15 farmers are determined by purposive sampling ie farmers belonging to the groupdevelopment of science and technology for the region. Data analysis used is descriptive analysis.

III. RESULTS AND DISCUSSION

Sangkub district has an area of about 30.58 percent of area of North BolaangMongondow Regency. The agricultural family amounts to 70.93% and 30.13% of family, is a farming family, that is, the family whose members are farm laborers. The agricultural sector is prime mover of the region's economy so that its development becomes government's priority. The number of cattle each farmer ranged from 2-6 to head with a total of 43 head.

The results showed that cattle consume corn waste about 8-10 kg and grass about 5-10 kg per head per

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day.AccordingNurdiati et al (2012), development of local cattle is done by utilizing agricultural waste. However, high quality forage is needed to increase productivity of cattle. According to Dianita et al (2014), sustainable forage production is an important factor in cattle production systems. Constraints that are often encountered in cattle farming is low productivity of cattle due to quality of feed that is not in accordance with nutritional needs of livestock (Lamid et al 2014). Based on results of the research, introduction of forage through the planting of dwarf grass under coconut tree (Figure 1).

Fig.1. Grasses Developed Under Coconut Trees in Sangkub District

The introduction of forage referred to as integrated cattle development. These developments according to Walia and Kaur (2013), Suroyo et al (2013), Baba et al (2014), and Wahyuni (2015), are known as integration systems of cattle-crop. Munandar et al (2015) stated that the farming system integration is an alternative to climate change mitigation.

IV. CONCLUSIONS AND SUGGESTIONS

Based on results of the research can be concluded that the introduction of cattle feed has been done and responded well by farmers. Suggestions submitted, need to socialize about the development of forage with business orientation and environmentally friendly.

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Future Turtle Management: Opportunities for Habitat Restoration Governance in East Java, Indonesia

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Abstract**—** *Turtles are species that lived on earth since millions of years ago, and are capable of annual migration, within thousands of kilometres between feeding areas and laying places. The current condition of turtles in Indonesia is threatened with extinction due to the uncontrollable exploitation of turtles and eggs. It is caused by greedy human behaviour. Turtles are protected by the Law of the Republic of Indonesia number 31 of 2004, although sea turtle conservation programme have been encouraged by the recent discovery of important new nestling beaches. The method used for future turtle management opportunities for habitat restoration governance is to use descriptive analysis. The current turtle management analysis was conducted with a literature review of various field and laboratory studies at the representative sites assessed for Indonesia. The results showed that turtle populations experienced a decline caused by turtle slaughter and harvesting of turtle eggs that led to turtle extinction. Therefore, it is necessary to manage the habitat of turtle habitat restoration which not only covers the technical aspect of captive breeding, but also covers the aspect of perfection of laws and regulations, economic and institutional aspects and community participation.*

Keywords**— uncontrollable exploitation, descriptive analysis, habitat restoration.***

I. INTRODUCTION

Gomez, *et al* (2011) stated that biodiversity is very important to increase economic activity in order to improve the prosperity of the community. However, the decline of biodiversity has a very serious impact on economic, social and environmental conditions. Destroying mangroves and coral reefs has social consequences, impacting on people's livelihoods and lifestyles — including the unmeasured cost of losing cultural traditions. Indonesian waters have a diversity of biological resources and habitat conditions that offer a suitable situation for the life of most marine turtle species. However, the current condition of turtles is threatened with extinction due to the uncontrollable exploitation of turtles and eggs caused by greedy human behaviour regardless of environmental balance. Beside, the threat to turtles is the trade of meat and eggs still exists, coupled with the emergence of demand for plastron for the international market.

To understand the condition of turtles and hatchlings in Indonesia, it will be done with 2 (two) approaches, namely the ecological approach of turtle habitat and approach of diseases suffered by sea turtles caused by infected fungi and bacteria. Both approaches are attributed to anthropogenic factors and predatory threats that are responsible for the decline of turtle populations in Indonesia. To get a more comprehensive understanding, it is taken as an example of three areas in East Java province consisting of Trenggalek regency, Jember district and Banyuwangi district.

For example, once abundant turtles, especially the Chelonia mydas species scattered throughout Indonesia, the current status becomes protected because of its existence (population) decreases, while the eggs laying period have to wait for about 3-4 years to spawn. All eggs can develop into hatchlings (turtle child) because of the threat from outside very many and various threats.

In Indonesia there are 6 of 7 types of turtles in the world. Of the 6 species of turtles, four of them are: leatherback turtle (*Dermochelys coriacea*), green turtle (*Chelonia mydas*), hawksbill (*Eremochelys imbricate*), and turtle (*Lepidochelys olivacea*) are known to breed in Indonesia, while other species, turtles crock (*Caretta caretta*) allegedly also breed here (Salm, 1984, salm and Halim, 1984, Silalahi *et al*, 1990). According to Suwelo, *et al* (1992) that turtle habitats in both nesting habitats and turtles foraging need to be protected and managed in the

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form of nature reserves or nature conservation parks. These natural conservation areas must be sufficiently large that at least 70% of the turtles can safely lay their eggs on the nesting beaches, the eggs have the opportunity to hatch and the hatchlings freely leap into the sea.

The turtle population has declined in number in Indonesia. The meat is thick and good taste to eat and the colour of the carapace is so beautiful. They make the turtle as a hunt by humans. In addition, coastal environmental conditions due to increased community activity, as well as reduced vegetation density caused by coastal abrasion and anthropogenic factors have resulted in less room for turtle nesting. The results of investigations conducted by Pro Fauna Indonesia (2005) states that the trade in sea turtles and turtle-containing products still occur freely on the southern coast of Java Island. This trade includes trade meat, eggs, preserved turtles and souvenirs made of turtle carapace (turtle products). Most (98%) of turtles traded were eggs, then turtle products (1.3%), preserved turtles (0.11%) and turtle meat 0.01%. Allegedly in one year there were about 60 turtles caught unintentionally by fishermen on the southern coast of Java Island. The abducted turtle is mostly consumed by the fishermen themselves. Fishing nets fisherman proved unsafe for turtles.

Table.1: Prices of turtles are preserved in Teluk Penyu Cilacap, East Java

No	Species of turtles	Price (year 2005)*) IDR	Estimated Price (year 2017) IDR	Location
1.	Hawksbill turtle (hatchling)	35,000 – 50,000	36,750-52,500	Situbondo
2.	Small hawksbill	60,000 – 80,000	63,000-84,000	Situbondo
3.	Medium hawksbill	80,000 – 500,000	84,000-525,000	Situbondo
4.	Big hawksbill	500,000-1,500,000	525,000-1,575,000	Situbondo
5.	Medium Olive ridley sea turtle	60,000 – 80,000	63,000-84,000	Situbondo
6.	Big Olive ridley sea turtle	80,000 – 150,000	84,000-157,500	Situbondo
7.	Medium green turtle	100,000-200,000	105,000-210,000	Situbondo
8.	Big green turtle	200,000-300,000	210,000-315,000	Situbondo

*) ProFanua (2005)

To protect turtles, the Government of Indonesia has issued a policy on turtles. Sea turtles in Indonesia are protected by Law No. 5 of 1990 on the conservation of biological natural resources and their ecosystems in Government Regulation No. 7 of 1999 concerning preservation of plant and animal species which states that the following turtles including their eggs are protected animals by country. Protection and utilization opportunities have been regulated through captive arranged by Government Regulation no. 8 of 1999 on the use of wild plants and species. Turtle protection is regulated in Law number 5 of 1990 and Law no. 31 of 2004 and government regulation no. 7 and 8 of 1999. The Minister of Marine Affairs and Fisheries has issued Circular Letter No. 526 / MEN-KP / VIII / 2015 on the implementation of the protection of turtles, eggs, body parts and / or derivative products.

The problem is that various governments regulations have been issued, but they still decreases the turtle population. The question is how to effectively address the turtle problem, so that the turtle protection policy for the future can be effective in its implementation.

II. MATERIALS AND METHODS

2.1 Description of turtle laying eggs

Turtle nesting intervals are affected by sea water temperatures. The higher the temperature of the seawater, the spawning interval tends to be shorter. Conversely, the lower the temperature of sea water, then the spawning interval tends to be longer. The best interval is the long spawning time, because the number of eggs hatched by the parent more and more. Pancaka (2000) states that the behaviour of turtles from ascending to return again need time 81.6 minutes (1.36 hour) with the detail as follows: a) the time taken for the coastal turtle averaged 13.41 minutes, while the turtle time makes the average hole is 16.87 minutes; b) Based on the average observation of turtle nesting time is 22.41 minutes; c) The turtle shell closed the average hole was 17.66 minutes and the average time to return to sea 11.25 minutes; d) The average nest temperature is 30^0C, with an average air temperature of 28^0C, the average air humidity is 17%; e) Turtle turtles lay eggs with an average number of eggs 104 grains; f) The nest of vegetation averages 3.09 meters; g) The nest distance from the highest tide averaged 14.16 meters; h) The depth of the nest averaged 43.41 cm; i) Turtle landing between at 20:35 pm and 00:15 am at half pairs. The physical condition of Marengan beach is suitable as a place to lay turtle eggs with slope, sand type, and vegetation condition.

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The turtle nesting is currently undergoing its initial change since the turtle rises to the shore until it returns 1 hour 36 minutes, currently undergoing a change in both the landing frequency and the return to sea. A decrease in the frequency of green turtles that landed in the beach might be caused by variation of nesting activity, the number of eggs, and hatching success. Another threat to the population of nesting green turtles changes in turtles habitat at Pangumbahan beach, the level of lighting of the beach, the increasing number of buildings villa, and social condition (Haryanti, 2014)

2.2 Data turtle in National park of Alas Purwo in Banyuwangi regency and National park of Kili-Kili in Trenggalek regency

Changes in nesting habitat, villa buildings, and the number of tourists will affect the instinct of green turtles to lay their eggs. Data on tourist numbers, number of villa buildings, and habitat biophysical change can be shown in following table.

Table.2: Biophysical Parameters Beaches in TNAP and TKK KB

Parameters	Measurement results (average) in TNAP *) for turtles (Lepidochelys olivacea) in Ngagelan beach	Measurement results (Average) in TNAP **) for turtles (Lepidochelys olivacea) in Sukamade beach	Measurement results (Average) in TKK KB ***) for turtles (Lepidochelys olivacea)
1. Width supratidal	14.01meters	17.5 meters	15.60 meters
2. Slope	5.33^{O}	$9,53^{O}$	6.01^{O}
3. Sand texture	Max diameter average 82.02 % (with diameter sand range from 0.21 - 0,50) Min diameter average 0.97 % (with sand diameter ranging from 1.00 – 2.00)	Medium sand 91.2 % (with diameter range from 0.25 -0.50)	Fine sand 82.67 %
4.Percentage of vege tation cover	Pandanus tecturius, Baringtonia asiatica, Manilkara kauki, Terminalia catappa, Hibiscus tiliaceus, Nypa fructicans	Barringtonia asiatica, Terminalia cattapa, Thespesia populnea, Pandanus tectorius, Rafflesia zollingeriana dan Buchanania arborescens.	Pandanus tectorius 1400 ind/ha

Note:
TNAP : National park of Alas Purwo in Banyuwangi regency;
TKK KB: National park of Kili-Kili in Trenggalek regency
*) source: Dumasari (2014);
**) Source: Yudhistira (2013).
***) Source:Prasetyo (2015).

Based on table 2 above, that the correlation between physical characteristics are obtained by correlation between turtle nest distance with the highest tide and supra tidal beach width that is equal to 0,921. This value indicates a strong correlation. Therefore, it is concluded that the farther the nest of the highest tides, the more distant the outer vegetation distance of the nest with the highest tides.

Dumasari (2014) by using Principal Component Analysis (PCA) shows that physical characteristics of nesting habitat contribute to axis F1 consisted of beach length of 14.34%, intertidal beach width of 8.99%, supratidal beach width by 11.30%, nest temperature of 10.93%, substrate type of very fine sand 14.99%, substrate type of fine sand of 14.45%, very coarse sand substrate type of 9.06% and the nest distance with the highest tide of 11.13%. While axis F2 contributed to slope 20.19 %, medium sand substrate type 24,49% and very coarse sand substrate type 22.59%.

Yudhistira (2013) analyzed using principal component analysis shows that on the one axis (F1) contributes 78.18% with the root character of 8.556. On the 2nd axis

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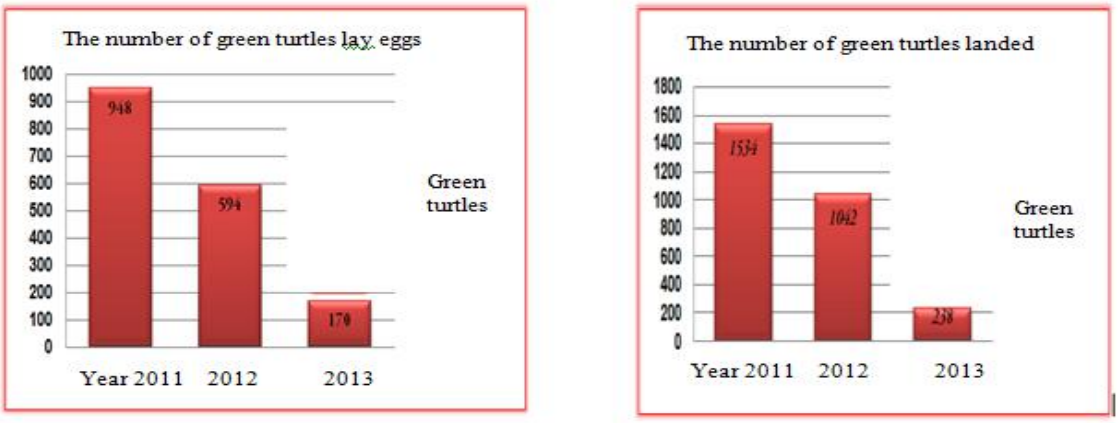
(F2) contributes 72.15% with the root character of 4.734. Meanwhile, on the 3 axis (F3) contributed 49.67% with the root character of 3.710. Axis 1 (F1) has a strong correlation with physical parameters such as coastal supratidal width, nest sand temperature, depth of sand nest, coarse sand, medium sand, fine sand, sand and dust. For axis 2 (F2) has strong correlation with coastal intertidal width, coastal slope, moist sand humidity, pH of sand nest, diameter of sand nest and very coarse sand, fine sand, nest distance from highest tide and clay. The main component analysis results show that most of the information is centred on two major axes ie F1 axis and F2 axis, where each axis describes 50.33% and 27.85% of the total variety. The result of data processing shows that the variables affecting the habitat of nesting green turtle on Sukamade Beach are beach width, sand texture consisting of: very coarse sand, coarse sand, medium sand, fine sand and very fine sand. While the sand fraction consists of sand, clay and dust.

Table.3: Number of turtles landed and Spawn in TNAP Year 2010 - 2012

Types of turtles	Year		
	2010	2011	2012
Green Turtle			
a. Number of turtles landed	13	5	2
b. Number of Turtles spawn	10	2	1
Hawksbill			
a. Number of turtles landed	5	3	3
b. Number of Turtles spawn	5	3	3
Leatherback turtle			
a. Number of turtles landed	2	1	1
b. Number of Turtles spawn	1	1	1
Turtles			
a. Number of turtles landed	1.506	1.457	1.450
b. Number of Turtles spawn	1.503	1.455	1.446
Amount			
a. Number of turtles landed	1.526	1.466	1.446
b. Number of Turtles spawn	1.519	1.462	1.449

Source: Reporting of TNAP (2013)

Table 3 above shows that some turtle populations experience decline both on landing and laying eggs. Decrease in the frequency of green turtles that landed in the beach might be caused by variation of nesting activity, the number of eggs, and hatching success. Another threat to the population of nesting green turtles changes in turtles habitat at TNAP beach, the level of lighting of the beach, the increasing number of human activities, and social condition. The data presented by TNAP is different from the data presented by Yudhistira (2013) based on his observations in the field. Green turtles observed based on data from 2011 to 2013 are shown in Figure 1 below.



Sources: Yudhistira (2013)

Fig 1: The number of green turtles landed and lay eggs

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Data shown figure 1 is indicated that on the period of 3 years from January 2011 to March 2013 the number of green turtle populations decreased. In 2011 the number of green turtles laying eggs 948 of the number of green turtles that landed 1534. In the following year in 2012, the number of turtles laying eggs are decreased to 594 from the number of green turtles that landed as many as 1042. Then in 2013, the number of sea turtles laying eggs decreased by 170 from total turtles that landed as many as 238 turtles. The decline in the number of turtles is due to: 1). extreme weather is a strong wind that is sometimes accompanied by a storm and occurs when the west season winds. Strong winds cause large waves and grains of sand flying along the beach. High rainfall causes the nesting areas to be harder and harder to dig. This causes the green turtle to delay the laying process. Lightning light can affect the turtles not to landing because turtles are very sensitive to light and moving objects that are considered predators; 2) Turtle Conservation Management Unit officers are very limited staff factors that have an impact on the monitoring of green turtles laying eggs in the morning and evening.

Bacterial parasites and fungi are disease that agents often infect turtles in conservation areas. Therefore, the decline in the number of turtles is not only caused by human hunting, but also caused by various diseases that threaten the survival of turtles. The disease is caused by viruses, bacteria, and environmental pollution. Pollution and blooming algae are the factors causing turtle disease. The role of diseases suffered by both hatchling and adults turtle will be given examples in Meru Betiri national park in Jember regency and Alas Purwo in Banyuwangi regency. Primaoktosa research results in Meru Betiri national park. The total of this national park is 58,000 Ha. There are 8 villages surrounding this park. The population of 8 villages as a buffer zone of Meru Betiri National Park is 62,145 people from 19,535 households.

Primaoktasa (2013) conducted a study on the study of ectoparasite distribution on green turtle (Chelonia mydas) in Meru Betiri national park, East Java. The results showed that the number of parasites found in each of the hatchlings in the maintenance pond showed the body parts covered with mushrooms include: forefoot, plastron, carapace and neck. The types of parasites found are Aspergillus sp, and geotrichum sp. While the parasite found in adult turtles is Chelonibian testudinaria. Aspergillus sp arises due to poor water conditions and it contains a lot of decomposition of organic material, so that the fungus grows as a polluter. According to Oros et al (2004) that aspergillus sp was found to have a turtle jellyfish flipper (Caretta caretta) due to poor water quality management and it caused the disease to have a hatchling. Diseases caused by Aspergilus spp are related to the circumstances of the hatchling environment, the environment with temperatures tends to be low and can help the growth of this type of fungus. The mushrooms attack the hatchling by utilizing the nutrients that are in the body of the hatchling slowly to breed and inhibit the imum response. In addition, the remaining feed in the breeding pond is one of the factors that make mushrooms grow. According to Phillott et al (2001), the mushroom invasion does not kill the newly hatched eggs, but gradually exploits the nutrients within the egg embryo network by penetrating the inorganic and organic tissues of the shell.

Whereas, geotrichumsp is highly resistant to oxygen and to carbon dioxide reduction. (Hudecova et al, 2009). Chelonibian testudinaria is a living organism attached to its host without giving any benefit to its host or is called Epibiont. This epibiont does not attack the immune system of the turtle, but it sticks to the skin and it causes the speed of the turtle to swim off. Meanwhile, Algadri (2014) studied the bacteria and fungi on hawksbill (Eretmochelys imbricata) in the hatchling phase in Alas Purwo Banyuwangi National Park, East Java. He researched on 15 samples of both hatchlings and turtles adults. He stated that the bacteria was found to include gram-positive, anaerobic and aerobic and fungi species identified as aspergilus spp, fusarium spp, geotricum spp and scolecobasidium spp. Fusarium is one of the pathogenic fungi causing damage to eggs and hatchlings in the conservation area. this type of fungus utilizes the nutrient source in the organism by sticking to the skin surface, then enter and start the infection stage in the organism. Whereas, scolecobasiidium is a pathogenic fungus that causes Pulmonary mycoses disease that causes infection in turtles.

Table.4: Comparison of Parasites in TMMB, TNAL and TKK KB, East Java

No	Type of parasites
1.	TMMB:*) for green turtle (*Chelonia mydas*) a. Aspergillus sp (in hatchlings); b. Geotrichum sp (in hatchlings); c. Chelonibian testudinaria (Adults turtle)
2.	TNAP**) for green turtle (*Chelonia mydas*) and hawksbill (Eretmochelys Imbricata)

a. Aspergilus spp;
b. Fusarium spp;
c. Geotricum spp;
d. Scolecobasidium spp.

3. TKK KB ***) for turtle turtles (*Lepidochelys Olivacea*) and Hawksbill turtle (*Eretmochelys Imbricata*)
a. Aspergillus sp;
b. Geotrichum sp;
c. Fusarium sp;
d. Gliocladium sp.

Note:
TMMB: National park of Meru Betiri in Jember regency;
TNAP : National park of Alas Purwo in Banyuwangi regency;
TKK KB: National park of Kili-Kili in Trenggalek regency
*) Researched by Primaoktasa (2013);
**) Researhed by Algadri (2014);
***) Reserached by Fitalaya (2015)

2.3 Methods

The methods used to develop future turtle management based on opportunities for restoration governance habitats, are used the policy analysis method of Dunn version (Nugroho, 2012). The information used the several researchers in the three districts in East Java province as described above. The policy analysis method incorporates five general procedures: definition, prediction, prescription, description and evaluation. In this paper only evaluation is used. Thus, the evaluation process focuses on the information already submitted by researchers both related to turtle habitat ecology, as well as by diseases suffered by the turtles themselves. The basic questions stated before doing evaluation as follows:

1. What is the essence of the turtle problem?
2. What is the result of the turtle policy that has been made to solve the problem ?
3. How significant are these results in solving the problem of turtles and hatchlings?
4. What is the best alternative for turtle management?
5. What results are expected?

Table 5: modified evaluation model

type of criteria	Questions
effectiveness	Whether the results of research conducted by turtle researchers in the three districts of East Java province have shown sufficient results and can be used as basic information on sea turtle population decline analysis in Indonesia.
efficiency	Is the policy that has been done to protect turtles to yield positive or otherwise harm?
adequacy	How far the achievement of desired results has solved the problem of turtle population decline?
Equity	Equality of turtle management with other protected fauna?
responsiveness	Are the results of research conducted by researchers able to provide policy input for decision makers?
accuracy	Whether the research results can reconstruct future turtle-handling policies.

III. RESULTS AND DISCUSSIONS

3.1 Results of the study

The existence of turtles population declined due to the impacts of human activities and natural factors. Human factors are influential not only the rapidly growing population, but also the need for residence land, business activities and ecotourism areas. In addition, the role of the government in determining a national park area does not involve community participation. As a result, land conflicts arise. Qodim (2012) mentioned that the pattern of social relations between Balai Meru Betiri National Park and the parties, especially with the community of the buffer village is the result of the construction of political policy and the pressure from the government on the importance of the conservation area.

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The government's policy has put the buffer villagers as weak communities in conservation and as if the community do not need nature conservation. The government, with its own authority, without any dialogue and other social processes with the community directly establishes zoning of the region. With the authorities establishing zone, the government is pushing the enclave societies with social stigma as illegal settlements and limiting the movement of non-enclosed buffer villages to access natural resources. Estuary of the process zoning areas in which already established areas of community life, forming a pattern of relations dimensioning long-term conflict, sustainable and broad spectrum.

The location map of the observation for the analysis of the physical characteristics of sea turtle habitat and the study of the diseases suffered by turtles is shown in the map below.

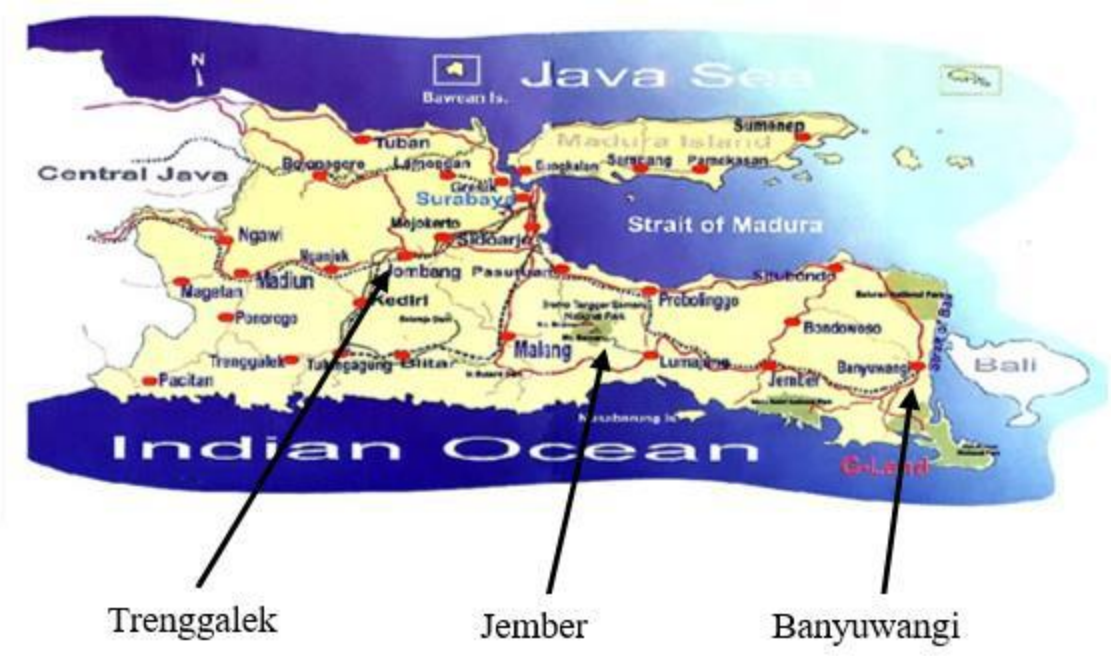

Fig 2 Map of Research Location

The condition and productivity of TNAL, TNMB and TKK KB as well as the flora and fauna associate with them are largely driven by these weather patterns and climatic events. It is reasonable to expect that such national parks will be sensitive to climate change. Therefore, the goal of turtles habitat management on units of the three national parks System are to ensure the long-term maintenance and where possible, restoration of healthy populations of native turtles, wildlife, plants, and their habitats.

The cause of the decline in turtle populations caused by the disease, actually due to human activities itself that do not follow the procedures in raising turtles and hatchlings. Ignorance of national park managers about turtle breeding, especially about water management, causes turtles and hatchlings to infect some fungi. Diseases that settle in hatchlings and turtles between adult parks Meru Beriri in Jember district and in Alas Purwo national park in Banyuwangi district have similarities about the diseases suffered by turtles in both national parks. The main cause is the management of water in the turtle breeding is not done properly and correctly.

Captive breeding of turtles aimed at saving and raising hatchlings including turtles from predatory threats has yet to show successful turtle breeding. Captive breeding of turtles originally intended to: a) perform maintenance and research for the survival of turtles; b). meet the demand for turtle shells; c) use it as a tourist attraction. Based on research by Primaoktasa (2013) and Algadiri (2014) that turtle breeding is not done properly and correctly that will accelerate the death of hatchling.

Based on the analysis with the approach of turtle habitat and approach of diseases suffered by sea turtles caused by infected fungi and bacteria, then it is done by conducting a study of Dunn model matrix as in table 6 below:

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Table 6: Modified Evaluation Model for Turtles

type of criteria	Questions
Effectiveness	Whether the results of research conducted by turtle researchers in the three districts of East Java province have shown sufficient results and can be used as basic information on sea turtle population decline analysis in Indonesia. The research results of the researchers in the three districts in East Java province showed a happy result. This is because the government is still limited to present data related to the condition of turtles and hatchlings. For example, the data provided by TNAP in Table 3 and Figure 1 shows very wide data gaps. In all national parks studied, of course, provide accurate information. Although it is still acknowledged that research by researchers has not provided a complete picture of the condition of turtles and hatchlings as a whole. Thus the results described above are very effective to help the government to reconstruct the turtles policies. The impact of the above sea turtle research suggests that turtle conservation is a fixed price and cannot be negotiable.
Efficiency	Is the policy that has been done to protect turtles to yield positive or otherwise harm? Based on the technical guidance of turtle conservation management issued by the Directorate of Conservation and Marine National Park, Directorate General of Coastal and Islands Marine Affairs, Ministry of Marine Affairs and Fishery of the Republic of Indonesia in 2009, stated that the technical management of turtle conservation consists of seven stages starting from: a) Technical Turtle nesting and egg nesting monitoring: b) Technical Breeding; c) Technical Monitoring; d) Technical Tagging; e) Technical Rescue of Turtles in Migration Areas; f) Technical Patrols; g) Technical Habitat Coaching; h) Technical Management of Turtle-Based Tourism. In addition, there are other regulations and policies that protect various types of turtles. Decree of the Minister of Forestry Number 882 / Kpts / II / 92 on the status of protection for turtles (Natator depressus), Decree of the Minister of Forestry Number 771 / Kpts / II / 96 on protection for hawksbill (Eretmochelys imbricata), Government Regulation Number 7 years 1999 on the preservation of plant and animal species in Article 4 paragraph 1 stated that the types of plants and animals are stipulated on the basis of: a) protected plants and animals; b) unprotected plants and animals. This Government Regulation states that all biological species in the annex include all types of sea turtles in accordanc Despite numerous laws, government regulations and various ministerial decrees to protect turtles, the reality on the ground shows that the turtle population is declining.
Adequacy	How far the achievement of desired results has solved the problem of turtle population decline? The protection of turtles has been declared in various forms of laws and regulations by both central and local governments. In each area that has turtles have created a management institution called the Turtle Conservation Management Unit. But the condition of turtle populations still decreased. Observations in the field indicate that the illegal harvesting of turtles is still taking place, such as the theft of turtle eggs, turtle hunting and harvesting of marine natural resources which are turtle feed. In addition to human disturbance, the turtle also has a disturbance of natural predators of lizards, raptors, ants, rats, wild pigs. Other disturbances that occur in the habitat of coastal abrasion and deflection of the river so as to make changes to the nesting beach.
Equity	the equality of turtle management with other protected fauna is the same. The difference lies in the treatment tailored to each character. For the management of turtles that need attention is on increasing the institutional capacity of turtle management, which includes the number of supervisors, facilities and infrastructure including operational funds that are currently limited.
Responsiveness	Are the results of research conducted by researchers able to provide policy input for decision makers? The use of evidence-based policy (evidence-based policy) today is increasingly considered very important and a demand. One of the basic evidence of policy that is able to document policy is the result of research. The use of inaccurate research results in policy making can lead to policy failure. The paradigm shift in evidence-based policy-making provides a great opportunity for researchers to participate in policy-making in cooperation with policymakers, but there is still a need to ensure that research is accessible to policy makers so that research results can be used more effectively. Therefore the government needs to create a forum for dialogue with turtle researchers, on the need for future public policy reconstruction
Accuracy	Whether the research results can reconstruct future turtle-handling policies. Many policies are based on

ideology, intuition, experience, public opinion, or made on the basis of political interests. Even some cynical figures about policymaking, Keynes says "There is nothing more hated by a government than complete and detailed knowledge, because it makes the process of getting to decisions far more complex and difficult." While Cook states "The main objective of politicians is to be re-elected rather than to respect the evidence", and Kogan conveyed "The government will seek to legitimize its policies by referring to the idea of evidence-based decision-making, but the government uses only research evidence if such evidence supports priorities, their politically driven priorities ".

The message of the above matrix are: a) Input of research result as material to reconstruct turtle policy; b) The importance of public participation in the preparation and determination of policies on national parks and conservation areas; c) The addition of infrastructure and cost in the management of the park area; d) Maintenance of turtle habitat should be seriously done both on land and sea; e) Sanctions for sea turtle habitat / breakers, as well as hunter turtles including trafficking will be subject to sanctions in the payment of environmental fines; f) Strict bans and penalties for those who trade turtles.

Therefore it is necessary as a material to develop turtle habitat restoration with the formulation of turtle management policy in national park as turtles habitat restoration governance, includes:

1. Effort to Improve shorelines conditions to keep shoreline as natural as possible, including removing things like retaining walls (If there is) to make them suitable for turtles;
2. In keeping with this simple, “go natural” philosophy, it is very important to plant native shrubs and trees;
3. Turtles love to bask on old logs or large rocks, so let that fallen log lie on the shore to become part of the natural habitat;
4. All stake holders, interns, and volunteers must hard at work monitoring and protecting turtles nesting beaches;
5. It is critical for both turtles and the other aquatic inhabitants of coastal, lakes and ponds that the water in which they live be kept free of harmful chemicals. So avoid using fertilizers and pesticides to the farmers;
6. Pile up all the huge wooden debris that has been stranded on the beach, then cleaned so as not to disturb the turtle's journey to spawn or travel back to shore;
7. Strengthening the monitoring process on a regular basis and carried out the recording and documented as a material evaluation of leadership;
8. The management of turtles in the national park prioritizes the principles of transparency, participation and accountability to the public;
9. Planning, implementing and evaluating the turtle condition should focused on the formal and informal actor participation.

3.2 Propose programme for Sea Turtle Conservation & Management in East Java province

In order to conserve sea turtle and to restore their habitats, the main policy recommendation as follows:

1. Inventory of marine turtle nesting potential, including its habitat at sea;
2. Take decisive action against anyone who becomes a predator for turtles, whether done by humans or by fauna. Action will be taken against who is involved in collecting turtle eggs and killing the tortoise for any purpose;
3. Coastal and turtle island nesting places will be preserved and protected from turtle hunters;
4. National park areas that have been designated as turtle protection areas will be closely monitoring, and local communities are invited to participate in conserving turtles;
5. The turtle breeding will be built in the right areas and the old captivity needs to be rejuvenated with due regard to the principles of good and proper management;
6. Turtles and hatchlings need to be scheduled for release to the sea for the sustainability of increased turtle resources;
7. Intensify the promotion of cooperation between national parks in turtle management and regional cooperation of Asia and International with a focus on management activities;

3.3 Constraints

Constraints encountered by each national park in East Java are: a) There are still many untrained supervisors handling turtle management when moving turtle eggs to captive breeding, including managing water properly. Supervisory skills in each national park are becoming an important issue; b) The difficulty of supervisors to control and regulate turtle eggs away from surveillance sites, so that eggs are vulnerable to destruction by predators; c) Observation in each national park is a matter of transport to control the existence of turtle nest that spreads in very distant areas, including communication issues between supervisors with main office, or between supervisors and communities who find violations of turtles and hatchling; d). Suitable research methodology has not been found to

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hatch eggs with high success rates, so traditional methods are still performed with a high risk of death; e). Information on the research ever conducted in national parks either from colleges, NGOs are only registered, without any attempt to review. Review actions are essential as materials for improving turtle management.

IV. CONCLUSION

The objective to be achieved is to manage both existing and undiscovered turtle habitats throughout the turtle's national parks to promote the inherent ecological diversity and integrity (both flora and fauna) around turtle habitats existing on land and at sea.

Goals Management turtle habitats through restoration is emphasizing the preservation of native flora and fauna over the next 10 years to achieve the following conditions: (1.)The balance of existence strategy between native species and non-invasive species. Even degraded indigenous genes, however, are of high value with regard to ecological diversity and management efforts should strive to provide equilibrium tips that support the turtle community; (2). The main threats of turtles in Indonesia are identified as (a) destruction / modification of the breeding habitat, related to intensification or alteration in coastal exploitation, (b) drought and climate change during the rainy and dry seasons poses a clear threat to the existence of both current and which will come; (c) any hunting or exploitation of turtles shall be terminated and the offender shall be punished accordingly; (d) any person who disturbs the habitat and life of the turtle shall pay an environmental fine subject to the degree of damage; (e) Predators of both human and animal life assessed to incriminate the life of the turtle need to be closely monitored, especially during reproduction, and the turtle species suffers from low productivity and ultimately encounter mortality rates in both adult and turtle turtles;

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Tulsi (*Ocimum sanctum*), excellent source of phytochemicals

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Abstract**—** *Ocimum sanctum also known as Tulsi or Holybasil is an aromatic plant and it belongs to the family Lamiaceae. It is widely used as medicine to cure various ailments. The objective of the study was to analyse different phytochemical components of tulsi leaf. The dried powder of Tulsi (50g) was placed in the thimble of Soxhlet apparatus and the experiment was done separately for methanol, ethanol and distilled water. The percentage yield was 8%w/w,7%w/w, and 5%w/w respectively. The study reveals that various secondary metabolites such as carbohydrate, tannin, flavonoids, saponins, glycoside, terpenoid, fatty acids and phenol are present in tulsi leaf extract. From the quantitative analysis it was found that high amount of phenols are present in Tulsi leaf ranging from 1.6 to 7.6 percentages. Consequently the amount of alkaloid and flavonoids ranged from 0.91 to 1.28 and 1.56 to 2.24 percentages respectively. From the GC-MS analysis of methanolic extract three compounds were identified as major constituents viz., Eugenol , Benzene, 1, 2-dimethoxy- 4-(2- propenyl), α - Farnesene and Cyclohexane, 1, 2, 4-triethenyl. Thesephyto-chemicals are known to possess antiseptic, analgesic, anti-inflammatory, antimicrobial, antistress, immunomodulatory, hypoglycemic, hypotensive and antioxidant properties. Hence it is more beneficial to use tulsi asan herbal medicine as compare to chemically synthesized drug.*

Keywords**— *Ocimum sanctum, phytochemical, medicine, GC-MS.***

I. INTRODUCTION

The plant kingdom is an excellent source of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Medicinal plants are rich source of different types of medicines and produce various bioactive molecules. Herbal plant extracts are very useful and are the major sources of medicine which play vital role in controlling various types of pathogens (Doss, 2009) and as growth promoters. These are the cheaper source for therapeutics and viable solution for various pathogens.The medicinal plants extract have now emerged as a good alternative as they are rich in a wide variety of secondary metabolites such as tannins, phenolics, alkaloids and flavonoids etc which enhances growth, innate immune response and disease resistance against pathogenic bacteria in human as well as in different organisms (Edoga *et al*., 2005). About 80% of individuals from developed countries use various medicinal plants as traditional medicines as anticancer drugs (Dewick, 1996), antimicrobial drugs (Phillipson, 1996), antifungal and in various proposes. The medicinal plants are rich sources of secondary metabolites which are chemically and taxonomically extremely diverse compounds with obscure function. A large number of phytochemicals are widely uses in human therapy, agriculture, veterinary, various scientific researches and in different areas (Vasu *et al*., 2009) along with inhibitory effects on all types of microorganisms in vitro (Cowan, 1999).

Ocimum sanctum L. commonly known as holy basil (Tulsi) is an herbaceous perennial, belongs to family Lamiaecae and is considered as one of the most important source of medicine and drugs with many secondarymetabolites and essential oils recommended fortreatment of malaria, diarrhoea, bronchial asthma,dysentery, bronchitis, skin diseases, arthritis, painfuleye disease, chronic fever and eye diseases etc 5,6. Inaddition, Ocimum *sanctum* also showsanticancerous, antifungal, antimicrobial, antifertility,hepatoprotective, antispasmodic, cardio protective,antiemetic, antidiabitic, analgesic, adaptogenic, anddiaphoretic properties6-9.The pharmacological studies reported in the present research confirm the therapeutic value of *O. Sanctum*. Therefore, the present study looks into the extraction and preliminary phytochemical analysis of *O. Sanctum* leaves.

II. MATERIALS AND METHODS

Collection of plant material: Leaves of *Ocimum sanctum* L. (tulsi) were collected from different sites of Dibrugarh District, Assam, washed with sterile water and dried in shades. Then the samples were powered in mechanical grinder.

Aqueous, methanol and ethanol extract: The dried tulsi (50g) powder was placed in the thimble of Soxhlet

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apparatus and 500- 700 ml of distilled water, methanol and ethanol was used for extraction procedure and the experiment was done separately for all the two solvents and distilled water. The extraction was continued till clear solvent or water was seen in the thimble. The extract was concentrated using rotary evaporator. Then the extract was dried in a digital water bath till a dark green residue was obtained. The percentage yield of the extract was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Final weight of the dried extract}}{\text{Initial weight of the powder}} \times 100$$

All the three extracts were kept in separate vials in the refrigerator till further use.

Qualitative phytochemical analysis: The extract was tested following standard biochemical methods as described below.

Test for proteins:

Biuret's test: 2ml of Biuret reagent was added to 2ml of extract. The mixture was shaken well and warm for 5 min. Appearance of red or violet colour indicated presence of proteins.

Million's test: Crude extract was mixed with 2ml of Millon's reagent, if precipitate appeared which turned red on gentle heating confirmed the presence of protein.

*Ninhydrin test:*Crude extract was mixed with 2 ml of 0.2% solution of Ninhydrin and boiled for some time, if violet colour appeared indicating the presence of amino acids and proteins.

Test for carbohydrates:

Fehling's test: Equal amount of Fehling A and Fehling B reagents were mixed and 2ml of it was added to the plant extract and then gently heated the sample. Appearance of brick red precipitate indicated the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Molisch's test: 2ml of Molisch's reagent was added to 0.5 ml of crude extract and the mixture was shaken properly. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Appearance of a violet ring at the interface indicated the presence of carbohydrate.

Iodine test: 2ml of iodine solution was mixed with 0.5 to 1 ml of crude extract. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for phenol: 2 ml of alcohol and 2-3 drops of ferric chloride solution was added to 1 ml of crude extract, blue-green or black coloration indicated the presence of phenols

Test for tannin: 1 ml of distilled water and 2-3 drops of ferric chloride solution was added to 0.5 ml of crude extract. A black coluration indicated the presence of tannin.

Test for flavonoids

Shinoda test: Crude extract was mixed with small amount of magnesium and concentrated HCl was added drop wise. Appearance of pink scarlet colour after few minutes indicated the presence of flavonoids.

Alkaline reagent test: 0.5 ml of crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for saponins: 1ml of crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides

Liebermann's test: Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. If colour change from violet to blue to green which indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test: 2ml of chloroform was mixed with crude extract. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of glycoside.

*Keller-kilani test:*0.5 ml of crude extract was mixed with 2ml of glacial acetic acid containing 2-3 drops of 2% solution of FeCl3. Then 2ml of concentrated H_2SO_4 was poured into the mixture. A brown ring at the interface indicated the presence of cardiac glycosides.

Test for steroid

(i) 2ml of chloroform was added to the crude extract of Tulsi. Then 2ml of each of concentrated H_2SO_4 and acetic acid were added into the mixture. The presence of steroids was indicated by appearance of a greenish coloration in the reaction mixture.

(ii) Crude extract was mixed with 2ml of chloroform and gently added concentrated H_2SO_4. A red colour was seen in the lower layer this indicated the presence of steroids.

Test for terpenoids: Crude extract was mixed in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. Presence of terpenoids was indicated by a greyish colour at the interface.

Test for alkaloids: 2ml of 1% HCl was mixed with crude extract and heated gently. After heating, Mayer's And Wagner's reagents were added to the mixture. If precipitate was observed in the reaction mixture which indicated the presence of alkaloids.

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Test for anthraquinone: 5ml of chloroform and 5 ml of ammonia solution was added to 0.2 gm of plant extract. Appearance of pink, red or violet colour indicated the presence of anthraquinone.

Oils & Fats: A small quantity of crude extract was pressed between two filter papers separately. An oily appearance on filter paper indicated the presence of fixed oil and fats.

Test for lactones

*Baljet's test:*Crude extract was treated with sodium picrate solution. Presence of lactone was observed by appearance of yellow to orange colour in the mixture.

Quantitative analysis of phytochemical in the plant extract:

Determination of total phenolic contents (Singleton *et al*., 1999): The amount of total phenol for aqueous, methanol and ethanol extract were determined by Folin-Ciocalteu reagent method. 2.5 ml of 10% Folin- Ciocalteu reagentand 2 ml of 2% Na_2Co_3 were added to 0.5 ml of plant extract. The mixture was then incubated at room temperature for 30 minutes. Gallic acid was used as standard (1mg/ml). The absorbance of the sample was measured at 765nm. All the tests were done in triplicates and the results were determined from standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound).

Determination of alkaloid (Harborne, 1973): 5 g of the sample was taken and 200 ml of 10% acetic acid in ethanol was added to the sample and allowed to stand for 4 hours. Then the solution was filtered and the extract was concentrated on water bath Conc. $NH_4(OH)$ was added drop wise and the whole solution was allowed to settle and the precipitate was then washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed and this was the amount of alkaloid present in the plant material

Determination of flavonoids (Bohm & Kocipai-Abyazan, 1994): 10 g of plant sample was taken and extracted repeatedly with 100ml 80% methanol. Then the solution was filtered and the filtrate was transferred into an empty crucible and evaporated into dryness over water bath and weighed. The final weight dry weight was amount of flavonoids in the plant sample.

Preparation of stock solution

The extracts were reconstituted in methanol. Methanolic extracts (1 µl) were injected for GC-MS analysis.

Gas Chromatography-Mass Spectrometry analysis

Methanolic extract of the leaves of *Ocimum sanctum*was subjected to GC-MS analysison a GC- MS Clarus 500 Perkin Elmer systemcomprising a AOC- 20i autosampler and gaschromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the followingconditions: Restek RtxR – 5, (30 meter X 0.25 mm)(5% diphenyl / 95% dimethyl polysiloxane), runningin electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of1ml/min and an injection volume of 2.0 µl wasemployed(split ratio of 10:1); injector temperature280^{0}C. The oven temperature was programmedfrom 50°C (for 1 min.), with an increaseof 6 ^{0}C / min to 280 ^{0}C, then ending with aisothermal for 15min at 280°C. Mass spectra weretaken at 70 eV; a 0.5 seconds of scan interval andfragments from 40 to 550 Da. Total GC runningtime was 60 minutes.

Identification of Compounds

Interpretation on mass spectrum GC-MS was conducted using the database of department of Chemistry; Dibrugarh University. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the department of Chemistry library.

III. RESULTS

The yield of residue after Soxhlet extraction and evaporation of 50 gm dried plant leaves in methanol, ethanol and water were as follows:

Table.1: Amount of plant extracts yield percentage in different solvents

Extract	Yield amount (%) W/W
Aqueous	5%
Methanol	8%
Ethanol	7%

The phytochemicals analysis in *Ocimum sanctum* (Tulsi) leave extracts in the two solvents and aqueous conditions were summarized in Table 2. Various bioactive molecules were found in Tulsi leaf extract from the phytochemical screening. The amount of extraction is more in case of organic solvent then that of water. From the quantitative analysis it was found that high amount of phenols are present in Tulsi leaf ranging from 1.6 to 7.6 percentages. Consequently the amount of alkaloid and flavonoids ranged from 0.91 to 1.28 and 1.56 to 2.24 percentages respectively.

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Table.2: Qualitative phytochemical screening methanol extract of tulsi leaf

Phytochemicals	**Aqueous extract**	**Methanol extract**	**Ethanol extract**
Protein	-	-	-
Carbohydrate	-	+	+
Phenol	+	+	-
Tannin	-	+	+
Flavonoid	+	+	+
Saponin	-	+	+
Glycosides	+	+	+
Steroid	-	-	-
Terpenoid	-	+	+
Alkaloid	+	+	+
Anthraquinone	-	-	-
Fixed oils and fatty acid	-	+	-
Test for lactones	-	-	-

"+"present, "-" absent

Table.3: Percentage of total phenolic, alkaloid and flavonoid contents in plant extract

Extract	**Phenolic**	**Alkaloid**	**Flavanoid**
Aqueous	1.61±0.56	0.91±0.66	1.56±0.64
Methanol	7.61±0.55	1.28±0.03	2.24±1.02
Ethanol	4.61±0.56	0.94±0.58	1.91±0.56

Each value is the average of three analysis and ± standard deviation.

Table.4: Chemical constituents and the activity of some of the phytocomponents of Ocimum sanctum

Sl. No	Retention time (unit?)	Name of the compounds	Molecular weight	Molecular formula	Activity**
1.	7.20	Eugenol	164	$C_{10}H_{12}O_2$	Anti- inflammatory, antioxidant, anticancer, Acaricide, Antibacterial,Antispasmodic, Antiviral, Insecticide
2.	7.70	α - Farnesene	93	$C_{15}H_{24}$	Acaricide, allergenic, analgesic, anaesthetic, antibacterial, anti-inflammatory, antiedemic, antioxidant, antiviral, antitumor, antiulcer
3.	7.50	Benzene, 1, 2-dimethoxy-4-(1-propenyl)	178	$C_{11}H_{14}O_2$	Insect-attractant, perfumery, flavour antibacterial, nematicide
4.	13.36	Cyclohexane,1,2,4-triethenyl	162	$C_{12}H_{18}$	Antibacterial, anti-inflammatory, antiedemic, antispasmodic

**Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

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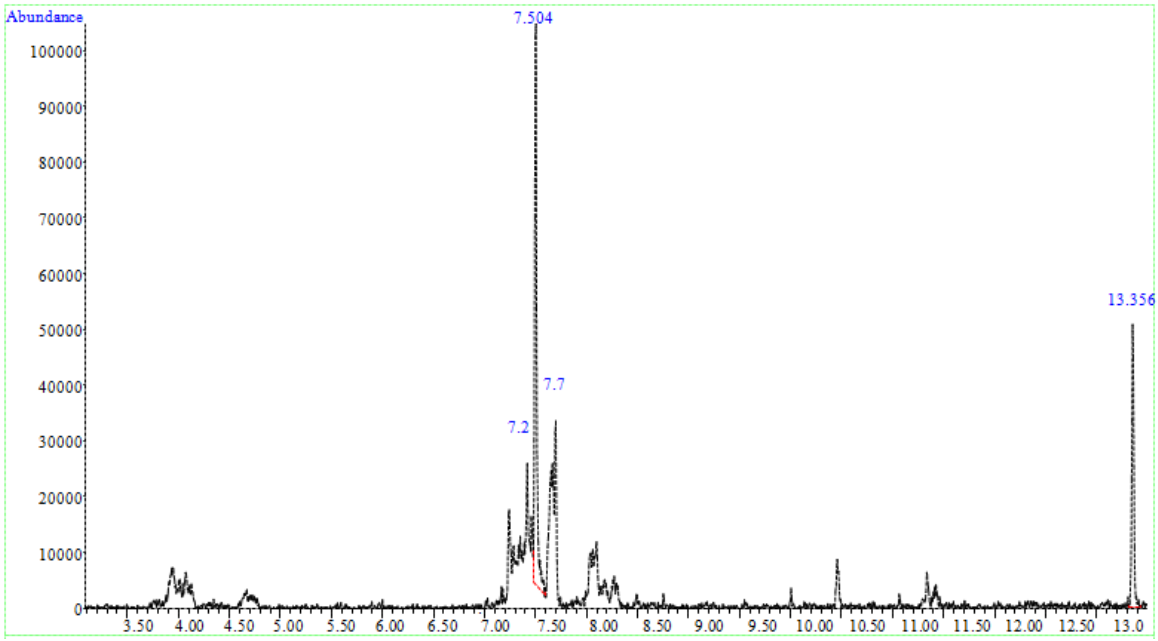

Fig.1: GC- MS chromatogram of the methanolic extract of the leaves of Ocimum sanctum

IV. DISCUSSION

Ocimum sanctum has various properties such as antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective and antioxidant (Williamson, 2002, Tanwar *et al*., 2015). Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituents present in *O. sanctum* have been found to be largely responsible for the therapeutic potentials (Sailaja *et al*., 2010). This plant has various properties such as antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective and antioxidant (Williamson, 2002, Tanwar *et al*., 2015). Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituents present in *O. sanctum* have been found to be largely responsible for the therapeutic potentials (Sailaja *et al*., 2010).

The study reveals that various secondary metabolites such as carbohydrate, tannin, flavonoids, saponins, glycoside, terpenoid, fatty acids and phenol are present in tulsi leaf extract. Leaves of *Ocimum sanctum* contain water-soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophylllene that may act as an immunostimulant. Saponins act as anti-hyperlipedemic, hypotensive and cardiodepessive properties (Bairwa*et al*., 2012). The phytochemical constituents such as alkaloids, steroids, flavanoids, tannins, phenols and several other aromatic compounds of plants serve a defense mechanism against predation by many microorganisms, insects and other herbivore (Bonjar *et al*., 2004) Glycosides can act as cardiostimulants in cases of cardiac failure (Sood et al., 2005). Tannins have anti diarrheal and haemostasis properties (Asquith *et al*., 1986). Flavanoids are responsible for antioxidant and immunostimulatory properties. According to Cragg *et al*., 1999 and Khanna *et al*., 2003 alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants and these antibiotic principles are actually the defensive mechanisms of the plants against pathogens.

GC-MS chromatogram of the methanolic extract of *Ocimum sanctum* showed four major peaks (Figure.1) and has been identified after comparison of the mass spectra with the department of Chemistry library, DU, indicating the presence of four phytocomponents. It was observed that presence of Eugenol (Synonym: 2-Methoxy- 4-(2-propenyl) phenol), Benzene, 1, 2-dimethoxy- 4- (2-propenyl) - (synonym: Methyl- Isoeugenol), α - Farnesene and Cyclohexane, 1, 2, 4- triethenyl were the major components in the extract. The phytochemicals that contribute to the medicinal property of the plant leaves is listed in Table No.1. Benzene, 1, 2-dimethoxy- 4-(1-propenyl) (Methyl-Isoeugenol) has the property of Antifungal activity (Kurita *et al*., 1981), Nematicidal activity (Park *et al*., 2003) and Antifeedant activity (Katsumi, 1987).Eugenol is reported to possessAntimycotic (Azzouz *et al*., 1982) Antiviral (Bishop, 1995) Desinsection (Konstantopoulou *et al*., 1992) Antiparasitic (Pandey *et al*., 2000) Antioxidant (Ou *et al*., 2006) Anticancer (Hussain *et al*., 2011) and Ant insect activities(Pessoa *et al*., 2002).

Leaves extract of *O. sanctum* affected both specific and non-specific immune responses and disease resistance against fungal and bacterial infection(Santra *et al*., 2017). It stimulated both antibody response and neutrophil activity. The experimental studies have shown

that methanolic extract of *Ocimum sanctum* has anticancer effect by inhibition of nitric oxide synthesis (Kim *et al*., 1998). The use of medicinal plants acts as a source of antimicrobial agent also for aquaculture. In *Macrognathus pancalus*, the extract of *O. sanctum* was found to enhance the antibody response (Dugenci *et al*., 2003). The different leaf extracts of Tulsi (*Ocimum sanctum)*, shows antimicrobial activity against three human pathogens *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. (Subramanian et al.,2014). Tulsi oil showed significant anti-inflammatory, analgesic, antipyretic and antimicrobial effects. It has also shown memory enhancing, antifertility, anticataract, antithyroid, antiulcer, antidiabetic, antiarthritic, antiamnesic, antihelmenthic, anticataract, hepatoprotective and no tropic activity (Rajesh *et al*., 2013). Alcoholic extract increased step down latency and acetyl cholinesterase inhibition and so used in the treatment of cognitive disorders. *Ocimum sanctum* has been widely employed in traditional medicines. Hence phytochemicals from this plant can be used in variety of disorders afflicting mankind. The herbs are cheap, available in large quantity around us and they pose no danger to the living organisms, the environment and the consumers and hence greatly helpful for living organisms.

V. CONCLUSION

The presence of various bioactive compounds in the tulsi leaves justifies the uses for various ailments by living population. The results confirm the use of *Ocimum sanctu*m plant as traditional medicinal properties andsuggest that some of the plant extracts possess compounds with antimicrobial properties that can be used asantimicrobial agents in new drugs for the therapy of infectious diseases caused by various pathogens. It is more beneficial to use tulsi asan herbal medicine as compare to chemically synthesized drug.

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Agricultural and biomedical application of Silver Nanoparticles synthesized by *Halimeda gracilis* Harvey ex J. Agardh

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***Abstract*—** *The present work is focused on the biosynthesis of the Silver (Ag) Nanoparticles using an aqueous extract of the green seaweed Halimeda gracilis. The visible colour change indicated the biosynthesis of Silver Nanoparticles and the specific peak produced within the UV-Vis spectrum confirmed the biosynthesis of Silver Nanoparticles. The possible functional groups were identified with Fourier Infrared Spectroscopy. The morphological characterization of biosynthesized Silver Nanoparticles was done by Scanning Electron Microscopy and Dynamic light scattering measurements and Zeta potential. The average size distribution of Ag-Nanoparticles were 295.9 (d.nm) and fairly stable with a zeta potential value of -28.6 mV. The size of biosynthesized Ag-Nanoparticles was also measured with X-ray diffraction assay. Due to agglomeration, the size difference of biosynthesized Ag-Nanoparticles in case of SEM and EDX occurred. The biosynthesized Ag-Nanoparticles were assayed for their antibacterial activity against some human pathogens and for their potential on seed germination of Abelmoschus esculentus and Raphanus sativus var. longipinnatus. The antibacterial activity of biosynthesized Ag Nanoparticles was the highest against Proteus mirabilis (2.33±0.2 cm), followed by Klebsiella pneumoniae, (1±0.0 cm). The effect of biosynthesized Ag-Nanoparticles on the seed germination of Raphanus sativus var. longipinnatus was excellent as the germination rate was 100 percent for Ag-Nanoparticles treated seeds, which was better than normal seaweed extract and seaweed liquid fertilizer treated seeds. The seed germination was also good for Abelmoschus esculentus with the treatment of seaweed mediated Ag-Nanoparticles as germination rate was 60 percent. This work proved that seaweed synthesized Ag-Nanoparticles are Phyto-friendly in nature and in future nano-bio fertilizer may be used as the growth promoter and eco-friendly Nano-bio-fertilizer.*

Keywords*— *Green synthesis, seaweed, Silver nanoparticles, seed germination, antibacterial activity.

I. INTRODUCTION

The drug resistance is a serious medical problem, as the strains are rising fast and mutated consequently, so the treatment of some diseases such as diarrheal diseases, malaria, Urinary tract infections and Tuberculosis (TB) required the new drugs. The pathogenic bacteria due to their multidrug resistance properties, they are gradually becoming resistant against many market available antibiotics. It may be due to uncontrolled and improper applications of various antibiotics, which resulted multidrug resistant bacteria (Manikandan et al. 2011). Some resistant strains such as Vancomycin Resistant Enterococci (VRE) (Gold, 2001), extended Spectrum Beta lactamase resistant Enterococci (Bhattacharya, 2006) had been reported previously. So, there is need to find out the better antibacterial compounds. Some seaweed compounds had been reported as antimicrobial agents (Pérez et al. 2016) but all seaweeds are not yet assayed for their bactericidal effect, likewise silver nanoparticles from some seaweeds had also been reported for its antimicrobial activity (Kumar et al. 2013, Gandhi et al. 2016, and Vivek et al. 2011). The major problem in agriculture is the use of chemical fertilizer, which gradually increased the chemical toxic effect in the soil by destroying the soil quality and also effecting on the production of crops and vegetables. Now a day, seaweeds were used as bio-fertilizer to save the environment by practicing organic farming, the effect of liquid fertilizer of *Stochiospermum marginatum* was analysed to brinjal plants which revealed promoting effect on growth and productivity of brinjal plants (Sivasangari Ramya et al. 2015). So, on the basis of the mentioned problem, I selected *Halimeda gracilis* for synthesis of Silver nanoparticles synthesized and investigated for its bactericidal effect and the potential for seed germination. This green seaweed and its mediated Silver nanoparticles, not yet investigated for the above mentioned applied fields for its applications.

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II. MATERIALS AND METHODS

Biosynthesis of Silver Nanoparticles:

Seaweed extracts preparation:

The fresh seaweed *Halimeda gracilis* had been collected from Olaikuda (09°18.309'N and 079°20.076'E), Rameshwaram (Fig. 1), southeast coast of India. Seaweed was identified with the standard taxonomic key of CMFRI. It was washed with *in-situ* sea water and distilled water thrice. Then, 20 gm of seaweed *Halimeda gracilis* (Fig. 2) was cut into very small pieces and ground to make it powder and was dissolved in 100 ml of distilled water and boiled for 10 minutes. The crude extract of seaweed was filtered with Whatman No. 1 filter paper and repeatedly filtered with a thin layer of cotton to get the clear seaweed extract. This crude seaweed extract was stored in 4˚C for further use.

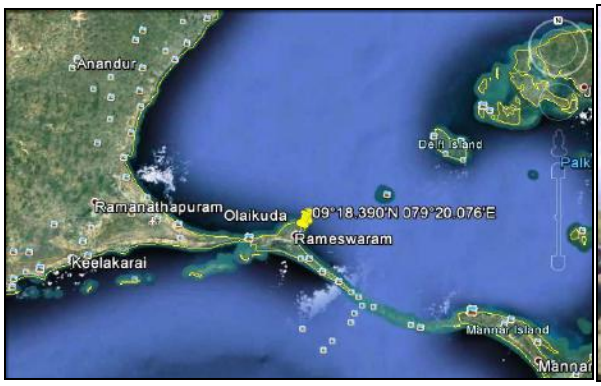

Fig 1: Map showing the sampling location. Fig 2: Halimeda gracilis, (Division-Chlorophyta, Class-Chlorophyceae, and Family - Halimedaceae)

Preparation of seaweed extract:

The collected seaweeds were washed repeatedly to properly clean for further use. The fresh cleaned seaweeds were cut into small pieces and grinded in mortar and pastel to make as paste, and then mixed well with 100 ml distilled water. The mixture was boiled in water bath for 10 minutes. The solution was cooled and filtered and the filtrates were kept in freeze at 4˚ C for biosynthesis of Silver Nanoparticles.

Biosynthesis of Silver Nanoparticles:

The aqueous 1 mM $AgNO_3$ solution was prepared with distilled water mixed with silver nitrate powder. The 10 ml seaweed extract as prepared above was taken and the Silver Nitrate solution of 90 ml was mixed in a conical flux for biosynthesis of Ag-Nanoparticles. The conical flux was placed in mechanical shaker at 120 rpm for 72 hours at continuous shaking. The solution colour was gradually change to reddish brown to red which is may be due to biosynthesis of Silver Nanoparticles.

Preparation of seaweed liquid bio-fertilizer:

The 10 gm of fresh chopped seaweed was boiled with 10 ml of distilled water and boiled for 1 hour in water bath and this solution was filtered through muslin cloth. The solution was kept at room temperature to cool completely and solution was filtered with what man filter paper of pore size 20-25µm and the extracted seaweed liquid fertilizer was kept in 4ºC freeze for future used.

Collection of seeds:

The market available seeds of ladies finger, *Abelmoschus esculentus* (L.) Moench, belong to family Malvaceae and *Raphanus sativus* var. *longipinnatus* belong to family Brassicaceae were used for the present seed germination experiment.

Characterization of biosynthesized Silver Nanoparticles:

UV-Visible Spectrophotometer:

The biosynthesized Silver Nanoparticles after 3 days of shaking, distilled water and silver nitrate solution was scanned from 300 nm to 700 nm at spectrophotometer (SHIMADZU).

Fourier Transform Infrared (FT-IR) Spectroscopy:

The biosynthesized Silver Nanoparticles solution was made a pellet of dry powder, after drying of collected residue which was collected after centrifugation for 30 minutes at 5000 rpm. The adequate amount of dry powder of Silver Nanoparticles and powder KBr was mixed well to prepare pellet for analyse to FT-IR spectroscopy for the identification of possible functional groups present in the biosynthesized Silver Nanoparticles.

Scanning Electron Microscopy:

The scanning electron microscopic images were used for analysing the morphological structure of biosynthesized Silver Nanoparticles. The powder of biosynthesized Silver Nanoparticles sprayed on grid for preparation of a thin film and images were taken under

Dynamic Light Scattering (DLS):

The size distribution of Silver Nanoparticles was analysed by dispersed it in Milli-Q water, similarly zeta potential of solution of Silver Nanoparticles was measured by Malvern method. For measurement, the

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Silver Nanoparticles was dispersed in measurement cuvette and analysed for 60 seconds at room temperature.

XRD Measurements:

The dried biosynthesized Silver Nanoparticles were sprayed on measurement cuvette and measured under BRUKER D8 ADVANCE POWDER X-ray diffractometer. The samples were analysed from 20 to 80˚ theta (θ) range and the operating voltage was 20 KV.

Test for potentiality for seed germination:

For surface sterilization of seeds, seeds were kept in 5% Sodium hypochlorite solution for 15 minutes. Then, seeds were dipped in biosynthesized Silver Nanoparticles solution over night. The seeds were also kept in normal water for control treatment. In sterile Petri plate, one piece of sterile filter paper was placed which was wetted with biosynthesized Silver Nanoparticles, then all seeds were kept within Petri plates and 5 ml of biosynthesized Silver Nanoparticles was added to the Petri plates. Then, seeds were incubated for 12 hours in room temperature. After germination of seeds, the seed germination parameters such as germination index, relative root elongation and relative seed germination along with the major parameters of seed germination i.e. seed germination percentage, seed germination rate and the mean seed germination time was calculated according to the standard equations used by Barrena *et al*., 2009; Thakkar et al. 2010 and Taga *et al*. 1984. The equations are as mentioned below.

Germination Percentage (GP %) = $(G_f/n) \times 100$ (1).

The G_f in equation is representing the total number of germinated seeds within the complete experiment duration.

Mean Germination Time (MGT) = $\Sigma Ni\ Di\ /n$ (2).

n= total seeds of experiment, Ni = at i^{th} day, the total germinated seed, and Di denoted as total number of the day at of experiment.

Germination Rate (GR) = $\Sigma\ Ni/\ \Sigma\ Ti.$ (3)

Where Ni is denoted total germinated seeds at time Ti.

GR = (a/1) + (b-a/2) + (c-b/3) +.....+ (n-n-1/N) (4)

Relative root elongation (E)

= (Mean root length with NPs) / (Mean root length with control) ×100

Germination index (GI)

= (Relative seed germination) × (Relative root elongation) / 100

Where, Relative seed germination

= (Seeds germinated with NPs) / (Seeds germinated with control) ×100

Antibacterial activity of biosynthesized Silver Nanoparticles

Antibacterial assay:

Antibacterial activity of the biosynthesized Silver Nanoparticles using aqueous seaweed extracts of *Chaetomorpha antennina*, *Chlorodesmis hildebrandtii*, *Halimeda gracilis*, *Amphiroa anceps*, and *Sargassum cinctum* was assayed by agar disc diffusion method against six human pathogenic bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, and *Proteus mirabilis* which were collected from Department of Medical Microbiology, Raja Muthiah Medical College, Annamalai University. Each pathogen was taken from pure culture and inoculated into freshly prepared nutrient broth which was sub-cultured from pure culture. After 24 hours of culture, each bacterial culture was inoculated into the agar plates and kept for 24 hours. The market available Chloramphenicol antibiotic was used as positive control. The 500 mg powder Chloramphenicol was dissolved in 100 ml autoclaved distilled water to a concentration of 5 mg/ml. The silver nitrate solution (1 mM) was considered as negative control. The beads of filter paper were soaked with 20 µl of antibiotic solution, silver nitrate solution and biosynthesized Silver Nanoparticles solution. The beads were placed in Petri plates with bacterial culture. The diameter of inhibitory zone was measured after 24 hours with technical measuring scale.

Statistical Analysis:

Each bacterial culture was inoculated in triplicates. The diameters of developed zone of inhibition were measured and from three zones of inhibitions, mean of zone of inhibition with standard deviation were measured.

III. RESULTS AND DISCUSSIONS

Biosynthesis of Silver Nanoparticles from *Halimeda gracilis* Harvey ex J. Agardh and its effect on seed germination:

Biosynthesis and characterization of Silver Nanoparticles:

The change of colour from white to dark brownish of the mixed solution indicated the biosynthesis of Silver Nanoparticles (Fig. 3). The broad bend at 425 nm to 430 nm produced in UV-Visible spectrum confirmed the biosynthesis of Silver Nanoparticles (Fig. 4).

The SEM images, of seaweed synthesized Silver Nanoparticles indicated the presence of cubical, hexagonal and irregular shaped scatter and well distributed Nanoparticles with size less than 100 nm (Fig. 5). For aqueous extract of seaweed, the 3420.83 cm^{-1} bend

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indicated the presence of amine (N-H) groups. The alkenes groups may be present due to the broad bend at 1636.70 cm^{-1} and several peaks narrow peaks at 1507.76 cm^{-1}, 1522.23 cm^{-1}, 1541.26 cm^{-1} produced due to aromatic group (C=C) and nitro (N-O) group, amine group, and consequently the stretches at 1490.41 cm^{-1}, 1458.09 cm^{-1} and 1384.06 cm^{-1} recorded the functional groups alkanes (C-H), aromatic (C=C) group, nitro (N-O) group, ester group. The stretches around 500 cm^{-1} to 400 cm^{-1} indicated the presence of alkyl halides (Fig. 6). The biosynthesized Silver Nanoparticles and the aqueous extract of seaweed were analysed for the characterization of their functional groups and their properties to FTIR Spectroscopy. For synthesized Silver Nanoparticles, the presence of bend at 3421.24 cm^{-1} indicated the presence of amine (N-H) group, 2924.25 cm^{-1} and 2851.32 cm^{-1} stretches reported the presence of alkanes (C-H) and aldehydes (C=O), (C-H) functional groups. The stretches at 1636.75 cm^{-1}, 1384.58 cm^{-1}, 1021.95 cm^{-1} and 527.63 cm^{-1} was recorded the presence of alkanes (C=C) stretch, N-O (nitro), ether, alkyl halides such as (C-I, C-Br) groups (Fig. 7). The peaks produced in X-ray Diffractometer spectrum demonstrated the formation of hexagonal and cubical biosynthesized Silver Nanoparticles as the peaks have broader base and the narrower apex which indicated the presence of reduced crystal size Silver Nanoparticles. The observed peaks were found at 2θ values at 28.25˚, 32.10˚ and 46.25˚. The grain size (D) was 30.10 nm. The equation used for analysis of the grain size as followed - $\beta = \pi/180\times$ width (x); $D = k\lambda/\beta\cos\theta$ (nm) where X= 0.21246, 2θ= 32.10˚ (Fig. 8). The Z-average size distribution of Ag-Nanoparticles was found 295.9 d. nm and particles were well distributed in the water solution (Fig. 9). The zeta potential value of Ag Nanoparticles, (-28.6) mV, indicated the high stability of Silver Nanoparticles (Fig.10).

Biosynthesis & characterization of Silver Nanoparticles by *Halimeda gracilis*

Fig. 3: Showing the biosynthesis of Ag- Nanoparticles from Halimeda gracilis.

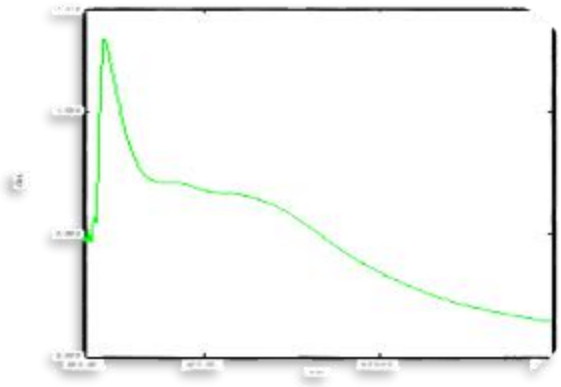

Fig. 4: Showing UV-Vis spectrum of biosynthesized Ag-Nanoparticles.

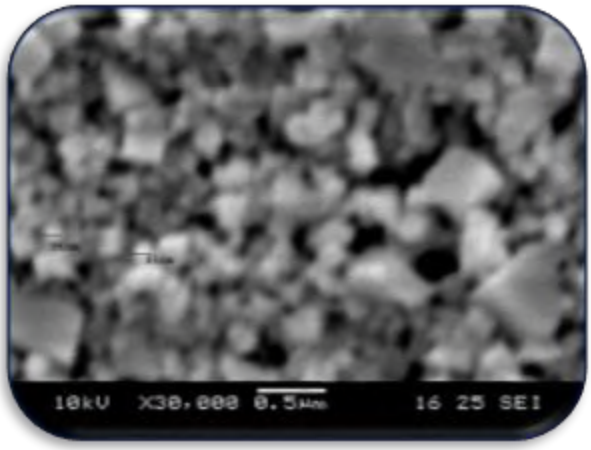

Fig. 5: Showing SEM image of biosynthesized Ag-Nanoparticles.

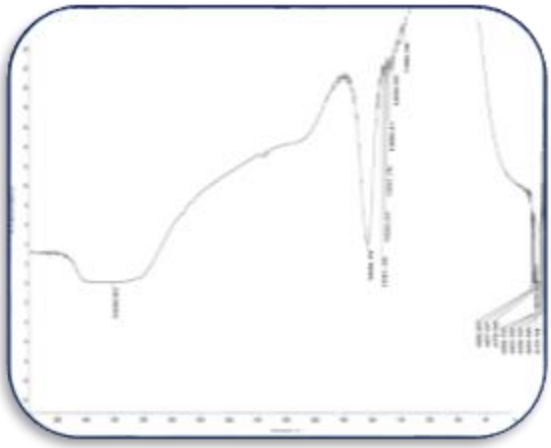

Fig. 6: Showing FT-IR spectrum of aqueous extract o of Halimeda gracilis.

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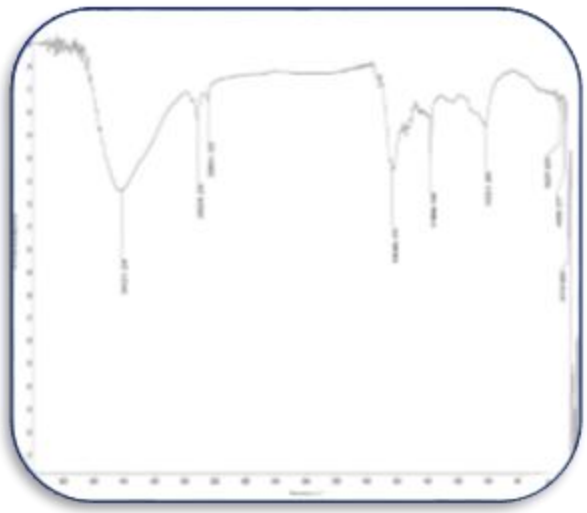

Fig. 7: Showing FT-IR spectrum of biosynthesized Ag-Nanoparticles.

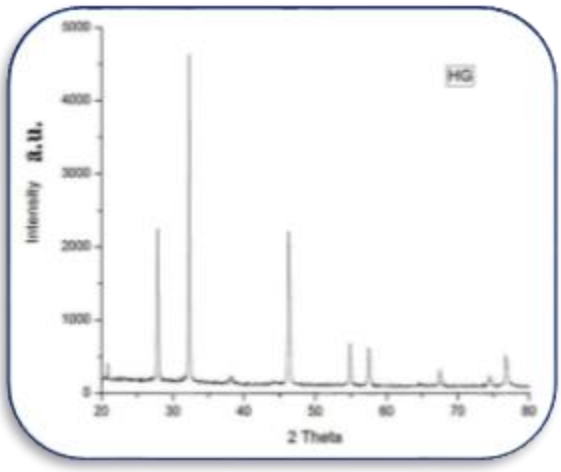

Fig. 8: Showing the XRD pattern of biosynthesized Ag-Nanoparticles.

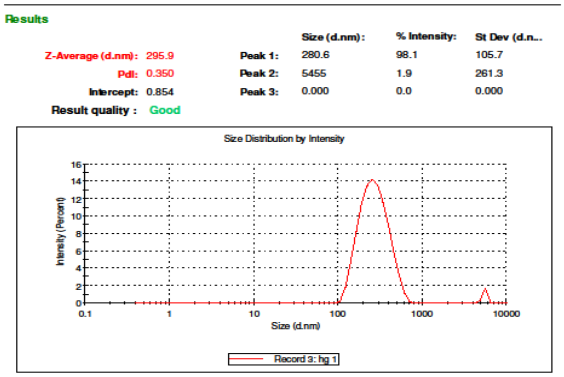

Fig. 9: Showing the size distribution of Ag-Nanoparticles.

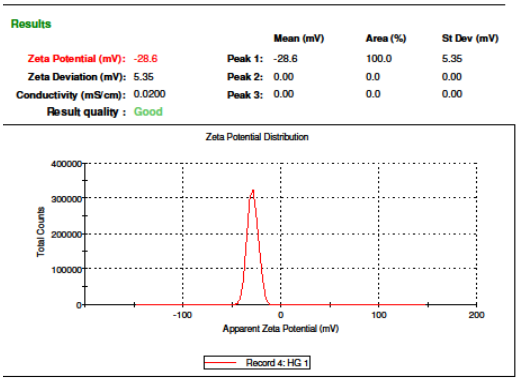

Fig. 10: Showing the Zeta potential of Ag-Nanoparticles.

Effect of biosynthesized Ag-Nanoparticles on seed germination of *Abelmoschus esculentus*:

The germination percentage of the seeds treated with Ag-Nanoparticles was 60% which was the highest at 24 hours and 48 hours as compared to the control seeds which was 40% but at 96 hours seed germination percentage was equal for seeds treated with Silver Nanoparticles and normal water which was 60% [Fig.11 (a)]. The mean germination time was the highest at 96 hours for both control and Ag-Nanoparticles seeds. The seeds germination, mean germination time was lower for seeds treated with Ag-Nanoparticles as compared to control at 24 hours and 48 hours [Fig. 11 (b)]. The germination rate was high at 24 hours for both Ag-Nanoparticles treated seeds and also controls. The germination rate was gradually decreased with time for both control and Ag-Nanoparticles treated seeds [Fig. 11 (c)]. The *Abelmoschus* seeds which were treated with Ag-Nanoparticles had the highest germination index of 273.80 at 48 hours [Fig.11 (d)] and relative root elongation was gradually increased from 24 hours to 96 hours and the relative root elongation was 146.34 at 24 hours, 182.53 at 48 hours and 208.33 at 96 hours.

The seed germination time was minimum for normal water treated seeds in compared to the biosynthesized Ag-Nanoparticles treated seeds after 24, 48 and 96 hours of treatment [Fig. 11 (e) and (f)].

Effect of biosynthesized Silver Nanoparticles on seed germination of *Abelmoschus esculentus:*

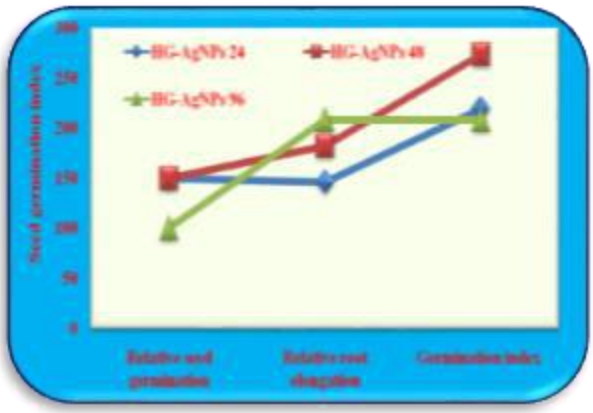

Fig. 11 (a): Showing seed germination. index.

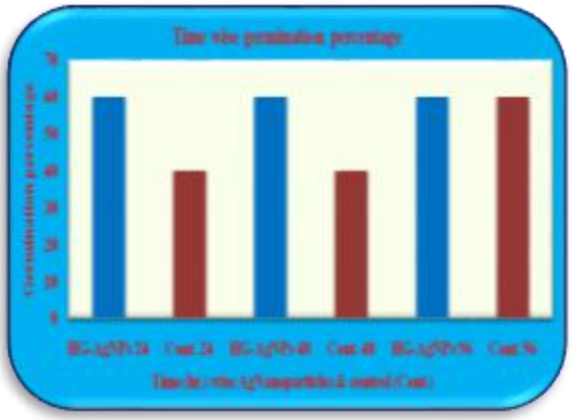

Fig. 11 (b): Showing seed germination. percentage.

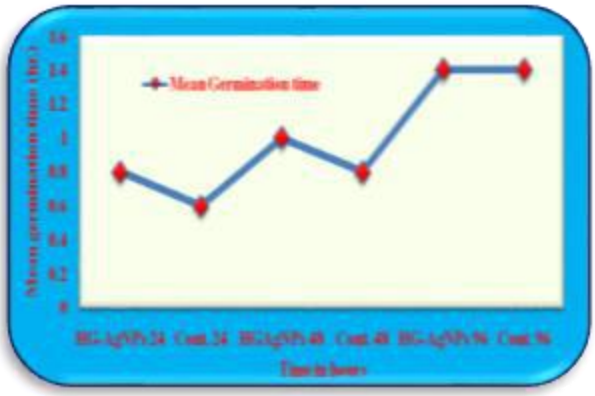

Fig. 11 (c): Showing mean seed germination time.

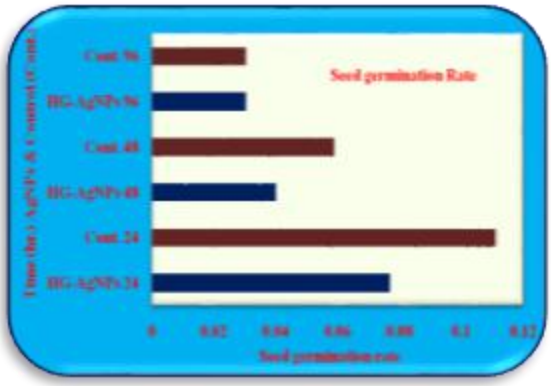

Fig. 11 (d): Showing seed germination rate.

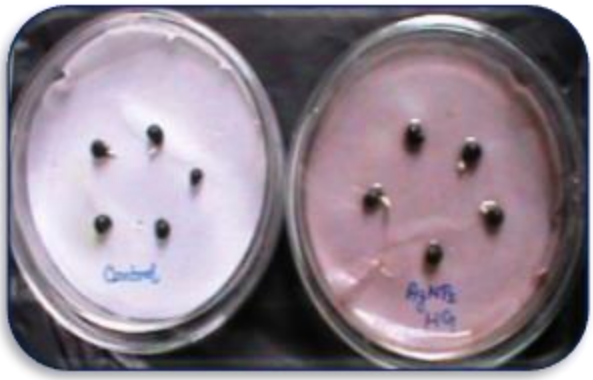

Fig. 11 (e): Showing seed germination.

Fig. 11 (f): Showing seedling of ladies finger.

Effect of biosynthesized Ag-Nanoparticles on seed germination of *Raphanus sativus* var. *longipinnatus*:

For *Raphanus sativus* var. *longipinnatus* the seed germination index was 1466.66 which were the highest at 24 hours including the highest relative seed germination and relative root elongation, followed by germination index of 204.77 at 48 hours and 243.85 at 96 hours [Fig 12 (a)]. The germination percentage was 100% at 24, 48 and 96 hours, it indicated that all seeds were germinated with the treatment of seaweed synthesized Ag- Nanoparticles; but the germination percentage was 40 % at 24 hours and 60 % at 48 and 96 hours for the normal water treated seeds [Fig. 12 (b)]. The mean germination time was gradually increased with increase of incubation time; at 24 hours mean germination time was minimum which indicated that germination rate was high at 24 hours for Ag-Nanoparticles treated seeds. The germination rate was 0.17 seed/hour and the seeds treated with Ag-Nanoparticles had maximum germination at 24 hours in comparison with normal water treated seeds [Fig. 12 (c), (d) (e), and (f)].

Effect of biosynthesized Silver Nanoparticles on seed germination of *Raphanus sativus var. longipinnatus.*

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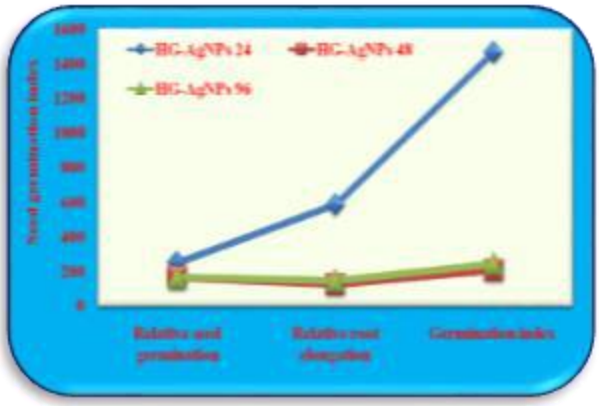

Fig. 12 (a): Showing seed germination index.

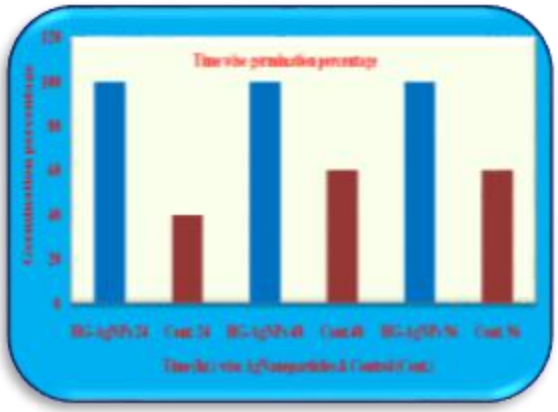

Fig. 12 (b): Showing seed germination percentage.

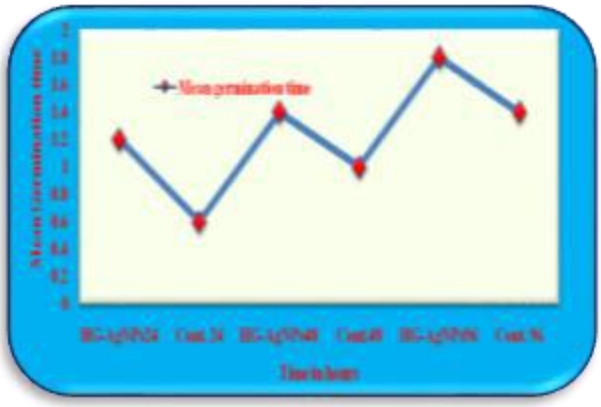

Fig. 12 (c): Showing mean seed germination time.

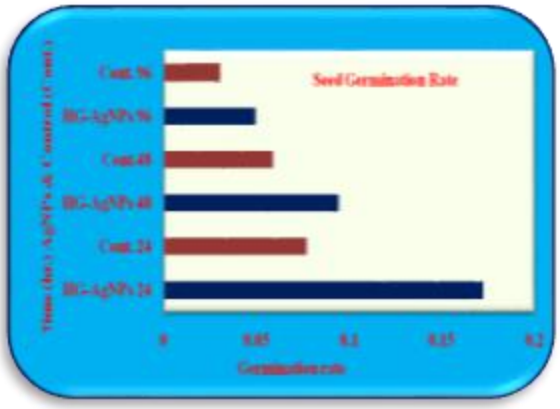

Fig. 12 (d): Showing seed germination rate.

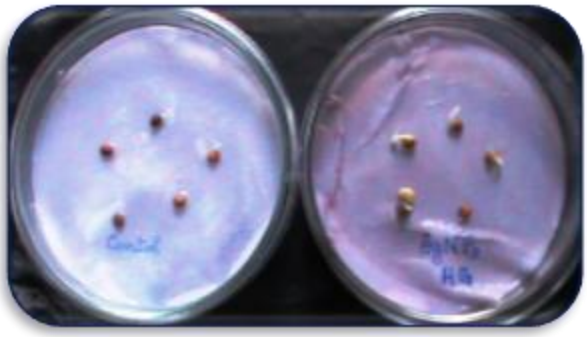

Fig. 12 (e): Showing seed germination.

Fig. 12 (f): Showing seedling of radish.

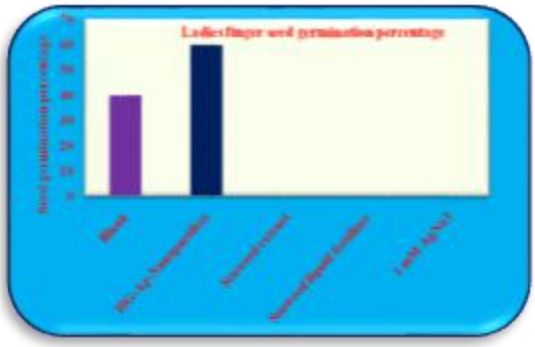

Fig. 13 (a): Showing seed germination percentage of ladies finger.

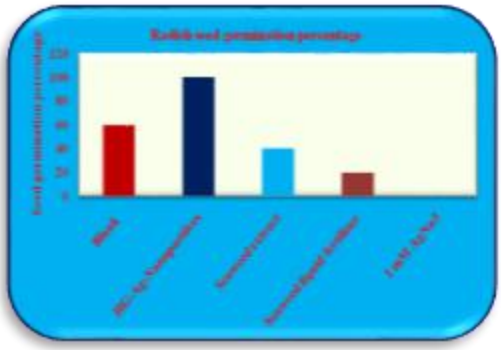

Fig. 13 (b): Showing seed germination percentage of radish.

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Effect of silver nitrate solution, seaweed liquid fertilizer and seaweed extract on ladies finger and radish seed germination:

The 1 mM $AgNO_3$ solution was used as negative control to test its effect on seed germination in comparison with normal water for both seeds and radish and ladies finger. The seed germination was 40% for ladies finger and 60% seeds of radish in normal water but no germination occurred for seeds treated with 1 mM $AgNO_3$ solution. It proved that 1 mM $AgNO_3$ solution was toxic to both *Abelmoschus* and *Raphanus* seed germination. Both seeds were also treated with normal seaweed aqueous extract and seaweed liquid fertilizer of *Halimeda gracilis* for one week but no seeds germination occurred in case of the seeds treated with seaweed extract and seaweed liquid fertilizer for *Abelmoschus* but for *Raphanus*, 40% seeds germinated with the treatment of seaweed aqueous extract and 20% seeds germinated with treatment of seaweed liquid fertilizer. The seeds treated with biosynthesized Silver Nanoparticles showed maximum germination and the growth of seedling which was better than normal water treated seeds and seaweed liquid bio-fertilizer and seaweed extract treated seeds [Fig. 13 (a) and (b)].

It had been previously reported that seaweed synthesized Ag-Nanoparticles by *Sargassum cinctum* had a quite good effect on *Abelmoschus esculentus* seed germination and the seedling growth. It had been reported that in comparing to normal water, seaweed Ag-Nanoparticles had excellent potential for promoting seed germination and seedling growth (Roy et al. 2017).

Antibacterial activity of biosynthesized silver nanoparticles:

The inhibition of biosynthesized Silver Nanoparticles was 2.33 ± 0.2 cm against *Proteus mirabilis*, 1 ± 0.0 cm against *Klebsiella pneumoniae* which was high as compare to Chloramphenicol (5mg/ml) and silver nitrate solution (1 mM). But the inhibition was less against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* (Fig. 14). Similarly, the biosynthesized Silver Nanoparticles by green seaweed *Caulerpa prolifera* had high antibacterial activity against *Bacillus subtilis* (Ismail *et al.* 2016).

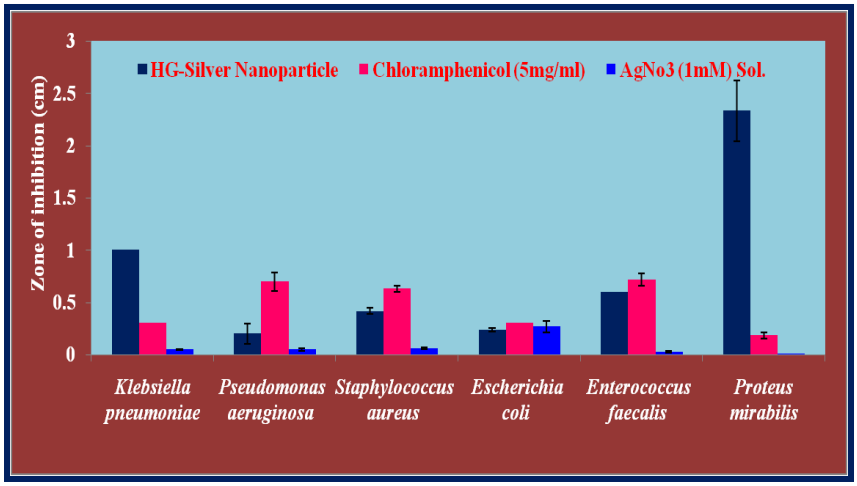

Fig. 14: Showing the zone of inhibition of biosynthesized Silver Nanoparticles (Halimeda gracilis), antibiotic and silver nitrate.

IV. CONCLUSIONS

It can be concluded that the Ag-Nanoparticles of *Halimeda gracilis* showed the highest resistance against two pathogenic bacteria such as *Klebsiella pneumoniae* and *Proteus mirabilis*, so it may be used as a source of antibiotic production in future after further analysis. The Ag-Nanoparticles synthesized from *Halimeda gracilis* had maximum growth promoting effect and the highest seed germination in case of *Raphanus sativus* var. *longipinnatus*. It may be due to the easy penetration of Ag-Nanoparticles for its nano-size. The similar results were also found previously for the biosynthesized Ag-Nanoparticles by *Chaetomorpha antennina* (Roy et al. 2017) and *Amphiroa anceps* (Roy et al. 2018). The biosynthesized Ag-Nanoparticles by *Sargassum Ilicifolium* (Roy et al. 2018) and *Chlorodesmis hildebrandtii* (Roy et al. 2018) also showed the same potential for seed germination of above mentioned both seeds. So, for circulation of nutrients and minerals very fast this nano-sized particles are suitable, so, fast germination and the highest seedling growth was observed in case of both plants *Abelmoschus esculentus* and *Raphanus sativus* var. *longipinnatus*. In future, Ag-Nanoparticles will be used as phyto-friendly nano-bio fertilizer.

ACKNOWLEDGEMENTS

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Conflict of interest: There are no conflicts of interest to be declared.

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Effect of some biocontrol agents against root-knot nematode (*Meloidogyne incognita* race2)

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Abstract— *Culture filtrate of four rhizospheric fungi and four biocontrol agents were studied in vitro for their efficacy against Meloidogyne incognita race 2. The per cent mortality and egg hatching inhibition was proportional to the concentration of culture filtrate and the duration of exposure period. Culture filtrates of Trichoderma viride, Trichoderma harzianum, Trichoderma sp., Fusarium sp., Penicillium sp. and Aspergillus sp. significantly induced inhibition of egg hatching and mortality of Meloidogyne incognita race 2.The highest percentage of inhibition of egg hatching and juvenile mortality was recorded in Trichoderma harzianum followed by Trichoderma viride and Trichoderma sp.*

Keywords— Biocontrol agent, culture filtrate, egg hatching, juvenile mortality, root-knot nematode.

I. INTRODUCTION

Root-knot nematode (*Meloidogyne* spp.) is an important plant pathogen affecting crop production throughout the world. Since, indiscriminate use of nematicides is responsible for environmental and human health concerns; the search for new microbial strains as nematode control agents is relevant. As fungi cohabit together with nematodes in the rhizosphere, their toxic metabolites may be responsible for keeping a low level of nematode populations [1]. The search for nematotoxic or antagonistic compounds in culture filtrates has greatly intensified in recent years, due to the number of toxins, enzymes or compounds derivable from their metabolites [2-7]. Assays with culture filtrates may provide first information about the role of a fungus in the plant rhizosphere, as *in vitro* studies showed toxic and inhibitory effects of several filtrates toward plant parasitic nematodes [8]. Toxic effects of fungal culture filtrates on *M. incognita* have been studied by several workers [9-16] and had showed different levels of efficacy [17-20]. Due to the differences of soil ecological types and climate, a broad range of fungi remains far unexplored. Therefore, present study was made to isolate rhizospheric fungal associations of root knot nematode infected plants and evaluate the potential of some isolated fungi and already recognized biocontrol agents (against insect pests and diseases) on hatching of eggs and mortality of second-stage juveniles of *Meloidogyne incognita* race 2 *in vitro*.

II. MATERIALS AND METHODS

Collection of samples

Soil samples were collected in different localities around Jorhat, Assam comprising an area approximately 1000 ha, in order to identify the root-knot nematode infection. To isolate the fungal antagonists from rhizosphere soils of infested cucurbits, tomato, brinjal, okra, cabbage, citrus, banana and tea, a total of 100 soil (500 g each) were collected. Samples were stored at 15^0C for not more than one week.

Fungal isolation and identification

Soil mycoflora was isolated by serial dilution pour plate technique [21, 22]. One g of rhizosphere soil was dispensed in 9 ml sterile water, from the 10^{-5} dilution, 50 µl were inoculated over Petri plates containing PDA media. The plates were incubated at room temperature 24±2°C for 48 hrs. Materials of the pure culture were mounted in Lactophenol, stained with Cotton blue and the morphological observations of hyphae, sporangiophore/conidiophores and conidia were done with the help of a Compound light microscope at 400X magnification [23, 24]. Axenic cultures of the fungi were obtained by single spore isolations [25] and the cultures were maintained on PDA slants. *Trichoderma viride*, *Trichoderma harzianum*, *Beauveria bassiana*, *Metarhizium anisopliae* were procured from the Department of Plant Pathology, AAU, Jorhat, Assam.

Nematode inoculum and mass culturing

The inoculum of root-knot nematode *M. incognita* race 2 was collected from naturally infested tomato crop in field and single egg mass was used to raise pure culture. Mass culturing of nematodes was done on tomato variety Sel 7, in order to get regular supply of the inoculums for the experiment. One month old tomato seedlings were

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inoculated with small volume of egg suspension approximately consisting of 2000 eggs of *M.incognita* race 2. These pots were watered and kept in glasshouse at temperature 28-35°C.

Preparation of fungal culture filtrates

To evaluate the nematicidal potential of the cell free fungal culture filtrate the most frequently occurring isolates belonging to the genera of *Trichoderma, Aspergillus, Penicillium and Fusarium* were selected. *Beauveria bassiana, Metarhizium anisopliae, Trichoderma viride and Trichoderma harzianum* were procured from the Department of Plant Pathology, AAU, Jorhat, Assam. These strains were inoculated on to Petri plates containing Potato Dextrose Agar medium and incubated for 7 to 10 days at 27°C. From these actively growing cultures, one disc each of 0.5 cm diameter was transferred to 250 mL Erlenmeyer flask containing 50 mL Potato Dextrose broth. These flasks were incubated at 27±1°C for 15 days. The culture was filtered through two layers of Whatman filter Paper No.1. Filtrates thus obtained were designated as standard solution (100%). Different dilutions (50%, 25%, and 10%) of each fungal filtrate were prepared by adding required amount of sterilized distilled water.

Hatching test

To determine the effect of culture filtrate on the hatching of eggs of *M. incognita* sterilized Petri dishes of 5 cm dia were separately pipette two ml of culture filtrate. Five sterilized healthy egg masses of nearly uniform size of *M.incognita* were transferred to each dish. The egg masses placed in culture medium served as control. All Petri dishes were kept at 28±2°C in completely randomized design, replicated thrice. Observations were recorded on every 24 h interval up to 72 h with the aid of stereomicroscope. The per cent egg hatch was calculated by the following formula and mean of three replications was presented in Table.2.

$$\text{Hatching \%} = \frac{\text{No. of hatched juveniles}}{\text{No. of hatched + unhatched eggs}} \times 100$$

Mortality of second stage juveniles (J2)

For determining the effect of fungal filtrates on juvenile mortality of *M.incognita* race 2, egg masses were collected from an infested root and allowed to hatch in distilled water with aeration. The hatched J_2 were collected in a beaker. One hundred freshly hatched second stage juveniles were transferred to 5 cm dia Petri dishes containing 2 ml filtrates of different dilutions of each fungus and medium separately. Equal number of J_2 was also transferred to separate Petri dishes containing culture medium to serve as control. Petri dishes were kept at 28±2°C temperature in completely randomized design, replicated thrice. Observation on the number of dead J_2 for every 24, 48 and 72 h of exposure was recorded with the aid of stereomicroscope and per cent mortality of juveniles was calculated. The J_2 were considered dead when they did not move when probing with a fine needle Mean percentage of dead J_2 was estimated using the following formula and presented in the Table 3.

$$\text{Per cent mortality} = \frac{\text{Total number of dead juveniles}}{\text{Total number of juveniles}} \times 100$$

STATISTICAL ANALYSIS

Per cent egg hatch and per cent mortality data was subjected to statistical analysis using the three factorial completely randomized design statistical package. The critical differences in main effects i.e. isolates, concentration, and time of exposure as well as in their interactions were tested at P=0.05.

III. RESULTS

A total of four isolates of different genera of fungi were isolated from the soil rhizosphere of *M.incognita* race 2 infected plants. *Trichoderma sp. Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. was isolated from rhizospheric soil of banana, cowpea, brinjal and cucumber respectively.

The results presented in Table 1 revealed significant differences among isolates (biocontrol agent) (T), concentration of culture filtrate(C) and exposure period(t). The culture filtrate of *Trichoderma harzianum* followed by *T.viride, T.sp. Aspergillus* sp., *P.* sp. and *F.*sp. adversely affected the larval hatching of *M.incognita* race 2. Irrespective of concentration of culture filtrate (C) and time of exposure period (t), *T.harzianum* was the most effective bioagent followed by *T.viride* and *T.*sp. as the hatching of *M.incognita* was suppressed. Similarly, irrespective of isolate (biocontrol agent) (T) and concentration of culture filtrate (C), time of exposure(t) also affected the larval hatching. With increase in exposure period up to 72 hours there was a correspondingly increased in egg hatching. With increase in the dilution of culture filtrate, the cumulative hatching was increased irrespective of isolate (T) and time of exposure period (t). Highest inhibition in hatching was obtained in 100% concentration of each fungal culture filtrates. The percentage hatching of *M.incognita* was 18.35% during 72 h exposure in the 100% concentrations of culture filtrates of *Trichoderma viride* followed by *T.harzianum* with percentage hatching 20.36%. *Beauveria bassiana* and *Metarhizium anisopliae* showed negligible

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effect on inhibition of egg hatching of *M.incognita*. Hatching percentage was 59.34%, 58.35% respectively at 10% concentration of culture filtrate during 72 h exposure period.

The data showed in the Table 2 revealed that all the culture filtrates of isolates were having nematicidal effect of varying degree on *M.incognita* race 2. Per cent mortality of nematodes was directly proportional to the concentration of culture filtrate and the period of exposure. Irrespective of concentration of culture filtrate (C) and duration of exposure(t), six isolates namely *Trichoderma harzianum, Trichoderma viride, Trichoderma* sp., *Fusarium* sp., *Aspergillus* sp. and *Penicillum* sp. were exhibited nematicidal effects on *M. incognita* J_2. The activity of *Trichoderma harzianum* was the highest, with juvenile mortality 82.66%, 85.33%, 89.33% for 24, 48 and 72 h, respectively at 100% concentration of culture filtrate. This was followed by *T.viride* with juvenile mortality 79.00%, 81.66%, 85.66% for 24, 48 and 72 h, respectively at 100% concentration of culture filtrate. All the new isolate namely *Trichoderma* sp., *Penicillum* sp., *Aspergillus* sp., *and Fusarium* sp. displayed more than 50% juvenile mortality during 24 h exposure time at 25% concentration of culture filtrate. On the other hand, *Beauveria bassiana* and *Metarhizium anisopliae* showed the lowest toxicity, caused only 67.66% and 51.65% juvenile mortality at 50% concentration of culture filtrate during 72 h exposure time.

IV. DISCUSSION

Culture filtrates of many fungi possess nematicidal activity against nematodes, due to the production of toxic metabolites [26].Variable effect of fungal filtrates on hatching and mortality of root-knot nematode *M.incognita* race 2 observed in the present study may be attributed to the varied nature of toxic metabolites produced by different fungi. Species of *Trichoderma, Fusarium, Paecilomyces, Aspergillus, Penicillium* are known to produce toxins and antibiotics like viridian, fusaric acid, lilacin, oxalic acid and penicillic acid [27, 28]. Various mechanisms have been suggested for the biocontrol activity of *Trichoderma* spp. against phytopathogenic fungi: antibiosis, competition, mycoparasitism, and enzymatic hydrolysis [29, 30]. *Trichoderma* spp. is utilized in the production of a number of antibiotics, such as trichoderin, trichodermol A and harzianolide. *Trichoderma* produces molecules such as 6-pentyl α-pyrone, VOCs and enzymes [31] that can attack the cuticle of nematodes. Also, its hyphae form a physical barrier, which is a difficult step for nematodes, since the fungus grows along with the plant roots. Successful parasitism of the nematode by *Trichoderma* requires mechanisms to facilitate penetration of the nematode cuticles or eggshells. The involvement of lytic enzymes has long been suggested and demonstrated in *Meloidogyne* parasitism [32]. Besides direct antagonism, other mechanisms involved in *Meloidogyne* control by *Trichoderma* spp. include production of fungal metabolites and induced resistance [33-35]. *Trichoderma harzianum* has been found to be an effective biocontrol agent for the management of root-knot and other nematodes [36-39]. Direct interactions between *T. harzianum* and the potato cyst nematode *Globodera rostochiensis* were demonstrated *in vitro* by Saifullah and Thomas [40].The fungus penetrated the cysts and the eggs in those cysts, resulting in larval death. *Beauveria bassiana* has a repressive action on nematodes of the genus *Meloidogyne* spp. [41-47]. *B. bassiana* may have more than a single bioactive metabolite that are responsible for nematicidal activities, and each metabolite may act on a different site. Ghayedi and Abdollahi [48] purified the isolated fungus and also they showed the biocontrol potential of the isolate on *Heterodera avenae*, with 47.1% of larval mortality. Chen *et al*., [49] found that *B. bassiana* showed little parasitism of nematode eggs but reduced hatch of *Heterodera glycines*. Studies have shown that *Beauveria* can produce beauvericin and oosporin. Beauvericin has a weak activity against *M. incognita* [50-53]. The percentage mortality and inhibition of hatching of root-knot nematode were directly proportional to the concentration of culture filtrates of *B.bassiana* [54]. Biocontrol potential of *M. anisopliae* against some species of root knot nematodes has been shown [55-58]. The lethal effect of *M. anisopliae* culture extract has been also reported [59].Some species of *Metarhizium* has root colonization ability [60, 61]. Some isolates of *M. anisopliae* have endophytic behavior [62]. The fungus produces sticky conidia that attach to nematode cuticle [63]. The conidia germinate, parasitize and kill the cadaver, by direct penetration and producing the infective hyphae inside the nematode body. The fungus produces some cyclopeptides and destruxins which may play an important role in its pathogenicity [64]. Prior to any direct attack to the host, the fungus produces destruxin A and destruxin B that can kill the host [65].

V. CONCLUSION

It is clear from this work that, in plant rhizosphere, there are many fungi that have potentialities for controlling root-knot nematode. Among four fungal isolates and four bioagents, *Trichoderma harzianum* was exhibited the highest

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production of nematicidal activities against root-knot nematode (*M. incognita* race 2) *in vitro*.

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Table.1: Effect of culture filtrate of some fungal bioagent on egg hatching of Meloidogyne incognita race 2.

Isolate(T)	Culture filtrate concentration (C) (%)	Period of exposure(t)			Isolate (T) Mean	Culture filtrate concentration (C) Mean
		24h	48h	72 h		
Trichoderma sp.	10	24.35(29.53)	32.33(34.64)	40.35(39.38)	24.19 (29.06)	40.11(39.22)
	25	20.34(26.79)	28.34(32.14)	34.35(35.84)		34.82(35.93)
	50	16.33(23.70)	22.35(28.17)	26.32(30.86)		28.32(31.74)
	100	10.30(18.66)	12.30(20.53)	22.65(28.41)		20.91(26.39)
Penicillium sp.	10	28.32(32.11)	34.30(35.82)	46.36(42.89)	25.34 (30.33)	
	25	20.35(26.79)	30.65(33.62)	38.32(38.21)		
	50	18.34(25.15)	24.30(29.54)	28.30(32.15)		
	100	10.30(18.71)	14.32(22.20)	20.30(26.73)		
Aspergillus sp.	10	28.30(32.06)	34.33(35.85)	43.34(41.16)	24.74 (29.92)	
	25	22.35(28.17)	28.34(32.04)	38.35(38.22)		
	50	16.35(23.70)	24.35(29.49)	26.33(30.86)		
	100	10.33(18.66)	12.30(20.49)	22.32(28.19)		
Fusarium sp.	10	26.32(30.81)	34.30(35.85)	44.36(41.74)	26.16 (30.53)	
	25	22.32(28.17)	30.35(33.37)	38.32(38.23)		
	50	16.30(23.73)	24.35(29.54)	28.35(32.14)		
	100	12.32(19.91)	14.32(22.20)	22.34(28.17)		
Trichoderma viride	10	25.33(30.18)	30.30(33.41)	42.30(40.58)	23.74 (28.63)	
	25	20.34(26.78)	28.35(32.08)	36.32(37.05)		
	50	12.30(20.53)	22.32(28.19)	26.35(30.85)		
	100	8.30(16.53)	14.35(22.20)	18.35(22.44)		
Trichoderma harzianum	10	22.30(28.17)	30.30(33.40)	36.34(37.05)	22.00 (27.59)	
	25	18.34(25.12)	26.30(30.85)	30.35(33.36)		
	50	14.35(22.19)	20.35(26.77)	22.35(28.19)		
	100	10.35(18.66)	12.35(20.42)	20.36(26.79)		
Beauveria bassiana	10	33.60(35.45)	42.32(40.58)	59.34(50.39)	33.53 (35.12)	
	25	28.30(32.11)	38.32(38.23)	46.32(42.89)		
	50	24.32(29.54)	34.30(35.85)	32.30(34.64)		
	100	18.62(25.33)	20.30(26.77)	24.34(29.53)		
Metarhizium anisopliae	10	33.65(35.45)	42.32(40.58)	58.35(49.79)	33.60 (35.16)	
	25	30.32(33.40)	36.32(37.05)	46.34(42.89)		
	50	26.30(30.85)	32.35(34.64)	32.35(34.64)		
	100	16.30(23.70)	20.35(26.77)	28.36(32.12)		
Culture broth	10	51.00(45.57)	74.34(59.57)	84.37(66.70)	64.41 (53.74)	
	25	50.00(44.98)	72.65(58.52)	79.38(62.96)		
	50	48.33(44.04)	67.34(55.15)	77.30(61.58)		
	100	45.00(42.12)	55.65(48.24)	67.65(55.34)		
Period of Exposure (t) Mean		23.36(28.27)	31.19(33.47)	38.60(38.22)		
CV=6.92 CD(P=0.05): Treatment(T):1.06; Concentration(C):0.71; Period of exposure(t):0.61; T ×C: 2.13;T×t: 1.84; C×t:1.23;T×C×t: 3.69						

Figures in the parentheses are Arc-Sine transformed values

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Table.2: Effect of culture filtrate of some fungal bioagent on juvenile mortality of Meloidogyne incognita race 2.

Isolate (T)	Culture filtrate concentration (C) (%)	Period of exposure(t)			Isolate (T) Mean	Culture filtrate concentration (C) Mean
		24h	48h	72 h		
Trichoderma sp.	10	44.66(41.92)	47.62(43.65)	51.60(45.95)	65.11 (54.08)	45.00(41.98)
	25	61.60(51.75)	65.64(54.14)	71.66(57.86)		56.98(49.07)
	50	63.62(52.94)	69.65(56.62)	77.64(61.80)		60.72(51.39)
	100	68.65(55.97)	77.66(61.86)	81.33(64.42)		66.09(54.96)
Penicillium sp.	10	38.67(38.42)	44.66(41.93)	50.60(45.38)	55.36 (48.13)	
	25	51.66(45.95)	54.66(47.67)	57.66(49.40)		
	50	54.66(47.67)	59.62(50.57)	61.60(51.75)		
	100	57.33(49.22)	65.60(54.13)	67.64(55.39)		
Aspergillus sp.	10	41.67(40.19)	50.66(45.37)	53.66(47.09)	62.71 (52.52)	
	25	60.34(50.96)	63.66(52.93)	67.66(55.34)		
	50	61.34(51.56)	65.66(54.14)	69.66(56.62)		
	100	67.33(55.24)	73.66(59.14)	77.33(61.56)		
Fusarium sp.	10	39.33(38.82)	43.66(41.35)	49.66(44.80)	63.71 (53.23)	
	25	60.33(50.98)	67.66(55.36)	69.66(56.65)		
	50	64.66(53.54)	69.33(56.38)	75.66(60.47)		
	100	69.66(56.58)	75.33(60.36)	79.66(63.44)		
Trichoderma viride	10	54.00(47.29)	59.66(50.65)	61.66(51.74)	71.77 (58.22)	
	25	67.33(55.15)	69.66(56.69)	73.66(59.13)		
	50	74.00(59.35)	77.66(61.81)	77.33(61.59)		
	100	79.00(62.75)	81.66(64.69)	85.66(67.75)		
Trichoderma harzianum	10	55.33(48.05)	57.66(49.42)	61.66(51.78)	72.25 (58.69)	
	25	69.35(56.38)	69.60(56.68)	71.60(57.86)		
	50	77.60(61.81)	71.33(57.80)	75.65(60.47)		
	100	82.66(65.42)	85.33(67.67)	89.33(70.95)		
Beauveria bassiana	10	32.66(34.83)	41.60(40.19)	55.66(48.25)	51.67 (46.03)	
	25	45.30(42.31)	46.66(43.08)	59.64(50.61)		
	50	47.33(43.46)	51.30(45.76)	67.66(55.35)		
	100	49.65(44.79)	55.00(47.86)	67.62(55.79)		
Metarhizium anisopliae	10	32.00(34.43)	37.00(37.41)	43.66(41.35)	45.50 (42.40)	
	25	42.33(40.58)	45.66(42.50)	49.65(44.78)		
	50	43.00(40.96)	47.62(43.64)	51.65(45.95)		
	100	46.30(42.89)	49.64(44.80)	57.60(49.40)		
Culture broth	10	14.00(21.83)	24.00(29.23)	28.30(31.93)	26.72 (30.86)	
	25	18.00(24.93)	28.00(31.93)	30.00(33.14)		
	50	22.00(27.95)	30.00(33.19)	32.40(34.39)		
	100	24.00(29.32)	34.00(35.65)	36.00(36.82)		
Period of Exposure (t) Mean		52.26(46.29)	57.18(49.34)	62.19(52.42)		
CV=5.83, CD(P=0.05): Treatment(T):1.33; Concentration(C):0.88; Period of exposure (t): 0.76; T×C: 2.66; T×t: 2.30; C×t:1.53; T×C×t: 4.60						

Figures in the parentheses are Arc-Sine transformed values

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Influence of Various Concentration of Aral Sea Salt on Germination of Seeds and Morphometric Characteristics of Agricultural Groups

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Abstract**—** *This article presents the results of studies of the chemical composition of the salt of the Small Aral Sea and their phytotoxicity for agricultural crops. The high toxicity of salts with a high content of chloride ions is established. They cause changes in the morphometric parameters of plants. The tested plant species differ in their response to the action of high concentrations of salts.*
Keywords— sea salt, phytotoxicity, morphometric parameters, chloride ions.

I. INTRODUCTION

The ecological disaster of the Aral Sea remains the main negative factor for the ecosystems of Central Asia. At the bottom of the dried sea formed a new desert that is called Aralkum. It consist a mixture of sand and salt. Intensive dust storms carry this mixture to the depth of the mainland to 300 km and lead to gradual degradation of historically formed phytocenoses [1]. Already irretrievably destroyed many natural plant communities and more than 2 million hectares of arable land have become unsuitable for agricultural purposes. The reason for this process is a gradual increase in soil salinity in the coastal zone of the Aral Sea. Sea salt is heterogeneous in its chemical composition in various parts of the dried bottom. Its main part is represented by chloride and sulphide compounds, which also contain other toxic substances and pesticides. These toxicants came to the sea with surface wastewater from the vast agricultural lands of the Central Asian republics of the former Soviet Union. Therefore, large reserves of sea salt remain a source of environmental pollution on a hugeterritory.

At present, the dried up Aral Sea is divided into northern and southern parts. The Northern Aral is located on the territory of the Republic of Kazakhstan. Therefore, the salvation of the Northern Aral Sea and restoration of phytocenoses of coastal areas is the main task of the Republic of Kazakhstan [2,3].Restoring the plant communities of the shoreline will serve as a natural barrier to the onset of sand, and help reduce the spread of salt. Many research projects of recent years are devoted to solving this problem. Scientists are carrying out studies to study the salt tolerance of both traditional and completely new crops and wild-growing species for the region. Using the salt-tolerant crops can facilitate the return of lost land to agricultural use and improve the socio-economic situation in the region. And the identification of salt-tolerant species of wild vegetation makes it possible to hope to restore a degraded terrestrial ecosystem. In this regard, the study of the reaction of some crops on the effect of different samples of the salt of the Aral Sea has become the goal of our studies.

II. MATERIALS AND METHODS OF RESEARCH

The materials of the study were 13 samples of the salt of the dried bottom of the Small Aral Sea, which differ from each other in the specific gravity of the chloride ions. The tested plant species were *Medicago sativa* L., Amaranthus tricolor L. and *Festucapratensis* Huds. In case of successful results, these species can be used in forage production. A substrate for experiments used vermiculite. The experiment was carried out at a temperature of 22-23 oC.and with an illumination of 2500 lux. Plant seeds were sown to a depth of 2-3 cm. 1%, 5%, 10% saline solutions of 13 test salt samples were used as irrigation. Observations started from the 5th day of the experiments.

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During the experiments we took the counts of influence of the concentration of salts on the germination of seeds and the morphometric indices of the plants.

In determining the chemical composition of salt were used standard methods of chemical analysis of sea water (Manual on methods for the chemical analysis of sea water, 1977) [4]. In addition to analytical methods for determining the main components of the salt composition, control measurements were made on an ion chromatograph. The concentrations of potassium and sodium ions were determined by ion-selective and chromatographic methods, as well as by the method of flame photometry [5-7].

Definitions of the relative electrical conductivity were carried out on a high-precision salt generator of the 601 MK III model produced by the Japanese company WATANABE KEIKI MFG. CO., LTD (an analogue of the Australian salt maker from YEO-KAL ELECTRONICS PTY LTD). For thermo stating the samples, a water precision thermostat TVP-6 from the Alma-Ata Experimental Factory "Etalon" was used with a working temperature range of -10 to 95 ° C. The measurements were carried out at temperatures of 15, 20, 25, 30, and 34 ° C.

III. RESULTS AND DISCUSSION

The results of analyzes of the chemical composition of the salts showed that all 13 samples from the Lesser Aral Sea belong to chloride salts. In 11 of these samples (samples No. 2,3,4,5,6,8,9,10,11,12,13), the specific total NaCl is 77.9 ± 4.4%, sodium sulfate (Na_2SO_4) 1 , 7500 ± 0.002%, insoluble residues 0.11 ± 0.001%. Other macro- and microelements is 20.24 ± 0.3%. The chemical composition of the other two samples (No.1 and No.7) turned out to be slightly different: NaCl - 97.9 ± 1.5%, sodium sulfate (Na_2SO_4) -0.7800 ± 0.001%, insoluble residues - 0.12 ± 0.001% and the share of other macro- and microelements is 1.2 ± 0.001%.A study of the toxicity of these salt samples for the tested plant species showed that the first 11 of them, with a lower content of chloride ions, are less aggressive than the last two. Their 1% concentration had a stimulating effect on the germination of plant seeds, the seed germination of all three plant species was within 85.5 ± 2.6% -96.52.8% (control parameter: 81.5 ± 3.1% - 94.3 ± 3.3%). However, an increase in the salt concentration to 5% led to a sharp decrease in the germination of seeds. In addition, inhibition of growth processes led to an extension of the plant germination period by 15 days compared to control and trial versions with 1% salt concentration. The seed germination rate was 18.9 ± 0.65% -32.4 ± 1.11%. At 10% concentration of seedlings, the test cultures were not observed. The results of other experiments showed that samples of salt No.1 and No.7 differ the most potent phytotoxicity. In our experiments, their 1% concentration strongly inhibited the growth processes of the seeds of *F. pratensis* Huds. Seed germination was only 15.5 ± 0.01%, while the control variant was 83.6 ± 3.8%. With an increase in the salt concentration to 5%, seed germination decreased to 1.3 ± 0.01%, and at 10% concentration the seed germination was completely absent. It should be noted that *M. sativa* L. and *A. tricolor* L. compare to *F. Pratensis* Huds. were the most sensitive. In this experiment the seed germination of these species at 1% salt concentration did not exceed 1.5 ± 0.001%, and at 2% concentration was absent completely. In general, analysis of the results of these experiments showed that the most phytotoxic samples are salts with an increased content of chloride ions, which is consistent with the results of early studies. From literature sources it is known that chloride salts are more aggressive ions in the composition of sea salt, while sulfate is less harmful [8].The lesser toxicity of sulphate salinity is in particular due to the fact that unlike the Cl ion, the $S0_4$ ion is required in small amounts for normal mineral nutrition of plants, and only its excess is harmful [8]. The effect of salinization on plant organisms is associated with two causes: a deterioration of the water balance and a toxic effect of high concentrations. Salinization leads to the creation in the soil of low water potential, while the flow of water into the plant is difficult. Under the influence of salts, the functions of all ultra-structural elements of cells occur. This is particularly pronounced in chloride salinity [9]. All chloride ions are toxic. Cl- ions are bound to hypothetical salts in the sequence: NaCl, $MgCl_2$, $CaCl_2$. They also have the greatest migration capacity, which is explained by their good solubility, poorly expressed ability to sorption on suspensions and consumption by aquatic organisms, which explains the nature of their phytotoxicity. Due to the fact that in our experiments the test salt concentrations were close to extreme, the study of the effect of salt toxic effects on the morphometric parameters of plants was carried out only in variants of the experiment with a reduced content of chloride ions and only at 5% concentration. Table 1 shows the results of morphometric studies of plants of F. pratensis Huds., M. sativa L. and A. tricolor L., grown in an experiment with a 5% salt content of the specific gravity of the substrate. In this experiment the salts of sample No. 5 were used. It was found that the 5% salt concentration of this sample affects the variability of the morphometric parameters of the studied plant species in different ways. The least changes in comparison with the control variant were found in F. pratensis Huds. His morphometric indicators were practically at the level of the control variant. While M. sativa L. and A. tricolor L., the length of the roots, stems, number and area of the leaves, compared to the control variant, underwent significant

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changes. Thus, the root length of M. sativa L. and A. tricolor L. was 8.9 ± 0.34 cm -12.3 ± 0.54 cm, and the length of the stems was 14.6 ± 1.0 cm -17.3 ± 1.1cm shorter than the control. Similar changes in the direction of decrease were established for the number of leaves and the total area of leaf blades on the plant.

Table.1: Mean values ±SD (n-5-6) of root and stem length, leaf number and total leaf area of FestucapratensisHuds.,MedicagosativaL. and AmaranthustricolorL.grown at 5% salt in the soil (salt sample №5).

Plant species	Root length [cm]	Stem length [cm]	Leaf number	Leaf area, [cm^2]
Festuca pratensis Huds.				
Control	38±2,13	59,6±2,81	7,3±0,11	45,9±2,30
5% 5% concentration of salt	36,6±2,11	58,6±2,15	7,2±1,11	44,3±2,10
Medicago sativa L.				
Control	39,8±1,19	45,5±2,2	15,8±1,10	115,8±7,21
5% 5% concentration of salt	30,9±1,70	30,9±2,10	9,8±1,00	74,8±1,11
Amaranthus tricolor L.				
Control	27,9±0,12	34,8±1,14	8,7±0,12	136,7±4,4
5% concentration of salt	15,6±0,12	17,5±0,14	4,9±0,11	65,7±2,4

The analysis of the results of the conducted studies suggests that the salt accumulations of the dried bottom of the Small Aral Sea are not homogeneous in their chemical composition. There are salt reserves with a moderate and high content of chloride ions. For this reason, they have a different degree of phytotoxicity. Within the limits of the 13 salt samples studied by us, the specific gravity of chloride ions varies within the limits of 77.9 ± 4.4% -97.9 ± 1.5%. The most phytotoxic are samples of salt with a high content of chloride ions, 1% of their concentration causes a strong inhibition of growth processes in the studied plant species. The toxic effect of sea salt ions negatively affects the morphometric indices of *F. pratensis* Huds.,*M. sativa* L. and *A. tricolor* L. The last two species of plants studied showed a more sensitive reaction to the effect of 5% salt concentration compared to *F. pratensis* Huds. Identified species of plants with the reaction of resistance and sensitivity in salt stress are of practical interest for studying the molecular mechanisms of salt tolerance in plants.

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The Effect of Methanotrophic Bacteria Application on Paddy Growth and Methane Emission in Rainfed Rice of Kupang Regency, East Nusa Tenggara, Indonesia

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Abstract*—Rice productivity in province of East Nusa Tenggara (ENT) is low due to the soil condition. One of the rice-producing regency in ENT is Kupang Regency with rainfed rice type. Paddy fields have also become a major source of methane emissions (CH_4) as one of important greenhouse gases. This research aims to know the effect of methanotrophic bacteria application on paddy growth and methane emission at rainfed rice. Bacteria that used is Methylocystisrosea BGM 1, Methylobacter sp. SKM 14, Methylocystispalvus BGM 3 and Methylococcuscapsulatus BGM 9. This research used completely random design with threatment: (1) NPK 100% (P1), (2) NPK 50% (P2), (3) without fertilizer (P3), (4) NPK 100% + methanotrophic (P4), NPK 50% + methanotrophic (P5), and methanotrophic bacteria (P6). Gas sampling using closed chamber method.The application of methanotrophic bacteria increased the rice production. Treatment NPK 50% + methanotrophic (P5) from that rice field produced 7.0 t ha^{-1} dry grain weight and methanotrophic bacteria treatment without NPK (P6) with improved 6.6 t ha^{-1} dry grain weight, higher than controls of 4.9 ha^{-1} dry grain weight without any addition of synthetic fertilizer.The inoculation of methanotrophic bacteria increase rice production of 1.7 t ha^{-1}.Result of methane flux measurement showed that application of methanotrophic bacteria may decrease methane emission in treatment of 100% NPK + methanotrophic (P4) (30 DAP) and treatment of 50% NPK + methanotrophic (P5) (60 DAP), -6.27 $mg/m^2/d$ and -23.87 $mg/m^2/d$, respectively.*

Keywords*—*Kupang regency, Methane emission, Methanotrophic, Rainfed rice.

I. INTRODUCTION

Rice is a basic requirement of Indonesian society, including the province of East Nusa Tenggara (ENT). Rice productivity in ENT belongs low because the soil is less fertile and arid climate with rainfall between 201-300 mm (BMKG 2017). One of the rice-producing regency in ENT is Kupang Regency. In the year 2013 produced rice as much as 60.469 t, 13.846 ha of which is rainfed rice (BPS 2013). Farmers in the Regency of Kupang still using synthetic fertilizers to increase crop production. Practices will further lower soil fertility due to damage to physical, chemical, and biological soil condition (Havlin*et al*. 2005). In addition, the use of inorganic fertilizers also has an impact on global warming.

Wetlands such as paddy fields have also become a major source of methane emissions (CH_4) as greenhouse gases. The activity of methanogenesis by methanogen bacteria on paddy fields produce CH_4 gas (Le Mer and Roger, 2001). The global warming potential of methane gas is 25 times greater than CO_2 (IPCC, 2007). According to Conrad and Rothfus (1991), as much as 80% of methane gas in the rice fields can be oxidized by the methanotrophic bacteria. This can be a solution in mitigating the emission of methane gas in the paddy fields.

Some of the methanotrophic bacteria has been succesfully isolated from paddy fields in Sukabumi and Bogor (Hapsari, 2008). Isolates *Methylocystisrosea* BGM 1 and *Methylobacter* sp. SKM 14 are known to have *pmo*Agene whereas isolates BGM 9 have the *mmo*Xgene (Rusmana and Akhdiya, 2009). Isolates *Methylocystispalvus*BGM 3 and *Methylococcuscapsulatus*BGM 9 known to have *nif*H and *nif*D genes these play a role in the nitrogen fixation (Bintarti*et al*. 2014). Methanotrophic bacteria have been tested on organic and inorganic paddy fields. The trial reduced methane gas to 20.47% when compared with the control and improved the vegetative phase of rice growth (Pingak e*t al*. 2014; Sutanto*et al*. 2014). Trials have also been conducted on paddy fields in the lowlands. The trial

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reduced of methane gas and increased the growth of vegetative phase on rice and the generative phase (Sukmawati*et al*. 2015). This research aims to know thepaddy growthand methane emissions in the application of methanotrophic bacteria at therainfed rice.

II. METHODS

2.1 Culturing Bacterial Isolates

Methanotrophic bacteria isolates i.e. BGM 1, 3, 9, and SKM 14 were cultured in NMS (Nitrate Mineral Salt) plus 1% methanol (v/v), incubated at room temperature (± 28^{o}C) for 7-10 days and shakedup to reach 10^8 CFU cell/mL.

2.2 Seedling and Plantation

Seeds of paddy variety Ciherang were germinated for 48 h. After that, the seed was sowed in the field for 20 days to make seedling. Before transplanting, the seedling was dipped in a mixture of methanotrophic bacteriafor 15-20 minutes, then plantated with a distance of 20 x 20 cm which 3 seedling in every hole. Five plants selected from every plot of treatment for measurement of growth parameters.

2.3 Experimental Design

The experimental design used was completly random design with one factor i.e. fertilization. The treatment consists of: (1) NPK100% (P1), (2) NPK 50% (P2), (3) without fertilizer (P3), (4) NPK 100% + methanotrophic (P4), NPK 50% + methanotrophic (P5), and methanotrophic bacteria (P6). Each treatment has 4 replications.

2.4 Measurement of Growth Parameters

Paddy growth was observed at 30, 60, and 90 day after plant(DAP). During the vegetative growth plant height and number of tillers was measurement. The shoot dry weight, number of panicles per plants, grains per panicle, empty grain, weight 1000 grain, and the dry grain weight was measured of the harvest.

2.5 Gas Sampling and Measurement Methane Fluxes

Gas sampling was using closed chamber method. Gas sampling is done at 30, 60, and 90 day after plant DAPwith time taking between 06.00-11.00 am. Gas sampling was done every 10 minutes from 0 to 30 minutes. Methane fluxes were calculated as follows by IAEA (1993) :

$$E = \frac{dc}{dt} \times \frac{Vch}{Ach} \times \frac{mW}{mV} \times \frac{273{,}2}{(273{,}2 + T)}$$

E = CH_4 emission rate(mg/m^2/d)
dc = Difference concentration (ppm)
dt = Time interval (min)
Vch = Volume of the chamber (m^3)
Ach = Basal area of the chamber (m^2)
mW = Molecular weight
mV = Molecular volume
T = Temperature (^{o}C)

2.6 Data Analysis

Data was analysed using microsoftexel software and software SAS 9 portable at the confidence level of 95%. The data showed a significant difference, was tested with Duncan multiple range test (DMRT).

III. RESULTS

3.1 Paddy Growth and Production

Observation of plant height and number of tillers were at 30, 60, and 90 DAP (Table 1 and Table 2). The observations showed that the treatment combination of NPK with methanotrophic bacteria was not significantly different from the treatment without combinations, but all treatment was significantly different with control without fertilization (P3). Treatment of NPK 100% + methanotrophic (P4) and treatment of methanotrophic bacteria (P6) without fertilizer higher showed plant height than other treatments at 30 DAP. Treatment NPK 100% + methanotrophic (P4) showed the highest plants height on 90 DAP than other treatment, while treatment of methanotrophic bacteria (P6) showed the lowest plant height.

Observation of the number of tillers showed that the treatment combination of NPK with methanotrophicbacteria was not significantly different with the treatment without the combination at 30 and 60 DAP, but all treatment was significantly different with the treatment without fertilization (P3). Treatment NPK 50% + methanotrophic (P5) was not significantly different with the control treatment without fertilization (P3) on 90 DAP. Treatment of methanotrophic bacteria (P6) was significantly different with the control treatment without fertilization at 60 and 90 DAP.

Table.1: Plant height at 30, 60, 90 DAP. (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. Methanotrophic + NPK 100%; P5. Methanotrophic + NPK 50%; P6. Methanotrophic)

Treatment	Plant Height (cm)*)		
	30 DAP	60 DAP	90 DAP
P1	49.35ab	89.40a	88.40a
P2	49.30ab	85.90a	90.00a
P3	45.70b	80.70b	81.85bc
P4	50.90a	87.50a	90.60a
P5	48.15ab	89.25a	85.65ab
P6	50.70a	81.15b	79.10c

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**) Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT (α = 0.05*

Table.2: Number of tillers at 30, 60, 90 DAP. (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. Methanotrophic + NPK 100%; P5. Methanotrophic + NPK 50%; P6. Methanotrophic)

Treatment	Number of Tillers*)		
	30 DAP	60 DAP	90 DAP
P1	31.25a	29.75a	27.60a
P2	27.05ab	26.00ab	26.50ab
P3	22.00b	23.20b	21.00b
P4	32.30a	24.80ab	25.35ab
P5	28.05a	24.15ab	21.65b
P6	28.35a	14.60c	14.95c

**) Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT (α = 0.05*

Harvest parameters observation showedin Table 3. Observation of shoot dry weight showed the treatment combination of NPK with the methanotrophic bacteria was not significantly different with the treatment without the combination, while the treatment NPK 50% + methanotrophic(P5) and treatment of methanotrophicbacteria (P6) was not significantly different with control without NPK (P3). Average shoot dry grain weight of P5 and P6 treatment was higher than treatment of P3.Treatment of 100% NPK (P1)produced the highest number of panicles per plants, while treatment of methanotrophic bacteria (P6) produced the lowest panicles per plants. Treatment of NPK 50% (P2) and treatment of NPK 100% + methanotrophic (P4) was not significantly different with treatment of 100% NPK (P1), whereas treatment of NPK 50% + methanotrophic (P5) was not significantly different with the treatment of 50% NPK (P2), control without NPK (P3), and treatment of NPK 100% + methanotrophic (P4).

All the treatments were not significantly different in the number of grains per panicle parameter. But treatment combination of NPK with methanotrophic bacteria produced the number of grains per panicle higher than treatment without the combination. Treatment of NPK 50% + methanotrophic (P5) produced the highest number of panicles, followed by treatment of methanotrophic bacteria (P6) and treatment of NPK 100% + methanotrophic (P4). Although it produced the highest number of grains per panicle, treatment NPK 50% + methanotrophic(P5) has highest empty grain, while treatment of methanotrophic bacteria (P6) produced the lowest empty grain. Weight 1000 grain measurements were not significantly different in all treatments.

Treatment of NPK 50% + methanotrophic (P5) produced highest dry grain weight, followed by treatment of 100% NPK (P1) and treatment of NPK 100% + methanotrophic(P4). Treatment of methanotrophic bacteria (P6) produced dry grain weighthigher than the control without NPK (P3).

Table.3: Measurement of harvest parameters (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. Methanotrophic + NPK 100%; P5. Methanotrophic + NPK 50%; P6. Methanotrophic)

Treatment	Shoot Dry Weight (g)	No. of Panicles per Plants	Grains per Panicle	Empty Grain	Weight 1000 Grain (g)
P1	114.03a	27.16a	97.94a	20.00ab	18.75a
P2	118.83a	23.55ab	98.11a	21.49ab	20.25a
P3	64.43b	19.16b	99.27a	13.08b	20.00a
P4	115.33a	24.33ab	99.47a	18.16ab	20.25a
P5	95.25ab	20.74b	109.80a	24.46a	20.00a
P6	97.57ab	13.93c	108.80a	12.63b	20.50a

Table.4: Dry grain weight parameters (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. Methanotrophic + NPK 100%; P5. Methanotrophic + NPK 50%; P6. Methanotrophic)

Treatment	Dry Grain Weight (t ha^{-1})
P1	6.8ab
P2	5.6bc
P3	4.9c
P4	6.7ab
P5	7.0a
P6	6.6ab

**)Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT (α = 0.05*

3.2 Methane Flux

The highest methane flux was shown in 30 DAP. Treatment of NPK without inoculation of methanotrophic bacteria showed highest emissions. Treatment of 50% NPK (P2) emmited 60.69 CH_4 mg/m^2/d, followed by treatment NPK 100% (P1) of 54.72 mg/m^2/d. Treatment of NPK with bacterial inoculation of P5 (NPK50% +methanotrophic) emitted 61.60 CH_4mg/m^2/d and treatment of methanotrophic bacterial alone without fertilizer (P6) produced 18.97 CH_4 mg/m^2/d.

Significant methane absorption (sink) was showed in the treatment of NPK100% + methanotrophic (P4) and

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emmited -6.27 mg/m^2/d at 30 DAP , treatment of 50% NPK (P2) of -10.72 mg/m^2/d at 60 DAP, and treatment of NPK 50%+ methanotrophic (P5) of -23.87 mg/m^2/d at 60 DAP. All the treatments showed a low methane flux on 90 DAP. This because of low rainfall so there was no formation of anaerobic environment as a habitat of methanogenic bacteria that produce methane gas.

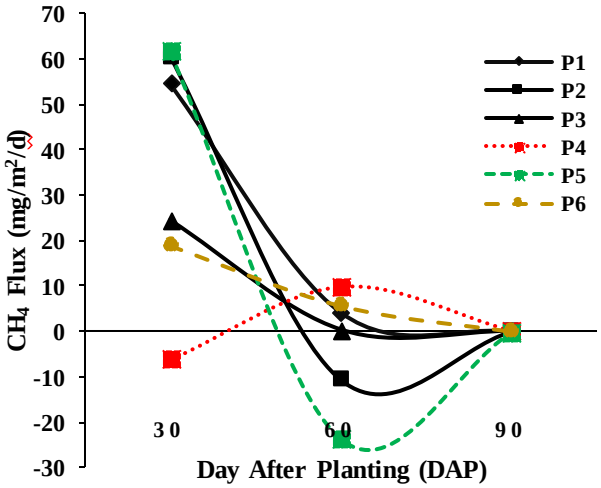

Fig.1: CH_4 Flux at 30, 60, 90 DAP. (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. NPK 100% + Methanotrophic; P5. NPK 50% + Methanotrophic; P6. Methanotrophic)

IV. DISCUSSION

Generally, the combination of methanotrophic bacteria and NPK have no effect in stimulating the growth of paddy in the vegetative phase, based on plant height parameters (Table 1) and the number of tillers (Table 2). According to Suparthaet *al*. (2012) treatment of solid organic fertilizers and organic liquid fertilizer has no effect against paddy height. Plant height and numbers of tillers has decreased at each observation. This is because of the low fertility of the soil. According to Lamberset *al*. (2008) plant height and the formation of tillers is an indicator of growth as a result of the interaction of the processes of photosynthesis, respiration, and nutrient transport.

Observations on harvest parameters generally do not indicate a difference between the treatment and control treatment. The results obtained in contrast to previous research by Sukmawatiet *al*. (2015) and Hadiantaet *al*. (2014). Both of these studies showed the application of methanotrophicbacteria effective in improving crop parameter. This is because of the content of soil chemical imbalance on every patch of the experiment. According to Zeigler and Puckridge (1995), the soil chemical imbalance to be another major constraint to the productivity of rainfed lowland rice. Most rainfed lowlands, particularly in Southeast Asia, have soils with potentially major fertility constraints. They list the main soil problems to be salinity, alkalinity, Fe toxicity, P deficiency, Zn deficiency, and organic and acid sulfate conditions.

There are differences in the parameters of dry grain weight. Treatment of NPK 50% + methanotrophic (P5) can produce 7.0 t ha^{-1}, whereas the methanotrophic bacteria treatment without NPK (P6) produces 6.6 t ha^{-1}. This indicates that the application of methanotrophic bacteria effective in increasing production in rainfed rice. Methanotrophic bacteria which applicated is a consortium of several isolates (Hapsari, 2008) i.e. *Methylocystisrosea* BGM 1,*Methylobacter* sp. SKM 14, *Methylocystispalvus* BGM 3 and *Methylococcuscapsulatus* BGM 9. Isolates *Methylocystispalvus* and *Methylobacter* sp. known to have *nif*H and *nif*Dgenes, the role gene in nitrogen fixation (Bintartiet *al*. 2014). This makes those methanotrophic bacteria can increase the availability of nitrogen for paddy growth. Nitrogen acts as a constituent of chlorophyll which is involved in the process of photosynthesis thus can increase the amount of productive grain, increase the percentage of protein and was instrumental in the preparation of the essential components of plant organs (Chaturvedi, 2005; Nettoet *al*. 2005; Watanabe and Kitagawa, 2000).

The Intergorvenmental Panel on Climate Change (IPCC) guidelines for compiling national inventories of greenhouse gas emissions (IPCC, 1997) distinguish between rice fields that are (1) permanently flooded and (2) those with unstable flooding regime. Rainfed rice belongs to the latter category (Wassmannet *al*. 2000). According to Phillips *et al*. (2009), one of the key factors that affect the production and consumption of methane is fertilization. Input of NPK emmited methane gas emissions range between 54.72 - 61.60 CH_4 mg/m^2/d at 30 DAP, higher than control without NPK ranging from 18.97-24.44 CH_4 mg/m^2/d. Setyantoet *al*. (2000) report the range of methane emissions in rainfed rice between 19-123 mg/m^2/d. The highest methane emissions occur at the beginning of the growth period and the decline in reproductive phase and the maturation phase. The intensity of the rain on the vegetative phase of 371 mm and declined on the reproductive phase and maturation phase, 10 and 11 mm, respectively. Rainfall is higher in the early growth period in rainfed rice trigger high methane emissions (Wassmannet *al*. 2000). Methane formed by the anaerobic conditions was temporary stay stuck on flooding condition. When drying, most methane is trapped will be oxidized, however, most will escape into the atmosphere as soon as flooding recedes and macro pores aerated (Neueet *al*. 1995).Strong rainfall triggered high emissions in the rainfed plots while relatively dry periods resulted in lower emission rates (Setyantoet *al*.2000). This causes the emission of methane

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gas was low in the maturation phase from 0.0072--0.15 mg/m^2/d.

The use of methane (sink) showed in the treatment of NPK 100% + methanotrophic (P4) at 30 DAP of -6.27 mg/m^2/d and treatment of NPK 50% + methanotrophic (P5) at 60 DAP of -23.87 mg/m^2/d. Methanotrophic bacteria including obligate aerobic bacteria that can use methane as a source of carbon and energy for growth (Roslev and King, 1994). According to Dubey (2005), methanotrophic bacteria is the only biological system which acts as a reservoir of methane. Methanotrophic bacteria capable of transforming CO_2 into methane oxidation process by using the enzyme methane monooxygenase (MMO). Methane oxidation can occur in the microenvironment aerobic condition on rooting zone and toxic part in the surface layer of the soil (Semrau*et al*. 2010).

Synthetic fertilizer can increase methane emission. Based on the observation, methane flux was increased in treatment with addition of synthetic at 30 DAP. Treatment of methanotrophic bacteria without NPK (P6) produced the lowest methane flux in 30 DAP (18.97 mg/m^2/d), followed by control without fertilization (P3) (24.44 mg/m^2/d). Inorganic fertilizer enhanced soil porosity by increasing regular and irregular pores and caused a priming effect of native soil organic matter (Tiquia*et al*. 2002) ultimately affecting CH_4 and N_2O emissions (Ge *et al*. 2010).

V. CONCLUSION

The application of methanotrophic bacteria (*Methylocystisrosea* BGM 1, *Methylobacter* sp. SKM 14, *Methylocystispalvus* BGM 3, *Methylococcuscapsulatus* BGM 9) increased the rice production in rainfed rice. Treatment NPK 50% + methanotrophic (P5) from that rice field produced 7.0 t ha^{-1} dry grain weight and methanotrophic bacteria treatment without NPK (P6) with improved 6.6 t ha^{-1} dry grain weight, higher than controls of 4.9 ha^{-1} dry grain weight without any addition of synthetic fertilizer.The application of methanotrophic bacteria may decrease methane gas emissions at rainfed rice. Treatment 100% NPK + methanotrophic (P4) emmited -6.27 mg/m^2/d at 30 DAP and NPK treatment 50% + methanotrophic (P5) emmited -23.87 mg/m^2/d at 60 DAP.

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Effects of micronutrient and spacing on growth and chlorophyll content of rice

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***Abstract*—** *An experiment was carried out at the research field of the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU).There were four nutrient treatments i.e., E_1= NPKS recommended dose; E_2= NPKS + Zn 5 Kg ha$^{-1;}$ E_3= NPKS + Zn (5 Kg ha^{-1}) + B (3 Kg ha^{-1}) E_4= NPKS + Zn (5 Kg ha^{-1}) + B (3 Kg ha^{-1}) + Mo (2 Kg ha^{-1}) and three spacing S_1= 20 x 10 cm^2; S_2 = 20 x 15 cm^2 and S_3 = 20 x 20 cm^2.Micronutrient and spacing combined had a distinct positive response in crop growth attributes and chlorophyll content of rice. The tallest plant height (147.0 cm) and root length (13.50 cm) highest panicle length (22.56 cm) was attained in the treatment E_2S_3 but the maximum tillers per hill (14.95) and effective panicle per hill (14.17) were recorded in treatment E_2S_2. Physiological parameter i.e., LAI, CGR, RGR, NAR, total chlorophyll content of rice also responded significantly and the appropriate combination was E_4S_2treatment. Based on vegetative growth, physiological parameters and yield attributes the treatment combination E_4S_2 showed the best performance.*

Keywords*— *Growth, chlorophyll, yield attributes and nutrients.

I. INTRODUCTION

Rice is the main food for the people of Bangladesh. Bangladesh is the 4th largest country in Asia with respect to rice production (BBS, 2004). It occupies 74% of the total cropped area, accounts for 70% of the value of crop output and contributes 20% to GDP (BBS, 2001). The average yield of rice in Bangladesh is around 2.74 tons per hectare (Anon, 2007) which is so lower than the world average of 4.25 tons per hectare. Peoples of Bangladesh have been facing shortage of rice yield for a long time. The horizontal expansion of rice area in the country is not possible due to increasing population pressure. Khan *et al*.(1999) reported thatimproper use of fertilizers and no use of micronutrients are limiting factors towards the higher rice yield. Micronutrients statuses have been decreasing day by day and finally fertility status of Bangladesh soils become declining. Micronutrients play a vital role in the yield improvement (Rehm and Sims, 2006). Micronutrients deficiency is widespread in many Asian countries due to the calcareous nature of soils, high pH, low organic matter, salt stress, prolonged drought, high bicarbonate contents in irrigation water and imbalanced application of NPK fertilizers. Micronutrient deficiency has become a major constraint for crop growth. Micronutrients help in chlorophyll formation (Reddy, 2004). Farmers of Bangladesh are habituated with the use of macro-nutrients for crop production. Kumar *et al*.(2002) stated that an optimum plant density is an important factor to achieve better growth of different rice varieties. Hamidul*et al*.(2002) rported thatthe growth and yield of rice plant is known to be affected quantitatively and qualitatively by plant spacing. So, the only option left to increase rice production is use of improved varieties and optimum spacing. Research on the use of micronutrients and spacing in increasing rice production is limited in Bangladesh.So due to lack of proper information on spacing the farmers are not getting proper yield. Considering the above mentioned facts, the present study was designed to ascertain - the combined effect of different micronutrient in presence of N, P, K, S and spacing on growth of rice, to find out suitable micronutrient combination along with N, P, K, S and spacing for rice production.

II. MATERIALS AND METHODS

An experiment was conducted at the research field of the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. Soil of this experimental site was a silty clay loam under the Salna series of Shallow Red Brown Terrace.The experimental design was split plot having three replications. Experimental variables were consisted different combination of three micronutrients

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along with N, P, K and S arranged as main plots and three spacing as sub-plots for rice production. Micronutrients and spacing were arranged as follows-

Micronutrient treatments (Main Plot)

E_1= NPKS recommended dose, E_2= NPKS + Zn 5 Kg ha^{-1}, E_3= NPKS + Zn (5 Kg ha^{-1}) + B (3 Kg ha^{-1}), E_4= NPKS + Zn (5 Kg ha^{-1}) + B (3 Kg ha^{-1}) + Mo (2 Kg ha^{-1})

Spacing treatments (Sub- Plot)

S_1= 20 x 10 cm^2, S_2 = 20 x 15 cm^2 and S_3 = 20 x 20 cm^2

A blanket dose of 65 kg N ha^{-1} as Urea, 7 kg P ha^{-1} from TSP, 28 kg K ha^{-1} as MP and 8 kg S/ha as Gypsum were applied to each treatment. All fertilizers applied as base dose except N fertilizer and N fertilizer applied as installments.Five hills per plot were selected randomly in the net plot and tagged for recording observations at four stages (30th, 60th, 90th day after transplanting and at harvest).For computing leaf area, numbers of tillers per hill were counted. The length and maximum width of each leaf on the middle tiller was measured and leaf area of each leaf was computed as follows.

Leaf area per hill (sq.cm) = Total leaf area of middle tiller × total number of tillers per hill

It was recorded for five hills separately and averaged to get leaf area per hill.

This physiological growth parameter was computed by using the following formulae-

LAI = Leaf area of plant / Land area covered by the plant.

CGR (g $m^{-2}day^{-1}$) = $W_2 – W_1 / T_2 – T_1$ X 1 / GA

Where;W_1 = Dry weight at time T_1, W_2= Dry matter at time T_2, $T_2 – T_1$ = Time interval between second and first measurement, GA = ground area of sample.

RGR (g g^{-1} day^{-1}) = ln W_2 - ln W_1 / $T_2 – T_1$

NAR (mg m^{-2} day^{-1}) = ($W_2 – W_1$) / ($T_2 – T_1$) X (ln LA_2 – LA_1) /($LA_2 – LA_1$)

Where, ln = natural logarithm, W_1 = Dry weight at time T_1, W_2 = Dry weight at time T_2, LA_1= Leaf area at time T_1, LA_2 = Leaf area at time T_2, ($T_2 – T_1$) = Time interval between second and first measurement.

III. RESULTS AND DISCUSSION

Combined effects of different Micronutrient and spacing on rice have been tested which deals with the presentation of the experimental results along with their interpretation and discussion.

Plant height

Plant height indicates the influence of various nutrients on plant metabolism. The plant height of rice was significantly unaffected due to the application of different treatment combinations (Table 1).However; it was found that application of micronutrient along with macronutrient increased the plant height over macronutrients when applied separately. But maximum plant height (147.0 cm) was obtained in E_2S_3. These results were statistically similar with the treatment E_4S_3(Table 1). The lowest plant height was recorded for only macronutrients application for all spacing. The increase in plant height in response to combined application of macro and micro nutrients along with different spacing is might be due to enhanced availability of macro nutrients as well as micro nutrients. These results are supported by the findings of Islam *et al*. (2010) who reported that the use of secondary and micronutrients maximized the plant growth and yield of T. aman.

Root length

Applications of micronutrients along with macronutrientsand spacing had significant effect on the root length of rice (Table 1). The maximum root length (13.50 cm) was obtained from the treatment E_2S_3.The lowest root lengthmaintained by the application of macronutrient only in all spacing. This result was very close with the finding of Alam*et al*. (2010).

Tiller number per hill

Number of tillers per plant or per unit area is the most important component of yield. More the number of tillers, especially fertile tillers, the more will be the yield. Tillering capacity of a plant depends on the genotype and environment. The data pertaining to number of tillers revealed that micronutrients alone with macro nutrients and spacing had positive effect on number of tillers (Table 1). Among various treatments, the treatment E_2S_2 produced the maximum number of tillers per hill (14.95) which was followed by the treatment E_3S_1(14.83).The minimum number of tillers was recorded in solely macronutrient application among the three spacing.So, these finding suggests that micronutrients had a positive influence on the increase of tillering number of rice (Sohel*et al*. 2009).

Panicle number per hill

The panicle number per hill was appreciably increased due to addition of micronutrients along with macronutrients and variation of spacing (Table 1). The maximum panicle (14.17) was recorded inE_2S_2 treatment which was statistically similar with all other treatments except E_1S_3. However, the lowest panicle per hill (10.17) was recorded in E_1S_3.Rahman et al., (2008) found that application of S and Zn had a significant impact on the panicle number of rice.

Panicle length

Panicle length responded significantly to micronutrients

along with macronutrients and variation of spacing (Table 1). Among different treatments, the treatment E_2S_3 produced the highest panicle length (22.56 cm) which was statistically similar with the second highest treatment E_2S_2 (22.14 cm).The lowest panicle length (16.53cm) was observed in the treatment E_1S_1. Rahman *et al*., (2008) found that the treatment containing 100% of the recommended dose of S and Zn produced the highest panicle length and the control did the lowest.

Number of grains panicle^{-1}

One of the basic yield components of rice is the number of grains penicle^{-1} which is affected by various factors including balanced nutrition. As shown in Table 1, micronutrients application along with basal dose of NPKS and spacing substantially improved the number of grains penicle^{-1} in rice. Maximum number of grains per panicle (98.70) was produced in the treatment E_4S_3 which was statistically similar with E_2S_2 and E_2S_3 with 97.50 and 95.85 grains penicle^{-1}. Since micronutrient is responsible for the translocation of food materials in plants therefore it played vital role in grain setting as well as higher number of grains in rice. Present results are in line with Uddin *et al*. (2008) who obtained higher number of grains by the application of boron @ 2 kg ha^{-1}. Minimum number of grains (52.40) was recorded in treatment E_1S_1. Similar finding was reported by the Hamid *et al*., (2011) that highest plant spacing gave the maximum number of grain per panicle.

Filled grain panicle^{-1}

Filled grain per panicle of rice was highly accelerated by the micronutrients application along with basal dose of macronutrients and spacing (Table 1). Among different treatments, the treatment E_2S_2 was produced the maximum filled grain per panicle (87.62) which was statistically similar with E_2S_3 (87.05) and E_4S_3 (85.43). The minimum grain per panicle (45.15) was recorded in the treatment E_1S_1. This results agreed with the finding of Nadim*et al*., (2011) that with application of micronutrient along with basal dose of macronutrient provide the maximum grain number per panicle.

1000-grain weight (g)

The data presented in Table 1 revealed that micronutrients application and spacing had significant effect on the grain weight. Maximum 1000 grain weight (12.07g) was recorded in the treatment E_2S_2 which was statistically similar at par (11.37g) and (11.17g) with grain weight obtained in E_2S_1 and E_2S_3 treatment respectively. The minimum grain weight (10.12g) was recorded in E_1S_1treatment. This might be due to zinc and proper spacing enhanced accumulation of assimilates in the grains, which resulted in heavier grains of rice.

Table.1: Effect of micronutrient and spacing on Yield attributes of rice.

Treatment	Plant height (cm)	Root length (cm)	Tiller No./ hill	Panicle No./hill	Panicle length (cm)	Kernel/plant	Filled kernel /plant	1000 seed weight (g)
E_1S_1	131.5	11.17bc	11.17	10.33ab	16.53d	52.40cd	45.15d	10.12b
E_2S_1	135.7	12.00abc	12.33	11.83ab	18.30bcd	65.15bcd	55.23bcd	11.37ab
E_3S_1	133.8	12.00abc	14.83	13.33ab	21.24ab	77.85abc	63.90abc	10.28b
E_4S_1	134.2	11.83bc	14.17	12.67ab	17.53cd	65.36bcd	55.65bcd	11.62ab
E_1S_2	131.7	10.83bc	12.83	12.17ab	17.63cd	62.90cd	46.85cd	10.27b
E_2S_2	136.3	12.17ab	14.95	14.17a	22.14a	97.50a	87.62a	12.07a
E_3S_2	140.2	11.83abc	13.50	12.67ab	19.71abcd	76.45abc	64.73abc	10.97ab
E_4S_2	139.8	12.00abc	13.17	12.83ab	18.62bcd	88.40ab	68.12abc	10.88ab
E_1S_3	133.5	10.83bc	10.67	10.17b	19.46abcd	69.00bcd	58.72bc	10.03ab
E_2S_3	147.0	13.50a	13.33	12.33ab	22.56a	95.85a	87.05a	11.17ab
E_3S_3	142.2	12.00abc	12.33	12.33ab	20.13abc	80.95abc	67.75abc	10.53b
E_4S_3	143.1	11.00bc	12.50	12.00ab	19.65abcd	98.7a	85.43a	10.15b
CV(%)	8.21	8.94	22.12	20.23	8.97	19.68	19.51	7.48
SE (±)	6.51	0.60	1.65	1.42	0.99	8.53	7.14	0.47

Leaf area index (LAI) at 45 and 90 days after Transplanting

The ratio of total leaf area to ground cover is termed as LAI. It is typically increases to maximum after the crop emergence (Reddy, 2004). The data presented in Fig.1. revealed that micronutrients and spacing had significant effect on leaf area index at 45 and 90 DAT. The maximum LAI (0.33and 3.53) was recorded in treatment E_4S_2 at 45

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and 90 DAT respectively. The lowest LAI was observed in solely macronutrient and closer spacing. In general, the application of Micronutrient especially boron and medium spacing had boosted up the tissue formation with better plant growth which increases its concentration in leaves and results in higher leaf area index.

Crop growth rate (g m-2 day-1)

Crop growth rate is the dry matter production per unit time. The data in Fig.3. revealed that combined effect of micronutrient and spacing significantly affected the crop growth rate. Micronutrients application enhanced the plant growth through increased plant photosynthesis and other physiological activities whereas, proper spacing has positive influence on nutrient uptake of plant. Among various treatments, E_4S_2 accelerated crop growth rate (33.78 g m-2 day-1). The use of micronutrient and proper spacing helped the plants to better utilize the available nutrients with increased leaf area, high photosynthesis and dry matter accumulation which enhanced crop growth rate. These results satisfy the findings of Asad and Rafique (2002) who reported that boron fertilization increased the dry matter production of wheat. The minimum crop growth rate (24.43) was recorded in macronutrient application with closer spacing (E_1S_1).

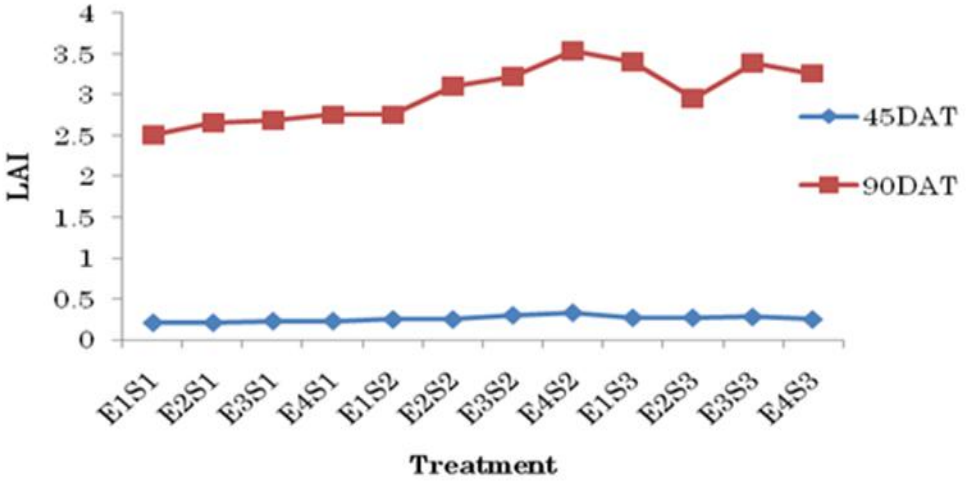

Fig. 1: Effects of different micronutrients and spacing on leaf area index of rice

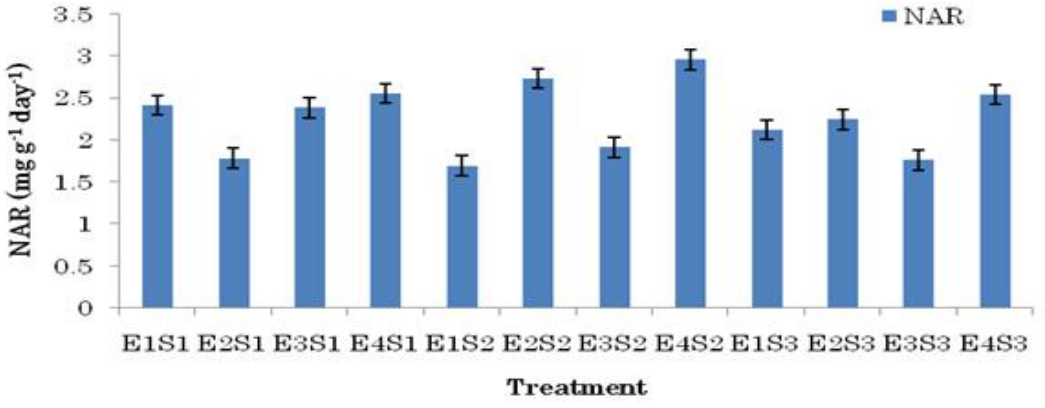

Fig.2: Effects of different micronutrients and spacing on net assimilation rate (NAR) of rice

Relative growth rate (mg g-1 day-1)

Relative growth rate (RGR) expresses the dry weight increase in time interval in relation to the initial weight. Since crop growth rate is an absolute measure of growth, similar values could be expected for different initial weights (Reddy, 2004). The data presented in Fig.4. revealed that

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application of different micronutrients and spacing had significant effect on the relative growth rate of rice. Maximum RGR (88.45 mg g-1 day-1) was produced in treatment E_4S_2 which was followed by (87.58, g g-1 day-1) E_2S_2. The reason might be the high concentrations of boron and zinc in the leaves increased plant food accumulation which resulted in more relative growth rate (Card *et al*. 2005). The sole application of macronutrient (E_1S_2) produced the minimum relative growth rate (76.30 mg g^{-1} day^{-1}).

Net assimilation rate (mg m-2 day-1)

The plant capacity to increase dry weight in terms of area of its assimilatory surface expresses the net assimilation rate. The data given in Fig. 5 revealed that different micronutrients and spacing had significant effect on net assimilation rate. Among various treatments, E_4S_2 produced the significantly maximum net assimilation rate (2.95 mg m-2 day-1) which was statistically closer with E_2S_2 treatment. Shukla and Warsi (2000) also obtained the highest net assimilation rate with the application of Zn along with NPK. The minimum net assimilation rate of 1.91 mg m^{-2} day^{-1} was produced at E_1S_2 treatment.

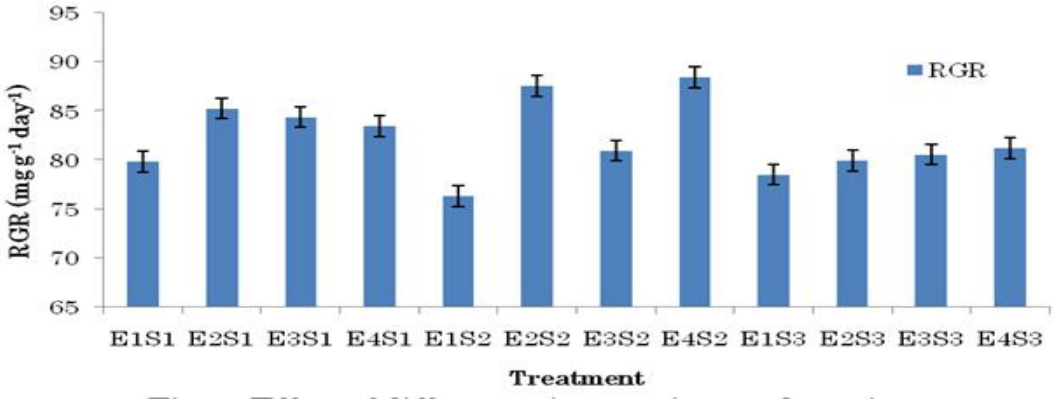

Fig. 3: Effects of different micronutrients and spacing on relative growth rate (RGR) of rice

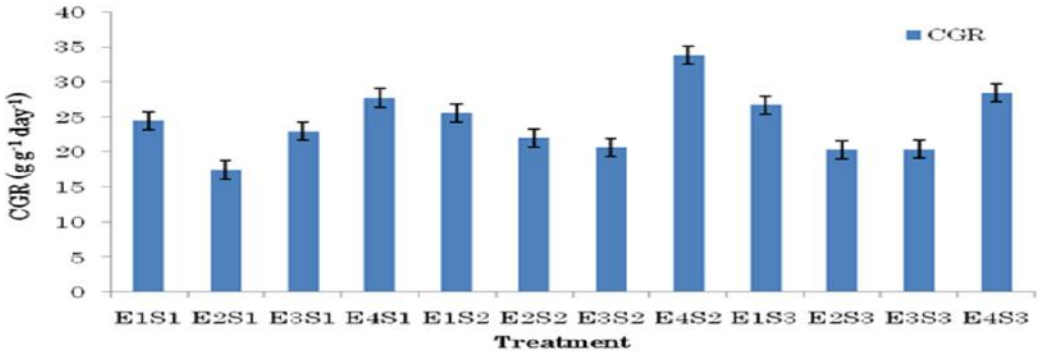

Fig.4: Effects of different micronutrients and spacing on crop growth rate (CGR) of rice

Chlorophyll Content (mg/g)

The response of growth and yield parameter depends upon the photosynthetic rate, which in turn is dependent on chlorophyll contents. In the present study, a significant increment in chlorophyll contents (a, b and total chlorophyll) was recorded in combined effects of micronutrient and spacing along with macronutrient. The chlorophyll "a" and "b" contents was found to be correlated with each other and the treatment Zn @ 5kg ha–1, B @ 3kg ha–1, Mo @ 2kg ha–1 along with different macronutrients along with 20x 15 cm^2 spacing (E_4S_2) showed highest. However, the treatment contains solely macronutrients with lowest spacing (E_1S_1) showed the lowest chlorophyll content. The chlorophyll "a" and "b" contents varied from 1.98 to 1.37 mg g-1 and 0.69 to 0.46 mg g-1, respectively with different combination of micronutrient and spacing. The highest chlorophyll contents (a, b and total) was recorded in (E_4S_2) treated plant. However, all other

treatments also had increased chlorophyll contents significantly (Table 2). The chlorophyll "a", "b" and total chlorophyll contents increased up to 33.78, 30.19 and 32.34%, respectively for the treatment Zn @ 5kg ha–1, B @ 3kg ha–1 and Mo @ 2kg ha–1 along with different macronutrients along with 20x 15 cm2 spacing (E_4S_2) over the similar spacing control. This trend was observed because the chlorophyll contents increased considerably in Zn and B treated group of plants (Hatwar*et al*.2003).

Table.2: Effect of Micronutrient and spacing on Chlorophyll content (mg/g) of rice.

Treatment	Chlorophyll Content (mg/g)		
	Chl. a	Chl. B	Total Chl.
E_1S_1	1.15h	0.36g	1.51f
E_2S_1	1.55d	0.47f	2.02d
E_3S_1	1.37g	0.49ef	1.86e
E_4S_1	1.41fg	0.46f	1.87e
E_1S_2	1.48e	0.53de	2.01d
E_2S_2	1.50e	0.57cd	2.06d
E_3S_2	1.63c	0.56cd	2.19c
E_4S_2	1.98[a]	0.69[a]	2.66[a]
E_1S_3	1.40fg	0.47f	1.87e
E_2S_3	1.78b	0.64ab	2.43b
E_3S_3	1.78b	0.61bc	2.39b
E_4S_3	1.54ef	0.48f	2.02d
CV(%)	2.03	4.20	2.08
SE (±)	0.02	o.02	0.03

IV. CONCLUSION

The tallest plant height (147.0 cm), longest root length (13.50 cm) and highest panicle length (22.56 cm) were attained in the treatment E_2S_3, though the maximum tillers per hill (14.95) and effective panicle per hill (14.17) were obtained in the treatment E_2S_2. Although, the maximum number of grains per panicle (98.7) was produced in the treatment combination E_4S_2, the maximum filled grains per panicle (87.62) was observed in the treatment E_2S_2. The maximum LAI, CGR, RGR, NAR and total chlorophyll content were produced by the E_4S_2 treatment. Based on vegetative growth, crop growth attributes treatment combination E_4S_2 may be specified as the best performer.

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Rearing of all Male Tilapia (Oreochromis Niloticus) Fingerlings with Chicken Manure and Mixture of Chicken Manure and Commercial Diet in Fibre Glass Tank

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Abstracts— *Mono-sex population of Oreochromis niloticus fingerlings were collected from Nigerian Institute for oceanography and Marine Research Sapele station Hatchery and acclimatized for a week and randomly stocked at density of 300 fish/tank in nine fibre glass tank. The fingerling were fed daily 800h and 1600h with three different types of feed (Dry chicken manure, mixture of chicken manure and commercial diet, and commercial (coppens) only.*

Each treatment was replicated three times. The water quality parameters are within tolerant limit. The growth response in all the treatment wee generally satisfactory. Though treatment T3 Tanks fed with commercial diet only (coppens) had better growth performance compared with treatment T2 and T1, chicken manure and mixture of chicken manure and commercial diet respectively.

The results demonstrate the feasibility of rearing all male tilapia (O. niloticus) fingerlings in fiber glass tanks.

Keywords— Fibre Glass, Chicken Manure, Fish farmers.

I. INTRODUCTION

Fish farmers in Nigeria use a variety of production systems with different level investment, different management requirements and production potentials. Thus farmers have several option for entering fish farming depending on their physical and financial resources.

As input cost rises (feed, energy, transportation) and yet the selling price of fish remains the same. Fish farmers are forced to find ways to reduce costs. Many farmers are forced to find ways to reduce costs. Many farmers try to find cheaper feed as this represent up to the 60 – 70% of operating cost. This has led to the alternative use of poultry manure (chicken waste) in place of compounded diets. Chicken manure contain considerable quantities of nutrients for fish production with ranges of between 10 and 30% for protein 0.45 – 5.86mg/kg for energy as well as high level of soluble vitamins. It also contains non-digested feed metabolic excretory products and residues resulting from microbial systems which can be utilized to replace reasonable quantities of feedstuffs used in conventional fish feed thereby reducing production cost (Falayi 1998), Fashakin et al 2000). Findings related to feed poultry waste at higher levels has been reported by several workers (, Harmon et al 1975b, Kamal et al2008. Boyd 1976,The aim of this study was to cut natural food chain and make chicken manure the only source of food for fish in order to determine the differences in fish growth, under these two different situations; and compare the effect of rearing tilapia (*O. niloticus*) with dried chicken manure and mixture of dried chicken manure and commercial diet.

Key Word: Tilapia. *Oreochromisniloticus* , Dry chicken manure, Commercial diet. (Coppens).

II. MATERIALS AND METHODS

Study Area: The study was carried out at Nigerian Institute for Oceanography and Marine Research Sapele out station Delta State Nigeria. ($N05^0$ $54^{'}03$"E 005^0 39' 56.4"). The experiment was conducted for 3 month using 9 circular fiberglass tanks each with a capacity of $3.08m^3$ of water (figure 1) between September 2017 and November 2017. The tanks were mounted out door in a row.

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Fig.1

Experimental tanks and stocking rate

Nine (9) circular fibre glass tanks used in this experiment were identical in shape and size, tanks capacities were 3.08m^3 and the flow of each tank drained to the centre drainage of the tank was on the outside via 100mm PVC pipes and gate valves. Each tank received water from a borehole passing through a water treatment plant to correct the pH.

Fingerlings of All male tilapia (*O. niloticus*) were used as specimen for this study. The fish were provided by the Nigerian Institute for Oceanography and Marine Research Sapele out Station hatchery. Each tank was watched, cleaned and disinfected with sodium chloride Nacl after which the tanks were filled with water to a depth of 60.5m and allowed to settled for a day before introducing the fish. Each tank was stocked with All male tilapia fingerlings of average weight of 0.90 – 0.97g and cultured for a period of 12 weeks at a stocking density of 300 fish per tank which translate to 158 fish per m^3. Three types of feed were used to feed the fish, these were dried chicken manure,dried chicken manure with commercial diet (coppens) and commercial diet (coppens) as control. The daily feeding rate was 5% of the total stocked biomass, thereafter the fish were sampled every two weeks to obtain information for adjustment of the feeding rates. Uneaten feed and feaces of the fish were siphoned off and $^1/_3$of the water was replaced every day there was no feed during the night time. Analysis of crude protein fibre ether extracts, ash and moisture content were done in triplicate generally following AOAC(1990) procedures for dried chicken manure and for the commercial diet (coppens).

III. RESULTS AND DISCUSSION

During the experimental period (September 2017 – November 2017) temperature in the fibre tanks ranged between (26 - 28^{0}C averaging 28.7^{0}C.Gui et al (1989)found that an average temperature of 28oc was optimal for growth of Nile tilapia fingerlings Dissolved Oxygenranged between 3.95 and 13.85ml/l DO normally remain above3ml/l with the low value at 3ml/l (before water exchange)Denser (1968), AIT(1968)and Hassan et al(1997) reported that 2.3mg/l is above the normal tolerance level of tilapia. The pH ranged between (6.77-9.41) Boyd (1998) reported that water with a pH range of 6.5 -9 are the most suitable for fish production. The average concentration of unionized ammonia (NH3) was 0.50, 0.60, 0.50mg/l for treatment T1, T2 and T3 respectively.Some studies showed the same trend for lower ammonia concentration; Diana and Lin (1998) reported ammonia concentration 0.374 - 0.410mg/l in pond fertilized with chicken manure. this low

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concentration of ammonia may be attributed to ammonia utilization by phytoplankton Boyd (1998), the average value of secchi disk reading were19.37 ± 2.96,27.57 ± 2.35,32.56 ± 1.19 (cm) for T1, T2, and T3 respectively. The significant decrease in secchi disk reading less than 20cm for T1 (fed chicken manure only) indicates that fiber glass tank is too turbid , which may due to either phytoplankton of suspended solid particle (Boyd (1998).Total Dissolved solid ranged between (102.58 – 182.550) averaging (107.21). The value of Electric conductivity ranged between 208.10 +23.87 – 273.3 + 20.74 µmhos/cm .the above results shows that all parameters of water quality were in a suitable range (Boyd, 1979)

Table.1: showed change in body weight after rearing for 12 weeks with chicken manure and commercial diet (coppens)

Feeds		**Initial fish weight (10g)**	
	Month	**Final fish weight**	**Average weight (gm)**
Dried chicken manure		**TA1 TA2 TA3**	
	1	40.04 42.06 48.10	
	2	80.36 84.38 88.56	84.46
	3	122.64 128.16 126.46	
		81.0184.8187.55	
Dried chicken manure plus commercial diet		**TB1 TB2 TB3**	
	1	62.26 64.40 65.52	
	2	121.32 128.92 126.54	111.11
	3	140.28 141.42 149.38	
		107.95111.58 113.81	
Commercial diet only (coppens)		**TC1 TC2 TC3**	
	1	73.82 72.72 78.40	
	2	148.00 145.58 148.04	147.92
	3	220.06 217.94 226.78	
		147.29145.41151.07	

Table.2: Showing average water quality parameter during the experimental period (12 weeks) in fibre glass tank stocked with all male tilapia fingerlings.

	TREATMENTS		
PARAMETERS	**T1**	**T2**	**T3**
Temperature ^{0}C	26 ± 0.77	25.77 ± 0.75	28.414 ± 0.75
Dissolved oxygen mg/l	3.95 ± 0.88	10.46 ± 0.62	13.850 ± 0.72
pH	6.77 ± 0.13	9.024 ± 0.13	9.101 ± 0.13
NH_3	0.50 ± 0.09	0.60 ± 0.09	0.50 ± 0.04
Secchi disc cm	19.37 ±2.96	27.57 ± 2.35	32.56 ± 1.19
TDS (ppm)	136 ± 16.11	128 ± 20.08	104 ± 11.61
EC µmhos/cm	273 ± 20.74	258 0 ± 17.99	208.10 ± 23.87

T1 = Chicken manure, T2 = Mixture of chicken manure and coppens, T3 = Coppens only

Growth performance

The growth response of fish in all the treatments were generally satisfactory as shown in table (1), the average body weight of all male tilapia (O. niloticus) fingerlings increased from10g to 84.46g, 111.11g and 147.92g for T1, T2 and T3 respectively. It is obvious that T3 (fed commercial diet coppens) recorded higher P>0.05 final body weight than the manure mixed with coppens and manure only T2 and T1 respectively. The same trend was obtained with regard to weight gain. The observation in the low weight gain in manure tans compared with control fed diet reported in similar studies (Daiana et al 1994, 1996, Brown et al 2000) indicates that either phytoplankton may not be enough to meet protein requirement of fish or that fish could not efficiently assimilate the produced phytoplankton in these tanks. Similar findings were

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reported (Colman et al 1990) they recorded poor fish growth in fertilized concrete tank and attributed it to the predomination of the green algae.

In conclusion, based on obtained results and the high cost of fish diet, it can be concluded that the use of chicken manure in fibre glass tanks could be recommended for producing all male tilapia.

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Extending Shelf-life of Different Cut-flowers under Cold Room Conditions

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Abstract- *Uniform and healthy Rose cv. 'Dutch'; Gerbera cv. 'Lexington'; Gladiolus cv. 'Top Secrate'; Tuberose cv. 'Bizet' and Carnation cv. 'Liberty' were used for the study in September 2016. Cut flowers were harvested at 7.00 am at proper stage, transported within 1.30 hours by AC car to the Agricultural Research Laboratory of Ecofrost Technologies Pvt. Ltd., Pune and then immediately prepared for post-harvest treatment and storage. The aim of this study was to determine the effectiveness of different storage conditions, i.e. room and cold storage conditions (10°C + 93 % RH) on the longevity of the cut flowers. The two treatments viz., holding flowers at room temperature (RT) (T_1) and at cold room conditions (T_2), were replicated twice. The result showed that keeping cut-flowers at cold storage in a holding-solution of tap water recorded the maximum storage-life (days) compared to room conditions.*

Keywords- Rose, Gerbera, Gladiolus, Tuberose, Carnation, Storage-life, Ecofrost.

I. INTRODUCTION

Cut-flowers are often harvested at the horticultural stage, so flowers need a large amount of soluble carbohydrates for proper opening and long life. Treatment with sugars, such as Sucrose and Glucose in combination with some germicides/biocides extend the vase-life of many cut flowers and can affect ethylene production and up-regulation of sugars accumulated in floral organs (Ichimura *et al*., 2006).

While production of high-quality flowers is important, it is critical to handle the flowers properly after they are harvested from the field. There are reports which suggest that improper post-harvest handling accounts for 20 to 30% of cut-flower loss during marketing (Jadhav *et al*., 2014).

The objective of this study was to determine the effect of different storage conditions like room conditions and cold room on the storage-life of Rose cv. 'Dutch'; Gerbera cv. 'Lexington'; Gladiolus cv. 'Top Secrate'; Tuberose cv. 'Bizet' and Carnation cv. 'Liberty'.

II. MATERIAL AND METHODS

The present study was carried out in the Agri Research Laboratory of Ecofrost Technologies Pvt. Ltd. Tathawade, Pune (MH), India in September 2016. Rose cv. 'Dutch'; Gerbera, Lexington; Gladiolus, Top Secrate; Tuberose, Bizet; and Carnation, Liberty flowers were cut in the early morning of 19th September 2016. After transportation, stems of cut-flowers of different flower crops were trimmed in the laboratory except Gerbera. Almost all cut-flowers had different stem lengths. Cut-flowers were put immediately in tap water containing 5% sucrose with Silver Thiosulphate (STS) @ 200 ppm for 4 hours under room conditions. Cut flowers (T_2) after postharvest treatment were subjected to cold storage treatment and cut-flowers of different flower crops after post-harvest treatment were stored at room conditions (T_1). Each treatment had two replications (R-2) and ten flowers per replication. After every two days, Rose cv. 'Dutch'; Gerbera, Lexington; Gladiolus, Top Secrate; Tuberose, Bizet and Carnation, Liberty flower stems were checked for fungal infection which could impede water uptake. Then, each flower stem was re-cut under the water to eliminate air bubbles or emboli, which can decrease flower life and lead to premature wilting of the bloom of Rose cv. 'Dutch'; Gladiolus, 'Top Secrate'; Tuberose, 'Bizet' and Carnation, 'Liberty' flowers. Precautions were also taken to remove foliage below the water line to prevent bacterial proliferation. Ten flowers of each crop were treated replication-wise, under post-harvest treatments. The period of initial pre-treatment solution was 4 hours under room conditions. The treated soaked Rose cv. 'Dutch'; Gerbera, 'Lexington'; Gladiolus, 'Top Secrate'; Tuberose, 'Bizet' and Carnation, 'Liberty' flower stems were transferred to a tap-water solution on 19th September 2016 inside cold room (T_2) and in room conditions (T_1). The level of the tap water was maintained as 2.5 to 3.0cm at the bottom of the bucket.

The storage-life was recorded by observing the number of days that were taken between the time of harvest and end of longevity that occurs in ways such as bending of the floral axis just below the flower head (bent-neck in rose),

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flower closure, wilting or abscission, changing color of petals prior to wilting or abscission.

Observations recorded:

The observations regarding the post-harvest parameter of the storage-life of Rose cv. 'Dutch'; Gerbera, 'Lexington'; Gladiolus, 'Top Secrate'; Tuberose, 'Bizet' and Carnation, 'Liberty' were recorded for each treatment replication-wise and cumulative data was subjected to analysis.

III. RESULTS AND DISCUSSIONS

The highest storage life (days) was noticed in Rose cv. 'Dutch'; Gerbera, 'Lexington'; Gladiolus, 'Top Secrate'; Tuberose, 'Bizet' and Carnation, 'Liberty' inside cold room (T_2) compared to room conditions (T_2) (Table 1; Photo 1 and 2).

Table.1: The storage-life (days) of different cut-flowers at room temperature and inside cold room (10°C and 93% RH)

Sr. No.	Cut-flowers of different crops	Storage-life (days) At room conditions	Storage-life (days) inside cold room	Post-storage life (days) at room conditions
1	Rose, 'Dutch'	3.5	5.5	1.0
2	Gerbera, 'Lexington'	3.0	5.0	1.0
3	Gladiolus, 'Top Secrate'	4.0	5.5	1.0
4	Tuberose, 'Bizet'	4.0	5.5	1.0
5	Carnation, 'Liberty'	4.0	5.5	1.0

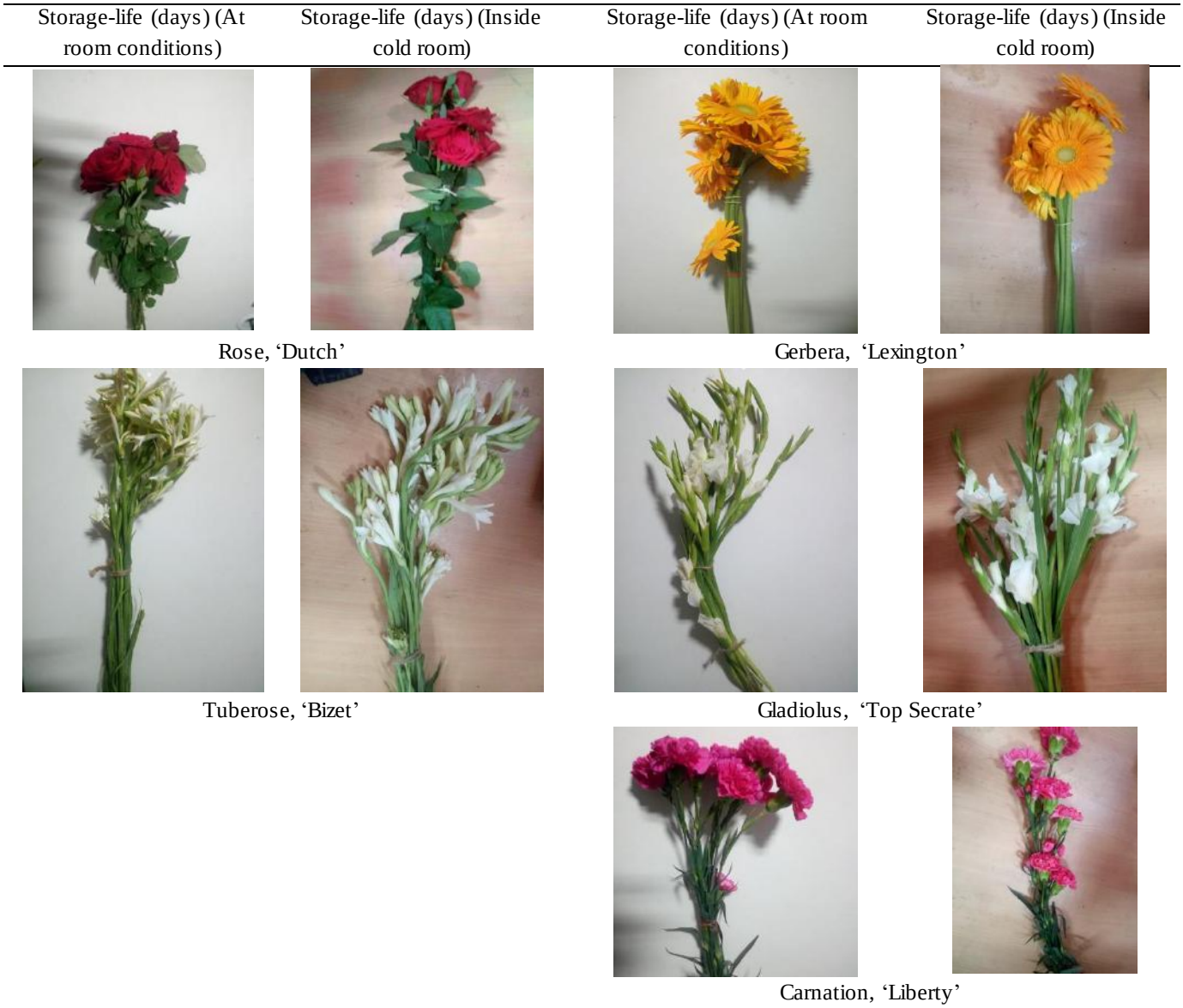

Photo.1: General view of different cut-flowers after two days storage at room conditions and inside cold room of Ecofrost (10°C and 93% RH).

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Storage-life (days) (Inside cold room) (10°C and 93% RH)

Rose, 'Dutch'	Gerbera, 'Lexington'	Gladiolus, 'Top Secrate'	Tuberose, 'Bizet'	Carnation, 'Liberty'

Photo.2: General view of different cut-flowers storage inside cold room on 8th day.

Sucrose serves as a source of energy to make up for the loss of the functioning of leaves and ensures continued development and longevity of the flower. The treatment of cut-flowers with Sucrose was found beneficial in delaying senescence process. When cut flowers are pulsed overnight it results in faster flower opening; longer the stem, longer the vase-life (Jadhav *et al.*, 2014 & Jadhav *et al.*, 2014). Low temperature was effective in delaying senescence process.

IV. CONCLUSION

In conclusion, cold storage conditions can extend the life of Rose cv. 'Dutch'; Gerbera, 'Lexington'; Gladiolus, 'Top Secrate'; Tuberose, 'Bizet' and Carnation, 'Liberty' cut-flowers'.

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Study on different potato continuous cropping ways on rhizosphere soil nutrients and enzyme activities

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Abstract**—** *To address the problem of food security, China produced potatoes as a staple food in 2015.However, there are increasing problems with continuous cropping production methods, potato continuous cropping has been inevitable.So it is necessary to research under the different potato continuous cropping ways, potato rhizosphere soil nutrients and enzyme activities which can direct potato fertilizer and ease potato continuous cropping obstacle. A two-growing season investigation was carried out during the spring and autumn of 2014 and 2015 to determine the different ways of potato continuous cropping on the overall growth of potatoes, soil nutrients, and enzyme activities. During continuous cropping nitrogen (N) content of rhizosphere soil was reduced; available potassium (Kav) was significantly reduced($p \leq 5\%$), especially in spring and autumn continuous cropping; and total phosphorus (P_{tot}) was reduced during the growth stage. However, the total potassium (K_{tot}), available phosphorus(P_{av}), and organic carbon (C_{tot}) increased before they decreased. For rhizosphere soil enzyme activities, urease initially increased and then decreased, and was lower in continuous cropping than multiple continuous cropping; in spring of 2015, invertase was the highest with continuous cropping. Catalase and polyphenol oxidase decreased initially before increasing. Continuous cropping in spring and autumn consumed more nutrients, especially potassium (K) than in spring. Therefore, potatoes planted in both spring and autumn enhanced the problems of continuous cropping. However, multiple continuous cropping that eased rhizosphere soil nutrient absorption and effectively improves soil nutrients and enzyme activities could provide an effective method for managing the negative impacts associated with continuous cropping.*

Keywords**— potato continuous cropping, rhizosphere, soil nutrients, soil enzyme activities, polyphenol oxidase.**

I. INTRODUCTION

Follow the rice, wheat and maize, potato (*Solanum tuberosum L.*) is the fourth-most important food crop worldwide. It is one of the major crops in China as well. To date, the planting acreage and production of potato in China ranked as top one worldwide. In 2015, the potato staple strategy are put forward in our country, by advancing the potato food development, adjusting measures to local conditions enlarges planting area, from the current $5.3*10^4 hm^2$ to expand to $1.0*10^6$ hm^2(Schirring, 2015). However, due to the limitations of different crops in different ecological conditions and authentic, and optional rotation crop limitations such as low efficiency and the urgency of the economic demand, makes the potato continuous cropping pattern is inevitable.

In Gansu, Sichuan and Inner Mongolia, due to rapid development of potato industry, its planted area extended to more than 65% of the country (Long et al., 2013), the potato continuous cropping patterns have been inevitable under the condition of limited arable land. under the condition of several years of planting potato model continuously has inevitable. Especially with the comparative benefits the improvement of potato industry, potato planting area in the northwest China increases year by year. Indingxi area, Gansu province, the potato continuous cropping has amounted to more than five years in some part of arable land, the potato continuous cropping is common, thus resulting in increasing problems for effective agricultural management in China. These problems include an increase of pests and diseases (Jeffrey et al., 2015), and a negative impact on soil physiochemical properties, soil microbes, and allelopathy(Rice,1985; Zhang et al., 2015). Previous researchers reported that continuous cropping decreases yield, quality, and causes diseases and imbalances of soil ecology (Wu at al., 2009; Zhang et al., 2010); thus, seriously affecting the production and economic benefits of potatoes.

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It is worth mentioning that with development of China, the potato continuous cropping is inevitable. So explore the different ways of continuous cropping patterns, research rhizosphere soil nutrient and enzyme activity is significant.

The negative impacts of continuous cropping have become a major constraint in potato cultivation (Meng et al., 2012). The negative impacts of continuous cropping were mainly caused by an increase of soil-borne diseases (Robert et al., 2014; Lai et al., 2011), the degradation of soil physical and chemical properties (Liu et al., 2009; Zhang et al., 2007), and root secretion and residue decomposition resulting in autotoxicity (Cheng et al., 2013; Wang et al., 2005). The negative impacts from continuous cropping of different crops are considerably different, but mainly affect the soil.Most studies have focused on vegetables (Wu et al., 2007; Xiao et al., 2012), melons and fruit (Zhao et al., 2008), soybeans (Miao et al., 2007), and Chinese medicinal herbs (Zhang et al., 2010; Hao et al., 2008). In continuous cropping systems, previous research reported that soil nutrients and enzyme activities were affected, and this effect was sustained in later growing seasons (Zhou et al., 2011). However, how the rhizosphere soil nutrients and soil enzyme activities at different growing stages are affected by continuous cropping needs to be determined. There has been limited research on overcoming the negative impact of continuous cropping and other production practices.

Although at present there have been lots of research about the potato continuous cropping obstacle, but the potato production practice of continuous cropping is not practically solved. This study based on the potato continuous cropping, under the different modes of the potato continuous cropping research onrhizosphere soil nutrient and enzyme activity of comparative analysis, aimed at the production of continuous cropping potatoes offer ideas and solutions.The objectives of this study were 1) to evaluate the effect of the continuous potato cropping on rhizosphere soil nutrients and enzyme activities on the growth characteristics of potatoes; and 2) to show the negative impacts of potato continuous cropping, direct the potato production. The results from the present study should provide a greater understanding of the negative impacts of continuous cropping and contribute to the improved management of potato cultivation.

II. MATERIALS AND METHODS

2.1 Site descriptions

The experiment was conducted in 2014 and 2015 in the field at the experimental station in the college farm, southwest Sichuan Agricultural University, Chengdu, Sichuan Province (southwest China, N 30° 67 ', E 104° 06'). The soil chemical characteristics were: pH (1:1 water) 5.08, organic carbon (C_{tot}) 18.72 mg/kg, total nitrogen(N_{tot}) 2.68 g/kg, total phosphorus (P_{tot})0.58 g/kg, total potassium(K_{tot}) 13.00 g/kg, available nitrogen(N_{av}) 138.68 mg/kg, available phosphorus(P_{av}) 18.72 mg/kg, available potassium(K_{av}) 126.31mg/kg. The plant site before the experiment had been previously planted with a potato crop in 2013. The present study included A (multiple continuous cropping), B (potato continuous cropping in spring), and C (potato continuous cropping in spring and autumn) cultivation.All three cropping were conducted in a randomized block design of three repetitions in two years and two growing seasons. About 30 g whole potato tubers, cultivar Chuanyu 117, were planted at a depth of 8 cm.Six potatoes were planted into the compartment with control soil from each pot (60 × 40 × 35 cm). Before potato planting, experimental plots were uniformly tilled and a compound fertilizer 750 kg·ha^{-1} (N-P2O5-K_2O 15-15-15) was initially used as a base fertilizer. Fertilizer was not added again. Standard agronomic practices were equally performed in all treatments. The sampling periods were before seeding, flowering, tuber bulking, and maturity. In autumn 2014 and 2015, the land of A and B was idle, and C was planted with potatoes (its index was not measured because planting in autumn was for enhancing the negative impacts of continuous cropping).

2.2Rhizosphere soil nutrients

The initial soil samples were ground by mortar and pestle to pass a 0.15 mm sieve to remove plant residues and stones prior to chemical analysis. Total nitrogen(N_{tot}), phosphorus (P_{tot}), and potassium(K_{tot}) were measured using the method described by Bao (2005).Soil organic carbon (C_{tot}) was determined using the Walkley-Black method; total nitrogen(N_{tot}) was assayed using the Kjeldahl method;total phosphorus(P_{tot}) was measured by phosphomolybdate-blue spectrophotometry; total potassium(K_{tot}) was measured by NaOH molten-flame photometry; organic matter was measured by heat dilution using potassium dichromate volumetry; alkali-hydrolyzablenitrogen(N_{av}) was measured by alkali-hydrolyzed reduction diffusion; available phosphorus(P_{av}) was measured using 0.5 mol/L $NaCO_3$; and available potassium(K_{av}) was measured by NH_4OAc leaching and flame spectrophotometry.

2.3Rhizosphere soil enzyme activities

Soil enzyme analysis was performed on the same samples that were air-dried,ground, passed through a 1-mm mesh, and maintained at room temperature (Guan, 1989). Invertase activity was determined using 3,5-dinitrosalicylic acid colorimetry; urease activity was measured using phenol-sodium hypochlorite sodium colorimetry; polyphenol oxidase was measured using phenol colorimetry; and catalase was assayed using a titration method described by Guan (1989).

2.4Statistics analysis

The analysis were carried out using Microsoft Office Excel 2007, variance for the data was performed by DPS. Means were separated by LSD at the 0.05 probability level.

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III. RESULTS AND DISCUSSION

3.1 Nitrogen contents of rhizosphere soils

In 2014, compared with before seeding, in maturity stage, the content of totalnitrogen(N_{tot}) in rhizosphere soil was reduced by 4.41, 14.55, and 14.71%, for A1, B1, and C1, respectively. However, except for C2, the content of totalnitrogen(N_{tot}) varied smoothly in spring of 2015, the A2 was reduced by 1.01%, and B2 and C2 increased by 0.51 and 16.16%, respectively. The C content decreased with continuous cropping years. In maturity stage of 2014, the alkali-hydrolyzablenitrogen(N_{av}) content reduced by 22.74, 26.10, and 20.26%, for A1, B1, and C1, respectively; and in A2 and B2 increased by 28.89, and 18.46%, however, C2 decreased by 9.15%. In tuber bulking stage, the content of alkali-hydrolyzablenitrogen(N_{av}) changed rapidly. Alkali-hydrolyzablenitrogen(N_{av}) content showed little difference in different continuous cropping ways in the spring of 2014, and were reduced in 2015. The variation of totalnitrogen(N_{tot}) showed the opposite trend.

Table.1: Nitrogen contents of rhizosphere soils

		Before seeding	Flowering	Tuber bulking	Maturity
Total Nitrogen (g/kg)	A1	2.72 ± 0.09a	2.67 ± 0.11c	2.81 ± 0.23a	2.60 ± 0.05ab
	B1	2.68 ± 0.03a	2.75 ± 0.26a	2.42 ± 0.21bc	2.29 ± 0.13c
	C1	2.72 ± 0.13a	2.72 ± 0.05a	2.42 ± 0.03bc	2.32 ± 0.04c
	A2	1.99 ± 0.02a	1.94 ± 0.23ab	1.78 ± 0.23ab	1.97 ± 0.16a
	B2	1.97 ± 0.04a	1.95 ± 0.32a	1.87 ± 0.20ab	1.98 ± 0.32a
	C2	1.66 ± 0.00b	1.96 ± 0.04a	1.76 ± 0.12ab	1.98 ± 0.06a
Alkali-hydrolyzable nitrogen (mg/kg)	A1	168.63 ± 1.52a	166.43 ± 1.92ab	132.77 ± 2.83cde	129.97 ± 1.65de
	B1	170.97 ± 2.72a	167.93 ± 1.30ab	136.73 ± 2.14cd	126.35 ± 0.93e
	C1	160.39 ± 8.55b	164.27 ± 5.76ab	137.89 ± 2.88c	127.89 ± 7.23e
	A2	105.00 ± 0.00h	121.33 ± 8.08cde	135.33 ± 4.04a	135.33 ± 4.04a
	B2	108.33 ± 5.77fgh	123.67 ± 4.04bcd	115.50 ± 9.26def	128.33 ± 4.04abc
	C2	116.67 ± 4.04def	130.67 ± 4.04ab	114.33 ± 8.08efg	106.00 ± 1.73gh

A1, B1, C1 stand for A, B, C in 2014, A2, B2, C2 stand for A, B, C in 2015; In the same year, values within a column followed by different lowercase letters are significantly different at the 0.05 probability level, as determined by Fisher's least significant difference test; n = 3.

3.2Phosphorus contents of rhizosphere soils

From table 2, total phosphorus(P_{tot}) was decreased with growth as the continuous cropping years increased. These three treatments showed the significant effects on the total phosphorus(P_{tot}) . A1 increased by 3.32%, and B1 and C2 were reduced by 15.49 and 14.53%, respectively. In 2015, the content of total phosphorus(P_{tot}) was reduced by 13.11, 23.07 and 15.71% for A2, B2, and C2, respectively. Throughout the growth stages, there was a considerable change in total phosphorus(P_{tot}) content. In maturity stage, the total phosphorus(P_{tot}) content ranged from 0.500 to 0.600 g/kg. The variation of available phosphorus(P_{av}) content is different from total phosphorus(P_{tot}). In spring of 2014, available phosphorus(P_{av}) was reduced by 46.99, 39.56, and 39.42% for A1, B1, and C1, respectively. In contrast to that in 2015, it increased by 28.35, 25.03, and11.96% for A2, B2, and C2, respectively. Even though the total phosphorus(P_{tot}) in the whole growth period fluctuated largely, its content did not significantly reduce. In the spring of 2014, phosphorus content increased, total phosphorus(P_{tot}) was insignificantly different from multiple continuous cropping, and the available phosphorus(P_{av}) content was more reduced than multiple continuous cropping in the spring of 2015.

Table.2: Phosphorus contents of rhizosphere soils

		Before seeding	Flowering	Tuber bulking	Maturity
Total phosphorus (g/kg)	A1	0.572 ± 0.007abc	0.571 ± 0.020abc	0.547 ± 0.003bcd	0.591 ± 0.010a
	B1	0.594 ± 0.009a	0.549 ± 0.058bcd	0.510 ± 0.051de	0.502 ± 0.022e
	C1	0.585 ± 0.007ab	0.542 ± 0.004cde	0.517 ± 0.003de	0.500 ± 0.007e

	A2	0.610 ± 0.010c	0.580 ± 0.010d	0.527 ± 0.006e	0.530 ± 0.0170e
	B2	0.659 ± 0.005a	0.638 ± 0.025ab	0.576 ± 0.009d	0.507 ± 0.004e
	C2	0.630 ± 0.006bc	0.579 ± 0.034d	0.566 ± 0.008d	0.531 ± 0.011e
Available-phosphorus (mg/kg)	A1	18.92 ± 0.26bc	16.80 ± 0.24d	16.77 ± 0.15d	10.03 ± 0.11f
	B1	20.35 ± 0.76a	19.23 ± 0.12b	17.06 ± 0.03d	12.30 ± 0.54e
	C1	20.09 ± 0.11a	18.35 ± 0.42c	17.01 ± 0.43d	12.17 ± 0.18e
	A2	16.86 ± 1.59cd	17.37 ± 0.17cd	24.14 ± 6.15a	21.64 ± 1.19ab
	B2	14.86 ± 0.77d	18.90 ± 0.32bc	21.43 ± 0.86ab	18.58 ± 0.65bc
	C2	18.90 ± 0.61bc	19.22 ± 0.22bc	20.39 ± 0.37ab	21.16 ± 2.79ab

A1, B1, C1 stand for A, B, C in 2014, A2, B2, C2 stand for A, B, C in 2015; In the same year, values within a column followed by different lowercase letters are significantly different at the 0.05 probability level, as determined by Fisher's least significant difference test; n = 3.

3.3Potassium contents of rhizosphere soils

As for total potassium(K_{tot}), in maturity stage, there was minimal difference between each treatment. Total potassium(K_{tot}) increased before it decreased. Eventually, the contents were reduced by 5.39, 1.85, 1.57%, for A1, B1, and C1, respectively, and then increased by 4.00, 4.37, and 0.00% for A2, B2, and C2, respectively. Available potassium(K_{av}) was reduced by 5.54, 23.92, and 24.46% for A1, B1, and C1, respectively, but thereafter increased by 30.55, 126.47, and 21.02% for A2, B2, and C2, respectively. From Table 3, total potassium(K_{tot}) content in rhizosphere soil was relatively stable, but the available potassium(K_{av})content varied greatly.potato continuous cropping in spring and autumn was lower than multiple continuous cropping year by year. Potatoes, as a high potassium crop, required more potassium for self-growth. In present study, potato continuous cropping consumed a small amount of total potassium(K_{tot}), but large amounts of available potassium(K_{av}). This can directly affect the application of fertilizer in production.

Table.3: Potassium contents of rhizosphere soils

		Before seeding	Flowering	Tuber bulking	Maturity
Total potassium (g/kg)	A1	13.55 ± 0.05ab	14.41 ± 1.53a	13.42 ± 0.09b	12.82 ± 0.55b
	B1	13.51 ± 0.04ab	13.52 ± 0.16ab	12.95 ± 0.74b	13.26 ± 0.20b
	C1	13.36 ± 0.22b	13.21 ± 0.04b	12.81 ± 0.23b	13.15 ± 0.14b
	A2	13.00 ± 0.74cd	11.47 ± 0.37e	12.54 ± 0.28d	13.52 ± 0.16bc
	B2	12.60 ± 1.15d	15.05 ± 0.46a	14.25 ± 0.97ab	13.15 ± 0.19cd
	C2	13.06 ± 0.19cd	11.40 ± 0.11e	13.03 ± 0.21cd	13.06 ± 0.05cd
Available-potassium (mg/kg)	A1	99.59 ± 0.95c	131.03 ± 6.13b	75.38 ± 2.94f	94.07 ± 4.78cd
	B1	103.03 ± 7.55c	141.88 ± 14.38a	88.54 ± 3.99de	78.39 ± 2.36ef
	C1	103.77 ± 6.44c	140.95 ± 5.82ab	93.78 ± 2.05cd	80.91 ± 4.21ef
	A2	137.88 ± 14.97c	149.22 ± 9.93c	166.76 ± 6.45b	180.00 ± 5.99a
	B2	50.20 ± 1.56h	122.75 ± 3.77d	104.11 ± 5.44e	113.69 ± 2.89de
	C2	56.10 ± 4.27h	90.95 ± 3.59f	84.95 ± 2.90f	67.89 ± 2.74g

3.4 Organic carbon contents of rhizosphere soils

The annual organic carbon (C_{tot}) content of rhizosphere soils decreased before increasing. In spring of 2014, the organiccarbon (C_{tot})content in maturity stage was reduced by 36.25, 25.26 and 24.84% for A1, B1, and C1, respectively, than before seeding. In 2015, it increased by 17.10, 31.13 and 67.27% for A2, B2, and C2, respectively. This implies that the higher the initial organic carbon (C_{tot}) content of soil, the faster it decreased. Organic carbon (C_{tot}) is the main source of soil nutrients; however,in this experiment, the

organic carbon (C_{tot}) content of potato continuous cropping in spring and autumn was higher, decreased lower, and increased faster than multiple continuous cropping and continuous cropping in autumn.

Table.4: Organic carbon contents of rhizosphere soils

		Before seeding	Flowering	Tuber bulking	Maturity
Organic matter (g/kg)	A1	19.17 ± 1.26bc	19.99 ± 0.97abc	21.65 ± 2.82a	12.22 ± 0.05e
	B1	20.55 ± 1.94abc	20.86 ± 2.30ab	18.42 ± 0.47c	15.36 ± 1.83d
	C1	20.29 ± 0.35abc	20.78 ± 0.57ab	19.24 ± 0.65bc	15.25 ± 0.55d
	A2	15.56 ± 2.40def	15.96 ± 0.96cde	12.10 ± 0.61g	18.22 ± 2.20bc
	B2	14.10 ± 1.66efg	14.50 ± 0.61defg	13.57 ± 2.11efg	18.49 ± 0.83b
	C2	13.44 ± 0.61fg	18.75 ± 1.05b	16.89 ± 0.23bcd	22.48 ± 2.05a

3.5 Enzyme activities of rhizosphere soils

Table 5 showed that urease steadily increased in t 2014, with the maximum level in flowering stage. In maturity stage, it increased by 34.00, 22.37, 21.71%, for A1, B1, and C1, respectively. and it increased by 20.33, 9.24, and 5.83%, for A2, B2, and C2, respectively. Although over both years urease increased, it was lower in 2015 than that in 2014.The rhizosphere soil invertase activity in maturity stage increased by 52.07, 7.65, 11.24%, for A1, B1, and C1, respectively.In spring of 2015, A2, and B2 in maturity stage decreased by 52.92 and 19.39%, respectively, whereas C2 increased by 25.47%. For catalase, B1 and C1 were reduced by 13.68 and 8.62%, whereas A1 increased by 13.86%. In 2015, catalase increased by 9.88, 6.13 and 11.88%, for A2, B2, and C2, respectively. In present study, there was little difference between the treatments in 2014, with only small variations. In maturity stage, A1 increased by 3.67%, and B1 and B2 were reduced by 10.05 and 1.66%, respectively. However, in 2015, polyphenol oxidase activity changed considerably, especially in tuber bulking stage. Eventually, the activity of polyphenol oxidase increased by 27.97, 25.28, 5.24%, for A2, B2, and C2, respectively.

Table.5: Enzyme activities of rhizosphere soils

		Before seeding	Flowering	Tuber bulking	Maturity
Urease (mg/g)	A1	1.50 ± 0.02e	1.52 ± 0.04e	1.77 ± 0.04c	2.01 ± 0.02a
	B1	1.52 ± 0.00e	1.51 ± 0.00e	1.58 ± 0.00d	1.86 ± 0.03b
	C1	1.52 ± 0.01e	1.51 ± 0.00e	1.58 ± 0.00d	1.85 ± 0.00b
	A2	1.23 ± 0.19cde	1.50 ± 0.08a	1.33 ± 0.11bcd	1.48 ± 0.03ab
	B2	1.19 ± 0.04de	1.41 ± 0.11abc	1.23 ± 0.03cde	1.30 ± 0.11bcd
	C2	1.23 ± 0.19de	1.32 ± 0.07bcd	1.10 ± 0.07e	1.30 ± 0.11bcd
Invertase(mg/g)	A1	19.07 ± 0.25ef	18.60 ± 0.36f	27.03 ± 1.10b	29.00 ± 1.11a
	B1	18.70 ± 0.70f	18.57 ± 0.15f	22.93 ± 1.47c	20.13 ± 1.34de
	C1	18.32 ± 0.29f	20.36 ± 1.17d	22.92 ± 0.30c	20.38 ± 0.43c
	A2	31.97 ± 2.24b	15.18 ± 1.25f	20.27 ± 0.90d	15.05 ± 1.26f
	B2	24.09 ± 1.37c	29.43 ± 1.87b	17.68 ± 0.09def	19.42 ± 4.02de
	C2	24.89 ± 2.23c	36.35 ± 1.11a	16.94 ± 0.74ef	30.57 ± 1.84b
Hydrogen peroxidase (mg/g)	A1	1.01 ± 0.01ef	1.09 ± 0.02bcd	0.98 ± 0.02f	1.15 ± 0.03ab
	B1	1.17 ± 0.07a	1.08 ± 0.03cd	1.09 ± 0.04bcd	1.01 ± 0.04ef
	C1	1.16 ± 0.03a	1.08 ± 0.02cd	1.12 ± 0.02abc	1.06 ± 0.04de
	A2	1.62 ± 0.04cd	1.58 ± 0.01de	1.64 ± 0.02c	1.78 ± 0.03ab
	B2	1.63 ± 0.02cd	1.57 ± 0.03e	1.81 ± 0.01a	1.73 ± 0.05b

Polyphenol oxidase (mg/g)	C2	1.60 ± 0.07cde	1.60 ± 0.03cde	1.73 ± 0.02b	1.79 ± 0.01a
	A1	1.91 ± 0.13ab	2.00 ± 0.22a	1.88 ± 0.32ab	1.98 ± 0.22a
	B1	1.89 ± 0.09ab	1.88 ± 0.04ab	1.76 ± 0.04ab	1.70 ± 0.11b
	C1	1.81 ± 0.09ab	1.80 ± 0.15ab	1.82 ± 0.03ab	1.78 ± 0.11ab
	A2	2.36 ± 0.61bcde	2.03 ± 0.48de	3.98 ± 0.67a	3.02 ± 0.45b
	B2	1.78 ± 0.15e	2.24 ± 0.27cde	2.30 ± 0.08cde	2.23 ± 0.15cde
	C2	2.48 ± 0.38bcd	2.80 ± 0.52bc	2.30 ± 0.21cde	2.61 ± 0.27bcd

Table.6: The tuber yield of all three A, B, C variants ($kg \cdot ha^{-1}$)

	A	B	C
2014	--	6985	7019
2015	7190	6215	6343

IV. DISCUSSION

It is generally hypothesized that the soil nutrients decreased annually with continuous cropping (Xiao et al., 2012). But in this research, the available phosphorus(P_{av}),the available potassium(K_{av}) contentand organic carbon (C_{tot}) content were different. This illustrated that the different crops, the difference of continuous cropping ways and seasons, the content of potato root nutrients, and root secretions from the toxic effect and residues, had different effects on soil nutrients. When plant roots and soil are in close contact, a large variability of soil area is formed, and the micro-ecological environment of the potato rhizosphere soil changes. Therefore, under the condition of constant continuous cropping, this inevitably affects plant growth and development.

Soil enzyme activities play an important role in the soil ecosystem, and can reflect the changes in soil quality. Soil enzyme activities are the key to overcome the problems associated with continuous cropping and other agricultural practices that destroy the soil health (Yim et al., 2013). In the present study, compared with potato multiple continuous cropping, the activities of urease, polyphenol oxidase, and invertase were lower in the spring of 2014 but higher in 2015. Catalase activity was lower and then little different. Simultaneously, as for continuous cropping years, urease enzyme activity was reduced, and invertase,catalase, and polyphenol oxidase activities increased. In tuber bulking and maturity stage, rhizosphere soil enzyme activities increased significantly. During the tuber-bulking and maturity stage, the potato root system shows high activity, utilizing excessive rhizosphere soil nutrients, which increases the rhizosphere soil enzyme activity.

Polyphenol oxidase (PPO) is secreted by the plant roots, increasing soil microbial activity and the decomposition of plant and animal residues, releasing compound enzymes (Claus et al. 2010; Yoruk et al., 2003). These are biodegradable soil phenolics that slow down the allelopathy between plants, thereby creating advantageous conditions for plants to expand their habitats. In the present study, rhizosphere soil polyphenol oxidase activity increased with increasing years of planting. The research results are similar to the research on *Caraganakorshinskii* (Cao et al., 2008), where polyphenol oxidase activity of continuous cropping in spring and autumn is higher than that in spring. The soil enzyme activity of sucrase, urease, and catalase has been related to soil fertility, butonly less data is available on the activity of polyphenol oxidase. In the present study, there were significant differences in polyphenol oxidase activity under continuous cropping. Therefore, soil polyphenol oxidase can be considered as an indicator of the negative impact of continuous potato cropping, but requires further verification.

As for the result of the tuber yield, there is a certain relationship between the tuber yield and continuous cropping years, the method of cultivation pattern. But a small difference soil change between multiple continuous cropping(A) and potato continuous cropping in spring (B) andcontinuous cropping in spring and autumn (C) in the short term of continuous cropping for two years, there is a significant difference between them for continuous cropping over three years. Potato continuous cropping consumed more nutrients, especially available nutrients. We think potato continuous cropping for two years is possibly the threshold for the local ecological environmental conditions and cultivation. For potato growing, to meet production requirements and the economic benefits of continuous cropping, the most efficient cultivation period for the process would be two years, and plans to extend it would require a reasonable arrangement to provide a practical basis. Meanwhile, continuous cropping in spring and autumn

increased the negative impact. Multiple continuous cropping reduced the rhizosphere soil nutrient absorption, but negated the negative impacts of continuous cropping.

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Effect of peak performance nutrients on soil chemical properties and nutrient uptake by rice (*oryza sativa* L.)

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Abstract: *The effects of peak performance nutrient (PPN) in combination with different fertilizer doses on soil properties and nutrient uptake by rice were investigated in a field experiment from seedling to maturity during the period of July to November 2014 in T. aman season. The experiment comprised of twelve treatments laid out in a randomized complete block design with three replications. The treatments were: T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD +PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK+PPN, T_7: 75 % P+100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}:75 % NPK+ PPN, T_{12}: 50% NPK+PPN. Experimental results reveal that the highest nutrient uptake (N and K) uptake by rice grain and straw was recorded in the treatment T_7. However, P uptake by rice grain and straw were higher in treatment T_3 that was statistically similar with treatment T_7. Initial and postharvest soil sample analysis indicated that most of the studied soil properties including soil pH and organic matter contents were increased in T_7. Therefore, the treatment combination PPN along with 75 % P+100 % NK (T_7) was found to be more suitable compared to other treatment combinations for improving soil quality as well as enhancing nutrient uptake by rice. Thus peak performance nutrient (PPN) showed a positive response both on improvement of soil health as well as nutrient uptake by the crop.*

Keywords— peak performance nutrients, rice, soil fertility, nutrient uptake.

I. INTRODUCTION

Rice is the main staple food and dominant crop in Bangladesh. It is grown on more than three fourths (81%) of the total cultivable land area and the per capita rice consumption is about 166 kg/year (BBS, 2010). The country is now producing about 25.0 million tons of rice to feed her 160 million people. This increased rice production has been possible largely due to the adoption of modern rice varieties on around 66% of the rice land, which contributes to about 73% of the country's total rice production. However, there is no reason to be complacent while the population of Bangladesh is still growing by two million every year and may increase by another 30 million over the next 20 years. Thus, Bangladesh will require about 27.26 million tons of rice for the year 2020. During this time total rice area will also shrink to 10.28 million hectares because of new settlements, industries and roads. To meet the current and future food requirements of increasing population and their rising dietary needs it is necessary to boost up crop yields adopting best management practices in agriculture (Gao *et al*., 2010). Therefore, yield boosting agronomic techniques such as application of certain plant growth regulators, foliar feeding need due attention.

Peak performance nutrient (PPN) can be a panacea to mitigate this problem because it is an eco-friendly mineral nutrient supplement serving as a liquid concentrate of natural assortment for agricultural crops. PPN contains a plenty of water-soluble nutrients in a balanced way, which is carefully extracted from the natural sources. It stimulates growth, yield, quality, nutrient concentration and taste of agricultural crops (rice, okra, tomato, eggplant, cabbage etc). The pH of PPN is around 9.0; it will progressively elevate the pH after each harvest. Therefore, utilization of PPN in acid soil might show significant effect to increase the soil pH and thereafter enhance the availability of plant nutrients. Sultana *et al*. (2001) revealed that foliar spray of nutrient solution partially minimized the salt-induced nutrient deficiency, increased photosynthesis, dry matter accumulation, number of fertile spikelet in the panicle and

grain yield. Since higher inorganic nutrient levels alone deteriorate soil health, an integration of nutrient solutions from botanical extracts and inorganic nutrients is the best solution for sustaining the soil health as well as enhancing the nutrient uptake by rice grain and straw. Considering the facts as stated above, the present investigation was undertaken to quantify the influence of PPN on soil properties and nutrient uptake by rice in acid soil.

II. MATERIALS AND METHODS

Experimental site

The experiment was performed in the research field of the Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur. The site is located at 24.09 N latitude and 90.26 E longitude with an elevation of 8.4 meters from sea level (Anonymous, 1989). The experimental field belongs to the Salna series under the Madhupur Tract (AEZ - 28). The soils belong to the general soil type Shallow Red Brown Terrace Soil and maintain the soil order Inceptisol under USDA Soil Classification System (FAO, 1988).

Materials Used in the experiments

The recommended high yielding and short duration Aman variety, BRRI dhan49 was used as the test crop. Rice seeds were collected from variety Bangladesh Rice Research Institute (BRRI) Gazipur, Bangladesh. The variety is photosensitive and blast disease resistant with lower affinity to insect and other disease invasion. The seeds germinated in water for 3 days were broadcast uniformly on puddle seedling nursery on 28 June 2014. Twenty-five days old seedlings were transplanted in the experimental plots maintaining a distance of 25 cm from row to row and 20 cm from plant to plant. The plot size of each treatment was 4m × 3m = 12 m^2. Three seedlings were used in each hill. The selected peak performance nutrients (PPN) were collected from China through representative country dealers.

Experimental design and treatment exposure

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The treatments were randomly allotted in each block. The experiment was comprised of twelve treatments including absolute control. The treatments were: T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD +PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK+PPN, T_7:75 % P+100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}:75 % NPK+ PPN, T_{12}: 50% NPK+PPN. The fertilizer treatments used in the experiment were based on soil analysis. In the 100% recommended fertilizer 220 kg ha^{-1} urea, 34 kg ha^{-1} triple super phosphate (TSP), 91.2 kg ha^{-1} MoP and 44 kg ha^{-1} gypsum was applied. The required amount of urea, TSP, MoP and gypsum as source of N, P, K and S respectively were calculated based on the following equation of Bangladesh Agricultural Research Council (FRG, 2012): $F_r = U_f - C_i / C_s \times (S_t - L_s)$

Where

F_r is the fertilizer nutrient required for given soil test values

U_f is the upper limit of the recommended fertilizer nutrient for the respective interpretation (STVI) class

Ci is the units of class intervals used for fertilizer nutrient recommendation

C_s is the units of class intervals used for STVI class

S_t is the soil test value within STVI class

L_s is the lower limit of the soil test value within STVI class

The full dose of TSP, MoP and gypsum were applied at the time of final land preparation. Urea was applied at three different splits at 20 days after transplanting (DAT), 40 DAT at maximum tillering stage and 60 DAT at panicle initiation stage. Peak performance nutrients (PPN) solution was applied both in nursery bed and main plot. Peak performance nutrients (PPN) solution was prepared by diluting 80 ml concentrated PPN in 18 L water per 1000 m^2 plot. Seeds were soaked with PPN solution before broadcasting to the nursery bed. PPN nutrient solution was applied to the main plots before transplanting of the rice seedlings and after seedling establishment at 10 days interval until 80 days of transplanting.

Intercultural operation

Preparation of land was done as per treatment. After the establishment of seedlings, various intercultural operations were accomplished for better growth and development of the rice plants. Proper intercultural operation facilities e.g. gap filling, weeding, irrigation and drainage, application of pesticides were provided whenever necessary.

Harvest

At maturity 6 subsamples (hills) in each were harvested for grain and straw nutrient analysis on 11 November 2014. After threshing Grain and straw samples were air dried and then put in an oven at about 65°C for 48 hours. Then ground in a grinding mill to pass through a 20-meshsieve. The ground plant materials (grain and straw) were stored in small paper bags and placed in desiccators.

Nutrient status evaluation of initial and residual soil Sample

For assessment of initial and residual soil properties, soil samples were collected from 0-15 cm depth before staring and after the experimentation respectively. During soil sampling, composites soil samples were collected from each plot following standard techniques as prescribed in FRG (2012). The collected samples were then air dried and ground to pass through a 2 mm sieve and stored in a clean

plastic container for analysis. The physical and chemical properties of soil sample were analyzed by maintaining standard protocols. The textural class of the initial soil sample was assessing by using the methods of Bouyoucos, (1962). Bulk and particle density of the soil were analyzed by core sampler and pycnometer method respectively (Page *et al*., 1989). The pH of the soil sample was examined according to Jackson (1973). Organic carbon and organic matter was measured by using wet oxidation method (Walkey and Black, 1965). Ten ml of 1 N $K_2Cr_2O_7$ oxidized one gram of soil sample in presence of H_2SO_4 and the amount of organic carbon was calculated by the titrated value against ammonium ferrous sulphate (AFS) solution. Total N content in soil was determined by the Kjeldahl method (Black, 1965). Available phosphorus in the soil samples was extrated with the combination of HCl with ammonium fluoride extract solution (pH below 6.8) and color intensity developed by ascorbic acid was measured by atomic absorption spectrophotometer at 890nm wavelength (Bray and Kurtz, 1945). Exchangeable potassium of the soil sample was measured according to the ammonium acetate solution method (Jackson 1973). Available sulphur of the studied sample was calculated by the calcium dihydrogen phosphate solution extraction method (Thomas, 1982). The studied physical and chemical properties of initial soil sample were representing in the Table 1 & 2.

Table.1: Initial physical soil properties of the experimental site

Soil Characteristics	Analytical Value
Physical Properties	
Sand	17.8%
Silt	45.6%
Clay	36.6%
Textual class	Silty clay loam
Bulk density	1.4 g/cm^3
Particle density	2.6 g/cm^3

Table.2: Initial chemical soil properties of the experimental site

Chemical Properties	
Soil pH	6.2
Total N (%)	0.058
Organic C (%)	0.8
C : N ratio	13.79
Available P (ppm)	10.5
Exchangeable K (meq/100g)	0.14
Exchangeable Ca (meq/100g)	8.75
Exchangeable Mg (meq/100g)	2.46
Exchangeable Na (meq/100g)	0.65
Available Sulphur (ppm)	15
Available Boron (ppm)	0.20
Zinc (ppm)	1.1

Plant samples analysis

Plant samples collected from the field experiment were analyzed for N, P and K contents. Grind grain and straw sample were used for chemical traits analysis. Total nitrogen concentration of grain and straw sample was measured by the Kjeldahl method. For the determination of nitrogen, 0.1 g oven dried ground sample was digested with concentrated H_2SO_4 at 360^{o}C for 2 hrs in presence of catalyst mixture (K_2SO_4: $CuSO_4.5H_2O$: Se =100:10: 1) and titrated against H_2SO_4. Phosphorus and potassium concentration of the grain and straw sample was analyzed by the di-acid mixture (HNO_3: $HClO_4$ = 5: 1) digestion method.

Statistical analysis

Data were analyzed statistically with the help of computer package STATISTIX 10. The mean differences of the treatments were observed by least significant difference (LSD) test at 5% level of probability for the interpretation of results (Gomez and Gomez, 1984).

III. RESULTS AND DISCUSSION

Nutrient uptake by grain and straw as influenced by Peak Performance Nutrient (PPN) and different fertilizer combinations

Nitrogen uptake by grain and straw

Nitrogen uptake by grain and straw varied significantly due to the effect of different fertilizer doses with Peak Performance Nutrient (PPN) treatments (figure 1 and 2). Results displayed in figure- 1 indicated that the N uptake by grain varied from 36.84 to 98.36 kg ha^{-1}. The highest N uptake (98.36 kg ha^{-1}) by grain was recorded in the treatment T_7 (75 % P + 100 % NK + PPN) which was statistically superior to all other treatments. The lowest N uptake (36.84 kg ha^{-1}) by grain was obtained in the treatment T_1 (control) that was statistically different from all other treatments. In straw, the N uptake ranged from 12.23 to 26.20 kg ha^{-1}(Fig1). The highest N uptake (26.20 kg ha^{-1}) by straw was observed in the treatment T_7 (75 % P + 100 % NK + PPN) which was statistically identical with treatment T_3 (100 % NPK + PPN) with the value 0f 25.70 kg ha^{-1}. The lowest N uptake (12.23 kg ha^{-1}) by straw was recorded in the treatment T_1 (control) treatment that was statistically inferior to all other treatments (figure 2). It has been reported that foliar application of nutrients in rice increases the nutrient content in rice grain (Rabin *et al*., 2016) which might enhance the higher nutrient uptake due to PPN application.

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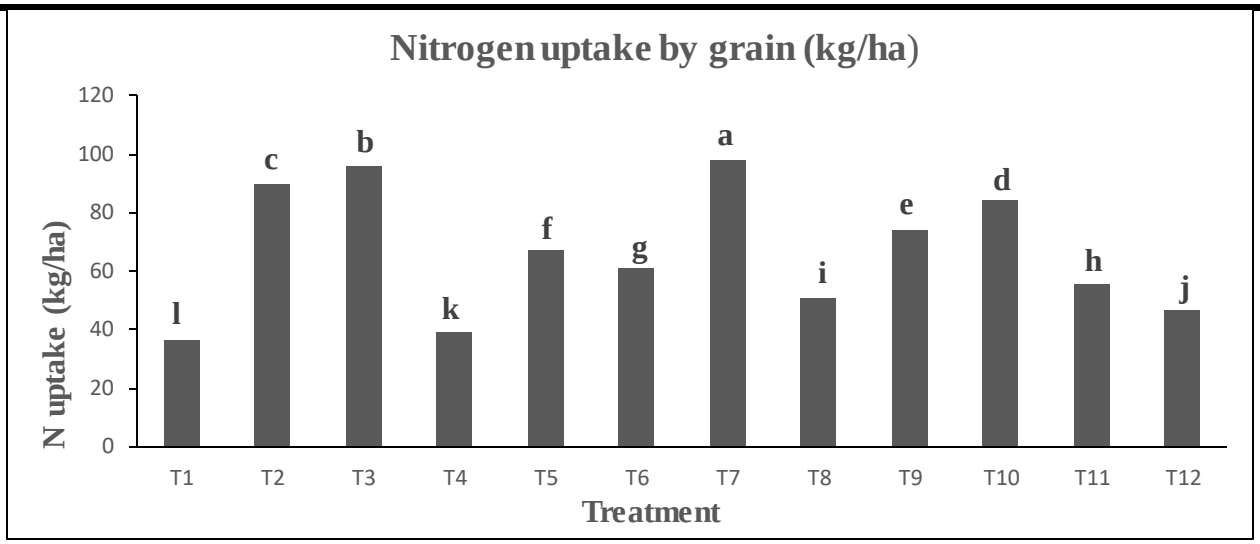

Fig.1: Effects of Peak Performance Nutrient (PPN) and different fertilizer treatments on N uptake by grain

Legends,

T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD + PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK + PPN, T_7: 75 % P + 100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}: 75 % NPK + PPN, T_{12}: 50% NPK + PPN.

Phosphorus uptake by grain and straw

The phosphorus uptake by grain and straw of aman rice (BRRI dhan49) was significantly influenced by different fertilizer combinations along with Peak Performance Nutrient (PPN) solution (figure 3 and 4). Phosphorus uptake by rice grain varied from 4.638 to 12.44 kg ha^{-1} (figure 3). The maximum P uptake (12.440 kg ha^{-1}) by grain was recorded in the treatment T_3 (100 % NPK + PPN) and it was statistically identical with treatment T_7 (75 % P + 100 % NK + PPN) with a value 12.290 kg ha^{-1}. On the other hand, the minimum P uptake (4.638 kg ha^{-1}) by grain was observed in the treatment T_1 (control) but it was statistically similar with the treatment T_4 (0 % RD +PPN only) having the value of 4.863 kg ha^{-1}. In case of rice straw, the P uptake ranged from 3.633 to 8.430 kg ha^{-1} (Fig 4). The highest P uptake by straw (8.430kg ha^{-1}) was documented in the treatment T_3 (100 % NPK + PPN) and it was statistically identical with

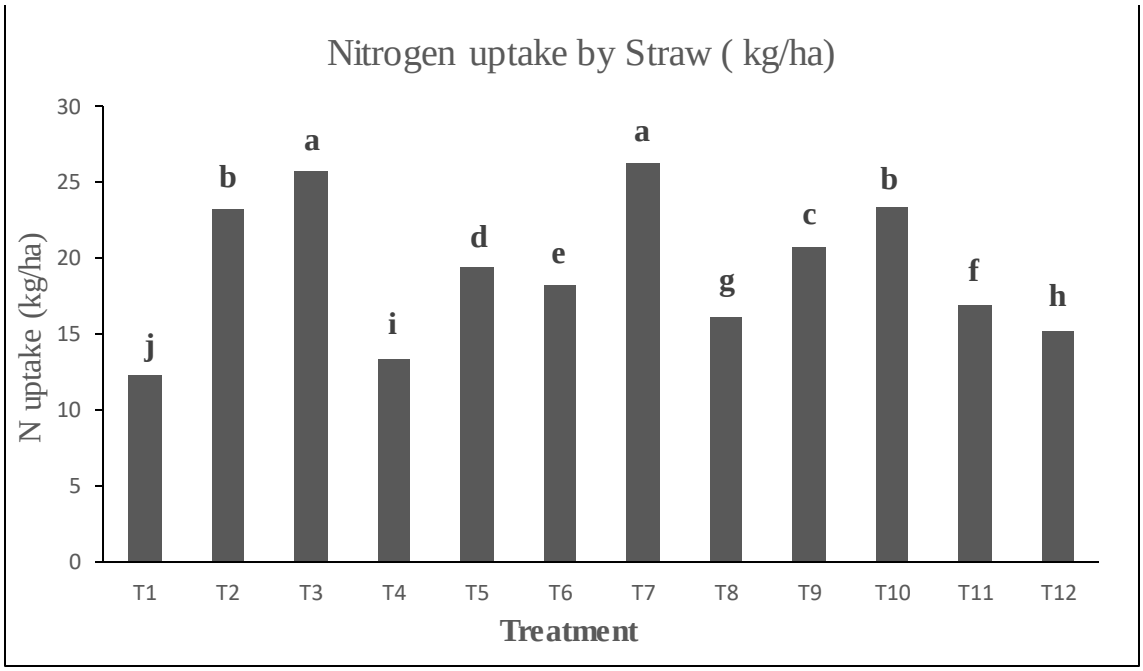

Fig.2: Effects of Peak Performance Nutrient (PPN) and different fertilizer treatments on N uptake by straw

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Legends,

T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD + PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK + PPN, T_7: 75 % P + 100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}: 75 % NPK + PPN, T_{12}: 50% NPK + PPN.

Treatment T_7 (75 % P + 100 % NK + PPN) with a value 8.350 kg ha^{-1}. The lowest P uptake (3.633 kg ha^{-1}) was found in the T_1(control) treatment, which was statistically similar to the treatmentT_4 (0 % RD +PPN only) with the P uptake of 3.666 kg ha^{-1} (figure 4). Experimental results indicated that P uptake by grain and straw respond differently by different treatment combinations. Due to the application of PPN in soil, the pH value in acid soil increased which ultimately might enhance more P uptake by the plants.

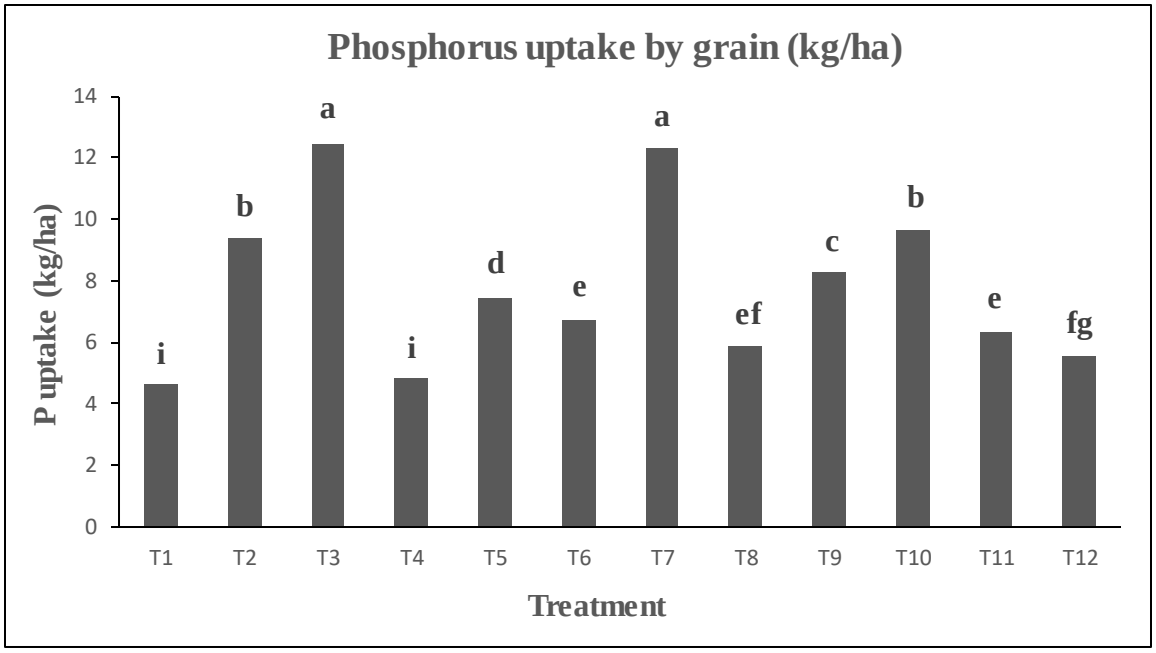

Fig.3: Effects of Peak Performance Nutrient (PPN) and different Fertilizer treatments on P uptake by grain

Legends,

T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD + PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK + PPN, T_7: 75 % P + 100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}: 75 % NPK + PPN, T_{12}: 50% NPK + PPN.

Potassium Uptake by grain and straw

Potassium uptake by BRRI dhan49 in both grain and straw was significantly influenced by various treatments of different fertilizer doses with Peak Performance Nutrient (PPN) treatments in this experiment (figure 5 and 6)). From the figure 5, it appears that the K uptake by grain varied from 14.540 to 32.443 kg ha^{-1}. The highest K uptake (32.443 kg ha^{-1}) by grain was noted in the treatment T_7 (75 % P + 100 % NK + PPN) that was superior from all other treatments. The lowest uptake value of K (14.540 kg ha^{-1}) by grain was obtained in the treatment T_1 (control) (figure 5). In straw, uptake values of K ranged from 24.327 to 52.380 kg ha^{-1}(figure 6). The highest K uptake value of 52.380 kg $ha^{-1}$was observed in the treatment T_7 (75 % P + 100 % NK + PPN) that was statistically superior from all other treatments. The lowest K uptake (24.327 kg ha^{-1}) by straw was obtained in the treatment T_1 (control).

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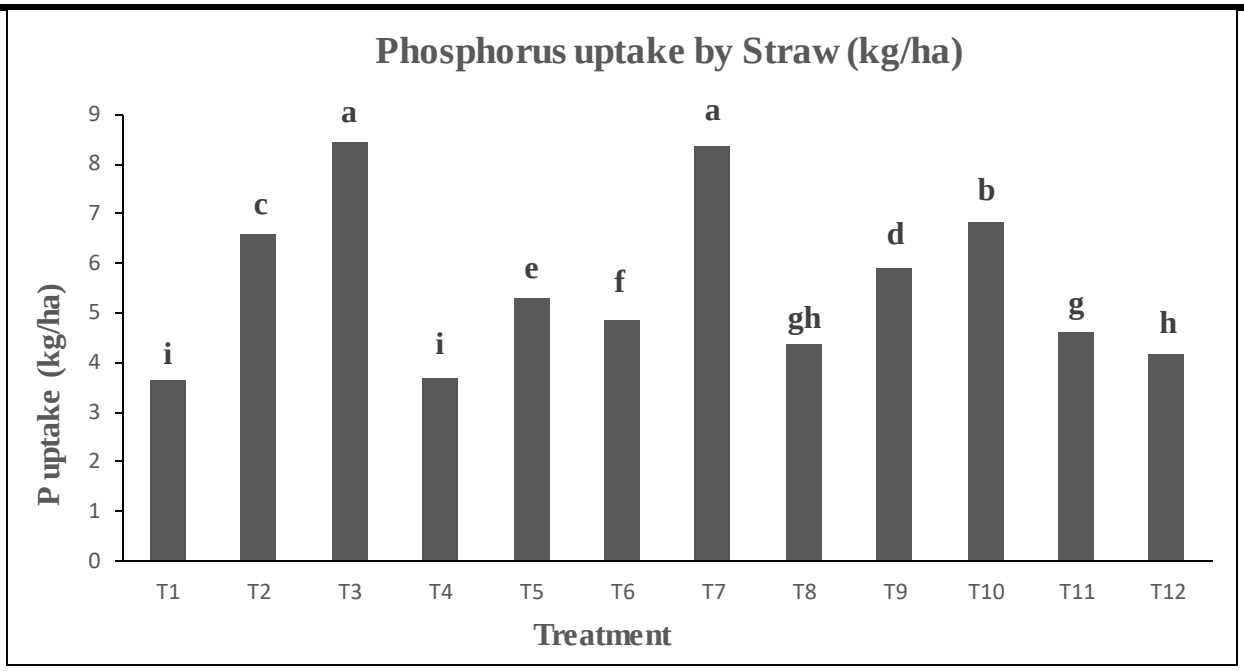

Fig.4: Effects of Peak Performance Nutrient (PPN) and different Fertilizer treatments on P uptake by straw

Legends,

T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD + PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK + PPN, T_7: 75 % P + 100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}: 75 % NPK + PPN, T_{12}: 50% NPK + PPN.

These results revealed that the K uptake by rice straw was much higher than that of K uptake by rice grain. It indicates that treatment T_7 (75 % P + 100 % NK + PPN) had pronounced effect on K uptake in both grain and straw. The results were in agreement with the findings of Sachdev *et al*. (1991).

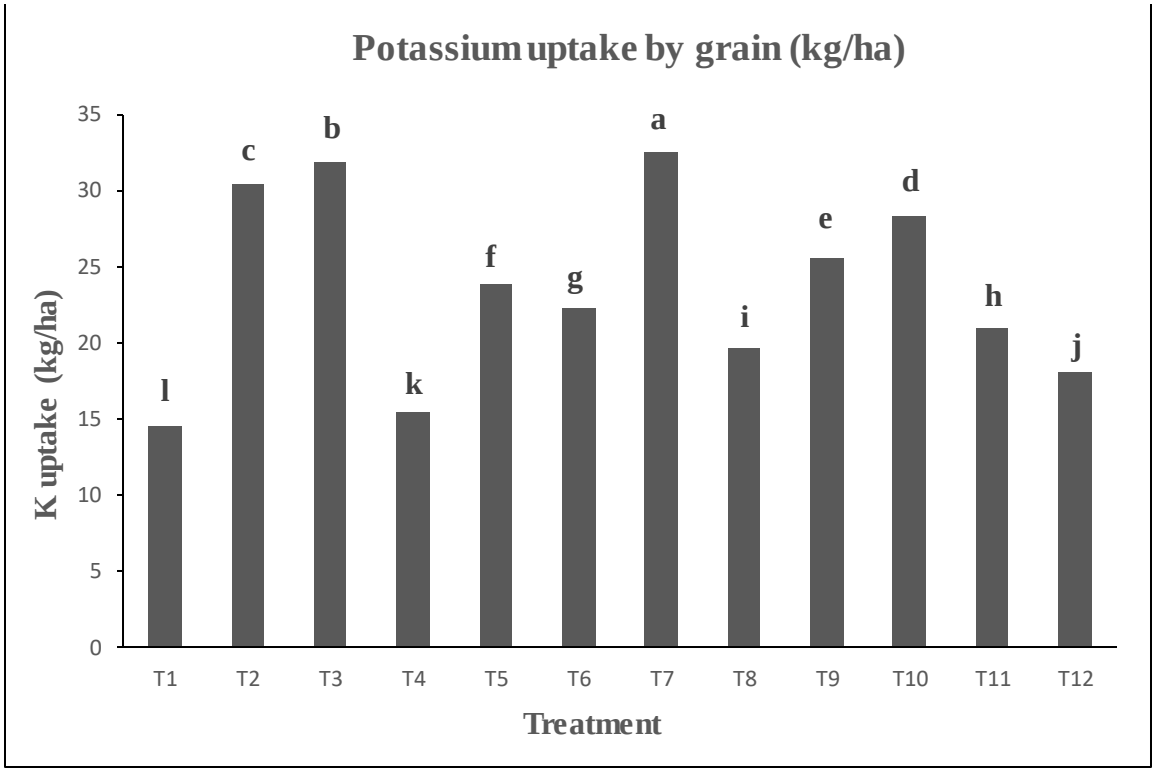

Fig.5: Effects of Peak Performance Nutrient (PPN) and different Fertilizer treatments on K uptake by grain

Legends,
T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD + PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK + PPN, T_7: 75 % P + 100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}: 75 % NPK + PPN, T_{12}: 50% NPK + PPN.

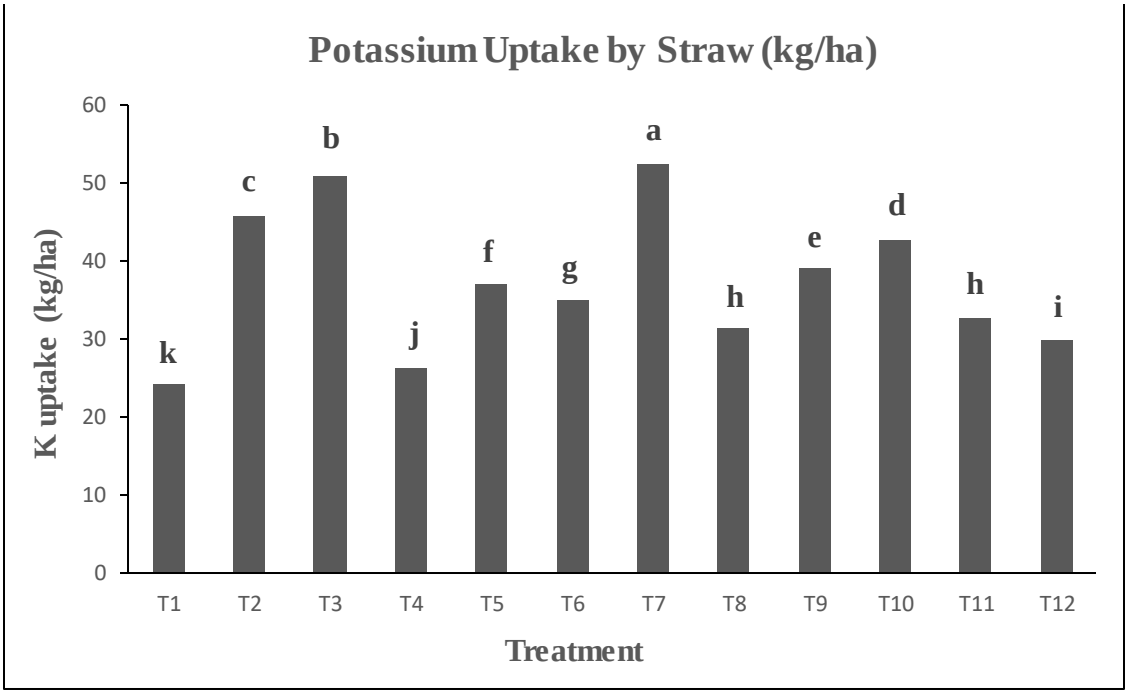

Fig.6: Effects of Peak Performance Nutrient (PPN) and different Fertilizer treatments on K uptake by straw

Legends,
T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD + PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK + PPN, T_7: 75 % P + 100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}: 75 % NPK + PPN, T_{12}: 50% NPK + PPN.

Effect of Peak Performance Nutrient (PPN) and chemical fertilizers on the nutrient status of Postharvest soil

Soil pH

There was a significant effect of different fertilizer doses with Peak Performance Nutrient (PPN) treatments on the pH of soil after harvest of aman rice (BRRI dhan49) (Table-3). Among the different treatments, highest pH (6.5) was found in T_7 (75 % P+100 % NK + PPN), which was statistically identical with the treatment T_3 (100 % NPK + PPN) with a value of 6.47 and superior from all other treatments. The lowest pH (6.04) was found in T_1 (control) treatment. Soil pH value was increased in the treatments that received the PPN solution. This result is very likely as the pH value of the PPN solution was high (more than 8.0). Therefore, PPN solution might be very effective to raise the pH of the acidic soil.

Organic carbon in soil

A significant variation ($p<0.05$) was recorded in the organic carbon content of the postharvest soil samples where the Peak Performance Nutrient (PPN) along with different fertilizer doses were incorporated in soil (Table- 3). Among the different fertilizer doses with Peak Performance Nutrient (PPN), T_7 (75 % P+100 % NK + PPN) treatment showed the highest organic carbon content (1.12 %) after the harvest of rice which was statistically similar with the treatmentT_3 (100 % NPK + PPN) with a value of 1.1 %. On the other hand, the lowest organic carbon content (0.852%) was observed in treatmentT_1 (control). Similar results were obtained by kamal *et al.*, (2002) who explained that soil organic carbon increased when organic fertilizers were supplied instead of NPK fertilizers.

Total nitrogen content in soil

Significant ($p<0.05$) variation was recorded in the nitrogen content of the postharvest soil samples as influenced by different treatment combinations (Table- 3). The highest total nitrogen (0.158%) was found in T_7 (75 % P+100 % NK

+ PPN), which was statistically superior from all other treatments. The lowest (0.085%) was found in treatment T_1 (control). This result might be attributed due to no use of fertilizers and nutrient solution in the control treated plots.

Table.3: Chemical properties of post-harvest soil as influenced by Peak Performance Nutrient (PPN)

Treatments	Nutrient content in soil				pH	% OC
	% N	P (ppm)	K meq/100g soil	S (ppm)		
T_1	0.085j	13.347e	0.132k	10.733i	6.040j	0.852i
T_2	0.142c	15.577cd	0.253b	17.567b	6.100i	1.060b
T_3	0.154b	18.453a	0.272a	19.800a	6.473a	1.100a
T_4	0.093i	15.007d	0.153j	12.067h	6.186h	0.882h
T_5	0.112f	15.973bc	0.228d	16.200cd	6.300de	0.980e
T_6	0.103h	16.250bc	0.220e	15.527de	6.266f	0.960e
T_7	0.158a	18.000a	0.276a	20.350a	6.500a	1.120a
T_8	0.118e	15.693bcd	0.207f	14.733f	6.286ef	0.920fg
T_9	0.123d	15.653bcd	0.234c	16.633c	6.373c	1.005d
T_{10}	0.138c	16.567b	0.200g	17.400b	6.426b	1.030c
T_{11}	0.108g	16.010bc	0.185h	15.087ef	6.320d	0.932f
T_{12}	0.096i	15.493cd	0.168i	13.867g	6.220g	0.910g
% CV	1.94	3.49	1.45	2.62	0.30	1.26
$LSD_{0.05}$	.003	0.94	.005	0.70	0.03	0.02

In a column figures having similar letter (s) do not differ significantly whereas figures with dissimilar letter (s) differ significantly as per LSD at 5% level of significant.

Legends,

CV= Co-efficient of Variation

T_1: Control (no fertilizer and no PPN), T_2: 100 % recommended dose of NPK, T_3: 100 % NPK + PPN, T_4:0 % RD + PPN only, T_5: 75 % N + 100 % PK+PPN, T_6: 50 % N + 100 % PK + PPN, T_7: 75 % P + 100 % NK + PPN, T_8: 50 %P + 100 % NK + PPN, T_9: 75 % K+ 100 % NP + PPN, T_{10}: 50 % K + 100 % NP + PPN, T_{11}: 75 % NPK + PPN, T_{12}: 50% NPK + PPN.

Available phosphorous in soil

Phosphorous content of postharvest soil showed significant ($p<0.05$) variation in the available phosphorus content of the postharvest soil samples (Table-3). Combined effect of different fertilizer doses and Peak Performance Nutrient (PPN) recorded significant variation in available phosphorous content of postharvest soil. Among the different treatments, the highest available phosphorous (18.453 ppm) was found inT_3 (100 % NPK + PPN) treatment which was statistically similar with the treatmentT_7 (75 % P+100 % NK + PPN) with a value of 18.0 ppm. The lowest (13.347 ppm) was found in T_1(control) treatment. Guan, (1989) reported that the application of organic fertilizers increased the availability of phosphorous in soil. As the PPN increased the pH of the acidic soil, therefore it increases the availability of phosphorus in soil.

Exchangeable Potassium content in soil

Significant ($p<0.05$) variation was recorded in exchangeable K content in soil after harvest of the rice in the (Table-3). The highest K (0.276 meq/100g soil) found in treatmentT_7 (75 % P+100 % NK + PPN) treatment, which was statistically similar with the treatmentT_3 (100 % NPK + PPN) with a value of 0.272 (meq/100g soil). On the other hand, the lowest K (0.132 meq/100g soil) was found in T_1 (control).

Available Sulphur in soil

Available sulphur content in the postharvest soil samples was affected significantly ($p<0.05$) variation due to the application of different fertilizer doses and Peak Performance Nutrient (PPN), which is shown in the Table-3. The highest sulphur (20.35 ppm) was found in treatment T_7 (75 % P+100 % NK + PPN) treatment that was statistically similar with the treatmentT_3 (100 % NPK + PPN) with a value of 19.8 ppm. The lowest available sulphur (10.733 ppm) was found in the control treatment.

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Experimental results revealed that nutrient solution had significant influence on the available sulphur content of the postharvest soil samples.

IV. CONCLUSIONS

Based on the findings of the present study on the response of Peak Performance Nutrient (PPN) and different fertilizer doses on post-harvest soil properties and nutrient uptake by aman rice, it might be concluded that the treatment T_7 (75 % P + 100 % NK +PPN) showed better performance in acid soil. Moreover, the findings reveal that combined application of Peak Performance Nutrient (PPN) and chemical fertilizer could reduce the application of 25% P from the recommended dose.

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Comparative Quality Evaluation of Oven-Roasted and Honey-Coated Cashew (*Anarcadium occidentale, L.*) Nut produced using Locally Fabricated Cashew Nut Processing Machine in Nigeria

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***Abstract*—** *Raw cashew nuts were processed to obtain cashew kernels. Part of the kernels was roasted using mud oven while another part was honey coated and dried. The two samples were subjected to chemical, microbial and sensory analysis to compare their quality attributes. Differences were observed in some chemical compositions of the two samples; moisture content value of the oven roasted kernel was significantly ($p<0.05$) higher than that of honey-coated kernel but generally below the maximum level of 8% for safe storage of kernels. Protein, crude fibre, ash and energy value of the honey coated sample were significantly ($p<0.05$) higher than that of oven roasted sample, while there were no significant ($p>0.05$) differences in the crude fat and fatty acids. Sodium, potassium, calcium and magnesium were significantly higher ($p<0.05$) in the oven roasted than honey coated cashew samples. However, phosphorous in honey coated kernel was significantly ($p<0.05$) higher than that of oven roasted kernel. Total viable count of the samples are within the standard limits of 10^4 to less than 10^6 cfu/g of ready-to-eat food products, Yeast and mould counts of the two samples are within the maximum standard of 10^4 sfu/g. From the sensory evaluation results, honey coated kernel was more acceptable than oven roasted kernel. This study revealed that there was significant difference in the chemical and sensory attributes of the oven roasted and honey coated cashew kernel and that, coating of cashew kernel with honey has an added nutritional advantage compared with oven roasted ready-to-eat cashew kernel.*

***Keywords*—Cashew kernel, Honey coated, Microbial, Oven roasted, raw cashew nut.**

I. INTRODUCTION

Adequate nutrition has been generally accepted as being central to good and healthy living [35]. In recent times, people have been increasingly aware of the quality, nutritional composition and health-promoting components of foods, proper processing and value addition of such food is necessary in other to obtain or retain maximum nutritional benefits from it [52; 47]. Nuts played an important role in diets of many cultures and civilizations for centuries due to its high energy and nutritional value as well as its huge variety of flavors and unique taste. It has also been recognized as a class of food that are rich in important nutrients including proteins and unsaturated fatty acids [45; 10]. As a good source of chemical constituents and certain vital bioactive compounds of important health benefits in human beings. Consumption of tree nuts had been linked with several health benefits during the last years [53; 30; 43]. Among tree nuts, cashew nuts ranks third in worldwide production, Vietnam was the world's largest individual producer in 2013 with 1.1 million tons while Nigeria was the largest African exporter with 0.95 million tons and second in the world [6]. However, as of 2014, rapid growth of cashew cultivation in Côte d'Ivoire made this country the top African producer with109, 583 MT [48].

Cashew is held with great esteem in many customs and cultures, it is often eaten roasted on its own, lightly salted or sugared, or covered in chocolate [6].Globally, cashew kernel (among other nuts) is an esteemed and highly priced food delicacy valued because of its pleasant taste and flavor [24]. Scientific investigations have shown that the nut kernels have beneficial effects on health, particularly on chronic diseases such as hypertension and obesity, coronary heart

disease and diabetes due to their and high content of unsaturated fatty acids[33;29].

Roasting is a cooking method that uses dry heat, whether an open flame, roaster or other heat source. Roasting can enhance flavor\ through caramelization on the surface of the food. Roasting uses indirect diffused heat and is suitable for slower cooking of nuts in a larger, whole piece [9]. Temperature range of 185^0C to 190^0C is ideal for roasting since it is sufficiently hot to exceed the decarboxylation temperature [16]. Roasting from time in memorial remains one of the common processing methods for nuts. During the process, the nuts become more crunchy and brittle leading to an overall increase in palatability [37; 44; 2].

Honey is a natural sweet substance produced by honey bees, from the nectars of plant flowers and honey dew [12]. Honey is a supersaturated solution of sugars, of which fructose (38%) and glucose (31%) are the main contributors. Honey has a wide range of phytochemicals including polyphenols which act as antioxidants. A wide range of minor constituents is also present in honey, many of which are also known to have antioxidant properties. Bee honey is one of the few virtually totally non-allergic foods that body easily assimilates. It contains nutrients especially as energy provider [42]. It is a high-energy carbohydrate food (80–85%) and the honey sugars are easily digestible as those in many fruits [51]. In an effort to combat free-radical activity, scientists are studying the effects of increasing individuals' antioxidant levels through the diet and dietary supplements. Honey appears to act as an antioxidant in more ways than one. Antioxidant compounds found in honey includes glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins [8; 38]. In the body, honey can take up free radicals and contribute to better health and when used in foods, the compounds produced when honey is heated can prevent rancidity in some products [7]. Honey is primarily a high-energy carbohydrate food whose distinct flavors cannot be found elsewhere, thus, it is an enjoyable treat. The honey sugars are largely the easily digestible “simple sugars,” similar to those in many fruits. Honey can be regarded as a good food for both infants and adults [13]. Due to honey's pleasing taste, it may be more readily consumed by individuals reluctant to ingest plant derived anti-oxidants. Honey which is also sweetener can be a flavorful, supplementary source of antioxidants [25]) compared to sucrose that has no anti-oxidant value. This has prompted the coating of cashew kernel with honey as a tool for food value addition for increased utilization, improved economic gain and enhanced nutritional benefits of the nuts.

Generally, food processing and preparation methods have been reported to have a direct effect on the quality characteristics (physical, chemical and sensory) of most foods [47; 26]. Due to the nutritional requirement and necessity of the population, there is need to critically asses both the mud- oven roasted cashew and coating of the nuts with honey after drying as a means of nutritional enhancement. Therefore, this study is aimed at comparing the effects of mud-oven roasting and honey coating on the chemical, sensory and microbiological qualities of the cashew kernels.

II. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The raw cashew nuts samples used for this work were obtained from Cocoa Research Institute of Nigeria's cashew plantation at Ochaja sub-station in Kogi State, Nigeria. Some preliminary quality tests (floatation, cutting test, total useful kernels and kernel out- put ratio) were done to ascertain the wholesomeness of the raw cashew nut samples.

2.2 Methods

2.2.1 Cashew nut processing, Roasting and Coating process

Cashew nuts were processed using the modified method of [26] with the use of locally fabricated set of Cashew nut processing machine, consisting of Steamer, Hand operated shelling machine, Mud oven and vacuum packaging machine, designed and fabricated by an indigenous company (Annacardium Engineers Ltd, Saki, Oyo State, Nigeria).

Raw cashew nuts were steam-boiled using a steam boiler at a pressure of 4.5kg/cm for 20 mins. The steamed nuts, after cooling for 24 hrs, were shelled using hand operated shelling machine. The kernel was then removed from the shell using a small metal knife. The kernels were then pre-dried in a mud Oven at temperature of 50 - 70°C for 4 hrs to allow for the easy removal of the peels from the Kernel using the small metal knife. The peeled kernels were then further dried in the mud Oven for 5 hrs at 50 - 70°C.Part of the dried nuts were oven-roasted using the mud oven at 135 to 145°C for about 20 - 30mins, until the kernel is fully brown, while the other dried kernels were coated with honey using dip method. The two samples were then packaged in plastic bottles, air-tight sealed and stored at ambient condition [28 ± 3°Cand 78 ± 2% (relative humidity)

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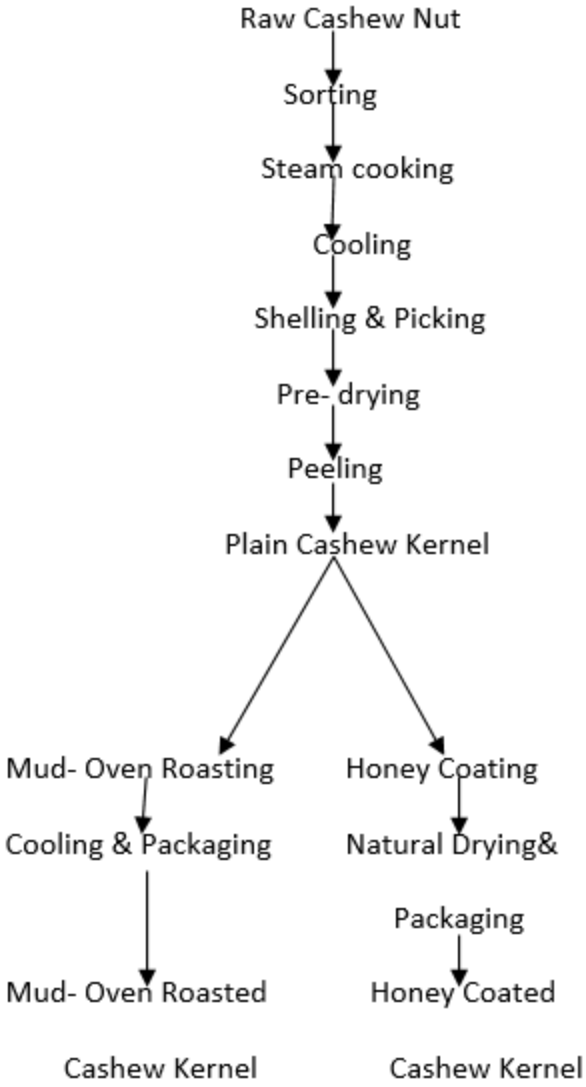

Fig. 1: flow chart for the production of mud oven roasted and honey coated cashew kernels

2.3 Chemical Analysis

2.3.1 Proximate analysis: Proximate compositions (moisture, protein, fat, ash and total carbohydrates) of the samples were determined using the methods recommended by the [1]. A. The nitrogen content was determined by the micro Kjeldahl method which was subsequently converted to protein by multiplying by a factor of 6.25 while the Carbohydrate content was determined by difference.

Fatty acid and metabolizable energy (KJ/100 g) were calculated using the formula described by [5]) as shown below:

Calculated fatty acids = (0.86×crude fat)

Calculated metabolizable energy (KJ/100 g) = ((Protein×17) + (Fat×37) + (Carbohydrate×17))

2.3.2 Mineral analysis: The minerals were analyzed by dry ashing the samples at 550°C to constant weight and dissolving the ash in volumetric flask using distilled, de-ionized water with a few drops of concentrated hydrochloric acid. Calcium, magnesium, iron and phosphorous were determined by Atomic Absorption Spectro-photometry [3], while sodium and potassium were determined using flame photometry [11] using NaCl and KCl to prepare the standards.

2.3.3 Microbiological enumeration**:** Both oven roasted and honey coated cashew kernels samples were subjected to total bacteria count, yeast and mould count and coliform count tests as described by [20].

2.4 Sensory analysis: The two cashew kernel samples were subjected to sensory evaluation to determine consumer preference among them. Parameters such as color, taste, crispiness, flavor and overall acceptability were determined using a 9-point hedonic scale where (9) represented 'liked extremely' and (1) representing 'disliked extremely' [28]. The samples were presented in coded identical plates and the samples were tested individually. The order of presentation of the two samples

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was completely randomized and water was provided to enable the panelists rinse their mouth after tasting each sample in order for each samples attributes to be noticeably distinct before inference could be drawn.

2.5 Statistical analysis: The data were analyzed using SPSS (Statistical Package for Social Scientist) version 15.0. The mean of the analyses were calculated and Analysis of Variance (ANOVA) was performed to determine the level of significant differences (5% significance level) between different mean of cashew kernel samples using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Table 1 show the results of the chemical composition of the Oven roasted cashew (ORC) and Honey coated cashew (HCC) kernels. The ranges of the chemical compositions are moisture content (%)7.58 to 6.04, protein (%) 18.25 to 17.37, fat (%) 43.66 to43.28, crude fibre (%)1.38 to 2.27, carbohydrate (%) 26.92 to 28.03, fatty acids(%) 37.55 to 37.22 and metabolizable energy(KJ/100g) 2157.24 to 2148.94.These results of the proximate composition obtained from the two cashew kernels samples shows comparison to those reported by [27] but slight differences were observed from that of [32] who has slightly higher values of protein and fat.

Table.1: Chemical composition (%) of plain roasted and honey coated cashew kernel samples

Parameter	HCC (%)	ORC (%)
Protein	18.25[a]	17.37[b]
Fat	43.66[a]	43.28[a]
Crude Fibre	2.27[a]	1.38[b]
Total Ash	2.53[a]	1.38[b]
Moisture Content	6.04[b]	7.58[a]
Dry Matter	93.96[a]	92.42[b]
N.F.E	26.92[b]	28.03[a]
F.Acids	35.5[a]	37.22[a]
Energy Value (KJ/100g)	2157.2[a]	2148.94[b]

[a,b,] mean values in a column having different superscripts are significantly different (p<0, 05)
Note: HCC- honey coated cashew, ORC- oven roasted cashew

Generally, the proximate composition of the two cashew nut kernels samples obtained in this study was comparable to those reported by [49]. However, some significant differences were observed in some chemical compositions of the two cashew kernel samples. The moisture content value of the Oven roasted kernel was significantly ($p<0.05$) higher than that of honey-coated kernel. However, the moisture content of the two cashew nut kernel samples were generally below the maximum moisture content level of 8% for safe storage of kernels as stated by both [40] and [22]. This is important especially for honey coated cashew kernel, since moisture content of bee honey represents a major importance to its stability against fermentation and granulation, the low moisture content protects honey from microbiological activity and thus it can be preserved for longer periods [4]and [15].

The protein content of the Honey coated cashew kernel (18.25%) was significantly ($p<0.05$) higher than that of Oven roasted cashew kernels (17.37%).These results are within the range stated by[37] as the protein content range of quality cashew nut. According to [42], the honey contains little protein, indicating that honey is not a good source of protein. However, the higher protein

content of the honey coated kernel sample compared to oven roasted kernel may be as a result of the coating of the sample with honey which is a way of protecting and enhancing the nutritional value of cashew nut.

Fat is an essential nutrient with a number of important functions. It carries fat-soluble vitamins and supplies essential fatty acids as well as contributes to overall energy value of foods [46] without adding to the bulk of the diet. They also play a structural role in providing fatty acids and cholesterol for the formation of cell membranes in all organs and much of fat necessary for the formation of these tissues are essential fatty acids. The total amount of fat in the diet and the amount of the different fatty acids in the diet can influence health. There was no significant ($p>0.05$) difference in the fat contents of the Oven roasted and Honey coated cashew kernels. This result is in agreement with that of [27]. This result implies that coating of cashew nut with honey does not have any significant effect on the fat content of the cashew. The fatty acid contents of the two samples also followed the same trend as that of crude fat content. This is expected since the fatty acids are the composition of crude fat.

The crude fibre is a measure of the quantity of indigestible sugars like cellulose, pentosans, lignins e.t.c that are present in foods and it helps to improve gastrointestinal function through maintenance of peristaltic movement of the intestinal tract thus, preventing constipation.[14]. Comparing the results of crude fibre of the two samples studied, the crude fibre of the honey coated sample was significantly ($p<0.05$) higher than that of oven roasted sample as shown in Table 1. The result of this finding does not agree with that of a similar study reported by [5]. However, this result indicates that coating of the dried cashew nut has affected the fibre content of the nut, thus, improving its nutritional value.

The results of ash content of honey coated sample, as shown in Table 1, was significantly ($p<0.05$) higher than that of oven roasted sample. This suggests that small amount of ash found in honey as reported by [49] had a positive effect on the ash content of the honey coated cashew samples.

The gross energy in a food is defined as total chemical energy measured from complete combustion of the food in a bomb calorimeter. The gross energy value of Honey coated kernel was significantly ($p<0.05$) higher than that of oven roasted sample as shown in Table 1. This result has shown conformity with the study of [51], who found that honey, is also a high-energy carbohydrate food (80–85%) with the honey sugars being easily digestible as those in many fruits. This has made it a good source of energy as stated by [49]. This served as an improved nutritional value for cashew nuts whereby the energy level has been boosted by the coating of plain cashew with honey.

Table.2: Mineral Analysis (mg/100g) of Oven roasted and honey coated cashew kernel samples

Parameter	HCC	ORC
Sodium	770.0	880.0
Potassium	710.0	780.0
Phosphorus	207.0	147.0
Iron	19.70	20.80
Calcium	3780.0	4470.0
Magnesium	530.0	650.0

[a,b,] mean values in a column having different superscripts are significantly different ($p<0, 05$)

Results of mineral analysis as shown in Table 2 indicated that minerals like; sodium, potassium calcium and magnesium are significantly higher ($p<0.05$) in the oven roasted than Honey coated cashew kernel. However, Phosphorous content of Honey coated kernel was significantly ($p<0.05$) higher than that of oven roasted kernel. This result is similar to the findings of [51].

Table.3: Populations of microorganism isolated from Honey coated and Plain roasted cashew nuts samples

S/N	Isolated microbes	Samples/Populations	
		HCC	ORC
1	Yeasts and Mould count	$6.7x10^4$sfu/g	$5.37x10^4$sfu/g
2	Total bacterial count	$1.89x10^5$cfu/g	$6.43x10^4$cfu/g
3	*E. coli*	0.00cfu/g	0.00cfu/g

HCC- Honey Coated Cashew, PRC- Oven Roasted Cashew

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Nuts and nut products in general are highly susceptible to microbial contamination primarily due to their high nutritional content as well as their pH which is conducive for microbial growth and activities [17]. Contamination of cashew nuts by moulds may also occur early in the field, and deterioration could develop during prolonged storage [19]. According to [40] most stored agricultural products, including Cashew nuts are hygroscopic and will absorb moisture from the surrounding atmosphere until they reach equilibrium, and thus the storage environment is an important concern in preserving this food. This coupled with the high ambient temperature and relative humidity in the absence of proper processing might lead to problem of microbial growth in processing nut and nut products [31].

Mesophilic bactera counts on roasted and salted Brazillian nuts ranged from 5.3 x 10^3 and 1.2 x 10^4 cfu//g [18]. These values do not differ widely with the findings in this study as shown in Table 3, considering the fact that the counts of total bacteria for HCC was1.89x10^5cfu/g while that of ORC was6.43x10^4 cfu/g.

Yeasts and mould count of 6.7x10^4sfu/g was recorded for HCC while 5.37x10^4sfu/g was obtained for ORC, these values are in consonance with the results of a similar study conducted by [34], but defers from the findings of [27] who reported lower counts of yeasts and mould of 7.2×10^2 and 6.8×10^2. However, it was observed that yeast and mould count of the two samples were within the maximum standard of 10 ^{4}/g set by [23] as acceptable for packaged nuts fit for human consumption. The result of the total viable count of the cashew kernel samples are within the microbial limits/standard of 10^4 to less than 10^6 cfu/g of ready-to-eat food products as reported by Fylde Borough Council in the manual of [39].

Table.4:- Mean Sensory Evaluation Scores of the Samples

Samples	Attributes				
	Colour	Taste	Flavour	Crispiness	General Acceptability
HCC	6.75b	7.10a	6.40b	6.55a	7.30a
ORC	7.15a	6.95b	6.65b	6.45b	6.65b

[a,b],mean values in a column having different superscripts are significantly different (p<0, 05)
HCC= Honey Coated Cashew, ORC=Oven Roasted Cashew.

The Sensory evaluation results obtained in Table 4 showed that there were significance difference in the colour, Taste, crispness and overall acceptability of the oven roasted and Honey coated Cashew kernels, while there was no significant difference (p>0.05) in flavor of the two samples. However, HCC was significantly (p<0.05) more acceptable than ORC.

III. CONCLUSSION

This study revealed that, there is significant difference in the chemical and sensory characteristics of oven roasted and honey coated cashew kernels. Also, honey coated cashew kernel has added nutritional advantage as ready-to-eat kernel, and was significantly more acceptable than oven roasted cashew by the sensory panelists.

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Inventorization of Abandoned Mines and quarry Pits in Taraba State, Nigeria

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***Abstract*—** *Although there have been so many studies on mining activities and their environmental impacts over the years, it is only recently that the issues of mine closure and rehabilitation have been included in mining discourse as contained in the Minerals and Mining Act 2007. Abandoned mine pits and their associated mine features constitute significant risk and environmental hazards to local communities and the natural ecosystem. This study undertook inventorization of abandoned mine sites in the state and classify the extent to which they negatively impact on the environment. This will facilitate screening of the abandoned mine sites in the state for reclamation and rehabilitation. Field identification of sites and assessment of mine features was carried out. Field assessment include measurement of mine pits dimension, quantity, volume, surface area, distance using GPS and description of the types of materials or products removed from the site. Physical hazard assessment approach was adopted. A hazard ranking system, or prioritization index (PI), was used to prioritize the mine site for reclamation and rehabilitation. The study listed 189 abandoned mine and quarry sites and visited 105 sites for detailed study. The study identified 8 abandoned mine sites that falls within the high risk hazard that were ranked for prioritization and reclamation. Most of the mining and quarry sites were abandoned as a result of communal conflicts, lack of modern equipment, inability to acquire licence and too much water in mine pond. The study recommends the need to enforce mining regulations and initiate the process of reclamation of the abandoned mine sites to reduce the dangers posed to host communities.*

Keywords*— *Abandoned mine, Inventorization, Prioritization, Quarry pits, Reclamation and Taraba State.

I. INTRODUCTION

Mining is the process of extracting minerals from the earth surface in an environmentally friendly manner (Davou, 2013). Mining can also be regarded as the extraction of minerals occurring naturally such as coal, ores, crude petroleum and natural gas. Mining industries have been viewed as key drivers of economic growth and the development process (Bradshaw, 2005), and as lead sectors that drive economic expansion which can lead to higher levels of social and economic wellbeing (Bridge, 2008). Artisanal and small scale mining is one important livelihood activity that can help reduce poverty and achieve rural economic renewal through the development of non-farm income generating opportunities. Previous studies have shown that solid mineral exploitation constitute more than 1% of Nigeria's GDP as most of the mining activities are still mainly carried out by the informal sector with over 95% of mining activities carried out by artisanal and small scale miners, out of which 95% are illegal (Uzoka, 2001). The Federal government generate large revenue from mining activity from mine licence fees, mining permits, rents and royalties from mining companies. This money is deposited in a special account and later shared among the different states. Each state presents documents of mining activities taking place within its territory which is used as a basis in sharing the revenue among the component states in the country. However, mining operations oftentimes leave the affected environment severely degraded, physically and socially. Degradation commonly occurs at all stages of mining activities from exploration to mine closure, resulting from both large and small scale artisanal mining operations (Walde, 1992).

Taraba state is well endowed with abundant mineral resources. Many of these mineral resources have been explored and worked on in the past decades. Mining in Taraba state is dominated by artisanal and small scale miners (informal mining activities undertaken by individuals or groups who rely heavily on manual labour, using simple implements and methods). The small scale and artisanal miners' and the large scale industrial miners indiscriminately carryout extensive mining activities without any consideration to the environment and other users. These mining operations are mostly surface mining carried out with little or no advanced technology to manage the environment degraded by the mining operations (Oladipo, 2006). Increasing environmental damage is made worst by the fact that most of the miners undertake their operations illegally and have no official permission and their areas of operations are not known to government officials (Ahmed, 2013). This makes it difficult for the

government to monitor their operations and also to enforce environmental regulations on them.

Mining of mineral resources is under the exclusive list of the Federal Government of Nigeria. This means that all mineral resources in the country is owned by the Federal Government of Nigeria and no State or Local council has right to explore, prospect or exploit mineral resources found in their territory without licence from the Federal Government. The 2007 mining law requires that anyone going into mining operation must apply and obtain mining licence or rights permitting them to do so. This has resulted to increasing numbers of illegal miners. Most of the illegal miners are uneducated and doesn't understand the rudiments of applying for mining licence which is usually from the Federal Ministry of Mines and Steel development, located in Abuja, the Federal Capital Territory.

Although there have been so many studies on mining activities and their environmental impacts over the years, it is only recently that the issues of mine closure and rehabilitation have been included in mining discourse as contained in the Minerals and Mining Act 2007. Most of the mines or affected mining pits were abandoned or left in an inadequate reclamation status. These abandoned mine pits and their associated mine features constitute significant risk and environmental hazards to local communities and the natural ecosystem. The activities of this artisanal and small scale miners litter the state with abandoned mine pits, 'lottos', trenches, ponds and mining dumps that pose serious danger to grazing livestock and human beings (Owolabi, 2013). It has been reported that the activities of the artisanal and small scale miners result in conflicts between livestock rearers, farmers and foresters on one hand and miners on the other hand (Ahmed, 2013).

Despite the widespread distribution of these unrehabilitated abandoned mines and their impact on communities in the state, there is limited knowledge about their existence, location and characteristics. Since the creation of the state in 1991, no effort has been made to inventorize these mine lands in the state. Inventorization will allow for remedial measures to be undertaken to rehabilitate and reclaim the sites in the state (based on zones).

Today, the Federal Government of Nigeria is faced with the challenges of reclaiming the abandoned mine lands and reducing their hazards at a time when the country is facing serious financial constrain, budget cut, land issues (crisis/conflict) and lack of comprehensive inventory of this abandoned mine pits. The first step in reclaiming these abandoned mines is inventorization. Inventorization is the process of identification of the abandoned mine sites, its location (longitude and latitude), type of mine sites, length, width, height and depth of mine pits or mine waste dumps among others. It is a process of generating information on the features of the abandoned mines. It is very important so that the extents of the problems are understood and commensurate effort put into the reclamation process. Unfortunately, despite the Federal government award of contract for a nationwide inventorization of these abandoned mine pits in 2006 (Ashawa, 2007), the government does not have adequate records of land degraded during these past operations in parts of the country where this artisanal and small scale mining took place (Ahmed, 2013). The report identified 1,200 abandoned mining sites across the country in its 2008 report (Premium Times, 2014). The report did not capture many of the abandoned mine sites in the state, only 18 abandoned mine sites was reported. This inadequate information makes future planning and policies of reclaiming, restoring and managing the abandoned mine sites difficult. After the nationwide inventorization of 2006, new mine sites have been worked and abandoned, hence the need for this inventorization.

This study is significant because it attempts to inventorize abandoned mine sites in the state and classify the extent to which they negatively impact on the environment. This will facilitate screening of the abandoned mine sites in the state for reclamation and rehabilitation. This study also provides a baseline data which include careful documentation of the locations, extent of impacts, nature of minerals mined, the type of mining methods used, mine pits dimension, overburdens and scraps of equipment/machines of the mine sites in the state. The main reasons for inventorization of abandoned mine sites usually include;

i. An indication of the quantity of minerals mined in the state and its resultant contribution to the Nigerian Economy since all revenue generated are sent to the Federal Government account.
ii. A basis of sharing of revenue generated from the State by the Federal government according to the mineral revenue sharing formula by the Revenue Mobilization, Allocation and Fiscal Commission (RMAFC).
iii. A licence data for the state to apply for its own revenue share from the Federal Government.
iv. Assist the State and Federal Government in creating database for degraded mine sites.
v. Prioritization of the mine sites for reclamation when the need arises.
vi. Reduce in future, the fund to be spent in carrying out the inventorization exercise.

The baseline data generated from the study will help in the formulation of mining and environmental policies for the state to reclaim, restore and rehabilitate environments degraded by past mining activities. The study will enrich existing literature on the activities of artisanal and small scale miners, and also the large scale miners in the state.

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Description of Study Area

Taraba State is found in the north eastern part of Nigeria. It is located between latitude 6º25'N and 9º30'N and between longitudes 9º30'E and 11º45'E. It is bordered on the west by Nassarawa and Plateau States, to the north by Bauchi and Gombe States and by Adamawa State to the northeast (Figure 1). The population of Taraba State, according to the 2006 National Population Census, was 2,300,736 (1,199,849 (52.2%) males and 1,100,887 (47.8%) females). There were 98,962 more males than females. The state has a population growth rate of about 3.1% per annum. Taraba State is the most ethnically diverse state in the Federation with over 80 different ethnic groups. Ethnic groups found in the state include Mumuye, Ichen, Wurkun, Mambila, Kuteb, Chamba, Jukun, Tiv, Yandang, Fulani, Jenjo, Kunini, Ndoro, Kambu, Kaka, Bandawa, Munga, Zo and Banbuka.

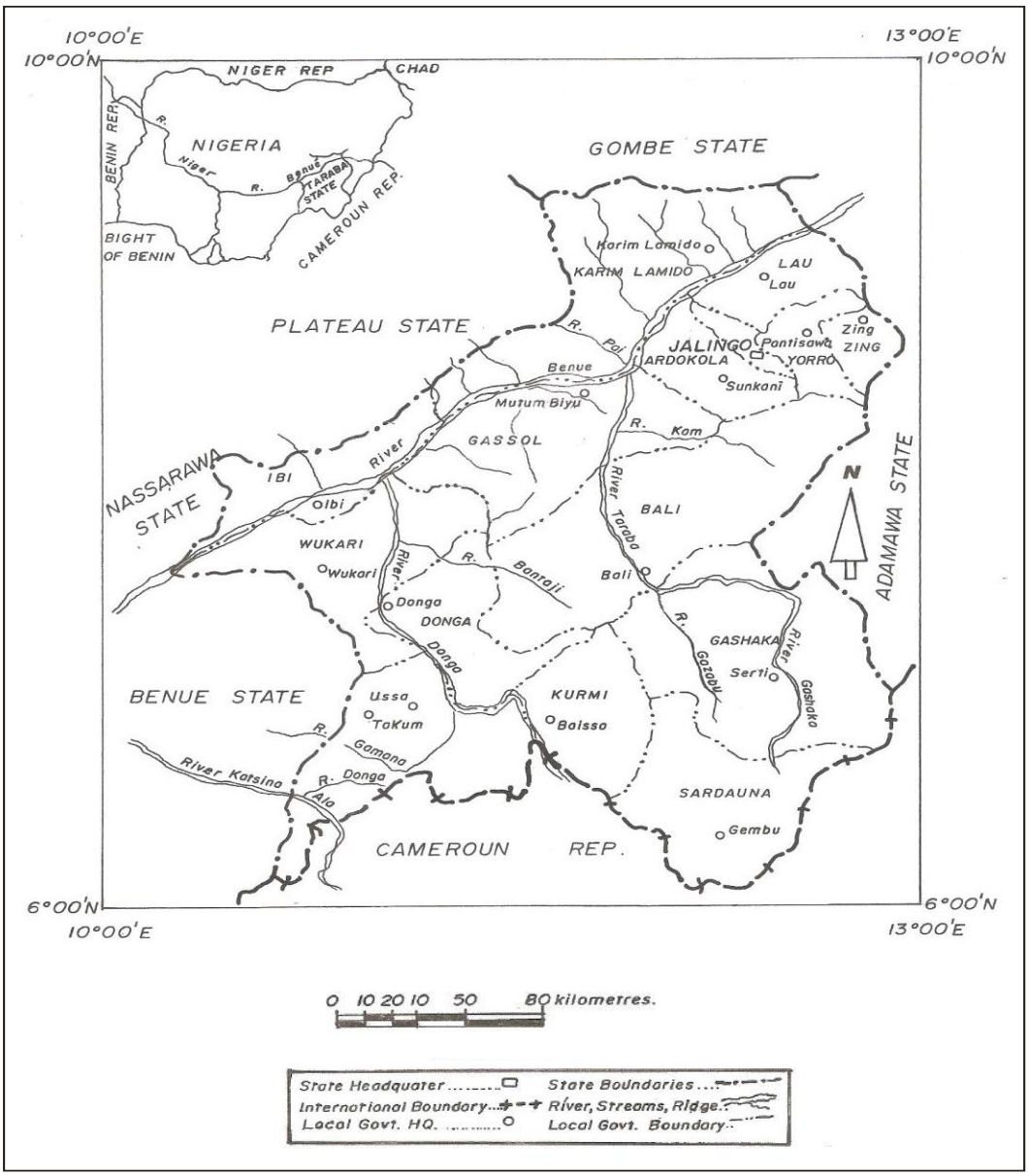

Fig.1: Map of the Study Area

The State has climatic types that ranges from northern equatorial type in the southern part of the state (Kurmi, Ussa and Takum LGAs) to the Tropical hinterland type (Donga, Gashaka and Wukari LGAs) to Tropical continental type of climate in northern part of the State (Yorro, Zing, Lau, Jalingo and Ardo Kola LGAs). The Mambilla area is a montane climate type. This climatic types have greatly influenced the vegetation types in the state. The vegetation types ranges from the tropical rainforest around Kurmi, Ussa and Takum LGAs to Guinea savannah in the central part of the state and Sudan savannah type in the northern part. Montane vegetation is found on the Mambilla plateau and Shebshi mountain and flood plain complex is found along the major rivers in the state.

Geologically, Taraba State is underlain by Basement Complex and sedimentary rocks, each occupying a very distinctive part of the state. The Basement Complex rocks

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occupy the greatest part of the state (above 80%), while the sedimentary rocks are found along the valleys of River Benue and its major tributaries such as Rivers Donga and Taraba. The geological formation of the state provided the basis of the rich solid mineral resource potentials that the state is known for.

The state is an agrarian state with about 80 percent of the populations directly engaged in agriculture while 20 percent are engaged in other economic activities including white collar job (TSEEDS, 2004). Common crops cultivated include cereals, legumes and tubers such as maize, rice, groundnut, beans, cassava and yam. Tree crops in the area include palm oil, banana/plantain and orange. Cash crops produced in the state include coffee, tea and groundnut. The State's agricultural sector is dominated by small scale rural farmers. The state has over 52 discovered solid mineral resources with the highest hydroelectricity power potential in the whole country. It is a tourist haven with the largest National park in West Africa (Gashaka Gumti National Park).

II. METHODS OF DATA COLLECTION

The method of carrying out inventorization of abandoned mines varies across different countries depending on the scale and peculiarity of the local mining environment. The inventorization of abandoned mines in the state was carried out in three stages:

i. Desktop review and consultation of some miners to identify abandoned mine sites in the state which were legally or illegally mined and the damaged environment.
ii. Field assessment of identified sites.
iii. Prioritization of sites for reclamation.

Stage I – Field Identification of Sites

The first task was identification and collation of list of abandoned mine sites. Primary and secondary data collection methods were employed in doing this. Secondary data involved desk review of existing literature and archival records from relevant government agencies and organizations. The researchers liaised with staff of the Federal Ministry of Mines and Steel Development in Jalingo and the State Bureau of Solid Mineral Resources in obtaining some list of abandoned mine sites in the state. Six (6) officials of government agencies were interviewed. Interviews was also carried out with seven (7) traditional leaders, 15 host community members and nine (9) renowned licensed small scale miners in the state to obtain additional list of abandoned mine sites. Interviews were preferred to the use of questionnaire due to limited number of small scale miners and other key informants. All the people interviewed were men who have knowledge of the mining activity in the area. The renowned small scale and artisanal miners and traditional village Heads assisted in directing the researchers to the abandoned mine sites. Most of the abandoned mine sites are found close to some active mining sites which were illegal and as such the miners react aggressively to any visitor to the site perceived to be government officials. There were cases of illegal miners attacking government officials on field survey to mining sites. Thus, the company of these old miners and village Heads assisted greatly in easing tension and providing direction to the location of abandoned mine sites in their communities. The study focused on the physical environment and description of features of the abandoned mines. Abandoned mine site assessment parameters were developed.

Stage II – Field Assessment

The large number of abandoned mine sites identified in step one above and the lack of accessibility to some of the sites, limited the field assessment to only sites that are accessible by means of transportation and trekking distance. The field assessment involved accessing the mine site, observation of mine features; measurement of mine pits dimension and photography. The recording of observations focused on quantifiable details such as quantity, volume, surface area and distance and descriptions of the types of materials or products removed from the site. Measuring tape was used to measure the extent of degraded areas as a result of past mining operations. For very large areas where measuring tape was not adequate, the Garmin Global Positioning System (GPS) was used. The volume of the areas of physical damage was calculated in the field. The choice of this method was because of its precision and ease of application. Only the GPS, measuring tape, ropes and pegs was required. This helped in the description of the features of the various abandoned mine sites in the state. The coordinate data of the locations of abandoned mine and quarry sites visited during the fieldwork was georeferenced to Minna datum, UTM zone 32N. This was overlay to satellite data obtained from the United State Geological Survey (USGS) online open resources. The locations of abandoned mine and quarry sites were identified by attribute and spatial query. This enables us to map out the locations of the abandoned mine and quarry sites.

Stage III – Risk Assessment and Prioritization of Mine Sites

The hazard risk assessment approach adopted in this study is the physical hazard assessment approach. The approach was adopted to provide an objective basis of prioritization of abandoned mines and quarries which is a major pre-requisite for resource allocation in mine reclamation exercise. The 2 broad group of risk usually associated with mining sites are human safety risk and environmental risks. Reference is usually given to human safety risk, while the environmental risk requires time, more resource and time to achieve good results. Information on the physical hazards collected during field inventory work consist of direct measurement and observations of physical conditions that reveals environmental and public safety

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conditions of the abandoned mine and quarry sites visited. The public safety data include identifying dilapidated mine structures and equipment, mine openings, pits/lottos, high walls and sink holes.

The study adapted the risk assessment approach that is modified from USEPA's source-pathway-target. This is a numerically derived system of assigning numeric values (scores) to abandoned mine site base on quantifiable measures of hazardous physical features and associated exposure potential of the mine sites (Ashawa, 2007). This method provides for relatively rapid, uniform and objective prioritization of abandoned sites. The hazard risk index used in this study are

i. Accessibility (such as distance, degree, and type of road development in the area)
ii. Number of mine openings in an area
iii. Closeness of mine site to major road
iv. Location of mine site close to high residential areas and
v. Pollution of pond water etc.

The prioritization for reclamation was based on public safety and environmental concerns. Here the study considers dangers posed by the mine site in arriving at its prioritization for reclamation. These dangers include its location close to high residential areas, farmlands/grazing lands. The mines were scored numerically based on their site features, proximity to high residential areas and accessibility, and then ranked by their scores. Higher scoring areas are higher risk and higher priority for reclamation. The approach adopted some simple ceteris paribus assumptions that are grounded in common sense (Rohrer and Smith, 2008). They include; all things being equal, an area with multiple mine openings is a higher risk than an area with only one or very few opening, all things being equal, areas close to high population is at higher risk than one located in area of low population and all things being equal, a mine in an area close to major roads is at higher risk than a mine in an area without road. All hazards observed were rated by the researchers on the site. The hazards rating was reviewed with the help of video clips and photographs. The information on hazard rankings was used to prioritize the mine site for reclamation and rehabilitation. The data generated from the field were collated and analysed using descriptive statistics.

III. RESULTS/DISCUSSIONS

The study listed 189 abandoned mine and quarry sites with 28 different minerals (Table 1). However, because of security challenges and inaccessibility, only 105 mine/quarry sites (Fig.2) associated with 13 minerals were visited during field inventory works (Table 2). The summary of the minerals associated with the listed mine sites is presented in Table 1.

Table.1: Types of Minerals Associated with listed Sites in the State

S/N	Types of Minerals	Frequency
1	Aquamarine	3
2	Alluvial Gold	7
3	Amethyst	7
4	Barite	22
5	Bauxite / aluminium	2
6	Beryl	9
7	Calcite	1
8	Columbite	1
9	Copper	3
10	Dolomite	1
11	Feldspar	1
12	Galena	17
13	Gemstone	9
14	Granite	2
15	Graphite	2
16	Quartz	1
17	Industrial Nickel	1
18	Laterite	45
19	Lead	11
20	Limestone	1
21	Molybdenum	3
22	Sapphire	12
23	Shillite	1
24	Tantalite	5
25	Tin	4
26	Topaz	2
27	Tourmaline	7
28	Uranium	6
	Total	186

Source: Fieldwork, 2017

Table.2: Types of Mineral Sites Visited

S/N	**Types of Minerals**	**Frequency**
1	Alluvial Gold	1
2	Amethyst	1
3	Barite	16
4	Copper	2
5	Galena	16
6	Gemstone	7
7	Granite	2
8	Laterite	45
9	Lead	4
10	Molybdenum	2
11	Sapphire	4
12	Tourmaline	2
13	Uranium	3
	Total	105

Source: Fieldwork, 2017

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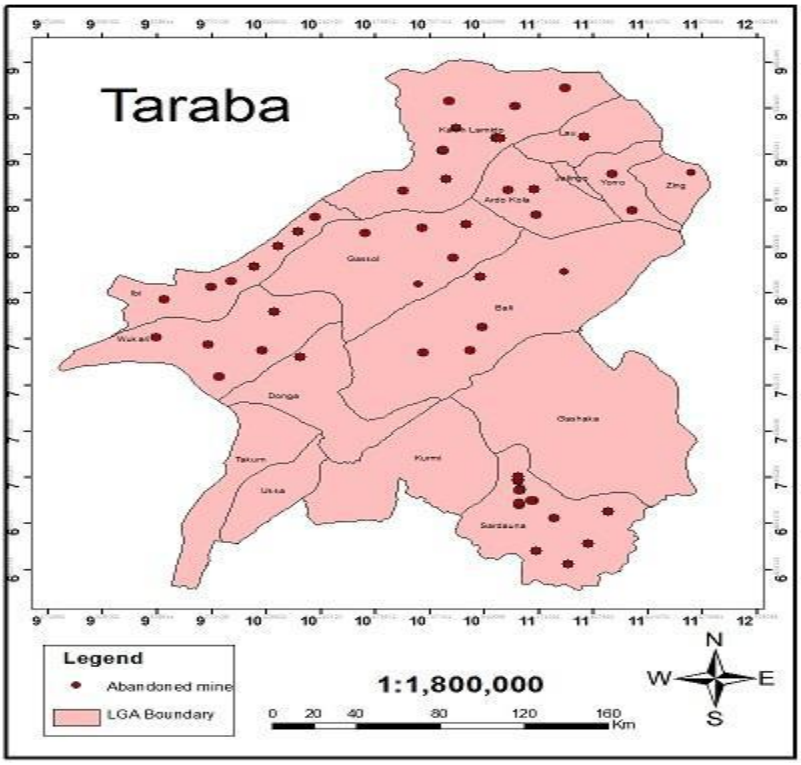

Fig.2: Abandoned Mine and Quarry Sites in Taraba State

An inventory of quarrying and mining titles in Nigeria in 2012 by the Federal Ministry of Mines and Steel Development reported 182 titles issued out from Taraba state (Table 3).

Table.3: Mining Leases in Taraba State

S/No.	Type of Title	Number	Type of Mineral
1.	Mining lease	42	Barites
2.	Mining lease	7	Gemstone
3.	Exclusive Prospecting licence	104	Barytes
4.	Exclusive Prospecting licence	27	Gemstone
5.	Exclusive Prospecting licence	2	Limestone
6	Total	182	

Source: MSMD (2012)

Of the 182 mining lease and titles issued out from Taraba State, 172 were from 4 LGAs, Ibi (40), Karim Lamido (44), Sardauna (37) and Wukari (51). In 2015, there were 77 mining titles reported in the inventory of quarrying and mining titles issued by the Federal Ministry of Mines and Steel Development from Taraba state as shown in Table 4.

Table.4: Mining Title in Taraba State (2015)

S/No.	Type of Title	Number
1.	Mine lease	6
2.	Quarry Lease	14
3.	Small Scale Mine Lease	18
4.	Exploration Lease	39
5.	**Total**	77

Source: MSMD (2015)

Findings from the study revealed that there are many small scale and artisanal mining that are not illegal contrary to previous speculations by some scholars. There is now an appreciable awareness among the small scale and artisanal miners and the mining communities that you cannot exploit a mineral resource without an approved mining licence/lease from Federal Ministry of Mines and Steel Development. Most of the small scale and artisanal miners on discovering a mineral deposit will look for a mining firm or individual who has a mining licence to lease the

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area of deposit (mine) to him. Most communities are becoming more conscious of protecting the mineral resources in their communities. The approved mining licence is the only document that allows the prospective miners the right to exploit the mineral deposit and the people's cooperation in this regard. One of the conditions of obtaining a mining lease or licence is payment of compensation of property to local communities. Findings of the study shows that large quantities of mineral ore deposits have been mined out in the state and large numbers of abandoned mine sites exist as a result of past mineral exploration /exploitation in the form of test pits, lotos and open ponds.

Mining Methods Employed in Abandoned mines

Findings of the study shows that most of the abandoned mine sites in the state are surface mining with open pits/cast. There are only 2 underground mining tunnels at Jukun and Ameche communities in Karim Lamido LGA (Plate 1).

Plate.1: Underground mining Tunnel at Jukun Village in Karim Lamido LGA

The open pits mining method is a common choice because of the mining implement and characteristics of the ore deposit (e.g. location of mineral veins close to the surface) which makes the removal of the overburden (i.e. host rock overlying the mineral laden ore) cost effective. It is the most economical method of mining in the area because it involves use of simple crude implements. The method involves excavation of an area of overburden and removal of the ore exposed in the resulting pit (USEPA, 2000). The history of poor open cast mining practices in the state and lack of post-mining closure or reclamation measures have left large expanse of wasteland in the state. The total size of the mine openings in the sites visited is 57,196,942 m^3. The largest of this mine opening are found in Wukari, Gassol, Donga and Ibi LGAs. Most of the abandoned mine openings were mainly laterite excavated pits used for road construction in the area. Ibi LGA has large abandoned open barite mine openings with large water collection used for domestic and animal consumption. The distribution of these mine openings is presented in Figure 2.

The largest quantities of wastes generated by mining operations are mine water and overburden which is generated at surface mines and with time washed away by erosion. Overburden is the surface material (i.e. topsoil and rock) removed during surface mining operations to expose the ore deposit beneath. The total volumes of overburden materials generated as a result of mining in the area is about 745,984 m^3. The distribution of the overburden materials is presented in Figure 3.

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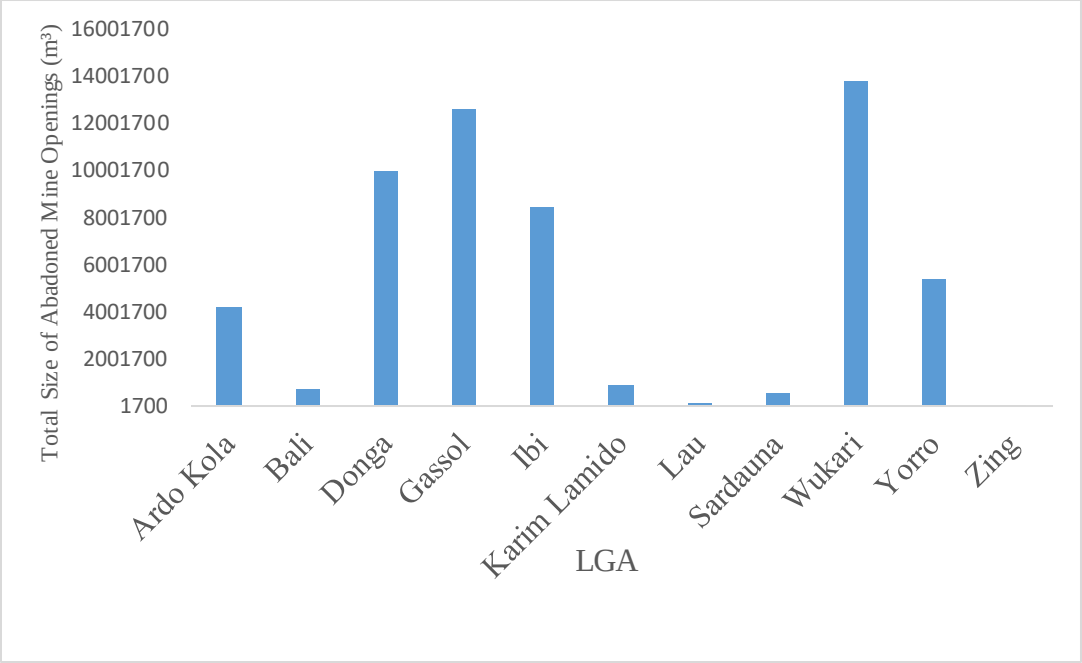

Fig.2: Magnitude of Abandoned Mined Openings in the State

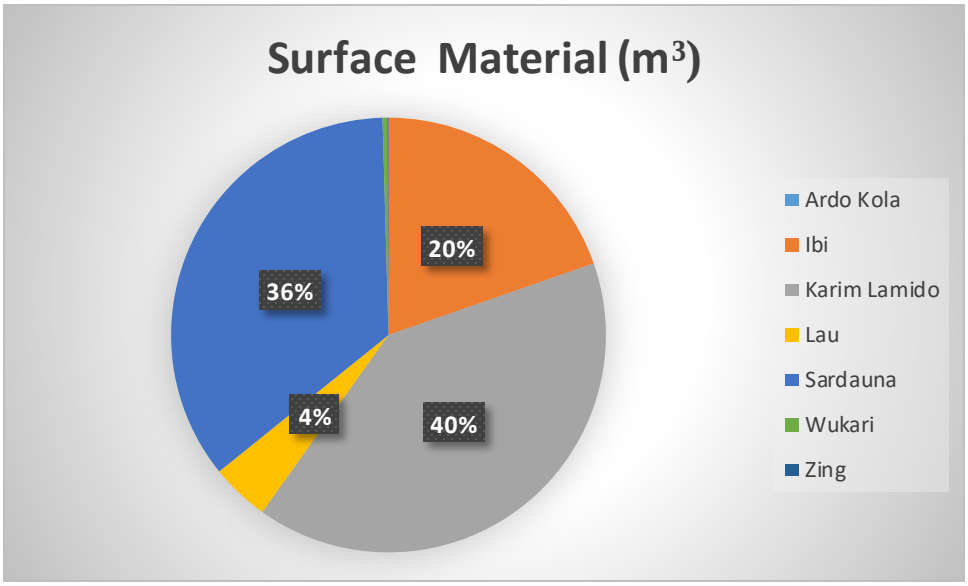

Fig.3: Volume of overburden materials at the abandoned mine site.

The availability of these wastes dumps at the abandoned mine site is very important in the description of the mine site features as it indicates the availability of materials for reclamation of the site and the less cost of the mine reclamation. If there are no overburdens at the site, the cost of reclamation would be expensive as materials needed would have to be obtained from other available locations and transported to the sites.

Causes of mine abandonment

Abandoned mines are those mines for which the owner cannot be found or for which the owner is financially unable to carry out reclamation after mining activities. The cause of mine abandonment varies across the country. However, some of the causes of mine abandonment in the study area include;

i. **Lack of Regulations/Policies** - many mining sites were abandoned in the past because there were no laws stipulating that those sites should either be reclaimed or rehabilitated whenever they were no longer active. The first mining law of 1946 has no detailed provisions about mine reclamation. This was reviewed in the Mining Act 2007 which prescribed certain standards for mining site reclamation. The new mining Act stipulate obligation on the mineral title holders to reclaim the site before they vacated a mining site. However, the mining law is not fully circulated

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for the communities to know their right. This is further made worst by the lack of capacity on the part of government to enforce the new mining regulations. This contributes to the increase in the number of abandoned mine sites.

ii. **Economic** – fluctuation in the prices of mineral resources which leads to sudden drop in the prices of mineral products could lead to abandonment of mining activities.

iii. **Small Scale mining** – the uncoordinated and uncontrolled occupation of mine sites by small scale and artisanal or illegal miners in Nigeria (who usually practice seasonal mining) has often led to a site been abandoned (Ashawa, 2007). This often leaves the government with the responsibility of reclaiming such abandoned mine site. Some mine and quarry sites are abandoned because some miners lacked the licence to mine such site. Thus, abandoned for fear of arrest by government and security officials of the state.

iv. **Technical** – the common method of mining in the area which is open cast method have led to the destruction of many mining sites and subsequent abandonment of such sites because of excessive soil tillage. Miners lacked modern equipment to mine the deposits. Many mine and quarry sites are also abandoned because of excess water level in the mine pond which often over-powered the miners and their equipment especially during the rainy season.

v. **Conflicts** - communal conflicts and clashes between crop farmers and cattle herders, different ethnic and religious groups among others has led to the abandonment of mining sites as observed in Ibi and Sardauna LGAs.

Many other mines were abandoned as a result of insufficient minerals in the area, while others were abandoned when it was discovered that the mining is becoming unprofitable. Some abandoned mine sites have large mineral deposits but the miners lacked the required equipment to mine the deposits. Thus, some abandoned mine sites are rich mineral deposits sites that could be exploited in the future.

Effects of abandoned mines

The issue of abandoned mines is important because it represents many former mining sites abandoned and that continued to pose real or potential threat to human/animals safety, health and/or environmental damage. In many areas this is considered a negative legacy of the mining industry and is important because it both demonstrates a lack of care and planning in past practice and adherence to regulations that were inadequate because of the lack of detailed understanding (MMSD, 2002). Abandoned mines are associated with many effects on the physical environments and human beings which can be grouped into environmental, socio-economic and health concerns.

i. **Environmental** – these includes: unused open pits, deforestation, degraded landscape, mine shafts, impoverished soils, degradation and contamination of groundwater; pollution of surface water by sediments or overburdens, changes in groundwater regime, air pollution from dust or toxic gases and risk of falls into open pits, contamination of soils and aquatic sediments among others.

ii. **Socio-economic** - the human related problems include conflicts, loss of arable lands, loss of income and employment opportunities as a result of decline in business activities within local communities, population migration and pressures on infrastructural facilities.

iii. **Health** - the existence of abandoned mine and quarry sites provide breeding grounds for disease vectors and a hiding place for dangerous reptiles. Many abandoned mine pits contain water (pond) which is used by the community for human and animal consumption. Water from abandoned mines may contain significant concentrations of heavy metals and total dissolved solids and may have elevated temperatures and altered pH, depending on the nature of the ore body and local geochemical conditions. These waters may become acidic over time when exposed to oxygen and, if present, pyrites or other sulfide minerals. The acidic water may also solubilize with the metals contained in the mine and mined materials, creating high concentrations of metals in solution. These acidic metal-laden waters may contaminate down-gradient ground-water and surface water resources (USEPA, 2000). Some of the ponds at the sites were now being used for irrigation farming, fishing and water supply for domestic and industrial purposes. Although some of the abandoned mine pond water is used for domestic consumption, the water is not good for drinking as it is untreated. This constitutes a health hazards/challenge even though it solves a lot of local water needs problem.

iv. The existence of abandoned mine sites has serious effect on crop yield. This is especially for crops grown in mine sites where the minerals are poisonous to humans. This can result to food poisoning from contaminated crops and low crop yield.

The findings of the study show that there are some sites that are not posing problems contrary to earlier assumptions. Many other sites are risk sites to humans and

animal lives, death traps especially in the sapphire mining areas where mining pits with lotos killed humans and animals. There are few sites with large quantity of equipment, whereas there are numerous sites with only a few equipment items left behind on the site.

Effort of government can be oriented towards those few sites containing such dangerous products and evaluate the opportunity to clean up and reclaim the other problematic sites according to clearly identified criteria such as potential danger to the environment and human health and the proximity between the sites and the communities.

Abandoned mine workings may be an environmental hazard because of the possibility of subsidence or collapse. They can also channel contaminated ground water flow, particularly if they are located in sulphide mineral bearing rock and contain water.

Prioritization of Abandoned Mine

Prioritization of abandoned mine for reclamation is the main objective for carrying out inventorization of abandoned mine. The abandoned mine sites identified were ranked and listed by priority based on health and environmental risk and threats associated with the sites to humans and livestocks. The rating of sites was based on the potential degree of their human and environmental hazard. The findings of the study show that about 8 mine and quarry sites falls within the high hazard risk as shown in Table 5.

Table.5: Hazard ratings of abandoned mines and quarry sites

S/No.	Mine Site	Location	Mine Feature	Average Risk Score	Ranking for prioritization
1.	Jauro Yinu - Ardo Kola LGA	08°53'42''N 11°16'56''E	Located within residential area, children swim in ponds and use for farming	72	3
2.	Iware - Ardo Kola LGA	08°49'23''N 11°05'52''E	Located within residential area. The pond is deep and constitute high hazard risk in the area	80	2
3.	Dan Anacha – Gassol LGA	08°13'57.1''N 10°20'01.6''E	Found in residential area and high hazard risk	68	5
4.	Gindin Waya – Ibi LGA	08°05'51''N 09°46'59.8''E	Mine was abandoned due to too much water and difficulty of draining it out. Serious hazard because of depth	58	8
5	Jukun – Karim Lamido LGA	09°05'09.1''N 10°45'46.4''E	Large mine pond surrounded by farmlands/grazing lands. A woman died in the pond while trying to rescue her child.	84	1
6	Maisamari Sardauna LGA	07°08'36''N 11°05'05''E	Mine pits with horizontal lotos dangerous for humans and grazing animals	66	7
7	Akwana – Wukari LGA	07°47'01''N 09° 09'30''E	So many open mine pits within farmlands/grazing lands	68	5
8	Jankwani – Monkin, Zing LGA	08°46'42''N 11°43'16''E	Very deep open pit around residential areas that can serve as death trap for animals and humans	72	3

Source: Fieldwork, 2017.

Limitations of the Study

Studies of this nature are fieldwork based and required physically visiting all the listed abandoned mining site and observing the features of such mine/quarry sites. Most of the abandoned mine sites were located in remote and inaccessible locations by any means of transportation. This will require long trekking for several kilometres and hours through bush path which will amount to more time and cost. This is also made worst by insufficient information on the location of most abandoned mine sites especially those carried out illegally.

Secondly, the recent economic hardship in the country has made it difficult for most people to avail researchers with useful information without demanding for token payment. Thus, the researchers have to pay local guides to the mining site and the Village Head for assistance. Any researcher

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attempting to go to an abandoned or active mine site without the knowledge, permission or company of the Village Head, local miner or Villager stand the risk of been attacked by the local community. This development has increased the cost of this research and made it difficult for the researcher to reach all the listed abandoned mining sites in the state.

The study was carried out during rainy season between June to September 2018, because this was the time that funds were made available to the researchers. The researchers started work immediately. This increased the challenges encountered during the study. The depth of most of the open mine pits were estimated instead of actual measurement because they were filled with waters at the time of the study. The rainy season also led to suspension of mining activities in most active mine sites because of the difficulties of dewatering the pits as a result of high rainfall.

The recent crisis on Mambilla plateau made it difficult for the researchers to access some important sapphire mine sites. Some mine pits (especially those with lottos) were used as dumps for human/animals during the crisis, which may lead to serious health hazard considering the topography of the area as a result of underground water movement in the area. This also made it difficult for the research team to visit some abandoned mine sites at hot spot of the conflict. The local communities are becoming very conscious of movement of strangers into their communities because of the recent security challenges in the state and region particularly between the herdsmen and farmers. Thus, the researchers spent much time explaining their mission to local communities to get their consent to visit the mine sites.

IV. CONCLUSION

This study has inventoried abandoned mines and quarry sites in Taraba state. The study has assessed the hazard risk associated with the investigated abandoned mine and quarry sites and ranked them for prioritization. The study ranks 8 of the identified high risk hazard sites for prioritization and reclamation. The study has thus provided an insight into effects of abandoned mine and quarry sites and baseline data that could help in the reclamation of the abandoned mine and quarry sites in the state. The study reveals the problems posed by the lack of data on most of the abandoned sites to issues of reclamation in the future in the state. Although there is new mining exploration and exploitation regulation in the country, this inventory suggests that a lot remains to be done to redress negligence from the past, and to harmonise future economic development with environmental concerns. The potential costs of reclamation, the lack of clearly assigned responsibility, the absence of criteria and standards of mine reclamation and other factors have continued to delayed action by governments and stakeholders.

V. RECOMMENDATION

Based on the findings of the study, the following recommendations were made;

1. There is need for the Federal Ministry of Mines and Steel to work hand in hand with the Taraba State Bureau for Solid Mineral resources. This will help in the enforcement of the new mining regulation and reduction in the level of land degradation related to illegal mining.
2. There is need for the state government to commission a research into inventory of the abandoned mine and quarry sites that will extend to areas that were not covered by the present study.
3. There is need for the government to clearly define the criteria and standards of mine reclamation and assign responsibility to stakeholders concerned.
4. It is important that the State government liaise with the Federal government in initiating the process of reclamation of the identified abandoned mine and quarry sites to reduce the dangers posed to communities in the area. This is important since mining is under the Federal exclusive legislative list and mining involves taking over lands in the states.
5. Establishment of mine reclamation unit in the State Bureau of Solid Mineral Resources. This unit will be charged with the responsibility of carrying out reclamation activities in the state when the need arises especially emergency reclamation exercises that is of manageable scope and specification.
6. Severe sanctions should be meted to individuals/groups and organizations that operate and abandoned a mine or quarry site without proper reclamation as stipulated in the new mining guidelines of 2007.
7. There is need for the government to come up with Environmental Protection and Rehabilitation Fund which will be funded through contributions from mineral title holders over a period of time. This fund can be used to carry out reclamation of the abandoned mines and quarry sites, given priority to high risk hazard sites.

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Influence of Different Energy-Proteins on Performance and Blood Hematological on Three Types of Local Chicken

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***Abstract*----** *Indonesia is rich in germplasm, including local chickens. Three types of superior local chickens are Sentul-Warso Chicken, Chicken Kampung-Unggul, and Chicken Local-Jimy. Chickens are relatively diverse growth and nutrient needs are also variations, especially energy and protein content. The research has been conducted at Test Farm cage, Faculty of Animal Husbandry, University of Padjadjaran, Sumedang, and West Java-Indonesia. The objective of the study was to determine the energy-protein requirements of the ration, which resulted in the highest production performance and optimal hematologic blood values in three types of local chickens (Sentul-Warso Chicken, Chicken Kampung-Unggul, and Chicken Local-Jimy). Research using experimental method in laboratory. The experimental design was a Completely Randomized Design, consisting of five treatment rations with different energy and protein levels and each repeated four times. The treatment consisted of: R1 = EM 2750 kcal / kg and PK 15%; R2 = EM 2750 kcal / kg and PK 17%; R3 = EM 2750 kcal / kg and PK 19%; R4 = EM 2950 kcal / kg and PK 15%; and R5 = EM 2950 kcal / kg and PK 17%. The data were analyzed by means of variation and the differences between treatments were tested with Duncan Multiple Range Test. The result showed that ration with metabolic energy content 2,750 kcal / kg and 17% crude protein resulted in optimal production and hematological blood value in local chicken. The performance of Chicken-Jimy's production is higher than Sentul-Warso chicken and the lowest Kampung-Unggul chicken. The hematological value of chicken blood is in the normal range.*

Keywords*--- *energy, hematological, local chicken, performance, protein.

I. INTRODUCTION

The problems that arise in the development of livestock are generally related to the provision of quality feed, the guaranteed continuity of feed ingredients, and the quality of livestock (genetic aspects). While the results of previous research, about rations or feed ingredients may not be used as a reference in the preparation of broiler rations in Indonesia. However, at least the results of previous research can be used as a point of departure or reference of thought to conduct research in accordance with the conditions in Indonesia.

Indonesia's local broiler is a national genetic resource and contributes significantly to both food security and rural income [5]. The chicken has a social and cultural function for certain communities [12] and is part of Indonesian history [17]. In addition, local chicken as part of Animal Genetic Resources through its genetic diversity is an asset for future food needs [4, 6]. Local chickens have more genetic variation and adaptive properties than exotic chicken breeds. In addition, local chickens require relatively smaller inputs in the maintenance process.

Domestic broiler commodity demand from year to year continues to increase, although it has experienced a decrease caused by information eradication fluburung. But after that demand increased again until now. High demand, the reality can not be responded by local poultry breeders, although genetic resources and feed sources are available.

Chickens consume most rations are to meet the needs of protein and energy. The content of protein in the ration is very influential on the achievement of body weight. Protein content in the ration is required for tissue growth, tissue repair, and management of production and part of the enzyme structure so that protein is known as one of the principal constituents of body cells and

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tissues [3]. This suggests that proteins play an important role in achieving the desired final weight.

Provision of ration with a good nutrient quality and balanced course can affect the rate of growth and development of chickens. The resulting weight gain is a description of the quality of the ration given. The increase in body weight results from the consumption of good proteins. High protein quality will affect the protein intake into the meat so that amino acids are fulfilled in the body. The increase of body weight is caused directly by the availability of amino acid forming tissue so that consumption of protein ration is directly related to growth process.

Protein is a key element in the formation of blood erythrocytes [7]. Protein has been absorbed for body use in the form of amino acids, so that if during the process of formation of erythrocyte deficiency of amino acids it will lower blood erythrocytes levels. The erythrocyte membrane has a glucose carrier, which is one of the energy-producing nutrients. Energy needed erythrocytes such as for the maintenance of erythrocytes and membrane structure. Thus energy and protein are needed erythrocytes as a material for erythrocyte activity in the blood. Proteins are also needed for hemoglobin synthesis. Proteins, especially amino acids glycine and mineral Fe are the components of hemoglobin forming. [11].

Amino acids glycine is a nonessential amino acid that can be formed in the body. However, non-essential amino acids can not be formed when essential amino acid deficiencies can not be formed in the body. Thus essential amino acids must be available in the diet. Therefore the prepared feed is cultivated to contain sufficient essential amino acids in order that the synthesis of nonessential amino acids is not disturbed. This will be related to the synthesis of hemoglobin that can run smoothly if the constituent material is needed sufficiently during the process. Therefore, the amount of hemoglobin produced is proportional to the amount of constituent material for hemoglobin synthesis.

Blood can indicate the physiological condition of cattle because blood is a component that plays an important role in the physiological regulation of the body. One that affects the physiological condition of the livestock body is the ration. The ration that is not in accordance with the needs of livestock will lead to stress so that physiological changes that resulted in the hematological status decreased. This stress can be avoided one of them by giving the right energy-protein rations.

The right energy-protein content of the ration is expected to have a positive effect on the production performance and hematology of local chicken blood. The purpose and objective of the study was to establish the energy-protein requirements of the ration, which resulted in the highest production performance and optimal hematological blood values in three types of local chickens (Sentul-Warso Chicken, Chicken Kampung-Unggul, and Chicken Local-Jimy).

recording, and chickens are kept until the age of 10 weeks.

II. MATERIALS AND METHODS

Livestock Experiments

Livestock used in this research is DOC Sentul-Warso Chicken, Chicken Kampung-Unggul, and Chicken Local-Jimy, each of 100 tails. Before put in the cage, the chicken is first weighed the initial body weight (coefficient of variation weight 8.28%, 13.99%, and 13.65%). Chickens are given wingtag to facilitate the

Trial Rations

The ingredients used for the preparation of the ration consist of corn, fine bran, soybean meal, fish meal, $CaCO_3$, fish meal, and bone meal. The rations used during the study were a mash-shaped ration, with nutrient content and metabolic energy rations presented in Table 1.

Table.1: Nutrient Content and Metabolizable Energy Research Ration

Nutrient and Energy	Treatment Rations				
	R1	R2	R3	R4	R5
Metabolizable Energy (kcal / kg)	2750	2750	2750	2950	2950
Crude protein (%)	15.00	17.00	19.00	15.00	17.00
Crude Fat (%)	6.66	6.54	6.19	7.,01	6.99
Crude Fiber (%)	4.89	4.75	4.62	4.09	3.97
Calcium (%)	1.05	1.25	1.34	1.01	1.24
Phosphorus (%)	0.58	0.67	0.72	0.55	0.67
Lysin (%)	0.97	1.18	1.35	0.94	1.16
Methionin (%)	0.35	0.40	0.44	0.35	0.40
Methionine + Cystine (%)	0.67	0.74	0.,80	0.64	0.72

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Trial Cage

Chickens are randomly divided into 60 units of cages, each cage containing 5 tails. The enclosure used is a cage enclosure made of bamboo and ram wire. Cage Size 75 cm x 75 cm x 75 cm. Each cage is equipped with feeding and drinking water, and at the beginning maintenance is equipped with a lamp that serves as a heater.

Observed Variables

Performance of production, including:

1. Consumption of rations (grams / birds).
2. Increase in body weight (gram / birds)
3. Convertible rations (Index)
4. Weight carcass (gram / birds)
5. Income over feed and chick cost (Rupiah / birds)

Hemotological blood, including:

1. Blood Erythrocytes (million / mm^3)
2. Haemoglobin Blood (g / dL)
3. Blood Hematocrit (%)
4. Blood Protein (g / dL)
5. The fragility of Blood Cells (% hemolysis)

Experimental design

Research using experimental method in laboratory. The experimental design was a Completely Randomized Design, consisting of five treatment rations with different energy and protein levels and each repeated four times. The treatment consisted of: R1 = EM 2750 kcal / kg and PK 15%; R2 = EM 2750 kcal / kg and PK 17%; R3 = EM 2750 kcal / kg and PK 19%; R4 = EM 2950 kcal / kg and PK 15%; and R5 = EM 2950 kcal / kg and PK 17%.

Data analyses

Analysis of variance was applied to the data using statistical package programme of SPSS version 19. Significantly differed means were separated by a Duncan's multiple comparison test at 0.05, respectively. Measured production performances were feed consumption, weight gain, feed conversion, carcass weight, and Income over feed and chick cost (IOFCC) Sentul Warso Chicken, and the data are presented in Table 2.

III. RESULTS AND DISCUSSIONS

Effect of Energy-Protein Rations on Performance of Sentul-Warso Chicken Production

Table.2: Effects of Ration Energy-Proteins on Consumption of Rations, Weight Gain, feed conversion ratio, Heavy Carcass, and Income over Feed and Chick Cost (IOFCC) of Sentul-Warso Chicken

Treatment	Consumption of Rations (g/b)	Weight Gain (g/b)	Feed Conversion Ratio (index)	Heavy Carcass (g/b)	IOFCC (Rp./kg)
R1	1652.08 ^{NS}	578.27^{NS}	2.88^{NS}	529.97^{NS}	10,719
R2	1761.47 ^{NS}	596.38^{NS}	2.98^{NS}	569.73^{NS}	12,647
R3	1479.13 ^{NS}	568.98^{NS}	2.61^{NS}	548.63^{NS}	10,468
R4	1659.94 ^{NS}	534.24^{NS}	3.12^{NS}	535.22^{NS}	11,881
R5	1550.40 ^{NS}	570.00^{NS}	2.75^{NS}	548.48^{NS}	11,536

Description: R1 = CP 15%, and ME 2750 kcal / kg; R2 = CP 17%, and ME 2750 kcal / kg; R3 = CP 19%, and ME 2750 kcal / kg; R4 = CP 15%, and ME 2950 kcal / kg; R5 = CP 17%, and ME 2950 kcal / kg.
NS: Not significant

The average consumption of Sentul-Warso chicken ration was between 1479.13 g / b (R3) to 1761,47g / b (R2), weight gain between 534.24 g / b (R4) to 596.38 g / b (R2), the conversion of rations between 3.12 (R4) to 2.61 (R3), carcass weight between 529.97 g / b (R1) to 569.73 g / b (R2), and gains (IOFCC) between Rp. 10,468 / kg (R3) up to Rp. 12,647 / kg (R2). Based on the result of statistical analysis, the five treatment rations did not give significant effect (P> 0.05) to the production performance of Sentul-Warso chicken. The difference of metabolic energy of ration of 200 kcal / kg and protein content of ration by 4% did not affect the production performance of Sentul-Warso chicken. Sentul-Warso Chicken is relatively stable against changes in nutrient content in rations (protein and energy).

The performance of a livestock is determined by its genetic ability and adaptability to the environment. Each offspring has different abilities in growth. This may be due to differences in the genetic potential of each heredity and the ability to adapt to different environments in each individual [2]. The high body weight gain can be caused by the balance of nutrient content of the formula of rations used, where the nutrient content of energy and protein in the treatment of R2 has a better energy and protein level than other treatments. According to [24] that the balance between energy and protein and other food substances contained in the ration plays a significant role

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in the rate of growth. Furthermore, Siregar [1] added that the addition of body weight increase depends on a number of nutrients consumed by livestock.

Sentul-Warso Chicken is more responsive to changes in nutrient content in rations (protein and energy). Rations with a metabolic energy content of 2750 kcal / kg and 17% crude protein, support the production performance, and generate the highest profit (IOFCC).

Influence Energy-Protein Rations on Performance of Chicken Kampung-Unggul

Ration consumption, weight gain, feed conversion, carcass weight, and Income over feed and chick cost (IOFCC) Chicken Kampung-Unggul, and the data are presented in Table 3.

Average consumption of Chicken Kampung-Unggul ration between 1211.19 g / b (R4) to 1581.69 g / b (R2), weight gain between 443.65 g / b (R4) to 510.31 g / b (R2), feed conversion between 3.12 (R2) to 2.45 (R5), carcass weight between 413.26 g / b (R1) to 486.04 g / b (R2), and the gain (IOFCC) between Rp. 8,632 /Kg (R4) up to Rp. 11,096 / kg (R2).

Table.3: Effects of Ration Energy-Proteins on Consumption of Rations, Weight Gain, feed conversion ratio, Heavy Carcass, and Income over Feed and Chick Cost (IOFCC) of Chicken Kampung-Unggul

Treatment	Consumption of Rations (g/b)	Weight Gain (g/b)	feed conversion ratio (index)	Heavy Carcass (g/b)	IOFCC (Rp./kg)
R1	1402.16 ^{ab}	458.96^{NS}	3.07^{a}	413.26^{NS}	10,439
R2	1581.69 ^{a}	510.31^{NS}	3.12^{a}	486.04^{NS}	11,096
R3	1409.88 ^{ab}	453.02^{NS}	3.11^{a}	424.43^{NS}	9,583
R4	1211.19 ^{b}	443.65^{NS}	2.74^{ab}	419.05^{NS}	8,632
R5	1238.50 ^{b}	507.92^{NS}	2.45^{b}	458.22^{NS}	9,879

Description: R1 = CP 15%, and ME 2750 kcal / kg; R2 = CP 17%, and ME 2750 kcal / kg; R3 = CP 19%, and ME 2750 kcal / kg; R4 = CP 15%, and ME 2950 kcal / kg; R5 = CP 17%, and ME 2950 kcal / kg.

a, b: means with no common superscript differ significantly, SEM: standard error of means: $p<0.05$; NS: Not significant

Based on the result of statistical analysis, the consumption of ration and conversion of real cross section ($P<0.05$), but the weight did not show significant difference to the performance of Kampung-Unggul chicken production. The difference of the metabolic energy of ration is 200 kcal / kg and the protein content of ration is 4% to the consumption and conversion of Kampung-Ungg chicken ration. Chicken Kampung-Unggul relatively unstable to changes in nutrient content in rations (protein and energy).

Growth is an interaction between genetic and environmental factors. Contribution of genetic factors to growth is smaller than environmental factors, environmental factors are more dominant influence on growth. Therefore, the resulting results show the type of chicken to the production performance [15].

Income over Feed and Chick Cost is a barometer to see the cost of feed which is the biggest cost in the livestock business [8]. Factors that affect food income and chicken costs are final body weight, price / kg ration and selling price per kg of live weight. Chicken Kampung-Unggul is more sensitive to changes in nutrient content in the ration. Ration with metabolic energy content of 2750 kcal / kg and 17% crude protein, contributes to production performance, and highest profit yield (IOFCC), although the conversion value of the ration is relatively larger.

The Influence of Rice-Protein Energy on Local Chicken Production Performance-Jimy

Ration consumption, weight gain, feed conversion, carcass weight, and Income over feed and chick cost (IOFCC) Chicken Local-Jimy, and the data are presented in Table 4.

Table.4: Effects of Ration Energy-Proteins on Consumption of Rations, Weight Gain, feed conversion ratio, Heavy Carcass, and Income over Feed and Chick Cost (IOFCC) of Local Chicken-Jimy

Treatment	Consumption of Rations (g/b)	Weight Gain (g/b)	feed conversion ratio (index)	Heavy Carcass (g/b)	IOFCC (Rp./kg)
R1	1698.23 ^{NS}	590.13 ^{NS}	2.88 ^{bc}	520.54 ^{NS}	12,207
R2	1816.46 ^{NS}	631.19 ^{NS}	2.89 ^{bc}	551.72 ^{NS}	13,088

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R3	1662.38 NS	629.38 NS	2.64 c	516.33 NS	13,036
R4	1887.56 NS	571.94 NS	3.30 a	509.99 NS	10,952
R5	1886.55 NS	617.31 NS	3.07 ab	527.89 NS	11,214

Description: R1 = CP 15%, and ME 2750 kcal / kg; R2 = CP 17%, and ME 2750 kcal / kg; R3 = CP 19%, and ME 2750 kcal / kg; R4 = CP 15%, and ME 2950 kcal / kg; R5 = CP 17%, and ME 2950 kcal / kg.

a, b: means with no common superscript differ significantly, SEM: standard error of means: $p<0.05$; NS: Not significant

Average local consumption of Chicken-Jimy chicken ration between 1662.38 g / e (R3) to 1887.56 g / e (R4), weight gain between 571.94 g / e (R4) to 631.19 g / e (R2), feed conversion between 3.30 (R4) to 2.64 (R3), carcass weight between 509.99 g / e (R4) to 551.72 g / e (R2), and the gain (IOFCC) between Rp. 10.952 / kg (R4) up to Rp. 13.088 / kg (R2). Based on the statistical analysis, the five ration treatments did not show a significant difference (P> 0.05) on the performance of Chicken-Local Jimy. The difference of metabolic energy ration of 200 kcal / kg and the protein content of ration by 4% did not affect the production performance of local chickens-Jimy. The Local Chicken-Jimy is relatively stable against changes in nutrient content in the diet (protein and energy).

Rations containing 2950 kcal / kg of metabolic energy and a crude protein of 15% (R4) resulted in the lowest productive performance in Local Chicken-Jimy. Income over Feed and Chick. Rations with a metabolic energy content of 2750 kcal / kg and 17% crude protein, support the production performance, and generate the highest profit (IOFCC).

Effect of Energy-Protein Rations on the Hematology of Sentul-Warso Chicken Blood

The hematologic blood measured was the amount of erythrocytes, blood hemoglobin, hematocrit values, blood proteins, and the fragility of the Sentol Warso Chicken cell, and the data presented in Table 5.

Table.5: Effect of Energy-Protein Rations on Number of Erythrocytes, Blood Hemoglobin, Hematocrit Value, Blood Protein, and Fragility of Sentul-Warso Chicken Blood Cells

Treatment	Erythrocytes (million / mm^3)	Haemoglobin (g/ dL)	Hematocrit (%)	Blood Protein (g/ dL)	The fragility of cells (% hemolysis)
R1	2.98 NS	6.59NS	29.25 NS	4.50 NS	2.94 NS
R2	3.01 NS	6.78 NS	18.06 NS	4.92 NS	2.74 NS
R3	3.18 NS	7.17 NS	24.50 NS	5.40 NS	3.00 NS
R4	3.02 NS	6.25 NS	19.88 NS	4.72 NS	2.67 NS
R5	3.21 NS	7.01 NS	27.75 NS	4.46 NS	3.06 NS

Description: R1 = CP 15%, and ME 2750 kcal / kg; R2 = CP 17%, and ME 2750 kcal / kg; R3 = CP 19%, and ME 2750 kcal / kg; R4 = CP 15%, and ME 2950 kcal / kg; R5 = CP 17%, and ME 2950 kcal / kg.

NS: Not significant

The mean erythrocytes of Sentul-Warso chicken were between 2.98 million / mm^3 (R1) to 3.21 million / mm^3 (R5), blood hemoglobin levels of 6.25 g / dL (R4) to 7.17 g / dL (R3), blood hematocrit values between 18.06% (R2) to 29.25% (R1), blood protein content between 4.50 g / dL) (R1) to 5.40 g / dL (R3), R3) and the fragility of blood cells between 2.67% (R4) to 3.06% (R5). Based on the statistical analysis, the five ration treatments did not show a significant difference (P>0.05) to the hematologic blood of Sentul-Warso chicken. The difference of metabolic energy of ration of 200 kcal / kg and protein content of ration by 4% did not affect the hematological value of Sentul-Warso chicken blood. Sentul-Warso Chicken is relatively stable against changes in nutrient content in rations (protein and energy).

Protein is a major element in the formation of blood erythrocytes. Enzyme protease in the body is an extracellular enzyme that serves to hydrolyze proteins into amino acids the body needs. So nutrients in the form of proteins that have been digested then will be converted into amino acids absorbed by the body can be used one of them for the formation of erythrocytes. In addition to protein, energy as a result of carbohydrate, protein and fat metabolism is also needed during the process of erythrocyte formation. The amount of erythrocytes is influenced by the nation and animal species, sex, age, body condition, daily variation, physical activity, ambient temperature and stress conditions [23]. Erythrocytes basically have three functions, namely the transport of oxygen (O_2) to the body tissues, transport of carbon

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dioxide (CO_2) to the lungs and buffers of hydrogen ions (H^+) [13].

The formation of red blood cells from the reticuloendothelial system formed endothelial specialized in the birth of megaloblast. Megaloblast was born erythroblast and started formation of hemoglobin. Stem cells form a proerytroblast which is the mother of erirocytes. Toward the formation of hemoglobin, erythroblasts can be seen with alkaline staining, so people call it basofil erythroblasts. When hemoglobin has been formed a lot, the basophilic substance mixes with hemoglobin so that the eosin staining can begin to be absorbed and the cell is now called polychromafil eritroblast. In the course of the nucleus is smaller and called normoblast. Over time the nucleus diffuses and hemoglobin continues to be formed so that the cytoplasm no longer absorbs the basophile color and hemoglobin continues to form until it reaches about 34% and forms the reticulocyte. In the poultry core is maintained until the form of erythrocytes [17].

Effect of Energy-Protein Rations on Hematologic Blood Chicken-Superior Villages

The amount of erythrocytes, blood hemoglobin, hematocrit values, blood proteins, and fragility of Kampung-Unggul chicken blood cells, and are presented in Table 6.

Table.6: Effects of Energy-Protein Rations on Number of Erythrocytes, Blood Hemoglobin, Hematocrit Value, Blood Protein, and Fragility of Chicken Cells Kampung-Unggul

Treatment	Erythrocytes (million / mm^3)	Haemoglobin (g/ dL)	Hematocrit (%)	Blood Protein (g/ dL)	The fragility of cells (% hemolysis)
R1	3.01 ^{NS}	6.60 ^{NS}	29.45 ^{NS}	5.51 ^{NS}	4.13 ^{NS}
R2	3.05 ^{NS}	6.82 ^{NS}	25.61 ^{NS}	4.72 ^{NS}	3.68 ^{NS}
R3	3.30 ^{NS}	7.21 ^{NS}	24.00 ^{NS}	5.05 ^{NS}	4.09 ^{NS}
R4	3.04 ^{NS}	6.31 ^{NS}	28.88 ^{NS}	5.08 ^{NS}	4.30 ^{NS}
R5	3.18 ^{NS}	7.02 ^{NS}	27.75 ^{NS}	4.70 ^{NS}	3.60 ^{NS}

Description: R1 = CP 15%, and ME 2750 kcal / kg; R2 = CP 17%, and ME 2750 kcal / kg; R3 = CP 19%, and ME 2750 kcal / kg; R4 = CP 15%, and ME 2950 kcal / kg; R5 = CP 17%, and ME 2950 kcal / kg.

NS: Not significant

Average erythrocytes of Kampung-Unggul chicken between 3.01 million / mm^3 (R1) to 3.30 million / mm^3 (R3), blood hemoglobin levels between 6.31 g / dL (R4) to 7.21 g / dL (R3), blood hematocrit values between 24% (R3) to 29.45% (R1), blood protein content between 4.70 g / dL) (R5) to 5.51 g / dL (R1), and fragility blood cells between 3.60% (R5) to 4.30% (R4). Based on the statistical analysis, the five ration treatments did not show a significant difference (P> 0.05) to the hematological blood of Kampung-Unggul chicken. The difference of metabolic energy of ration of 200 kcal / kg and protein content of ration by 4% did not affect the hematological value of chicken blood of Kampung-Unggul.

Energy and protein have their respective portions of the erythrocyte composition as well as the erythrocyte formation process. Erythrocytes are surrounded by a plasmalemma. Plasmalemma is a cell membrane composed of approximately 40% lipid (phospholipids, cholesterol, glycolipids and so on), 50% protein and 10% carbohydrates. Inside, erythrocytes contain 33% of hemoglobin [16]. Components in the body are determined from the composition of rations consumed by livestock, this is in accordance with the opinion of [14] that the body of livestock is constructed from substances derived from food rations that are consumed.

Decreased hemoglobin levels can occur due to the disorder of erythrocyte formation (erythropoesis). Erythropoesis will increase in blood when iron reserves are reduced. According to [19] factors that affect the amount of erythrocytes in the circulation include the hormone erythropoietin which serves to stimulate the formation of erythrocytes (erythropoiesis) by triggering the production of proeritroblas from hemopoietic cells in the bone marrow. According to [10, 20] can also be caused by the disruption of amino acid synthesis, especially glycine so that hemoglobin synthesis is impaired. According to [11] that proteins, especially amino acids glycine and mineral Fe are the components of hemoglobin forming. So that the suspected decrease in hemoglobin levels can be caused due to disturbances during erythropoesis, and when the erythropoesis occurs there is a disruption of the work of the hormone erythropoietin during the process, it can also be caused by nutrients in the form of proteins that have been digested to be converted into amino acids to be absorbed by the body experiencing interference and this will have an effect on the synthesis of hemoglobin in which the amino acid is required for the formation process. According [21, 24] other factors affecting hemoglobin levels are the age of animals, species, environment, feed, presence or absence

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of erythrocyte defects, and blood handling during examination.

Effect of Energy-Protein Rations on Hematologic Local Chicken Blood-Jimy

The amount of erythrocytes, blood hemoglobin, hematocrit values, blood proteins, and the fragility of the local Chicken-Jimy blood cell, and the data are presented in Table 7.

Table.7: Influence of Rice-Protein Energy on Number of Erythrocytes, Blood Hemoglobin, Hematocrit Value, Blood Protein, and Local Chicken Blood Cell Cellity-Jimy

Treatment	Erythrocytes (million / mm^3)	Haemoglobin (g/ dL)	Hematocrit (%)	Blood Protein (g/ dL)	The fragility of cells (% hemolysis)
R1	3.01 ^{b}	7.60 ^{NS}	30.75 ^{NS}	4.,88 ^{NS}	2.22 ^{b}
R2	3.52 ^{a}	7.31 ^{NS}	34.06 ^{NS}	4.79 ^{NS}	2.10 ^{b}
R3	3.58 ^{a}	7.63 ^{NS}	34.63 ^{NS}	5.40 ^{NS}	2.75 ^{b}
R4	3.35 ^{ab}	6.89 ^{NS}	33.34 ^{NS}	4.87 ^{NS}	4.11 ^{a}
R5	2.98 ^{b}	6.38 ^{NS}	30.54 ^{NS}	4.80 ^{NS}	4.01 ^{a}

Description: R1 = CP 15%, and ME 2750 kcal / kg; R2 = CP 17%, and ME 2750 kcal / kg; R3 = CP 19%, and ME 2750 kcal / kg; R4 = CP 15%, and ME 2950 kcal / kg; R5 = CP 17%, and ME 2950 kcal / kg.

a, b: means with no common superscript differ significantly, SEM: standard error of means: $p<0.05$; NS: Not significant

The mean erythrocytes of local chickens-Jimy between 2.98 million / mm^3 (R5) to 3.58 million / mm^3 (R3), blood hemoglobin levels between 6.38 g / dL (R5) to 7.63 g / dL (R3), blood hematocrit values between 30.54% (R5) to 34.63% (R3), blood protein content between 4.88 g / dL (R1) to 5.40 g / dL (R3), and the fragility of blood cells between 2.10% (R2) to 4.11% (R4). Based on statistical analysis, the number of erythrocytes and the fragility of blood cells showed a significant difference ($P <0.05$), but hemoglobin, hematocrit values, and blood protein levels did not show any significant difference in the local chickens. The difference of metabolic energy of ration of 200 kcal / kg and the protein content of ration by 4% have an effect on the amount of erythrocytes and the fragility of blood cells in Chicken-Jimy Local.

The liver synthesizes and releases more than 90% plasma proteins [22]. According to [13] there are three major fractions of proteins in the blood, namely albumin, globulin and fibrinogen. Albumin, fibrinogen, and globulin (50-80% globulin) are synthesized in the liver, while the rest of the other globulins are formed in lymphoid tissue.

Total protein is a very important organic compound, one part of a very important total protein is the plasma protein. Plasma proteins consist of a very complex mixture of simple proteins and conjugate proteins such as glycoproteins and various forms of lipoprotein [9]. Several plasma protein functions are proposed by [8] as a function of transport, immune function, buffer function, and maintaining osmotic pressure. The importance of plasma proteins causes the total protein in the blood to be distributed evenly to the needs of the organs so that the total protein present in the blood increases with the increase in protein content in the ration.

Factors that may affect the total protein concentration are physiologically affected by age, growth, hormonal, gender, nutrition, environment and fluid loss [13]. The environment can cause stress on the cattle so that the cattle will lose their body fluids. Protein is also important to regulate the body's water balance. Plasma proteins such as albumin serve to maintain osmotic pressure in the blood. Therefore, the protein serves to help spread the body fluids evenly between the blood and body tissues.

There are several factors that can affect the physiological erythrocyte fragility, according to [23] that nutritional status, environmental temperature, and genetics can affect the erythrocyte fragility. Nutritional status affects the composition of erythrocyte membrane constituents, such as the views of [24] that the erythrocyte composer consists of components of phospholipids, glycolipids, cholesterol, and proteins (glycoproteins), which are highly dependent on nutritional status consumed by animals. Reported [19] that animals in warmer environments have lower erythrocyte fragility than animals living in wetlands. Furthermore [17] stated that the blood storage in refrigator and anticoagulant use of Ethylene Diamine Tetra Aceticacid (EDTA) can increase erythrocyte fragility.

IV. CONCLUSIONS

1. Ration with a metabolic energy content of 2,750 kcal / kg and 17% crude protein, resulting in optimal production and hematological blood values in broiler chickens.

2. The performance of Chicken-Local production of Jimy (consumption of ration = 1816.46 g / e; weight gain = 631.19 g / e; conversion of rations = 2.89; carcass weight = 551,72 g / e; and IOFCC = (Ration consumption = 1761,47 g / e) weight gain = 596,38 g / e; conversion of ration = 2,98; carcass weight = 569,73 g / e and IOFCC = Rp 12,647 / kg), and the lowest Kampung-Unggul chicken (consumption of ration = 1581.69 g / e; weight gain = 510.31 g / e; ration conversion = 3.12, carcass weight = 486.04 g / e; and IOFCC = Rp 11,096 / kg).
3. Hematological value of chicken blood Local-Jimy (blood erythrocytes = 3.52 million / mm^3, blood hemoglobin = 7.31 g / dL; hematocrit blood = 34.06%; blood protein = 4.79 g / dL; blood cells = 2.10% haemolysis), chicken Sentu-Warso (blood erythrocytes = 3.01 million / mm^3, blood hemoglobin = 76.78 g / dL; hematocrit blood = 18.06%; blood protein = 4.92 g / dL, and the fragility of blood cells = 2.74% hemolysis), and Chicken Kampung-Unggul (blood erythrocytes = 3.05 million / mm^3, blood hemoglobin = 6.82 g / dL; hematocrit blood = 25.61% blood = 4.72 g / dL, and the fragility of blood cells = 3.68% hemolysis) is in the normal range.

V. SUGGESTION

The preparation of ration formulas for local broilers is recommended using the metabolic energy content of 2750 kcal / kg with crude protein of 17%. Chicken Local-Jimy is more suitable to be developed in Indonesia to generate greater profit.

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Prebiotics^{BAS} (*Bacillus sp.*, *Aspergillus n.*, and *Sacharomyces c.*) as Feed Supplement on Nutrients and its Effects on Digestibility Value of Fish Feed

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Abstract— *Feed quality shown from level of digestibility can affect fish growth.Some of omnivorous fish have complete digestive organs as a place to live abiotic and biotic ecosystems in the form of living microflora. Growth performance can be improved through the addition of exogenous microflora as feed supplements to help produce simpler components of food substances (amino acids, fatty acids, simple sugars, organic vitamins and minerals). The microflora tested consisted of bacteria Bacillus sp. and fungi (Aspergillus niger and Saccharomycescereviseae) with optimization of its prebiotic bioprocess conditions (bioprocess temperature, inoculum dose, and bioprocess time). Furthermore, to see the quality and value of benefits of feed supplement products, measurements were taken of their digestibility.The experiment was carried out experimentally in a laboratory in two stages. The first stage, using a nested design (3×3) which was repeated three times. The second stage used a completely randomized design, consisting of six ration treatments and repeated four times. The variables observed in the first stage: nutrient content (crude protein, crude fibre, extract ether, calcium and phosphorus) of prebiotics^{BAS}; second stage: digestibility of dry matter and crude protein.The data were subjected to analysis of variance, and the differences between treatments were tested by Duncan's multiple range test.Conclussion: The following results were obtained the best bioprocess conditions for making Prebiotics^{BAS} from Bacillus sp. was a dose of 2% with temperature of 45°C, and fermentation time 2 days, while Aspergillus niger 2% at a temperature of 35°Calong 2 days, and Saccharomyces cereviseae 2% with a temperature of 35°C, and fermentation time 2 days. The use of a mixture of three types of microbial each a much 1.5% in the ration, resulting in the best digestibility value in fish. The dry matter and crude protein digestibility valueof Prebiotics^{BAS} were respectively 76.07%, and 75.28%.*

Keywords— BAS (Bacillus sp. Aspergillus niger, Saccharomyces cereviseae), digestibility of fish feed,optimization of bioprocess, Prebiotics.

I. INTRODUCTION

Fish growth is influenced by feed quality, because it has a simple digestive tract which is a small tube that extends. The performance of the digestive tract can be increased through the addition of exogenous microflora as feed supplements (prebiotics) to help increase digestibility and feed efficiency. The mechanism of prebiotics that is quite beneficial is that it can stimulate add enzymes related to the digestive process of complex substances or enzymes that are not present in the digestive tract; and synthesize essential substances that are not enough in quantity from food [3,6]. The prebiotics tested consisted product of bacteria (*Bacillus sp.*), and fungi (*Aspergillus niger*and *Saccharomyces cereviseae*), and their mixtures. The combination of these cultures is expected to be able to support each other (synergism) in excellence and cover up each other's shortcomings, so as to improve the performance of microflora that live in the intestines of fish, which in turn can increase the digestibility of food substances [7, 9]. To get quality feed additives,optimization of Prebiotic bioprocess conditions (inoculum, duration, and bioprocess temperature) was carried out. Furthermore, to see the quality and value of benefits of feed additives products, measurements were taken of their digestibility.

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II. MATERIALS AND METHODS

Producing Prebiotics^{BAS}(Optimization of Bioprocess Conditions)

The first stage of the experiment was to obtain product optimization, namely: Inoculum doses of *Bacillus sp*., *Aspergillus niger* and *Saccharomycescereviseae*, long and bioprocess temperatures that produce the best nutritional content.Fermented media (shrimp skin, rice flour and molasses), *Bacillus sp*. bacteria, molds of *Aspergillus niger*, yeast *Saccharomyces cerevisiae*, nutrient agar and standard mineral solutions. Other ingredients used were distilled water, glucose, yeast extract, technical glucose, tryptone, NaCl, NaOH, azo-casein reagent, borate buffer, phosphate buffer, citrate buffer, bicarbonate buffer, TCA, oxygen gas and Bovine Serum Albumin. The tools used are stainless jars (reactors), water heaters, autoshakerbath, autoclaves, goblets, Bunsen burners, petri dishes, porcelain cups, centrifuges, funnels, pH-meter Knick, spectrophotometer, test tubes, furnaces, HPLC, and grinding machines.

Stages of Making the Prebiotics

Make a starter inoculum by culturing microbes in 125 ml Erlenmeyer containing 50 ml of sterile Luria broth, pH 7, incubated in an incubator (2 days at 30-35^{o}C), and the number of colonies is calculated using the Total Plate Count (TPC) method (minimum number of colonies is 109 per ml or per g).Bioprocess media (shrimp skin, rice flour, and molasses, 0.5% (b/v) yeast extract; 0.5% (b/v) KH_2PO_4; 0.1%(b/v) $CaCl_2$; 0,5% (b/v) NaCl, and 0.05% (b/v) $MgSO_4$ was inoculated by *Bacillus sp*., *Aspergillus niger*, and *Saccharomyces cereviseae*, and a standard mineral solution was added.Bioprocess in auto-shakerbath (temperature 25^{o}C; 35^{o}C; 45^{o}C, dose 1%; 2%; 3%, time 1 day, 2 days, 3 days for each treatment.

Experimental Design

Experiments used a completely randomized design (7×3) for each process condition for each microbe used. From the combination of treatments, the variables he observed were; nutritional content of the product (crude protein, extract ether, crude fiber,calcium, and phosphorus. The selected treatment was used for the second phase of the study.

Table.1: Composition of Ration and Nutrient Content (%)

Treatments of Ration	CP	EE	CF	Ca	P
	……..……..……..…….. %				
R0 (Basal of ration)	30,02	6,90	7,56	1,51	0,87
R1 (97% R0 + 3% FSPB)	30,06	6,83	7,59	1,59	0,91
R2 (97% R0 + 3% FSPA)	30,00	6,84	7,56	1,58	0,90
R3 (97% R0 + 3% FSPS)	29,98	6,85	7,56	1,58	0,90
R4 (97% R0 + 1,5% FSPB+1,5 FSPA)	30,03	6,83	7,57	1,59	0,91
R5 (97% R0 + 1,5% FSPB+1,5 FSPS)	30,02	6,84	7,58	1,58	0,90
R6 (97% R0 + 1,5% FSPA+1,5 FSPS)	29,99	6,84	7,56	1,58	0,90
R7 (97% R0 + 1% FSPA+1 FSPS+ 1% FSPS)	30,02	6,84	7,57	1,58	0,90

FSP = feed supplement of prebiotics (B : *Bacillus sp*.; A : *Aspergillus niger*; S : *Saccharomyces cerevisiae*).

Second Phase Experiment (Determination of Digestibility Value)

Materials and Tools

Tested fish: 240 red tilapia fish with 200 ± 10 g body weight.

The tools used in this study are: 1m^3 volume of fiber-blower tubes, aerator,thermometer analytical scales O-thirst scales, pH meters and spectrophotometers "Milton Roy Spectron, gloves, wipes, tweezers, threads, and scalpels, ovens and aluminum foil, pellet printing machine, and installation of lignin testers and protein testing installations the Kjehdahl method.

The experiment was carried out in three stages

Adaptation stage to familiarize fish with the test feed and estimate the length of feed in the digestive tract which is indicated by the initial discharge of feces, and determine the frequency of feeding.

collection of feces (2 weeks): Feed is given ad libitum, on the last day of the study fish were dissected and feces were taken.Stool analysis phase, which includes: fresh weight, dry weight, and oven dryness, protein analysis and feed lignin content.

The variables observed

Consumption of dry ingredients and ration lignin (grams); drymatter and faecal lignin (gram) and calculated [10, 13] formula as follows:

$$\text{Digestion coefficient} = 100\% - 100\left(\frac{\%\ ligninfeed}{\%\ ligninfeces} \times \frac{\%nutrientsfeces}{\%nutrientsfeed}\right)$$

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The experiments were performed in the laboratory using acomplete randomized design, consisted of 8 treatments of Prebiotic each of which was repeated four times. The data obtained were analyse byVariance (Test F) and differences between treatments were tested by Duncan Test.

III. RESULTS AND DISCUSSION

Nutritional Contents of Prebiotics^{BAS}

Observations on the bioprocess temperature conditions were carried out at the specified time and dose, i.e. for 2 days at a dose of 2%. While the observation of the dose and time is carried out at the selected temperature. The results of the Analysis Variance showed that various levels of temperature, dose, and time had significant (P <0.05) content of crude protein, crude fat, crude fibre, calcium and phosphorus both in *Bacillus sp*., *Aspergillus niger*and *Saccharomyces cerevisiae* products. To find out how much difference in effect between treatments, Duncan's multiple distance test was carried out which results can be seen in Table 2, Table 3, and Table 4.

Table.2: Effect of Bioprocess Temperature on Product Nutritional Content

Microbes	Doses	Nutrients				
		CP	EE	CF	Ca	P
		……..(%)……..				
Bacillus sp.	S1	27.59 A	5.04 B	9.33 A	3.88 A	1.69 A
	S2	28.09 A	4.76 AB	9.00 B	4.05 AB	1.83 A
	S3	30.91 B	4.63 A	8.74 B	4.16 B	2.09 B
Aspergilus niger	S1	27.89 A	5.07 B	9.07 A	3.70 A	1.56 A
	S2	29.19 B	4.85 AB	8.14 B	4.04 B	1.91 B
	S3	29.23 B	4.70 A	7.93 B	4.08 B	1.97 B
Sacharomyces cerevisiae	S1	27.76 A	5.20 B	8.22 A	3.67 A	1.70 A
	S2	28.48 A	5.06 AB	8.16 A	3.74 A	1.86 B
	S3	28.52 A	4.89 A	7.99 A	3.83 A	1.88 B

S1 = 25ºC; S2= 35ºC ; S3 = 45ºC

Table 2 shows that the increasing treatment temperature tends to increase protein, calcium and phosphorus products, both in bioprocess *Bacillus sp*., *Aspergillus niger* and *Saccharomyces cerevisiae*. This is supported by the opinion of [15], that protein and mineral content of bioprocess Prebiotics microbiologically will experience an increase in line with the increase in temperature to some extent. The use of 45 ^{0}C (S3) temperature in the Prebiotic bioprocess of *Bacillus sp*. significantly affected the highest crude protein (30.91%) compared to the temperature (25^{0}C) S1 and 35^{0}C (S2). This shows that *Bacillus sp*. is more effective at working on substrate at a temperature of 45 ^{0}C. The results of this study are in line with opinion [8], that *Bacillus sp*. is thermophilic and has a maximum growth temperature of 50-55^{0}C. The Effect of Temperature on Bioprocess *Bacillus sp*. and *Aspergillus niger* on changes in other nutritional composition, are: a decrease in crude fibre content, an increase in fat content, and an increase in calcium levels which produce at a temperature of 45 ^{0}C produce the greatest changes. Bioprocess of *Aspergillus niger* can be done at 35ºC. Whereas in the Prebiotic S. cerevisiae bioprocess, all three temperature treatments did not show significant differences in protein, crude fibre and calcium content. According [5], *Aspergillus niger* fungi grow well in the temperature range of 32-33ºC, with a pH of 2.8-8.8 and humidity of 80-90%. Whereas *Bacillus sp*. has a maximum growth temperature of 50-55ºC, and *Saccharomyces cereviseae* can grow at room temperature [8]. However, these three temperature treatments can change the substrate into a product whose nutritional content is better. Observation of the 2condition of bioprocess dosage was carried out at the selected temperature, namely *Bacillus sp*. 45ºC, *Aspergillus niger* 35ºC and*S. cereviseae* 25ºC; with bioprocess for 2 days. The number of microbes planted determines bioprocess products. The dosage level of the inoculum and time is related to the size of the microbial population that has the opportunity to determine the speed of microbial development in producing enzymes to remodel the substrate, which in turn affects the final product. In this study, D1 turned out to produce the lowest nutritional content, meaning that the inoculated microbial population was not enough to be used to remodel the substrate to its full potential. From the results of this study almost in total doses of 2% inoculum (D2) produced a nutritional content that was not significantly different (P <0.05) with a dose of 3% inoculum (D3), although the crude fibre content of the product feeds on Prebiotic supplements at lower D3. D2 is an effective inoculum dose to produce optimal crude protein content of Prebiotic^{BAS} products. In accordance with the opinion [17], that the number of

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microbes that are too much can cause sporulation that is too fast so that some of the energy is not used to multiply cells, and vice versa, the number of microbes that are too few causes optimal growth.

Table.3: Effect of Bioprocess Dose on Nutritional Content Product

Microbes	Doses	Nutrients				
		CP	EE	CF	Ca	P
		……..……..……..……..……..(%)……..….				
Bacillus sp.	D1	27.74 A	5.00 B	9.33 A	3.88 A	1.54 A
	D2	31.19 B	4.27 AB	8.84 AB	4.23 B	1.91 B
	D3	30.98 B	4.13 A	8.66 B	4.22 B	2.11 B
Aspergilus niger	D1	27.70 A	5.08 B	8.77 A	3.61 A	1.57 A
	D2	29.36 B	4.63 AB	7.12 B	4.17 B	1.86 B
	D3	29.54 B	4.51 A	6.90 B	4.22 B	1.97 B
Sacharomyces cerevisiae	D1	26.72 A	5.26 B	8.25 A	3.66 A	1.62 A
	D2	28.65 B	5.14 AB	7.13 B	3.89 B	1.79 AB
	D3	28.39 B	4.96 A	7.14 B	3.99 C	1.96 B

D1 = 1%; D2= 2% ; D3 = 3%

Observation of the condition of the time when bioprocess was carried out at the specified temperature and dosage, i.e. for each *Bacillus sp*. bacterium 45ºC,*A.niger* 35ºC and *S. cerevisiae* 25ºC; with a dose of 2%. Table 4 shows that time has a significant effect on increasing the nutritional content of the prebiotic products of the three types of microbes (bacteria and fungi). Bioprocess time 1 day (W1) produces the lowest protein, calcium and phosphorus. The size of the three nutrients can show the quality of nutrients in terms of chemistry. Similarly, W1has the highest crude fibre content, which means that the crude fibre component in the substrate has not been optimally converted into simple sugars. Whereas bioprocess time 2 and 3 days did not show a significant difference in nutrient content. As with other bioprocess conditions (inoculum dose level), the length of time the microbiological fermentation process is related to the size of the microbial population that has the opportunity to determine the speed of microbial development in producing enzymes to remodel the substrate so that in turn affects the nutritional content of the final product. Table 4. Duncan's Multiple Distance Test Effect of Bioprocess Time on Product Nutritional Content.

Table.4: Duncan's Multiple Distance Test Effect of Bioprocess Time on Product Nutritional Content

Microbes	Time	Nutrients				
		CP	EE	CF	Ca	P
		……..……..……..……..(%)……..….				
Bacillus sp.	W1	26.77 A	4.72 B	9.22 A	3.81 A	1.64 A
	W2	31.59 C	4.24 A	8.35 B	4.29 B	2.14 B
	W3	28.18 B	4.17 A	8.26 B	4.11 B	1.92 AB
Aspergilus niger	W1	26.97 A	5.13 B	8.74 A	3.74 A	1.65 A
	W2	29.47 B	4.53 A	6.93 B	4.10 B	2.08 B
	W3	30.44 B	4.47 A	6.80 B	4.31 B	2.05 B
Sacharomyces cerevisiae	W1	27.08 A	5.38 A	8.74 A	3.59 A	1.62 A
	W2	29.64 B	5.22 A	7.43 B	4.13 B	1.95 B
	W3	29.17 B	5.05 A	7.20 B	3.90 AB	2.01 B

W1 = 25 ºC; W2= 35 ºC ; W3 = 45 ºC

Sum of colonies andNutrient content, Before andAfter Bioprocess.

Table.5: Nutrients content of Substrate and PrebioticsBAS Product of Bioprocess.

No		Sum of colonies	CP	EE	CF	Ca	P
		× 10^9CFU	……..……..(%)……..….				
1	Initial Bioprocess	4,01-4,42	22,19	5,91	12,82	3,41	1,44
2	Product *Bacillus sp.*	15,22	31,23	4,38	8,64	4,22	2,05

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3	Product *A.niger*	11,92	29,34	4,67	7,40	4,10	1,95
4	Product *S.cerevisiae*	10,66	28,68	5,18	7,59	3,90	1,77

In Table 5 it appears that there is a change in the composition of the bioprocess substrate in the manufacture of Prebiotic products. This is in line with the opinion [14], which states that in bioprocess there will be changes in complex molecules or organic compounds such as proteins, carbohydrates and fats into simpler molecules. The protein content of Prebiotic B products contains the highest protein because *Bacillus sp*. is a species of bacteria that is able to produce relatively high amounts of protease [8], and multiply rapidly so that it becomes the microbial protein (from 12.82% to 7.4%). According [12], *Aspergillusniger* is one of the fungi that is reported to be capable of producing cellulase enzymes. Cellulase derived from *Aspergillus niger* is in the form of a cellulase complex and is capable of being produced in sufficient quantities. Fish use protein as an energy source compared to other types of livestock, and tend to be less able to utilize carbohydrate sources, especially those with high crudefibres[2]. The use of microbes has changed the composition of the substrate to be more qualified as a process of digesting "outside the body" and a source of microbial enzymes. *Aspergillus niger* produces cellulase enzymes which can degrade cellulose (a component of crude fibre) into glucose (a source of energy for fish) as well asS. cerevisiae can work to break down starch to be simpler.

Digestibility of Feed Supplements

Supplements on Digestion Bioprocess results were selected in stage 1, used as supplement feed (supplementary feed) containing Prebiotics.Results of biological tested for effectiveness through measurement of digestibility value at tilapia fish, can be seen in Table 6.

Table.6: The Effect of Feed Supplement Prebiotics BAS on Digestibility Value of Dry Matter and Protein.

RationTreatments	Digestibility Value	
	DM Dig.	Protein Dig.
	..... (%).....	
R0 (Basal Ration; without prebiotic)	65,83 E	64,17 F
R1 (97% R0 + 3% Prebiotic^{B})	70,11 D	69,10 D
R2 (97% R0 + 3% Prebiotic^{A})	70,16 D	68,82 DE
R3 (97% R0 + 3% Prebiotic^{S})	69,11 D	67,68 E
R4 (97% R0 + 1,5%Prebiotic^{B} + 1,5% Prebiotic^{A})	74,52B	73,64 B
R5 (97% R0 + 1,5%Prebiotic^{B} + 1,5% Prebiotic^{S})	74,07 B	72,34 C
R6 (97% R0 + 1,5%Prebiotic^{A} + 1,5% Prebiotic^{S})	72,35 C	71,21 C
R7 (97% R0 + 1% Prebiotic^{B}+ Prebiotic^{A} +1% Prebiotic^{S}	76,10 A	75,39 A

Table 6 shows that the value of digestibility of dry matter and crude protein in treatment R0 was lower ($p<0.05$) compared to the treatment of rations containing feeds of Prebiotic supplements. The low digestibility value in the R0 treatment was caused by the fact that rations without using supplement feeds were not sufficiently supportive to improve the performance of the digestive tract, even though the ration contained protein that was in accordance with the nutritional needs of red tilapia. Especially in R0 as well as other treatment rations containing crude fibre that exceeds the 4% tolerance limit according [16], so that in the absence of Prebiotics as a source of exogenous enzymes and intestinal microflora balancer, it does not support the effectiveness of the digestive tract.

High digestibility with R3, while R2 shows no significant difference with R3 and R1 treatments. *Bacillus sp*. is proteolytic so it helps digest protein [11], so that it can help protein digestibility more than other microbes. Protein digestibility which contains a combination of 1.5% Prebiotics and 1.5% Prebiotics (R4) was significantly higher than the combination of two other types of Prebiotics. This is because *Bacillus sp*. is a protein remover, *Aspergillus niger* is a rough fibre remover so that both are synergistic. Decrease in crude fibre content will have an impact on the digestibility value, which in turn will also affect digestibility. In line with the opinion [18], which states that crude fibre is one of the food substances that affect digestibility. The use of feed supplement combination of the three types of bacterial bioprocess, mold and yeast products resulted in the highest dry matter digestibility and protein digestibility compared to a combination of two types of Prebiotics, and differed significantly when compared with the use of one type of prebiotics. This can be understood because *Bacillus sp*. is proteolytic so it helps digest protein [11], *A. niger* is cellulolytic and amylolytic [19], so it helps to degrade carbohydrates; whereas *S. cerevisiae* is amylolytic and stimulates appetite [1, 19].

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The combination of these cultures is expected to be mutually supportive (synergism) in excellence and cover up for each other's deficiencies, because according [4]Prebiotics can improve the performance of living microflora and intestinal ecosystems in fish intestines, which in turn can increase the digestibility of nutrients.

IV. CONCLUSIONS AND SUGGESTIONS

Conclusions

1) The temperature of 45^0C in the Prebiotic bioprocess *Bacillus sp*. is the best bioprocess condition to increase the protein content of the product. While the Prebiotic*Aspergillus niger* can be carried out at temperatures of 35^0C and 45^0C. Preparation of *Saccharomyces cerevisiae*Prebiotics can be carried out at a temperature of 25-45^0C.
2) The effective dose in making Prebiotics^{BAS}is 2%, with bioprocess time for two days. Bioprocess feed supplement The Prebiotics^{BAS}product produces an increase in the number of colonies and nutrient content of the substrate. The initial substrate protein content is 22.19%; and the bioprocess results obtained protein content of Prebiotic^{B} products of 31.23% higher thanPrebiotic^{A}(29.34%); and Prebiotic^{S}(28.68%).
3) The use of a mixture of three types of microbes (bacteria, and yeast) from Prebiotics^{BAS} Products can increase the value of dry matter digestibility and crude protein digestibility of basal rations (without using Prebiotics^{BAS} supplement feed). Value of dry matter digestibility and crude protein basal ration, that is equal to 65.83% and 64.17%; each increased to 76.10% and 75.39%.

Suggestions

Further research is needed to determine the effectiveness of the use of Prebiotics^{BAS} as feed supplements to growth, feed conversion, composition of intestinal microflora and feed efficiency through experiments on feeding red tilapia fish starting from the seed stage.

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Use of bioremediation for the removal of petroleum hydrocarbons from the soil: an overview

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Abstract**—** *Large amount of organic and inorganic compounds are released constantly in the environment as a consequence of human activity and technological and industrial advancement. Environmental pollution by petroleum and petrochemicals, such as petroleum hydrocarbons (PHCs), is considered one of the most serious hazards today due to its worldwide distribution. Contamination by these pollutants causes degradation of global environment and a substantial reduction in biodiversity. In addition, a deep removal of the pollutants is often required to prevent their migration into the water, air and therefore threaten human health. In this way, the search for ecologically sustainable approaches to repair contaminated environments have been of great concern in society. Bioremediation is a technique, based on the metabolic activity of living organisms, which aims to reduce, degrade and/or remove contaminants from the marine and terrestrial ecosystems. It is a more economical and more efficient process to minimize waste, compared to the usual physical-chemical treatment methods. Historically, bioremediation has been used to restore environments polluted by PHCs, where microbial communities play a key role during this course, either by the direct degradation of pollutants or by interaction with other microorganisms. Finally, this review discusses about the soil contamination by PHCs, the role of living organisms in this mechanism and their recent application in bioremediation process.*

Keywords**— PHCs, pollutants, environment, remediation, microorganism.**

I. INTRODUCTION

The pollution of the environment increases at an alarming rate. Large amounts of organic and inorganic compounds are released into the environment continuously, as a consequence of human activity, technological advance, and indiscriminate use of agricultural practices [1, 2]. Crude oil contains a range of compounds toxic to humans and to the environment, including PHCs. Some compounds that are classified as PHCs are better known as BTEX (benzene, toluene, ethylbenzene and xylene). In terms of harmful effects on health, benzene is one of the most concerning compounds due to its carcinogenic effect. Long-term exposure to benzene may cause bone marrow abnormalities. Ethylbenzene is recognized as potentially carcinogenic since its inhalation increases the incidence of renal, testicular, and liver tumors. Toluene and xylene exhibit acute and chronic toxicity in the central nervous system of humans and animals [3, 4].

1.1 Bioremediation

Bioremediation is a technique that exploits the ability of living organisms to reduce, degrade and/or remove contaminants from marine and terrestrial ecosystems, thereby minimizing the risk to human health by restoring the ecosystem to its normal condition [1, 5, 6]. The fundamental principles of bioremediation involve reducing the solubility, redox reactions and the adsorption of contaminants from the polluted environment [7]. The success of this technique also depends on the nature of the pollutant, which may be hydrocarbons, heavy metals, agrochemicals, greenhouse gases, nuclear waste, sewage [8].

Bioremediation technologies are based on chemical oxidation/reduction reactions. These reactions modify the chemical composition, from the addition of reagents, generating an increase of the degradation and extraction of contaminants, converting them into less toxic, less mobile or inert compounds [7].

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Depending on the application site, bioremediation techniques can be classified *ex situ* or *in situ*. *In situ* bioremediation implies a cleaning treatment of pollutants in their place of origin. It does not require excavation, therefore it results in little or no disturbance in soil structuring. This bioremediation has been successfully used in the treatment of hydrocarbons, heavy metals and dyes [7]. *Ex situ* bioremediation involves the excavation of the pollutants and subsequently transporting it to another treatment site. To choice the suitable technique which should be used, certain variables such as the type of pollutant, the degree of pollution and the geology of the contaminated site must be considered [8].

The usual *in situ* and *ex situ* physico-chemical treatments for remediation of PHCs involve costly strategies, and often result only in the incomplete decomposition of the pollutants. Thus, in the last two decades, alternative remediation techniques based on biological methods have been progressively accepted as a standard practice, since they are more efficient in minimizing waste and preserving natural resources, as well as being more cost-effective [6, 9].

Environmental pollution by oil and PHCs is considered one of the most serious current problems [10, 11]. Historically, bioremediation has been used for restoration of environments polluted by PHCs [5, 6, 12]. Bioremediation has become an alternative method of remediation of oil-contaminated areas, where microbial communities play a key role during the process, either by direct degradation of pollutants or by interaction with other microorganisms added [13]. However, environmental conditions such as temperature, substrate availability, presence of suitable microorganisms, pH and humidity, directly influence the growth and metabolism of microorganisms, making these the main limiting factors for the success of bioremediation [7].

Many bacterial strains have been described capable of degrading PHCs, among them the species of *Pseudomonas*, *Acinetobacter*, *Mycobacterium*, *Haemophilus*, *Rhodococcus*, *Paenibacillus* and *Ralstonia* [14]. This ability is attributed to the presence of genes and enzymes that use chemical complexes present in petroleum as vital sources of energy. In some situations, these bacterial enzymes need the plants to remove the pollutants, a process that is called phytoremediation [15]. Phytobioremediation is an alternative of remediation of organic pollutants and contaminants of heavy metals, using plants and associated microorganisms, to metabolize and degrade contaminants found in the most varied habitats. Phytobioremediation is considered an efficient method because it is ecologically correct and economical, being a good alternative for the effects generated by the growth of the petroleum industry [16, 17]. However, the expected success depends on the level of contamination, amount of contaminating metal, as well as the absorption capacity of heavy metals by plants and microorganisms [6].

The treatment of contaminated soils through bioremediation involves two main strategies, biostimulation and bioaugmentation. Biostimulation consists of the manipulation of environmental variables, introducing essential nutrients or biosurfactants, in order to increase the degradation of PHCs by native microorganisms [5, 13]. The bioaugmentation is based on the increase of the microbial population with degradative capacities, through the addition of oil degrading microorganisms to the contaminated soil matrix. Bioremediation is ideal for circumstances in which native microorganisms cannot perform pollutant degradation. Both techniques can be applied separately or in combination [5, 18]. The diversity and abundance of microorganisms present in polluted environments directly affect the success of the remediation technique employed. Once the PHC removal process has started, the availability of the free contaminant and its potential to penetrate the membrane of the organism have determined the rate in which the contaminant can be absorbed by the microorganism [8].

II. OIL HYDROCARBONS

During the process of extraction, refining, storage and transport of PHCs, considerable amounts of this product are released into the environment, with spillage being the main route of contamination [5, 19]. The need of oil and other compounds as alternative sources of energy has contributed to the increase in pollution resulting from this class of pollutants [8].

PHCs are organic pollutants of great concern due to its toxicity and extensive worldwide distribution. They are classified into two categories, diesel range hydrocarbons (DRHs) and gasoline-range hydrocarbons (GRHs). DRHs include longer chain alkanes and hydrophobic chemicals, such as polycyclic aromatic hydrocarbons (PHCs). GRHs include hydrocarbons, such as ethylbenzene, benzene, xylenes and toluene [6].

For more than half a century, procedures in the petrochemical industry have indiscriminately caused the release of hydrocarbons and related pollutants, causing degradation of the environment and a considerable reduction in soil biodiversity. It is not only a social and sanitary issue, but it is also an economic issue, by way of these problems are harmful to local populations living on agriculture, an important pillar for the world economy [3]. In addition, deep cleaning is often necessary in order to prevent the migration of contaminants into the water, air and hence threaten human health [5, 20]. Prolonged

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exposure to PHCs can impair the central nervous system and endocrine system, increasing the risk to develop bladder, kidney, lung and skin cancers [21-23]. The attempt to create an appropriate and effective protocol for decontamination by petrochemicals remains an enigma [24, 25]. Factors such as the geological formation, composition and types of hydrocarbons found in different regions should be considered. Therefore, a single and unvarying approach does not seem appropriate. In view of this, the search for new ecologically sustainable approaches to repair environments contaminated with PHCs is of major importance [3]. Over the years, various researches have been published with technologies available to deal with contaminated soils. Bioremediation based on the metabolic activity of microorganisms for restoration of environments contaminated by a range of contaminants presents certain advantages. Nonetheless, a successful application for PHCs remains a challenge.

III. BIOLOGICAL SOLUTIONS FOR CONTAMINATION

The ability of certain microorganisms to utilize PHCs as a carbon source in their metabolism has been proven for about 80 years [18]. For the purpose of promote an efficient bioremediation of PHCs, it is necessary to add fertilizers rich in nitrogen (N) and phosphorus (P), as these elements promote the growth of the local microbial community [6, 26].

The degree of susceptibility of PHCs to different bioremediation techniques can be evaluated by understanding three parameters: (a) microbial properties (regulation and gene expression, metabolic diversity, adhesion mechanisms, metal tolerance, chemotaxis); (b) environmental factors (nutrient availability, salinity, pressure, temperature, pH, water availability); and (c) hydrocarbon substrate properties (solubility, concentration, hydrophobicity, volatility, molecular mass) [6, 27, 28].

Among the microorganisms with the capacity to restrain the hydrocarbons in the soil, bacteria and fungi stand out.

3.1 Bacteria

Bacteria are efficient microorganisms in the petroleum degradation process [29]. Certain bacteria such as *Bacillus* sp., *Pseudomas* sp. and *Chomobacterium vinosum* reduce the total hydrocarbons by the secretion of lipases that can hydrolyze the constituent fatty acids [30].

The P-1 strain of *Pseudomonas* sp. was able to degrade crude oil and hydrocarbons such as hexadecane. Bacteria of this genus are known for the ability to produce biosurfactants, which, together with the degradation capacity of PHCs, make them advantageous options in the bioremediation of contaminated soils [31]. Four species of biosurfactant-producing *Pseudomonas*: P. *acidovorans*, P. *cepacia*, P. *picketti* and P. *fluorescens*, had the ability to remove 80% of motor oil present in the soil [32]. The strains of *Bacilli*, *Bacillus stratosphericu*, *Bacillus subtilis*, *Ochrobactrum* sp. and *P. aeruginosa*, isolated from the soil contaminated by creosote, present great potential for hydrocarbons degradation by the production of biosurfactants [33]. *Bacillus subtilis* besides producing surfactants, also has the ability to degrade hydrocarbons [34]. Both bacteria, *P. aeruginosa* sp. and *B. subtilis*, use PHCs as a source of carbon and energy. However, some studies found a greater activity when both were previously exposed to the pollutant [35, 36]. Furthermore, these bacteria are used in tropical countries to treat contaminated soils, since they are thermophilic microorganisms [37]. Other studies reported the association of bacteria as a potent bioremediator. Two species of *Sphingomonas*, B0695 and EPA505 were evaluated for their bioremediate effect separately and in combination. When hydrocarbons are exposed to bacteria individually, only low-weight hydrocarbons (naphthalene, phenanthrene and fluoranthene) are degraded. However, all contaminants were degraded when exposed to both bacterial species simultaneously, indicating that the use of the associated species brings advantages in the bioremediation of contaminated soils [38, 39].

3.2 Fungi

Fungi play an important role in bioremediation due to their metabolic activity, ability to secrete enzymes and to survive in extreme environmental conditions. Thus, the use of fungi in bioremediation seems to be the sustainable and economical choice for the treatment of soils contaminated by toxic organic compounds [40]. *White-rot* fungi have a great potential for bioremediation. These fungi produce lignolytic enzymes, responsible for the adsorption of dyes, allowing their application in places that contain dyes in degradation and discoloration of organic pollutants. *Coriolus versicolor, Hirschioporus larincinus, Inonotus hispidus, Phanerochaete chrysosporium, Phlebia tremellosa* are some examples of these fungi. The evaluation of the activity of thirteen lignolytic fungal strains was performed and it was verified that the degree of degradation of aromatic hydrocarbons varied according to the amount of lignolytic enzymes [41]. Moreover, fungi are efficient in the reduction of phenolic compounds, and can be useful in the recovery of soils contaminated by PHCs. Fungi are excellent bioremediators of toxic compounds due to their ability to produce enzymes, such as lipases, that have the capacity to breaks-up highly stable aromatic rings presented in PHCs. Among fungi with potential for degradation of these compounds are *Aspergillus*, *Curvularia*, *Drechslera*, *Fusarium*, *Lasiodiplodia*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma* [42, 43]. Low

molecular weight PHCs are easily degraded by *Aspergillus* sp., *Fusarium oxysporum* and *Trichocladium canadense*. On the other hand, the high molecular weight ones are degraded with higher potential by *T. canadense*, *Aspergillus* sp., *Verticillium* sp. and *Achememonium* sp. [44]. *P. ostreatus* also demonstrated the ability to remove PHCs, such as phenanthrene and pyrene [45]. The fungi present an intracellular network of cytochrome P450 (CYPs) that can be used as catalytic agent of the hydrocarbon oxidation process, being an important mechanism for the successful removal of PHCs [46]. Another mechanism of action of fungi is the production of laccases. Laccases belong to the group of blue oxidases, which use extracellular copper as cofactor and oxygen as co-substrate. Furthermore, they are able to oxidize phenolic and non-phenolic compounds, being observed with a higher activity in fungi [47]. Due to its activity upon a variety of substrates, laccases are considered an ideal catalyst for different industrial applications, such as the use of these enzymes for bioremediation, even in extreme conditions of salinity, permitting their application in the bioremediation of polluted soils and seas [48]. *Phanerocheate chrysosporium* and *Aspergillus niger* fungi produce enzymes that act on hydrocarbon substrates, allowing their efficient application in the process of removing residues from diesel contaminated soils [10].

3.2 Earthworms

Earthworms have been applied in the bioremediation since 1976, after having survived the ingestion of toxic products that leaked after explosion of a factory in Italy. Since then, researches have evaluated the potential of earthworms in bioremediation of soils contaminated with crude oil and by-products. *E. eugeniae* was not the only microorganism able to survive to the high concentration of contaminating diesel, it also reduced the concentration of heavy metals, PHCs and benzene, as well as completely eliminated toluene, ethylbenzene and xylene, adapting to the new soil conditions [3]. A similar result was observed when using *Eisenia foetida* in pyrene bioremediation [49]. Degradation rates increase when *E. fetida* was added to soil contaminated with heavy crude oil [50]. A study evaluating *Eudrilis eugeniae* in soil contaminated with hydrocarbons showed that a greater reduction in hydrocarbon content was obtained when compared to samples that were not exposed to the worm [24]. Soil contaminated with PHCs, phenanthrene and fluoranthene were rapidly bioremediated after exposure to the earthworm species *Lumbricus rubellus* when compared to microorganisms [51].

Nanomaterials: an alternative method to remove pollutants

The use of nanosystems to remove pollutants has important advantages. Nanomaterials have a much larger surface area compared to their total volume, which increases the area of interaction between the substances, the reactivity of reaction and the efficiency in the degradation of toxic compounds, reducing the amount of activation energy and shortening the latency after remediation [52].

Nanomaterials of different shapes and sizes can be applied for environmental remediation [52]. TiO_2 nanotubes are useful in the degradation of pentachlorophenol (PCP), a fungicide and herbicide, toxic to humans [53]. Polyamidoamine dendrimers (PAMAM) are used for the treatment of water as they are considered efficient and harmless to human health [54]. Magnetic iron oxide nanoparticles (IONPs) can be linked to enzymes, such as trypsins and peroxides, to protect these enzymes from oxidation, increasing their shelf life from hours to weeks for them to act as catalysts for remediation process [55]. Laccases, short half-life enzymes capable of catalyzing the oxidation of a series of phenolic compounds, have already been encapsulated in nanoparticles to provide stability of these enzymes over a wide pH and temperature ranges [47]. In this way, polymeric nanoparticles consisted of amphiphilic polyurethane (APU) and containing laccases have high capacity to remove PHCs from the soil [48].

IV. CONCLUSION

This review highlighted the effects of PHC contamination by certain human activities on the environment, possible health risks after long exposure, and characteristics of bioremediation process. The review also emphasized the potential use of microorganisms as biological tools, providing a more economical and efficient alternative to minimize waste and preserve natural resources, intend to be a promising solution to one of the crucial problems of modern society.

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Perceived Role of Agricultural Extension Services in Promoting Cooperative Entrepreneurship among Farmers in Ahiazu Mbaise Local Government Area, IMO State

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***Abstract*—** *Cooperative entrepreneurship avails participating entrepreneurs the opportunity to combine different skills and competencies to set up an enterprise. This study assessed the perceived roles of agricultural extension services in promoting cooperative entrepreneurship among farmers in Ahiazu Mbaise Local Government Area, Imo State. Data was collected from 120 respondents with the aid of a well-structured questionnaire. Results revealed that farmers in the study area were engaged in different entrepreneurial activities. They perceived the roles of agricultural extension services as effective in promoting cooperative entrepreneurship with the provision of vocational/skill training; sanitation activities; provision of storage/processing facilities; procurement of agricultural input and information on credit sources. The perceived constraints militating against extension service delivery to the respondents were inadequate finding; lack of technical support and poor infrastructure in communities. It was recommended that government and non-governmental organizations should provide adequate funding and technical support to extension personnel to enable them deliver agricultural extension services geared at promoting cooperative entrepreneurship to farmers.*

***Keywords*— Role, Agricultural Extension Services, Cooperative, Entrepreneurship, Imo State.**

I. INTRODUCTION

Given the prevailing economic downturn in Nigeria and the high rate of unemployment among the potential labour force, entrepreneurship is a veritable tool to reduce poverty, especially in rural communities where over 70% are farmers. The development of entrepreneurship among the rural areas is a vital tool for achieving economic growth (Mba, 2014). To encourage rural farmers to harness economic potentials that may be available to them within their locality, they need to engage in cooperative entrepreneurship. Cooperatives are a sustainable way of achieving equitable distribution of wealth. Cooperative entrepreneurship is a form of joint entrepreneurship. It involves more than one person and the participating entrepreneurs have the opportunity of combining different skills and competencies to set up an enterprise (McDonnell, MacKnight and Donnelly, 2012).

Cooperatives are being considered useful mechanisms to manage risk for members in agriculture and other sectors, help wage earners save for the future through a monthly contribution that is deducted from source, acquire what might be difficult for individuals to own by their efforts, strengthen the communities in which they operate through job provision and payment of local taxes (Dogarawa, 2005). Agricultural cooperatives play an important role in supporting men and women smallholder farmers and marginalized groups by creating sustainable rural employment. Cooperatives offer market opportunities to smallholder farmers as well as provides them with services such as better training in resource management, better access to information, technologies, innovations and extension services (FAO, 2011). Communal cooperative plays a major role in the collection, preservation and dissemination of technical and cultural knowledge (Odubanjo, 2010).

Extension is a process aimed to teach both the rural and urban clientele how to determine their problems and be

able to rise to such problems using their own resources (Asiabaka, 2002). Kristin (2009) defined agricultural extension as the entire sets of organizations that support people engaged in agricultural production and facilitate their efforts to solve problems; link to markets and other players in the agricultural value chain; and obtain information, skills and technologies to improve their livelihoods. According to this definition, extension goes beyond the traditional view of extension as technology transfer. One of extension's major activities over time has been adult and non-formal education (Agwu and Irohibe, 2013). This role of agricultural extension service is important today in promoting cooperative entrepreneurship among farmers.

Agricultural extension service encompasses the operational process, structure and facilities tailored towards achieving a voluntary out of school education system of transferring useful information and tangible improved farm technologies from their source to affected population. It is a service or system which assists farm people, through educational procedures, in improving farming methods and techniques, increasing production efficiency and income, improving their level of living and uplifting the social and educational standards of rural life (Maunder 2009). Extension plays a very vital role in promoting the development of business plans of entrepreneurs. Extension specialist can assist entrepreneurs in three ways which includes understanding the essential components of a business plan, pro-forma financial analysis and legal issues that affect the industry, operations, cost, market access and future opportunities (Brodsky, 2009). Extension specialist can assist farmers to develop budgets and analyze the cost and benefits of various enterprises.

Increased agricultural productivity at the rural farm level can only be achieved through the provision of agricultural extension services to resource poor of farmers. This will in turn improve the overall quality of life in the rural areas where farmers are predominately domiciled. The need for cooperative entrepreneurship as a sustainable way to create wealth among rural farmers calls for agricultural extension service delivery to these farmers to create awareness on cooperative entrepreneurship as well as educate them on the benefits of joining/forming one. This study therefore assessed the perceived role of agricultural extension services in promoting cooperative entrepreneurship among farmers in Ahiazu Mbaise Local Government Area, Imo State. The specific objectives were to; describe the socio-economic characteristics of the respondents, determine entrepreneurial activities engaged by the respondents; ascertain the perceived roles of agricultural extension in promoting cooperative entrepreneurship as well as to identify the perceived constraints to the discharge of these roles by extension agents.

II. METHODOLOGY

This study was carried out in Ahiazu Mbaise Local Government Area of Imo State. Ahiazu Mbaise Local Government Area is one of the 27 local governments that make up Imo State. It shares common boundaries with Ehime Mbano Local Government Area on the north, Aboh Mbaise Local Government Council on the South and on the West and east are Ikeduru and Ezinihitte Mbaise Local Government Council respectively (IMSG 2009). Ahiazu Mbaise is made up of 27 autonomous communities which includes Amuzi, Umuokirika, Eziama, Mpam, Okirikama, Ihieteaforukwu, Umuocheze. There are two distinct season namely rainy and dry seasons, the annual rainfall is between 26'c and 28'c and relative humidity is about 98%.

The people of Ahiazu Mbaise are predominantly subsistence farmers. Crops cultivated in the area includes yam, cassava, vegetables, maize, plantain, cocoyam, banana, groundnut etc. Livestock's reared are goat, sheep, pig and poultry.

A multi-stage sampling technique was used to select respondents for the study. The first stage involved the selection of twelve (12) communities from Ahiazu Mbaise Local Government Area. The second stage involves the selection of two (2) villages from each of the twelve (12) communities to give a total of (24) villages. The third stage involves the selection of five (5) respondents from each of the 24 villages, to give a total sample size of one hundred and twenty (120) respondents. Primary data was collected for the study using well structured questionnaire. Data were analyzed using both descriptive and inferential statistics.

III. RESULTS AND DISCUSSION

3.1 Socio-economic characteristics of the respondents

The socio-economic characteristics of the respondents investigated were age, sex, household size, level of education, monthly income and membership of co-operative organization. The results of the socio-economic characteristics of the respondents is presented in Table 1. The distribution of the respondents by sex shows that most of the respondents were male (60.0%) with a mean age of 43 years. About 50% were married, and a majority (95%) had formal education. This implies that majority of the respondent were literate. Asiabaka (2017) opines that education remains a vital tool for acceptance of technology by respondents. The mean household size was 8 persons, implying that the household size is relatively large. The mean years of farming experience was 5 years. Entrepreneurs with business experience are better informed on the intricacies of establishing an enterprise.

Hence, their involvement in co-operatives to enable them pool their resources together. Experiences provide veritable information that could be an asset to managing a co-operative enterprise. The average monthly income was ₦ 39, 579.13. It can be concluded that majority of the respondents were low income earners since their earning is below the poverty line of $1 a day (World Bank, 2006). Majority, 96.7 percent of the respondents belong to a co-operative organization. Membership in formal organization satisfies the social needs of the respondents. They could engage in economics of sealed by pooling their resources together.

Table.1: Socio-economic characteristics of the respondents

Socio-economic characteristics	Frequency	Percentage	Mean
Sex			
Male	72	60.00	
Female	48	40.00	
Age			43 years
20 - 29	22	18.34	
30 - 39	21	17.50	
40 - 49	30	25.00	
50 - 59	40	33.33	
60 - 69	7	5.83	
Marital status			
Single	35	29.17	
Married	60	50.00	
Divorced	5	4.16	
Separated	20	16.67	
Level of education			
No formal education	6	5.00	
Primary education	24	20.00	
Secondary education	56	46.67	
Tertiary education	34	28.33	
Household Size			
1 - 3	7	5.83	
4 - 6	35	29.17	
7 - 9	44	36.67	
10 - 12	31	25.83	
13 - 15	3	2.50	8 persons
Business experience			
1-5	43	35.8	5 years
6 - 10	38	31.7	
11 - 15	22	18.3	
16 - 20	17	14.2	
Monthly income			
11, 000 - 20, 000	15		
21, 000 – 30, 000	19		
31, 000 – 40, 000	28		
41, 000 – 50, 000	27		
51, 000 – 60, 000	21		
61, 000 - above	10		₦ 39, 579.13
Member	116	96.7	
Non-member	4	3.3	

Source: Own computation from field survey data, 2017.

3.2 Farmers' involvement in entrepreneurial activities

Table 2 shows the farmers involvement in entrepreneurial activities in the study area. About 70.0% of the farmers engage in poultry production with about 68.3% owning oil palm plantation. Cassava processing accounts for

65.8% and piggery production 56.7%. from the results obtained, the farmers were already involved in entrepreneurial activities and need to be educated on how to pool their resources together to form cooperative enterprises that would be of mutual benefit to the cooperators.

Table.2: Involvement of farmers in entrepreneurial activities

Entrepreneurial Activity	Frequency	Percentage**
Oil Palm Plantation	82	68.3
Plantain/Banana Plantation	42	35.0
Pineapple Orchard	38	31.7
Cassava Production	78	65.0
Snailry (Heliculture)	28	23.3
Poultry Production	84	70.0
Melon Production	41	34.2
Fishery Production	53	44.2
Warehousing	10	8.3
Livestock Feed Production	65	54.2
Groundnut Processing	8	6.7
Cassava Processing	79	65.8
Maize Processing	72	60.0
Vegetable (*Telferia Ooccidentalis*) Production	75	62.5

** Multiple responses

Source: Own computation from field survey data, 2017.

3.3 Perceived roles of agricultural extension service in promoting co-operative entrepreneurship activities

The perception of the respondents on the role of agricultural extension in promoting cooperative entrepreneurship is presented in Table 3. From the results, five activities were perceived as effective by the farmers using the discriminating index of 2.5 for acceptance and rejection of items. Activities with mean score above the discriminating index were; vocational/Skill training; sanitation activities; provision of storage/processing facilities; procurement of agricultural input and information on credit sources. This implies that the farmers perceived that when extension agents perform these roles in the study area, it will be effective to promote cooperative entrepreneurship in the study area.

Table.3: Perceived roles of agricultural extension in promoting co-operative entrepreneurship

Extension Activities	Very Effective		Effective		Fairly Effective		Not Effective		Mean	Remark
	F	%	F	%	F	%	F	%		
Rural feeder roads	14	11.7	22	18.3	4	3.3	80	66.7	1.76	Not effective
Recreation centers	2	1.7	3	2.5	0	0	115	95.8	1.11	Not effective
Acquisition of capital equipment	6	5.0	10	8.3	14	11.7	90	75.0	1.43	Not effective
Vocational/Skill training	28	23.3	46	38.3	40	33.3	6	5.0	2.8	Effective
Sanitation activities	48	4.0	60	50.0	10	8.3	2	1.7	3.29	Effective
Borehole Construction	28	23.3	32	26.7	8	6.7	52	43.3	2.29	Not effective
Provision of storage/ Processing facilities	56	46.7	58	48.3	5	4.2	1	0.8	3.4	Effective
Procurement of agricultural input	52	43.3	60	50.0	8	6.7	0	0.0	3.36	Effective
Information on Credit sources	58	48.3	47	39.2	10	8.3	5	4.2	3.32	Effective

Source: Own computation from field survey data, 2017.

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3.4 Perceived constraints of extension agents in promoting cooperative entrepreneurship

Table 4 shows the distribution of the respondents according to the perceived constraints of agricultural extension in providing services that would promote cooperative entrepreneurship among the farmers in the study area. The perceived constraints were ranked according to the perceived extent of effect on agricultural extension service delivery to the farmers. Inadequate finding (68.3%) was ranked first. This implies that inadequate funds to the agricultural extension personnel would constrain them from providing adequate extension service to promote cooperative entrepreneurship development among the farmers in Ahiazu Mbaise Local Government Area Imo State. Lack of technical support and poor infrastructure in communities were ranked second and third. So to encourage easy work and effectiveness of agricultural extension activities government and non-governmental organization should tackle these constraints.

Table.4: Perceived Constraints of Extension Agents in promoting cooperative entrepreneurship

Perceived constraints	Frequency	Percentage **	Rank
Inadequate funding	82	68.3	1st
Lack of technical support	73	60.8	2nd
Poor transportation	72	60.0	3rd
Poor infrastructure in communities	72	60.0	3rd
Weak/poor linkage between extension and general knowledge institution	62	51.6	5th
Fear of failure	60	50.0	6th
Lack of training opportunities	58	48.3	7th
Inadequate staffing	51	42.5	8th
Poor management of resources	50	41.7	9th
Poor working environment	42	35.0	10th
Government policies	35	29.2	11th
Language barrier	25	20.8	12th
Socio-cultural factors	20	16.7	13th
Inadequate in-service training	16	13.3	14th

** Multiple responses

Source: Own computation from field survey data, 2017.

IV. CONCLUSION AND RECOMMENDATION

The perceived roles of agricultural extension services in promoting cooperative entrepreneurship among farmers in the study area includes the provision of vocational/skill training; sanitation activities; provision of storage/processing facilities; procurement of agricultural input and information on credit sources. It was recommended that government and non-governmental organizations should provide adequate funding and technical support to extension personnel to enable them deliver agricultural extension services geared at promoting cooperative entrepreneurship to farmers.

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Effects of Household Waste Generation, Disposal and Management on Farmers' Health in Owerri Metropolis of IMO State, Nigeria

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Abstract— *The study investigated the effects of household waste generation, disposal and management on farmers' health in Owerri metropolis. It specifically ascertained the socio-economic characteristics of the farmers, identified the types and sources of waste in the study area, examined the waste disposal and management methods in the study area examined the effects of inappropriate waste disposal and ascertained the appropriate waste disposal methods used in the study area. A multi stage sampling technique was used to select one hundred and eight farmers from the three Local Government Area in Owerri metropolis. Data were collected using a validated questionnaire and were analyzed using descriptive statistics tool such as mean, frequency, percentage, and mean scores. Results show that waste is majorly generated from markets and residential homes. Waste disposal methods were mainly burning, landfills and open dumping. Its effects includes destroys the beauty of the environment, blocks gutters and drainage system, pollutes the environment among others. Subsequently, appropriate waste disposal methods in the study area includes burning of waste (29.17%), placing of bins at appropriate places (16.07%). The study recommends amongst others that waste management environmental agency should make waste dumps or receptacles accessible to residents, public campaigns should be embarked upon to educate the citizens on ills of dirty environment.*

Keywords— Farmers' health, household waste generation, waste disposal, waste management.

Contribution to knowledge: *This study is one of the few studies that evaluated effects of waste generation disposal and management on farmers' health. It also found that wastes are majorly generated from markets and residential homes, and disposed through burning, landfills and open dumping, which destroys beauty of the environment, blocks drainages and pollutes the environment.*

I. INTRODUCTION

The day to day activities of man generally draw inputs from the natural base in his environment. This may be by way of raw materials for industrial production or by direct utilization of the resources from the reserve in land, water and air. However, the use of these resources in turn results in the generation of various classes of unwanted, useless, damaged and discarded materials termed "waste" (Anurigwo, 2000). Waste is defined as any useless, unwanted and discarded material that is no longer needed and therefore must be thrown away (Ruchi and Avinash (2007). Waste therefore, is any unavoidable material resulting from industrial, household, and/or commercial activity for which there is no economic demand by the owner and which must be disposed of (Ofodile, 2002).

In every human activity, waste is generated and man continues to generate waste all his life. Waste generation is unavoidable by-product of many aspects and types of human activities and households. Household wastes are those unwanted materials (which must be discarded), produced in the kitchens or by any other activities of households or homes (Attah, 2003). They include food and packaging materials, leathers, metals, bottles (glasses),

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plastics, polythene (sachet water and polythene bags), clothes, papers, ceramics, and vegetables/leaves and construction materials among others (Egun, 2012). Waste generation is a common feature in urban and rural households. With annually generated solid waste in Nigeria been more than 25 million tonnes of 0.66 kg/cap/day in urban areas and 0.44 kg/cap/day in rural areas (Adeniran, (2005) and Babatunde et al., 2013). Population pressure on the available living areas, people's poor attitude to waste disposal, the shift from agriculture to manufacturing, resulting in the use of more plastics, glasses, metals, polythene and others, is an indication that we are certainly heading to a crises stage if unmanaged and as such make waste disposal practices an important topic of discourse if man has to live in harmony with his environment.

One of the challenging environmental problem facing urban centers worldwide, particularly in developing countries is the improper management of municipal solid waste (APO, 2007). Solid waste comes from residential, institutional, commercial, agricultural or even industrial discards while municipal solid waste has emerged as one of greatest generated hazards (Benjamin, Emmanuel & Gideon, 2014; ABUJA-CITISERVE, 2004). The Management of municipal solid waste consist of practices involving waste generation, collection, sorting, storage, transport, transfer, processing and disposal (Habib, Abdolhossinpari & Hamed, 2014), which can lead to environmental pollution like land degradation, vector breeding ground, offensive odours, emissions of toxic gases and groundwater contamination if not managed effectively in urban areas as noted by Farasat et al., (2015) and even the formation of leachates which contains heavy metals, microorganisms and radioactive elements (Egharevba, Amengialue, Edobor & Omoigberale, 2013) especially in dumped open landfills (Olusegun, 2013). Thus municipal waste disposal as reported by Iyanda, Titilope & Olaniyi (2014) have reached a crucial point in major towns and cities in Nigeria including Owerri urban.

Again, differences in the wealth of communities and countries degree of urbanization and industrialization, and intensity of agricultural activities account for the significant differences in waste treatment and disposal problems faced by developed and developing countries, and between urban and rural areas. In Nigeria, 25million metric tonnes of solid wastes are generated yearly (Ogwueleka, 2009). About 2.2 million people in developing countries die yearly from diseases associated with lack of safe drinking water, lack of adequate sanitation and poor hygiene (Haryanto, and Sutomo, 2012). Expired materials that have no useful value or materials that have outlived their life spans, unwanted substances, scraps that await disposal or recycling, remain a source of environmental degradation and threat to public health in cities worldwide (Faccio et al., 2011). Again, Sanitation deficiency causes environmental and health threats in developing countries. Managing sanitation properly contributes to reducing mortality from diarrhea diseases by 65% and morbidity by 26% (Haryanto, and Sutomo, 2012). All this is as a result of inappropriate waste disposal practices. Waste are dumped into the drainages that block the free flow of runoff water and this practice gives rise to flooding and the communities are adversely affected, some people dumped their waste to the road side, thereby reducing the width of the road and aesthetics of the cities especially in Nigeria. This is evident as one walk across the nook and the crannies of Nigeria; you find heaps of refuse littering the entire landscape, road sides, parks, gardens, commercial centres and other land use (Danbuzu, 2011, Imam et al, 2007). Little attention is given to waste management practices as it is common to see heaps of waste in the major cities littering the streets, dumped indiscriminately in drainages, vacant plots and open space especially in the developing world. This has contributed not only to the spread of communicable diseases in the affected areas; it has effect on flooding and other environmental problems (Wilson et al, 2009; Babalola et al, 2010). Health issues related to waste are complex and gives rise to numerous debates (Department for Environment, Food and Rural Affairs 2004). Given the diversity of pollutants present, management methods and routes of exposure, knowledge remains imperfect and still need to be improved. The uncollected or illegally dumped wastes constitute a starting point for disaster of human health and the environmental degradation. Apart from the increasing quantities, the waste composition and characterization evolves, incomes and changing consumption habits have also been affected by globalization (EPA, 2011). Knowledge of the type and sources of solid waste, method of waste disposal, its effect on farmers' health along with data on the composition of waste generation is basis to the design and operation of the functional element associated with the management of solid waste.

II. METHODOLOGY

The study was conducted in Owerri, Imo state. Owerri is the capital of Imo State in Nigeria. Owerri consists of three Local Government Areas including Owerri Municipal, Owerri North and Owerri West. It has an estimated population of about 401,873 as of 2006 (Federal Republic of Nigeria Official Gazette, 2007) and is approximately 100 square kilometres (40 sq mi) in area. Owerri is bordered by

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the Otamiri River to the east and the Nworie River to the south. Its coordinate consists of 5°29′06″N 7°02′06″E/5.485°N 7.035°E. Owerri has a tropical wet climate according to the Köppen-Geiger system. Rain falls for most months of the year with a brief dry season. The Harmattan affects the city in the early periods of the dry season and it is noticeably less pronounced than in other cities in Nigeria. The average temperature is 26.4 °C. Owerri sits in the rain forest and produces many agricultural products, such as yams, cassava, taro, corn, rubber and palm products. Owerri also sits on huge crude oil and natural gas reserves like most of the Igbo land areas. Important educational institutions in Owerri include Imo State University, Federal University of Technology Owerri, Imo State Polytechnic Umuagwo, Federal Polytechnic, Nekede, African Institute of Science and Technology (AIST CCE Owerri), and Federal College of Land Resources Oforola.

Multi stage sampling technique was used to select the respondents. At the first stage, the three local government areas were purposively selected viz-a-viz Owerri West, Owerri North and Owerri municipal. At the second stage, six (6) communities were randomly selected from Owerri West and Owerri North, which includes; Obinze, Ihiagwa, Nekede, Uratta, Emekuku, and Nazi. In the case of Owerri Municipal no community was selected at this stage because of the fact that their settlements are not known as communities but as villages and residential areas. There are five villages, which include, Umuororonjo, Amawom, Umuonyeche, Umuodu and Umuoyima and the residential areas include, Ikenegbu, Aladinma, World bank housing estate etc. At the third stage, 2 villages were randomly selected from the communities selected from Owerri West and Owerri North namely; Umueje, Umuanunu, Umuelem, Nakaramoche, Umudibia, Umualum, Ezeogba, Ezedibia, Akwakuma, Amakohia, Umuakali, and Umuezuo, while the five (5) villages and one residential area was selected from Owerri Municipal. At the final stage, 6 farmers were randomly selected from the villages making it a total number of one hundred and eight (108) respondents. Structured questionnaire was used to collect primary data from the field. Data analysis was achieved using descriptive statistics like mean, frequency, and percentage.

III. RESULTS AND DISCUSSION

3.1 Socio-economic Characteristics of farmers.

The socioeconomic features of the farmers are presented in table 1.

Table.1: Distribution of farmers according to their Socioeconomic Characteristics

Socioeconomic Characteristics	Frequency	Percentage	Mean
Sex			
Male	48	55.56	
Female	60	44.44	
Age (years)			
21-30	20	19.05	
31-40	35	32.41	
41-50	29	26.85	
51-60	17	15.74	
61-70	7	6.48	32.69
Marital Status			
Single	22	20.37	
Married	49	45.37	
Widowed	20	19.05	
Divorced	17	15.74	
Household size (number of persons)			
1-3	22	20.37	
4-6	47	43.52	
7-9	39	36.11	5.47
Farm size (hectare)			
1-3	57	52.78	
4-6	31	28.70	
7-9	20	18.52	3.97

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Farming experience (years)			
11-20	17	15.74	
21-30	22	20.37	
31-40	40	37.04	
41-50	17	15.74	
51-60	12	11.11	34.11
Education level (years)			
0 (No formal education)	26	24.07	
1-6	42	38.89	
7-12	30	27.78	
13-18	10	9.26	9.2
Income (naira)			
11000-20000	26	24.07	
21000-30000	43	39.81	
31000-40000	21	19.44	
41000-50000	7	6.48	
51000-60000	11	10.19	29388.89
Social organization membership			
Yes	51	47.22	
No	57	52.78	
Extension visits			
Yes	52	48.15	
No	56	51.85	
Number of times visited by extension agents			
Weekly	11	21.15	
Forth nightly	14	26.93	
Monthly	27	51.92	

Source: Field Data, 2017.

The result in table 1. showed that 55.56% of the farmers were female while 44.44% of them were male. This implies that majority (44.44%) of waste managers at household level are female. It has been hypothesized that women demonstrate great enthusiasm about environmental issues than men (Hampel et al, 1996). The mean Age of the farmers' was 32.69 years which indicates that the Farming population of the study area is quite young and active in Farming. This further strengthens the findings of Bamiro, Otunaiya and Idowu (2012) who said that most active group among rural farmers' falls within 30-50 years of age. It was found that 45.37% of the farmers were married, 20.37% of them were single, 19.05% of them were Widowed while 15.74% of the farmers were Divorced. This supports the finding of Sanful and Darko (2010), who stated that the majority of the farmers (74.5%) are faced with responsibilities of taking care of their family. The result also revealed that 43.52% of the farmers in the study area had an approximate average household size of 6 persons indicating that the farmer's household contributes greatly to Farm labour. This agrees with Baruwa and Oke 2012 who stated that the size of household is a good indicator of labour available for farm work. The mean Farm size was 3.97 ha which implies that farmers in the study area are small holder farmers operating small farm holdings. This supports the study of Ekong (2005) who stated that small holders farmers still persist in Nigeria based on Inheritance and prone to fragmentation. Average Farming experience was 34.11 years indicating that with more experience, a farmer can become less averse to the risk implied by adopting a new technology (Nwaobiala, 2014). It was further observed that 38.89% of the respondents spent 1-6 years in school, 27.78% of them spent 7-12 years in school, 24.07% of them had no formal education, while only 9.26 of them had Tertiary education and spent 13-18 years in school with mean level of education of 9.2years, indicating that the farming population in the study area is quite literate and have acquired one form of education or the other. This further support the statement by Obinne (1991), who stated that education is advantageous to farmers as it will lead to increased adoption of innovation. The mean income is N29, 388.89. According to Adedibu and Okekunle (1990)

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personal income influences waste generation due to its impact on individual consumption pattern. In addition, the rate of solid waste generation per capital increase as the standards of living improves (UNCHS, 1992). It revealed that 52.78% of the respondents do not belong to social organisation, while 47.22% of them belong to one social organisation or the other. The result further revealed that 51.85% of farmers in the study area were not visited by extension agents while 48.15% of them were visited. This shows that Extension contact in the study area is relatively poor. Though a good number of farmers were visited. It is hypothesized that contact with extension workers and adequate information on production techniques will increase farmer's likelihood of adoption of improved agricultural practices (Salau et al., 2014). Majority (51.92%) of the farmers visited by the extension agents were visited on a monthly basis, 26.93% of them were visited on a forth nightly basis, while, 21.15% of them were visited weekly.

3.2 Types and Sources of waste Generated by Households.

The distribution of the types and sources of waste generated by households in the study area is presented in tables 2. and 3. respectively.

Table.2: Distribution of farmers according to Types of Waste

Types of waste	Frequency	Percentage (%)
Biodegradable	51	47.22
Non-biodegradable	57	52.78
Total	108	100

Source: Field Data, 2017.

Table 2. shows the distribution of farmers according to type of waste generated in the study area. The result indicates that 52.78% of the farmers said the waste generated in the study area were Non-biodegradable waste while 47.22% of them said it was Biodegradable waste. This is an implication that both biodegradable and non-biodegradable wastes are present in the study area. The economic and social status of the city could be the reason for the greater percentage (52.78%) of non-biodegradable wastes generated in the area. This finding disagrees with earlier work by Oil Resources and Allied Investment Limited (2008) which gave the figures of biodegradable and non-biodegradable wastes as 54% and 46% respectively.

Table.3: Distribution of Farmers according to Sources of Waste

Sources of waste	Frequency**	Percentage (%)
Residential homes	72	23.92
Markets	101	33.55
Hospitals	48	15.95
Farms	43	14.29
Business places	37	12.29

**Multiple responses recorded
Source: Field survey data, 2017.

Investigation into the major sources of solid waste generation presented markets (33. 55%) and residential homes (23.92%) as the main sources of waste in the study area as seen in table 3. The table also indicates that 15.95% were generated from hospital, 14.29% of them generated from Farms, while 12.29% of them were generated in business places. It is certain that markets are centre's of commercial activities in any city and accommodate greater number of people than any other sector at any given time. The increased human population and activities in both markets and residential homes could account for the huge quantities of different kinds of wastes around the municipality and its environs. This finding concurs with the view of Ogwueleka (2009) who found that markets and residential homes are the major sources of wastes.

3.3 Methods of waste disposal and management

The methods of waste disposal and management in the study area are presented in Table 4.

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Table.4: Distribution of Farmers according to Methods of Waste disposal and Management

Waste disposal methods	Frequency **	Percentage (%)
Landfills	102	26.91
Burning	106	27.97
Open dumping	98	25.86
Ocean dumping	42	11.08
Grinding and discharge in sewers	31	8.17
Waste management		
Recycling		
Incineration	47	27.48
Composting of waste	52	31.52
Plasma gasification	37	22.42
Waste to energy	0	0
Waste minimization	10	6.06
Ploughing in Field	15	9.09
	4	2.42

** Multiple responses recorded

Source: Field Data, 2017

Table 4 showed that 26.91% of the farmers dispose waste through landfills, 27.97% through Burning, 25.86% of them through open dumping, 11.08% of them through Ocean dumping or dumping in river bodies and flowing/running water. It further shows that 31.52% of farmers carry out Incineration as management practices of waste, 27.48% do Recycling, 22.42% compost waste, 9.09% do waste minimization, 6.06% convert waste to energy which 2.42% of them plough the waste in the field especially as a result of mechanized Farming. The result implies that farmers adopt different methods of waste disposal and uses several strategies to manage waste the best way possible to them in the study area. The reasons for adopting open dumping as the main waste management method could be unavailability of adequate man-power and equipment, inaccessibility to final dumpsites, people's poor attitude towards environmental sanitation as well as the method being cheap but not cost effective. This finding agree with those of Olafusi (2004) and Iman et al., (2007) who reported that in most cities of Nigeria and other developing countries, the greater percentages of waste generated are dumped on the surface of the ground along major roads, streets and open spaces. The finding was further confirmed by the information obtained from field trips and direct observations which revealed the existence of more than 250 dumpsites of different sizes within the municipality and its environs.

3.4: Effect of Inappropriate Waste disposal on Farmers' Health

The effect of inappropriate waste disposal on farmers' health is presented in table 5.

Table.5: Distribution of farmers according to effects of inappropriate waste disposal

Effects	Mean	Standard deviation
Foul odour	4.204	0.992
Disease outbreak	4.370	0.718
Attract animals like rats	4.037	1.013
Pollutes the environment	4.426	0.726
Blocks gutters and drainage system	4.444	0.777
Destroys the beauty of the environment	4.602	0.528
Disrupts free movement to and from the farm	4.129	1.014
Soil infertility		
Farm flooding	4.306	1.008
	4.1481	1.151

Mean <3.0: Not accepted; Mean ≥3.0: Accepted

Source: Field Data, 2017.

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The result in Table 5 showed that all the effects listed are observed as effects in the study area, however some effects are ranked higher because of its prevalence in the study area. As observed effects such as destroys the beauty of the environment (M= 4.602), Blocks gutters and drainage system (M =4.444), Pollutes the environment (M =4.426), disease outbreak (M =4.370), soil infertility (M = 4.306) are major effects as seen in the study area. The result therefore revealed that Owerri metropolis is associated with poor looking environment as a result of inappropriate waste disposal. The standard deviations for all the items were low indicating high concentration around the mean and this could have implication on the farmer's heath. This agrees with the study by Madukwe et al., (1996) that the heap of the dump site creates obnoxious odour and encourage the growth of pathogens and insect pests lay their eggs on the heap.

3.5 Appropriate Waste Disposal Methods

The appropriate waste disposal methods identified in the study area is presented in table 6.

Table.6: Distribution of farmers according to appropriate waste disposal methods in the study area.

Methods	Frequency**	Percentage (%)
Placing bins at designated places	54	16.07
Burning of waste	98	29.17
Recycling of waste	32	9.53
Composting of waste	37	11.01
Residents should be educated on appropriate waste disposal	48	14.28
Separate bins for decomposable and non-decomposable waste		
Educating households on appropriate waste disposal methods	20	5.95
	l	
	47	13.98

**Multiple responses recorded.

Source: Field Data, 2017

Table 6 showed that 29.17% of the farmers exercise opening burning of waste, 16.07% uses bins placed in appropriate places, 14.28% of them reveal that Residents should be educated on appropriate waste disposal, 13.98% also reveal that households should be educated on appropriate waste disposal, 11.01% compost waste while 9.53% recycle waste, only 5.95% suggested the use of separate bins for decomposable and non-decomposable waste. This implies that there are several appropriate measures available in the study area that household could adopt for proper waste disposal to reduce waste effect on farmers. The present finding is in consonance with the study of Ugboaja (2002) who reported that a greater percentage of the residents did not sort waste into biodegradable and non-degradable. This accounts for large volume of waste deposited at the central positions in the municipal council. The finding is also in line with that of Chukwu (2012) who reported that many cities in Nigeria today are suffering from sudden increase in solid waste and poor disposal practice.

IV. CONCLUSION AND RECOMMENDATION

From the findings it was observed that due to rapid urbanization in Owerri metropolis with its numerous hotels, fast food centers and markets the volume of waste generated is on the increase and has become a major concern for farm households and the government at large. Inappropriate waste disposal practices have resulted in environmental degradation with serious health implications on farmers. Households should conceive appropriate waste disposal practices as a crusade against poor living environment. It is implicit that Owerri metropolis spends huge sums of money in waste management. Therefore its residents need to be educated on efficient waste disposal practices to reduce health hazard on farmers., waste management environmental agency should make waste dumps or receptacles accessible to residents and appropriate sorting of waste into decomposable and non-decomposable components should be practiced by the residents.

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Starch films for agronomic applications: comparative study of urea and glycerol as plasticizers

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***Abstract*—*This work aims to study the effects of urea, glycerol and their mixture as plasticizers for cassava starch films, regarding their impact on the material structure, water susceptibility, barrier and mechanical properties. All plasticizers were compatible with starch-based matrices, without detecting migration at the plasticizers level tested. In general water related properties were not affected. Plasticizer-polymer interactions as well as those involving water molecules were evidenced by ATR-FTIR spectra. Urea resulted the most efficient plasticizer, since it lowers glass transition temperature values and enhances mechanical properties. The co-plasticization of the starch films with glycerol and urea mixture resulted in poorer mechanical performance, though with higher light absorption which is relevant considering the potential film applications as mulching functionalized cover material.*

Keywords*—*cassava starch, barrier properties, plasticizer interaction, mechanical properties, urea.

I. INTRODUCTION

Research related to functionalised biodegradable materials with active compounds is highly demanded for biomedical and pharmaceutical uses. However, the applications reported in the agronomic area are scarce, being these mainly focused on fertilisers' encapsulation [1, 2]. Likewise, the great plastics consumption for agronomic purposes has triggered the development of biodegradable materials [3-5]. In this regard, the inclusion of fertilisers to biodegradable films could help diminishing pollution as well as increasing crop efficiency and decreasing agrochemical use; thus, providing a greener alternative. The addition of urea, a common fertiliser, could not only functionalise but also plasticise the film matrix being this later released to the soil.

In general, plasticizers are included in material formulations for two main purposes: as processing aid agents and as final product properties modifiers [6]. In the first case, plasticizers lower the processing temperature, reduce sticking in moulds and enhance wetting. In the second one, they increase the temperature range of usage; increase flexibility and toughness; and lower the glass transition temperature. There is a consensus in the scientific community that plasticizers reduce intermolecular forces along the polymer chains, thus increasing the free volume and chain movements. However, the plasticizer selection depends on its compatibility, efficiency and permanence in the polymer matrix [6]. Moreover, plasticisation is particularly important on biopolymer films, since the dehydration of these structures produces strong cohesive films with poor mechanical and barrier properties [7]. Since most plasticizers contain hydrophilic groups, these compounds can interact by means of hydrogen bonds not only with polymer matrix but also with water molecules, increasing therefore films moisture absorption [8]. As regards starch-based materials, many studies have been carried out on different plasticizers to evaluate their performance, being polyols -especially glycerol- the most commonly used [9-23].

In comparison to polyols, urea exhibits a strong hydrophilicity due to its chemical structure -containing two amino groups and one carbonyl group- and a tendency to crystallise. It has been used for plasticisation of starch [17, 24-26], as well as cellulose [27], poly(vinyl alcohol) [28] and soy protein [29].

In respect of external plasticization, hydrocolloid-based films admit a maximum amount of plasticizer that is limited by their migration towards the film surface. As regards glycerol plasticised-films migration is evidenced by the oily appearance of films surface [30, 31], whereas when urea is used superficial crystallisation can occur [26]. Therefore, several studies on plasticizer content effect have been reported, being 30%w/w of dry basis the maximum concentrations reported for both glycerol and urea in starch-based materials [20, 26, 30, 32]. In addition, references on plasticizers-mixtures to extend migrations limits can been found, for instance urea/formaldehyde and urea/ethanolamine [24, 25]. The use of glycerol-urea, in particular, blends has been reported in thermoplastic starch (TPS) films [2, 33]. Nonetheless, no research addressing urea and glycerol mixture effect on cassava starch films obtained by casting has been published hitherto.

In this paper, we are therefore attempting to reveal the structure and behaviour of urea, glycerol and their mixture as plasticizers for cassava starch films, regarding the effects on the water susceptibility, barrier and mechanical properties of the films considering potential agronomic applications.

II. MATERIALS AND METHODS

2.1 Materials

Native cassava (*Manihot esculenta*) starch was purchased from Cooperativa de Productores de Jardín América Ltda. (Misiones, Argentina). Reagent grade glycerol (CAS# 56-81-5, Anedra, Argentina) and urea (CAS# 57-13-6, Biopack, Argentina) were used as plasticizers.

2.2 Film preparation

Native cassava starch films were prepared by casting and plasticised with glycerol, urea or its half-and-half mixture (w/w). Aqueous suspensions of 3 %w/w starch were gelatinised at 90 ºC during 20 min. Plasticizers were added after gelatinisation in a ratio of 25:100 of plasticizers to starch (w/w). A control film without any plasticizer (C) was also prepared as matrix reference.

Approximately 20 g of the film-forming suspensions were cast onto Petri dishes (diameter 8.7cm) and later dried in a ventilated oven (GMX 9203A PEET LAB, USA) at 50 ºC for 4 h; films were removed from the plates and stored at 20ºC and 65 % relative humidity (RH) for at least 48 h.

2.3 Film properties

2.3.1 Wettability and water content

2.3.1.1 Water content

Films moisture content was determined gravimetrically by measuring the weight loss of films upon drying in an oven at 105 ºC until constant weight. Reported values correspond to the mean value of three determinations.

2.3.1.2 Water sorption

Water sorption was measured gravimetrically on 2 cm × 2 cm films exposed to 100 % constant relative humidity at 20 ºC. Films were previously dried to constant weight in an anhydrous $CaCl_2$ atmosphere with an accuracy of ± 0.0001 g. Water uptake curves were fitted to the experimental model of Elizaldey col. [34]:

$$q = (Q\ t)/(B + t) \qquad (1)$$

Where q and Q are water taken up at time (t) and at equilibrium respectively and B is the time needed for samples to gain half of equilibrium value. Water content are given on dry basis; therefore, the samples dry matter was determined gravimetrically by oven drying at 105 ºC. At least three replicates were measured for each sample.

By differentiation of Eq. (1) a specific rate of water uptake constant (K) was determined as follows:

$$K = 1/(Q\ B) \qquad (2)$$

2.3.1.3 Wettability

Films wetting was evaluated through static contact angle measurements by the sessile drop method, using a Ramé-Hart Model 250 Standard Goniometer (USA). A 2 - 3 µL doubly distilled and deionized water droplet was released on the film surface, then the contact angle was calculated from a digital picture taken as soon as the droplet had reached the sample to avoid the anomalous behaviour of swelling. The contact angle (θ) was determined from the angle made between the baseline representing the film surface (liquid-solid interface) and the tangent to the droplet surface curvature (liquid-air interface). The mean value of ten replicates were taken on each film sample.

2.3.2 Optical and barrier properties

2.3.2.1 Water vapour permeability (WVP)

Water vapour permeability (WVP) tests were conducted using ASTM Standard Method E96 with several modifications according to [30]. After steady-state condition was reached, the acrylic permeation cells were weighed (0.0001 g) at initial time and at 1h interval over 8hs. The WVP (g/m s Pa) was calculated considering the thickness of each tested film, as well as the cell area and the water vapour partial pressure difference across the film at 20 ºC. Samples were analysed at least in triplicate.

Additionally, a digital coating thickness gauge for non-conductive materials CM-8822 (SolTec, Argentina), was used to evaluate the films thickness. Ten measurements were randomly taken at different locations for each specimen and the mean value was reported.

2.3.2.2 Optical properties

To evaluate the films light barrier capacity the absorbance spectrum (200 – 700 nm) was recorded using a HITACHI U-1900 Spectrophotometer (Japan). Films were cut into rectangles (3 cm × 1 cm) and placed on the internal side of a quartz spectrophotometer cell. Film opacity and UV-barrier capacity (AU ×nm) were defined as the area under the recorded curve between 400 – 700 nm and 200 – 400

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nm, respectively, as described by Castilloy col. [35] and the standard test method for haze and luminous transmittance of transparent plastics recommendations ASTMD1003-00 Standard.

2.3.3 Film microstructure and mechanical properties

2.3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of plasticised films were measured in a FTIR Nicolet-iS10 Thermo Scientific Spectrometer (USA) with Attenuated Total Reflection (ATR) accessory. Spectra were taken in the wavenumber range: 4000 – 500 cm^{-1} by accumulation of 64 scans at 4 cm^{-1} resolution. Data was analysed by using the Software Omnic 9 (Thermo Scientific, USA). The spectral deconvolution of the data was performed using curve fitting algorithms within the following regions: 3700 – 2800 cm^{-1}, 1700 – 1500 cm^{-1} and 1200 – 900 cm^{-1}, as described in a previous work [8].

2.3.3.2 Differential Scanning Calorimetry (DSC)

Thermal properties of plasticised films were analysed by DSC employing a Q100 TA Instruments DSC equipment (USA) controlled by a TA 5000 module, with a quench cooling accessory, under a N_2 atmosphere (20ml/min). Film samples (5 - 6 mg) were weighed in aluminium pans and hermetically sealed, using an empty pan as a reference. Samples were analysed between −80 and 12 ºC, at a 10 ºC/min heating rate. Glass transition temperature (Tg, ºC) was determined using the Universal Analysis V1.7 F software (TA Instruments, USA). All measurements were performed at least by duplicate.

2.3.3.3 Mechanical testing

Mechanical performance of the studied films was evaluated by tensile tests using a texturometerTA.XT2i-Stable Micro Systems (UK) with a tension grip system A/TG. Ten probes of 7 mm × 60 mm were assayed for each sample and stress-strain curves were recorded. Maximum tensile strength (R_{max}), elongation at break (E_{max}), elastic modulus (Ec) and tenacity (Eg) were calculated according to the ASTM D882 - 00 Standard. At least ten replicates were measured, and the mean value was reported. Additionally, films thickness was determined as described previously.

2.3.3.4 Scanning Electron Microscopy (SEM)

Besides, some tested probes were mounted on bronze stubs and coated with a gold layer (40 – 50 nm) to be studied by SEM with a FEI QUANTA 200 SEM (Japan) with Apollo 40 electron detector. All samples were analysed using an accelerating voltage of 10 kV, under high vacuum mode.

2.3.4 Statistical analysis

Multifactor analyses of variance were performed using InfoStat Software [36]. Differences in the properties of the films were determined by Fisher's Least Significant Difference (LSD) mean discrimination test, using a significance level of $\alpha = 0.05$. In addition, in order to analyse the interdependence and variability of the results obtained a Principal Components Analysis (PCA) was carried out. The software (InfoStat) was used for the analysis considering two main components. From the analysis performed, the Biplot graph and the cophenetic correlation coefficient were reported, the latter being indicative of the efficiency of the variable clustering.

III. RESULTS AND DISCUSSION

I.1. Wettability and water content

Since both plasticizers evaluated present a hydrophilic nature, films water susceptibility was studied by different simple tests: water sorption, moisture content and contact angle.

Films water uptake behaviour was similar for all plasticised films. A relatively good fit ($r^2 > 0.920$) of the experimental data to the model of Elizaldey col. [34] was observed (Fig.1), from which parameters Q, B and K were obtained (Table 1).

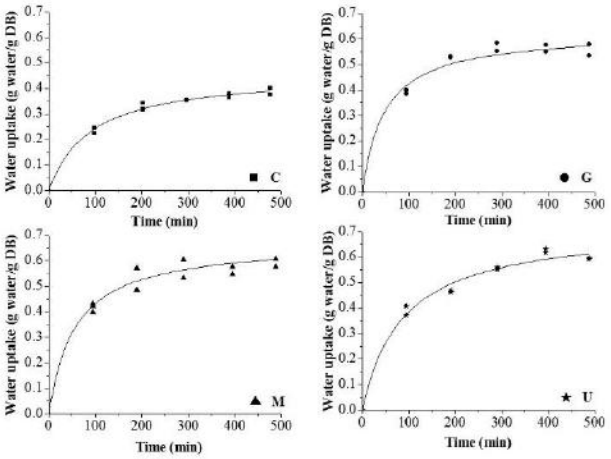

Fig. 1: Predicted (solid lines) and experimental (single points) sorption curves at 100 %RH of cassava starch-based films: unplasticized (C) and plasticised with glycerol (G), urea (U) and the mixture of both (M). (DB = dry basis)

Films containing glycerol (G, M) reached similar equilibrium water uptake (Q) and time needed to gain half of equilibrium value (B), while films plasticised only with urea (U) presented significantly higher values ($p<0.05$) in both parameters though showing lower water uptake rate (K). Control films (C), on the other hand, gave intermediate K values compared to those of plasticised films however showing significantly lower Q values

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Table 1. Water uptake kinetic parameters, water content and wettability of control cassava starch films (C) and films plasticized with: glycerol (G), urea (U) and their mixture (M).

Film	Water uptake			Water content (%)	Contact angle (Θ)
	Q (g water/g DB*)	B (hr)	K (g DB/g water hr)		
C	0.45 ± 0.02 ^{a}	1.5 ± 0.02 ^{b}	1.5 ± 0.07 ^{ab}	13.4 ± 0.8 ^{a}	38.2 ± 4.3 ^{a}
G	0.64 ± 0.01 ^{b}	0.8 ± 0.01 ^{a}	2.0 ± 0.01 ^{b}	21.1 ± 0.4 ^{d}	48.9 ± 2.9 ^{b}
M	0.67 ± 0.01 ^{bc}	0.9 ± 0.05 ^{a}	1.7 ± 0.10 ^{b}	17.7 ± 0.9 ^{c}	46.6 ± 3.6 ^{b}
U	0.71 ± 0.02 ^{c}	1.4 ± 0.11 ^{b}	1.05 ± 0.10 ^{a}	15.8 ± 0.9 ^{b}	46.5 ± 3.3 ^{b}

Reported values correspond to the mean ± standard deviation.
*DB = dry basis

($p<0.05$). As outlined by Elizaldey col. [34] at RH > 90, water uptake represent mostly multilayer water, water held
in voids, crevices and capillaries. In the absence of plasticizer films present a more compact structure [6], therefore holding less water at the same equilibrium moisture than plasticised films.

The water content of the plasticised polymer samples ranged between 15.8 and 21.1 %, with significant differences ($p<0.05$) among the three samples tested, and significantly higher than unplasticized films (C) water content (Table 1).Even though all samples contained the same amount of plasticizer (25 %w/w), the addition of urea resulted in lower moisture content, indicating differential interaction among polymer-plasticizer-water depending on the plasticizer type.

The contact angle measurement is a useful tool to determine the hydrophobic or hydrophilic character of a film surface: low contact angle values ($\Theta<90°$) correspond to surfaces that are more wettable, on the contrary, hydrophobic surfaces show high values ($\Theta>90°$) of this parameter [37]. All films presented low contact angle values (Table 1). Nevertheless, a slight but significant ($p<0.05$) increase with respect to unplasticized films (C) was observed with plasticizers inclusion independently of their nature or concentration (G, M and U). Correspondingly, reported values of corn starch based films confirm the hydrophilic nature of these materials, though the comparison of contact angle values results difficult, since it strongly depends on additives and film [38, 39].

I.2. Barrier properties

Even though G films plasticised presented higher mean WVP values than those plasticised with M and U there were no significant differences ($p>0.05$) among plasticised films (Table 2), yet these resulted significantly ($p<0.05$) lower than C films WVP. These results are in accordance with other published works, considering that all samples contained the same total amount of plasticizer. Plasticizers interfere with polymeric chain association decreasing the rigidity of the network, producing a less ordered film structure, such an effect has great impact on films WVP [6]. In comparison to unplasticized cassava starch films, WVP decreases significantly ($p<0.05$) with 25 % of plasticizer addition, since more homogeneous and compact films are obtained without pores or cracks evidenced by SEM [30, 32].

Table 2. Light barrier capacity and WVP of control cassava starch films (C) and films plasticized with: glycerol (G), urea (U) and their mixture (M).

Film	WVP (g/m s Pa × 10^{10})	Opacity (AU × nm)	UV-barrier (AU × nm)
C	1.53 ± 0.3 ^{b}	32.69 ± 2.1 ^{c}	40.84 ± 1.3 ^{a}
G	1.03 ± 0.1 ^{a}	28.83 ± 2.9 ^{bc}	39.43 ± 3.1 ^{a}
M	0.99 ± 0.1 ^{a}	25.03 ± 3.0 ^{ab}	46.00 ± 3.2 ^{b}
U	0.93 ± 0.1 ^{a}	23.45 ± 2.5 ^{a}	46.90 ± 0.94 ^{b}

Reported values correspond to the mean ± standard deviation.

With regard to films light barrier capacity, the UV barrier capacity (200 – 400 nm) was higher for films plasticised with urea, due to its characteristic absorption peak. On the contrary, unplasticized films showed higher absorption in the visible region (400 – 700 nm) attributed to a more compact structure, hence the addition of plasticizer significantly reduced ($p<0.05$) films opacity being glycerol influence lower than that of urea.

I.3. Film microstructure and mechanical properties

I.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for pure components: starch; glycerol and urea, and the 50:50 mixture of the later are shown in Fig.2. Urea exhibits characteristic absorption bands in two main regions: 3700 – 3000 cm^{-1} (N-H amide stretching) and 1700 – 1300 cm^{-1} (N-H amide bending and carbonyl group stretching), presenting three characteristic peaks located at 1675, 1618 and 1585 cm^{-1}. Glycerol, on the contrary, has a broad band between 3700 – 3000 cm^{-1} (O-H stretching and bending) and the characteristic peaks of C-H bonds in the region of 3000 –

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2800 cm^{-1} and 1500 – 1200 cm^{-1}. Urea and glycerol mixture showed characteristics absorption bands of both pure components, though maximums shift towards higher wavenumbers were observed on the 3700 – 3000 cm^{-1} and 1700-1300 cm^{-1} regions, representative of H-bridges interactions between both compounds. Moreover, native cassava starch spectrum presents a wide band between 3700 – 3000 cm^{-1} corresponding to the O-H stretching and bending, another peak at 1643 cm^{-1} associated to the O-H stretching in water molecules clusters with moderately strong H-bonded, and the characteristic bands of the C–O-C stretching vibrations and C-O-H bending vibrations in glycoside and pyranose rings in amylose and amylopectin at 1200 – 900 cm^{-1} [40, 41].

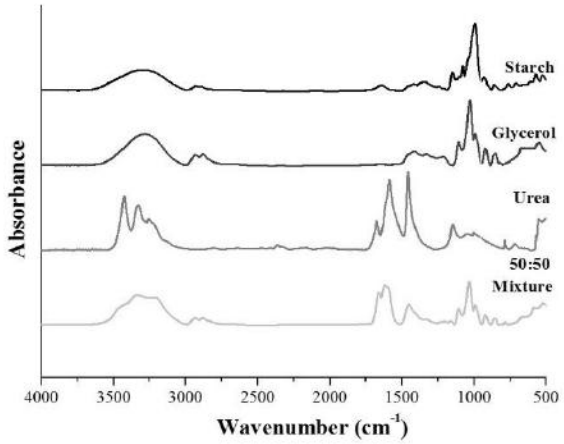

Fig.2: ATR-FTIR spectra of pure film components: starch, glycerol, urea and its 50:50 mixture.

Likewise, FTIR spectra of plasticised films with glycerol, urea and the mixture of both revealed the characteristic bands of the pure components. Nonetheless, variations in intensity and maximums shifts were observed indicating distinctive interactions among components. Main differences were displayed in 3700 – 3000 cm^{-1}, 3000 – 2800 cm^{-1}, 1700 – 1500 cm^{-1} and 1200 – 900 cm^{-1} regions (Fig.3). Table 3 shows the most important absorption peaks in each spectral window.

On the one hand, C and G films revealed a broad and intense absorption band in 3700 – 3000 cm^{-1}region centred at 3281 cm^{-1}, which is assigned to O–H stretching and bending vibrations. Films containing urea on the other hand, presented three important contributions in this region: about 3200, 3345 and 3452 cm^{-1} (Fig.3a). The latter are attributed to the characteristic amide N-H stretching peaks of urea (3254, 3327 and 3427 cm^{-1}), although the significant shifts observed indicate -as expected- that these N-H groups are involved in the H-bridge interactions within the matrix. The individual contributions of each of these peaks to the band depend on the urea content,resulting relative areas of 3200 and 3345 cm^{-1} peaks the most affected (Table 3).

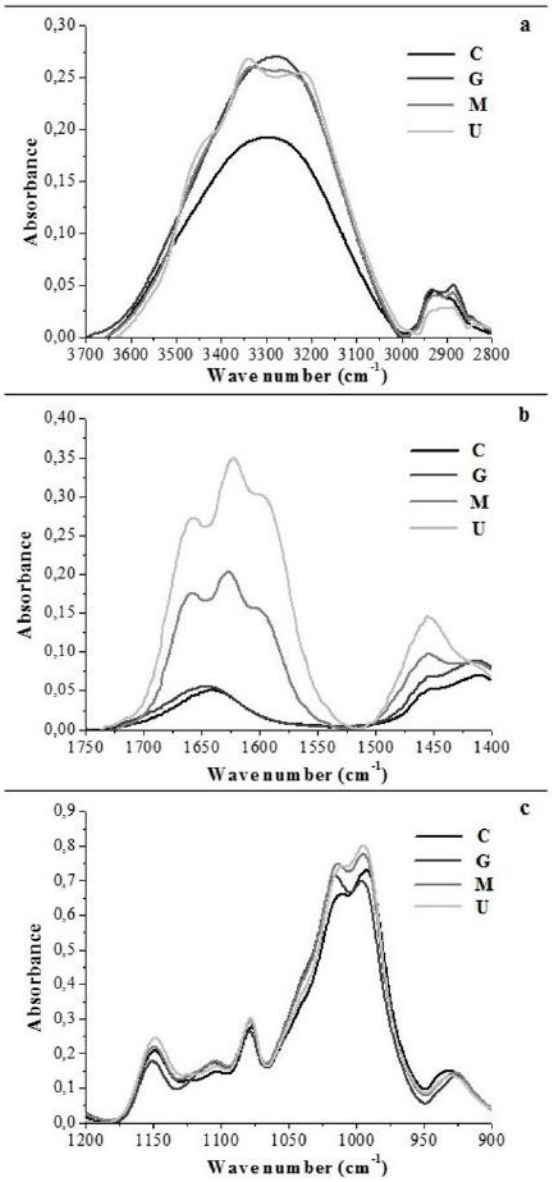

Fig.3: ATR-FTIR spectra of cassava starch-based films: unplasticized control (C), plasticised with 25% glycerol (G), 12.5% glycerol and 12.5% urea (M) and 25% urea (U). Three main regions are shown: a) 3700-2800cm^{-1}; b) 1750-1400cm^{-1}; and c) 1200-900cm^{-1}.

In the spectral region between 3000 – 2800 cm^{-1} urea does not reveal any band, whereas glycerol has a band with two clear peaks at 2937 and 2875 cm^{-1}. Cassava starch C and G films exhibit an analogous band though relative intensities shift due to the presence of glycerol's methylene groups (Table 3). Moreover, in films containing urea this band unfolds into three contributions with similar relative intensities (Table 3), indicating that the environment of C-

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Table 3. FTIR band deconvolution peaks in the analysed spectral windows of control cassava starch films (C) and films plasticized with: glycerol (G), urea (U) and their mixture (M).

Film	C	G	M	U
3700-3000 cm^{-1}	3297.0 (1)	3281.9 (1)	3203.4(0.41)	3199.6 (0.48)
	--	--	3346.2(0.41)	3343.2 (0.34)
	--	--	3467.1(0.18)	3458.8(0.18)
3000-2800 cm^{-1}	2886.0 (0.36)	2884.4 (0.58)	2881.3 (0.43)	2884.1 (0.40)
	--	--	2914.6 (0.26)	2914.0 (0.30)
	2931.1 (0.64)	2936.3 (0.42)	2938.1 (0.31)	2940.2 (0.30)
1700-1500 cm^{-1}	--	--	1593.8 (0.25)	1587.6 (0.32)
	1643.7 (1)	1651.5 (1)	1628.5 (0.45)	1623.8 (0.40)
	--	--	1664.8 (0.30)	1663.9 (0.28)
1200-900 cm^{-1}	928.7 (0.07)	923.8 (0.06)	925.6 (0.06)	927.8 (0.06)
	989.7 (0.34)	993.9 (0.35)	992.9 (0.36)	992.4 (0.36)
	1016.9 (0.23)	1017.3 (0.17)	1016.5 (0.19)	1016.1 (0.22)
	1045.9 (0.12)	1040.1 (0.20)	1041.2 (0.17)	1043.1 (0.13)
	1078.8 (0.06)	1079.8 (0.05)	1079.3 (0.05)	1079.0 (0.06)
	1101.8 (0.06)	1103.6 (0.07)	1102.9 (0.06)	1102.6 (0.05)
	1124.6 (0.03)	1122.5 (0.03)	1123.4 (0.03)	1123.8 (0.03)
	1152.0 (0.09)	1151.1 (0.07)	1150.3 (0.08)	1149.7 (0.09)

In the case of deconvoluted bands each informed value corresponds to the peak position and in between brackets its relative contribution to the total band area.

H groups' change in the presence of urea affecting its vibrational transition.

In the FTIR spectra, films containing urea present three similar signals in terms of peak position and relative contribution in the region comprised among 1700 – 1500 cm^{-1} yet with higher absorbance in the U samples (Table 3). The observed shifts with respect to pure urea peaks indicate that both plasticizer-matrix and plasticizer-water interactions occur in the material. Similar results were published by Wangy col. [26] in oxidized corn-starch films plasticised with urea, being the peak at 1659 cm^{-1} attributed to C=O stretching (amide-I region) and the one at 1626 cm^{-1} assigned to N-H bending (amide-II region). In both C and G films, a single contribution could be seen in this region, associated with the O-H bonds of the water molecules that interact with the matrix (Table 3). Shifts observed in C and G films compared to those of pure components (Fig.2 and 3b) proved to be less important than that seen in films containing urea (M and U). From these results, it is clear that urea-matrix interactions are stronger than glycerol-matrix ones. MayYu [17] have exhaustively analysed the effect of plasticizers containing amide groups on the properties of thermoplastic starch, revealing that the hydrogen bond-forming abilities with starch was higher for urea than for polyols. Thus, in films containing urea the reaming hydrophilic groups available for interaction with free water are reduced, leading to lower film water content as shown in Table 1.

An increase in urea content led to an increase in the intensity of the peak located at 1455 cm^{-1}, which correlates with the C-N bond stretching in urea structure. Wangy col. [26] associated the absorbance ratio of this peak to that of 2930 cm^{-1} (A_{1455}/A_{2930}) to the superficial urea content of the material. The authors had observed a significant increase in A_{1455}/A_{2930} ratio when its concentrations exceeded 30 %w/w at the same time migration of this plasticizer to the surface of potato starch films had been detected by SEM. Despite the fact that A_{1455}/A_{2930} doubled from M (3,5 ± 0,6) samples to U (8 ± 0,6), urea superficial crystallisation was not observed in either samples probably because urea contents assayed were below those reported by Wangy col. [26] for urea migration to occur. Nonetheless, such differences can only be attributed to the greater concentration of urea molecules, and therefore C-N bonds, in films with 25 % of urea.

As shown in Fig.3c, in the fingerprint region of the spectrum all films components absorb, presenting distinctive peaks and intensities. Since in this region the major contributions are related to the starch matrix no major spectral variations were observed. Besides, the bands in this region of the infrared spectrum result mainly from C-O and C-C vibrational modes that are highly coupled, therefore the assignment of individual bands results difficult [42]. The main contributions found are shown in Table 3.

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Table 4. Glass transition temperature, thickness and tensile resistance properties of control cassava starch films (C) and films plasticized with: glycerol (G), urea (U) and their mixture (M).

Film	Tg (ºC)	Thickness (µm)	R_{max} (MPa)	E_{max} (%)	Ec (MPa)	Eg (kJ/m^3)
C	68.3 ± 1.8 [c]	75.8 ± 2.4 [a]	61.8 ± 2.9 [b]	6.1 ± 0.8 [a]	1936 ± 376 [b]	1995 ± 344 [c]
G	37.8 ± 0.5 [b]	75.9 ± 5.3 [a]	2.2 ± 0.6 [a]	47.3 ± 9.6 [b]	14 ± 3 [a]	1091 ± 73 [a]
M	35.0 ± 1.2 [b]	74.5 ± 5.8 [a]	2.9 ± 0.3 [a]	68.9 ± 4.5 [b]	7 ± 2 [a]	977 ± 122 [a]
U	13.6 ± 1.0 [a]	74.9 ± 3.1 [a]	3.0 ± 0.4 [a]	81.8 ± 7.2 [c]	29 ± 7 [a]	1740 ± 99 [b]

Reported values correspond to the mean ± standard deviation.

The peaks between 990 and 1030 cm^{-1} were attributed to the anhydroglucose ring C–C, C–O, C–H bonds stretching and C-O-H bending modes [25, 43],while those at around 1150 and 1080 cm^{-1} were assigned to C–O–H stretching in starch [44]. The slight deviations detected in peaks maximums were attributed to plasticizers-starch interaction.

In addition, many authors have emphasised that both the absorbance ratios of the peaks at 994 and 1047 cm^{-1} relative to that of 1022 cm^{-1} could be indicative of the degree of crystallinity of starch [42, 43]. In line with these studies, C films presented A_{990}/A_{1022} and A_{1047}/A_{1022} ratios 15% lower than native starch, since in C films starch had been gelatinised and therefore present a rather amorphous gel structure [45]. Likewise, films plasticised with U showed similar results than C films. Films containing glycerol in their formulation (M and G) presented significantly higher A_{990}/A_{1022} and A_{1047}/A_{1022} ratios ($p<0.05$), that should be indicative of more crystalline regions in the films structure. Nonetheless, these unusual results could be attributed to the band at 995 cm^{-1} that correspond to the vibration of the skeleton C–C and the peak at 1045 cm^{-1} associated to the stretching of the C–O linkage in C1 and C3 in glycerol [46]. Consequently, this criterion would not be adequate to estimate the crystallinity of plasticised starch films.

I.3.2. Differential Scanning Calorimetry (DSC)

The DSC measurements served to determine the relaxation transitions of starch films (Table 4). The registered glass transition temperature (Tg) value for C films proved to be noticeably higher than that reported for films including plasticizer. Such results are in agreement with transition temperature registered for unplasticized starch films by other authors in literature [47, 48]. Moreover, a significant decrease ($p<0.05$) in the Tg of cassava starch films was observed in films plasticised with urea (U), being this effect less important in the co-plasticisation with glycerol (M). Lowering of Tg is regarded as an indicative of plasticisation efficiency [6], therefore these results imply that urea is a more efficient a plasticizer than glycerol and their mixture.

I.3.3. Mechanical testing and SEM characterisation

Furthermore, the mechanical behaviour of starch-based films depends heavily on their composition and thickness, yet all studied films had an average thickness circa 75 µm with no significant differences ($p>0.05$) among samples (Table 4).

Mechanical tensile resistance parameters of cassava starch-based films are shown in Table 4. From the results, it is clear that unplasticized films (C) present a brittle behaviour due to the strong cohesive forces among amylose and amylopectin chains [6]. The addition of 25 %w/w of plasticizer had a substantial impact on films maximum resistance (R_{max}) and elongation (E_{max}) as well as their elastic modulus (Ec), regardless of their nature (Table 4). Plasticised films mechanical profiles are shown in Fig.4.

As expected, the films mechanical behaviour was markedly affected by the type of plasticizer. Films plasticised with urea (U) resulted more flexible and resistant than that plasticised with the same content of glycerol (G). A significant increase ($p<0.05$) was observed in the elongation at break and tenacity of the material, although no significant differences were observed in the maximum tensile strength at rupture (Table 4). The materials elastic modulus follow a similar tendency (Table 4), thus it could be concluded that in terms of mechanical performance urea is a more efficient plasticizer than glycerol, most probably due to molecular size difference between both plasticizers [6]. In this regard, the higher mechanical resistance of U films is attributed to the stronger interactions between urea and the starch matrix, which was evidenced by FTIR analysis. With regards to film flexibility, Ivaničy col. [33] had revealed an opposite behaviour studying native corn starch films plasticised with urea and glycerol, reporting higher Tg values for urea plasticised starch films; therefore, in their study films were in a vitreous-amorphous state at ambient temperature which would explain their brittleness. Differences are attributed to processing conditions since in this work plasticizer was incorporated before starch gelatinisation.

When both plasticizers were added to the matrix, the resultant mechanical profile is in between that of a flexible and a flexible-tenacious material, exhibiting

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though poorer characteristics than that of films with a single plasticizer. In spite the fact that there are no significant differences ($p>0.05$) in matrix elongation at break between U and M samples, both the maximum strength and the tenacity of the latter are significantly lower ($p<0.05$) than those of films plasticised either with urea or glycerol (U and G, respectively). Presumably, H-bridges interactions could be favoured among urea and glycerol molecules rather than interactions with the polymer matrix, negatively affecting the films mechanical properties.

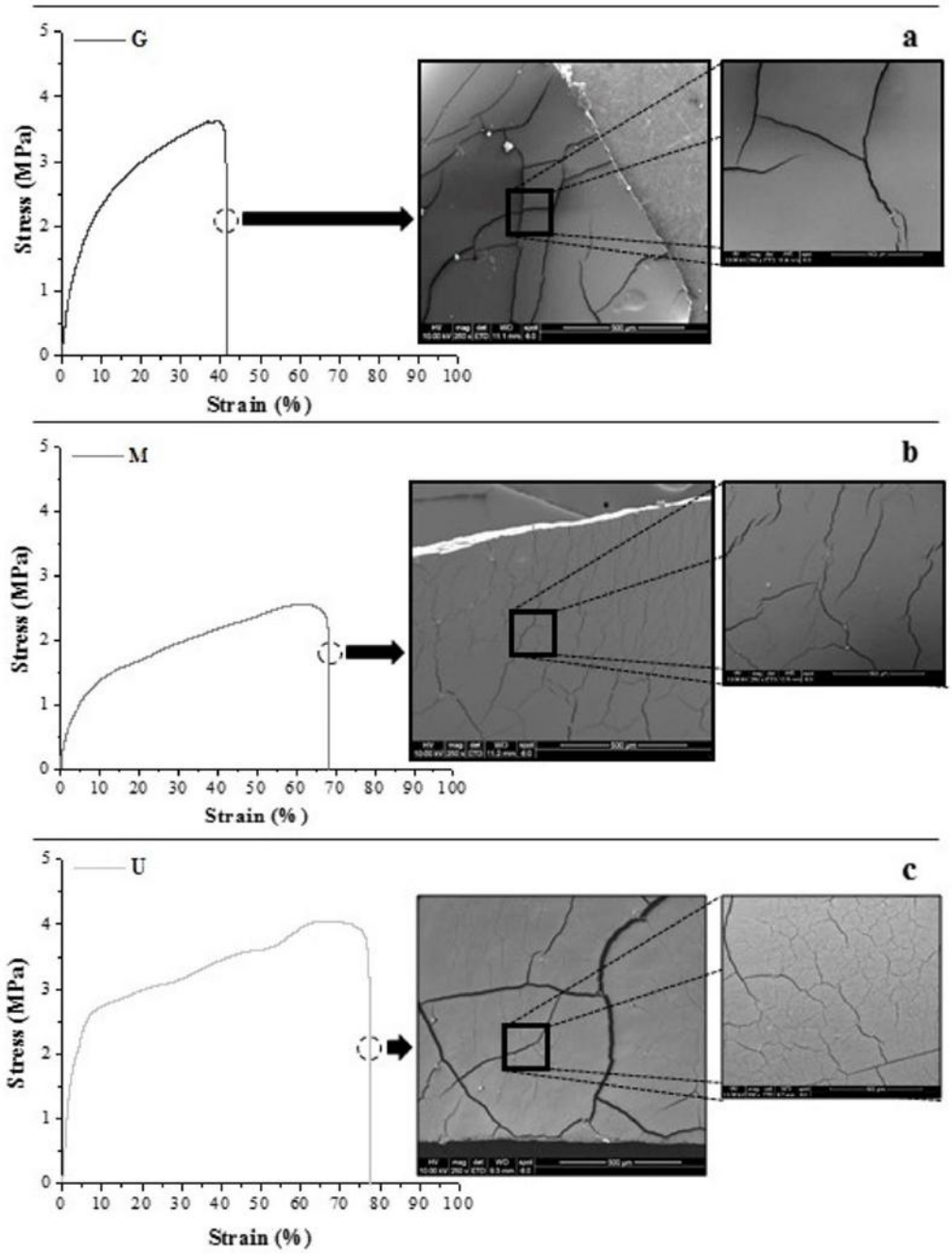

Fig.4: Tensile stress-strain curves and SEM micrograph of fractured plasticised cassava starch films with: a) 25% glycerol (G); b) 12.5% glycerol and 12.5% urea (M); and c) 25% urea (U). The SEM micrographs correspond to the cross section and a close-up image of the films surface.

Moreover, the topography of the surface and cross sections of the plasticised films subjected to tensile rupture test were studied using the SEM technique. Fig.4 shows the films probe rupture cross section and surface close-up of cassava starch films containing different types of plasticizer. Cracks or micro-cracks were observed on the surface of the materials in the direction in which the fracture of the specimen was propagated. However, films

with the addition of urea presented smaller cracks in every other direction probably because of the amorphous structure of the plasticised matrix. Besides, it should be remarked that no superficial urea migration, nor crystallisation, was detected. The micro-cracks observed result from the non-elastic elongations of films containing urea (detail box in Fig.4b and 4c). These results proved again the more flexible and resistant structure developed in U films, due to urea-starch interactions development as was confirmed by FTIR.

I.4. Principal Components Analysis (PCA)

In order to illustrate the aforementioned effects of the plasticizers on the polymer matrixes a PCA was carried out (Fig.5). Two separate analysis were done: one comparing plasticised and unplasticized samples all together (Fig.5a), and another one comparing only plasticised films to evaluate the effect of plasticizer type (Fig.5b). Both analyses gave a *cophenetic correlation coefficient* value circa 1, showing that such data grouping is representative of the experimental variables studied.

In the first case, it is clear that the first main component (CP1) -that explains the 70.2% of the total variance- represents the plasticisation effect on starch films. In comparison, considering the plasticised films (left side of Fig.5a) it can be seen that U films generate more important changes in films properties, since it is further away from the centre axis. Considering that C and M samples were not considerably affected by the second main component (CP2), in this case representing the 25.7% of the total variance, it was therefore attributed to the presence of urea or glycerol as single plasticizers.

Similarly, in the second case (Fig.5b) this analysis showed that: the first main component (CP1) that explains 76.1% of the total variance, associated with the presence of glycerol in the film matrix; whereas the other main component (CP2) attributed to single or co-plasticisation, accounts for the remaining 23.9%. The latter effect correlates with that seen in the first case, indicating that overall co-plasticisation with 50:50 urea and glycerol mixtures have a lower impact on films end properties than single compound plasticisation.

This analysis summarizes the previously detailed results, indicating a stronger influence and efficiency of urea as plasticizer of the starch matrix.

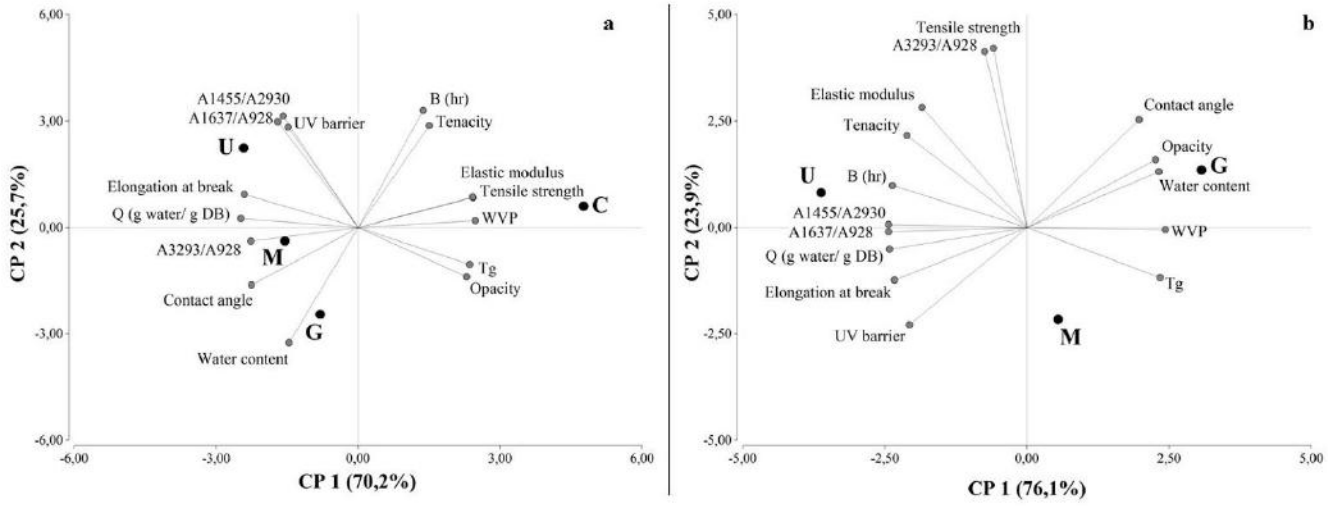

Fig.5. Principal components analysis (PCA) considering films properties with significant differences for cassava starch films, comparison of: a) plasticised (G, M and U) and unplasticized (C) films, and b) films plasticized with 25 %w/w of glycerol (G), urea (U) and their 50:50 mixture (M).

IV. CONCLUSION

In conclusion, urea, glycerol and their mixture were compatible plasticizers for cassava starch-based matrices, not detecting surface migration within the plasticizer's content tested (25 %w/w). Even though all samples contained the same amount of plasticizer, the addition of urea resulted in lower film moisture content. Films containing urea showed enhanced optical properties, especially the UV barrier capacity, since this plasticizer exhibited an electronic transition in this spectral region; though films containing a mixture of both plasticizers presented the highest UV-Vis absorption (200 - 700 nm).

Plasticizer-polymer interactions as well as those involving water molecules were evidenced by peak shifts observed in ATR-FTIR spectra: in particular, the detected shift to lower frequencies at 3300-3000 and 1700-1300 cm-1 regions, suggested stronger H-bonding interaction between starch O-H groups and N-H and C=O groups in urea than those between O-H pairs. In addition, the higher plasticizing efficiency of urea was demonstrated by both

the decrease in Tg values and the mechanical properties enhancement. On the contrary, the co-plasticization of the starch films with glycerol and urea 50:50 mixture resulted in lower mechanical resistance. As discussed, this behaviour could be ascribed to H-bridges interactions among urea and glycerol molecules -which were evidenced by FTIR of the mixture- reducing the plasticizers' interactions with the polymer matrix. Hence, the latter's use for starch- based films plasticization should be discouraged.

The obtained results provide a starting point for the study of applications of starch-based biomaterials as active compounds for controlled-release systems, particularly for agronomic purposes considering that urea is a commonly used fertilizer. In this regard, further research on co-plasticization with urea should be encouraged.

V. ACKNOWLEDGEMENTS

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Characterization, classification and suitability ratings of soils for rainfed rice production in Rukubi, Doma, Nasarawa State, Nigeria

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***Abstract*—** *Rice is an important annual crop in Nigeria. It is one of the major staples, which can provide a nation's population with the nationally required food. The objectives of this study were to characterize, classify and determine the suitability ratings of some soils of Rukubi for rainfed rice production. All the soil units were deep (150 – 199 cm), unit III soils were well drained, while units I and II soils were somewhat poorly drained. The soils had textures ranging between sandy clay loam and clay loam. The soils were well structured (strong coarse sub-angular blocky). Soil reactions were slightly acid (pH 5.12 – 7.15 in H_2O). The organic carbon content of the soils were moderately low to high (1.03 – 1.62 %) in the surfaces, while low in the sub-surface horizons (0.50 – 1.60 %). The total nitrogen was low at the surface horizons and ranged between 0.01 and 0.16 %. The soils were dominated by Ca and Mg with values varying from 1.10 – 4.021 cmol/kg and 0.05 – 3.89 cmol/kg respectively. The available phosphorus was relatively high in the surfaces (4.10 – 11.8 mg/kg), but, much lower in the sub-surfaces (14.15 – 9.85 mg/kg). The percentage base saturation of the soil ranged from 47 % to 98 %. Based on the physical and chemical characteristics, the soils of unit I 1were classified as TypicEndoaquepts/ AndicFluvisols; unit II was classified as EutricEndoaquepts/ AndicCambisols and unit III as ArenicEndoaquepts/ EutricFluvisols. The characteristics of the soil units were compared with the land requirements for rice production. On suitability rating, all of the soil units highly suitable for rainfed rice production.*

Keywords*— *Rainfed rice, suitability ratings, soils.

I. INTRODUCTION

Cereals are one of the important foods for growing population of human. Approximately 50% of consumed calories by the whole population of humans depend on wheat, Rice and maize (Gnanamanickam, 2009). Although rice has the second place because of planted area but it serves as the most important food source for Asian countries mainly in south-east parts where it is an economic crop for farmers and workers who grow it on millions of hectares throughout the region (Gomez 2001). Historically, rice was cultivated l0000 years ago in the river valleys of South and Southeast Asia and China since it served as the most important food for people. Although Asia is the main place of rice cultivation but it was harvested in other continents like Latin America, Europe, some parts of Africa and even USA (Gnanamanickam, 2009).

The rice sector in Nigeria is one of the most important remarkable agricultural developments over the decades. It is the most consumed staple food by Nigeria's over 174 million people across states and geo-political zones. There is lopsidedness in the level of production of rice in Nigeria as compared to its consumption pattern. The implication is that, to meet up with the high demand for its consumption, the rice has to be imported and these have been on the high side and it is inelastic.

In the light of this, Frederic *et al*. (2003) observed that, with rice now being the structural component of the Nigerian diet, and rice imports making up an important share of Nigeria's agricultural imports, there is considerable political interest in increasing the consumption of local rice. This has made rice a highly political commodity.

Akpokodje*et al*. (2001) maintained that, a comprehensive and up to date picture of rice sector in Nigeria in general and rice production, processing and consumption in particular is lacking. It can be seemingly noticed that, despite its agricultural potentials, Nigeria is yet to harness its vastland resources suitable for agriculture, to not only improve its export on rice, but even to cater for its domestic consumption which will invariably serve for sufficient food security. This is evident from the fact that, rice consumption in Nigeria increases over decades and in alarming rates.

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Although, the total rice production is increasing recently due to high demands; the recorded increase however, have not been sufficient to meet the increasing demand from the rapidly growing population; estimated at over 174 million people.

Osagie (2014) observed that Nigeria currently spends about a billion Naira daily importing rice. The Nigerian government recently came up with a policy decision to ban rice importation completely by 2015. The question is how prepared is the Nigerian government towards ensuring that, this policy intentions are actualized? Considering the fact that, the United States Department of Agriculture reveals that, Nigeria's rice imports in 2012 to 2013 alone were estimated to reach about 3 million tones. This is mainly because, the projected increase in rice production in 2012 to 2013 falls short of consumption requirements.

II. MATERIALS AND METHODS

The study area is Rukubi, located at about 81 km South-west of Lafia and 40 km North-west of Makurdi town. The area lies between Latitudes $7^{0}19^{1}28^{11}$ and $7^{0}55^{1}45^{11}$N, Longitudes $8^{0}30^{1}56^{11}$ and $8^{0}18^{1}20^{11}$E, and the altitude of 252 m above sea level (asl). The area experiences distinct wet and dry seasons with the mean annual rainfall of about 1307 mm falling between April and October of most years. The mean average temperature is about 27.4^{0}C.The monthly minimum temperature is between 16.2^{0}C - 17.2^{0}C.

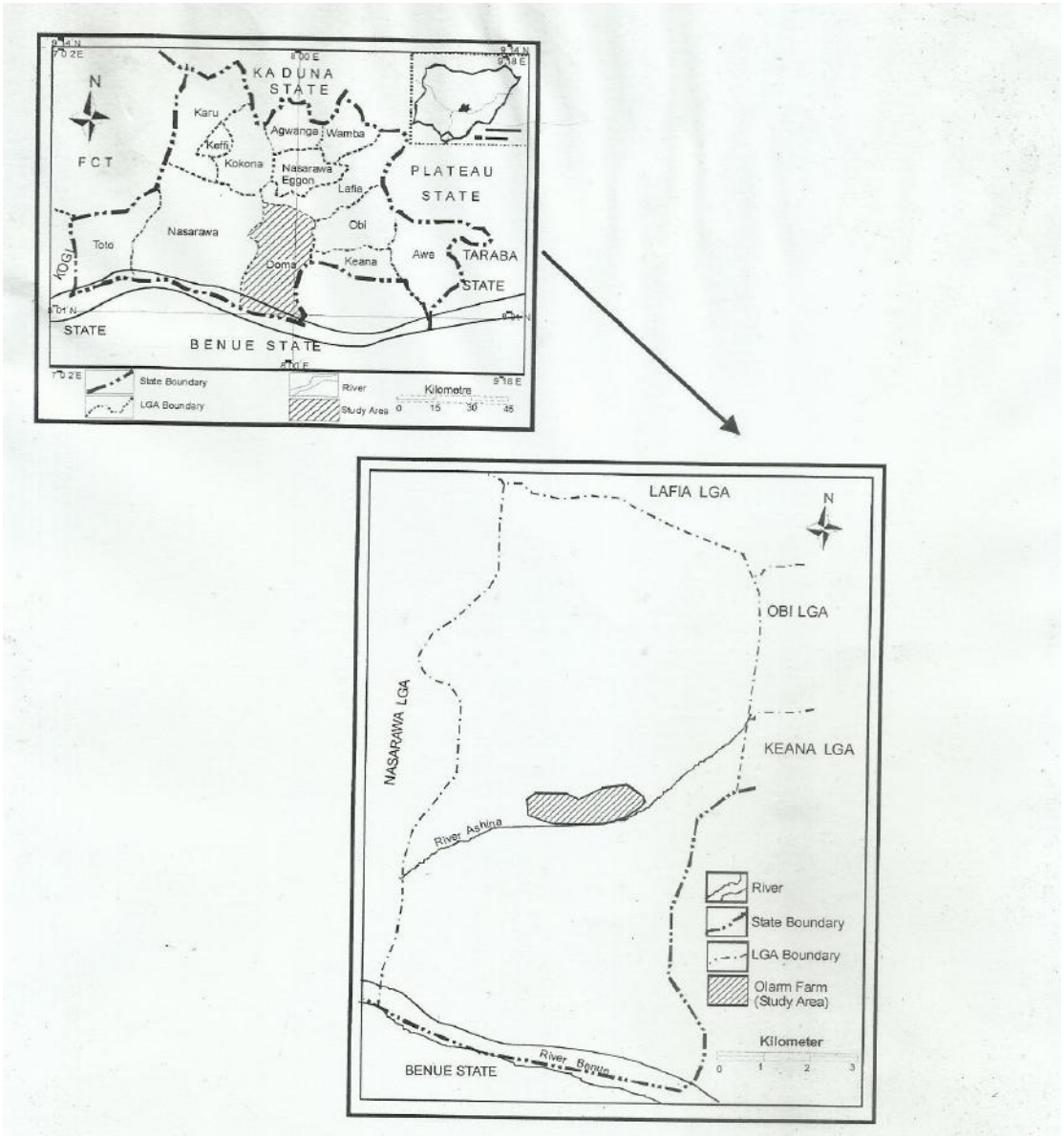

Fig.1: Map of Nasarawa State showing the study area

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The geomorphology of the area shows that the rock types are making up the components of Nigerian geology (Basement, Younger Granites and Sedimentary rocks). The basement complex cover up to 60% of the total area of the area, while the remaining 40% is made of sedimentary rocks of the middle Benue Trough.The area is composed of undulating lowlands and network topography with little or no rock outcrops (Nyagba, 1995).

Field Studies

About 300 ha of the extensive farmland at Olam were soil-surveyed using grid method with traverses cut at 200m perpendicular to the baseline. Auger point investigations were conducted at 200 m interval. Different soil types were identified using morphological characteristics such as colour, texture, structures, topography, consistence, and surface characteristics as differentiating features for delineating soil boundaries. Two pits will sunk in each soil unit, described using the guidelines of soil profile descriptions (soil survey staff, 2014). Sampling were done for each identified horizon

The soil samples from each representative soil unit were collected into polythene bags, neatly labeled and taken to the laboratory for physical and chemical analysis. Based on the data obtained from the soil survey, the soils at Rukubi were subsequently characterized, classified and mapped.

The soil samples were air-dried, gently crushed and sieved to obtain the fine earth fraction (<2 mm). Soil bulk density were determined by the undisturbed core sampling method after drying the soil samples in an oven at 105 ^{0}C to constant weights, while particle density were measured by the pycnometer method (Black, 1965). Percentage pore space was computed from the values of bulk density and particle density (Brady and Weil, 2002) as total pore space (percentage) = (1-BD/PD) x 100.

The laboratory analysis was carried out included particle size distribution using hydrometer method as described by Day (1965). Soil pH was determined by electrometer method as described by Hesse (1971). Soil organic carbon was determined by Walkey Black method based on the oxidation of organic matter by potassium dichromate (Hesse, 1971). Total nitrogen was determined using macro Kjeldahl procedures. Available phosphorus determined using Bray 1 method (IITA, 1979). The exchangeable bases were extracted using neutral NH_4OAC as displacing solution. Calcium and Magnesium were read on atomic absorption spectrophotometer, while Potassium and sodium were read on flame photometer. Exchange acidity was determined using Barium Chloride Triethanolamine as described by Peech (1965). Effective cation exchange capacity was calculated as the sum of exchange acidity and exchangeable bases. The percentage base saturation was calculated as total exchangeable bases divided by effective cation exchange capacity multiplied by 100.

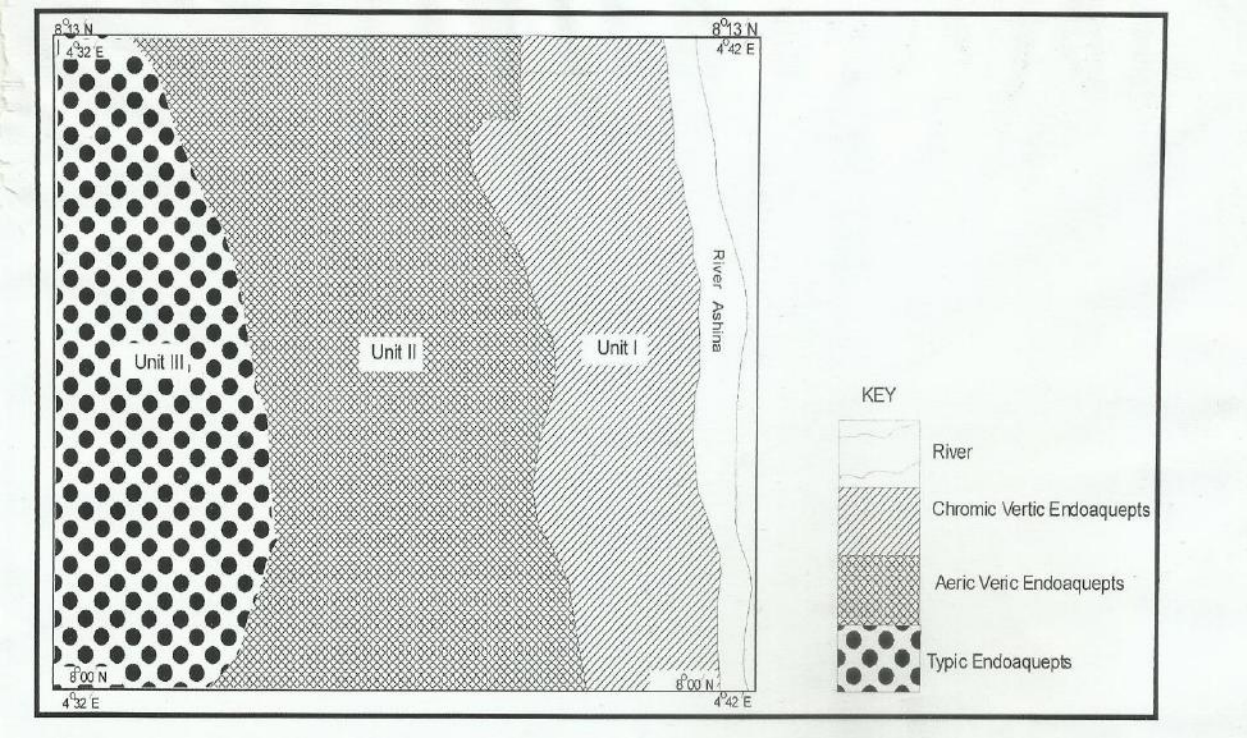

Fig.2: Soil Map of Rukubi

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III. RESULTS AND DISCUSSIONS

Soil morphological characteristics

Figure 1 shows distribution of the three mapping units. The soils were generally poorly drained. They were fine textured with clay content ranging from 10.7 % and 40.0 %, this could be as result of the shale parent material. The clay and sand content distribution was irregular across the horizons except for sand in unit III were the sand content decreased with increase in depth. This could be as result of intense disturbances caused heavy machinery used in farming by Olam rice farm. More so, the irregular pattern in most of the units could be due to different types of sediment with varying textures deposited annually in accordance to their source. The structure of the surface horizon in all the soil units was strong coarse sub- angular blocky possibly as a result of high organic and clay contents.

Soil chemical properties

The pH value of the soils of the study area as shown in Table 1 indicates that the soils were slightly acid in reaction. Soil pH of surface horizon ranged 5.12 and 7.1. These figures mostly decreased with depth probably due to the effect of nutrient biocycling (Ogunwale *et al*., 2002) the percentage organic carbon was highest 1.62% in pedon 3 probably due to incorporation of the crop residues to the soil. The total nitrogen values of the soils ranged between 0.01 to 0.70 %. These nitrogen levels are very low for surface horizons of soils as rated by (IRRI, 1995) and probably due to release from plant tissues, gaseous loss and volatilization (De Datta, *et al*., 1991).

The available phosphorus values were relatively high (11.8 cmol/kg). The values decrease with increasing depth. This is perhaps due to the relationship of organic carbon with phosphorus (Miura *et al*., 1997).

Exchangeable Ca and Mg dominated over K and Na in the exchange complex. This is in agreement with earlier finding of Fagbami and Akamigbo, (1986). The values of exchange acidity were generally low and ranged between 0.88 to 1.80 cmol/kg. Effective cation exchange capacity values were low and ranged between 3.39 cmol/kg and 9.70 cmol/kg of soil, which is rated as low to moderate (FAO, 1983). This may probably be due to the contribution of clay (Idoga and Azagaku, 2005). The percentage base saturation was rated from moderate to high, ranging from 47 % to 98 %. This could be linked to the active plant litter decomposition process, which incorporates cations from the litter into the soil surface (Malgwi, 1979).

The USDA soil taxonomy (soil survey staff, 2014) and the WRB, (2006) were used in classifying the soils of the area. Both field and laboratory studies of the soils of the area indicate an increasing trend in the amount of clay with depth (especially unit III) and also high degree of aggregation. The clay distribution pattern shows that there is argillic horizon in all the profiles studied. This clay distribution pattern corresponds with the high base saturation status of the soils therefore qualifying them as Alfisols. The presence of mottles mostly near the soil surface, a chroma of 2 in some horizons qualifies the soils as Aqualfs. Soil units II and III show evidence of episaturation by the presence of mottles at or near the soil surface and the dominant hue of 10YR and 2.5Y; they therefore qualify as Epiaqualfs. They further qualify as AericVerticEndoaqualfs and ChromicVerticEndoaqualfs because of the presence of cracks narrower than 2 cm and shallower than 50 cm, as well as the dominance of hue of 7.5YR, and 10YR. All the soil units showed irregular but decreasing clay content with depth. They therefore classified as *Inceptisols*. This inferred ustic soil moisture regime of the area places these soils into the suborder ustept. The clay distribution pattern of the soils places them in the great group Haplustepts. They further classified as AquicHaplustepts because of aquic soil moisture regime within 70 cm of soil surface.

In the WRB (2006), the soils have high clay content in the subsoil than in the topsoil as a result of pedogenic processes (elluviation and Illuviation) leading to an Argic subsoil horizon. All soil units have evidence of redoximorphic features caused by subsurface water, which periodically wet both the topsoil and the subsoil over a considerably period of time leading to the formation of mottles. For these reasons, they are classified as Luvisols (WRB, 2006). They further qualify as VerticLuvisols because of vertic properties within 100 cm of soil surface and higher clay content of the subsoil as well as the high base status of the soils.

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Table.1: Physical and Chemical Characteristics of the Soils

Horizon	Depth	Bulk Density	Particle size analysis			Textural Class	pH (1:1)		Organic Carbon (%)	Avail. P (mg/kgp)	Total N (%)	Exchangeable Cations				Exch. Acidity	ECEC	BS (%)
			Sand	Silt	Clay		KCl	H_20				Ca	Mg	K	Na			
			→	%	←							→	Cmol/kg		←			
Soil Unit I: Pedon 1 Chromic VerticEndoaqualfs/ AndicLuvisols																		
A	0-15	2.73	69.2	2.60	28.2	SCL	4.58	5.63	1.60	4.10	0.70	3.80	3.40	0.34	0.36	1.08	8.63	92
B	15-30	2.82	25.5	40.6	33.9	CL	4.50	5.76	1.10	4.50	0.56	3.76	3.30	0.34	0.38	1.03	8.97	89
B_1	30-87	2.87	39.1	42.0	18.9	L	4.40	5.74	1.10	4.40	0.56	3.63	3.20	0.32	0.34	1.02	8.51	88
B_2	87-130	2.79	40.8	32.0	27.2	L	4.87	5.84	0.87	4.20	0.56	3.61	3.18	0.32	0.34	0.99	8.44	89
C	130-150	2.90	41.3	29.9	28.8	CL	4.53	5.92		4.15	0.49	3.52	3.06	0.29	0.33	0.72	7.92	91
Soil Unit I: Pedon 2 Chromic VerticEndoaqualfs/ AndicLuvisols																		
A	0-30	2.93	73.5	15.7	10.7	SCL	5.07	7.15	1.44	4.80	0.56	4.10	3.60	0.36	0.40	1.12	9.52	88
A_1	30-70	2.74	31.5	37.7	30.8	L	5.04	6.40	1.32	4.65	0.56	4.00	3.89	0.34	0.37	1.10	9.70	89
B	70-116	2.82	64.2	18.3	17.5	SL	4.93	6.38	1.00	4.65	0.49	3.92	3.37	0.33	0.34	0.98	8.94	89
C	116-199	2.84	61.3	25.0	13.5	SL	4.65	5.93	0.84	4.20	0.49	4.00	3.50	0.35	0.38	0.88	9.11	90
Soil Unit II: Pedon 3AericVerticEndoaqualfs/ VerticLuvisols																		
A	0-10	2.74	33.5	36.0	30.5	CL	4.49	5.91	1.62	4.40	0.59	3.86	3.30	0.30	0.34	1.13	8.93	87
AB	10-49	2.79	21.5	41.3	37.2	CL	4.41	5.86	1.60	4.50	0.56	3.88	3.17	0.31	0.35	1.10	8.81	88
B	49-99	2.94	35.1	36.4	28.5	CL	4.54	5.92	0.87	4.40	0.56	3.51	3.00	0.30	0.34	1.06	8.21	71
Bt_2	99-140	3.02	44.8	35.0	20.2	L	4.54	5.88	0.68	4.30	0.59	3.40	3.10	0.28	0.32	0.98	8.08	88
C	140-154	3.11	28.9	40.0	31.1	CL	4.56	5.78	0.60	4.25	0.70	3.31	3.00	0.28	0.29	0.81	7.69	90
Soil Unit II: Pedon 4AericVerticEndoaqualfs/ VerticLuvisols																		
A_1	0-13	2.70	37.1	38.4	24.5	L	4.69	6.69	1.12	4.65	0.56	3.84	3.40	0.34	0.56	1.14	8.28	98
B_2	13-57	2.98	34.8	42.0	23.2	L	5.60	6.62	1.07	4.50	0.56	3.74	3.18	0.32	0.36	1.04	8.64	88

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B_3	57-96	2.84	40.8	40.0	19.2	L	6.03	6.54	0.87	4.30	0.70	4.02	3.60	0.37	0.40	1.00	9.39	89
C	96-156	2.88	73.1	10.9	16.2	SL	5.62	6.92	0.62	4.75	0.63	3.86	3.60	0.36	0.40	0.94	9.32	88
Soil Unit III: Pedon 5 Chromic VerticEndoaqualfs/ VerticLuvisols																		
A	0-28	2.52	40.8	18.2	40.0	SC	4.50	5.98	1.56	11.8	0.59	2.22	1.47	0.31	0.17	1.50	5.67	74
B	28-49	2.67	45.2	19.2	35.6	CL	4.42	5.96	1.06	9.85	0.52	1.14	1.61	1.36	0.12	1.50	5.73	73
Bt_1	40-70	3.00	44.6	23.2	33.2	CL	4.33	5.68	0.92	9.12	0.51	1.10	1.54	0.84	0.10	1.35	4.37	81
Bt_2	70-93	3.03	46.8	20.0	33.2	CL	4.00	6.51	0.56	8.22	0.43	1.07	1.46	0.44	0.05	1.20	4.22	72
Bt_3	93-150	3.09	48.8	20.0	31.2	SCL	4.12	5.12	0.62	8.0	0.04	1.05	1.33	0.48	0.02	0.88	3.76	77
Soil Unit III: Pedon 6 Chromic VerticEndoaqualfs/ VerticLuvisols																		
A	0-22	2.78	48.5	15.5	36.0	SC	4.88	5.99	1.03	10.5	0.10	2.81	2.53	0.30	0.16	1.50	7.30	79
B	22-67	2.77	44.6	21.0	34.4	CL	4.62	5.88	1.00	9.01	0.09	1.92	1.29	0.28	0.12	1.65	5.26	69
Bt_1	67-93	3.20	48.2	20.8	31.0	SCL	4.81	5.91	0.67	8.51	0.07	1.74	1.04	0.27	0.15	1.60	4.30	74
Bt_2	93-138	3.38	50.0	20.0	30.0	L	4.72	5.82	0.58	8.50	0.05	1.53	0.89	0.24	0.10	1.80	4.36	63
Bt_3	138-160	3.40	39.7	22.3	38.0	L	4.91	5.82	0.50	7.00	0.01	1.23	0.05	0.21	0.10	1.80	3.39	47

Source : Field Studies

Table.3: Summary of Soil of Soil Type and Their Suitability Ratings

Soil Units	Pedons	Taxonomic classes	Suitability ratings
I	1 and 2	Chromic VerticEndoaqualfs/ AndicLuvisols	S_2
II	3 and 4	AericVerticEndoaqualfs/ VerticLuvisols	S_2
III	5 and 6	Chromic VerticEndoaqualfs/ VerticLuvisols	S_2

Where S_2- moderately Suitable

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Suitability ratings for rainfed rice production

Suitability ratings derived from the results of soil survey work (Fagbemi and Akamigbo, 1986). The interpretation of soil survey work itself is a statement of prediction of performance. Suitability ratings is therefore, carried out by comparing the characteristics of the soils with the requirements of the crop in this case, rice. The chemical characteristics of the soils such as pH, organic matter, exchangeable bases, effective cation exchange capacity, and exchange acidity are found to be conducive to rice production or can be mended by individual farmers and therefore cannot be permanent limitations.

All mapping units were very deep (>120cm) and all are considered suitable for the production of rice. However, all the pedons have characteristic mottling at the subsurface through to the last horizon. This probably accounted for the observed redoximorphic condition in all the soil units indicated by the presence of few fine medium to coarse and distinct to prominent mottles occurring within the horizons. However, the soil may not have been under permanent water saturation for a period longer than few weeks as indicated by soil colour which ranged from the texture of the soils ranged from clay loam to sandy clay loam. According to Sys (1991, 1993), rice require loamy clay to sandy loam clay for optimum yield. Thus, the soils in pedon unit I and III present a very slight limitation to rice yield, while soil unit II seems to possess no textural limitation to rice production, hence, it is highly suitable. The structure of the soils ranged between fine sub angular blocky to coarse sub angular blocky. Coarse sub angular blocky is regarded as highly suitable for rice production (Sys, 1991). The structures of all the mapping units were considered highly suitable for rice production. The soil chemical properties, which could affect their suitability for rainfed, rice production are acidity, salinity, and fertility. The reactions of the soils ranged from slightly acidic to neutral (pH 5.12 to 7.15). Although this pH level may not pose serious problem for P uptake, pH above 6.0 may limit the availability of micronutrients such as Fe, Zn, Mn and Cu which form metallic cations that precipitate into low solubility compounds at high pH levels. Total exchangeable acidity ($H^+ + Al^+$) ranged between 0.72 cmol/kg and 1.80 cmol/kgindicating that the level of exchangeable aluminium was below toxic range (Tanaka and Yoshida 1970). The soils have low to medium levels of exchangeable Ca, Mg, K, Na and of N and medium to high levels of Bray-1 P. All with the exception P, the major nutrient content in the soil were lower than the critical requirement for rice production (De Datta, 1989). The result of the survey revealed that the levels of organic matter, nitrogen, exchangeable cations and Mn were below the critical requirements for rice production (Adeoye, 2002).

Appendix A: Land Requirement for Suitability Classes For rain-fed Rice Production

Land Qualities	$S1_1$	$S1_2$	S2	S3	N1	N2
CLIMATE						
Annual Rainfall	>1000	900-1000	800-900	600-800	600-500	<500
Mean annual temperature(^{O}C)	>25	22-25	20-22	18-20	16-18	<16
Relative Humidity (%)	>75	70-75	65-70	60-65	<60	
Topography: Slope (%)	<2	3-4	5-6	7-8	9-10	>10
DRAINAGE (s):						
Wetness	WD (ID) †	MWD (ID) †	MD	ID (WD) †	PD (WD) †	PD (WD) †
Flooding	Fo	Fo	F1	F1	F2	F3
SOIL PHYSICAL PROPERTIES						
Texture	L (LC) †	Lfs (SLC) †	LS (SL) †	S	S	S
Structure	Cr (SAB) †	C (SAB) †	SAB (Cr) †	SAB (Cr) †	Col (Cr) †	Col (Cr) †
Coarse fragment (%) (0-45cm)	<3	3-5	5-10	10-15	>15	
Soil Depth (cm)	>75	65-70	50-65	35-50	30-35	<30
FERTILITY (F)						
pH	5.5-6.5	5.0-5.5	4.5-5.0	4.0	4.5	<4.0
Base Saturation	>80	70-80	50-70	40-50	25-35	<25

Organic Carbon (%) (0-30cm)	>2.0	2.0-1.5	1.2-1.5	1.0-1.2	1.0	<1.0
MACRO-NUTRIENTS						
Nitrogen (%)	>2.0	1.5-2.0	1.0-1.5	0.5-1.0	<0.5	
Phosphorus (mg kg^{-1})	>20	15-20	8-15	5-8	3-5	<3
Potassium (cmol kg^{-1})	>0.5	0.3-0.5	0.2-0.3	0.1-0.2	<0.1	
MICRO-NUTRIENTS						
Iron (Fe) (mg kg^{-1})	>4.5	3.5-4.5	2.5-3.5	1.5-2.5	1.0-1.5	<1.0
Zinc (Zn) (mg kg^{-1})	2.0-2.5	1.5-2.0	1.0-1.5	0.8-1.0	0.6-0.8	<0.6
Manganese (Mn) (mg kg^{-1})	1.5-1.7	1.0-1.5	0.8-1.o	0.6-0.8	0.5-0.6	<0.5

***Source:* Sys *et al.*, *(*1991, 1993*);* De Datta (1989)**

†= ratings for lowland rice production; SAB= Sub-Angular Blocky; Col= Columnar; Cr= Crumb; WD= Well Drained; MWD= Moderately Well Drained; ID= Imperfectly Drained; PD= Poorly Drained; L= Loamy; SL= Sandy Loam; LS= Loamy Sand; Lfs= Loamy fine sand; SCL= Sandy Clay Loam; Fo= Rarely flooded; F1= Flooding expected; F2= Irregularly Flooded; F3= Regularly Flooded

The climate of the studied area is quite favourable for the production of rice. The mean annual temperature (27^0C-30^0C), average sunshine hours (>5 hours), total annual rainfall and distribution pattern (>1000) and relative humidity during cropping season (>75%) are all adequate by the standard of Sys (1993). The topography of the toposequence is also considered adequate (Slope between <1-2%).

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Productivity of dwarf elephant grass (*Penisetum purpureum* cv. Mott) and coconut (*Cocos nucifera*) in Coconut-Beef Cattle Integrated Farming System (Coco-Beef IFS) in South Minahasa, Indonesia

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***Abstract*—** *This study as a phase I research was carried out on farm (in situ) in the farmer's coconut (Cocos nucifera) land with different designs every year. In this study, the treatments are dwarf elephant grass (Penisetum purpureum cv. Mott) planting and the use of organic fertilizer processed from cattle manure and coconut waste. The variables that become the parameters of technical productivity were measured in first year of three years of research, namely the amount of fresh forage production of dwarf elephant grass, the amount of nuts per coconut bunch, and the stocking rate of forage forages in coconut fields in the Coconut and Beef Cattle Integrated Farming System (Coco-Beef IFS). The results of the study as follows: (1) Productivity of Pennisetum purpureum cv. Mott in coconut field that has been fertilized with cattle manure based compost, produces the yield of fresh forage per year can reach 661,947.64 kg per hectare per year, then the stocking rate of grass* Pennisetum purpureum *cv. Mott per hectare of land under coconut trees can be given to around 45.34 heads of adult cattle a year; and (2) Production of coconut (Cocos nucifera) around 7.88±2.44 nuts or in average about 8 nuts per bunch in land planted with Pennisetum purpureum cv. Mott and fertilized with cattle manure-based compost. Coconut productivity is seen in two parameters (around the bunches and number of coconuts) in the first year of study is still low. The results of the influence of cattle manure-based compost fertilizer on several parameters of coconut productivity are expected to increase in the second or third year of research to be conducted later.*

***Keywords*— cattle, compost, forage, manure, nut.**

I. INTRODUCTION

Nowadays farmers' heavy dependence of cattle on natural pasture for grazing has resulted in the emergence of a range of grazing systems and ecosystem challenges (Lawal-Adebowale et al. 2018). Farmers generally only rely on local forage from vegetation of natural pastures, both grasses and legumes which according to Paat and Taulu (2012) and Osak et al. (2018) that the farmers generally only cultivate forage crops in the lands, fields and on the edge of the irrigation canal lands.

Prawiradiputra and Priyanti (2009) stated that in almost all cattle production areas in Indonesia, smallholder farmers have problems providing and supplying forage sources that are effective and available throughout the year, especially the limited area of forage crops. Whereas in the areas of coconut production centers there are lands that are generally only monocultures of coconut plants that can be intercropped with forage crops.

Coconut plant is one of plantation plants that are able to adapt to the environment, growing in tropics and can be found both in lowlands and highlands (Salendu et al. 2018). Paat and Taulu (2012) explained that in coconut fields if planted with superior grass such as dwarf elephant grass (Pennisetum purpureum cv Mott) and intensively applied fertilizer will be able to increase carrying capacity forage up to more than 30 head per ha.

The land under coconut trees is only overgrown with vegetation for wild pastures both grass and local legume that grows wild, although the yield and quality of these types of forages are low and some of them are low edible for cattle, but due to lack of forages then the farmers are forced to feed or provide feed for local species. Land under a coconut tree if used by planting quality grass, the

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income earned by the farmer household will be higher (Salendu and Elly 2012).

However, the introduction of forage fodder grasses on coconut plantations can lead to competition in the absorption of soil nutrients between forage and coconut plants, so it is necessary to intensify the use of fertilizers to meet the needs of both types of plants. Mantiquilla et al. (1994) suggested that fertilizer used in coconut fields could be chemical fertilizers (in organic fertilizer), organic fertilizers or a combination of both.

The use of inorganic fertilizers continuously and tends to be excessive can cause a lot of agricultural land in Indonesia to be in sick condition. Based on this condition, manure as an organic fertilizer has been glimpsed to substitute inorganic fertilizers, where organic fertilizer based on livestock manure and crop waste can improve soil physical properties. The recycling of precious organic manure wastes might have been responsible for conserving ecosystem and thus increasing the fertility of soil and keeping the environment free from pollution hazards (Ramrao, et al. 2006)

Forage planting on coconut fields and the use of compost based on livestock manure on coconut and forage crops can save fertilizer costs, by eliminating chemical fertilizers so as to increase forage and coconut products that are more productive. Integration of pasture and cattle in coconut plantation is expected to increase the value of the land productivity (Anis et al. 2014). Likewise the integration of plants and livestock in the coconut area can increase coconut production almost twice as much, through the use of livestock manure as organic fertilizer (Polakitan 2012). And according to Ramrao et al. (2006) that the farmyard manure available from the animal was used for fertilizing of crops and 30-35% savings in fertilizer use could be affected in mixed farming system.

Today's mixed farming system is known as Integrated Farming System (IFS), where according to FAO (2001) consist of components such as crops and livestock that coexist independently from each other. The integration system of cattle and plantation is often considered as a step forward in farming practices that are environmentally friendly and sustainably, and as an alternative approach to diversifying sustainable agricultural production that profitable mutually and simultaneously (Osak et al. 2015 and Osak et al. 2016). For this reason, the need for research on the productivity of forage especially dwarf elephant grass (*Penisetum purpureum* cv. Mott) and coconut (*Cocos nucifera*) for Coconut - Beef Cattle Integrated Farming System (Coco-Beef IFS) in South Minahasa, Indonesia.

II. MATERIALS AND METHODS

The study was carried out in a coconut plantation land owned by farmers (in situ) in South Minahasa Regency, Indonesia. Materials and tools used in this study are: coconut land covering 0.5 hectares, dwarf elephant grass seeds, cattle manure, coconut waste (coconut water, dry leaves, and coconut husks), scales and other auxiliary equipment.

This research was conducted in May – August 2018, as a Phase I study in the first year of the three years plan, which was carried out on the farmer's coconut land (*in situ*) with different designs and applications each year. In the Phase I study, the application of dwarf elephant grass (*Penisetum purpureum* cv. Mott) and the use of organic fertilizer processed from cattle manure and coconut waste. The variables that become the parameters of technical productivity were measured in year I, namely the amount of fresh forage production of dwarf elephant grass, the amount of nuts per coconut bunch, and the level of stocking rate for forage in coconut fields in the integration system of coconut – beef cattle and (coco-beef IFS).

The data collected was analyzed descriptively, where according to Lawal-Adebowale et al. (2018) that the descriptive tools such as frequency counts and standard deviation in tables form the basis for summarizing the data collected in relation to the research goals. The descriptive methods of data analysis can be used to identify a new and smaller assembly of non-correlated variables (Gabor, 2012).

III. RESULTS

Based on the results of interviews with cattle farmers in the research location (*in situ*) information was obtained that the management of cattle feeding was still simple with the type and composition as it was in accordance with the availability on their land. Characteristics of farmers showed that the average amount of forage grass fed for a cattle is only about ±17-20 kg/day, while the feeding of rice or corn bran will be given to cattle if available.

Farmers are only able to raise cattle as much as 1-4 heads or <10 heads only, and forage feeding is not yet in accordance with the needs of the existing cattle, because of the low availability of forage livestock owned by farmers. While coconut production is still around 4-15 nuts per bunch or an average of about 8 nuts per bunch, with around the stem of the coconut bunches only about 12 cm, even though the larger it is around the bunches, the production of coconut per bunch is higher.

Training and demonstration activities have been carried out for farmers for the com

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posting process. Cattle manure is used for the processing of manure-based organic fertilizer, which is used as fertilizer for grass forages planted in coconut fields. Farmers are trained in compost processing by utilizing cattle manure from the feedlot, which has been done in compost hut. Processing procedure: initially a box made of beams and bamboo measuring 2x1x1 m in compost hut. Then the plant waste is stacked in the box as high as 15 cm and then put cow dung that has been dried while trampled to make it solid. Then watered or sprinkled with a mixture of coconut water with sugar. So the stages are repeated until the box becomes full and solid. After the box is full, the wall of the box is opened / released, and then the compost material is covered with tarpaulin and tied. A week later the compost was reversed and this was repeated over four weeks. Furthermore, the composting box is opened and aerated by field cooperators. A good compost fertilizer is one that has experienced enough weathering and is characterized by a color that is different from the color of the constituent material, odorless, low moisture content and room temperature.

The land is carried out perfect tillage and then planted with *Pennisetum purpureum* CV. Mott with a planting distance of 100x50 cm, the number of potential clumps are 20,000 clumps per hectare, while cattle manure-based compost is used as much as 10 tons of wet. Coconut land used is 0.5 hectares, so that the number of seeds used are 10,000 cuttings and 5 tons of wet cattle manure-based compost.

Table.1: Productivity of Pennisetum purpureum cv. Mott on coconut land and fertilized cattle manure-based compost in Phase I of coco-beef IFS research

Parameters	Productivity		
	Amount		Std. Dev.
1. Plant height (cm)	201.68	±	34.33
2. Number of tillers per clump (buds)	16.31	±	0.79
3. Stem production per clump (kg)	2.72	±	0.19
4. Leaf production per clump (kg)	2.26	±	0.11
5. Fresh forage production per clump (kg)	4,98	±	0.27

The results of the production of *Pennisetum purpureum* cv. Mott in coconut field fertilized with cattle manure-based compost can be seen in Table 1. Plant height reached 201.68±34.33 cm each 45 days after crop (d.a.c) or days after first harvest, with number of tillers per clump amounting to 16.31±0.79 buds each 45 days after crop (d.a.c), production of stems per clump weighing 2.72±0.19 kg each 45 days after crop (d.a.c) and production of leaves per clump weighing 2.26 ± 0.11 kg each 45 days after crop (d.a.c), so the fresh forage of stems and leaves per clump weighing 4.98 ± 0.27 kg each 45 days after crop (d.a.c). Then several parameters such as neutral detergent fiber (NDF), acid detergent fiber (ADF), feed convertion ratio (FCR) and carrying capacity of *Pennisetum purpureum* cv. Mott and other forages will be observed in the following research phases in the second and third years.

Tabel.2: Two parameters of coconut productivity (Cocos nucifera) on land planted with Pennisetum purpureum cv. Mott and fertilized cattle manure-based compost in Phase I coco-beef IFS research

Parameter	Productivity		
	Amount		Std.Dev.
1. Circle stem of bunches on 11 coconut trees (cm)			
- bunch 1 (bottom bunch)	11.70	±	1.73
- bunch 2 (second bunch after bottom)	10.89	±	1.27
- bunch 3 (third bunch after bottom)	11.11	±	1.65
Average around the bunches in 11 trees	*11.23*	±	*1.18*
2. Coconut production per bunch in 11 coconut trees (nuts)			
- bunch 1 (bottom bunch)	7.18	±	2.27
- bunch 2 (second bunch after bottom)	8.09	±	3.42
- bunch 3 (third bunch after bottom)	8.36	±	3.93
Average number of nuts per bunch in 11 trees	*7.88*	±	*2.44*

In this phase I study, coco-beef was only observed in two coconut (*Cocos nucifera*) productivity parameters in the land planted with *Pennisetum purpureum* cv. Mott and fertilized cattle manure-based compost, which are around the bunches and the number of coconut production per bunch in 11 sample coconut trees, as can be seen in Table 2, while other parameters will be observed in the next phase of research in the second and third years.

The results of the influence of cattle manure-based compost fertilizer on several parameters (such as nuts per bunch, total weight per nut, nut meat weight, and copra weight, protein content, reducing sugar content, fat content, galactomannan content and phospholipid content of fruit meat) of coconut productivity are expected to increase in the second or third year of research to be conducted later.

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IV. DISCUSSION

The above forage production data is still the first data in the Phase I study for three years of research. Closer data to be used in stocking rate calculation is data based on the second harvest in 45 days after crop (d.a.c) or 120 d.a.p., and so on every 45 days after crop (d.a.c) interval. Therefore, phase II research and so on in the following years, must still be carried out to obtain more accurate and systematic production data by rotating forage crops for both grasses and legumes according to the needs of the number of cattle raised. In the next year research will be observed based on ten feeder cattle that will be farmed, where in the second year and third rotational forage harvest based on the needs of the ten feeder cattle.

Based on the results of the Phase I study, the second crop or cut of forage after plant age 45 d.a.c (days after crop) or 120 d.a.p (day after planting) of *Pennisetum purpureum* cv. Mott, produced stem weight per clump every 45 days after crop (d.a.c) is 2.72±0.19 kg and leaf weight per clump is 2.26±0.11 kg so that the total feed for fresh forage is 4.98 ± 0.27 kg per clump in 45 d.a.c or 120 d.a.p., with a spacing of 100 x 50 cm or 20,000 clumps of plants per ha, minus 20 percent of ineffective land overgrown with forages in coconut land, then the number of forages is only 16,000 clumps of plants per ha, so the potential for fresh forage at the first harvest is 79,752.73 kg per harvest.

After harvesting at the first devoliation at 75 d.a.p (day after planting) with a devoliation distance of 45 days, there are 8.3 harvests in a year, so the annual forage yield is 661,947.64 kg per hectare per year. The amount of consumption per adult cattle per day is 40 kg fresh forage, then the stocking rate or availability level of *Pennisetum purpureum* cv Mott per ha of land under coconut tree can be given to a total of 45.34 animal unit (AU) cattle a year.

Coconut (*Cocos nucifera*) production on land planted with *Pennisetum purpureum* cv. Mott and fertilized cattle manure-based compost showed that the total production of coconut fruit is 7.88±2.44 or about 8 nuts. Coconut productivity in the first year is still low, or still like the initial data from interviews with farmers around 4-15 nuts per bunch or about 8 nuts per bunch on average. These results are due to the coconut fruit being only based on natural soil fertility, where the results of cattle manure-based compost fertilizer are expected to increase coconut productivity in the second or third year during future research.

Organic fertilization based on cattle manure is indeed a versatile component in the integration system of cattle and coconut-based agriculture. This organic fertilization not only affects the soil, forage crops, cattle and coconut plants, but also for farmers as an important element in the system in a practical, efficient and effective.

There are still challenges that make organic fertilizer less acceptable, especially bulkiness, relatively low nutritional content, need more labor, stinging odors, and indirect effects on plants. However, intensive research and development efforts will be able to make organic farming more attractive to farmers, and benefit technically, economically and ecologically (environmentally friendly). In addition to forage and coconut productivity, also the production of cattle product that are more relevant will be conducted in the second or third year of the following research will be conducted later. It is expected that there will be continuous efforts that can provide other options acceptable to farmers, in producing high yields and high-quality agricultural products as a result of sustainable integration farming system management.

V. CONCLUSION

1. Productivity of *Pennisetum purpureum* cv. Mott in land under coconut (*Cocos nucifera*) trees was fertilized with cattle manure-based compost in phase I of coco-beef IFS study, the potential yield of *Pennisetum purpureum* cv. Mott fresh forage per year can reach 661,947.64 kg per hectare per year, then the stocking rate of per ha of land under coconut trees can meet the need for cattle feed of about 45.34 heads mixed-age cattles for a year.
2. Coconut (*Cocos nucifera*) production in phase I of coco-beef IFS research on land planted with *Pennisetum purpureum* cv. Mott and fertilized cattle manure-based compost produce around 7.88 ± 2.44 or about 8 nuts per bunch. Coconut productivity in the first year is still low, where the results of fertilization with cattle manure-based compost are expected to increase coconut productivity in the second or third year of following research will be conducted later. In addition those productivity, also the production of cattle product that are more relevant will be conducted in the second and third year of the following research will be conducted later.

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Dung beetle (Coleoptera: Scarabaeinae) community structure across a forest-agriculture habitat ecotone in South Western Ghats

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***Abstract*—** *Ecotones are zones of transition between biomes or ecosystems. Ecotones, natural or anthropogenic, can greatly affect insect community structure across habitats. Scarabaeinae dung beetles are ideal biological indicators that are used to study effects of habitat modification, fragmentation and edge effects on biodiversity. Dung beetle community structure across a forest-agriculture habitat ecotone in South Western Ghats, a biodiversity hotspot in India was studied. Dung baited pitfall traps were used to collect dung beetles from forest, ecotone and agriculture habitat. Community attributes such as species richness, abundance, diversity, indicator and detector species were recorded in the study sites. Species composition varied between the three habitats. Greater similarity in species composition was observed between forest and ecotone. This is attributed to the presence of heliophilic species in the region, adapted to survive in forest and the open edge. Though forest recorded higher abundance, ecotone and agriculture habitat recorded higher species richness and diversity. Low diversity in forest resulted from decreased equitability in the overall forest assemblage resulting from increased dominance of few species such as Onthophagus furcillifer and O. pacificus. Higher species richness in ecotone and agriculture habitat was associated with heliophilic species that responded positively to disturbance, whereas stenotopic species adapted to closed canopy such as Ochicanthon mussardi was negatively affected in the region. Onthophagus furcillifer, the indicator species in the forest and ecotone was also the detector species in agriculture habitat. Presence of such species in the region that are adapted to survive in widely different habitat types is a result of decades of forest degradation and fragmentation in the Western Ghats which led to the establishment of heliophiles and synanthropic species in the region. Such increase in species richness in disturbed habitat is not considered a positive attribute, as original species composition is altered to favor disturbance adapted species in the region.*

Keywords*— *Agriculture habitat, community structure, dung beetles, ecotone, forest, heliophiles, synanthropic species, South Western Ghats.

I. INTRODUCTION

Deforestation over the past half century, has resulted in the loss of more than a third of all forest cover worldwide (Hansen *et al*., 2013). Nearly 70% of the world's remaining forests, lies within 1km of an edge and is in close proximity to human modified landscapes. These forest ecosystems are influenced by human activities, altered microclimate, and non-forest species invasion (Haddad *et al*., 2015). Reduced fragment area, increased isolation, and increased edge, initiate changes in the forest ecosystems which can have unpredictable outcomes (Haddad *et al*., 2015).

Anthropogenic edges created by habitat fragmentation affects biodiversity across ecotones (Laurance, 2000; Murcia, 1995; Risser, 1995). Ecotones are zones of transition between biomes or ecosystems (Hansen and di Castri,1992). Ecotones can be sharp or gradual and is characterized by unique sets of environmental conditions dissimilar from the adjacent habitats, collectively called edge effects (Murcia, 1995). The intensity and direction of edge effects on population level of organisms can be extremely variable across species. Different species respond positively, negatively or neutrally to edges (Murcia, 1995; Baker *et al*., 2002).

Invertebrates such as insects has important functional role to play in an ecosystem. Ecotones natural or anthropogenic, can greatly affect insect abundance and diversity (Didham *et al*.,1996); faunal movement (Yahner, 1988; Wiens *et al*.,1995, 1997; Desrochers and Fortin, 2000); population dynamics (Leopold, 1933); species interactions and

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community structure (Didham *et al*.,1998). Scarabaeinae dung beetles are a group of predominantly dung feeding detritivorous beetles, abundant and widely distributed in the terrestrial ecosystems (Halffter and Mathews, 1966). Through their dung feeding and dung burial activities, they increase soil fertility (Bertone, 2004; Bang *et al*., 2005; Losey and Vaughan, 2006), soil permeability (Bang *et al*., 2005); plant growth (Galbiati *et al*., 1995, Bang *et al*., 2005); seed dispersal (Andresen and Levy, 2004) and control populations of disease causing parasites (Hingston, 1923; Miller *et al*., 1961).They are ideal biological indicators that are effectively used to study the effects of habitat modification, fragmentation and edge effects on biodiversity (Duraes *et al*., 2005; Feer, 2008; Filgueiras *et al*., 2015; Klein, 1989; Nichols *et al*., 2008; Spector and Ayzama, 2003).

The Western Ghats in the Indian subcontinent is one of the 34 biodiversity 'hotspots' of the world (Myers, 2003; Mittermeier *et al*., 2004). Nearly three-fourths of the natural vegetation in the ecoregion are cleared or converted. Due to their fragility, biological richness, high rates of endemism and multiple anthropogenic threats, the remaining severely fragmented forests of the Western Ghats are of major conservation priority on a global scale (Pascal, 1991). There is very limited information on effects of habitat fragmentation and creation of anthropogenic edges on ecologically important insect communities in the region. In the present study, dung beetle community structure attributes such as species richness, abundance, species composition and diversity was investigated across a forest-agriculture habitat ecotone in South Western Ghats. We hypothesize that dung beetle community structure attributes will vary across the habitats.

II. MATERIALS AND METHODS

2.1 Study site

The study site Nelliampathi is located on the "edge" of Palghat gap in South Western Ghats (Pearson and Ghorpade, 1989). The collection site Kaikatty in Nelliampathi is located at 10^0 31'N longitude and 76^0 40'E latitude, at an elevation of 960 msl (Fig. 1). Though extensive in area, Nelliampathi forests presents a fragmented landscape interspersed by large number of plantations, dams, and roads. It is an ecologically high sensitive area forming a corridor for the movement of long ranging species such as *Panthera tigris* Linnaeus, 1758 (tiger), *Panthera pardus* Linnaeus, 1758 (leopard), *Bos gaurus* Smith, 1827 (wild gaur), and is also a crucial migratory route for *Elephas maximus* Linnaeus, 1758 (elephant) (Sukumar and Easa, 2006).

The vegetation in the forest habitat is characterized by west coast semi-evergreen forest consisting of a mixture of evergreen and deciduous trees (Kerala Forests and Wildlife Department, 2004). Mammalian fauna in the region consists of *Elephas maximus* Linnaeus, 1758 (elephant), *Bos gaurus* Smith, 1827 (gaur), *Cervus unicolor* Kerr, 1792 (sambar deer), *Sus scrofa scrofa* Linnaeus, 1758 (wild boar), *Semnopithecus sp*.(langur), *Macaca silenus silenus* Linnaeus, 1758 (lion tailed macaque), *Martes gwatkinsii* Corbet and Hill, 1992 (Nilgiri marten), *Petinomys fuscocapillus* Jerdon, 1847 (small Travancore flying squirrel), *Herpestes fuscus* Thomas, 1924 (brown mongoose), *Viverra megaspila* Blyth, 1862 (Malabar civet) (Kerala Forests and Wildlife Department, 2004). The study sites consisted of a 971 hectare reserve forest, 372 hectare agriculture habitat of banana and orange plantations and a well-defined ecotone separating the two habitats, characterized by scattered trees and less undergrowth. Traps were placed in the reserve forest, ecotone and in the portion of the agriculture habitat with the banana plantation (Fig. 2).

2.2 Sampling

Dung beetles were collected using dung baited pit fall traps in the year 2007-08. Three collections were made during the study period (monsoon, presummer, summer). Each collection effort involved placing ten traps each in the three habitats (forest, ecotone and agriculture habitat). Traps were placed along ten transverse transects. Each transect was composed of three traps, one trap was placed in forest, one in ecotone and one in agriculture habitat. The traps were separated by a distance of 50 m. Each transect was separated by a distance of 50 m. Traps were baited with 200g fresh cow dung. A 25 x 25 cm plastic sheet was set over each trap to protect it from rain and sun. The trap contents were collected at 12 h interval (6:00-18:00h and 18:00-6:00h). The collected beetles were identified to species levels using taxonomic keys available in Arrow (1931) and Balthasar (1963 a, b) and also by verifying with type specimens available in the Coleoptera collections of St. Joseph's College, Devagiri, Kozhikode.

2.3 Data analysis

For the purpose of data analysis, the diurnal and nocturnal collections and the three seasonal collections for each habitat were pooled. Sample based species accumulation curves were plotted for each habitat to assess sampling adequacy (Gotelli and Colwell, 2001). Nonparametric species richness estimator Chao 2 was used to compare observed species richness (Sobs) to estimated species richness (Gotteli and Colwell, 2001). Estimate Sv9 was used for both analyses. Indicator and detector species for each habitat was selected

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by Indicator Value Method (IndVal) (Dufrêne and Legendre, 1997). Shannon-Weaver diversity index (H') (Shannon and Weaver, 1949) was computed for each habitat. Bray-Curtis similarity coefficient (Bray and Curtis 1957) was used to quantify and compare the similarity of dung beetle species composition among habitats. SIMPER analysis was performed to assess the average percent contribution of individual species to dissimilarity between habitats (Clarke, 1993). Analysis of similarities (ANOSIM) was used to test differences in species composition between habitats. PAST 3 was used to compute all diversity analysis. Patterns in species composition of dung beetle assemblages were analysed by constructing species-abundance plot for each habitat (Whittaker, 1965). These graphs are also useful to explore attributes of the assemblage, such as species richness (number of points), evenness (slope) and number of rare species (tail of the curve).

All data used for statistical analysis were tested for normality using Anderson-Darling test. Since the data was not normally distributed, non-parametric statistics, Kruskal-Wallis H tests was used to test the significant levels of variation in abundance and diversity between habitats (Sachs, 1992). Differences with a p-value <0.05 was compared using Mann-Whitney Test. Statistical analysis was performed using Megastat version 10.0 (Orris, 2005).

III. RESULTS

A total of 1425 dung beetles were collected from the three habitats during the study period; 622 beetles from forest, 460 from ecotone and 343 from agriculture habitat. Twenty one species and seven genera were collected from forest; 25 species and eight genera were collected from agriculture habitat; and 25 species and eight genera were collected from ecotone (Table 1). Species accumulation curve for forest did not reach an asymptote (Fig. 3). Chao 2 values for ecotone and agriculture habitat showed 86% inventory completeness but for forest only 44.6% inventory completeness indicating that more species could be collected in forest with additional sampling effort. Overall abundance varied significantly between habitats (H= 11.31, df=2, p=<0.05).Abundance between forest and ecotone; ecotone and agriculture habitat showed no significant difference (p=>0.05) but between forest and agriculture habitat showed significant difference (p=<0.05). *Onthophagus furcillifer* and *O. pacificus* were the indicator species in forest; *O. furcillifer* in edge and *O. fasciatus* in agriculture habitat. *Copris repertus* and *Paracopris cribratus* were the detector species in forest, *Onthophagus bronzeus, O. pacificus* and *Copris repertus* in edge and *Caccobius meridionalis* and *Onthophagus furcillifer* in agriculture habitat (Fig 4).

Shannon-Weaver diversity (H') values did not vary significantly between habitats but were highest in ecotone and lowest in forest (H= 3.24, df= 2, p=>0.05) (Table 1; Fig.5). Bray Curtis similarity coefficient showed highest similarity between the dung beetle assemblages of forest and ecotone (77.30%) followed by ecotone and agriculture habitat (56.59%) and least similarity between agriculture habitat and forest (45.80%) (Fig.6). Percentage contribution of each species towards dissimilarity between habitats is provided in Table 2. Highest average dissimilarity was observed between forest and agriculture habitat (54.20%) contributed mainly by the species *Onthophagus pacificus* (13.79 %), *Caccobius meridionalis* (11.03%) and *Onthophagus fasciatus* (10.12%). Ecotone and agriculture habitat showed a dissimilarity of 43.38%, largely contributed by *Caccobius meridionalis* (13.32%) and *Onthophagus fasciatus* (10.80%). Forest and edge showed a dissimilarity of 22.69% principally contributed by *Onthophagus pacificus* (14.32%). Composition of assemblage varied significantly between habitats (ANOSIM; R= 0.34, p = 0.0001). Rank abundance plot in all the three habitats showed a steep slope as a result of dominance of few species and a long tail of several rare species (Fig.7).

IV. DISCUSSION

In the present study, species composition varied between habitats. Ecotone shared species with forest and agriculture habitat, and least similarity existed between forest and agriculture habitat. Similarity in species composition and abundance between forest and ecotone is in contrast to results of earlier studies done across a forest-savanna ecotone in Bolivia (Spector and Ayzama, 2003), forest-cerrado ecotone in Brazil (Duraes *et al.*, 2005), bushland and agriculture habitat in Tanzania (Nielsen, 2007), forest-savanna edge and forest-roadside edge in French Guiana (Feer, 2008) and forest-pasture edges in Los Tuxtlas Biosphere Reserve (Diaz *et al.*, 2010), where species composition and abundance varied between forest and edge with significant decrease in abundance observed in edge.

Forest edges have a relatively higher temperature, lower humidity and is exposed to higher solar radiation when compared to forest interior and this impacts organisms (Kapos, 1989; Brown, 1993). Though ecotone in Nelliampathi had less shade and higher sun exposure, such microclimatic conditions did not deter forest dung beetles in the region from colonizing the edge habitat. Decades of anthropogenic pressures such as fragmentation, logging and

habitat conversion exerted on the forests in the Western Ghats (Sukumar and Easa, 2006; Latha and Unnikrishnan, 2007; Prabhakaran, 2011) had led to the establishment of heliophilic species in the forest of the region which are adapted to tolerate the warmer microclimatic conditions of the edge. Earlier studies done in forest and modified habitats had revealed the presence of heliophilic species in the region (Vinod, 2009; Sabu *et al*., 2011, Venugopal, 2012). In addition, intrusions of wild animals from forest into the edge provides adequate food resource for dung beetles of ecotone. This is because the forests in the region is fragmented, this results in frequent incursions of long ranging herbivorous mammals such as elephant, gaur into forest edges and even agriculture habitats in the region.

High species richness and Shannon-Weaver diversity in ecotone and agriculture habitat when compared to forest is in contrast to records from Borneo (Davis *et al*., 2001), Neotropics (Avendaño-Mendoza *et al*., 2005), Southeast Asia (Shahabuddin *et al*. 2005), Africa (Nielsen, 2007), and Wayanad (Vinod, 2009). Studies have shown that increase in species richness in disturbed habitats is associated with species that respond positively to disturbance whereas stenotopic species adapted to closed canopy are negatively affected (Davis *et al*., 2001, Janzen, 1987). Such increase in species richness in disturbed habitat is not considered a positive attribute, as original species composition is altered to favor disturbance adapted species in the region (Davis *et al*.,2001).

Nelliampathi is a mosaic of forest fragments and agriculture habitats. Decades of habitat degradation in the region has negatively affected the community attributes of dung beetles in the forest habitats of Nelliampathi. High species richness and diversity in ecotone and agriculture habitat is attributed to arrival of tourist species, adapted to disturbance, from remnant forest habitats into ecotone and agriculture habitat. Such species are *Catharsius molossus, Copris repertus, Onthophagus amphicoma, O. andrewesi, O. bronzeus O. ensifer, O. favrei, O furcillifer, O. insignicollis, O. laevis, O. manipurensis, O. pacificus, O. turbatus, Paracopris cribratus, Tibiodrepanus setosus*. In addition, synanthropic species with preference towards cow dung, such as *Caccobius meridionalis, C. gallinus, C. ultor, Onthophagus fasciatus* and *Paracopris davisoni* were absent in forest but recorded from agriculture habitat and/or ecotone. Such movement of tourist species (Avendaño-Mendoza *et al*., 2005, Estrada *et al*., 1998, Filguieras *et al*.,2015, Quintero and Rosalin, 2005; Quintero and Halffter, 2009) and establishment of synanthropic species in a region were observed in forests of Colombia (Escobar, 2004), in guamil patches of Gautemala (Avendano-Mendoza *et al*., 2005), in pastures of Central America (Horgan, 2007), isolated fragmented forest and disturbed forests of Belize (Latha *et al*., 2016 a, b). Low diversity values in the forest is due to decreased equitability in the overall assemblage resulting from increased dominance of certain species (Davis *et al*., 2001) such as *O. furcillifer* and *O. pacificus* in the forest of Nelliampathi whereas stenotopic species adapted to closed canopy such as *Ochicanthon mussardi* was negatively affected in the region.

The indicator species selected for each habitat are highly specific to that particular environment (McGeoch *et al*., 2002), and are therefore more susceptible to changes in a habitat while detector species possess moderate specificity, with different degrees of preference among various ecological states (McGeoch *et al*., 2002).The presence of *O. furcillifer*, as the indicator species for both forest and ecotone and detector species in agriculture habitat indicates the establishment of heliophilic beetles tolerant to open habitat in the forests of Nelliampathi.

V. CONCLUSION

The is the first reported study on the effects of habitat fragmentation and creation of anthropogenic edges on dung beetle community structure across habitats in South Western Ghats. Decades of anthropogenic disturbance in the region has resulted in the establishment of heliophiles and synanthropic species. Further deterioration of the forests can lead to species loss in the region (Sabu *et al*., 2011). Hence, it is recommended to conduct similar studies to fully understand the effects of anthropogenic disturbance on biodiversity of the South Western Ghats, as such studies assists to plan adequate conservation strategies for the region in the future.

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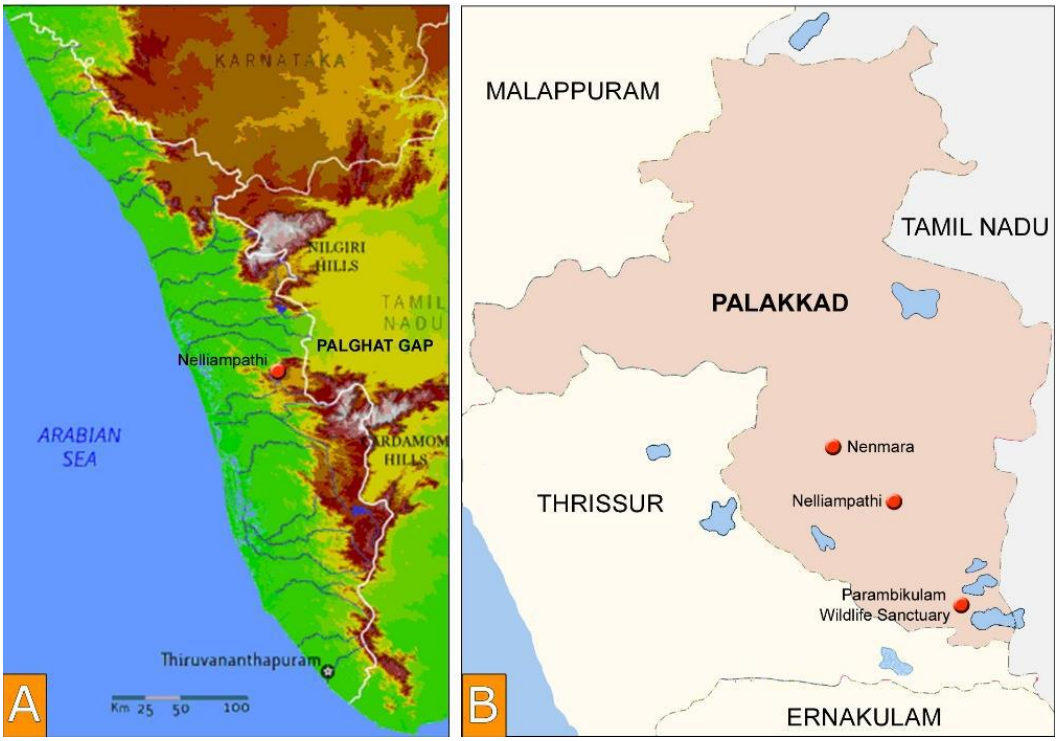

Fig. 1: A. Map showing Western Ghats; B. Map of study region Nelliampathi.

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Fig. 2: Habitat types under study in Nelliampathi in South Western Ghats, A. Semi-evergreen forest; B. Ecotone; C. Agriculture habitat.

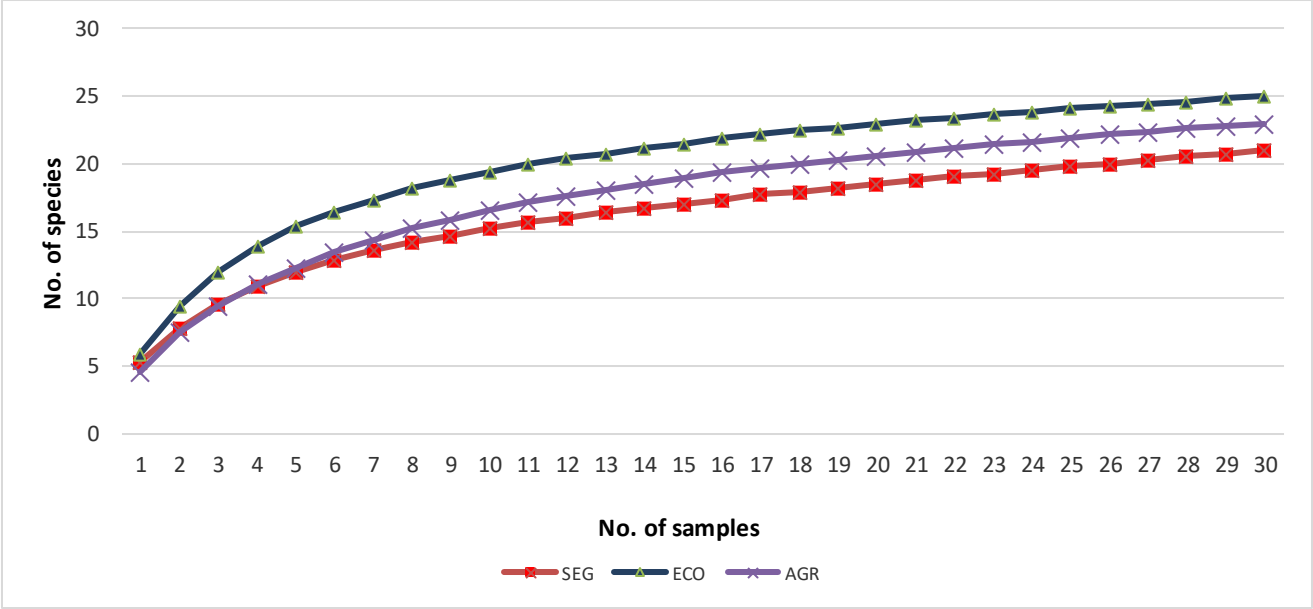

Fig. 3: Sample based species accumulation curve (Mao Tau) for dung beetles collected from a semi-evergreen forest (SEG), ecotone (ECO) and agriculture habitat (AGR) of Nelliampathi in South Western Ghats for the 2007-08 study period.

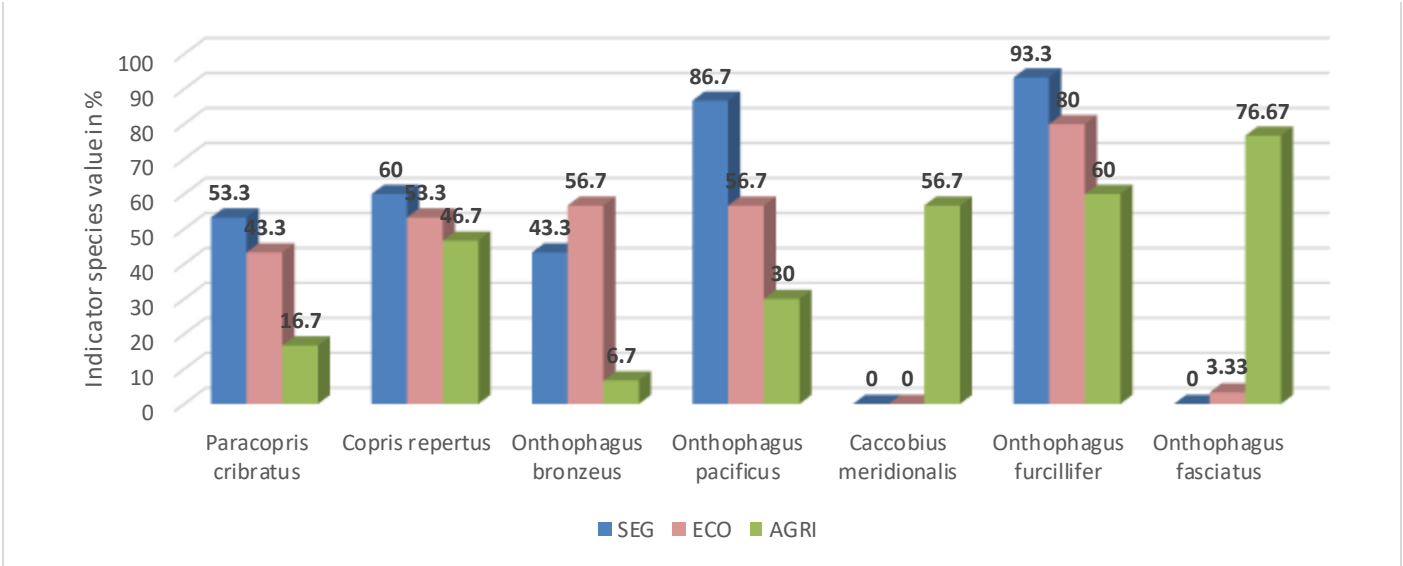

Fig. 4: Indicator and detector species of dung beetles in a semi-evergreen forest (SEG), ecotone (ECO) and agriculture habitat (AGR) of Nelliampathi in South Western Ghats for the 2007-08 study period.

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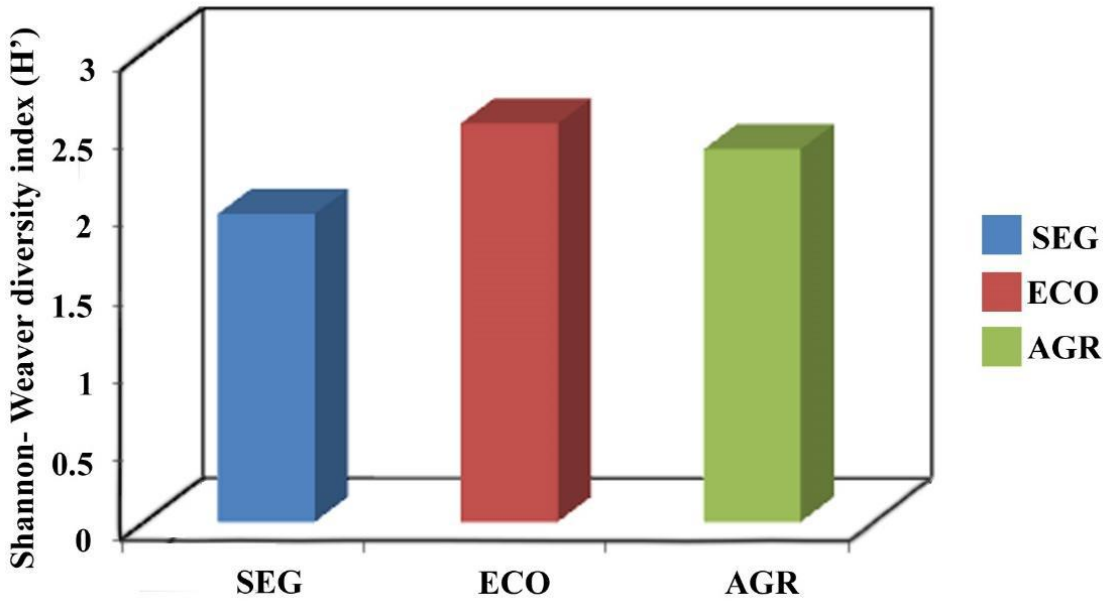

Fig. 5: Shannon-Weaver diversity Index (H') values in a semi-evergreen forest (SEG), ecotone (ECO) and agriculture habitat (AGR) of Nelliampathi in South Western Ghats for the 2007-08 study period.

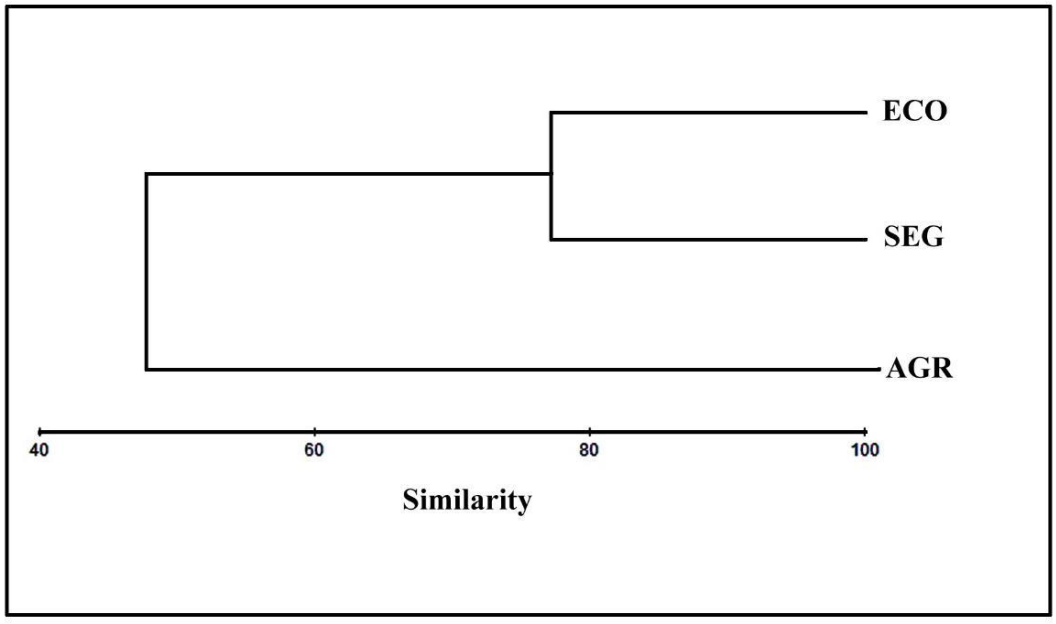

Fig.6: Cluster diagram of Bray Curtis Similarity Index between semi-evergreen forest (SEG), ecotone (ECO) and agriculture habitat (AGR) of Nelliampathi in South Western Ghats for the 2007-08 study period.

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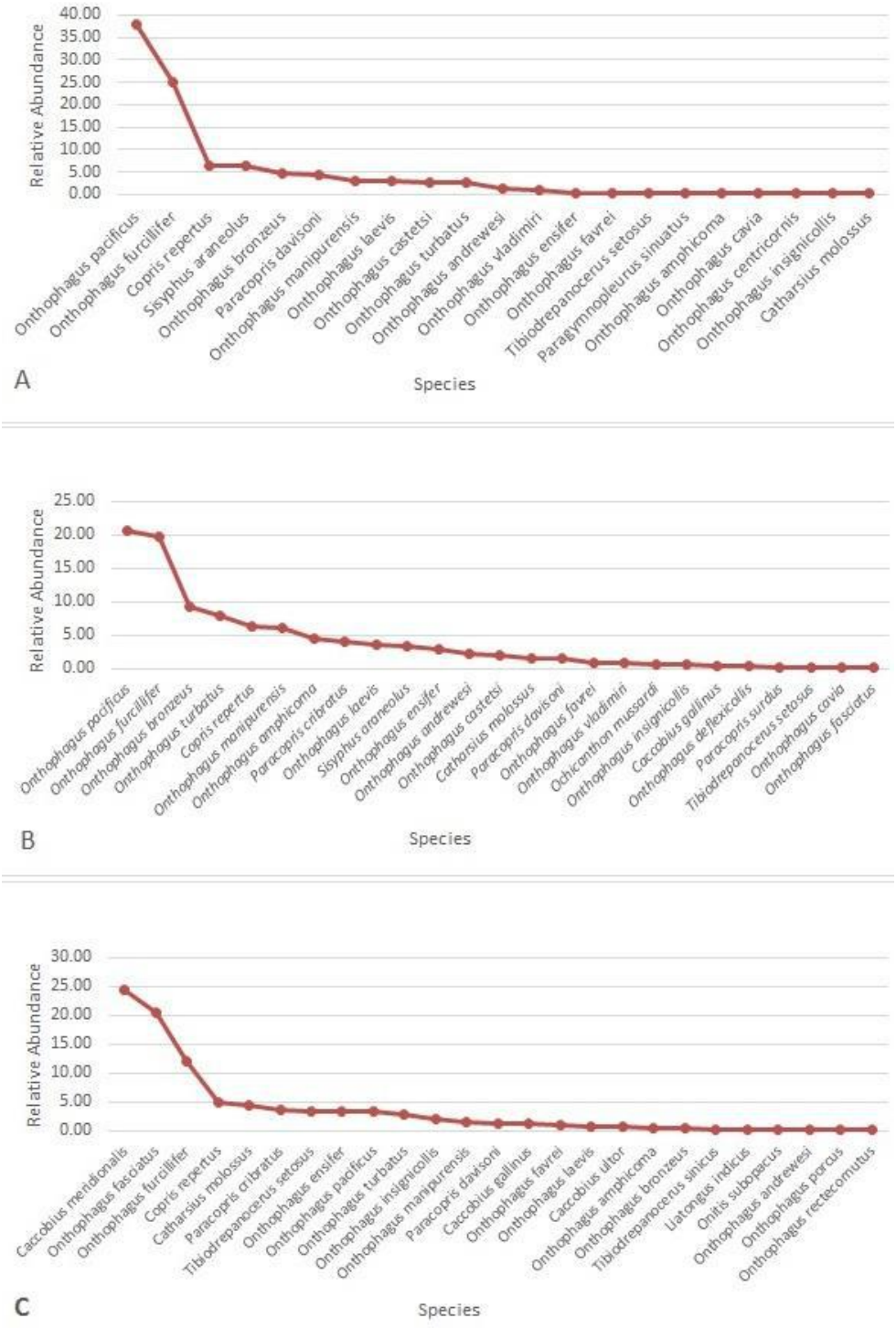

Fig. 7: Species abundance curve for dung beetle species in a semi-evergreen forest (SEG), ecotone (ECO) and agriculture habitat (AGR) of Nelliampathi in South Western Ghats for the 2007-08 study period.

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Table 1: Dung beetle species abundance, overall abundance, species richness, Chao 2, Shannon-Weaver diversity index (H') values in a semi-evergreen forest (SEG), ecotone (ECO) and agriculture habitat (AGR) of Nelliampathi in South Western Ghats for the 2007-08 study period.

Species	SEG	ECO	AGR
Caccobius gallinus	0	2	5
Caccobius meridionalis	0	0	88
Caccobius ultor	0	0	3
Catharsius molossus	1	7	12
Copris repertus	28	29	27
Liatongus indicus	0	0	1
Ochicanthon mussardi	0	3	0
Onitis subopacus	0	0	1
Onthophagus amphicoma	1	21	3
Onthophagus andrewesi	8	7	1
Onthophagus bronzeus	29	39	2
Onthophagus castetsi	16	9	0
Onthophagus cavia	1	1	0
Onthophagus centricornis	1	0	0
Onthophagus deflexicollis	0	2	0
Onthophagus ensifer	3	13	12
Onthophagus fasciatus	0	1	74
Onthophagus favrei	2	6	5
Onthophagus furcillifer	155	91	44
Onthophagus insignicollis	1	2	2
Onthophagus laevis	18	17	4
Onthophagus manipurensis	19	28	8
Onthophagus pacificus	235	96	13
Onthophagus porcus	0	0	1
Onthophagus rectecornutus	0	0	1
Onthophagus turbatus	16	36	12
Onthophagus vladimiri	7	4	0
Paracopris cribratus	40	18	7
Paracopris davisoni	0	7	6
Paracopris surdus	0	1	0
Paragymnopleurus sinuatus	1	0	0
Sisyphus araneolus	39	15	0
Tibiodrepanus setosus	1	1	10
Tibiodrepanus sinicus	0	0	1
Abundance	622	460	343
Species Richness	21	25	25
Chao 2	44.68 (47%)	2903 (86%)	28.8 (86.8%)
Shannon-Weaver diversity (H')	1.97	2.55	2.3

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Table 2: Percentage contribution of species towards dissimilarity between a semi- evergreen forest, ecotone and agriculture habitat of Nelliampathi in South Western Ghats for the 2007-08 study period.

Species	Semi-evergreen forest v/s Ecotone	Ecotone v/s Agriculture habitat	Semi-evergreen forest v/s Agriculture habitat
Caccobius gallinus	3.63	1.17	2.63
Caccobius meridionalis	0	13.32	11.03
Caccobiu sultor	0	2.46	2.04
Catharsius molossus	4.22	1.16	2.9
Copris repertus	0.24	0.27	0.11
Liatongus indicus	0	1.42	1.18
Ochicanthon mussardi	4.44	2.46	0
Onitis subopacus	0	1.42	1.18
Onthophagus amphicoma	9.19	4.05	0.86
Onthophagus andrewesi	0.86	3.07	2.15
Onthophagus bronzeus	3.01	7.3	4.67
Onthophagus castetsi	2.56	4.26	4.7
Onthophagu scavia	0	1.42	1.18
Onthophagus centricornis	2.56	0	1.18
Onthophagus deflexicollis	3.63	2.01	0
Onthophagus ensifer	4.8	0.2	2.04
Onthophagus fasciatus	2.56	10.8	10.12
Onthophagus favrei	1.5	0.34	0.97
Onthophagus furcillifer	7.46	4.13	6.84
Onthophagus insignicollis	1.88	0.45	0.49
Onthophagus laevis	0.62	2.84	2.64
Onthophagus manipurensis	2.39	3.5	1.8
Onthophagus pacificus	14.32	8.72	13.79
Onthophagus porcus	0	1.42	1.18
Onthophagus rectecornutus	0	1.42	1.18
Onthophagus turbatus	5.13	3.6	0.63
Onthophagus vladimiri	1.66	2.84	3.11
Paracopris cribratus	5.34	2.27	4.33
Paracopris davisoni	6.79	0.28	2.88
Paracopris surdus	2.56	1.42	0
Paragymnopleurus sinuatus	2.56	0	1.18
Sisyphus araneolus	6.08	5.5	7.34
Tibiodrepanus setosus	0	3.07	2.54
Tibiodrepanus sinicus	0	1.42	1.18

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Evaluation of the Efficiency of Aqueous Extact of Neem Fruits on Insect Pest of Rice in Rice Agroecosystem of Maga in the Far North Region of Cameroon

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Abstract**—** *The chemical fight against insects pest causes many problems on the biodiversity of ecosystems, destabilizes the trophic level of the ecosystem and has harmful effects on the on health human. Mean while the biological fight using plants extractions can equally play the same role of killing pest, reason why the present study which was carried out in the irrigated perimeters of Maga in the Far North region of Cameroon, have as principal objective to evaluate the aqueous extraction of neem fruit on the insects pest of rice. The specific objectives were to know the biological diversity of insect pest in the irrigated perimeters of Maga, and their repartitioning in the phenological stages, again, to see the effects of the aqueous extractions of the neem fruits on the insects pest per variety and in function of the phenological stages, also to evaluate the damages cause by insects pest during the talling stage in function of the varieties, finally, to evaluate loss cause by the insects pest. The study was made on two rice varieties which were IR46 and NERICA3 in a split plot disposition. The capturing of the insects was done with the help of a sweep net and the identification of the species was done with the help of an entomological buttle, the identification key of insects by Heinrich (1993), Hill (1983), Heinrichs and Barrion (2004) and the families recognition keys by Delvare and Aberlenc (1989).The method of Breniere permited the estimation of loss of output at the talling and harvesting stages of rice caused by the insect pest. The analysis of variance of the result was done using SPSS 20. In the class of insects, twenty two species of insects fall in twenty families divided in seven orders were collected. Among the captured insects, we investigated fourteen insects which were pest. The biological fight have shown an effectiveness in the nursery, talling, and a positive and non negligible effects on the reduction insects pest in the heading and maturation stages and thus has permitted the reduction of damages from insects on the rice plants.*

Keywords**— Biology fight, insects pest, rice, neem, agro ecosystem.**

I. INTRODUCTION

1. Context

Rice (*Oryza sativa* L.) constitude the most feed aliment in the whole world (Guigaz, 2002). According to FAO, world rice consomation soppose to has risen between 2012 and 2022 at least 1% per year against 1,7% for the years 90- 2010. The average consomation per habitat soppose to have increase slightly to 58, 2 kg per person (FAO, 2013). In Africa, rice is produced and consium in 39 countries (Sanni *et al*., 2009). The culture of rice is an important activity for the population of some zones in West and Central Africa assuring food security to about 20 million producers and makes about 100 million persons to live if we make the averages of five persons in peasant famillies (ADRAO, 2002). For the period of 2000 to 2005 Africa has produce about 17,4 millions of tones of paddy rice whereas in 2006 to 2009 this production increase to 22 millions tones of paddy rice (FAO, 2011). The demand for rice in West and Central Africa had increase to 6 % per year ; more faster than anywhere in the world mean while in the same time production increase to 4 % (ADRAO, 2004 ; Sanni*et al*.,2009).

Faced with this situation, the populations of these regions are forced to imports in order to meet their needs. While the world paddy rice production is 745709788 tonnes on a surface of 1 647 216 663 ha (FAO, 2013) thus a yield of 4 527,1 kg/ha, Africa has a production of 29

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318 488 tonnes on a surface of 10 931 051 ha with a yield of 2 682,1 kg / ha (FAO, 2013), which is twice as small compared to the world yield. Cameroon for its part has a production of 194094 tonnes for a cultivated surface of 166734 ha or a return of 1164, 1 kg/ha (FAO, 2013), which is three to four times smaller than the world and two times smaller than the yield in African. Cameroon is rank 64th in the world and the 16th in Africa behind Chad. Cameroon imports more than two thirds of its rice needs. The annual production of Cameroon oscillates between 100,000 and 170,000 tons. Too little to satisfy its annual requirements estimated at 600,000 tons approximately. The slightest disruption in producing States (floods or) are drought, or social unrest) is automatically felt in the basket here. Besides the food crisis of February 2008, dubbed "riots of February 2008" is a consequence of this situation. Yet Cameroon has the resources (soil, favorable climate, water) needed to be the leader in production of rice in the subregions of Central Africa. The public power and the research are expected to play a major role to boost production, which is faced with many problems. These constraints include the non-competitiveness of the local rice against the imported rice including countries in Asia, and the technical improvement of routes especially the fight against pests such as insects. Arhent and *al*. (1983), felt that among the 41.1 percent of the total losses of rice, 27.5% were due to insects. They are famous for the damage they cause to crops and diseases which they are vectors (Breniere, 1983). Woin and *al*., (2004) four species of insects transmit the virus of variegation yellow rice plants in three major irrigated rice Lagdo, Maga and Yagoua in northern Cameroon and of lowland rice paddies. These are among other *Chnootriba similis*, *Chaetocnema pulla*, *Trichispa sericea* and *Locris rubra*. Wilhelm and *al*., (2008) fourteen species of insect pests and vectors of the (RYMV) rice yellow mottle virus are associated with irrigated and rain-fed rice cultivation in the region of the far North. Among these insects four species are found in abundance throughout the region of the far North. These include *Nephotettix nigropictus* (Hemiptera: Cicadellidae), *Cofana spectra* (Hemiptera: Cicadellidae), *Diopsis thoracica* (Diptera: Diopsidae) and *Sogatella furcifera* (Hemiptera: Delphacidae).

The use of insecticides to control insect pests of rice is source of many problems and the phytosanitary industry uses all arguments to convince them of the merits of the use of its herbicides, insecticides and other pesticides. Facing these speeches, the facts are still there: industrial accidents, pesticides banned or outdated shipped in developing countries, water contamination, end, weigh definitely favour a reduction in the use of pesticides. Especially since alternatives exist.

2. Problem

Improvement and security of agricultural production and the difficulties associated with the insect attacks have pushed researchers and farmers towards the use of synthesis chemical insecticide. But these insecticides have proved its ineffectiveness against certain insect pests, according to Brevault and *al*. (2007), the whitefly, *Bemisia tabaci* (Gennadius), and aphid, *Aphis gossypii* have acquired resistance to organophosphate insecticides characters in Cameroon. In addition these pesticides are extremely stable and persistent in the environment, accumulate in living organisms and food chains, are toxic to humans and animals and cause chronic effects such as dysfunction at the level of the reproductive and immune and endocrine systems, as well as cancers, and are propagated in the environment over long distances to remote locations of the sources of emissions (IOMC, 2002).

Yet there are alternatives to the use of insecticides such as substances of plant origin. *Azadirachta indica* Juss is a tree in the Meliaceae family, native to India (Formad environment, 2013). It is used and known for its insecticide, fungal properties and medicinal (Huang and *al*., 1995; Valladares and *al*., 1999; Carpinella and *al*., 2002). This tree is present in the far North region of Cameroon, however the use of the aqueous extract of the fruit has never been experienced on insect pest of rice. Yet the work of Kosma and *al*., (2010) on the properties chemical seeds of neem on nematodes of plantain were effective more, the work of Djile, (2010) bode on the use of neem seed extracts have shown efficiency of the cake of neem on fungal diseases of Cowpea and still the experimental work of Abu Togola, (2010) using oil of neem on insect pests of rice shown very useful.

In view of all this knowing the properties of the neem tree, it is necessary before use of this plant against insect pests, to assess the effectiveness of these substances on insects before using, to see what variety is more efficient, what is its economic significance on two main varieties of rice grown in the far north region of Cameroon in an agroecosystem finally, to see the variety that is best suited for beneficial conclusions can be drawn for food security.

3. Objectives

The main objective is to assess the effectiveness of the aqueous extract of fruit of neem on insect pests of rice in the rice-growing perimeter of Maga, specifically our work ambition are:

-Know the biological diversity of insect pests in the irrigated perimeter of Maga and their distribution by phenological stage.

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-See the effect of the aqueous extract of fruit of neem on insect pests by variety and according to the phenological stages.
-Evaluate damage during talling stage according to variety
-Evaluate the losses due to drillers' insects during harvesting

II. MATERIALS AND METHODS

1. Materials

1.1. Localisation of studied area

The study was conducted in the irrigated rice area of the company of Expansion and modernization of the rice of Yagoua (SEMRY) on the site of Maga.
Maga is located in the Division of the Mayo-Danay, region of the far North, and between 10 ° 9' and 10 ° 50' latitude North and 14 ° 57' and 15 ° 12' longitude. Maga is limited:
-To the North by the Borough of Logone-Birni,.
-To the West by the Borough of Bogo,
-To the South by the Borough of Kaikai,
-The East by the river Logone.

1.2. Biological Materials

1.2.1. Cultivars of rice

The cultivar of rice used in this study will be *Oryza* sp. The two varieties of rice used are IR46 and Nerica 3.

1.2.2. The fruits of neem

Neem fruits have been picked up in the town of Maga at the level of the premises of the SEMRY, the collected fruits are those found in the ground below the neem tree, and then they are dried.

1.3. Experimental dispositive

Experimental design was a split plot consists of three blocks, divided into basic plots of 7 m x 4 m; in each block, two types of treatments (biological control and control) were applied on two varieties of rice (IR 46 and NERICA 3) with three replicates. The dimensions of each basic plot are 7 m x 4 m, between the basic plots are walkways of 1 m wide and between blocks the aisles of 2 m wide.

1.4. Materials for collections of insects

The collection equipment used here is the sweep net.
The "sweep net" is a net that is used to collect the insects that live on plants (Goldstyn, 2003). There are different types of nets for the capturing of flight, capture to the ground and mowing, but all consist of three parts: a circle (or RIM), a Pocket (or purse) and a handle. These three parts can be adapted to specific hunts types, for example in the water or in the air. The net used in this study is characterized by the length of his pocket which is approximately twice the diameter of the circle. The diameter of the circle is 40 cm, Pocket about 80 cm and the handle is long (more than 1 m). The Pocket quite fine-mesh fabric, offers little resistance to air. The net is used to mow by Rapids lateral movements of comes and goes.
Insects will be caught using net "sweep net" depending on the case at the rate of 25 double sweeps (50 sweeps) on each of two (02) perpendicular medians in each plot during periods of collections.

2. ethods

2.1. Obtention of the aqueous extract of neem fruit

The principle of obtaining extracts from seeds of the neem tree has been described since 1975 by Jacobson and Kumar (2003). Extracts from seeds of the neem tree are formed by powder, meal, aqueous and alcoholic seed extracts. The extraction of these substances from seeds of the neem tree can be done in various ways, mechanical and basis of alcoholic compounds such as ethanol and methanol. This principle will be amended to adapt it to our context which is the fruit of aqueous neem extraction

2.2. Aqueous extract of the fruit of neem

The fruits of neemier picked up are dried at a temperature of 40 ° C, for two days. We take a quantity of 2 kg of the fruit, grinds it, envelope in a canvas. The canvas is immersed in a container of 5 liter water, immersion lasts one night. Removing water canvas and spins its contents, finally we add the liquid water to have 15 litre of water then we sieve. The aqueous solution obtained is the extract. Before use is added to a wetting agent such as the detergent previously dissolved in water of 5 to 10 mg and everything is thoroughly mixed. The prepared solution is used directly in the following hours.

2.3. Application of the aqueous extract of fruit of neem

The application of aqueous extract of seed by spraying on rice plants is performed during an interval of two weeks after the nursery of plants until the last collection of insects in maturity or 0.84 per litre for 28 m 2.

2.4. Method of collecting insects

2.4.1. Rhythm of the sampling of insects after the application of the aqueous extract of the fruit of neem by sweep net

On each elementary plot regardless of the treatment applied to the basic plot, the insects will be caught using net "sweep net" depending on the case at the rate of 25 double sweeps (50 sweeps) on each of the parcels elementary so as to cover all the surface by positioning itself on two (02) perpendicular medians in each plot at two week intervals from the fifteenth day after planting or transplanting.

2.4.2. Period of collection of insects

The sampling was carried out on the following four phenological stages of rice: nursery, talling, inflorescence and seed maturation.

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2.5. Technique of observation and identification of specimens

The keys for the identification of insects from Heinrich (1993), Hill (1983), Heinrichs and Delvare (2004) and the recognition of Delvare and Abbasi families of key (1989) will be used to identify different collected species.

2.6. Measurements and data analysis

The larvae, pupae and adults will be counted as the representatives of the strength of the species on each variety, according to variety, from the application of the extract of neem and untreated plots. This allowed us to classify various specimens collected in different orders, families, genera and species and then determine the number of each of these specimens compared with the phenological stages of the plant.

The data used was the Excel software and submitted to statistical analysis using the software SPSS 20. Averages will be compared using ANOVA test to the 5% threshold.

2.7. Assessment of losses due to insects at harvest time

The methodology for this evaluation is that of Breniere (1982).

Fifteen days before the beginning of the harvest, we take 20 clumps of rice on each elementary parcel to examine. Sampling is done randomly. To do this, we simply stretch a rope with knots spaced all across the rice fields of 2 m, removed the nearest clump of each node. All stems carrying panicles are separated from each other, until we get a total of 200 stems (stop at this number). We opens then each stem with a penknife, and are classified in:

-panicles without attacks from borers in the stem: n1;

-panicles with attacks of borers in the stem (insects present or not): n2.

After threshing of grains of each batch, we get:

-p1, weight of n1.

-p2, weight of n2.

On the same location, estimate the number of panicles per square metre. To do this, we uses a rigid framework of 1 m² asked to randomly on the ground (do ten repetitions to get an average value). The following formula expresses with a fairly good approximation (by weight of grain per hectare) loss due to attacks by Drillers from the heading

$$P = \frac{200\,\frac{p_1}{n_1} - (p_1 + p_2)}{200} \times 10\,000\,N$$

P= Weight loss of the grain in ha

n_1=Panicles without attack of borers in stems;

n_2=Panicles with attack of borers in stems; (insects present or not);

p_1= Weight of n1;

p_2= Weight of n2;

N= Numbers of panicles in m^2

This data can then be converted into monetary value. There will be care in category n1, stems whose tassels would be altered by causes other than Drillers (blast to the neck for example). If their number is not too important, the benefit expected by the fight against the Drillers would be reduced even. Note that this method of calculation does not take account of losses (dead hearts) during tillering. Because of these inaccuracies, this assessment - is usually the actual loss. It can therefore be regarded as a minimum usable for the study of the profitability of the fight. The method is quite laborious but without difficulty.

2.8. Assessment of damage from insects during talling

To achieve this, it was noted on each parcel:

-Nt: average number of fruiting stems / m2 control plots;

-N: average number of fruiting stems per meter square plots. These values are obtained by averaging a few surveys (at least five) conducted at random in each plot using a rigid framework of 1 m².

If, on the other hand, surveys intended for the assessment of the loss of harvest due to the stem borers after tillering was conducted (using the above method) on plots of couples, the formula indicates the loss before bolting:

$$P_a = \frac{P_1}{n_1} \quad X\ 10\ 000\ (N - Nt)$$

n_1= Numbers of stems without attack of borers

p_1= Weight of grains of n_1

P_a= Loss of harvest cause by insects before heading.

Applied to each control plot, this formula is used to calculate the average value of Pa of all couples (treated plots and untreated) representative of the rice-growing perimeter. The expected results will be reliable if the area concerned is relatively homogeneous and if found not in the presence of certain pests that are characterized by heterogeneous infestations: sampling, then, is more really representative of reality. All of this is generally feasible in situations of strongly framed rice which has begun investments (irrigated) requiring the guarantee of high productivity.

III. RESULTS, ANALYSIS AND DISCUSSIONS

1. Biological diversity of insects in the paddy field of Maga

1.1. Inventory of the biodiversity of insects and classification

The Table 1 show the species collected in our experimental plot in the irrigated perimeter of Maga.

Table 1 presents the species collected in our experimental plot in the irrigated perimeter of Maga, Classes, orders,

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families, genera species classification and along with their status.

Table.1: Status and classification of species caught in the rice fields of Maga

CLASS	Orders	Famillies	Gender/species	Status according to Heinrich et *al*., (2004)
insects	Diptera	Otitidae	*Physiphora clausa F*	Scavenger
		Micropezidae	*Glyphodera mantis*	Scavenger
		Culicidae	*Culex robinotus*	Scavenger
		Diopsidae	*Diopsis sp*	Scavenger
		Syrphidae	*Microdon johannae*	Predator
			Allongnota nasuta macquar	Predator
			Paragus dolichorus	Predator
	Coleoptera	Lagridae	*Lagria gesquierie*	Scavenger
		Apionidae	*Apion africanum* gull	Scavenger
		Coccinellidae	*Cheilomenes lunata*	Predator
			Xanthadalia effusa	Predator
		Staphilinidae	*Paeserus fucipes* curtis	Predator
	Hemiptera	Cicadellidae	*Nephottetix nigropictus*	Scavenger
		Pentatomidae	*Diploxys dipunctata*	Scavenger
			Agonocelis harolldi Beigs	Scavenger
		Alydidae	*Stenocoris claviformis*	Scavenger
	Héminoptera	Braconidae	*Bracon sp*	Parasitoïds
			Apanteles rufierus	Parasitoïds
	Lepidoptera	Noctuidae	*Sesamia calamistis*	Scavenger
		Pyralidae	*Maliarpha separatella*	Scavengers
	Orthoptera	Avididae	*Cussyrtus bivittatus*	Scavenger
		Tettigoniidae	*Conocephalus maculatus*	Predators and Scavengers occasionaly
	Odonata	Lestidae	*Lestes sp*	Predator
		Libellulidae	*Palpopleura sp*	Predator
Arachnida	Araneae	Araneidae	*Araneus sp*	Predator
		Tetragnidae	*Tétragnatha juculator*	Predator

It appears from this table 1 that we have surveyed 26 species of arthropods divided into two classes, the class of insects and the class Arachnida. 24 species belong to the class of insects divided into 7 orders and 18 families, and 2 species belong to the class of Arachnids in an order and two families. 13 inventoried insect species have the status of pests, 9 the status of predators and 2 species of parasitoids status. 2 arachnid species have the status of predator of insects. Insect pests of rice are diverse and numerous, addressing almost all parts of the rice plant. They are in the numbers of 14 and 4 insect pests cause damage to plants, according to Chaudhary and *al*., (2003) of *Sesamia calamistis*, *Diopsis* sp, *Nephottetix nigropictus*, *Maliarpha separatella*. By the same author: *Nephottetix nigropictus* apart from the food damage caused by suction and resulting in a shortened culture, growing in these early stages of development. It is a vector of viruses from stunting rice, nanissante jaundice, transitory yellowing, tungro of disease of the yellow sheet - orange and stunting to wales of rice.

• *Sesamia calamistis*, *Diopsis* sp, *Maliarpha separatella* are stem borers. Damage caused by these borers are boring and are carried out by the larva in the leaf sheath, generate from large longitudinal zones discolored and whitish on the feeding sites. But lead rarely wilting and drying of leaf boundaries, about a week after hatching, the larva stops feeding from leaf sheaths and hollow inside the rod to feed on the parenchyma of the stems. Such a mode of feeding often results in a rupture of the apical parts of the plant above the location of damage when this type of damage occurs during the vegetative stage of the plant, the Central whorl of leaves do not open, becomes brownish and dry, while the leaves lower remain healthy and green. This State is known as of dead heart and affected tillers die without producing tassels. Larvae feeding on the panicles sometimes cause dead hearts but

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if no further damage occurs cut parts are repelled by the new growth.

These results compared to that done by Wilhelm and *al*., (2013) in the same ecosystem. Shows us that there are some insects that are more present as *Chaetocnema pulla*, *Chnootriba similis*, *Locris ruba*, *Cofana spectra*, *Sogatella furcifera*, *Nilaparvata lugens*. This can be caused by climatic factors which are not favourable to the outbreak of these insects during this period. Compared to the result of Ondo and *al*., (2014) our biodiversity is less rich in species, this can is hard to different ecosystems, the method of collection of various insects and different varieties of rice.

1.2. Inventory of insects captured in different phenological stages and classification

The Table 2 show the inventory in different stages shows that insect pests vary in richness (number of species), in abundance (number of each species), based on different stages.

Table.2: Insect pests inventoried in the nursery

Class	Orders	Famillies	Genders/Species	IR46	N3
Insecta Hexapoda	Diptera	Otitidae	*Physiphora clausa F*	83	94
		Culicidae	*Culex robinotus*	183	176
		Micropezidae	*Glyphodera mantis*	196	211
		Diopsidae	*Diopsis sp*	1	4
	Coleoptera	Lagridae	*Lagria gesquierie*	0	1
		coccinellidae	*Cheilomenes lunata*	0	1
	Hemiptera	Cicadellidae	*Nephottetix nigropictus*	48	56
	Lepidoptera	Noctuidae	*Sésamia calamistis*	0	1
Total	**4**	**8**	**8**	**511**	**544**

IR46: Total number of insects collected in plots IR46

N3: Total number of insects collected in plots NERICA3

This table shows the number of insects captured on control plots, rice varieties IR46 and NERICA 3 nursery depending on their class, order, family, genus and species. The insects captured at the nursery stage vary in numbers and species. They are spread over 4 orders and 8 families of Diptera, the order that has the most species, and the most abundant species is *Glyphodera mantis*.

At the nursery stage, the plants has characteristic of being very young and has well developed leaves bodies which promotes the development of phytophagous insects as defoliators insects and sucking biting of the leaves and it is what will justify the presence of Diptera pests and bugs. The table 3 shows the number of insects captured on control plots, rice varieties IR46 and NERICA3 during talling.

Table.3: Inventory of insect pests at the talling stage

Classe	Orders	Famillies	Genders/species	IR1	N1
Insect hexapoda	Diptera	Otitidae	*Physiphora clausa F*	342	316
		Culicidae	*Culex robinotus*	132	133
		Micropezidae	*Glyphodera mantis*	212	427
		Diopsidae	*Diopsis sp*	0	2
	Coleoptera	Lagridae	*Lagria ghesquierie*	3	0
			Cheilomenes lunata	2	2
	Hemiptera	Cicadellidae	*Nephottetix nigropictus*	92	249
	Lepidoptera	Pyralidae	*Maliarphas eparatella*	1	2
		Noctuidae	*Sesamia calamistis*	0	0
Total	**4**	**9**	**9**	**784**	**1131**

IR46: Total number of insects collected from plots IR46

N3: Total number of insects collected from plots NERICA3

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This table 3 shows the number of insects captured on control plots, rice varieties IR46 and NERICA3 during talling depending on their class, order, family, genus and species.

At the talling stage insect pests that we have captured, we divided them into 4 orders, 9 families and 9 species.

At the talling stage the plants are young, well developed, bushy so insects grow best because the environ is conducive for their outbreak. So, defoliating, biting and sucking phytophagous insects belonging to the orders of Diptera and hemipteran especially develops. According to Grist and *al*., (1969), Hemiptera *Nephotettix* sp were indeed recognized as vectors of serious diseases of Tungro, Yellow dwarf and the Grassy Stunt Virus including the extension seems to expand with the development of highly productive varieties and high talling.

The table 4 shows the number of insects captured on control plots, rice varieties IR46 and NERICA3 in heading stage.

Table.4: Inventory of insect pests in heading

Class	**Order**	**Famillies**	**Genders/species**	IR1	N1
Insecta Hexapoda	Diptera	Otitidae	*Physiphora clausa F*	255	413
		Micropezidae	*Glyphodera mantis*	182	260
		Culicidae	*Culex robinotus*	15	15
		Diopsidae	*Diopsis sp*	21	15
	Coleoptera	coccinellidae	*Cheilomenes lunata*	1	0
		Lagridae	*Lagria gesquiere*	0	1
	Lepidoptera	Pyralidae	*Maliarpha separatella*	18	24
		Noctuidae	*Sesamia calamistis*	6	4
	Hemiptera	Cicadellidae	*Nephottetix nigropictus*	111	129
		Alydidae	*Stenocoris clariformis*	1	1
	Orthoptera	Avididae	*Cussyrtus bivittatus*	0	1
Total	**5**	**11**	**11**	**610**	**863**

IR1: Total number of insects collected in plots IR46

N3: Total number of insects collected in plots nerica3

This table 4 shows the number of insects captured on control plots, rice varieties IR46 and NERICA3 in heading stage depending on their class, order, family, genus and species.

At the heading stage we caught 11 species of insect pests in 5 orders and 11 families the order Diptera was dominant and abundant. Insects caught in this stage are defoliators and also sucking biting, we note the presence of a grain pest, Stenocoris clariformis

At the heading stage the plant has the characteristics of tillering but the difference of the output of the panicle is warranting that they are almost of the same type of insect pests and paniculaire initiation the granivores call where the presence of *Stenocoris clariformis*.

The table 5 shows the number of insects captured on control plots, rice varieties IR46 and nerica 3 at the stage maturite.

Table.5: Inventory of insect pests in mature

Class	**Order**	**Famillies**	**Gender/species**	**IR1**	**N1**
Insect	Diptera	Otitidae	*Physiphora clausa F*	14	33
		Culicidae	*Culex robinotus*	7	10
		Micropezidae	*Glyphodera mantis*	1	16
		Diopsidae	*Diopsis sp*	43	34
	Coleoptera	Coccinellidae	*Cheilomenes lunata*	0	0
		Apionidae	*Apion africanum gull*	1	0

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		Pentatomidae	*AgonocelisharolldiBeigs*	1	2
			Diploxys dipunctata	2	2
		Alydidae	*Stenocoris claviformis*	0	1
	Hémiptera	Cicadellidade	*Nephottetix nigropictus*	12	13
Total :	**3**	**9**	**10**	**81**	**111**

IR1: Total number of insects collected in plots IR46

N3: Total number of insects collected in plots NERICA 3

This table 5 shows the number of insects captured on control plots, rice varieties IR46 and NERICA3 at the stage maturite function of their class, order, family, genus and species.

At the maturity stage we captured 10 species of insects. Divided in 3 levels and 9 families, insect pests are less abundant and are of sucking biting of seeds which are dominate.

The mature stage is marked by the senescence of leaves and stems. Insects are struggling to feed on the foliage for insects phylophages, also on the stem to stem borers. But the training and seed development promotes granivores insect outbreaks of the justifying the increase in species of Hemiptera much.

1.2.. Population dynamics

- **Population dynamics of insects on the IR46 varieties**

The figure 1 shows the dynamics of insect phenological stages.

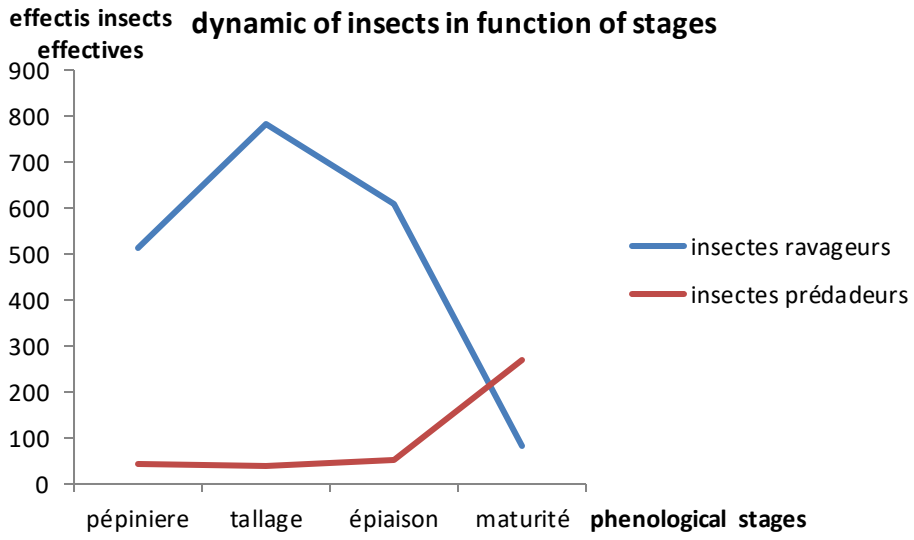

Fig.1: Curve of the dynamics population of insects on the IR46 variety

- **Dynamic of the population of insects on NERICA 3 variety**

The curve of insect pests has almost the shape of a Bell, the size of the population of insects at the talling stage has the highest number followed by the heading and the nursery, insect pests are more numerous than the predators except at the end of maturity where the predators are more numerous than insect pests.

Insect pests are more numerous at the tillering stage because at this stage the plant is young and well developed. Combined with climatic factors this stage promotes the overgrowth of insect pests, at the nursery stage pests such as stem borers are in the larval stage which makes a very difficult conditions for their capture by the sweep net.

- **Dynamics population of insects on NERICA 3 variety**

The figure 2 shows the dynamics population of insects on NERICA 3 variety

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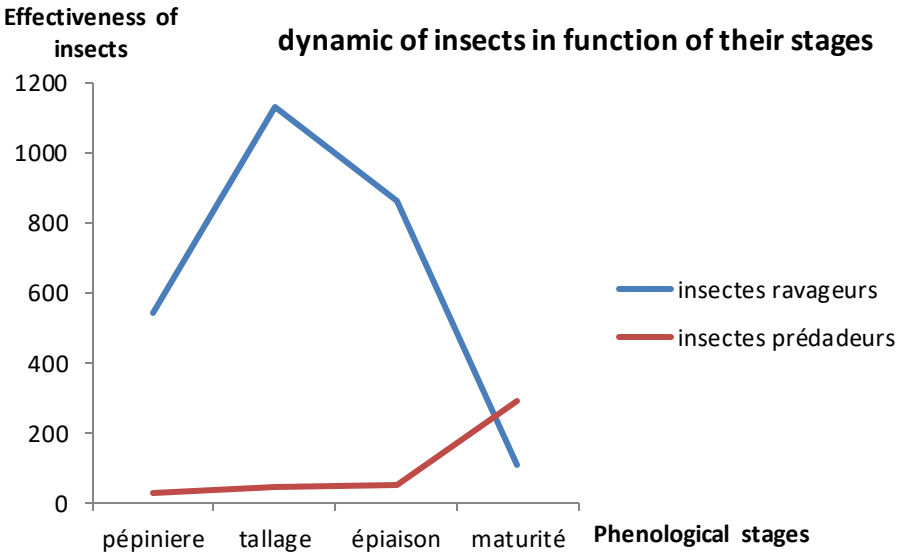

Fig.2: Dynamic of the population of insects on NERICA 3 variety

2 Evaluation of the aqueous extract of neem fruit on rice variety

The curve of pest insects in the shape of a Bell (figure 2), she believed from the nursery until the tillering reaches an optimum and decreases to the maturation. This means that throughout the growth of the rice, the population of insects varies according to the phenological stages and varieties. Tillering is the stage or insects are more likely followed by the heading, nursery and finally maturation. The curve of Predatory insects believes slowly. The nerica3 variety has more bugs than the variety IR46 no matter the phenological stages. The variation of the insects throughout the growth of rice can be explained because each phenological stage has a peculiarity and involves some specific insects, and play on their density in relation to the ecological environment. During the talling stage the plant are bushy well-developed, phytophagous insects like defoliators of leaf sucking biting, and also of stem borers are present in abundance. The duration of this status and the ecological conditions for the development of insects can explain significant outbreak of insects in this stage.

During the heading stage, the quality and quantity of SAP contained in the leaves and parts of the plants declines, leaves are sparse and poor quality this justifies the decline in population during this stage.

The maturity stage is marked by the senescence of leaves and stems so insects do feed on the vegetative parts of the plant, the maturity stage is especially marked by advanced defoliation and senescence. All this helps to reduced numbers of insect pests, the abundance of predators in this stage can be explained by the effect that the maturity stage coincides with the arrival of rains which promotes the Tettigonidae outbreaks.

Given this dynamic of insects from the rice phenological stage we realize that insects have much more visited the heading, nursery and talling stage and it more on during talling, nurseries and early heading stages that should be considered to fight against insect pests. In mature climatic conditions may favour the outbreak of insect predators.

3. Assessment of the effect of the aqueous extract of the fruit of the neem on insects of rice

- **Nursery**

The figure 3 shows the diagram of the strength of insects, collected in the different treatments by the variety at the nursery stage.

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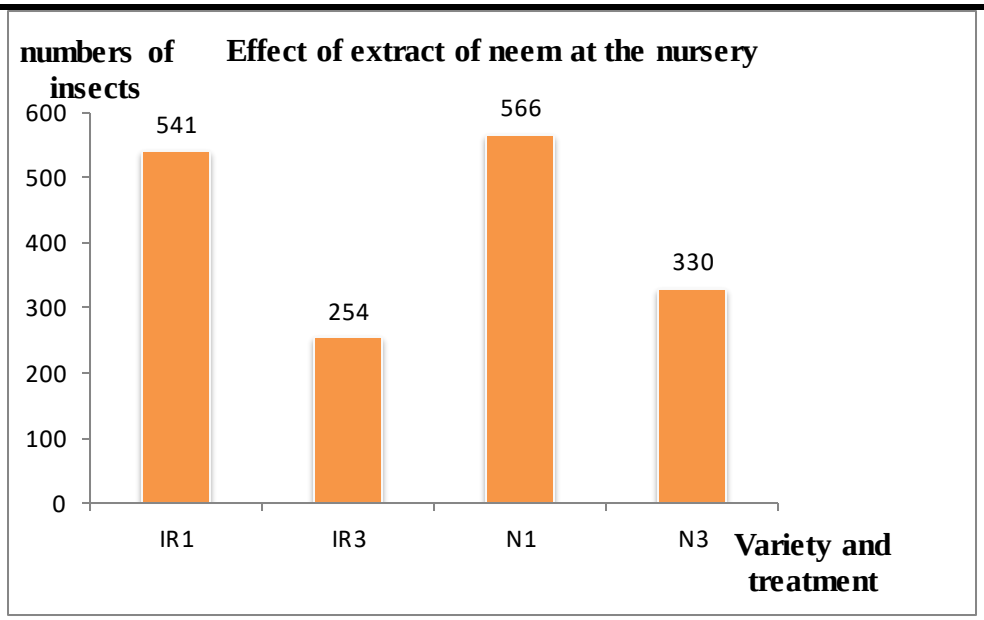


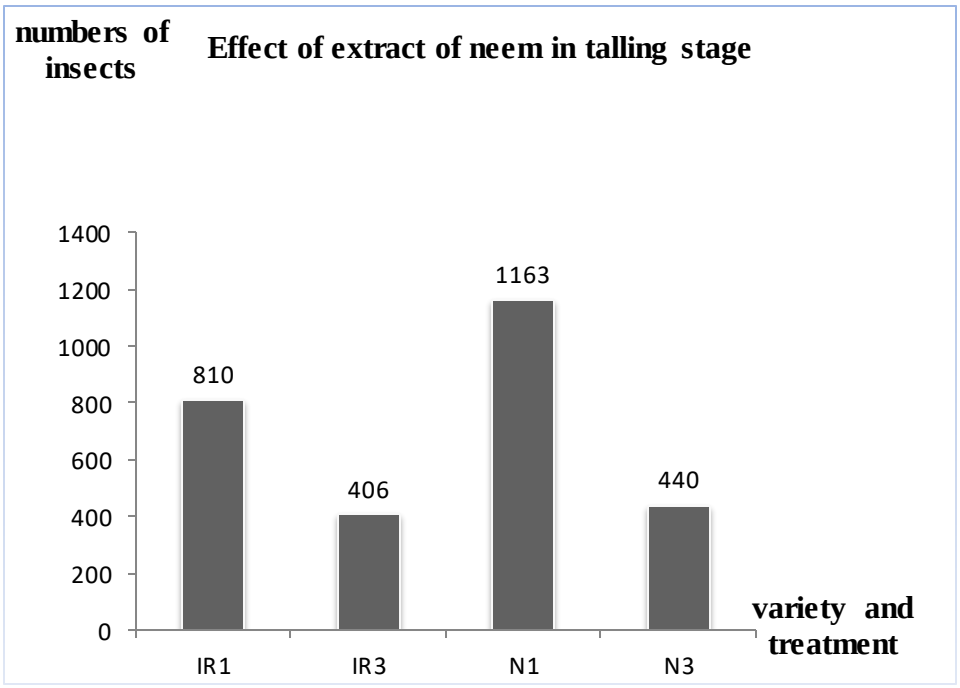
IR1: control IR46, IR3: Treatment of IR46 with aqueous extract of neem seeds, N1 control NERICA3, N3: treatment of NERICA 3 with aqueous extract of neem seeds.

Fig.3: Effect of biopesticide in the nursery

One notes that the number of insect pests on the control is larger, than the number of insect pests on plots treated with aqueous neem fruit extract regardless of the variety of rice. On the threshold of 5% biological treatment is significant for the variety IR46 compared with control IR46, and organic NERICA3 treatment is significant as compared to the nerica3 control. This means that the treatment has been effective insect pests affect hard rice by hunting and by inhibiting food intake. Jacobson (1984), demonstrates the inhibition of food intake of the flea beetle of *Podagrica uniforma* and development of larvae of the beetle and the Epilarchnacrysoelina melon lemon *Papiliode modocus*.

- Talling

Figure 4 shows the diagram of the strength of insects, collected in the different treatments by variety at the tillering stage.



IR1: control IR46, IR3: Treatment of IR46 with aqueous extract of neem seeds, N1 control NERICA3, N3: treatment of NERICA 3 with aqueous extract of neem seeds

Fig.4: Effect of biopesticide in talling

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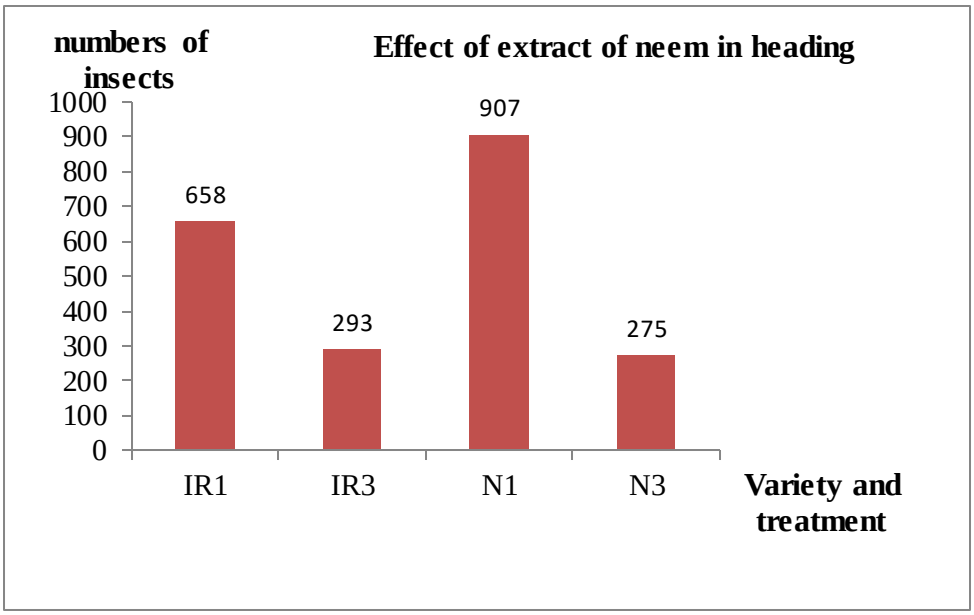
Numerically the number of insect pests on witnesses is larger, than the number of insect pests on plots treated with aqueous neem fruit extract regardless of the variety of rice. On the threshold of 5% biological treatment of Nerica is significant on the control Nerica treatment and IR46 biological treatment has a positive effect on insects from the control of IR46. The insecticidal action of the aqueous extract of neem fruit had an effect on insects pest by reducing their numbers on treated plots according to Gaby (1997) the action of the neem tree is at least 100 species of pests including rice leafhoppers, flies etc...

- **Heading**

The figure 5 shows the diagram of the strength of insects, collected in the different treatments by variety at the heading stage.



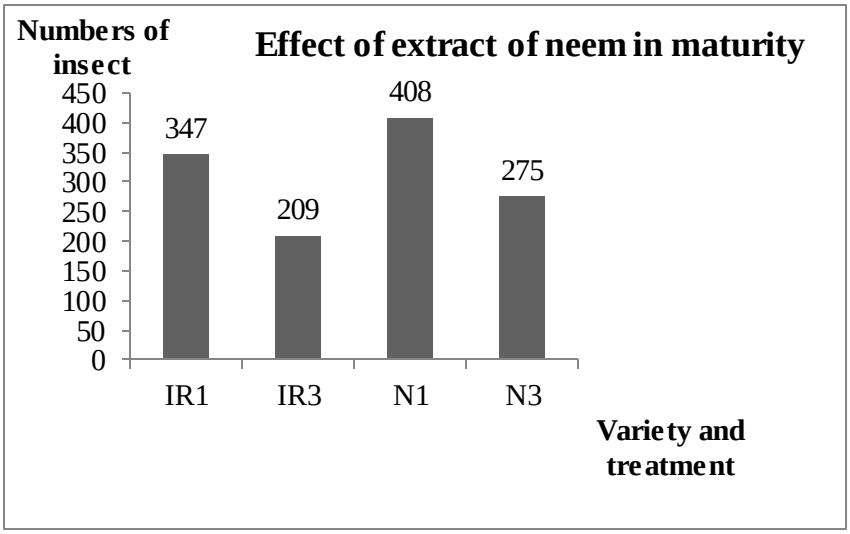
IR1: control IR46, IR3: Treatment of IR46 with aqueous extract of neem seeds, N1 control NERICA3, N3: treatment of NERICA 3 with aqueous extract of neem seeds

Fig. 5: Effect of biopesticide in heading

On the threshold of 5% biological treatments had effects on control the action of the aqueous extract of neem on insects, it is not significant because the dose was not sufficient at this point or Sunshine which quickly denatured the product during heading.

- Maturation

The figure 6 shows the diagram of the strength of insects, collected in the different treatments by variety at the maturity stage



IR1: control IR46, IR3: Treatment of IR46 with aqueous extract of neem seeds, N1 control NERICA3, N3: treatment of NERICA 3 with aqueous extract of neem seeds

Fig.6: Effect of biopesticide in maturity

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The strength of the insect pests on plots of varieties IR46 and NERICA3, is significantly higher compared to plots treated with aqueous extract of fruit of neem in the maturity stage. But on the threshold of 5% biological treatments are not significant. This can be due to climatic conditions which are not conducive to the application of the aqueous extract of neem fruit. Because the maturity stage coincides with the onset of the rains. Jacobson (1997), advocate that the aqueous extract of neem fruit may protect rice during two week to condition that does not rain.

➢ **Effect of the varieties on insects**

The figure 7 shows diagram of the population of insects on IR46 and NERICA3 varieties

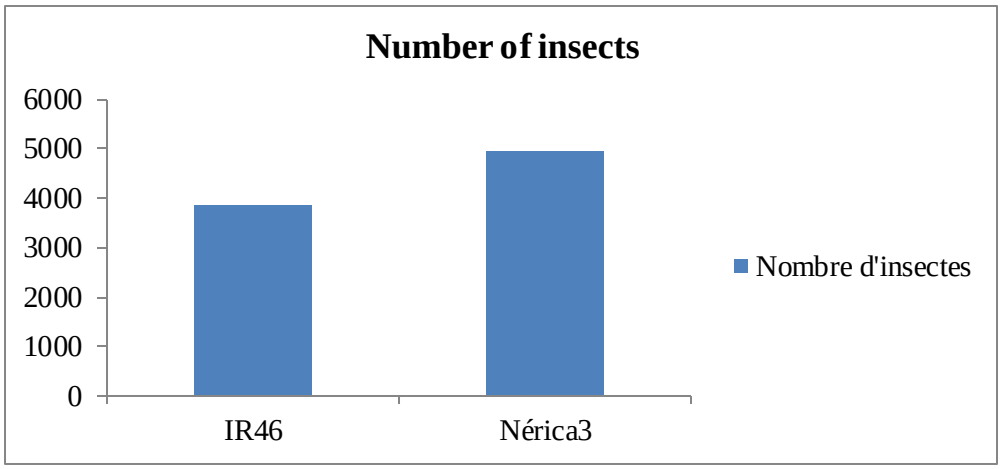

Fig.7: Diagram of the population of insects on IR46 and NERICA3 varieties

Insects are many on the NERICA3 variety IR46 variety but statistical analysis on the threshold of 5% is not significant on the presence of insects on the different varieties.

2. Assessment of damage during talling

The table 6 presents the average number of fruiting stems per square metre on plots of witness, the average number of fruiting stems per square metre on the treated plots, the weight of grain of n1 and the loss of harvest due to insects acting before bolting.

Table.6: Components of the estimate of the damage during talling

IR1	N1
n_1=93 P_1 =0,11 N= 365,5 Nt= 358	n_1=104 P_1 =0,082 N= 480 Nt= 480
Pa=88,71 Kg	Pa=520,38 Kg

N = number of stems without attacks by Drillers
P1 = n1-grain weight
N1 = number of stems without attacks by Drillers
PA = loss of crop due to insects acting before bolting
NT: average number of fruiting stems / m2 control plots;
N: average number of fruiting stems per meter square of plots
IR1: control of IR46 plot
N1: control of NERICA 3 plot

The estimate of damage caused by insects pests before bolting for the control of variety IR46 parcel is Pa = 88, 71 Kg, and for the control of the nerica3 variety plot is Pa = 520, 38 Kg. These results indicate that if rice plant is not treated with the aqueous extract there will be losses due to insect pests before bolting of Pa = 88, 71 Kg for the variety IR46 and Pa = 520, 38 Kg for the variety of rice NERICA3 in this rice agroecosystem. The aqueous extract of neem fruit has a positive effect on the yield of rice, in the sense that it prevents insect pests to their devastating actions on rice.

3. Evaluation at the time of harvest of losses due to borers

The table 7 presents the results of 200 fruiting stems counted on the 20 clumps of rice plants, collected samples of our basic plots, depending on the treatment and the variety. The panicles of the 200 primocanes are divided in panicles without attack of stem borers and panicle with attack of stem borers.

Table.7: Enumeration of the 200 primocanes by treatment

	T1	T3
IR46	n_1= 107 n_2= 93	n_1=93 n_2=107
NERICA3	n_1=106 n_2=94	n_1= 107 n_2=93

T1 : control T3: biological treatment

The table 8 shows another component of the formula for the evaluation of losses.

This table 8 shows average fruiting stems per square metre and the weight of panicles without attacks and attacks.

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Variety and treatment	weight of panicle(Kg)	Average of fruitified stem in m^2
IR1	p_1=0,122 p_2=0,1	375
IR3	p_1=0,08 p_2=0,09	365,5
N1	p_1=0,092 p_2=0,053	558
N3	p_1=0,01 p_2=0,075	358

IR1: control IR46, IR3: Treatment of IR46 with aqueous extract of neem seeds, N1 control NERICA3, N3: treatment of NERICA 3 with aqueous extract of neem seeds

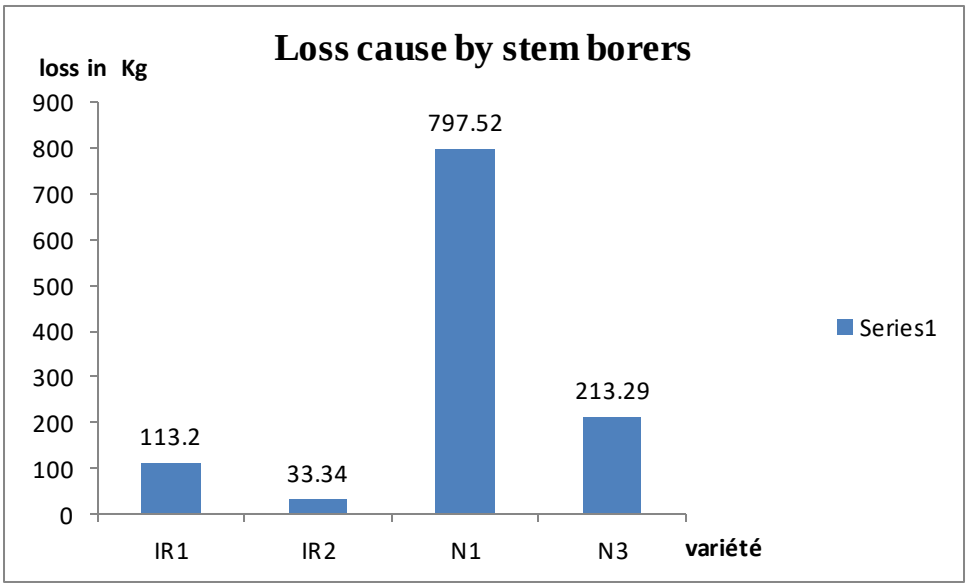
.

P1: weight of panicules without attacks from borers in the stem.

P2: weight of panicles with attacks of borers in the stem (insects present or not).

The fruitful shoots average per square meter is higher for the control compared to the treated plots. This is due to anti parasite and insecticides of the aqueous extract of fruit of neem on borers, which consequently reduces their action on the rice plant by preventing the formation of dead hearts.

The figure 8 shows the damage caused by the action of insect borers on the rice plant.



IR1: control IR46, IR2: Treatment of IR46 with aqueous extract of neem seeds, N1 control NERICA3, N3: treatment of NERICA 3 with aqueous extract of neem seeds.

Fig.8: losses caused by stem borers on the basis of treatments

Losses caused by stem borers at harvest for control of IR46 is 113,20 Kg, biological treatment of IR46 is 33, 34 Kg, control of nerica 3 797, 52 Kg, biological treatment of nerica 3 213, 29 Kg. The control recorded more losses in the aqueous extract of fruit of neem in treated plots, but the Nerica variety, had more loss than the variety IR46.

The aqueous extract of neem fruit has an effect on insect borers, this influence reduces their damage to the rice plant and causes the increase in efficiency.

IV. CONCLUSION

Rice (*Oryza sativa l.*) is the base food of over half of the population of the globe (Guigaz, 2002). But this culture faced many obstacles to its production including attacks from insect pests, control by use of synthetic insecticides and have negative impacts on the biodiversity of the ecosystems and human health yet nature gives us ways to biocontrol through crop protection of crops as the aqueous extract of neem seed against these insects was at issue for us in this study to evaluate the effectiveness of the aqueous extract of fruit of neem on

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insect pests of rice. To get there we set specific objectives:

- Know the biodiversity of insects in the area irrigated by Maga left by phenological stage of the rice plant.
-To see the effect of the aqueous extract of fruit of neem on insect pests by variety and according to the phenological stages.
-Evaluate insect on the plant during talling damage according to variety
-To assess losses due to insect borers.

As indicated in this study that there are two classes of arthropods, class Arachnida, and the insecta. The class of Arachnids is represented by an order, two families, and two species of insects, the class of insects is distributed in seven orders, twenty families, and twenty-four species. All species of spiders are predators. Among the captured insects we identified 13comon insect pests, of which four are classified as the fearsome enemies to the cultivation of rice. They registed a damage of up to 100% yield loss, it's *Sesamia calamistis*, *Diopsis* sp, *Nephottetix nigropictus*, *Maliarpha separatella*. Insect predators include 9 species and parasitoids are numbers 2 species of insects.

The distribution of insect pests by phenological stage varies in abundance in each phenological stage. The most visited stadium by insects is the talling stage. Because at this point the rice plant is well developed and shaped tuft, favourable to the development of insects. The insects were much more abundant on the variety of rice NERICA 3 on the variety of upland rice IR 46.

The aqueous extract of fruit of neem shown to be effective against insects in the nursery and talling stages and had effects on reduction and the action of insects on the rice heading and ripening plant.

The aqueous extract of neem fruit resulted in a reduction of the yield losses caused by insects, and therefore it has improved the yield of rice. Despite to kill insects and reduce the losses caused by them, the aqueous extract of neem seed is more efficient than the use of insecticides synthetic insecticides on insects but contributed enormously to the respect for the environment by decreasing its impact on biodiversity ecosystems.

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Development of RAPD, DAMD and ISSR markers for authentication of medicinal plant *Cassia auriculata* and its adulterant *Cassia surattensis*

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***Abstract* —** *Cassia auriculata is an important traditional medicinal plant commonly used in many Ayurvedic formulations, meant for diabetes, rheumatism, conjunctivitis, infertility and etc. But due to similar morphological characters and misidentifications, adulteration from Cassia surattensis has been observed. Since safety and efficacy of herbal products has become a major concern due to adulterations, DNA profiling which is an effective and cheap method can be used to solve this problem by discriminating the genuine material. However, no reports about the genetic identification for these species are available to our knowledge. Therefore, the present study was devoted to developing Random Amplified Polymorphic DNA (RAPD), Direct Amplification of Minisatellite-region DNA (DAMD) and Inter Simple Sequence Repeat (ISSR) DNA profiles to authenticate C. auriculata and C. surattensis. As the first step, fresh leaf samples from both plant species were randomly collected from Gampaha district, Sri Lanka and genomic DNA were extracted using modified Cetyltrimethylammonium Bromide (CTAB) protocols. Four short arbitrary primers, two core primers and two SSR primers were used respectively with three different Polymerase Chain Reactions (PCR) based molecular markers which were RAPD, DAMD and ISSR to develop DNA profiles. Out of eight primers, three arbitrary primers, OP-F03, OP-U10 and OP-U20 and one core primer, HBV(5) yielded clear and reproducible amplification products. These results clearly discriminated the medicinal plant C. auriculata and the adulterant C. surattensis providing a complementary tool for quality control of plant derived herbal medicinal products. However, both SSR primers couldn't authenticate two plants and further work is needed to develop ISSR DNA profiles for the authentication.*

***Keywords* — CTAB, DNA profiles, herbal medicines, microsatellites, minisatellites.**

I. INTRODUCTION

Cassia auriculata (Tanners Cassia / Ranawara), is a legume shrub belongs to the large plant family Fabaceae. It prefers drought and dry habitats, therefore can easily found in the tropical climates in India, Sri Lanka and Myanmar [23]. Since it is usually used as a medicine for diabetes, it has a high economic value. Other than in diabetes, *C. auriculata* is widely used in traditional medicine for rheumatism, conjunctivitis, diarrhea, female infertility, leprosy and also for skin diseases [17].

Species such as *Cassia surettensis* and *Cassia divericata* are morphologically indistinguishable from *C. auriculata*, since flowers of these species are more or less similar in morphology to each other and therefore, easily adulterated specially during harvesting. And also adulterations can arise due to the price pressure, increased demand, limited availability, deceitful substitution using less valuable species and indistinct taxonomy due to confusion between Latin nomenclature and local terminology. Importantly, adulterants do not have medicinal values as in *C. auriculata* and some contains toxic compounds [04]. Therefore, an accurate, effective and reliable method is essential for the authentication of the *C. auriculata* plant to avoid adverse effects on herbal medicine consumers.

Morphological based authentication of medicinal plants may not provide the correct identification due to similarities in morphology between original plant and adulterants, and also due to geographical variations in plants of same species. Chemical based methods cannot also use due to variations in the chemical composition arising from age and geographical variations.

Since the genetic composition is unique to each species and it is not affected by age, physiological and geographical factors, DNA based molecular authentication is the most desirable way to authenticate those medicinal

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plants. Therefore, DNA markers and PCR based DNA profiling techniques can be used [02].

DNA profiling is a technique which is used to identify an individual from a DNA sample by looking at the unique DNA banding patterns which result from unique DNA markers after PCR and gel electrophoresis [07]. It can be done in two ways. Those are PCR based single-locus method and PCR based multi-locus method. Among them multi-locus method is mostly and efficiently used with genomes where genome sequence data are scantly or lacking. Multi-locus method uses single oligonucleotide primers with universal arbitrary sequences to produce DNA markers from genomic DNA resulting in multi-locus banding patterns after gel electrophoresis. RAPD, ISSR and DAMD PCR profiling methods are popular variants of this method [13].

In RAPD-PCR profiling, short arbitrary random synthetic oligonucleotide primers are used to amplify multi locus DNA markers which have distributed within the genome [24]. In ISSR-PCR method, DNA segment present in between two identical Simple Sequence Repeat (SSR) regions (Microsatellite regions) oriented in opposite directions is amplified [14] using SSR regions as primers. These primers target multiple loci within the genome to amplify Inter Simple Sequence Repeats (ISSRs) of different sizes, producing multi locus dominant markers. Most of the minisatellite sequences which have dispersed among many organisms share common central motifs which are known as core sequences or core units. These core sequences can be used to design universal primers to use in DAMD-PCR profiling of previously unknown genomes. Core sequence of minisatellite regions is used as a single primer and it can anneal with two adjacent core sequences which have located in opposite directions within two minisatellite regions. Therefore, amplified PCR products are rich in minisatellite repeats and show polymorphism due to site specific length variation in the inter repeat region as in ISSR-PCR method [16, 24].

C. auriculata and its adulterants do not have well defined genomes and therefore, this study was undertaken to authenticate *C. auriculata* plant from its adulterant *C. surattensis* plant using RAPD, ISSR and DAMD DNA profiling.

II. MATERIALS AND METHODS

2.1 Sample Collection

Fresh, young and healthy leaf samples of *C. auriculata* and *C. surattensis* were randomly collected respectively from five and three locations in Gampaha district, Sri Lanka. Plants were identified by Dr. (Ms.) T. D. Ramanayake (DAMS). Collected leaf samples were washed well with tap water and surface sterilized with 70% Ethanol followed by three serial washings with sterilized distilled water. Then leaves were allowed to air dry on sterilized filter papers. After drying, midribs of leaves were removed using a sterilized scissor and remaining leaf parts were measured using analytical balance (CY 720-Citizen Scale, USA) as portions of 1.00 g. Each portion was covered with an aluminium foil and stored at -80 °C until further use.

2.2 DNA extraction

Plant genomic DNA were extracted from all samples according to the protocol described by Sahu *et al.* (2012) after several modifications.

After all modification events two different CTAB extraction buffers have to use with two plant species to obtain highly purified genomic DNA. CTAB extraction buffer consisting 2% (w/v) CTAB, 100 mM Tris HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA (pH 8.0), 3% (w/v) PVP and 2% β-mercaptoethanol was used with *C. auriculata* plant samples and buffer containing 2% (w/v) CTAB, 100 mM Tris HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA (pH 8.0), 4% (w/v) PVP and 2% β-mercaptoethanol was used with *C. surattensis* leaf samples. Both CTAB extraction buffers were preheated at 65 °C in a water bath for 30 minutes. Frozen leaf samples (1.00 g of each) were crushed using ice cold mortar and pestle. Finely ground tissues were added into microfuge tube (2 mL) which contained relevant pre heated extraction buffer (1.00 mL). Then they were mixed vigorously and incubated at 65 °C for 30 minutes in a water bath with occasional swirling. After incubation tubes were cooled to room temperature and centrifuged (Hettich Mikro 200 Zentrifugen, Germany) at 13,000 rpm for 5 minutes and the supernatants were transferred into new microfuge tubes. Equal volumes of chloroform: isoamyl alcohol (24:1) were added and mixed gently by inverting 5 minutes. Then the tubes were centrifuged at 13,000 rpm for 2 minutes and the supernatants were collected to new microfuge tubes. Re-extraction with chloroform: isoamyl alcohol (24:1) was done and the aqueous supernatants were transferred to new microfuge tubes. Ammonium acetate (7.5 M, 100 µL) followed by ice cold absolute alcohol (1.00 mL) was added to the transferred supernatants and tubes were gently inverted few times. Then the tubes were incubated at -80 °C for 2 hours. After that tubes were centrifuged at 13,000 rpm for 5 minutes to precipitate the DNA and then the supernatant was removed. The DNA pellet was washed with 1.00 mL of Ethanol (95%) and was agitated vigorously to release the pellet. Then tubes were centrifuged at 13,000 rpm for 5 minutes to pellet out the DNA again. Then the supernatant was removed and microfuge tubes were air dried on filter paper for 10 minutes. Then, the DNA pellet was resuspended in 50–100 µL of TE buffer. Then RNase A (2.00 µL, 10µg/ml) was added and incubated at 37 °C in dry bath for 1.5 hour. Finally, extracted DNA samples were stored at -20 °C

until further use. DNA concentration and purity were determined by measuring the absorbance of 25 times diluted DNA solution at 230 nm, 260 nm and 280 nm respectively using ORION Aquamate 8000 UV-VIS spectrophotometer. The quality of the genomic DNA was checked on 0.8% agarose gel stained with Ethidium Bromide.

2.3 PCR reactions

Eight primers which can be used in PCR amplifications were selected according to the research that Ranade and Farooqui has conducted to authenticate medicinal plant Neem (*Azadirachta indica*) in year 2002 (Table 1).

Table.1: The microsatellites, minisatellites and arbitrary sequences used as primers in PCR amplification reactions.

No.	Primer name	Primer sequence (5'- 3')
1	TATC-6	TAT CTA TCT ATC TAT CTA TCT ATC
2	GATA-6	GAT AGA TAG ATA GAT AGA TAG ATA G
3	33.6	GGA GGT TTT CA
4	HBV(5)	GGT GTA GAG AGA GGG GT
5	OP-F02	GAG GAT CCC T
6	OP-F03	CCT GAT CAC A
7	OP-U10	ACC TCG GCA C
8	OP-U20	ACA GCC CCC A

2.3.1 RAPD-PCR

Four arbitrary primers of OPF and OPU series were used in RAPD analysis. The reactions were carried out in 25 µL volume in a tube using four RAPD primers which were OP-F02, OP-F03, OP-U10 and OP-U20. Each reaction tube contained 0.5 µL template DNA, 1× GoTaq® Flexi Buffer, 2.5 mM $MgCl_2$, 200 µM of dNTPs, 0.4 µM of primer and 0.5 units of GoTaq® DNA polymerase. Amplification reaction was performed in a thermal cycler (Veriti® 96-Well Thermal Cycler, Applied Biosystems, USA), using following conditions: pre denaturation at 94 ºC for 2 minutes followed by 45 cycles of denaturation at 94 ºC for 30 s, annealing at 35 ºC for 30 s and extension at 72 ºC for 1 minute and final extension at 72 ºC for 7 minutes.

2.3.2 ISSR-PCR

Two SSR primers, TATC-6 and GATA-6 were used. Pilot experiments using a range of annealing temperatures were performed to optimize the annealing temperature for each SSR primer, along with different DNA volumes. The annealing temperature range was determined in the range of 2-10 °C lower than the denaturation temperature (Td) of each primer. As mentioned by Ranade and Farooqui (2002), the Td was calculated by adding 2 °C for each A or T and 4 °C for each G or C in the primer sequence. According to that calculation, the Td of primer TATC-6 is 60 °C and Td of GATA-6 primer is 64 °C. Therefore a temperature gradient PCR which includes 52 °C, 54 °C, 55 °C, 57 °C and 58 °C combined with a range of DNA volumes (0.50 µL, 1.00 µL, 2.00 µL and 5.00 µL) was carried out with primer TATC-6. Another temperature gradient PCR which includes 54 °C, 55 °C, 57 °C, 60 °C and 62 °C combined with the same range of DNA volumes as in the above reaction was used with primer GATA-6. Other reagents in the reaction mixture (25 µL final volume) were 1× GoTaq® Flexi buffer, 3.0 mM $MgCl_2$, 200 µM of dNTPs, 0.4 µM primer and 0.5 units of GoTaq® DNA polymerase. PCR cycles were carried out with pre denaturation at 94 ºC for 5 minutes followed by 25 cycles of denaturation at 94 ºC for 30 seconds and extension at 72 ºC for 1 minute and final extension at 72 ºC for 7 minutes in the thermal cycler.

2.3.3 DAMD-PCR

Two DAMD primers named HBV(5) and 33.6 were used for DAMD analysis. The PCR reaction mixture of 25 µL contained 5.00 µL of genomic DNA, 1× GoTaq® Flexi Buffer, 3.0 mM $MgCl_2$, 200 µM of dNTPs, 0.4 µM of primer and 0.5 units of GoTaq® DNA polymerase. During the amplification reaction in the thermal cycler, pre denaturation step of 5 minutes at 94 ºC was followed by 35 PCR cycles (denaturation at 94 ºC for 30 seconds, annealing at 55 ºC for 1 minute, extension at 72 ºC for 1 minute). A final step of 7 minutes at 72 ºC was carried out for the polishing of PCR products.

In all PCR amplification reactions a sample without template DNA was used as a negative control to check the presence of contaminations.

2.4 Gel electrophoresis

The reaction products obtained after PCR were electrophoresed on 1.5% agarose gel stained using ethidium bromide in 1× TBE buffer at 50 V for 1.5 hours using a horizontal electrophoresis apparatus (APPLEX PS 9009 TX, France). Electrophoretic profile was visualized under UV transilluminator and documented through a Gel Documentation System (QUANTUM ST5, Germany). The sizes of DNA fragments were estimated by comparison with a 100 bp or 1 kb molecular weight marker (Promega Corporation).

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III. RESULTS AND DISCUSSION

"Herbal drugs" is a main part of the traditional medicine especially in developing countries. According to surveys, about 75% of the world population depends on traditional medicine [02]. The traditional medicine system basically depends on large number of medicinal plants, which represents a huge biodiversity. Rapid and accurate authentication of medicinal plants is difficult to obtain, at the scale of international trade. Especially because, many medicinal plant based commercial products are sold either in dried form or as processed material, leading to their authentication by morphological methods very difficult or impossible. But, DNA based methods such as DNA-profiling can be used to identify adulterated raw materials more accurately and effectively. PCR based DNA-profiling method has been successfully used as a molecular authentication system of several medicinal plants. Neem [16], Oregano [11], Saffron [06] and *Acorus calamus* [15] are some successful examples.

In this research, a medicinal plant *Cassia auriculata* was chosen for the authentication through PCR based DNA-profiling. To the best of our knowledge, this is the first DNA profiling based molecular authentication experiment which has done with *C. auriculata*.

Cassia surattensis can be found as adulterant in *C. auriculata* based herbal medicinal products which is available in the market. *Cassia divericata* also can be used as an adulterant, but it is a rare plant in Sri Lanka, therefore it is impossible to use it as an adulterant in the market samples. Due to these reasons, in this study *C. surattensis* plant samples were also used for differential identification.

When moving steps into molecular authentication, the initial step which have to do is DNA extraction. Molecular authentication is a downstream application of DNA extraction. Therefore, high quality and highly purified genomic DNA free from proteins, RNA and other secondary metabolites such as polyphenols, polysaccharides and terpenes should be isolated from both *C. auriculata* and *C. surattensis* species to obtain successful final result from molecular authentication. Generally OD260/280 ratio of pure dsDNA should be around 1.8 and OD260/230 ratio should be between 2.0 and 2.2 [03]. It means that, OD ratio values which obtained other than these standard values indicate the presence of contaminations in extracted genomic DNA. When extracting genomic DNA from *C. auriculata* leaf samples, optimization of DNA extraction protocol was essential, since initial DNA extraction protocol [18] used in this study ended up with low quality DNA with good yield from 1.00 g of fresh leaf sample. After the modifications in the initial DNA extraction protocol extracted genomic DNA had a 1.82 ± 0.03 of OD260/280 ratio and a 1.85 ±0.07 of OD260/230 ratio with a yield of 740.00 ± 47.55 µg/mL. According to Semagan (2013), OD260/230 is a secondary measure of nucleic acid purity and therefore that ratio of 1.8–2.2 is acceptable for downstream applications such as PCR. Therefore DNA extractions from *C. auriculata* leaf samples collected from all five locations were carried out after the modifications of CTAB protocol.

Then, this optimized CTAB DNA extraction protocol was used to extract genomic DNA from adulterant *C. surattensis* leaves. Extracted genomic DNA resulted in low OD ratio values while providing good yield. Therefore, modifications were needed with this extraction also. After several optimization events, genomic DNA with OD260/280 ratio of 1.85 ± 0.04 and OD260/230 ratio of 2.00 ± 0.20 was obtained with 1085.83 ± 492.38 µg/mL yield. Both obtained values were in the standard OD ratios. Therefore, DNA extraction from *C. surattensis* leaf samples collected from all three locations were carried out after the modifications in CTAB protocol.

The next step which have to do is molecular authentication of those two plant species using extracted high quality DNA. In that case DAMD-PCR profiling, RAPD-PCR profiling and ISSR-PCR profiling were used.

DAMD-PCR technique was first described by Heath *et al.* (1993) and has been applied with various plant species including *Triticum* [01] and *Capsicum* [09]. In this PCR technique, a single primer from a minisatellite core sequence is used to direct PCR by amplifying regions rich in minisatellites. These regions may have core sequences involved in inversions between successive minisatellites on both strands in opposite orientations, allowing the primer annealing and amplification of minisatellite regions [24]. HBV(5) [12] and 33.6 [10] are famous core sequences which have been used as DAMD primers. *Homo sapiens* (Human) genome is the source of these primers, but they are conserved across many other species making them as universal primers [12]. Therefore, when conducting DAMD-PCR reaction in this study, these two primers were used. For any PCR, it was necessary to standardize the genomic DNA concentration which need to contain in the PCR mixture, since higher DNA amounts can inhibit PCR reaction and lower DNA concentrations cannot produce sufficient amount of amplicons to be visualized. Therefore, a pilot experiment was conducted with a series of genomic DNA volumes from DNA stocks as 1.00 µL, 2.00 µL and 5.00 µL to check the best amplification. All DNA dilutions provided DNA profiles with primer HBV(5), but the best profiles with clearly visible bands were obtained with 5.00 µL genomic DNA volume in the PCR mixture (Fig. 1).

But, a profile with faint bands was observed with the Sample no. 7. It can be due to many practical errors. However, the amplification products of profiles were in the range of 450–1250 bp and those bands had an equal

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degree of polymorphism and stability. But the scoring of those markers was difficult due to low separation. According to Ince *et al.* (2008), this problem could be reduced by using Agarose gels such as 25–30 cm in length with a long running time. With the primer 33.6 no profiles were obtained even with any genomic DNA volume. It may be due to the absence of that particular core sequence in minisatellite regions within the genomes of these two species.

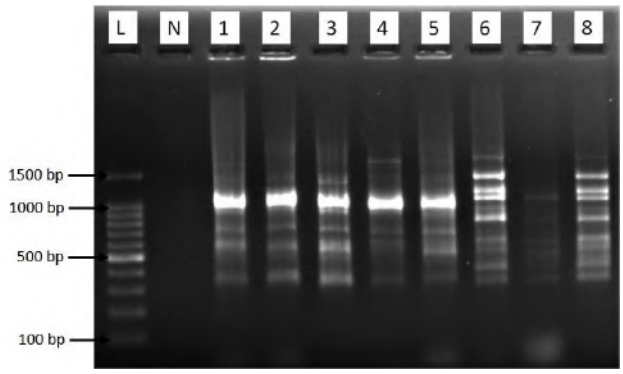

Fig. 1: DAMD-PCR profiles of C. auriculata and C. surattensis for primer HBV(5). Starting from the left, Lanes: L, 100 bp DNA ladder; N, negative control; 1-5, C. auriculata; 6-8, C. surattensis. The lane numbers mentioned correspond to the samples collected from different locations.

RAPD-PCR technique was first described by Williams *et al.* in the year 1990. Thereafter, this technique has been successfully used with many plant species including different medicinal plants such as *Panax* [20] and *Aloe* [21]. In this technique DNA polymorphism is determined based on the amplification of random DNA segments in the genome using single primers of arbitrary nucleotide sequences. In this study, four arbitrary primers OP-F02, OP-F03, OP-U10 and OP-U20 were used. In these reactions also, it was necessary to standardize the genomic DNA concentration which has to contain in the PCR mixture. Therefore, a pilot experiment was conducted with a series of genomic DNA volumes from DNA stocks as 0.50 µL, 1.00 µL and 2.00 µL to check the best amplification. After gel electrophoresis, smears were observed with 1.00 and 2.00 µL DNA volumes and it can be due to high concentrations of DNA. But with 0.50 µL genomic DNA volume, good banding patterns were observed with all four primers. The amplification products of those profiles were in the range of 350–1600, 350–2000, 450–1000 and 350–1600 with primer OP-F02 (Fig. 2. A), OP-F03 (Fig. 2. B), OP-U10 (Fig. 3. A) and OP-U20 (Fig. 3. B), respectively. Profiles with clear and well distinct bands were observed with three primers other than the primer OP-F02. The profile obtained with primer OP-F02 contained faint bands, with some plant samples such as Sample no. 2, 3, 4, 5 and 6. It can be due to the low reproducibility of that particular primer OP-F02. Due to low reproducibility, other three RAPD profiles obtained from this experiment are more suitable to differentially authenticate these two plant species successfully.

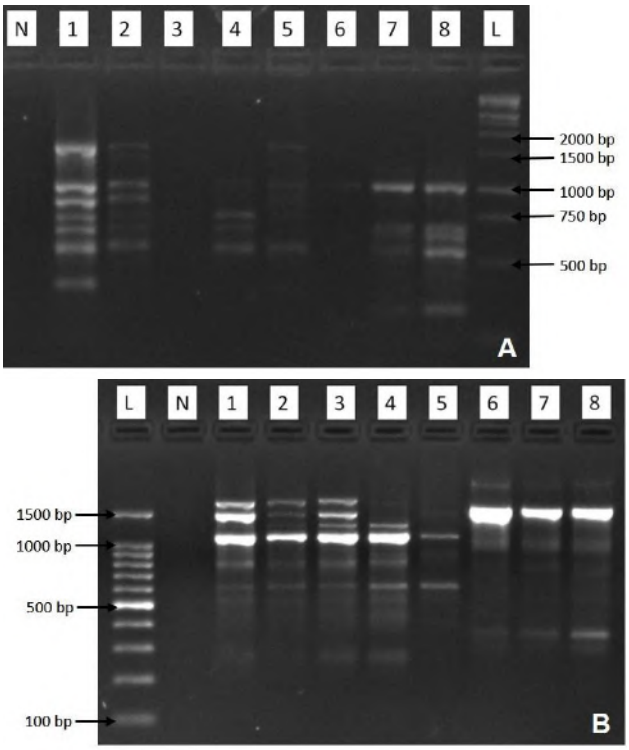

Fig. 2: RAPD-PCR profiles of C. auriculata and C. surattensis for ***A,*** *primer OP-F02 and* ***B,*** *primer OP-F03. Starting from the left, Lanes: L, 100 bp/1 kb DNA ladder; N, negative control; 1-5, C. auriculata; 6-8, C. surattensis. The lane numbers mentioned correspond to the samples collected from different locations.*

ISSR-PCR technique also has been successfully used to identify some plant species including *Citrus* cultivars [05], *Pseudotsuga menziesii* and *Cryptomeria japonica* [22]. ISSR-PCR involves a single primer composed of microsatellite / SSR sequence. This primer can target microsatellite regions and amplify the intervening region between the two microsatellite regions in opposite orientations [07]. In this research, two ISSR primers have been used which are TATC-6 and GATA-6 but, there were no standardized annealing temperatures for these primers. Therefore, pilot experiments using a range of annealing temperatures were performed to optimize the annealing temperature for each SSR primer along with a range of template DNA volumes to determine the optimum DNA volume which should be in the PCR mixture. But, no profiles were observed for any SSR primer. It can be due to the lack of these primer sequences within the *C. auriculata* and *C. surattensis* plant genomes. Also according to Ranade and Farooqui (2002) most of the SSR

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primers with tetra-nucleotide repeats could not result in any amplification only result in a smear or no profile as in this study and tri-nucleotide SSR primers were more effective and resulted in clear distinct amplification profiles. This can be due to the low annealing ability of these primers. Therefore, tri-nucleotide SSR primers such as CAC-5 and GAA-6 which have succeeded in PCR-profiling of medicinal plant Neem as mentioned by Ranade and Farooqui (2002), may be successful with these two plant species also.

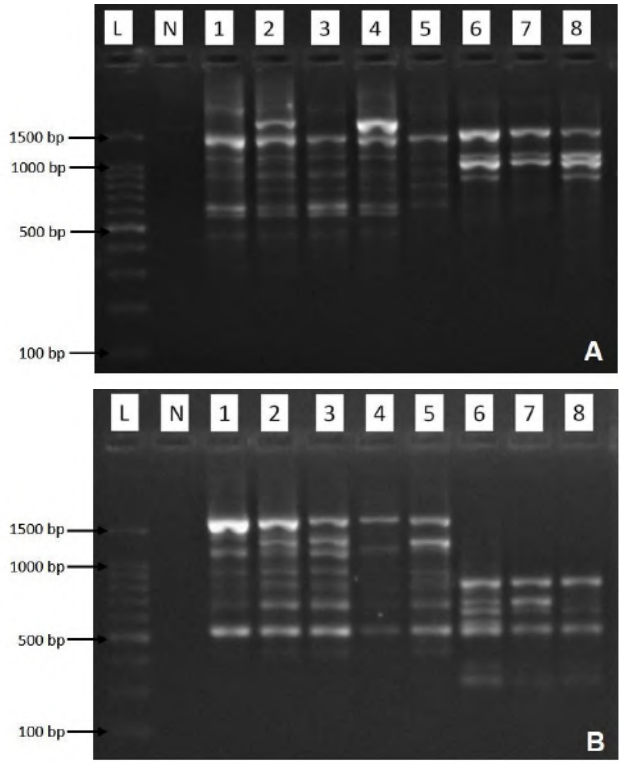

*Fig. 3: RAPD-PCR profiles of C. auriculata and C. surattensis for **A,** primer OP-U10 and **B,** primer OP-U20. Starting from the left, Lanes: L, 100 bp DNA ladder; N, negative control; 1-5, C. auriculata; 6-8, C. surattensis. The lane numbers mentioned correspond to the samples collected from different locations.*

According to this study, though RAPD-PCR profile with primer OP-F02 is not suitable and ISSR-PCR profiling cannot be used to authenticate *C. auriculata* and *C. surattensis* plants effectively, other four DNA profiles which obtained with primer OP-F03, OP-U10, OP-U20 and HBV(5) can be used to differentially identify *C. auriculata* and *C. surattensis* plants effectively. Unique DNA banding patterns specific to each *Cassia* species with different primers have summarized in Table 2. Those unique banding patterns can be either used to authenticate two plant species or those plant derived herbal medicinal products which is available in the market.

Table.2: Species specific bands (bp) obtained with five different primers in PCR amplifications.

Primer	Species specific bands (bp)	
	Cassia auriculata	***Cassia surattensis***
HBV(5)	-	1250
	-	1100
	1000	-
	750	-
	-	650
	-	450
OP-F03	-	2000
	1650	-
	1100	-
	-	1000
	800	-
	600	-
	-	350
OP-U10	-	1000
	900	-
	-	800
	750	-
	600	-
	550	-
	450	-
OP-U20	1600	-
	1250	-
	950	-
	-	720
	700	-
	-	350

IV. CONCLUSIONS

To the best of our knowledge this is the first attempt of the genomic based authentication of medicinal plant *C. auriculata* and its adulterant *C. surattensis*. RAPD and DAMD PCR markers developed in this study provide guidance to authenticate two plant species effectively and reliably. Nonetheless, further studies are required to introduce another SSR primer to develop ISSR PCR markers for this authentication.

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Effect of zaï and micro dose on root biomass and the grain and straw yield so sorghum at Tangaye in the North region in Burkina Faso

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***Abstract*—** *Faced with rainfall variation and the poor performance of farming practices, the North region of Burkina Faso often observed cereal deficits. Sorghum, the main staple food crop in this region, provides relatively low yields (1000 kgha^{-1}). Furthermore, in the area, the density of the population is one of the highest in the country. In order to increase sorghum yields, a study has been carried out in the village of Tangaye by combining the water management practice through mechanized and manual zaï techniques with fertilization by microdose of NPK fertilizer. The experimental design of the study was a split-plot with three replications and four treatments set on a crusty bear soil "Zipellé".The mechanized zaï and the manual zaï have been compared with and without applying mineral NPK fertilizer by a micro dose. The effects of these techniques have been evaluated on the soil and the root system by the method of taking monoliths. The grain and straw yields of sorghum have been evaluated for each treatment. The results showed that the greatest roots system development was obtained on the mechanized zaï plot with the application of micro dose of NPK fertilizer. This treatment also has the highest grain yield (2910 kgha^{-1}) compared to manual Zaï (1620 kgha^{-1})*

***Keywords*— Soil tillage, manual and mechanized Zaï, sorghum, root, yield.**

I. INTRODUCTION

Agricultural production in Burkina Faso is dominated by cereals (sorghum, millet, maize, rice and fonio), which cover 69.9% of the area devoted to food crops. The physical and sufficient availability of food cereals to meet the needs of populations remains a challenge for agricultural policies. Among food cereals, sorghum, [*Sorghum bicolor* (L.) Moench] is the most widely used in Burkina Faso. Its cultivation covers 1.73 million hectares, which represent 43.2% of cereal areas and 30.2% of crop production areas (MAAH, 2016). Most of sorghum-based cropping systems are extensive. The increase in grain production is more related to that of the areas. In 2016, the sorghum growing areas were increased up to 7.6% with regard to the average of the past five years, with an average increase of 3.5% in grain production, showing the extensive character of the production (MAAH, 2016).

In the North region of Burkina Faso, situated in the Sub-sahelian zone between 500 and 700 mm of rainfall, 95% of the population depend on agriculture and livestock. Cereal productivity is low. Among the main reasons of the low productivity are the rainfall constraints, the physical and chemical degradation of soils and the inadequacy of cultural practices (Bado, 2002). Thus, a large proportion of rural households regularly live in food insecurity (MAAH, 2016). A study conducted in Burkina Faso showed that food security improves when households depend on their own production (Thiombiano *et al.*, 2014). In 2016, in the North region, cereal production losses due to drought were estimated at 104,466 tons which represent 15.6% of losses of all the 13 regions of Burkina Faso.

Nicou *et al.* (1987), Zougmoré*et al.*(2002), Barro *et al.* (2005) showed in Burkina Faso that water management techniques combined with fertilization allowed to have high yields in farmer's fields. Palé *et al.* (2009) have also shown that scarification associated with mineral fertilization by micro-dose leads to increase sorghum grain yields of 25%. Dimtsu *et al.* (2018) reported in Ethiopia that soil degradation has an impact on crop productivity.

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Studies conducted in Zondoma province in the North region showed that the phenomenon of soil degradation in this province slowed down but not stopped (PDCL, 2007). To improve the productivity of degraded soils and increase sorghum productivity, a study was conducted in the village of Tangaye located in Zondoma province to assess the effect of water management techniques using manual and mechanized zaï and micro dose of fertilizer (NPK) on the development of sorghum root system, as well as on grain and straw yields.

II. MATERIAL AND METHODS

2.1The study site

The study was conducted in Sub-sahelian zone (isohyet 600-700 mm), in the village of TangayeFig. 1),Zondoma province in the North region of Burkina Faso. Tangaye is located at 15 km East of Gourcy, at 13°33 'N and 2°33'W with an altitude of 338 m. The rainfall is characterized by a strong interannual variation. The climate is Sahelo-soudanian with two seasons: a long dry season from November to June alternating with a rainy season from July to October (Fontes andGuinko, 1995; Kaboré, 2013). The average annual rainfall over the last 30 years has been 650 mm.

The natural vegetation is significantly degraded by human activities (demographic and land pressure, etc.)combined to climate change (droughts and floods). The most common species are: *Khaya senegalensis, Annogeissus leiocarpus, Acacia penata, Mitragyna inermis, Tamarindus indica, Ficus sp., Accacia ssp., Combretum ssp., Butirospermum paradoxum, Guiera senegalensis, Boscia senegalensis, Zizyphus mauritiana and Piliostigma ssp*. Theherbaceous layer mainly includes *Pennisetum pedicellatum, Schoenfeldia gracilis, Loudecia togoensis and Andropogon Sp*.(Fonte andGuinko, 1995).

Most of the soils are lixisoil types on gravels (FAO, 2006). This type of soil represents 26.2% of the country's areas (Zougmoré andBarro, 2002). They are poor in mineral nutrients; their depth and water reserve are low; in the case of high population density like Tangayethese soils are exploited. The soils of Tangaye are particularly exposed to water erosion because of their physical characteristics (sensitive structures and textures favoring encrusting, low organic matter content) and the length of the slopes (sometimes greater than 2 km) (Roose, 1981).

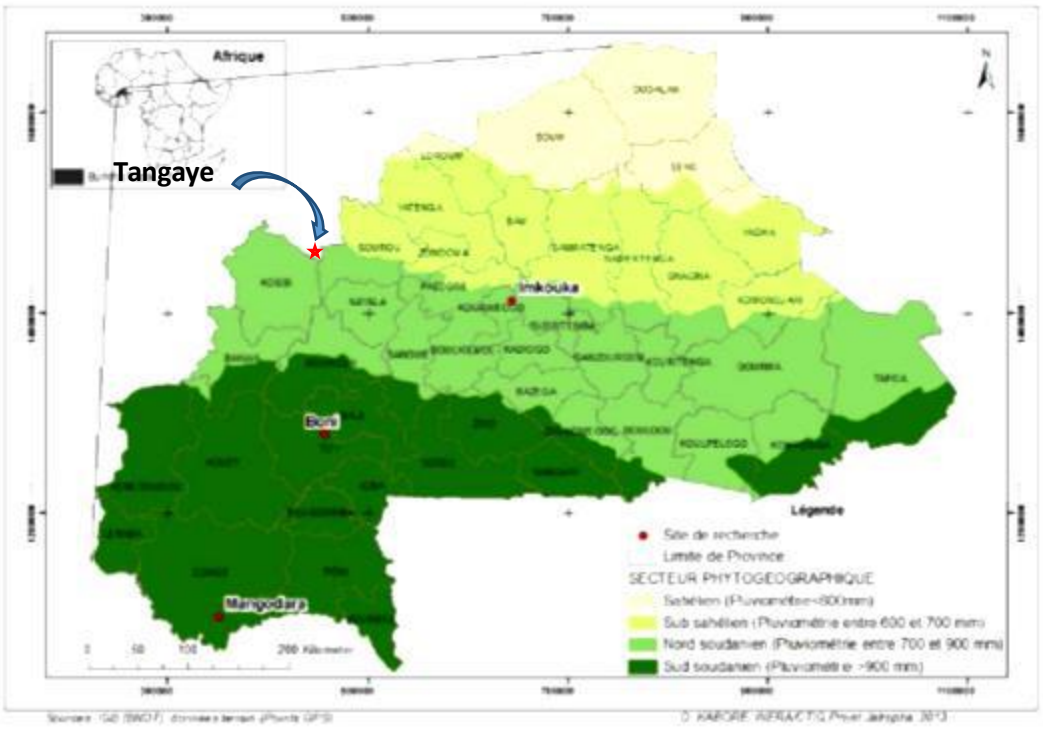

Fig. 1: Location of the study site in Burkina Faso

2.2Plant material

The plant material used in this study was sorghum, the Kapèlga (SCHV 168) variety. Kapèlga belong toguinea botanical race, an improved local sorghum variety released by INERA sorghum program. This sorghum is particularly interesting for its white grain, its short cycle duration (90 days) and it's good vitreous grain well suited to local dishes. Kapèlga is largely cultivated in many areas, even in the humid zones of Burkina Faso (900-1000 mm).

2.3Experimental design

The experimental plots were set on a crusty bear soil "Zipellé". Thedesignwas a split plotwith two factors

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studied in three replications. The main factor was the soil tillage with two modalities: mechanizedzaï andmanual zaï. The secondary factor was fertilization with two modalities: the application of the microdose of NPK fertilizer and the no-application of the micro-dose. The manual and mechanized zaï practices were carried out in dry soil conditions before the beginning of rainy season2016.The plots of soil tillagewere14 m long and 10 m wide. The sowing was carried out on 3rd July 2016.

The soil tillage modalities:

- *The mechanized* zaï was carried out with two-oxenhitch with 80 cm between the rows and 40 cm between the seed holes.
- *The manual zaï* was carried out manually with a hoewith 80 cm between rows and 40 cm betweenthe seed holes. The seed holes were arranged in staggered from row to row.

For each seed hole ofmanual ormechanizedzaï, the quantity of compost applied was 300 g that mean 9.375 tha^{-1}. The thinning was carried out with two plants for each seed holes.

The fertilizationmodalities:

- *The application of the micro dose of* NPK (14-23-14, 6S-1B) was carried out on the plots of manualand mechanized zaï 3weeks after the sowing. The dose applied was 2 g/seed hole at 2-3 cm upslope from the seed hole and at 2 or 3 cm deep. This corresponds to 62.3 $kgha^{-1}$of NPKfertilizer.
- *The treatmentwithout applying fertilizerby micro dose:*there was not application of micro dose of NPK fertilizeron the plots ofmanualandmechanizedzaï.

2.4Data collection and analysis

- *The soil resistance topenetration*

The soil resistance to penetrationwas measured withthe percussion cone penetrometeron theplots at the beginning of therainy seasonwith 5.3% of soil moisture.

- *The soil moisture*

The soil moisture was measured on theplots 31stJuly(28 days after sowing) and 29th September(88 days after sowing) from 0to 60 cm deep at each 10 cm.

- *The rootbiomass*

The rootbiomasswasevaluated by the method of takingmonolithswith three-side box(Chopart andSiband, 1999). The weight of the roots was measured after washing the earth of the monoliths.The measurements were carried out atsorghumflowering stage.Threerepetitionswerecarried out for eachplotin the layers 0-20 cm and 20-40 cm.The dry weight of the sorghum rootsis then measured afterstove drying.

- *The grain and strawyields*

The grain and strawyields were calculated from the grain and straw dry weight. The data were analyzed withXLSTAT software version 2016.02.27444 by the ANOVAmodule.The means comparison was carried out by Newman and Keuls test at $\alpha = 0.05$.

III. RESULTS

3.1 Chemical and physical characteristics of plot soil

The chemical characteristics of experimental soil showed a low content of nitrogen (N) and organic matter (table 1). Granulometric analysis showed in the first 20 centimeters that the soil is a sandy-loam (US textural classification), (table 2). This soil is rather sensitive to erosion due to its high level of silt and fine sand content. With such a texture made up of 74.5 % of sand and fine sand, the soil resistance to penetration cannot be high.

Table1:Some chemicalcharacteristicsofthe experimentation soil

pH_{H2O}	pH_{kcl}	Carbon (%)	OM (%)	N (%)	C/N	P_total (mg/kg)	P_available(mg/kg)	K (mg/kg)
5.7	4.7	0.43	0.74	0.05	9	70.00	0.43	0.30

Legend: OM: Organic matter, N: nitrogen; P phosphorus; K: potassium

Table.2: Experimentation soiltexture (0-20 cm)

Fractions	Loam	Fine silts	Coarse Silts	Fine Sand	Coarse Sand
Rate (%)	10.8	6.3	14.9	46.8	21.4

3.2 The soil resistance to penetration

Fig.2 shows the variation insoil resistance to penetration depending onthe depth. The profilesof the soil resistance to penetration (1, 2and 3) show a weak valueon the layer0-15 cm. This resistance increases quickly to reach 800 to 1000 (Pa)around 25 cm deep. Only the profile 4shows that resistances of 500 Pa can be reached in the first 5 cm layer.

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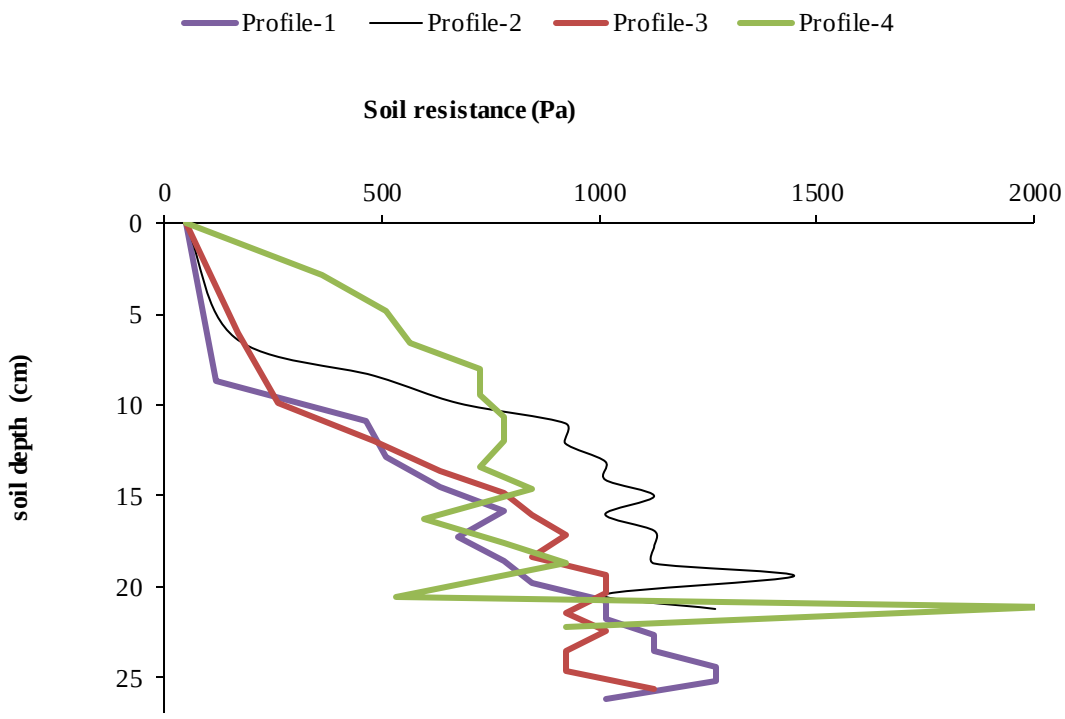

Fig.2:Variation of the soil resistance to penetration atthe beginning of the cropping season

3.3 Zaï holes sizes

The ANOVA on the sizes of the holesof manual and mechanized zaï in the plots showed that there is no statistical difference between them(table 3).

Table.3:Sizes of zaï holes

Soil tillage	Depth (cm)	Wide (cm)
Mechanized zaï	10.8	35.2
Manual zaï	10.1	36.0
Probability	0.23	0.52
Signification	NS	NS

Legend: NS:Not-significant

3.4 Soil moisture

The ANOVA on the soil moisture at the beginning and at the end of plants cycle showed a highly significant difference between the two dates of the measurement (31st July and 29th September). The interaction between dates and soil depths is also significant (table 4). On 31stJuly (28 days after seedlings),the soil moisture is high on all plots. It varies from 14.5 to 17.3% in the first 20 cm layer.On29thSeptemberat the end of the plants cycle, the soil moisture is low in the first 20 cm layer (10.1 to 10.5%);but between 20 and 60 cm depth, it remains as high as the moisture levels of 31stJuly.The Fig.3 shows the soil moisture variation between the two dates.

Table.4: Probabilities from ANOVA on the soil moisture

Source	DDL	Probability (soil moisture)
Date	1	0.009
Soil tillage	1	0.677
depth	5	0.018
Date* soil tillage	1	0.986

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Date * depth	5	0.032
Soil tillage * depth	5	0.364
CV (%)		17.3

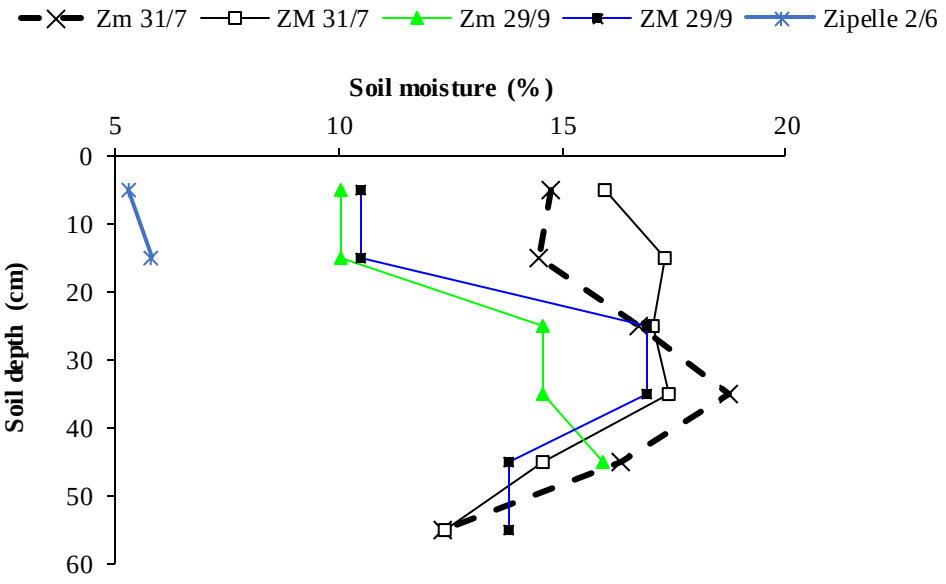

Fig. 3: Soil moisture variation on the zaïplots

Legend:Zm:manual zaï; ZM:mechanized zaï; zipellé:control

3.5 Sorghum root biomass

The ANOVA showed a highly significant difference for the root biomass between the soil tillage practices. The differences were also highly significant for mineral fertilizer as well as its interaction with soil tillage (table 5).Regarding the dry weight of sorghum roots per hectare in the soil layer 0-20 cm and 20-40 cm, there was a significant difference between treatments. The highest root biomass was provided by the mechanized zaï treatments with micro dose or not, but, for manual zaï the micro dose led to increase root biomass (Fig.4).

3.6 Sorghum grain and straw yields

The results of ANOVA on sorghum grain yield were highly significant for soil tillage as formineral fertilizer (table 5).The difference between the treatments of mechanized zaï and manual zaï was very highly significant with a probability of 0.001;the same trend was observed between the treatments with application of micro dose and without micro dose with a probability of 0.000. The mechanized zaï with micro dose leads to an increase of sorghum grain yield of + 89.8% compared to manual zaï with the micro dose. Regarding the mechanized zaï without micro dose, it leads to sorghum grain yield gain of +79.6% compared to manual zaï without the micro dose .For the mechanized zaï the application of fertilizer by micro dose gave an average grain yield of 2910 $kgha^{-1}$(+ 114.7% compared to mechanized zaï without micro dose). For the manual zaï the application of fertilizer by micro dose gave an average grain yield of 1620$kg.ha^{-1}$ (+ 126.9% compared to manual zaï without micro dose), (Fig.5). Regarding the straw yield, there was no statistical difference between the treatments. The yields have varied from 3413 to 6225 $kg.ha^{-1}$(Fig 6).

Table.5:Probabilities from the varianceanalysison the rootbiomass,grain and strawyields

Source	DDL	Roots biomass	Grains yield	Straw yield
Soil tillage	1	< 0.0001	0.001	0.704
Fertilizer	1	0.004	0.000	0.087
Soil tillage * Fertilizer	1	0.003	0.078	0.658
CV (%)		21.6	50.6	39.0

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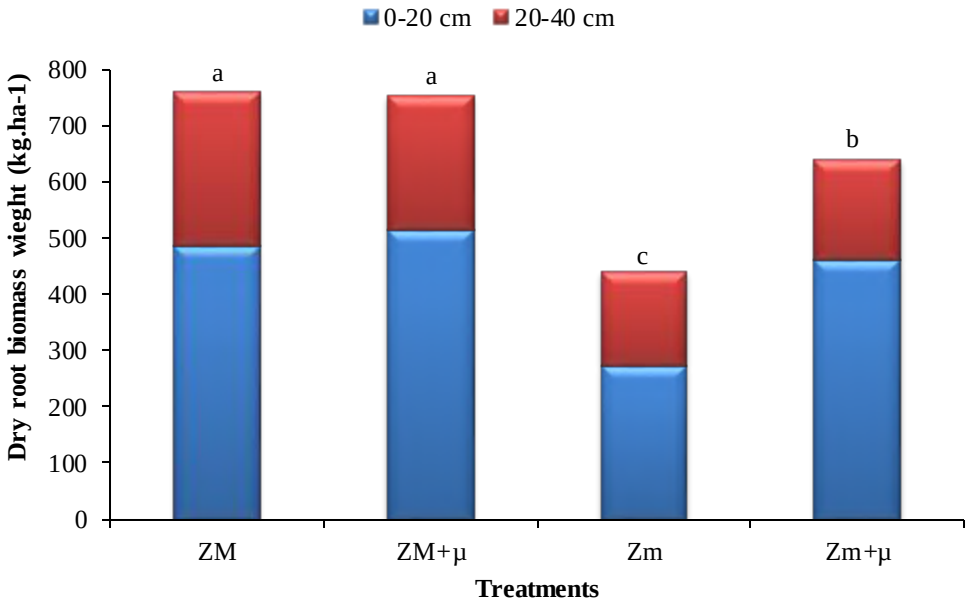

Fig. 4: Sorghum roots biomass in two layers of soil

Legend: ZM: mechanized zaï; Mm: manual zaï; µ: micro dose of NPK

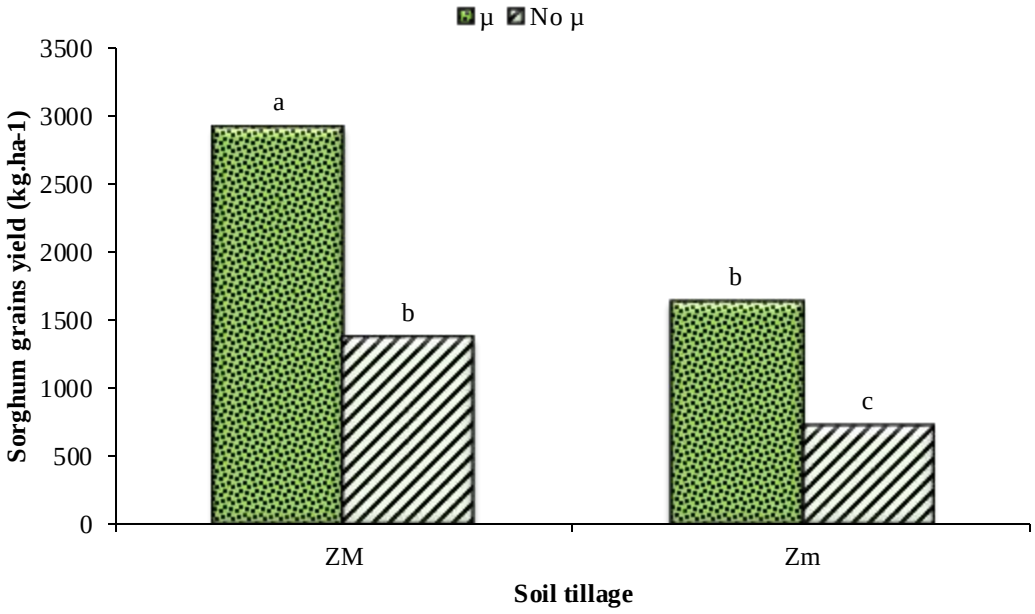

Fig. 5: Sorghum grains yields comparison

Legend: ZM:mechanized zaï; Zm: manual zaï; µ: micro dose of NPK

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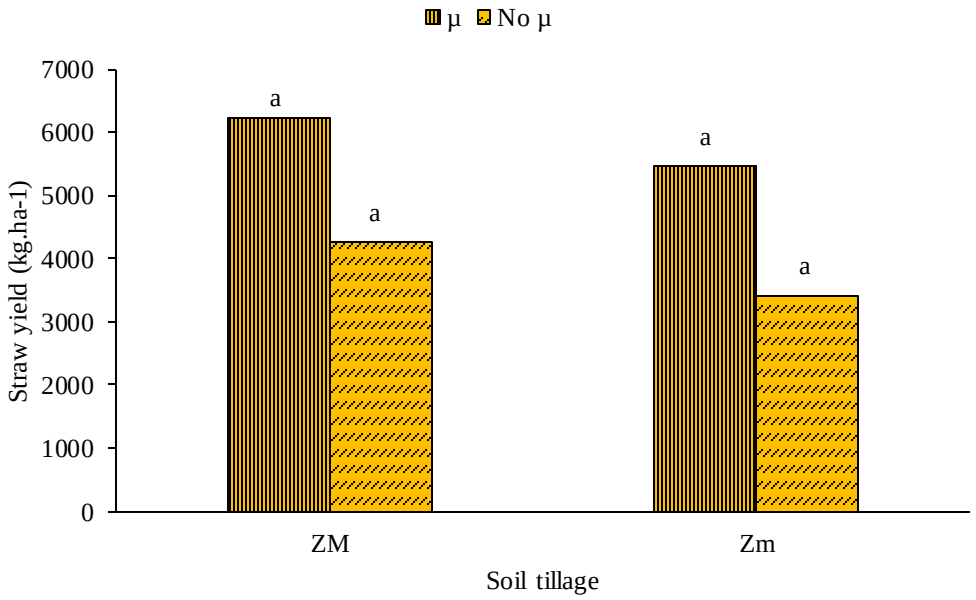

Fig. 6: Sorghum straw yield

Legend: M: mechanized zaï; Mm: manual zaï; µ: micro dose of NPK

IV. DISCUSSION

The depths and widths of the mechanized zaï holes and those of the manual zaï in this study were the same. Indeed, this soil was of low resistance to penetration and the operators of the realization of the manual zaï did not have trouble during holes digging. In the first 6 cm on this site, the soil resistance to penetration was on average 202 Pa. At 10 cm depth the soil resistance to penetration was 524 Pa. The holes were 10.8 cm for the mechanized zaï and 10.1 cm for the manual zaï. Hole digging was done in the area of low soil resistance to penetration. The low soil resistance to penetration can be explained in part by the high soil sand content of the site (68%) (Table 2). This "zipellé" type of soil is less resistant to penetration compared to that found by Barro *et al*. (2005) atPougyango (in the Passoré province) where the penetration resistance was 500 Pa in the first 5 cm, showing the differences between "zipellé"typeof soil.

The higher soil moisture on the plots on July 31 is explained by the fact that it was the beginning of the rainy season,r ainfall was important while plant water requirements were not yet raised. The cumulative rainfall at that time was 257 mm against 172 mm in normal years of rainfall. The water demand of the plants increased gradually until the end of their cycle at the end of September corresponding to the period of the end of the rainy season in the study site. Six (6) mm of rain was received seven days before soil moisture measurement (September 29), explaining the reduction of soil moisture in the 0-20 cm layer (10.1 to 10.5%), while the moisture in the 20-40 cm layer remained high (15.0 to 17.0%). The fact that the soil moisture in the 20-40 cm layer for the mechanized zaï was greater than that of the manual zaï could be explained by the reaction of the dry soil during the action of the tool that breaks while creating cracks that allow the infiltration of water and also the effectiveness of the dry soil preparation tool in improving soil moisture, such as the ripper used by Ayad *et al*. (2018).

The manual zaï plot with the micro dose produces more root biomass than the one that did not receive the micro dose. This is linked to the supply of NPK mineral fertilizers which contains 23 units of phosphorus (14.26 kg ha^{-1}). Mollier *et al*. (1999) showed that phosphorus is a favorable nutrient for the development of the maize root system. Mohammed *et al*. (2018) found that 5.72 g kg^{-1} of P in soil induced high yield with wheat. The low level of phosphorus in soils is one of the major constraints of production in Burkina Faso (Compaoré *et al*., 2003; Bonzi*et al*., 2011). Hien (2004) showed that compost had a low level of phosphorus. For manual zaï plots, the applied of the micro dose of NPK fertilizer increases roots biomass by 68%. This increase the ability of plants in these plots to get nutrients. For mechanized zaï plots in the 0-20 cm layer, the difference between the root biomass of the two plots is smaller. The intake of micro dose of fertilizer only leads to an increase of 5%. This can be explained in part by the action of the tool during dry tillage which by creating cracks in the soil and reduces its resistance to penetration consequently promoting the development of the root system. In the 0-40 cm layer, the highest root biomass was ranged from 620 to 760 kg ha^{-1}. This is a parameter of soil fertility sustainability for the

following years because the roots contain NPK mineral nutrients because Myers (1980).

For grain yield, the plot of mechanized zaï with micro dose supply had almost double the yield of the same plot without micro dose (Fig. 5). This was due to nutrients brought by fertilization by micro dose including phosphorus which is also important in fruiting. This is in line with IDRC's 2014 results in Burkina Faso, where micro-dose intake has led to increased grain yield of sorghum. The decrease in soil resistance to penetration has led to an increase in the amount of root in the soil. With a good moistening of the soil the contribution of the micro dose lead to a high grain yield on the plots. Moreover, the production of manual zaï with micro dose is in the same order as that of the mechanized zaï plot without micro dose. The mechanical action of the tool used for mechanized zaï on the ground, generates a yield equivalent to the same yield as that obtained with a micro dose. The straw yields, although having the same tendencies, are not statistically different (Fig. 6).

V CONCLUSION

Mechanized zaï and micro dose of NKP fertilizer gave high level of root biomass on sorghum (760 kg ha^{-1} in the layer 0-40 cm). This roots system is favorable to sorghum nutrient and water uptake.The root system development on thesesplots providesa potential for sustainable production, because the organic matter of the roots will evolve in the soil to improve its structure and enrich its nutrients content. The practice of sorghum cropping by themechanizedzaï combined with the application of mineral NPK fertilizerby micro doseshowed a great potential of sorghum yield increase (+238% compared with mechanized zaï without mineral fertilizer).

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Factors Affecting Youth Generation Interest on Agricultural Fields (Case Study in Deli Serdang District)

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Abstract*—Indonesian agriculture has a serious problem with the decline in interest of the younger generation towards businesses in agriculture, especially food crops. This is shown in a period of 10 years there has been a decline of nearly 15% of farmer households engaged in agriculture (BPS Data 2013), but on the other hand the need for food continues to increase along with the increase in the population of Indonesia. Indonesian agriculture also faces the problem of decreasing the quality of agroecosystems, foreign product competition, productivity and land conversion. Paddy rice cultivation is becoming increasingly less attractive to the younger generation, especially for several years, due to a decline in income levels. The purpose of this study was to understand the factors that influence the interest of young people in rice farming. This research was conducted in Deli Serdang District. Determination of the research location is based on the potential area of rice cultivation. Research methods using linear regression survey and analysis methods. The results of this study indicate that internal and external factors (age, gender, education, marital status, expectations, wide land ownership, socialization and technology) have a significant influence on the interest of the younger generation.*

***Keywords*—*Youth generation, interest, rice farming, Deli Sedang, Sumatera*.**

I. INTRODUCTION

Until now the agricultural sector still has a strategic role in national development with its role as a food provider for the population of Indonesia, which amounts to nearly 260 million people (BPS 2016). But on the other hand Indonesian Agriculture is experiencing serious challenges. Not only from the decreasing quality of agro-ecosystems, the destruction of imported products, the stagnation of production, but also the decline in the number of farmers. These conditions indicate that the agricultural sector is currently less attractive to the younger generation. Similar conditions also occur in developing economies, where the number of farmers will continue to decrease. "There is no reintegration at the age of farmers because the percentage of young farmers under 35 years of age continues to shrink. It was seen from 2003 to 2013, the number of farming families was reduced. BPS data records that within 10 years, 2003-2013, the number of farmer households decreased by 5 million. This figure is quite large and has implications for the sustainability of the agricultural sector. Because our agricultural model is a family farming model that has been proven to be able to maintain the production and sustainability of the life of farmers. In addition to the reduced number of farmers, other problems are related to the age and productivity of the farmers themselves. The age structure of farmers is old, ie 60.8% above 45 years with 73.97% to only the elementary level, and the capacity to apply new technology is low.

Agricultural problems are not only old-age farmers, but also problems related to Hard and Resources of human resources in agriculture, namely PPL (Field Agriculture Extension) and POPT (Observers of Plant Disturbing Organisms) most of which have entered old age, namely 70% over 50 years and approaching retire. This certainly affects the performance, and even the sustainability of the national agricultural system. The low level of young age groups in the agricultural sector is not a new phenomenon. We have been faced with this situation for a long time and continue to increase in degrees. There are many reasons that young people can be reluctant to return to agriculture. The main reason is of course related to the economy. Farmers are still seen as a profession that is not promising, gives no hope, does not provide big profits, businesses are at high risk due to crop failure caused by pests and diseases, natural disasters and unclear price fluctuations so farmers often experience losses, and wrestle with poverty. With this stigma, the agricultural sector is not a sector that can attract the attention of

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young people. They would prefer to work as factory workers or work in the city.

Interst and participation of young people in agriculture continue to decline. There are a number of causes, such as agriculture being considered unable to sustain the future, limited access to land and capital, and a lack of other support for the younger generation. Based on data from the Agriculture Service Office of Deli Serdang District, the average worker working in the agricultural sector has been more than 45 years of age. The low level of young people in the agricultural sector causes no regeneration in agriculture. Agriculture as a supplier of food for humans is possible not to experience development because the younger generation as a generation that is rich in little ideas plunged into the world of agriculture. The imbalance in the agricultural sector will affect the decline in the amount of food produced. The interest of the young generation in the Coal Regency to work in the agricultural sector in general is still low at present, this is supported by the opinion of Herlina in Herawati (2017), which states that currently many young people have advanced cultural value orientations and choose jobs outside the agricultural sector in urban areas, to gain wealth and glory.

Some facts in Deli Serdang District show that the younger generation is starting to be reluctant to try in the field of agricultural business. This has proven that the younger generation prefers to work in the industrial sector, preferring to work in non-agricultural fields such as construction workers, porters, online motorbike taxi drivers, hair barber and so on. As an excuse for not choosing to work in the agricultural business because the selling price of agriculture is not fixed, uncertain price fluctuations that often cause losses and the assessment of the younger generation that to be able to make money from the sale of agricultural products requires a long time, lack of encouragement or support from the government for socialization the importance of young people to the world of agriculture.

Based on the description above, it is very necessary to do research with the title "Factors Affecting Interest in Young Generation Against Enterprises in the Field of Agriculture in Deli Serdang District".

The Aim of Research

From the identification of the problems that have been raised above, it can be explained that the purpose of this study is:

1. To examine the interest of the younger generation in the business in agriculture in Deli Serdang Regency.
2. To assess what factors influence the interest of the younger generation in business in agriculture in Deli Serdang Regency.

II. IMPLEMENTATION METHOD

This research was conducted for 4 months (August-December 2018) in Batang Kuis Subdistrict and Hamparan Perak District, Deli Serdang District, North Sumatra Province. This study used descriptive quantitative methods. Research conducted to collect information by compiling a list of questions submitted to respondents. In this study, surveys were used to examine the symptoms of a group or individual behavior (Sujawerni, 2014 in Herawati, 2017).

Each variable tested is independent (X) and dependent (Y) using ordinal data types and using a Likert scale. The quizaries tested are developed based on predetermined indicators. Internal Factors (X) consisting of Variables X1.1 (Education), X1.2 (Gender), X1.3 (Marital Status), X1.4 (Age), X1.5 (Desire and Hope) and X1. 6 (Needs) and External Factors (X2) consist of X2.1 (Socialization), X2.2 (Land), X2.3 (Technology) and X2.4 (Attractiveness of Other Jobs). All variables tested are cured by using a Likert Scale with 4 levels of scale and the type of data used is ordinal data. Variable Y (Interest in Young Generation) is measured based on the indicators specified. Variable measurement in this study uses a Likert scale. What will be measured is translated into a variable indicator and the indicator is used as a starting point to compile instrument items that can be statements or questions. The measurement of the variables causing the effectiveness of farmer group management can be seen in table 1 below.

Sampling was conducted on 73 randomly selected farmers from the young generation. To find out the factors that influence the interest of young people towards businesses in agriculture, multiple linear regression analysis is performed with the following mathematical formula.

$$Y = a + b1X1 + b2X2 + b3X3 + b4X4 + b5X5 + b6X6 + b7X7 + b8X8 + b9X9 + b10X10 + \mu$$

Information :

Y: Interest in the Young Generation
X1: External factors
X1.1: Education
X1.2: Gender
X1.3: Marital Status
X1.4: Age
X1.5: Desire and Hope
X.16: Needs
X2: Internal factor (X2)
X2.1: Socialization
X2.2: Land
X2.4: Technology
X2.5: The attractiveness of another job

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To determine the suitability of the analysis models of these factors used coefficient of determination (R2) and F test (overall test). The value of determination (R2) is to determine the accuracy of the model used showing the ability of the independent variable to explain its effect on the dependent variable, which is expressed by what percentage of the dependent variable is explained by the independent variables included in the regression model. R2 values range from 0-1 and if the results obtained are close to 1, the model is said to be good.

Koefisien determinasi diformulasikan sebagai berikut:

$$R^2 = \frac{SS_{REg}}{SS_{Tot}} \quad \text{or} \quad R^2 = \frac{\sum(\hat{Y}-\bar{Y})^2}{\sum(Yi-\bar{Y})^2}$$

Noted:

Y '= The results of estimating the value of the dependent variable

Y = Average value of the dependent variable

Yi = value of observation

R2 = Coefficient of Determination

The F test is used to determine the level of influence of all independent variables (X) together on the dependent variable (Y) or to find out whether the independent variable (X) together affects the dependent variable (Y).

Ftable = (k-1), (n-k): α

Information

R2 = coefficient of determination

k = Number of regression coefficients

n = Number of samples

α = Critical value

III. RESULTS AND DISCUSSIONS

Table.1: Analysis of Factors Affecting Interest in Young People Against Enterprises in Agriculture in Deli Serdang Regency.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,769[a]	,606	,416	3,46548

Predictors: (Constant), education, gender, marital status, age, desires and hopes, needs, socialization, land, technology, attractiveness of other occupations.

Source: Primary Data Analysis (2016)

Regression models can be explained using coefficient of determination (KD = R Square x 100). The greater the value, the better. Based on table 1, the value of R Square is 0.606. So in this case the determination coefficient value obtained is 60.6%. This means that the variable X has a contribution effect of 60.6% on the Y variable and another 39.4% is influenced by other factors outside the variable X (predictors). In addition, the R value which is a symbol of the correlation coefficient is obtained at 0.769. This value is interpreted that the relationship between variables X and Y in this study is categorized as strong.

a. Simultaneous Effect Test (Test F)

F test is used to determine whether the independent variable (X) simultaneously affects the dependent variable (Y). The results of the F test are presented in the following table.

Table.2: Simultaneous Effect Test (Test F)

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	83,501	10	10,538	9,435	,000[a]
	Residual	95,423	62	11,326		
	Total	178,924	72			

a. Predictors: (Constant), education, gender, marital status, age, desires and hopes, needs, socialization, land, technology, attractiveness of other occupations.

b. Dependent Variable: interest of young generation

Source: Primary Data Analysis (2016)

Based on table 2 it can be seen that the value of Fcount (9.435)> Ftable (2.62) and the significance value of 0.000 <0.05 then H0 is rejected and H1 is accepted. This means that variable X simultaneously has a significant effect on variable Y. The second hypothesis states the factors of education, gender, marital status, age, desires and expectations, needs, socialization, land, technology, attractiveness of other occupations have a significant effect on interest the younger generation of businesses in agriculture in the Deli Serdang Regency is accepted.

b. Partial effect of variable X on Y (t-test)

To test variable X partially (individually) t test is used. T test results obtained inform the regression equation model with constant coefficients and variable coefficients in the Unstandardized Coefficients B column. The regression equations obtained will be presented in the following table 3 below.

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Table 3. Results of Multiple Linear Analysis

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	10,308	3,662		2,814	,006
- Formal education - ($X_{1.1}$)	,322	,227	,206	2,412	**,003**
- Gender ($X_{1.2}$)	**-,506**	**,198**	-,101	-1,856	,102
- Marital status ($X_{1.3}$)	,053	,205	,028	1,304	,762
- Age ($X_{1.4}$)	,355	,152	,042	1,330	,202
- And Hope ($X_{1.5}$)	,218	,183	,140	2,528	**,004**
- Need ($X_{1.6}$)	,201	,181	,136	1,114	,268
- Socialization ($X_{2.1}$)	,179	,169	**,283**	3,063	**,001**
- Land ($X_{2.2}$)			,167		
- Technology ($X_{2.3}$)	,175	,177	**,232**	2,198	**,005**
- Other job appeal ($X_{2.4}$)	.217	,235	,102	2,721	**,002**
	,347	,176		1,613	,106

a. Dependent Variable: minat generasi muda

$Y=\alpha+b_1x_1+b_2x_2+b_3x_3+b_4x_4+b_5x_5+b_6x_6+b_7x_7b_8x_8 b_9x_9+b_{10}x_{10}$

Y = 10,308 + 0,322 X1 + 0,506X2 + 0,053X3 + 0,355X4 + 0,218X5 + 0,201X6 + 0,179X7 + 0,175X8 + 0,217 X9 + 0,347 X10

Based on Table 3 it can be explained that of all the variables tested (X1) with 6 sub-variables and (X2) there are 4 sub-variables, the results show that 5 sub-variables have a significant effect on Y. This is evidenced by the sign value <0.005. namely the Formal Education variable (X1.1) sign value (0.003), Desire and hope (X1.5) sig value (0.004), Socialization (X2.1) sign value (0.002), Socialization (X2.1) sign value 0.005 , Land (X2.2) sign value (0.005), Technology (X2.3) sig value (0.002), while independent variables that do not have a significant effect on Y (young generation's interest) also have 5 variables namely Gender (X1. 2) Marital status (x1.3), Age (X1.4), Needs (X1.6) and other job appeal (X2.4). this can be seen in the sign value> 0.005.

1. Effect of Formal Education (X1.1) variables on Y

Based on the results of the t-test obtained the value of tcount is (2.412) <ttable (1.999), which means that the formal education variable (X1.1) has a significant influence on the interest of the younger generation towards business in agriculture. This is strengthened by the significance value (0, 003) <(0.05). To see the magnitude of the contribution of the value of formal education variables (X1.1) to the interest of the younger generation (Y) is 20.6%. This is evidenced from the results of multiple linear regression analysis with the Standardized Coefficients Beta value of 0.206. This is because the agricultural world is not only a young man or someone who graduated from elementary school, but even those who graduated from high school and even graduate graduates also liked working in the agricultural sector. Even undergraduate graduates who are majors outside of agriculture just switch to the agricultural sector. In addition, recently the faculty or university of agriculture has been flooded with students who want to study agriculture, more and more students who want to enter the faculty of agriculture show one of their emerging interests in the agricultural sector. This is also reinforced by the opinion of Eryanto (2013), formal education is an effort to lead to the achievement of developments that can stimulate a rational, creative and systematic way of thinking.

2. Effect of gender variables (X1.2) on Y

The t-test results obtained by the value of t count as (1.856)> t table (1.999) which means that the formal education variable (X1.2) has no significant insignificant effect on the interest of the younger generation towards businesses in agriculture. This is strengthened by the significance value (0.102) <(0.05). To see the magnitude of the contribution of the value of formal education variables (X1.1) to the interest of the younger generation (Y) is 10.1%. This is evidenced by the results of multiple linear regression analysis with the Standardized Coefficients Beta value of 0.101.

Herlina (2002) suggests that youth perception of work is also influenced by gender differences. This is indicated by the perception in the community of employment in the agricultural sector as a tiring and destructive performance, so that it is inappropriate for unmarried girls to work in the agricultural sector.

3. Effect of Marital Status (X1.3) on Y

The t-test results obtained t-count value of (1.304) <ttable (1.999) which means that the marital status variable (X1.3) has no significant insignificant effect on the interest of the younger generation towards business in agriculture. This is reinforced by the significance value (0.762)> (0.05). To see the magnitude of the contribution of the value of formal education variables (X1.1) to the interest of the younger generation (Y) is 2.8%. This is evidenced by the results of multiple linear regression

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analysis with the Standardized Coefficients Beta value of 0.101.

Herlina (2002), married young people have a good perception of employment in the agricultural sector when compared to youth who have never married. Youth who have never been married have the notion of working in the agricultural sector as heavy and dirty work, as well as a low social status in the eyes of society. Meanwhile, married young men are faced with demands to fulfill their family's income, so they have to work even though the work is heavy, so this is the cause of married youth who have a better perception of agricultural work than unmarried youth.

4. Effect of Age (X1.4) on Y

The t-test results obtained by the value of tcount is (1,330)> t table (1,999) which means that the age variable (X1.4) has no significant insignificant effect on the interest of the younger generation towards business in agriculture. The magnitude of the contribution of the age variable (X1.4) to the interest of the younger generation (Y) is 4.2%. This is evidenced by the results of multiple linear regression analysis with a Standardized Coefficients Beta value of 0.042. The younger generation is also active in activities outside the agricultural sector, they also wrestle outside the agricultural sector such as in the industrial sector, trade and so on. This is reinforced by the opinion of Lionberger (1960), that younger age usually has the enthusiasm to want to know what they do not know, so that they try to adopt innovations more quickly even though they have not experienced the adoption of these innovations.

Tjakrawati in Amelia (2005) factors driving the lack of involvement of young workers in the agricultural sector are caused by the assumption in the individual that states that at a young age they are looking for other jobs outside the agricultural sector which are more challenging and in accordance with their interests. They will do agricultural work later if they have collected money from working outside the agricultural sector to work in the agricultural sector in old age. In addition, they are encouraged to work outside the agricultural sector, there can be positive results they will get later.

5. Effect of Desire and Hope (X1.5) on Y

The results of statistical analysis show that the variables of desire and expectation have a significant effect on the perception of generation shown by the sign value of 0,000> 0.05. This is evidenced by the t-count (2,528)> of the t-table (1,999) at a 5% error rate. The expectation and desire variables affect the interest seen from the sign value. 0.004 <0.05 at a 5% error rate. Expectations and desires will influence the interest in farming because there is a belief that it will succeed when planting rice and hoping to get a profit that can meet the family's living needs. Another hope is that the government helps in the success of farming.

6. Effect of needs (X1.6) on Y

The results of statistical analysis show that the need variable has a significant effect on the perception of generation is indicated by the sign value of 0.268> 0.05. This is evidenced by the t-count value (1.114 <of t-table (1.999) at an error rate of 5%. The variables of desire and hope have no effect on interest seen from the sign value 0.268> 0.005 at an error rate of 5%.

7. Effect of Socialization (X2.1) on Y

The results of statistical analysis show that the socialization variable has a significant effect on the interest of the younger generation to work in agriculture. This has proven the sign value of 0.001> 0.05. This is evidenced by the t-count value (3.063> of t-table (1.999) at an error rate of 5%. The expectation and desire variables affect the interest seen from the sign value 0.001 <0.05 at an error rate of 5%.

The socialization of farming efforts is generally obtained by youth from families, newspapers, brochures, magazines, television and radio and sometimes from extension activities organized by extension agents or related institutions. The role of families in socializing farming activities greatly influences the perception of family members that will shape the attitudes and views of the younger generation towards agriculture in general and try to plant rice in particular. In certain times the young generation is involved in farming activities because in general their parents are farmers.

Sucipto in Chandra (2008) the process of socialization is cultural development and development takes place in the form of activities involving young people in a series of learning processes and appreciation of cultural values prevailing in the community with teachings, guidance, exemplary from the family.

8. Effect of Land Area (X2.2) Against Y

To see the magnitude of the contribution value of the farming area variable (X2.2) to the interest of the younger generation (Y) is 16.7%. This is evidenced by the results of multiple linear regression analysis with the Standardized Coefficients Beta value of 0.167. From the results of statistical analysis, the effect of farming land area variables on the interest of the younger generation on business in the agricultural sector has a significant effect. This is evidenced by the value of t-count (2.198> from t-table (1.999) at an error rate of 5%. The results of this study illustrate that the extent of the effect of farming has

a significant effect on the interest of young people towards businesses in agriculture. the higher the area of farming, the greater the interest of the younger generation in the business in agriculture.

According to Luntungan's opinion (2012), farming is usually interpreted as the study of how to allocate existing resources effectively and efficiently for the purpose of obtaining high profits at a certain time. It is said to be effective if farmers or producers can allocate the resources they have as well as possible and are said to be efficient if the resource utilization results in output that exceeds input.

9. Influence of Technology (X2.3) Against Y

The results of statistical analysis show that the socialization variable has a significant effect on the interest of the younger generation to work in agriculture. This proved that the sign value was 0.002 <0.05. This is evidenced by the t-count value (2.721> of t-table (1.999). The magnitude of the effect of the Technology Variable on the interest of the younger generation is 23.2% at an error rate of 5%.

The nature of the technology used in farming will affect the interest of young people towards businesses in agriculture where the easier the technology is implemented and easy to do throughout the year and does not require large costs will be more easily accepted. The use and ownership of technology affects youth perception of agriculture. Usually technology ownership is only owned by workers who have money because this technology is expensive. Youth who do not have land eventually become farm laborers. Cultivators prefer their own land to be cultivated so that farm laborers do not have income.

10. Effect of Attractiveness of Other Jobs (X2.4) Against Y

The results of statistical analysis show that the variables of other job attractiveness have no significant effect on the interest of the younger generation to work in agriculture. This proved to be a sign value of 0.106> 0.005. This is also evidenced by the t-count value (1.613 <of t-table (1.999) at an error rate of 5%. Variables Another attraction of labor gives a 10.2% effect on interest seen from the Standardized Coefficients Beta value of 0.102 at the level 5% error.

Simamora in Andriani (2017) states that prospects are individuals, groups or organizations that are considered potential marketers and want to be involved in a business exchange. In short, prospects are prospective buyers who have a desire for a particular product or service. Datad in the field shows that the interest of the younger generation in other jobs does not affect the interest of the younger generation so that the younger generation chooses jobs that are easily obtainable and have more understanding. also to get profit or profit.

IV. CONCLUSIONS AND RECOMMENDATIONS

A. Conclusion

1. All variables tested are Internal (X1) with sub-variables of education, gender, marital status, age, hope and desire, needs and External (X2) with sub-variables of socialization, land area, technology and other work attractiveness Simultaneously significant impact on young people's interest in business in agriculture (Y).
2. Partially the factors of formal education, desires and expectations, socialization, land area, and technology have a significant effect on the interest of the younger generation on businesses in agriculture and the more dominant variables that influence the interest of the younger generation are the socialization sub-variables (X2.1) namely 28.9%.

B. Suggestions

To increase the interest of the younger generation in the agricultural sector is done by:

1. Socialization of rice farming through families, communities, extension workers, media and agricultural institutions.
2. The use of technology that has characteristics that are easy to implement, requires cheap (efficient) costs and can be done throughout the year.

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The Impact of Invasive Alien species on Forage and Pasture Genetic Resource Diversity in Pastoral Area of Afar National Regional State, Northeastern of Ethiopia

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Abstract**—** *Prosopis juliflora is one of the most invasive species in arid areas of Afar region and protected area of Awash national park. The invasion of Prosopis juliflora increased at alarming rate devastating forage and pasture abundance and diversity that makes livestock rearing difficult; which ultimately affects the Afar pastoral livelihoods. The study focused on the impact assessment of impact of Prosopis juliflora on the forage and pasture diversity in the pastoral area of the region. Even though, the plant has several uses, it becomes out of control, deteriorates and reduced the diversity of forage and pasture species of the Region especially Amibara, Gewane and Buri Mudaitu Weredas by reducing their abundance, distribution and by changing grazing land ecosystem to prosopis thickets. This resulted in decrease in number of livestock per head of the pastoralists due to shortage of feed, decreased productivity of the livestock in terms of milk, meat and other products, migration of the pastoralists and their livestock to another place in search of feed source. Several control measures such as mechanical control, producing charcoal and collection of pods for feeding of the cattle has been implemented in the areas but lacks proper prevention and management methods of the plant. Thus, proper management and control of the invasive species and conservation of forage and pasture species are urgent issues for sustaining the livelihoods of the pastoral communities of the area.*

Keywords— biodiversity, conservation, Prosopis juliflora, pastoral community, native trees.

I. Introduction

The Ethiopian pastoral areas are estimated to occupy about 61-65% of the total area of the country and are home to 12-13% of the total population. In addition, out of the total estimated livestock population of the country, the pastoral areas constitute approximately 30% of the cattle, 52% of the sheep, 45% of the goats, and 100% of the camels[1].

However, the pastoral areas are affected by recurrently occurring drought accompanied by mismanagement is leading to dramatic threats of the natural vegetation, deterioration of pasture both in quality and quantity and hence unable to sustain livestock production, which is the major occupation of the inhabitants. The more recent serious phenomenon is encroachment of native ranges and ecosystems in dry lands areas by exotic invasive alien species.

In the Afar region of North-eastern Ethiopia, *Prosopis juliflora*, *Azadirachta indica*, *Melia azadirach*, *Parknsonia aculeata*, and *Delonix regia* are some of exotic species introduced to the area. *Prosopis juliflora* was intentionally introduced as an agroforestry species in Awash River Basin. Since recent years, the invasive nature of *Prosopis juliflora* has been revealed in the pastoral areas, protected area in Awash National Park and threatening the lives of the pastoral community and expanding at an alarming rate in the Region. The species spreads in a sporadic nature including in the wooden huts of the pastoral community. The major problems posed by *Prosopis juliflora* include invasion of the pasture lands which prohibited both the grazing and watering areas of the pastoral people, destroying grasses and displacing native trees and reducing the productivity of rangelands [2].This puts heavy pressure on the remaining pasture and browse able trees which leaves pastoral communities under frequent conflicts in the course of utilization [3].

Thus, there is a need to gather and compile the available information on status and impact of alien invasive species of *Prosopis juliflora* on forage and pasture diversity of the areas before it is wiped out. Therefore the objectives to assess, generate and compile information on the influence

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Prosopis juliflora on forage and pasture diversity in the pastoral areas.

II. Materials and Methods

The study was conducted in Amibara, Gewane and Buri Modeitu weredas of Afar National Regional State that were selected based on infestation of invasive alien species and treat level on the forage and pasture genetic resource with particular interest on *Prosopis juliflora*. The study was undertaken in collaboration with the Ministry of Agriculture and Rural Development offices of the areas and Awash National park.

To capture the information on the invasive alien species and the threat to the forage and pasture genetic material, a semi structures questionnaires and informal interviews with locally known elder pastoralists were conducted. Three kebeles were selected from each Weredas of Amibara, Gewane and Buri Mudaitu of the Afar Region and five locally known elder pastoralists were selected from each kebele.

III. Result and Discussion

Dispersal of *Prosopis juliflora*

The local known pastoralists told that *Prosopis juliflora* propagated through seeds and dispersed by domestic and wild animals. Different research findings confirmed their idea that *Prosopis juliflora* is propagated through seeds, root suckers and hardwood cuttings [4].The most important reasons for fast invasion of *Prosopis juliflora* in the area is due to the role of domestic animals (camel, cattle, sheep, goat and donkeys) and wild animals (monkey, warthog, rabbit, rodent and birds) in dispersal of seeds. Cattle are the major dispersers of *Prosopis juliflora* seeds followed by warthog, camels and goats. During the investigation, it was observed that areas previously used for grazing land were invaded by *Prosopis juliflora* but conserved areas were not invaded by the plant (Figure 1a and b).

Fig.1a: Elfora conserved grazing land

Fig.1b. Area invaded by Prosopis juliflora which is out of Elfora conserved grazing Camp

Uses of *Prosopis juliflora*

Fire Wood and Charcoal

The local communities and the urban dwellers are using *Prosopis juliflora* for cooking and heating. The local communities produce charcoal and sell the products to the urban dwellers even transported to major cities and used as a source of income. *Prosopis juliflora* is a good source of fuel wood with specific gravity of 0.7 or higher and the wood has been termed "wooden anthracite" because of its high heat content, burning slowly and evenly and holding heat well[5].

Fodder, Shade and Fence

The leaves and pods are used as forage for cattle, Goat, Sheep, Camel and wild animals such as Warthog, Monkey, etc.. Foliage of *Prosopis juliflora is* rich in protein and minerals and is highly digestible, but the general unpalatability of the leaves to livestock severely limits the utilization of this resource as an animal feed. This is due to condensed tannins known to be present in the leaves of *Prosopis juliflora*. These are thought to be the primary determinants of leaf palatability for browsing ruminants [6].

The value of leaves as browse depends on livestock species, which is palatable to goats, sheep and camels in decreasing order. However, during dry seasons or droughts when alternative sources of fodder are lacking, all livestock types will browse the foliage. Leaf age has a marked effect on intake, with leaf buds and young leaves being most palatable, possibly due to the low levels of tannins found in juvenile material. Palatability decreases as leaves mature and undesirable tannins, polyphenols and flavonoids are synthesised[7].

Prosopis juliflora pods are used as a feed mainly for cattle but also for sheep, goats, camels and pigs. Pods are mainly used as forage, browsed directly from the tree or the ground below, rather than as a fodder, where the pods are collected and fed to stalled stock. Especially during the dry season, the pods of *Prosopis juliflora* are the main source of forage for livestock. However, the ingestion of pods alone over long period of time will result in death to cattle. Research

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undertaken on consumption of rations containing up to 45% *Prosopis juliflora* pods was 1.5% of cattle body weight in India, with acceptable live weight gains [8]. Other studies have indicated that cattle rations containing less than 50% *Prosopis juliflora* pods lead to no adverse effects on consumption, digestibility, nutrient balance and animal health. However, there are several records of pods causing ill effects in livestock when used alone as a feed. This was assumed to be due to the regression of rumen bacterial cellulase activity due to the high sugar content of the pods. Rations containing *Prosopis juliflora* pods have been recommended for lactating animals, with milk production often said to improve following inclusion of pods in the ration. No effects on milk flavor were noted at less than 50% pods in the ration, though as a sole feed some taste change has been suggested [9].It may be seen that pods have a valuable role either as forage for grazing animals, or as part of milled rations for stalled livestock. The tree is also useful for soil protection and as wind break. The local people use it as live fences, shade (both for human and livestock) as well as for ornamental purposes.

Negative Impacts of *Prosopis juliflora*

Forage and Pasture Diversity

Natural grazing lands (grasses, bushes and herbaceous plants) are the major source of feed for livestock in Afar region. According to pastoralists interviewed in Amibara, Gewane and Buri Mudaitu, forage grasses, browse trees and legume species were found abundantly before the invasion of *prosopis juliflora*. Currently, the natural pasture is decreasing in amount and some of the species are extinct form these areas. This is due to the invasion of *Prosopis juliflora* which covers the plains of grazing lands found in the Middle Awash following the Awash River banks. According to report obtained from Pastoralist Agriculture and Rural Development office of the area, all 9 kebeles of Gewane, 15 out of 18 kebeles of Amibara and 14 out of 19 kebeles of Buri Mudaitu Weredas are invaded by *Prosopis juliflora*. Only the hilly and mountainous areas are remained uninvaded by *Prosopis juliflora* in these areas.

There are different species of grasses, browse trees and legumes grown in the area. Among grasses/herbs *Cenchrus cilliaris, Chrysopogon spp., Crymbopogon spp., Andropogon canaliculatus, Eragrostis spp*. and *Dactyloctenium spp*. were affected by *Prosopis juliflora* invasion in the areas. Grasses such as *Crymbopogon spp.* and *Andropogon canaliculatus* are also used for other purposes like roofing the traditional thatch houses. Similarly, *Acacia hecatopyla, Acacia nilotica, Acacia mellifera, Acacia oerofea, Grewia spp., Dobera glabra, Acacia tortilis, Cadaba rotundifolia* and *Salvadoria persica* are major affected browse tree species. Among browse tree species, fruits of *Grewia spp., Dobera glabra, Cordia sinensis* and *Balanites aegyptica* are eaten by human. According to information obtained from interviewed pastoralists of weredas, the invasion of *Prosopis juliflora* started 20 years ago and the invasion was increased at alarming rate and invades the natural grazing lands by destroying and displacing the above mentioned under growing grasses and browse trees and changes the area to unusable land which forms thick thickets of *Prosopis juliflora* population (Figure 2a and b). The areas of grazing lands invaded by *Prosopis juliflora* will be very difficult and costly (in terms of time, money and logistic sources) to turn back to its original state. [10]reported that *Chrysopogon plumulosus, Cymbopogon pospischilii, Andropogon canaliculatus, Eragrostis cylindriflore* and *Terapogon cenchriformis* were frequently mentioned grasses to have been affected by *prosopis juliflora* invasion in the region. Also among the indigenous trees, *Salvadora persica, Acacia tortilis, Acacia senegal, Cadaba rotundifolia* and *Acacia nilotica*, which are browseable, were perceived to be affected more than others the invasion of *Prosopis juliflora* (Table 1).

Table.1: Plant species perceived to be threatened by Prosopis juliflora[10].

Grass/Herbs		**Tree/Bush**	
Scientific name	Frequency	Scientific name	Frequency
Terapogon cenchriformis	77 (54.2)	*Acacia senegal*	81 (57.0)
Tribulus zeyher	35 (24.6)	*Salvadora persica*	93 (65.5)
Setaria acromelaena	41 (28.9)	*Cadaba rotundifolia*	79 (55.6)
Eragrostis cylindriflore	49 (54.8)	*Acacia tortilis*	86 (60.6)
Chrysopogon plumulosus	97 (68.3)	*Acacia oerfota*	67 (47.2)
Ipomoea sinensis	38 (26.8)	*Dobera glabra*	47 (33.1)
Cyndon dactylon	69 (48.6)	*Grewia tenax*	44 (31.0)
Cymbopogon pospischilii	96 (67.6)	*Acacia nilotica*	76 (53.5)

Sedge species	35 (24.6)	*Cordia Sinensis*	25 (17.6)
Andropogon canaliculatus	78 (54.9)		
Cenchrus cilaries	39 (27.5)		
Ipomoea aquatica	6 (28.6)		
Cyprus spp.	18 (85.7)		
Vossia cuspidata	19 (90.5)		

* Note: The numbers in bracket show the percentage

Therefore, the invasion of *Prosopis juliflora* deteriorate and reduced the diversity of forage and pasture species of the Region especially Amibara, Gewane and Buri Mudaitu Weredas by reducing their abundance, distribution and by changing grazing land ecosystem to prosopis thickets. This resulted in decrease in number of livestock per head of the pastoralists due to shortage of feed, decreased productivity of the livestock in terms of milk, meat and other products, migration of the pastoralists and their livestock to another place in search of feed source. The pastoralists of Amibara, Gewane and Buri Mudaitu Weredas migrated to the border of Somali National Regional State which caused conflicts between Ethnic groups in the past and economic and social crises of the pastoral communities that resulted from forced displacement from their home and grazing lands.

Fig.2a: Area invaded by Prosopis juliflora

*Fig.2b: Previously conserved g*razing land found in Amibara wereda

Control Methods of *Prosopis juliflora*

Mechanical Control

Manual clearance of *Prosopis juliflora* either in groups or individually is practiced by majority of pastoralists and they claimed that this practice aggravate the invasion. This is due to the fast regenerating nature and ability of the plant from the remnant root and stem stock and covering a lot of area in short period of time have discouraged the local people to continue their controlling activity. [4]Found out those individuals stumped 10 cm below ground did not regenerate after a couple of months, however, those individuals cut at any height above ground had high regeneration. Hence, cutting individual plants above ground may aggravate the invasion by *Prosopis juliflora* unless proper management such as repeated clearance is employed. On the other hand, information obtained from Worer Agricultural Research Center revealed that cutting the plant 20 cm below the ground was effective in controlling the invasion of *Prosopis juliflora* population.

Producing Charcoal

The interviewed pastoralists of Amibara, Gewane and Buri Mudaitu Weredas told that *Prosopis juliflora* was introduced to Middle Awash especially around Worer areas and dispersed to Gewane and Buri Mudaitu weredas. In these areas, the *Prosopis juliflora* strongly established itself and form thick thickets and serve as seed bank for the areas. In order to control the invasion of *Prosopis juliflora*, the Regional Government gave permission of producing charcoal for individual and group of people in these weredas (Figure 3). Since it was started in limited areas, no much effect was seen on the control of the invasion. On the other hand, there was deforestation leftover of *acacia spp*. from the area and are used for charcoal producing.

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Fig.3: Charcoal produced from Prosopis juliflora in Gewane wereda

***Porosopis juliflora* Pod Collection**

Seeds retained in intact pods are the main source of dispersal and propagation of *Prosopis juliflora*. In order to prevent and decrease the dispersal of seeds in the areas, Sideha Fage Cooperative association was established before four years in Amibara wereda of Melka Sedi town and the association comprises of 90 members. According to information obtained from the members, the association focuses on the activities that control the invasion of *Prosopis juliflora* by producing Charcoal from *Prosopis juliflora* and collecting pods from their surroundings. The association has two pods milling (one big and one small) and sells 1 kg of grinded *Prosopis juliflora* pods at 2.75 birr to the pastoral communities and urban dwellers (Figure 4 a and b). The members explained that the grinded *Prosopis juliflora* has no negative effect on the health condition of the livestock. But, these activities are limited Melka Sedi town and its surrounding and have not brought visible change on the prevention and control of *Prosopis juliflora* invasion.

Considerable research has been undertaken on the use of milled pods in livestock rations, particularly in Brazil and India. Pods would be ground or milled to secure the full nutritive value as most of the protein rich seeds, otherwise pass undigested through the digestive tract of livestock. Whole pods *Prosopis juliflora* were found to provide 7% digestible crude protein and 75% total digestible nutrients on a dry matter basis. The digestibility of crude protein from *Prosopis juliflora* pods was 50-60%, with the average digestibility of ether extract being 70%, crude fiber 80%, nitrogen free extract 79% and organic matter 74% [8].

Fig.4a: collected Prosopis juliflora pods

Fig.4b: Grinded pods of Prosopis juliflora

IV. Conclusion

The invasion of *prosopis juliflora* especially in Amibara, Gewane, and Buri Mudaitu Weredas of Afar Region continue at alarming rate due to lack of proper prevention and management methods. Despite of their use, the invasive species brought huge loses of grass and browse biodiversity in the area and displacing pastoralists from their areas. It is advisable to set priority to the areas where forage and pasture genetic resources are under extinction like the Afar Region because of invasive species which are prevailing all over the Regions. Since the advert of this exotic species, a great numbers of cattle, goats, sheep and camels as well as considerable number of wild life have been lost due to shortage of feed and the poisoning of the plant. Therefore, the possible attention must be given to this hazardous instance to alleviate and mitigate the disastrous phenomenon of invasion and conservation of threatened species of forage and pasture biodiversity.

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Evaluation of Earthworm Species and Bedding Material Collected from Tea Plantations for Vermicomposting in Sri Lanka

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Abstract*—Earthworms has the ability to convert organic waste into compost and this process is known as vermicomposting. This study was conducted to evaluate the suitability of three common earthworm species and four waste material collected from tea plantations as bedding material for earthworms in producing vermicompost. Three experiments were conducted and the experimental design was a Complete Randomized Design with three replicates.*

In the first experiment, four bedding materials that is leaves of Gliricidia (Gliricidia sepium) and Mana (Cymbopogon confertiflorus), tea prunings and refuse tea were composted using earthworm species Eudrilus euginea. Each waste material was mixed with cow dung and poultry manure, separately before using them as bedding material. The results showed that these material can be used for producing high quality vermicompost. In the second experiment three earthworm species Eisenia foetida, Periyonix excavator and Eudrilus euginea which were commonly recorded from Sri Lankan soils were evaluated for vermicomposting. Vermicomposting has increased the quality of organic material but the mean differences in nutrient levels in relation to earthworm species were non-significant (P=0.05). This indicates the suitability of all three species for vermicomposting. In the third experiment three soil amendments, vermicompost produced using Eudrilus euginea, garden compost and inorganic fertilizer were compared using tomato as an indicator crop. Vermicompost applied treatment showed significantly higher (P<0.05) fruit weight and number of branches when compared to other two treatments showing its usefulness as a soil amendment. This study shows that all factors are in place for producing vermicompost successfully in Sri Lanka.

Keywords*— *Earthworms, Eudrilus euginea, Periyonix excavator, Eisenia foetida, bedding material, vermicompost.

I. INTRODUCTION

In the recent years earthworms have been identified as a major tool for producing high quality compost due to their ability to convert organic waste into valuable plant nutrients and organic matter (Sharma, 2009). In the food web earthworms are regarded as decomposers, detritivores and diggers. They swallow mineral particles and plant debris, partially pushing through the soil and partially eating its way. They are mixed in their gut and pass out as casts on the surface of the soil (Nardi, 2007). Many authors have stated about the process of "feeding high and burrowing low," carried out by the earthworms to physically improve soil structure by enabling pathways for aeration, water infiltration and root penetration. Earthworms are known as "living plow", "best tiller", "farmer's friend", "intestine of earth", "pulse of the soil" as it digest earth and litter leaving behind a rich humus layer, most probably the nature's best fertilizer or known as "black gold" (Ansari and Ismail, 2012; Megraw, 2012; Siddaraju *et* al, 2013; Lorson, 2016). This was also highlighted by the Charles Darwin in his last manuscript written in 1881 titled "The Formation of Vegetable Mould, Through the Action of Worms*"*. Darwin observed and recorded the habits of the earthworms and its effect on soil formation. Darwin learned that worms literally move the earth in the process of their meanderings. Their passage through the earth aerates the soil and the natural chemistry of their guts renders soil and plant matter into fertile pellets. As a by-product of their movements, worms deposit new soil on the surface, causing whatever was on top to slowly submerge" (Megraw, 2012).

About 3,627 species of terrestrial earthworms have been reported from different parts of the world and 63 species of earthworms from Sri Lanka (Reynolds, 1994). A recent study has revealed 22 species of earthworms belonging to 16 genera and 9 families in 17 natural and agricultural sites in the wet and intermediate zones of Sri Lanka

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(Samaranayake, 2008). Earthworms categorized into three ecological types, namely epigeic, endogeic and anecic based on morphological features, habits and location in soil (Bouche, 1977). Epigeic earthworms are adapted to living close to the soil surface and are capable of eating and decaying organic material such as garbage and litter. They have a short life cycle and small segmented body. *Eudrilus euginea*, *Periyonix excavator* and *Eisenia foetida* are some common epigeic earthworm species found in Sri Lankan soils (Samaranayake and Wijekoon, 2010).

This burrowing and feeding activity of earthworms have numerous beneficial effects on overall soil quality for crop production. Typical earthworm populations can easily consume 5 tons of dry matter per ha per year, partly digesting and mixing it with soil. It is also estimated that for a single acre of cultivated land, earthworms move 8 tons of earth in a year, enough to form a new layer of earth 2 inches thick, rich in nitrogen, phosphorus and calcium (Megrow, 2012). Earthworm casts have higher available N, P, K and Ca contents than surrounding soil, as well as a higher cation-exchange capacity (Jones, 2013; Aladesida *et al*, 2014; Sherman, 2017). The excrement of earthworms are rich in micronutrients, such as Zn and B through chelation. Earthworms also excrete material that has high concentrations of beneficial microbes that help decompose crop residue. The increase in porosity created by earthworms facilitating quick water infiltration into the soil and reduce the effects of compaction is highly advantageous in the no-till systems of farming. pH buffering action of organic molecules produced in the gut of worms is another advantage of earthworms (Jones, 2013; Aladesida *et al*, 2014; Sherman, 2017).

Decomposition of waste materials using earthworms is known as vermicomposting (Sharma, 2009; Ansari and Ismail, 2012). Vermicmpost is accepted as humus, bio fertilizer, soil fertility booster, soil activator and soil conditioner with required plant nutrients, vitamins, enzymes, growth hormones and beneficial microbes like nitrogen fixing, phosphate solubilizing, denitrifying and decomposing bacteria.

Tea industry provides several biodegradable waste or by-products during field operations as well as during tea manufacturing. They include refuse tea, instant tea wastes, shade tree loppings, tea prunings and weeds. In Sri Lanka about 4-6 percent of the total product of made tea goes as refuse tea (Rupasinghe, 2006).

The past research has reported that quality of vermicompost is influenced by the earthworm species and the bedding material (Manaf *et al*, 2009; Bisen *et al*, 2011; Yadav *et al*, 2013). *Eudrilus euginea*, *Periyonix excavator* and *Eisenia foetida* are three common earthworm species recorded in Sri Lanka and are being been used for composting worldwide especially in India (Suthar and Singh, 2008; Manaf *et al*, 2009; Bisen *et al*, 2011; Anandharaj *et al*, 2013; Siddaraju *et* al, 2013; Perera and Nanthakumaran, 2015). The objective of this study was to examine the suitability of three common earthworm species and some waste materials generated in tea estates as bedding material for earthworms for producing vermicompost.

II. MATERIALS AND METHODS

Experimental site

Three experiments were conducted during this study to achieve objectives. First and second experiments were conducted at the Erin Tea Estate, Galaha during August to September, 2016. The estate is located in the mid country wet zone (agro-ecological region WM_2). The Mean minimum and maximum temperature during the research time period was 17^0C and 27^0C, respectively. The third experiment was conducted at the University Experimental Station, Dodangolla, Kundasale during September to November, 2016. It is located in the mid country intermediate zone (agro-ecological region IM3a). The soil group of this area is Immature Brown Loam and Reddish Brown Latasolic. The Mean minimum and maximum temperature during the research period was 20.6^0C and 29^0C, respectively.

Multiplication of Earthworms

Earthworm species *Eudrilus euginea* and *Periyonix excavator* were collected from cow dung heaps in Erin Tea Estate, Galaha. *Eisenia foetida* were collected from partly decomposed vegetable refuse from the Nuwara Eliya area. Multiplication of earthworms were done using a mother compost media stored in a 30cm x 25cm x 45cm plastic pots. Plastic pots were filled with 3cm layer of stones, 3cm layer of brick pieces, 5cm layer of soil and 20 cm layer of bedding material kept from bottom to top and placed under closed room conditions. Mixture of vegetable refuse, gliricidia leaf, wild sunflower leaf, banana stem and cow dung were used as the bedding material. After filling the pots with bedding material they were kept for two weeks to allow partial decomposition before introducing the earthworms. Top of the Plastic pots were covered with one layer of newspaper. Watering was done on a need basis and kept for one month for multiplication.

Experiment 1: Evaluation of bedding material for Vermicompost production

In the first experiment four waste materials commonly occur in tea plantations, the leaves of *Gliricidia sepium* and Mana (*Cymbopogon confertiflorus*), tea prunings and one month old refuse tea was composted using earthworm species *Eudrilus euginea*. Each bedding material were mixed either with one week old cow dung or one year old

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poultry manure at 1: 2 ratio to have eight treatment combinations. The experimental design was a Complete Randomized Design with eight treatment combinations and three replicates. Watering was done on a need basis and kept for two months for vermicompost production.

Experiment 2: Evaluation of Earthworm Species for Vermicompost production

In the second experiment *Eudrilus euginea*, *Periyonix excavator* and *Eisenia foetida* were evaluated for vermicomposting with respect to N, P and K level in the produced compost. The waste material mixture consisting Gliricidia leaf, tea prunings, mana leaf and refuse tea was blended with either cow dung or poultry manure before using as a bedding material. Hence there were six treatment combinations. The experimental design was a Complete Randomized Design with three replicates. Watering was done on a need basis and kept for two months for vermicompost production.

Measurements - Experiments 1 and 2

Total Nitrogen, Phosphorus and Potassium content, Electrical Conductivity and pH values of bedding material were measured before adding earthworms and also after two months of vermicomposting. Total Nitrogen, phosphorus and potassium contents were measured using micro kjeldhal method, spectrophotometer and flame-photometer, respectively. Conductivity meter and pH meter were used to measure EC and pH, respectively. Earthworms weighing fifty grams were added to 100g of mother compost at each of the experimental units during experiment 1 and 2.

Experiment 3: Evaluation of Vermicompost as a soil amendment.

Vermicompost produced from tea waste + poultry manure with *Eudrilus euginea* (T1) was compared with garden compost (T2) and inorganic fertilizer (T3). Twenty one day old same sized tomato plants from *Thilina* variety were used as the indicator crop in the experiment. Plastic pots were used for this experiment and they were filled with soil 5 days prior to planting of tomato plants. Both vermicompost and garden compost were added at the rate of 400g per pot. The fertilizer recommendation of the Department of Agriculture for tomato was applied under the inorganic fertilizer treatment. Application of water was done on a regular basis. Staking was practiced on 14th day after planting. Plant Height, Number of Leaves, Number of Leaflets, Number of Flowers, Number of branches, Number of fruits were measured at 1st, 2nd, 3rd and 4th week after planting. The experimental design was a Complete Randomized Design with three treatments and three replicates.

Data analysis

Data were analyzed using Statistical Analysis System (SAS) software package. Parametric data were analyzed using analysis of variance (ANOVA) and non-parametric data with categorical data analysis procedures. Significant means of treatments were separated using the Least Significant Difference Test (LSD).

III. RESULTS AND DISCUSSION

Experiment 1: Evaluation of bedding material for Vermicompost production

Total Nitrogen, Phosphorus, Potassium, Electrical Conductivity and pH values of bedding materials and vermicompost produced by the earthworm species *Eudrilus euginea* is given in the Table 1. The results indicates that vermicomposting has increased Nitrogen, Phosphorus, and Potassium contents and also the Electrical Conductivity. pH has decreased as a result of earthworm activity. It was also revealed that mean differences in N, P and K contents among different treatments were non-significant (P=0.05). Hence, it can be concluded from this study that the biodegradable waste and by-products generated during field operations and tea manufacturing such as refuse tea, shade tree loppings, tea prunings and weeds can be used as suitable bedding material for earthworms to produce high quality compost.

Table.1: Quality of vermicompost produced using Eudrilus euginea and different bedding materials.

Trt..	N (mg g^{-1})		P (mg g^{-1})		K (mg g^{-1})		PH		EC (mS cm^{-1})	
	BM	VC	BM	VC	BM	VC	BM	VC	BM	VC
T1	7.03	7.40	0.93	0.97	7.69	7.73	8.38	8.06	2.17	2.47
T2	8.90	8.90	0.93	0.96	8.59	8.61	8.06	7.85	3.19	3.77
T3	4.20	4.47	0.50	0.54	7.90	7.92	8.19	7.72	2.48	2.91
T4	6.97	7.30	1.70	1.73	9.10	9.13	8.55	8.23	3.38	3.93
T5	5.00	5.40	0.71	0.74	7.91	7.94	8.95	8.82	2.47	2.87
T6	7.07	7.37	1.31	1.33	7.77	7.79	8.06	7.49	3.51	3.85
T7	4.17	4.43	1.14	1.16	10.69	10.72	8.15	7.12	2.87	3.17
T8	4.47	4.67	0.62	0.65	11.63	11.66	8.22	7.16	3.91	4.18

Key: Trt-Treatments, BM-Bedding material, VC-Vermicompost

Treatments: T1 - Gliricidia + Cow dung, T2 - Tea prunings + Cow dung, T3 - Mana + Cow dung, T4 - Refuse tea + Cow dung, T5 - Gliricidia + Poultry manure, T6 - Tea prunings + Poultry manure, T7 - Mana + Poultry manure, T8 - Refuse tea + Poultry manure.

Experiment 2: Evaluation of Earthworm Species for Vermicompost production

Total Nitrogen, Phosphorus, Potassium, Electrical Conductivity and pH values of bedding materials and vermicompost produced by the earthworm species *Eudrilus euginea*, *Periyonix excavator* and *Eisenia foetida* are given in the Table 2. The results indicates that vermicomposting has increased Nitrogen, Phosphorus, and Potassium contents and also the Electrical Conductivity.

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pH has decreased as a result of earthworm activity. It was also revealed that mean differences in N, P and K contents in the vermicompost produced by applying different treatments were non-significant (P=0.05). Hence, the study confirms that all three earthworm species evaluated in this study are suitable for producing high quality vermicompost.

Table.2: Quality of vermicompost produced by the earthworm species Eudrilus euginea, Periyonix excavator and Eisenia foetida.

Trt.	N (mg g $^{-1}$)		P (mg g $^{-1}$)		K (mg g $^{-1}$)		PH		EC (mS cm $^{-1}$)	
	BM	VC	BM	VC	BM	VC	BM	VC	BM	VC
T1	10.16	10.64	0.57	0.87	8.94	9.12	8.67	7.07	0.75	1.85
T2	10.01	10.34	1.62	1.92	12.08	12.33	8.73	6.83	3.20	5.40
T3	10.20	10.52	0.69	0.96	10.08	10.42	8.50	7.00	1.21	3.58
T4	10.84	11.22	1.54	1.95	12.52	12.82	8.73	6.80	2.72	5.61
T5	10.71	11.03	0.33	0.66	8.84	9.18	8.43	7.23	1.42	2.38
T6	9.65	10.06	2.17	2.61	10.57	10.95	8.67	6.93	2.53	4.80

Key: Trt-Treatment, BM-Bedding material, VC-Vermicompost

Treatments: T1 – *E. euginea* + Cowdung, T2 – *P. excavator* + Cowdung, T3 – *E. foetida* + Cowdung, T4 – *E. euginea* + Poultry manure, T5 – *P. excavator* + Poultry manure, T6 – *E. foetida* + Poultry manure

Experiment 3: Evaluation of Vermicompost as a soil amendment.

In this experiment vermicompost produced from tea waste + poultry dung with *Eudrilus euginea* was compared with garden compost and inorganic fertilizer by taking tomato as an indicator crop. Vermicompost applied treatment showed significantly higher (P<0.05) fruit weight (g/plant) and number of branches per plant when compared to other two treatments (Table 3). This shows that vermicompost is a useful soil amendment for producing vegetables such as tomato.

Table.3: Number of branches produced at weekly intervals and mean fruit weight (g/plant) of tomato in relation to three treatments.

Treatment / WAP	Number of branches			Mean fruit weight (g/plant)
	2WAP	3WAP	4WAP	
Vermicompost	5.46^{c}±0.49	5.77^{b}±0.70	6.38^{b}±0.49	363.9^{b}±52.8
Garden compost	1.27^{a}±0.12	1.27^{a}±0.12	1.69^{a}±0.26	128.3^{a}±9.48
Inorganic fertilizers	2.68^{b} ±0.43	2.68^{a}±0.43	2.99^{a}±0.38	134.7^{a}±15.00
LSD	1.32	1.65	1.35	111.3

Key: WAP-Weeks after planting, LSD-Least Significant Difference.

Difference of means denoted by same letter are non-significant.

IV. CONCLUSIONS

The results of the experiment shows that waste material generated from field operations of tea plantations and tea manufacturing such as Gliricidia leaf, tea prunings, mana leaf and refuse tea after mixing with cow dung or poultry manure can be used successfully as bedding material for earthworms for producing vermicompost. The study also shows that *Eudrilus euginea*, *Periyonix excavator* and *Eisenia foetida* are suitable earthworm species for vermicomposting. Further it is observed that vermicompost is a useful soil amendment when compared to garden compost and inorganic fertilizer for the successful culture of tomato. Similar results have been reported by many authors including Chanda *et al*, (2011), Tringovska and Dintcheva, (2012) and Basheer and Agrawal, (2013) when vermicompost was used in the growing media for tomato.

Based on the results of this study it can be concluded that there is a high potential for developing vermitech as a technology for improving soil fertility for crop production systems in Sri Lanka.

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Market Structure of Yam in Selected Market in Ibadan, Oyo State, Nigeria

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Abstract**—** *This study carried out Market Structure of Yam in selected markets in Ibadan. Oyo State, It specifically determined profitability of Yam marketing and examined the market structure for yam in the study area. Five markets centres were covered and simple random sampling techniques wereused to select a total of 120 yam sellers in the study area. Gross margin and gini-coefficient techniques were used to analyse the data collected. The profitability analysis showed that yammarketing in Bodija is more profitable as the seller on the average realizes a net profit of N111,075. The result of the Gini coefficient for yam sellers obtained in the study area was 0.572 which implies that yam market in the study area is imperfectly competitive with the market structure inclining towards monopoly.*

Keywords— yam, market structure, profitability, gross margin, gini coefficient.

I. INTRODUCTION

Yam (Dioscorea species) is a premium crop in the Nigeria food system and Nigeria is the world's largest producer with an aggregate annual output in excess of 50% of yam produced worldwide in 2000. Nigeria alone accounted for 26million tonnes followed by Ghana and Cote'dovoire with 3million and2.9million tonnes respectively [I]. Yam tubers are the consumable product of yam crop, and the tubers are sources of carbohydrate. The tubers can be prepared for consumption by boiling and eating with stew, roasted and eating with stew, boiling and pounding, eating with stew, as porridge, yam balls, sliced and fried into yam chips [2]. [3], enumerated that some basic decision needs to be taken on the food market structure that would leads to more effective market performance. Since production is not complete until the product get to the hands of the final consumers, this study therefore carried out market structure of Yam in selected markets in Ibadan, Oyo State. Specifically, it (i) determined the profitability of yam marketing in the study area and (ii) examined the market structure for yam in the study area.

II. RESEARCH METHODOLOGY

The study was conducted in Ibadan capital of Oyo State, Nigeria. Ibadan is located in south western Nigeria, 128km in land northern of Lagos and 530km southwest of Abuja, the federal capital, and is a prominent transit point between the coast region since the day of British colonial rule, and part of the cities ancient protective walls still stand to this day. Ibadan has rainfall of about 1250mm minimum and 1800mm maximum, it lies between latitude 70N and 900N of the equator and longitude 20E and 50E of the Greenwich meridian. Ibadan has two distinct seasons; Rainy season between April and October; and the dry from November and March. The temperature ranges between 270C and 380 C with relative humidity of about 25% to 90%.

Five markets centers were covered namely Bodija market, Oje market, Dugbe market, Oja'oba market, and Oritamerin market. Each market was purposively selected in each local government where different species of plank can be found in Ibadan. Simple random sampling techniques was used to select a total of 120 yam sellers consisting of 45 yam sellers in Bodija market, 20 yam sellers in Oje market, 15 yam sellers in Dugbe market, 25 yam sellers in Oja-oba market and 15 yam sellers in Oritamerin market on the basis of the size of each market in the study area. The data for the study was collected using structured questionnaire. Personal visit were made to the market to obtain firs hand information on other relevant market issues that could not be captured by the questionnaire. Gini co-efficient as used by [4] was adopted to measure the relative degree of income inequality among yam sellers. The model specification is as follows;

Gini co-efficient= 1 – ΣXY Where X= the percentage of sellers, Y= the cumulative percentage of total sales, ΣXY= the summation of XY. The grossmargin and marketing margin analysis as adopted by [5] was used to measure the profitability of yam marketing. The market performance of any particular product is usually determined by taking method of storage, transportation, grading and standardization into consideration. Gross

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margin analysis is to estimate the profitability of yam marketing as represented below.

GM= GI - TVC

Where; GM= Gross Margin, GI = Gross Income, TVC=Total Variable Cost.

III. RESULTS AND DISCUSSION

Table.1: Income Distribution of Yam Sellers in the study Area and Computation of Gini coefficient for yam sellers in Ibadan

Sales[N]	No of sellers	% of sellers(X)	Cumm. Frequency	% cumm. Frequency sellers	Total sales/month	% total sales	% cumm. Of total sales[Y]	ΣXY
1-100,000	9	7.5	9	7.5	730,000	1.5	1.5	0.001125
100,000-200,000	21	17.5	30	25.0	3,580,000	7.4	8.9	0.015575
200,001-300,000	23	19.2	53	44.2	5,641,000	11.7	20.6	0.039552
300,001-400,000	10	8.3	63	52.5	3,430,000	7.1	27.7	0.022991
400,001-500,000	13	10.3	76	63.3	6,261,000	13.1	40.8	0.044064
400,001-500,000	6	22.5	82	68.3	3,550,000	7.4	48.2	0.0241
600001-700000	27	9.2	109	90.8	17,102,000	35.5	83.7	0.188325
700,001-800,000	11	100	120	100	7,845,000	16.3	100	0.092
	120				48,139,200			0.4277

Source:Field survey, 2015

The yam market concentration was determined by means of Gini coefficient. Table 1 shows that 7.5% (N0 - 100,000) of yam sellers accounted for 1.5% of the total monthly sales,17.5% (N 100,001-200,000) accounted for 7.4% of total monthly sales, 19.2% (N 200,001- 300,000) accounted for 11.7% of total monthly sales, 8.3% (N 300,001-400,000) accounted for 7.1% of total monthly sales, 10.8% (N 400,001- 500,000) accounted for 13.1%, 5% (N 500,001- 600,000) accounted for 7.4% of total monthly sales, 22.5% (N 600,001-700,000) accounted for 35.5% of total monthly sales, while 9.2% (N 700,001 - 800,000) accounted for 16.3% 0f the total monthly sales. The result of the Gini coefficient for yam sellers obtained in the study area was 0.572.This implies that yam market in the study area is imperfectly competitive with the market structure inclining towards being a monopoly. This is in contrast with study by [6] on the analysis of the fundamentals in palm oil marketing in Osun state, Nigeria, which shows that Ginicoefficient of 0.4277, meaning low levelincome inequality.

Gini coefficient = 1 -EXY

= 1 -0.428 = 0.572.

Table.2: Market Performance of Yam Marketing in the Study Area

Market	No of Sellers	Gross Revenue	Total Variable cost	Gross Income (₦)	Return/Naira Invested
Dugbe	15	170,100.00	119,100.00	51,200.00	1.4
Bodija	45	555,375.00	444,300.00	111,075.00	1.3
Oje	20	409,095.24	356,714.29	52,380.95	1.2
Ojaoba	25	430,273.68	371,894.74	58,378.95	1.2
Oritamerin	15	550,000.00	494,100.00	55,900.00	1.1

Source: Field survey, 2015

Table 2: Shows the market performance of yam marketing in the study area, the performance of any particular product is usually determined by taking methods of storage, transportation, loading and offloading, and standardization into consideration. Which shows that yam marketing in Bodija is more profitable as the sellers are

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on the average realizes a net profit of N 111,075 in a year. This is because the market consists of the highest number of sellers and different varieties of yam were found in the market. The marketing efficiency using (Total Revenue/ Total variable cost) for Dugbe, Bodija, Oje, Ojaoba, and Oritamerin were 1.4, 1.3, 1.2, 1.2 and 1.1 respectively. This means that yam marketing in the study area were highly efficient since the value were more than 1. This is in conformity with [7] who says that values that are less than 1 are notefficient. Analysis of Gross and Marketing Margin, One of the indicators of marketing performance is the marketing margin. Marketing margin is the cost of performing marketing function. It is the difference between what the consumer pays for the final product and the amount the producer receives. Gross margin on the other hand is the difference between the Gross Income (Sales) and Total Variables Cost.

IV. CONCLUSSION AND RECOMMENDATION

The business of yam in the study area shows that the business is profitable. Bodija market is more profitable as the seller on the average realizes a net profit of N111, 075. This is because the market consists of the highest number of sellers and different varieties of yam were found in the market. Government agencies like the ministry of agriculture should try to organize workshop and seminars on a regular basis for the wholesalers and retailers so as to impact them more knowledge on yam marketing. This is necessary for the easy flows of the product from the farm available to the consumers.

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Processing and Development of Dragon Fruit Wine

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***Abstract*—** *The research project was conducted to establish protocols in the production of dragon fruit wine from unmarketable and surplus harvest of dragon fruits. The products were characterized in terms of physico-chemical and sensory properties. Acceptable semi-sweet dragon fruit wine was produced from fermenting must with 250g sugar and 2t yeast (Saccharomyces cerevisiae) per kg of dragon fruit. Dragon fruit wine produced has moderately clear, moderately brilliant yellow color, moderately complex detectable aroma, fair texture, good balance of a number of detectable flavors and smooth and rich taste that lingers in the mouth after swallowing. The dragon fruit wine was given unanimous acceptable ratings without any negative acceptable rating based on the results of evaluation using 100 consumer-type judges.*

***Keywords*— Dragon fruit, physico-chemical, properties, sensory, wine.**

I. INTRODUCTION

Dragon fruit (*Hylocereus undatus Haw. Britton & Rose)*, also known as pitaya, has been successfully grown in the Philippines and has an average annual production of 25 tons /hectare. The seasonality of fruiting and high rate of loss due to peel damage in dragon fruit are the primary factors which may hinder its development and increased popularity in the country. To maximize the utilization of dragon fruits during peak season, July to October, and to utilize peel-damaged dragon fruits, processing technologies for dragon fruit have been generated [1],[2]. Among the products developed from the fruit are the dragon fruit jam, jelly, juice and puree, which are currently undergoing pilot testing [2].

Being a tropical country, there are wide variety of fruits that are produced in the Philippines, most of which are seasonal which results in oversupply and under utilization during peak season. During such season of abundance, prices for the particular fruit are low which suggests timely processing and preservation of the fruit. Once processed, fruits can be made available even during off-season, especially in areas where supply may be limited or where the fruits are not available at all. Processing of peel-damaged dragon fruits also convert non-marketable raw materials to high-value product like wine .

For centuries, the Philippines had its own tradition of fermenting and drinking wines which are produced in different parts of the country. As there is an abundance of a number of readily available tropical fruits all year round, a variety of fruit wines produced either for home consumption or commercial purposes may be found everywhere, suggesting that Philippine fruit wine industry is highly feasible. In addition, Filipinos have learned and develop the culture of wine drinking, specifically, for high-end members of the society.

Colored fruit wines are gaining much popularity because of their powerful antioxidant activity due to the naturally-occurring pigments. In addition, the pigments in colored fruit wines have potential health effects against cancer, aging, neurological diseases, inflammation, diabetes and bacterial infections. The exotic and well-blended sweet, sour and alcoholic tastes of fruit wine easily curb one's appetite.

In addition to the developed dragon fruit products, wine is considered to be a high-value product which may be produced from dragon fruits. Utilization of dragon fruit in fruit wines can possibly stimulate development of local wine manufacturing and may help reduce importation of alcoholic beverages.

II. MATERIALS AND METHODS

2.1 Research Design

Fully mature peel-damaged but not rotten fruits supplied by Silan Agri Farm, Philippines were used in the production of wine.

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To determine fruit to sugar to yeast ratio, the experiment was arranged in a 4 x 3 factorial experiment in Completely Randomized Design (CRD) with level of sugar as Factor A and different amounts of yeast as factor B. The experimental units were replicated three times in storage after single preparation.

Factor A, level of sugar (LS) per kilogram dragon fruit

LS1 = 0.25 kg
LS2 = 0.50 kg
LS3 = 0.75 kg
LS4 = 1.0 kg

Factor B, Amount of Yeast (AY) per kilogram dragon fruit

AY1 = 1 tsp
AY2 = 2 tsp
AY3 = 3 tsp

The following treatment combinations were used:

T		LS	Sugar, kg		AY	Yeast, tsp
T1	=	LS1	0.25	x	AY1	1
T2	=	LS1	0.25	x	AY2	1
T3	=	LS1	0.25	x	AY3	1
T4	=	LS2	0.50	x	AY1	2
T5	=	LS2	0.50	x	AY2	2
T6	=	LS2	0.50	x	AY3	2
T7	=	LS3	0.75	x	AY1	3
T8	=	LS3	0.75	x	AY2	3
T9	=	LS3	0.75	x	AY3	3
T10	=	LS4	1.00	x	AY1	4
T11	=	LS4	1.00	x	AY2	4
T12	=	LS4	1.00	x	AY3	4

2.2 Processing of Dragon Fruit Wine

Four kilos of dragon fruits per treatment were used. Fruits were washed in running water and drained in stackable plastic baskets. Each fruit was cut, peel on, into quarters lengthwise and pulp was cut into 1-inch thick slices crosswise before finally peeling off, separating the sliced cubes in a container. Prior to blending, the pulp was diluted with water at 1:2 pulp to water ratio. Pulp was blended until homogeneous mixture is achieved.

The prepared must was allowed to stand for 30 minutes to let pulp to float over juice. The juice was extracted by scooping and separating the pulp. Sugar was added to the extracted juice according to treatments.

Five milliliters of 10% sodium metabisulfite per gallon of must was added to sterilize the mixture, allowing 24 hours holding time. Dry yeast was added according to treatments. Fermentation of must with cotton plug cover was done for 24 hours to allow yeast incubation and multiplication. Fermentation bottles were then closed with fermentation locks. Fermentation was allowed to proceed for 3-4 weeks, until gas formation ceased.

Wine was harvested by siphoning carefully, filtered and clarified, transferred to ageing bottles and allowed to age for 3 months.

Wine was packed in clear wine bottles and pasteurized for 15 min at 75°C.

2.3 Product Evaluation

Soluble solids and alcohol content of dragon fruit wine samples were determined right after fermentation. Total soluble solid content was determined using refractometer and expressed in °Brix. Total acidity was determined by titration method and alcohol content of the distillate was measured using a hydrometer.

Sensory properties of the dragon fruit wine were determined by a trained panel. The wine samples were presented in shot glasses to the panel. Unstructured 5-point scale for blind testing of wines was used (Table 1).

Table.1: Unstructured score sheet for blind testing of wines [3]

ATTRIBUTE	SCORE				
	5	4	3	2	1
Appearance	Clear, appropriate color, brilliance, no off colors				Cloudy, off colors
Aroma	Complex, many detectable aromas, intense				Little or no aroma, off aromas
Body	Perfect texture and weight feel in the mouth				Too little or too much texture or weight feel in the mouth
Taste	Good balance, structure, several flavors detected				Little balance and structure, few flavors
Finish	Flavors linger after swallowing, smooth and rich after taste				Taste and flavors end abruptly, no after taste

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Consumer acceptability of wine samples was evaluated by 100 consumer-type panel using the following scale:

_____ Highly acceptable
_____ Moderately acceptable
_____ Acceptable
_____ Slightly acceptable
_____ Unacceptable

III. RESULTS AND DISCUSSION

3.1 Process Standardization

Based on wine classification [3], fermentation of one kilo of the fruit with the addition of 250g sugar produced semi-sweet wine while addition of 500 g sugar produced sweet wine (Table 2).

Further reduction of added sugar below 250g/kg fruit may indicate that it can probably produce a dry wine, 16-19% alcohol, an alcohol content which is considered much too high for a table wine [4]. At low initial sugar level, the yeast assumes optimum condition for their activity without any inhibition due to very fast conversion rate of sugar to alcohol as a result of very high initial sugar content. At this condition the yeasts can efficiently and completely convert the low initial sugar content into alcohol. The resulting wine should, therefore, contain high alcohol and negligible residual sugar which are the properties of dry wines.

Increasing the initial sugar content of the fermenting must to 750g and 1 kg sugar resulted to a fermented beverage which did not fall to any wine classification due to the resulting very high residual sugar and low alcohol content. The drink was very sweet, hence, was not be classified as wine.

Bread yeast tolerate up to about 5% alcohol. Beyond this alcohol level the yeast cannot continue fermentation. The level of alcohol tolerance by yeast varies from 5% to about 21% depending on yeast strain used in the alcoholic fermentation.

It can also be observed that addition of 2t yeast per kilo of the fermenting must consistently produced wine with both higher alcohol content and soluble solids (T_2 and T_4). This indicates that although alcohol production is high, the fruit wine was able to maintain high sugar content to provide higher flavor intensity to the wine.

Low alcohol production in T_7 to T_{12} can be due to the very high initial sugar concentration that could have inhibited yeast activity during incubation and actual alcoholic fermentation. Very small amount of the sugars was converted to alcohol, hence, very sweet wines, associated to low quality wines, were produced.

Table.2: Wine classification of the different treatments based on soluble solid and alcohol content

TREATMENT	DESCRIPTION (Sugar:yeast Per kg pulp)	SOLUBLE SOLIDS (ºBrix)	ALCOHOL CONTENT (% v/v)	WINE CLASSIFICATION
T1	250g: 1t	7	14.96	Semi-sweet*
T2	250g: 2t	7.1	15.36	Semi-sweet*
T3	250g: 3t	7	13.8	Semi-sweet*
T4	500g: 1t	20	10.35	Sweet**
T5	500g: 2t	25	12.82	Sweet**
T6	500g: 3t	17	11.36	Sweet**
T7	750g: 1t	31.2	6.39	Low quality
T8	750g: 2t	34	5.36	Low quality
T9	750g: 3t	35	5.6	Low quality
T10	1kg: 1t	36	5.04	Low quality
T11	1kg: 2t	36	4.9	Low quality
T12	1kg: 3t	36	1.76	Low quality

* (14-16% alcohol) [4]
** (10-13% alcohol) with residual sugar [4]

3.1.2. Standardized Process

A standard process for the production of dragon fruit wine is described by this study (Table 3).

Washing of fruits should be done with running water with agitation to loosen and remove dirt, soil, dry scales and peels. This can significantly prevent contamination of pulp with microorganisms from the peels. Quartering and slicing of fruits before peeling also prevents further contamination.

Dilution of pulp with water facilitates blending and juice extraction and at the same time provides enough solvent to facilitate dissolving of added sugar to the juice.

Standing of blended juice for 30 min facilitated juice recovery by allowing the pulp to float for easy removal from the mixture.

For the production of semi-sweet dragon fruit wine, 250 g of sugar and 2 t of yeasts must be added per kilo of pulp used. For the production of sweet wine, 500 g of sugar and 2 t yeast must be used.

Addition of five mL of 10% metabisulfite is enough to sterilize a gallon of must in the fermentation jar. This is important to make sure that there would be no contaminant during fermentation. Contaminants may compete with the desirable yeasts in terms of nutritional requirements and other conditions which may result to inefficient alcoholic fermentation.

Upon inoculation, the yeasts must be given initial aerobic conditions for 24 hrs to allow incubation and yeast multiplication. Thereafter, anaerobic condition must be provided to focus yeast activity on converting sugar to alcohol rather than increasing the number of yeast cells. Anaerobic condition is provided by using fermentation locks which allow release of gases produced by alcoholic

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fermentation but at the same time preventing entry of oxygen.

Wine is harvested by siphoning to prevent sediments form being disturbed so that clearer wine can be recovered. Further filtration produces clear, brilliant wines. The wines are then transferred to another glass container for ageing, a process for aroma and flavor development.

Table.3: Process flow and specifications used in the preparation of dragon fruit wine

PROCESS FLOW	SPECIFICATION
Washing	Wash with running tap water. Drain in stackable plastic baskets
↓	
Slicing and Peeling	Cut into quarters lengthwise. Cut across quarter to 1 inch slices. Separate pulp slices from peels and place in a container.
↓	
Blending	1 part pulp: 2 parts water, 15 sec blending or until mixture is homogenous.
↓	
Juice extraction	Stand blended pulp for 30 min – 1hr to allow pulp to float over juice and then scoop the pulp.
↓	
Addition of sugar	250 g/ L for semi-sweet dragon fruit wine 500 g/ L for sweet wine
↓	
Yeast activation	2 t yeast per kg dragon fruit, 24 hr incubation
↓	
Must preparation	Add 5 mL of 10% sodium metabisulfite per gallon of must in empty fermentation bottle, pour the must half full, stand for 24 hrs
↓	
Addition of starter to must	Ferment covered with cotton plug for 1 day at 25-30oC.
↓	
Fermentation	Ferment 3-4 weeks with fermentation lock in dark fermentation room
↓	
Harvesting	Siphon wine using tygon tubing, filter
↓	
Yeast inactivation	Add 5 mL of 10% sodium metabisulfite per gallon of wine
↓	
Clarifying	Use gelatin
↓	
Ageing	3-4 months
↓	
Bottling	Clean wine bottles, cover with cork and cap seal
↓	
Labeling	

3.2 **Sensory Properties of Dragon Fruit Wine**

No significant differences were observed among the semi-sweet and sweet wines in terms of the sensory attributes evaluated (Table 4). The wine samples were characterized by moderately clear, moderately brilliant yellow color, moderately complex detectable aroma, fair texture, good balance of a number of detectable flavors and smooth and rich taste that lingers in the mouth after swallowing.

Table.4: Mean sensory scores for dragon fruit wine samples

ATTRIBUTE	MEAN SCORE*						AVE
	T_1	T_2	T_3	T_4	T_5	T_6	
appearance	3.3	3.4	3.2	3.4	3.3	3.2	3.3
Aroma	4.2	4.3	4.3	4.3	4.4	4.3	4.3
Body	4.2	4.2	4.4	4.1	4.3	4.2	4.2
Taste	4.7	4.6	4.7	4.8	4.6	4.7	4.7
Finish	4.6	4.7	4.6	4.7	4.8	4.7	4.7

*no significant difference at 5% probability level

Equivalency of scores

appearance: 5 = clear, appropriate color, brilliance, no off colors
1 = cloudy, off colors

aroma: 5 = complex , many detectable aromas , intense
1 = little or no aroma, off aroma

body: 5 = perfect texture & weight in the mouth
1 = too little or too much texture or weight on feel in mouth

taste: 5 = good balance, structure, several flavors detected
1 = little balance and structure, few flavors

finish: 5 = flavors lingers after swallowing, smooth and rich taste
1 = taste and flavors end abruptly , no after taste

3.3 Consumer Acceptability

The consumer acceptability of the dragon fruit wine sample can be considered very promising. The relatively new product received unanimous acceptable ratings without any negative acceptable rating based on the evaluation results of 100 consumer-type judges. The relatively high percentage of evaluators who considered the wine sample as moderately acceptable and highly acceptable can confirm the market potential of the dragon fruit wine (Table 5).

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Table.5: Frequency distribution of scores for general acceptability of dragon fruit wine

ACCEPTABILITY LEVEL	FREQUENCY	PERCENT
highly acceptable	35	35
moderately acceptable	60	60
acceptable	5	5
slightly unacceptable		
highly unacceptable		
TOTAL	100	100
Mean score for acceptability	4.3	

Rating scale = 5 - highly acceptable to 1- highly unacceptable

IV. SUMMARY AND CONCLUSION

Protocols for the production of acceptable dragon fruit wine was established. Initial sugar content of the fermenting must at 250g and use of 2 t yeast per kg of fruit are required in the production of acceptable semi-sweet dragon fruit wine. The use of 500 g sugar and 2 t yeast is required in the production of acceptable sweet dragon fruit wine.

Dragon fruit wines produced by the generated technology has moderately clear, moderately brilliant yellow color, moderately complex detectable aroma, fair texture, good balance of a number of detectable flavors and smooth and rich taste that lingers in the mouth after swallowing. The dragon fruit wines have unanimous acceptable ratings without any negative acceptable rating based on the evaluation results of 100 consumer-type judges.

V. RECOMMENDATION

The generated technology for the production of dragon fruit wine developed new product which are proven to have high consumer acceptability and can provide additional income. Since the study was conducted on a laboratory scale, it is being recommended that pilot testing and studies for process mechanization for dragon fruit wine be conducted prior to commercialization. In addition, further nutritional evaluation of the products should be conducted for nutritional labeling. Also, health benefits of dragon fruit should be verified to support any medicinal of health promotion claims which can be used later as marketing and promotion tools.

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Antibacterial and antibiofilm activities of quercetin against clinical isolates of *Staphyloccocus aureus* and *Staphylococcus saprophyticus* with resistance profile

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Abstract *— The aim of this study was to determine the antibacterial and antibiofilm properties of quercetin against clinical isolates of Staphyloccocus aureus and Staphylococcus saprophyticus with resistance profile. The antibacterial activity of quercetin was performed by the determination of the minimum inhibitory concentration (MIC) through the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI). The percentage of inhibition of Staphylococcus spp. biofilm, after treatment with sub-inhibitory concentrations of quercetin (MIC/2 and MIC/4), was evaluated by the violet crystal assay. Quercetin showed an antimicrobial activity against clinical isolates of methicillin-susceptible S. aureus (MSSA) (MIC = 250 µg/ml), methicillin-resistant S. aureus (MRSA) (MIC = 500 µg/ml), vancomycin-intermediate S. aureus (VISA) (MIC = 125 and 150 µg/ml), S. saprophyticus resistant to oxacillin (MIC = 62.5 to 125 µg/ml), vancomycin-resistant S. aureus (VRSA) and S. saprophyticus resistant to oxacillin and vancomycin (MIC = 500 to 1000 µg/ml). At MIC/2 and MIC/4 the quercetin inhibit 46.5 ± 2.7% and 39.4 ± 4.3% of the S. aureus biofilm, respectively, and 51.7 ± 5.5% and 46.9 ± 5.5% of the S. saprophyticus biofilm, respectively. According to the results of this study, it was noticed that the quercetin presented an antibacterial activity against strains of Staphylococcus spp. with resistance profile and also inhibited the bacterial biofilm production even in sub-inhibitory concentrations.*

Keywords*— *Resistance; biofilm; quercetin; antibacterial activity; antibiofilm activity.

I. INTRODUCTION

Staphylococcus aureus is one of the most important pathogens causers of infections in humans due to its prevalence in hospital and community contaminations [1]. In general, *S. aureus* is associated with superficial and deep infections in skin and soft tissues, as well as toxin-mediated diseases such as staphylococcal scalded skin syndrome, toxic shock syndrome and bacteremia with abscess formation that could lead, often, to the death of patient [1-3].

Resistant staphylococcal strains were observed shortly after the use of penicillin G in the medical clinic, in 1941. A few years later, in 1950, about 80% of the hospital samples of *Staphylococcus* were resistant to penicillin G, due to the production of penicilinases enzymes that inactivate this drug. Methicillin, oxacillin and its derivatives, as well as the first and second generation cephalosporins were used aiming to treat infections caused by *Staphylococcus* with resistance profile [4,5]. The resistance to these antimicrobials is increasing, mainly in hospital environments, which presents 50% of bacterial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Another alarming factor is that the resistant strains of *S. aureus* are widely distributed around the world [3,6-8].

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Staphylococcus saprophyticus is also a species of the genus *Staphylococcus* that has a wide clinical importance. *S. saprophyticus* composes the normal microbiota of the skin and urinary and genitals tracts. However, when there is an imbalance in the microbiota, occurs the begining of urinary infections [2,3]. The resistance to methicillin in the *S. saprophyticus* strains has also reached a global distribution. Many studies defend that the main mechanism related to the acquisition of resistance to methicillin, in *S. saprophyticus*, is through the transfer of resistance genes present in the strains of MRSA or methicillin-resistant *S. epidermidis* [3,9].

The ability of some microorganisms to produce biofilm is another global public health concern. Biofilms are biological communities with a high degree of organization, in which microorganisms form structured, coordinated and functional communities. In addition, these biological communities are capable of produce polymeric matrices, wherein they are immersed and adhered to a biotic or abiotic surface [10,11]. Biofilm-producing microorganisms are responsible for most of the human bacterial infections, once they have colonization with greater structural stability and longevity. The biofilm promotes a protective barrier between bacteria and the environment, acting like an important virulence and pathogenicity factor, making these bacteria highly resistant to antimicrobials and host immunity [11,12]. In this way, it is important to conduct studies to identify the bacterial resistance phenotype, in order to contribute to epidemiological surveillance, especially of the genus *Staphyloccocus*, one of leading causes of nosocomial infections.

The dissemination, especially in hospital environments, of these pathogens resistant to antimicrobial agents and biofilm producers, represents a serious threat to public health, implying in the therapeutic failure of many infectious diseases [13,14]. Despite of the development of new antimicrobials by pharmaceutical industry in the last three decades, infections caused by bacteria of genus *Staphylococcus* are still an alarming health problem. Therefore, it is necessary to discover new therapeutic options with antimicrobial and antibiofilm activity [13-16].

The flavonoids, secondary metabolites of the polyphenols class, are found in vegetables, fruits, nuts, honey, stems and flowers. Quercetin, 3,5,7,3'-4'-pentahydroxy flavone, is the most abundant flavonoid present in the human diet and represents about 95% of the total ingested flavonoids. This molecule is one of the most studied flavonoids due to its biological activities, such as antiviral, antimicrobial, antioxidant, antithrombotic and antitumoral. Some studies have described its antimicrobial activity against some microorganisms, such as *Bacillus subtilis*, *Micrococcus luteus* and *Aspergillus flavus* [17,18]. Despite of the existence of studies that already report its antimicrobial activity, there are no researches regarding its antimicrobial and antibiofilm activity against clinical isolates of *Staphylococcus* spp. resistant to vancomycin.

In this way, the aim of this study was to evaluate the antimicrobial and antibiofilm activities of quercetin against *Staphylococcus* spp. clinical isolates with resistance profile.

II. MATERIAL AND METHODS

2.1 Identification of clinic isolates

Staphylococcus spp. clinical isolates were provided by a university hospital of Pernambuco, in the period from January to March 2017. The isolates were seeded in nutrient Agar (AN) for subsequent identification of bacteria. After that, the samples were seeded in Baird Parker Agar (BPA) base supplemented with 2% Egg yolk Tellurite emulsion (Hi-Media), incubated at 35 ± 2 °C for 48 h. The typical colonies of *S. aureus* (shiny black with an opaque ring, surrounded by a clear halo) were submitted to gram stain, catalase assay, coagulase, mannitol salt Agar assay and DNAse for *Staphylococcus aureus* identification. The colonies that did not presented typical aspects were submitted to gram stain, catalase assay and novobiocin sensitivity tests (5 µg), to identify *S. saprophyticus* (resistant to novobiocin) or *S. epidermidis* (sensitive to novobiocin) [19,20]. Methicillin-sensitive *Staphylococcus aureus* (MSSA) ATCC 29213 and MRSA ATCC 33591 were used as control strains.

2.2 Identification of resistance profile of the clinical isolates

The identification of resistance profile of the *Staphylococcus* spp. clinical isolates was conducted according to *Clinical and Laboratory Standards Institute* [21]. For the identification of MRSA, vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Staphylococcus aureus* (VRSA) and *S. saprophyticus* resistant to cefoxitin, oxacillin and vancomycin were submitted to the method of disk diffusion with cefoxitin, oxacillin and vancomycin; microdilution method with oxacillin and vancomycin; as well as *screening* for oxacillin and vancomycin [21].

For the disk diffusion method, inocula of microorganisms were adjusted to 0.5 of the McFarland scale and seeded in Müeller Hinton Agar (MHA). Then, cefoxitin, oxacillin and vancomycin were deposited on the plates and incubated at 35 ± 2 °C for 24 h. After incubation, the inhibition halos were measured and analyzed following the CLSI cutting points [21].

The minimum inhibitory concentration (MIC) was determined by the microdilution method according to the CLSI [21]. Initially, 95 µl of Müeller Hinton Broth

(MHB) was added to all plate wells. After, oxacillin and vancomycin were added in concentrations range from 0.5 to 256 µg/ml or 0.0625 to 32 µg/ml, respectively. Bacterial suspensions were adjusted to 0.5 of the McFarland scale, diluted and added in the wells to obtain a final concentration of 2–5 x 10^5 CFU/well. Subsequently, the plates were incubated at 35 ± 2 °C for 24 h. The MIC was determined as the lowest concentration of the standard drug able to inhibit >90% of the microbial growth through spectrophotometry at 620 nm.

The minimum bactericidal concentration (MBC) was determined after the obtained results of MIC. An aliquot of the wells with no microbial growth was inoculated in MHA and the plates were incubated at 35 ± 2 °C by 20-24 h. After this period, the MBC was determined as the lowest concentration with no microbial growth. The samples were analyzed following the CLSI cutting points [21].

In the *screening* test, initially, plates with Müeller Hinton Agar containing 4% NaCl and 6 µg/ml of oxacillin and plates with Brain Heart Infusion Agar (BHIA) containing 4% NaCl and 6 µg/ml of vancomycin were prepared. Then, microorganism inocula were adjusted to 0.5 of the McFarland scale and seeded in the plates. Finally, the plates were incubated at 35 ± 2 °C for 24 h. The plates were carefully observed against the light and any growth after 24 h was considered resistant to oxacillin and/or vancomycin [21].

2.3 Phenotypic characterization of biofilm production

2.3.1 Congo Red Agar test

The qualitative determination of biofilm production by clinical isolates was carried out according to the method of Congo Red Agar [22]. The isolates were adjusted to 0.5 of the McFarland scale (10^8 CFU/ml) in BHIA, incubated at 35 ± 2 ºC for 24 h and seeded in plates containing Congo Red Agar. Subsequently, they were incubated in aerobic environment at 35 ± 2 ºC for 48 h. After this period, the colonies with blackened coloration, with dry or rough consistency, were considered as biofilm-producers. Colonies of red color, with mucous consistency, were considered as not biofilm-producers. The experiment was performed in triplicate and in 3 different days.

2.3.2 Violet crystal staining

The quantitative determination of biofilm production was performed by the method of violet crystal staining [23]. Initially, the bacterial isolates were seeded in AN and incubated at 35 ± 2 °C for 18-24 h. Inocula were incubated in Tryptone Soy Broth (TSB) with 1% glucose for 24 h. Every culture was adjusted to 0.5 of the McFarland scale (10^8 CFU/ml) in the TSB with 1% glucose and the adjusted bacterial suspension was added to 96 wells plate with flat bottom. The plates were incubated at 35 ± 2 °C for 48 h. Then, the wells content were aspirates and washed with phosphate buffer (pH 7.4). Next, 200 µl of 99% methanol was added and incubated. After 15 minutes of incubation, the content was discarded. Subsequently, a solution of 1% of violet crystal stain was added in the wells and the plates were kept at room temperature for 30 minutes. The wells content was removed and washed with phosphate buffer. A solution of 33% glacial acetic acid was added and the optical density (OD) was measured by spectrophotometry at 570 nm (Multiskan microplate photometer FC, Thermo scientific, Madrid, Spain). Wells containing only the culture medium were used as control. The strains were classified into four categories, based on the values of ODs of bacterial biofilms, in comparison with value of the ODc (optical density of the control). The strains were classified into non-adherent if $OD \leq ODc$; weak biofilm producer if $ODc < OD \leq 2 \times ODc$; moderate biofilm producer if $2 \times OD \leq 4 \times ODc < ODc$; or strong biofilm producer if $4 \times ODc < OD$ [23]. The experiment was performed in triplicate and in 3 different days.

2.4 Antimicrobial activity of quercetin

The antimicrobial activity of quercetin (Sigma-Aldrich®) was performed by the microdilution method, already described previously, according to the CLSI [21]. The range of concentration of quercetin used in this study was 2 to 1000 µg/ml. The experiment was performed in triplicate and in 3 different days.

2.5 Biofilm formation-inhibition test

The antibiofilm activity of quercetin was carried out according to Das, Yang and Ma [24]. Initially, inocula were adjusted to 0.5 of the McFarland scale (10^8 CFU/ml) in TSB with 1% glucose and diluted to obtain bacterial cells concentration of 10^5 CFU/ml. These inocula weredistributed in 96 plate flat-bottom wells and incubated at 37 ± 2 °C for 24 h. Later, the wells content was removed and quercetin was added in MIC, MIC/2 and MIC/4. The plates were incubated at 35 ± 2 °C for 24 h. Then, the wells content was aspirated and the violet crystal stain method was performed, as described in section 2.3.2. The experiment was performed in triplicate and in 3 different days.

III. RESULTS AND DISCUSSION

3.1 Identification of species and phenotypic resistance profile

The identification of microorganism's prevalence in a given region is essential for the implementation of containment measures of infections caused by these bacteria. In addition to the knowledge of the species that cause infection, the identification of the resistance profile is of great importance for infections treatment caused by

these microorganisms [14]. The prevalence of resistant bacteria of genus *Staphylococcus* in hospital and community infections, especially in immunosuppressed individuals, makes these bacteria important subjects in research studies [3,6].

Bacteria of the genus *Staphylococcus* are recognized for their ability to develop drug resistance, prolonging the patient's treatment time and causing high morbidity and mortality rates [3-6]. One of the main bacterial resistance profiles of the genus *Staphylococcus* is the resistance to oxacillin [5.6], which was identified in most *S. aureus* strains and in all *S. saprophyticus* strains of the present study.

Sina et al. [25] analyzed 1904 urogenital samples and isolated, about, 80 strains of *Staphylococcus* spp.. *Staphylococcus aureus* was identified in 30% of the samples and 70% as species of coagulase-negative *Staphylococcus*. Among these 70%, 50% were identified as *S. saprophyticus*. The proportion of resistance to methicillin was 54.17% for *S. aureus* and 52.50% for *S. saprophyticus*.

Vancomycin, an antimicrobial of the glycopeptide class, is, practically, the only option of treatment for infections caused by methicillin-resistant *Staphylococcus* strains. Although, vancomycin is currently demonstrating inefficiency in some cases [26,27]. The arising of clinical isolates with intermediate resistance or resistant to vancomycin is one of the reasons that worries the worldwide organizations related to public health, as well as an alert to health professionals [27].

Studies indicate that the appearance of the antibiotic resistance phenotypes of VISA is related to hospitalization and persistent infection [26,27], and may arise when a single colony of bacterial cells, formed mostly by cells that do not have resistance to vancomycin (MIC ≤ 2 µg/ml), has an antibiotic-resistant subpopulation at intermediate level (MIC = 4 to 8 µg/ml) [26]. The first cases of vancomycin resistance were only described in the year of 2000, in Rio de Janeiro and 2002 in Japan [28].

Almeida et al. [28] analyzed *S. aureus* clinical isolates from infections in patients of a university hospital in the city of Londrina, from 2001 to 2004, where 70% of the strains were resistant to oxacillin and none of them showed resistance to vancomycin. Moreira et al. [29] performed phenotypic tests in samples of *Staphylococcus aureus* from patients and members of the nursing team of a tertiary hospital to verify their resistance profile to oxacillin and vancomycin. In their study, 75% of the strains were MRSA and all were sensitive to vancomycin.

Tiwari and Sen [30] conducted an epidemiological study that estimated the presence of vancomycin resistance in samples of patients with *S. aureus* and coagulase-negative *Staphylococcus*, from a hospital in northern India. The group analyzed 783 strains of *S. aureus*, where 10 of them showed resistance to glycopeptides, 8 of these strains were resistant to vancomycin. Although this study was performed 11 years ago, the increasing incidence of *Staphylococcus* spp. with a resistance profile turns evident the worrying in the recent years, bringing the reflection that the resistance phenotype VRSA can be as frequent as the phenotype MRSA in the present day.

Hannan et al. [31] evaluated the resistance profile of 240 clinical isolates of *S. aureus*, obtained from 4 tertiary hospitals in Pakistan from July to December 2014. The study showed that 215 (89%) of the *S. aureus* strains were sensitive to vancomycin, at concentrations ranging from 1.0 to 2.0 µg/ml, while 25 (11%) of the strains exhibited MIC > 2 µg/ml.

Table.1: Identification of the resistance phenotypic profile of Staphylococcus aureus clinical isolates.

Sample identification	Inhibition halos (mm)			MIC (µg/ml)		*Screening*		Resistance profile
	OXA	CFO	VAN	OXA	VAN	OXA	VAN	
MSSA ATCC 29213	20.2 ± 1.7	29.5 ± 1.3	18.2 ± 0.6	1	2	-	-	MSSA
LMB 150	18.8 ± 1.1	21.4 ± 1.2	17.7 ± 1.2	1	2	-	-	MSSA
LMB 151	16.2 ± 0.9	17.2 ± 1.1	21.1 ±1.6	1	1	-	-	MSSA
LMB 152	20.2 ± 2.1	30.2 ± 1.6	21.3 ± 0.5	2	1	-	-	MSSA
MRSA ATCC 33591	0	12.5 ± 1.2	25.2 ± 0.6	> 256	1	+	-	MRSA
LMB 153	0	14.3 ± 1.7	22.4 ± 1.3	8	2	+	-	MRSA
LMB 154	0	11.1 ± 0.6	23.6 ± 1.7	> 256	2	+	-	MRSA
LMB 155	0	17.7 ± 1.5	11.4 ± 0.2	16	8	+	-	VISA
LMB 156	0	19.3 ± 0.6	11.8 ± 0.6	16	4	+	-	VISA
LMB 157	0	18.6 ± 0.6	10.4 ± 0.2	> 256	16	+	+	VRSA
LMB 158	0	20.1 ± 0.8	0	> 256	> 32	+	+	VRSA

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LMB 159	0	0	0	> 256	> 32	+	+	VRSA
LMB 160	0	18.2 ± 1.2	0	> 256	> 32	+	+	VRSA
LMB 161	0	0	8.1 ± 0.5	> 256	> 32	+	+	VRSA
LMB 162	0	16.4 ± 2.1	7.2 ± 0.8	> 256	> 32	+	+	VRSA

MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*; VISA: vancomycin-intermediate *S. aureus*; VRSA: vancomycin-resistant *Staphylococcus aureus*; ATCC: American Type Culture Collection; MIC: Minimum Inhibitory Concentration; LMB: Laboratory of Microbiology; OXA: Oxacillin; VAN: Vancomycin.

Table.2: Identification of the resistance phenotypic profile of Staphylococcus saprophyticus clinical isolates.

Sample identification	Inhibition halos (mm)			MIC (µg/ml)		*Screening*		Resistance profile
	OXA	CFO	VAN	OXA	VAN	OXA	VAN	
LMB 163	17.3 ± 1.5	24.0 ± 2.3	8	> 256	2	+	-	*S. saprophyticus* resistant to OXA and CFO
LMB 164	0	21.0 ± 1.4	0	> 256	4	+	-	*S. saprophyticus* resistant to OXA and CFO
LMB 165	0	21.0 ± 1.3	0	32	2	+	-	*S. saprophyticus* resistant to OXA and CFO
LMB 166	8.2 ± 0.9	12.1 ± 0.7	2	> 256	> 32	+	+	*S. saprophyticus* resistant to OXA, CFO and VAN
LMB 167	0	17.4 ± 2.1	0	> 256	> 32	+	+	*S. saprophyticus* resistant to OXA, CFO and VAN
LMB 168	0	18.3 ± 1.1	0	> 256	> 32	+	+	*S. saprophyticus* resistant to OXA, CFO and VAN
LMB 169	0	12.8 ± 0.7	0	> 256	> 32	+	+	*S. saprophyticus* resistant to OXA, CFO and VAN
LMB 170	0	10.1 ± 0.2	0	> 256	> 32	+	+	*S. saprophyticus* resistant to OXA, CFO and VAN
LMB 171	0	0	0	> 256	16	+	+	*S. saprophyticus* resistant to OXA, CFO and VAN

MIC: Minimum Inhibitory Concentration; LMB: Laboratory of Microbiology; OXA: Oxacillin; VAN: Vancomycin; CFO: Cefoxitin.

3.2 Phenotypic characterization of biofilm production

In the Congo Red Agar test, all 22 *Staphylococcus* clinical isolates were characterized as biofilm-producers (fig. 1). In the violet crystal method, all strains were characterized as biofilm-producers, being 1 classified as a low producer (4.5%), 10 as strongly biofilm-producer (45.5%) and 11 as moderately biofilm-producer (50%) (Table 3). This compatibility in the results for quantitative and qualitative methods that evaluated the biofilm production by bacteria of the genus *Staphylococcus* has been described in other studies [32,33].

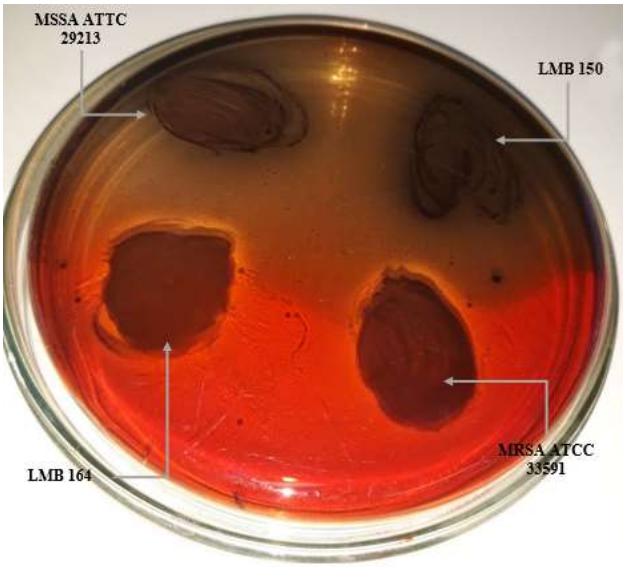

Fig.1: Evaluation of biofilm production by Congo Red Agar test.

MSSA: Methicillin-sensitive *Staphylococcus aureus*; MRSA: Methicillin-resistant *Staphylococcus aureus*; LMB: Laboratory of Microbiology.

According to the national health institutes publications, microorganisms that produce biofilm are related to more than 65-80% of the bacterial infections [32-35]. Hassan et al. [32] evaluated the ability of biofilm production in 110 clinical isolates of pathogenic bacteria, of different species, by the method of violet crystal staining. The obtained results were similar to our results that showed production of biofilm in all strains (100%), of which, 22.7% were classified as strongly producers, 41% moderate producers and 36.3% were weak producers. Shrestha et al. [33] noticed the biofilm production in 82% of 71 clinical isolates of the genus *Staphylococcus*.

3.3 Antibacterial and antibiofilm activities of quercetin

In the evaluation of antimicrobial activity of quercetin against *S. aureus* and *S. saprophyticus*, with different resistance profiles, it's observed that this molecule has a bacteriostatic effect against all microorganisms tested. Quercetin exhibit MIC values ranged from 250 to 1000 µg/ml for *Staphylococcus aureus* (Table 4) and 62.5 to 1000 µg/ml for *Staphylococcus saprophyticus* (Table 5). In addition, the molecule was able to inhibit the biofilm production by these bacteria, even when analyzed in sub-inhibitory concentrations (Tables 4 and 5).

Quercetin showed MIC of 250 µg/ml, 500 µg/ml and 125 to 250 µg/ml against MSSA, MRSA and VISA, respectively. The best inhibitory activity of quercetin was against the *S. saprophyticus* strains resistant to oxacillin and cefoxitin (MIC = 62.5 to 125 µg/ml). The lower inhibitory activity of quercetin was observed against the VRSA strains and *S. saprophyticus* resistant to vancomycin, oxacillin and cefoxitin (MIC = 500 to 1000 µg/ml).

To show a good antibacterial activity, the molecule has to present MIC < 100 µg/ml, moderate activity with MIC between 101 and 500 µg/ml, weakly active when MIC is between 501 and 1000 µg/ml, and is inactive when MIC > 1001 µg/ml [36]. So, quercetin, in general, presented moderate antibacterial activity against the clinical isolates tested, except for VRSA and *S. saprophyticus* resistant to vancomycin, oxacillin and cefoxitin, where this molecule showed a weak activity.

Studies evaluated the antimicrobial activity of quercetin against bacterial strains using the disk diffusion or Agar diffusion method. Rauha et al. [37] observed that quercetin presented antimicrobial activity at concentration of 500 µg/ml against ATCC strains of the species: *Aspergillus niger, Bacillus subtilis, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Staphylococcus aureus* and *Staphylococcus epidermidis*, determined by the disc diffusion method. Gatto et al. [17] found no antibacterial activity of this flavonoid, in the concentration of 100 µg/ml, in any of the tested bacteria (*Staphylococcus aureus, Bacillus subtilis, Listeria ivanovi, Listeria monocytogenes, Listeria serligeri, Escherichia coli, Shigella flexneri, Shigella sonnei, Salmonella enteritidis* and *Salmonella tiphymurium*).

Nitiema et al. [38] evaluated the antibacterial activity of quercetin, at a concentration of 1000 µg, through Agar diffusion method, and did not observe any activity of this molecule against bacterial strains causers of gastroenteritis. Studies that use qualitative and less precise methods, such as disk diffusion and Agar diffusion, are able to identify the antibacterial activity of quercetin, but they cannot determine the minimum inhibitory concentration. Thus, quantitative methods are important for a future *in vivo* drugs application, because they help in the determination of the dose that will be used in the treatment of infection, in humans and animals [16].

Table.3: Biofilm production from clinical isolates of the genus Staphyloccocus.

Sample identification	Bacteria identification	Congo Red Agar test	Violet crystal staining assay
MSSA ATCC 29213	*S. aureus*	+	Strong
LMB 150	*S. aureus*	+	Strong
LMB 151	*S. aureus*	+	Strong
LMB 152	*S. aureus*	+	Strong
MRSA ATCC 33591	*S. aureus*	+	Moderate
LMB 153	*S. aureus*	+	Strong
LMB 154	*S. aureus*	+	Weak
LMB 155	*S. aureus*	+	Moderate
LMB 156	*S. aureus*	+	Moderate
LMB 157	*S. aureus*	+	Strong
LMB 158	*S. aureus*	+	Moderate

LMB 159	*S. aureus*	+	Moderate
LMB 160	*S. aureus*	+	Moderate
LMB 161	*S. aureus*	+	Strong
LMB 162	*S. aureus*	+	Strong
LMB 163	*S. saprophyticus*	+	Moderate
LMB 164	*S. saprophyticus*	+	Moderate
LMB 165	*S. saprophyticus*	+	Moderate
LMB 166	*S. saprophyticus*	+	Strong
LMB 167	*S. saprophyticus*	+	Strong
LMB 168	*S. saprophyticus*	+	Strong
LMB 169	*S. saprophyticus*	+	Moderate
LMB 170	*S. saprophyticus*	+	Moderate
LMB 171	*S. saprophyticus*	+	Moderate

MSSA: Methicillin-sensitive *Staphylococcus aureus*; MRSA: Methicillin-resistant *Staphylococcus aureus*; ATCC: American Type Culture Collection; LMB: Laboratory of Microbiology; (+): production of biofilm.

Table.4: Antibacterial and antibiofilm activities of quercetin against Staphylococcus aureus clinical isolates.

Sample identification	Resistance profile	Biofilm production	MIC of QUER (µg/ml)	% of biofilm inhibition		
				MIC	MIC/2	MIC/4
MSSA ATCC 29213	MSSA	Strong	250	49.4 ± 1.2	43.4 ± 3.1	34.6 ± 1.4
LMB 150	MSSA	Strong	250	47.3 ± 0.9	44.7 ± 0.8	36.3 ± 3.9
LMB 151	MSSA	Strong	250	49.4 ± 2.1	44.1 ± 2.3	33.1 ± 2.0
LMB 152	MSSA	Strong	250	48.4 ± 1.7	45.4 ± 2.2	37.8 ± 1.4
MRSA ATCC 33591	MRSA	Moderate	500	52.8 ± 0.6	49.7 ± 1.4	42.2 ± 0.9
LMB 153	MRSA	Strong	500	48.4 ± 1.5	43.9 ± 3.9	35.5 ± 0.5
LMB 154	MRSA	Weak	500	58.3 ± 1.4	52.4 ± 1.5	46.1 ± 1.6
LMB 155	VISA	Moderate	250	55.3 ± 2.4	48.9 ± 0.6	41.5 ± 1.3
LMB 156	VISA	Moderate	125	54.6 ± 2.0	48.6 ± 0.8	44.5 ± 2.3
LMB 157	VRSA	Strong	1000	47.1 ± 1.7	44.7 ± 1.7	36.6 ± 0.9
LMB 158	VRSA	Moderate	500	57.2 ± 1.8	47.4 ± 2.2	43.7 ± 1.1
LMB 159	VRSA	Moderate	500	55.5 ± 0.8	46.6 ± 2.4	43.9 ± 1.8
LMB 160	VRSA	Moderate	500	58.5 ± 2.9	49.5 ± 0.5	44.5 ± 0.8
LMB 161	VRSA	Strong	1000	46.7 ± 0.9	44.7 ± 0.6	36.4 ± 1.7
LMB 162	VRSA	Strong	1000	47.7 ± 1.4	43.8 ± 1.7	35.2 ± 3.1

MSSA: Methicillin-sensitive *Staphylococcus aureus*; MRSA: Methicillin-resistant *Staphylococcus aureus*; VISA: Vancomycin-intermediate *Staphylococcus aureus*; VRSA Vancomycin-resistant *Staphylococcus aureus*; ATCC: American Type Culture Collection; LMB: Laboratory of Microbiology; MIC: Minimum Inhibitory Concentration; QUER: Quercetin.

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Table.5: Antibacterial and antibiofilm activities of quercetin against S. saprophyticus clinical isolates.

Sample identification	Resistance profile	Biofilm production	MIC of QUER (µg/ml)	% biofilm inhibition		
				MIC	MIC/2	MIC/4
LMB 163	*S. saprophyticus* resistant to OXA and CFO	Moderate	62.5	60.2 ± 1.4	55.5 ± 1.8	49.1 ± 1.2
LMB 164	*S. saprophyticus* resistant to OXA and CFO	Moderate	125	59.6 ± 0.9	54.8 ± 0.6	48.4 ± 1.3
LMB 165	*S. saprophyticus* resistant to OXA and CFO	Moderate	125	59.7 ± 1.7	53.2 ± 0.8	47.9 ± 2.1
LMB 166	*S. saprophyticus* is resistant to OXA, CFO and VAN	Strong	500	50.4 ± 0.6	43.8 ± 1.2	39.2 ± 1.5
LMB 167	*S. saprophyticus* resistant to OXA, CFO and VAN	Strong	500	52.3 ± 2.4	45.1 ± 1.3	40.3 ± 0.8
LMB 168	*S. saprophyticus* resistant to OXA, CFO and VAN	Strong	1000	50.9 ± 1.4	44.6 ± 0.9	40.6 ± 1.1
LMB 169	*S. saprophyticus* resistant to OXA, CFO and VAN	Moderate	500	62.8 ± 0.7	56.4 ± 0.9	52.2 ± 2.3
LMB 170	*S. saprophyticus* resistant to OXA, CFO and VAN	Moderate	1000	60.7 ± 3.1	56.4 ± 1.1	51.1 ± 0.5
LMB 171	*S. saprophyticus* resistant to OXA, CFO and VAN	Moderate	1000	61.5 ± 0.8	55.9 ± 2.4	53.4 ± 1.3

LMB: Laboratory of Microbiology; MIC: Minimum Inhibitory Concentration; QUER: Quercetin; OXA: Oxacillin; VAN: Vancomycin; CFO: Cefoxitin.

Additionally, researches show the potential of quercetin combined to other drugs for bacterial infections treatment caused by *Staphylococcus* spp.. Hirai et al. [39] analyzed the activity of quercetin in combination with other antimicrobials against MRSA strains. Quercetin, in the concentration of 50 µg/ml, enhanced *in vitro* antibacterial activity of ampicillin (0.5 µg/ml), erythromycin (8 µg/ml), gentamicin (0.5 µg/ml), oxacillin (0.8 µg/ml) and vancomycin (0.125 µg/ml).

Regarding the quercetin antibiofilm activity, this molecule reduces the bacterial biofilm of *S. aureus* at MIC, MIC/2 and MIC/4, when compared to the negative control ($p < 0.05$). Quercetin, at MIC, reduced 53.2 ± 5.0%, 59.7 ± 5.5%, 51.6 ± 0.4% and 56.5 ± 5.8% against MRSA and VRSA, *S. saprophyticus* resistant to OXA and CFO and *S. saprophyticus* resistant to OXA, CFO and VAN, respectively. At MIC/2, quercetin reduced 48.67 ± 0.61%, 45.7 ± 2.0%, 54.5 ± 1.1% and 50.5 ± 5.9% the bacterial biofilm of MRSA, VRSA, *S. saprophyticus* resistant to OXÅ and CFO; and *S. saprophyticus* resistant to OXA, CFO and VAN, respectively. At MIC/4, quercetin reduced 42.2 ± 5.3%, 40.2 ± 4.4%, 45.9 ± 0.8% and 48.4 ± 6.7% the bacterial biofilm of MRSA, VRSA, *S. saprophyticus* resistant to OXA and CFO, and *S. saprophyticus* resistant to OXA, CFO and VAN, respectively. Lee et al. [40] evaluated the ability of quercetin to inhibit the formation of biofilm of *S. aureus* ATCC 6538, through the method of violet crystal staining and verified 80% of inhibition on bacterial biofilm in the concentration at 50 µg/ml.

The relevance of our results in the evaluation of the antibiofilm activity of quercetin was to prove that this molecule, even in sub-inhibitory concentrations, is able to inhibit the formation of biofilm. This is an important fact, because some commercial drugs, such as macrolides acetilisovaleriltilosin tartrate and erythromycin, when used at lower concentrations than the values of MIC, stimulates the formation of biofilm in *Staphylococcus* strains, inducing resistance in clinical isolates of the genus *Staphylococcus* [6,41].

IV. CONCLUSION

In this study, we showed that the *S. aureus* is the major cause of bacterial infection in genus *Staphylococcus*, followed by a high incidence of *S. saprophyticus*. In addition, there is a concern on the incidence of resistant bacterial strains among patients of this hospital in Pernambuco, evidenced by the occurrence of vancomycin-resistant strains and the high incidence of strains that are strongly biofilm producers. In this way, we emphasize the need for identification of the resistance profile of clinical isolates, as well as the ability of this isolates to produce biofilm, once that these two factors are important to bacteria survival and could explain the inefficiency of many treatments. According to our results of antimicrobial and antibiofilm activities of quercetin, we can affirm that this molecule exhibited a promising

antibacterial activity against VISA and *S. saprophyticus* strains resistant to OXA and CFO and weak activity against VRSA strains and *S. saprophyticus* resistant to OXA, CFO and VAN. Regards the antibiofilm activity, even at sub-inhibitory concentrations, quercetin inhibited, approximately, 50% of the biofilm produced by isolates of *S. aureus* and *S. saprophyticus* vancomycin-resistant and, in consequence, reduced the resistance that could be caused by the increase in bacterial biofilm formation. Finally, further studies must be conducted in order to analyze the *in vivo* antibacterial activity of quercetin in infections caused by *Staphylococcus* species.

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