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Review

Viruses infecting common bean (*Phaseolus vulgaris* L.) in Tanzania: A review on molecular characterization, detection and disease management options

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Common bean (*Phaseolus vulgaris* L.) is a major legume crop, serving as a main source of dietary protein and calories and generating income for many Tanzanians. It is produced in nearly all agro-ecological zones of Tanzania. However, the average yields are low (<1000 kg/ha), which is attributed to many factors including virus diseases. The most important viruses of common bean in Tanzania are *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) but other viruses have also been reported. There has never been a review of common bean virus diseases in the country, and the lack of collated information makes their management difficult. Therefore, this review focuses on (1) occurrence of different viruses of common bean in Tanzania, (2) molecular characterization of these viruses, (3) detection tools for common bean viruses in Tanzania and (4) available options for managing virus diseases in the country. Literature and nucleotide sequence database searches revealed that common bean diseases are inadequately studied and that their causal viruses have not been adequately characterized at the molecular level in Tanzania. Increased awareness on common bean virus diseases in Tanzania is expected to result into informed development of strategies for management of the same and thus increased production, which in turn has implication on nutrition and income.

Key words: *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), common bean viruses, Tanzania, virus molecular detection.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a diploid (2n = 2x = 22) self-pollinating species that can also out-cross, albeit at very low rates (Ferreira et al., 2000; Gepts, 2001). It originates from Mesoamerica (Bitocchi et al., 2012). It is documented that common bean was introduced in coastal areas of East Africa, especially Tanzania, in the 16th century by the Portuguese and that further spread in inland areas occurred through the Arab slave traders (Wortmann et al., 2004). Common bean is an essential source of proteins and nutrients to over 500 million people in Africa, Latin America and the Caribbean (Singh, 2005; Cortés et al., 2013). It plays a key role in reducing malnutrition as well as generating income for otherwise low-income households in the developing world.

In Tanzania, beans are commonly cultivated as intercrops with other crops such as banana and maize. They are grown in mid- to high-altitude areas of the country, which experience more reliable rainfall and cooler temperatures (Hillocks et al., 2006). Specifically, areas suitable for cultivation of beans are the northern zone (Arusha, Kilimanjaro, Manyara and Tanga Regions), eastern zone (Morogoro Region), southern highlands zone, western zone (Kigoma Region) and the north-western regions of Kagera and Mara around Lake Victoria. Although mostly a subsistence crop in many areas of Tanzania, some regions such as Kilimanjaro and Arusha commercially produce the crop (Hillocks et al., 2006).

The estimated mean dry weight yield of common bean for Tanzania is 982.5 kg/ha (FAOSTAT, 2014), which is lower than the potential yield of >1500 kg/ha (Nchimbi-Msolla, 2013). Such low yields are attributed to both abiotic and biotic factors, namely drought, pests and diseases (Hillocks et al., 2006; Mourice and Tryphone, 2012). Some of the diseases that constrain bean production in Tanzania are angular leaf spot (caused by *Phaeoisariopsis griseola*), anthracnose (caused by *Colletotrichum lindemuthianum*), root rot (caused by *Pythium* spp. and *Fusarium* spp.) and common bacterial blight (caused by *Xanthomonas axonopodis* pv. *phaseoli*). Other important diseases of common bean in Tanzania are bean common mosaic disease (caused by *Bean common mosaic virus*, BCMV; and *Bean common mosaic necrosis virus*, BCMNV), Ascochyta blight (caused by *Phoma* spp.), halo blight (caused by *Pseudomonas savastanoi* pv. *phaseolicola*) and leaf rust (caused by *Uromyces appendiculatus*) (Hillocks et al., 2006; Akhavan et al., 2013; Figure 1).

Studies have addressed some of these biotic constraints in Tanzania and solutions found for some (Fivawo and Msolla, 2011; Mourice and Tryphone, 2012; Langwerden, 2014; Kusolwa et al., 2016). There have also been reviews on different aspects of common bean production in the country (Hillocks et al., 2006; Tryphone et al., 2013) but none focused on common bean virus diseases, which can cause 100% yield loss (Worrall et al., 2015). Particularly, previous reviews have not focused on the common bean virus diseases concerning their areas of incidence, distribution and approaches for detecting their causal agents. Briefly, there has been lack of organized and collated information on virus diseases of common bean in the country and this was a motivation for this review. Given the economic importance of potyviruses in common bean production, special emphasis is on BCMV and BCMNV; however, for the first time gaps in knowledge of other common bean viruses are highlighted. Where specific information is lacking, we briefly point out information available from studies conducted in countries neighbouring Tanzania under the assumption that there are similarities in agro-ecologies and possibility of cross-border spread of virus diseases through vectors and anthropogenic activities. The aim of this review is to stimulate studies on viruses causing common bean diseases, including (1) occurrence of different viruses of common bean in Tanzania, (2) molecular characterization of the viruses that infect common beans, (3) diagnostic tools for common bean viruses and (4) options in management of common bean virus diseases in the country. Increased awareness on common bean virus diseases in Tanzania is expected to result into informed development of strategies for management of the same and thus increased production, which in turn has implication on nutrition and income.

VIRUSES AND VIRUS DISEASES OF COMMON BEAN

Worldwide, both pathogenic and non-pathogenic viruses infect common bean crops. They include BCMV, BCMNV, *Bean golden mosaic virus* (BGMV; *Begomovirus*), *Cowpea mild mottle virus* (CPMMV; *Carlavirus*), *Cowpea aphid-borne mosaic virus* (CABMV; *Potyvirus*), *Phaselous vulgaris endornavirus* 1 (PvEV-1; *Endornavirus*) and *Phaseolus vulgaris endornavirus* 2 (PvEV-2; *Endornavirus*), *Southern bean mosaic virus* (SBMV; *Sobemovirus*), *Cucumber mosaic virus* (CMV; *Cucumovirus*), *Bean yellow mosaic virus* (BYMV; *Potyvirus*) and Squash yellow mild mottle virus (SYMMoV; *Begomovirus*). Some of these viruses have been detected in Tanzania and the isolates sequenced as reviewed subsequently.

BCMV and BCMNV

BCMV and BCMNV are positive-sense single-stranded RNA viruses belonging to the genus *Potyvirus* in the family Potyviridae. The genomic RNA of BCMV and BCMNV translate into a single polyprotein that auto-

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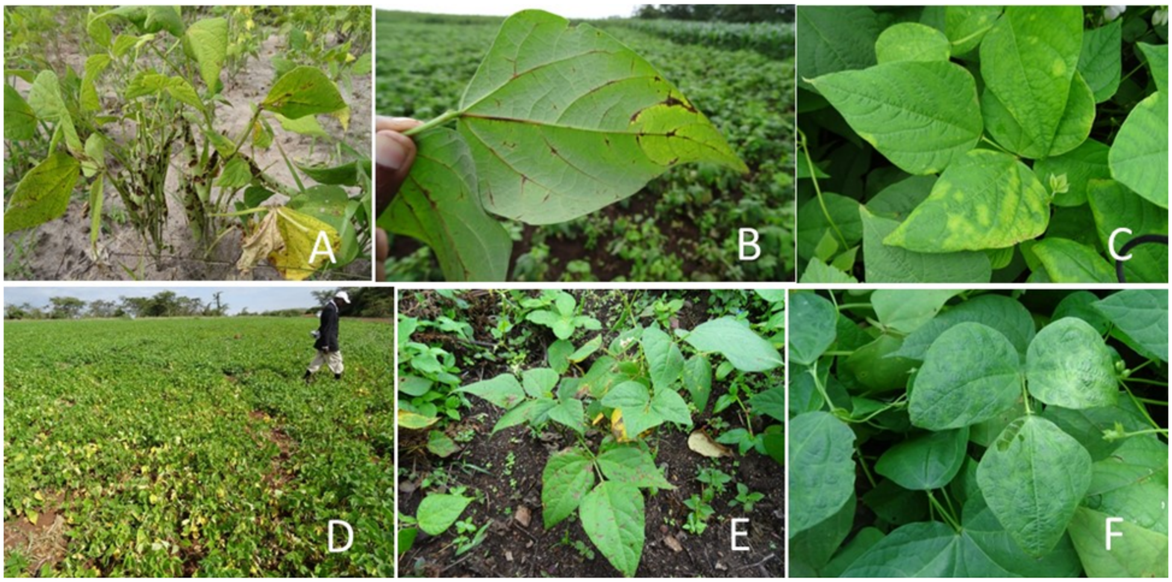

Figure 1. Symptoms of selected diseases of common bean in Tanzania. Anthracnose symptoms on common bean pods (**A**) and leaves (**B**) in southern highlands zone in Nkasi District; halo blight in northern zone in Karatu District (**C**); root rot in northern zone in Siha District (**D**); angular leaf spot in northern zone in Lushoto District (**E**); and virus-like mosaic symptoms in north western Kagera (**F**). All photos were taken in a country-wide common bean virus disease survey conducted during May to November, 2015.

catalytically cleaves into 10 mature proteins: The first protein (P1), helper component proteinase (HC-Pro), third protein (P3), first 6-kDa protein (6K1), cytoplasmic inclusion (CI), second 6-kDa protein (6K2), genome linked viral protein (VPg), nuclear inclusion a (NIa), Nuclear inclusion b (NIb) and coat protein (CP). An additional short open reading frame known as Pretty Interesting Potyviridae ORF (*PIPO*) has been described in the P3 cistron (Chung et al., 2008). BCMV and BCMNV are transmitted in common bean seeds by several aphids in a non-persistent manner (Spence and Walkey, 1995). Aspects of taxonomy and transmission of these viruses were recently reviewed (Worrall et al., 2015) and are beyond the scope of this review.

The history of BCMV and BCMNV traces back to 1917 when all strains of these viruses were considered as pathogenically identical and in literature they were assigned different names including bean mosaic virus, bean virus 1 and phaseolus virus 1 (Worrall et al., 2015). According to Kulkarni and Muguga (1973), viruses causing bean common mosaic disease were reported in Kenya and Tanzania in 1936. There was interest in surveying for this disease in the years that followed.

There are several published reports of detection of viruses that caused common bean mosaic disease symptoms on common bean in Tanzania in the 1980s and 1990s (Vetten and Allen, 1991; Spence and Walkey, 1994, 1995; Myers et al., 2000). According to Vetten and Allen (1991), BCMNV and BCMV, which were then referred to as serotypes A and B, respectively, already existed in Tanzania and occurred in single or dual infections. Serological studies indicated that most isolates at that time reacted strongly to the antiserum raised to "BCMV" strain NL5 which led the authors to suspect that most isolates collected from Tanzania (also Burundi, Rwanda, Uganda and Zambia) belonged to serotype A or what is today known as BCMNV (Vetten and Allen, 1991). Isolates belonging to this group were associated with systemic vascular necrosis (also known as black root; Grogan and Walker, 1948) in common bean plants. A report of surveys and enzyme linked immunosorbent assay (ELISA) detection of BCMNV conducted during 1993 to 1998 in Tanzania, Uganda and Kenya showed that 15% (n = 1378 bean and wild legumes) of the samples were infected with potyviruses, with BCMNV accounting for 54% of this (Myers et al., 2000). The results of Myers et al. (2000) showed that incidences of BCMNV were low in Southern Tanzania but high in Northern Tanzania and even higher in samples collected from Uganda and Kenya, suggesting that distribution of BCMNV was related to altitude.

In a collaborative research project between the Sokoine University of Agriculture (SUA) and Washington State University, an isolate of the serotype A virus from common bean seed produced in Tanzania was characterized and shown to cause systemic vascular necrosis (black root) at a temperature range of 23 to 27°C (Silbernagel et al., 1986). This was the first published account of occurrence of the temperature insensitive necrosis-causing strain of BCMV in Tanzania.

This strain has since been named TN1 (Silbernagel et al., 1986; Worrall et al., 2015). To date, serotype A isolates have been reclassified as BCMNV but serotype B isolates are BCMV (Worrall et al., 2015). BCMNV occurs at higher incidences compared with BCMV and its origin is believed to be wild plants in central and southern Africa (Spence and Walkey, 1995). The strains known to occur in Tanzania as determined using differential cultivars are NL1, NL3, NL5, NL8, TN1, TN2 and TN3 (Vetten and Allen, 1991; Spence and Walkey, 1994; Njau and Lyimo, 2000).

Incidence of BCMV and BCMNV in seeds

From the early days of discovery, BCMV strains were shown to be transmitted through infected seeds (Morales and Castaño, 1987). The rates of transmission of BCMV and BCMNV strains depend on common bean genotypes (Morales and Castaño, 1987) and can be as high as 83% (Bos, 1971). Transmission of BCMNV, however, is not possible in common bean plants that have the dominant ***I*** gene because of the black root symptom, which results in plant death and thus no seeds for the next cropping season (Grogan and Walker, 1948). There are few published studies on seed transmission of BCMV and BCMNV in Tanzania. A comprehensive study was conducted about 17 years ago and aimed at determining the incidence of BCMV and BCMNV in seeds collected from farmers, public markets and Agricultural Research Institutes (ARIs) and wild legumes (Njau and Lyimo, 2000). There were 10,300 seeds collected in this study, representing 341 and 30 seed lots of common bean and wild legumes, respectively (Njau and Lyimo, 2000). The seeds were grown in an insect proof screen-house and the incidence of BCMV and BCMNV determined using ELISA. The two viruses were detected only in bean seed samples collected from northern and eastern Tanzania (Njau and Lyimo, 2000) and were not detected in wild legume seeds. The virus infections were more common in bean seeds collected from ARIs (in 20 out of 59 seed lots) and rare in bean seeds collected from farmers and public markets (in four out of 282 seed lots). The highest incidence for BCMNV was 36.6%, whereas it was only 12.4% for BCMV. The average incidences for both viruses were less than 8% (Njau and Lyimo, 2000).

Alternative hosts of BCMV and BCMNV

Plant viruses, including potyviruses, can infect a wide range of hosts. BCMNV and BCMV, for instance, infect plants in at least six families (Bos and Gibbs, 1995; reviewed in Worrall et al., 2015). In Tanzania, surveys for alternative hosts of BCMNV were conducted within 1993 and 1998 (Myers et al., 2000). In their survey, Myers et al. (2000) found that several wild legume species were hosts of BCMNV: *Centrosema pubscens*, *Neonotonia wightii*, *Senna* spp., *Crotolaria* spp. and *Rhynchosia zernia*. Moreover, in artificial mechanical inoculations, BCMNV and BCMV infected five of the six legumes studied (Njau and Lyimo, 2000). The legumes infected were *Senna occidentalis*, *Senna obtusifolia*, *Cassia floribunda*, *Crotalaria* spp. and *Rhynchosia minima*. Njau and Lyimo (2000) showed that these viruses were systemic in four of the six infected plants. In Uganda, Sengooba et al. (1997) reported natural occurrence of BCMNV in *C. pubescens*, *Crotalaria incana*, *Lablab purpureus*, *Phaseolus lunatus*, *Senna bicapsularis*, *Senna sophera*, *Vigna vexillata* and also in an unidentified *Crotalaria sp*. Alternative hosts of BCMNV and BCMV including *Glycine max*, a natural host of BCMNV have been reported previously (Spence and Walkey, 1995; Worrall et al., 2015).

Occurrence of other common bean virus diseases in the country

Worldwide, common bean is infected by a large number of both single- and double-stranded DNA and RNA plant viruses. In addition to BCMNV and BCMV, other important viruses infecting common bean are CPMMV (Mink and Keswani, 1987; Chang et al., 2013), CMV (Davis and Hampton, 1986; Njau et al., 2006), CABMV (Bashir et al., 2010), SBMV (Verhoeven et al., 2003), BGYMV (Karkashian et al., 2011), SYMMoV (Karkashian et al., 2011), PvEV-1 (Okada et al., 2013; Khankhum et al., 2015), PvEV-2 (Okada et al., 2013), BGMV and *Calopogonium golden mosaic virus* (CalGMV) (Diaz et al., 2002; Karkashian et al., 2011). Viruses that infect common bean in Tanzania and elsewhere are shown in Table 1.

MOLECULAR CHARACTERIZATION OF ISOLATES FROM TANZANIA

Developing strategies to manage common bean virus diseases requires knowledge of the transmission mechanism and molecular characteristics of the causal viruses. There is scanty information on molecular characterization of common bean viruses for Tanzanian isolates. Sometimes isolates are sequenced and sequences submitted in nucleotide databases without publication. Therefore, the nucleotide sequence databases were searched for availability of sequences of isolates collected from Tanzania. However, there was only one sequence for BCMNV and none for any other viruses infecting common bean in Tanzania (Table 2). This contrasted with availability of information on molecular characterization of common bean viruses in

Table 1. Some viruses known to infect common bean worldwide.

Virus	Abbreviation and taxonomy	Genome type[b]	Reference*
Bean common mosaic virus[a]	BCMV (*Potyvirus; Potyviridae)*	+ssRNA	Njau and Lyimo, 2000
Bean common mosaic necrosis virus[a]	BCMNV (*Potyvirus*; *Potyviridae)*	+ssRNA	Njau and Lyimo, 2000
Cowpea mild mottle virus[a]	CPMMV (*Carlavirus; Betaflexiviridae)*	+ssRNA	Mink and Keswani, 1987; Njau et al., 2006
Cucumber mosaic virus[a]	CMV (*Cucumovirus; Bromoviridae)*	+ssRNA	Davis and Hampton, 1986; Njau et al., 2006
Cowpea aphid-borne mosaic virus[a]	CABMV (*Potyvirus; Potyviridae)*	+ssRNA	Bashir et al., 2010; Patel and Kuwite, 1982
Southern bean mosaic virus	SBMV (*Sobemovirus;* unassigned to family)	+ssRNA	Verhoeven et al., 2003
Bean golden yellow mosaic virus	BGYMV (*Begomovirus; Geminiviridae)*	ssDNA	Karkashian et al., 2011
Calopogonium golden mosaic virus	*CalGMV* (*Begomovirus; Geminiviridae)*	ssDNA	Diaz et al., 2002; Karkashian et al., 2011
Squash yellow mild mottle virus	SYMMoV (*Begomovirus; Geminiviridae)*	ssDNA	Karkashian et al., 2011
Phaseolus vulgaris endornavirus 1	PvEV-1 (*Endornavirus; Endornaviridae)*	dsRNA	Okada et al., 2013; Khankhum et al., 2015
Phaseolus vulgaris endornavirus 2	PvEV-2 (*Endornavirus; Endornaviridae)*	dsRNA	Okada et al., 2013; Khankhum et al., 2015
Bean golden mosaic virus	BGMV (*Begomovirus; Geminiviridae)*	ssDNA	Kim et al., 1978

[a]These viruses have been detected in common bean or other crops in Tanzania. *References shown are examples only and not exhaustive lists, [b]ssRNA, dsRNA, ssDNA and dsDNA stand for single stranded ribonucleic acid, double stranded ribonucleic acid, single stranded deoxyribonucleic acid and double stranded deoxyribonucleic acid, respectively.

other bean growing locations, notably in Asia, Latin America, USA and Europe.

BCMV

A search for BCMV in the NCBI nucleotide database in December 2016 resulted in 347 genomic sequences. Individual examination of each of these sequences showed that only 288, some unverified, were submitted as being of BCMV. The remaining sequences were stated as being of Azuki bean mosaic virus, Blackeye mosaic virus, Peanut stripe virus and Dendrobium mosaic virus, which are regarded as strains of BCMV (Worrall et al., 2015). Of the 288 BCMV sequences, assuming no isolate was assigned duplicate accession numbers, only 52 sequences were complete genomes (about 10 kb) that were generated by either Sanger or next generation sequencing (NGS) techniques. Although there were isolates whose origins were not mentioned (Worrall et al., 2015), it could not be established that any of these partial or complete genomic sequences were of BCMV isolates from Tanzania.

BCMNV

There were 34 BCMNV nucleotide sequences in the sequence databases. Of these, only nine sequences translated to yield complete polyproteins, each of which could be predicted to yield 10 mature proteins upon autocatalytic cleavage (Adams et al., 2005). One of these nine sequences (KX302007), however, was only nearly complete because a few nucleotides (12 nt) were missing at the 5′-end of the 5′-untranslated region. Moreover, only one complete sequence (Accession number HQ229995) of BCMNV isolates was originally collected from Tanzania (Larsen et al., 2011). Additionally, there was a coat protein and 3′-UTR partial sequence of what was referred to as strain TN1 (Accession number U37076) but the country of origin was not stated. However, this partial sequence was 100% identical to the corresponding genomic region in the TN1 strain of Tanzanian origin, suggesting that the sequences were the same isolate.

Previous phylogenetic analysis using these nine complete genomes of BCMNV showed that they were closely related (Worrall et al., 2015). Sequences of BCMV isolates are known to be very diverse (Worrall et al., 2015) and contain evolutionary signatures of frequent recombination events (Zhou et al., 2014). Their complete nucleotide sequence identities were computed

Table 2. The number of nucleotide sequences of selected viruses of common bean in GenBank in December 2016.

Virus name	Total number of sequences	Complete sequences	Tanzanian sequences
Bean common mosaic virus[a]	288	52	0
Bean common mosaic necrosis virus[a]	35	9	1
Cowpea mild mottle virus[a]	38	8	0
Southern bean mosaic virus[a]	13	5	0
Cowpea aphid-borne mosaic virus[a]	90	7	0
Cucumber mosaic virus[a]	3019	489	0
Bean golden mosaic virus[a]	173	168	0
Phaseolus vulgaris endornavirus 1[b]	5	3	0
Phaseolus vulgaris endornavirus 2[b]	2	2	0

[a]Pathogenic common bean virus. [b]Non-pathogenic common bean viruses that are highly transmitted in common bean seeds and potentially spread all over the world.

using the BioEdit software (Hall, 1999). The nucleotide sequence identities between isolates of different strains of BCMNV were in the range of 92.5 to 100%. The sequence of BCMNV isolate from Tanzania (strain TN1) was 94.4 to 98.4% identical to complete nucleotide sequences of other BCMNV isolates (Table 3).

Studies using a differential symptoms approach have consistently placed BCMNV and BCMV into seven pathogenic groups: I to VII. Despite tight clustering of BCMNV isolates in phylogenetic analysis, they cause different symptoms on differential cultivars. However, there is very little information regarding molecular characteristics of BCMNV and BCMV isolates in Tanzania. Consequently, such aspects as selection pressure and recombination events which drive evolution of plant viruses, including common bean viruses (García-Arenal et al., 2001; Larsen et al., 2005; Feng et al., 2014; Zhou et al., 2014), have remained largely unstudied. The lack of such information means it has not been possible to use PCR-based methods for reliable detection of both viruses in plants in past and continuing breeding programmes. With evidence of high genetic variation and frequent recombination events in BCMV isolates (Larsen et al., 2005; Feng et al., 2014), the availability of many sequences from outside East Africa may be insufficient for development of strain-specific diagnostic primers. Therefore, in some studies on common bean virus diseases that were conducted in Tanzania, researchers in ARIs and universities have relied on ELISA assays to detect viruses (Vetten and Allen, 1991; Njau and Lyimo, 2000; Njau et al., 2006).

CMV

CMV, a seed-borne virus (Davis and Hampton, 1986; Zitter and Murphy, 2009), is one of the most important plant viruses and is known to have a wide range of hosts including common bean (Davis and Hampton, 1986; Zitter and Murphy, 2009; Amayo et al., 2012). In Tanzania, CMV has been detected by ELISA in a common bean leaf sample collected from Arusha (Njau et al., 2006) and in *Vigna unguiculata*, *Cucumis sativus*, *Citrullus lanatus*, *Cucurbita pepo*, *Cucumis hystrix*, *Luffa aegyptiaca* (Sydänmetsä and Mbanzibwa, 2016) and *Solanum lycopersicum* (Nono-Womdim et al., 1996). CMV has also been detected in common bean and *P. lunatus* in samples from Zambia (Vetten and Allen, 1991) and Ethiopia (Spence and Walkey, 1995), respectively. There were no nucleotide sequences of CMV for isolates from Tanzania (Table 2).

CABMV

CABMV is known to occur in East Africa (Bock, 1973; Orawu et al., 2013) and in Tanzania has been detected in cowpea (*Vigna unguiculata* (Patel and Kuwite, 1982; Patel et al., 1982) and common bean (Sengooba, 2001). There were 90 nucleotide sequences in the nucleotide sequence database. None of these nucleotide sequences were indicated as being of isolates from Tanzania.

CPMMV

CPMMV belongs to the genus *Carlavirus* and is transmitted by whitefly in a non-persistent manner. It was detected in common bean plants in Tanzania in the 1980s (Mink and Keswani, 1987). Since it was only detected in and near plots of imported germplasm at the SUA, the researchers involved surmised that it was introduced from India through infected mung bean (*Vigna radiata*) (Mink and Keswani, 1987). There were nine complete (about 8 kb) and 30 partial nucleotide sequences of CPMMV in the database and none of these

Table 3. Nucleotide sequence identities of the nine complete genomes of BCMNV isolates.

Accession/strain	AY282577	KX302007	U19287	HQ229995*	HQ229994	HQ229993	AY138897	AY864314	NC_004047
AY282577	**XX**	96.3	99.8	**98.4**	96.5	99.4	99.9	95.6	99.8
KX302007		XX	96.1	**96.0**	97.8	96.1	96.2	92.5	96.1
U19287			XX	**98.3**	96.3	99.2	99.8	95.4	100
HQ229995*				XX	**96.3**	**98.3**	**98.4**	**94.4**	**98.3**
HQ229994					XX	96.3	96.5	92.6	96.3
HQ229993						XX	99.4	95.4	99.2
AY138897							XX	95.6	99.8
AY864314								XX	95.4
NC_004047									XX

*Accession number of the only sequenced isolate of BCMNV from Tanzania. Authors and determined or genetically related strain for each sequence shown in the Table are indicated in parenthesis after each accession number: AY282577 (Unpublished; NL-3), KX302007 (Maina et al., 2016; NL-8), U19287 (Fang et al., 1995; strain NL-3), HQ229995 (Larsen et al., 2011; strain TN-1), HQ229994 (Larsen et al., 2011; strain NL-8), HQ229993 (Larsen et al., 2011; strain NL-5), AY138897 (Unpublished; NL-3), AY864314 (Larsen et al., 2005; NL-3) and NC_004047 (Fang et al., 1995; NL-3).

isolates was from Tanzania.

PvEV-1 and PvEV-2

Two high-molecular mass dsRNA viruses, named PvEV-1 and PvEV-2 and belonging to the genus *Endornavirus* (family Endornaviridae), were recently discovered in common bean (Okada et al., 2013). These viruses are efficiently (nearly 100%) transmitted through seeds (Okada et al., 2013). The viruses have been characterized at molecular level with a total of seven sequence submissions (Accession numbers NC_023678, AB719398, AM284175, X16637, AB719397, KT456288 and KT456287) in the database but none come from Africa. Of the seven sequence submissions, two were partial sequences (Accession numbers AM284175 and X16637) closely related to the corresponding genomic region in the sequence of an isolate PvEV-1 (Accession number AB719397). There has been no survey for PvEV-1 and PvEV-2 in Africa despite availability of a molecular detection tool (Segundo et al., 2008). The lack of mention of these viruses in literature of common bean virus diseases in Africa may be because they are considered non-pathogenic and therefore of no economic significance. Moreover, it may also be due to the fact that no NGS technique has been used to detect viruses in common bean plants in Africa.

SBMV

There are no reports of SBMV infections in common bean plants in Tanzania. Likewise, a NCBI search for SBMV sequences of Tanzanian origin showed no submissions (Table 3). This is consistent with previous reports from plant pathologists in the region (Allen et al., 1989). However, occurrence of this virus may be overlooked because it usually causes mild symptoms on common bean.

BGMV

BGMV (*Begomovirus*) is a single-stranded DNA virus with two components (A and B) and is transmitted by whitefly (*Bemisia tabaci* Gennadius). It has been reported to infect and cause symptoms on common bean plants in the New World (Bonfim et al., 2007). Searching the nucleotide sequence databases under the search terms “bean golden mosaic virus”, followed by examining each sequence, revealed 173 sequences for BGMV. Of these, 168 were complete sequences of BGMV for DNA components A and B. There was no information about occurrence of this virus in Tanzania and searching the nucleotide databases did not reveal any sequenced isolate of this virus from Tanzania. The highest number of sequences for this virus was generated in a study conducted in Brazil (Sobrinho et al., 2014).

METHODS FOR DETECTION OF COMMON BEAN VIRUSES

Reliable and cost-effective detection of plant pathogens, including viruses is a crucial step in management of plant diseases. There is a wide range of methods used to detect plant viruses worldwide; however, they can be categorized into two major types: Serological and molecular methods. The two most commonly used virus detection methods in laboratories across the world are ELISA and PCR (Boonham et al., 2014). There is also the use of electron microscopy for morphological identification and indicator plants in bioassays. According to Boonham et al. (2014), the adoption of the technique may be based on detection sensitivity, repeatability and reproducibility, detection in fields and resource poor locations, simultaneous detection of multiple pathogens and power to discover new pathogens. Plant pathology laboratories in Africa are normally resource poor, experience problems acquiring reagents and equipment, and have erratic supply of electricity, even with good funding, for geographical and institutional reasons.

PCR

PCR is commonly used in detection of common bean viruses (Xu and Hampton, 1996; Melgarejo et al., 2007; Tavasoli et al., 2009; Petrović et al., 2010). A universal primer pair for detection of the potyviruses that infect common bean plants was recently published (Zheng et al., 2010). The PCR detection is more accurate and sensitive compared with ELISA and its sensitivity may be improved through use of immunocapture reverse transcription PCR for RNA viruses (Udayashankar et al., 2012). In detection of common bean viruses, PCR detection followed by sequencing of PCR products directly or after cloning in plasmids is mostly used to confirm viruses detected by ELISA (Petrović et al., 2010).

Molecular-based methods have not been used to detect common bean viruses in Tanzanian laboratories. Nevertheless, the numbers of laboratories using molecular techniques (that is, PCR) are increasing in East Africa although lagging far behind laboratories in the developed world. Of the 16 national agricultural research centres in Tanzania, PCR machines (including for quantitative PCR) have been installed at only one institute, the Mikocheni Agricultural Research Institute. However, Tanzania-based international agricultural research centres such as the International Institute of Tropical Agriculture have state-of-the-art laboratories with equipment for molecular detection of plant pathogens. Through collaborative arrangements, researchers based at national agricultural research centres can access the facilities in these laboratories. Moreover, PCR can be performed at the University of Dar es Salaam, SUA and the Nelson Mandela African Institution of Science and Technology. Training, mainly since the late 1990s, has led to availability of agricultural researchers capable of running laboratories that employ molecular-based techniques in detecting plant pathogens. Moreover, sequencing facilities have been installed at different institutes in the country. Examples are sequencing facilities at Mbeya Zonal Referral Hospital Laboratory and at SUA in the College of Veterinary and Medical Sciences. Sending samples there for sequencing reduces both costs and time required to send samples outside the country. In-country sequencing reduces time for results delivery from about a week to three working days. This allows for prompt interventions in management of plant diseases as well as timely completion of laboratory-based experiments that aim at generating sequence data.

Several factors have hindered application of molecular techniques in detection of common bean viruses in the country. There has been inadequate funding, which may be attributed to more attention paid to diseases of root and tuber crops, cassava and sweet potato, compared with other crops in the region. Moreover, although BCMV and BCMNV are known to be among the main constraints to common bean production in the country, there are other pathogens that constrain its production even more (Hillocks et al., 2006). Another reason is, as shown in this review, that there are no published sequences from Tanzania for designing primers to be used in PCR detection of viruses that infect common bean in the country. It is also likely that the known high genetic diversity between isolates of BCMV, which is considered the most important virus of common bean, hinders development of molecular-based diagnostic primers. However, this may not be true for the genetically closely related BCMNV strains.

PCR detection has been shown to work for nucleic acids (both DNA and RNA) extracted from dry leaf samples (Aloyce et al., 2013). Use of dry leaves as a starting material for nucleic acid extraction is paramount for virus detection in developing countries; many leaf samples collected from fields in Tanzania are delivered to the laboratory after pressing in the herbarium. Unfortunately, the detection of common bean viruses has not been demonstrated from dry leaf samples in the country, but it is likely that viral RNA will be well preserved if leaves are carefully pressed. There are alternative means for collection of samples for nucleic acid extraction. For example, fast technology for analysis (FTA) cards have been used to preserve nucleic acids and used in fields to collect samples for laboratory analysis (Ndunguru et al., 2005; Owor et al., 2007). Moreover, leaf samples can be kept in silica gel or $CaCl_2$ for desiccation (Vetten and Allen, 1991). Freeze drying ensures high-quality materials for RNA and DNA extraction, and for mechanical transmission of viruses to indicator plants, but is difficult to implement at the field level.

NGS-based detection

In a large country like Tanzania, with common bean produced in nearly all places, the chances of occurrence of many known and unknown viruses is high. This makes it difficult to apply conventional PCR in detection of viruses that infect common bean because this would require that viral genomic sequences are known for all viruses and that several different pairs of primers are designed. Moreover, plant viruses have genomes, which are either single- or double-stranded or circular, or linear DNA or RNA. Thus, in the past, researchers devised methods to target viruses depending on the nature of their genomes (Haible et al., 2006; Balijja et al., 2008). To overcome the limitations associated with different methods in detection of plant viruses, NGS techniques have been developed. These methods are robust in detection of plant viruses from different families and have led to discovery of novel plant viruses (Adams et al., 2009; Kreuze et al., 2009; Boonham et al., 2014; Zheng et al., 2017).

Some studies conducted in or using plant samples from Tanzania have employed NGS to detect plant viruses but not those infecting common bean (Mbanzibwa et al., 2014; Ndunguru et al., 2015). Elsewhere, viruses infecting common bean have been detected and sequenced using NGS (Kehoe et al., 2014; Maina et al., 2016). In detection of plant viruses infecting crops other than common bean in Tanzania, either sequencing was done on viral small RNAs, naturally generated by plants as they defend against invading viruses, or on intact RNA (Mlotshwa et al., 2008; Mbanzibwa et al., 2014; Ndunguru et al., 2015). The NGS studies on Tanzanian plant RNA samples enabled detection of viruses not previously known to occur in sweet potato (Mbanzibwa et al., 2014) and the sequencing of complete genomes of *Cassava brown streak virus* (*Ipomovirus*) and *Ugandan cassava brown streak virus* (*Ipomovirus*), and hence studies on evolution of these viruses (Ndunguru et al., 2015). Following generation of information on these viruses, plant breeders have targeted specific species in their breeding programmes and have diagnostic tools to confirm viruses with which they challenge their breeding materials. Moreover, this has allowed development of diagnostic tools that have in turn helped pathologists to distribute clean planting material to farmers in the country.

Despite exciting opportunities in use of NGS to universally detect viruses in plants, there are challenges associated with its use in detection of plant viruses in developing countries. Firstly, there is nearly always a need for collaboration with scientists from advanced laboratories in developed countries. Finding collaborators interested in the same projects as scientists in the developing countries is not easy, but such collaboration is required in order to have access to supercomputing machines and also for backstopping in NGS data analysis. However, for small-data NGS analysis studies, such as assembly of plant virus genomes, it is possible to overcome the problem of supercomputer access through purchase of computers with relatively high computation capability, 8 TB hard disk drive (HDD) computers are sufficient for most virus genome assembly work. Moreover, installing a Linux virtual machine in 1 TB HDD or less capacity computers is sufficient for analysis of NGS data using some computer programs such as VirusDetect program (Zheng et al., 2017).

Although it is possible to assemble and map plant virus genomes using commercial packages (Kehoe et al., 2014), it is expensive to purchase such software as CLC genomic workbench and Geneious whose costs may be around USD6000 and 300, respectively, or even more depending on terms and conditions (Smith, 2014). There are also costs associated with updating of software (Smith, 2014).

Secondly, sequencing service are outsourced from outside the continent (e.g. at Fasteris in Switzerland). This requires the shipping of RNA samples on dry ice or manipulating of RNA, which may compromise results. Unfortunately, all low-cost international couriers operating in Tanzania do not accept packing using dry ice and specialist couriers that accept nucleic acids on dry ice charge over USD3000, an amount sufficient to cover vehicle hire costs for disease surveys covering a distance greater than 3000 km.

Thirdly, comprehensive countrywide surveys would require collection of many samples and their sequencing is likely to cost a huge amount of money (but some argue that it reduces costs as explained below) despite recent decreases in sequencing costs (Boonham et al., 2014). As an example, as of December 2016, sequencing of two samples cost about EUR1185 (with a 5% discount on sequencing and library preparation). Therefore, working with individual plant samples is not possible for countrywide surveys of plant virus disease incidence; however, pooling an equal amount of RNA extracted from up to 100 individual plants has enabled detection of viruses in many samples at lower cost. Unfortunately, pooling of RNA extracted from many individual plants complicates the exercise of assembling genomes of individual isolates. This is because it cannot be determined which isolates the sequences obtained relate to, unless PCR is separately done on cDNA or DNA of individual plants whose RNA was pooled in a single tube.

Otherwise there is a likelihood of assembling the sequences in a manner that will create artificial recombinant sequences.

Among laboratories, the issue of costs associated with use of NGS is perceived differently. (1) Using NGS reduces the time required to detect viruses in many samples and thus reduces costs associated with laboratory work (e.g. salaries). (2) Only viruses detected by NGS can be specifically targeted in the steps that follow. Thus, no money is wasted on ordering primers or different antibodies to target many suspected viruses that may not be infecting samples. (3) Timely identification of a pathogen enables rapid intervention and thus may prevent spread of disease or damage to crops, which has cost implications. Therefore, the cost challenge on use of NGS may not apply in some cases and when decisions on its use are rationally made.

ELISA method

ELISA has been used widely in detection of common bean viruses. It has been used to detect viruses in seeds (Klein et al., 1992; Arli-Sokmen et al., 2016) and leaf samples (Tremaine et al., 1985; Shahraeen et al., 2005; Peyambari et al., 2006; Petrović et al., 2010; Arli-Sokmen et al., 2016). In Tanzania, ELISA is the only method that has been used to detect common bean viruses in common bean, mung bean and wild plants (Mink and Keswani, 1987; Vetten and Allen 1991; Myers et al., 2000; Njau and Lyimo, 2000; Njau et al., 2006).

ELISA detection uses a polystyrene plate capable of binding antibodies with association of the enzyme-substrate reaction (Jeong et al., 2014). When performed using nitrocellulose and nylon membranes, it is known as a tissue blot immunoassay (Jeong et al., 2014) or dot immunobinding assay. With ELISA, it is possible to detect viruses in many leaf or seed samples (cost effective) in a relatively short period (normally six hours to two days). Costs and time of screening for viruses in leaf samples may be reduced further by using a mixture of antibodies in a single well for simultaneous detection of co-infecting viruses and also by detecting viruses in pooled plant leaf samples (Njau et al., 2006). However, ELISA may be less accurate and sensitive compared with molecular-based diagnostic techniques. It can fail to distinguish between strains of the same virus (Boonham et al., 2014) and in some instances the antibody may react with plant constituents. Moreover, ELISA is only useful when the virus that causes the disease is known and there are antibodies for the virus or its strains. Despite these shortcomings of ELISA, BCMNV and BCMV isolates have been consistently classified into two distinct serotypes using ELISA and these groups have been confirmed using molecular techniques. The method will inevitably continue to be used because of its simplicity (no requirement for highly trained personnel) and cost effectiveness.

Many laboratories in developing countries have the human capacity and facilities to apply ELISA for detection of plant viruses: refrigeration, incubators and spectrophotometers. Commonly, ELISA is done on fresh leaf samples; however, keeping leaf samples fresh may be difficult in large surveys for common bean viruses in Tanzania. Following surveys, it may be a long time before scientists return to laboratories for sample processing and ELISA detection. The commonly used approach in storage of collected leaf samples is placing them between print sheets and pressing in an herbarium. Lister et al. (1985) demonstrated that a *Barley yellow dwarf mosaic virus* (*Luteovirus*) survived different environmental conditions after being air-dried. Dry leaf samples of common bean have been used for ELISA detection of common bean viruses (Spence and Walkey, 1994, 1995).

Indicator secondary hosts of viruses

Indicator secondary plants are used in bioassays and host range studies. A good example of use of indicator plants in detection of plant viruses is the detection of virus infections in sweet potato using *Ipomoea setosa*. Other commonly used secondary hosts include *Nicotiana* spp., *Petunia* spp. and *Datura* spp. Virus infections in common bean have also been detected using indicator plants (that is, bioassays). Petrović et al. (2010) identified BCMV, BCMNV, CMV and *Alfalfa mosaic virus* based on the reaction of *Glycine max*, *Lupinus albus*, *Datura stramonium*, *Zinnia elegans*, *Nicotiana glutinosa*, and *Nicotiana tabacum* var. *samsun*. In other studies, *Vigna unguiculata* var. *sinensis* was also included (Lee et al., 2015). In Tanzania, Njau and Lyimo (2000) determined reaction of selected leguminous plants to BCMV and BCMNV. Mechanical inoculation is the most commonly used approach in transmission of viruses between bean plants and secondary hosts.

Indicator plants must be carefully characterized for their reactions to a given virus isolate. The indicator plants should also be able to accumulate sufficient virus that detection is possible even for common bean material with mild symptoms or asymptomatic virus infections.

COMMON BEAN VIRUS DISEASE MANAGEMENT STRATEGIES

Use of disease-free seed

In areas with low disease pressure and with appropriate timing to avoid high populations of vectors, use of disease-free, certified seed leads to increased yields, all other things being equal. In Tanzania, the use of clean

Table 4. Areas in Tanzania where farmers are growing QDS as of January 2017.

Agricultural zone	Region	District	Varieties grown as QDS
Lake	Kagera	Bukoba Rural, Missenyi, Karagwe and Muleba	Jesca, Lyamungu 90 and Njano Uyole
	Arusha	Arumeru, and Mondulu	Lyamungu 90
Northern	Manyara	Babati and Karatu	Jesca and Lyamungu 90
	Tanga	Kilindi and Lushoto	Jesca, Lyamungu 85, Lyamungu 90 and Selian 94
	Iringa	Iringa and Makete	Njano Uyole and Uyole 96
	Mbeya	Mbeya	Njano Uyole and Uyole 03
Southern Highland	Njombe	Njombe and Wanging'ombe	Calima Uyole, Njano Uyole, Resenda, Uyole 03 and Uyole 96
	Rukwa	Sumbawanga and Nkasi	Calima Uyole and Njano Uyole
	Songwe	Mbozi and Momba	Calima Uyole, Njano Uyole, and Uyole 96

seeds is increasing in areas where there are seed companies or where farmers are being sensitized and supported by bean-related projects like Tropical Legume III (TLIII) and non-governmental organizations such as World Vision-Tanzania and Farm Africa. The International Center for Tropical Agriculture is supporting Tanzanian organizations and private seed entrepreneurs (e.g. Meru Agro-Tours and Consultants Co. Ltd in Arusha) to produce and market bean seeds with the aim of sustaining a bean seed delivery system. Because of these efforts, quality declared seeds (QDS) is used by farmers in three zones in Tanzania: The Lake and northern and southern highlands zones (Table 4). The commonly used QDS varieties are Jesca, Lyamungu 90, Lyamungu 85, Selian 97, Selian 94 and Uyole 03. A simple market survey conducted across the three zones showed that a kilogram of breeder seed costs around USD3.7 (TZS 6000 to 8000/-), whereas that of QDS costs USD1.2. This is affordable compared with the price of farm-saved seed of USD0.8. It should be noted that, in the past, involvement of inexperienced farmers in seed production led to seed degeneration and affected viability of the seed trade (Hillocks et al., 2006). Moreover, most farmers in Tanzania use farm-saved bean seeds from the previous harvest or purchase them from fellow farmers at local village markets, a consequence of the failure of the formal seed sector to meet the needs of smallholders for high-quality seed (Hillocks et al., 2006).

Planting of resistant materials

Several improved cultivars, which are tolerant or moderately or completely resistant to BCMV or BCMNV have been released in Tanzania and include Canadian Wonder, Uyole 94, Uyole 96, Lyamungu 85, Jesca, Selian 97, Rojo, Mshindi, Selian 05, Selian 06 and Cheupe (Tryphone et al., 2013). Kusolwa et al. (2016) also registered the red kidney bean germplasm line (AO-1012-29-3-3A) that has multiple virus and bean weevil (storage pest) resistances. This bean germplasm line has *I* and *bc-12* genes that confer complete resistance to BCMV and BCMNV. Many other breeding programmes are being implemented in Tanzania; a good example is an on-going programme that aims at developing multiple-resistance (including BCMNV and BCMV) bean varieties based on Mshindi, which is an improved variety obtained in crosses that involved the variety Kablanketi (Nchimbi-Msolla, 2013). Breeding for resistance against other viruses is rare. Although there has been no intensive screening for resistance against many different common bean viruses in Tanzania (but see Njau et al., 1994) there are landraces that remain symptomless in fields that also contain other plants with severe virus-like symptoms. This seems to indicate that local landraces might have resistance trait(s) to some viruses that exist in Tanzania. Screening for resistance against BCMNV (serotype A, as then called) showed that there were lines in the SUA germplasm collection that were resistant to isolates of BCMNV (Njau et al., 1994). BCMNV can be prevented from spreading through seeds by planting varieties with the dominant *I* gene. Plants with this gene are killed by BCMNV through the black root syndrome and so cannot contribute to the next generation (Grogan and Walker, 1948). Use of resistant materials is the most effective way to manage plant virus diseases worldwide. However, in most cases this is complicated by the ability of viruses, especially plant RNA viruses, to evolve so rapidly that they overcome resistance faster than breeders can release new varieties.

Control of vectors

Common bean viruses are transmitted by insect vectors such as aphids (potyviruses), whiteflies (CPMMV and BGMV) and beetles (SBMV). Being an annual crop, vector transmission of viruses in common bean is possible through occurrence of plants infected by seed-borne viruses and viruses harboured in alternative host plants, especially leguminous weeds. Indeed, low incidence of BCMNV in Malawi and southern parts of Tanzania was attributed to low vector population densities due to the high altitudes of these areas (Myers et al., 2000). Management of common bean vectors, when possible, would therefore prevent virus infections of plants grown from virus-free common bean seeds. However, the most devastating virus diseases of common bean in Tanzania are caused by BCMV and BCMNV, which are transmitted by aphids in a non-persistent manner (Vetten and Allen, 1991; Hillocks et al., 2006). The aphid vectors take a very short time to transmit these viruses to plants such that application of insecticides is not effective. The same is true for the non-persistently whitefly transmitted CPMMV (Brito et al., 2012), which has been detected in common bean in Tanzania (Mink and Keswani, 1987; Vetten and Allen, 1991). Conversely, pesticide use may prevent or reduce infection by persistently transmitted viruses.

There are cultural practices that can prevent vectors from transmitting viruses to common bean plants. For instance, planting early in the season helps the plants escape the high aphid population period (Buruchara et al., 2010). Mulching can reduce potyviruses infections and it is thought that the lack of bare soil reduces aphid landings during crop emergence and before the canopy has fully formed (Kirchner et al., 2014 and references therein).

Field sanitation and avoidance of alternative hosts

Vector-mediated transmission of viruses between alternative hosts and common bean plants has been studied, although not in Tanzania (Spence and Walkey, 1995). The inoculation of BCMV and BCMNV isolates from common bean onto other legume plants caused disease symptoms on the latter (Njau and Lyimo, 2000). This indicated that the BCMV and BCMNV isolates from common bean successfully infected certain wild legumes and could be transmitted back to common bean plants by vectors. Moreover, Myers et al. (2000) reported BCMNV natural infections in several cultivated and uncultivated legumes in Tanzania. Recently, CMV was detected in cucurbits (Sydänmetsä and Mbanzibwa, 2016). Therefore, transmission of viruses between common bean plants and alternative hosts is possible. Indeed, aphid transmission of BCMV and BCMNV from wild legumes to common bean, secondary hosts and wild legumes has been demonstrated (Spence and Walkey, 1995). It is reasonable to assume that avoidance of leguminous weeds in and around common bean fields and proper handling of crop residues would reduce the spread of diseases within and between fields. Indeed, removing weeds and non-common bean weeds has been recommended for management of bean diseases (Buruchara et al., 2010). Management of alternative hosts for common bean viruses is likely to be complicated by farmers' preferences for mixed cropping and the medicinal value attached to some potential alternative hosts. Field sanitation is, however, easy to achieve in areas where farmers produce QDS for marketing because QDS production involves, field isolation by distance and regular inspections by seed industry regulators, the Tanzania Official Seed Certification Institute.

CONCLUSION

This review aimed at assembling the information on virus diseases of common bean and the molecular characterization and detection of the responsible viruses in common bean in Tanzania. Also reviewed was the progress on management of common bean virus diseases. The writing of this review was mainly motivated by the fact that most studies on common bean virus diseases are inaccessible, fragmented and some are of unknown date; however, the information contained therein would be helpful in management of common bean virus diseases. It is acknowledge in the study that some literature might have been missed during writing this review but the information presented can guide in development of integrated disease management strategies. It is anticipated that this review will revitalize interest in studying common bean virus diseases beyond the common mosaic diseases (that is, BCMV and BCMNV) by identifying the gaps in research on common bean virus diseases, the neglected but economically important viruses such as CPMMV, CMV and SBMV)

It is evident from the information obtained from literature and stakeholder consultation concerning viruses and virus diseases of common bean in Tanzania that there is scanty information concerning distribution, occurrence, characterization and detection for nearly all viruses known to cause diseases of common beans in many other countries. Although viruses that infect common beans in other countries have been characterized at the molecular level and diagnostic tools developed, in Tanzania such information is rare and when available, for example for BCMNV (Silbernagel et al., 1986), is too old (30 years) and involves very few isolates. A sequence of common bean virus collected from Tanzania may have been missed because the origins of isolates were not supplied for all nucleotide sequence submissions; however, this would not appreciably change the results from the few sequenced isolates from Tanzania. Molecular characterization of viruses depends on availability of funds, which is affected by national priorities, political drivers as well as private donors' funding interests.

There was also scant information on alternative hosts, incidence and distribution of common bean viruses in the country. Such information is required to develop integrated pest management strategies. For instance, such information would guide deployment of specified resistant planting materials for different agro-ecological zones because information on occurrence of viruses and their strains would determine the appropriate cultivar to distribute to farmers in each area.

In addition, availability of information on genetic diversity would equip common bean breeders with the tool for decisions in their breeding programmes. Whereas several common bean viruses are known to cause indistinguishable symptoms on common bean plants, different genotypes of common beans may respond differently to viruses or strains of the same virus (Feng et al., 2014). Literature searching revealed a lack of knowledge on molecular characteristics of the common bean virus isolates in Tanzania; there was only one sequence and that was for BCMNV. Consequently, current identification of virus isolates for evaluating resistance of bean genotypes is based on response of differential cultivars. There is an inherent danger in this because viruses can cause unexpected phenotypes (Feng et al., 2014).

Since molecular characterization has not been done for viruses that infect common bean in Tanzania, it was recommended that NGS be considered and used in any future surveys for these viruses. This will enable detection of both known and unknown, DNA and RNA viruses. This can be followed by the use of ELISA and development of specific and degenerate primers for detection of specific or group of viruses using the sequences generated using NGS techniques.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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The decision making process on the adoption of innovations in the Brazilian Serra Gaúcha hills vitiviniculture: The case of Wine Producers Association of Altos Montes, at Serra Gaúcha/Rio Grande do Sul/Brazil (APROMONTES)

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This article discusses the decision-making process of Wine Producers Association of Altos Montes, at Serra Gaúcha/Rio Grande do Sul/Brazil (APROMONTES) winegrowers and winemakers, in their process of adopting innovations to increase their competitiveness. To do that, we used the Prospect Theory. Data collection was conducted through interviews with 12 wineries, 25 winegrowers of APROMONTES wineries, and, as witnesses, seven winegrowers, members of the Rural Labor Union (STR), from Flores da Cunha, Rio Grande do Sul/Brazil. The results point to the fact that both the wineries and winegrowers support their decisions on heuristics of judgment, and, wine producers use mainly the affect, representative, anchoring and adjustment heuristics, while the wineries used representative and affect heuristics. In this perspective, we identified winegrowers as "affective decision makers" whose decisions are based primarily on emotional aspects. On the other hand, the wine makers as "copier decision makers" whose decisions are supported primarily by observing the decisions of other wine producers: The practice of overflow.

Key words: Decisions, judgment heuristics, wine production sector.

INTRODUCTION

The Agro industrial System (SAG) Vitiviniculture located in the Serra Gaúcha/Rio Grande do Sul/Brazil hills products are facing strong price competition from products from other countries and even other producing regions in Brazil. Therefore, generating demand for innovations in the production processes and management, and also, the creation of new products that can increase the sector's competitiveness (Protas et al., 2002; Visão, 2025, 2006).

However, by adopting such innovations, the SAG sets

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new trading levels between the various links of the supply chain, since such innovations have - for winegrowers - the vineyards conversion character, planting specific varieties, in addition to the adoption of new production methodologies. These innovations result in higher levels of specific assets and greater complexity in the transactional process between winegrowers and winemakers, which could generate more uncertainty, due to the increased risk perspective. This can lead these agents not making the investments necessary to the innovation process, which may decrease the chances of SAG in a highly competitive environment (Paiva and Lentz Jr., 2011).

An important asset to this investigation is the discussion raised by Simon (1972, 1977, 1980) of rationality as a theory of human behavior. Simon (1977) moves the actions of global rationality model towards a bounded rationality perspective. This author (1977) proposes three levels of analysis: (a) To list all possible behavioral alternatives; (b) To determine all the future consequences to adopt each of these alternatives; (c) To compare these alternatives, which should be evaluated by the sets of consequences following each one of them. Torres Junior and Moura (2011) complement Simon's proposal by suggesting the following steps: (i) To understand the problem, its context and possibilities; (ii) To establish the criteria to be chosen according to the scope of the decision-maker/organization; (iii) To establish the alternatives as well as the influences of these alternatives in the defined objectives; (iv) To collect information to evaluate the consequences of each alternative as well as the possibilities to generate new alternatives; (v) To evaluate the alternatives considering the costs, estimated results, risks and other objectives; (vi) To choose and implement the defined actions and (vii) To follow the results, acting in the correction of problems, returning, if necessary, to the first step (understand the problem).

Looking at Simon's (1977) and Torres Junior and Moura's proposals (2011), we begin to understand how the decision-making process is cognitively complex. Therefore, another theory may be brought into picture to unveil the decision-making process on the adoption of innovations: The Prospect Theory.

The Prospect Theory

In an attempt to clarify the in and outs of Prospect Theory, Kahneman (2011) makes use of a working model of the human mind consisting of two systems: System 1 (fast) and system 2 (slow). The system 1 works automatically and therefore quickly, with minimum effort, and no perception of voluntary control. It has innate abilities, common in other animals, such as the ability to perceive the world, to identify objects, to drive attention to prevent loss and fear, to incorporate repetitive activities, associating ideas, to read and understand the nuances of social situations. All these processes are stored in our memory and are accessed unintentionally and without effort. Some automatic activities: Distinguish distances between objects, recognize a docile nature.

System 2 takes notice of effortful mental activities that require complex calculations. It is typically lazy, slow and logical, therefore, works on complex issues. It can build thoughts by ordering series of steps. These activities have common features. They require much attention and are suspended when that attention is diverted. Some examples: filling in the form of income tax, count the occurrence of the letter "a" on a page.

We can visually describe Kahneman's proposal (2011) as follows (Figure 1). Kahneman (2011) describes the associative process as another system 1 skill, whereas when we see, hear or feel something, in the end, we suffered some stimulus, we have as a result the called associative activation, in which ideas that were memorized activate many other ideas, like a twister shaking the brain, which psychologists call associative memory.

Kahneman (2011) points out that the system 1 is characterized by creating stories with causal connections from the perception of information which we are exposed and this is an automatic operation. This story with its causal connections is achieved by system 2 that accepts it.

The quickness of system 1 has already been approached but it is important to treat how this occurs and what is its consequence. Quick conclusions would have no major problems if there was a high probability that they were not wrong.

The stories we have built are the System 1's responsibility. They rely on activated ideas and are not recovered by the memory - as if they never existed. In other words, there is no possibility of using them. Kahneman (2011) highlights the fact the quantity and quality of information whereupon the story will be settled are negligible. Faced with this information, the system 1 is quick to draw its conclusions.

The author points out that the attitude would be towards the search for more information about the problem, what would be important to build a conception of the subject-matter being investigated. The first attribute is enough for system 1 to deduct and issue its conclusion, with all cognitive comfort and, when this system seeks congruence with system 2, which is slow, it finds out that the system 2 will secure the intuitive beliefs generated by the system 1. Kahneman (2011) created an expression to refer to the perception of disability beyond what is evident to System 1: WYSIATI (what you see is all there is).

Regarding the judgments we make about certain people or situations, Kahneman (2011) clarifies that we are capable of answering a multitude of questions that we are made by others or by ourselves. These questions are

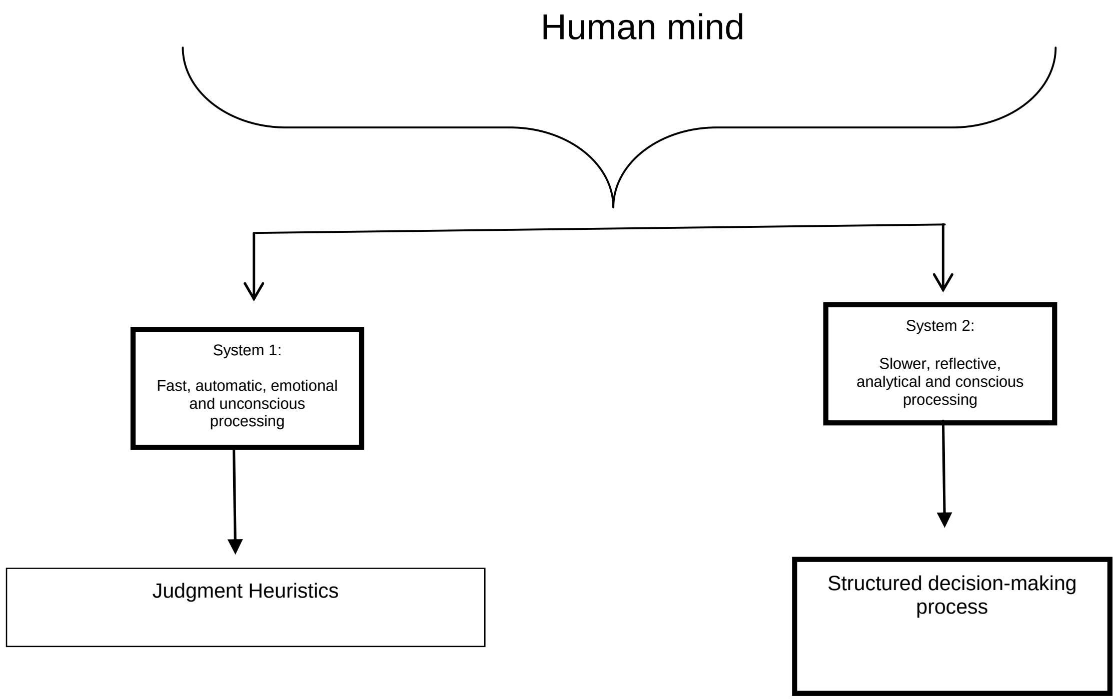

Figure 1. Mental Model based on Kahneman (2011).

sent to the system 2, which will conduct an investigation inside the memory in search of answers. But the system 1 proceeds continuously monitoring what is happening in the mind and beyond, continuously, generating ratings of the various features of the situation without specific goals and inferring little effort into it. It is precisely this basic assessment that is responsible for the intuitive judgment (heuristics), which takes the place of the most difficult issues.

Kahneman (2011) defines, technically, heuristics as a simple procedure that helps getting appropriate responses, even incomplete, for complex issues. Among the heuristics, the author lists the representation, where the probability in which things, people and events are evaluated, following the criterion of the degree to which is representative of, or similar to something is already known. That is, studies prove that events that should be assessed according to their probabilities are evaluated by the similarity and representativeness.

This type of trial, according to Kahneman and Tversky (1974), leads to significant errors, given that neither the similarity nor the representativeness is influenced by factors that interfere on the probabilities of course trials. That is, they are not settled in data probabilistic basis.

According to Kahneman (2011), the availability heuristic is a cognitive procedure (trial heuristics) in which people estimate the frequency of a class or the probability of an event by the easiness in which instances or occurrences can be brought to mind. It is the confidence in which the elements come to mind. When we encounter a specific feature, any figure immediately comes to mind that represents these characteristics. This availability may be favored by the frequency that such events are presented, like the media exposure for instance.

Imagination is also responsible for a significant bias in the probability assessment in real situations, because if some difficulties are perennials in our mind, it is possible that the situation could be impregnated by these difficulties. However, these difficulties do not have the actual probability of their occurrence.

Another device of availability which can lead to a bias is illusory correlation, in which two events that have a high mental frequency can occur simultaneously via the associative bonding, leading to an idea that there is a correlation between them.

There are situations in which the evaluation process for decision making is given by estimation of the initial values that ranges toward a final result. This initial rate can take values depending on the questioning or a partial calculation to provide that value. Moreover, these different starting points yield different estimates, which produce bias towards the initial values. Kahneman and Tversky (1974) call this process the anchoring phenomenon or heuristic adjustment and anchoring. This phenomenon can be seen when the individual is exposed to a value or is based on some incomplete computing at the beginning of its assessment, which can lead the individual to insufficient adjustments.

Another important issue, addressed by Kahneman

(2011) are the emotions, because they surround our daily lives and our decisions. The author leads us to reflect on the fact that, as the effect of emotions, there is a more pronounced dominance of the findings on the arguments. The author presents the psychologist Paul Slovic, who proposed the affect heuristic.

In this heuristic, people allow their empathy and repulsion to influence their beliefs about the world. logically system 2 can intervene in this process by the self-criticism, but, according to Kahneman (2011), the system 2 acts more as a protector of system 1 than as a critical analyst of emotions involved. System 2 searches for information, but this search turns more to the endorsement of beliefs than to an intention to evaluate them in all respects.

Another aspect addressed by Kahneman (2011) is the halo effect, that is to assign greater weight to first impressions, disregarding, in many cases, subsequent information, or selected information that may corroborate with those who received greater weight.

But the framing effect was first described by Kahneman and Tversky (1984) and later worked by Kahneman (2011). The emotional aspect is very important in the framing of questions. To exemplify that Kahneman (2011) describes Amos Tversky's experiment, conducted at the Harvard Medical School. There, doctors received statistical results on two different lung cancer treatment: (a) surgery and (b) radiation. The survival rates were higher after surgery although riskier than radiation within a five-year treatment. Half of the doctors were given the following information: "The survival rate in the first month after surgery is of 90%" and the other half received: "The mortality rate in the first month after surgery is of 10%".

The result was that 84% of the doctors in the first group decided for the surgery against 50% in the second group. Kahneman (2011) argues that the information of survival is more reassuring than the information about the mortality rate even for trained professional such as doctors. In other words, we tend to focus on what generates less emotional damage.

Therefore, this author points out that different ways of presenting different information usually recall different emotions. Kahneman (2011) highlights that reframing is laborious and system 2, which is responsible for this alternative, is slow and "lazy."

In terms of decision based on structured processes to adopt innovations, scope of this investigation, we follow the perspective in Figure 2.

Considering this perspective, this paper discusses how the decision-making processes take place in the productive relationship that includes both wineries and winemakers, using the prospect theory as a theoretical benefit.

MATERIALS AND METHODS

In this paper, we adopted a qualitative methodology (PATTON, 1986) that allowed capturing the stories of life experiences, especially with regard to production and industrial processes surrounding the agribusiness systems, in this case, the wine industry of the Brazilian Serra Gaúcha, in its aspects of negotiation and decision to invest in technological innovations. Data collection was personally conducted by researchers in the region spanning the APROMONTES (Montes Altos Wine Producers Association), in the Serra Gaúcha hills, Rio Grande do Sul/Brazil, where the wineries are and winemakers are located (Tonietto et al., 2013).

Definition of the sample

This study had two phases. In the first phase, we conducted semi-structured interviews with the leaders of each of the wineries associated with the APROMONTES (Montes Altos wine Producers Association). In the second one, we raffled two winegrowers linked to each of the wineries, with two exceptions: (a) One winery whose grape production is verticalized, and (b) The winery V12. In this second case (V12), we interviewed not two but four winegrowers because of their proximity with the winemakers. Besides, we also interviewed winegrowers associated to the Rural Workers Union (STR) randomly as they entered the STR agency in the city of Flores da Cunha, Rio Grande do Sul, for the payment of their health plans.

Our sample resulted in the completion of 43 (forty-three) interviews with a total duration of 45 (forty-five) hours, distributed in twelve (12) wineries surveyed, totalizing 16.61 h. Twenty-five winegrowers associated to each of the wineries of APROMONTES answered the questionnaire totalizing 24.41 h. And, as witnesses were heard seven (7) members of the winegrowers STR (Rural Workers Union) of Flores da Cunha and Nova Padua) with whom we spent 3.96 h.

Analytical procedures

To process the data collected in this study, we used NVivo software (QSR, 2011). According to Ames (2013), the basic structures of NVivo are the Document System, where the sources of the data collected in our research are located and the Index System which contains nodes, according to a pre-defined classification. According to Ames (2013), these features make possible to insert, organize and sort our data by generating matrices, charts, analysis and consultation models, either by source, by node or by key words.

In the classification of the information collected, the sources were stratified into three categories: (a) Wineries that bring together the twelve winery members of APROMONTES; (b) Linked winemakers, representing the wine growers who provide grapes for each of the twelve wineries that were chosen, and; (c) Randomly chosen winegrowers in the STR (Rural Workers Union of Flores da Cunha) who had no relation to any of the twelve wineries linked to Apromontes.

The review process was owed to the hearing of the interviews and the coding of the relevant passages in their respective category analysis (node).

Finally, the encodings were evaluated according to their density as a percentage of the total of the interviews, allowing their analysis and subsequent link of their references.

The following tables shown are from the general framework of discourse density, aiming a better explanation of each of the subjects covered. The general framework for discourse density has a 100% density in each of the corresponding columns to the wineries, winemakers and winegrowers linked to the STR. To identify the participants and their discourse in this study, we use the following index system proposed by NVivo software:

<Internal \\ V062-Winegrower> - § 2 coded references [5.81%

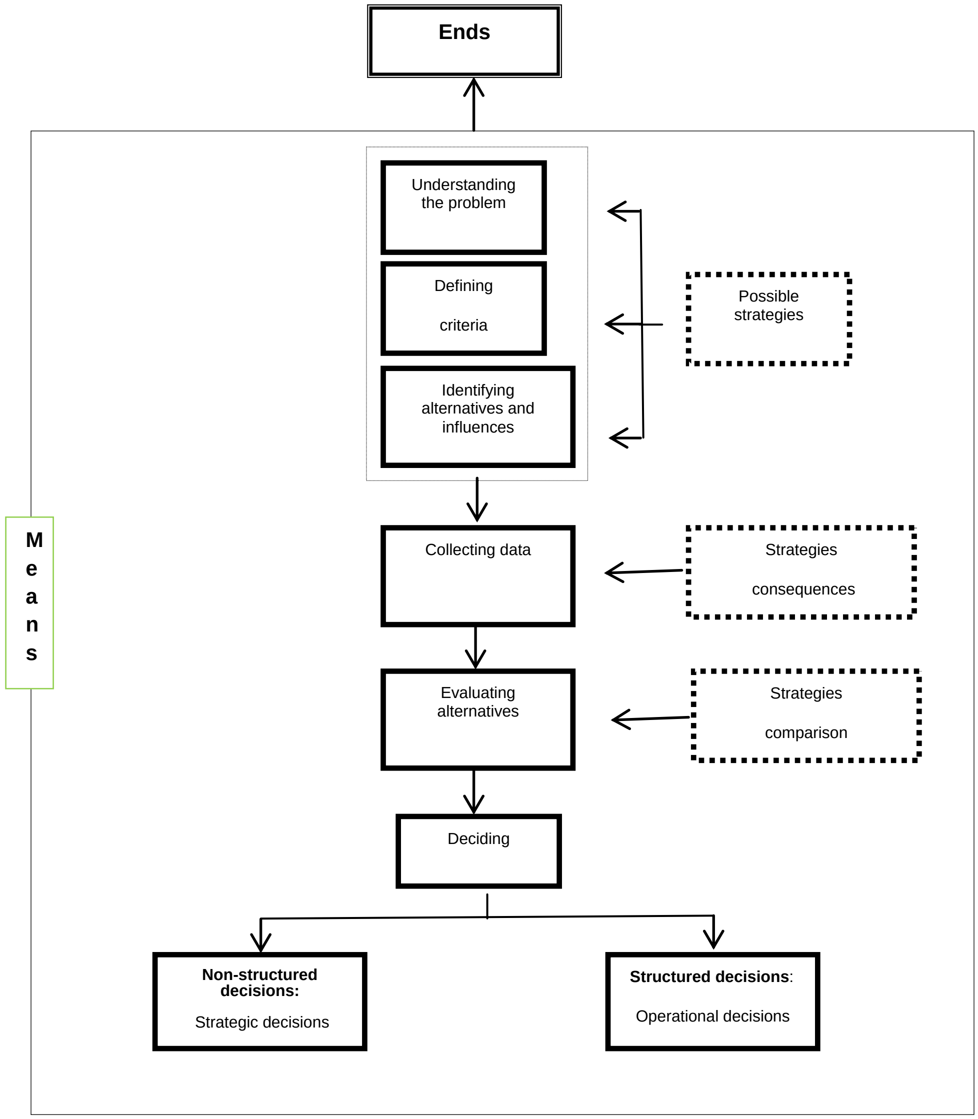

Figure 2. Decision-making model based on Simon (1972, 1977, 1980) and Torres Junior and Moura (2011).

Coverage]
Reference 1 to 4.09% Coverage

This index system can be read as follows:

<*Internal*: The data (interviews) inserted in and processed by the software
V062-Winegrower: The winegrower number 2 from the winemaker 6
§ 2 coded references [5.81% Coverage]: Two coded discourses which cover, in terms of time, 5.81% of the participant' discourse
Reference 1 to 4.09% Coverage: The first reference (discourse) covers, in terms of time, 4.09% of the participant's discourse.

RESULTS

The general results in the decision-making process on the adoption of innovations in the action area of the wine industry of APROMONTES can be seen from Table 1.

One can observe that there is a predominance of the affect heuristic in both groups of winegrowers at very close levels, and the winegrowers linked to the wineries have a value of 35.46% and STR 35.87%, while the wineries present a number of 14.29%. Examples of how to define emotional issues decisions in this type of heuristic can be viewed in the transcript of V062 and then V2:

<Internal \\ V062-Harvesters> - § 2 coded references [5.81% Coverage]
Reference 1 to 4.09% Coverage:

Mr. John Doe does not need to sign anything to me, if he

Table 1. Decision heuristic and structured decision.

Description	STR Winegrowers (%)	Wineries (%)	Winegrowers linked (%)
Affect heuristic	35.87	14.29	35.46
Anchoring and adjustment heuristic	33.51	7.99	28.63
Representativeness heuristic	28.7	16.48	22.5
Availability heuristic	1.92	4.74	1
Understanding the Problem	0	30.21	6.22
Identifying alternatives and Influences	0	11.36	4.36
Collecting Information	0	4.44	1.29
Selecting criteria	0	10.48	0.55

tells me to take the grapes there I take them. I do what they ask me to. They have asked me to do things to increase the quality and I believed them. Our part is done, we produced quality, but the problem is that we delivered grape with 15 degree that should get a price of R$ 0.57, but they paid R$ 0.49 or R$ 0.50.

<Internal \\ V2 Winery> - § 3 coded references [11.82% Coverage]
Reference 1 to 1.73% Coverage:

My relationship with producers is good because we are all neighbors and its a long term relationship. Of course there are cases that we have to negotiate the prices because the table wine is R$ 0.70; we cannot afford to pay R$ 0.57 for the grape.

We can see that even the winemakers are used to affect heuristic, although at lower levels (14:29%), but one can still verify the occurrence of this decision-making procedure, as it can be seen in the winery V2's discourse already presented.

Fragments of discourses previously presented fit the contributions of Kahneman (2011) related to the affect heuristic, in which people consent that their emotions influence their assertions about a situation. The author points out that there is the possibility of the individual performing a self-criticism, seek information, but these are almost always selected to ratify the decision influenced by the load of affection.

A second decision-making heuristic identified in the interviews is the Anchoring and adjustment heuristic, which presented more relevance among winegrowers, whether associated or STR, with 28.63 and 33.51%, respectively. The wineries presented a number of 7.99%. The following is an excerpt from V031:

<Internal \\ V031-Harvesters> - § 1 coded reference [8.62% Coverage]
Reference 1 to 8.62% Coverage:

To adopt this innovation, we made a test; we did a piece to see how it worked. The climate is complicated, so we have to have these alternatives. A problem in one year prejudices the other years because the vine gets weak. Every year we do something different, planted pieces of new varieties to see how it works and produce different things and then apply to the whole if it works.

The above references point to situations in which the decision is based on the estimation of values ranging towards a result. The point is that this initial value is related to questioning the capacity or the existence of information that allows partial calculations that lead to value. Thus, different starting points yield different estimates, which produce biased toward the initial values. It is precisely this process that Kahneman and Tversky (1974) call the anchoring phenomenon, which can be verified by the extent that the individual is subjected to an incomplete value or at the start of their evaluation, which can take one to insufficient adjustments.

By analyzing the discourses of winegrowers, we notice that their anchoring and adjustment heuristic became intensified with regard to their vineyards, since the decisions are given at a time "t" and the results will be checked in a "t + x", where "x" is characterized by a period of more than a year, which is typical of perennial crops. Besides, as the winegrower (STR1) pointed out, climate variations favor or require adjustments and docking procedures. In turn, the wineries do not present, over their discourses, evidence that the decision to rely on anchoring and adjustment process is in the same proportions as the winemakers, and the discourse density is around 25.72% of the average observed on both associated winegrowers and on STR.

Another operative heuristics verified throughout our interviews was the representativeness heuristic. Although it was the third in the scale percentage of density greatness, it showed a higher proportion comparing the wineries to the average of winegrowers (associated and STR), getting around 64.38%, while in the foregoing heuristics this ratio was 40.07 and 25.72% for heuristics affection and adjustment, and anchor respectively. In our view, it is relevant because it shows that the wineries rely strongly in the heuristics of representativeness, which can be seen in Table 1, in which the associated winegrowers had a 22.5% density for heuristic representation, while the STR growers showed 28.7%. Already the wineries had 16.48%. Here are some references (the STR3, V022 and V11) on the representativeness heuristic:

<Internal \\ STR3-Winegrower> - § 2 coded references [15.88% Coverage]
Reference 1 to 4.61% Coverage:

There is no information regarding that, I hear that's bad market, but who knows. My decision to cut the Coder is because everyone is cutting because it is worthless.

Reference 2 to 11.27% Coverage:

I bought the equipment because the neighbors bought it. What I saw in the neighbor and I thought it would be nice is irrigation, but we do not have water. I saw this covered grape in the neighborhood, Niagara, we are thinking about it ... but still we did not speak with any buyer to see if there is any interest. But I will do the same because the way we are ... I'm observing that those who planted are doing well, who knows, you know, by the time I plant it can go wrong, it seems that we are always late.

<Internal \\ V022-Winegrowers> - § 2 coded references [14.63% Coverage]
Reference 2 to 6.56% Coverage:

We did a study of various alternatives and chose one. But we always observe the others to see if it works. We started Irrigation because we have used it in other cultures (garlic) so we did it for the vine.

<Internal \\ V11-Winery> - § 2 coded references [9.86% Coverage]
Reference 2 to 3.85% Coverage:

In our region, there is a culture of copying and being larger than the other. On one side it is good, but it can be dangerous as it can lead to take a step longer than the legs. But I have to agree that it does go forward. We thought enough alternatives, not just specific things to solve a specific problem. It does not always work.

We can observe that, in the discourses presented earlier, in which we demonstrate the representativeness heuristic, the evaluation criteria of things, people and events are based on a representative system, or do not follow a probabilistic criterion, often, not even follow simple return analysis criteria.

This type of heuristic decision, according to Kahneman and Tversky (1974), can lead to significant biases as similarity and representativeness are not good elements, given that they are not influenced by factors that, of course, interfere in probability judgments because they are not supported by data probabilistic basis. This can be seen in the V12 discourse (Reference 2 to 4.09%):

<Internal \\ V12-Winery> - § 3 coded references [8.81% Coverage]
Reference 2 to 4.09% Coverage:

Many wineries do not even have a cost sheet let alone the search for this kind of information. There are many wineries that plant focused on volume and insist on entering the high-quality wine niche. This at first cannot be achieved.

The initiative to access vinifera markets is an example of representativeness heuristic, in which the decision was made by the idea of similarity and representation, as illustrated by V12 discourse (Reference 2 to 4.09% coverage). There is no use of data to support the decision, only perceptions that generate evidence for others, thus forming a halo effect, which takes proportions that generate cyclical crises, as seen highlighted in V2 (Reference 1 to 3.14%).

<Internal \\ V2 Winery> - § 3 coded references [15.66% Coverage]

Reference 1 to 3.14% Coverage:

In 1995 the vitiviniculture exploded and they planted, planted. Many who planted vinifera grapes are having to sell for common grape, they will finish spoon and cut out. Then they begin to grow again. See that there is a mismatch between the planting and the market. That is cyclical, every 10 to 15 years that happens. There is a disruption between the wineries and producer. When the market begins to improve, it is time to plant new vineyards.

Kahneman and Tversky (1974) refer to the issue of representativeness, in which there is insensitivity to predictability, which stems from the way people anticipate situational descriptions. These predictions happen by representation, for example, in the case of a neighbor whose description is favorable, and harvest well and makes money from the culture, which indicates that the activity is promising. On the other hand, if the description of the neighbor is not favorable, their initiatives will not be considered. There is a tendency to ignore the degree of reliability of this description, making people rely only on the suitable aspects of the information, generating previsions that do not guarantee the accuracy of the results.

By analyzing the data from our research, the availability heuristic has not been identified over the discourses with relevant intensity, compared with the previous ones (Affect, Anchoring and adjustment and representativeness), for both the group of associated

Table 2. Presence of information.

Description	STR winegrowers (%)	Wineries (%)	Winegrowers linked (%)
Fairs	0	1.2	0.28
Business trip	0	0.38	0

winegrowers and the STR, the density percentages were around 1 to 2%, a weighted average of 1.47%, whereas this heuristic (availability) was observed in the group of wineries 4.74%, which also is not a significant number. However, this value (4.74%) is three times higher (3.22) than the average of winegrowers (1.47%).

This information is at least curious because, conceptually, the availability heuristic, according to Kahneman (2011), is defined as a cognitive procedure in which people estimate the frequency of a class or the probability of an event by the easiness in which cases or occurrences can be brought to mind. In other words, the confidence in which the elements come to mind. When we encounter a specific feature, we think immediately of any figure that represents these characteristics. This availability may be favored by the frequency in which such events are presented to us, and this is directly linked to the informational level we have. Let's see some references given by V5 on the subject:

<Internal \\ V5> - § 3 coded references [10.13% Coverage]
Reference 3 to 1.38% Coverage:

The idea of attracting by the package came from travelling, where we found that the packaging appeal segments the market, each package for a different market and wine compatible with every type of consumer. For example, we launched the LA Young Wine, using wine of fresh years and low alcohol content to reach young consumers and prepare them for other types of wine.

The V5 discourse (Reference 3 to 1.38% Coverage) shows that the wineries have access to different types of information from the winegrowers and this allows them to develop different mindsets in number and complexity. Table 2 obtained from the analysis of discourse density shows that the wineries have 81.08% density of participation in fairs while winegrowers had a density of only 18.92%. The Excerpt from V10, below, shows an example of participation in fairs.

<Internal \\ V10> - § 3 coded references [9.35% Coverage]
Reference 3 to 1.45% Coverage:

Every two years, we go to Italy, to the Simei show and to the Vinitaly. Anyway, we are always looking for information. We search in Germany, Italy and France about machines because there we have the best in the beverage industry.

When we look at the business trip participation, the wineries have a 100% density. Let us look at the references regarding the V1 and V5 trips:

<Internal \\ V1> - § 1 coded reference [0.85% Coverage]
Reference 1 to 0.85% Coverage:

I get informed through contacts with producers in Europe.

<Internal \\ V5> - § 2 coded references [14.06% Coverage]

Reference 1 to 5.43% Coverage:

I have just been to Italy and there, there is this vertical integration. Most industries produce their grapes to produce their wine. This is a culture that comes from the past. The few winemakers have organized themselves into cooperatives to produce the wine. So, Europe is different. The Argentine is a mixture.

Reference 2 to 8.63% Coverage:

We seek these innovations visiting other countries. For example, when we visited Spain, I saw that they trimmed soon after harvest. When I came here I met with our technician and discuss the importance of leaving the branches on the vine until August. He said no. I asked what was the problem of cutting now, after the harvest? He said he thought it was okay, so we made a test. That is how innovation happens. It resulted in a good product, hand labor rationalization, cost reduction and specialization of staff. Now Embrapa [Brazilian Government Reseach Company] is researching the subject. With the bottles was the same thing, our staff had been researching and came up with the idea. We have to understand that the wine industry in Europe has more than 1000 years, and their reality is different from ours.

The fragments of discourses presented above serve as evidence wineries are more exposed to different information provided by participation in fairs and business negotiations. These activities allow the wineries to adopt three times more availability heuristic than the winemakers, because the information collected along these experiences assume the conformation of cognitive frames that are more easily accessed and incorporated into its heuristic decision processes.

We can go back to the Structured Decision-Making Processes which are: (a) Understand the problem; (b) Define the selection criteria; (c) Identify the resolution alternatives of the problem and its influences; (d) Collect information, and (e) Evaluate alternatives.

We can see that when we look at the density of the wineries discourse, 30.21% of the time is geared to understanding the problems to which the wineries are exposed. See example of V10:

<Internal \\ V10> - § 2 coded references [4.20% Coverage]
Reference 1 to 2.02% Coverage:

Every innovation we want is discussed in the winery and in the sector. The tendency is that the winery will seek for vertical integration of the high-quality products or look for suppliers that are legalized as a company.

Reference 2 to 2.17% Coverage:

We want to be a small winery with great potential.

We emphasize that the criteria to qualify the discourse as a problem of understanding lies on the clearness in which the individual sees the situation, regardless of whether it would be the ideal explanation. In this sense, the linked winegrowers had a 6.22% density, as demonstrated in the examples of V102:

<Internal \\ V102> - § 4 coded references [7.60% Coverage]
Reference 3 to 1.49% Coverage:

One issue that would have to work is to adjust the workforce to the area of the vine. What is happening is that there are not enough workers, then we hire. Besides the fact the workers hired are expensive, they are also unqualified and because they do not harvest or prune right, the profit that area would earn is lost. So, there is no use in having that area anymore if you do not have manpower to work on it. Then you should reduce the area to match the volume of available workforce.

Regardless of the nature of the problem of winemakers, references presented demonstrate that they can identify it clearly. However, it is a small value compared with the decision heuristic. In the case of the STR winegrowers, it was not identified any of these issues in their discourses.

Further, we can see the most formalistic part of a structured decision process consisting of the definition of the chosen criteria, the identification of alternatives to solve the problem and the evaluation of the consequences. Again, these are procedures identified with greater density in the discourse of the wineries. However, in the discourse of the associated winegrowers, these values are much smaller.

In wineries, the collection of information is 4.44% *of the density of their* discourses, the survey of alternatives and influences *are 11.36% of the density of their* discourses *and the definition of the chosen criteria is 10.48% of the density of their* discourses. In turn, associated winemakers present in the same sequence, 1.29, 4.36 and 0.55%. We have not observed any of these categories in the discourses of the winegrowers of STR.

Another possibility of seeing Table 1 framework data would be checking the density of discourses horizontally, view which shows the density percentage when we see the sum of the interviewees talking time for each category of analysis. This can be confirmed by the information presented in Table 3.

We can identify, through the density of discourses, that the winemakers have their decision-making processes based on heuristics, including affect heuristic that has higher density, demonstrating that 75.39% of the time dedicated to this category exposed winemakers. The wineries showed a percentage of 24.62%. It is important to notice the supremacy of the values of associated winegrowers in relation to the STR winegrowers. There is evidence via discourse density that STR winemakers interviewed are using heuristic decision making.

However, lower values related to its associated pairs can be explained because they are apparently not as articulated to expose their views as the associated.

One can see that the anchoring and adjusting heuristic is a decision-making process very present in the winegrowers' discourse, with the highest density of 81.89% of the time. The representativeness heuristic also has a strong impact on the decisions of winegrowers, spending 63.61% of the time devoted to it. Although the horizontal analysis of the availability heuristic ratifies the vertical analysis, which demonstrated the increased use of this heuristic by the wineries: 77.30% against 22.7% of winegrowers. It is worth having in mind that the values in the vertical analysis were not significant.

Concerning the structured decision-making process, if we use simple average, the vertical analysis of the discourse density, we can see that the wineries respond for 56.49% of the discourse density of these categories while winegrowers respond for 12.42%. However, we must emphasize that from these 56.49%, 53.48% are related to the category understanding the problem (30.21%). Alternatives and influences appear with 11.36% and criteria selection with 10.48%. However, the collection of information comes up with 4.44% of the interviewees talking time. The categories of analysis that constitute the formal part of the structured decision-making process are not supported by a consistent information process.

Table 3. Decision heuristics and structured decision (analysis in the line).

Description	STR winegrowers (%)	Wineries (%)	Winegrowers linked (%)	∑ Winegrowers (%)
Affect heuristic	10.81	24.62	64.58	75.39
Anchoring and adjustment heuristic	13.28	18.11	68.61	81.89
Representativeness heuristic	11.08	36.39	52.53	63.61
Availability heuristic	5.49	77.31	17.21	22.7
Understanding the problem	0	82.11	17.89	17.89
Identifying alternatives and Influences	0	71.14	28.86	28.86
Collecting Information	0	76.52	23.48	23.48
Selecting criteria	0	94.74	5.26	5.26

Table 4. Supporting elements in heuristic decisions.

Description	Halo effect	Framing effect	Neglect of the rate base	Overconfidence
Affect heuristic	32.43	37.71	31.24	56.81
Availability heuristic	5.73	0	2.58	0
Representativeness heuristic	42.21	32.47	36.34	32.41
Anchoring and adjusting heuristic	19.63	29.82	29.84	10.78

If there is not a set of information available to support decision-making process what is left is what Kahneman (2011) calls WYSIATI (What you see is all there is), a term created by the author and which reflects the perception of what is evident in System 1 (autonomous System), as it provides consistency and cognitive comfort, which leads the individual to accept an assertion as the truth, leading to a plausible attitude. This lead the way towards the heuristic decisions.

Supporting elements in heuristic decisions

According to Kahneman (2011), jumping to conclusions can be effective to the extent that there is a significant probability that the conclusions are correct, and if the costs of a possible error are plausible and if this assumption saves time and effort. Presuming is dangerous in unfamiliar situations because there is much to be lost in the game and there is no time to gather more information. This is an intuitive scenario where errors are likely to happen. They can be avoided with a deliberate intervention of system 2 (system consideration). However, by the lack of information of a comprehensive context, the system 1 (automatic system) generates its own context, supported on experience. It is like a bet, in which predominates the rule of support on recent events that have a higher weight in the understanding of a new situation.

In this regard, Kahneman (2011) also points out that, contrary to what the philosophers of science (who propose the testing of hypotheses to reject them), people and the scientists themselves sometimes get hold of data that have greater possibilities of being reconcilable with the beliefs that they have at that time. However, Kahneman (2011) points out that this decision process generates some biases, some effects (Table 4).

None of these supporting elements are relevant to the availability heuristic, which may have been compromised by the low density observed in this category, as has been shown previously. Regarding the neglect of the rate base, it has a slightly lower density in the anchoring and adjusting heuristic (29.84%), a fact that, in a way, brings surprise, because even if it is a heuristic working with small numbers (gradual adjustments), we found a considerable degree of negligence about the trends of information series.

The overconfidence aspects are also more observable in the affect and representativeness heuristics, with a density of 56.81 and 32.41% respectively, which is predictable because, according to Kahneman (2011), the overconfidence as well as the halo effect and the effect of environment are also a manifestation of WYSIATI. This happens because when we estimate a quantity or situation, we rely on the information that comes to mind and build a coherent story in which the estimate makes sense. This is because it is impossible to admit information that does not come to mind for specification of that information.

This overconfidence is not something that primarily affects only people with no information or little information. Kahneman (2011) presents numerous cases of recognized experts from the financial world, medical professionals and the legal world. The author argues that training activities have been tried to mitigate the problems of overconfidence, however, does not consider as something promising. An example Kahneman (2011) quotes is the case of judges who were encouraged to consider competing hypotheses. However, the author points out that overconfidence are a result correlated to the System 1 (automatic), which can be suppressed but not eliminated. This is because overconfidence is based on the coherence of the story that the individual built and not by the quality and quantity of information that supports it.

DISCUSSION

The survey results point to a preponderance of affect, anchoring and adjustment and representativeness heuristics by the winegrowers, which is also present in the decision making of the wineries. This is a predictable result, according to the ideas of Kahneman and Tversky (1979), and Kahneman (2011), because the heuristics are simple procedures that help get satisfactory answers, even if imperfect, to complex questions. That is the case of the decision to adopt certain innovations.

These decision heuristic (judgment) often serves as a reasonable approximation of reality. However, they can generate, as previously mentioned, biases caused by the halo effect, which is characterized by a tendency to like (or dislike) everything that relates to how we see people and situations, even those that were not observed. Biases can also be generated by overconfidence, due to the neglecting of evidence, because what counts are the beliefs that are related to the quality of the narrative, which takes into account what is seen, even if it is little.

In this study, we observed biases arising from the framing effect, they are different ways to display the same information and repeatedly evoke different emotions in addition to the neglecting of the rate base. This leads one to ignore statistical facts to consider issues such as the neglect case of information about the historical sequence of production and product demand (either grape or wine), information on grapevines planted area.

These are biases that were significant in our research, leaving evidence of heuristic decision cognitive processes undertaken by winegrowers when negotiating with winemakers. They also have their own heuristic decision-making processes, which leads to incompatibilities that may hinder the decision to adopt a desired innovation, since the framings of these parts are not aligned.

Although Kahneman, Tversky (1979) and Kahneman (2011) did not work on which heuristics generates certain biases, we found in our work, a strong alignment between the halo and framing effects and the affect and representativeness heuristics, which seems to have a logical, given that "like" or "dislike" relates to the affection and the way we see certain things become representations of reality. These are valuable issues in our research, because, like we discussed in the analysis, the decisions of winegrowers and winemakers are constituted primarily as affect and representativeness heuristics and they suffer direct influence of "liking someone or something" process, as in the case of a winegrower that likes or dislikes certain winemaker, or the degree he likes his activity or some form of production.

This issue of "liking" a way of producing, when contrasted with the high incidence of path dependence, elucidates the fact that the choices are conditioned by the choices made previously, which becomes familiar and representative in the subsequent periods. According to Kahneman (2011), our automatic system (System 1) will remove the ambiguity and, unconsciously, will build a story that is as consistent as possible in order to endorse the decision towards the actions or people already known. If there is no message immediately disapproving the previous concept, the association of this with the past or the first impression about the person will spread as if the message was true.

The conjunction between heuristic decision (in our case the affect and representativeness) with the path dependence defines a vision of technological opportunity restricted to issues of quality of the raw material (framing), which, by the way, is sought by the wineries as the key to increase competitiveness, which is being adopted by the winemakers. However, other technological, production and management opportunities are not addressed and, when they are, the participants of this research come up with some kind of "story" to justify excluding such opportunities.

The observed decision heuristics are also enhanced by the practice of overflow (spillover) and in this case, it revealed the halo effect because the concept that a winemaker or a winegrower has within the community creates the conditions for the others to follow him, even before the specific characteristics that, perhaps, might exist. According to Kahneman (2011), even though the situation remains ambiguous, it will be interpreted in a way that makes them consistent in the context.

Another issue that seems to support the affect and representativeness heuristics regards the specific characteristics of the assets, which, in this case, focus on the physical characteristics, because the terrain and weather restrict production to the vine and to the human specificity. This is because there is a tacit knowledge (know-how) that gravitates around the vine, acquired primarily from learning by doing. However, we are proposing here extending human asset specificity, incorporating an affective dimension, personified in the bond of the "individual" to its production object (vine) because, during the research, we found this bond through discourses like "the vine is very clever, more than you, because it teaches you to prune " and "The vine is as a person, she talks ...".

This type of asset specificity appears to be a kind of catalysis when adopting affect heuristic in either accepting or rejecting decisions to innovate. We add that the affective dimension of human specificity favors a greater or lesser degree of representativeness heuristic, because this is an observable sense in other winegrowers, which provides that the winegrowers identify with each other when making a decision.

Affect heuristic proved to be so intense during the research that we could observe that construct “trust” was adapted by the operant actors in the sector to account for the necessary transactional relationships. This adaptation is polarized in trust for the payment because the participants recognize that what is discussed in terms of payment and product delivery will be fulfilled and the existence of distrust to establish a fair price for the grape, understanding that there is an opportunistic behavior in that dimension. This conceptual adaptation is a "story" suggested by Kahneman (2011), in which one creates a context that considers an observable reality. This is due to the fact that there is a need to transact the production. However, the transactions require the presence of trust, but that does not exist in its scope. Therefore, it is necessary a mental construct that gives greater cognitive comfort when making decisions and this is made possible by the two trust dimensions presented: (a) Allowing the transaction (I trust that they will pay) and (b) One that can be "saved" not to mess with the transaction (they take advantage in price). This is a procedure also checked with the winemakers, who trust that winegrowers will do what is asked of them in vineyard. However, they do not trust that the grapes will be fully delivered to the winery. Another important aspect regarding the use of heuristics decision relates to the primacy of one category over the other, because we observed that one does not exclude the other. The heuristics may occur simultaneously or sequentially. We see this fact in using affect heuristic in a position to adopt innovations, followed by anchoring and adjustment heuristic, whose meaning is to correct observed deviations in the initial decision supported by he affect heuristic and, once implemented, creates a new starting point (anchor) for subsequent phases. Taking up the issue of decision-alignment on the adoption of innovations among winemakers and their winemakers, we notice that although the wineries also support their decisions to innovate on heuristics, their understanding of the problem to be faced is superior to wine producers in a ratio of about five to one (30.21 vs. 6.22%), in addition to that, wineries articulate better the survey of alternatives and the influences they cause and decide, considering better defined criteria for choosing than the winegrowers. However, the lack of gathering and handling information weaken the decision-making process.

On the other hand, the understanding of what is the problem to be attacked creates a mismatch between the decision-making processes of winemakers, in relation to its winemaker suppliers of raw material (grape), because this disparity generates a framing effect that is, if not divergent, in a different level. Thus, any movement towards adoption of innovation to improve competitiveness by the wineries is understood by winemakers as something centered only on production quality. What is worse reduced to produce grapes with glucometric degree measured in Babo scale degrees. This divergence frame (framing) creates an environment of distrust, both by the wineries in relation to winegrowers as winegrowers in relation to wineries. This can be verified as we review the density of this lack of confidence, which ranges from 5% by the wineries to 3.6% by the winegrowers. This environment creates side effects that also interfere in the decision to adopt innovations.

The considerations presented here refer to the idea of classifying both winegrowers and winemakers in the decision-making process in terms of adopting innovations. In this sense, we suggest that the winegrowers are "affective decision makers" whose decisions are based primarily on personal emotional aspects or by recurrent processes of how to develop a particular activity. On the other hand, the winemakers are characterized as "copier decision makers" whose decisions are supported primarily by observing the decisions of other winemakers, that is, by the practice of spillover, without disregarding the existence of the innovative winemaker, following the Schumpeterian (1985) innovative ways that will anchor the others. This classification enables us to reflect what kind of action should be adopted to implement a new technological trajectory that enables an increase in the competitiveness of the Brazilian gaúcha wine industry, given that there is a need for a decision-making alignment between winegrowers and winemakers in planning adoption of innovations, since the vertical integration of production by the wineries is prohibitive because of the restriction of the areas for implementation of new vineyards in the Serra Gaúcha region that concentrates more than 90% of the wineries of the Rio Grande do Sul State. Therefore, the prospect that winegrowers would be more emotional in their decisions leads us to act exploring more this peculiarity. At the same time, actions aiming the winemakers should take into account that their decisions are taken supported by the observations of what the other winemakers are doing.

Conclusion

Qualitative researches are often criticized for producing biased analysis. However, a previously tested method may minimize this risk. In our case, we invested on software for data analysis - Nvivo (QSR, 2011). This software provided the organization of data in an independent way for each of the investigated information sources.

Other limitation this investigation had to handle was the fact this is a case study (Yin, 2010). Case studies have the disadvantage of reducing the possibilities of generalizing results. In this study, we have made an attempt to compensate this limitation by applying an ethnographical investigation process. The first author of this study worked as a consulting technician for APROMONTES during a period of five years, sometime before starting this investigation. This immersion in the context helped deepen the contextual knowledge and the analysis and interpretation of results.

Considering the contributions, this study seems to have explored both empirical and theoretical dimensions of science. In terms of empirical contributions, we have described the decision-making process adopted by APROMONTES vitiviniculture and the phenomena which influenced these decisions, especially on the adoption of innovations. In terms of theoretical contributions, we highlighted the intersection of theories such as path dependence in the constitution of decision-making affect heuristics. We suggest the development of more qualitative studies to verify the connections and intensity of the categories analyzed. Future investigations on the strength of each category in the decision-making process may help the wine sector plan its actions on the adoption of innovations seeking the development of the sector.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Composition of different composts and vermicompost and effects of their application rates on growth parameters of pot grown tomato

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Two sets of pot experiments were conducted to investigate the effect of different compost types (market compost, home compost and vermicompost) and their rates on growth of pot grown tomato. During the first experiment, two compost types (market and home composts) each applied at four rates (0, 200, 400 and 800 g/pot) were evaluated while during the second experiment all the three compost types including vermicompost each applied at five rates (0, 200, 400, 600 and 800 g/pot) were evaluated. The chemical compositions of the composts were analyzed using appropriate laboratory procedures. Results revealed that market compost had the highest available P, exchangeable K and total S content whereas vermicompost had the highest organic matter and total N content followed by market compost. Growth parameters increased with increasing rates of home compost and vermicompost, but decreased with increasing rate of market compost. Shoot nutrient content varied much between compost types than compost rates. Shoot nitrogen and potassium concentration was higher with the application of market compost; phosphorus concentration was higher with the application of vermicompost while sulphur concentration was the highest with the application of home compost. Growth reduction at the highest market compost application could be ascribed to higher pH and total soluble salt content, especially chloride and potassium.

Key words: Compost, salt stress, tomato, toxicity, vermicompost.

INTRODUCTION

Disposal of organic waste is a serious challenge, especially in big cities due to absence of appropriate disposal sites and thus utilization of these wastes through composting is, now a days, given due attention as an alternative solution to the difficulties of organic waste disposal. Compositing of organic waste is a low external energy input microbial decomposition process that produces mineral ions and stabilized humus like

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substance (Huang et al., 2000; Stocks et al., 2002). Vermi-composing is on the other hand, a biotechnological process of composting in which certain species of earthworms are used to enhance waste conversion and produce a better product called vermicompost (Khan and Ishaq, 2011). Vermicomposts (*VC)*, which are produced by the fragmentation of organic wastes by earthworms, have a fine structure and contain nutrients in forms that are readily available for plant uptake (Atiyeh et al., 2000). Vegetable wastes and non-edible portions that are usually discarded during harvesting, marketing and processing can be used as very good composting materials.

Both composts and vermicomposts are being used in agricultural/horticultural production to improve soil fertility as they significantly enhance soil organic matter content (Nguyen et al., 2012). The application of composts and vermicompost promotes soil aggregation and stabilizes soil structure (Khan and Ishaq, 2011), increases soil water availability and hence plant nutrient uptake (Nguyen et al., 2012). Vermicompost contains 5 times high N and 7 times higher K and 1.5 time higher Ca than the first 15 cm top soil (Parkin and Berry, 1994). According to Khan and Ishaq (2011), the exchangeable potassium content of vermicompost is 58 times higher compared to garden soil. Different types of composts may have different pH and Lazcano et al. (2009) observed higher pH for compost (9.3) than for vermicompost (6.5). Likewise, Hernandez et al. (2010) also observed higher pH (8.5) for compost than for vermicompost (7.3). The P and K contents of vermicompost were 2 and 3 times higher, respectively than that of composts.

The application of quality compost and vermicompost increased growth and yield of many horticultural crops including tomato (Bahrampour and Ziveh, 2013; Ansari, 2008; Najar and Khan, 2013). Number of leaves/plant, plant height, root length and root weight of pea grown in vermicompost media were significantly higher compared to those pea plants grown in garden soil (Khan and Ishaq, 2011). Composts improved plant growth parameters such as number of leaves/plant and leaf area, at lower doses (up to 20%) while vermicompost improved growth at higher doses (up to 100%) (Lazcano et al., 2009). On the other hand, Joshi and Pal Vig (2010) observed increased growth and yield parameters in tomato (stem diameter, plant height, leaf number/plant, total biomass/plant, yield/plant), due to vermicompost application although these parameters did not increase beyond 15% VC soil mix. Bahrampour and Ziveh (2013) also reported better growth, higher yield, fruit quality and higher tissue P, K, Fe, Zn contents of tomato grown in vermicompost compared to the ones grown in the control.

Although composts and vemicomposts, besides improving soil fertility/nutrient availability and various soil properties, are also believed to improve plant growth, the amount to be applied, however, depends on the potentially toxic nutrient ions contained within the compost since some compost become toxic when applied at higher dosage (Lazcano et al., 2009). Higher pH was one of the serious problems with using higher doses of compost and addition of phosphoric acid was recommended to minimize the detrimental effect of such composts at higher application rates (Sorgona et al., 2011).

The aim of this investigation was to determine the nutrient composition of composts and vermicompost and the optimum rates of each compost type to be applied to raise healthy and vigorous tomato seedlings.

MATERIALS AND METHODS

Source of the composts

Home and market composts were obtained from development project administered under the association of EEG (Ethiopians Educated in Germany). Market compost was composted from fresh vegetable leftover collected from Piassa/Atikltitera, in Addis Ababa city whereas home compost was composted using the leftover of vegetables used for home consumption collected from individual households in Addis Ababa city. Vermicompost was obtained from Ambo Plant Protection Research Center, division of Agronomy. The vermicompost was made from donkey faeces casted using an earthworm called *Eisenia fetida* also known as the red wiggler, brandling worm, dung worm, or the tiger worm.

Experiment one

The treatments consisted of factorial combination of two different compost types (Market compost and Home compost) and four application rates (0, 200, 400 and 800 g/pot). A pot of 5 L size was used for the experiment. The treatments were replicated three times and were arranged in Randomized Complete Block Design (RCBD) in the glasshouse of Ambo University. Data on plant height, number of leaves per plant and shoot and root weights were recorded.

Experiment two

The treatments consisted of factorial combination of three different compost types (Home compost, Vermicompost and Market compost) and five application rates (0, 200, 400, 600 and 800 g/pot). A similar pot size used in the first experiment was also used (5 L size) during the second experiment. The treatments were replicated 3 times and were arranged in RCBD. Data on number of leaves per plant, plant height and shoot and root weights were collected.

Plant raising and determination of plant parameters

About 8 to 10 seeds of tomato variety called Melka shola was sown in each pot and after emergence the seedlings were thinned to 2 seedlings per pot. All the plants were being irrigated as required to avoid moisture stress. After 45 days of sowing, the above ground part (shoot) was removed and the fresh weight was determined. After oven drying the same shoots at a temperature of 65°C for 48 h the dry weight was determined. The roots were extracted from the pot after washing under tap water and the fresh and dry weight were also recorded.

Measurement of the physico-chemical properties of the composts and vermicompost

Determination of pH

The pH of the composts and vermicompost was measured in 1:2.5 compost: water suspension as well as in $CaCl_2$ solution using a portable pH meter (Portamess®).

Determination of available P and K

Available phosphorus content in the composts and vemicompost was determined using both CAL method (Schüller, 1969) in which Calcium Acetate and Lactate (composed of calcium lactate pentahydrate, calcium acetate hydrate and acetic acid) solution was used as an extractant and the Olsen method (Olsen et al., 1954) in which sodium bicarbonate (0.5 M $NaHCO_3$ at a pH=8.5) was used as an extracting solution. Phosphorus concentration in the extract was measured using spectrophotometer (µQuant MQX200) at 405 and 882 nm for CAL and Olsen methods, respectively following the addition of appropriate color reagents. Likewise, the potassium content of the soil was determined both by extracting the potassium with Calcium Acetate and Lactate (CAL) solution as well as by 1 M ammonium acetate solution and was measured using flame photometer (Eppendorf Elex 6361 Flammenphotometer).

Determination of total N and S

The total nitrogen and sulphur content was determined by using CNS autoanalyzer (Elementar Vairo EL III) in which 20 mg compost sample was put in an aluminium foil, wrapped up and was heated up to a temperature of 1200°C using Tungston (III) oxide as a reaction catalyzer in the presence of oxygen supply. Additionally, the total nitrogen was also determined using Kjeldahl method (Kjeldahl, 1883).

Determination of organic matter (OM)

The organic matter (OM) content of the soil was determined using two methods, Walkley and Black (Walkely and Black, 1934) and CNS methods. Organic matter content using the first method was determined at the Department of Chemistry, Ambo University, Ethiopia whereas OM with CNS method was determined at the Institute of Plant Nutrition, Leibniz University of Hannover, Germany. With the Walkley and Black method, the amount of Ferrous sulphate solution used for back titrating the excess dichromate was recorded and used for carbon estimation while with the CNS method, 20 mg compost sample was put in an Aluminium foil, wrapped up and heated to a temperature of 1200°C using Tungston (III) oxide as a reaction catalyzer in the presence of oxygen supply. The amount of carbon in the sample was determined using CNS autoanalyser (Elemetar Vario III). The organic matter content was calculated from the carbon content value obtained by multilying with 1.724 with the assumprion that organic matter contains 58% C.

Determination of total salt and salt components

Total soluble salt was estimated from EC measurement using an EC meter. Salt components such as Na^+ and K^+ was measured using flame photometer after extracting with 1 M ammonium acetate solution. Chloride ion (Cl^{-1}) was measured through back titration with $HgNO_3$., Ca^{++} and Mg^{++} were determined using inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) and NO_3^- and NH_4^+ was measured using nitrogen auto analyzer (*SAN*$^{++}$ *SYSTEM*). Except NO_3^- and NH_4^+, which were extracted using 0.1 M KCl and Na^+ and K^+, which were extracted using 1 M ammonium acetate solution, all the other salt components were extracted using distilled water (1:2 compost: water ratio).

Plant sample analysis

After the shoot dry weight was measured, the dried plant samples were ground, and 50 mg of the ground samples was ashed overnight at 500°C and analysed for various nutrient concentration after extracting the ashed content using 1:3 nitric acid and measured using ICP-MS.

Statistical analysis

Data was analysed using SAS statistical software version 9.3 following PROC GLM procedure. When the Analysis of Variance (ANOVA) shows significant effects of main factor or their interaction at a probability level α=0.05, appropriate mean separation was carried out using Tukey test.

RESULTS

Chemical composition of different composts

pH

The market compost had the highest pH value (10 in water and 9.8 in calcium chloride) compared to both vermicompost (7.1 in water and 6.9 in calcium chloride) and home compost (8.7 in water and 8.4 in calcium chloride). The pH of the composts determined using $CaCl_2$ was lower by 0.2 to 0.3 compared to the pH of the composts determined in water (Table 1).

Organic matter

The organic matter content (OM) of the composts determined using CNS auto analyser was 1.3 to 1.8 fold higher than the OM content determined through Walkley and Black method. The organic matter content was higher for vermicompost followed by market compost. The organic matter content of vermicompost was nearly 4 to 5 folds higher than that of the farm soil while the organic matter content of market compost was 2.5 to 3.5 times higher than that of farm soil. Vermicompost had 1.9 fold higher and 1.4 fold higher organic matter content than home compost and market compost, respectively.

Available phosphorus

Home compost had the lowest available phosphorus (Av.P) content compared to the other two compost types (market compost and vermicompost). The available

Table 1. Physicochemical characteristics of the composts and farm soil.

S/N	Types of composts	Av.P (mg/kg soil)-CAL	Av.P (mg/kg soil)-Olsen	Exc. K (mg/kg soil)-CAL	Exc. K (mg/kg soil)-Amm.acetate	OM%(CNS autoanalyser	OM% (Walkelay and Black)	pH (water/$CaCl_2$)	Bulk density (g/cm^3)	Total N (%) CNS	Total N (%)Kjeldahl	Total S (%)	C/N ratio
1	Home compost	2161.3	620	21060	18780	22.1	12.2	8.7/8.4	0.81	1.14	0.8	0.38	11
2	Vermicompost	4942.7	1190	15430	15240	41.3	32.0	7.1/6.9	0.64	2.02	1.8	0.36	12
3	Market compost	6876.8	3824	48330	43000	29.8	20.3	10/9.8	0.83	1.62	1.1	0.52	11
4	Farm soil	87.5	39.1	537	830	8.8	8.1	5.5/5.0	1.38	0.48	0.4	0.11	10

phosphorus content of market compost was three-fold higher (P-CAL) and six-fold higher (P-Olsen) than that of home compost. The available phosphorus content of vermicompost (both CAL and Olsen) was two-fold higher than that of home compost (Table 1). Compared with farm soil, the available phosphorus content of market compost was 79 to 98 fold higher while that of vermicompost was up to 30 to 56 fold higher depending on the P determination method used. Likewise, the available phosphorus content of home compost was by 16 to 25 fold higher compared to that of the farm soil.

Available potassium

The available/exchangeable potassium content (both CAL-K and Ammonium Acetate extractable-K) of market compost was three-fold and that of vermicompost was on average 1.3 fold higher than that of home compost (Table 1). Compared to the farm soil, the exchangeable potassium content of market compost was up to 50 to 90 folds higher while that of home compost was up to 22 to 39 fold higher. Likewise, the exchangeable potassium content of vermicompost was by 18 to 29 folds higher than that of the farm soil.

Total nitrogen

The total nitrogen determined through CNS auto analyzer was generally higher compared to that of Kjeldahl method. Vermicompost had 1.8-fold and 1.2-fold higher total nitrogen content than home compost and market compost, respectively (Table 1). Compared to the farm soil, the total nitrogen content of vermicompost was by 4-fold higher and that of market compost was by 2.8 to 3.5 times higher.

Total sulphur

Unlike the total nitrogen, the total sulphur content was determined only through CNS auto analyzer. Market compost had higher total sulphur content (0.52%) than both home compost (0.38%) and vermicompost (0.36%). Compared to the farm soil, the total sulphur content in market compost, home compost and vermicompost was by 4.7, 3.5 and 3.3-fold higher.

Interaction effect of compost types and rates on growth of tomato

The analysis of variance showed that the shoot fresh and dry weights, root fresh and dry weights as well as plant height and number of leaves per plant were significantly affected by the main effect of compost types and rates as well as by their interaction effects. For home compost, the shoot fresh and dry weights significantly increased with the increase in the application rate (Figures 1 and 2) during both experimental periods. It also increased with the increase in the application rate of vermicompost during the second experiment (Figure 2). To the contrary, for market compost the shoot fresh and dry weights decreased significantly with the increase in the application rate (Figure 1A and B) during the first experiment. During the second experiment, increasing the application rate of market compost beyond 400 g/pot, also tended to reduce shoot weights but the shoot weights obtained at the application rate of 200, 400 and 600 g/pot did not significantly differ while application at the highest rate (800 g/pot), resulted in the lowest shoot fresh and dry weight (Figure 2A and B).

For home compost and vermicompost, the root fresh and dry weights increased with increasing the application rates (Figures 5 and 6). However, with market compost, the root weights tended to increase with increase in the rates of application from 0 to 400 g/pot and sharply declined with increasing the market compost rate from 600 to 800 g/pot during both experiments. At the highest market compost rate (800 g/pot), nearly the plant did not produce extractable roots (Figures 5 and 6).

For home compost and vermicompost, increasing their application rates from 0 to 800 g/pot tended to increase the number of leaves per plant as well as plant height, while these parameters tended to decrease with an

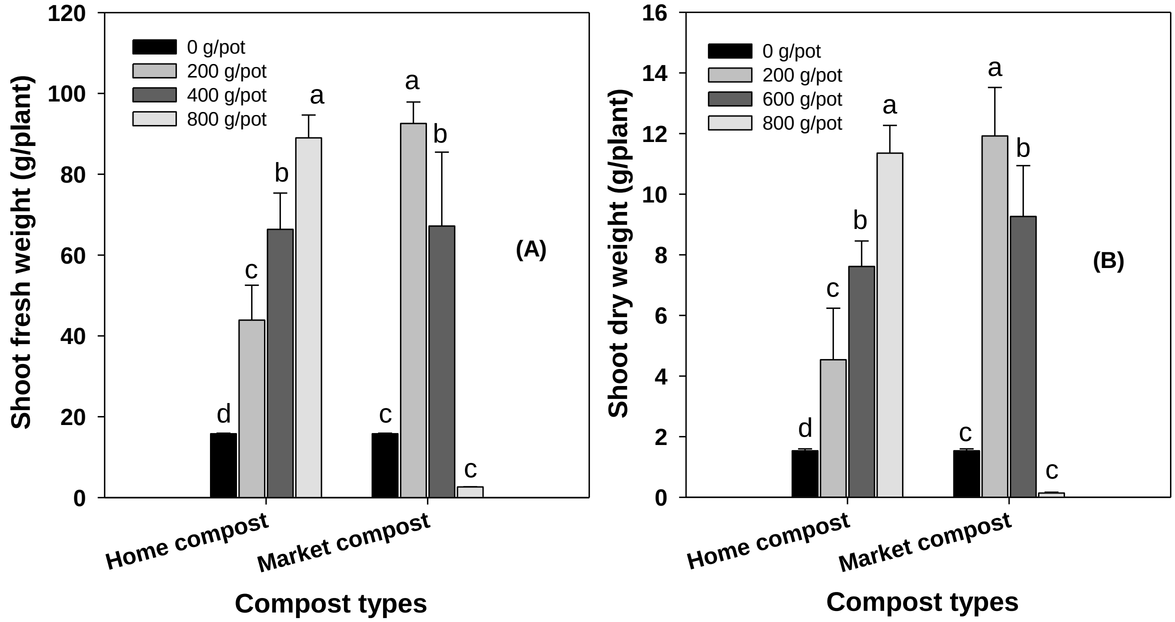

Figure 1. Interaction effect of compost types and rates on shoot fresh (A) and dry weight (B) of tomato during the first experiment (for the same compost type. Means followed by similar letters are not significantly different from each other.

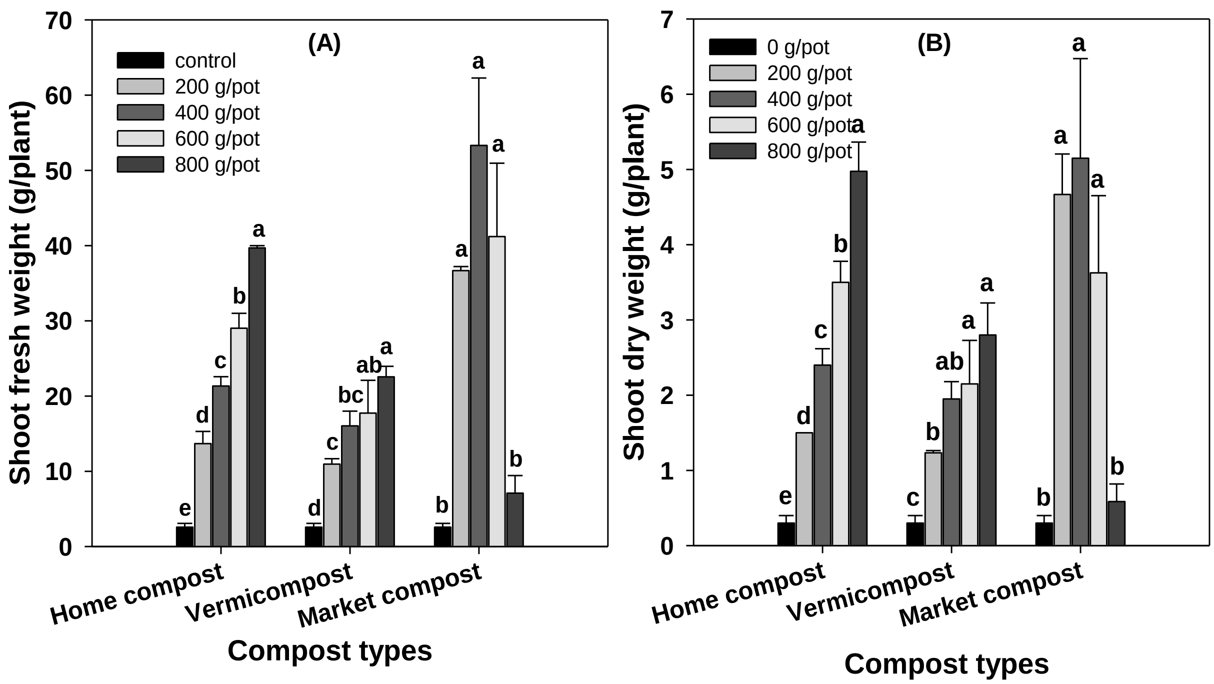

Figure 2. Interaction effect of compost types and rates on shoot fresh (A) and dry weight (B) of tomato during the second experiment (for the same compost type means followed by similar letters are not significantly different from each other).

increase in the application rate of market compost (Figures 3 and 4). At the highest market compost rate (800 g/pot), the plants was as short as that of the control where no compost was applied (Figures 3 and 4).

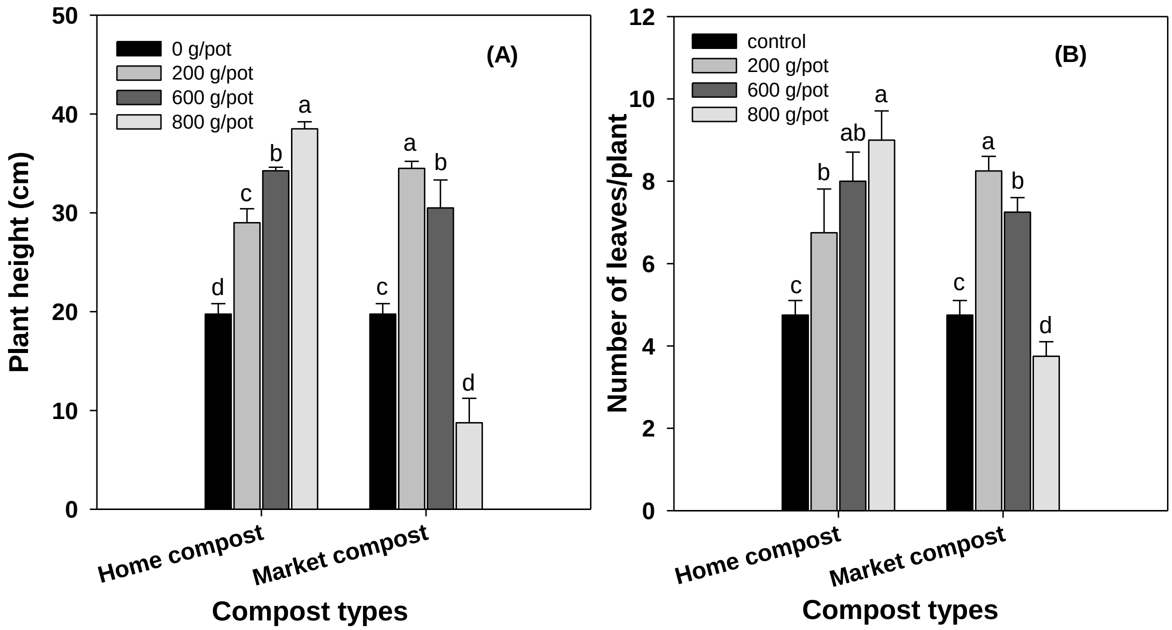

Figure 3. Interaction effect of compost types and rates on plant height (A) and number of leaves per plant (B) of tomato during the first experiment (for the same compost type means followed by similar letters are not significantly different from each other).

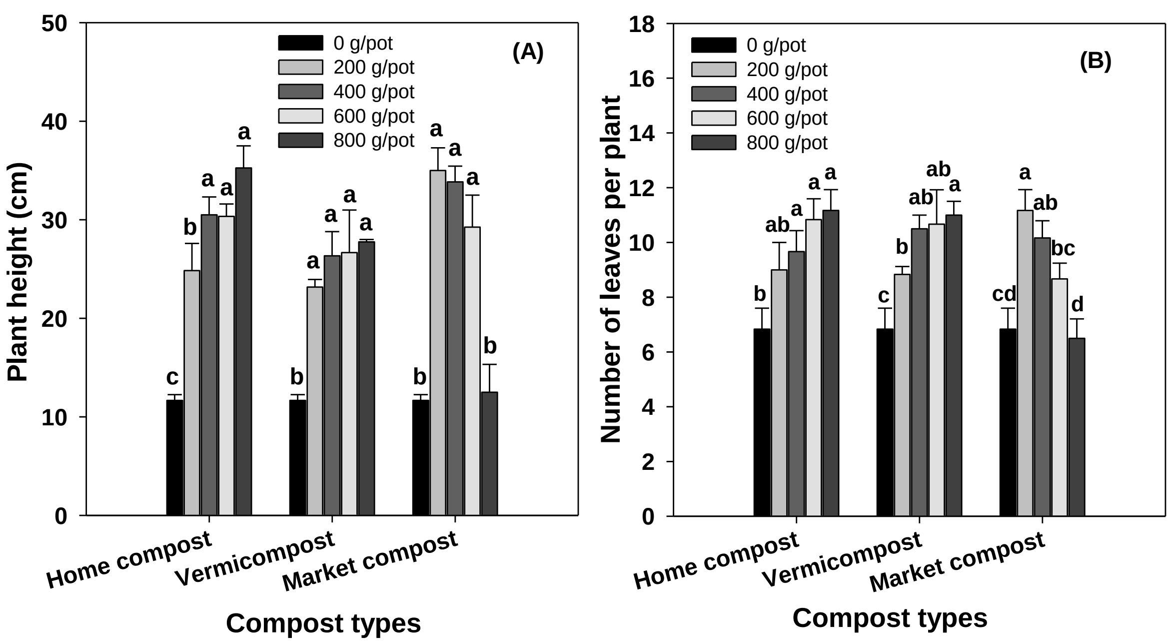

Figure 4. Interaction effect of compost types and rates on plant height (A) and number of leaves per plant (B) of tomato during the second experiment (for the same compost type means followed by similar letters are not significantly different from each other).

Interaction effect of compost types and rates on shoot nutrient concentration

The analysis of variance showed that the shoot nitrogen, sulphur, phosphorus, potassium, sodium and zinc concentration were significantly affected by the main effects of compost types and rates as well as by their interaction effects. For home compost and vermicompost,

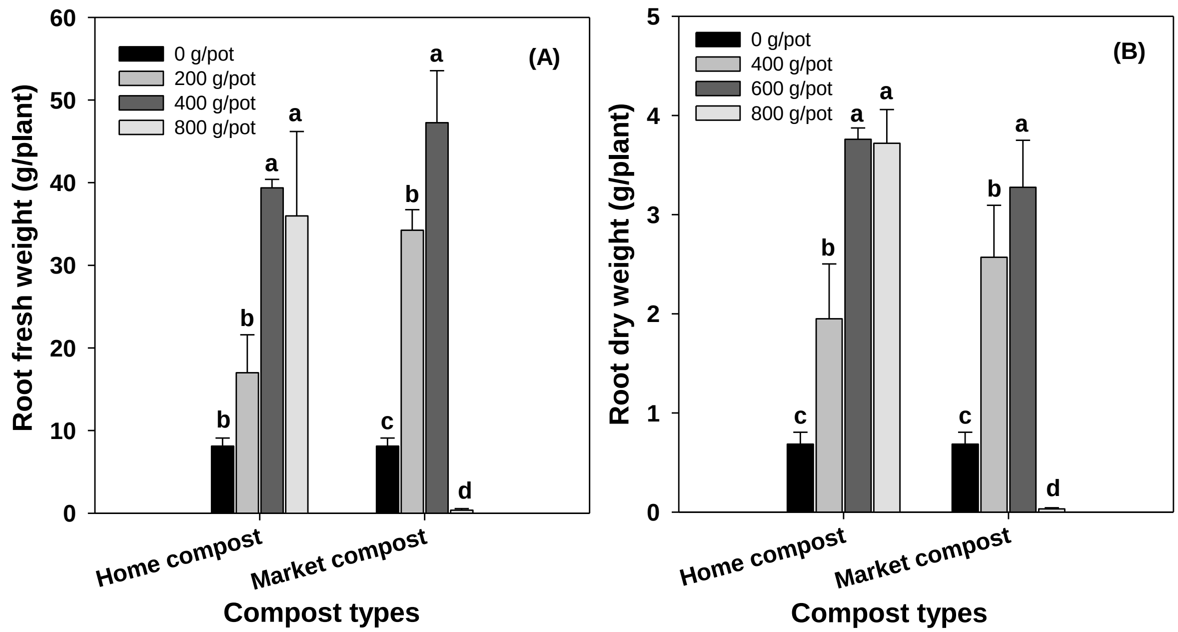

Figure 5. Interaction effect of compost types and rates on root fresh (A) and dry (B) weights of tomato during the first experiment (for the same compost type means followed by similar letters are not significantly different from each other).

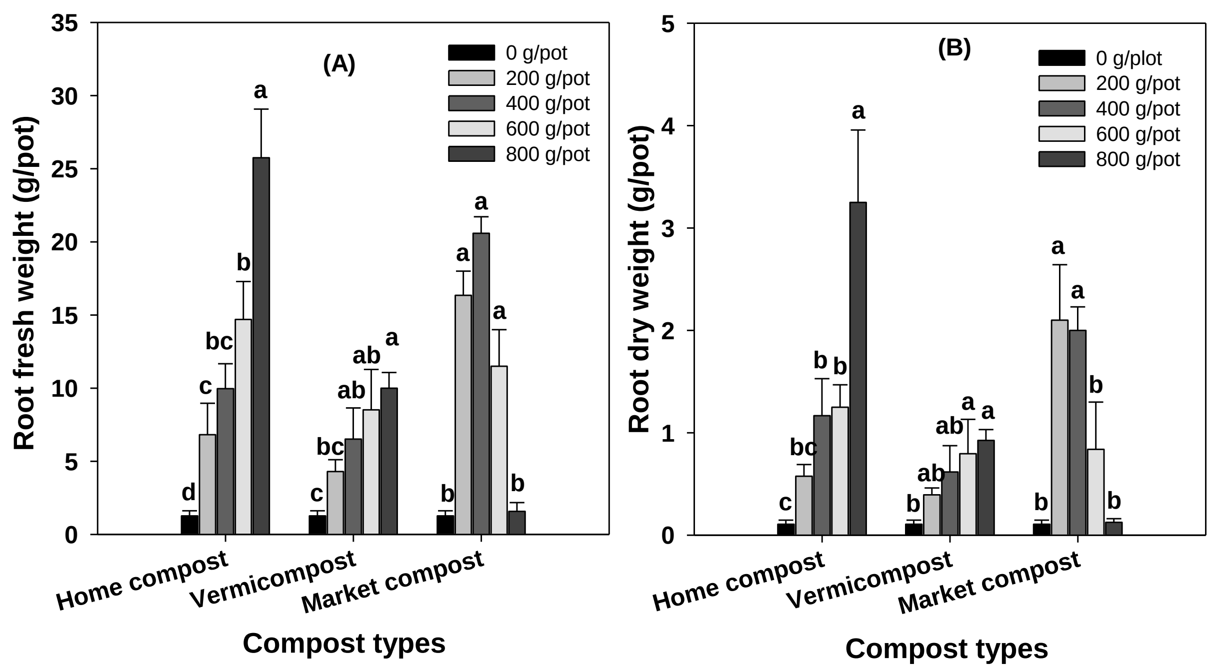

Figure 6. Interaction effect of compost types and rates on root fresh (A) and dry (B) weights of tomato during the second experiment (for the same compost type means followed by similar letters are not significantly different from each other).

the shoot nitrogen concentration did not vary between the application rates while for market compost the shoot nitrogen concentration was significantly higher for the tomato plants grown at higher market compost rates of 400, 600 and 800 g/pot. With both home compost and vermicompost, the application significantly increased the shoot sulphur concentration over the control (Figure 7). The shoot sulphur concentration was higher for the

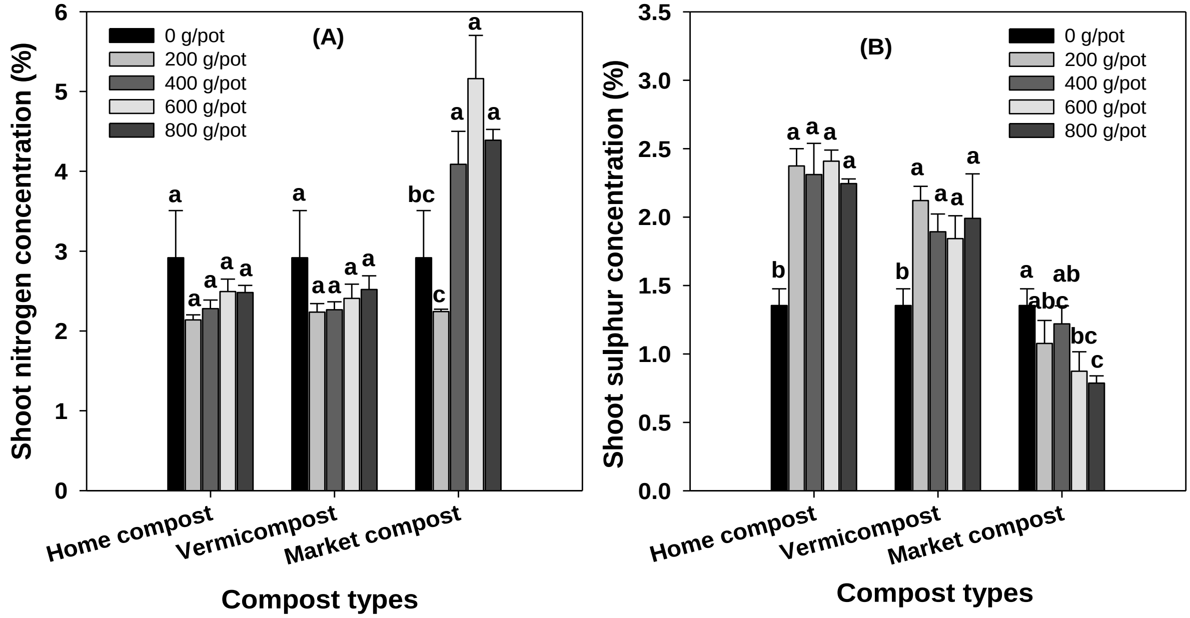

Figure 7. Interaction effect of compost types and rates on shoot nitrogen (A) sulphur (B) content of tomato during the second experiment (for the same compost type means followed by similar letters are not significantly different from each other).

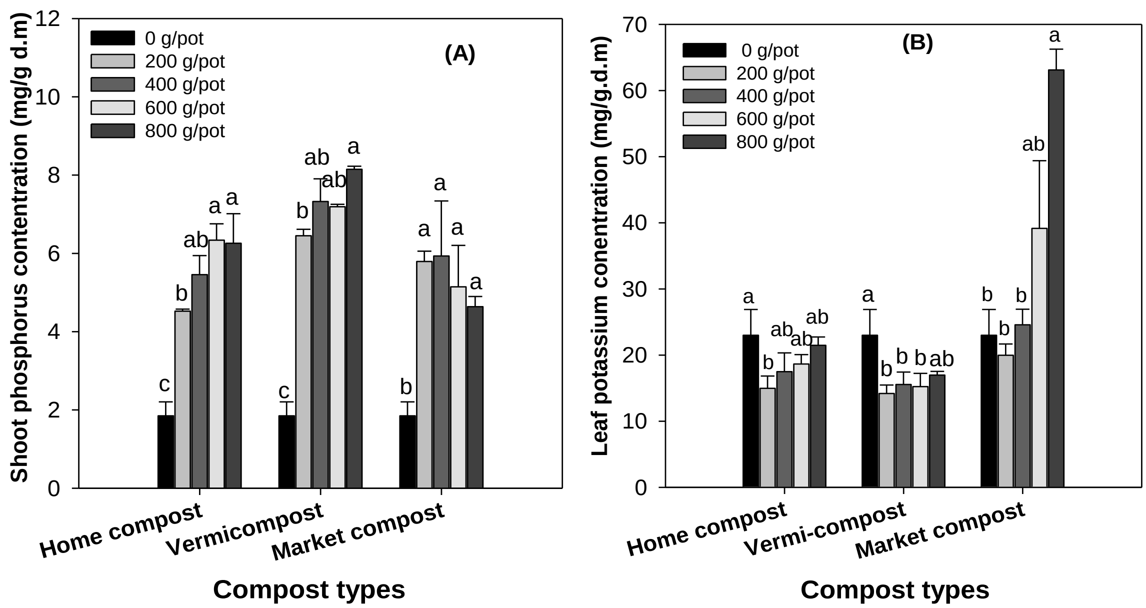

Figure 8. Interaction effect of compost types and rates on shoot phosphorus (A) potassium (B) content of tomato during the second experiment (for the same compost type means followed by similar letters are not significantly different from each other).

tomato plants grown in home compost and vermicompost and was lower for the tomato plants grown in the market compost. Sulphur uptake was highly constrained at the highest market compost rate (shoot sulphur concentration <1.5%) while nitrogen uptake was not at the application of similar market compost rate (Figure 7). For all application rates, the shoot phosphorus concentration was generally higher (6 to 8 mg/g d.m) for vermicompost than for home compost (4.5 to 6 mg/g d.m) and market compost (4.5-5 to 5 mg/g d.m) (Figure 8A). However, the amount of available P in market compost was much higher than that of vermicompost and home compost. At

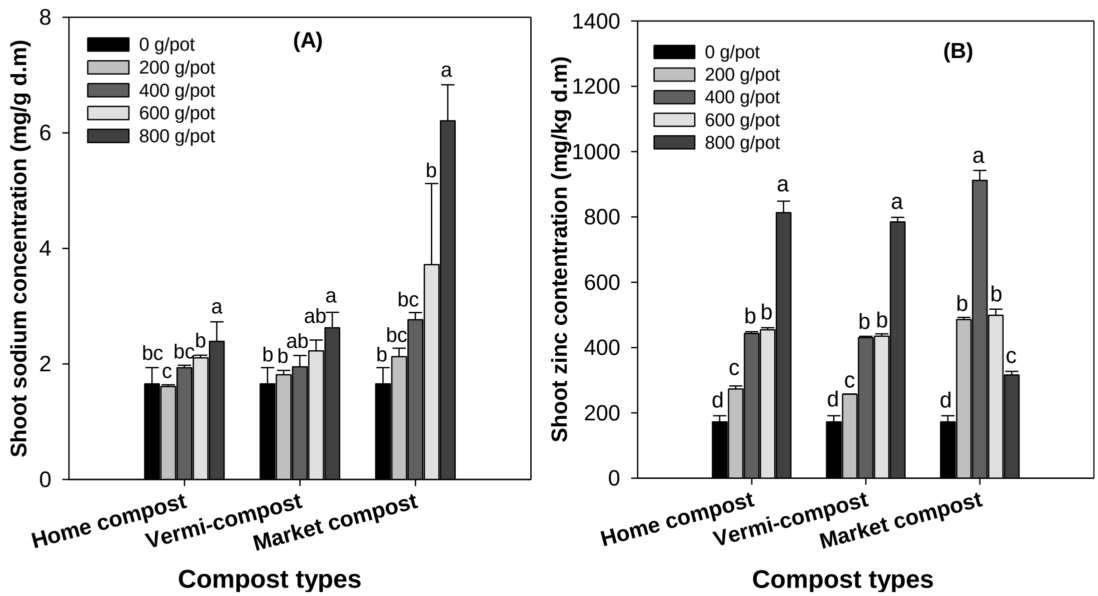

Figure 9. Interaction effect of compost types and rates on shoot sodium (A) zinc (B) content of tomato during the second experiment (for the same compost type means followed by similar letters are not significantly different from each other).

all application rates, the tomato plants accumulated sufficient P concentration in their tissue except the plants in the control pots. The K concentration in tissues of plants grown at different application rates of both home compost and vermicompost was lower or comparable to the plants grown in the control pot (Figure 8B). The K concentration in the tissues of plants grown at the highest application rate of market compost (800 g/pot) was three-fold higher to the K concentration in tissues of plants grown in the same compost at the lower application rates (200 and 400 g/pot).

The shoot sodium concentration was extremely higher (6.6 mg/g d.m) for the plants grown at the highest (800 g/pot) market compost application rate (Figure 9A). The shoot sodium concentration of plants grown at 200 and 400 g/pot of both home compost and vermicompost were not different from that of the control plants. Shoot sodium concentration tended to increase with the increase in the compost application rates and the increase was much more pronounced in the case of market compost. For the tomato plants grown in home compost and vermin-compost, the shoot zinc concentration tended to increase with the increase in the application rate. However, for the tomato plants grown in the market compost, the shoot zinc concentration decreased with the increase in the application rate beyond 400 g/pot (Figure 9B).

DISCUSSION

Nutrient composition of composts

Market compost had higher pH, which might have adversely affected tomato growth at higher doses of 800 g/pot. Similarly, Sorgona et al. (2011) also reported reduced growth rate in tomato and zucchini seedlings at higher doses of compost application due to higher pH and EC. However, at lower rates (200 and 400 g/pot) of market compost application, such detrimental effect of higher pH was not observed, probably due to the fact that the high pH from the compost might have been buffered by the lower pH of the large proportion of the mixed soil. In the current study, from among the three compost types, vermicompost had the lowest pH value (7.1). Hernandez et al. (2010) also observed lower pH value for vermicompost (7.3) than for compost (8.5), which is in line with the present observation. The pH of vermicompost reported by Bahrampour and Ziveh (2013), which was 7.7 and by Kmetova and Kovacik (2013), which was 7.4 are also in agreement with what was observed in the present study. Similarly, Lazcano et al. (2009) also reported higher pH of 9.3 for compost and lower pH of 6.5 for vermicompost.

The organic matter (OM) content of all the composts was higher than that of the farm soil, which ultimately accounted for better tomato growth compared to the control. The organic matter content of vermicompost reported in the present study is higher (41.3% for OM determined with CNS auto analyser and 32.0% for OM determined with Walkley and Black method) than what was reported by Bahrampour and Ziveh (2013) for vermicompost (25.8%). Lazcano et al. (2009) reported similar organic matter content of relatively higher value (61%) in both compost and vermicompost while Hernandez et al. (2010) reported an OM content of 41.4% for vermicompost and 36% for compost, which are in agreement with the current study. The slight difference in the organic matter content value reported by different authors could be attributed to the difference in the materials used for composting (vegetable leftovers in the case of the present study and cow manure by Lazcano et al. (2009) and vermicomposting (which was donkey manure in the case of the present study and pig manure by Lazcano et al. (2009).

The available P, exchangeable K and total N and S contents of the composts used in the present study were also quite higher compared to that of the farm soil and have largely contributed to better tomato growth in compost and vermicompost treatments than in the control treatment.

The exchangeable K content of home and market composts as well as vermicompost were about 21, 48 and 15 g/kg, respectively with market compost having higher K content. Kmetova and Kovacik (2013) also reported an exchangeable K content of 14 g/kg for vermicompost, which is quite comparable with the K content observed for vermicompost in the current study (15 g/kg). Hernandez et al. (2010) also reported lower potassium content in vermicompost than in compost, which supports the present observation. However, Hernandez et al. (2010) observed slightly higher potassium content values for both composts and vermicompost compared to the results observed in this investigation probably due to the difference in the material used for vermi-composting/composting. Similar to the present investigation, Bahrampour and Ziveh (2013) also reported higher potassium content (10 g/kg) in vermicompost as compared to soil (31-fold higher). However, the exchangeable K content they measured was slightly lower (10 g/kg) compared to what was observed in this study (15 g/kg). In the current study, the exchangeable potassium content of market compost, home compost and vermicompost was up to 50 to 90 fold, 22 to 39 fold and 18 to 29 fold higher than that of farm soil, respectively which is in line with the results of Khan and Ishaq (2011), who also reported a 56 to 58 fold higher potassium content between compost/vermicompost and garden soil. Likewise, Kmetova and Kovacik (2013) reported 47.5-fold higher exchangeable K content of vermicompost compared to farm soil which further supports the present observation.

The total N content in this study was higher for vermicompost than for the other compost types (Table 1). Similar to the present study, Hernandez et al. (2010) also observed higher total N for vermicompost than for compost although the actual value in the current study was higher (2.02% for CNS auto analyser, 1.8% for Kjeldahl method) than what he reported for vermicompost (1.6%). The difference in the total N content value between the current study and the authors' report might be accounted to difference in the material used for vermicomposting, cattle manure in the case of the authors and donkey manure in the case of the current study. Bahrampour and Ziveh (2013) also reported lower total N (1.3%) content in vermicompost compared to what was observed in the current study and the lower total N value he reported could be due to the difference in vermicomposting material used, sheep manure by the authors and donkey manure in the case of the current study. Compared to the farm soil, the total nitrogen content of vermicompost in the current study was by 4-fold higher, which is supported by the report of Kmetova and Kovacik (2013) who also observed 10-fold higher total N content in vermicompost compared to soil. In terms of the actual total N content, Kmetova and Kovacik (2013) reported a total N content of 2.94% for vermicompost which is closer to the current result of 2.02% total N content (Table 1).

Compared with farm soil, the available phosphorus content of market compost was 79 to 98 fold higher, that of vermicompost was up to 30 to 56 fold higher while that of home compost was by 16 to 25 fold higher depending on the P determination method used. The available P content of both compost and vermicompost in the present investigation was quite higher compared to what was observed by Hernandez et al. (2010), which were 0.14 g/kg for vermicompost and 0.16 g/kg for compost. Although there was tremendous difference in available P value between the two observations, the difference in available P content could partly be due to difference in materials used for composting/vermicomposting (cattle manure) in the case of the authors' and donkey manure in the case of the present study. On the other hand, the available P content of vermicompost in the current investigation was lower (4.9 g/kg P-CAL and 1.2 g/kg P-Olsen) compared to what was reported for vermicompost by Bahrampour and Ziveh (2013), which was 13 g/kg, a relatively higher value. Kmetova and Kovacik (2013) also reported an available P content of 5.6 g/kg in vermicompost, which was 215-fod higher compared to that of the farm soil which supports most other reports as well as the present observation.

Effect on plant growth

For home compost and vermicompost, increasing the application rates from 0 to 800 g/pot increased shoot fresh and dry weights, leaf number per plant and plant height. Similarly, Joshi and Pal Vig (2010) also observed an increase in plant height and leaf number with an increasing rate of vermicompost. But increasing the application rate of market compost above 400 g/pot reduced the above growth parameters (Figures 1 and 3). Similar to what is currently observed with market compost, higher proportion of some composts relative to soil (50% each) resulted in reduced growth and even in plant mortality, while the application of higher doses of vermicompost resulted in a significantly higher plant growth compared to the application of lower doses

Table 2. Components of total soluble salt in different composts.

Compost types	Total soluble salt/KCl content (g/kg)	Chloride content (g/kg)	Sodium content (g/kg)	Calcium content (g/kg)	Magnesium content (g/kg)	NO_3-N content (g/kg)	NH_4^+-N content (g/kg)	Potassium content (g/kg)
Home compost	22	4.6	1.86	0.34	0.19	0.24	27	11.08
Vermicompost	18	2.7	1.27	0.27	0.30	0.81	152	6.65
Market compost	35	5.3	1.65	0.85	0.28	0.56	112	22.16
Farm soil	0.8	0.7	0.04	NM	NM	NM	NM	NM

NM=not measured

(Lazcano et al., 2009; Bahrampour and Ziveh, 2013; Najar and Khan, 2013; Ansari, 2008). The reduction in growth of tomato plant grown at the highest market compost rate of 800 g/pot could be related to salt stress and specifically to chloride and potassium toxicity (Table 2). The higher sodium accumulation in the tissue (Figure 9) might also be another reason for growth reduction at higher market compost application.

Effect on tissue nutrient concentration

Surprisingly, the potassium concentration in plants grown at all levels of both home compost and vermicompost was not different from that of the control (Figure 8B). Absence of such difference in tissue K concentration, despite the huge difference in the exchangeable K content between the farm soil and home and vermicompost, cannot be easily explained. However, the potassium concentration in plant grown at the highest MC application rate of 800 g/pot was considerably higher (three-fold) than that of the control (Figure 8B) and hence might have resulted in toxicity and hence reduced plant growth as suggested above. The other weird thing is that the treatment in which the shoot nitrogen concentration was higher, the shoot sulphur concentration was lower and vise versa. These relationships usually occur with nutrient having antagonistic effects and the reason for the current observation with N and S is not clear and is not easily justifiable.

In a nut shell, results of the present investigation showed that the reduction in growth parameters of tomato at the application of 800 g/plot MC seems to be related to the higher total soluble salt content (35 g/kg soil) and higher pH of the market compost (pH=10) as opposed to the other two compost types. Further investigation of the soluble salt components revealed that MC contained higher potassium and chloride content than the other compost types. This was further confirmed by the highest K concentration in the shoot tissue. Apart from the potassium, the shoot sodium concentration was also extraordinarily higher for the same treatment. Thus, reduction in growth of plants grown at 800 g/pot of MC application could also be due to salt stress and especially due to chloride and potassium toxicity and also perhaps due to Na^+ toxicity.

Conclusions

Vermicompost is richer in OM and total N content than the other composts while market compost is richer in available P and exchangeable K as well as total sulphur content. Increasing the rate of market compost from 200 to 800 g/pot resulted in reduction of growth of tomato plant. However, with both home compost and vermicompost, increasing the rate from 0 to 800 g/pot resulted in increased shoot fresh and dry weights, root fresh and dry weights, plant height and leaf number per plant. Unlike home compost and vermicompost, market compost, therefore, should not be applied at higher rate since it results in reducing plant growth. The reduction in growth of pot grown tomato plant with the application of 800 g/pot MC could be due to effect of higher pH and salt stress (chloride, potassium as well as sodium toxicity).

In summary, the application of compost is essential for improving growth of tomato plant however, since some compost could have toxic effects such composts as market compost should be applied at lower rate to give effective result whereas for home compost and vermicompost higher rates of application favours tomato growth.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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the analysis of the composts and plant samples.

Abbreviation

HC, home compost; VC, vermicompost; **MC,** market compost; **OM,** organic matter; **N,** nitrogen; **Av.P,** available phosphorus; **Exch. K,** exchangeable potassium; **S,** sulphur.

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Prevalence of equine lungworm and associated risk factors in Sudie district, Oromia region, south eastern Ethiopia

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A cross-sectional study was carried out from November 2014 to March 2015 to determine the prevalence of *Dictyocaulus arnfieldi* and to identify associated risk factors in equines in Sudie district, south eastern Ethiopia. A total of 384 faecal samples were collected randomly from horses (n = 128), donkeys (n = 217) and mules (n = 39) for coprological examination. Isolation of *D. arnfieldi* was performed using a modified Baermann technique. The overall prevalence of *D. arnfieldi* was 164 (42.7%) with infection rates of 22.7, 57.6 and 22.7% in horses, donkeys and mules, respectively, with statistically significant (P<0.05) variation. High prevalence of lungworm infection was recorded in the age group of ≤4 years (50.9%) followed by the age group of 4-10 years (42.3%) and ≥10 years (40.46%), however, statistically non significant. Observed prevalence of lungworms in female equines was 37.1% and in males was 47.0% with no statistically significant difference (P>0.05). In this study, animals with poor body conditions were found to be highly infested (50.9%) compared to medium (41.6%) and good body conditions (21.3%) with statistically significant difference (P<0.05). The prevalence in non-dewormed equines was 53.2% and dewormed equines were 26.2% with significant difference (P<0.05). From this study, it can be concluded that body condition can be considered as one of the important factors which influence the occurrence of lung worm parasite in equines. It is recommended that owners should be trained to improve the management system, especially in terms of the level of nutrition so that the animal can have good body condition that confers some level of resistance against lung worm infection. In addition, strategic deworming should be implemented using broad spectrum anthelmintic drugs in the study area.

Key words: Equine, lung worm infection, prevalence, risk factors, Sudie.

INTRODUCTION

Ethiopia is one of the developing countries in Africa, which is predominantly an agricultural country with over 85% of its population engaged in agricultural activity (FAO, 1999; EARO, 1999). The country has the highest

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equine population probably with the highest density per square kilometer in the world (Alemayehu, 2004) and it has a total of 6.9 and 42.4% in the world and Africa equine population, respectively (Wilson, 1991).

Ethiopia has 21.7 million horses, 5.57 million donkeys (second largest in the world next to China) and 380 thousand mules (CSA, 2009). Equines are one of the most important and mostly intimately associated with man. They have enormous contribution through their involvement in different social and economic sectors. Equines play an important role as working animals in many parts of the world, for packing, riding, carting and ploughing. Equine power is very crucial in both rural and urban transport system. This is because of its cheapness and availability and so provides the best alternative transport means in places where the road network is insufficiently developed and the landscape is rugged and mountainous and in the cities where narrow streets prevent easy delivery of merchandise (Feseha et al*.,* 1991).

In Ethiopia equines have been as animals of burden for long period of time and still render valuable services mostly as pack animals throughout the country particularly in areas where modern means of transportation are absent, unaffordable or inaccessible (Abayneh et al*.,* 2002).

In some areas of North West Kenya and Southern Ethiopia, donkey meat is a delicacy and the milk believed to treat whooping cough (Fred and Pascal, 2006).

Even though mules and donkeys have often been described as sturdy animals, they succumb to a variety of diseases and a number of other unhealthy circumstances. Among these, parasitic infection is a major cause of illness (Sapakota, 2009). Lungworms are widely distributed throughout the world providing nearly perfect conditions for their survival and development but are particularly common in countries with temperate climates, and in the highlands of tropical and subtropical countries*.* Dictyocaulidae are known to exist in East Africa and South Africa (Hansen and Perry, 1996).

Dictyocaulus arnfieldi is the true lungworm affecting donkeys, horses, ponies and zebras and is found throughout the world (Smith, 2009). Donkeys and their crosses (Mules) are the natural hosts for lungworm and the condition in horses is usually found in those that have been in the company of donkeys and mules (Rose and Hodgson, 2000).

The pathogenic effects of lungworm depend on their location within the respiratory tract, the number of infective larvae ingested, the animal immune status, the nutritional status and age of the host (Fraser, 2000; Blood and Radostits*,* 1989). Larvae migrating through the alveoli and bronchioles produce an inflammatory response, which may block small bronchi and bronchioles with inflammatory exudates. The bronchi contain fluid and immature, latter adult worms and the exudates they produce also block the bronchi. Secondary bacterial pneumonia and concurrent viral infections are of the complication of Dictyocaulosis (Howard, 1993).

Despite the prevalence of patent *D. arnifieldi* infection in donkeys, overt clinical signs are rarely seen; however, on close examination, slight hyperpnoea and harsh lung sounds may be detected. Donkeys usually show no disease signs and can be silent carriers and shedders of this parasite, which causes clinical signs in horses (Johnson et al*.,* 2003).

Despite the huge numbers of equine population and the increasing importance of equines in the Ethiopian economy, very little research relating to equine lungworm has been carried out in Ethiopia. And therefore, the aims of this study were to estimate the prevalence of lung worm infection in equines and to investigate the association between intensity of lung worm infection and risk factors area in Sudie district Oromia region, southeastern Ethiopia.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Oromia Regional State, Arsi zone, Sudie district which is located at a distance of 216 km south east of Addis Ababa and 93 km east of Asela (the capital of the zone) at altitude of 1500 to 2750 m.a.s.l. The area covers 112400 hectare in range lands. The area is comprised of twenty seven peasant associations and is bordered by different districts such as, Cholle by north, Robe by south, Diksis by west, and Seru and Cholle by east. Topographically, it has 7.41% highland, 70.37% midland and 22.27% lowland. It receives bimodal rainfall occurring from March to April (a short rainy season) and from July to October (long rainy season). It receives an annual range of rain fall from 880 to 1100 mm, and the annual range of temperature varies from 15 to 25°C. The equine population of the area is found to be 26560 (CSA, 2009).

Study animals

The study animals were horses, donkeys and mules in Sudie district, Arsi zone. Faecal samples were directly collected from the rectum of 384 equids of all age and sex groups. They were all local breeds, kept under extensive management system used for packing and transportation. The age of selected equines was determined using deciduous and the incisor teeth eruption times, wear, tear by Crane (1997) and Svendsen (1997) and by asking owners. Equines were grouped into three age categories namely equines under two years were classified as young (n=55), those in range of two to ten years were classified as adult (n=156) and those beyond ten years were classified as old (n=173). Body condition score was assessed subjectively using a scale from 1 (emaciated), 2 (thin), 3 (average), 4 (fat), to 5 (very fat) (Svendsen, 1997). The body condition of animals was classified as poor (emaciated and thin), medium (average) and good (fat and very fat) body condition scores. Equines those dewormed within 3 months interval and not dewormed within 3 months interval were selected for this study.

Study design and sampling procedure

A cross-sectional study design has been employed and the study animals were selected using simple random sampling method and the origin, age, sex, body condition scores and species of the animals were taken into consideration.

Sample size

The sample size required for the study was determined using the following formula given by Thrustfield (2005):

$n= (1.96)^2 pexp\ (1-pexp)/d^2 = (1.96)^2\ 0.5(1-0.5)/(0.05)^2$

n =384

To calculate the sample size 95% confidence level, 50% expected prevalence and 5% of desired absolute precision (d=0.05) was used. Where n = required sample size, pexp = expected prevalence, d^2= desired absolute precision at 95% confidence level. According to the formula, 384 equines were sampled.

Faecal sample collection and examination

Faecal samples were collected per-rectum or but some samples (especially from temperamental animals) were collected from fresh deposits using plastic gloves in clean plastic bags, labelled and kept in icebox and transported to Asela Regional Veterinary Laboratory and each sample was processed by Baerman and modified Baerman techniques as described by Charles and Robinson (2006). While collecting faecal sample, the species of the animals, sex, age, overt clinical signs of lungworm infection, and date of sampling were properly recorded. The larvae were then identified under lower power microscope (10X objective), based on the shape and number of gut cells, relative size and shape of larvae’s tail.

Modified Baerman procedure used in the laboratory

(1) The faecal samples were prepared with the necessary materials
(2) Place a double layer of cheese cloth or gauze on a disposable paper towel or equivalent on the bench. Using a spoon or spatula, weigh or measure approximately 5-10 g of faecal material. Place the faecal material in the center of cheesecloth. Form a pouch containing the faecal material by holding the four corners of the cheesecloth together and moulding around the faecal material.
(3) Using a rubber band or length of string close to the cheesecloth pouch. Push the sticks or short metal rod under the rubber band or string so that the pouch can be suspended.
(4) Place the pouch containing the faecal material in the plastic cone. Trim off the excess cheesecloth using scissor. Fill the plastic cone with lukewarm water. Make sure the faecal material is covered. Leave the apparatus to stand for 24 h.
(5) The supernatant was discarded and sediment was taken.
(6) Use a Pasteur pipette to transfer a small droplet of the sediment fluid from the petridish to a microscope slide. Add drop of iodine to fix the larvae and gently place a cover slip over the drop.
(7) Let examine under compound microscope at 10x magnification.
(8) Using the Pasteur pipette, remove a drop of sediment at the bottom of the tube and place it on microscope slide for examination. Be careful not to resuspend the sediment before you take a sample from it.

Data management and analysis

All raw data generated were entered into Microsoft excel spread sheet and statistical analyses were conducted using SPSS statistical software version 20.0 and multivariate logistic regression model. In all cases, 95% confidence intervals and P<0.05 were set for significance.

RESULTS

Over all field prevalence under coproscopic examination

Of the total 384 animals examined, the overall prevalence of *D. arnfieldi* was 164 (42.7%) in the study area.

The prevalence of lung worm infection with different risk factors

Of the total horses (128), donkeys (217) and mules (39) examined, 22.66, 57.6, and 25.64 of horses (*equus cabalus*), donkeys (*equus assinus*) and mules were positive for *D. arnfieldi*, respectively and statistically with significant difference (P<0.05).

Age wise prevalence of the parasites was observed and its rate was 50.9, 42.3 and 40.5% in young, adult and old equines, respectively. And the prevalence was found to be statistically not significant (P>0.05). Out of 217 male equines, 102 (47.0%) of them were positive for *D. arnfieldi* and from 167 female equines, 37.1% were affected by *D. arnfieldi*. The sex of animals have no significant difference (P>0.05) on the prevalence of *D. arnfieldi.* Body condition scores of equines in Sudie district showed that the prevalence of *D. arnfieldi* were 59.6, 41.46 and 21.3% in poor, medium and good body condition scores, respectively with statistically significant difference (P<0.05).

Deworming history of animals with some types of anthelementics had a significant variation among the groups. Out of 235 equines specie without any deworming history 125 (53.2%) of them were found to be positive for *D. arnfieldi* and from 149 dewormed equines 39 (26.2%) were positive for *D. arnfieldi* statistically with significant (P<0.05) difference between the two groups (Table 1).

DISCUSSION

The overall prevalence of lungworm infection in this study was 42.7% that is higher than the previous findings of Yitna et al. (2015), who reported a prevalence of 37.5% in Lode Hetosa district, south eastern Ethiopia. This difference could be due to the differences in environmental conditions and management practice favouring the survival of the larvae of the parasite.

In the current study, the infection rate was compared among equine species and the highest prevalence was recorded in donkeys (57.6%). This result is in line with the reports of Yitna et al. (2015), Pandy (1980) and Lyons

Table 1. The prevalence of lung worm infection with different risk factors.

Factors	Group	N	Positive No. (100%)	CI (95%)	OR	P-value
Species	Donkey	128	29 (22.7)			
	Mule	217	125 (57.6)	2.8-7.6	4.64	0.000
	Horse	39	10 (25.6)	0.51-2.7	1.18	
Age	≤4	55	28 (50.9)			
	4-10	156	66 (42.3)	0.83-2.81	1.53	0.394
	≥10	173	70 (40.5)	0.69-1.67	1.08	
Sex	Male	217	102 (47)	0.441-1.01	0.666	0.053
	Female	167	62 (37.1)			
Body conditions	Poor	114	68 (59.6)			
	Medium	190	79 (41.46)	2.85-10.53	5.478	0.000
	Good	80	17 (21.3)	1.435-4.85	2.64	
Deworming history	Dewormed	149	39 (26.2)	2.05-5.01	3.205	0.000
	Non-dewormed	235	125 (53.2)			

et al. (1985), who described prevalence of 57.81, 48 and 54% Lode Hetosa district, south eastern Ethiopia, in Morocco and Kentucky, USA, respectively. On the contrary, Feseha et al. (1991) and Hassan et al. (2004) had reported higher (83 and 70.5%) prevalences of *D. arnfieldi* in donkeys in Ethiopia and Sudan, respectively. And extremely higher prevalence (87.5 and 93%) of larvae of *D. arnfieldi* in Denmark and central Kentucky, USA were found in donkeys by Andersen and Fogh (2010) and Lyons et al. (1985), respectively. One study also indicated that, in an examination of fecal samples from part of a donkey herd, 85 of 90 donkeys were positive for lungworm larvae (Junquera, 2014).

However, extremely lower prevalence of 3.6 and 9.67% of *D. arnfieldi* were reported in donkeys by Nuraddis et al. (2011) and Inasi and Mustafa (2009) in Hawassa town, Ethiopia and in the Central Black Sea region, Turkey, respectively. In several studies, 50 to 80% of donkeys have been found infected with *D. arnfieldi* (Clayton and Duncan, 1981; klei, 1986).

The present prevalence of lungworm infection in mules (25.64%) is in agreement with previous finding of Tihitna et al. (2012) who reported 29.26% in and around Jimma town and Klei (1986), who reported prevalence of 29.3% in North America, respectively. The current prevalence is higher than finding of Ram (2009), who reported 14.10% in Nepal. On the contrary, higher prevalence of lungworm infection in mules (54%) was reported by Lyons et al. (1985) in central Kentucky, USA and 45.31% prevalence reported by Yitna et al. (2015) in Lode Hetosa district, south eastern Ethiopia. These differences may be due to agro-ecology of the study areas, management, season and sample size.

In this study area, the prevalence of *D. arnfieldi* in horses (22.66%) was higher than the previous findings of Saeed et al. (2010), Tihitna et al. (2012), Yacob and Ashenafi (2013), Tilahun et al. (2014) and Yitna et al. (2015) who reported 2.5, 4.26, 0.5 and 3.7% in Lahore (Pakistan), Jimma town, Arsi-Bale highlands of Oromia region, Hawassa town, Ethiopia and in Lode Hetosa district, south eastern Ethiopia, respectively. Before the advent of anthelmintic, Ivermectin, the prevalence of *D. arnfieldi* infection in horses in Kentucky at necropsy was approximately 11%, while it was 2% in live horses at the same time and at the same region, based on fecal examination (Lyons et al., 1985a, 1985b). This difference may suggest that the difficulty in antemortem diagnosis of *D. arnfieldi* in horses. However, patent infections have been found in horses, resulting in disease occurrence in closed herds with no exposure to donkey or mules (Lyons et al., 1985b; Claytons and Duncan, 1981).

In the present study, prevalences of 50.9, 42.3 and 40.5% were recorded in young, adult and old age groups, respectively and the prevalence was found to be statistically non-significant (P>0.05). This result was not in agreement with Tihitna et al. (2012) and Yitna et al. (2015).

Body condition scores were found to be a major risk factor (P<0.05) in the prevalence of equine lung worm infection was in agreement with finding of Yitna et al. (2015). The prevalence according to body condition grade was 59.6, 41.46, and 21.3% in poor, medium, good body condition scores of the equines, respectively. In addition, in different species of equids, (donkey, horse and mules) body condition scores were considered as risk factor and was statistically significant (P<0.05). This might be due to the fact that poorly nourished animal appears to be less competent in getting rid of infection, although, it is unusual for well fed animals to succumb to the disease provided the right environmental conditions are available (Kimberling, 1988).

Sex was not found as a major risk factors (P>0.05) in the prevalence of equine lungworm infection. A prevalence of 47.0 and 37.1% were recorded in male and female equines, respectively that match with the insignificant prevalence reported by Tihitna et al. (2012) and Yitna et al. (2015). However, the prevalence in male equine was higher as compared to female.

Deworming history of animals with anthelmintic usage was found as a major risk factors for statistical variation (p<0.05) in the prevalence of the parasite. Higher prevalence (53.2%) of the *D. arnfieldi* was recorded in equines with non-dewormed history than dewormed (26.2%). The reason why dewormed equines infected with *D. arnfieldi* might be either due to the anthelmintic used in the area for the treatment only temporarily suppress egg production of the adult worms or parasite may become resistance to anthelmintic used. It may also be related to the poor quality of anthelmintic used in the country. In contrast, 46.8% of none dewormed animals were not infected by lungworm; this might be due to development of acquired immunity from previous exposure (Blood and Radostits, 1999; Urquhart et al., 1996) and it may also be due to no exposure to the infective stages of *D. arnfieldi.* Similarly, at the time of examination, the adult parasite might not shed eggs because either it is in the prepatent period which lasts about 4 weeks or larvae in the lungs may become arrested (dormant, hypobiotic, inhibited) for up to 5 months when there is unfavorable condition.

Conclusion

The result of current study clearly indicated that equine lungworm is highly prevalent in donkeys. It also demonstrated the abundance and distribution of lungworm parasitism in different age groups and body condition scores of equines. The findings of current study suggest that emphasis be given to lungworm parasitism in the study area and in the country as whole.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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Full Length Research Paper

Fuzzy simulation of bioclimatic indexes environments with and without cover for Santa Inês sheep farms

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This study aimed to analyze bioclimatic indexes: black globe temperature and humidity index (BGHI) and radiant thermal load (RTL) in Santa Inês sheep farms, with and without coverage to develop a fuzzy logical computational model to forecast variables analyzed experimentally. Ten Santa Inês sheep were placed in two cover paddocks, and one without cover. Data recorded were: air temperature schedules and black globe (°C), relative humidity (%) and wind speed (m s^{-1}), and BGHI and RTL were calculated. Computational models were developed using fuzzy logic which had as input variables, air temperature (°C) relative humidity (%) for the output variables BGHI and RTL. Based on the experimental data, there were also certain relevance curves that conform more to the results for the model generation via Fuzzy logic. The Mandani inference method was used for the preparation of rules and defuzzification of the center of gravity method was applied. The results showed that in the critical periods, 12 to 14 h, coverage was insufficient to differentiate between stress environments. It was also found that the Fuzzy models compared with the experimental data were highly correlated with R^2 equal to 0.99, proving it is suitable for implementation in practice.

Key words: Computational model, heat stress, shelter, simulation.

INTRODUCTION

In Brazil, sheep farming in the northeast is done in small farms, represents a source of resource generation (Ribeiro et al., 2008), being exploited with a low level of technology. Santos et al. (2011) reported that in this region, most sheep are raised in pastures with little or no shade and analyzing the effect of shading on the comfort of Santa Inês sheep, showed that the shade provided by the trees reduced the radiant heat load by up to 44.7%, providing a better thermal environment for the animals. Relative to the comfort of sheep Santa Inês, Neves et al.

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(2009) found the maximum temperature of 35°C, being above the thermal comfort zone (30°C) recommended for sheep, with values of black globe temperature index and humidity characterizing the situation of thermal discomfort. High BGHI values in the semi-arid region are also cited by Oliveira et al. (2013) and Leitão et al. (2013).

According to Pandorfi et al. (2012), considering the current demands, it is no longer possible to consider animal production or production chain, without considering the concept of well-being in production. Thus, the uses of new information technology tools favor the accuracy of research and the development of expert systems for decision making.

Several computational modeling techniques to predict animal-related variables from a mathematical knowledge base are being used (Schiassi et al., 2015). Among the possible models to be developed, those based on artificial intelligence, specifically, the Fuzzy methodology has been shown to be more adequate. The Fuzzy sets logics have been applied in estimating the comfort and well-being of animals confined by several researchers (Ferreira et al., 2010).

The objective of this research was to analyze the bioclimatic indexes, BGHI and RTL in environments for Santa Inês sheep breeding, with and without cover, and to develop a model using the Fuzzy set theory capable of predicting such variables.

MATERIALS AND METHODS

This research was carried out in two phases: the first one was an experiment in the field, in which 10 male Santa Inês sheep, average of 24 months old, were confined, distributed equally in two pickets of equal area of 24 m² each, one discovered (Treatment 1) and another provided with covering of asbestos cement tiles with 3 m ceiling (Treatment 2). The experimental data were used to develop the model able to predict the black globe temperature and humidity index (BGHI) and radiant thermal load (RTL).

This stage was performed in Caturité-PB, Brazil, (7° 25 '12' 'S and 36° 1' 3 '' O) with semi-arid climate (BSh) characteristics according to Köppen-Gieger classification with maximum temperatures of 37°C and the minimum of 16°C and annual rainfall of 500 mm. The experiment was carried out in April and May 2012, with a pre-experimental period of 77 days to adapt the animals to the test conditions and 46 days of collection. The animals were confined to rations composed of sorghum silage and balanced concentrate, based on soybean meal and corn, fed twice daily at 7 and 16 h. Water was supplied *ad libitum*.

In both treatments, air temperature (AT), relative air humidity (RH), black globe temperature (BGT) and wind speed (WS) were recorded. The climatic data were collected every 60 min during the 24 h of each day of experiment, and the values were analyzed in the interval of 7 to 17 h. The AT and RH data were obtained by means of electronic sensors (datalloger HT500), the WS was collected by means of a digital thermohygranemometer and for collecting the BGT, black balloons calibrated with the standard globe, positioned in both environments, at the height corresponding to the center of mass of the animals were used. Thus, was possible to calculate the black globe temperature and humidity index (BGHI) for the environments, by the formula proposed by Buffington et al. (1981). The RTL was calculated using the Esmay (1969) methodology.

In the second stage of the research, the data for each treatment were tabulated and used to calculate the BGHI and RTL, which were used in the validation of the proposed mathematical model. The computational model was based on the theory of Fuzzy sets, having as input variables, the values of TA and UR and as output data, the bioclimatic variables BGHI and RTL for the covered and discovered environment. Figure 1A and B correspond to the BGHI and RTL pertinence curves for the covered environment, respectively, whereas Figure 1C and D correspond to the variables of the environment exposed to direct solar radiation.

The intervals adopted for the input and output variables were represented by triangular pertinence curves, as used by several authors such as Ponciano et al. (2012) and Tolon et al. (2010), and the intervals were defined based on the ranges established during the conduction of the experiment and through consultation with specialists.

In order to simulate the data, the Mamdani inference method was used, as proposed by Li and Gatland (1996) and also used by Ponciano et al. (2012) and Schiassi et al. (2015), response, a Fuzzy set originated from the interaction of the input values with their respective degrees of pertinence, through the minimum operator and then through the superposition of the rules, through the maximum operator. The Fuzzy number is the number of points in the center of gravity. A comparison of the output data of the model with the data acquired through the experiment was made to evaluate the accuracy of the proposed Fuzzy models using Xfuzzy software.

RESULTS AND DISCUSSION

The highest averages observed for ITGU were in the uncovered environment at 13 and 14 h, equal to 90.8 and 90.4, respectively. For the indoor environment, the highest values were recorded from 12 to 14 h and 81.7 (Figure 2).

When studying the comfort of Santa Inês sheep raised in uncovered environment and shaded with polypropylene tiles in the Petrolina region, PE, Brazil, Oliveira et al. (2013) found a significant effect between the two environments and shifts of the day for the BGHI in the dry season with values varying from 82 to 85 for the shaded and uncovered environment, respectively, where they concluded that values above 82 represented an alert condition for sheep Santa Inês.

Leitão et al. (2013) found ITGU values above 94 when keeping Santa Inês sheep in paddocks deprived of coverage in Juazeiro, BA, Brazil, in the dry period, concluding that the existence of a cover can have a significant effect on the reduction of the stress condition for sheep raised in the semiarid. This fact was corroborated by the present research since a reduction of up to 10% in the BGHI value between the two environments was found. However, according to Oliveira et al. (2013), although the coverage significantly reduced the BGHI values as compared to the uncovered environment, the authors did not observe a reflection of this in the behavioral parameters as feeding, rumination or idle activity when compared with the treatment without shading for sheep Santa Inês.

In the present research, the highest air temperature

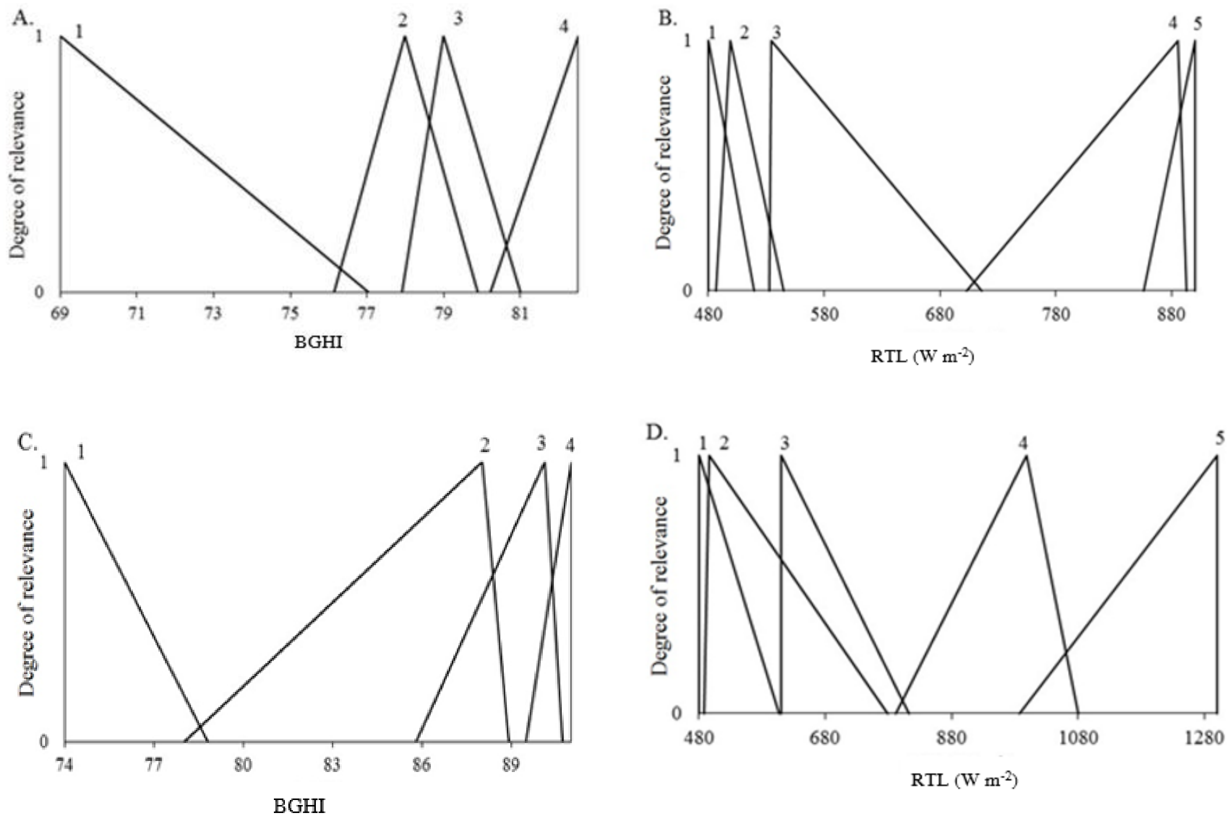

Figure 1. A. Relevance curves for BGHI; B. Relevance curves for RTL for the indoor environment; C. Relevance curves for BGHI; D. Relevance curves for RTL for the discovered environment.

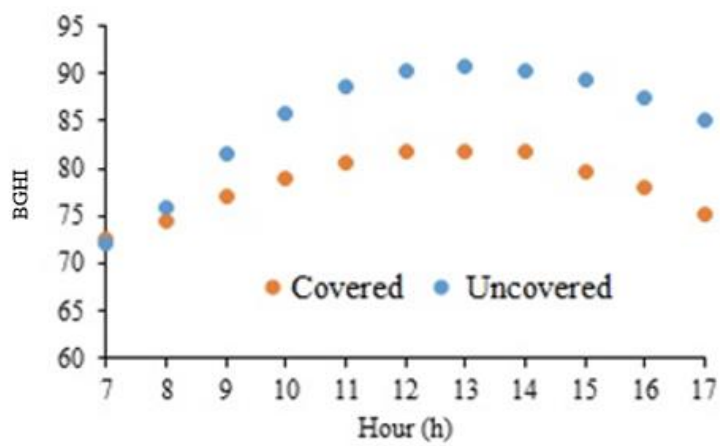

Figure 2. Globe and humidity temperature index in the two environments.

value was found for the uncovered environment at 12 pm (34.2°C), being close to the temperature predicted as a stressor of 35°C found by Neves et al. (2009) for Santa Inês sheep raised in the agreste region of Pernambuco, Brazil, which confirms an alert situation, independent of the studied environment.

The maximum radiant thermal Load values observed were 956.2 and 887.3 $W.m^{-2}$ for the uncovered and covered environments, respectively, both at 12 pm (Figure 3). Ribeiro et al. (2008) evaluated the RTL in environments covered with ceramic tile in the confinement of native sheep in São João do Cariri, PB, Brazil, and

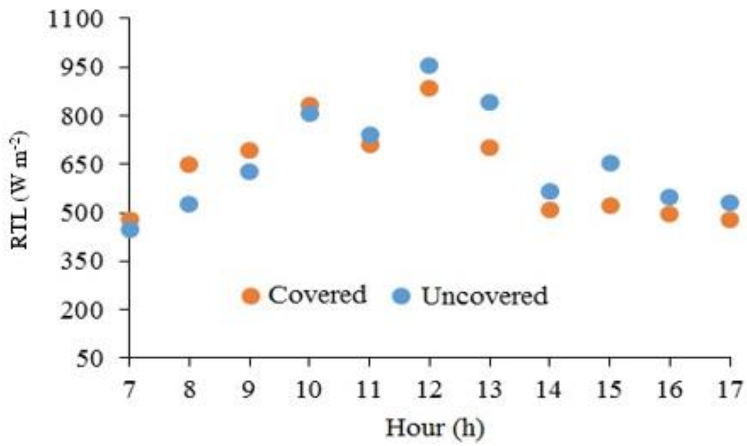

Figure 3. Radiant thermal load in both environments.

found at 15 h the highest value equal to 543.5 W m^{-2}. This means that even considering the asbestos cement tile with higher values of absorptivity and thermal transmittance than the ceramic tile, the RTL values of the present research still present stressors, especially for the uncovered environment.

When studying RTL variation between the internal and external environment in reduced models with different coverages, Cardoso et al. (2011) concluded that the existence of cover can dampen up to 40% of the radiant heat energy during the hottest times of the day, which is not corroborated by the present research since the reduction of RTL to the internal environment was only 8%.

This can be explained by the existence of side walls in the covered environment that may have acted as a barrier to ventilation, resulting in increased storage of thermal energy throughout the day and consequently increasing the black globe temperature. Another factor that is important to highlight was the existence of vegetation cover in the soil in the experiment carried out by Cardoso et al. (2011) and the lack of this in the current research, which certainly contributed to higher values of RTL and smaller differences between the environments analyzed.

The values of BGHI and RTL observed and simulated by the Fuzzy model are shown in Figure 4, as well as the regression equations and determination coefficients to estimate these indices. No research was found on the creation of Fuzzy models for bioclimatic prediction of sheep facilities and, therefore, the results found here were compared to the models developed for other species.

When analyzing Figure 4, it is possible to notice that the values of the determination coefficient for BGHI in covered and uncovered environment was 0.99 and 0.99, respectively. For the RTL in covered and uncovered environment, in this order, R^2 values were 0.97 and 0.99. It can be observed that for the two treatments and bioclimatic indices, the proposed model was shown with good precision, being able to realistically portray the same as a function of the temperature and relative humidity of the air.

Similar results were found by Marques et al. (2016) where, when developing a Fuzzy model to predict values of BGHI and RTL in an environment destined to confinement of quails, in the Brazilian semi-arid region, they verified R^2 values equal to 0.97 and 0.98, respectively. These authors also adopted, for the output variables (BGHI and RLT), intervals represented by triangulated pertinence curves, used by several authors (Ponciano et al., 2012).

It can be noticed that the adjustment of the four proposed models to the bioclimatic data were distributed approximately, with coefficients of determination (R^2) higher than 0.97. Thus, the R^2 report that the Fuzzy models explain more than 97% of the variance for all the evaluated situations, which means the numerical reliability as a specialist model to be used as a decision-making mechanism in the control of bioclimatic variables in facilities for sheep.

Conclusions

At the critical times between 12 and 14 h, the thermal environment was considered as an alert situation for both environments and the existence of asbestos cement cover in one of the environments was insufficient for the damping of the radiant thermal load. In the simulation models reproduced with fidelity, the experimental values with coefficients of determination superior to 0.97 indicate that Fuzzy logic can be applied with efficiency in the prediction of BGHI and RTL in covered environment and discovered as a function of the temperature and relative humidity of the air. Thus, it can be implemented in

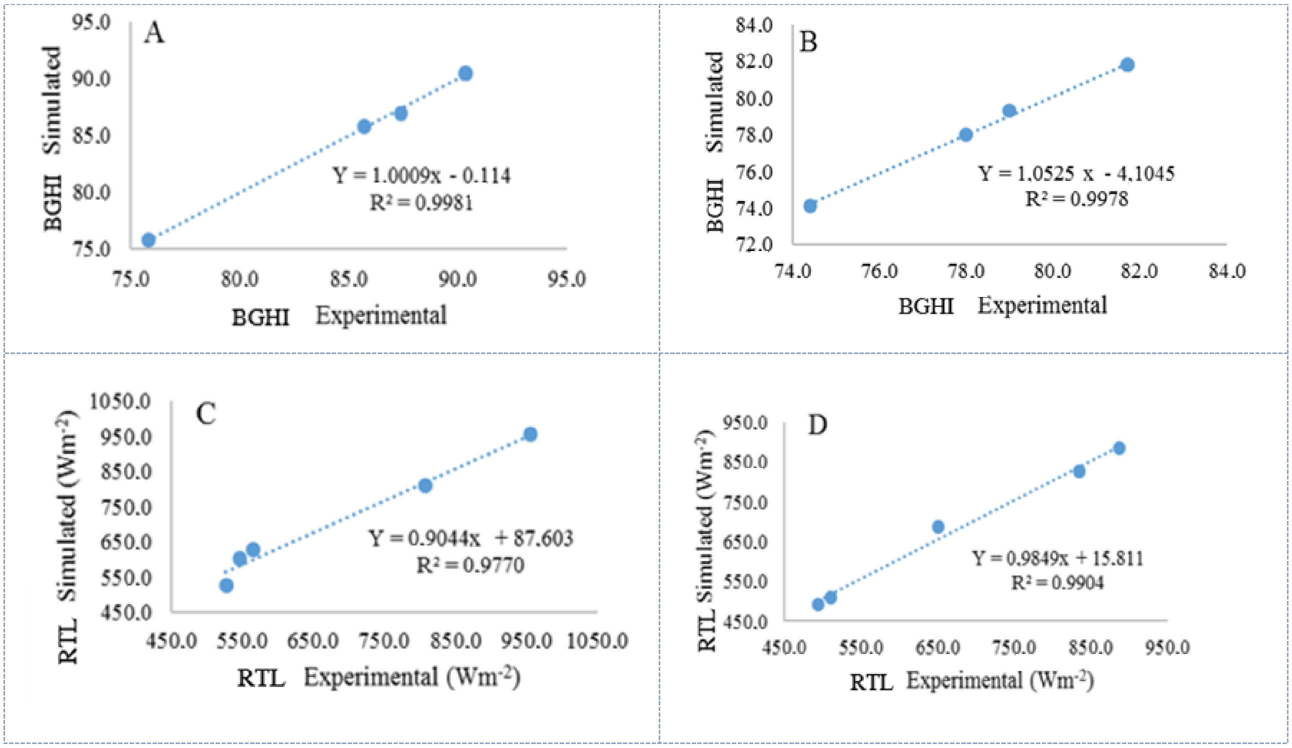

Figure 4. Simple linear regressions for the output variables: (A) BGHI environment covered; (B) BGHI uncovered environment; (C) RTL covered environment; (D) RTL uncovered environment.

systems of automation and control of zootechnical facilities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Abreviations

BGHI, Black globe temperature and humidity index; **RTL,** radiant thermal load; **AT,** air temperaure; **RH,** relative humidity; **BGT,** black globe temperature; **WS,** wind speed.

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Full Length Research Paper

Soil fertility status of seasonally closed wetland ecosystem (*ondombe*) in north-central Namibia

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In the Cuvelai Seasonal Wetland System (CSWS) of North-central Namibia, there are widespread manifestations of seasonally flooded river and seasonally closed wetland ecosystems (ponds). These wetlands are called *oshana* (seasonally flooded river wetland) and *ondombe* (seasonally closed wetland) according to the local language. This study was initiated to find out the soil fertility status of *ondombe*s and whether they could be utilized for agricultural purposes unlike the present situation. Soil salinity and sodicity were determined to find out impact of such adverse conditions on possibility of food production. A total of 70 representative *ondombe*s were identified from three selected villages. A total of 210 soil samples were collected from upper, middle and lower positions adjacent to *ondombe*s, and 15 soil samples from each 5 upland fields in the three villages and 102 soil samples from different spots of the flood plain in the three *oshana*s for comparison. The results indicated that the mean soil pH (H_2O) in *ondombe* was 6.3, the means of organic C and total N were 6.28 and 0.41 g kg^{-1}; respectively, the mean of available P was 4.81 mg P kg^{-1}. The means of exchangeable Ca, Mg, K, and Na in *ondombe* were 2.31, 1.44, 0.21, and 0.61 cmol$_c$ kg^{-1}, respectively. Most soil nutrients were higher in lower *ondombe* positions than on upper and middle positions. Organic C, exchangeable Mg, and clay at the *ondombe* soils were significantly higher than those at the croplands. The means of electrical conductivity of saturation extract (ECe) and sodium adsorption ratio (SAR) in *ondombe* soils were 0.62 ds m^{-1} and 7.32, respectively; even though most of the *ondombe* soils did not exhibit salinity and sodicity problems. Hence, one can conclude that an *ondombe* soil has an appropriate condition for agriculture, and may only be prone to sodicity whenever the sodium content is high, as sometimes observed.

Key words: Soil fertility, seasonal wetland, soil salinity, sodicity, Cuvelai Seasonal Wetland System (CSWS).

INTRODUCTION

Semi-arid ecosystems in tropical regions exhibit high climatic variability, where food security is threatened by frequent drought (Steiner and Rockström, 2003). North-central Namibia is a semi-arid area in southern Africa

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which is dry through most of the year receiving very little rainfall. There is frequently occurring drought, and performance of cereal production fluctuates greatly (Shifiona et al., 2016). In north-central Namibia, during the rainy period, a specific seasonal wetland system is usually experienced, which is commonly referred to as Cuvelai Seasonal Wetland System (CSWS).

In the CSWS, there are widespread manifestations of seasonally flooded river and seasonally closed wetland ecosystems (ponds). These wetlands are called *oshana* (seasonally flooded river wetland) and *ondombe* (seasonally closed wetland) according to the local language (Figure 1). The *ondombe* water is shallow and the water level fluctuates during December to May. *Ondombe*s include different-scale seasonal ponds. Most of them usually range between 500 and 1000 m^2 in size and the water depth of *ondombe* is less than 1.0 m. Efficient utilization of *oshanas* and *ondombes* during rainy seasons, may alleviate problems associated with drought and food production in Namibia. *Ondombe*s are usually situated on a gentle slope having some vegetation. Closer examination has also revealed the prevalence of different soil types at different slope positions.

In principle, the local farmers do not often utilize *ondombe* for irrigation purposes because they lack water pumps to draw the water from *ondombe*. The area surrounding *ondombe* is usually left for grasses to grow, for livestock grazing, or for obtaining roof materials and the *ondombe* water is used for livestock drinking and washing clothes. Through the Rice-Pearl Millet Namibia-Japan project, efforts have been made to utilize *ondombe* for rice production and other crops such as pearl millet and sorghum (Iijima et al., 2016a). Iijima et al. (2016b) found that mixed-planting pearl millet or sorghum with rice improved the photosynthetic and transpiration rates and biomass under O_2 deficient solution culture conditions. Awala et al. (2016) reported that mixed planting with rice could alleviate flood stress under field flood conditions.

Lowlands with wetlands generally have a high agricultural production potential (Andriesse et al., 1994; Rodenburg et al., 2014). Lowland soils are usually fertile because they receive transported materials from adjacent uplands. Soil fertility characteristics of lowlands were reported in West Africa (Issaka et al., 1996; Buri et al., 1999). Although limited studies on soil fertility of seasonally flooded wetlands (*oshana*s) are available (Watanabe et al., 2016), there appears to be significant paucity of information on *ondombe*s.

A lot of croplands in arid areas have a problem of soil salinity and sodicity. It is well established that salinity reduces water availability to plants and sodicity results in sealing of soil pores and reduction of air and water exchange. Semi-arid catchments usually experience salinization and are degraded by intensive agricultural use (Moreno-Mateos et al., 2010). The irrigation and drainage mass balance is important for control of salinity effects on agriculture in semi-arid areas (Tedeschi et al., 2001; Causapé et al., 2004). Therefore, reclamation and utilization of arid wetlands should consider salt accumulation, and maintenance of food production.

This study was hence initiated to find out the soil fertility status of soils around *ondombe*s and whether the *ondombe* could be utilized for agricultural purposes unlike the present practices. The investigation of the soil physicochemical properties and salt accumulation was done to confirm the possibility of crop production from the seasonal wetlands.

MATERIALS AND METHODS

Study area

The study focuses on *ondombe* wetlands in CSWS area in north-central Namibia. Mean annual rainfall where *ondombe*s are observed, ranges from 269 to 914 mm, and an average monthly temperature from a minimum of 9.1°C in June to a maximum of 36.6°C in October, from 2003 to 2015 (Ondangwa station, Metrological service division Namibia). The vegetation can be broadly classified into the following major associations; mixed woodland of the deep aeolian sands, the Palm tree savanna, Mopane woodland and Mopane savanna, Sclerocarya-Ficus savanna, and Various scrub Mopane-Acacia (Moller, 1997). The soils are classified into three major groups: Cambic Arenosols, Eutric Cambisols, and Haplic Calcisols (Mendelsohn et al., 2002). Many people benefit from the seasonal wetlands. Fishing and grazing are common practices in the wetland areas. Three villages, two in Omusati (Oshiteyatemo; 17°28'29.57"S, 15°20'19.41"E and Onamundindi; 17°45'59.17"S, 15°15'0.25"E) and one in Oshana (Afoti; 18° 0'55.20"S and 15°19'58.76"E) regions, with altitudes ranging from 1090 to 1110 m above sea level, were selected for the study of *ondombe* (Figure 2). A total of 70 representative *ondombe*s (Oshiteyatemo, 24; Onamundindi, 24; Afoti, 22) were identified in the three villages.

Soil sampling

Soil samples were collected from the plow layer (0-15 cm) during 2014 to 2015. Soil samples were collected from upper, middle and lower positions adjacent to *ondombe*s. Fifteen soil samples from 5 representative upland fields in the three villages, and 102 soil samples from different spots of the flood plain in the three *oshana*s were collected for comparison (Watanabe et al., 2016). At each spot of *oshana*, 3 sub-samples were collected along topographical setting (lower, middle upper) from 0-15 cm depth and composite sample was used for chemical/physical analysis (Watanabe et al., 2016). At the soil sampling points, the vegetation observed were few grasses, and the prevailing slope was gentle (less than 1%). The soil samples were collected and stored in plastic bags. The soil samples were air-dried and were ground and sieved to remove all materials above 2 mm diameter, before laboratory analyses.

Laboratory analysis

The samples were subjected to different physical and chemical analyses. The physical analysis involved particle size determination using the pipette method. The glass electrode method was used to

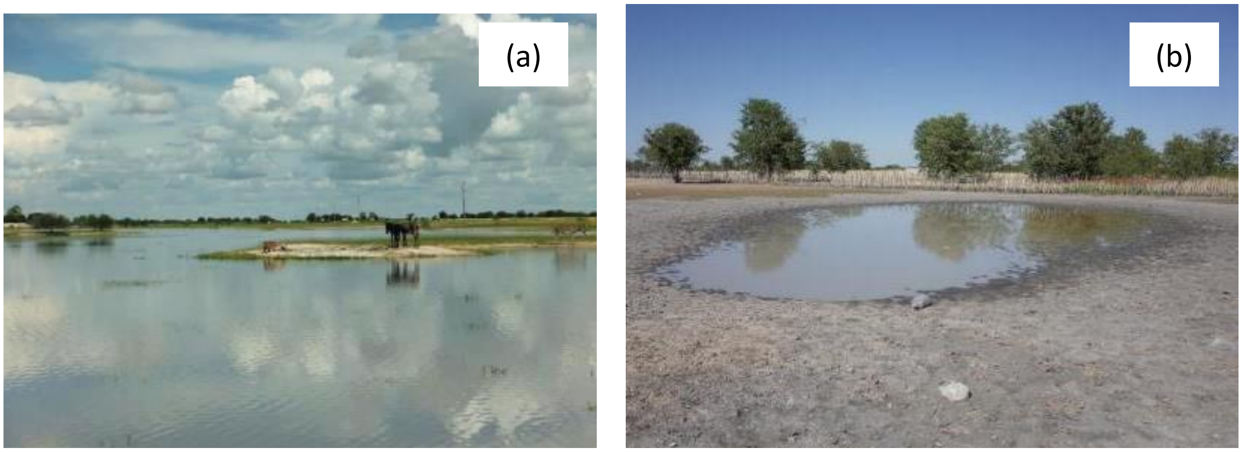

Figure 1. Picture of *oshana* (a) and *ondombe* (b).

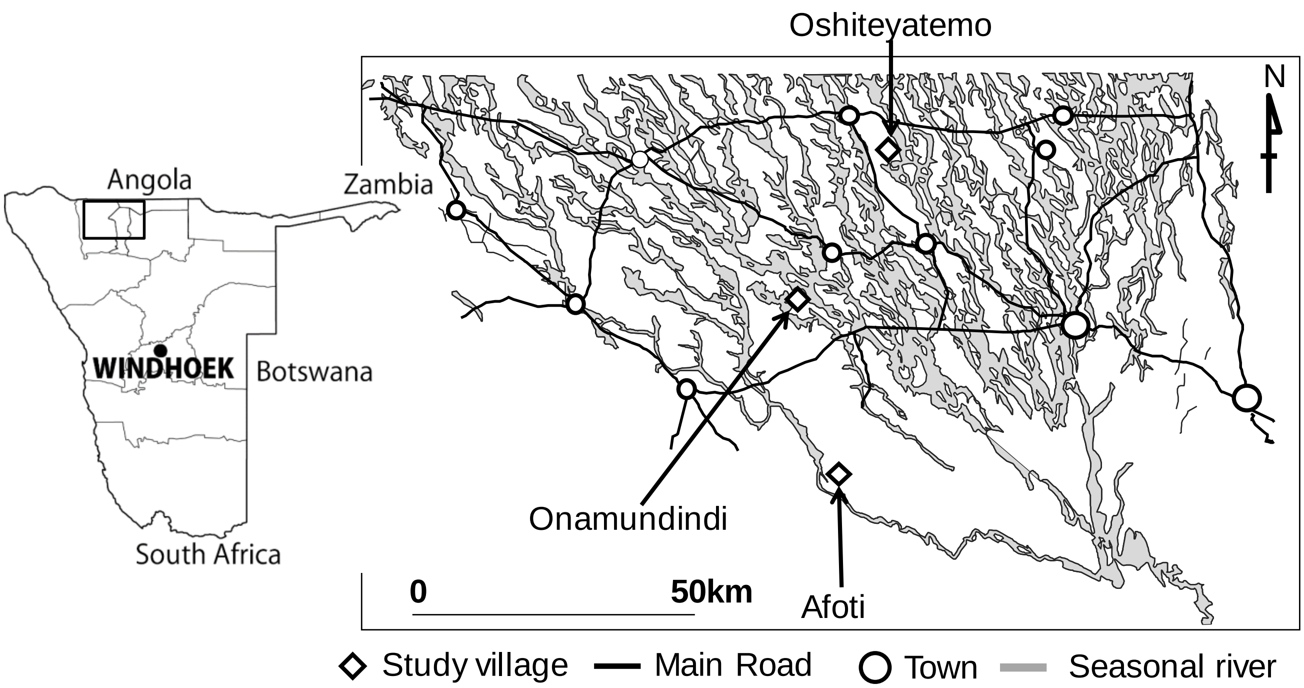

Figure 2. Location of the study sites in north-central Namibia.

determine soil pH in water (soil: H_2O, 1:2.5), and hereafter shown as pH (H_2O). Organic carbon content was measured by the Walkley Black method. Total nitrogen content was measured by the modified Kjeldahl method (salicylic acid added to the sulphuric acid). Available phosphorus (P) was extracted by the Olsen method followed by colorimetric measurement using an Ultraviolet-Visible (UV/VIS) spectrophotometer (Spectrophotometer; UV mini 1240, Shimadzu Corporation, Kyoto, Japan). Exchangeable calcium (Ca), magnesium (Mg), and potassium (K) and sodium (Na) were extracted from the soil with 1 mol l^{-1} neutral ammonium acetate, and were subsequently determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Optima 7000 DV, Perkin Elmer Inc., U.S.A.). The pH saturated, the electrical conductivity of the saturation extract (ECe) and the concentrations of soil Ca, K, Mg and Na were determined in the extract from saturated paste of the soil samples and were subsequently determined using a pH-mV and conductivity meter (MultiLab 540; WTW Wissenschaftlich- Technische Werkstätten GmbH Weilheim i. OB, Germany), and an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Optima 7000 DV, Perkin Elmer Inc., U.S.A.). Soil salinity is expressed by the soil electrical conductivity of saturated paste extract (ECe). The adsorption of sodium by the soil is expressed by the sodium adsorption ratio of the saturated paste extract (SAR). This ratio was used as an indicator of sodicity and was defined as follows:

$$SAR = Na^{+} / \sqrt{(Ca^{2+} + Mg^{2+})/2} \quad (1)$$

Table 1. Physicochemical properties, salinity and sodicity in *oshana*, cropland and *ondombe* soils.

Variables	pH (H_2O)	Organic carbon	Total N	C/N ratio	Av. P	Ex. Ca	Ex. Mg	Ex. K	Ex. Na
		(g kg^{-1})			(mg P kg^{-1})	(cmolc kg^{-1})			
Oshana	7.61^{a}	1.95^{a}	0.27^{a}	7.61^{a}	3.49^{a}	1.49^{a}	0.41^{a}	0.45^{a}	4.88^{a}
Cropland	7.57^{a}	2.62^{a}	0.32^{ab}	8.00^{a}	9.55^{b}	3.58^{b}	0.76^{a}	0.25^{ab}	0.12^{b}
Ondombe	6.30^{b}	6.28^{b}	0.41^{b}	17.80^{b}	4.81^{a}	2.31^{b}	1.44^{b}	0.21^{b}	0.61^{b}

Variables	Sand	Silt	Clay	pH saturated	ECe	Ca2+	Mg2+	K+	Na+	SAR
		(g kg^{-1})			(dS m^{-1})		(mmolc L^{-1})			
Oshana	852^{a}	45	103^{a}	6.55^{a}	5.47^{a}	1.92^{a}	2.08^{a}	1.29^{a}	54.06^{a}	39.84^{a}
Cropland	932^{b}	37	31^{b}	7.32^{b}	0.42^{b}	1.57^{ab}	0.75^{ab}	1.11^{ab}	1.73^{b}	2.08^{b}
Ondombe	864^{a}	53	83^{a}	6.54^{a}	0.62^{b}	0.55^{b}	0.61^{b}	0.66^{b}	4.94^{b}	7.32^{b}

Different letters indicate significant differences between treatments at 5 % significance level using Tukey-Kramer test.

Data analysis

All results are reported as the mean ± standard error. One-way ANOVA test was used to compare the soil physicochemical, salinity, sodicity characteristics between different land conditions. Pearson's correlation coefficients were used to compare soil fertility parameters originating from *ondombe* soils. All statistical analyses were performed using Excel Statistics Version 2015 software (Social Survey Research Information Co., Ltd., Japan).

RESULTS

General soil fertility conditions

Data of mean soil physicochemical properties of the *ondombe* soils are shown in Table 1. Soil pH (H_2O) from extracted soil solution in *ondombe* ranged from 4.6 to 8.8 with a mean soil pH of 6.3. Most *ondombe* soils were neutral, but some were acidic and alkaline. The soil organic C and total N in *ondombe*s ranged from 6.18 to 54.27 and 0.11 to 3.31 g kg^{-1} with a mean 6.28 and 0.41 g kg^{-1}, respectively. Available P ranged from 0.24 to 91.04 mg P kg^{-1} with a mean of 4.81 mg P kg^{-1}. The means of exchangeable Ca, Mg, K, and Na in *ondombe* were 2.31, 1.44, 0.21, and 0.61 cmol$_c$ kg^{-1}, respectively. Calcium is the cation which occupies the greater part of the exchange sites. The means of sand, silt, and clay in *ondombe* were 864, 53 and 83 g kg^{-1}, respectively. These results show that *ondombe* soils are sandy with little clay contents.

The mean values for soil salinity and sodicity of the *ondombe* soils are shown in Table 1. Based on determinations from saturated extract solution, the pH saturated of *ondombe*s ranged from 4.6 (acidic) to 8.7 (alkaline) with a mean soil pH saturated of 6.5. The ECe and SAR in *ondombe* soils ranged from 0.03 to 9.38 ds m^{-1} and 0.80 to 110.60 with a mean of 0.62 ds m^{-1} and 7.32, respectively. In principle, soils with ECe and SAR of more than 4 and 13 are judged as saline and sodic soils, respectively (Soil Science Society of America, 2008). The results show that most of *ondombe* soils do not have saline and sodic problems. In a few instances, high ECe and SAR values have been observed. The mean values for Ca, Mg, K, and Na determined from saturated extract solution were 0.55, 0.61, 0.66, and 4.94 mmol$_c$ l^{-1}. Sodium was greater than the other extracted elements.

The result of soil physicochemical properties of the *ondombe* soils from upper, middle and lower positions are shown in Figure 3. The lower positions at *ondombe* happen to have a relatively higher amount of nutrients, compared to upper and middle positions. Organic C, total N, available P, exchangeable Ca, Mg, K Silt, and Clay for the lower positons of the *ondombe* soils were significantly higher than that of the upper and middle positions. The results of soil salinity and sodicity of *ondombe* soils at different slope positions are shown in Figure 4. Soil pH saturated at upper position was significantly higher than that of middle and lower positions. Other salinity and sodicity properties were not significantly different along slope positons.

Comparison of soil physicochemical properties of *ondombe* with cropland and *oshana*

The result of mean soil physicochemical properties of the *oshana,* cropland*,* and *ondombe* soils are shown in Table 1. Soil pH (H_2O) at the *ondombe* soils was significantly lower than the croplands. The C/N ratio at all positions of the *ondombe* soils was significantly higher than that of the croplands. Organic C, exchangeable Mg, and clay at the *ondombe* soils were significantly higher than those at the croplands. Soil available P and sand at the *ondombe*s were significantly lower than that of the cropland soils. ECe and SAR, salinity and sodicity indicators, for *ondombe*s were not different from the croplands.

Soil pH (H_2O) at the *ondombe* soils was significantly lower than the *oshana*s (Table 1). Soil organic C, total N,

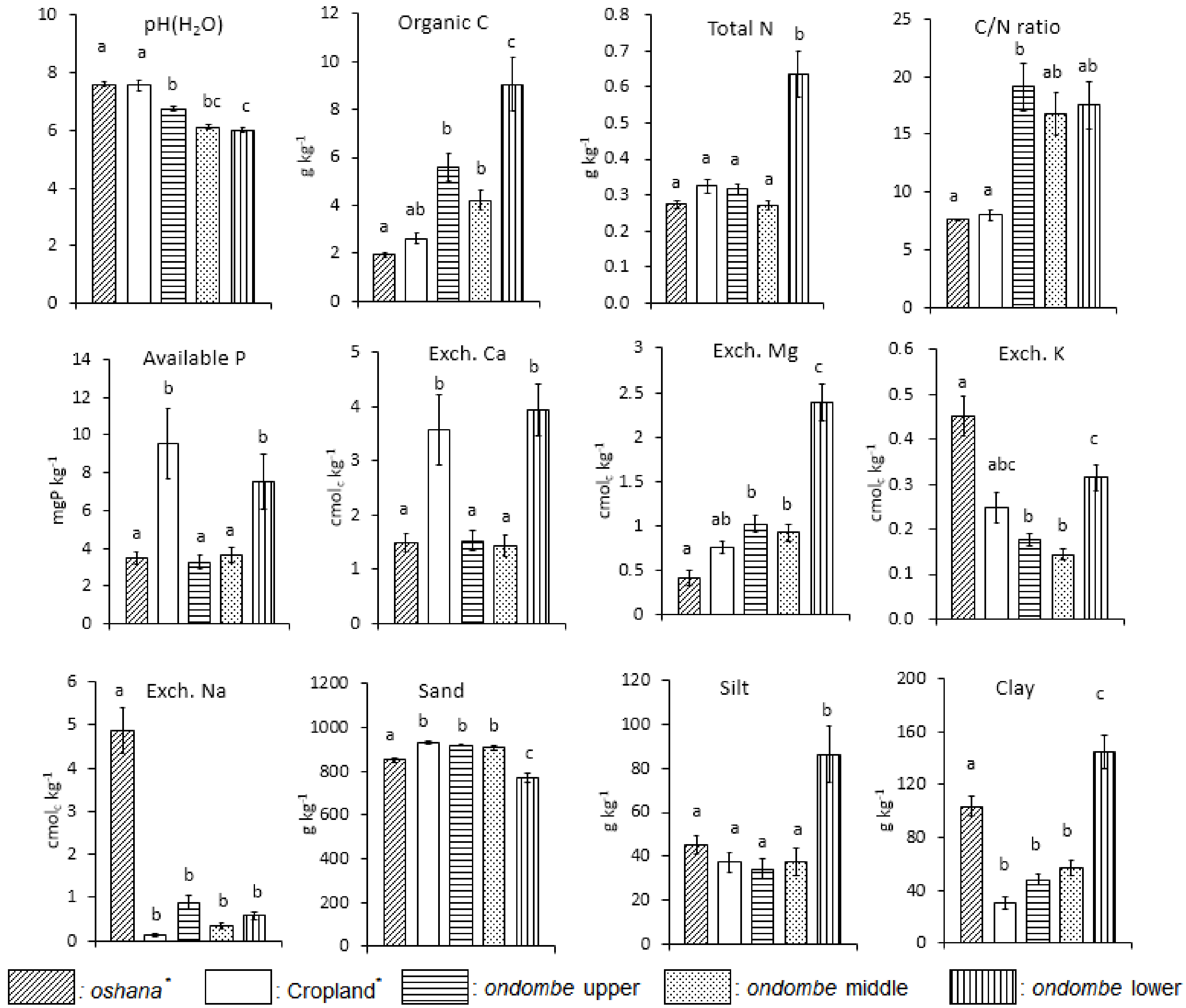

Figure 3. Soil physicochemical properties for *ondombe* soils at different slope positions, croplands, and other wetland soils. Error bars represent the standard error of mean. Different letters indicate significant differences between treatments at 5% significance level using Tukey-Kramer test. *Watanabe et al., 2016. Reference methodology (Watanabe et al., 2016) is same as the method of this paper.

C/N ratio, and exchangeable Ca and Mg at the *ondombe*s were significantly higher than the *oshana* soils. Exchangeable K and Na at the *ondombe*s were significantly lower than the *oshana* soils. ECe, Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, and SAR for *oshana* soils were much higher than that of *ondombe*s.

Correlation between soil physicochemical properties in *ondombe*

Table 2 shows the correlation matrix of the physicochemical and saline-sodic parameters in different slope positions. The organic C was positively correlated with available P in upper and middle positions. It was correlated with exchangeable Ca, Mg and K in lower positions. The clay content was positively correlated with total N, and exchangeable cations at all slope positions, and it was correlated with ECe and SAR in upper position. Although available P was correlated with organic C, C/N ratio and exchangeable Mg in upper and middle positions, it was only correlated with C/N ratio in lower position. Among exchangeable cations, exchangeable Ca, Mg and K were positively correlated with each other.

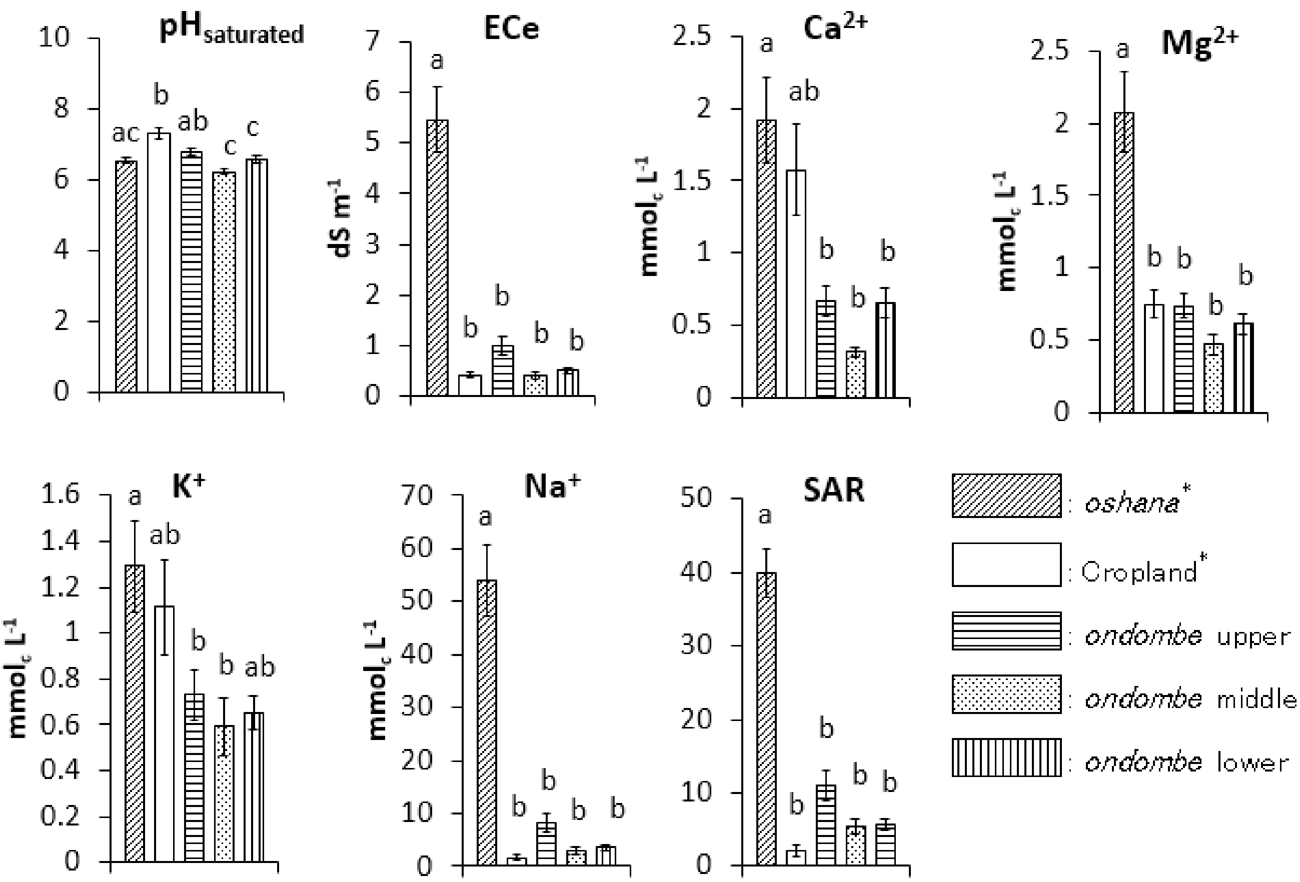

Figure 4. Soil salinity and sodicity of *ondombe* soils at different slope positions, croplands, and other wetland soils. Error bears represent the standard error of mean. Different letters indicate significant differences between treatments at 5% significance level using Tukey-Kramer test. *Watanabe et al., 2016. Reference methodology (Watanabe et al., 2016) is same as the method of this paper.

Exchangeable Na was strongly correlated with ECe and SAR. ECe was strongly correlated with SAR in all positions. SAR was positively correlated with pH (H_2O).

DISCUSSION

General soil fertility conditions of *ondombe*s

Soil pH (H_2O) of *ondombe*s was significantly lower than soil pH (H_2O) of the croplands and *oshana*s (Table 1). Organic C in *ondombe* soils is higher than the cropland and the *oshana*s soil (Table 1). High organic matter in *ondombe* soils can increase soil productivity and mitigate soil alkalinity by influencing soil pH (H_2O). Organic matter can associate H^+ irons, and can dissociate in high pH conditions, thereby modifying soil pH (Brady and Weil, 2014).

The organic carbon contents in *ondombe* soils are quite high (Table 1). The clay contents in lower positions of *ondombe* soils are also high, signifying the potential of high nutrient retention (Figure 3). *Ondombe*s are usually used for livestock grazing. Livestock come to drink water and drop their dung. A lot of studies reported that livestock manure affected and increased soil organic carbon and nitrogen (Jokela, 1992; Wani et al., 1993; Agele et al., 2005). The high plant biomass at some spots in the *ondombe*s is likely due to the high soil organic matter contents of *ondombe* soils and the dung added by animals. Under this study, organic C, total N, silt, and clay contents at the lower position of *ondombe* soils were significantly higher than that of the cropland soils (Figure 3). Soil organic C and total N of *ondombe* soils were significantly higher than the *oshana* soils (Table 1). Organic matter tends to accumulate in lower positons of *ondombe* soils. Other studies also confirm that wetlands have long-term soil organic carbon stabilization mechanisms (Cui et al., 2014). Soil organic matter and clay are well known to influence soil physicochemical properties, which as a matter of fact is suitable for agriculture. Soil organic matter reduces the plasticity and cohesion of soils, and increases water holding capacity while soil organic matter and clay hold nutrient cations (Brady and Weil, 2014). Clay content is an important

Table 2. Correlation matrix of selected physiological parameters in upper (a), middle (b), and lower (c) ondombe slope positions.

(a) Upper slope Position

	pH (H2O)		OC		Total N		C/N		Av. P		Ex. Ca		Ex. Mg		Ex. K		Ex. Na		Sand		Silt		Clay		Ece		SAR
pH	1.00																										
OC	-		1.00																								
Total N	0.08		0.02		1.00																						
C/N	-		**0.93**	**	**-0.24**	*	1.00																				
Av. P	0.07		**0.56**	**	0.00		**0.50**	**	1.00																		
Ex. Ca	0.04		0.16		**0.58**	**	-0.06		0.15		1.00																
Ex. Mg	**0.28**	*	0.06		0.21		-0.08		**0.27**	*	**0.42**	**	1.00														
Ex. K	**0.37**	**	0.13		**0.43**	**	-0.03		**0.42**	**	**0.29**	*	**0.55**	**	1.00												
Ex. Na	**0.59**	**	**-0.27**	*	0.06		**-0.29**	*	-0.04		-0.13		**0.34**	**	**0.45**	**	1.0										
Sand	**-**	**	-0.02		**-0.39**	**	0.15		-0.13		**-0.59**	**	**-0.82**	**	**-0.55**	**	**-**	**	1.00								
Silt	-		0.22		**0.30**	*	0.09		0.03		**0.37**	**	**0.34**	**	0.23		0.0		**-0.48**	**	1.00						
Clay	**0.44**	**	-0.07		**0.31**	*	-0.21		0.13		**0.50**	**	**0.77**	**	**0.52**	**	**0.5**	**	**-0.92**	**	0.09		1.00				
Ece	**0.34**	**	**-0.24**	*	0.08		**-0.27**	*	-0.03		-0.09		0.17		**0.36**	**	**0.8**	**	**-0.32**	**	0.09		**0.32**	**	1.00		
SAR	**0.43**	**	**-0.25**	*	-0.02		**-0.24**	*	-0.07		-0.21		0.10		**0.33**	**	**0.8**	**	**-0.28**	*	0.03		**0.30**	*	**0.86**	**	1.00

(b) Middle slope Position

	pH (H2O)		OC		Total N		C/N		Av. P		Ex. Ca		Ex. Mg		Ex. K		Ex. Na		Sand		Silt		Clay		Ece		SAR
pH	1.00																										
OC	0.07		1.00																								
Total N	-		0.14		1.00																						
C/N	**0.29**	*	**0.62**	**	**-0.24**	*	1.00																				
Av. P	0.17		**0.63**	**	**0.25**	*	**0.36**	**	1.00																		
Ex. Ca	0.06		0.11		**0.60**	**	-0.20		**0.31**	**	1.00																
Ex. Mg	0.12		0.08		**0.68**	**	**-0.29**	*	**0.27**	*	**0.82**	**	1.00														
Ex. K	-		0.16		**0.67**	**	-0.21		0.17		**0.46**	**	**0.52**	**	1.00												
Ex. Na	**0.67**	**	0.06		0.12		-0.12		0.18		0.11		**0.32**	**	0.17		1.0										
Sand	-		-0.12		**-0.80**	**	**0.30**	*	**-0.25**	*	**-0.84**	**	**-0.91**	**	**-0.58**	**	-		1.00								
Silt	0.05		0.18		**0.77**	**	**-0.28**	*	0.18		**0.62**	**	**0.71**	**	**0.48**	**	0.0		**-0.87**	**	1.00						
Clay	0.10		0.08		**0.73**	**	**-0.28**	*	**0.26**	*	**0.88**	**	**0.93**	**	**0.57**	**	**0.3**	*	**-0.96**	**	**0.71**	**	1.00				
Ece	**0.54**	**	0.07		-0.05		-0.21		0.15		-0.08		0.02		0.05		**0.8**	**	0.01		-0.11		0.05		1.00		
SAR	**0.59**	**	0.02		-0.08		0.13		0.10		-0.17		-0.04		0.06		**0.8**	**	0.05		-0.10		-0.02		**0.93**	**	1.00

(c) Lower slope Position

	pH (H2O)		OC		Total N		C/N		Av. P		Ex. Ca		Ex. Mg		Ex. K		Ex. Na		Sand		Silt		Clay		Ece		SAR
pH	1.00																										
OC	0.04		1.00																								
Total N	**-**	**	**0.42**	**	1.00																						
C/N	**0.29**	*	**0.62**	**	**-0.24**	*	1.00																				
Av. P	0.15		0.20		0.00		**0.36**	**	1.00																		
Ex. Ca	-		**0.27**	*	**0.61**	**	-0.20		0.00		1.00																
Ex. Mg	-		**0.29**	*	**0.62**	**	**-0.29**	*	-0.01		**0.75**	**	1.00														
Ex. K	-		**0.29**	*	**0.66**	**	-0.21		0.02		**0.55**	**	**0.72**	**	1.00												
Ex. Na	**0.39**	**	0.00		0.01		-0.12		0.00		-0.01		**0.38**	**	**0.30**	*	1.0										
Sand	0.08		-0.14		**-0.53**	**	**0.30**	*	0.05		**-0.69**	**	**-0.85**	**	**-0.72**	**	-		1.00								

Table 2. Contd.

Silt	0.07		0.07		**0.39**	**	**-0.28**	*	-0.07		**0.52**	**	**0.74**	**	**0.59**	**	0.2		**-0.87**	**	1.00						
Clay	-		0.17		**0.55**	**	**-0.28**	*	-0.03		**0.71**	**	**0.83**	**	**0.72**	**	0.1		**-0.97**	**	**0.72**	**	1.00				
Ece	**0.31**	*	-0.10		0.00		-0.21		-0.01		0.02		0.19		0.09		**0.7**	**	-0.09		-0.01		0.14		1.00		
SAR	**0.30**	*	-0.01		-0.23		0.13		0.11		**-0.36**	**	-0.19		-0.05		**0.5**	**	**0.25**	*	**-0.29**	*	-0.21		**0.62**	**	1.00

*, ** = significant at 0.05 and 0.01 probability levels, respectively; OC = organic carbon; Av. P = Available P (Olsen method); Ex. Ca, Ex. Mg, Ex. K., Ex. Na = exchangeable cations, respectively; ECe = Electrical conductivity of the saturation extract; SAR = Sodium Adsorption Ratio of the saturated paste extract.

factor for land productivity and drought tolerance (He et al., 2014). At our study site, organic C and clay contents were correlated with many soil fertility parameters (Table 2a to c). These results suggest that *ondombe* soils, especially at lower positions, have appropriate condition for agriculture.

The amount of available P at the lower positons in the *ondombe* soils, which is significantly higher than that of the upper and middle positions (Figure 3), can improve the amount and quality of crop production. Balkcom et al. (2005) reported that landscape position affected soil P concentration. It was considered that phosphorus accumulated by erosion and deposition during flooding from higher to lower positions in *ondombe*s. The available P at cropland was significantly higher than that of soils along *oshana*s and *ondombe*s. Generally, high P fertility on the floodplain would only be expected to arise if long-term floodplain P deposition was the dominant process affecting P fertility (Ogden et al., 2007). Phosphorus deficiency is a major constraint to crop production in sub-Sahara Africa due to inherently low P in the parent material and no use of fertilizer (Nziguheba et al., 2016). These soils have high iron and aluminum oxides and hydroxides, and resultantly also have high P sorption (Bekunda et al., 2010). These compounds are the least soluble and the least available sources of phosphorous. However, phosphorus is released and made available under wet conditions. Therefore, soil in lower *ondombe* position has higher available phosphorus content, due to solubility by water, and that position is a very important spot in semi-arid Africa. Available soil P, exchangeable Ca and Mg, silt and clay at the upper and middle positions of *ondombe*s were lower than that of lower position of *ondombe*s. The soil fertility of inland valley bottom in West Africa is similarly enriched by soil deposition of organic C, available P, exchangeable Ca and Mg, silt and clay (Ogban and Bebalola, 2003). Exchangeable Ca and Mg increased downslope positions (Brubaker et al., 1993). Our results suggest that available P, exchangeable Ca and Mg, silt and clay materials tend to be removed by erosion from upper parts of *ondombe*s and accumulate in bottom land positions.

Soil salinity and sodicity conditions of *ondombe*s

The average ECe and SAR in the *ondombe* soils were 0.62 ds m^{-1} and 7.36, respectively (Table 1). These values were less than the threshold of saline and sodic soils which was defined by Soil Science Society of America (2008). Although most *oshana* soils are saline-sodic soils (Watanabe et al., 2016), problems of salinity and sodicity do not so much appear to be in *ondombe* soils. In fact, ECe, and SAR values at all positions of the *ondombe* soils were significantly lower than the *oshana*s (Figure 4). In understanding, semi-arid landscapes, sodic soils begin to appear from the footslope position (Dye and Walker, 1980). Clays and salts are transported downslope from uplands by runoff and accumulate at the footslope (Sumner, 1993). Hence, the *oshana* soils are likely to accumulate salts. Humphries et al. (2011) reported that soil salinity and sodicity are related with salt contents in groundwater. It was considered that soil formation processes are different around *oshana*s compared to *ondombe* soils. Therefore, only *ondombe* soils generally have low saline and sodic levels. According to the mean values of saturated extract solution, the Na^+ (sodium) content is much higher than the Ca^{2+}, Mg^{2+}, and K^+, contents in other solutions (Table 1). The sodium extracted from saturated extract solution signifies that Na is main factor for soil sodicity. These results indicated that the *ondombe* soils are likely prone to sodicity in the long run. It is evident that sodic soils tend to be worse than saline-sodic soil; in terms of detrimental factors such as poorer physical soil conditions which include low permeability to air and water than saline-sodic soil (Brady and Weil, 2008). Therefore, it is important to monitor soil management from such adverse soil conditions.

Conclusion

*Oshana*s and *onbombe*s are both seasonal wetlands in north-central Namibia, although the surrounding soils are significantly different in their physicochemical properties. Most of *ondombe* soils did not exhibit salinity and sodicity problems. They contained reasonable amount of essential nutrients and favorable pH. *Ondombe* soils also have higher organic C and clay due to erosion from higher to lower positions. *Ondombe*s consist of a good amount of water for a period of about 5 to 6 months through the year. From this study, it can be concluded that *ondombe* soils have far better favorable conditions for crop production than *oshana* soils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Mathematical modeling of the pulp drying curves murici (*Byrsonima crassifolia)*: The foam layer drying

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The murici is a fruit of the Brazilian cerrado, with striking aroma and nutritional value. It is known that the fruit drying process for obtaining the murici dry contributes to the preservation of the product, the content of nutrients and enables your addition in formulations, emphasizing the sensory and nutritional quality of food. A drying method that has been expanding in Brazil is the method of drying foam layer, where it promotes a faster drying, being used in juices, purees and pulps. The aim of this work is to carry out the pulp drying murici per layer of foam (*foam mat drying*), aimed at establishing a mathematical model for your drying. The study of drying of murici in layer of foam in the conditions in which the experiment was developed, it was found that the drying temperature exerts influence on the speed of drying of the product and the mathematical model that adapted the drying conditions murici was the logarithmic.

Key words: Foam mat drying, temperature, modeling.

INTRODUCTION

The muricizeiro is a shrubby plant, medium, native to the Cerrado, which can reach 5 m in height. Stem cylindrical, dark shell features, rough and narrow cup with rigid sheets and bright. The flowers are yellowish, forming clusters of 10 to 15 cm, and the seeds germinate in loamy substrate, requiring local shaded, with slow development. Its flowering begins in late August, and fruiting at the end of September. According to the incidence of rainfall, the fruit can be in mid-January or March. The harvest is done manually because of the ease of fall of ripe fruits, being that your maturation occurs in the plant and are picked when presenting yellowing, com characteristic aroma and flavour (Emater, 2010).

The murici is a fruit with a strong smell, similar to rancid cheese, typical of the Brazilian cerrado, being found from September to March, in the mountain regions of the southeast, in the Cerrado of Mato Grosso, Goiás, and in the North and Northeast of Brazil (2006). When mature, yellowish, with a diameter of 1.5 to 2 cm, each plant produces on average 15 kg of fruit per year (Emater, 2010). The pulp is fleshy and soft, and can be consumed fresh or in the form of juices, jams, ice cream, liqueurs and the dried product can be used in cereal bars (Alves

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and Franco, 2003). Despite all the information provided, there are few projects, involving studies on this fruit, damaging the knowledge of its physical and chemical properties, chemical and biological weapons, as well as minimize their business potential.

Drying fruit for murici dry contributes to the preservation of the product, the content of nutrients and enables addition in formulations, emphasizing the sensory and nutritional quality of food. The conservation drying mechanism is based on the fact that all the metabolic mechanism requires some amount of water for their activities. With the reduction in available water, therefore the activity of water and the speed of chemical reactions in the product, as well as the development of microorganisms will be reduced (Christensen and Kaufmann, 1974).

In recent decades, many studies have been conducted regarding the dehydration of fruit and fruit pulp (Pereira et al., 2009; Mosquera et al., 2010; Osorio et al., 2011), geared mainly to increase the retention of the nutritional and sensory properties of the product through amendments to the existing processes or application of new techniques. Among them, we highlight the foam mat drying method, which in Brazil is also called foam mat, foam bed drying or drying foam layer.

This method is widely used for drying of heat-sensitive foods. Although it has been developed to promote rapid drying of liquid food, such as juices, and has been widely used for food products such as purees and fruit pulp (Bag et al., 2011). The process consists basically of three steps: transformation of juice or pulp in stable foam through the use of additives (emulsifiers, stabilizers, thickeners); dehydration of thin-layer material till constant mass and dry mass disintegration in scales and, subsequently, powder (Furtado et al., 2010).

The aim of this work was to carry out the pulp drying murici per layer of foam (foam mat drying), aimed at establishing a mathematical model for your drying.

MATERIALS AND METHODS

The fruits were harvested in production properties murici in city of Britain, Goias State. The experiments were carried out in the drying laboratory in the State University of Goiás.

For samples of the fruits, they were selected manually on the uniformity of m Stadium old-fashioned gutting it out and absence of defects. In the lab, the fruits of murici (*Byrsonima crassifolia)* were removed and stored in cold Chamber to 10°C during 24 h, targeting the reduction of metabolism.

The water content of the product is determined by the standard method of the greenhouse, the 105 ± 3°C for 12:00 am in three repetitions (Brasil, 2009). For foam layer drying pulp, murici, was used as a product with features sparkling, the Emustab (distilled Monoglycerides based product, sorbitana monostearate and polysorbate, 60) was added to the mass, and the pulp of Afzal was the percentages by weight of 5%. After that, all quantities were mixed in the mixer in time of 15 min, thus forming a pulp foam murici.

Pulp + emulsifier formulations were arranged in circular shaped stainless steel trays (RADIUS 150 mm and height 5 mm) and placed in an oven with forced air convection, temperature 60, 70 and 80 ± 1°C, and kept in the equipment until the dough became constant. The reduction of the water content is determined by weighing the product in digital scale (Homis, DS-2000), accurate to two decimal places, at the beginning of the drying and, later, in 1 h intervals until constant weight. The dry material was removed from the trays with the aid of plastic spatulas, showing fine granulometry, but not homogeneous.

For the calculation of the humidity ratio (RU), during the dryings in different temperatures, Equation 1 was used:

$$RU = \frac{U - U_e}{U_i - U_e} \quad (1)$$

where RU is the humidity ratio, dimensionless; U is the average water content at time t,% b.s.; Ue is the water content of balance,% b.s.; and Ui is the initial water content,% b.s.

The magnitudes of the coefficient of determination (R^2), the average relative error (P) and the estimated average error (SE) were used to verify the degree of fit of the models, which were calculated according to the equations:

$$P = \frac{100}{n} \sum_{i=1}^{n} \frac{|Y - \hat{Y}|}{Y} \quad (2)$$

$$SE = \sqrt{\left(\sum_{i=1}^{n} (Y - \hat{Y})^2\right) / GLM} \quad (3)$$

where Y: observed experimentally; $\hat{Y}$: value estimated by the model; n: number of experimental observations; and GLM: degrees of freedom model (number of observations minus the number of parameters of the model).

Four mathematical models were adjusted for the experimental data of drying the pulp of Afzal. For the adjustment, nonlinear regression analysis was used, through the STATISTICA software (Table 1).

RESULTS AND DISCUSSION

Drying curves

The initial humidity of the product is determined by the standard method of the greenhouse and was 22.9% b.s. Figure 1 demonstrates the behavior of the curve of the foam layer drying of pulp murici with the addition of emulsifier in the concentrations of 5%, at different temperatures. The influence that it had on the times of dryings was observed.

It was found that the time required for drying the pulp of the murici in foam layer was 2.5, 2.7 and 1.7 h to temperatures of 60, 70 and 80°C, respectively. The temperature of 80°C influenced directly on the drying time of the product 5% emustab.

Tables 2, 3 and 4, show the coefficients of each mathematical model in modeling of the pulp drying curves murici in temperatures of 60, 70 and 80°C. Table 5 shows the values of the coefficients of determination, average on errors and estimate for the four models adjusted.

Among the models that performed best were the determination coefficients of logarithmic and page,

Table 1. Mathematical models used to predict the phenomenon of pulp drying murici.

Name	Equation	
Page	$RU = a\exp(-k\,t^{n})$	4
Henderson and Pabis	$RU = a\exp(-k\,t)$	5
Logarithmic	$RU = a\exp(-k\,t) + c$	6
Thompson	RU = exp ((-a – (a^2 + 4 b t)0,5) / 2 b	7

RU: Product humidity ratio, dimensionless; t: drying time, h; k, k0, k1: coefficients of drying, h^{-1}; a, b, c, n: in the models.

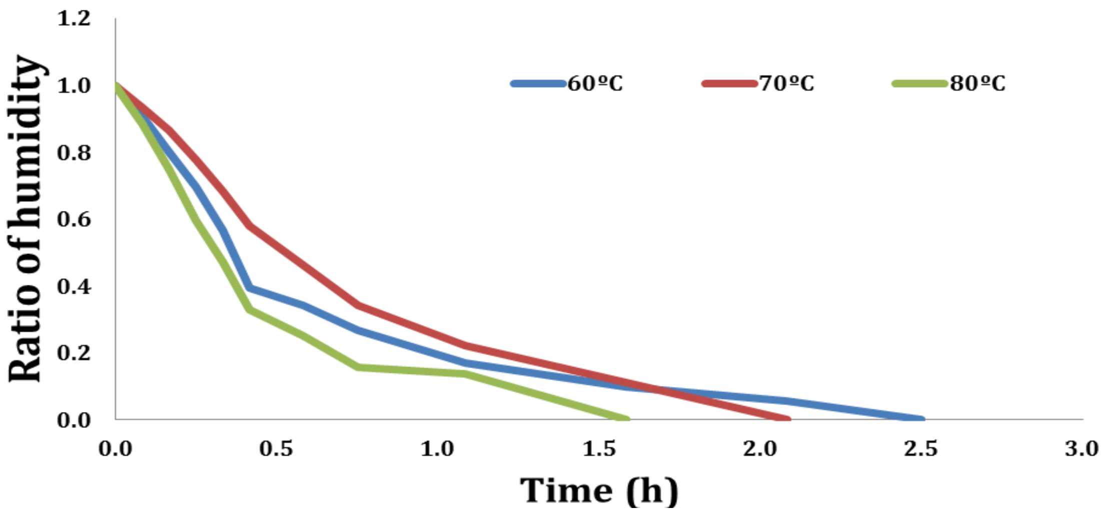

Figure 1. Experimental drying curves in pulp foam layer murici for temperature of 60, 70 and 80°C.

Table 2. Mathematical models adjusted Coefficient of drying curve of the murici (*Byrsonima crassifolia*), to the temperature of 60ºC.

Equation	Coefficients				
	A	B	C	n	k
Page	-	-	-	1.480307	0.096802
Henderson and Pabis	1.515268	-	-	-	1.812262
Logarithmic	1.475391	-	-0.072144	-	0.175859
Thompsom	1.710000	0.652000	-	-	-

RU: Product humidity ratio, dimensionless; t: drying time, h; k, k0, k1: coefficients of drying, h^{-1}; a, b, c, n: in the models.

Table 3. Mathematical models adjusted Coefficient of drying curve of the murici (Byrsonima crassifolia), for temperature of 70°C.

Equation	Coefficients				
	a	B	C	n	k
Page	-	-	-	1.2921	0.09642
Henderson and Pabis	1.1122	-	-	-	0.1821
Logarithmic	1.3480	-	-0.861	-	0.8788
Thompsom	1.543	1.406	-	-	-

RU: Product humidity ratio, dimensionless; t: drying time, h; k, k0, k1: coefficients of drying, h^{-1}; a, b, c, n: in the models.

Table 4. Mathematical models adjusted Coefficient of drying curve of the murici (Byrsonima crassifolia), for the temperature of 80°C.

Equation	Coefficients				
	a	B	c	n	k
Page	-	-	-	0.625	0.1609
Henderson and Pabis	1.544	-	-	-	3.8672
Logarithmic	0.7386	-	0.2235	-	0.1051
Thompsom	-1.0363	0.4303	-	-	-

RU: Product humidity ratio, dimensionless; t: drying time, h; k, k0, k1: coefficients of drying, h^{-1}; a, b, c, n: in the models.

Table 5. Coefficients of determination (R2%), Significant errors (P%), estimated (If decimal) to the four models analysed, for drying pulp murici in temperatures of 60, 70 and 80°C.

Equation	60°C			70°C			80°C		
	R^2	P	SE	R^2	P	SE	R^2	P	SE
4	99.25	22.03	0.02	99.48	7.94	0.03	99.44	32.45	0.03
5	97.87	52.97	0.04	97.89	45.03	0.04	98.85	32.98	0.02
6	99.91	12.01	0.01	99.97	13.41	0.01	99.98	10.02	0.01
7	98.89	20.44	0.02	99.88	4.54	0.02	99.85	12.45	0.01

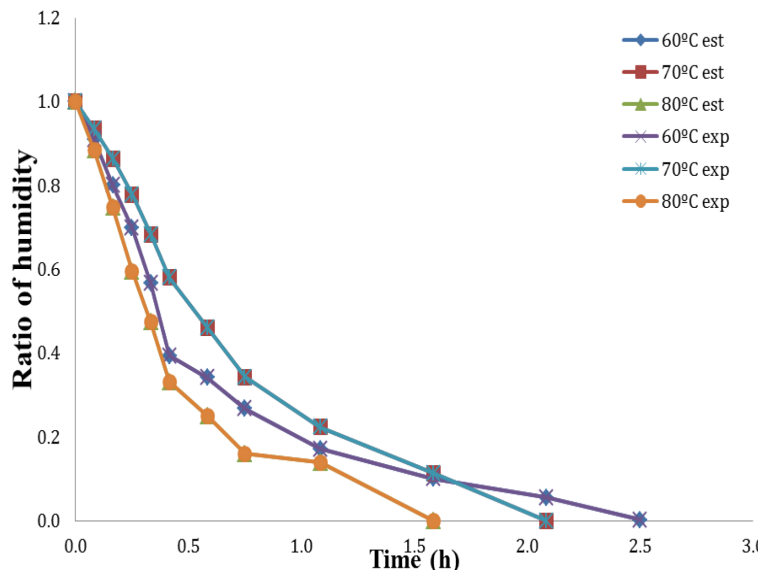

Figure 2. The pulp drying curves murici experimental and estimated mathematical model logarithmic time for temperatures under study.

agreeing with Bridges et al. (2009) in the drying of pepper. Since the logarithmic model presented for temperature of 60°C, R2=99.91, P=12.06, SE=0.01, to a temperature of 70°C, R^2=99.97, P=13.41, SE=0.01 and para 80°C, R^2=99.98, P=10.02, SE=0.01.

It was found that the Page templates, Logarithmic and Tompsom can meet the needs and be used to represent the drying process of the formulation with excellent precision, by coefficients of determination (R^2) greater than 0.98 and SE estimated less than 0.04 reinforcing studies of Midilli et al. (2002) (Figure 2).

Conclusion

For the study of drying of murici in layer of foam in the conditions in which the experiment was developed, it was found that the drying temperature exerts influence on the speed of drying of the product studied, being less drying time with increasing temperature.

The mathematical model that best adapted to dry conditions was the logarithmic model, showing values of R^2 = 99.91, 99.97 and 99.98, for use at temperatures of 60, 70 and 80°C, respectively.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Dynamics and adaptation of agricultural farming systems in the boost of cotton cropping in southern Mali

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Integration of crop and livestock production systems constitutes an important engine for agricultural development and enhancement of smallholder farmers' livelihoods in the least developed countries. For the last forty years, the Malian cotton sub-sector has recorded mixed growth trends, having been initially successful before declining and then catching up. The growth dynamics in the cotton sub-sector has permitted smallholder farmers to endow and improve their living condition. The purpose of this study was to establish smallholder farmers' dynamics of integrating crop and livestock production systems as well as classification of the systems and their trajectories. The study was carried out in different agro-ecological zones in Southern Mali. A multistage sampling technique was used to select the area of study. Stratified random sampling technique was then used to select 134 smallholder farming households from three villages. A panel datasets was used from the Malian Company of Textile Development (CMDT) from 1961 to 2014 and 1974 to 2014. Based on explanatory structure variables, principal component analysis (PCA) and ascending hierarchical classification (AHC) were used to distinguish smallholder farmers' dynamics as well as classify them into different classes or groups. Five types of smallholder were distinguished. Type 1 was super large families representing 14% of the total smallholder farmers. Type 2 consists of large families, and constituted of 28% of the smallholder farmers. Type 3 consists of medium-sized families which represented 28% of the total smallholder farmers. Type 4 and type 5 were small and young families with 19 and 11% of smallholder farmers respectively. Notably, small family farming was low in Southern Mali, and so some agricultural options can be developed such as the milk and meat value chains among others.

Key words: Dynamics, agricultural farming family, integrated crops and livestock, multivariate analysis, Southern-Mali.

INTRODUCTION

Crop and livestock integrated systems constitute one of the major activities of the rural population by contributing to food security, income generation, organic matter and draught power. In the least developed countries, integration of crops and livestock is considered as the primary source of organic manure, compensating for low use of chemical fertilizer by smallholder farmers.

In Sub-Saharan Africa (SSA), the association of crops, livestock and forestry systems is the centre of options for arriving at sustainable development goals for agricultural and food production (Poccard et al., 2014). To understand the agricultural family farming system, an analysis of the structure and functioning of the systems is essential (Giller et al., 2006; Sanogo et al., 2010; Falconnier et al., 2015).

In cotton production belt of Mali, cash crop farming was favored by increasing farm size, herd size, tools for production and intensifying food crops (maize, sorghum, millet) and fodder crops for animal feeding (Ridder et al., 2015). Furthermore, smallholder farmers in the cotton growing region in Mali have been practiced and developed livestock keeping system using oxen as draught power for cultivation and cows for breeding (Ba et al., 2011).

Thus, technology has facilitated the association of crops and livestock (Vall et al., 2006; Diarisso et al., 2015), and also increased herd size and farm sizes. Institutional support in terms of inputs and equipment in the cotton producing areas in Mali has enabled farming families to gradually change and diversify their livelihoods and production objectives (Benjaminsen, 2001; Baquedano et al., 2010).

This has necessitated the classification of agricultural families at different production levels in order to understand and make decision and assessment for further development interventions (Robles et al., 2005). Ezeaku et al. (2015) argues that smallholder farmers' dynamics in an integration production system improves global food productivity, assures soil fertility and enhances peasants' income. However, adoption of new technologies by agricultural farming families in Sub-Saharan Africa requires not only resource, but also structural and functional support (Mbetid-bessane et al., 2003; Tittonell, 2013).

Indeed, the number of smallholder farmers in the cotton growing zone in Africa has grown rapidly over time causing fluctuation in farm size, herd size, and draught tools. This change is accentuated today by fragmentation of large ancient family land and deteriorating food security situation, malnutrition, and poverty. In addition, deflation of purchase price of cotton over time in the international market has led to market uncertainty for smallholder farmers producing primarily for export (Fok, 2010; Theriault et al., 2013).

Agricultural landscape in the least developed countries is dominated by diverse crop and livestock systems. Agriculture is familial and is still managed by the head of extended families who also contribute most of the family expenditure (Djouara et al., 2006).

Worryingly, cotton companies in Africa are monopolists. Companies' charges on inputs, transportation, and extension services are exorbitant (Baffes, 2007; Theriault et al., 2013). Benjaminsen (2001) reiterated this in the context of the Malian cotton production. Many studies have been done to characterize smallholder farmers in SSA based on different objectives such as soil fertility in East Africa Kenya and Uganda (Tittonell et al., 2010). On the other hand, changing in behavior and agricultural practices allows options for smallholder farmers to improve livelihood, food security and income generation (Descheemaeker et al., 2010; Sissoko et al., 2011).

In the cotton growing zone of Mali, smallholder farmers are diverse and complex. They switch over time due to several production constraints such as the effects of climate change (Callo-Concha et al., 2013; Traore et al., 2015; Descheemaeker et al., 2016).

Cash crop farming in Mali was favoured by investing surplus of cotton revenue in draught tools and livestock. Although dominated by food crops (maize, millet, sorghum), more than 95% of agricultural farming families possess a herd of cattle or small ruminants. However, indigenous chicken also constitutes a current account for certain smallholder farmers and form part of the daily expenditure and protein. But most importantly, breeding in cotton growing areas of west and central Africa is mainly practiced for draught power. It plays an integral part in the integrated crop and livestock system. Nowadays, almost all agricultural families are abandoning hand work with the poorest farmer also hiring draught tools and draught power without expending much money as a fee to the owner.

Sanogo (2011) argued that in the early 2000s, more than 85 % of agricultural farming families possessed complete yokes which were expected to increase year by year according to farming practices. As a result, there is degradation of the ecosystem which has rendered soils infertile. In this context, pressure on natural resources associated with an increase in smallholder farming activities has threatened the timing of land fallowing and pastures (Gigou et al., 2004; Kante, 2001).

On the other hand, sustainable development for crop and livestock production systems in the cotton belt continues to be a challenge in relation to climate change and demographic transition. Since 1996, smallholder farmers in Southern Mali have been classified into four types. The classification is based on structured variables such as equipment, oxen ownership and breeding bovine.

Moreover, the classification does not permit an understanding and forecasting of the dynamics in the cotton belt. Despite the numerous research and development interventions and extension services, the smallholder farmer classification typology has not been updated in the face of new context of agricultural

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development. There is need to understanding cotton production dynamics of mixt systems crops and livestock to understand the fluctuation in terms, area under cotton and number of agricultural farming families involved in the system over period of time. Moreover, the classification does not permit an understanding and forecasting of the dynamics in the cotton belt.

Despite the numerous research and development interventions and extension services, the smallholder farmer classification typology has not been updated in the face of new context of development. The typology also uses explanatory variables based on structure and functioning of smallholder farmers. The objectives of this study were:

1. To establish smallholder farmers' dynamics in crop and livestock integration in the cotton growing zone of Mali
2. Update smallholder farmers' trajectories in the new context of population growth and constraints and
3. Making decision for future intervention.

METHODOLOGY

Study area

The study was conducted in three villages in the cotton growing area of Mali. Cotton zone constitutes a strategic area that the government invests much effort to improve agricultural productivity. The zone supplies the rest of country in terms of cereals and horticultural products. In a nutshell, Southern Mali plays a critical role in ensuring food security in the country. The three study villages are located in different agro-ecological zones from the northern part to southern part of cotton growing zone of Mali (Figure 1).

Beguene is located in Northern part of the cotton growing zone. It is situated at -5, 84498 longitude and 12, 81824 latitude and corresponds to old cotton basin. The area is characterized by strong pressure on natural resource because of one of the saturated zones in Southern Mali. The climate is typical of the Sudano–Sahelian region. Average annual rainfall is around 850 mm, with a high inter-annual variability. The rainy season lasts from June to October with rainfall peaks in August. The dry season comprises a relatively cold period from November to February and a dry period lasting from March to May. The average maximum temperature is 34°C during the rainy season and 40°C during the hot dry period.

Ziguena is located in middle to the intermediary area of the cotton growing zone and lies at -5,8924 longitude and 11,6376 latitude. It has a weak pressure on natural resources. During the rainy season (June to October), rainfall averages 1000 mm. Daily average temperature varies between 22°C cold season from November to February and 38°C hot and dry season from March to May.

Nafegue located in Southern part of the cotton production zone. It lays on -5.9658 longitude and 10.5017 latitude. It corresponds in the Sudano-Guinea region. Average annual rainfall is more than 1200 mm per year. The rainy season starts by June up to October and the remaining months are dry and hot. Average temperature fluctuates between 22°C and 35°C. Nafegue is more favorable than other two villages but tends to show a sign of pressure.

The soils are mainly Ferric Lixisols with low clay content (<10%) in the top soil. Soils are in general moderately acidic with a pH of around 6. Soil nutrients (N, P, K) in depth of 0 to 20, are 0.30%, 3.45mg /kg and 0.07me/100g respectively (Table A Appendix).

Cropping systems in the three villages are dominated by cash crop (cotton) and food crops (maize, millet, sorghum) which are the staple foods. Groundnut and rice are increasingly being adopted in the three villages. In addition, we find also some secondary crops at family level such as cowpeas, soybean, and sesame. Livestock system is dominated by cattle, small ruminants (sheep and goats) and also indigenous chicken.

The integrated agricultural production system is based on the use of organic matter by the majority smallholder farmers. The farmers apply manure mainly on cotton and to some extent on maize. Residues from crops are used for animal feeding.

Chemical fertilizer is applied mainly on cotton and maize and sometimes on millet and sorghum. Livestock constitutes one of the main activities of smallholder farmers and is an important source of income. The three selected villages are a representation of agricultural and agro-ecological practices from northern part to southern part of cotton belt in Mali

Research design

The study used multiple approaches for data collection. Structured questionnaire was designed and administered to the smallholder farmers while focus group discussion were held at village level. Data was collected on the number of equipment (plough, donkey cart and ox cart), livestock possessed by smallholder farmers (ox, bull, cow, heifer, calves, small ruminants, indigenous chicken) and organic matter. The institutional analysis was carried out using two scales. That is an analysis of the company in charge of cotton production and village-level cotton cooperatives

Focus group discussions

Focus group discussions were conducted in each village in order to obtain supplementary information. The discussions involved a limited number of persons. Discussions were about production system in each village. Information collected was related to land ownership and management, constraints in production system (crops and livestock), environment, sources of income and off farm activities.

Sample design and data collection

Multistage sampling technique was used for the study. It involved a combination of purposive, stratified and simple random sampling procedures. The research unit was the agricultural farming families. The study used two sources of data. Primary cross sectional data was collected through field surveys of the three villages.

On the other hand, panel data was obtained from Malian Company of Textile Development (CMDT), a company in charge of cotton production in Mali. The first panel dataset spanned from 1961 to 2014 and contained information on the total cultivated area under cotton and yield.

Another panel dataset spanned from 1974 to 2014 and provided information on the number of agricultural farming families involved in cotton production. Three districts were purposively selected at the first stage, then three communes at the second stage and finally one village was selected from each commune. In total, 134 agricultural farming families were randomly selected following the stratified typology that was established by the research institute Institute for Energy Research (IER) and the CMDT based on the level of equipment and cattle owned Table 1.

Choice of structure for explanatory variables of agricultural farming families

In classifying the smallholder farmer dynamics, some key variables

Table 1. Typology of smallholder farmers used in Southern Mali (CMDT).

Types	Structured variables (criteria)	Explanations
A	Plough, cart, number of oxen with or without breeding bovine	2 pairs of ploughing and more than 10 breeding bovine
B	Plough, cart, number of oxen with or without breeding bovine	1 pair of ploughing and less than 10 breeding bovine
C	Plough, cart, number of oxen with or without breeding bovine	incomplete possesses one plough or ox
D	Plough, cart, number of oxen with or without breeding bovine	no equipment, hand worker

have been selected based on their functional weight on smallholder farmers' endowment. For that purpose, ten explanatory variables have been selected as well as describe well the structure of agricultural farming families in Southern Mali. They constitute the principal factors of agricultural assets in Southern Mali. They include age of agricultural farming family's head, family size (population), equipment (ploughs, carts, seeders), herd size expressed in Tropical Livestock Unit (TLU), number of oxen possessed: 1 Unit for (bull and ox), 0.8 for cows, 0.5 for (heifer and bull-calf), 0.2 for calf and 0.2 for small ruminants, total farm size (hectare), allocated hectare for cash crop (cotton), allocated hectare for food crops (maize, millet and sorghum), organic matter production and number of workers. The variable of education has been omitted in the analysis as heads of families in this research have not received formal education. Gender is not considered here due to non-female headed farming families in Southern Mali

Data analysis

We used the structured variables identified earlier as determinants of agricultural farm dynamics. In order to distinguish and group similar farmers, Multivariate Analysis (MVA) and ade4 have been used. The analysis is run using R3.3.2 software through Principal Component Analysis (PCA). We used a histogram of proper values to determine the contribution of variables to form plan factorials axes. The first three proper values explain about 72.96 percent of the variation in the structure of information. Other proper values (variables) contribute limited information.

PCA is a method used to describe the variability of correlated variables by smaller set. It allows graphical characterization of smallholder farmers using quantitative values through information continuity in the dataset. It also allows understanding of how the individuals are related and distinguished.

Ascending Hierarchical Classification (AHC) or Clusters Analysis (CA) is a method which regroups a group of homogenous smallholder farmers. In this research, we use AHC in order to have a group with resemblance and can be represented graphically in dendrogram or clusters.

Description of structured variables used

Explanatory variables can be divided into two categories. The structured variables include the total population, herd size, farm size, number of oxen, tools, area allocated for cotton, area allocated to food crops, functional variables, the number of workers and organic matter.

Agricultural farming family

This constitutes an extended family with the head of family, one or more than one household, working on the same plot and eating together. The principal feature is family labour (men and women) and the decision-making process. The chief decision maker is usually the head of the family.

Age of family head

The oldest person in the family is the family head, and is a key person in the agricultural decision-making process. The age of family head also has a link with livelihood diversification strategies.

Total population/family size (the number of mouths to feed)

The more the number of people in the family the more resources are diversified.

Herd size

This is a key factor of resource endowment of farming families. It is expressed in Tropical Livestock Unit (TLU). Having a large herd size means insurance and source of diversification for income.

Total farm size

It refers to the total cultivated land area in hectares either self-owned or owned by the family. A large farm size allows crop diversification which guarantees high income.

Number of oxen

In the context of this research, we differentiate oxen and herd size because numerous agricultural farming families keep oxen only for ploughing.

Number of workers/labour

It is the human capital. Labour is an important asset in rural areas.

Agricultural tools for cropping

Plough, seeder, donkey cart and ox cart are the main asset in Southern Mali, and this allows farmers to intensify their production systems. Area allocated for cotton and food crops is expressed as a percent of the land under crop rotation system.

Organic matter

This shows the degree of integration of crops and livestock, and capacity of farms to mobilize important quantity of manure.

RESULTS

Institution analysis at CMDT level

Cotton production is an important and well-organized value chain in Mali. This allows the poor farmers to access services and products from the company in charge of cotton. Indeed, it is cultivated by poor smallholder farmers under vertical integration. In 1974, CMDT owned 60% of the shares whereas French Company for Textile Development (CFDT) held the remaining 40%.

Badiane (2002) states that for West and Central Africa in CFA currency zone, the cotton sub-sector is under the restriction of unique company. This fact limits the provision of inputs and others services to farmers as it operates as a single buyer and seller of the cash crop. Malian cotton sector assumes main activities of cotton production such as extension services, production and marketing.

CMDT has a unique responsible to supply cotton producers with inputs on credit until harvesting. It supplies fertilizer for cotton and maize production, seeds, pesticides, draught tools, and oxen. It also empowers farmers on cotton production techniques through regular training and extensions services. It offers guaranteed purchasing price, transportation, and marketing (Tefft, 2004).

CMDT holds monopoly power in the cotton production system. In addition, the Malian cotton sector is sustained by the collaborative effort of institutes of research such as National Institute of Rural Economics (IER) and International Research Centre Agricultural Research Centre International Development (CIRAD). The support is based on agronomist aspects such as varietal breeding, soil fertility and bio-pesticides (Benjaminsen, 2001; Theriault et al. 2013).

In the least developed countries, information and technologies transfer in agriculture passes through field experimentation. Although most of the smallholder farmers are uneducated, they are rich in local knowledge. Asmah (2011) argues that habitual technique of transfer of knowledge in the agricultural sector is based on trial and field school through extensional services.

Cotton producers' cooperatives at village level (CPC)

At villager level, the CPC assume the role of CMDT by acting as field agents. They are based in cotton producing areas only. The CPC do an inventory of the area allocated to cotton and maize before the start of every season. The inventory is based on the declaration made by the farming families' heads and is critical in estimating the quantity of inputs needed. Each CPC is in charge of supply of fertilizer, cotton seed, pesticides, credit, animal feeds to all members.

Furthermore, CPCs are also in charge of cotton marketing. At marketing time, all cotton output is weighed and valued before the costs of all inputs supplied to each agricultural family are subtracted. After marketing, farmers wait for payment. After receiving the money from CMDT, each cooperative is in charge of distributing the appropriate amount to its members.

The CPC apply unwritten rules, which are based on retaining some kilogram from each ton supplied. The revenues generated from the retained cotton are used to manage the common pool resources and infrastructure in the village such as the construction of schools, health center and payment of instructors under commune contract.

Focus group at villagers scale

Land management in the rural areas in Mali is such that land belongs to the first families that come and settle in the area. The families have the custom right to use it. There are no written formal rules to distribute the land for the new people coming into the village. Traditional rules (unwritten) are based on non-planting trees, non-well and sometimes non-constructions for new settlers. In order to symbolize that the land is not your property, at the end of harvesting the occupier offers some basket of millet or maize or sorghum to the initial land owner. In the cotton growing area in Mali and everywhere, the rural lands have no titles but are well governed under local authorities. This has led to land and food insecurity in the rural areas of Mali. Table 2 describes constraints and assets in the study area.

Cotton dynamics, declining and catching up later

Cotton was produced by smallholder farmers before independence under the traditional form. Figure 1 shows some different steps of dynamics of cotton production after the creation of CMDT. From independence in 1960 to the creation of CMDT in 1974, yield per hectare of cotton was between 225 and 731 kg ha^{-1}. It corresponded to the usage of some agricultural equipment such as ploughs, seeders, non-industrial crop and non-improved cottonseed. Cotton was mainly cultivated for traditional clothing purposes, not as marketable products.

During the industrial time, the total area of arable land increased from 69311 ha in 1974 to 200368 ha on average in 1994, an increase of 65%. At the same time, yield by hectare rose from 731 to 1199 kg ha^{-1}, an average increase of about 39 percent. The increase in cotton production can be explained by the quality of soil fertility, rotation system and long land fallowing. This period also corresponded to cotton dynamics in Southern Mali as sustained by the development of animal traction, mastering the technique for applying fertilizers, pesticides

Table 2. Major constraints and assets in production system in southern Mali.

Villages	Constraints	Assets	Sources
Old basin Beguene	Climate change, low fertility of soil, no pasture space, degradation of natural resources, lack of animal feeds, soil acidity, low yield of all crops, soil erosion, difficulty to access improved seeds, low income, malnutrition, no tractors, food insecurity, late payment from CMDT, low yielding of livestock, high price of fertilizers and pesticides, low selling price for food crops	Importance of livestock, diversity of crops, extension services (research institute, National services, NGOs,..), integration of crops and livestock, equipment (draught tools)	According to farmers perceptions (focus group at village level)
Intermediary zone Ziguena	Climate change, low fertility of soil, degradation of natural resources, soil erosion, striga (weeds), insufficient quantities of fertilizer for cereals, no value chain for mangoes, conflicts with transhumance,	Diversity of crops, importance of livestock, extension services (research institute, NGOs, National services,), integration of crops and livestock, importance of potatoes , equipment (draught tools and tractors), diversity of source of income	According to farmers perceptions (focus group at village level)
Sub-humid zone Nafegue	Climate change, low yielding for cereals, lack of improved seeds, conflicts with transhumance from the North, no value chain for milk	Diversity of crops, equipment (draught tools), availability of other cash crop (Cashewnut)	According to farmers perception (focus group at village level)
	No market for cashew	-	-

Source: Survey, 2016, Author.

and other technique of cash and food crop production. The number of smallholder farmers involved in cotton production increased by 41% between 1974 and 1994.

Thus, smallholder farmers started to obtain complete draught tools (plough, seeder, donkey cart and oxen cart) and drought power. The income from cotton was invested in livestock during this period which steadily led to crop intensification and crop, and livestock integration. This increased yield per hectare as a result of improved soil fertility and agricultural practices such as crop rotation and long fallowing of land. The second important period in cotton production in Mali was at the end of 1994.

The currency, CFA, diminished in its value by two, corresponding to the global currency devaluation period. This caused a decline in terms of yield per hectare despite the increase the area of arable land under cotton. Cotton production further dropped in 2001 as a result of cotton producers' strike which translated into non-sowing of cotton. Arable land increased in 2002 reaching 532163 ha before decreasing steadily in 2009 by 196779 ha with the yield still decreasing over time.

The decrease corresponded to the declining period and also the international crisis combined with the high price of agricultural inputs. Despite the start of cotton production in the western part of the country, the yield per hectare was still decreasing. This crisis affected all agricultural sectors and, particularly, the smallholder farmers' income in the least developed countries due to agricultural taxation and subsidy pattern. Cotton producers have since reduced the area allocated to cotton and increased area under food crop. Also, other smallholder farmers have shifted from cotton production to non-farm activities. That shifting is not only attributed to the deflation of cotton price in the international market but also to climate variability.

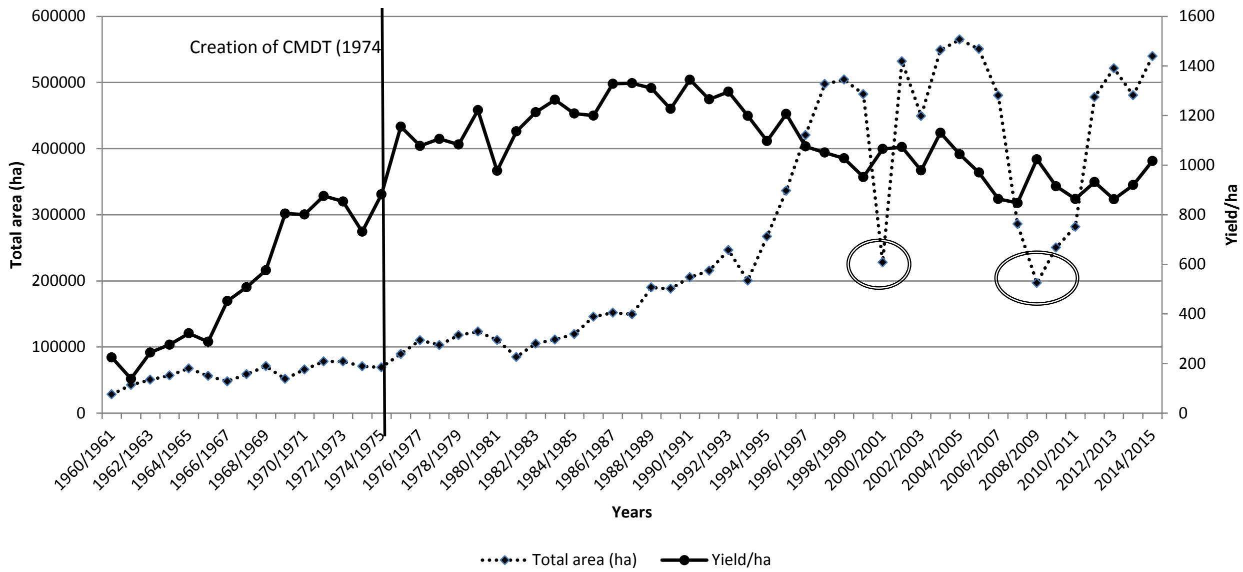

Figure 2. Evolution of cotton production from 1960-1961 to 2014-2015. Source: Data from Malian Company of Textile Development (CMDT), 2016.

Second important period in cotton production zone, is at the end of 1994. The currency (CFA) diminished in its value by two, and corresponded to devaluation time and there was a decline in terms of yield per hectare despite the arable land still increasing. It dropped in 2001 (producers of cotton were on strike) translated by the non-sowing of cotton. Then arable land increased in 2002 reaching 532163 ha and decreased steadily in 2009 by 196779 ha with the yield still decreasing over that period of time. The decrease corresponded to the declining period and also the international crisis combined with high price of agricultural inputs: fertilizers and pesticides.

Despite the entrance of a new region in Western part of country into cotton production, the yield per hectare was still decreasing. This crisis affected all agricultural sectors and particularly, the smallholder famers' income in least developing countries due to agricultural taxation and subsidy pattern. Cotton producers have since reduced the area allocated for cotton and increased food crop areas while others shifted from cotton cropping to non-farm activities or only growing food crops. That shifting is not only attributed to the deflation of price of cotton in international market but also the fluctuation of rainfall pattern and climate change.

A third important change corresponds to strengthening and catching up of cotton production as a result of increasing farm gate price. By 2011, the price of a kilogram of cotton was 185 CFA, and it increased to 255 CFA a kilogram in 2012. This triggered an increase in the area allocated for cotton. However, this did not translate into an increase in yield per hectare. This was attributed less subsidization of agricultural inputs such as fertilizers and pesticides. On the other hand, maize production has also increased in terms of the area allocated and yield per hectare. This has been sustained through access to fertilizer provided to cotton producers. The slowed catching up coincides with numerous factors such as climate change, low soil fertility, over cultivation and low quantity of organic matter applied. Most importantly, the population growth rate and herd size has constrained the catching up of the cotton sub-sector. This is in the backdrop of Mali being projected to be the leading producer of cotton in West Africa by 2018 (World Bank) (Figure 2).

Dynamics of number of farmers and area under cotton

The number of agricultural farming families involved in cotton production and the area under cotton cultivation increased between 1974 and 1994 (Figure 3). In 20 years, the number of agricultural farming families has increased by 41%. The expansion of cotton production to the western part of the country in 1995 increased the number of producers. This increased the cultivated land area under cotton by the year 2000. The number of producers and area under cotton went down due to the boycott by producers in 2001.

The input prices skyrocketed as the price per kilogram of cotton plummeted. The crisis in cotton growing area started, but the number of producers still increased until the beginning of new price spikes. Afterward, both the number of agricultural farming families and area under cotton declined in 2008 to 2009 due to the international market crisis, high price of cotton inputs and low farm gate price of cotton per kilogram. These factors accompanied by severe climatic conditions in cotton

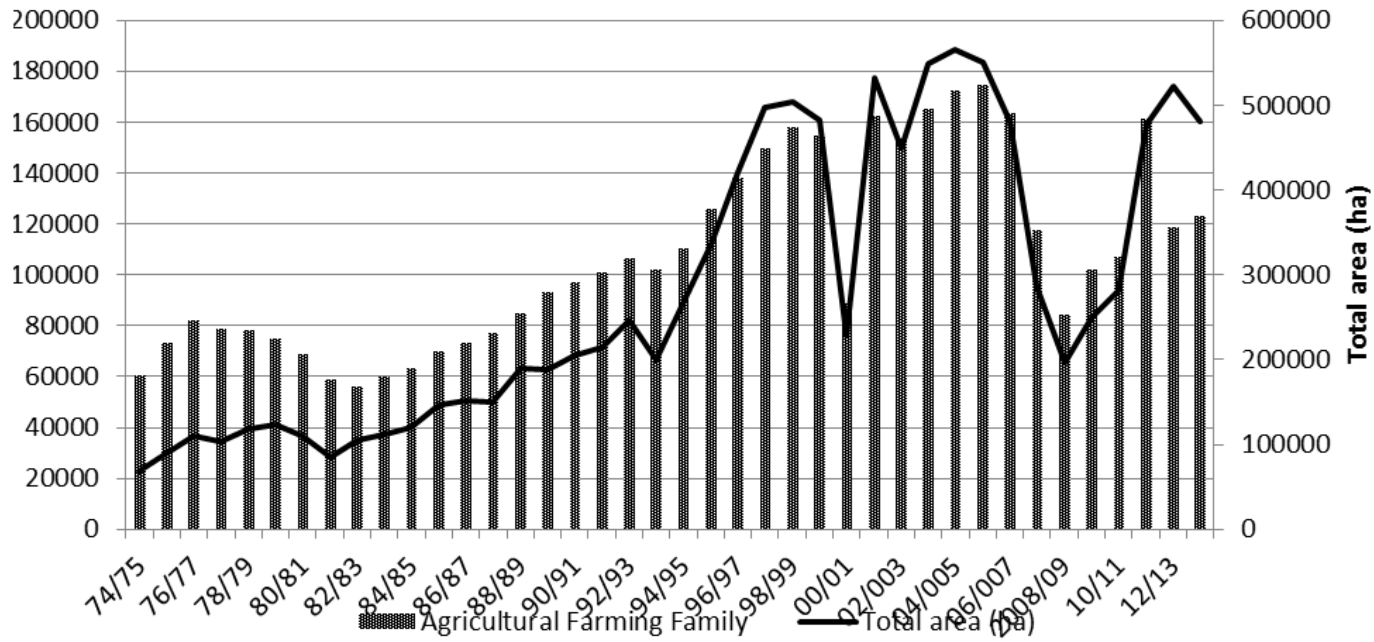

Figure 3. Evolution of number of agricultural farming and total are (1974 -2014) (Source: Data from Malian Company of Textile Development (CMDT), 2016).

growing area, worsened cotton production and marketing. Despite the crisis, the number of producers and land area under cotton cultivation went up causing further spikes. Famers cultivated cotton in order to access cotton inputs with or without subsidy and other opportunities associated with cotton production. Cotton constitutes the heart of socio-economic development and livelihood of the farming families. It is a unique and guaranteed source of income for the poor farmers and allows them to invest the surplus income in livestock and diversify sources of their livelihoods. This dependency on cotton in a closed market causes situational poverty, malnutrition and food insecurity.

Livestock (cattle) in the agricultural production system

Livestock is the heart of agricultural growth in the cotton production belt. The perceptions of agricultural farming families were analyzed based on the main functions of livestock in cotton belt. The functions are shown in Figure 4. About 67% of the farmers identified draught power as the main function of livestock.

Possessing oxen allows farmers to access credit and insurance as well as to plough early at the onset of rains, which guarantees food security. Farmers in Nafegue have limited access to tractor power, indicating less use fuel-powered machinery among smallholder farmers. The second function of livestock keeping, Nafegue village is milk production as indicated by 18% of the respondents.

Milk production is not well-developed due to the market and informational constraints. Although it offers important protein and reduces malnutrition in rural area, the milk production system is still considered non-value added. The last functions of livestock keeping are for organic matter production and revenue generation at 9 and 6 percent respectively.

These functions are considered not directly important in rural area. However, selling one head of cattle involves many members of the farming family in decision making. Organic matter production depends on family organization and is motivate by the need to produce an important quantity and reduce the quantity of chemical fertilizer applied on the farm.

Farmers' point of view on livestock (cattle) keeping shows that drought power also constitutes the most important function at 64% (Figure 5). Having drought power in Southern Mali indicates the priority in having a large herd size. It also means being self-sufficient in terms of labour, income, organic matter among many more others. Animal power allows farmers to increase farm sizes and also invest crop income in cows or bulls.

On the other hand, about 18% of farmers pointed out that they keep livestock for milk production purposes. Despite the numerous interventions in milk value chain by researchers, extensional service providers, and non governmental organisations (NGOs), farmers uptake of cattle keeping for dairy purposes is still low in the cotton production belt.

In other words, livestock keeping is not aimed at milk production despite milk forming part of families' daily sources of income and proteins. . This observation is a confirmation of the low consumption of milk in many rural

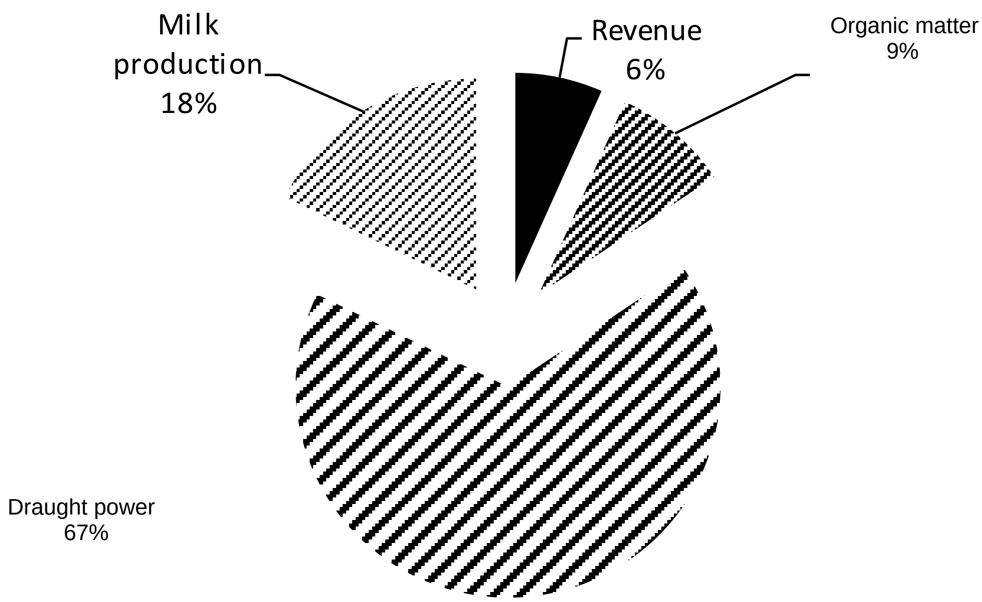

Figure 4. Objective of livestock (cattle) keeping in Nafegue. Source: Author, 2016

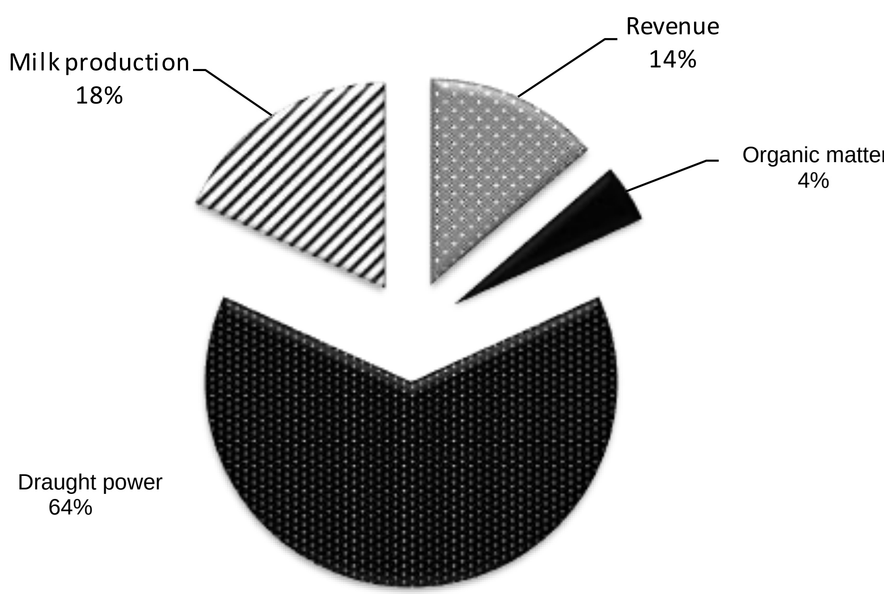

Figure 5. Objective of livestock (cattle) keeping in Ziguena. Source: Author, 2016.

areas in Southern Mali. The last two parameters, revenue and organic matter, were rated at 14 and 4% respectively by farmers as being important pillars for livestock keeping. These findings reiterate animal power as the main function of livestock keeping in cotton growing areas in Southern Mali since mechanical equipment are not accessible or affordable to the poor farmers.

The old basin zone was the first cotton growing area that extensively used draught power in agricultural production system Figure 6. About 71% of the agricultural farming families surveyed rely on draught power as an important role development of their livelihood. Nowadays, that zone is characterized by intense human pressure, degradation of environment and reduction of pasture space. Due to over-cultivation of arable land, yield per hectare of almost all crops is gradually going down. The

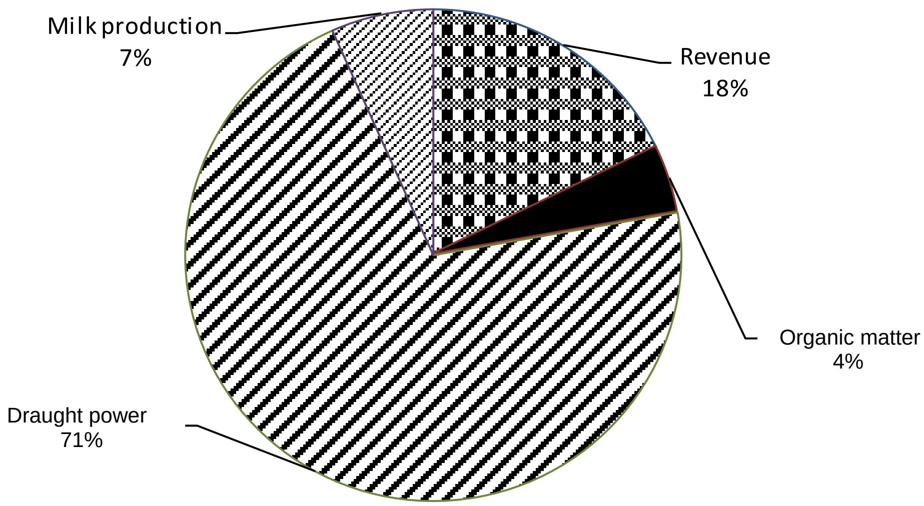

Figure 6. Objective of livestock (cattle) keeping in Beguene (Old basin zone) Source: Author, 2016.

adverse climate condition further exacerbates poor crop performance. The old basin zone is always under threat of food insecurity, malnutrition and poverty. Farmers keep draught power at home and the rest of the herd migrates towards a favourable area for feeding. Livestock migration affects milk and organic matter production.

The second function of livestock keeping in Buguene village from farmers' point of view is the revenue generation. Oxen are often sold for to cater for any family or social events. Milk and organic matter production were ranked as the third and fourth important livestock functions at 7 and 4%, respectively. The migration of important part of livestock for six or seven months negatively influences the quantity of organic matter produced and the quantity of milk that is produced, consumed and sold.

Furthermore, the milk value chain is not well-developed due to low investment and lack of market information. Milk is considered as a non-marketable commodity, which discourages specialization in milk production. Lastly, milk production in Beguene is also constrained by unavailability of improved fodder available. These occur against the background of the cotton belt being renowned for practicing crop and livestock integration and intensification.

To define the different homogenous groups or cluster of smallholder farmers, we used AHC estimator in R analytical software Figure 6. Automatically, 134 agricultural farming families form the homogenous class or type according to their characteristics.

Visual examination of the branches of dendrogram allows cutting off the place chosen based on the functioning of most homogenous smallholder farmers. This typology represents the diversity and dynamics of the sampled agricultural farming families. Thus, we chose five classes or groups for this research to describe the dynamics based on structured variables. We then compared the topology to the current typology used by researchers, CMDT and NGOs in Southern Mali.

Agricultural farming family dynamics

Structured and functional variables describing smallholder farmers' dynamics were classified using the PCA. Five classes or types were identified, and are provided in Table 3 (Figure 7).

Type 1: Super large families (n= 19)

It represents 14% of the sampled agricultural farming families. These types of families are found in all the three villages. This type corresponds to old families that invest the surplus of cotton income in livestock and farm equipment. The number of mouths to feed in such families is averagely 54 people.

The average total land area under cultivation is around 26 ha and the draught tools (plough, seeder, donkey, ox cart...) are an average of 9 types of tools. Livestock, an important asset for crop intensification, is owned by 55 percent of agricultural farming families.

Approximately 33 percent and 49 percent of the total cultivated land areas is under cotton and food crop production, respectively. Despite the importance of cotton in terms of income and supporting others crops, super large families prefer food crops in order to reduce their dependency of food purchases. However, the quantity of organic matter (manure, compost and domestic waste) applied is only 1835 kg ha^{-1} under cotton, which is a very

Table 3. Characteristics of Agricultural farming family.

Variable	Units	Type1super large families (n= 19)		Type 2 large families, (n=37)		Type3 medium families (n=38)		Type4 small families (n= 25)		Type5 Young and small families (n= 15)	
		Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV
Age	Year	68	15	63	14	50	11	55	15	43	9
Population	Person	54	30	26	9	16	8	12	3	8	4
Tools	No.	9	2	7	2	5	2	4	2	1	1
Farm size	Ha	26	13	19	7	12	4.02	7	3	4	2
Workers/ha	W/ha	1.38	0.58	0.88	0,25	0.99	0.43	0.92	0.33	1.48	0.57
Cotton %	Percent	33	0.16	44	0.10	35	0.08	25	0.10	11	0.13
Food crops %	Percent	49	0,13	39	0.08	44	0.09	56	0.09	76	0.15
Total TLU	TLU	52	38.71	24	13.82	7.02	6.32	6.54	4.30	4	7.14
Org Matter	Kg	1835	1565	1062	538	790	402	2138	851	772	585
Oxen/h a	Ox/ha	0.31	0.12	0.27	0.08	0.26	0.25	0.28	0.18	0.32	0.50

TLU= Tropical livestock unit of 250 kg; Ha= Hectare; Kg= Kilogram; No.= Total number of equipment; W/ha= worker per hectare (Source: Survey result, 2016, Author).

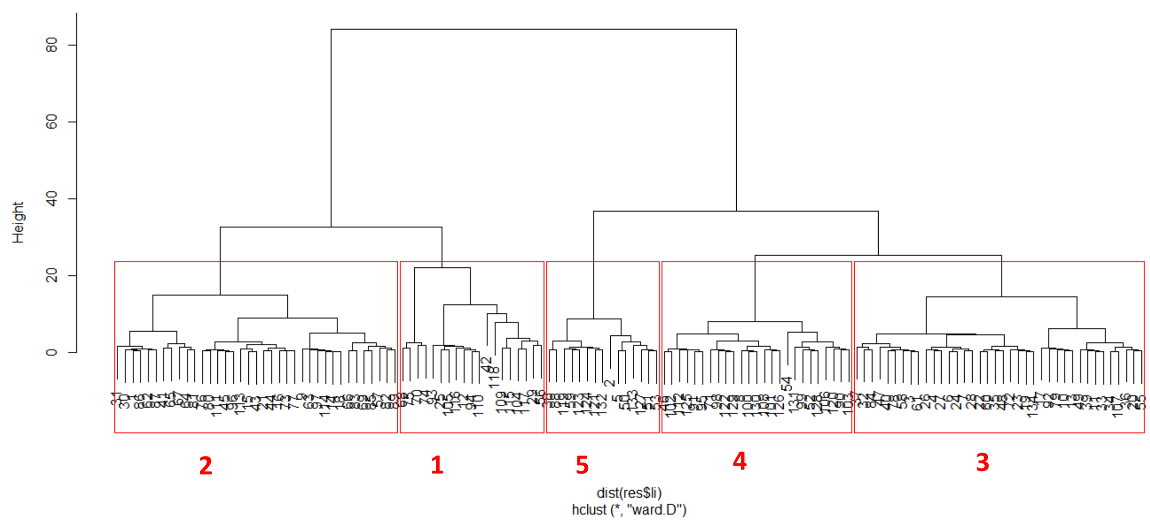

Figure 7. Dendrogram of ascending hierarchical classification (Source: Survey result, 2016, Author).

low quantity with reference to the number of livestock, availability of labour, owned and draught tools.

The super large families have a large labour force mainly composed of children and older people. These types of families also practice crop diversification, with the rainy season rice, groundnuts and potatoes being the most common crops that they prefer to grow in order to diversify their daily food expenditure.

Type 2: Large families (n=37)

About 68% of the sampled farming families are classified as large families. These types of farming families have an average of 26 people. On average, these types of families allocate about 44 and 39% of the total cultivated land areas to cotton and of food crops respectively. Large families practice cash crop farming in order to support the families' daily expenditure.

In terms of age, it is similar to the type 1 and different in terms of composition. Large families are presently the most dominant type of agricultural farming families in Southern Mali. This type of agricultural families usually increases the share of land under cash crop in response to increases in farm gate prices and input subsidies. Furthermore, large families are well-endowed with arable land.

On average, large families cultivate 19 ha, which is 7 ha less than the super large families. About 26 % of the large agricultural families keep livestock compared to 55 percent of the super large families. The quantity of organic matter applied is low at about 1062kg ha^{-1} despite the availability of technology for making organic matter and important assets such as draught tools, labour, and livestock.

Despite the number of livestock owned and the productive resource endowment, large families are less specialized in intensive milk and meat production. Livestock production system is mostly extensive.

Type3: Medium families (n =38)

Type 3 is characterized by a medium number of people. This type has an average of 16 mouths to feed. About 28% of the sampled agricultural farming families are medium-sized. Medium-sized families are also equipped in terms of draught tools and total cultivated land areas.

Averagely, a medium-sized family cultivates 12 ha of land. The main feature that distinguishes medium families from large families is the number of people, livestock owned, the area allocated to food crops and quantity of organic matter applied per hectare. The share of land allocated to cotton is about 35 percent of the total cultivatable area.

Cotton production is the primary activity and the principle source of income for this type of farming family. Income from cotton is invested in livestock such as draught animal and breeding cows. The food security status of this type of families is an important driving force of the size of land that is allocated to cotton and food crop enterprise.

However, it is not a primary feature that distinguishes it from type 2 families. Moreover, all agricultural farming families possess an almost identical ratio of labour except type 5. The ratio of oxen per hectare is identical for all types. Notably, farmers in Southern Mali use draught animal power for agricultural tasks. The quantity of organic matter applied per hectare by medium families is low, yet the equipment they possess is sufficient to produce an important quantity of organic matter coupled with technologies available.

Type4: Small families (n= 25)

It represents 19% of sampled agricultural farming families. The type is composed of an average of 12 people. It is considered as a small family in Southern Malian. This type can be distinguished from the first three types based on the land area allocated to food crops and the quantity of organic matter applied per hectare. Small families allocate 25% of their arable land to cash crop in order to benefit from advantages of cash crop production such as the provision of fertilizer for cereals and access to equipment from the company.

Furthermore, they direct much effort to produce significant quantities of organic matter, averagely 2138 kg ha^{-1}, in order to compensate for the low quantities of chemical fertilizer offered by the company. About 56% of small families produce important and staple food crops such as maize, sorghum and millet. This type of farming family prioritizes food security and the surplus food crops are sold and used to meet the daily expenditure. However, type 3 farming family is less market orientated. They are well equipped compared to the first typology as established by IER and CMDT. This type of farming families owns an average of 7 hectares of land and 4 draught tools. They possess a few head of cattle mainly composed of oxen for drought power

Type5 Young and small families (n = 15)

This represents 11% of the sampled agricultural farming families. It is the youngest type of families in terms of age and not the cropping system. This type has many different features or characteristics from others types. The major differences are in terms of the number of people, draught tools, livestock owned, the quantity of organic matter and area allocated to cash crops. This type of agricultural farming families is oriented towards ensuring food security.

Hence, 76% of arable land is allocated to food crops. About 11% of the owned land areas is rotationally allocated to the cash crop. These families underutilize draught tools (incomplete) for agricultural tasks. The ratio of workers per hectare is quite high than the others four types. The total arable land cultivated is an average of 4 hectares. The quantity of organic matter produced is very low as a result of lack of tools for transporting manure and harvesting the waste.

The small land size that is allocated to cash crop indicates orientation towards food security rather than the market. Young families cultivate cash crop in order to access small quantities of chemical fertilizers which are diverted and used on food crops, particularly maize. Most of the young families detach from the extended family because of issues associated with management of common pool of resources and migration of new families into the villages.

DISCUSSION

Analysis of village-level focus group discussion responses reveals numerous opportunities and constraints of the production systems. Farmers identified the declining yields, lack of significant land fallowing, degradation of soil, low fertility of soil as the major constraints in rural area.

In addition, demographic growth and climate change are the biggest challenge to integrated agricultural production systems. These results are consistent with earlier findings by Descheemaeker et al. (2016), Jones and Thornton (2008), Traore et al. (2015).

However, farmers diversify their sources of income from cotton and food crops to off-farm activities in response to the constraints and challenges. Worryingly, the migration workers to the traditional mining sector are negatively affecting labour provision to the agricultural production activities. From a livestock point of view, feeding system constitutes the main problem in cotton belt.

Pasture lands are hardly increasing to cope with the increasing herd sizes. This forms a major source of conflicts among farming families. Fodder is developed by extension service providers and research institutes. However, the uptake of fodder crops is decreasing as a result of rampant intercropping. Farmers indicated that due to extensive livestock keeping, they loss organic matter and milk.

PCA was employed to establish agricultural farming family dynamics in Southern Mali based on the structure of their agricultural systems and the perceived functions of livestock. PCA has been used in Europe, Asia and Africa in the past to classify and differentiate types of smallholder farmers and also to define their development (Alvarez-Lopez et al., 2008; Rao et al., 2014; Robels et al., 2008; Todde et al., 2016).

This statistical method has been used to simplify the classification of a large number of smallholder farmers into types or classes that are easily understandable. A similar method was used to describe the level of equipment ownership and socio-economic characteristics of dairy farmers (Pienaar and Traub, 2015; Robles et al., 2005).

On the other hand, Faruque (2014) applied PCA to differentiate production systems crop, livestock and fishery production systems in different locations in Bangladesh. The categorization of smallholder farmers and agricultural production systems in the least developed countries is useful in understanding, intervening and making future decisions with regard to research and investment. For example, PCA has been used to classify different farm activities in urban and semi-urban agricultural systems in Nigeria, Burkina Faso and Mali (Dossa et al., 2011).

The typology of smallholder farmers in cotton growing zone that was established by IER and CMDT in twenty two years ago is still being used for research and development purpose. However, IER and CMDT classification only use equipment and cattle owned to classify the farming families. However, with rapid demographic change and the level of equipment used in agricultural production, there is need to develop a new classification of agricultural farming families in the cotton growing area in Mali. In this study, agricultural farming families have been classified into five types based on ten explanatory variables.

Results of this study reveal that differences in farmer dynamics are largely as a result of difference between the typology established by IER and CMDT as illustrated in Table 1 and the newly proposal topology as illustrated in Table 3. Only the type 5 is still operating on incomplete draught tools and it represents 11 percent of sampled farming families. The new classification is largely different from the ancient CMDT type in terms of drought tools, the number of livestock owned as expressed in Tropical Livestock Unit, total cultivated land area, family size and more other factors (Tittonell et al., 2010; Tittonell, 2013) reported that types of farmers varied in terms of resources endowment such as land, livestock, equipment and labour. Others researchers sought to classify smallholder farmers according to the income generated from agricultural activities (Djouara et al., 2006; Koutou et al., 2015; Nubukpo and Keita, 2006). Mbetid-bessane (2003) classified smallholder farmers in the cotton production system based on the structure and functioning of their integrated farm systems in order to understand their trajectory.

Sakana (2012) also established smallholder farmers typology in wetland zones in Kenya and Tanzania based on their production systems. Douxchamps et al. (2016) described and classified smallholder farmers into different groups based on their agricultural technology adoption patterns and food security in three Western African countries. The quantity of organic matter applied on crops by smallholder farmers varies between 772 to 2138 $kgha^{-1}$ in this study.

Blanchard (2010) and Falconnier et al. (2015) reported almost the same quantities, 1600 to 2500 $kgha^{-1}$, as being applied in the old basin. The variability in the

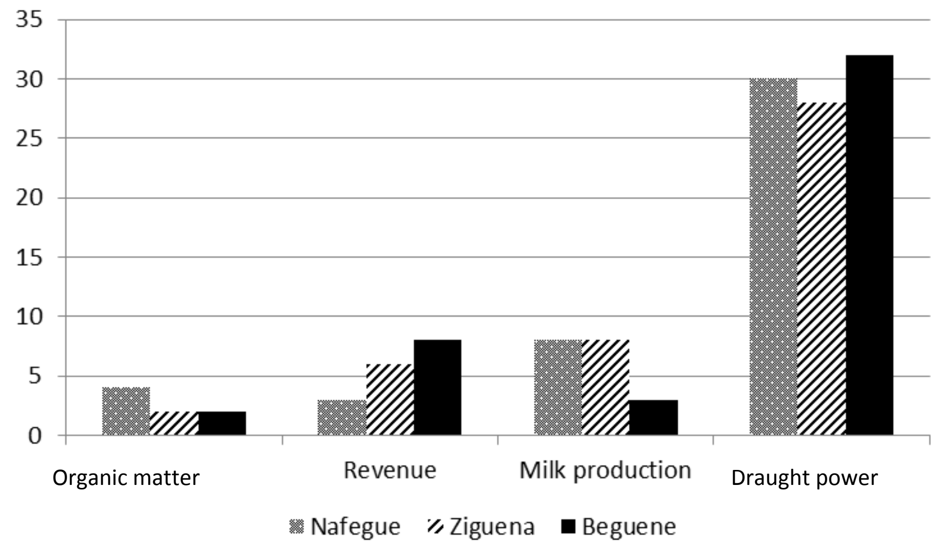

Figure 8. Main function of livestock per village (Source: Survey result, 2016, Author).

quantity of organic matter application can be explained by non-standardized estimation of the weight of a cartload of organic matter.

In another study conducted in Uganda, (Okoboi and Barungi, 2012) observed that the variability in the use of organic matter and chemical fertilizer could be explained by several constraints such as access to agricultural inputs and market information that smallholder farmer face. Vall et al. (2006) also argued that organic matter applied on cotton by smallholder farmers in cotton growing zone of Burkina Faso varied widely from one type of smallholder farmer another. The applied quantities of organic matter are far below the recommended rate of 5000 kg ha^{-1}.

Organic matter is specifically important for reclaiming and improving soil fertility of over-cultivated land. According to Rufino (2007) and Giller et al. (2010), the use of organic matter is critical in improving crops yields per land area in SSA. For this reason, farming families with an important herd size should not only produce significant quantities of organic matter but also utilize it for crop production. Furthermore, the share of cash crop in the rotation varied among farming family types. Agricultural farming family types 1, type 2 and type 3 allocated 33, 35 and 44% of the cultivated land area on cotton in the crop rotation system respectively.

A study conducted by Mujeyi (2013) in Zimbabwe showed a similar trend, where farmers allocated almost 34% of the total cultivated land to cotton. Djouara (2006) also found about 42 and 30% of the cultivated land were allocated to cotton by large and medium families respectively in Southern Mali. Small families in the cotton belt engage in cotton production in order to benefit from chemical fertilizer supply from CMDT.

However, increase in the area allocated to cotton by the five types farming families may be linked to population growth and market orientation due to increases in farm gate prices. Daloglu et al. (2014) explained that farm typology is essential in making decision in a diverse of production system. Dynamics in agricultural farming families and the diverse production systems offer multiple options for agricultural development in SSA.

Economically associated crops and livestock

Crop and livestock production are major activities and sources of income in rural areas of least developed countries. There is limited use of farm machinery and therefore, smallholder farmers rely on animal power to expand their farm size as they attempt to maximize farm profit.

In addition, integrating crop and livestock enterprise areas offers higher income to smallholder farmers as compared to those who own isolated crop or livestock enterprises (Bakhsh et al., 2014). Moreover, livestock is an important asset for smallholder farmers because it is used to perform different farm or cropping operations. Animal power in the cotton growing zones is a major driver of food security, and plays an important role in poverty alleviation. Figure 8 shows that agricultural farming families rise cattle for drought power.

Moreover, drought power is related to herd size and quantity of manure produced. Randolph et al. (2014) argued that livestock rearing is essential in improving human health status by ensuring dietary diversity for both young and older household members. Other functions are also potential in certain cases or countries where animal power plays a little or feeble value addition on income. Smallholder farmers do not consider milk production and organic matter as the main objective for livestock keeping in SSA.

Although smallholder farmers integrate crop and livestock, such production systems are not sufficient in technical and economic terms because only two products, that is milk and manure, are produced (Okoruwa et al., 1996; Schiere et al., 2002). Although, revenue is generated by smallholder farmers by selling old oxen and old cows to renew herd size by fattening, it contributes to a large variety of expenditure within the family.

Although farmer generate revenue by selling old oxen and cows, a large proportion of the revenue is used to renew the herd, leaving little for family food and non-food expenditure The surplus is invested in new draught tools and transportation equipment and also spend on marriages, payment of caretaker, taxes and human health (Barrett, 1991; Ba et al., 2011).

This is opposed to the economic and nutritional roles of milk and organic matter production in other areas in SSA. For instance, smallholder farmers in Western Kenya keep livestock with a purpose of milk production, meeting household daily nutritional requirement, and contributing to households' economic well-being (Rufino et al., 2007).

Herrero et al. (2009) argued that there are many functions of livestock keeping. They encompass employment, nutrition and traction. The last function is the main objective for livestock keeping in SSA. Livestock keepings allow farmers to expand cultivated land area and reduce timing for work. An agricultural farming family is diverse and complex to understand its practices. Livestock rearing (cattle), being the heart of agricultural development in Southern Mali should be continuously promoted and supported (Figure 8).

Conclusion

The Malian cotton sub-sector has been affected by numerous fluctuations in terms of farm gate prices input subsidies, and also declining areas under cultivation. However, increases in farm gate prices and input subsidies have allowed cotton production to catch up. The number of agricultural farming families is steadily increasing, but cotton yields per hectare are still stagnant. This is mainly explained by over-cultivation of the land and low soil fertility (Figure 1)

Analysing agricultural farming family dynamics in an integrated crop and livestock system in an SSA context is complex. However, it can offer a global view of smallholder farmers' endowment and open up intervention in the agricultural sector for alleviating malnutrition and extreme poverty. The study was carried out in the representative zones of Southern Mali from the saturated zone in the North (old basin) to centre (intermediary zone) and Southern part (sub-humid zone).

These zones represent the real picture of cotton belt in Mali. Smallholder farmers' dynamics were established and classified into five types using structured and functional variables. Type 1 represented 14% of the sampled agricultural farming families. Most of type A in CMDT typology has tended to change to another type by being endowed with large herd size, more draught power, draught tools and more labour.

Agricultural farming families that constitute Type 2 represented 28% of the sampled families. Some of type A are also represented in this category as they move towards large families that are well equipped in terms of herd size, draught power, draught tools and cultivated area. Type 3 is the most important and the most dominant in the cotton growing areas. It represented about 28 percent of smallholder farmers.

Former type A, B, and C are represented in this type of smallholder farmer type. On the other hand, these types tend to move towards medium agricultural farming families that are well-endowed just like type A in the CMDT typology. They allocate 35 and 44% of their land to cotton and food crop production respectively and possessing important herd sizes.

Type 4 represents 19% of the sampled agricultural farming households. It overtakes former type A in terms of the number of draught tools, draught power, herd size and the area allocated to food crops. The last type, type 5, represents 11% of the farming families. It is equivalent to CMDT's type C. They operate on incomplete tools and have some livestock. It is composed of the young families and families that migrate into the village. Type 5 families attempt to endow themselves and are not market oriented.

Food crops represent 76% of crop rotation. However, the quantity of organic matter produced by all types is very low despite the availability of technologies to produce organic matter in large quantities and good quality. About 67% of smallholder farmers in cotton producing areas in Southern Mali keep livestock primarily for animal power.

Milk production and revenue follow at 14 and 13% respectively. Lastly, only 6 % of smallholder farmers keep livestock for organic matter production. Regarding the diversity of agricultural farming family, their dynamics offer multiple options for agricultural development in SSA.

The results of this study can be extended by further assessment of smallholder farmer dynamics. The study methodology can also be applied in all agricultural production systems research. Farmers in the cotton growing region in Mali have over the years gradually endowed themselves in terms of farm resources. The typology that was established in 1996 should be updated to capture the current situations by taking into accounts some relevant variables. There are several and alternative development interventions can be used to improve the livelihoods of the rural population.

The study recommends interventions such as the development and modernization of milk, meat and horticulture value chains in Southern Mali. There is also the need to extend the study to cover the entire Southern Mali so as to contribute to the updating of smallholder farmer classification topology in the cotton growing region. Lastly, the findings of this study may assist policymakers and future researchers in designing measures for achieving the sustainable development goals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDIX

Table A. Soil composition in southern Mali.

Variable	Units	0-20 cm	20-40 cm
Clay	%	4.84	7.35
Silt	%	11.22	9.93
Sand	%	83.94	82.72
pH (water)	-	5.81	5.75
Organic matter	%	0.79	0.57
Organic carbon	%	0.46	0.33
Total N	%	0.30	0.24
C/N		15.24	14.18
Available P (Olsen-Dabin)	mg/kg	3.45	3.60
Ca exchangeable	me/100g	0.89	0.83
Mg exchangeable	me/100g	0.43	0.47
K exchangeable	me/100g	0.07	0.05
Na exchangeable	me/100g	0.01	0.02
Al exchangeable	me/100g	0.01	0.02
Mn exchangeable	me/100g	0.09	0.03
H exchangeable	me/100g	0.01	0.02
S(Ca, Mg, K, Na)	me/100g	1.40	1.36
CEC	me/100g	1.79	1.73

Source: Soil composition in southern Mali (IER/CMDT Sissoko et al. (2014)).

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Effect of replacing inorganic with organic trace minerals on growth performance, carcass characteristics and chemical composition of broiler thigh meat

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Micro minerals (Trace minerals) are required for normal growth and development in broilers. The present study was undertaken to compare the effect of replacing inorganic with organic trace minerals on growth performance, carcass characteristics and chemical composition of broiler thigh meat. A corn soybean based diet supplemented with organic trace minerals (OTM) (x) and an inorganic trace mineral (ITM) (y) was prepared. Four hundred eighty birds were stratified by weight and randomly assigned to 6 dietary treatments with four replicates per treatment (20 birds/replicate pen). The treatments were A) Positive control group diet (x) supplemented for whole period (0-5 wk). B) Fed diet (x) during starter and grower phase (1-4 wk) and diet (y) was offered in finisher phase (5 wk). C) 1st 3 weeks were fed diet (x) and last two weeks were given diet (y). D) First 2 weeks were fed diet (x) while, diet (Y) offered in the last three weeks. In the treatment (E), diet (x) offered during initial phase and nourished with diet (y) during grower and finisher phase while in treatment (F), complete diet (y). The result showed that organic trace mineral supplementation did not affect growth performance in the first and last week of trail but during 2nd, 3rd and 4th weeks organic trace mineral supplementation showed better growth performance than that of inorganic trace mineral supplementation. Chicken fed with organic trace mineral supplemented diets had the better growth performance which differed from that of inorganic supplemented diet groups. Additionally, organic trace minerals supplementation did not affect dressing percentage and giblets weight but shank and keel lengths were improved. While, OTM supplementation did not alter dry matter, ash and moisture content in thigh meat; however, crude protein content was improved in thigh meat. Overall r**esults demonstrated that the quality of broiler chicken meat in high organic trace mineral supplement increased relative to the low supplemented groups. It is concluded that addition of organic trace mineral to feed can improve the growth performance of broiler particularly during growing phase.**

Key words: Carcass, dry matter, growth, performance, replacing.

INTRODUCTION

It is well recognized that demand of poultry meat and meat products has increased due to shortage of red meat supply (FAO, 2010) and price phenomena. Annual broiler global meat production was 84.6 million tons in 2013 (USDA, 2003) and covered 33% of global meat demand (FAO, 2010). The genetic advancement continuously elevated the broiler growth potential; broiler can attain mature body weight by consuming less feed (Mckay, 2009) and in shorter period of time. It is well established that trace minerals are important for broiler normal growth and development (Kratzer and Vohra (in press)). However, due to relative less economic importance, trace minerals sector failed to attracts poultry scientist attentions. The current interest in trace minerals nutrition has been vigorously undertaken due to better bioavailability of trace minerals (Wedekind et al. 1992, Oyagi and Baker, 1993) and concerns of environmental pollution (Lesson, 2003).

Poultry nutritionists balance the trace minerals requirements according to National Research Council (NRC, 1994) recommendations (NRC, 1994). However, these recommendations have not supported well due to many factors such as FAO, (2010) Broilers growth potential elevated trace minerals requirement, (USDA, 2003) negative interactions between the inorganic forms of trace elements (Du et al. 1996), Mondal et al. 2007). Therefore nutritionists increased trace minerals supplementation levels much more than that of NRC (1994) recommendations (Inal et al. 2001; Lesson, 2005). The studies conducted in last decades have shown that organic trace minerals supplementation significantly improved livestock production (Paik 2001). The bioavailability of various forms of the organic trace elements is superior to that of inorganic element due to their better absorption rate (Lesson, 2003; Nollet et al., 2005; Van Der Klis et al., 2002). Organic trace minerals make complexes with organic molecules and attaining structure which can easily pass through intestinal mucosa (AAFCO, 1997; Kincaid, 1989; Nelson, 1988).

Pakistan has an agriculture based economy. About 70% of the total population is directly or indirectly involved in wide range of agriculture businesses. Livestock contributes about 55% of total the agriculture GDP and poultry sector has been expanding its wing as most integral and dynamic component of national economy (Anonymous, 2008). Commercial poultry sector is on the stage of rapid boom with annual growth rate of 20 to 25%. Kamal (2010), Pakistan Poultry industry has recommended the addition of organic trace minerals in poultry feed. However, industry is reluctant due to price phenomena and availability. Keeping in view the result of available literature, the present study was planned to determine the effect of replacing the inorganic with organic trace minerals on growth performance, carcass characteristic and composition of thigh meat of broilers.

MATERIALS AND METHODS

The present study methodology and protocols were approved by the Institutional Animal Care and Use Committee of University of Veterinary and Animal Science, Lahore (UVAS, Lahore). A total of 480 unsex broilers birds were randomly divided into six treatment groups (n=80 per group) and were grown over 35 days. Each treatment group is further divided into four replicate while each replicate was comprised of 20 chicks. Diet consisted of a corn Soy based ration supplemented with organic trace minerals (OTM) (x) and inorganic trace minerals (ITM) (y). Inorganic trace minerals were purchased from local market while Bioplx Cu, Bioplx Fe, Bioplex Zn, Bioplex Mn, and Sel-Plex were provided by Alltech Inc USA. The basal diet (Table 1) was formulated according to NRC (1994) recommendations. Analyzed by the AOAC (2000) and both feed were procured from commercial feed mill. The diets were fed *adlibitum* throughout experimental period. Dietary treatments were divided in different replicate pens A) x fed diet throughout trial period), B) fed diet (x) in week 1-4 and diet (y) was offered in last week. C) Diet (x) served weeks 1-3 followed by diet (y) week 4-5. D) First 2 weeks were fed diet (x) while, diet (y) offered in the last three weeks. In the treatment (E), only first week were fed diet (x) and remaining last 4 weeks were fed diet (y) while in treatment (F), complete diet (y) was fed to experimental birds. The initial temperature of house was maintained at 95°F at chick level and was reduced by 5°F every week to maintain the final temperature at 75°F.

Growth performance

The feed intake (F.I) and body weight (B.W) were recorded on day 7, 14, 21, 28 and 35 per pen. Feed conversion was calculated as feed to gain ratio. Livability was recorded daily and calculated as percent within the pen.

Carcass characteristics

At the end of trail feeding period, two birds close to mean body weight were selected from each replicate and slaughter. After skinning dressing percentage were recorded and giblets were collected and weight while shank and keel bone length were measured after removing meat.

Composition of thigh meat

Whole thigh meat were collect from every slaughter bird that were slaughtered at the end of feeding period and preserved in deep freeze at 4°C until further analysis. They were at room temperature and oven dried at 100 C^0 for 24h and ground it for proximate

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Table 1. Detail chemical composition of basal diet

Ingredients	Quantity (%)
Maize	33.87
Rice tips	30
Soybean meal	32.30
DCP	1.80
Salt	0.25
Lime stone	1.21
Supplement micro minerals (Bioplex, Alltech)	0.20
DL- Met. 99%	0.17
L- Lys-HCL. 78.4%	0.2
Total	100
Nutrients	**Composition**
Crude protein (%)	19
ME (Kcal)/kg	2900
Crude Fiber (%)	3.05
Ether Extract (%)	2.76
Total P%	0.73
Available P%	0.42
Calcium %	1.02
Lysine %	1.25
Methionine %	0.48
Methionine + Cystine %	0.82
Threonine%	0.80
Tryptophan%	0.25
Arginine%	1.39
Isoleucine%	0.93
Na%	0.18

Each 2 kg of Vit. and Min. Mixture contains: Vit. A 12000,000 IU, Vit. D3 2200,000 IU, Vit. E 10,000 mg, Vit. k3 2000 mg, Vit. B1 1000 mg, Vit. B2 5000 mg, Vit. B6 1500 mg, Vit. B12 10 mg, Pantothenic acid 10,000 mg, Niacin 30,000 mg, Folic acid 1000 mg, Biotin 50 mg, Manganese 100,000 mg, Zinc 80,000 mg, Copper 10,000 mg, Iron 50,000, Iodine 1000 mg, Selenium 300 mg, Cobalt 100 mg, Ca CO3 to 2,000 g.

analysis.

Statistical analysis

The data obtained on growth performance; carcass characteristics and meat composition were analyzed for Analysis of Variance (ANOVA) technique under Completely Randomized Design (Steel et al., 1997). Level of significance was kept at $P<0.05$. Means were compared for significance of difference with Duncan’s Multiple Test (Duncan, 1955).

RESULTS

Growth performance

The supplementation of OTM is a possible solution to fulfill the modern broiler trace minerals requirement by using the NRC (1994) recommendations. Dietary supplementation of organic trace minerals did not affect feed intake, body weight gain and Feed conversion during first and last week of trail, suggesting that ITM replacement with OTM did not affect broiler performance during starter and finisher phase (1-7 days, 29-35 days). However, during growing phase (8-28 days), as OTM supplemented diet level increase weight gain by in taking less feed and FCR was improved. In the finisher phase (29-35 days) no significant differences were observed in feed intake and weight gain among all treatments groups (Tables 2, 3 and 4). In this study, overall growth performance values were slightly lower than those already reported studies, because the present study conducted in august and September month during these month temperature and relative humidity is much higher in Pakistan, these unfavorable conditions on the period of present study may be caused to lower BWG which may worse overall growth performance. Mortality rate among different experimental groups was also recorded and shown in Table 3. Non-significant difference was observed among 6 treatments groups. However, highest mortality rate was observed in group which was fed inorganic trace mineral supplemented diet throughout trail period. Overall mortality was within the acceptable ranges.

Carcass characteristics

Non-significant difference was observed between different treatments groups with little bit better dressing percentage were observed when feeding diet supplemented with ITM level. Table 4 present data on carcass characteristic. Organic trace minerals supplemented diet was found to have effect on shank and keel length, however it was not significant. as ITM diet level increased at 35 days trail, The shank length various treatment groups was 5.48, 5.45, 5.44, 5.43,5.39 and 5.40 cm respectively, and keel length measurement was 14.04, 14.01, 13.98, 13.95, 13.93 and 13.92 cm, respectively. Overall, organic trace mineral has effect on shank and keel bone length growth. While, organic trace mineral supplementation has no effect on weight of liver, spleen, heart but organic trace minerals supplementation has improved gizzard (gm) weight than that of ITM supplemented diet (Table 5). Table 6 shows the data on the variable presenting the chemical composition of thigh meat. Feeding OTM did not have any significant effect on DM, fat, ash, and water content in thigh meat. However, CP content was improved in the groups fed with OTM supplemented diets than those of ITM supplementation.

DISCUSSION

Growth performance and carcass characteristics are the main parameters which normally used to evaluate the broiler performance. Since last 70 years, the success of

Table 2. Average weekly feed intake.

T	7	14	21	28	35
A	158.6^{a}±3.2	434.3^{c}±0.2	624.2^{c}±2.6	915.2^{a}±11.5	1145.5^{a}±15.5
B	154.5^{ab}±0.3	438.0^{b}±1.9	632.1b^{c}±6.9	916.3^{a}± 1.9	1162.0^{a}±17.3
C	150.1^{b}±2.8	439.5^{b}± 1.2	649.1^{ab}±2.6	930.5^{a}± 11.9	1164.7^{a}±17.6
D	154.0^{ab}±1.1	440.8^{ab}±0.6	658.2^{a}±7.1	927.9^{a} ± 12.0	1166.1^{a}±17.8
E	151.5^{b}± 1.0	441.1^{ab}±0.7	648.1^{ab}± 6.2	929.8^{a} ± 11.8	1164.8^{a}±16.4
F	155.2^{ab}±0.2	442.9^{a} ±0.6	664.5^{a}±9.0	944.4^{a} ± 13.8	1166.7^{a}±16.5

Table 3. Average weekly weight gain.

T	7	14	21	28	35
A	161.7^{a} ±1.49	356.7^{a}± 0.85	607.5^{a}± 1.25	1108.0^{a}±20.6	1603.5^{a}±14.5
B	150.2^{bc}±4.13	353.2^{ab}±1.79	586.5^{b} ± 1.55	1133.5^{a}± 11.8	1614.7^{a}±31.0
C	145.4^{c}±3.015	352.0^{b}± 1.95	563.0^{c} ± 1.22	1087.7^{ab}±18.0	1547.2^{a}±17.7
D	153.5^{b}±1.90	352.0^{b}± 0.91	550.0^{dc}± 2.85	1084.5^{ab}±13.7	1566.2^{a}±17.5
E	148.0± 0.81	349.0^{b}±0.91	548.0^{dc}±10.59	1014.0^{c}±19.3	1586.5^{a}±29.1
F	147.7±0.85	337.5^{c}±1.32	539.7^{d}± 11.30	1049.5^{b}±14.3	1577.2^{a}±3.6

Table 4. Average weekly feed conversion ratio.

T	7	14	21	28	35
A	1.0^{a}±0.0	1.7^{b}±0.0	2.2^{a}±0.1	1.9^{c}±0.0	2.0^{c}±0.0
B	1.0^{a}±0.1	1.7^{ab}±0.0	2.2^{a}±0.1	1.9^{c}±0.0	2.1^{bc}±0.0
C	1.0^{a}±0.0	1.7^{ab}±0.0	2.2^{a}±0.0	2.0b^{c}±0.0	2.1^{a}±0.0
D	1.0^{a}±0.0	1.7^{ab}±0.0	2.2^{a}±0.1	2.0^{c}±0.0	2.1^{ab}±0.0
E	1.0^{a}±0.0	1.7^{ab}±0.0	2.2^{a} ±0.1	2.2^{b}±0.1	2.1^{abc}±0.0
F	1.0^{a}±0.0	1.7^{a}±0.0	2.3^{a}±0.1	2.1^{ab}±0.0	2.1^{ab}±0.0

Table 5. Carcass characteristics of meat.

T	Shank length	Keel length	Dressing %	Spleen weight	Liver weight	Heart weight	Bursa weight	Gizzard weight
A	5.5^{a}+0.0	14.0^{a}+0.0	61.4^{a}+1.0	1.8^{a}+0.2	46.2^{a}+2.7	11.9^{a}+1.6	2.3^{c}+0.0	70.2^{a}+3.4
B	5.4^{b}+0.0	14.0^{b}+0.0	60.2^{a}+2.0	2.3^{a}+0.3	55.2^{a}+5.2	10.1^{a}+0.4	2.3^{b}+0.0	62.9^{ab}+3.0
C	5.4^{b}+0.0	14.0^{c}+0.0	62.7^{a}+2.3	2.2^{a}+0.2	48.2^{a}+5.2	9.1^{a}+ 0.6	2.3^{b}+0.0	52.9^{b}+4.4
D	5.4^{b}+0.0	13.9^{d}+0.0	63.9^{a}+2.0	2.0^{a}+0.4	48.4^{a}+2.1	10.4^{a}+ 0.4	2.3^{ab}+0.0	63.1^{ab}+5.6
E	5.4^{c}+0.0	13.9^{e}+0.0	47.6^{a}+15.6	1.8^{a}+ 0.4	51.0^{a}+1.6	8.9^{a}+ 0.5	2.4^{ab}+0.0	60.5^{ab}+ 5.1
F	5.4^{c}+0.0	13.9^{e}+0.0	66.2^{a}+ 0.7	1.5^{a}+0.2	53.4^{a}+4.6	10.4^{a}+1.2	2.4^{a}+0.0	63.6^{ab}+4.3

higher broiler performance has been successfully achieved through genetic improvement which demands better management and appropriate nutrition, especially in trace element nutrition. It is well established that dietary requirement of trace minerals are negligible; however, it is important to note that these negligible element are important for broiler enzymes system, metabolism, growth and reproduction (Berger and Cunha, 2006).

In this study, we evaluated the effect of replacing ITM with OTM on growth performance, carcass characteristics and meat composition of thigh meat. We observed non significance difference in growth performance parameters between different treatment groups during 1^{st} week of trial

Table 6. Proximate analysis of thigh meat.

T	D. M.%	C. P.%	M%.	Ash%
A	26.0^{a}±1.043	77.8^{a}±0.3	74.0^{a}±1.0	3.6^{a}±0.2
B	24.9^{a}±0.35	77.2^{ab}±0.4	75.0^{a}±0.3	2.9^{a}±0.2
C	24.8^{a}±0.32	77.4^{bc}±0.3	75.1^{a}±0.3	2.9^{a}±0.3
D	24.2a±0.19	76.9^{abc}±0.2	75.8^{a}±0.2	3.0^{a}±0.3
E	23.9^{a}±1.32	76.3^{bc}±0.3	76.1^{a}±1.3	3.0^{a}±0.2
F	26.4^{a}±1.14	76.3^{c}±0.3	73.6^{a}±1.1	3.2^{a}±0.2

which are completely in line with previous reports (Bao et al., 2007). However, we found differences in production performance of various treatment groups, these result are consistent with a pervious observations (Xia et al. 2004; Abdallah et al. 2009; El-Hussein et al., 2012). Current study findings were supported in their subsequent investigation in which they reported that OTM supplementation according to NRC (1994) recommendations is reasonable for highly growing broilers due to their inherent better bioavailability (Fly et al., 1989). However, inorganic trace element does not fulfill modern broiler trace element requirements due to their less bioavailability and negative interaction (Du et al. 1996; Mondal et al., 2007). However during 5th week non-significant difference was identified, these finding are in agreement with Bao and Choct (2009) and Smith et al. (1995) who reported that OTM efficacy decrease as birds age increased (Bao et al. 2009; Smith et al. 1995).

Current study observations about carcass characteristics parameters are in line with the finding of Lu et al. (2006) who reported that broiler intake diet supplemented with organic Mn had shown lower percentage of abdominal fat, further OTM supplemented diet had no significant effect on the weight of liver (g), heart (g), and spleen (g). Our result are completely in agreement with the studies of Bao and Choct (2007) (2009) and Zhao et al. (2010) who reported that organic trace mineral had no effect on dressing percentage and giblets weight. In the present study, organic trace minerals supplementation improved the growth of shake and keel bone length. Osama et al. (2012) reported that organic Zn, Cu, Mn has improved the Tibia weight and length. In 2009, a study was conducted on turkeys and researcher observed that organic trace minerals supplementation improved biochemical properties of bone (Ferket et al. 2009). Similar observation has been reported on equine in which improvement of bone growth has been observed in yearling feeding organic trace mineral supplemented diet than those fed inorganic trace mineral supplemented diet (Ott and Johnson. 2001).

Poultry nutritionist are conscious about organic trace minerals supplementation due to price phenomena, however, we found that organic trace mineral supplementation become inexpensive due increased weight gain with better feed efficiency. The observation of present study are completely in line with the studies conducted by Osman and Raga (2007) and Abdallah et al. (2009), Osama et al. (2012) and El-Hussein et al. (2012) who reported that broilers fed diet supplemented with organic trace minerals has improved profit by decreasing cost of production.

Proximate analysis of thigh meat after skinning indicate that OTM supplementation did not affect significantly on DM, ash and Moisture content, however, crude protein content was improved among OTM fed groups than that of ITM supplemented group. This higher content was may be due to OTM forming complexes with organic compounds which are more soluble and mobile to the cell membranes and easily absorbed (Kincaid, 1989; Nelson, 1988).

Conclusion

1) It is proved that OTM supplementation according to NRC (1994) recommendation could be fully satisfy high genetic broilers requirement.
2) Feeding broiler diet with organic trace mineral during growing phase is improved broiler growth performance.
3) Feeding the diet supplemented with organic trace mineral has improved broiler thigh meat quality and overall economic efficiency of flock.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Abbreviations

OTM, Organic trace mineral; **ITM,** inorganic trace mineral; **CP,** crude protein; **DM,** dry matter; **NRC,** National Research Council; **BWG,** body weight gain; **g,** gram.

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Evapotranspiration and control mechanisms in managed Amazonian forest in, Pará, Brazil

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This work examines whether management causes changes in evapotranspiration (ET) surface conductance (g_s), aerodynamic conductance (g_a) and the decoupling factor (Ω) in managed and natural forest sites in a tropical rain forest in the Amazon. The study was conducted in the Tapajós National Forest (FNT) in managed (logged) and natural (unlogged) forests, which have micrometeorological towers for data capture. For ET estimation, the Penman-Monteith (PM) and Eddy Covariance (EC) equations were used. The models were significantly different only for unlogged (PM 134.9±15.9 mm.month^{-1} and EC 100.9±11.1 mm.month^{-1}), while the means of the logged site were PM 111.1±15.7 mm.month^{-1} and EC 108.5±18.3 mm.month^{-1}. Each area has different characteristics for the surface variables, g_a, g_s and Ω, and therefore the sites were different from each other for the study variables. However, logged ET did not differ for the PM, while EC decreased in the year after the management intervention, and was then followed by an increase.

Key words: Tapajós, water vapor, surface, aerodynamic conductance, decoupling factor.

INTRODUCTION

The Amazon has a key role in regional and global climate systems, in large part due to contributions to evapotranspiration (ET) of the surface and therefore for the global carbon cycle. However, the Amazon forest currently faces risks due to deforestation pressure and climate change (Randow et al., 2014).

Forest management is a method of selective logging that limits damage to the forest by cutting lianas, doing road planning, using skidders with articulated wheels and conducting directional felling (Palace et al., 2007).

Historically, forest management research emphasized mainly silvicultural aspects and changes in species composition, but little has been investigated on post-operation changes in biogeochemical cycles and their

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effects on forest productivity (Hall et al., 2003).

ET is an essential component of the water cycle, due to the control it exerts on energy exchanges and biogeochemical cycles and the interaction of these with the atmosphere and terrestrial ecosystems (Katul et al., 2012; Wang and Dickinson, 2012), and globally ET returns more than 60% of annual precipitation to the atmosphere (Liu et al., 2013).

Changes in ET due to land cover changes or other land use practices have received special attention in the last ten years because of their potential effects on climate and water resources (Boisier et al., 2014).

The arboreal contribution to Amazonian regional hydrologic cycling represents a considerable portion of the regional water balance, therefore, changes in vegetation cover due to deforestation, leading to decreased ET, modified hydrologic balance, with consequences in the Amazon and neighboring regions (Correia et al., 2007).

Each type of vegetation has a different structure, and these differences effect gas exchange processes, so it is important to know whether interventions through forest management cause changes in the structure of vegetation that change ET. Thus, this work aims to determine the hydrologic aspects in a tropical rain forest in the Amazon, examining whether management creates differences in ET, surface and aerodynamic conductance, and decoupling factors for managed and natural forest sites.

MATERIALS AND METHODS

Study area

The study area is located in the Tapajós National Forest (FNT) - (3,017º S; 54,970º W) in the State of Pará, located about 70 km south of the city of Santarém, with an area of 6 × 10^5 ha and an average canopy height of 36 m.

The climate is classified as Ami (Köppen), with an average annual temperature of 25.5°C. The rainy season occurs between January and May, resulting in an average annual rainfall of 1,820 mm. The local relief is slightly hilly, with gently undulating wavy topography. The soil that is predominant in the study area is a dystrophic Yellow Latosol. The vegetation is classified as dense forest, characterized by the dominance of individual trees of large size (IBGE, 2012).

The FNT is limited by the Tapajós River to the west, and the Santarem-Cuiaba Highway (BR-163) to the east, extending from 50 to 150 km south of the city of Santarem, Brazil, Pará. The area of forest management (logged) is located at km 83 of the BR-163 and has a 67 m tall micrometeorological instrumental tower located 6 km west of the BR-163 highway and 6 km east of the Tapajos River. The site used as control (no forest management – unlogged) is an undisturbed primary forest that extends for tens of kilometers to the north and south located close to the FNT entrance at km 67 of the BR-163 and also has a 67 m tall micrometeorological instrumental tower located at 54°58 'W, 2º51' S.

The study site was an area of 18 ha that was harvested in three phases between August and December, 2001. The first phase occurred starting on August 18th with the felling of trees near the micrometeorological instrumental tower. These trees were felled in order to prevent accidental treefall onto the tower once harvesting activities were initiated and probably had little if any effect on subsequent measurements of the variables in this study. The second phase occurred during the month of September and was comprised of harvesting in an area that included nearly the entire 18 ha intensive harvesting area which was situated 1 km to the east, 0.1 km to the west, 0.1 km to the north, and 0.7 km to the south of the micrometeorological instrumental tower. The third phase occurred in November and December and included an area that extended up to 3 km from the measurement tower and encompassed the remainder of the 18 ha harvest area not included in the September harvest activities (Figueira et al., 2008).

Calculation of ET and surface variables

The vortices correlation technique (Eddy Covariance - EC) uses sonic anemometers and infrared gas analyzers (IRGA) for scalar measurements at high frequency involved in determining the turbulent flow and mass exchange (carbon dioxide and water vapor) and energy (latent heat and sensible heat) between the biosphere and atmosphere (Baldocchi, 2003).

The data collected from micrometeorological towers generated 30 min, 24 h and monthly averages calculated from the fluxes of momentum, heat, water vapor and carbon dioxide, and carbon dioxide storage in the air column (Miller et al., 2009).

ET is calculated by Eddy system as follows: ET and latent heat flux (LE) are both based on the water vapor flow measurements (M/H_2O) and represent the sum of surface evaporation, condensation and transpiration by plants. LE is calculated as the product of the latent heat of vaporization and the measurement of fluid stream of water vapor (F / H_2O) in $W.m^{-2}$. ET is the sum of half hour averages of F / H_2O in $mm.day^{-1}$, from evaporative transpiration (Hutyra et al., 2007).

ET calculations using the Penman Monteith equation (Kume et al., 2011) was done as follows:

$$ET=\frac{\delta(\mathrm{Rn}-G)+\rho_a c_p\frac{(e_s-e_a)}{r_a}}{\delta+\gamma\left(1+\frac{r_s}{r_a}\right)} \quad (1)$$

Where: ET = actual daily evapotranspiration (mm day^{-1}); δ is the slope of the saturated vapor pressure curve of water (k Pa $°C^{-1}$); Rn = net radiation (W m^{-2}); L = heat flow in soil (W m^{-2}); ρa = mean density of air (1.292 kg m^{-3}); cp = specific heat of air at constant pressure (J kg^{-1} C^{-1}); γ = psychrometric constant (k Pa C^{-1}); r_s = stomatal resistance (s m^{-1}); r_a = air resistance (s m^{-1}); and = actual vapor pressure (k Pa); s = saturation vapor pressure (k Pa).

ET is influenced by the power available at the surface, by the vapor pressure gradient between the water surface and the air and the resistance to vapor transfer. The canopy exchange process with the atmosphere are characterized by biotic and abiotic factors, such as surface conductance (g_s), aerodynamic conductance (g_a) and the decoupling factor (Ω) (Pinto Jr. et al., 2009).

The control exerted by the stomata of the water flow path between the mesophyll in the leaf and the atmosphere is represented by stomatal conductance (g_s) (Costa et al., 2010) in Equation 2:

$$g_s=(r_s)^{-1}=\left\{\frac{\rho_a c_p \mathrm{DVP}}{\gamma \mathrm{LE}}-\frac{1}{g_a}\left(1-\frac{\delta \mathrm{H}}{\gamma \mathrm{LE}}\right)\right\}^{-1} \quad (2)$$

Where: g_s is the stomatal conductance; DPV vapor pressure deficit (k Pa); ρa is the density of air (1.292 kg m^{-3}); cp is the specific heat of moist air (1,013 J Kg^{-1} $°C^{-1}$); γ is the psychrometric constant (k Pa $ºC^{-1}$); ra is the aerodynamic drag (s m^{-1}).

The aerodynamic conductance (g_a) is the inverse of aerodynamic resistance (r_a), which was calculated in accordance with Allen et al.

(1998), as shown in Equation 3.

$$(g_a)^{-1}=r_a = \frac{\ln\left\{\frac{z_m-d}{z_{om}}\right\}\ln\left\{\frac{z_h-d}{z_{oh}}\right\}}{k^2U_z} \quad (3)$$

Where: zm is measured wind height (m), zh is moisture height measurement (m), d is zero plane displacement height (m), Zom is length of roughness governing the time of transfer (m), Zoh is length of roughness governing heat transfer and steam (m), k is the von Karman's constant, 0.41, Uz is the wind velocity at height z (m s^{-1}).

Atmospheric conditions were determined as in Campbell and Norman (1998), from the convection rate that produces mechanical air turbulence which can be used to estimate the atmospheric stability parameter (ζ) described in Equation (4).

$$\zeta = -\frac{0{,}4gzH}{\rho_a c_p T_k u^3} \quad (4)$$

Where in g is the acceleration of gravity (9.8 m s^{-1}), z is the surface height, H is the sensible heat flow, ρ is the density of air (k^{-3}), T_k is the air temperature (K), u is the air friction velocity (m s^{-1}), cp is the specific heat of moist air.

The atmospheric stability was used in the correction factor for the momentum flow (ΨM) and sensible heat flux (ΨH) as in Equation 5. For stable atmosphere (ζ ≥ 0):

$$\Psi_M=\Psi_H = 6\ln(1+\zeta) \quad (5)$$

For unstable atmosphere (ζ <0):

$$\Psi_H = -2\ln\left[\frac{1+(1-16\zeta)^{\frac{1}{2}}}{2}\right] \qquad \Psi_M = 0{,}6\Psi_H \quad (6)$$

The control of tree transpiration by the stomata is commonly described by the decoupling factor (Ω), which reflects the extent to which the canopy of trees is coupled to the atmosphere (Kumagai et al., 2004). The coefficient Ω of decoupling ranges between 0 and 1 (Wullschleger et al., 2000; Han et al., 2011).

A Ω value closer to 1 indicates that transpiration is more dependent on the radiation balance; Ω values closer to 0 indicate that transpiration is more greatly controlled by the prevailing weather conditions that affect the physiological control of plants (Souza Filho et al., 2005).

$$\Omega = \frac{1}{1+\left(\frac{\gamma}{\delta+\gamma}\right).\left(\frac{g_a}{g_s}\right)} \quad (7)$$

Where Ω is the decoupling factor; g_a is aerodynamic-conductance (s.m^{-1}).

Statistical analysis

R version 3.2.3 was used to process and analyze data. Statistical analysis compared the managed and control forests as well as whether there are differences between the models and if each model differs between seasons. The data were submitted to the Kolmogorov-Smirnov normality test and comparison between models were made using ANOVA. The probability level of α = 0.05 was used for all comparisons, and for the post-hoc mean separation the Tukey test was used.

RESULTS AND DISCUSSION

Forest management causes a reduction of the impact on vegetation structure, and it can aid in faster return to evapotranspiration values of a primary forest reduced impact logging. The average ET calculated by Penman Monteith (PM) was 111.06 ± 15.71 and 108.52 ± 18.29 mm.$month^{-1}$ in the logged area. In the statistical analysis, data were normal, and when ET was compared the PM and EC data using a two-way ANOVA the two did not differ (F = 0.025, p = 0.87), but the fixed effect of seasons (wet and dry) showed a difference between models (F = 9.88; p <0.01); however, by the Tukey test these models did not significant differ (p = 0.87) for the logged site.

Annual average PM-ET was 1,322.98 mm while rainfall was 1,480.19 mm for 2001 to 2003 at the logged site. Similarly, Kume et al. (2011), working in a Malaysian tropical forest measured an annual average PM-ET of 1,323 mm. This shows that the level of ET for the current study area could be considered to be equal to those in an unmanaged tropical rain forest, and this result is important in order to evaluate the effect of forest management in the Amazon on ET levels.

The PM varied for seasonal periods, while the EC had a tendency to increase over time. This may be related to the features of each model used to calculate the ET (Figure 1). In the application of PM, the aerodynamic and surface resistances are two important parameters. When the canopy resistance is estimated, this model provides satisfactory results even if the ET is subject to changes, such as harvest history (Ebisu and Ogawa, 1993).

The PM results from 2000 to 2001 were 3.16 ± 0.28 mm day^{-1} and 3.85 ± 0.33 mm day^{-1} for the rainy and dry seasons, respectively, and the annual average was 3.51 ± 0.75 mm day^{-1} (Rocha et al., 2004) and 3.50 ± 0.46 mm day^{-1} for this study. In Manaus, in the Jarú Reserve, Costa et al., (2010) obtained ET values of 3.58, 3.57 and 3.11 mm day^{-1}. These data are similar to those from the current study and show that even with the loss of a few trees the managed site is able to maintain a level of ET equal to that of unlogged forest areas.

In the current study, PM ET had an average of 134.89 ± 15.94 mm $month^{-1}$ for the unlogged site, and there was an increase in the evapotranspiration rate for the dry season each year, while the EC had a more regular distribution with ET 100.89 ± 11.05 mm $month^{-1}$ (Figure 1).

The PM and EC showed a significant difference in two-way ANOVA (F = 162.87; p> 0.01) for the unlogged site, and the Tukey multiple comparison test showed that the models had significant differences. The seasons are different between models (wet and dry F= 45.08; p <0.01), and comparing season within the model, EC not differ between seasons (p = 0.91), while the PM had a difference (p <0.01). The global level of ET is around 1,500 mm (Kume et al., 2011), and at the unlogged site annual average ET was 1,618.71 mm for 2002 to 2005.

ET estimated by EC significantly decreased during the year after forest harvest (2001-2002) and then increased up to 2003. Since this technique is based on available energy, after forest harvest there was a decrease in

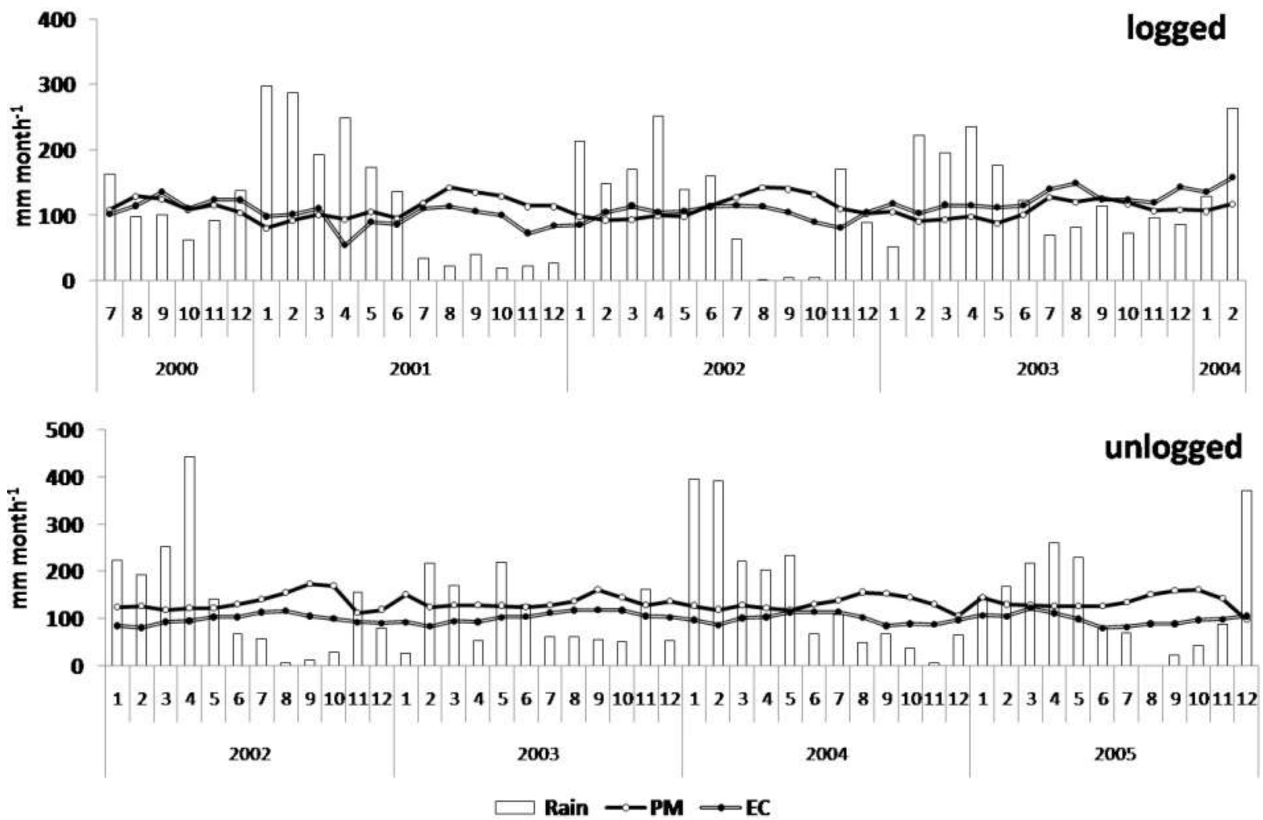

Figure 1. ET from PM, EC and precipitation for the logged and unlogged sites.

monthly average LE (121.09±7.9 and 100.68±12.85 $W.m^{-2}$) during the final five months comparing 2000 and 2001, which implied a reduction in ET. Afterward the forest recuperated LE and ET values and these levels increased during the next few years.

At the unlogged site the PM and EC had averages of 4.50±0.53 and 3.32±0.42 $mm.day^{-1}$, respectively. At the same site Hutyra et al. (2007) used water vapor flux to determine ET and the daily average during 2002 to 2005 was 3.07 $mm.day^{-1}$. This result demonstrates the extent to which model characteristics influence ET rates, and this is very important when studying forests that have different vegetation structures.

The PM ET rate for the unlogged site was 4.5 $mm.day^{-1}$ and was EC 3.32 $mm.day^{-1}$. Costa et al. (2010), using an energy balance analysis for both the logged and unlogged sites, found a value for ET of 3.49 $mm.day^{-1}$, and Vourlitis et al. (2008), calculating ET based on sap flow found a value of 3.01 $mm.day^{-1}$. ET values using PM in the current study were higher than those from most other reported studies probably due to the fact that the model aggregates variables such as r_s and r_a that influence vegetation interaction with the atmosphere, a relevant caveat when comparing distinct forests.

In the precipitation correlation analysis PM ET had an r = -0.63 in the logged site and r = -0.80 in the unlogged site; the EC ET showed no correlation (r = -0.06 and r = -0.21, logged and unlogged sites respectively). The precipitation in the Tapajós National Forest had an annual average of 1480.2 $mm.year^{-1}$ (logged site from 2001 to 2003) and 1618.71 $mm.year^{-1}$ (unlogged site from 2002 to 2005), and seasonal variations are very well defined in the region of the Amazon. Precipitation and ET are meteorological elements that move in opposite directions, expressed in millimeters of rainfall (Thornthwaite, 1948), so this trend is justified by the PM method. ET in the dry season is higher than in the rainy PM due to environmental conditions that favor the process. But as the real ET requires water availability, it can be considered that the ET values for the dry season would be the potential values and the real values are from the rainy season.

Comparison of sites

The distribution of ET over the two year period for the PM sites had the highest ET (unlogged) compared to the dry period. In the ANOVA sites had differences for the years of study (F = 10.78 and p ≤ 0.01), and the Tukey test showed that sites had significant differences in seasonality. In the one-way ANOVA for ET EC was not

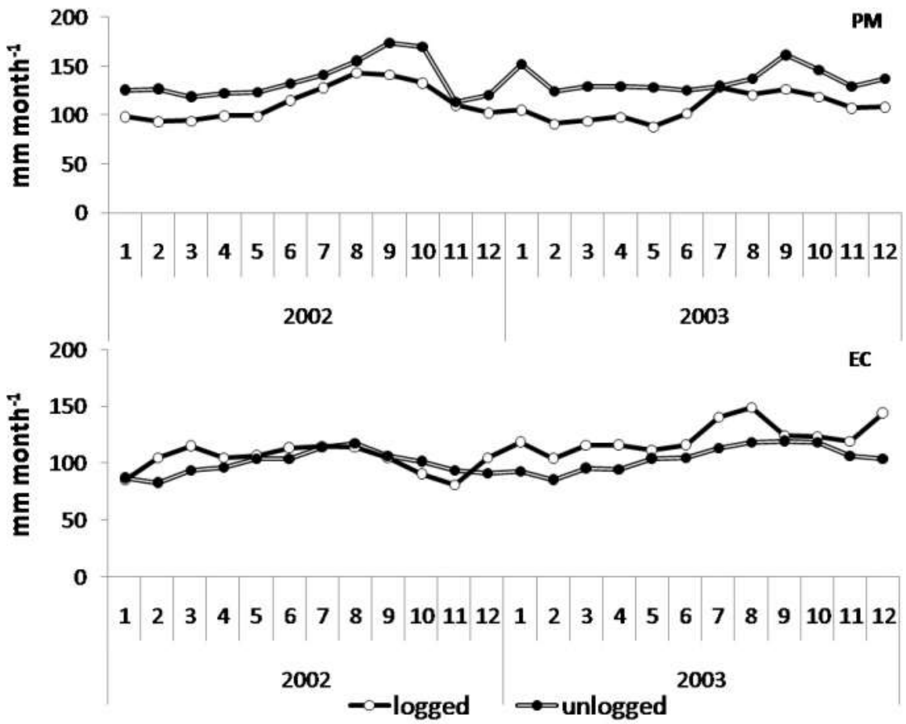

Figure 2. ET logged and unlogged for the period 2002 and 2003, using the PM and EC.

different (F = 0.025; p = 0.87) (Figure 2).

Seasonal comparisons showed that the dry period at the logged site in 2002 had lower average ET than in the rainy period. In the unlogged site the forest had a greater evapotranspiration in the dry season, and the variation of differences over the period was higher for the logged site.

The distribution of ET (PM) in comparison to the two sites has an unchanged distribution following the rainfall trend for respective sites. The peak in the distribution at the unlogged site in month 1 of the year 2003 is due to an extension of the summer season, wherein the average monthly rainfall was 52.3 mm.

When used for comparison of EC sites, the logged ET attained higher rates than in the unlogged (Figure 2), and also had higher rates of LE in the logged (113.00 ± 13.12 W m^{-2}) compared to unlogged (85.62 ± 8.16 W m^{-2}).

EC averages between study areas showed that the unlogged site was 101.82 ± 11.05 mm $month^{-1}$ for ET, while the logged was 113.16 ± 16.21 mm $month^{-1}$ with a difference of +11.34 mm $month^{-1}$ for the logged compared to the unlogged. The major variable involved in ET is the energy available at the surface and the way it regulates the transpiration of vegetation through their stomata (Avissar and Werth 2004). In the logged site high latent heat values were essential for obtaining higher monthly average than the unlogged. Therefore we can infer that the EC ET depends more on solar radiation balance than the ET process.

PM for the unlogged site had an ET rate higher than that of the logged site, with an annual average for the period of 135.03 ± 16.32 mm $month^{-1}$, while the other site was 110.35 ± 16.11 mm $month^{-1}$. This is due to the different structure of the canopy that influences atmospheric and physiological parameters, and aerodynamically regulates ET through features such as the activity of the stomata and roughness of the canopy (Matsumoto et al., 2008).

Using PM the daily average for the rainy season ranged from 4.1 ± 0.15 to 4.4 ± 0.34 mm day^{-1}, and the dry period from 4.8 ± 0.84 to 4.6 ± 0.40 mm day^{-1} for the unlogged site for the years of study. At the logged site the daily average for the rainy season was 3.3 ± 0.26 and 3.2 ± 0.21 mm day^{-1} and dry season had averages of 4.2 ± 0.55 and 3.9 ± 0 29 mm day^{-1}. ET ranged from 3.6 to 5.1 mm day^{-1} using the PM 2007 to 2009 in a tropical rainforest in Costa Rica (Cadol et al., 2012). The variation in the TNF is similar to that of other tropical forests when applying the PM model, since high temperatures and radiation create an evaporative demand in the Amazon region, which is usually 3 to 4 mm day^{-1} (Fisher et al., 2008).

Research conducted in tropical forests shows that the benefits of forest management are limited by the intensity of exploitation (Van Der Hout, 1999). Thus, one of the main impacts that can affect the ET is the opening of the canopy from the felling of trees.

The post-harvest growth rate was higher in the logged site with diameter increment of 0.6 $cm.year^{-1}$ before

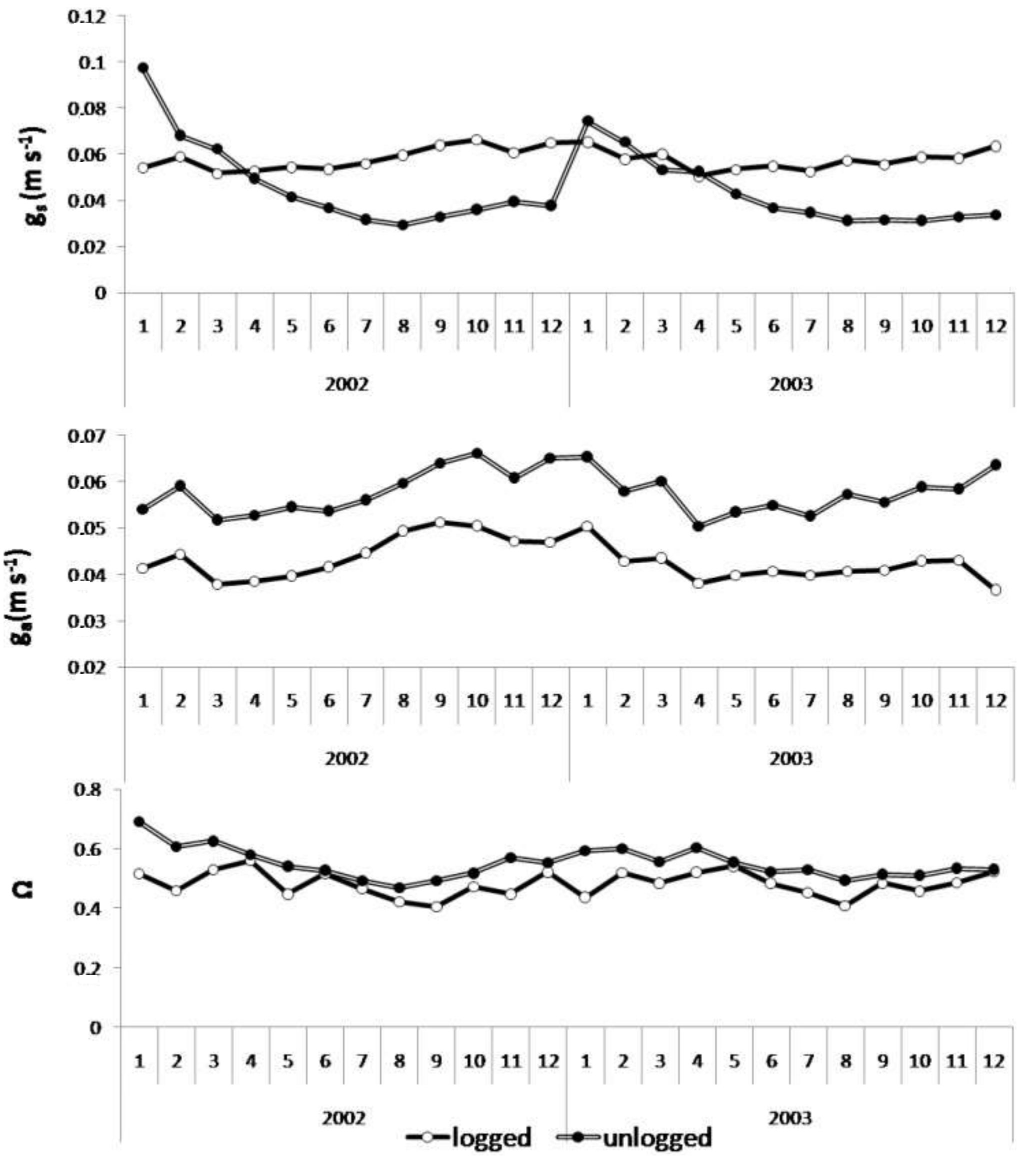

Figure 3. Surface conductance (g_s), aerodynamics (g_a) and decoupling factor (Ω) for the logged and unlogged for the period 2002 and 2003.

management to 1.21 cm.year^{-1} after three years; while the unlogged site diameter increment ranged from 1.04 to 1.34 cm.year^{-1} (Miller et al., 2011). The logged site had a greater increase, which is linked to factors such as the ecology of the species involved in colonization and canopy openness, which should consequently influence biogeochemical processes of forests, such as evapotranspiration.

Control mechanisms

Surface conductance

The gas fluxes in the study areas were different, in which the logged has a regular distribution over the years, while the unlogged has high values for the first half of the year (Figure 3). In ANOVA, this variable was significant for a difference between the sites ($F = 12.06$ and $p \leq 0.01$) and was significant according to the Tukey test at $\alpha = 0.05$. The sites had means of 0.058 ± 0.005 m s^{-1} for the logged site and 0.045 ± 0.017 m s^{-1} for the unlogged site for the years 2002 to 2003. The logged site had an annual average of 0.058 ± 0.005 m s^{-1} for 2002 and 0.057 ± 0.004 m s^{-1} for 2003 and the unlogged site had 0.047 ± 0.02 and 0.043 ± 0.014 m s^{-1} for the respective years. The conductance (g_s) to the boundary layer ranged from 0.01 to 0.02 m s^{-1} in Sarawak, Malaysia (Lim et al., 2009), values lower than those from this work. Thus, by analyzing the parameter for the g_s (logged), it can be inferred that the canopy structure provides smaller variations throughout the study period (minimum 0.050 m s^{-1} and a maximum of 0.066 m s^{-1}). In contrast, the unlogged site has well defined peaks in the distribution with peaks at the start of each year and having higher values with a minimum of 0.029 m s^{-1} and a maximum of 0.097 m s^{-1}.

The vegetation of the two sites in question have different structural characteristics, due to changes in forest cover from harvest activities, which causes changes in physiological and structural characteristics of the vegetation (Arora, 2002). To calculate the ET, it must be taken into account that the different composition of the forest canopy will influence the variables used to calculate the g_s.

As the unlogged site (2002 to 2003) has an average of g_s 0.045 m s^{-1} at 0.057 m s^{-1} the logged site can thus be said to exert a greater control on transpiration in the high g_s area. The difference between the gs for unlogged and logged sites can be influenced by the proximity of the unlogged tower to the plateau slope. For the Flona Caxiuanã the g_s values were 0.060 m s^{-1} for the rainy season and 0.045 m s^{-1} for the dry season (Souza Filho et al., 2005). For a mangrove forest in the Amazon region the g_s ranged from 0.01 to 0.04 m s^{-1} (Rodrigues et al., 2011). Since this variable represents the canopy surface roughness it can give different responses for different vegetation types, and this is important in order to define the level of interaction between the forest and the atmosphere.

In the state of Mato Grosso a tower located in an area covering intact forest, selectively logged forest, and pasture, the average g_s value was 0.006 ± 0.002 m s^{-1} for the period from August 1999 to July 2000 calculated from EC Covariance (Vourlitis et al., 2002). Since the study area is surrounded by a larger area with intense human disturbance, the value for g_s in Vourlitis et al. (2002) was lower than that found in the current study in the TNF. Thus, the conductance of the canopy surface features of the exchange process with the atmosphere is controlled at various times by biotic and abiotic factors (Souza Filho et al., 2005).

Conductance aerodynamics

The sites had different averages (ANOVA, F = 131.60 and p≤0.01) and these were significant (Tukey). The unlogged site had higher values than the logged site (Figure 3). g_a in the study by Rocha et al. (2004) in the logged site from July 2000 to June 2001 was 0.0287 ± 0.0073 m s^{-1} while the current work for the same period had an average of 0.041 ± 0.005 m s^{-1}. According to Rennó (2003), Monteith suggests a value of 0.1 m s^{-1} for a very rough surface such as foliage of forests. In the TNF the average values were 0.043±0.004 m.s^{-1} for the logged site and 0.057±0.004 m.s^{-1} for the unlogged site from 2002 to 2003.

For an area subject to grazing management for the years 1993 and 1994, the g_a values were in the range of 0.034 and 0.027 m s^{-1} for the respective periods (Dirks and Hensen, 1999). In the present study the g_a levels were higher for the forest (0.057 m s^{-1} in unlogged and 0.043 m s^{-1} for logged).

For the rainy and dry seasons, respectively, the values for the year 2002 in the unlogged site were 0.054 m s^{-1} and 0.062 m s^{-1} and at the logged site they were 0.040 m s^{-1} and 0.048 m s^{-1}. In an intact forest in Mato Grosso g_a was 0.046 m s^{-1} and 0.052 m s^{-1} for the rainy and dry seasons, respectively (Pinto Júnior et al., 2009). With this typical seasonal variation, during the rainy season g_a is generally greater, and therefore changes in atmospheric variable will also cause different responses of g_a for each study site. This seasonal trend is the same for other work done in the Amazon region (Souza Filho et al., 2005; Vourtilis et al., 2008).

In an ecosystem of transition between the Cerrado and the Amazon in Sinop, Mato Grosso, the average g_a for the rainy season was 0.042 and 0.048 m s^{-1} for the dry season (Silva and Sanches, 2011). In the Atlantic Forest, a fragment of forest had average g_a of 0.099 m s^{-1} (Pereira et al., 2010), higher than other tropical forests.

Factor decoupling

The variation of Ω (Figure 3), had different monthly averages for the study area. ANOVA showed a difference between sites (F = 24.67 and p ≤ 0.01) and the Tukey test showed differences in means (p<0.05). In this figure, it can be seen that the decoupling sites have different patterns indicating that the conditions of interaction of the vegetation with the atmosphere are not the same for the two areas. Thus, the logged site, with an average of Ω = 0.48, depends more on solar radiation than the ET process. The unlogged site had a Ω = 0.55 meaning that this area is more decoupled from the atmosphere. The Ω for the rainy season was higher than the dry season in the unlogged forest (Table 1).

The Ω the annual figures tend to vary for forests in the two sites of the FNT, and the logged site obtained Ω = 0.48 ± 0.05 and Ω = 0.48 ± 0.04 for 2002 and 2003, while in the unlogged site the values were Ω = 0.55 ± 0.06 and Ω = 0.54 ± 0.03 for the years in question. In a forest of *Pinus sylvestris* L., for a period of 11 years, there were annual maximum and minimum values of Ω = 0.43 to Ω = 0.19 with a final average of Ω = 0 32 (Launiainen, 2010). Thus, the sites have different decoupling characteristics with the atmosphere, wherein these depend on different conditions for evapotranspiration; while the logged site depends more on the vegetation, the control site depends more on weather conditions.

The range of maximum and minimum for the managed site was Ω = 0.56 to Ω = 0.40 for study years compared

Table 1. Monthly average for rainfall data (P) mm, ET (PM, EC) (mm month^{-1}), g_a (m s^{-1}), g_s (m s^{-1}), Ω and standard deviation (SD) for the logged and unlogged sites.

Years	Month	Logged						Unlogged					
		P	PM	EC	Ω	g_a	g_s	P	PM	EC	Ω	g_a	g_s
2002	1	213.11	98.27	85.17	0.52	0.041	0.054	223.01	125.72	86.31	0.69	0.054	0.097
2002	2	147.72	93.29	104.71	0.46	0.044	0.059	192.53	126.67	82.09	0.61	0.059	0.068
2002	3	169.67	94.01	114.32	0.53	0.038	0.052	252.48	118.54	93.85	0.63	0.052	0.062
2002	4	251.46	99.39	104.54	0.56	0.039	0.053	442.47	122.48	96.33	0.58	0.053	0.049
2002	5	139.95	99.09	106.04	0.45	0.039	0.054	141.22	123.44	103.94	0.54	0.054	0.041
2002	6	160.02	114.90	112.96	0.52	0.041	0.054	68.83	131.86	104.01	0.53	0.054	0.037
2002	7	64.01	127.78	114.79	0.47	0.044	0.056	58.17	141.41	114.08	0.49	0.056	0.032
2002	8	1.52	142.90	114.16	0.42	0.049	0.060	6.86	155.47	117.44	0.47	0.060	0.029
2002	9	3.81	140.95	104.69	0.41	0.051	0.064	13.46	173.92	106.46	0.49	0.064	0.033
2002	10	5.08	132.63	89.74	0.47	0.050	0.066	29.46	169.98	100.82	0.52	0.066	0.036
2002	11	170.94	110.05	80.84	0.45	0.047	0.061	155.19	113.35	93.48	0.57	0.061	0.039
2002	12	88.39	102.47	104.86	0.52	0.047	0.065	79.76	120.07	91.16	0.55	0.065	0.038
2003	1	52.32	105.47	117.89	0.44	0.050	0.065	27.69	152.36	92.95	0.59	0.065	0.074
2003	2	222.50	90.83	103.41	0.52	0.043	0.058	217.42	124.47	84.66	0.60	0.058	0.065
2003	3	195.83	94.12	115.85	0.49	0.043	0.060	169.67	129.40	95.08	0.56	0.060	0.053
2003	4	234.95	97.97	115.48	0.52	0.038	0.050	52.83	129.42	94.47	0.60	0.050	0.052
2003	5	176.28	88.26	111.72	0.54	0.040	0.053	220.22	128.12	103.26	0.55	0.053	0.043
2003	6	123.70	101.67	115.50	0.48	0.041	0.055	129.29	125.40	104.82	0.52	0.055	0.037
2003	7	69.85	128.19	140.41	0.45	0.040	0.052	62.23	129.52	113.12	0.53	0.052	0.035
2003	8	81.79	120.56	148.78	0.41	0.041	0.057	61.72	137.36	118.56	0.49	0.057	0.031
2003	9	113.79	126.28	124.19	0.49	0.041	0.056	55.88	161.51	118.79	0.51	0.056	0.031
2003	10	71.88	118.69	123.41	0.46	0.043	0.059	51.05	146.30	118.35	0.51	0.059	0.031
2003	11	97.03	107.03	119.23	0.49	0.043	0.058	161.04	129.10	106.21	0.53	0.058	0.033
2003	12	85.85	108.42	143.42	0.52	0.036	0.063	54.36	136.87	103.66	0.53	0.063	0.034
Mean		122.56	110.13	113.17	0.48	0.04	0.06	121.95	135.53	101.83	0.55	0.06	0.05
SD		73.35	16.12	16.21	0.04	0.00	0.00	101.32	16.32	11.05	0.05	0.00	0.02
Total		2941.47	2643.20	2716.13	11.57	1.03	1.38	2926.84	3252.75	2443.90	13.19	1.38	1.08

to the control site, which despite having an average near to that of a pine forest (Launiainen, 2010) the minimum value was greater and there was less amplitude between the data. Furthermore, Vourlitis et al. (2002) found a Ω of 0.27 ± 0.07 in Sinop, Mato Grosso, Brazil.

The control site in 2002 to 2003 averaged 0.51 ± 0.02 in the dry season and 0.58 ± 0.04 for the rainy season. In the logged site the values for the respective seasons were 0.32 ± 0.03 and 0.33 ± 0.04. The variation of Ω for the dry season was 0.2 to 0.3 and 0.5 for a rainy semi-deciduous forest in Mato Grosso, Brazil (Vourlitis et al., 2008), and in Rodrigues et al. (2014), working in a Mato Grosso, Brazil, the Ω averaged 0.48 during the rainy season and declined to a low of 0.21. In an ecosystem of transition between the Cerrado and the Amazon in Mato Grosso, Silva and Sanches (2011) described a Ω = 0.4 for the rainy season and Ω = 0.2 in the dry season. This demonstrates that as with other control mechanisms, Ω is influenced by seasonal changes at these study sites.

Generally, the forest has an Ω value indicative of the importance of the energy available to the process of ET during the wet season, wherein there is relatively less importance of stomatal control, because the leaf area index (LAI) is high (Wullschleger et al., 2000).

Typical values for Ω range from 0.1 in conifers, while for stomatal conductance this increases to 0.5 or greater in broadleaf trees, and values are higher in dense herbaceous vegetation (Wood et al., 2008). In rainforest in French Guiana Granier et al. (1996) obtained a value of Ω = 0.38. In the current study, the uncoupling of the unlogged site (Ω = 0.55) and logged site (Ω = 0.48) is consistent with hardwood vegetation. The monthly averages for 2002 and 2003 are shown in Table 1.

Conclusions

The PM ET did not change after forest management over the years, while EC increased for the logged site over the years, and this may be related to variation in LE. When compared to the unlogged site, values were higher for

PM and did not differ in EC. The PM model results capture the influence of rainfall in these study areas, and therefore the PM model can be considered better at evaluating the interaction of the forest structure with the atmosphere. As the forest is growing after the management operations, it should not decrease ET rates due to higher growth rates provided by opening of the canopy, which increases the incidence of light on the trees in the lower strata. When the surface parameters such as g_a, g_s and Ω were analyzed, it was revealed that the sites have different structural characteristics that influence the forest ET process.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Opportunities and constraints of beekeeping in Wolaita and Dawro zones, Southern Ethiopia

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This study was conducted in districts of Wolaita and Dawro zones, Southern Ethiopia with objectives of assessing constraints and opportunities for beekeeping production. Multistage purposive sampling procedure was employed for 180 respondents. Data were analyzed using SPSS version 20. The results showed that majority of the respondents kept honey bees for consumption and income generation, while very few reported they kept honey bee for consumption, income generation, teaching and income generation. Major of the constraints for beekeeping included but not limited to honeybee pests and diseases, lack of modern equipments, absconding, swarming, bee predators, lack of skilled man power, lack of appropriate apiary site, finance limitation, lack of market, agro-chemicals, shortage of water availability and shortage of bee forages. On the other hand, major opportunities for beekeeping were reported to be suitable climate, availability of bee forages, sufficient rainfall, water availability as well as bee colony and race availability. Regardless of the presence of a number of constraints facing beekeeping such as pests and diseases and limitation to modern equipments' input, there are also opportunities which, proves their importance. It was therefore, recommended that modern beekeeping production system should be introduced to farmers in order to help them improve the quantity and quality of honey production.

Key words: Assessment, beekeeping constraints, honey, honeybee, opportunities.

INTRODUCTION

Bee keeping is one of the agricultural sub-sectors that most suits the rural poor people. It is simple and relatively cheap to start, as it requires a very low level of inputs such as labor, capital and knowledge (Gemechis et al., 2012). Beekeeping does not depend directly on soil and it can be a single means of living for families with very little or no soil. The bee keeping sub-sector has a lot uses for improvement of the livelihood of communities as it creates job for many people who engage in the production, trading and processing of bee products at different levels of market linkage and industry cottages (ARSD, 2000; Gezahegn, 2001; Gemechis et al., 2012). Moreover, beekeeping has contributions in sustainability and balancing the natural resources by assisting plants pollination and in turn, the activity in bee keeping is environmentally friendly and has no impact on the environment, rather it stabilizes fragile areas and helps in retrieving degraded lands and increases biodiversity

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balance (Gemechis et al., 2012).

In bee keeping, Ethiopia has a huge potential. There are various conducive ecological zones which, owns over 7000 species of flowering plants, such zones have supported the existence of large number of bee colonies in the country (Beyene and David, 2007; MoARD, 2007; CSA, 2009). It has also the largest bee population in Africa with over 10 million bee colonies, out of which about 5 to 7.5 million are estimated to be hived while the remaining exist in the wild (MoARD, 2007; CSA, 2009).

Due to constraints of skilled manpower and training institutions, low level of technology used, poor quality of honey harvesting, absconding, drought, poor society awareness about beekeeping practice, shortage of bee flora, pesticides, poisoning, honeybee diseases, shortage of bee colonies, shortage of modern bee hives, and marketing problems among others factors (Abadi et al., 2016; Nebiyu and Messele, 2013; Chala et al., 2012; Arse et al., 2010; Kerealem et al., 2009; Gidey and Mekonen, 2010; Workneh et al., 2008), the honey productivity has been very low from the expected potential. This has led to low utilization of hive products domestically and relatively low export earnings. Thus, the beekeepers have benefited less and the contribution of beekeeping sub-sector to the state gross domestic products has been limited (Tessega, 2009). To mitigate such hindering factors and to improve honey production and to increase household livelihoods with modern beekeeping, and honey production, technical training and equipment to local bee keepers have to be provided to support their transition from traditional beekeeping to more modern techniques of beekeeping (Eugenia, 2016).

An attempt research is necessary to improve both the production and quality of honey, since it enables to identify some of the major constrains that are obstacles to the possible maximization of benefits by producers and the whole national economy. Moreover, research can help in transforming and addressing technologies on the beekeeping system, processing and marketing bee products. It is also the fact that research can play an important role in designing and establishing the appropriate bee keeping development strategies and policies in the country particularly in the study region. Accordingly, policy makers would detect the possible intervention area of bee keepers to improve the overall performance of honey yield in terms of quality and quantity.

MATERIALS AND METHODS

Description of the study areas

The study was conducted in Wolaita and Dawro zones of Southern Nations, Nationalities and Peoples Region (SNNPR).). Wolayita is located at 390 km to southwest from the capital city of the country, Addis Ababa along the main road that passes through Shashamane to Arbaminch It is bordered on the south by Gamo Gofa, on the west by the Omo River which separates it from Dawro, on the northwest by Kembata Tembaro, on the north by Hadiya, on the northeast by the Oromia Region, on the east by the Bilate River which separates it from Sidama, and on the south east by the Lake Abaya which separates it from Oromia Region. Wolaita zone has three agro-ecologies namely Dega (3%), Weina-dega (57.96%) and Kolla (40%); an altitude ranging from 1200 to 2950 m above sea level; average annual temperature of 15.1°C and mean annual rainfall ranging from 1200 to 1300 mm. With regard to land utilization, 261,000 hectares (ha) was used for cultivation, 5318 ha for grazing, 8261 ha is Bush-land and the remaining 35382.5 ha is a cultivable land. The total population of the zone is estimated to be about 1721339 with a density of 385 inhabitants per square kilometer (CSA, 2007).

Dawro Zone has a total land area of 4,814.52 sq km and is located at about 500 km in south western of Addis Ababa, the capital of Ethiopia and 275 km of Hawassa, the capital of SNNPR. It is bordered to the south by Gamo Gofa, to the west by the Konta special woreda, the Gojeb River which, defines its boundary with the Oromia Region is found to the north. Dawro Zone has a total population of 489,577, out of which 249,263 are men and 240,314 women. Dawro has a population density of 101.69, whereby, 35,044 (7.16%) are urban inhabitants, and 14 individuals are pastoralists. A total of 89,915 households were counted in this zone, which resulted in an average of 5.44 persons per household, and 86,642 housing units (CSA, 2007).

Sampling size and sampling techniques

Multistage purposive sampling methods were employed in undertaking this study. Wolaita zone has twelve woredas and Dawro zone has five woredas. In consideration of the number of woredas in each zones and potentiality of honey bee production, Offa and Boloso -Sore woredas and Tocha woreda were selected purposively from Wolaita and Dawro zones, respectively. In the same manner, two kebeles from each selected woredas were selected purposively; thereby, a total of six kebeles were obtained. From each selected kebeles, 30 households were selected purposively, giving the total sample size of the study as 180 households.

Data collection

Both primary and secondary sources were used for data collection. Structured and semi-structured questionnaires were employed. The questionnaires were pre-tested before the actual data collection implementing so as to evaluate the clarity of the questions, and interpretation of the questions by the farmers and time required for an interview. Results from the pre-test were used to re-frame the final questions. The interviews were conducted by trained research assistants under close supervision by researchers. Moreover, direct observation, focus group discussions and key informant interviews were also conducted with beekeepers, extension workers and bee experts. Enumerators who have know-how on beekeeping were recruited and trained to collect data using the interview schedule, under the close supervision of the researchers. The researchers monitored the enumerators during data collection. In the survey study, parameters like bee keeping production, constraints and opportunities were studied. Secondary data were collected from different sources such as books, research publications, journals and office reports/unpublished data.

Data analysis

Depending on the type of information collected, different analysis methods were applied using SPSS statistical package (version 20). Qualitative data were presented using descriptive statistics such as percentages and frequencies, while quantities data were analyzed

Table 1. Household size, land size and beekeeping purposes.

Parameters	Category	Boloso-Sore (N=60%)	Offa (N=60%)	Techa (N=60%)	Total (N=180%)
	Male	3.383±.274[a]	3.7±.274[a]	3.267±.274[a]	3.450±.158
Household size	Female	3.1±.202[a]	3.932±.203[b]	3.211±.207[a]	3.414±3.182
	Total	6.483±.406[ab]	7.567±.406[b]	6.317±.406[a]	6.789±.235
Land size		0.708±.145[a]	0.721±145[a]	2.035±.145[b]	1.154±.084
	For consumption	6.7	8.3	11.7	8.9
	For income generation	1.7	1.7	10	4.4
Purpose	For consumption and income generation	91.7	90.0	76.7	86.1
	For teaching and income generation			1.7	0.6

and presented using general liner model. The differences between means were separated via Ducan test.

RESULTS AND DISCUSSION

Household size, land size and beekeeping purposes

Table 1 presents family and land size and beekeeping findings. In average, the households had 6.789 (±.235) and 1.154 (±.084) households size and land size, respectively and these were significantly different among districts in the study area. Of the study districts, Offa and Techa woredas revealed significant average households size and land size, respectively. However, in Burie, Amhara Region Ethiopia, Tessega (2009) reported the average land size of 1.77 hectares and national average households land holding of 1.0 - 1.5 hectares.

Result further showed that most of households kept honey bee for the purposes of both consumption and income generation (86.1%), while the rest kept if for consumption (8.9%), income generation (4.4%) as well as teaching and income generation (0.6%). These result were supported by Nebiyu and Messele (2013) who reported on the uses in Gamo Gofa zone of Southern Ethiopia as income generation (16%), home consumption (9%) and both for income and households consumption (75%). Findings from Tessega (2009) indicated the main reasons for involvement of the farmers in beekeeping in Burie district, Amhara region, in Ethiopia as income-generating activity (79.2%), easy to perform with other agricultural activities (10.0%), source of income used for different house expenses (5%), inheritance from parents (4.2%) and due to training (1.6%).

Opportunity of beekeeping in the study area

Beekeeping is highly significant in sustainability of natural resources and agriculture, since this sub-sector can assist in conserving native habitats and by increasing the yield of crops and forage production through efficient pollination. Honeybees also have great contribution in maintaining the equilibrium of the nature by interacting with different biotic ecosystem (Kerealem et al., 2009; Mekonen et al., 2011). Major opportunities of beekeeping (Table 2), included suitable climate, bee forage plants availability, sufficient rainfall, available of water, bee colony as well as race availability. Supporting beekeeping sector by non-government and government institutions through marketing access, modern equipment access, skilled manpower, extension service and credit access were also said as major opportunities by some respondents in the current study area. In Gamo Gofa Woreda Southern region, Ethiopia beekeeping production has good opportunities since the government has advocated self-contained program to beekeepers; there is expansion of micro finance institution for credit facility and beekeeping training center; availability of local bee hives and suitable environment for beekeeping. There is also a high market demand for crude honey for domestic consumption and export (Nebiyu and Messele, 2013).

Moreover, study in Kewet district of Amhara Region, Ethiopia by Beyene (2015) has found presence of natural resources like a place to keep bee colony, biodiversity and environmental conditions and human capital, beekeepers indigenous knowledge, cultural practices and local innovations, and marketing expertise, water availability, establishments of beekeeping association and government attention to improve beekeeping sub-sector as major opportunities. In Negelle and Shashemene districts, Oromia Region in Ethiopia, the major opportunities for honey bee production included: access to new technologies, vegetation coverage potential, high demand for bees' products in markets, good government and non-government sector policy (Mekonen et al., 2011).

Constraints of beekeeping in the study area

Critical constraints affecting honey production in the study area were reported to be honeybee pests and

Table 2. Opportunities of beekeeping (multiple responses)

Opportunities	Boloso-Sore (N=60%)	Offa (N=60%)	Techa (N=60%)	Total (N=180%)
NGO support	16.7	5	10	10.5
Availability of water	66.7	73.3	70	69.6
Skilled manpower	26.7	3.3	25	18.2
Extension service access	41.7	13.3	36.7	30.4
Marketing access	50	58.3	38.3	48.6
Credit access	36.7	8.3	28.3	24.3
Modern equipments access	40	11.7	5	18.8
Suitable climate	100	88.3	90	92.3
Bee colony and race availability	61.7	60	66.7	62.4
Good government policy	31.7	51.7	38.3	40.3
Bee forage plants availability	71.7	75	81.7	75.7
Sufficient rainfall	93.3	60	65	72.4

Table 3. Constraints of honey bee production in the study area (multiple response)

Constraints	Boloso-Sore (N=60%)	Offa (N=60%)	Techa (N=60%)	Total (N=180%)
Honeybee pests and diseases	88.3	85	88.3	86.7
Honey bee forage	28.3	25	18.3	24.3
Water availability	33.3	26.7	30	30.4
Absconding	86.7	81.7	65	77.3
Swarming	76.7	71.7	81.7	76.2
Finance limitation	68.3	41.7	58.3	55.8
Marketing limitation	50	41.7	61.7	51.4
Manpower skill	61.7	46.7	83.3	63.5
Bee predators	76.7	63.3	68.3	69.1
Bee colony	38.3	40	33.3	37.6
Lack of labour	16.7	28.3	6.7	17.1
Modern equipments in put	60	88.3	95	81.2
Bee colony agro-chemicals	50	46.7	43.3	46.4
Beehives (modern)	46.7	55	48.3	49.7
Apiary site limitation	41.7	60	70.0	56.9

diseases (86.7%), modern equipment input limitation (81.2%), absconding (77.3%), swarming (76.2%), bee predators (69.1%), lack of manpower skill (63.5%), apiary site limitation (56.9%) and finance limitation (55.8%). Others were marketing limitation (51.4%), lack of modern beehives (49.7%), bee colony agro-chemicals (46.4%), bee colony (37.6%), water availability (30.4%), honey bee forage limitation (24.3%) and lack of labour (17.1%) (Table 3).

Due to different predators like ants, wax moth, bee lice, beetles, spiders, wasps, lizards, snakes, birds and monks of bee colony, the yield of honey is highly affected because such predators reduce survival powers of bee colonies and sometimes kill them. Moreover, swarming and absconding of bee colonies were commonly observed in the study area. This could be either due to delay in harvesting the honey yields; or when they face deficit of water and bee forage especially during dry season; when frequently disturbed while harvesting honey, predators interference or sometimes due to improper inspecting manner by human.

Limitations of modern equipments like wax, wax printer, kenya/Germany hives model form; trained man power and other inputs to transform and scale-up the honey beekeeping into modern production system were the major challenges. Lack of good marketing place, selling of honey at low price in local market by farmers and many of them used traditional honeybee production. To some extent, limitation of bee colony, apiary site, bee forage and water, bee colony agro-chemicals were also major challenges reported in the study area.

Supporting the above findings, Arse et al. (2010), in West Arsi zone of Oromia, Ethiopia reported on the shortage of honeybee forages, shortage of honeybee colonies, poisoning of agro-chemicals, shortage of modern hives, prevalence of honeybee enemies and market problems. They also reported on the shortage of improved bee equipments, absconding and swarming problems, prevalence of honeybee diseases, lack of knowledge of the right harvest time and theft problems are the major beekeeping. Chala et al. (2012) in Gomma District, South Western Ethiopia also reported that lack of beekeeping knowledge, shortage of trained manpower, shortage of beekeeping equipment, pests and predators, fires, pesticide threat and inadequate research works to support development programs were major constraints that affected apiculture production in area. The prevailing production constraints in the beekeeping development in the country were found to be complex and to a large extent varied between agro-ecological zones and production systems. Variations of production constraints also extended to socio-economic conditions, cultural practices, climate (seasons of the year) and behaviors of the bees (Adjare, 1990; Kerealem et al., 2009).

HBRC (1997) and Edessa (2002) reported the major constraints in the beekeeping sub-sector as unpleasant behaviours of bees (aggressiveness, swarming tendency and absconding behaviors); lack of skilled manpower and training institutions; low level of technology used; high price of improved beekeeping technologies, drought and deforestation of natural vegetation; poor post-harvest management of beehive products and marketing constraints and indiscriminate application of agro-chemicals. Other constraints according to them were honeybee disease, pest and predators; poor extension services; absence of coordination between research, extension and farmers; absence of policy in apiculture; shortage of records and up to date information, and inadequate research institutions to address the problems.

Conclusion

Beekeeping is highly significant in sustainability of natural resources and agriculture. It has also great contribution in maintaining the equilibrium of the nature by interacting with different biotic ecosystem. Households kept bee honey mainly for consumption and income generation. The major constraints of beekeeping were honeybee pests and diseases, modern equipments input limitation, absconding and swarming. Regardless of availability of constraints, beekeeping was found to have a number of opportunities which demands for the sub-sector to be encouraged in the study area.

RECOMMENDATIONS

Based on the above findings, it was recommended that:

1. The regional government, bureau of animal and fishery production, and NGOs should formulate policy with regard to honeybee pests, diseases and predators control, introducing/dissemination of modern equipments, absconding and swarming control.
2. Moreover, inspiring training on modern beekeeping practice should be given frequently for farmers and health care professionals.

CONFLICT OF INTERESTS

The authors did not declare any conflict of interests.

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Occurrence and distribution of cucumber mosaic virus in cucurbits in Karanganyar, Central Java, Indonesia

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***Cucumber mosaic virus* (CMV) is an important pathogen in agricultural crops which is spread throughout the world with a wide host range. In Indonesia, CMV is also an important pathogen that infect various high economic value crops. Karanganyar regency is a horticultural production centers in Central Java. Its foremost products are cucurbits incuding cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita moschata*), chayote (*Sechium edule*), angled luffa (*Luffa acutangula*) and bitter melon (*Momordica charantia*). Recently, cucurbits crops in the area suffered from disease with symptoms of virus infection, especially CMV, that is, mosaic, vein clearing, vein banding, malformation, etc. The disease is detrimental to farmers. This research aimed to determine whether the causal agent of disease in the crops is CMV. For that, the authors conducted a survey in the field, picked up sample crops showing symptoms, brought the sample crops to the laboratory, and checked for the presence of CMV by triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA). The results showed that of the 50 sample crops including 7 species of cucurbits showing symptoms of virus infection, 12 were detected to be infected by CMV. CMV was distributed evenly in cucurbits in Karanganyar regency.**

Key words: *Cucumber mosaic virus* (CMV), cucurbits, triple antibody sandwich enzyme-linked immunosorbent assay (TAS ELISA), Karanganyar, Indonesia.

INTRODUCTION

Cucumber mosaic virus (CMV), a member of *Bromoviridae* family is an important pathogen in agricultural crops which spreads throughout the world. The virus has a wide host range including 1200 species

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in over 100 plant families (Zitter and Murphy, 2009; Palukaitis et al., 1992). In agricultural crops, especially cucurbits (*Cucurbitaceae* family), CMV infection causes varied symptoms, commonly mosaic, vein clearing, vein banding and malformation (Zitter and Murphy, 2009). In Indonesia, CMV is also an important pathogen in agricultural crops. CMV was first detected in Indonesia in 1972 in Bogor, West Java which infected tobacco (Suseno and Lumanau, 1972). Hartana (1987) reported that CMV spread throughout Indonesia and infect cucurbits and other plants. Subsequent reports have shown that CMV was found widespread in various regions in Indonesia and infect a variety of agricultural crops. Outside Java island, CMV was reportedly detected in Sumatra (Bengkulu) to infect chili (Sutrawati, 2010; Sutrawati et al., 2012) and soybean (Damayanti and Wiyono, 2015), in Sulawesi to infect chili (Taufik et al., 2011) and in Bali to infect weeds (Pranatayana et al., 2014).

In Java island, CMV was detected infect soybean (Damayanti and Wiyono, 2015). In the Special Region of Yogyakarta Province, CMV was detected to infect cucurbits (Somowiyarjo et al., 1993; Daryono and Natsuaki, 2009) and cucumber (Septariani et al,, 2014). In West Java Province, CMV was detected to infect yardlong bean (Damayanti et al., 2009, 2010) and cucumber (Septariani et al., 2014). In Central Java province, CMV was detected to infect cucurbits in Klaten Regency (Daryono and Natsuaki, 2009), yardlong bean (Damayanti et al., 2009, 2010), and cucumber in Tegal and Sukoharjo Regency (Septariani et al., 2014).

Karanganyar Regency is a horticultural production center in Central Java. This area is a plateau with an average elevation of 511 m above sea level and located between 110° 40’ - 110° 70’ East Longitude and 7° 28’- 7° 46’ South Latitude. The horticultural commodities provide 40% of total regional income. The flagship product are cucurbits which include cucumber, melon, watermelon, pumpkin, chayote, angled luffa and bitter melon (Statistics Bureau of Indonsia, 2014). Recently, many cucurbits crops in the region suffered a kind of disease showing symptoms of viral infections such as mosaic, vein clearing, vein banding and malformation which are typical of CMV infection symptoms. The disease is very detrimental to farmers (Center for Monitoring Pests and Plant Diseases of Karanganyar, 2014), but so far, basic information and research on the existence and distribution of CMV infecting cucurbits in the region is not yet available.

This research aimed to find out the presence and distribution of CMV infecting cucurbits in Karanganyar Regency. Therefore, the authors conducted a survey of the field, then observed crops showing the symptoms as well as determined the disease incidence. They also picked up some samples of diseased crops representatively and brought them to the laboratory for detection of CMV using TAS ELISA. The results showed that CMV was detected in various species of cucurbits in Karanganyar Regency with evenly distribution.

MATERIALS AND METHODS

Study area

Karanganyar Regency consists of 17 sub-regions (districts), that is, Tasikmadu, Mojogedang, Colomadu, Gondangrejo, Kebakkramat, Jaten, Kerjo, Ngargoyoso, Jatipuro, Jenawi, Karanganyar, Karangpandan, Matesih, Tawangmangu, Jumantono, Jumapolo and Jatiyoso (Figure 1). During the survey (in the year 2014), cucurbits were found relatively evenly planted over the entire region. Cucurbits showing symptoms typical of CMV infection were also found distributed relatively evenly over the region. Firstly, 10 districts from where the data were collected were representatively determined. The districts were Tasikmadu, Mojogedang, Kerjo, Ngargoyoso, Karangpandan, Matesih, Tawangmangu, Jumantono, Jumapolo and Jatiyoso (Figure 1).

Selection of gardens, crops sampling, symptoms observation and visual assessment of disease prevalence and disease incidence

In each district, 5 field plots (gardens) of cucurbits crops having crops showing the symptoms of CMV infection were representatively determined as mentioned above. Disease prevalence was estimated according to Sydanmetsa and Mbanzibwa (2016) to obtain the percentage of the fields with at least one diseased crop as assessed visually (by symptoms observation). Then, disease incidence of each garden was estimated (computed) by dividing the number of crops with symptoms by the total number of crops on which observation were made per field. In each garden, one diseased crop was purposively determined. Then, each of the determined diseased crop was observed for symptoms and recorded.

Collection of leaf of sample crops

From each of the determined diseased crop in the above stage, young leaves showing the symptoms were cut off, inserted into plastic clip, labeled, and kept in ice box. After taking to the laboratory, the leaf samples were transferred to refrigerator prior next assay.

Detection of CMV by TAS ELISA

To ensure that the causal agent of the disease was CMV, sap of sample crops were immunoassay detected with CMV antiserum. Immunoassay was performed utilizing Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay (TAS ELISA) format using PathoScreen® Kit from Agdia Inc., following protocol from the manufacturer. Three leaf pieces of sample crops were grinded in extraction buffer at a 1:10 ratio (w/v). In brief, after sample wells were coated using polyclonal antibody, 100 µl of prepared samples were dispensed into sample wells. The plate was incubated for 2 h at room temperature, followed by 7 times washing with washing buffer. Here, monoclonal antibody mixture specific for general CMV was used as detection antibody. 100 µl of alkaline phosphatase conjugate was dispensed per well, then incubated for 2 h at room

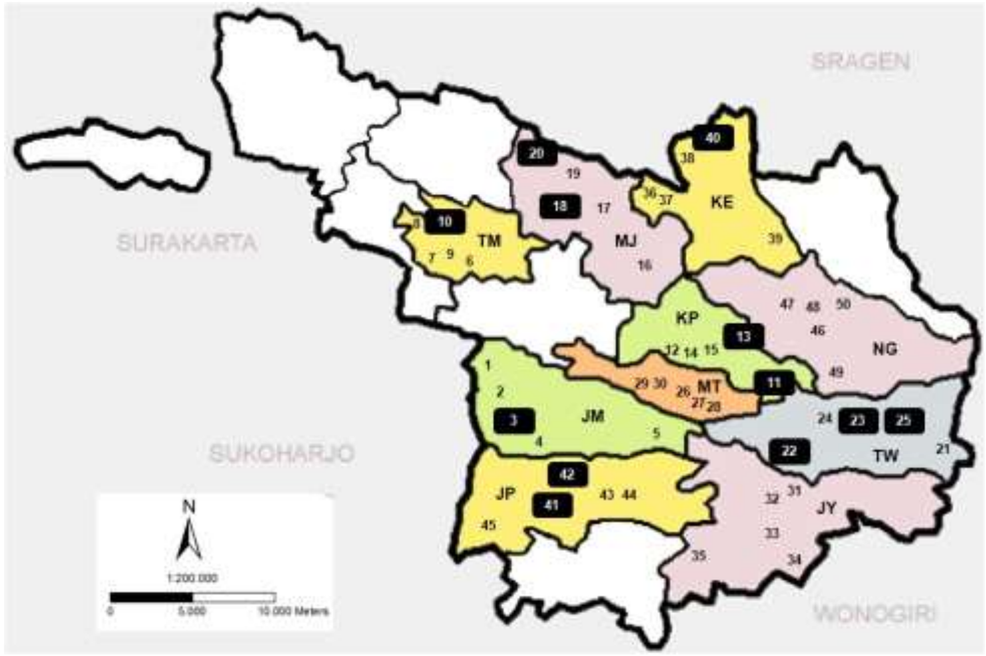

Figure 1. Map of Karanganyar regency and distribution of sample crops in the region. JM, Jumantono; TM, Tasikmadu; KP, Karangpandan; MJ, Mojogedang; TW, Tawangmangu; MT, Matesih; JY, Jatiyoso; KE, Kerjo; JM, Jumapolo; NG, Ngargoyoso. Numbers refer to serial number of sample crops or field plots. Numbers with dark backgrounds are sample crops in which CMV were detected (Table 1).

temperature, followed by 8 times washing with washing buffer. 100 µl of PNP substrate was dispensed into test well followed by incubation for 60 min (in dark). The developing color was observed by eyes, and the color intensity (absorbance value) was measured on an ELISA plate reader at 405 nm. The tested samples were stated as positively CMV infection if they have absorbance value of twice of the negative control.

RESULTS AND DISCUSSION

Variations of visual symptoms in the field

Fifty field plots (gardens) of cucurbits crops have been observed for their virus disease symptoms. The crops includes seven species of cucurbits, that is, cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita moschata*), chayote (*Sechium edule*), angled luffa (*Luffa acutangula*) and bitter melon (*Momordica charantia*). Based on visual observations in the field, the cucurbits crops showed variation of symptoms which include: mosaic, vein clearing, vein banding and malformation (Table 1 and Figure 3). The symptoms are typical of CMV infection in cucurbits (Zitter and Murphy, 2009). However, these symptoms can also be caused by infection with other viruses in cucurbits, such as *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini lethal chlorosis virus* (ZLCV), *Zucchini yellow mosaic virus* (ZYMV) and other viruses (Ullman et al., 1991; Yuki et al., 2000; Lima et al., 2012; Romay et al., 2014; Sydanmetsa and Mbanzibwa, 2016).

Based on visual observations (Table 1), it can be seen that the cucurbits crops showing symptoms of viral infections (including CMV) are widely spread in Karanganyar Regency. The virus-like disease symptoms were seen in cucurbits in all of 10 fields that were surveyed. According to Sydanmetsa and Mbanzibwa (2016), this represented a disease prevalence of 100% as was assessed as the number of fields with at least a diseased plant in the total number of fields.

Visual assessment of virus disease incidence

Of the 50 field plots of cucurbits crops, their virus disease incidence were assessed based on visual observation of disese symptoms in the field (Table 1). From Table 1, it can be seen that the disease incidence varied among the field plots, ranged from 0.6 to 64%. The lowest disease incidence was found in the field number 40 of bitter melon crops in district Kerjo. In this field, bitter melon crops showed malformation and mosaic symptoms. The

Table 1. Crops sampling and symptoms observation of cucurbits crops in the field and the results of TAS-ELISA.

District	Field plot and location	Sample crop and serial number	Symptoms	Number of crops in a field plot	Number of diseased crops in a field plot	Disease Incidence	TAS-ELISA results		
							Color	Absorbance value	Judgment*
Jumantono	1. Sukosari	1. melon	malformation, mosic	27	3	11.1	clear	0.316	-
	2. Sukosari	2. cucumber	vein banding	135	5	3.7	clear	0.439	-
	3. Tugu	3. melon	mosaic	300	17	5.7	yellow	0.649	+
	4. Tugu	4. melon	mosaic, vein clearing	300	17	5.7	yellow	0.539	+/-
	5. Tunggulsari	5. melon	mosaic, vein banding	3850	27	0.7	yellow	0.526	+/-
Tasikmadu	1. Suruh	6. melon	malformation, vein banding	7200	67	0.9	clear	0.062	-
	2. Kaling	7. melon	mosaic, vein clearing	96	2	2.1	clear	0.391	-
	3. Karangmojo	8. melon	malformation, mosaic	560	5	0.9	yellow	0.573	+/-
	4. Ngijo	9. pumpkin	mosaic	12	4	33.3	clear	0.282	-
	5. Pandeyan	10. bitter melon	mosaic	1110	157	14.1	yellow	1.428	+
Karangpandan	1. Salam	11. cucumber	mosaic, vein banding	300	6	2.0	yellow	0.816	+
	2. Doplang	12. cucumber	malformation, vein clearing	180	3	1.7	clear	0.421	-
	3. Harjosari	13. Angled luffa	malformation, mosaic	80	4	5.0	yellow	0.694	+
	4. Doplang	14. melon	mosaic	8100	126	1.6	clear	0. 330	-
	5. Dayu	15. melon	malformation	56	1	1.8	clear	0.406	-
Mojogedang	1. Mojogedang	16. chayote	malformation	10	4	40.0	clear	0.377	-
	2. Pendem	17. cucumber	mosaic, vein clearing	233	9	3.9	clear	0.454	-
	3. Gentungan	18. cucumber	mosaic	340	15	4.4	yellow	0.839	+
	4. Munggur	19. angled luffa	mosaic	60	17	28.3	clear	0.384	-
	5. Munggur	20. angled luffa	mosaic, vein banding	180	3	1.7	yellow	1.876	+
Tawangmangu	1. Gondosuli	21. chayote	malformation	10	2	20.0	clear	0.444	-
	2. Tawangmangu	22. chayote	mosaic	28	4	14.2	yellow	0.84	+
	3. Blumbang	23. pumpkin	mosaic	167	7	4.2	yellow	2.106	+
	4. Blumbang	24. pumpkin	mosaic	295	11	3.7	clear	0.308	-
	5. Kalisoro	25. cucumber	mosaic	560	4	0.7	yellow	0.742	+
Matesih	1. Karangbangun	26. cucumber	mosaic	430	8	1.9	yellow	0.595	+/-
	2. Karangbangun	27. cucumber	malformation, mosaic	250	9	3.6	clear	0.381	-
	3. Koripan	28. cucumber	mosaic	560	13	2.3	clear	0.362	-
	4. Matesih	29. cucumber	mosaic	148	6	4.1	clear	0.39	-
	5. Matesih	30. cucumber	mosaic	80	3	3.8	clear	0.375	-
Jatiyoso	1. Beruk	31. chayote	malformation	20	14	70.0	clear	0.358	-
	2. Beruk	32. chayote	malformation, mosaic	15	7	46.7	clear	0.347	-
	3. Wonorejo	33. chayote	mosaic	24	9	37.5	clear	0.343	-
	4. Wonokeling	34. chayote	mosaic	12	2	16.7	clear	0.357	-
	5. Jatiyoso	35. chayote	mosaic	18	5	27.8	clear	0.362	-

Table 1. Contd.

Kerjo	1.	Kutho	36.	watermelon	malformation	370	6	1.7	clear	0.395	-
	2.	Kutho	37.	watermelon	malformation	200	16	8.0	clear	0.313	-
	3.	Tawangsari	38.	watermelon	malformation	60	1	1.7	clear	0.353	-
	4.	Plosorejo	39.	bitter melon	mosaic	48	4	8.3	clear	0.439	-
	5.	Sumberejo	40.	bitter melon	malformation, mosaic	60	9	0.5	yellow	0.66	+
Jumapolo	1.	Kwangsan	41.	cucumber	mosaic	280	7	2.5	yellow	0.646	+
	2.	Bakalan	42.	cucumber	mosaic	440	13	3.0	yellow	0.85	+
	3.	Jumapolo	43.	cucumber	malformation, vein banding	44	1	2.3	clear	0.321	-
	4.	Jumapolo	44.	bitter melon	malformation	38	17	44.7	clear	0.326	-
	5.	Lemahbang	45.	watermelon	malformation	525	7	1.3	clear	0.302	-
Ngargoyoso	1.	Girimulyo	46.	chayote	mosaic	15	4	26.7	clear	0.378	-
	2.	Kemuning	47.	chayote	malformation, mosaic	25	16	64.0	clear	0.472	-
	3.	Segorogunung	48.	chayote	malformation, mosaic	18	6	33.3	clear	0.306	-
	4.	Berjo	49.	chayote	malformation, mosaic	10	1	10.0	clear	0.122	-
	5.	Segorogunung	50.	cucumber	malformation, mosaic	40	2	5.0	clear	0.219	-
Standard		CMV infected		nd*	nd	nd	nd	nd	yellow	0.539	+
		CMV free		nd	nd	nd	nd	nd	clear	0.326	-
		Extraction buffer		nd	nd	nd	nd	nd	clear	0.452	-

*Annotation: +, Positif; -, Negatif; nd, no data. Blue columns are that the sample was CMV detected by TAS-ELISA.

highest disease incidence was found in the field number 47 of chayote crops in district Ngargoyoso. In this field, chayote crops also showed malformation and mosaic symptoms.

From Table 1, it can be seen that the disease showing symptoms tipical of virus infection had suffered cucurbits crops in Karanganyar Regency with disease incidence of moderate level, referring to a scale of Sydanmetsa and Mbanzibwa (2016). This corroborates the reports of Statistics Bureau of Indonsia (2014) and Center for monitoring pests and plant diseases of Karanganyar (2014) which reported that disease with such symptoms of virus infection had recently been seen in cucurbits in the regoin and caused economic loss to farmers.

Presence of CMV in sample crops based on TAS ELISA

Of the 50 field plots of cucurbits observed, from each was taken the best to represent sample crop. The sample crops were then visually observed and the symptoms were recorded. From parts of each sample crop (leaf pieces) were detected the presence of CMV by TAS ELISA using antibody for general CMV. The results of ELISA are presented in Table 1 (Figure 2). The table shows that of the 50 sample crops showing CMV infection symptoms, there were only 12 sample crops in which CMV was detected. The details are as follows: 1 melon crop showing mosaic; 2 bitter melon crops showing mosaic and malformation; 5 cucumber crops showing mosaic and vein banding; 2 angled luffa crops showing malformation, mosaic and vein banding; 1 chayote crop showing mosaic, and 1 pumpkin crop showing mosaic (Figure 3).

From Table 1 and Figure 3, it can be seen that the symptoms may vary between crops in the

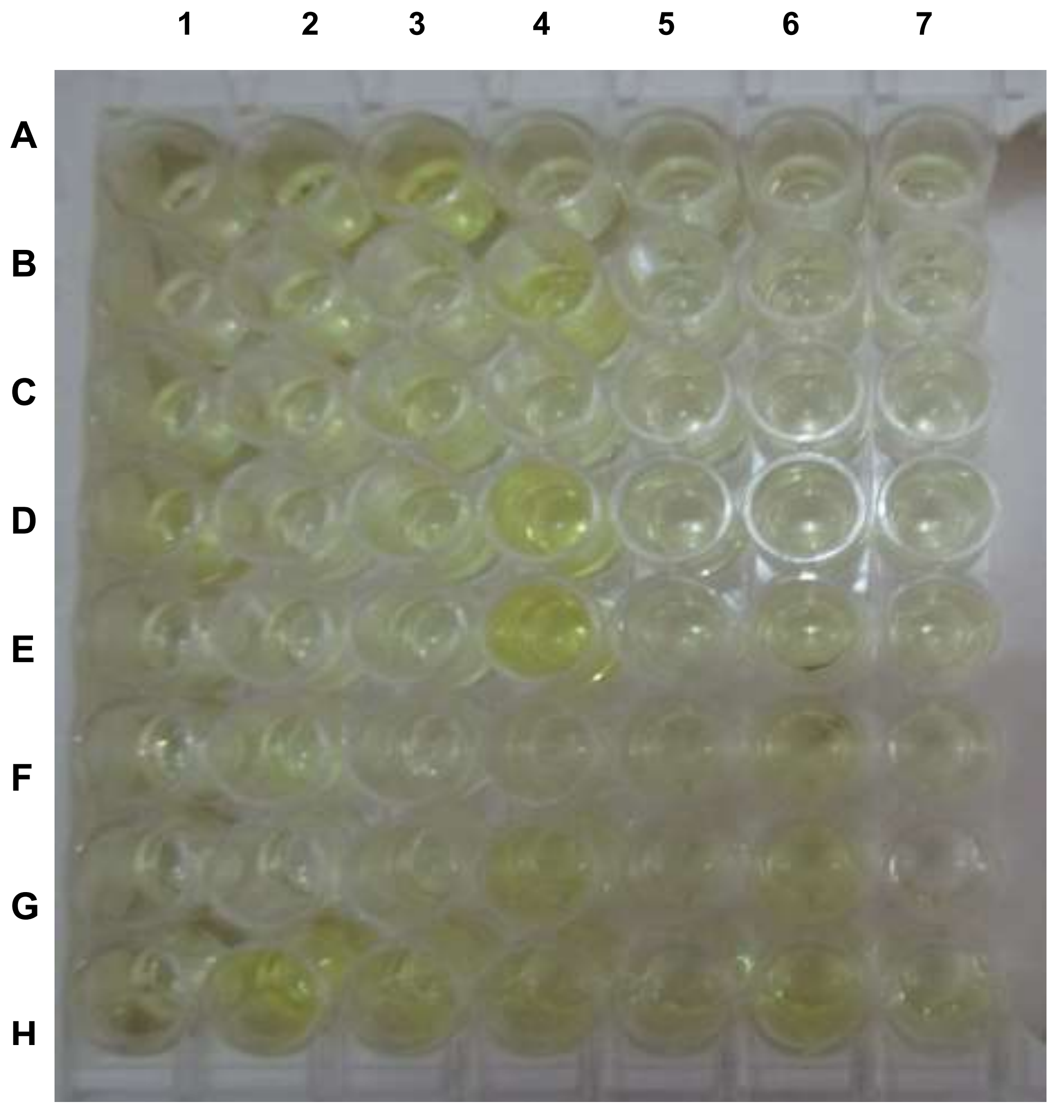

Figure 2. Visual appearance of TAS ELISA result. A1 (extraction buffer), B1 (negative control), D1 (positive control), G1 (1), H1 (2), A2 (3), B2 (4), C2 (5), D2 (6), E2 (7), F2 (8), G2 (9), H2 (10), A3 (11), B3 (12), C3 (13), D3 (14), E3 (15), F3 (16), G3 (17), H3 (18), A4 (19), B4 (20), C4 (21), D4 (22), E4 (23), F4 (24), G4 (25), H4 (26), A5 (27), B5 (28), C5 (29), D5 (30), E5 (31), F5 (32), G5 (33), H5 (34), A6 (35), B6 (36), C6 (37), D6 (38), E6 (39), F6 (40), G6 (41), H6 (42), A7 (43),B7 (44), C7 (45), D7 (46), E7 (47), F7 (48), G7 (49), H7 (50). Numbers in the brackets are the serial numbers of sample crops.

same species but similar symptoms may show in crops in different species. Several explanations could be given as follows. Theoritically, the appearance of viral infection symptoms of a crop in the field can vary depending on many factors. The main factors affecting the development of symptoms in the field include the virus strains, species/varieties of host crop, crop age and environmental factors (Green, 1984; Zitter and Murphy, 2009; Hull, 2014).

The other possible causes of variations in symptom is the presence of mixed infections. There were some reports of mixed infections in cucurbits causing variations of symptoms in the field. In Tanzania, Sydanmetsa and Mbanzibwa (2016) reported that mixed infections of three viruses, CMV, ZYMV and WMV on cucurbits caused varied symptoms that were mosaic, leaf curling, wrinkled leaves, stunted growth, green vein banding, yellow spots and yellow mottling. For examples, mixed infection in pumpkins caused mosaic, rugosity and green veinbanding; in cucumber caused green vein banding, yellowing and

Figure 3. Symptoms variation of various sample crops of cucurbits which was CMV detected. Numbers refer to the serial number of sample crops. The description of the symptoms are as follows: sample no 3, mosaic; sample no 10, mosaic; sample no 11, mosaic, vein banding; sample no 13, malformation, mosaic; sample no 18, mosaic; sample no 20, mosaic, vein banding; sample no 22, mosaic; sample no 23, mosaic; sample no 25, mosaic; sample no 40, malformation, mosaic; sample no 41, mosaic; sample no 42, mosaic (Table 1).

mosaic; whereas in watermelon, it caused leaf deformation. Similar phenomenon was also reported by Barbosa et al. (2016) in Brazil, in which mixed infection of CMV, WMV, ZYMV and PRSV on cucurbits showed a variety of symptoms in the field.

In Java and Central Java, there have also been reported mix infections of viruses in Cucurbits. Daryono and Natsuaki (2009) reported mix infection of CMV, *Cucumber green mottle mosaic virus* (CGMMV) and *Kyuri green mottle mosaic virus* (KGMMV) on melon and other cucurbits which induced a variety of mosaic symptoms. Septariani et al. (2014) also reported that a variety of symptom including mosaic, chlorotic spotting, leaf curling, blistering, vein banding, reduction and distortion of leaf and fruit were observed on cucurbit which had mixed infected with *Squash mosaic comovirus* (SqMV), ZYMV and CMV. Variations of symptoms on cucurbits with mixed infected by viruses has also been reported in Yogyakarta by Somowiyarjo et al. (1993).Not all plants showing typical symptoms of CMV was detected as having CMV. The first possibility is that the crops were infected by distinct strains of CMV. Zitter and Murphy (2009) reported that distinct strains of viruses could differ serologically. The second possibility is that the crops were infected by other distinct cucurbits viruses. Many authors reported that cucurbits could be infected by many viruses, commonly CMV, PRSV, ZYMV, WMV and others both in single or mix infection that induce a variety of symptoms including symptoms of CMV infection such as mosaic, vein clearing, vein banding and malformation (Zitter and Murphy, 2009; Romay et al., 2014; Barbosa et al., 2016; Sydanmetsa and Mbanzibwa, 2016).

Distribution of CMV in the field

The result of ELISA indicated that CMV was detected in 7 districts out of 10 selected districts (Table 1 and Figure 1). From Figure 1, it can be said that CMV is distributed evenly in Karanganyar Regency. The first explanation that could be given is that CMV is seed borne virus. In general, Zitter and Murphy (2009) and Palukaitis et al. (1992) reported that CMV is a seed-borne virus in many crops with varied transmission rate. In India, Abdullahi et al. (2001) reported the efficiency of CMV transmission through seed in beans was 30%. In Indonesia, Nurhaelena (2013) reported that CMV was seed transmitted in cucurbits such as cucumber, squash, melons with efficiency ranged from 2 to 12%. In Karanganyar where this survay was conducted, many farmers used seeds for growing cucurbits which were derifed from the previous cucurbits crops (Center for Monitoring Pests and Plant Diseases of Karanganyar, 2014). Thus, the infected crops are sources of inoculums for the next growing crops. The second explanation that could be given is that CMV is insect borne. CMV has many insect vectors and the vectors have a broad host range (including within cucurbits). Palukaitis et al. (1992) and Zitter and Murpgy (2009) reported that in general, more than 80 aphid species could transmit CMV. Transmission efficiency varies with the aphid species, virus strains, host plant species, environmental conditions and time of the year. The authors also reported that CMV is transmitted mainly by the green peach aphid, *Myzus persicae*, and by *Aphis gossypii*. In Karanganyar, from the rough sampling done during the survey, some species of insect vectors such as Aphids and Myzus (Unpublished data) were found.

From the aspect of host plant species, CMV also spread relatively evenly among the species of cucurbits. Of the seven species of host crops which were selected for observation, that is, cucumber, melon, watermelon, pumpkin, chayote, angled luffa and bitter melon, CMV was detected in all species except in watermelon. It is suggested that, in addition to the relatively few number of sample crop for watermelon (4 out of 50 sample crops), their distribution were also relatively clustered, this made the crops to get small probability to get virus through insect vectors.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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