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Full Length Research Paper

Molecular characterization of some brinjal genotypes (*Solanum melongena* L) using simple sequence repeat (SSR) markers

Farid Ahmed[1], Md. Golam Rabbani[2], Md. Rafiqul Islam[1], Mostafezur Rahman[2], Md. Abdul Malek[1], Mirza Mofazzal Islam[1] and Reza Mohammad Emon[1]*

[1]Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh.
[2]Department of Horticulture, Bangladesh Agricultural University, Mymensingh, Bangladesh.

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To analyze genetic variability of 48 brinjal genotypes by Simple Sequence Repeat (SSR) markers, four primers named smSSR01, smSSR03, smSSR11 and smSSR14 were selected for analyze the data. Four primers generated clear bands and a total of 44 alleles were detected among the 48 brinjal genotypes (9.0 to 14.0 alleles per locus with a mean of 11.0 alleles per locus). According to Nei's (1973), the highest level of gene diversity value 0.8672 was observed in loci smSSR11 and the lowest level of gene diversity value 0.6580 was observed in the loci smSSR14 with a mean diversity of 0.7847. As a measure of the informativeness of microsatellites, the PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660. The genetic distance (GD) between genotypes was computed from combined data for the four primers, ranging from 0.250 to 1.000. The highest genetic distance (1.000) was observed between Morich Begun (Small) vs. Laffa (Long, Violet), Morich Begun (Small) vs. BARI Begun -1 and many other genotype pairs and also the lowest genetic distance (0.250) was found in Morich Begun (Small) vs. Pahoza-2, Morich Begun (Small) vs. BAU Begun-1, Singnath (Long, Violet) vs. Laffa BAU (Long, Violet), Borka (Long, Green) vs. Purple Long and many other genotype pairs. The Unweighed Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis lead to the grouping of the 48 genotypes into seven major clusters and the genotypes that are derivatives of genetically similar type form cluster together. Maximum brinjal genotypes include cluster IV. The SSR markers used in the present study were able to differentiate forty eight brinjal genotypes genetically. The results of the study suggested that SSR can be used as a suitable genetic marker to identify the brinjal genotypes. However, further studies involving large number of genotypes and primers need to be conducted to get more precise information and help enhance the knowledge of students and researchers relating to molecular characterization of other solanaceious crops.

Key words: Brinjal, SSR, Genetic Diversity

INTRODUCTION

Brinjal (*Solanum melongena* L.), also known as eggplant or Aubergine (French name) is one of the most important, inexpensive and popular vegetable crops grown in Bangladesh.

Brinjal is grown on 17,28,271 ha with a total production of 4,31,73,989 tons (FAO, 2012). Asia has the largest eggplant production which comprises more than 90% of the world production area and 87% of the world production. It is cultivated and available year-round in Bangladesh with annual production areas of 124,214 ha and the total production was 450,136 tons in the year 2014-2015 (BBS, 2016).

As a popular vegetable in Bangladesh brinjal has a great potentiality. But due to limitation of land it is not possible to increase the area of the crop. Brinjal exhibits extensive variation in morphological and biochemical traits (Arivalagan et al., 2013). The study of genetic diversity and relationships of collections of local genotypes provides information of relevance for the breeding programmes. DNA marker technology and molecular characterization are immensely helpful in selective breeding from diverse parents to widen the breeding gene pool (Fu, 2006). Several molecular studies (Cericola et al., 2013; Prohens et al., 2005; Tumbilen et al., 2011) have shown that eggplant cultivar groups are genetically diverse. SSR markers indicated a strong genetic affinity of eggplant (*Solanum viarum, Solanum melongena* and *Solanum aethiopicum*; *Aculeatum* group) and also assayed informative for the potential to serve as perfect markers for studying variation (Adeniji et al., 2012). Genetic diversity and relatedness may be informative for the varietal identification and genetic improvement of brinjal (Sultana et al., 2018).

However, despite its widespread cultivation and nutritional and economic importance, the eggplant genome has not yet been extensively evaluated as for the other solanaceous vegetables such as tomato, potato and pepper, all of which have high density linkage maps (Barchi et al., 2007; Jacobs et al., 2004; Tanksley et al., 1992). Morphological, molecular and combined trait analyses consistently recognized the main groups of eggplants (cultivars and land races). It also exhibited higher variation compared to the landraces and cultivars. For eggplants landraces, morphological variability was moderately high but low diversity was observed on SSRs and combined data analyses (Caguiat and Hautea, 2014).

High degree of diversity of brinjal cultivars may be attributable to genetic improvement programme based on the molecular clustering patterns. It also provides support for selection of crossing combinations from parental genotypes and for broadening the genetic basis of breeding programs. Due to having some medicinal values particularly against cancer, diabetes and cardiovascular disease, marker assisted breeding and production of eggplant may contribute to enrich diets and bring health benefits (Sultana et al., 2018). The use of molecular markers in eggplant breeding has been limited compared to other relevant crops of the same family (Barone et al., 2009; Jo et al., 2010; Danan et al., 2006). A number of SSR markers have been identified in Solanaceae (Yi et al., 2006; Bindler et al., 2007), but the numbers are less in eggplant. Portis et al. (2018) developed an "Eggplant Microsatellite DataBase" (*EgMiDB*) which permits identification of SSR markers in terms of their location on the genome, type of repeat (perfect vs. imperfect), motif type, sequence, repeat number and genomic/gene context. It also suggests forward and reverse primers and also employed an *in silico* PCR analysis to validate these SSR markers, using as templates two CDS sets and three assembled transcriptomes obtained from diverse eggplant accessions.

The aim of the present study was to characterize eggplant genotypes collected from different geographical regions of Bangladesh using SSR markers and to assess the genetic diversity within these genotypes. Assessment of genetic diversity is important for breeding purposes, and the utilization of molecular markers helps accelerate the evaluation process. Therefore, the present study was conducted at molecular level with the following objectives: (i) to estimate the level of genetic diversity of brinjal genotypes by means of SSR markers; and (ii) to estimate the relationship among some brinjal genotypes of particular location or geographic origin.

MATERIALS AND METHODS

Forty eight brinjal genotypes were used in the study. In order to carryout SSR analysis, young leaves from each of the forty eight genotypes were collected randomly, which were used as the source of genomic DNA (Table 1).

After preparation of lands the seeds of brinjal were sown in lines to get uniform and healthy seedlings during July 2016. The seeds of Indian genotype, local exotic Zumka and Islampuri brinjal were exposed to gamma irradiation (^{60}Co) at different doses. The M_1 and M_2 generations were grown in two consecutive years and then selected mutants as $IndD_{150}L_8P_4$, $IndD_{150}L_1P_{27}$, $IndD_{300}L_{13}P_{11}$, $IndD_{300}L_{12}P_{12}$, $ZumD_{150}L_1P_{22}$, $ZumD_{150}L_{13}P_{21}$, $ZumD_{300}L_{12}P_5$, $ZumD_{300}L_{13}P_{12}$, $IslD_{150}L_{13}P_{22}$, $IslD_{150}L_{13}P_{14}$, $IslD_{300}L_5P_3$, $IslD_{150}L_{14}P_{23}$, $IslD_{150}L_2P_{25}$ and $IslD_{300}L_{12}P_{19}$. The M_3 populations were used for molecular characterization using SSR markers. For genomic DNA the seedlings were grown in the field of Horticulture Division, Bangladesh Institute of Nuclear Agriculture, Bangladesh Agricultural University Campus, Mymensingh. The uniform, viable, healthy, disease and insect free seeds of 48 brinjal genotypes were used for growing seedlings. Young, vigorously growing fresh leaf samples were collected for the SSR analysis from 20 days old seedling of each of the germplasm. Modified CTAB mini-prep method was followed to extract DNA from leaf samples of brinjal. PCR reactions were followed by Adeniji et al. (2012). After completion of PCR, the gel image (agarose and PAGE) resolution was adjusted using the camera setting. The gel was exposed to UV light and save the gel image as a tiff or jpeg file. Five primers of Simple Sequence

*Corresponding author. E-mail: emonbina@yahoo.com. Tel: +88-01720585124.

Table 1. Accession number of 48 brinjal genotypes.

S/N	Name of genotype	S/N	Name of genotype
01	Morich Begun (Small)	25	$IndD_{300}L_{13}P_{11}$
02	$IndD_{150}L_8P_4$	26	Purple Long
03	BARI Begun -1	27	$IndD_{300}L_{12}P_{12}$
04	Thama	28	Kaikka-N (Violet)
05	BARI Begun-7	29	BAU Begun-1
06	Thapa	30	$ZumD_{150}L_1P_{22}$
07	Indian-1	31	Darata (Round, Green)
08	Morich Begun	32	Dohazari (Long, Violet)
09	Khatkhatia Long (Green)	33	Laffa Long Violet-G
10	Uttara (Violet)	34	$ZumD_{150}L_{13}P_{21}$
11	Singnath (Long, Violet)	35	$ZumD_{300}L_{12}P_5$
12	Borka (Long, Green)	36	$ZumD_{300}L_{13}P_{12}$
13	China Long	37	$IslD_{150}L_{13}P_{22}$
14	Laffa (Long, Violet)	38	$IslD_{150}L_{13}P_{14}$
15	Kailkkah (Long, Green)	39	Dohazari (Green)
16	Laffa BAU (Long, Violet)	40	Pahoza-1
17	Islampuri BS	41	Salta
18	Pahoza-2	42	Apple Begun (Round, Violet)
19	Puta Begun	43	$IslD_{300}L_5P_3$
20	Longla Tal Begun	44	$IslD_{150}L_{14}P_{23}$
21	Irri Begun (Round, Green)	45	Jessore Local
22	Dhondol Begun (Long, Violet)	46	$IslD_{150}L_2P_{25}$
23	Khatkhetia Long (Violet)	47	Zumka
24	$IndD_{150}L_1P_{27}$	48	$IslD_{300}L_{12}P_{19}$

Table 2. Summary of microsatellite markers used for diversity study.

Primer name	Sequence		Product size (bp)	Annealing temperature (°C)
smSSR01	For. Rev.	GTGACTACGGTTTCACTGGT GATGACGACGACGATAATAGA	310	54.8
smSSR03	For. Rev.	ATTGAAAGTTGCTCTGCTTC GATCGAACCCACATCATC	145	54.8
smSSR04	For. Rev.	CTCTGCTTCACCTCTGTGTT CCATGAAAGAGAAGATCGAG	320	54.8
smSSR11	For. Rev.	AAACAAACTGAAACCCATGT AAGTTTGCTGTTGCTGCT	126	54.8
smSSR14	For. Rev.	ATACCACATCAATCCAAAGC CATCATCATCTTCACAGTGG	241	54.8

Repeat were screened on a sub sample of five randomly chosen brinjal genotypes, to test their suitability for amplifying brinjal SSRs that could be accurately scored. To confirm the reproducibility of SSR markers, the selected primers were screened five times on the same sample. The details of the primers are shown in Table 2.

Molecular weight for each amplified alleles was measured in base pair using Alpha Ease PC 4.0 software. The allele frequency data from Power Marker Version 3.25 (Liu and Muse, 2005) was used to export the data in binary format (allele presence=1 and allele absence=0) for analysis with NTSYS-PC Version 2.2 (Rohlf, 2002).

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, and Polymorphism Information Content (PIC) values were determining using Power Marker Version 3.25 (Liu and Muse, 2005). Alleles frequencies were calculated directly from the observed genotypes. Allelic variations and fit to Hardy-Weinberg proportions were estimated by the software POPGENE version 1.31 (Yeh et al., 1999). Expected (*He*) and observed heterozygosity (*Ho*) were also calculated (Nei, 1973) using the following formula and with the help of POPGENE version 1.31 (Yeh et al., 1999) computer package program. For the unrooted polygenic tree, genetic distance was calculated using the "CS Chord 1967" distance, Cavalli-Sforza and Fdwards (1967) followed by phylogeny reconstruction using neighbor-joining as implemented in Power Marker with tree viewed using the Tree view. The allele frequency dated from Power Marker was used to export the data in binary format (allele presence = 1 and allele absence = 0) for analysis with NTSYS-PC Version 2.2 (Rohlf, 2002). A similarity matrix was calculated with Simqual Subprogram using the Dice coefficient, followed by cluster analysis with the SAHN Subprogram using the UPGMA clustering methods implemented in NTSYS-PC used to construct a dendogram showing relationship among the genotypes. The similarity matrix was also used for principal coordinate analysis (PC_OA) with Dcenter, Eigen, Output and MX plat subprogram in computer program Numerical Taxonomy and Multivariate analysis system (NTSYS-PC). Nei's (1973) genetic distance value was computed using the formula as described in the POPGENE (version 1.31) software using manual (Yeh et al., 1999).

RESULTS

The analysis of genetic diversity is very important for brinjal improvement that can be obtained through DNA fingerprinting techniques. In this study, 48 genotypes of brinjal were analyzed using 4 primer pairs (smSSR01, smSSR03, smSSR11 and smSSR14). Amplified microsatellite loci were analyzed for polymorphism using Polyacrylamide Gel Electrophoresis (PAGE) and the results revealed that all the primer pairs detected polymorphism among the brinjal genotypes analyzed. The microsatellite loci were also multi-allelic (9.0 to 14.0 alleles per locus with a mean of 11.0 alleles per locus in the present study) and the alleles were co-dominant suggesting their relative superiority in detecting DNA polymorphism over some other markers.

Using 4 SSR markers, a total of 44 alleles were detected among the 48 brinjal germplasm. The average number of allele per locus was 11, with a range of 9 (smSSR03) to as many as 14 (smSSR01) (Table 3).

Comparing microsatellite markers with the different repeat motifs, those with high number of AGC repeats has the highest genetic diversity 0.8672, while those with high number of ATT, AT/GA and ACCAA repeats had the lower number of genetic diversity (0.8472, 0.7665 and 0.6580, respectively). According to Nei's (1973), the highest level of gene diversity value 0.8672 was observed in loci smSSR11 and the lowest level of gene diversity value 0.6580 was observed in the loci smSSR14 with a mean diversity of 0.7847. The value of pair-wise comparisons of Nei's (1973) genetic distance (GD) between genotypes were computed from combined data for the four primers, ranging from 0.250 to 1.000. SSR markers with ATT motifs show the maximum variation in allele size. As a measure of the informativeness of microsatellites, the PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660 (Table 3). The means of genetic distances between genotypes were used to evaluate the genetic diversity of different brinjal genotypes.

The number of alleles, their size range and allele frequency of different brinjal germplasm are shown in Table 4.

From the difference between the highest and lowest genetic distance value it was revealed that there were wide variability's among 48 brinjal genotypes. High genetic variability within genotypes and significant difference between genotypes indicate rich genetic material of a species. This study indicated genotypes that showed the highest genetic variation can be used as parental source for breeding line to improve brinjal varieties.

A dendogram was constructed based on the Nei's genetic distance calculated from the 44 SSR alleles generated from the 48 brinjal genotypes (Figure 1). All 48 brinjal genotypes could be easily distinguished. The Unweighed Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis leads to the grouping of the 48 germplasm into seven major clusters, three genotypes Thama, Kaikka-N (Violet) and BARI Begun-1 formed cluster I. BARI Begun-7, Kailkkah (Long, Green), Khatkhatia Long (Green), $IslD_{300}L_{12}P_{19}$, Indian-1, Singnath (Long, Violet) and Laffa BAU (Long, Violet) were grouped in cluster II in which sub cluster-1 includes BARI Begun-7 and Kailkkah (Long, Green) and sub cluster-2 includes $IslD_{300}L_{12}P_{19}$, Indian-1, Singnath (Long, Violet) and Laffa BAU (Long, Violet). Cluster-III includes $IslD_{300}L_5P_3$ and Jessore Local brinjal genotypes. In cluster IV $ZumD_{150}L_{13}P_{21}$, $IndD_{150}L_8P_4$ and Darata (Round, Green) formed sub cluster-1; Thapa, Morich Begun, BAU Begun-1, $ZumD_{150}L_1P_{22}$, Puta Begun, Laffa Long Violet-G, Morich Begun (Small), Borka (Long, Green), Pahoza-2, Purple Long, $ZumD_{300}L_{13}P_{12}$ and Zumka formed sub cluster 2 and $IslD_{150}L_{14}P_{23}$, $IslD_{150}L_{13}P_{22}$ and Apple Begun (Round, Violet). It is clearly observed that there is no genetic difference between Laffa Long Violet-G, Morich Begun (Small) and Borka (Long, Green). Cluster V includes Irri Begun (Round, Green), Laffa (Long, Violet) and Khatkhatia Long (Violet) genotypes. $IndD_{300}L_{12}P_{12}$, Dohazari (Long, Violet), $IslD_{150}L_{13}P_{14}$ and Dohazari (Green) formed cluster VI. In cluster VII, Dhondol Begun (Long, Violet), Laffa (Long, Violet), $ZumD_{300}L_{12}P_5$, Salta and China Long formed sub cluster-1; Pahoza-1, Islampuri BS, Longla Tal Begun, Uttara (Violet), $IndD_{150}L_1P_{27}$, $IndD_{300}L_{13}P_{11}$ and $IslD_{150}L_2P_{25}$ formed sub cluster-II. From this study, the dendrogram revealed that the genotypes that are derivatives of genetically similar type form cluster together. Maximum brinjal genotypes

Table 3. Data on the number of alleles, gene diversity, highest frequency allele and polymorphism information content (PIC).

Marker	Repeat motifs	Allele no.	Size range (bp)	Gene diversity	Highest frequency allele		PIC Value	Average PIC value
					Size (bp)	Frequency (%)		
smSSR01	(ATT)21	14	263-315	0.8472	293	14	0.8341	
smSSR03	(TA)9 (GA)8	09	144-156	0.7665	149	21	0.7350	
smSSR11	(AGC)6	10	124-133	0.8672	126	23	0.8536	0.7660
smSSR14	(ACCAA)3	11	187-268	0.6580	193	10	0.6413	
Mean	-	11	-	0.7847	-	-	0.7660	

Table 4. Size and frequency of alleles at 4 SSR loci of 48 brinjal genotypes.

Locus	Allele size (bp)	Allele frequency	Genotypes
smSSR 01	263	0.0208	Khatkhatia Long (Green)
	268	0.0208	Laffa (Long, Violet)
	269	0.0208	Indian-1
	286	0.0208	Kailkkah (Long, Green)
	288	0.0417	Uttara (Violet), IndD150L1P27
	290	0.0625	BARI Begun-7, Singnath (Long, Violet), Laffa BAU (Long, Violet)
	292	0.0625	Islampuri BS, IndD300L12P12, Dohazari (Green)
	293	0.1458	Thama, China Long, Dhondol Begun (Long, Violet), ZumD300L12P5, Pahoza-1, Salta, Apple Begun (Round, Violet)
	295	0.1042	ZumD150L1P22, Dohazari (Long, Violet), IslD150L13P14 IslD150L14P23, Jessore Local
	297	0.0208	BARI Begun-1
	298	0.0833	Irri Begun (Round, Green), IndD300L13P11, IslD150L13P22, IslD150L2P25
	300	0.0625	Longla Tal Begun, Kaikka-N (Violet), ZumD150L13P21
	315	0.0208	IslD300L12P19.
smSSR 03	144	0.0208	BARI Begun-7
	147	0.0417	IslD300L5P3, Jessore Local
	148	0.1875	Uttara (Violet), Irri Begun (Round, Green), Dhondol Begun (Long, Violet), Khatkhatia Long (Violet), IndD300L12P12, Dohazari (Long, Violet), ZumD300L12P5, IslD150L13P14, Dohazari (Green)
	149	0.2083	China Long, Laffa (Long, Violet), Islampuri BS, Islampuri BS, IndD150L1P27, IndD300L13P11, Kaikka-N (Violet), Pahoza-1, Salta , IslD150L2P25,
	151	0.0208	Laffa BAU (Long, Violet)
	152	0.1042	Indian-1, Khatkhatia Long (Green), Singnath (Long, Violet), Kailkkah (Long, Green), IslD300L12P19
	155	0.0208	BARI Begun -1
	156	0.0208	Thama
smSSR 11	124	0.0417	China Long, Pahoza-2

Table 4. Contd.

	125	0.1250	Laffa (Long, Violet), Islampuri BS, Longla Tal Begun, Irri Begun (Round, Green), Dhondol Begun (Long, Violet), Khatkhatia Long (Violet)
	126	0.2292	BARI Begun-7, Indian-1, Morich Begun, Khatkhatia Long (Green), Uttara (Violet), Singnath (Long, Violet), Kailkkah (Long, Green), Laffa BAU (Long, Violet), IndD150L1P27, IndD300L13P11, Purple Long
	127	0.1042	Thapa, IndD300L12 P12, BAU Begun-1, ZumD150L1P22, Darata (Round, Green)
	128	0.0833	BARI Begun -1, Thama, Kaikka-N (Violet), Dohazari (Long, Violet)
	129	0.1667	IndD150L8P4, ZumD150L13P21, ZumD300L12P5, ZumD300L13P12, IslD150L13P14, Dohazari (Green), Pahoza-1, Salta
	130	0.0417	IslD150L13P22, Apple Begun (Round, Violet)
	131	0.0417	IslD300L5P3, Jessore Local
	133	0.0833	IslD150L14P23, IslD150L2P25, Zumka, IslD300L12P19
smSSR 14	187	0.0417	Thama, BARI Begun-7
	189	0.0417	Thapa, Morich Begun
	190	0.0417	Khatkhatia Long (Green), Kailkkah (Long, Green)
	192	0.0208	Puta Begun
	193	0.1042	IndD150L8P4, BARI Begun -1, Irri Begun (Round, Green), Darata (Round, Green), ZumD150L13P21
	194	0.0625	Laffa (Long, Violet), Khatkhatia Long (Violet), Kaikka-N (Violet)
	258	0.0208	IslD150L13P14
	263	0.0208	Dohazari (Green)
	267	0.0208	Salta
	268	0.0625	Apple Begun (Round, Violet), IslD300L5P3, IslD150L14P23

include cluster IV.

DISCUSSION

The previous knowledge about the genetic relationships among breeding materials is documentary for the effective use of the genotypes in a breeding programme. Brinjal is an important vegetable in Bangladesh, but little information is available on genetic structure of brinjal genotypes. There are no specific markers found to remark different genotypes accurately, the SSR technique stated some degree of polymorphisms for investigating the genetic relationship among different brinjal genotypes.

Using 4 SSR markers, a total of 44 alleles were detected among the 48 brinjal genotypes. The average number of allele per locus was 11, with a range of 9 (smSSR03) to as many as 14 (smSSR01) which is supported by Vilanova et al. (2014), they found 2 to 11 alleles/locus by using 19 genomic SSRs for the molecular characterization of 30 eggplant accessions; the number of alleles ranged from 2 to 8 with a mean of 4.3 alleles per marker recorded by Caguiat and Hautea (2014), Adeniji et al. (2012) observed a total of 417 alleles amplified with the number of alleles ranging from 5 (EM 141) to 38 (EM 120 b), Verma et al. (2012) recorded the number of alleles per primer ranging from 2 to 10, with a mean of 4.67; Sunseri et al. (2010) found the number of alleles ranging from 2 to 7 with an average of 4.5. Nearly similar observation was found by Li et al. (2003) where they ranged from 2 alleles to 11 alleles with an average of 6.61 alleles per locus in chilli.

Comparing microsatellite markers with the different repeat motifs, those with high number of AGC repeats has the highest genetic diversity 0.8672, while those with high number of ATT, AT/GA and ACCAA repeats had the lower number of genetic diversity (0.8472, 0.7665 and 0.6580, respectively). This genetic diversity based on repeat motifs are compared from some previous analysis in brinjal such as Nunone et al. (2003a) who recorded that the AT repeat was the most common, representing 8.3% of the total SSRs

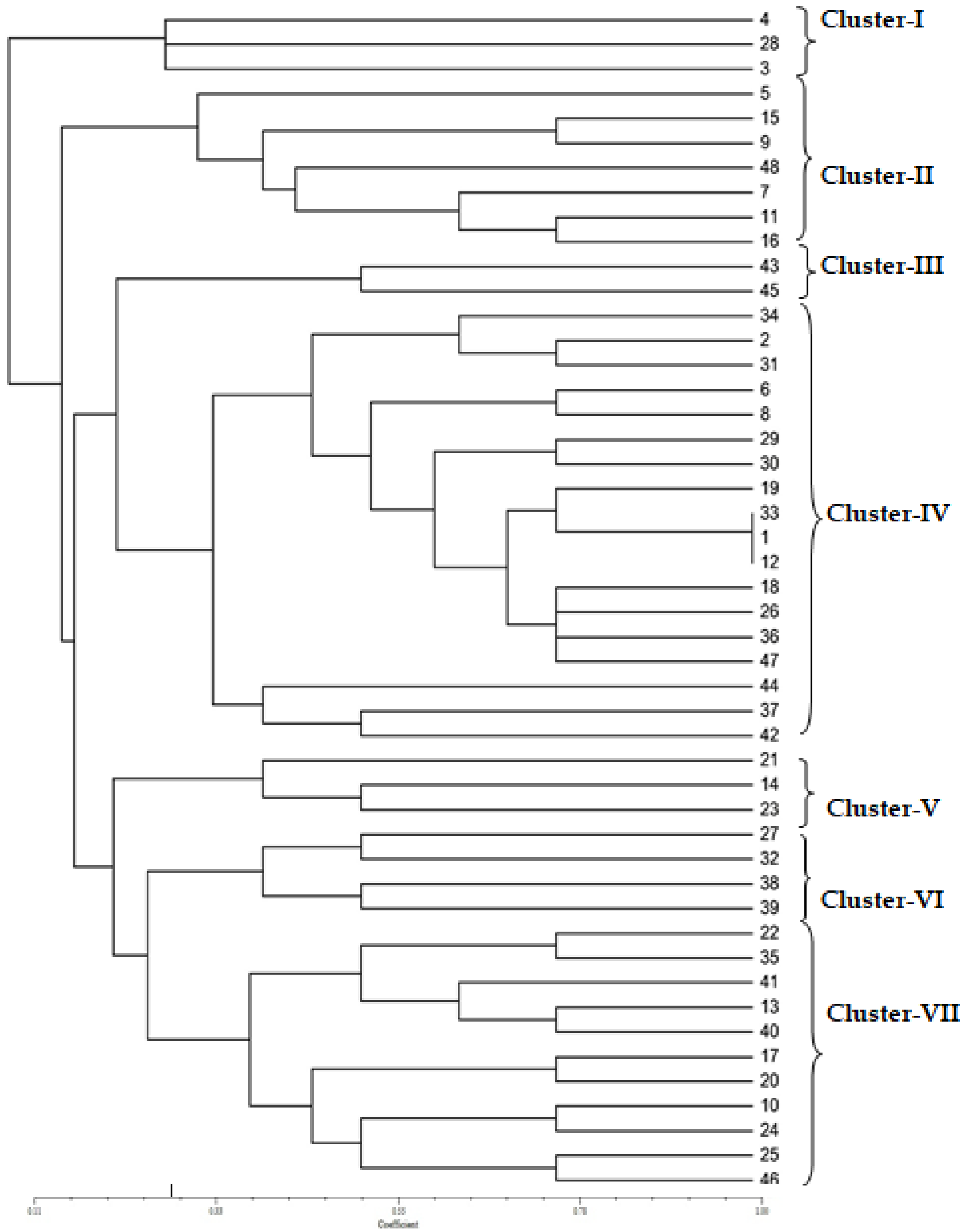

Figure 1. A UPGMA cluster dendrogram showing the genetic relationships among 48 brinjal genotypes detected by SSR markers. 01 = Morich Begun (Small), 02 = $IndD_{150}L_8P_4$, 03 = BARI Begun-1, 04 = Thama, 05 = BARI Begun-7, 06 = Thapa, 07 = Indian-1, 08 = Morich Begun, 09 = Khatkhatia Long (Green), 10 = Uttara (Violet), 11 = Singnath (Long, Violet), 12 = Borka (Long, Green), 13 = China Long, 14 = Laffa (Long, Violet), 15 = Kailkkah (Long, Green), 16 = Laffa BAU (Long, Violet), 17 = Islampuri BS, 18 = Pahoza-2, 19 = Puta Begun, 20 = Longla Tal Begun, 21 = Irri Begun (Round, Green), 22 = Dhondol Begun (Long, Violet), 23 = Khatkhatia Long (Violet), 24 = $IndD_{150}L_1P_{27}$, 25 = $IndD_{300}L_{13}P_{11}$, 26 = Purple Long, 27 = $IndD_{300}L_{12}P_{12}$, 28 = Kaikka-N (Violet), 29 = BAU Begun-1, 30 = $ZumD_{150}L_1P_{22}$, 31 = Darata (Round, Green), 32 = Dohazari (Long, Violet), 33 = Laffa Long Violet-G, 34 = $ZumD_{150}L_{13}P_{21}$, 35 = $ZumD_{300}L_{12}P_5$, 36 = $ZumD_{300}L_{13}P_{12}$, 37 = $IslD_{150}L_{13}P_{22}$, 38 = $IslD_{150}L_{13}P_{14}$, 39 = Dohazari (Green), 40 = Pahoza-1, 41 = Salta, 42 = Apple Begun (Round, Violet), 43 = $IslD_{300}L_5P_3$, 44 = $IslD_{150}L_{14}P_{23}$, 45 = Jessore Local, 46 = $IslD_{150}L_2P_{25}$, 47 = Zumka, 48 = $IslD_{300}L_{12}P_{19}$.

identified. The AT repeat has also been frequently identified in other genic and genomic SSRs in eggplant. Herrera et al. (2008) recorded SSR markers with ATT, GA, TAA and GTT motifs showed the maximum variation in allele size; James et al. (2000) observed that ATT motifs showed the maximum variation in allele size. Stagel et al. (2008) found that CCG/CGG and AGG/CCT are the most common monocotyledonous EST-SSR motifs (18,24,29) and were under-represented in dicotyledonous species as well as in the present dataset, which is less polymorphic than other motif. Adeniji et al. (2012) investigated polymorphism was fairly high (0.05 to 0.92) among SSR markers with high number of repeats.

According to Nei's (1973), the highest level of gene diversity value 0.8672 was observed in loci smSSR11 and the lowest level of gene diversity value 0.6580 was observed in the loci smSSR14 with a mean diversity of 0.7847. It was observed that there is minimum difference in loci smSSR11 and smSSR01 in terms of gene diversity. The maximum allele size range is 315 bp, which is in locus smSSR01 and showed the second highest genetic diversity. Considering the genetic diversity a number of SSR markers have been identified in Solanaceae (Yi et al., 2006; Bindler et al., 2007), but the numbers are less in eggplant. The development of SSR markers derived from SSR-enriched genomic library of eggplant has been reported by Nunome et al. (2003b, 2009). SSR markers have been used in determination of genetic diversity in eggplant (Nunome et al., 2003a, 2003b; Stagel et al., 2008; Nunome et al., 2009).

As a measure of the informativeness of microsatellites, the PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660 (Table 3). The results of the study supported by Vilanova et al. (2014), recorded the polymorphism information content (PIC) of SSR markers ranging from 0.07 to 0.77, with an average value of PIC=0.50. Also SSRs was selected by Caguiat and Hautea (2014) based on their high polymorphism information content (PIC) and the high quality of bands.

The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. Basically this value is an indication of their genetic dissimilarity. Genotype pair with higher value is more dissimilar than a pair with lower value. The value of pair-wise comparisons of Nei's (1973) genetic distance (GD) between genotypes were computed from combined data for the four primers, ranging from 0.250 to 1.000.

Considering the genetic distance values the result indicates that the genotypes of brinjal were used in the present study genetically different from each other. This proportion of genetic distance are compared from some previous analysis in brinjal such as Verma et al. (2012) recorded the maximum genetic distance of 1 was found between Pusa Bhairav and Green Long, Green Long and KS-224, Green Long and SL-195, Green Long and KS331 and between Pusa Kranti and SL-195 followed by 0.85 between Pusa Kranti and KS-224, and NDB-25 and Pusa Kranti. Demir et al. (2010) recorded that the genetic similarity according to SSR data was scaled between 0.15 and 1, suggesting the potential of SSR markers in discriminating among plants of close or distant genetic backgrounds.

From this study, the dendrogram revealed that the genotypes that are derivatives of genetically similar type form cluster together. Maximum brinjal genotypes includes cluster IV.

Considering the genetic similarity the result supports the previous findings in brinjal such as Ansari and Singh (2015) who recorded six cluster groups using SAHN cluster analysis UPGMA method which revealed that morphological characters *viz.*, shape, size and peel colour of fruits and plant type showed a positive relationship with the DNA based molecular analysis through SSR markers; Demir et al. (2010) found that UPGMA dendrograms were used to examine the genetic relatedness of the genotypes. Khorsheduzzaman et al. (2008) estimated genetic similarities of SSR profiles based on Jaccard's coefficient value. The dendrogram generated two clusters and they were clearly distinct and separated from each other. Cluster-I consisted of genotypes TURBO and BL009; and cluster-II comprised genotypes EG058, EG075 and ISD006. Genotype TURBO and BL009 were identified as the diverse genotype and showed a maximum of 17% dissimilarity from EG058, EG075 and ISD006. The similarity value ranged from 0.83 to 1.00 which indicated the presence of narrow range of genetic diversity at molecular level. It has been reported that SSR markers were ideal markers for constructing high resolution genetic maps in order to identify similarity between different species within a single genus (Provan et al., 1999). Nunome et al. (2003a) evaluated primer SSR for *S. melongena* and its related species and found them most suitable for brinjal. SSRs used in this study provided important information about the genetic diversity and relationships among brinjal genotypes.

Conclusion

Five random primers were initially screened and finally four primers smSSR01, smSSR03, smSSR11 and smSSR14 were selected for the analysis. The microsatellite loci were also multi-allelic (9.0 to 14.0 alleles per locus with a mean of 11.0 alleles per locus in the present study) and the alleles were co-dominant suggesting their relative superiority in detecting DNA polymorphism over some other markers. According to Nei's (1973), the highest level of gene diversity value 0.8672 was observed in loci smSSR11 and the lowest level of gene diversity value 0.6580 was observed in the loci smSSR14 with a mean diversity of 0.7847. As a measure of the informativeness of microsatellites, the

PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660. As a measure of the informativeness of microsatellites, the PIC value ranges from a low of 0.6413 (smSSR14) to a high of 0.8536 (smSSR11) and averaged 0.7660. All 48 brinjal genotypes could be easily distinguished. From this study, the dendrogram revealed that the genotypes that are derivatives of genetically similar type form cluster together. Maximum brinjal genotypes include cluster IV. These genotypes can be of a potential value for the breeders with wider genetic base that increase the present eggplant collection and to widen the genetic diversity of currently cultivated eggplant varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Milk production, marketing practices and qualities along milk supply chains of Haramaya District, Ethiopia

Mitiku Eshetu*, Mekdes Seyoum and Yesihak Yusuf Mummed

School of Animal and Range Sciences, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia.

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A study was conducted with the aim of evaluating the physicochemical properties and microbial qualities of raw cow's milk along the milk supply chain in Haramaya district. A total of 123 smallholder dairy producers, one dairy cooperative, 3 milk collectors, 6 milk retailers, 8 selling points and 18 consumers were interviewed using a semi-structured questionnaire. Sixty raw milk samples were used for physicochemical and microbiological analysis. Random sampling method was used to collect samples from producers and purposive sampling method was used to collect samples from other sampling sources. The overall mean values for temperature, pH, specific gravity and titratable acidity of marketed milk in the study area were 24.07°C, 6.32, 1.03, and 0.20, respectively, while the overall mean value of fat, protein, total solids, solids-not-fat, and lactose contents were 4.50, 3.24, 12.78, 8.28, and 4.27%, respectively. Significant differences (P<0.05) were found between the sources of milk samples on all measured parameters of chemical composition except fat content. The overall mean total bacterial, coliform, and yeast and mould counts were 5.48, 4.96 and 4.90 log cfu/ml, respectively. In conclusion, the result of this study indicated that milk samples collected from all sampling points were subjected to microbial contamination and did not meet quality standards set by Quality Standard Authority of Ethiopia. It is, therefore, recommended that awareness should be created among stakeholders involved in milk production and handling practices on method of quality milk production and marketing in the study areas.

Key words: Raw milk, milk value chain, physicochemical properties, microbial quality.

INTRODUCTION

Cattle are the main source of milk (95%) in Ethiopia (CSA, 2010). It is also an economically important farm commodity and investment option for smallholder farmers in the country (Zelalem et al., 2011). However, the Ethiopian per capital milk consumption was much lower (17 kg) compared to that of African average which was about 62.5 kg recommended as a minimum level to satisfy the need for a balanced diet and the world's per capital average which was about 100 L/year (FAO, 2010).

In Ethiopia, the milk marketing system is not well developed and for the majority of smallholder producers, access to market is limited. In year 2010, for instance, only less than 7% of the annual milk production was estimated to be marketed at national level. In 2009, there were 180 cooperatives involved in milk production and marketing in the entire nation, accounting for only 2% of

*Corresponding author. E-mail: mitikuguya@yahoo.com.

agriculture based cooperatives. These small proportions of existing dairy cooperatives were operating in areas that are accessible to transportation and market. This has resulted in the inability of substantial amount of milk to rarely reached the market in demand of the commodity (Zelalem, 2012).

In addition to low level of milk production, post-harvest handling of the product contributed to significant loss along the value chain. The quality of milk was compromised by not only milk producers but also by milk collectors and transporters, vendors and consumers across the supply chain (Mattias, 2013). Unhygienic conditions of milking, unclean conditions of handling equipment and the use of contaminated cleaning water were reported among the important sources of milk contamination (Zelalem, 2012).

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. It contains almost all nutrients required for the growth of newborn; and protein, fat, and lactose are the major component of milk. Because of its physicochemical properties, it needs strict hygienic condition to avoid contamination of milk with microorganisms. Therefore, examination of physicochemical properties and microbial load is a major factor in determining milk quality.

A research report at Harar Milk Shed in Eastern Ethiopia revealed that the physicochemical and microbiological properties of raw cow milk samples collected from various supply chain actors were not significantly different (Estifanos et al., 2015). But there is scanty information on the physical properties, chemical composition and microbial load of raw milk along the milk chain in the study areas. This research was therefore conducted to evaluate handling practices, physicochemical properties, and microbial qualities of milk along the market supply chain in Haramaya district, Eastern Ethiopia.

MATERIALS AND METHODS

Description of the study area

Haramaya district is located at 510 km east of Addis Ababa along the main road to Harar town. The altitude of the district ranges from 1400 to 2340 m above sea level. The area is located at 41°59'58"N latitude and 09°24'10" E longitude. The mean annual rainfall is 492 mm ranging from 118 to 866 mm. The district has mean annual temperature and relative humidity of 18°C and 68%, respectively. The district has two ecological zones of which 66.6% mid land and 33.3% low land (CSA, 2009).

Sampling techniques and data collection

The study has two parts, that is, field survey and laboratory analysis. The field survey was conducted to assess milk production and handling practices. For the survey part, 3 *kebeles* (Beftu Geda, Ifa Oromia, and Tuji Gebisa) were purposively selected from Haramaya District based on size of cattle population and availability of dairy cooperative. The required sample number of farmers was determined based on the formula suggested by Yamane (1967). Accordingly, 123 sample farmers were randomly selected from selected *kebeles* of Haramaya District having lactating cow on proportionality basis at 95% confidence level with degree of variability of 0.05 level of precision which was recommended to obtain a sample size, required to represent a true population. The total number of farmers having lactating cow was 178.

$$n = \frac{N}{1 + N(e^{2)}}$$

where n=sample size, N=population size and e=level of precision.

Milk samples were collected from a subset of farmers and purposively selected milk supply chain actors that include milk cooperative, milk collectors, retailers, selling points (who sold boiled milk) and consumers in the district.

Laboratory work

Milk samples were collected for the determination of the physical properties, chemical composition and microbial qualities. A total of 60 milk samples were collected for laboratory analysis directly from sample farmers, dairy cooperative (pooled milk), milk collectors, retailers, selling points and consumers. The sampling was done from the different sampling source (milk market chain actors) proportionally based on survey results (Table 1 and Figure 1).

A semi-structured questionnaire was developed, pre-tested and used to collect the required information from sample farmers. The questionnaires were administered through face to face conversation with sample farmers who had lactating animals during the time of data collection. While administering the questionnaires, the general cleanliness and hygienic practices of milk production and handling was also noted through personal observation. Interviews were conducted at the farm site and/or female and male headed household were involved. From smallholder producers the assessment focused on the hygienic handling practices during milk production such as barn type and cleaning practices, source of water used for milk utensils cleaning purpose, milker hygiene and milk utensils, type of container used for milk storage, transportation and marketing. Questionnaires survey was also used for data collection from cooperative, milk collectors and retailers, selling points and consumers.

The physical, major chemical composition and microbial load of the raw milk were analyzed at Plant Pathology and Dairy Technology Laboratory of Haramaya University. About 250 ml of raw milk samples were collected in a sterilize glass bottles. Samples were labeled and put in an icebox maintained at 4°C to limit microbial multiplication and transported to the laboratory and transferred into a refrigerator adjusted at a temperature of 4°C. Then, the milk samples were analyzed for microbial qualities within 24 h of sampling as described by APHA (1992). Samples from the farmers were collected during early morning. Milk samples from cooperative were collected at midday and samples from the milk collectors, retailers, and selling points were collected in the afternoon as milk reached the market. Samples from consumers were collected during morning until evening.

Physicochemical and microbial analysis

Physical quality of raw milk

The temperature of milk samples was determined at the collection point using thermometer while the pH of the milk samples was determined in the laboratory using a digital pH meter based on the procedure described by O'Connor (1995). To determine specific

Table 1. Sampling layout for survey and laboratory work.

Milk supply chain actors	Name of kebeles/cooperative	Number of respondent taken for field survey	Milk samples collected for laboratory analysis
	Beftu Geda	43	11
Milk producers	Ifa Oromia	48	13
	Tuji Gebisa	32	9
Cooperative	Jiru Siresa* Milk and Milk Product Processing and Marketing Cooperative	1	2
Milk collectors	-	3	3
Retailers	-	6	6
Selling points	-	8	2
Consumers	-	18	14
Total	-	159	60

*We get permission to use this name from the dairy cooperatives and interview was made with managing board of the cooperatives that were mainly involved in day to day activities of the cooperative.

gravity, fresh milk samples were filled sufficiently into graduate cylinder (100 ml capacity and a lactometer were held by the tip and inserted into the milk. The lactometer was allowed to float freely and then records were taken (O'Mahony, 1988). The following formula was used to calculate the specific gravity of the milk.

$$\text{Specific gravity} = \frac{L}{1000} + 1$$

where L=corrected lactometer reading at a given temperature. For every degree above 15.5°C, 0.2 was added to lactometer reading but for every degree below 15.5°C, 0.2 was subtracted from the lactometer reading (O'Mahony, 1988).

For the determination of titratable acidity, 10 ml of raw milk samples was pipetted into a beaker and three drops of 0.5% phenolphthalein indicator was added into the milk and then titrated with 0.1 N sodium hydroxide (NaOH) solution until faint pink colour persists (O'Mahony, 1988). Acidity was expressed as percent lactic acid (O'Connor, 1994) and calculated as:

$$\%\ \text{lactic acid} = \frac{ml\ of\ 0.1\ N\ NaOH * 0.009 * 100}{ml\ of\ milk\ sample\ used}$$

where ml = millilitres of NaOH and milk samples used.

Clot on boiling test was performed by boiling a small amount of milk in a test tube or any other suitable container. When there was coagulation or precipitation, the milk sample was not considered as fresh milk. The test is not sensitive to slightly sour milk (O'Connor, 1995). Alcohol test was done by mixing equal amounts of milk and 68% ethanol (usually 2 ml) in a small bottle or test tube. When there was coagulation or precipitation up on shaking the milk sample was not considered as fresh milk (O'Connor, 1995).

Chemical composition of milk

Fresh milk samples were analyzed for determination of the major chemical composition of the milk samples namely total-solid (TS), solids-not-fat (SNF), fat, protein and lactose using calibrated milk Milkoscan FT1 (Model MilkoScan™ FT1- FOSS, Hillerød, Denmark).

Microbiological analysis of milk

Milk samples were analyzed for total bacterial, coliform and yeast and mould counts following standard procedures (Richardson, 1985). All milk samples were kept cooled at 4°C until analysis. For bacterial count analysis, 1 ml of milk sample was diluted with 9 ml of peptone water. Each culture was constituted of 1 ml of the diluted solution poured on a Petri dish, on which 12 to 15 ml of Standard Plate Count Agar (SPCA) was added. The media were prepared according to the guidelines given by the manufacturers. When the solution in the Petri dish solidifies, it was put into incubator at 32°C for 48 h. After incubation, all colonies including those of pin point size in SPCA medium were counted. When the colonies found to be too many, compromising the accuracy of counting the same procedure was repeated using higher dilution levels as recommended by Francesconi (2006). For analysis purpose, only counts in the normal (25-300) colonies were taken directly (APHA, 1992). The following formula was used to calculate the counts for total bacterial and coliform counts.

$$\mathrm{N} = \frac{\sum c}{[(1 \times n1) + (0.1 \times n2)]d}$$

where N = number of colonies per ml of milk; Σ C = sum of all colonies on all plates counted; n1 = number of plates in first dilution counted; n2 = number of plates in second dilution counted; d = dilution from which the first counts were obtained.

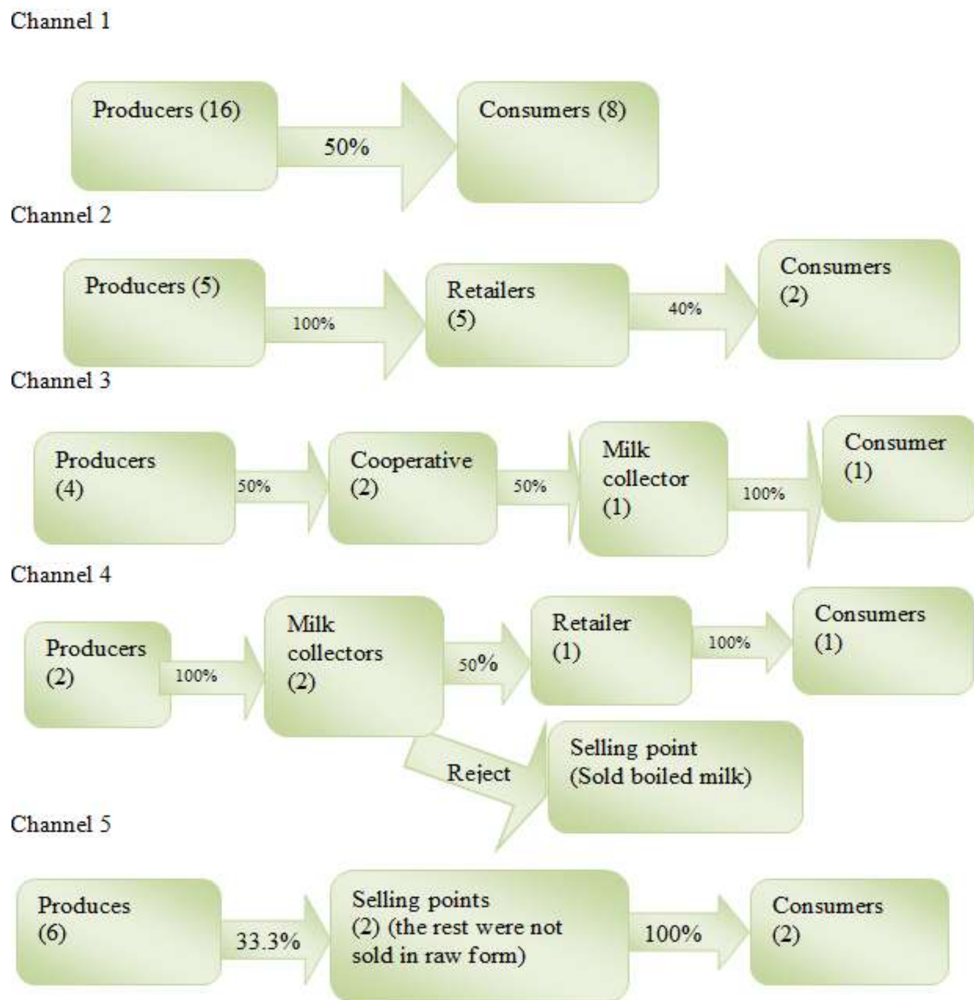

Figure 1. Sampling layout for laboratory work in each channel (numbers in parenthesis are number of milk samples).

For coliform count, 1 ml samples of milk were serially diluted with 9 ml of peptone water following similar methods as for total bacterial count but dilutions were surface plated on Violet Red Bile Agar and incubated at 32°C for 24 h, after which typical coliform colonies were counted. Purplish red colonies in VRBA medium were counted under colony counter less than 100 coliform colonies were recorded. For colonies beyond this count, the next dilutions were plated. For yeast and mould count, 1 ml milk samples were serially diluted with 9 ml of peptone water following similar methods as for total bacterial count but dilutions were surface plated on Potato Dextrose Agar (PDA) (Oxoid, Pvt. Ltd. MU 096: UK). The dried plates were then incubated at 25°C for 3 to 5 days. Colonies with a blue green color was counted as yeasts and moulds (Yousef and Carlstrom, 2003).

Statistical analysis

Survey data were analyzed using descriptive statistics such as means, frequency distribution and percentage using SPSS version 20.0 Software (SPSS, 2007). Microbiological counts were first transformed into logarithmic value (log10 cfu ml^{-1}) to get normally distributed data. Data on the physicochemical quality and log 10 transformed microbial counts were analyzing using General Linear Model (GLM) procedure of SAS (SAS, 2003). Mean comparison was done using the Duncan Multiple Range test for variables that the F-values showed significant difference at 5% significance level.

The statistical model used was:

$Y_{ij} = \mu + Mi + \varepsilon_i$.

where Y_{ij}= dependent variable which was milk quality parameters (microbiological qualities and physic-chemical qualities), μ=overall mean, Mi= milk sources (producers, cooperative, milk collectors, retailers, selling points and consumers), and ε_i= error term.

RESULTS AND DISCUSION

Household characteristics

Household characteristics of respondents in the study area are shown in Table 2. Most respondents in the present study were male (92.7%) as they were head of the family. The respondents in the study area had

Table 2. Household characteristics of respondents in the study areas.

Variable category	Beftu Geda (N=43)		Ifa Oromia (N=48)		Tuji Gebisa(N=32)		Overall mean [Total (N=123)]	
	N	%	N	%	N	%	N	%
Sex of family head								
Male	41	95.35	44	91.67	29	90.63	114	92.68
Female	2	4.65	4	8.33	3	9.38	9	7.32
Age category of respondents								
21-30	2	4.65	7	14.58	7	21.88	16	13.01
31-40	32	74.42	25	52.08	21	65.63	78	63.41
41-50	8	18.60	16	33.33	3	9.38	27	21.95
>50	1	2.33	-		1	3.13	2	1.63
Education level of the respondents								
Illiterate	40	93.00	42	87.50	28	87.50	110	89.43
Read and write	-		1	2.10	1	3.13	2	1.63
1-4	2	4.65	4	8.33	1	3.13	7	5.69
5-8	-		-		1	3.13	1	0.81
9-10	1	2.33	1	2.10	-	-	2	1.63
Religious education	-				1	3.13	1	0.81

different educational status with majorities being illiterate (89.4%). Most of the respondents (63.41%) were in age group of 31 to 40 years.

Feed resource and breeding practice in the study area

Feed resource and breeding practice in the study area are shown in Table 3. About 74% of the interviewed dairy farmers had grazing land which implied that grazing was the major sources of feed in the study area. Next to grazing on-farm improved forage and crop residues were supplemented by industrial byproducts. In mixed farming system, crop residues are mainly used as source of livestock feeds together with natural pastures. The dominant crop residue available and used as feeding options for dairy production includes maize and sorghum stovers (Kedija et al., 2008). Farmers in the study area also grow sorghum and maize as fodder for livestock by intentionally over sowing above the recommended seeding rate. From the factory outlets, farmers mainly purchase wheat bran and common salt. The major three feed resources mentioned earlier were reported as critical constraints of milk production and particularly the price of industrial by product was increased from year to year. It is becoming unaffordable for farmers and had impacted negatively the supply from smallholder side. This has restricted the supplementary concentrate to lactating cows only. Inability to formulate feed was the other limitation for improved feeding.

Gender roles in milk production and marketing

The role of gender in dairy herd management in the study area is shown in Table 4. Overall, dairy farm operation was mainly carried out by female members of the household. Milk marketing is a specialized activity for female members of the household. Only 1.64% of the respondents indicated that males take part in milk marketing provided that the woman was occupied with other activities and the milk was sold to indirect

Table 3. Feed resource and breeding practice in the study area.

Variable category	Beftu Geda (N=43)		Ifa Oromia (N=48)		Tuji Gebisa (N=32)		Overall mean [Total (N=123)]	
	N	%	N	%	N	%	N	%
Feed regularly used								
Grazing	39	90.70	37	77.08	15	46.88	91	73.98
Did not have grazing land	4	9.30	11	22.92	17	53.13	32	26.02
Breeding								
Natural	34	79.10	36	75.00	21	65.60	91	74.00
AI	1	2.30	8	16.70	7	21.90	16	13.00
Both Natural and AI	8	18.60	4	8.30	4	12.50	16	13.00

Table 4. The role of gender in dairy farm operation and herd management in the study area.

Activity	Household member						
	Total HH	Female		Male		Both	
		No.	%	No.	%	No.	%
Dairy farm operations							
Milking cows	123	123	100.00	0	0.00	0	0.00
Cleaning of milk containers	123	123	100.00	0	0.00	0	0.00
Barn cleaning	123	123	100.00	0	0.00	0	0.00
Milk marketing	122	120	98.36	0	0.00	2	1.64
Herd management							
Feeding of dairy animals	123	29	23.60	63	51.20	31	25.20
Health management	123	46	37.40	43	35.00	34	27.60
Live animal marketing	123	30	24.40	48	39.00	45	36.60
Buying dairy inputs	123	43	35.00	38	30.90	42	34.10

marketing channels. Sintayehu et al. (2008) indicated that in 60% of the cases housewives and/or other female household members were involved in milking operations in urban dairy production system of Shashemene and Dilla areas. Zewdie et al. (2016) also reported that milk marketing is a specialized activity for female members of the household in Fafem Zone, Ethiopian Somali Regional State. Regarding to herd management such as feeding of dairy animals, health management, live animal marketing and buying dairy inputs both male and female members were responsible. This indicated that the contribution of women in dairy production was important and promoting the women in the dairying practices and marketing can enhance productivity, effectiveness and efficiency of the sector.

Milk production

The overall average amount of milk produced by local cow breeds was 2.23 L/day. About 2.4% of the participants have three milking cows, 30.9% own two milking cows, and 66.7% own only one milking cow per household. As indicated in Figure 2, most of the milk produced (61.54%) was marketed and about 38.11% was retained for home consumption and only 0.35% milk was processed into other dairy products like *Ergo*. Most (72.13%) of the milk was sold directly to the consumers and the remaining 2.46% to milk collectors, 5.74% to cooperative, 8.2% to retailers and 11.48% to selling points. The marketing system of milk in the Haramaya District can generally be characterized as informal type.

Hygienic quality of milk during productions

Housing system and barn cleaning

About 85.37% of the respondents keep their cattle in separate barns from family house and 14.63% keep inside family house (Table 5). As observed during the field visit, all barns (100%) were not constructed to facilitate drainage of the farm wastes, which leads to soiling of dairy cows and contamination of milk. All respondents do not use bedding materials for the animals. Yitaye et al. (2009) reported a similar case for farms in

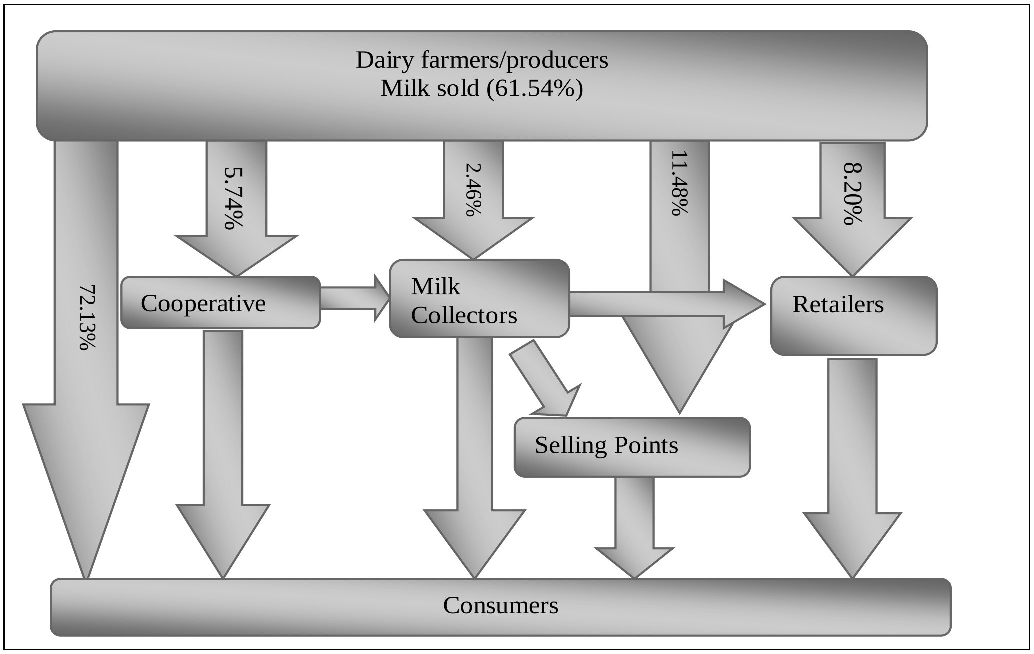

Figure 2. Actors involved in milk marketing of milk in the study area.

Table 5. Types of housing and barn cleaning frequency.

Variable	Beftu Geda (N=43)		Ifa Oromia (N=48)		Tuji Gebisa (N=32)		Total (N=123)	
	N	%	N	%	N	%	N	%
Type of housing								
Separate dairy house	36	83.72	41	85.42	28	87.50	105	85.37
Inside family house	7	16.28	7	14.58	4	12.50	18	14.63
Cleaning frequency								
Daily	32	74.42	36	75.00	28	87.50	96	78.05
Thrice/Week	7	16.28	8	16.67	2	6.25	17	13.82
Twice/Week	1	2.33	4	8.33	2	6.25	7	5.69
Once/Week	3	6.98	-	-	-	-	3	2.44

Northwestern Ethiopian highlands. Abebe et al. (2012) reported that 90.8% of households share the same house with their animals, while 9.2% of the households used separate houses for the cows in Ezha District of Gurage Zone. Practices that expose the teat end to organic bedding sources, wet and muddy pens increase the risk of occurrence of mastitis and milk contamination (Ruegg, 2006). Mbabazi (2005) reported that milking of cows in undesignated poorly maintained milking shades/parlors predisposes the milk to contamination and spoilage.

Most of the respondents (78.05%) reportedly remove manure daily (Table 5). Zelalem (2010) reported similar result for smallholder farms in Central Highland of Ethiopia in which smallholder farmers clean barns on daily basis (87%), once or twice a week (9%) and did not clean at all (4%). Maintaining the sanitary condition of the milking area is an important prerequisite for clean wholesome milk production, and hence daily removal of manure and cleaning of barn is recommended.

Hygienic practices during milking operations

The majority of the households (83.7%) reported that they did not clean animal shed before milking (Table 6). All the interviewed household milk cows using hand milking after either washing cow teats or after the calf suckle the dam to stimulate milk let-down. Hygienic practices are major pathways to produce safe and quality products for consumers thereby reduces microbial contamination and loss of product. Cleaning the udder of cows before milking is one of the most important hygienic

Table 6. General hygienic practices during milking operations in the study area.

Variable	Beftu Geda (N=43)		Ifa Oromia (N=48)		Tuji Gebisa (N=32)		Total (N=123)	
	N	%	N	%	N	%	N	%
Clean animal shed before milking								
Yes	7	16.30	6	12.50	7	21.90	20	16.30
No	36	83.70	42	87.50	25	78.10	103	83.70
Hand washing								
Wash hands before milking	40	93.02	45	93.75	31	96.88	116	94.31
No washing	3	6.98	3	6.25	1	3.13	7	5.69
Wash cow's teats before milking								
Warm water	7	16.28	11	22.92	1	3.13	19	15.45
Cold water	24	55.81	26	54.17	22	68.75	72	58.54
Both cold and warm water	3	6.98	2	4.17	1	3.13	6	4.88
No washing	9	20.93	9	18.75	8	25.00	26	21.14
Towel used for udder drying								
Common towel	2	4.70	1	2.10	1	3.10	4	3.25
No drying	41	95.30	47	97.90	31	96.90	119	96.75

practices required to ensure clean milk production. This is important since the udder of milking cows could have direct contact with the ground, urine, dung and feed refusals (Zelalem, 2010). Improper milking practices can predispose milk to contamination for instance milking can be done following calf suckling without cleaning the teats and same reports indicated that saliva from the calf mouth and unwashed teats increases bacterial counts of milk (Kurwijila, 1989).

Majority of respondent milk producers (94.31%) reported to wash their hands before milking in the study areas (Table 6) and the proportion was higher in Tuji Gebisa than that of Beftu Geda and Ifa Oromia. Similarly, Tadele et al. (2016) reported that in Eastern Hararghe, milk producers wash their hands before milking. However, none of them use warm water and detergent for hand washing but use cold water without detergent. Washing hands with cold water without detergent is not sufficient to remove germs and can serve as a major source of microbial contamination of milk. Therefore, milk handlers should always wash their hands with warm water and detergent, and then dry it properly with proper towels before start of milking, milk handling or preparation (Zelalem, 2012). Hand washing (especially in the developing countries) between milking, during, pre and post milking stages using safe disinfectants can reduce bacterial load and enhance production of safe fresh milk (Oliver et al., 2005). The milker can be an important source of milk contamination. In addition to keep good personal hygiene, milkers should be in good health during milking operation (Zelalem, 2010).

The use of individual towel and following essential cleaning practices during milking is important for the production of quality milk (Zelalem, 2010). However, about 96.75% of the households reported that they did not use towels for udder drying before milking but massage the udder with hand to stimulate milk let down, and only 3.25% of the respondents use common towel for udder drying. Milking in dry condition significantly reduces bacterial count. This is because there is no surplus water remains on the surface of the udder to drip into the milk and less chance of contamination by dirt and bacteria from udder, teats and hands into milk (Islam et al., 2009). In Ethiopia, there is no standard hygienic condition followed by producers during milk production. The hygienic conditions are different according to the production system, adapted practices, level of awareness, and availability of resources (Zelalem, 2003).

Milk equipment and sanitary practices

One of the major factors affecting the quality of dairy products is milking utensils. The interviewed milk producer households reported to mainly use traditional gourd vessels (67.48%) and plastic jerry can (28.45%) (Table 7). For transport purposes, traditional gourd vessels are dominantly used (59.02%) followed by plastic jerry cans (40.98%) at producer level. All sample milk collectors and selling points as well as 83.3% of retailer's reported to uses plastic jerry cans for milk collection and storage (Table 8). In general, plastic jerry cans were the dominant type of containers used for milk marketing and storage; while traditional gourd vessels are mainly used for milking. Such utensils can contribute for the rapid spoilage of milk, as plastic jerry cans cannot be cleaned

Table 7. Milk equipment used for milking, transportation and sanitary practices in the study areas.

Variable	Biftu Geda		Ifa Oromia		Tuji Gebisa		Total	
	N=43	%	N=48	%	N=32	%	N=123	%
Milk utensils used for milking								
Traditional Gourd vessel	27	62.80	38	79.17	18	56.25	83	67.48
Aluminum vessel	4	9.30	1	2.08	0	0.00	5	4.07
Plastic jerry can	12	27.90	9	18.75	14	43.75	35	28.45
Cleaning frequency of milk utensils								
Before every use	2	4.65	1	2.08	3	9.38	6	4.88
After every use	29	67.44	38	79.17	24	75.00	91	73.98
Before and after every use	12	27.91	9	18.75	5	15.63	26	21.14
Transportation	**N=42**	**%**	**N=48**	**%**	**N=32**	**%**	**N=122**	**%**
Traditional Gourd vessel	26	61.90	28	58.33	18	56.25	72	59.02
Plastic jerry-can	16	38.10	20	41.67	14	43.75	50	40.98

properly, due to their shape and narrow opening. This is in line with the findings of Yitaye et al. (2009), Teklemichael et al. (2015b) and Tadele et al. (2016) who reported that 83% of the surveyed urban dairy farms in Bahir Dar and Gondar, 75% of the surveyed farms in Dire Dawa Town and 87.5 to 97.5% milk producers, all milk collectors and transporters as well as vendors in Eastern Ethiopia were using plastic containers, respectively. The left-over of milk and other dirt particles within the container may result in contamination of the subsequent milk. Omore et al. (2005) also reported that lack of formal training and use of plastic containers are the main factors that contribute to the poor quality of raw milk sold by producers and informal milk traders. Since proper metal milk containers are expensive, milk producers use plastic containers which are difficult to clean and disinfect and thus it might contribute to poor quality of the milk. Non-food grade plastic cans, buckets and jerry cans are not appropriate thus must not be used for milk storage and transportation (Kurwijila, 2006). Aluminium containers are recommended because they do not have adhesive properties and therefore easy to clean as compared to plastic containers.

In the present study, the majority of the respondent in the study areas washed milk utensils after every use (Table 8). Moreover, all milk producers reported to smoke milk utensils. Smoking is done by using wood splinters of *'Ejersa' (Olea africana*). They assume that smoking is used to develop desirable flavor in the milk. In addition, smoking has anti-microbial activity and thus inhibits growth of microorganisms in milk (Mogessie and Fekadu, 1993).

Source of water for cleaning

The sample dairy cooperative, selling points (cafeteria and restaurants) and 66.7% milk collectors and 33.3% retailers reported to use tap water (Table 9). About 99.2% of the farmers and 22.2% of consumers reported the use of hand dung well water for cleaning. All of the respondents that reported to use water from non-tap sources were neither boil nor filter it before use. For production of quality milk, a good supply of clean water is essential. Water used for washing and rinsing milk equipment and milk containers should be of the same in safety and purity as drinking water (Younan et al., 2007). Jay (1992) also reported that water obtained from different sources such as wells, rivers and springs can easily be contaminated by human and animal organic wastes gaining entry by drainage.

Milk transportation and storage condition

All the respondent small-scale milk producers did not use cooling systems while storing milk before selling (Table 9). The sample dairy cooperative, 66.7% of milk collectors, and 75% of selling points kept milk in a refrigerator; while the rest stored at room temperature. Due to the absence of appropriate cooling systems at small-scale milk producer's level, milk is transported at ambient temperatures to selling points. FAO (2007) recommended that milk should be cooled below 4°C or processed and conserved well immediately after milking or processing. Therefore, it would be beneficial to have an access to cooling facilities for retarding bacterial growth in raw milk during collection and transportation to selling points.

Milk marketing

The sale of fresh whole milk is the common practice in

Table 8. Milk equipment and sanitary practices for traders and consumers.

Variable	cooperative (n= 1)	Milk collectors (n=3)	Retails (n=6)	Selling points (n=8)	Consumers (n=18)
	%	%	%	%	%
Types of containers used for milk collection, transportation and storage					
Traditional Gourd vessel	-	-	16.70	-	-
Plastic jerry can	-	100.00	83.30	100.00	-
Aluminum vessel	-	-	-	-	38.90
Plastic jerry-can and aluminum vessel	100.00	-	-	-	
Plastic water bottle	-	-	-	-	33.30
Plastic bag	-	-	-	-	27.80
Methods of cleaning milk containers					
Before every use	-	-	-	-	22.20
After every use	100.00	66.70	66.70	75.00	44.40
Before and after every use	-	33.30	33.30	25.00	33.40

Table 9. Water sources for cleaning and transportation condition at different actors in the supply chain.

Variable	Producers (%)	Cooperative (%)	Milk collectors (%)	Retailer (%)	Selling points (%)	Consumers (%)
Water source for cleaning purpose	**N=123**	**N=1**	**N=3**	**N=6**	**N=8**	**N=18**
Tap water	-	100.00	66.70	33.30	100.00	77.80
Hand dung well	99.20	-	33.30	66.70	-	22.20
Lake water	0.80	-	-	-	-	-
Storage condition						
At room temperature	100.00	-	33.30	100.00	25.00	77.80
Use of refrigerator	-	100.00	66.70	-	75.00	22.20

the study areas. Marketing system of milk is unorganized informal and is carried out through direct sellers (milk passes directly from the producer to the consumer) and indirect marketing channels where several agents operate between producers and consumers. The channel actors in marketing of milk in the study area include producers, milk collectors, retailers, milk cooperative, selling points (cafe and restaurants) and consumers. There were two different milk outlets identified under direct selling; namely traditional milk association group locally called *Faraqa Annanni* selling system and individual seller system. *Faraqa Annanni* is self-organized women groups who have milking cows and produce saleable milk. The number of women that participate in group ranges from 2 to 10. Members are organized to sell out whole fresh cow milk turn by turn. In the *Faraqa Annanni*, members contribute an agreed amount of milk on a daily basis and the collected milk is given to one of the member woman to sell out in the nearby market. The woman sells the milk and the daily income generated belongs to her. The cycle continues

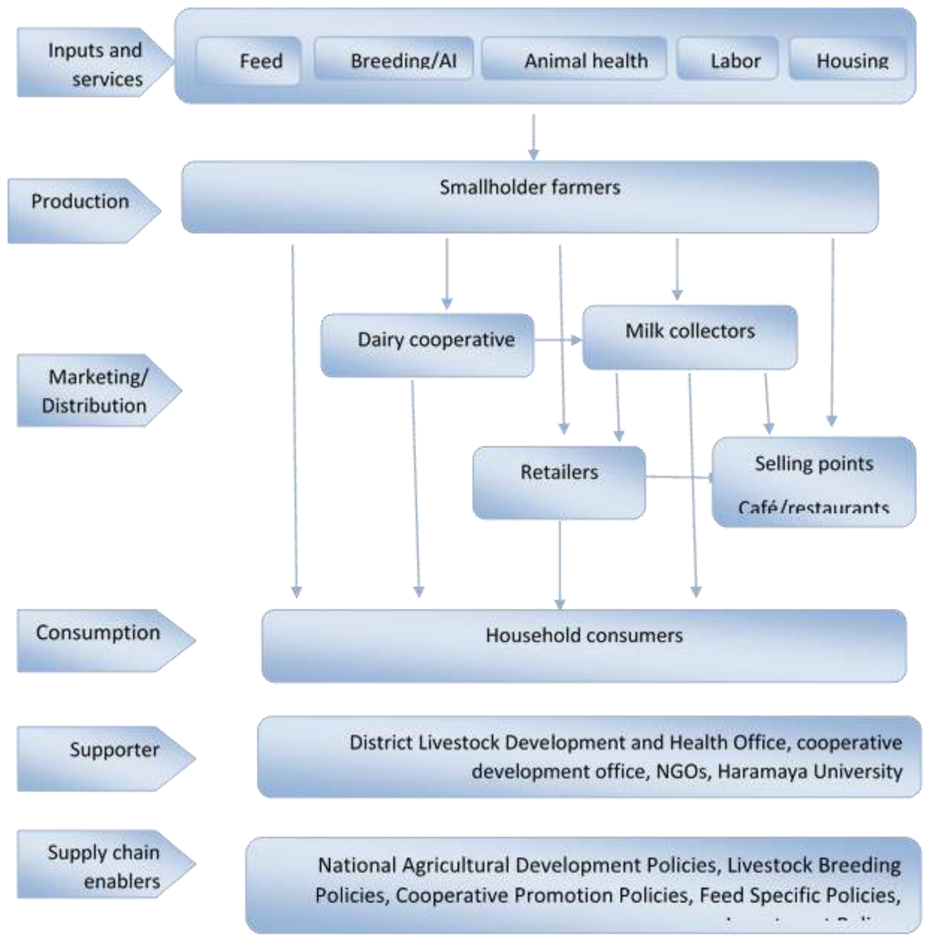

Figure 3. A generic schematic diagram representing fluid milk market chain at Haramaya district.

until every group member gets her share of the milk income. This system has several advantages. It saves time and labor spent because of daily travel to milk market places, which is mostly to nearby towns such as Bate, Haramaya, Awaday and Dengago. Moreover, it helps the women to save money since they obtain income on a weekly basis.

About 99% of the respondent sample households participate in milk marketing. In similar situation, Amistu et al. (2015) reported that 99% of the respondent sample farmers are involved in whole milk marketing in Holetta, Sebeta and Sululta area of Oromia Special Zone.

Fluid milk supply chain

A generic schematic diagram representing the fluid milk supply chain in the study areas based on the information gathered during the present assessment is sketched and presented in Figure 3. The main supply chain fractions identified are: input supply, production, marketing (distribution) and consumption. Similarly, the key actors along the chain include input suppliers, producers, distributor and consumers.

There are various milk marketing channels in the study areas through which smallholder dairy farmers channel their milk to dairy cooperative, milk collectors, retailers, selling points and consumers. About 94.3% of the sample milk producers channel milk through the informal marketing system. Retailers buy milk either from dairy farms or wholesalers/milk collectors. The retailers are involved in milk marketing to restaurants and cafeterias. Most of the dairy farmers in the study area prefer to sell milk through the informal chain where they get high price per liter of milk. In informal markets, sellers need to trade through bilateral bargaining so as to remain anonymous from the taxing authority whereas in formal markets, sellers can publicly advertise their prices and locations. Van der Valk and Abebe (2010) also reported that 98% of the milk produced in rural areas was sold through informal chain in Addis Ababa Ethiopia. Girma and Verschurr (2013) also reported 35% of the sample respondents to sell their milk both thorough the informal and formal channels; 25% of them through formal

Table 10. Results of alcohol and clot on boiling test of milk samples in the study areas.

Milk source	N	Positive results (%)	
		Alcohol test (%)	Clot-on-boiling test (%)
Producer level	33	12.10	3.00
Cooperative	2	50.00	50.00
Milk collector level	3	33.30	33.30
Retailer level	6	50.00	33.30
selling point	2	50.00	50.00
Consumers	14	64.30	57.10
Total	60	31.70	23.30

Table 11. Physical properties of raw cow milk samples in Haramaya district (Mean ± SD).

Milk source	Variable			
	Temperature (°C)	pH	Specific gravity	Titratable acidity
Producers	26.00±0.83[a]	6.66±0.26[a]	1.031±0.0036[a]	0.17±0.059[b]
Cooperative	24.50±0.71[b]	6.17±0.46[ab]	1.027±0.0007[b]	0.21±0.007[ab]
Milk collectors	24.30±0.58[b]	6.26±0.44[ab]	1.026±0.0010[b]	0.19±0.055[ab]
Retailers	24.00±0.89[b]	6.06±0.44[ab]	1.025±0.0019[b]	0.22±0.04[ab]
Selling points	21.00±0.00[c]	6.14±0.54[ab]	1.024±0.0014[b]	0.24±0.014[ab]
Consumers	19.90±0.77[c]	5.67±0.78[b]	1.026±0.0019[b]	0.26±0.019[a]

[abc]Different superscripts in the same column differ significantly (P<0.05).

marketing channels and the remaining 40% through the informal marketing channels in Ada'a District East Shawa Zone of Oromia Regional State.

Physicochemical properties

Alcohol and clot on boiling tests

From the total number of collected milk samples, 31.70 and 23.30% were positive to alcohol and clot on boiling tests, respectively (Table 10). The highest values for positive alcohol tests were recorded for samples collected at consumer level. This result shows that milk quality decline as the milk moves from producer to consumers. Rapid elevation of milk acidity is more than 0.21% acid results in coagulation of the milk proteins thus the milk is positive on alcohol test (Pandey and Voskuil, 2011). These observations support the view that alcohol test is more sensitive than the clot on boiling test (O'Connor, 1994). Zelalem (2010) reported that 21% of milk samples checked with alcohol test were positive, while only 14% of the samples were positive for clot on boiling test for samples collected from the Central Highland of Ethiopia. The report of Alganesh (2002) also indicated that 58 and 21% of cow milk samples collected from smallholder farmers in Eastern Wollega of Oromia Region were positive to alcohol and clot on boiling tests, respectively. Asaminew and Eyassu (2011) also reported that 51% of smallholder and dairy cooperatives milk sample clot by alcohol test and only 23% clot on boiling test in Bahir Dar Zuria and Mecha District.

Temperature and pH

The mean temperature of raw milk samples was significantly different ($P < 0.05$) among milk sample sources (Table 11). The temperature of milk samples collected from milk producer households was significantly higher than those collected from cooperative, milk collectors, retailers, selling points and consumers. This might be due to cooling of milk from cow body temperature to the ambient temperature while being transported from farm to milk markets. Fresh milk should be cooled to 4°C within 2 h after production, and inadequate cooling increases bacterial counts by creating a better environment for bacterial growth during storage (Reinemann et al., 2005).

The milk pH gives an indication of milk hygiene and freshness and it usually ranges between 6.6 and 6.8 (FAO, 1999). According to the result obtained in the present study, pH of milk samples from milk producer households (6.66) were within the range of fresh cow milk; while milk samples obtained from market (cooperative, milk collectors, retailers and selling points)

Table 12. Chemical composition of raw cow milk along the milk supply chain (Mean ± SD).

Chemical quality parameter	Mean value of chemical quality along milk value chain actors					
	Producers	Cooperative	Milk collectors	Retailers	Selling points	consumers
Fat	4.60±0.74[a]	3.59±0.05[b]	3.88±0.50[b]	4.45±0.78[a]	4.31±0.53[a]	4.59±0.70[a]
Protein	3.31±0.41[a]	2.40±0.08[b]	2.84±0.14[ab]	3.27±0.42[a]	3.25±0.08[a]	3.30±0.24[a]
SNF	8.50±0.54[a]	7.69±0.39[abc]	7.48±0.46[bc]	8.28±0.46[ab]	7.11±0.82[c]	8.17±0.46[ab]
TS	13.10±0.84[a]	11.27±0.34[b]	11.36±0.42[b]	12.73±0.47[a]	11.43±0.29[b]	12.76±0.63[a]
Lactose	4.07±0.33[bc]	4.83±0.16[a]	4.38±0.53[ab]	4.50±0.39[ab]	3.79±0.75[c]	4.59±0.43[ab]

[abc]Different superscripts in the same row differ significantly (P<0.05), SNF=Solid not fat, TS=total solid.

were not within the normal ranges. The pH of milk samples collected from consumers was lower than the normal pH value of fresh cow milk and significantly lower (P<0.05) than the pH of milk obtained from producers and other milk market actors in the supply chain. This result indicates that milk is clearly under fermentation resulting from bacterial multiplication during the time that elapsed between production and until it reaches consumers. Teklemichael et al. (2015a) indicated that in Dire Dawa Town the pH of milk samples collected from vendors was lower than milk obtained from dairy farms. The pH values higher than 6.8 indicates mastitic milk and pH values below 6.6 indicates increased acidity of milk due to bacterial multiplication (O'Connor, 1995).

Specific gravity

The specific gravity of normal milk ranges from 1.027 and 1.035 with an average value of 1.032 at 16°C (FAO, 1999). The mean specific gravity of raw milk samples was 1.031 (Table 11) for household milk producers which fall within this range. The specific gravity of raw milk samples obtained from other sources in a milk supply chain however is lower than that obtained from producers and also below the acceptable limit. These variations might be due to the different sources of milk mixed together that might have been adulterated with water. A similar result was also reported by Teklemichael et al. (2015a) where specific gravity of milk samples collected from vendors was significantly lower (P<0.05) than that obtained from dairy farms in Dire Dawa Town, Eastern Ethiopia. Teshome et al. (2015) however, reported no significant difference in specific gravity among milk samples collected from dairy cooperatives, milk collection centers, hotels, small milk shops and small-scale milk producers in Shashemene Town, Southern Ethiopia. Zelalem (2010) also reported that the specific gravity of most raw whole milk samples collected from Holetta and Selale areas fall within the range 1.028 and 1.032. The specific gravity of milk can be affected by various factors. For instance, the specific gravity of milk decreases by addition of water and addition of cream; while it is increased by removal of fat and reduction of temperature (O'Connor, 1995).

Chemical quality of raw cow's milk

The overall mean value of milk fat (4.5%) in the current study area was higher than that (3.50%) indicated in the Quality Standard Authority of Ethiopian (ES, 2009) for milk produced and marketed in Ethiopia regardless of cow breed (Table 12). The Food and Drug Administration (FDA) and Milk Ordinance and Code of USA recommended that acceptable milk fat contents require not less than 3.25% milk fat for fluid milk (Raff, 2011). In the current study, the fat content of milk was comparable with values reported by earlier findings of Estifanos et al. (2015) for milk collected from Harar Milk Shed in Eastern Ethiopia.

The average protein content of milk as observed in the current study was 3.24%. According to Ethiopian standards (ES, 2009) for protein content of unprocessed whole cow, milk should not be less than 3.20%. Therefore, the average protein content observed from all milk sampling source were within the recommended standard. The result of the present study is also consistent with that reported by Rahel (2008) and Zelalem (2010) for milk samples collected from smallholder farmers in Delbo area of Wollayta Zone and Central Highlands of Ethiopia, respectively.

According to Quality Standards Authority of Ethiopian, total solids content of unpasteurized cow milk should not be less than 12.80% (ES, 2009). The overall mean TS (12.78%) content obtained in the current study almost met this quality standard. The TS content of milk samples collected from cooperatives was significantly (P<0.05) lower than that sampled from other milk sources in the milk supply chain. Estifanos et al. (2015) reported higher (13.1%) TS value for milk samples obtained from Harar Milk Shed, Eastern Ethiopia. The values obtained in the present study are consistent with 12.58% reported by Teklemichael et al. (2015a).

According to Food and Drug Administration (FDA) as well as European Union (EU) quality standards, a minimum solid not fat (SNF) content of whole milk is 8.25% by Raff (2011). In view of that, the result obtained

Table 13. TBC, CC and YMC of raw milk samples collected along milk market chain (Mean ± SD).

Milk source along milk value chain actors	TBC	CC	YMC
Producers	5.17±0.39^{b}	4.64±0.41^{b}	4.58±0.38^{b}
Cooperative	5.57±0.73^{ab}	5.08±0.77^{ab}	5.14± 0.97^{ab}
Milk collectors	5.41±0.57^{b}	4.90±0.57^{b}	4.97±0.47^{ab}
Retailers	5.59±0.55^{ab}	5.10±0.54^{ab}	4.95±0.52^{ab}
Selling points	5.60±0.71^{ab}	5.13±0.69^{ab}	5.12±0.77^{ab}
Consumers	6.12±0.01^{a}	5.62±0.03^{a}	5.54±0.14^{a}
Over all mean	5.48±0.54	4.96± 0.56	4.90±0.55

^{abc}Superscripts in the same column differ significantly (P<0.05), TBC=Total bacterial count, CC=coliform count, YMC=yeast and mould count.

in the present study is in quality standards given by FDA and EU. But the overall mean SNF content (8.27%) obtained in the current study was lower than that reported by Teklemichael et al. (2015a) for milk obtained from dairy farms (8.75%) in Dire Dawa Town. Similarly, EU and FDA set that unprocessed whole milk lactose content should not be less than 4.2% (Tamine, 2009). Therefore, the average lactose content of 4.27% is within the recommended standard.

Microbial properties of raw milk

Total bacterial count (TBC)

The overall mean total bacterial count of raw milk produced in the study area was 5.48 log cfu/ml (Table 13). This value was higher as compared to acceptable level of 5 log cfu/ml of raw milk (O'Connor, 1994). This high level of contamination of milk might be attributed to initial contamination originating from the udder surface, cleaning water, and milking utensils. This implies that the sanitary conditions in which milk is been produced, handled, and sold were substandard.

The present result is comparable with that of Teklemichael et al. (2015b) who reported mean TBC of 5.84 log cfu/ml in milk sampled from Dire Dawa Town Dairy Farms. Estifanos et al. (2015) also reported mean TBC of 6.25 log cfu/ml for Harar Milk Shed, Eastern Ethiopia. The TBC observed in the current study is lower than the value (7.08 log cfu/ml) reported by Fikrineh et al. (2012) in Shashemene, Arsi Negele, Adami Tullu-Jiddo Kombolcha, Adama and Lume Districts at Mid Rift Valley of Ethiopia. Asaminew and Eyassu (2011) also reported higher value (7.58 log cfu/ml) for cow milk sampled from Bahir Dar Zuria and Mecha Districts. Comparatively, the lower total bacterial count in this study could be due to smoking of milk containers, which reduces microbial load in milk. This agrees with the finding of Mogessie and Fekadu (1993) who reported that smoking reduced the undesirable microbial contaminants that enhances the rate of fermentation.

Coliform count (CC)

The overall mean CC of milk produced in the study area was 4.96 log cfu/ml (Table 13). Coliform counts observed in the current study were higher when compared with the acceptable limit given by the American Public Health Service: <2 log cfc/ml for Grade A milk and 2-2.3 log cfc/ml for Grade B milk (APHA, 1992). The CC obtained in the present study is similar with that reported by Asaminew and Eyassu (2011) who found CC of 4.49 log cfu/ml in Bahir Dar Zuria and Mecha Districts. CC can indicate fecal contamination or contamination from equipment that has not been properly cleaned and sanitized (Bintsis et al., 2008; Biruk et al., 2009). Since it is not practical to produce milk that is always free of coliform, even at high level of hygienic condition, their presence in raw milk to a certain extent may be tolerated. However, their presence in large numbers in dairy products is an indication that the products are potentially hazardous to the consumers' health (Godefay and Molla, 2000).

Yeast and mould counts (YMC)

The overall mean YMC was 4.90 log cfu/ml samples collected from different sampling point in the milk market chain (Table 13). These values are higher than the acceptable value (<10 cfu/ml) (Mostert and Jooste, 2002). This might be due to lack of hygienic practices especially washing milking and milk storing utensils, improper sanitary conditions in milking area, poor personal hygiene of milkers and milk sellers, mixing of old and newly drawn milk and storing together. Milk from market had higher yeast and mould counts which were significantly different (P < 0.05) from milk producer households. Haile et al. (2012) also reported higher yeast and mould counts (4.65 log cfu/ml) for milk samples collected from storage containers and for milk samples collected from distribution containers (7.13 log cfu/ml) in Hawassa, Southern Ethiopia.

Conclusions

In the study areas, we found that milk marketing was the common practice in which 62% milk produced was marketed and this marketing was mostly done by women that organized into a traditional milk association group called *Faraqa Annanni*. This group consists of milk producing and selling women of two to ten individuals. This group of women could be an entry point for intervention to improve milk production and marketing in the study areas through organizing and providing necessary technical and financial supports. For example, in rural areas where there is no grid electricity supply solar milk cooling system could be used by *Faraqa Annanni* to cool milk up until marketing. This will ultimately reduce postharvest milk loss as well as increases income of the milk producing women. It is therefore recommended to encourage and empower *Faraqa Annanni* group for sustainable development of the dairy sector in the study areas. It is also equally important to create awareness among concerned actors about quality milk production and marketing as the hygienic and microbiological qualities of milk were poor and did not meet national as well international quality standards.

RECOMMENDATIONS

From results of the current study, the hygienic status of milk along the milk supply chain was sub-standard and both the physicochemical as well as microbial qualities of raw milk samples were poor. It is, therefore, recommended that farmers should be trained on proper hygienic milk production and handling; ensure implementation of good production and hygiene practices throughout the milk market chain; responsible authorities like Ethiopian Food and Drug Authority must ensure that existing rules and regulations are put into practice for screening of milk; and inspection of milk production facilities with physiochemical and microbiological controls.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of cocoa mirid (*Distantiella theobroma* Dist. and *Sahlbergella singularis* Hagl.) control practices in Côte d'Ivoire

Wouter Vanhove[1*], Luc Affoli N'Guessan Toussaint[2], Alexandre Kaminski[2] and Patrick Van Damme[1,3]

[1]UGent, Faculty of Bioscience Engineering, Laboratory of Tropical and Subtropical Agriculture and Ethnobotany, Coupure Links 653, Gent, Belgium.
[2]Barry-Callebaut, Société Africaine de Cacao SA• SACO, Zone 4 Site, 6, rue de Pierre et Marie Curie, 01 BP 1045, Abidjan 01, Côte d'Ivoire.
[3]Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamycka 129, Prague 6 – Suchdol, 165 21, Czech Republic.

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Rapid pod damage evaluations were performed in four cocoa producing regions in Côte d'Ivoire to assess the severity and regional variability of the mirid pest problem. We further tested the impact of three insecticide products at two different dosages, applied at two different periods on cocoa pod production and mirid infestation on sixty cocoa farms. It was found that in Côte d'Ivoire, cocoa production and mirid infestation levels vary greatly between and within regions. All applied insecticide products were effective, but combinations of products, dosages and application timings were found which resulted in significantly higher amounts of harvestable cocoa pods.

Key words: *Distantiella theobroma*, neonicotinoids, pyrethroids, *Sahlbergella singularis*, *Theobroma cacao* L.

INTRODUCTION

Mirids (Hemiptera, Miridae) are considered to be the most important pest problem in West African cocoa (*Theobroma cacao* L.) cultivation (Anikwe, 2010; Anikwe and Otuonye, 2015; Awudzi et al., 2016a; Babin et al., 2010). Mirid problems in West Africa are caused by several species of which two, *Distantiella theobroma* Dist. and *Sahlbergella singularis* Hagl. are the most important (Awudzi et al., 2016a; Babin et al., 2010; Leston, 1970; Wheeler, 2001; Youdeowei, 1973). Across West Africa, *S. singularis* is reported to be more damaging than *D. Theobroma* (Anikwe, 2010; Anikwe and Otuonye, 2015; Babin et al., 2011; Gidoin et al., 2014; Bagny et al., 2018). Mirid damage on cocoa trees is caused by the feeding activities of both mirid nymphs and adults on cocoa pods and young shoots (Anikwe, 2010; Babin et al., 2010; Babin et al., 2011). Mirids suck sap from these plant parts and inject histolytic saliva causing dark markings (lesions) on the tissue, leading to destruction of

*Corresponding author. E-mail: Wouter.Vanhove@UGent.be. Tel: +32 9 264 60 89.

foliage and young pods (Anikwe, 2010; Babin et al., 2010; Babin et al., 2011; Adu-Acheampong et al., 2017). Mirid attacks are usually lethal to cocoa pods that are less than three months old (Anikwe and Otuonye, 2015; Wheeler, 2001). Parasitic fungi may invade the lesions, leading to secondary infections such as cankers (Babin et al., 2010, 2011). Mirid damage, particularly in combination with secondary diseases cause physiological dieback and – when severe – can lead to a delay in first pod production or even death of young trees (Babin et al., 2011; Adu-Acheampong et al., 2017; Wood and Lass, 1985). Notwithstanding that apart from pest and disease pressure, cocoa pod production also depends on genetic and environmental factors, it is often claimed that cocoa yield losses as a result from mirid infestation can be as high as 30-40% (Anikwe, 2010; Anikwe and Otuonye, 2015; Awudzi et al., 2016a; Bagny et al., 2018; Anikwe et al., 2009b; Kouamé et al., 2014) although it is not clear how these estimates were made.

D. *theobroma* and *S. singularis* females bury eggs in the epidermal layer of cocoa pods, pod stalks, chupons and fan branches. Eggs hatch after 10-17 days and develop in 5 successive juvenile stages (nymphs), with a total duration of 18-30 days, into winged adults of 7-12 mm long (Wheeler, 2001; Wood, 1975). Although mirid population numbers in West Africa vary between countries or regions and between years, mirid population peaks are reported for the April until November period (Anikwe, 2010; Kouamé et al., 2014; Adu-Acheampong et al., 2014; Awudzi et al., 2016b), the period that in West Africa concurs with the most abundant cherelle and mature pod production of the main harvesting season and in which most of the annual precipitation occurs (Awudzi et al., 2016b). In Côte d'Ivoire, in the region of Haut-Sassandra, a second peak in mirid populations was observed in January, which coincides with a peak in cherelle production for the secondary harvesting season (with smaller volumes)(Kouamé et al., 2014).

In West African cocoa agroforestry systems, a negative correlation between shade density and mirid numbers was observed (Babin et al., 2010; Gidoin et al., 2014; Bisseleua et al., 2013). Also, more severe mirid damage can be concentrated in sunny patches, resulting from dead or degraded trees inside a cocoa plantation, the so-called mirid pockets (Anikwe and Otuonye, 2015; Wood and Lass, 1985).

In the 1950s and 1960s, organochlorides (lindane, dieldrin, DDT) were widely used in cocoa pest control (Wood and Lass, 1985; Entwistle, 1972) until mirid resistance, mostly to lindane (Dunn, 1963; Gerard, 1964) was reported. Later (1960s – 1990s). Carbamates (e.g. propoxur, promecarb) and organophospates (e.g. chlorpiryfos, diazinon) were used until they were banned because of environmental and health hazards (Bateman, 2015). Today, mirid control in West Africa is almost exclusively done using pyrethroids (such as bifenthrin, deltamethrin, cypermethrin and lambda-cyhalothrin) and neonicotinoids (such as imidacloprid and thiamethoxam) (Bateman, 2015; Anikwe et al., 2009a; Asogwa and Dongo, 2009). Although the latter insecticides have a lower (acute) toxicity than the earlier-used organochlorides, organophosphates and carbamates ((Bateman, 2015), their widespread use continues to pose human health hazards and risks to terrestrial and aquatic wildlife (Diakite et al., 2018; Jepson et al., 2014; Williamson, 2011). There is particular concern on the neurotoxicity of neonicotinoids on bees. Widespread use of neonicotinoids is linked with bee colony collapses (Blacquiere et al., 2012; Gill et al., 2012; Sanchez-Bayo and Goka, 2014). Thus, health and environmental concerns linked to pesticide applications in West African cocoa production, together with the labour and product costs of pesticide application urge for their rational application. However, West African cocoa producers apply an array of insecticides at frequencies that vary between 0 to 11 times per year (Mahob et al., 2011; Antwi-Agyakwa et al., 2015). Since the 1950s, West African governmental agencies have recommended (Awudzi et al., 2016b; Adu-Acheampong et al., 2014; Antwi-Agyakwa et al., 2015; Ahoutou et al., 2015) a calendar-based insecticide application scheme, using motorised knapsack mistblowers, targeting mirid populations when they are most abundant (August – November).

In Côte d'Ivoire, the largest cocoa-producing country in the world, the *Conseil Cacao-Café* (CCC), the government body regulating the commercialisation of cocoa and coffee recommends two applications per year (July – September and December – January) (Ahoutou et al., 2015), presumable on the alleged two mirid population peaks occurring in the country (Kouamé et al., 2014). Analysis of recommendations on labels of 16 different pesticide products (from 7 different brands) sampled from commercial shops in Abidjan (unpublished) confirmed the binary application periods recommended by the CCC.

It is nevertheless unclear if the recommended applications effectively control mirid infestation in Ivorian cocoa production. In order to shed more light on this issue, we evaluated the cocoa mirid infestation in Côte d'Ivoire using a rapid assessment method (March - April 2017) and performed experiments (January – March 2018) in which we evaluated the impact of different common insecticide products applied during different application periods within the recommended range and at a normal (that is, recommended) and reduced dosage (33% of recommended dosage), on cocoa production and mirid infestation in Ivorian cocoa farms. We hypothesize that insecticides containing only a systemic (neonicotinoid) insecticide, will have a different impact on mirid infestation than when products are applied that combine systemic with contact insecticides (pyrethroids). Moreover, assuming that mirid populations show indeed a peak in a certain period, applying insecticides at

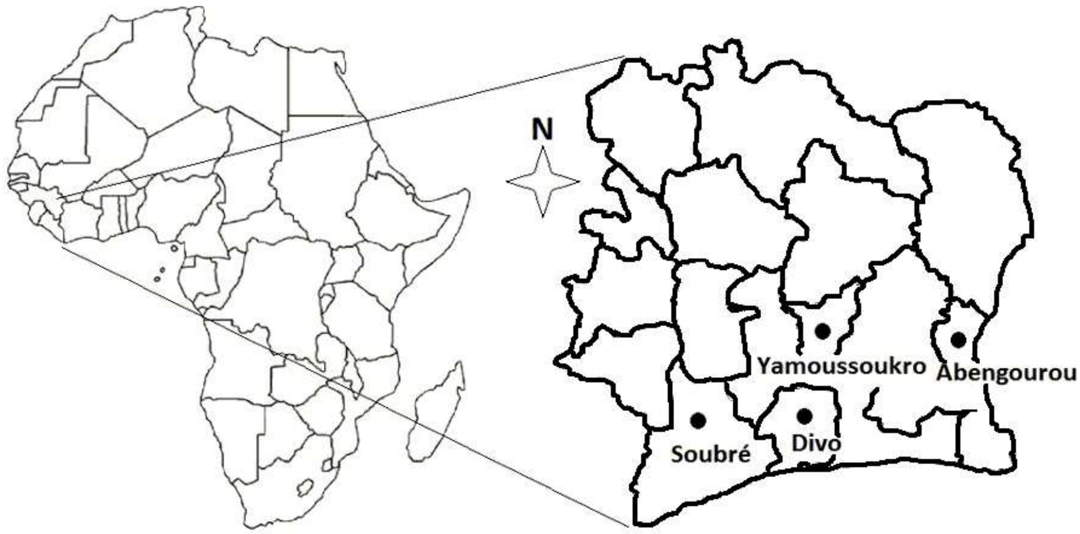

Figure 1. Location of Côte d'Ivoire in Africa (left) and the 4 cities within the country (right) around which the rapid mirid infestation assessment was performed.

different occasions within that period could have a different impact on mirid infestation symptoms after insecticide treatments. Pesticide companies' recommended application dosages might be above optimum rates for a certain cocoa region, because they usually guaranteed good results in company trials at several locations. We therefore hypothesize that a lower insecticide dosage can have the same impact on mirid infestation in cocoa production as the dosage recommended on commercial product labels.

MATERIALS AND METHODS

Rapid mirid infestation assessment

A rapid mirid assessment was performed between 20 March and 1 April 2017 in the Ivorian cocoa producing regions of Indénié-Djuablin, Lôh-Djiboua, Nawa and Belier, more specifically in the areas around the cities of Abengourou, Divo, Soubré and Yamoussoukro (Figure 1). These areas cover the Ivorian cocoa-producing area which ranges from the Southeast to the Southwest of the country. In each area, 6 cocoa farms from two different cocoa cooperatives (three per cooperative) were randomly selected for the assessment (Table 3). In order to assess the impact on mirid infestation of earlier insecticide treatments, farmers were asked if and which insecticide products they had used during the latest pesticide application period (December 2016 – February 2017).

Next, on each farm 30 cocoa trees that had at least one mature cocoa pod were randomly selected at the center of the plot and ensuring at least 3 unselected cocoa trees occupied the space between selected trees. Each tree was scored for the presence or absence (on at least 1 pod) of mirid infestation symptoms (black lesions). Next, 5 out of the 30 trees per farm were randomly selected for more detailed observations of mirid infestation. Total number of cherelles (defined as pods with a length < 10 cm), mature pods, cherelles with mirid infestation symptoms and infested mature pods were registered. Differences in the number of cherelles and mature pods and of the percentage of mirid-infested cherelles and of mirid-infested mature pods between farms that had used insecticide during the latest application period (December 2016 – January 2017) and those that had not, were was assessed by T-tests, using SPSS 26.0. Prior to ANOVA, percentage data were transformed by Arcsin[sqrt(×/100)].

Influence of dosage, timing and Côte d'Ivoire-used insecticide products on mirid infestation levels

Experimental design

Experiments were performed with a selection of cocoa smallholders of the SOCAS cocoa cooperative that has its administration office is in the community Blé (Divo Department, Côte d'Ivoire) (5° 53' 48.91" N, 5° 9' 29.46" W). The cooperative unites 532 farmers from 8 administrative subsections around Blé. Seventy farmers (10 from Ahouanou-II, 9 from Beheri, 9 from Bodo, 15 from Divo Nord, 4 from Kouassikankro and 23 from Obie subsections) were selected by the cooperative president based on i) their willingness to cooperate; ii) the earlier demonstrated ability of the farmer to adequately weed their plots and prune their cocoa trees; and iii) farm distance (that is, reachable on foot in less than 30 min beyond the point where a car could go no further). Experiments consisted of a fully randomized 3x2x2 factorial design with 5 replicates (60 farms) and with 10 untreated control farms (3 from Beheri and 7 from Obie subsections) in addition to the 60 treated farms. Factors considered were 'product', 'dosage' and 'timing'. Five farmer plots were thus entirely treated with the unique combination of one out of three tested insecticide products, at one out of two different dosages and at one out of two different application timings. Control plots (relying on farmer statements) had not been treated with insecticides since December 2016.

Tested products

A selection of commercially available insecticides was used, each containing a different systemic neonicotinoid insecticide, together with or without a pyrethroid contact insecticide (Table 1). Each product was administered to 20 different cocoa farms.

Table 1. Products used in the insecticide treatment efficiency test, with active ingredients and their concentrations in the bottles.

Product brand	Supplier	Neonicotinoid	Pyrethroid
Callifan Super BD	Callivoire	Acetamiprid (20 g/L)	Bifenthrin (20 g/L)
Caotop 30 SC	DMG	Imidacloprid (30 g/L)	None
Azudine 50 SD	RMG	Thiamethoxam (30 g/L)	Deltamethrin (20 g/L)

Table 2 . Standard and reduced dosage (L/ha) for each product tested.

Product	Standard dosage	Reduced dosage
Acetamiprid (20 g/L) + Bifenthrin (20 g/L)	0.50	0.15
Imidacloprid (30 g/L)	1.00	0.30
Thiamethoxam (30 g/L) + Deltamethrin (20 g/L)	0.50	0.15

Applied dosages

Anikwe et al. (2009) exposed *S. singularis* nymphs and adults to filter papers impregnated with various concentrations of the neonicotenoid thiamethoxam, during several exposure periods and found that a reduced dosage (50 or 0.01% instead of 0.02%) reduced mirid mortality from 100% after 90 min (dosage of 0.02%) to 13.3% after 90 min and to 33.3% after 120 min. Tan et al. (2012) defined lethal doses (LD_{50}) of the neonicotinoid imidacloprid to the mirid *Apolygus lucorum* at 6.70 ng of active ingredient (a.i.) per adult, whereas a 'low lethal dose' (LD_{25}) was found to be 1.96 ng a.i. per adult (= 29% of LD_{50}). Based on the latter studies, we compared the effect of spraying with the standard dosage (that is, the dosage recommended on the product bottle) with a lower dosage of (30%) of the standard dosage (Table 2). Application was done using Cifarelli Nuvola^{TM} 5HP mist blowers (77 cc single-cylinder two-stroke gasoline engine with a power of 3.6 kW, liquid tank capacity of 17 L) at a flow rate of 1.25 L per min. Application was done by diluting a quarter of the recommended standard or the reduced dosage per ha in 10 L of water and by applying 4 × 10 L of solution per ha to the cocoa field. The standard and reduced dosages were each applied by 30 farmers.

Timing

Applications were done in the recommended periods of July and August 2017 (presumed mirid peak) and January and February 2018 (alleged second mirid population peak in Côte d'Ivoire). For 30 farmers, treatments were performed early in these recommended period: i.e. from 1 until 20 July 2017 and from 3 until 10 January 2018 for the first and second applications, respectively (henceforth called 'early' treatment) whereas for another 30 farmers, treatments were done later in the recommended periods, that is, from 19 August until 1 September 2017 and from 20 February until 9 March 2018 for the first and second applications, respectively (henceforth called 'late treatment).

Treatments

Treatments were performed by spraying teams consisting of two persons who had been trained by the cocoa cooperative for safe and adequate pesticide application. Products were applied in the morning (before 10 am) by the two sprayers simultaneously until the whole farm plot (Min. 0.3 ha, Max. 14 ha; average area = 3.25 ± 0.39 (SE) ha) had been treated. Treatments were postponed until the next day in case of rain. Application was done while walking through each cocoa row, as recommended by Bateman (2015). Mist, which had a median droplet size of 90 µm, was aimed at the canopy and pods on the main branches and stems.

Evaluation

Two evaluations were performed: from 16 until 20 October 2017 (that is, after the first application) and from 3 until 25 April 2018 (that is, after the second application). Number of cherelles and mature pods, as well as mirid infestation levels on cherelles and mature pods were evaluated on 15 trees per farm following the same procedure as for the rapid mirid infestation assessment (§ 2.1). Main and interaction effects of products, dosages and timings were revealed by analysis of variance (ANOVA) in SPSS 26.0. Control farms were those that have not received any insecticide treatment at all, which implies that they act as a control for all three factors 'product', 'dosage' and 'timing'. In the analysis, we will consider the cherelle and mature pod numbers, and their mirid infestation levels recorded at the second evaluation (that is, after both treatments) as well as the changes in these parameter values between the first and the second evaluation periods. For the latter, we used per farm parameter averages as different trees were evaluated in the first and the second evaluation. Parameter differences between the first and the second evaluation were assessed using a paired samples T-test. Factor interaction effects were computed without considering the control plots, because there is no control for each individual factor. Prior to ANOVA, percentage data were transformed by Arcsin[sqrt(×/100)]. Means are always reported ± their standard error (SE).

RESULTS

Rapid mirid infestation assessment

Fourteen out of 24 sampled cocoa farms from 4 cocoa-growing regions had applied insecticides in the period December 2016 – February 2017. Insecticides applied were either i) only imidacloprid; ii) acetamiprid in combination with either bifenthrin, cypermethrin or

Table 3. Insecticides applied in the December 2016 – February 2017 period, by cocoa farmers from 8 cocoa cooperatives from 4 cocoa-growing regions in Côte d'Ivoire, selected for rapid mirid infestation evaluation.

Area	Cocoa Cooperative	Farm	Insecticide used	Active ingredient
Abengourou	CA CAADI	1	None	
		2	None	
		3	Phytocao	Acetamiprid (20 g/L) + Bifenthrin (20 g/L)
	CAGRAMIA	1	Phytocao	See above
		2	Phytocao	See above
		3	None	
Divo	SOCAS	1	None	
		2	Thiosulfan	imidacloprid (60 g/L)
		3	Phytocao	See above
	SCAC	1	None	
		2	Onex Super 40 EC	Acetamiprid (20 g/L) + cypermethrin (20 g/L)
		3	Phytocao	See above
Soubré	ECAO	1	None	
		2	None	
		3	None	
	CPAY	1	Grosudine Super 50	Imidacloprid (30 g/L) + Bifenthrin (20 g/L)
		2	Grosudine Super 50	See above
		3	Onex Super 40 EC	See above
Yamoussoukro	BINKADI	1	Toro	Acetamiprid (20 g/L) + Deltamethrin (20 g/L)
		2	None	
		3	None	
	COOPABIN	1	Phytocao	See above
		2	Phytocao	See above
		3	Phytocao	See above

deltamethrin; or iii) imidacloprid in combination with bifenthrin (Table 3).

Our rapid mirid survey of 4 cocoa producing areas shows that on all farms, the rate of mirid infested trees (that is, showing at least 1 infested pod or cherelle) was 40.6% in Soubré, 61.1% in Yamoussoukro, 61,7% in Abengourou, and 75.6% in Divo. The more detailed evaluation of 5 trees per farm revealed that trees contained on average 4.07 ± 0.53 cherelles and 15.03 ± 0.94 mature pods and 17.97 ± 2.46% and 24.73 ± 2.45% mirid infested cherelles and mature pods, respectively. There were no significant differences in the four parameters between trees on farms that had received insecticide treatments one year before the evaluation and trees on farms that had not.

Influence of dosage, timing and insecticide products on mirid infestation levels

In the second evaluation period (that is, after two pesticide applications), mean per-tree cherelle number was 2.55 (± 0.16), whereas mean per-tree number of mature pods was 10.8 (± 0.26). Main effects of products, dosage and timing of application were significant ($p < 0.01$) for all considered parameters (Table 4). Significant ($p < 0.05$) interaction effects were found between factors 'product' and 'dosage' for the number of mature pods and between 'product' and 'dosage', 'product' and 'timing' and 'dosage' and 'timing' for mean number of cherelles (Table 4).

Mean rates of infested cherelles and mature pods were always significantly higher in control plots than in plots treated with insecticides. The reduced insecticide dosage resulted in a significantly ($p < 0.0005$) higher rate of infested mature pods (29.12 ± 1.43) as compared with the normal dose (18.22 ± 1.19 mature pods). However, the latter values were both significantly ($p < 0.0005$) lower than the rate of infested mature pods in the control plots (64.38 ± 2.36) (Figure 2).

Considering only the treatments with imidacloprid, it was found that the reduced pesticide dosage resulted in a significantly lower number of mature pods (10.40 ± 0.69) ($p = 0.001$) and cherelles (1.90 ± 0.23) ($p = 0.003$), as compared to the normal dosage (14.27 ± 0.99 for mature pods and 4.19 ± 0.73 for cherelles, respectively). However, in cocoa trees treated with thiamethoxam + deltamethrin, a reduced dosage resulted in significantly($p = 0.001$) higher number of cherelles (4.44 ± 0.63) as

Table 4. Factorial ANOVA of tested products, dosages and application timing on average number of cherelles and mature cocoa pods as well as on the rate of mirid-infested cherelles and mature pods, recorded per tree in the second evaluation period; * Significant ($p < 0.05$) factorial main or interaction effects; control plot tree data was included in the factorial ANOVA for the main factors, but not in the analysis of interaction effects.

Variable	df	Total number of Cherelles		Total number of Mature Pods		Mirid-infested Cherelles		Mirid-infested Mature Pods	
		F	p	F	p	F	p	F	p
Product	3	6.19	0.000*	6.78	0.000*	78.4	0.000*	22.09	0.000*
Dosage	2	5.98	0.003*	4.99	0.007*	132.77	0.000*	32.39	0.000*
Timing	2	6.19	0.002*	4.95	0.007*	118.58	0.000*	32.16	0.000*
Product x Dosage	2	8.17	0.000*	13.75	0.000*	2.74	0.065	1	0.368
Product x Timing	2	2.92	0.054	7.08	0.001*	1.02	0.361	0.35	0.707
Dosage x Timing	1	0.2	0.654	45.2	0.000*	0.09	0.769	2.97	0.086
Product x Dosage x Timing	2	0.39	0.681	0.71	0.493	7.12	0.001*	2.94	0.054

compared to the normal dosage (2.08 ± 0.31), whereas the number of cherelles did not differ significantly between reduced and normal dosages for the other two products.

Furthermore, when imidacloprid was applied, late treatments resulted in a significantly ($p < 0.01$) higher number of cherelles (4.32 ± 0.81) as compared to early treatments with only 2.03 ± 0.25 cherelles. Different treatment timings of the other two products did not cause significant differences in cherelle numbers. Analysis of the interaction effect between 'timing' and 'dosage' reveals that when insecticides are applied early in the recommended period the mean number of cherelles was significantly ($p < 0.0005$) lower for the normal (1.42 ± 0.19) than for the reduced dosage (3.75 ± 0.42), whereas the opposite was found when insecticides are applied late in the recommended period, where 4.07 ± 0.55 cherelles were observed for the normal dose, which was significantly ($p < 0.0005$) higher than was the case with the reduced dose (1.77 ± 0.21).

As compared to the first evaluation (October 2017), during the second evaluation (April, 2018) the cocoa farms on average had produced significantly less cherelles (2.87 ± 0.52 as compared to 12.65 ± 0.73), and had a significantly higher rate of infested cherelles (36.39 ± 2.45% as compared to 21.51 ± 1.46%) and a significantly higher rate of infested mature pods (to 21.51 ± 1.46% as compared to 10.86 ± 1.15%). When reduced pesticide dosages were applied, the increase in the mean rate of infested mature pods was significantly ($p < 0.05$) higher (+16.30 ± 3.34 %) than when the normal dosages were applied (+3.51 ± 2.79%) (Figure 2).

DISCUSSION

Rapid mirid infestation assessment in 4 different cocoa producing regions in Côte d'Ivoire revealed that mirid infestation is highly variable, both between and within regions. In all regions, at least half of the cocoa farmers had used insecticides three months prior to our assessment. The latter period is also the recommended application period, that is, concurring with one of the reported mirid population peaks in Côte d'Ivoire (Kouamé et al., 2014). Products used are approved for use in cocoa cultivation in Côte d'Ivoire (Ahoutou et al., 2015). Also in Ghana (Awudzi et al., 2016a; Anikwe et al., 2009a; Adu-Acheampong et al., 2014; Antwi-Agyakwa et al., 2015), Cameroon (Mahob et al., 2011) and Nigeria (Asogwa and Dongo, 2009), after Côte d'Ivoire, respectively second, fourth and fifth largest cocoa-producing countries in the world, similar neonicotenoids (imidacloprid, acetamiprid) and pyrethroids (bifenthrin, deltamethrin and cypermethrin) are common in cocoa cultivation. The rapid mirid assessment did not reveal significant differences in tree infestation rates between plots that were treated with insecticides in the latest application period and those that were not. However, in the experiment on the impact of different products, dosages and application timings, all four parameters under consideration were significantly different on the treated farms from those on the control farms. These different findings can be due to the fact that in the latter experiment control farms not only were not treated with insecticides in the application period of December 2016 – February 2017, but continued not to be treated until the second evaluation of April 2018. In the former experiment (rapid mirid assessment), untreated plots might have been treated with insecticides in the year before December 2016. The effects of those treatments might still have had an effect on cherelles and pod production as well as on observed infestation rates.

In the experiment on the impact of different products, dosages and application timings, we used 4 proxy parameters (number of cherelles and mature pods, and rate of mirid-infested cherelles and mature pods per tree) for assessing the impact on mirid infestation. Since cocoa mirids are particularly harmful (and usually lethal) to

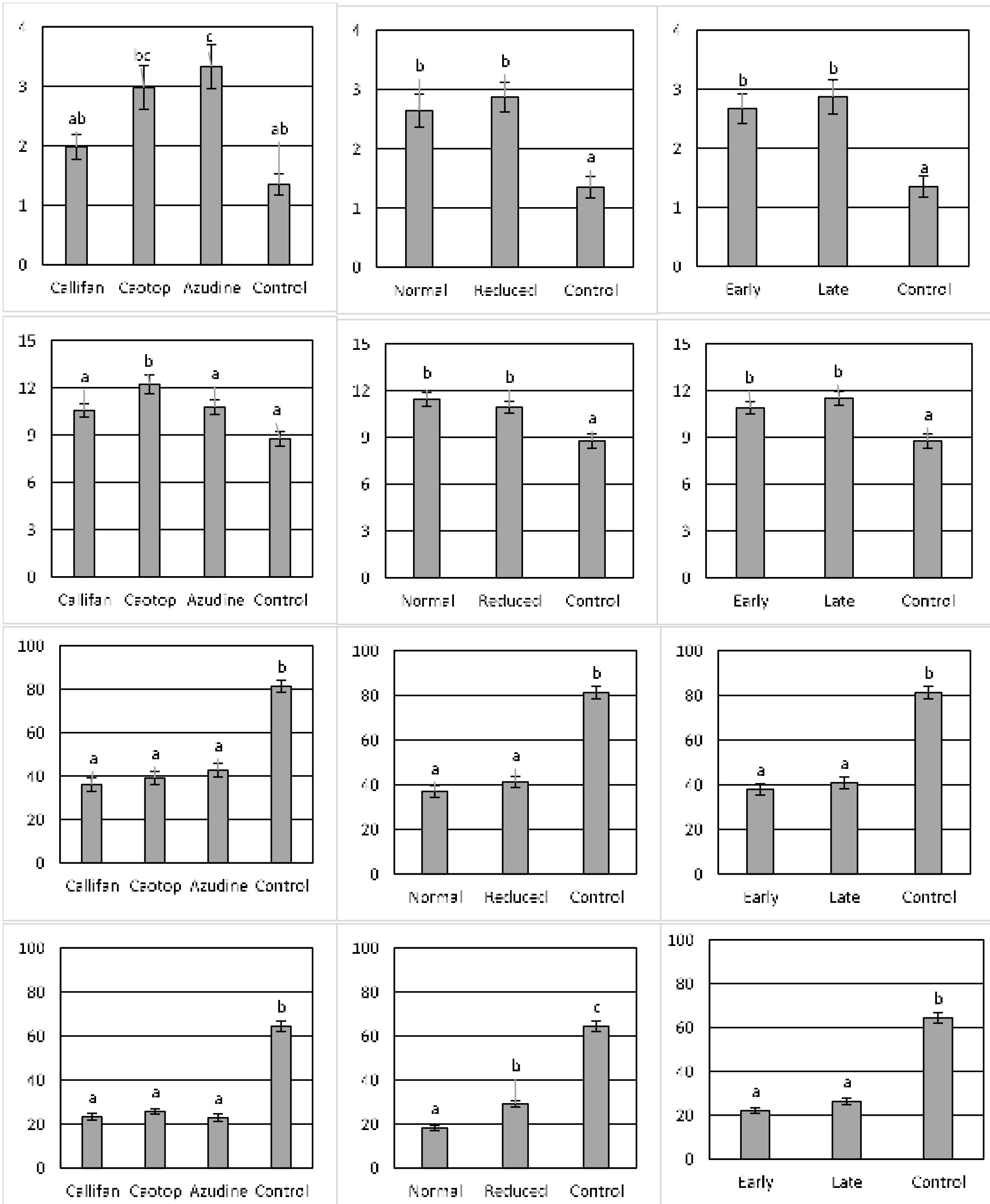

Figure 2. Average per tree number of cherelles (top row) and mature pods (second row) and rates (%) of mirid-infested cherelles (third row) and mature pods (fourth row) observed in the second evaluation after treatment with different products (first column), at different dosages (second column) and application timings (third column) as well as for untreated control plots. Different lowercase letters indicate significant ($p < 0.05$) differences in parameter values within each factor. Error bars show standard errors of the mean.

mirids are particularly harmful (and usually lethal) to cocoa cherelles (Anikwe and Otuonye, 2015; Wheeler, 2001), trees where lower cherelle numbers were recorded, were probably more affected by mirids than other trees. It is, however, also possible that differences in cherelle numbers are caused by differences in cocoa genotypes, soil nutrition or pollinator abundance between the studied cocoa farms, which will cause differences in physiological cherelle wilt, a natural fruit thinning mechanism in cocoa (Valle et al., 1990; Claus et al., 2017).

The effect of reducing the insecticide dosage to one third of the recommended level on total cherelle and mature pod production depends on the insecticide product used, as well as on the timing of application. Only when imidacloprid was used, the reduced dosage did significantly reduce the number of observed cherelles as well as mature pods. A hypothesis for the different effects of a decreased dosage is that when systemic insecticides such as thiamethoxam or acetamiprid are combined with contact insecticides such as deltamethrin or bifenthrin, not only mirids are killed, but also significant numbers of mirid predators are knocked down. As a result, reducing the dosage would decrease the impact of the contact insecticides on mirid predators leading to a higher amount of cherelles observed after treatments. Weaver ants *Oecophylla longinoda* and *Oecophylla smaragdina* have been suggested as key-predators to mirids in cocoa cropping systems in Cameroon (Babin et al., 2010; 2011, 2012,) and Australia (Forbes and Northfield, 2017), respectively. Those predatory ants will less likely be killed by insecticide products that do not contain pyrethroids and will consequently be able to maintain their populations to levels that are able to significantly reduce mirid numbers.

The effect of the reduced pesticide dosage further depends on the timing of application in the recommended periods. When applied early, the reduced dosage had a significantly positive impact on the number of observed cherelles, whereas a late application resulted in significantly less observed cherelles as compared to the normal dosage. Furthermore, only for imidacloprid we found a significant difference between the early and the late application period in the average number of cherelles and the average number of mature pods per tree. The later imidacloprid application resulted in significantly more cherelles ($p < 0.01$) than the earlier imidacloprid application. In the Haut-Sassandra region of Côte d'Ivoire, between 2009 and 2013, Kouamé et al. (2014) evidenced a first mirid population peak in January, after which mirid populations rapidly declined. It would thus be logical that a later application period that is, when mirid population numbers have declined due to natural reasons, irrespective of pesticide applications - would result in lower cherelle and mature pod numbers per tree, as application timing in that case would not coincide with mirid population peaks. The fact that only for imidacloprid the application period influences cherelle numbers, suggests that the absence of a pyrethroid – as opposed to the two insecticide combinations tested – probably has a higher impact on predator conservation than on mirid control in the application period.

The latter results could suggest that the normal imidacloprid dosage, applied in the latest two weeks of the recommended period, is the most optimum chemical mirid control method in our study region. Indeed, considering average values of the 15 evaluated trees on the 5 farms per unique factor combination, we found the highest mean number of cherelles (6.39 ± 1.33) after the application of a normal dosage of imidacloprid, late in the recommended application period (Annex 1). However, there are several reasons why such a recommendation cannot be generalized: i) although also a significantly higher number of mature pods (which is the objective of pest control efforts) is produced after normal imidacloprid application as compared with the reduced imidacloprid application, there were no interaction effects observed between factors 'product' and 'timing' or between 'dosage' and 'timing' for the number of mature pods; ii) experiments took place in an area of ± 120 km² around Blé, which might have a specific ecology, influencing mirid populations; iii) recommendation of a single insecticide would rapidly lead to mirid resistance (Bateman, 2015). With only 2 different neonicotinoid and 3 different pyrethroid applications observed during our rapid mirid infestation assessment (Table 3), mirid resistance is a potential risk in mirid control in Côte d'Ivoire (Bateman, 2015). Even though our results suggest imidacloprid as the most appropriate insecticide, the risk of resistance urges for rotation in the use of insecticides with different modes of action. A rotation scheme could be developed in which insecticides with active ingredients with different modes of action are used in different Ivorian cocoa areas, and in which after a number of years, insecticide applications are rotated between different areas, following e.g. a similar rotation scheme for acaricides to control *Tetranychus* spp. spider mites in cotton (*Gossypium hirsutum* L.) production in Zimbabwe (Tibugari et al., 2012).

We used a motorized mistblower to perform insecticide treatments, because this is the only pesticide application equipment that is able to project insecticides to the cocoa canopy, which in some cases in West-Africa (as a result of inadequate pruning) can be as high as 14 m (Bateman, 2015). Adequate pruning, keeping tree height below 4 m, is therefore an important part of mirid control in cocoa cultivation (Wood and Lass, 1985). The disadvantage of motorized mistblowers is that not all active ingredients reach their biological target due to spray drift (Graham-Bryce, 1977). Inadequate spraying (e.g. in rainy or windy conditions, or aiming at stems and soil, rather than at the cocoa canopy) will increase pesticide drift and will consequently require higher dosages to deposit the same amounts of active ingredients to cocoa leaves and pods as would be the case with appropriate spraying.

Conclusions

Our assessment of production, and mirid infestation of cherelles and mature cocoa pods in 4 regions in Côte d'Ivoire, revealed that mirid infestation is highly variable, both within as well as between regions. Although our insecticide treatments had a positive impact on the cocoa parameters considered as compared to untreated control trees, we found no significant differences between the main effects of the two different application timings (on any of the parameters) and between the normal and reduced dosage (all parameters except for the rate of mirid-infested mature pods). However, the observed interaction effects reveal that there are combinations of products, dosages and application timings that after two applications in one year result in a significantly higher amount of mature, harvestable cocoa pods.

Before these findings can be translated into general mirid control recommendations, more research is needed on the effect of neonicotinoids and pyrethroids on mirid ecology and population dynamics. Natural vegetation (Leston, 1970) as well as the presence of predators such as weaver ants (Bagny et al., 2018; Babin et al., 2012) can influence mirid prevalence and therefore interact with insecticide application. Further research should ideally be performed in a wider area than the one used in the present study and should be performed over multiple years. In order to avoid the development of mirid resistance in multiple-year trials, different insecticide products need to be applied in subsequent applications. In such trials, it is important to assess how insecticides with different modes of action have an impact, not only on mirids, but also on their potential predators. Given the observed significant interactions of dosage and timing of insecticide applications on the number of mature pods produced on cocoa trees, it would further be interesting to test the effect on mirid infestation of reduced dosages of insecticides applied at more than two occasions per year.

The large variability of mirid infestation and damage between and within regions further suggests that insecticide applications, rather than following a fixed biannual calendar scheme should better be based on observed presence of mirids. Experiments in Ghana have shown that mirid monitoring can be easily done using pheromone-based monitoring traps (Awudzi et al., 2016a; Mahob et al., 2011; Sarfo et al., 2018). Mirid monitoring systems might in future be used by West African cocoa smallholders as a decision tool for insecticide applications, following e.g. Cruz et al. (2012) who used pheromone traps as a decision tool for insecticide applications against the fall army worm (*Spodoptera frugiperda*) in Brazil. Today, insecticide applications by West African cocoa farmers are influenced by socio-economic, farm-specific and institutional factors but also by pest incidence perceptions (Danso-Abbeam and Baiyegunhi, 2018). As a result, successful adoption of integrated mirid control, using insecticide applications in periods and at dosages guided by mirid population numbers will require farmer training and applied research support to determine best control practices. When successful, such integrated mirid control can contribute to decreased overall pesticide use in the West African cocoa sector, increase cocoa farm profitability and improve farmer health and environmental sustainability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Annex 1. Mean number of cherelles and mature pods per tree and mean rate of mirid-infested cherelles and mature pods for each combination of factors 'product', 'dosage', 'timing' recorded in the second evaluation after treatment with different products, at different dosages and application timings as well as for untreated control plots.; N = number of trees evaluated per factor combination.

Product	Dosage	Timing	Number of				Rate of mirid-infested			
			Cherelles		Mature Pods		Cherelles		Mature Pods	
			N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Acetamiprid + Bifenthrin	Normal	Early	60	0.78 ± 0.15	60	9.02 ± 0.70	25	0.2067 ± 0.0707	60	0.1957 ± 0.0275
		Late	60	2.53 ± 0.45	60	10.83 ± 0.91	43	0.3532 ± 0.0584	59	0.2159 ± 0.0328
	Decreased	Early	60	3.22 ± 0.68	60	11.13 ± 1.00	40	0.3849 ± 0.0609	60	0.2075 ± 0.0277
		Late	75	1.49 ± 0.23	75	11.11 ± 0.77	47	0.4281 ± 0.0614	74	0.2976 ± 0.0301
Imidacloprid	Normal	Early	59	1.92 ± 0.42	59	13.31 ± 1.39	34	0.4585 ± 0.0732	59	0.1422 ± 0.0257
		Late	61	6.39 ± 1.33	61	15.21 ± 1.41	48	0.3266 ± 0.0455	61	0.2454 ± 0.0329
	Decreased	Early	90	2.1 ± 0.31	90	9.8 ± 0.78	53	0.3365 ± 0.0524	87	0.3412 ± 0.0354
		Late	45	1.51 ± 0.29	45	11.6 ± 1.35	26	0.5308 ± 0.0866	43	0.2627 ± 0.0366
Thiamethoxam + Deltamethrin	Normal	Early	75	1.55 ± 0.34	75	10.68 ± 0.88	37	0.4251 ± 0.0731	75	0.1571 ± 0.0240
		Late	45	2.98 ± 0.56	45	9.07 ± 0.92	32	0.4254 ± 0.0732	43	0.1259 ± 0.0294
	Decreased	Early	75	6.16 ± 1.01	75	11.8 ± 0.96	60	0.4110 ± 0.0453	75	0.2371 ± 0.0312
		Late	60	2.30 ± 4.71	60	10.63 ± 0.85	31	0.4614 ± 0.0764	60	0.3823 ± 0.0416
Control	Control	Control	135	1.35 ± 2.06	135	8.76 ± 0.46	69	0.8127 ± 0.0278	134	0.6438 ± 0.0233

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Appropriate fertilizer (NPK) rates for cassava (*Manihot esculenta* Crantz) production in the humid forest agro-ecological zone of Cameroon

TEMEGNE NONO Carine[1*], NGOME AJEBESONE Francis[2] and ETHE NGALLE[2]

[1]Department of Plant Biology, Faculty of Science, University of Yaounde I, P. O. Box 812 Yaounde, Cameroon.
[2]Institute of Agricultural Research for Development (IRAD), P. O. Box 2123 Yaounde, Cameroon.

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The objective of this study was to test for appropriate quantity of fertilizer (NPK 12-11-18) required to boost the production of cassava (variety TME 419) in the forest zone of Cameroon. The trial was conducted at the experimental farm of the Institute of Agricultural Research for Development (IRAD) in Mbalmayo. The experimental design was completely randomized block design in three replicates. The treatments were as follows: T0 (Control (No input)); T1 (200 kg.ha^{-1}); T2 (300 kg.ha^{-1}); and T3 (400 kgha^{-1}). The data were analyzed using the IBM SPSS Statistics 20 Software. The results showed that chemical fertilizer improves the growth (number of leaves, plant height, and petiole length) and yield (number of marketable root, yield) of cassava mainly because it enhances soil nutrient availability. Thus, NPK-fertilizer significantly ($p<0.001$) increased petiole length by 20.71, 27.83, and 31.62% for T1, T2, and T3, respectively at 5 months after planting (MAP). The highest plant height and number of leaves at 4 and 5 MAP was observed with T3 followed by T2 and T1. Similarly, the highest root yield (43.33±10.97 t.ha^{-1}) was observed with treatment T3. Hence, the fertilizer application rate of 400 kgha^{-1} of NPK 12-11-18 appears most appropriate for intensification of cassava production in the humid forest agro ecological zone of Cameroon.

Key words: Cassava, growth, NPK, root yield, soil fertility.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a food crop grown in all agro-ecological zones of Cameroon for its leaves and tuberous roots (Mouafor et al., 2016; Temegne et al., 2016; Fonji et al., 2017; Njukeng et al., 2017). TME 419 is a new cassava variety which is increasingly adopted by producers in Cameroon (Mouafor et al., 2016). Nevertheless, cassava is still considered a soil-depleting crop (Ngome et al., 2013) and its production on degraded soils of Cameroon requires fertilizer use.

Indeed, large areas of sub-Saharan soils including Cameroon are affected by various types of degradation including soil fertility decline (Ngome et al., 2011). Nutrient balances are largely negative and symptoms of nutrient deficiencies are widespread (Temegne et al., 2015). This phenomenon is exacerbated by intense cultivation (Nambiro, 2008), poor residue management (Ngome, 2006) and poor access to mineral fertilizer due to high unit cost and irregular supplies. Additionally, there

*Corresponding author. E-mail: nonocarine2003@yahoo.fr. Tel: +237 651 32 34 56.

is limited use of organic manure because of high land and labor requirements. Fertilizer application has a great potential to ameliorate the nutrient content of degraded soils and enhance plant nutrient uptake (Ngome et al., 2011). It is thus hypothesized that an increase in soil fertility could enhance crop productivity. Therefore the objective of this study was to investigate the appropriate amount of NPK fertilizer required to boost cassava production in the humid forest agro ecological zone of Cameroon, which is characterized by poor ferralitic soils.

MATERIALS AND METHODS

Experimentation site

The study was carried out between April 2018 and April 2019 at Mbalmayo (Figure 1), Nyong and So'o Division of the Centre Region of Cameroon. The area is found in the humid forest agro-ecological zone, characterized by poor ferralitic soils, a bimodal rainfall pattern, with four seasons: long rainy season from September to November, long dry season from December to February, short rainy season from March to June, and short dry season from July to August. The average daily temperature was estimated at 20 to 29°C and 1,700 mm was an average rainfall recorded during the experimental period (IRAD, 2018; IITA, 2018).

Soil analysis

Before seed-bed preparation, a composite sample of the top soil (between 0 and 15 cm depth) was obtained from a mixture of ten samples collected from the field with an auger following the transect method (Okalebo et al., 2002). Portions of the composite soil sample (about 200 g) were analyzed in the laboratory for physical and chemical characteristics. Soil sample was air-dried and ground to pass through a sieve (2 mm). For carbon (C) and nitrogen (N) analysis, soil was further fine ground to pass through a 0.5 mm sieve. Soil pH in water, was determined in a 1:2.5 (w/v) soil: water suspension. Exchangeable cations (Ca, Mg and K) were extracted using the ammonium acetate (NH_4OAC, pH: 7) and determined by flame atomic absorption spectrophotometry. Organic C was determined by chromic acid digestion and spectrophotometric analysis (Heanes, 1984). Total N was determined by a wet acid digest and analyzed by colorimetric analysis (Anderson and Ingram, 1993). P was extracted using Bray extractant and the resulting extract analyzed using the molybdate blue procedure (Ngome, 2006). This soil analysis was done in the Analysis Laboratory of Soils, Plants, Fertilizers and Waters of the Institute of Agricultural Research for Development (IRAD) of Nkolbisson-Cameroon.

The soil was acidic, low in organic carbon content and total nitrogen (Table 1).

Plant

The plant material used was an improved cassava variety namely TME 419. It is tolerant to drought, African cassava mosaic disease (CMD) and cassava bacterial blight (CBB) with an average yield between 21 and 52 t.ha^{-1} of fresh root, and a dry matter content of42% (IITA, 2018).

Experimental set-up

The experimental design was a completely randomized block that consisted of four treatments (four fertilizers levels): T0 (Control (No input)); T1 (200 kg NPK 12-11-18 ha^{-1}); T2 (300 kg NPK 12-11-18 ha^{-1}); T3 (400 kg NPK 12-11-18 ha^{-1}) in three replicates, with 12 experimental units of 25 m² (5 m × 5 m).

The experiment field spread over an area of 481 m^2 (13 m × 37 m) of which 300 m² for the test and 181 m² of border.

Implementation of the test

Cassava cuttings (25-30 cm in length) of the variety TME 419 were planted on the 3rd of April, 2018, following a 1 m × 1 m pattern at the rate of one per hole. 2/3 of the cutting was below ground and 1/3 above ground level with a 45° inclination. Two months after sowing, the different levels of mineral fertilizers [N: P: K] [12:11:18] were applied on experimental plots at 0 (0 g.plant^{-1}), 200 (20 g.plant^{-1}), 300 (30 g.plant^{-1}) and 400 kg.ha^{-1} (40 g.plant^{-1}) (Ukaoma and Ogbonnaya, 2013). Weeds were removed manually as required. Harvesting was done 12 months after planting (10th April, 2019).

Measurement of agronomic parameters

The number of leaves, plant height, and petiole length were measured at 3, 4, and 5 months after planting (MAP). At harvest (12 MAP), the number of commercial roots and total root yield (t.ha^{-1}) was evaluated.

Data analysis

The obtained data were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and for homogeneity of variances using Levene test. The collected data were treated by analysis of variance (ANOVA) with the IBM SPSS Statistics 20 software using one way test (one factor). Means comparison was done using Student-Newman-Keuls test at 5% threshold. Pearson correlation test was done to highlight the relationships between some studied parameters.

RESULTS

Effect of NPK (12-11-18) fertilizer levels on growth parameters of cassava

NPK fertilizer significantly improved ($p<0.001$) the production of leaves in cassava over time (Table 2), irrespective of the rate of application. Thus, 3 MAP, the number of leaves was significantly the highest at T3; it was followed by T2, then T1; T0 showed the lowest number of leaves. The same observation was made at 4 and 5 MAP.

The different levels of chemical fertilizer significantly ($p<0.001$) increased plant height over time (Table 2). Thus, T1, T2 and T3 were significantly higher than those of T0 at 3 MAP. However, there was no significant difference between T1, T2 and T3 treatments. The highest plant height was observed at T3 and T2, followed by T1 at 4 and 5 MAP.

Similarly, the application of chemical fertilizer (NPK) significantly increased ($p<0.001$) the leaf petiole length

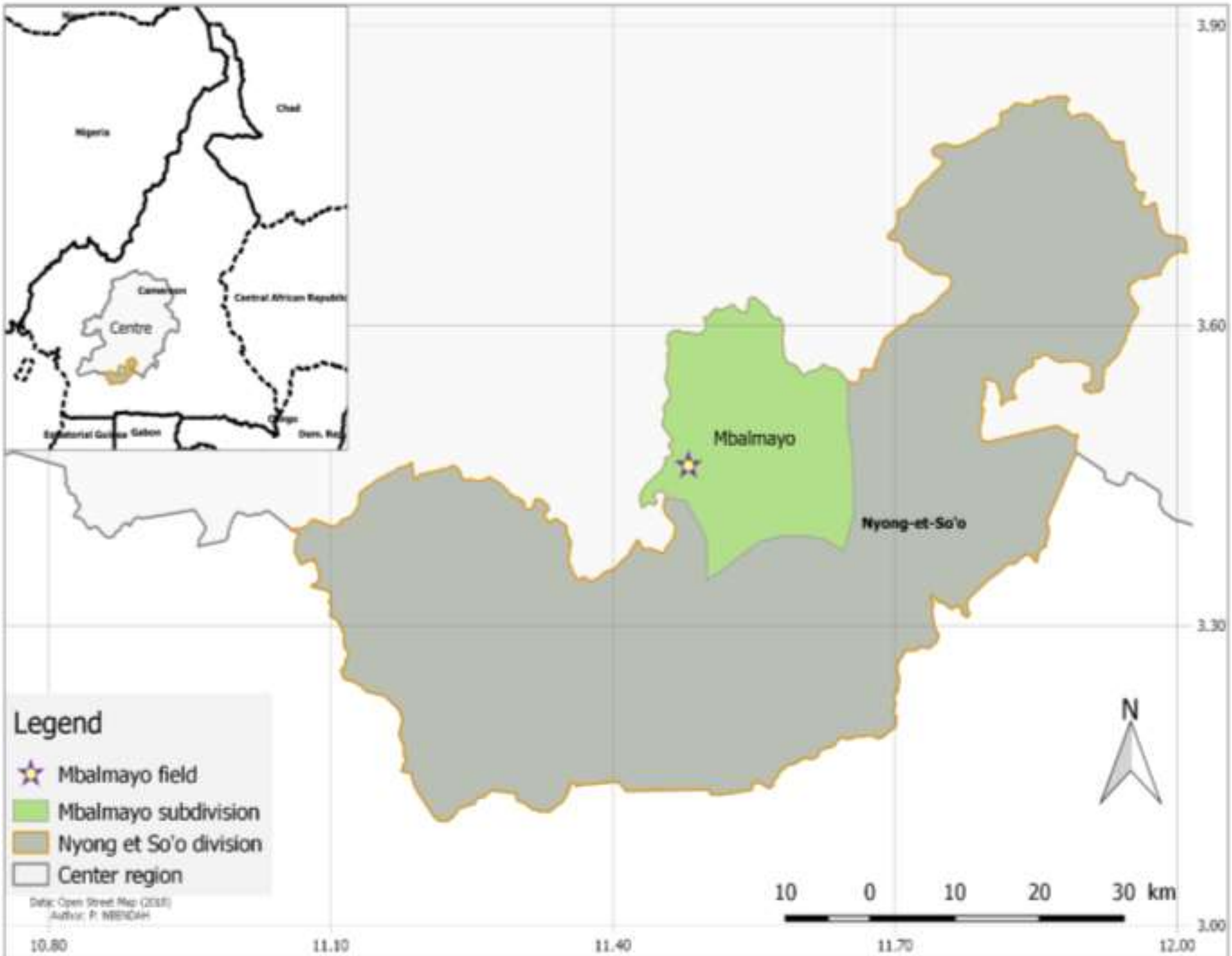

Figure 1. Study site.

Table 1. Soil physical and chemical characteristics of the experimental field.

Sand	Loam (silt)	Clay	pH	OC	N	C/N	P	Ca^{2+}	Mg^{2+}	K^{+}
	%		-	%		-	$\mu g.g^{-1}$		$cmol.kg^{-1}$	
50.55	9.59	39.86	6.76	1.89	0.16	11.96	7.55	8.37	2.13	0.28

over time (Table 2). Thus, at 3 MAP, T1, T2 and T3 treatment plants had significantly longer petioles than those of T0. However, there was no significant difference between T1, T2 and T3 treatments. At 4 MAP, the petiole length was greatest at T3 and T2, followed by T1; T0 gave the smallest petioles lengths. At 5 MAP, the NPK fertilizer use significantly increased the petiole length of 20.71, 27.83, and 31.62% for T1, T2 and T3, respectively (Table 2).

Effect of NPK (12-11-18) fertilizer levels on root yield parameters of cassava

Increasing levels of chemical fertilizer (NPK 12-11-18) significantly increased ($p<0.001$) the number of tuberous roots produced per plant compared to the control (T0) (Figure 2). The number of tuberous roots was highest at treatment T3 (400 kg NPK ha^{-1}); followed by T2 and T1. The number of tuberous roots varied from 6 (for T0) to 10

Table 2. Influence of fertilizer use on number of leaves, plant height and petiole length of cassava (TME 419).

Parameter	Month after planting (MAP)	Treatments				p value
		T0	T1	T2	T3	
N° leaves	3 MAP	22±5[a]	31±5[b]	35±5[bc]	37±7[c]	0.000
	4 MAP	33±8[a]	38±6[b]	43±5[bc]	45±8[c]	0.000
	5 MAP	56±13[a]	77±19[b]	108±23[c]	113±19[c]	0.000
Plant height (m)	3 MAP	1.45±0.1[a]	1.96±0.09[b]	1.96±0.1[b]	1.91±0.11[b]	0.000
	4 MAP	1.93±0.26[a]	2.21±0.25[b]	2.5±0.24[c]	2.6±0.27[c]	0.000
	5 MAP	2.77±0.4[a]	3.34±0.48[b]	3.72±0.19[c]	3.93±0.27[c]	0.000
Petiole length (cm)	3 MAP	24.37±2.74[a]	32.13±2.39[b]	31.87±1.43[b]	32.73±2.09[b]	0.000
	4 MAP	27.17±3.26[a]	34.4±3.27[b]	38.6±1.58[c]	38.1±1.37[c]	0.000
	5 MAP	30.9±1.97[a]	37.3±2.88[b]	39.5±2.25[bc]	40.67±4.48[c]	0.000

T0: control (no input), T1: 200 kg NPK (12-11-18) ha^{-1}, T2: 300 kg NPK (12-11-18) ha^{-1}, T3: 400 kg NPK (12-11-18) ha^{-1}. Values (Mean ± Standard deviation, n=15) with the same small letter for each measured parameter, at each month are not significantly different at the 0.05 probability level.

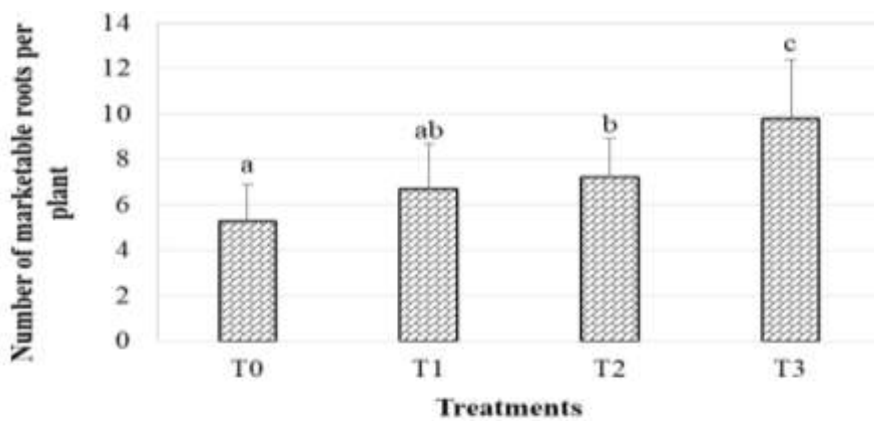

Figure 2. Influence of fertilizer amount on number of commercial roots per plant of cassava (TME 419). T0: control (no input), T1: 200 kg NPK (12-11-18) ha^{-1}, T2: 300 kg NPK (12-11-18) ha^{-1}, T3: 400 kg NPK (12-11-18) ha^{-1}. Values (Mean ± Standard deviation, n=15) with the same small letter for each measured parameter, at each month are not significantly different at the 0.05 probability level.

(for T3) (Figure 2).

The analysis of the obtained results (Figure 3) shows that the chemical fertilizer significantly improves ($p<0.001$) the yield of cassava. Compared with the control (T0), root yield was significantly higher by 71.05, 29.82 and 129.82% at T1, T2 and T3, respectively. The highest mean value (43.33 ± 10.97 $t.ha^{-1}$) was observed at treatment T3 (400 kg NPK ha^{-1}). However, the average root yield (T1: 32.17 ± 15.89 $t.ha^{-1}$, T2: 24.33 + 9.42 $t.ha^{-1}$) was higher at T1 (200 kg NPK ha^{-1}) than at T2 (300 kg NPK ha^{-1}).

Correlation test

Table 3 shows the correlations between the evaluated parameters. The number of leaves 5 MAP (month after planting) was significantly ($p < 0.01$) and positively correlated with plant height 5 MAP (r: 0.64), petiole length (r: 0.55), number of commercial root per plant (r: 0.52), and the yield of fresh tuberous root per hectare (r: 0.39).

DISCUSSION

The different levels of chemical fertilizer (NPK 12-11-18) significantly improve the growth (number of leaves, plant height, and petiole length), the number of commercial root per plant and the root yield of cassava. The control (no input) plants of cassava recorded the shortest plants, the lowest number of leaves with the least petiole length. Indeed, many studies showed that the cassava respond

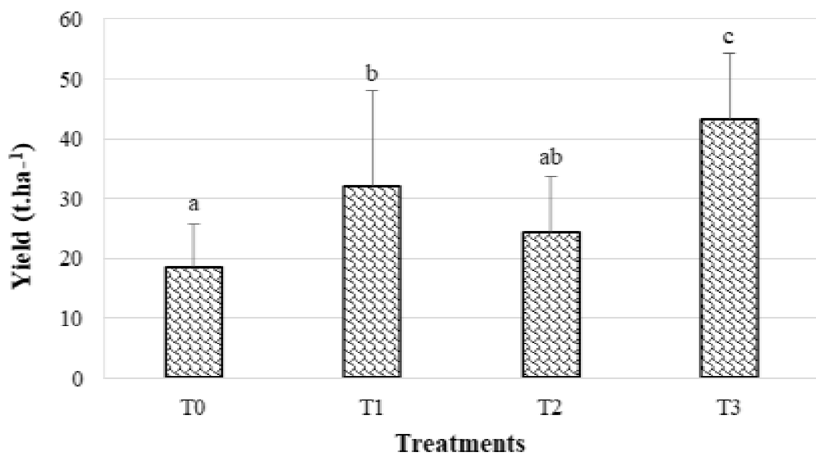

Figure 3. Influence of fertilizer amount on cassava (TME 419) yield. T0: control (no input), T1: 200 kg NPK (12-11-18) ha^{-1}, T2: 300 kg NPK (12-11-18) ha^{-1}, T3: 400 kg NPK (12-11-18) ha^{-1}. Values (Mean ± Standard deviation, n=15) with the same small letter for each measured parameter, at each month are not significantly different at the 0.05 probability level.

Table 3. Pearson correlation matrix of cassava measured parameters.

Correlation	No. of leaves 5 MAP	Plant height 5 MAP	Petiole length 5 MAP	No. of root per plant	Yield
No. of leaves 5 MAP	1				
Plant height 5 MAP	0.643**	1			
Petiole length 5 MAP	0.548**	0.674**	1		
No. of root per plant	0.516**	0.470**	0.191^{ns}	1	
Yield	0.389**	0.438**	0.322*	0.589**	1

MAP: Months after planting, ns: no significant, *: correlation is significant at the 0.05 level (2-tailed), **: correlation is significant at the 0.01 level (2-tailed).

to inorganic fertilizer use. The obtained results corroborates with those of Temegne and Ngome (2017) in Cameroon, Ukaoma and Ogbonnaya (2013) in Nigeria, and Akanza and Yao-Kouame (2011) in Côte d'Ivoire.

The positive response of growth traits to chemical fertilizer (NPK 12-11-18) is due to their role in cell multiplication and photosynthesis, resulting in an increase in the size and length of leaves and stems (Uwah et al., 2013).

The positive response of cassava root yield to high levels of N, P and K could be attributed to high starch synthesis and translocation activities impulse by the N, P and K intake (Uwah et al., 2013). Howeler (2002) states that N, P and K are essential for the initiation of cassava root, the increase in size and number of tuberous roots. The increase in cassava root yield could also be attributed to the increase in single root weight per stand.

The results obtained confirm the effectiveness of the fertilizer type (NPK 12-11-18) chosen for this test compared to others on the market (NPK: 20-10-10, 14-24-14, etc). Indeed, cassava plants consume more K than N and P (Temegne and Ngome, 2017). Adjanohoun (2006) noted that the amount of K extracted by cassava plants is more than twice that of N. A high N supply leads to an excessive foliage production and small roots by the plant. K plays an important role in photosynthesis, it also enhances the circulation of the ascending sap in the xylem and descends into the phloem and permits the transfer of assimilates (amino acids, sugars) to roots and reserve organs (grains, fruits, tubers). P also plays an important role in plant production. It promotes root development and increases the mass of roots, favoring nutrition and plant.

The average root yield was higher at T1 (200 kg NPK ha^{-1}) than at T2 (300 kg NPK ha^{-1}). This result could be explained by the fact that some tubers from one of the three plots allocated to T2 treatment were attacked by rots.

The highest amount of applied NPK (400 kg. ha^{-1}) fertilizer used in this study did not reduce the yield of cassava roots, contrary to those of Ukaoma and Ogbonnaya (2013), who found that a high fertilizer application may result in a reduction in root yield. This is possibly because the level of inherent fertility of the ferralitic soil was very low and thus required high application rates to raise the level of nutrients to optimal levels.

Conclusion

The results of this study showed that chemical fertilizer NPK (12-11-18) has a positive effect on cassava growth and yield. The application rate of 400 kg ha^{-1} of NPK (12-11-18) appears appropriate for the intensification of cassava production in the study area. However, it would be desirable to test in future work the effect of NPK rates above 400 kg.ha^{-1} on cassava yield. Given the very high prices of chemical fertilizers, future studies are also warranted to evaluate combined use of organic and chemical NPK fertilizer for cassava production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of fungicide treatments against Anthracnose in Calabrian (southern Italy) Olive Orchards during 2014-2015

Veronica Vizzarri[1*], Francesco Zaffina[1], Massimiliano Pellegrino[1], Tiziana Belfiore[1] and Laura Tosi[2]

[1]CREA Research Centre for Olive, Citrus and Tree Fruit CREA-OFA, 87036, Rende (CS), Italy.
[2]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy.

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In recent years, severe natural olive anthracnose infections have been observed in orchards of Southern and Central Italy, favoured by mild and rainy seasonal trends, making disease control with the sole use of copper fungicides difficult. Besides, European restrictions in the use of copper compounds for eco-toxicological aspects have forced researchers towards the use of available plant protection products. Thus, 2 year investigations (2014-2015) was carried out in a Calabrian olive orchard (Southern Italy) to evaluate the efficacy of different commercial fungicides (copper oxychloride, tebuconazole/ trifloxystrobin mixture and tebuconazole/trifloxystrobin mixture plus copper oxychloride) in controlling olive anthracnose on 2 CV. Ottobratica (highly susceptible) and Carolea (susceptible). In 2014, anthracnose incidence on Ottobratica ranged from 2% (April) to 21% (November), while in 2015 it was 15 and 29%, respectively. In both years, CV. Carolea showed a higher disease incidence ranging from 22% (April 2014 and 2015) to 56 and 46% in November, 2014 and 2015, respectively. In both years, 3 applications, in April, June and October, with the mixture of tebuconazole/trifloxystrobin and copper oxychloride were the most effective, significantly reducing anthracnose incidence on Ottobratica (11 and 6%) and Carolea (13 and 11%), respectively in November 2014 and 2015. Moreover, the mixture showed a satisfactory activity on primary and secondary infections. The results also showed that respecting fungicide decay curves, the residue levels in extra-virgin olive oil for all tested pesticides were well below European MRLs.

Key words: *Olea europaea*, *Colletotrichum* spp., leprosy, epidemiology, fungicides, Integrated Pest Management (IPM).

INTRODUCTION

Olive anthracnose (OA) is caused by different species of *Colletotrichum* spp.; fruit rot and mummification are the typical and severe symptoms that are responsible for a heavy decrease in olive yields of up to 80-100% and affecting oil quality (high acidity and peroxide values, reduction of polyphenols and α-tocopherol) (Carvalho et

*Corresponding author. E-mail: veronica.vizzarri@crea.gov.it. Tel: +39 0984 4052202.

al., 2008). Leaves, flowers and immature drupes are susceptible to *Colletotrichum* infections, but the pathogen remains latent on plant symptomless tissues for several months, becoming active when environmental conditions are favourable or when fruit begin to ripen. Infected olive trees also show chlorosis, necrosis of the leaves, defoliation and dieback of twigs and branches. OA occurs in many olive-growing areas around the world including Mediterranean countries (Italy, Spain, Portugal, Greece, Tunisia, Morocco), South Africa, South America (Uruguay, Argentina, Brazil), California, Australia and New Zealand (Cacciola et al., 2012; Talhinhas et al., 2018).

OA infections are variable both in incidence and severity depending on environmental conditions (mild temperatures and high rainfall), cultivar susceptibility and pathogen virulence. Since the first appearance of OA in some Mediterranean Basin countries (Portugal, Greece, Spain, Italy) (Petri, 1930), the pathogen pressure is very different, with epidemic outbreaks occurring in low and sporadic attacks. During the last decade, severe infections of OA occurred in Italy, especially in 2010, 2011, 2014, not only in Calabria and Apulia, regions where the disease became endemic after epidemic outbreaks, but also in Tuscany, Umbria, Latium and Sicily. Climate change and approximate cropping practices appear to have created relevant conditions which favoured the olive fruit fly (*Bactrocera oleae* Gmelin) (Gutierrez et al., 2009; Pautasso et al., 2012); larval throphic activity encourages *Colletotrichum* colonisation contributing to the spread of pathogen conidia. A similar effect is also caused by the olive moth (*Prays oleae* Bern.), which is often underestimated by olive-growers. Copper fungicides, commonly applied during pre-flowering and post-fruit set to control olive leaf spot [*Spilocaea oleagina* (Castagne) Hughes] and also to prevent OA infections, has failed to provide an effective OA control especially in regions where a high pathogen pressure and a mild and rainy seasonal trend favour the disease. Moreover, European restrictions in the use of copper compounds such as negative effects on soil and water and the regulations (128/2009/EC, 1107/2009/EC) on plant protection require a new and systematic approach in eco-sustainable integrated control strategies.

Since 2011, the Italian Ministry of Health has temporarily authorised a single application of pyraclostrobin, between June and August, to control OA epidemics, however, in calabrian olive orchards this treatment resulted as being ineffective in controlling OA (Cacciola et al., 2012; Talhinhas et al., 2018). In the following year, the Italian Ministry of Health, in accordance with the emergency situations in plant protection (article 53 of EC Regulation No 1107/2009), authorized a single application of Flint Max (Tebuconazole + Trifloxystrobin) for a period not exceeding 120 days up to pre-flowering for OA control, and therefore the aim of the present study is to evaluate the effect of different commercial fungicides recently registered for OA control under field conditions in Calabria (Southern Italy).

MATERIALS AND METHODS

Field trial location

The study was carried out during the 2014 and 2015 olive growing seasons. The trials were executed in a olive orchard, located at San Giorgio Morgeto (38° 23' N, 16° 5' E, 550 m a.s.l.), representative of the main commercial olive groves in the province of Reggio Calabria.

Field trial design

The experimental field has a soil texture mainly constituted by sand and siltose clays. The field has olive trees (CV. Ottobratica and Carolea) aged 25 years old which were selected because they have been seriously affected by OA and are spaced on 8 x 5 m grid. The olive orchard is not irrigated, and other integrated management practices are those recommended by the Region of Calabria (Disciplinari produzione integrata-Regione Calabria, 2014 and 2015) for commercial olive growers. The trial was laid out in a randomised-block design with 3 replicates and plot size of about 300 m^2. Each plot was separated from the next one, in the row, and from the adjacent rows by a buffer row of 3-5 olive trees.

The treatments and doses of applied fungicides are listed in Table 1. Treatments were applied with a water volume of 1200-1500 L ha^{-1}, depending on the size of olive trees. Each fungicide treatment was applied three times, during both years, specifically in April, June and October, using the growers' nozzle atomiser equipped with an extended lance to spray into the upper branches of olive plants. Control plots consisted of untreated olive trees. All plants were also treated with imidacloprid/Nuprid® Supreme SC, (Nufarm, Italia s.r.l.) (18.08.2014 and 16.08.2015) as recommended by regional olive management practices against olive fruit fly. Monthly average temperatures and rainfall, recorded during the two experimental years as well as the data series of the period 1916 (1924)-2016 were provided by Arpacal Centro Funzionale Multirischi (http://www.cfd.calabria.it).

Disease monitoring

The efficacy of the treatments on OA was assessed by determining the disease incidence before each fungicide treatment for the three application times in both years and at the end of the trials, 30 days after the last fungicide application to respect the safety interval before the olive harvest. OA incidence was evaluated by humid chamber to promote pathogen sporulation (acervuli). Particularly, at the beginning of each trial, on 5 plants per each replicate, ten twigs (approximately 15-20 cm) with leaves and flowers were randomly collected and then placed in Petri plates (∅150 mm), one twig per plate, containing filter paper moistened with deionised water. All plates were maintained at 20 ± 2°C for 5 days. Each twig was then examined with the aid of a stereomicroscope to observe the presence of acervuli formed on the tissues surface and disease incidence was expressed as a percentage of infected twigs per plant. Before the other 2 application times (June and October) and before the olive harvest (November), 100 drupes were randomly collected from each plant and then placed in Petri plates (20 drupes /plate) as described above. Drupes were then examined to determine pathogen sporulation. Disease incidence was expressed as % of infected fruits. Since fruit susceptibility increases with ripening and rot symptoms become visible, drupe samples collected in October and November, were also used to determine disease

Table 1. Fungicide treatments applied in the field trials during 2014 and 2015.

Treatment	Commercial product and formulation[a]	Active ingredient (%)	Commercial product rate (g hl^{-1})	Application dates[b] 2014	2015
Untreated control	↓-	-↓-	-↓-	-↓-	
Copper oxychloride	Cupravit® Blu 35, WG	35	350	3 April	13 April
Tebuconazole + Trifloxystrobin	Flint Max, WG	50 + 25	20	25 June	15 June
Copper oxychloride and Tebuconazole + Trifloxystrobin mixture	Cupravit® Blu 35 and Flint Max mixture	35 and 50 + 25	350 + 20	1 October	1 October

a = Commercial products are supplied by Bayer
b = Each fungicide treatment was applied at each application dates; for more details see Materials and methods.

severity by scoring OA infections according to a scale from 0 to 3 (0= no fruit rot; 1= 10-25% fruit rot; 2=26-50% fruit rot; 3 over 50% fruit rot).

After 1 month safety interval, drupe samples (about 2.3 kg) from plants of each replicate were randomly collected and transported to the laboratory to obtain extra virgin olive oil. Drupes were ground by an “Olio Mio 50” continous cycle mini-mill (Toscana Enologica Mori, Tavernella Val di Pesa, Florence, Italy). Drupes were crushed in cold and the malaxing was lasted for 20 min. The oil was then centrifuged at 7000 rpm for 3 min by a Sorvall centrifuge and stored in dark glass bottles at 9±2°C. Pesticide residue analyses and estimation on all oil samples were conducted by Centro Analisi Biochimiche s.a.s. (Rizziconi, Reggio Calabria), certified Accredia no.0859.

Statistical analysis

Data regarding disease incidence, expressed as a percentage of infected twigs or infected fruits, were subjected to the analysis of variance (ANOVA) followed by LSD post hoc test to separate the means and to evaluate the effect of treatments. Disease severity was calculated using the McKinney index (McKinney, 1923) by scoring OA infections according to a percentage scale expressing the fruit rot. Data were subjected to the analysis of variance and to the Tuckey test.

RESULTS

Examining the monthly average temperatures and rainfalls recorded during the two experimental years (2014-2015), it is possible to note that temperatures were generally similar to those recorded in the period 1924-2016 (Figure 1). On the contrary, the amount and the distribution of rainfalls were above the average of the years included in the period 1916-2016 (Figure 1). In particular, a variable distribution among months was observed in the years 2014 and 2015 and OA infections were favoured by mild and rainy autumns (October-November).

Indeed, olive anthracnose incidence on untreated control plants of both susceptible cultivars showed a progressive increase throughout the growing season during 2014 and 2015. In particular, in 2014 OA incidence on CV. Ottobratica (high susceptible) ranged from 2% (April) to 21% (November) while in 2015 OA incidence was 15 and 29%, respectively (Figures 2 and 3).

In both years, CV. Carolea (susceptible) showed a higher disease incidence ranging from 22% (April 2014 and 2015) to 56 and 46% in November 2014 and 2015, respectively (Figures 2 and 3). This apparent discrepancy between the two cvs could be explained by the larger size of Carolea drupes compared to Ottobratica fruits, and therefore they are more affected by punctures or exit holes of the olive fruit fly. Larval throphic activity encourages *Colletotrichum* colonisation. Moreover, under favourable environmental conditions the longer harvest period of Carolea, compared to Ottobratica (which takes its name as it is harvested in October), contributes to severe OA infections.

All the treatments applied during April-October generally reduced OA latent and secondary infections. In both years, two applications for each fungicide reduced disease incidence. The effects of these fungicides, after two applications, are shown in Figures 2 and 3. In particular, in October 2014 and 2015, Flint Max (Tebuconazole + Trifloxystrobin) and the mixture Flint Max + Cupravit® Blu 35 (Copper oxychloride) significantly reduced disease incidence on both cvs. In October 2014, for example, disease incidence on Carolea was 15 and 8% on olive plants that received two applications of Flint Max and the Flint Max + Cupravit® Blu 35 mixture, respectively compared with 47% of untreated plants. Flint Max and the mixture Flint Max + Cupravit® Blu 35 resulted particularly effective against OA latent infections while Cupravit® Blu 35 generally showed a modest effect in disease control and resulted in a significant OA incidence reduction only on CV. Carolea in both years.

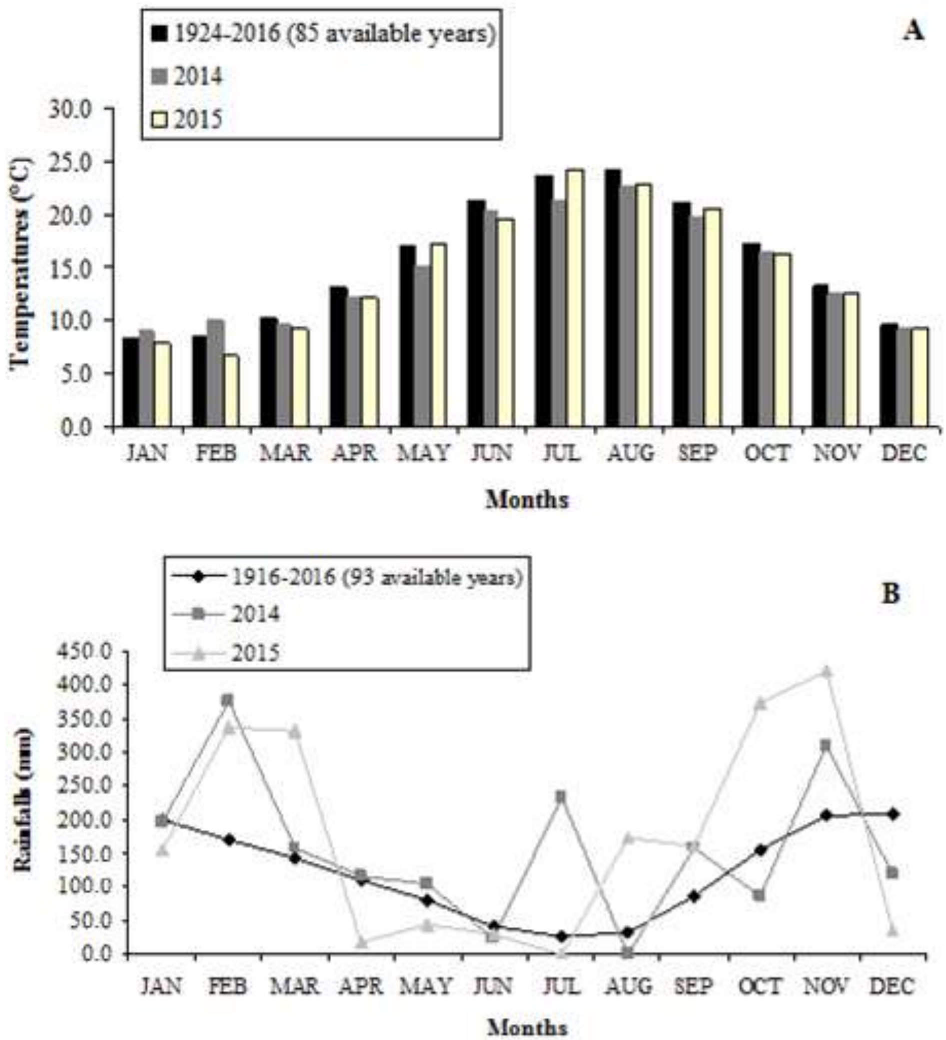

Figure 1. Monthly average temperatures (A) and rainfalls (B) recorded during 2014 and 2015.

In November, after the third application, Flint Max and the mixture Flint Max + Cupravit® Blu 35 resulted to be effective in reducing OA secondary infections compared to the untreated control plants (Figures 2 and 3). Considering all fungicide treatments, the best disease control was observed with the Flint Max + Cupravit® Blu 35 mixture which was effective on both cultivars and in both years.

The performance of each fungicide is also confirmed by the average OA severity observed during October-November in 2014 and 2015 (Figure 4). In particular, the application of the Flint Max + Cupravit® Blu 35 mixture showed a statistically significant higher efficacy compared to the untreated control for both cultivars. By the end of the field experimental trials (November 2014 and 2015), this fungicide treatment showed the lowest OA severity of 22 and 6%, respectively for CV. Ottobratica and of 17% for CV. Carolea compared with Ottobratica (42 and 23%) and Carolea (70 and 50%) untreated controls, respectively (Figure 4). A satisfactory disease control was observed with Flint Max. Modest results were achieved with Cupravit®Blu 35 which, however, resulted in a significant reduction in OA severity on CV. Carolea in both years, but disease control on CV. Ottobratica was unsatisfactory (Figure 4).

The results of fungicide residue analyses on all oil samples are shown in Table 2. Residue levels of the applied systemic fungicides (tebuconazole and trifloxystrobin) and the insecticide (imidacloprid) were determined by a multi-residue pesticide analysis. The average recovery of fungicides was determined at value of uncertainty k = 2 and confidence level 95%. The residue levels for all tested pesticides were well below European MRLs (Table 2). On the basis of the three recommended copper treatments to prevent olive leaf spot (*Spilocaea oleagina*), useful for their collateral action

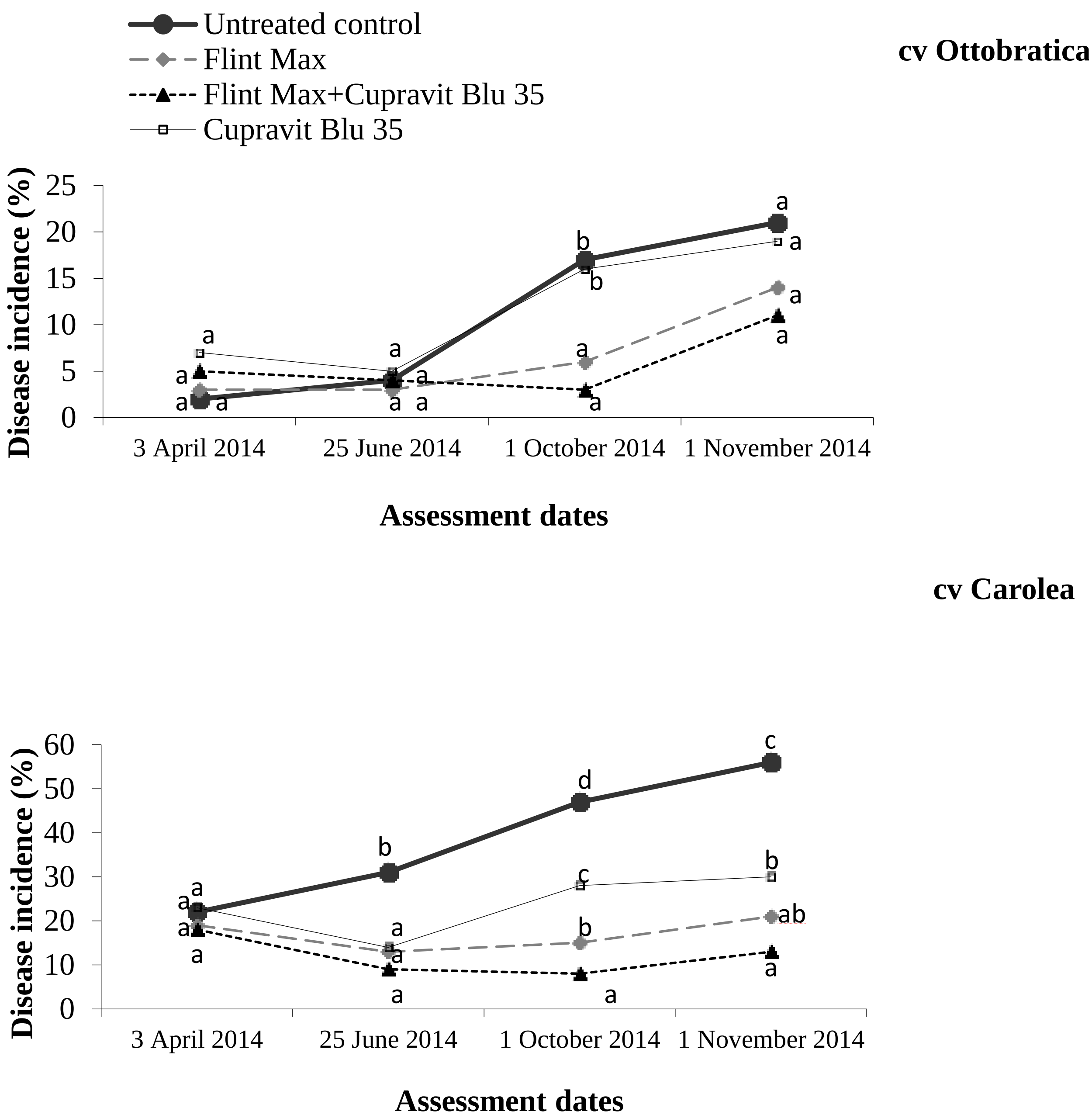

Figure 2. Effect of fungicide treatments on cvs Ottobratica and Carolea applied during April-October 2014 on olive anthracnose incidence. Within each assessment date, the values of incidence average with the same letter do not differ statistically from each other ($P < 0.05$).

against OA infections, in this study the residue level of copper was not determined as it was assumed that the copper MRL on olives was below European MRL (30 mg kg^{-1}, according to the European Regulation No 396/2005). Results of pesticide residue monitoring for olive and olive oil, show that the metal concentration was always found at a value lower than the MRL. Copper is concentrated in the olive water fraction and it is easily eliminated with the vegetation waters by centrifugation (Simeone et al., 2009).

DISCUSSION

The results obtained during the two-year investigations performed in Calabria on olive anthracnose and its chemical control confirmed the seriousness of this olive disease. Under natural conditions, OA incidence on untreated trees of both susceptible cultivars (Ottobratica and Carolea) increased during the growing season reaching highest incidence (56%) and severity (70%) on Carolea in November 2014. Weather conditions favoured

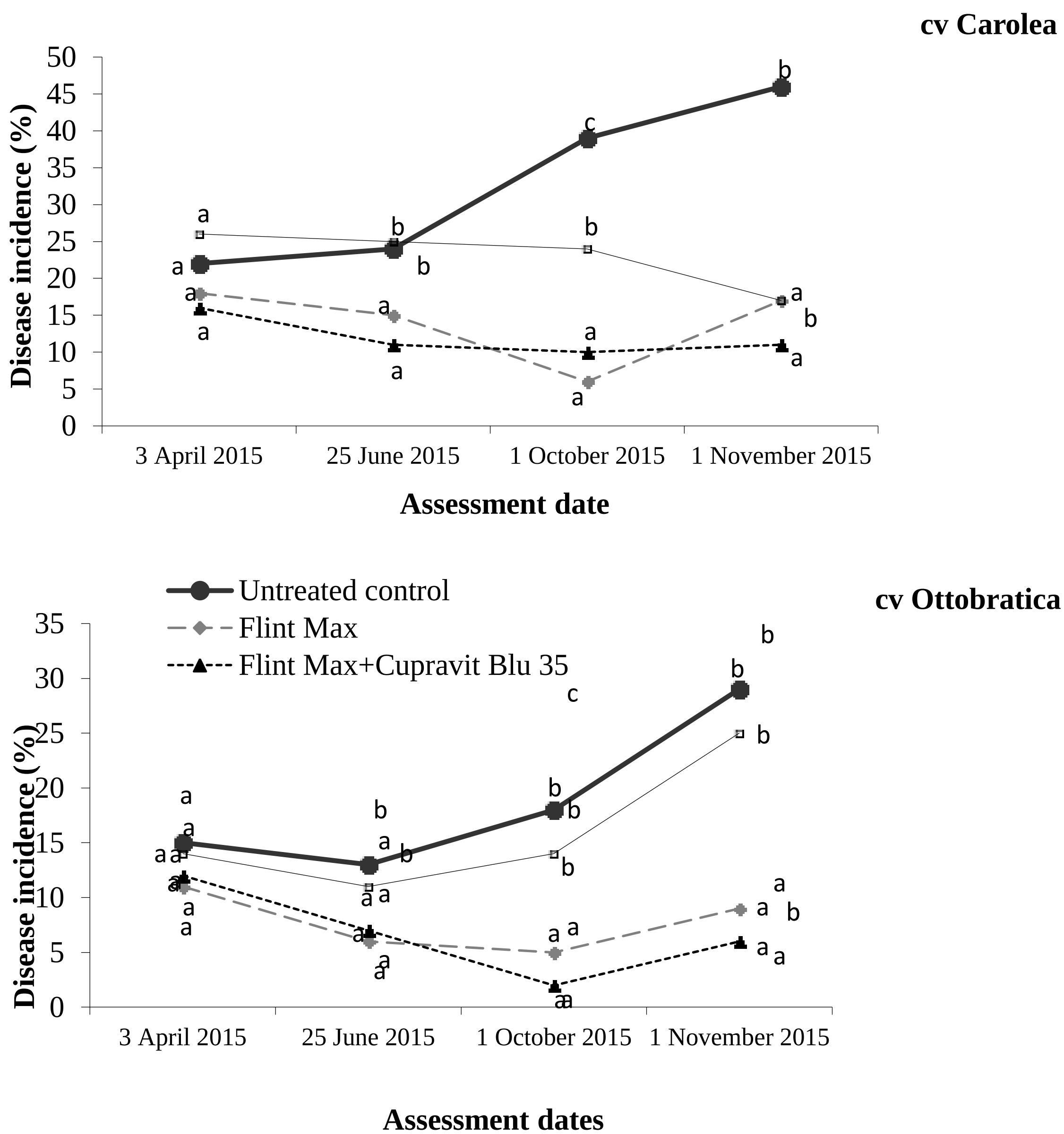

Figure 3. Effect of fungicide treatments on cvs Ottobratica and Carolea applied during April-October 2015 on olive anthracnose incidence. Within each assessment date, the values of incidence average with the same letter do not differ statistically from each other ($P < 0.05$).

the rapid increase of OA incidence, shaped disease severity among cultivars and also the virulence variability existing among *Colletotrichum* species populations (Talhinhas et al., 2009, 2015). Therefore, as a consequence of future changes in climate and also in current annual variability of weather conditions, chemical control is necessary to prevent serious OA epidemics.

Our results show that Flint Max (Tebuconazole + Trifloxystrobin) and the mixture Flint Max + Cupravit® Blu 35 (Copper oxychloride) were the most effective fungicides in reducing latent and secondary infections when sprayed in three applications (April, June and October). Moreover, the fungicide residue levels of the commercial products applied in accordance with the label recommendations were well below European MRLs. Furthermore, application of the Flint Max + Cupravit® Blu 35 mixture resulted as being very active in controlling the pathogen and reducing OA severity in both cultivars.

In consideration of eco-sustainable integrated protection strategies, the Italian Ministry of Health should authorise up to three application of the Flint Max only when weather conditions are very conducive for OA infections, depending on the cultivar susceptibility and the history of the disease in the previous years, to reduce epidemics in

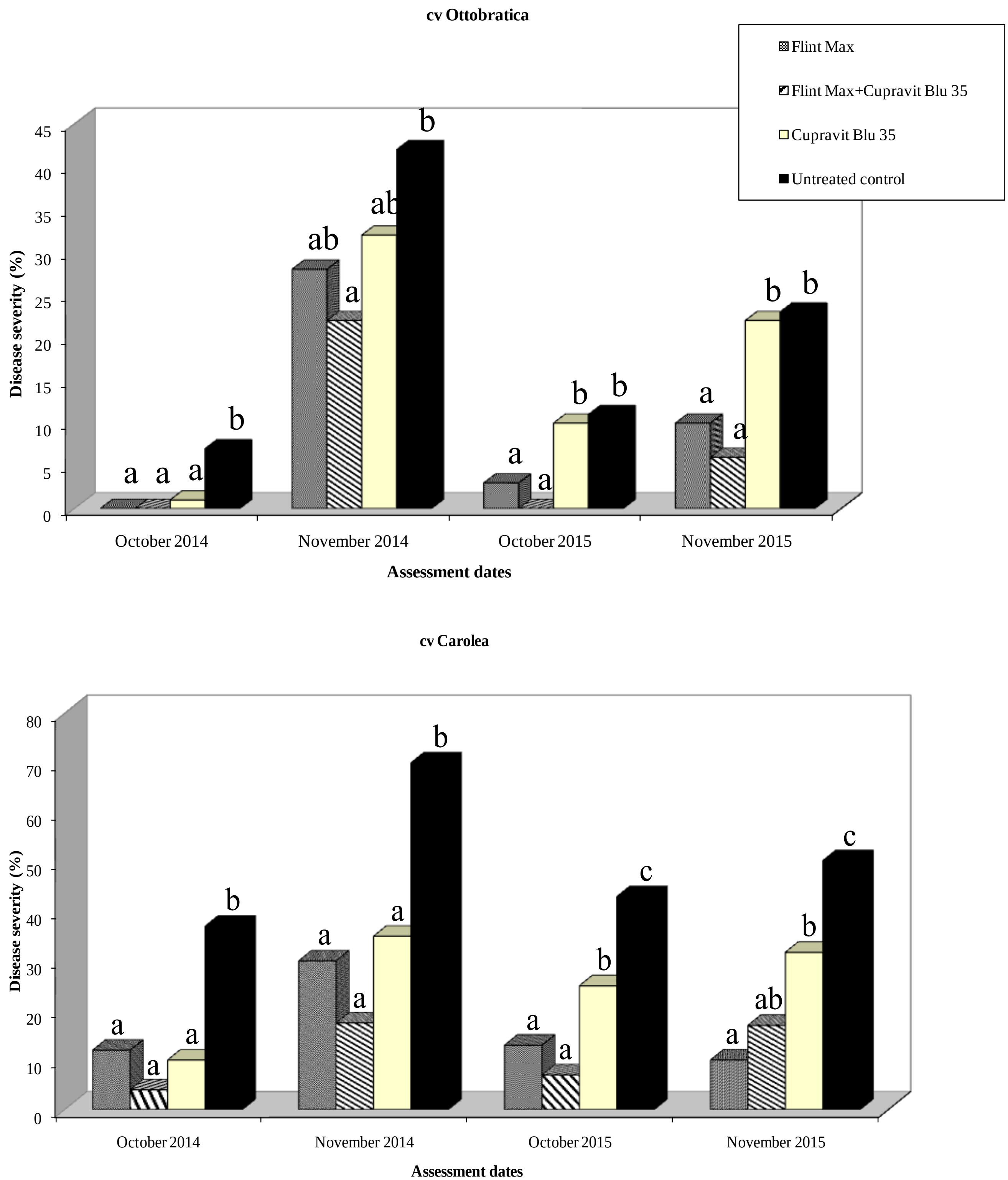

Figure 4. Olive anthracnose severity observed on cvs Ottobratica and Carolea sprayed with different fungicides during October-November in 2014 and 2015. Within each assessment date, columns with different letter are significantly different at (P <0.05).

accordance with the principles of good agricultural practice. Nevertheless, OA chemical control should necessarily be combined with cultural management such as balanced fertilization and pruning to remove infected

Table 2. Average residue levels of applied systemic pesticides recovered in olive oil samples collected in the field trials in November 2014 and 2015.

Commercial product	Active ingredient[a]	November 2014		November 2015		MLR[b] (mg kg^{-1})
		Ottobratica	Carolea	Ottobratica	Carolea	
Flint Max	Tebuconazole	0.061 ± 0.065*	0.058 ± 0.013	0.107 ± 0.024	0.119 ± 0.026	0.25[c]
	Trifloxystrobin	0.064 ± 0.014	0.014 ± 0.003	0.023 ± 0.006	0.020 ± 0.006	0.3
Nuprid	Imidacloprid	0.014 ± 0.003	0.014 ± 0.003	0.022 ± 0.006	0.012 ± 0.004	1.0
Flint Max + Cupravit® Blu 35	Tebuconazole	0.029 ± 0.006	0.060 ± 0.013	0.081 ± 0.019	0.157 ± 0.033	0.25[c]
	Trifloxystrobin	0.058 ± 0.002	0.029 ± 0.006	0.031 ± 0.008	0.052 ± 0.013	0.3
Nuprid	Imidacloprid	0.008 ± 0.002	0.022 ± 0.005	0.012 ± 0.004	0.014 ± 0.004	1.0

[a]LOQ max (mg kg^{-1}) for 0.01;
*concentration expressed as mg kg^{-1} ± standard deviation;
[b]MLRs according to the European Union legislation framework Regulation (EC) No 491/2014 for imidacloprid and trifloxystrobin;
[c]for tebuconazole, the MLR value of 0.05 [Regulation (EC) No 61/2014] must be rectify applying a correction factor for the pro-cessed products, as reported in Regulation (EC) No 400/2014 [*Virgin olive* oil (unless a specific oil processing factor is available, oil pro-cessing factor =5, taking into account an olive oil production standard yield of 20% of the olive harvest. Member States are requested to report the pro-cessing factors used in the 'National Summary report].

twigs and mummified fruits, to reduce potential source of infection, and to improve air movement in the canopy.

In organic olive orchards, where copper is the only preventive fungicide to control olive leaf spot and anthracnose, the adoption of efficient and reliable control measures becomes more strict and the cupric ion, due to its antibacterial activity, is also effective against olive knot (*Pseudomonas savastanoi* pv. *savastanoi*) and olive fruit fly because its repellent and anti-oviposition effects interrupt the bacterium-fly symbiosis and so jeopardize nutrition of the larval stages (Sacchetti et al., 2008; Caleca et al., 2010).

The uncertain future of copper-based fungicides, due to reduction of soil microbial biomass and potential influence on physical and chemical processes, and the resulting European regulatory restrictions make it necessary to develop effective alternatives such as selected biological agents, antifungal compounds, plant and compost extracts inducing plant resistance. Recently, the biocontrol potency of olive epiphytic and endophytic fungal communities was evaluated *in vitro* tests against *Colletotrichum acutatum* J.H. Simmonds (Preto et al., 2017) and two treatments with a pomegranate (*Punica granatum* L.) peel extract were very effective in reducing OA infections and bacterial populations, when applied under natural conductive conditions of Southern Italy (Pangallo et al., 2017). Moreover, further researches developping more eco-friendly anthracnose control alternatives are needed to replace synthetic and copper fungicides, contribute to a successful integrated and biological olive disease management systems and improve olive productions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of probiotic-treated rice straw on blood parameters and gut microbes of heifers

Abu Sadeque Md. Selim[1*], Mahbub Sobhan[1], Mueena Jahan[2], Md. Morshedur Rahman[3], Md. Kaosar Niaz Bin Sufian[4] and Shilpi Islam[1]

[1]Department of Animal Science and Nutrition, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur - 1704, Bangladesh.
[2]Department of Microbiology and Public Health, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh.
[3]Department of Dairy and Poultry Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh.
[4]Department of Genetics and Animal Breeding, Patuakhali Science and Technology University, Out Campus, Barishal, Bangladesh.

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The study investigated the effects of probiotic treatment on nutrient content of rice straw and its impact on blood parameters and gut microbes of heifers. Eight Holstein crossbred heifers with initial average body weight (BW) of 180.25±21 kg were used in this study. The experimental period lasted for 33 days of feeding, of which 5 days were adjustment period. The treatments included feeding of untreated rice straw (Control) and probiotic treated rice straw. In each treatment, four animals were randomly assigned. Feed were offered twice daily and ensured *ad libitum* water supply. Probiotic treatment improved crude protein (CP) level from 4.35 to 6.37%. Crude fiber content (acid detergent fiber, ADF) was decreased from 64.83 to 61.30% after treatment of straw using probiotic. Blood glucose, albumin and high density lipoprotein (HDL) level were significantly (P<0.05) increased from 65.62 to 125.08 mg/dl; 3.70 to 4.18 g/dl, and 106.28 to 165.28 mg/dl, respectively in probiotic treated straw fed animals. Low density lipoprotein (LDL) level was significantly (P<0.05) decreased from 8.77 to 0.79 mg/dl and 247.6 to 162.2 mg/dl in probiotic treated rice straw fed animals which are worthy for animal health. Probiotic treated group had a normal range of microbial community in the intestine. In conclusion, probiotic treated straw improves nutrient content of rice straw and maintains normal blood parameters and microbial gut flora of heifers.

Key words: Probiotic, rice straw, crude fiber, crude protein, acid detergent fiber, blood parameters, fecal microbiota.

INTRODUCTION

Rice straw is one of the agricultural by-products that are abundantly available in many tropical countries (Nguyen et al., 2017). Small holder farmers usually store it and use rice straw as ruminant feed throughout the year

*Corresponding author. E-mail: asmselim@bsmrau.edu.bd

especially in the dry winter season. Although, ruminants are able to convert low quality agriculture by-products into high quality protein but the use of rice straw as ruminant feed is hampered by its low nutritional value such as high neutral detergent fiber (NDF) and lignin content, low protein content and their low digestibility (Sarnklong et al., 2010). Various methods have been used to improve the nutritive value of fibrous feeds including physical, chemical, and biological treatments for ruminants. However, physical and chemical treatments can be expensive, harmful to users or unfriendly to the environment (Tuyen et al., 2012). Among them, biological treatment of rice straw through probiotic is the best alternative treatment because it is cost effective and ecofriendly and can improve its digestibility. Researchers in India (Gowher et al., 2017) have found significant improvement in nutrient degradability of DM, OM and NDF due to probiotics mix supplementation with maximum values at 3 g kg to 1 DM.

Probiotics are live microorganisms, beneficial to animal health when consumed in adequate quantities. Several *Lactobacillus* strains have been found to reduce total cholesterol (TC) and triglyceride (TG) concentrations. Probiotics have the ability to enhance intestinal health by stimulating the development of a healthy microbiota (predominated by beneficial bacteria), preventing enteric pathogens (Coliforms) from colonizing the intestine, increasing digestive capacity, lowering the pH, and improving mucosal immunity. Probiotics have the ability to enhance intestinal health by stimulating the development of a healthy microbiota, preventing enteric pathogens from colonizing the intestine (Yutaka et al., 2015). Probiotics compete with harmful gut flora, stimulate the immune system of the animal, and increase its resistance to infectious agents in order to promote growth (Kritas and Morrison, 2005). Intestinal bacteria play a vital role in digestion of feed and maintaining good health of animal. However, no information is available on the effect of probiotic on cattle diets on health and population of intestinal bacteria. The mode of action of the probiotic on nutrient improvement of rice straw is also poorly understood. To assess the beneficial effects of commercial probiotic treated straw, a thorough investigation is needed. Therefore, the objectives of this study were to evaluate the effects of probiotic on straw nutritive value, blood parameters and microbial community of heifers.

MATERALS AND METHODS

Collection and treatment of rice straw

Rice straw and probiotic (protexin) were collected from local market. The probiotic was a mix culture compost of *Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophiles* and *Enterococcus faecium.* The total CFU of the probiotic 1.8×10^8/g was determined against manufacturer claim 2.0×10^9 g^{-1}. Rice straw cut into 2 to 3 cm, water added to give moisture up to 65% and then mixed 0.5% probiotic with straw sealed in polythene bag and incubated for 2 days.

Feeding of experimental animals

Eight Holstein crossbred heifers with initial average body weight (BW) of 180.25±21 were used in this study which lasted for 33 days of which 5 days adjustment period. The Holstein crossbred heifers were grouped based on initial BW. Each dietary treatment was randomly assigned to each heifer in a block resulting in four replications per treatment. The treatments included feeding of untreated rice straw (Control T1) and treated rice straw T2. Composition of diet ingredients and chemical composition of rice straw and treated rice straw used in the experiment has been shown in Table 1.

The diets (Table 1) were fed to the experimental heifers twice daily in the morning and afternoon. The experimental animals had *ad libitum* access to water. Daily feed offers and refusals were recorded for each heifer to calculate daily feed intake. Samples of feed offers were taken on batches. Feed and refusals were sampled per animal and pooled for each treatment. Representative samples of both diets and refusals were kept and used for further analysis. The experiment was conducted at the Livestock and Poultry farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gzipur-1706, Bangladesh.

Collection of blood sample

Blood samples were collected from experimental animals at days 0, 7, 14, 21 and 28 of the research period. Five milliliters of blood was collected from the jugular vein of animals with the help of 5cc sterile syringe. In every case, a new sterile syringe was used to collect blood and after collecting blood, all syringes were disposed carefully. After collecting, the blood was kept in a heparinized tube to prevent clotting and transported to the lab for further use.

Determination of blood parameters

Albumin, glucose, high density lipoprotein (HDL) and high density lipoprotein (LDL) were determined using selective kits purchased from Elabscience® China and with the support of Double Beam Spectrophotometer, Aarson Scientific, India.

Collection of fecal sample and monitoring fecal microbiota

Fecal sample was collected at days 0, 7, 14, 21 and 28 using sterilized spatula and immediately transferred to the laboratory for further analysis. De Man, Rogosa and Sharpe agar (MRS), Eosin Methylene Blue (EMB) and Plate Count Agar (PCA) were used for the determination of lactobacillus, coliform and total bacteria in heifers at different periods incubating Petri dish at 37°C for 48 h.

The animals were maintained and monitored by university ethical committee.

Chemical analysis

DM was determined in two steps, the first step was according to Goering and Van Soest (1970) and during the second step, oven temperature was increased to 105°C. The CP and EE levels were determined according to AOAC (2005). The NDF and ADF contents were analyzed according to Van Soest et al. (1991).

Table 1. Composition of ingredients and chemical composition of rice straw and treated rice straw used in the experiment.

Treatment	Control (T1) (%)	Treated Straw (T2) (%)
Diet ingredients		
Green grass	52.26	52.36
Treated rice straw	-	23.63
Untreated rice straw	23.78	-
Wheat bran	11.72	11.74
Mustard oil cake	5.40	5.41
Rice polish	4.13	4.13
Broken rice	2.11	2.13
Salt	0.60	0.60
Parameter	**Untreated rice straw (%)**	**Treated straw (%)**
Dry matter (DM)	93.18	92.42
Crude protein (CP)	4.35	6.37
Acid Detergent Fiber (ADF)	64.83	61.30
Neutral Detergent Fiber (NDF)	76.46	77.68
Ether Extract (EE)	0.07	0.90
Ash	13.42	18.68

T1: Control; T2: Probiotic treated straw

Statistical analysis

Data were analyzed by analysis of variance using the General Linear Models procedure of SAS (Kuehl, 1994; SAS, 2000). Differences among treatments, when significant, were also ordered using Tukey's test (Kuehl, 1994). Statements of statistical significance were based on P<0.05 or P<0.01.

RESULTS

Improvement of nutritional value of rice straw after probiotic treatment

Table 1 shows that probiotic treatment improved crude protein (CP) content from 4.35 to 6.37%. This result is almost similar with Sariubang et al. (2002), Sembiring et al. (2002) and Antonius (2009) who observed CP content in the range of 5.63 to 11.25% fed starbio, starbio and pobion, respectively.

Acid detergent fiber (ADF) was 64.83% and after treatment it decreased to 61.30%. The reduction of ADF (4.89%) is similar with the results of Syamsu (2001) when rice straw was treated with chicken manure.

Effect of probiotic treated rice straw on blood parameters in heifers

Table 2 shows the effect of probiotic treated straw on blood albumin, glucose and LDL. These are the indications of animal health status. Table 2 shows that there was no significant difference (P<0.05) of albumin content in days 14 to 28 among treated and untreated group. However, a significant difference (P<0.05) of albumin content at days 0 and 7 was observed, may be due to individual variation of animals, not might be probiotic impact. The range of albumin content was observed between 3.70 and 5.69 g/dl in the current study. Glucose is a measurement of the blood sugar level. It was found that the range of blood glucose varied (48.41 vs. 125.8 mg/dl) (Table 2). The highest value (125.8 mg/dl) was found in treated straw at day 21 and lowest value was found in untreated straw at day 0. The certain fall of glucose (52.44 mg/dl) in untreated straw at day 21 was due to illness of two animals. Glucose level was increased after probiotic supplementation (65.62 vs. 115.43 mg/dl). This result is consistent with the results of Dlamini et al. (2017) who found 127.98 mg/dl. In other study, Ahmad et al. (2004) reported higher values in cyclic cross breed cow (50.72±1.12 g/dL).

Low-density lipoproteins (LDL), or "bad" cholesterol, may make arterial narrowing worse. The normal range of LDL in blood is 3 to 800 mg/dl. From the Table 2, it was found that there was a significant reduction (P<0.05) of LDL concentration in blood of experiment animal at day 28 fed probiotic treated straw compared to untreated straw. However, a significant (P<0.05) increased LDL concentration was observed in blood of animal at days 14 and 21 fed probiotic treated straw. Highest value was found at day 0 (247.6 mg/dl) and lowest value was found at day 28 (162.2 mg/dl) in treated straw compared to untreated straw group. However, in probiotic treatment, there was no difference in time points. LDL level was decreased after probiotic supplementation which is

Table 2. Effect of probiotic treated rice straw on albumin (g/dl), glucose (mg/dl), HDL (mg/dl) and LDL (mg/dl) concentration in heifers.

Parameter	Treatment	Days				
		0	7	14	21	28
Albumin (g/dl)	T1	5.69[a]	4.16[a]	4.05[a]	4.37[a]	4.24[a]
	T2	3.70[b]	3.80[b]	4.08[a]	4.15[a]	4.18[a]
	SEM	0.31	0.35	0.35	0.43	0.18
	P-value	0.0002	0.0052	0.9461	0.3817	0.7747
Glucose (mg/dl)	T1	48.41[b]	93.20[b]	100.6[a]	52.44[a]	123.01[a]
	T2	65.62[a]	95.07[b]	109.45[a]	125.08[b]	115.43[b]
	SEM	1.48	2.20	1.68	2.08	3.03
	P-value	0.0002	0.0637	0.0360	0.0001	0.0001
LDL (mg/dl)	T1	245.2[a]	241.7[a]	184.9[b]	195.8[b]	251.5[a]
	T2	247.6[a]	243.1[a]	233.5[a]	232.3[a]	162.2[b]
	SEM	7.05	9.30	6.18	4.89	5.30
	P-value	0.0637	0.0360	0.000	0.0001	0.0001
HDL (mg/dl)	T1	120.42[a]	30.29.7[a]	119.92[a]	135.75[a]	136.19[a]
	T2	106.63[a]	139.891[b]	148.46[c]	165.28[d]	148.09[c]
	SEM	6.50	2.30	3.18	3.89	2.30
	P-value	0.003	0.060	0.0043	0.0021	0.0031

[a-b]Mean values within a row with different superscripts are significantly different (P<0.05). T1=Control group, T2=treatment group, SEM=standard error mean, LDL=low density lipoprotein.

similar with the results of Petkova et al. (2008) who found 1.04 mmol/l. Similar findings were also observed by Kumar and Sharma (1993) in dairy cows and Jayachandranl et al. (2007) in buffaloes who found 2.55 and 3.45 mmol/l. After probiotic supplementation LDL (bad cholesterol) was greatly decreased which is good for animal health condition. HDL or “good” cholesterol protects the body against narrowing blood vessels. HDL interprets its levels in the opposite manner of LDL. Table 1 shows that there was no significant difference (P<0.05) of HDL concentration in the blood of treated and untreated straw fed animals. The highest value (165.28 mg/dl) was found at day 28 in treated straw and the lowest value (119.92 mg/dl) was found at day 14 in untreated straw.

Monitoring microbial status

Figure 1 shows the effect of probiotic treated rice straw on fecal microbiota in heifers. The number of lactobacilli was in a range of 4.8×10^5 to 6.0×10^5 cfu/ml after probiotic treatment. This result is consistent with the results of Mallo et al. (2010), who found similar concentration while *Enterococcus faecium* added to diet. This result was in agreement with the findings of Timmerman et al. (2005) who reported that fecal counts of lactobacilli was not different compared to control, while six *Lactobacillus* species was administered with one-week-old veal calves for eight weeks. The use of probiotic bacteria to inhibit *Escherichia coli* O157:H7 in cattle is a promising method to control this food-borne pathogen. Previous reports have shown the potential of beneficial *E. coli* strains and lactic acid bacteria to reduce O157:H7 fecal shedding or prevalence in cattle. Coliforms are pathogenic micro-organism and are also responsible for many diseases.

Figure 1 shows that the number of coliforms decreased (6.8×10^5-4.0×10^5 cfu/g) after probiotic treatment. This result is similar with the results of Zhao et al. (1998) who observed 3.2×10^4 cfu/ml after probiotic supplementation isolated from cattle feces. This result is consistent with the results of Mallo et al. (2010) who found 2.76×10^5 cfu/ml. But this result disagreed with the findings of Timmerman et al. (2005) who found 5.69×10^5 cfu/ml in dairy goats. Decreasing amount of coliform in feces is a positive sign for animal health. Figure 1 indicates that in general total number of total bacteria did not vary (8.4×10^{11} to 4.8×10^{11} cfu/ml) after probiotic treatment. This result is close to Srinivas et al. (2013) who found 1.8×10^{10} cfu/ml of total bacteria supplementing of yeast culture (Levucell SC 20) 0.5 g/animal/day in the diet of graded Murrah buffalo bulls.

DISCUSSION

Rice straw, a by-product of the rice production is mainly used as a source of feed for ruminant livestock. Rice

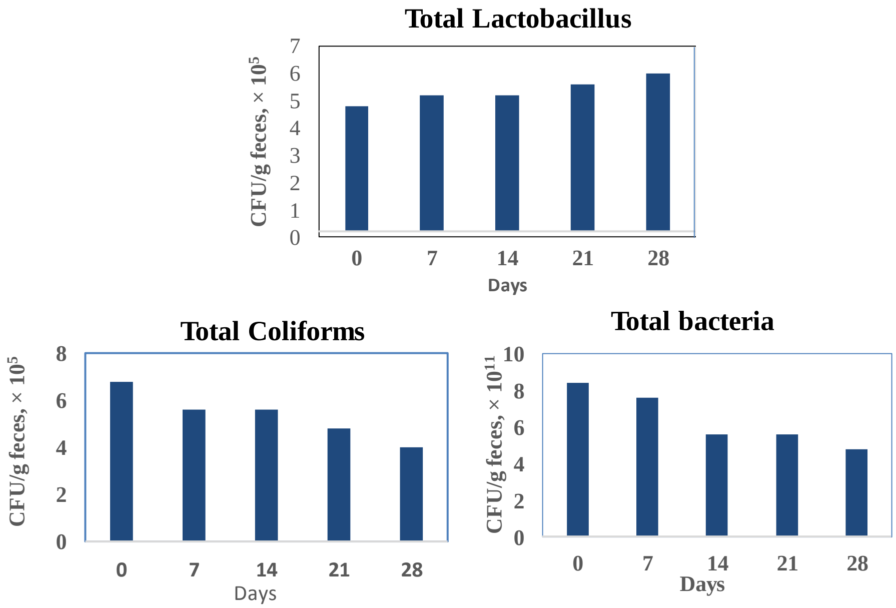

Figure 1. Effect of probiotic treated rice straw on faecal microbiota.

straw has very low nutritive values, especially its crude protein content and digestibility. Probiotic treatment increased CP content tremendously in the current study (Table 1). From the current findings, it was assumed that fermentation process with addition of microbes might improve crude protein content of rice straw. Sairubang et al. (2002) and Antonius (2009) strongly suggests this that animal may receive more digestible protein for their production performance. ADF concentration was also decreased in the present study (Table 1). This was a good sign of release of cellulose and hemicellulose from straw. The decreased crude fiber level of rice straw suggests that probotic microbes are able to penetrate the fibrolytic structure and cleave the binding of lignified carbohydrate and in some extent, degrade cellulose and hemicellulose. The addition of beneficial microbes in the straw seemed to have an effect in the breakdown of fiber materials in rice straw. The acid detergent fiber value refers to the cell wall portions of the forage that are made up of cellulose and lignin. Treating rice straw with effective microorganisms decreased the acid detergent fiber (ADF) component thus making it more digestible and animal will get more digestible energy (DE).

Albumin, glucose and LDL are important blood parameters of dairy cows. Blood albumin level was in a normal range (Table 2) when fed probiotic treated rice straw to the animals indicating a good health condition of those animals. A lower level of albumen was found compared to untreated straw group in the current study. The reasons of lower content of albumin in the present study may be due to individual variations of animal, breed or seasonal effect. The interesting thing is that probiotic treated straw feeding could not affect normal range of glucose, LDL and HDL concentration in the blood of animals. After probiotic supplementation, LDL (bad cholesterol) was greatly decreased (Table 2) that strongly suggest that probiotic straw feeding is not harmful for the animals. The present study stated that, there was an increasing tendency of HDL after probiotic treatment. These results are in accordance with results of Yu et al. (2004) who found 179 mg/dl of HDL concentration in blood after supplementation with direct fed microbes in cows. After probiotic treatment, HDL (good cholesterol) was significantly increased which is good for animal health status.

The beneficial effects include increased number of lactobacillus in feces on growth performance, nutrient retention, diarrhea reduction and for the balance of intestinal microflora. By this process, the health status of the animals will be increased.

The use of probiotic bacteria to inhibit *E. coli* O157:H7 in cattle is a promising method to control this food-borne pathogen. Previous reports have shown the potential of beneficial *E. coli* strains and lactic acid bacteria to reduce O157:H7 fecal shedding or prevalence in cattle. Coliforms are pathogenic micro-organism and are also responsible for many diseases. It was found that the number of coliforms decreased (6.8×10^5-4.0×10^5cfu/ml) after probiotic treatment (Figure 1). This result is similar with the results of Zhao et al. (1998) and Harman et al. (1996) who observed 3.2×10^4 cfu/ml after probiotic supplementation in buffalos. This result is consistent with the results of Mallo et al. (2010) who found 2.76×10^5 cfu/ml. But this result disagreed with the findings of Timmerman et al. (2005) who found 5.69×10^5 cfu/ml in dairy goats. Decreasing number of coliform in feces is a positive sign for animal health. Disease incidence related to coliforms may be reduced in animals after probiotic supplementation.

Conclusion

Pobiotic (protexin) treated rice straw improves crude protein content and decreases fiber content compared to untreated rice straw. An *in vivo* trial showed that blood metabolites such as glucose, albumin, LDL, HDL and fecal microbiota were almost in a normal range after probiotic treated straw feeding. Therefore, probiotic can be considered as a straw treating agent and an environment safe alternative to the hazardous chemicals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Indigenous rhizobia strains: The silver bullet for enhanced biological nitrogen fixation and soybean (*Glycine max* (L.) Merr.) yield under different soil conditions in South Kivu province, Democratic Republic of Congo

Ndusha Bintu Nabintu[1,2*], Onwonga Richard Ndemo[1], Nabahungu Leon Sharwasi[3], Mushagalusa Nachigera Gustave[2], Matendo Rehema Esther[2] and Keya Shellemia Okoth[1]

[1]Department of Land Resource Management and Agricultural Technology, University of Nairobi, Nairobi -Kenya, P. O. Box 29053-00100, Nairobi.
[2]Université Evangélique en Afrique, D.R. Congo, P. O. Box 3323, Bukavu Democratic Republic of the Congo.
[3]International Institute of Tropical Agriculture (IITA), P. O. Box 30772-00100, Democratic Republic of the Congo.

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Soybean is an important crop in the Democratic Republic of Congo, a country faced with high levels of war induced malnutrition but its productivity is limited by poor soil fertility coupled with low access to nitrogen mineral fertilizers. Commercial rhizobia strains introduced in 2010 failed to adapt and increase soybeans yields at desired level. We studied the performance of six indigenous rhizobia strains in enhancing soybean productivity compared to two commercial strains USDA110 and SEMIA5019. The study was carried out in the greenhouse and field of Kalambo station of International Institute of Tropical Agriculture (IITA), D. R. Congo during 2016/2017 cropping season. The treatments included: (1) N-, control without inoculation and N-fertilizer; (2) N+, non-inoculated control with 80 kg of N ha^{-1}; and inoculated with (3) commercial strain *Bradyrhizobium diazoefficiens* USDA110; (4) commercial strain *Bradyrhizobium elkanii* SEMIA5019; (5) local strains *Bradyrhizobium japonicum* NAC17; (6) NAC22; (7) NAC37, (8) NAC42 (9) NAC 46 and (10) NAC78. Greenhouse and field experiments were laid out as completely randomized design and randomized complete block design respectively. The best inoculation treatments across all experiments were the indigenous strains NAC46 and NAC17 which nodulated equally or better than the commercial strain USDA 110. In the field NAC46 and NAC17 increased soybean grain yield from 2.4 to 3.3 t ha^{-1} and 3.4 t ha^{-1}; indicating the increase of 68.7 and 70.8% respectively, over the commercial strain USDA110. The results demonstrated that indigenous rhizobia NAC46 and NAC17 would thus be the silver bullet to enhanced BNF and soybean yields in South Kivu province of Democratic Republic of Congo.

Key words: Inoculation, local rhizobia; soil fertility, USDA110.

INTRODUCTION

Soybean, *Glycine max* (L.) Merr. is an important crop worldwide and is becoming even more important and popular in South Kivu due to its potential to curb high malnutrition (Hartman et al., 2011). Soybean was introduced in Africa from Asia in the 19th century (Khojely et al., 2018) to address the need for cropping systems diversification dominated by maize (Giller et al., 2011). In these systems, soybeans enhance soil fertility through their ability to fix nitrogen (N) from atmosphere in symbiotic relationship with rhizobia bacteria through the iological Nitrogen Fixation (BNF) process (Collino et al., 2015). At the same time, this crop provides smallholders farmers the opportunity to increase their households' income while fighting malnutrition issues because of their important nutritional value in terms of protein, amino acid and micronutrient (Arslanoglu, 2011; Xu et al., 2015).

Since the last decades, in South Kivu province of Democratic Republic of Congo, where the economy depends largely on agriculture (Jeníček and Grófová, 2016; Maass et al., 2012), there is an increase of soybean demand due to the presence of market created by the development of livestock (Rudel et al., 2015) and industry of soybean processing (Bisimwa et al., 2012). The most common soybean based formula consumed in South Kivu province includes soy infant formula (Bahwere et al., 2016; Owino et al., 2011), soymilk, soy oil, soybean flowers, soybean biscuits, soybean spices, soybean meat, soybean bread and cakes and soybean waste industry used as animal feed (Shurtleff and Aoyagi, 2009). This crop is essentially cultivated by smallholders' farmers and maintained by women to improve nutrition and generate income for their households, and by youth to pay costs of their education (CIALCA, 2010).

In South Kivu, farmers generally plant legumes, including the soybean without adding mineral fertilizers because they are neither available nor affordable and less economic to them (Lambrecht et al., 2016; Pypers et al., 2011a; Vanlauwe et al., 2010). Therefore, soils have been depleted due to a continuous cropping without soil replenishment as consequence of population pressure (Bashagaluke, 2015). In that case, crop yield depends upon N fixation by native rhizobia that are not always effective (Ojo et al., 2015) thus obtaining low yields, estimated at 0.5 t ha^{-1} (FAO, 2018).

Inoculation of soybean with appropriate, highly effective, adapted and compatible rhizobia has been stated as the most economic (Chianu et al., 2011), productive (Saturno et al., 2017) and environment friendly (Collino et al., 2015) mean to improve crop yield. Two main approaches have been pursued by research international organizations to improve soybean yield: first, promiscuous soybean cultivars were developed to nodulate freely with native rhizobia (Tefera, 2011); second, inoculation with highly effective rhizobia strains has been promoted (van Heerwaarden et al., 2018). In that line, commercial inoculants, Biofix Legume inoculants, containing *Bradyrhizobium diazoefficiens* USDA110 strain, was introduced among South Kivu farmers by N_2 Africa program since 2010 (www.n2africa.org) and disseminated among farmers by agricultural extensions services and humanitarian organization.

From trials and farmer's fields results, the commercial inoculants increased legume yield from 500 to 1343 kg ha^{-1} (van Heerwaarden et al., 2018), but still not at desired levels in certain farms and with no increase in other farmers, while the potential soybean yield is above 5000 kg ha^{-1} (Salvagiotti et al., 2008; Zanon et al., 2016). That low improvement was attributed to the effect of environmental and edaphic conditions on the introduced commercial strains in addition to the failure to overcome the competition barriers opposed by native rhizobia (van Heerwaarden et al., 2018).

Numerous studies in Africa have shown the presence of effective rhizobia strains among indigenous rhizobia populations (Chibeba et al., 2017; de Almeida Ribeiro et al., 2015; Musiyiwa et al., 2005). In addition, indigenous rhizobia have been described by many studies as being persistent, well adapted to local conditions and therefore can compete successfully at the expense of exotic strains for nodule occupancy and N fixation (Fening and Danso, 2002). From past results on genetic diversity on indigenous rhizobia nodulating soybeans in Africa and in South Kivu, some indigenous rhizobia clustered together with the commercial strains USDA110 (bootstrap value: 99%) showing possible relatedness of indigenous strains with this commercial strain. There is need to test these indigenous strains related to the commercial strain and identify indigenous strains suitable for South Kivu environment and edaphic conditions. We tested six indigenous rhizobia strains for their competitiveness ability to improve soybean nodulation and yield compared to the commercial strains USDA110 and SEMIA5019 in order to characterize and isolate indigenous strains with potential to be included in soybean inoculants.

MATERIALS AND METHODS

Study area

The study was carried out in South Kivu province of Democratic Republic of Congo, in the greenhouse and station field of International Institute of Tropical Agriculture (IITA), Kalambo station. South Kivu is one of the 25 provinces of Democratic Republic of Congo, located in Eastern between 1°36' - 5° South and 26°49'-29°20' East and the surface is estimated to be 69,130 km^2 with 3.8 million people of population with the estimated density of 91 people per km^2 (Pypers et al., 2011). It is recognized as a high humid forest

*Corresponding author. E-mail: bintundusha@yahoo.fr. Tel: +254 703 201 363.

Table 1. Greenhouse and field soils characteristics.

Sampling site	pH	SOC	N	P	K	Ca	Mg	Rhizobia population
Walungu	5.45	3.32	0.21	7.70	245	1061	302	2×10^{2}
Kalehe	6.89	3.24	0.19	19.14	205	6980	258	5×10^{3}
Murhesa	7.9	3.33	0.21	22.95	455	3230	537	1×10^{3}

P: extractable P in mg/kg; K: exchangeable K in mg/kg; Ca: exchangeable Ca in mg/kg; Mg: exchangeable Mg in mg/kg; rhiz. pop.: rhizobia population in number of cells per gram of soils.

zone depicted by high vegetation diversity (Potapov et al., 2012) and highlands. Soils are mostly Dystric, Humic Nitisols and Humic Ferralsols (van Engelen et al., 2006; Eswaran et al., 1997). This region has a tropical climate, the Aw3 type according to Koppen classification with an average annual rainfall of 1500 mm and mean temperature of 18°C (Nash and Endfield, 2002).

Agriculture is the main activity contributing more than 50% to the GDP of this province (CIALCA, 2010). Most of the farms have the characteristics of smallholders farming systems characterized by the farm size ranging from 0.5-1 ha (Pypers et al., 2011). The main cultivated crops include cassava, common beans, maize and banana (Maass et al., 2012). Soybean crop has been promoted since 1990 to deal with high malnutrition caused by repetitive wars (Kismul et al., 2015) and since then its cultivation is increasing (FAO, 2018). This province has been the site of repetitive armed conflicts and rapes (Bartels et al., 2013).

Soils sampling and analysis

Selected field had no history of neither rhizobia inoculation nor soybean cultivation. Two weeks before sowing, twenty composite soil samples were collected from 0-20 cm depth along the field diagonal (Carter and Gregorich, 2008). Soil samples were pretreated (air dried and sieved through a 2 mm perforation size) prior for analysis. Total soil organic carbon was determined by the modified Walkley-Black (Okalebo et al., 2002), total N and available phosphorus were determined by the Kjeldahl and Olsen methods, respectively (Anderson and Ingram, 1993). Exchangeable potassium, Magnesium and Calcium were determined by Mehlich 3 method (Okalebo et al., 2002). Soil pH was determined using the 1:10 water method and measured by the seven compact, S210 Metler Toledo pH meter, after 60 min after agitation. The population of native rhizobia was determined by the plant infection technique (Somasegaran and Hoben, 1994) (Table 1).

Rhizobia culture and inoculant preparation

Six indigenous rhizobia and two commercial strains used in this study were obtained from IITA/Kalambo station rhizobiology laboratory selected based on their high effectiveness index (Ndusha, 2014) and high genetic similarity compared to the commercial strain USDA110 (Ndusha et al., in press). These rhizobia strains were streaked on Yeast Extract Mannitol broth (YMB) (Somasegaran and Hoben, 1994), incubated at 25°C until turbid until the concentration attained 10^{9} cells ml^{-1}. Inoculants were prepared from indigenous rhizobia cultures using sterilized peat as carrier material, incubated for two weeks and applied at the rate of 10 g kg seed^{-1} with 20% sugar-water (w/v) used as adhesive following the two-step inoculation method of Woomer (2011).

Trial management and experimental design

Three experiments were carried out to compare six outperformed strains from our past study (Ndusha, 2014) with two commercial strains USDA 110 and SEMIA5019: 1) Effectiveness testing in potted field soils in the greenhouse using two types of soils (Table 1), 2) on station field testing, and 3) finally assessing the growth rate and ability of utilizing different carbon sources on YMA media.

Indigenous rhizobia testing in controlled conditions

Greenhouse experiment was established in the greenhouse at Kalambo station of IITA; temperature in the greenhouse varied from 22 to 38°C. Two site soils; from Walungu and Kalehe village, were used as substrate in 3 L pot containers. The two villages were selected because they are all soybean production zones and their soils conditions are very different (Table 1). Sterilized 3 L capacity PVC pots were filled with 2.5 kg of soil and covered with a sterile plastic plate with limited access to limit contamination. Soybean seeds were surface sterilized using the pre described procedure (Somasegaran and Hoben, 1994), pre-germinated in agar plates; and 3 seeds per pot were sewed, thinned to 2 after emergence for appropriate spacing. Seeds were inoculated with 1 ml of broth pre cultured (described in section 2.3). For the mineral N control, urea was applied at a rate of 80 kg ha^{-1} (Pypers et al., 2011). Watering was done regularly at the frequency of 3 per week adjusted according to plant needs. After 7 weeks, at early flowering, plants were harvested; nodules counted, weighted and shoot weight determined by weighing after oven dried at 70°C for 48 h. A Completely Randomized Design consisting of 10 treatments including 6 indigenous rhizobia and 2 commercial strains (SEMIA5019 and USDA110), and non-inoculated pot with (N+) and without mineral N (N-), with 3 replicates was established. Promiscuous soybean (SB24) was used as the test crop, selected for their high adoption among farmers (Walangululu et al., 2014).

Indigenous rhizobia testing in field condition

A field experiment was established in the station field of IITA Kalambo located in Murhesa during 2015-2016 long rains (September to January). Soils characteristics of field were determined (Table 1). Six indigenous rhizobia strains were compared to 2 commercial strains USDA110 and SEMIA5019 on promiscuous soybean variety SB24. The experiment was laid out as Randomized Complete Block design with 3 replicates. The treatments included: (1) N-, control without inoculation and N-fertilizer; (2) N+, non-inoculated control with 80 kg of N ha^{-1}; and inoculated with (3) commercial strain *B. diazoefficiens* USDA110; (4) commercial strain *Bradyrhizobium elkanii* SEMIA5019; (5) local strains *Bradyrhizobium japonicum* NAC17; (6) NAC22; (7) NAC37, (8) NAC42 (9) NAC 46 and (10) NAC78. Each plot measured 6 m x 4 m, seeds were planted in rows 45 cm apart and at 5 cm intervals. To avoid cross contamination, plots were separated by four non-inoculated lines. Legume inoculants were prepared from isolates subsequently described in the paper.

The trial management was done according to known farmer's practice; weeding as per need before the canopy closure. The intensity of green color in leaves was measured at different growth

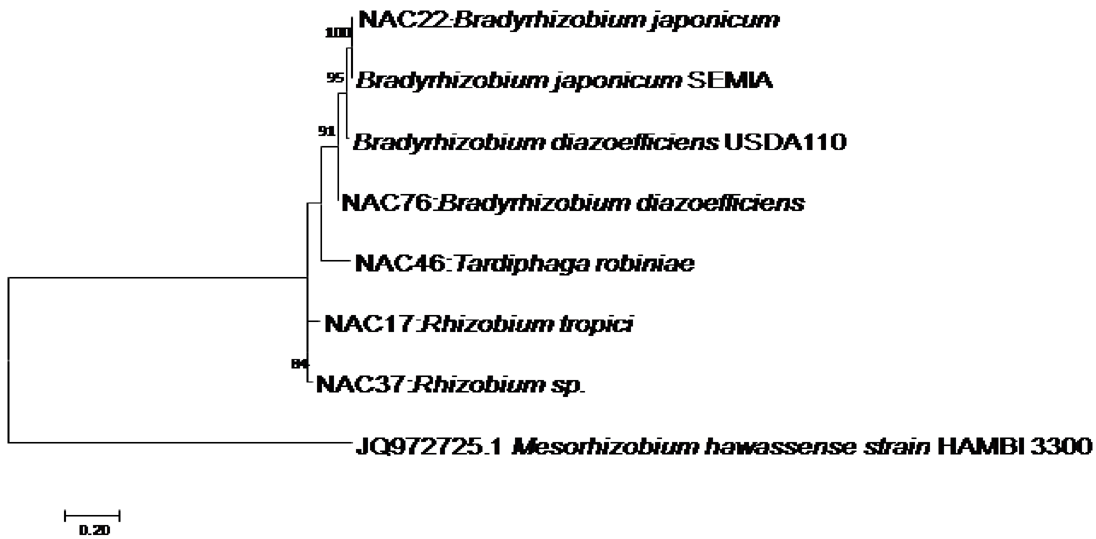

Figure 1. Phylogenetic relationship between tested indigenous strains and commercial strains USDA110 and SEMIA. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model. The percentage of replicate (1000 replicates) is shown next to the branches. *Mesorhizobium hawassense* was used as out-group.

stages using a chlorophyll meter (Dey et al., 2016). Plants were assessed for nodulation at flowering stage, seven weeks after planting. Plants were carefully uprooted, roots washed, nodules counted, oven-dried for 48 h at 70°C and dry weight recorded. Soybean grains were harvested at maturity (4 months), dried and dry weight recorded.

Data analysis

Data were subjected to analysis of variance (ANOVA) using the software R version 3.5.1. When differences between treatments were detected, Tukey test was used to compare means at $p > 0.05$ level of significance. Relative effectiveness (RE) was determined by dividing the shoot dry weight of treatment over that of the N+ treatment, in the same block (Chibeba et al., 2018).

RESULTS

Phylogenetic relationship between indigenous strains and commercial strains

Indigenous rhizobia isolated from Congo phylogeny was determined based on the housekeeping gene gnlII. The tested isolates and commercial strain USDA 110 tended to cluster together with bootstrap value of over 90% (Figure 1).

Nodulation and shoot dry weight of indigenous rhizobia recorded in the greenhouse

Nodules number reported in the greenhouse varied significantly among strains ($p=0.0001$) and between the two soils types ($p=0.0203$). The recorded nodules number varied from 3 nodules to 21 nodules per plant in Walungu soil while it varied between 1 to 36 nodules per plant in Kalehe soil. The highest nodules number in Walungu was recorded by the indigenous strain NAC46 and NAC76 (±21 nodules per plant), followed by commercial strains (USDA110 and SEMIA 5019) and indigenous strains (NAC22, NAC17 and NAC37). The lowest number of nodules in Walungu site soils was recorded by the treatment without inoculation but with mineral N fertilizer (N+), where recorded nodules number averaged only 3 nodules per plant (Table 2). The highest number of nodules in Kalehe soil was recorded with the same indigenous strains NAC46 and the control (N-) (average 34 nodules per plant), followed by NAC76 (21 nodules per plant) (Table 2). The lowest nodules number per plant was also reported with the treatment N+, where the nodule number averaged only 1.

The nodules dry weight (DW) recorded in the greenhouse experiment also varied between site soils and among strains ($p=0.0001$). The highest nodules dry weight was recorded in Kalehe soil (average 0.373 g per plant DW) while the lowest nodules weight was recorded in Walungu soil (average 0.284 g per plant DW). The inoculation with indigenous strains NAC46 produced the highest nodules weight followed by NAC37 and NAC76 in Walungu soil (Table 2). The lowest nodules weight was recorded with N+ control for both soils.

Shoot dry weight variation ($p=0.0012$) was recorded only in the Kalehe soils while in Walungu no difference was recorded among the inoculated strains. In Kalehe soils, the highest shoot weight was recorded by N+

Table 2. Nodule number, nodule dry weight and shoot dry weight recorded in the greenhouse by the effects of different rhizobia strains.

Treatment	Nodule number		Nodule weight(g)		Shoot dry weight(g)	
	Walungu	Kalehe	Walungu	Kalehe	Walungu	Kalehe
NAC46	21.0[a]	36.0[a]	0.66[a]	0.88[a]	8.00[a]	9.06[ab]
NAC76	20.0[a]	21.3[b]	0.30[bc]	0.26[ab]	6.63[a]	6.30[bc]
NAC22	11.0[b]	10.6[cd]	0.18[bc]	0.18[c]	6.23[a]	6.50[bc]
NAC17	10.0[b]	9.0[cde]	0.17[bc]	0.16[c]	6.10[a]	6.36[bc]
NAC37	9.3[b]	17.0[bc]	0.46[ab]	0.62[ab]	5.66[a]	5.90[c]
NAC42	8.0[bc]	7.0[de]	0.40[abc]	0.20[c]	5.80[a]	6.50[bc]
USDA110	12.6[b]	9.0[cde]	0.21[bc]	0.24[c]	6.10[a]	6.53[bc]
SEMIA5019	10.6[b]	8.3[cde]	0.15[bc]	0.20[c]	6.23[a]	7.33[abc]
N-	7.0[bc]	33.0[a]	0.20[bc]	0.60[b]	5.06[a]	6.30[bc]
N+	3.0[c]	1.3[e]	0.09[c]	0.15[c]	7.10[a]	9.60[a]
p-value	< 0.0001	< 0.0001	0.0001	< 0.0001	0.6439	0.0012

Table 3. Nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), leaf greenness (LG), plant height (PH) and crop yield recorded in the field by the effects of different rhizobia strains.

Treatment	NN	NDW (g)	SDW (g)	LG	PH (cm)	Yield (kg/ha)
NAC17	69.0[a]	1.40[a]	7.97[a]	43.83[a]	64.4[a]	3397[a]
NAC46	65.0[a]	1.10[a]	7.60[ab]	47.57[a]	55.2[d]	3409[a]
NAC76	65.0[a]	0.83[a]	6.04[ef]	34.71[d]	60.2[abc]	2342[ef]
NAC37	62.6[a]	0.93[a]	7.15[bc]	37.90[cd]	62.8[ab]	2924[bc]
NAC22	59.6[ab]	0.96[a]	6.43[de]	40.08[c]	62.2[ab]	2720[cde]
NAC42	46.6[c]	0.42[a]	6.46[de]	41.27[bc]	58.4[cd]	3148[ab]
USDA110	64.6[a]	3.19[a]	6.73[cd]	37.90[cd]	58.9[bcd]	2416[de]
SEMIA5019	62.0[a]	0.98[a]	6.64[cd]	39.32[c]	61.1[abc]	2768[bcd]
N-	50.6[bc]	0.38[a]	5.75[f]	35.55[d]	58.4[cd]	1543[g]
N+	30.0[d]	0.20[a]	6.63[cd]	39.72[c]	58.5[cd]	2012[f]
p value	< 0.0001	0.3426	< 0.0001	< 0.0001	< 0.0001	< 0.0001

control (9.6 g plant DW), followed by indigenous strain NAC46, and by the commercial strain SEMIA5019. The lowest shoot dry weight in Kalehe soils was recorded by the indigenous strain NAC37 (5.9 g plant DW) (Table 2).

Nodule number, nodule dry weight, shoot dry weight, leaf greenness, plant height and crop yield recorded in the field study

In the field, all treatments produced nodules but their number varied greatly across treatments ($p< 0.0001$). The nodules number varied from 30 to 69 nodules per plant. Even the non-inoculated and not fertilized (N- and N+) control plants nodulated abundantly (average 40 nodules per plant). The highest nodule number was recorded by the treatments of both indigenous and commercial strains, which did not differ among them, except for NAC42, with a lower number of nodules (Table 3).

Nodules dry weight did not vary with the treatments but shoot dry weight significantly varied among rhizobia strains ($p< 0.0001$). The highest biomass was recorded with the indigenous strain NAC17 (8.0 g plant DW), followed by NAC46, even higher than the commercial strains USDA110 and SEMIA5019. The lowest shoot dry weight was recorded by the treatment N- control (5.8 g plant DW) (Table 3).

The plant leaf greenness also varied among treatments ($p< 0.0001$). The highest intensity of green color measured on leaves was recorded by the indigenous strains NAC17 and NAC46 (Table 3). The lowest green color intensity was recorded by the control N- and the indigenous strain NAC76 (about 55) (Table 3).

Plant height also varied significantly across treatments ($p< 0.0001$). The highest plant height was recorded by the indigenous rhizobia strain NAC17 (64 cm), followed NAC22 and NAC37. The commercial strain SEMIA5019

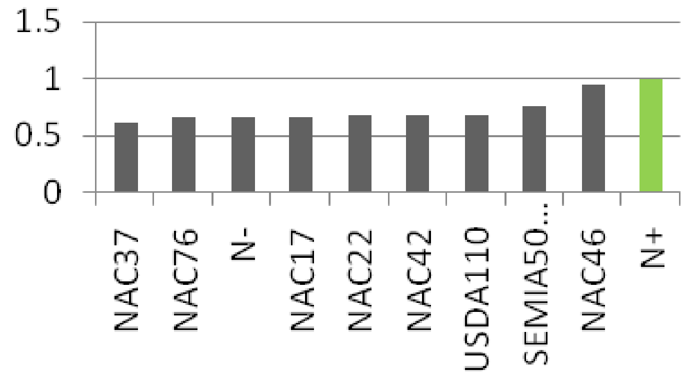

Figure 2. Relative index in the greenhouse.

took the third place while USDA 110 took the fourth position. The lowest treatment in terms of plant height was the treatment NAC46 (55 cm) (Table 3). Grain yield also varied across treatments. Yield improvement was recorded by the indigenous strains NAC17 and NAC46 that yielded 1.4 fold than the commercial strain USDA110 and 1.6 fold than the application of N (N+). The lowest grain yield was recorded by the treatment N-, followed by N+ (Table 4).

Relative effectiveness of indigenous rhizobia strains compare to the reference strains

In the greenhouse, the effectiveness of all tested isolates did not exceed the reference treatment N+ but the indigenous rhizobia NAC46 had a relative index higher than the commercial strains USDA110 and SEMIA 5019 (Figure 2). However, in the field, the relative effective index by the 3 indigenous rhizobia strains (NAC17, NAC46 and NAC37) exceeded both commercial strains and control with N (N+) (Figure 3.)

DISCUSSION

Effectiveness of indigenous strains under controlled environment

In the greenhouse (controlled environment), there was significant differences in nodulation among treatments in the two soils highlighting the need to inoculate the soils of the two sites. These differences may be explained by the low abundance or less effectiveness of native rhizobia population in the Walungu and Kalehe soils (Table 1). Sanginga and Okogun (2003) and Osunde et al. (2003) stated that inoculation responses are more likely to occur when there are less than 10^3 cells of indigenous or naturalized rhizobia per gram of soil or when the native rhizobia are less effective. The same results were found by Koskey et al. (2017) in their study on potential of native rhizobia in enhancing N fixation and crop yield of climbing beans in contrasting environments of Kenya, the yield improvement and nodulation of introduced rhizobia strains depended largely on the number of viable native rhizobia in the soil.

There was significant difference in nodulation between the two sites soils, higher nodules number was produced in Kalehe soils (Table 2). This is because of the differences in soils conditions (Table 1). Nodulation depends on a number of soils factors, especially the soil pH (Lapinskas, 2007), P availability and the indigenous rhizobia abundance and effectiveness (Sanginga et al., 1996; Singleton and Tavares, 1986; Slattery, 2004; Thies et al., 1991). Kalehe soil has better conditions for growth and survival of rhizobia bacteria namely the neutral pH and higher P content compared to Walungu soil (Table 1). This result is in concordance with other authors, for example Gyogluu et al. (2016) who assessed the symbiotic response of soybeans to inoculation by different *B. japonicum* strains at 3 experimental sites in Mozambique. They found response variation depending on different sites and suggested that there are specific effects of sites on nodulation and dry matter improvement by rhizobia. This observation is also in agreement with Boucho et al. (2019); they found that the response to inoculation is highly affected by soils conditions and for their case phosphorus availability in the soil promote nodulation and biomass. Therefore, this study suggests the improvement of soils conditions prior to inoculation such as liming where soil pH is low and phosphorus application should be considered for maximization of inoculation response.

A significant difference in shoot dry weight (biomass) improvement in the greenhouse was observed only in Kalehe soils. The non response in Walungu soils may be explained in terms of the low pH and low P levels, which limited the process of nitrogen fixation despite the presence of nodules. The same observation was done by a study conducted in Argentine by Collino et al. (2015); they observed variation of BNF depending on crop, soil and meteorological factors. This study also revealed differences among strains in both nodulation and shoot

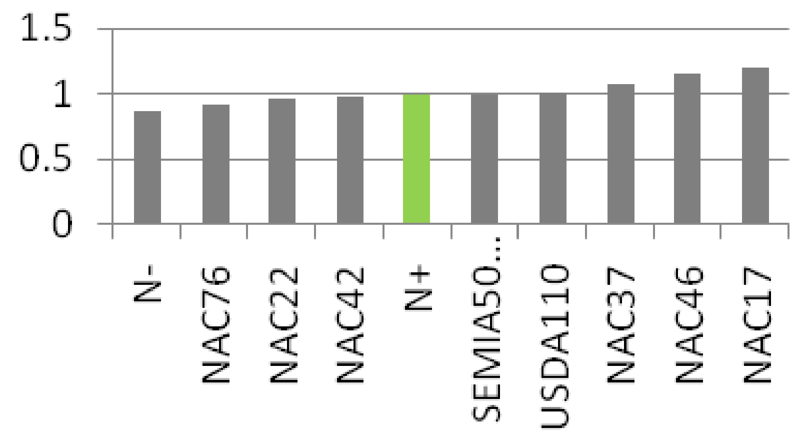

Figure 3. Relative index in the field.

dry weight improvement which is primarily due to their genetic differences. Other studies conducted in Africa have similarly reported consistent variation in symbiotic effectiveness among indigenous rhizobia strains (Abaidoo et al., 2007; Chibeba et al., 2017, 2018) and consequently this study further contributes to the evidence that effective rhizobia do occur in African soils.

Effectiveness of indigenous strains under field conditions

In the field, nodules were observed on all treatments including the non inoculated control without nitrogen N-; the non inoculated control produced even higher nodules number compare to the inoculated plots. This is due to the presence in soils of natives and compatible rhizobia. Many studies have demonstrated that effective rhizobia can be presented in some soils but they are less efficient (Abaidoo et al., 2007; Gyogluu et al., 2016; Jaiswal and Dakora, 2019; Wongphatcharachai et al., 2015). These less efficient indigenous rhizobia strains can be even more competitive than the inoculants and occupy a significant portion of the nodules, reducing the impact of the introduced inoculant strain on improving BNF (Batista et al., 2015). The same observations were made by Irisarri et al. (2019) who observed a higher nodules occupation by native rhizobia compared to introduced strains. This has important practical implication for agriculture to the effect that selection of rhizobia for improving BNF and legume productivity must be done among native population, and be provided in higher concentration through inoculants. Furthermore, the host plant show preference on native rhizobia compared to exotic strains (Osunde et al., 2003).

Only the N+ control produced very few nodules. This is attributable to the fact that the presence of mineral nitrogen inhibits the biological nitrogen fixation by inhibiting the nodules formation and nitrogenase activity. This result is in agreement with a study conducted by Ulzen et al. (2016) who reported very few nodules with the application of 100 kg of nitrogen. In addition, many authors have stated that N doses as low as 20-40 kg of N/ha may highly decrease nodulation and BNF, with no benefits to yield (Hungria et al., 2005, 2006; Saturno et al., 2017).

The differences in nodulation among tested strains in response to inoculation may be attributed to the fact that natives or naturalized rhizobia were less effective in one hand and on the other hand introduced strains were highly effective. This results are in line with those of Osunde et al. (2003) who tested the nodulation of two soybean promiscuous varieties by introduced elite rhizobia and indigenous rhizobia. The introduced rhizobia through inoculants were less competitive but highly effective compared to native rhizobia. The differences in nodulation may be ascribed to their genetic makeup expressed through symbiotic efficiency, competitiveness for nodule occupancy, compatibility with the host plant and adaptive ability to soil stress conditions (Tas et al., 1996). The indigenous rhizobia NAC17 and NAC46 produced higher number of nodules compared to the commercial strain USDA110 suggesting that these strains had higher symbiotic efficiency and higher adaptation ability to local conditions compared to the commercial strains.

The increase of leaves green color noted in the native strains compared to the control may be because of nitrogen being the major constituent of chlorophyl that confers green color to the plants. Inoculation improves nitrogen content in leaves and thus promotes the formation of chlorophyll which is also important for photosynthesis (Sinclair, 2004; Hakeem et al., 2012). The same results were observed by Abaidoo et al. (2007) who classified rhizobia isolates tested into four symbiotic phenotypic groups based on green color intensity. The less effective group was composed by isolates that recorded lower green color intensity on leaves of soybean genotypes.

In the field, the significant differences of shoot dry weight were observed among treatments as result of enhanced nodulation. Nitrogen is the component responsible for vegetative development; it has been demonstrated that up to 80% of the above-ground N accumulation in soybean is due to N fixation by rhizobia (Hungria et al., 2006). The N- control produced a higher number of nodules but lower shoot dry weight. This is as a result of the native rhizobia being less effective (Osunde et al., 2003). The same observation has been made by Chibeba et al. (2018) who observed that an appreciable proportion of rhizobia population in Mozambican soils was composed of ineffective rhizobia. The N+ control produced high shoot dry weight; this is mainly as a result of the mineral N being absorbed by the plant at early stages compared to the fixed N and thus improved vegetative formation (Saturno et al., 2017). This is in agreement with the findings of Kinugasa et al. (2012) who found that higher biomass production did not result in grain yield increase.

This study demonstrated significant differences in yields among treatments. There was yield improvement with inoculated plots compare to the plots where N had been applied and N-control. The indigenous strains NAC46 and NAC17 increased yields by 1.7 and 1.6 fold respectively compared to the N+ control, 2.2 folds compared to the N-control and 1.4 and 1.3 fold compared to the commercial strain USDA110.These yield gains are within the 3.2–14.5% interval of inoculation yield benefit reported in Brazil (Hungria et al., 2006) and in Mozambique (Chibeba et al., 2018). This study has further confirmed the findings of Chibeba et al. (2017, 2018) and Hungria et al., (2005, 2006) that BNF is the most efficient way of improving soybean productivity.

Conclusion

The best strains across all the experiments are NAC17 and NAC46 with average yields gain 60-70% over commercial strains and controls. These results suggest that these indigenous strains hold the best potential as commercial inoculants in South Kivu soils conditions. USDA 110 and SEMIA 5019 are also effective but it is preferable to use adapted and competitive strains. Therefore, the native strains are likely to adapt well not only in South Kivu, but also in other countries with similar agro-climatic conditions.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

An analysis of socioeconomic factors affecting avocado production in saline and flooded areas around Lake Victoria Basin of Western Kenya

Ouma George[1], George Duncan Odhiambo[2], Samuel Wagai[3] and Johnson Kwach[4*]

[1]Institute for Climate Change and Adaptation, University of Nairobi, P. O. Box 30197-00100, Kenya.
[2]Department of Applied Plant Sciences, P. O. Box 333, Maseno University, Kenya.
[3]Department of Botany, Rongo University Private Bag Rongo, Kenya.
[4]Faculty of Biological and Physical Sciences, Tom Mboya University Collage Homa bay, Kenya.

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Avocado (*Persia americana*) a very important crop worldwide nutritionally and economically. In Kenya avocado does not thrive well in soils with high salinity, flooded or waterlogged areas. Survey was conducted around Lake Victoria Basin in Kenya to establish socioeconomic factors and flooding that affects its production. A sample of 400 households was interviewed using structured questionnaire, focus group discussions and key informants. Data were collected and analysed using Statistical Package for Social Scientists. Objectives were to find how flood, human capital and other socioeconomic factors such as gender, age, farmer education affects avocado production in areas with saline soils and floods among other factors namely Busia, Muhoroni, Nyando and Rachuonyo counties in Kenya. Results indicated that the major impediments were water logging (76.2%), flooding (73.0%), soil fertility (62.5%) and soil salinity (42.9%), to agricultural development and affect the crop production. Farmers had limited access to information on avocado production and marketing 31.3%, and sourced information from fellow farmers. Majority of farmers owned indigenous or non-certified avocado trees; low fruits market prices also discouraged its production. Therefore, farmers should be sensitized on the impacts of climate change on agriculture, encourage majority of them to take samples of their plots for soil analysis. 65.5% expressed soil fertility problems in their farms yet 95.5% of them had not taken their plots for soil analysis. Increasing of human capital, farmer education, and extension services may contribute to farmer's abilities to adopt new technologies for increased avocado production in these areas and may positively contribute to improved livelihood through nutrition, income generation of the stakeholders. Farmers should be encouraged to plant certified avocado seedlings tolerant to water logging and salinity.

Key words: Avocado, climate change, Lake Victoria, Kenya, strategies.

INTRODUCTION

Avocado (*Persea Americana)* is generally cultivated in tropical and sub-tropical regions from 40°N and 40°S. The fruit has remarkably high nutritional values and contains 15 to 30% oil, similar in composition to olive oil, eleven vitamins (Vit A, B6, B12, K, C, E, Folacin, Niacin, etc.) and fourteen minerals. Its calorific value is exceptionally high, 123 to 387 gmcal /100 g edible avocado and has low sugar content (Bergh, 1991; Currier, 1991; Gaillard and Gregory, 1995; HCDA, 2010). They are eaten fresh, in salads with lemon juice, salt, etc.

Avocado is a complete food in terms of protein, containing nine essential amino acids. It can almost substitute butter and meat and is called in many countries as poor man's butter. The avocado fruit was once a luxury food reserved for the tables of royalty but is today enjoyed around the world by people from all walks of life. Avocado is one of the most nutritive fruits known. Further it has several uses; as a natural cosmetic, with advantage in rapid skin penetration and as a superior natural sunscreen (Bose and Mitra, 1996). Compared with almond, corn, olive and soybean oils, avocado oil has the highest skin penetration rate (Currier, 1991; Swisher, 1998; HCDA, 2010). Avocado oil is easily digestible and can have beneficial effects on the digestive system. The oil is largely unsaturated and as the sugar content is low (about 3%), the fruit can be recommended as a high-energy food for diabetics.

Most countries where agriculture drives their economies in terms of employment, foreign exchange, subsistence and contributes to Gross Domestic Product (G.D.P) are adversely affected by the climate change affecting food security and incomes of the people. Other effects of climate change are incidences of diseases such as malaria, genetic erosion, biodiversity loss and ecosystems disturbance among others. (Robinson, 2004).

Economic importance of Avocado

Avocado's world production of 3.2 million tones (FAO, 2004) makes it an important fruit crop internationally. Its main producers are North America and Central America whose production constitutes 80% of the world production. Other countries produce the remaining 20%. Currently, Avocado represents about 17% of the total horticultural exports from Kenya. In the year 2003 total Avocado exports from Kenya was approximately 39% of total Avocado's annual production of 70,000 tones (Griesbach, 2005).

Although Avocado is important in Kenya its production is limited by waterlogging or flooding and in poorly drained soils which encourages Avocado root rot (*Phytophthora cinnamon*). This is the most serious disease in nearly all avocado producing areas of the world (HCDA, 2010). In Kenya every effort has been made to rectify the situation by uprooting or treatment of affected trees. Hot water and fungicide treatment of seeds for propagation purposes is highly recommended and grafting on phytophthora-tolerant and/or resistant rootstocks have been included as control options. Flooding limits gaseous exchange in the soil because it affects oxygen availability Oxygen has a slow diffusion rate in water than air. Consequently, in a poorly drained or flooded soil the main factor causing root damage is lack of adequate oxygen for normal root respiration (Drew, 1977; Kramer and Boyer, 1995). Other accompanying changes are the increase in the ethylene precursor, ACC, (I-carboxylic,I-aminocyclopropane) (Kramer and Boyer, 1995).

Objectives of this study to carry a survey on avocado production and utilization in five Counties around the Lake Victoria Basin and assess the livelihood contexts, strategies and outcomes of small holder farmers in avocado production systems. To consider socioeconomic factors with special emphasis on flooding common in some parts of the counties, human capital and its implications in Nyando, Muhoroni, Rachuonyo, Bunyala and Samia. The outputs are useful future information that can be scaled up in any new areas with similar characteristics such as saline and water-logging where tolerant Avocado varieties may be recommended.

Conceptual framework approach

This study applied the Sustainable Livelihood Approach (Figure 1). The Sustainable Livelihoods Approach (SLA) has been developed to help understand and analyze the livelihoods of the poor. In addition to improving the understanding of livelihoods, the approach can be used in planning new development activities and assessing the contribution to livelihood sustainability made by existing activities (DFID, 2000). Sustainable livelihoods approach offers a conceptual framework for understanding causes of poverty, analyzing relationships between relevant factors at micro, intermediate and macro-levels and prioritizing interventions. The approach explicitly requires going beyond sectorial barriers, to look at more of the context in which people live (DFID, 2000; Ashley and Diana, 1999; Ashley, 2000). There are variations on the SLA, emphasizing different aspects with many common elements. The SLA considers five assets or types of capital namely natural, human, financial, physical and social. It also integrates vulnerability contexts and livelihood strategies and was, therefore, used in this study to understand the livelihood support system of local households.

Livelihood assets

Swisher (1998) indicated five assets or types of capital available to people namely natural, human, financial,

*Corresponding author. E-mail: kwachjk@gmail.com.

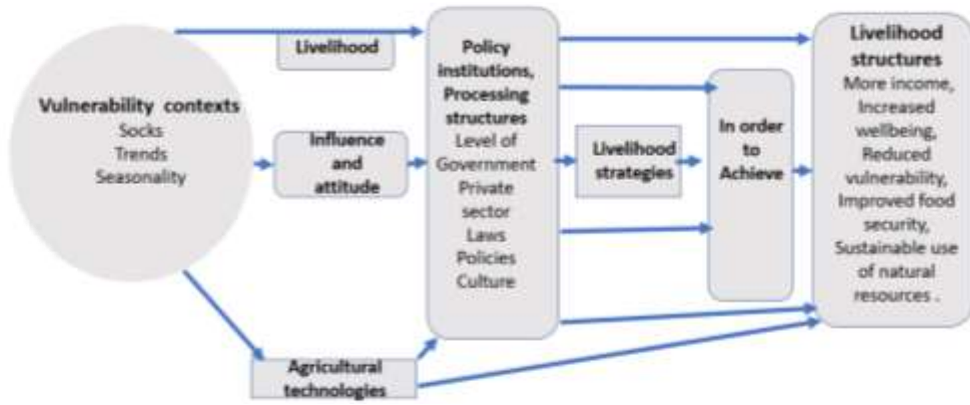

Figure 1. Sustainable livelihood framework (Adapted from DFID, 2000),

physical and social. These five forms of capital have different characteristics. People, according to the livelihoods approach, rely for their success on the value of services flowing from the total capital stock. Different households with different access to livelihood assets are affected by the diversity of assets, quantity and balance between assets. It is, therefore, worth investigating accessibility, quantity and balance of assets as benchmark information against which progress in the future can be measured from the changes brought about by the deployment of new innovations.

Natural capital

Natural capital refers to the biophysical elements such as water, air, soil, sunshine, woodlands and minerals the basic conditions for human existence. And it is the most fundamental of the core forms of capital namely manufactured, human and natural since it provides the basic conditions for human existence such as fertile soils, forests, productive land and seas, good quality freshwater and clean air (EU, 2013). A conceptual framework (Figure 1) involving interactions between socioeconomic and ecosystems in natural capital. Natural capital sets the limits for socioeconomic systems and is both limited and vulnerable (EU, 2013) was applied in this study.to exhaust information on ecosystem services to human wellbeing. The ecosystem services such as; biomass, water and fiber, regulating and maintenance services such as soil formation, pests and disease control and cultural services such as physical, intellectual, spiritual and symbolic interactions with ecosystems or landscapes (EU, 2013).These types of services are enhanced by supporting services such as nutrient cycling and are provided at a range of scales from global-climate regulation to local-flood protection. These are naturally occurring assets that are largely renewable. In this study, household land size and land under cultivation of fruits were analyzed to explore other livelihood contexts and outcomes as reported.

Human capital

Human capital is perhaps the most important livelihood support factor (Ashley, 2000). It is the people who are both the object and subject of development. Since this study was on smallholder farmers, their knowledge about agriculture, technology available, sources and levels of accessibility were investigated. Investigation was, of necessity, carried out on farmers’ exposure to agricultural extension.

Financial capital

Financial capital is the medium of exchange and, therefore, central to the functioning of a market economy. Its availability is critical to the successful utilization of the other factors or assets. The main analyzes in this section were farmers’ exposure to financial services such as savings and access to cash credit.

Physical capital

Physical capital refers to man-made assets such as productive assets, housing quality and consumer durables. An analysis was done on the number and status of productive assets within the households.

Social capital

Social capital according to Ashley (2000) is the productive capital making possible the achievement of certain ends that would not be attained in its absence. In the Sustainable Livelihood Approach (SLA), social capital entails the social networks and associations to which people belong. In this context, social capital is taken to mean the social resources upon which people draw in seeking to achieve their livelihood outcomes, such as networks, and connectedness that increase their trust and ability to cooperate, or membership to groups and their systems of rules, norms and sanctions. Social capital attributes were analyzed descriptively through simple statistics (frequencies). Attributes that were analyzed included: group belongingness in terms of group typology and mandate.

Vulnerability concept and livelihood strategies

Under SLA, people's livelihoods and availability of assets are fundamentally affected by critical trends (such as population, resource, technology, national and international economic, shocks (such as human health shocks, natural shocks, economic shocks, conflicts and crop/livestock health shocks), and seasonality of prices, production, health and employment opportunities) (Ashley and Diana, 1999; Ashley, 2000). In general, people tend to have limited or no control on the vulnerability contexts. The factors (trends, shocks and seasonality) that make up the vulnerability contexts impact directly on people's asset status and the options open to them in pursuit of beneficial livelihood outcomes. Local households' vulnerability to climate change and livelihood strategies and outcomes were analyzed.

MATERIALS AND METHODS

Study locations

The study covered four Counties around the Lake Victoria Basin, namely: Nyando, Muhoroni, Rachuonyo and Busia based on their edaphic and climatic conditions such as salinity and water-logging commonly affecting some parts of the named counties. In these areas screening avocado rootstocks for tolerance to salinity and water-logging adaptable to these conditions would increase the production of avocado as a food security crop and income generation would be of importance.

Nyando and Muhoroni Counties; Nyando and Muhoroni Counties are in Nyanza region of Kenya covering Upper Nyakach, Lower Nyakach, Nyando, Miwani and Muhoroni. Much of the area lies in the Kano Plains that is predominantly black cotton clay soils with moderate fertility and poor drainage. The rest of the County has sandy clay loam soils derived from igneous rocks. Altitude range 1100 to 1800 m, annual precipitation 600 to 1630 mm, bi-modal rainfall pattern and exhibit wide variation in distribution. Kano Plains in Nyando is prone to flooding and water overflow that has caused extensive erosion in its lower parts resulting in huge galleys (GoK, 1999).

Rachuonyo county

The County has a population of 307,126 and an area of 945 Km². with four sub Counties namely East Karachuonyo, Kabondo, Kasipul and Rachuonyo (GoK, 1999)

Busia county (Bunyala)

Bunyala in Busia County has a total population of 58,773 lies to the north of Lake Victoria near the Kenya-Uganda border. Rainfall is bi-modal per annum. The major season occurs in March to May while the minor season occurs in October to December. The period June to July is generally dry unlike other areas in western Kenya, which observe a major rainfall peak during the period. The months of January and February are also generally dry though occasional wet conditions may occur especially in January (GoK, 1999).

Sampling method

Four Counties (Muhoroni, Nyando, Rachuonyo, Bunyala) were purposively sampled based on their edaphic and climatic (salinity and water-logging) conditions. Two divisions were selected from each county for the study. Further, one administrative sub-location was selected from each of the selected divisions. Two villages were randomly sampled from each sub-location, from a list of all the villages in the selected sub-locations. The same procedure was used for selecting sample villages in all the three counties. The listing of villages was done during reconnaissance survey by the research team and FEWs (Frontline Extension Workers) through the assistance of the area assistant chiefs that availed the lists of all the households in each village from which sample households were selected for the study.

Sample size

Four steps were used to select the sample population. The first step involved developing a list of all villages in the selected sub-locations. The second step involved sampling four sample villages from the list developed. The third step involved making a list of all households in the sampled villages. The fourth step involved selecting the sample households from the village lists. Thus, using the 'lottery technique, four villages were randomly sampled from each county. Using systematic sampling technique, 25 households were selected from each village for the survey. Thus, one household was selected randomly from among the first five households in each village through the 'lottery technique'. The next and subsequent households were then selected based on the interval established. An appropriate sampling interval (I) was calculated by dividing the total village household size (N) by the required sample size (n) as follows:

$$I = {}^{N}/n$$

Where, I = the interval; N = the total village household population and n = the sample size.

All households were assigned sequential numbers from 1……..n for each County based on the village lists. If the first random household was, for example, 5, and the interval was 2 then the next 2nd household on the list of households was selected along with every following 2nd household until the required sample of 400 households was obtained.

Data collected and analysis

The collected data included; livelihood contexts, avocado production levels, vulnerability, capital assets from smallholder farmers by means of a structured questionnaire, Focus Group Discussion (FGD) where two FGDs per each county comprising of 20 participants were captured and Key Informant Interviews (KII) using a checklist. The structured questionnaire was administered by Field Extension Workers (FEWs) from local county Agricultural Offices and Research Assistants. The data was cleaned prior to analysis and subjected to Statistical Package for Social Scientists (SPSS) outputs interpreted and reported. The purpose of these tools was to assess farmers' opinion on: avocado production and livelihood contexts. Information collected through these tools were used for triangulation purposes, that is, cross-checking it with information that was collected through the structured questionnaire.

RESULTS AND DISCUSSION

Socio-economic and demographic characteristics of respondents

Demographic and socio-economic characteristics play a key role in determining the livelihoods of rural people. Variables such as age and education greatly influence how people perceive, use and dispose of assets (Usman et al., 2013). Normally, aged household heads are expected to have accumulated wealth of experience on various aspects of life. A farmer's experience, for instance, can generate or erode confidence. With more experience, a farmer can become more or less averse to agricultural risks by adopting new technologies and improved agricultural practices (Usman et al., 2013). All these falls under human capital whose results as reported and discussed below.

Human capital

Human capital represents the skills, knowledge, ability to labor and good health that together enable people to pursue different livelihood strategies and achieve their livelihood objectives (DFID, 2000). At the household level, it varies according to variables such as household size, skill levels, leadership potential and health status. Human capital appears to be a decisive factor in making use of other types of assets. Therefore, changes in human capital have to be seen not only as isolated effects, but as a supportive factor of the other assets. Human capital has been reported to increase agricultural productivity in Senegal (Ndouri, 2017), while in Iran (Mehdi, 2012), reported that experience and education increases agricultural productivity, additional year of education squared reduced level of inefficiency while additional years of experience squared increases famer's income (Jules and Fondo, 2012). In this paper physical capital, age, financial capital and membership to cooperatives were considered because they affect human capital since they interact with each other.

Age

Age can have a positive or negative effect on a farmer's decision to adopt technologies. An aged farmer, for example, may avoid a technology that is more labor involving. In China an increase in age lowered agricultural, similarly, in Nigeria majority of older farmers reduced scope and time hours in farming operations (Usman et al., 2013). Thus, age may reduce technical efficiency in crop productivity. Majority of the respondents interviewed were aged 41 and above. However, analysis across the counties indicated that Nyando County had the highest number of respondents, 76.2%, while Rachuonyo had the lowest, 56.3% (Table 1). During the study period most farmers were in the age groups of 31 to 40 years and greater than 60 years.

Level of education

Exposure to education increases a farmer's ability to obtain, process and use information relevant to adoption of improved technologies and hence, increase yields and influence wealth (Usman et al., 2013). A mean of 60.4%, of the framers, had completed primary level education. Muhoroni County had the highest at 72.5%, while Nyando County had the lowest at 51.4% (Table 1). In Kenya primary level of education is sufficient for farming but more education is even better (Usman et al., 2013).

Physical capital in relation to human capital

Physical capital comprises the basic infrastructure required to support livelihoods in a given environment (rural or urban). These basic infrastructures include adequate water supply, sanitation, environmentally friendly sources of energy, secure shelter, access to transportation and communication facilities (Asif et al., 2015) increases agricultural productivity and output since it determines agricultural production decisions (Moser, 1998; Putman, 1993; Bebbington, 1999) and also affects marketing of produce (Winters, 2000).

Mobile phones as a tool for change

Mobile ownership was considered as proxy indicator for household socio-economic status. The ownership of a mobile phone has nowadays become and essential household item in Kenya and lack of it in the household means that the household will struggling to acquire one. Mobile phones can facilitate flow of information along value chains among stakeholders from agricultural extension officers. They can also be used to manage livelihood shocks such as livestock deaths or harvesting problems (Aker and Mbiti, 2010; Sen and Chaudhery, 2011) or for savings, insurance services and for marketing (Sen and Chaudhery, 2011). Almost, 71.2% of the farmers owns, at least owned a mobile phone.

Table 1. Gender, education and age of respondents.

Characteristic		Number of respondents (%)					
		Muhoroni (%) (N=80)	Nyando (%) (N=80)	Rachuonyo (%) (N=80)	Bunyala East (%) (N=80)	Bunyala (%) West (N=80)	All counties (%) (N=400)
Gender	Male	72.5	71.2	71.2	66.2	58.8	68.0
	Female	27.5	28.8	28.8	33.8	41.2	32.0
Age (Years))	<18	1.2	Nil	3.8	Nil	Nil	1.2
	19-30	15.0	7.5	10.0	16.2	8.8	11.4
	31-40	23.8	16.2	30.0	25.0	21.2	23.1
	41-50	15.0	25.0	22.5	15.0	23.8	19.9
	51-60	20.0	20.0	13.8	21.2	10.0	17.1
	> 61	25.0	31.2	20.0	22.5	36.2	27.3
Schooling	None	13.8	20.0	8.8	23.8	17.5	16.9
	Primary	72.5	51.4	52.5	55.0	71.4	60.4
	Secondary	12.4	23.7	33.7	21.2	9.9	20.0
	College	Nil	3.8	5.0	Nil	1.2	0.5
	University	1.2	1.2	Nil	Nil	Nil	0.4

Therefore, passing information may be faster, this may be a good channel for change and may increase adoption of technology

Agricultural extension services

This study specifically focused on human capital in relation to agricultural activities. In this section, human capital was analyzed from the perspective of agricultural extension, decision-making on key household enterprises and time allocation by key household members to the enterprises. Extension is an important parameter of human capital. The extensive ownership of mobile phones in the study areas is a boost to the flow of agricultural information from agricultural extension officers as discussed earlier under physical capital. Both government and private sector extension services help farmers to access new technologies and demonstrations on how to apply the various guidelines (Thabitt and Suleiman, 2015; Usman et al., 2013; Akkad, 1990; Ndour, 2017). In addition, extension providers play an important role in monitoring and evaluation of these new technologies. Agricultural extension services play a major role in building the knowledge stock of farming communities (Jules and Fondo, 2012; Ouma et al., 2018). They help farmers to translate results into improvement in livelihoods. In Ethiopia Agricultural extension has helped farmers replace their local crop landraces with improved varieties thus increasing crop yields and food security (Biratu, 2008) while in Argentina it has resulted in increases in Grape yield, productivity and quality (Pedro et al., 2008). In Kenya it has been similarly reported that it increases yields but this depends on factors such as availability of labour, farmers' level of education, types of crops grown, farmers' experience, farm management abilities of the farmer and agroecological characteristics of the farm.

Visits by extension agents to farmers and farmers' participation in field days, seminars and/or agricultural shows are cost effective ways of reaching out with the new agricultural practices or technologies to a large number of farmers. The study revealed that agricultural extension contacts increases yields and incomes for farmers and particularly for farmers near cities where the officers stay, farm inputs available for purchase, younger farmers who are active and educated in addition to high incomes. The study revealed that overall 55.1% of the respondents' farmers indicated that they never sought extension advice for crops and livestock production, specifically, Bunyala East had 81.2%, and conversely, Nyando County had 60%, sought extension service. The main source of extension services was the public sector while in Rachuonyo County where private sector was the major source of extension services as "service providers". Some the reasons for not accessing extension services were reported as that the extension services were not available (Table 2). Thus, any undertaking on fruit production and especially, avocado production should address the issue of extension. By the farmers indicating that they sought advice from the public sector indicates that they have confidence in the sector and therefore any extension services should be channeled through the public sector. Some farmers would expect extension services to reach them as they fear that if the call for them there might pay for the services rendered. However, extension reforms are taking place in many countries to enable private agencies to be hired by farmers for the task (Rivera, 1996; Rivera et al., 2000; Carner, 1998; Feder et al., 1999).

Table 2. Sourcing of extension services.

Service type		Number of respondents					
		Muhoroni (%) (N=80)	Nyando (%) (N=80)	Rachuonyo (%) (N=80)	Bunyala East (%) (N=80)	Bunyala West (%) (N=80)	All Counties (%) (N=400)
Sought extension service	Yes	46.2	60.0	45.0	18.8	55.0	44.9
	No	53.8	40.0	55.0	81.2	45.0	55.1
Source of extension	Public extension agent	15.0	15.0	12.5	6.2	45.0	18.4
	Private extension agent	1.2	35.0	1.2	7.5	2.5	9.9
	Neighbor/farmer	13.8	Nil	7.5	2.5	2.5	5.2
	ASK show	2.5	3.8	1.2	1.2	1.2	2.0
	Input dealer	1.2	Nil	11.2	1.2	Nil	3.0
	Radio/TV	Nil	2.5	1.2	Nil	Nil	0.5
	Family/friend	1.2	1.2	Nil	2.5	3.8	1.7
	Farmer organization/Cooperative	2.5	Nil	2.5	Nil	2.5	1.5
	Field days/Demos	1.2	Nil	1.2	Nil	Nil	0.5
	NGO agent	10.0	1.2	6.2	Nil	Nil	3.5
	Research organization	Nil	Nil	Nil	1.2	Nil	0.2
	Other	Nil	Nil	1.2	Nil	Nil	0.2
Reasons for not seeking advice	Long distance	20.0	12.5	3.8	21.2	8.8	13.2
	Expensive	8.8	8.8	Nil	5.0	15.0	7.4
	Time consuming	Nil	1.2	1.2	3.8	Nil	1.2
	Extension agent not available	17.5	10.0	18.8	12.5	15.0	14.9
	Don’t need extension services	5.0	10.0	20.0	35.0	1.2	14.1
	Other	7.5	Nil	8.8	2.5	Nil	4.0

Payment for extension services

In the rapidly changing world, with demands on few resources, the provision of free extension services is becoming impracticable thereby necessitating cost-sharing in extension services with the farmers. The results indicated that 64.5% of the respondents were not paying for extension services with Rachuonyo topping the list with 80% followed by Muhoroni, 76%. The willingness to pay for extension service, were as follows: 56.1% said no. Bunyala East topped this list at 71.2% followed by Muhoroni at 65.0% of the respondents indicating that they would not be willing to pay for extension services. Willingness to pay for extension services appeared to have been affected by factors such as availability of skilled extension staff, farm productivity, size of farm, crop type; some crops such as fruits and vegetables are high value crops and can help the famer pay for extension services (Onoh et al., 2012). Other factors which may have influenced these results include cost of extension services, economic benefits, access of government extension services, subsistence farming, quality of extension services (Uddin et al., 2016), farm size, farmer’s level of education, can help the famer pay for extension services (Onoh et al., 2012). However, 66.2% of the respondents in Rachuonyo were willing to pay for extension service.

Table 3. Payment for extension services.

Service		Number of respondents					
		Muhoroni (%) (N=80)	Nyando (%) (N=80)	Rachuonyo (%) (N=80)	Bunyala East (%) (N=80)	Bunyala West (%) (N=80)	All counties (%) (N=400)
Paying for extension	Yes	3.8	47.5	7.5	3.8	1.2	12.9
	No	76.2	41.2	80.0	62.5	62.5	64.5
Willing to pay for extension for a fee	Yes	30.0	66.2	46.2	28.8	33.8	41.2
	No	65.0	32.5	52.5	71.2	60.0	56.1
Reasons for non-payment for extension service	Cannot afford	30.0	13.8	36.2	51.2	53.8	37.0

Conversely, the farmers gave various reasons why they would not be willing to pay for service. Topping the list was the feeling that the farmers cannot afford to pay for extension service. Yet others felt that it is the responsibility of the government to provide extension services (Table 3). The several implications were that was difficult to sell extension services as a cost-sharing venture in Rachuonyo and Muhoroni Counties and that any intervention aimed at scaling up extension services either on fruit production or any farming venture should aim at reversing the thinking that farmers cannot afford to pay for the services and/or that it is the responsibility of the government.

Decision making on agricultural production

Maize is as a staple crop grown by the farmers both for food and cash income in the survey areas. Avocado was studied as a fruit crop mainly for cash production. Decisions making of various crops in the farm by the farmers were influenced by age, farming experience, type of agricultural land, ecology, extension programs, attitude of cooperative society members, agricultural knowledge, level of full-time activity, professionalism, farm size, social status, knowledge, attitudes and social association (Mehdi, 2012). Other factor affects include labour availability and cost, market price, availability and cost of inputs, crop requirements and pests and diseases (Madhu and Chandargi, 2004). Gender roles in the household decision-making process are important in a baseline study before a new technology is deployed in an area. An understanding of the role of household members in making decisions about the utilization of resources guides the design of appropriate strategies, for the introduction of a new technology. Household members decide on the disposal of benefits from agriculture is important in order to predict who among the household members, the new technology would benefit most. Results from the survey indicated that most decisions on maize and fruit production were made by head of the household as indicated by 44.4 and 38.0% of the respondents respectively. Bunyala West had majority of the decisions on the two crops made by the head of the household as indicated by 76.5 and 77.5% for maize and fruit production, respectively (Table 4). Any intervention, therefore, aimed at decisions on crop production should address the issue of household headship as this dictate which enterprise the household would give priority.

Similar to maize and fruit production, the farmers indicated that it was the head of the household who made decisions on maize and fruit marketing as indicated by 26.3% for both maize and fruits respectively (Table 4). Factors which may affect marketing decisions are farmer attitudes, age and education of the farmer. A relatively young, educated and innovative farmer sells at farmer's markets while the older, less educated farmer sells at traditional markets. Other factors were farming experience, farming traditions in the family, farm size, number of

Table 4. Decision making on production of maize and fruits.

Crop		Number of respondents					
		Muhoroni (%) (N=80)	Nyando (%) (N=80)	Rachuonyo (%) (N=80)	Bunyala East (%) (N=80)	Bunyala West (%) (N=80)	All Counties (%) (N=400)
Maize production	Head	37.5	41.2	28.8	42.5	76.2	44.4
	Spouse	11.2	23.8	15.0	11.2	13.8	15.6
	Male children	1.2	3.8	Nil	1.2	Nil	1.2
	Female children	Nil	Nil	1.2	Nil	Nil	0.2
	Head and spouse	47.5	26.2	41.2	41.2	10.0	33.3
	Head/Spouse/Children	1.2	Nil	3.8	1.2	Nil	1.2
	Household non-member	Nil	1.2	Nil	Nil	Nil	0.5
Fruit production	Head	32.5	32.5	17.5	32.8	77.5	38.0
	Spouse	10.0	6.2	11.2	10.0	13.8	10.4
	Male children	1.2	3.8	Nil	7.5	Nil	2.5
	Female children	Nil	Nil	Nil	Nil	Nil	Nil
	Head and spouse	46.2	3.8	31.2	36.2	8.8	25.1
	Head/Spouse/Children	6.2	Nil	1.2	10.0	Nil	3.5
	Household non-member	Nil	Nil	Nil	Nil	Nil	0.2

products, higher price motivation, plans to continue farming, plans to develop farm infrastructure, external supports and cooperatives (Ouma et al., 2018). Other factors are market price, scale of operation, distance to the market, farm mechanization, institutional and agricultural markets ,access to finance, investment and infrastructure services, speed of payment, farmers age (Nwachukwi, 2013), market information, credit availability, availability of cooperatives, expertise on grades and standards, contractual agreements, availability of social capital and market infrastructure, communication infrastructure. For fruits such as Avocado the decision making depends on orchard characteristics, variety, fruit maturity, quality attributes. There are other crops in the farm which also influence decision making namely rice, groundnuts, sweet potatoes, tomatoes, vegetables, millet/sorghum (Nwachukwi, 2013). Where both male and female participate equally in decision making in a house hold a higher production were realized especially in a high valued crop such melon (Mohammad and Abdulquaris, 2012) that the involvement of both sexes in various field activities may be specific but they are complimentary as also confirmed for maize and beans in this study.

Households decision making on fruit and maize enterprises marketing

The decision making on fruit and maize enterprises marketing lies on the head of the household who makes decisions on how to market maize and fruits (Table 5). However, other family members can market in absence of household head.

Decision making on use of income from maize and fruits

When it came to the issue of use of income, the results further still indicated that it was the head of the household who made decisions on how to use income from sale of maize and fruits as indicated by 44.4 and 38.0% of the respondents, respectively. In both cases, Bunyala West was leading that it was the head of the household who made decisions on use of income from maize and fruits (Table 6).

Table 5. Households decision making on fruit and maize enterprises marketing.

N (Number of respondents)		Number of respondents					
Enterprise		**Muhoroni (%) (N=80)**	**Nyando (%) (N=80)**	**Rachuonyo (%) (N=80)**	**Bunyala East (%) (N=80)**	**Bunyala West (%) (N=80)**	**All Counties (%) (N=400)**
Maize marketing	Head	17.5	1.2	16.2	33.8	63.8	26.3
	Spouse	12.5	1.2	13.8	12.5	25.0	12.9
	Male children	2.5	Nil	Nil	3.8	Nil	1.2
	Female children	Nil	Nil	Nil	Nil	Nil	Nil
	Head and spouse	18.8	1.2	20.0	45.0	10.0	18.9
	Head/Spouse/Children	2.5	Nil	Nil	2.5	1.2	1.2
	Household non-member	1.2	Nil	Nil	Nil	Nil	0.5
Fruit marketing	Head	16.2	20.0	7.5	22.5	65.0	26.3
	Spouse	12.5	12.5	15.0	13.8	25.0	15.4
	Male children	2.5	2.5	Nil	10.0	Nil	3.0
	Female children	Nil	Nil	Nil	Nil	Nil	Nil
	Head and spouse	16.2	1.2	21.2	41.2	8.8	17.6
	Head/Spouse/Children	6.2	Nil	1.2	8.8	1.2	3.5
	Household non-member	1.2	1.2	Nil	Nil	Nil	0.7

Time allocation to household enterprises by household members

The household is the level at which most resource allocation decisions are made. Division of roles, time and responsibilities among different family members occurs naturally among men, women, youth and the elderly.

Allocation of labour varies if farm income is uncertain for reasons of farm product price variability and uncertain rainfall (Mishra and Godwin, 1997). Like any other resource in the household, time is not equally distributed across members. Time allocation is highly influenced by farm characteristics, individual member characteristics and market access (Ellis, 1993). Farm size is negatively related to amount of time allocated to off-farm activities since farmers undertake them due to constraints in getting access to farming land (Reardon, 1997). Usually, there are significant differences not just along gender lines but also by age, social status, wealth, etc. Time allocated to household (Table 6) activities can range from 24 h to days spent in various activities over a year. How much time one devotes to certain activities in the household may imply the importance the person attaches to the activity or its necessity as compared to farming. Family members may allocate their time budget between self-employment on their piece of land and local agricultural labour market (Escobal, 2001). Participation in farm activities may be influenced by labour availability and cost and economic status of the family (Madhu and Chandargi, 2004; Escobal, 2001). Some household members may not work off farm due to their low education, advanced age, gender and customs (Udry et al., 1995). Rural household members are motivated to enter non-farm sector due to factors such as risk in farming or lack of insurance. Under the assumption of perfect labour market farmers may not participate in the off-farm labour even if the reservation wage rate is less than the marginal value of labour (Blundell and Meghir, 1987). The actual participation of farmers in off-farm activities depends on the incentive and capacity to participate (Reardon, 1997), variables that raise the value of marginal product of labour

Table 6. Decision making on use of income from maize and fruits enterprises.

Crop		Number of respondents					
		Muhoroni (%) (N=80)	**Nyando (%) (N=80)**	**Rachuonyo (%) (N=80)**	**Bunyala East (%) (N=80)**	**Bunyala West (%) (N=80)**	**All Counties (%) (N=400)**
Use of income from maize	Head	15.0	3.8	16.2	33.8	63.8	44.4
	Spouse	2.5	Nil	13.8	12.5	25.0	15.6
	Male children	2.5	Nil	Nil	3.8	Nil	1.2
	Female children	Nil	Nil	Nil	Nil	Nil	.2
	Head and spouse	15.0	1.2	20.0	45.0	10.0	33.3
	Head/Spouse/Children	17.5	Nil	Nil	2.5	1.2	1.2
	Household non-member	2.5	Nil	Nil	Nil	Nil	.5
Use of income from fruits	Head	13.8	31.2	10.0	32.5	67.5	38.0
	Spouse	1.2	6.2	8.8	5.0	8.8	10.4
	Male children	2.5	Nil	Nil	1.2	Nil	2.5
	Female children	Nil	Nil	Nil	Nil	Nil	Nil
	Head and spouse	12.5	1.2	26.2	40.0	11.2	25.1
	Head/Spouse/Children	22.5	Nil	1.2	16.2	10.0	3.5
	Household non-member	2.5	Nil	Nil	2.5	2.5	0.2

in off-farm employment increase the probability and level of participation in off-farm. Therefore, family members whose real opportunity cost of time is lower than marginal productivity of labour work on the farm and vice versa (Reardon, 1997). Attention was given to the household head and their spouse as the key household members. The findings indicated that both the head of the household and spouse hadalmost similar time allocation to household farming activities as indicated by 42.4 and 38.7% of the respondents, respectively (Table 6). This agrees with past findings reported on effect of gender on time allocation. Males however been reported to increase chances of working off-farm but reduces time in farm activities (Abdullai and Delgado, 1999; Newman and Canagarajah, 2000) but contrasts with most scholars who reported that growth in non-farm activities would benefit women (Newman and Canagarajah, 2000).

Time allocation to household members

Time allocation on the scale of 100% to farming activities scored dismally across the head and the spouse (Table 7). This shows that the families were involved in off-farm activities due to their low economic status due to may be to poor crop yields or produce prices (Mishra and Godwin, 1997; Rose, 2001; Bandyopadhyay et al., 2012). The present study findings disagree with those of Adeyonu (2012) who reported that male allocated more time to farming than off-farm activities while female allocated more time to farming during rainy seasons than dry seasons. In Uganda it has been reported that education and road access positively affected time allocation to off-farm employment (Bagamba, et al., 2007). The age groups of the famers in the counties appear to have been middle aged since they are likely to work off-farm compared to young and old due to their higher education levels (Newman and Canagarajah, 2000).

Household membership to cooperative organizations

In this sturdy, the focus was on farmers' membership to cooperative organizations. Results

Table 7. Time allocation to household members.

N (Number of respondents)		Number of respondents					
Household		**Muhoroni (%) (N=80)**	**Nyando (%) (N=80)**	**Rachuonyo (%) (N=80)**	**Bunyala East (%) (N=80)**	**Bunyala West (%) (N=80)**	**All Counties (%) (N=400)**
Time allocation by household head	0	12.5	2.5	11.2	8.8	12.5	10.2
	25	50.0	60.0	20.0	56.2	27.5	42.4
	50	Nil	Nil	Nil	Nil	Nil	Nil
	75	27.5	32.5	47.5	27.5	37.5	34.2
	100	10.0	5.0	18.8	6.2	22.5	12.4
Time allocation by spouse	0	5.0	2.5	3.8	1.2	11.2	5.0
	25	42.5	51.2	18.8	45.0	35.0	38.7
	50	Nil	Nil	Nil	Nil	Nil	Nil
	75	28.8	18.8	40.0	26.2	27.5	28.3
	100	23.8	21.2	31.2	26.2	26.2	25.6

Table 8. Household membership to co-operatives.

Cooperative type	Number of respondents					
	Muhoroni (%) (N=80)	**Nyando(%) (N=80)**	**Rachuonyo (%) (N=80)**	**Bunyala East (%) (N=80)**	**Bunyala West (%) (N=80)**	**All Counties (%) (N=400)**
Producer cooperative	22.5	3.8	3.8	3.8	1.2	6.9
Multi-purpose cooperative	6.2	2.5	2.5	Nil	1.2	2.5
Savings and credit cooperative	10.0	15.0	10.0	3.8	5.0	8.9
Informal self-help groups	28.8	12.5	40.0	11.2	3.8	18.9
Out grower company	Nil	1.2	Nil	Nil	Nil	0.2
Other cooperative	1.2	1.2	Nil	Nil	1.2	0.7

revealed that the responses were evenly spread out across different associations including; informal self-help groups, (18.9%), savings and credit cooperatives, (8.9%) and producer cooperative (6.9%) among other associations (Table 8). Across all the Counties there was poor enrolment in cooperatives. This may have been due to their low education and lack of coordination and efficient distribution of resources to members (Abdullai and Delgado, 1999; Arcas-Lario and Hernadez Espallardo, 2003). Membership to cooperatives has been reported to be positively affected by farmer's level of education, communication, log of gross income and farm size. Small farmers are expected to join cooperatives than large farmers for input services (Karh and Celik, 2006). It is most probable that the farmers in these counties had low education, low gross income and poor awareness but were small scale farmers who needed this membership (Ouma et al., 2018). Factors such as age, farming experience, type of agriculture, agricultural land area and area of cultivated land, social status, knowledge and attitudes, facilities, attitudes of the cooperatives also could have led to this poor membership to cooperatives (Mehdi, 2012).

Conclusion

Farmers had limited access to information on avocado production and marketing as 31.3%, sourced information from fellow farmers, lack of certified avocado seedlings. Sensitizing farmers on the importance of knowing the soil fertility status of their farms by sampling their soils and taking to soil laboratory for analysis for appropriate recommendation of the required amendments would enhance profitable production. Increase in human capital, farmer education and extension services contribute positively to farmer's abilities to adopt new technologies. In water logging and salinity areas planting tolerant certified avocado seedlings are beneficial to farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Suitability of biosolids from university sewage ponds as a substrate for crop production

Peter Caleb Otieno*, Samuel Nyalala and Joseph Wolukau

Department of Crops, Horticulture and Soils, Egerton University, P. O. Box 536-20115, Egerton, Kenya.

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Currently, sewage waste management is a serious environmental problem and one of the major growing concerns for urban areas all over the world. Utilization of biosolids (BS) for crop production may be a sustainable waste management strategy. The present study evaluated the physico-chemical and biological characteristics of biosolids from sewage ponds at Egerton University, Kenya. This was to determine its suitability for crop production. Biosolids were evaluated separately then as mixture with forest soil at rates of 0, 10, 20, 30, 40, 50 and 60% and compared with tea compost (TC) and coco peat (CP) in a completely randomized design experiment with four replications. Data collected included: macro-elements, micro-elements, heavy metals, pH, electrical conductivity (EC), bulk density (BD), water holding capacity and biological properties. Results showed that total organic carbon (0.03%), total organic nitrogen (2.0%) and Molybdenum (22 mg kg^{-1}), in biosolids were significantly ($p < 0.05$) higher compared with forest soil, but not significantly different from tea compost. For heavy metals, Hg (0.33 mg kg^{-1}), As (5.9 mg kg^{-1}), Cr (31.1mg kg^{-1}), Cd (0.38 mg kg^{-1}), Ni (16.3 mg kg^{-1}) and Zn (127 mg kg^{-1}) were significantly ($p < 0.05$) higher in biosolids but within the allowable limits according to Environmental Protection Agency (EPA) standards. bulk density (1.2 to 1.5) g cm-3 and pH (5.4 to 5.8) units, but high organic matter (195 to 230) g kg-1, water holding capacity (35 to 42 %) and EC (2.6 to 5.4) µSm-1). For microbial load, total viable count (TVC) and colony forming units (CFU) registered 5 ×10-7 and 6.5×10-7 respectively. However, Escherichia coli, Salmonella sp. and Staphylococcus sp. were not detectable in the fully composted biosolids. Similar trend of these results were subsequently observed in the substrates formed in the mixture of biosolids and forest soil and this provide insight on the potential of biosolids as substrate for crop production and a reliable alternative to soil alone.

Key words: Biosolids, forest soil, organic amendment, substrate.

INTRODUCTION

Application of mineral fertilizer has been the norm of maintaining soil fertility because of its uniformity and ease of application. Hence, fertilizers have reduced the use of organic nutrient sources (Shaheen and Tsadilas, 2013). This massive use of such mineral fertilizers and other inappropriate cultivation practices, including stubble burning, has greatly reduced soil organic-matter content, subsequently, influenced the physical, chemical, and

*Corresponding author. E-mail: petercaleb68@gmail.com Tel: +254721327263

biological properties leading to soil degradation risk. Such agronomic practices could lead to the mineralization and desertification (Tejada et al., 2001).

The generation of sewage wastewater has been increasing with rapid world population increase and urbanization. Application of treated wastewater in landfill is generally considered the most economical and beneficial way of disposing biosolids (Haynes et al., 2009). According to Al-Gheethi et al. (2018), biosolids is a sewage sludge that has been treated by advanced processes including aerobic and anaerobic, heat or lime treatment, which has met standards required for beneficial use. The organic and inorganic contents of biosolids are essential for soil and plants (Nowak, 2007). So they are nutrient-rich with organic matter content of up to 50% (Qin et al., 2012). They are also rich in nitrogen, phosphorus, and other trace elements and present a good source of nutrients for plant growth (Sukkariyah et al., 2005). Application of biosolids has been observed to improve the physico-chemical and biological properties of soils, which in turn facilitates better growth of plants (Mtshali et al., 2014). Besides acting as a food source for microorganisms, organic matter is the major binding agent for soil aggregate formation and stabilization (Tisdall and Oades, 1982). The soil structure formed, in turn, improves many other important soil physical and chemical properties such as bulk density, porosity, water holding capacity, cation exchange capacity, aeration and drainage, microbial communities and soil fauna, thus contributing to disease suppression and reduced soil erosion. However, the use of biosolids depends on a number of factors such as food habits, culture, socioeconomic and climatic conditions (Abur et al., 2014). It varies not only from city to city but also within the same city (Gakungu et al., 2012). Therefore, before using them for crop production, it is always necessary to characterize biosolids.

For crop production, biosolid waste generally contains significant concentrations of organic matter, nitrogen, phosphorus and potassium and to a lesser extent, calcium (Ca), sulphur (S) and magnesium (Mg). According to Kirchmann et al. (2016), about 10% of the total nitrogen (N) in biosolid waste is present as ammonium nitrogen, which is plant accessible, while 90% is present in organically bound forms that need to be mineralized to become plant available. Biosolids also insure against unforeseen nutrient shortages by supplying essential plant nutrients such as sulphur (S), manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), molybdenum (Mo), and boron (B) that are seldom purchased by farmers because crop responses to their application are unpredictable (Sukkariyah et al., 2005). They can be applied on micronutrient deficient soils like alkaline soils (Moral et al., 2002) and sandy soil (Ozores-Hampton et al., 2011). Nutrient values of biosolids vary with sources of wastewater and wastewater treatment processes. Processes such as digestion or composting result in the loss of organic matter through decomposition increase concentrations of phosphorous and reduce trace elements (Mtshali et al., 2014). It also leads to a decrease in ammonium nitrogen by volatilization and a decrease in potassium by leaching. However, nutrient composition of biosolids is significantly altered by stabilization processes and mineralization.

Trace elements and heavy metals are of particular concern in regard to their effects on human and animal health (Qin et al., 2012). The USEPA (1995) analysed their risks to humans, animals, plants, and soil organisms from exposure to pollutants in biosolids via different pathways for land-applied biosolids. Nine trace elements: Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Selenium (Se) and Zinc (Zn), were deemed to be of sufficient risk to regulate. Land application of biosolids must meet the ceiling concentrations and cumulative loading rates for these nine trace elements, above which, the biosolids cannot be applied in agricultural land. There are also concerns about the pathogen contaminations in biosolids (Qin et al., 2012). Biosolids applied to the land for crop production, both for human or as fodder, should not show any unacceptable microbial level or have adverse impact on human health (NRMMC, 2004).The objective of the current study was to determine the suitability of biosolids from Egerton University sewage ponds as potting substrate in terms of its physico-chemical and biological properties.

MATERIALS AND METHODS

Experimental site

The work was done in Njoro sub-county in Nakuru, Kenya from January to February 2018. The site is located on latitude 0° 23' S and longitude 35° 35' E in the Lower Highland III (LH3) agro-ecological zone at an altitude of 2238 m above sea level (Jaetzold et al., 2012). The analyses of the samples were done in Soils and Food Science laboratories of Egerton University (Figure 1).

Biosolids and forest soil samples collection

Naturally dried biosolids samples were collected from the seventh pond of the Egerton University wastewater treatment plant (Figure 2). The wastewater treatment plant is made of seven open aerated ponds. Wastewater undergoes aerobic digestion, in an oxygen-rich environment lagoon aerated naturally. Every year, the dry spell occurs in the months of December to March, during which the sixth and seventh pond normally dry up, leaving dry biosolids ready for disposal (Figure 2). The biosolid samples were collected (Plate 1) and solarized for two month sunder clear polythene paper gauge 200 mm thick. After solarization process, the biosolids were further stored in a plastic greenhouse for 10 months and then comprehensively analyzed in the laboratory.

Forest soil (FS) on the other hand was collected from an indigenous forest surrounding Egerton University botanic garden. This was an area, which has not been subjected to any farming activity for the last 20 years (Plate 2). After collection, the soil was solarized two months and then taken to the laboratory for comprehensive physico-chemical analysis along with the biosolids, tea compost (TC) and coco peat (CP).

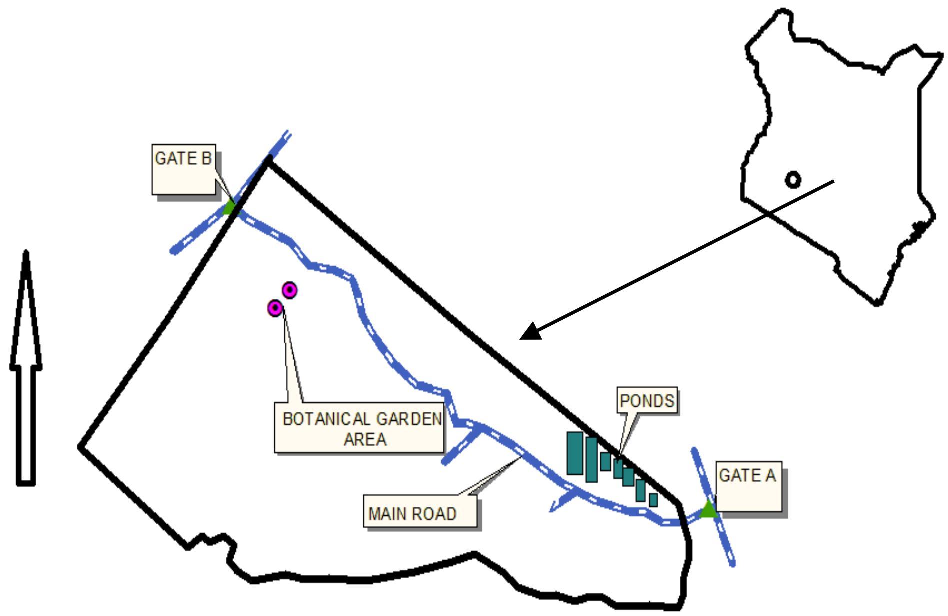

Figure 1. Map of the site of sampling forest soil and biosolids. Source: Agricultural Engineering Department, Egerton University (2019).

Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7
Effluent Water	Water	Water	Water	Water	Dewatered Biosolids	Dewatered Biosolids

Figure 2. The arrangement of lagoons at Egerton University wastewater treatment plant.

Preparation of biosolids as a substrate

Forest soil and biosolids were mixed at rates of 10, 20, 30, 40, 50 and 60% (v/v) and taken to the laboratory for comprehensive analysis of their physico-chemical characteristics. The physico-chemical characteristics of Tea compost (TC) and coco peat (CP) were also analyzed as reference commercial substrates. For microbial analysis, both water and dried biosolids portions were collected separately from different ponds (Figure 1) for presence or absence of the specified pathogens-*Salmonella* sp., *Escherichia coli* and *Staphylococcus* sp.

Determination of physico-chemical properties of the substrates

Electrical conductivity

Salinity was determined using conductivity meter with a conductivity bridge (Model CM-1 Mark V) for each growing medium. A ratio of 1:1 (substrate: water) suspension was prepared and filtered using a Buchner funnel with filter size 14. Each growing medium was filtered through Buchner funnel. After the filtrate was clear, it was transferred into a 50-ml bottle and the conductivity cell was immersed in the solution to take reading according to Okalebo et al. (2002).

Measuring pH of the substrates

The pH was measured using pH-meter (digital ion analyzer). Sample of 50 g of air-dried growing medium was taken into a 100-ml glass beaker; thereafter 50 ml distilled water was added using a graduated cylinder, mixed well and allowed to stand for 30 min. The suspension was stirred after every 10 min and pH determined according to the procedure described by Okalebo et al. (2002).

Organic matter content and organic carbon

One gram of air-dried growing medium was placed into a 500-ml beaker. Ten millilitres of 1 N potassium dichromate solution and 20 ml concentrated sulphuric acid was added in a beaker and swirled to mix the suspension. After 30 min, 20 ml of distilled water was added along with 10 ml concentrated orthophosphoric acid and the mixture was allowed to cool. Ten drops of diphenylamine indicator was added. The solution was titrated with 0.50 M ferrous ammonium sulphate solution and upon colour change from violet blue to

Plate 1. Harvesting of biosolids in the sixth (semi solid) and seventh (dry) lagoons**.**

Plate 2. Harvesting of forest soil

green, the reading was recorded, and organic matter content determined. Organic carbon was determined by method of Walkey and Black (1934).

Bulk density

A core ring of 5 cm diameter with known weight (W_1) and volume (V) was inserted 5 cm in the substrate to scoop the amount of substrate material of the same volume. It was then removed from the substrate and samples around the core was wiped and trimmed at the bottom and top using a knife. They were then placed in an oven at 105°C for two days after which they were allowed to cool and weighed (W_2), according to Okalebo et al. (2002).

Bulk density (g cm^{-3}) = (W_2(g) –W_1(g)/V (cm^3).

Where, W_1 was the fresh weight of the sample with the core and W_2 was the weight of the dried sample in the core and V was the volume of the core.

Water holding capacity

This is the maximum amount of water that freely drained soil can hold, estimated after saturated soil has been drained without allowing its moisture stores to be depleted by evaporation. The substrate was filled with water and free water allowed to drain off, then covered with plastic containers for 2 days. Moisture content was thereafter determined based on the initial and final weights and using the formula below:

Field capacity of substrate (%) =(W_2 –W_3)/(W_3-W_1)) x 100

Where, W_1 was the wet substrate in moisture container with known weight, W_2 was the total weight and W_3, was weight of the dry soil in moisture container (Okalebo et al., 2002).

Determination of nutrient and heavy metal elements

Total nitrogen (Kjeldahl method)

Substrate sample weighing 0.3 g was digested in digestion tubes using a digestion mixture comprising of N/140 HCl, HNO_3, Se and $CuSO_4$. The temperatures in the heating block was maintained at 360°C for two hours after which the samples were allowed to cool, transferred to 50-ml volumetric flasks and the volume made to the mark. The sample was then allowed to settle and 5 ml of the aliquot was put into the distillation bottle where 10 ml of 1% NaOH was added. It was then steam distilled into 5 ml 1% boric acid containing 4 drops of mixed indicator for 2 min, from the time the indicator turned green. Distillate was titrated using HCl and the end point was reached when the indicator turned green through grey to definite pink (Okalebo et al., 2002), modified by Juma et al., (2018). A blank experiment was prepared using the same procedure, according to the method described by Kirk (1950).

Total phosphorous

Total phosphorus in the substrate samples was determined by the method described by Juma et al. (2018). A substrate sample of 0.3 g was digested in digestion tubes using a digestion mixture comprising of HCl, HNO_3, Se and $CuSO_4$. The temperatures in the heating block were maintained at 360°C for two hours after which the samples were left to cool, transferred to 50-ml volumetric flasks and volume made to the mark. Five ml of the aliquot was transferred into the sample bottles with 1 ml of developing colour solution (Ammonium Vanadate and Ammonium Molybdate in the ratio of 1:1). The samples were left to stand for 30 min after which they were transferred to cuvettes. Readings (absorbance) were taken using a spectrophotometer at λ_{max}= 430 nm. Calibration curve was done using laboratory certified standards containing 0, 0.2, 0.4, 0.6, 0.8 1.0 and 1.2 ppm P respectively.

Potassium analysis

A substrate sample weighing 0.3 g was digested in digestion tubes using a digestion mixture comprising HCl, HNO_3, HF and $H_3 BO_3$. The temperatures in the block was maintained at 360°C for two hours, thereafter samples were cooled, transferred to 50-ml volumetric flasks and volume made to the mark. Calibration was done for each element using certified standards. Samples were analysed using Atomic Absorption Spectrophotometer (AAS), Varian spectra AA10 AAS machine.

Determination of Na, Ca, Mg, Fe, Zn, Cu, Mn, Pb, Cu and Cd

The determination of these elements in the substrate was done using double acid method of extraction. AAS was used for estimation of these available elements in the tested substrate. This followed the procedure of Okalebo et al. (2002).

Determination of biological properties of the substrates

This work was done to determine the presence or absence of faecal contaminants, specifically *Salmonella* sp., *Escherichia coli* and *Staphylococcus* sp., as microbial organisms of health concern on in dry biosolids ready for disposal.

Isolation of microbial cultures

Fifty grams of compost were added to 950 ml of normal saline and homogenized for 30 min. Ten-fold serial dilution (10^{-1} to 10^{-6}) was made from the homogenate. The homogenate was used for enumeration of bacteria of medical importance in the biosolids. For enumeration of bacteria, 1 ml of homogenate was aseptically transferred onto plate count nutrient agar (Oxoid, England) in triplicates. The plates were incubated at 37°C for 24 h under aerobic atmosphere. After incubation and isolation, the number of colonies was counted with a colony counter, recorded as colony-forming unit (CFU).g^{-1} and Total Viable Count (TVC).g^{-1} of the growing medium. The evaluation of cellular concentration in a substrate samples were determined by plate counting of serials dilutions. The presence or absence of microbial organisms of health concern such as pathogenic *Salmonella* sp., *E. coli* and *Staphylococcus* sp., was determined in each sample in triplicates.

Data analysis

Data for each variable measured were analyzed using the statistical model for completely randomized design with five treatments and four replications. Data analysis was carried out using Statistical Analysis System software statistical package version 9.1 (SAS Institute, Cary Inc., 2001). Shapiro Wilk test was used to check for normality of the data before analysis. Numerical data were subjected to analysis of variance (ANOVA) at $p \leq 0.05$ and means for significant treatments separated using Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$.

RESULTS

The results on quantity of macro-, micro- elements and heavy metals determined in the biosolids (BS), forest soil (FS), tea compost (TC) and coco peat (CP) substrates are shown in Tables 1 and 2.

Macro- and micro-elements and heavy metals

Forest soils (FS) was used as reference and TC and CP as commercial substrates to compare the suitability and potential of BS in crop production (Table 1). In the four substrates, total organic carbon (ToC) content was detected in the range of 0.02 to mg g-1), which was not significantly ($p < 0.05$) different among them. Biosolids (BS) was significantly ($p < 0.05$) higher in molybdenum (22 mg kg^{-1}) and total organic nitrogen (2.0%), which was not significantly ($p < 0.05$) different from that of tea compost (TC). In comparison to the four substrates, FS as reference material was significantly ($p < 0.05$) higher in Fe (7.2%), Mn (0.6% mg kg^{-1}) and B (81 mg kg^{-1}).

Heavy metals

Forest soil (FS) was higher than the rest of the

Table 1. Chemical characteristics of biosolids from Egerton University waste water treatment plant.

Substrates^{a}	ToN % (0.01)*	ToP % (0.007)	K % (0.07)	Mg % (0.07)	Ca % (0.1)	S %	Fe %	Mn % (0.002)	Mo (mg kg^{-1})	B (mg kg^{-1})
FS	0.26±0.02^{c}	0.13±0.01^{c}	0.52±0.01^{b}	0.14±0.01^{b}	0.56±0.01^{c}	1.72±0.01^{b}	7.15±0.20^{a}	0.61±0.02^{a}	9.35±1.30^{b}	81.25±2.30^{a}
BS	2.00±0.02^{a}	0.25±0.01^{b}	0.50±0.02^{b}	0.16±0.01^{b}	0.49±0.02^{c}	0.64±0.01^{c}	4.26±1.40^{b}	0.26±0.01^{c}	22.47±4.00^{a}	61.73±2.80^{b}
CP	1.01±0.09^{b}	0.16±0.02^{c}	0.83±0.02^{a}	0.27±0.02^{a}	1.02±0.02^{b}	ND	0.68±0.01^{c}	0.19±0.01^{c}	4.84±0.90^{c}	52.98±1.50^{c}
TC	2.10±0.20^{a}	0.87±0.01^{a}	0.01±0.00^{c}	0.25±0.02^{a}	4.27±0.60^{a}	55.56±5.70^{a}	0.47±0.01^{c}	0.44±0.01^{b}	5.04±1.20^{c}	88.77±7.60^{a}

Means ± standard deviation followed by the same letter within a column are not significantly different according to Tukey's HSD test at $p \leq 0.05$. *Maximum recommended values of plant nutrient in the soil/ substrate for tomatoes production (Sainju et al., 2003) modified. ^{a}N = 4 in the substrate analysis. TC = Tea compost, CP= Coco Peat, FS =Forest soil ND= Not detected.

Table 2. Heavy metal characteristics of biosolids from Egerton University waste water treatment plant.

Substrates^{a}	Pb (mg kg^{-1}) (150) *	Hg (mg kg^{-1}) (1)	As (mg kg^{-1}) (20)	Se (mg kg^{-1}) (100)	Cr (mg kg^{-1}) (100)	Cu (mg kg^{-1}) (100)	Cd (mg kg^{-1}) (1)	Ni (mg kg^{-1}) (60)	Zn (mg kg^{-1}) (200)
FS	46.15±2.73^{a}	0.04±0.00^{b}	5.73±0.35^{a}	16.12±1.23^{a}	22.52±2.75^{b}	9.24±0.97^{c}	0.39±0.04^{a}	12.01±0.47^{a}	66.41±4.21^{b}
BS	21.82±1.99^{b}	0.33±0.01^{a}	5.85±0.55^{a}	1.07±0.07^{b}	31.12±2.15^{a}	34.43±1.24^{b}	0.38±0.01^{a}	16.73±0.49^{a}	127.02±4.97^{a}
CP	3.27±0.03^{c}	0.03±0.00^{b}	0.87±0.01^{b}	0.79±0.03^{b}	35.63±1.25^{a}	63.22±3.57^{a}	0.08±0.00^{b}	7.05±0.05^{b}	127.05±1.56^{a}
TC	3.38±0.05^{c}	0.07±0.00^{b}	0.78±0.01^{b}	2.31±0.02^{b}	36.77±2.53^{a}	64.75±2.97^{a}	0.06±0.00^{b}	7.26±0.06^{b}	28.88±2.03^{c}

Means ± standard error followed by the same letter within a column are not significantly different according to Tukey's HSD test at $p \leq 0.05$.*Maximum ceiling values of heavy metals for agricultural land application according to New South Wales EPA, (2000). ^{a}N = 4 in the substrate analysis. ^{a}N = 4 in the substrate analysis. Key: TC = Tea compost, CP= Coco Peat, FS =Forest soil, BS= solarized for 2 months and stored for 10 months.

substrates in Pb (45 mg kg^{-1}), As (5.7 mg kg^{-1}), Se (16 mg kg^{-1}), Cd (0.4 mg kg^{-1}) and Ni (12 mg kg^{-1}) which was nevertheless not significantly ($p <$ 0.05) different from that of biosolids (16.3 mg kg^{-1}). Biosolids registered significantly ($p < 0.05$) higher Hg (0.33mg kg^{-1}), As (5.9mg kg^{-1}), Cr (31.1mg kg^{-1}), than the other substrates. On the other hand TC and CP recorded significantly ($p <$ 0.05) higher contents of Cr (36mg kg-1) and Cu (64mg kg-1) respectively (Table 2).

Substrate analysis

Forest soil was mixed with BS at different rates (V/V) and compared with FS, TC and CP as controls, to get the best BS combination with significantly higher macro- and micro-nutrient composition (Tables 3 and 4). Forest soil was significantly higher in Mg (130 mg kg^{-1}) and C:N ratio of 21. Biosolids at 30% was significantly higher in most of the elements analyzed: ToN (13 mg g^{-1}), ToP (101 mg kg^{-1}), K (428 mg kg^{-1}), Mg (119 mg kg^{-1}) and ToC (114 mg g^{-1}). Both BS at 10 and 20% were higher in K, Mg and C, but not ToN and ToP. Biosolids at 40% was significantly higher in K (422 mg kg^{-1}), ToC (122 mg g^{-1}). On the other hand BS at 50 and 60% were significantly higher in Na 350 mg kg^{-1} and 376 mg kg^{-1}, respectively. In comparison, TC (commercial substrate) was significantly higher in ToN (16 mg g^{-1}) and ToP (116 mg kg^{-1}) but was not significantly different from BS at 30%. Both commercial substrates TC and CP were significantly ($p < 0.05$) higher in Ca (44 mg kg^{-1}) and (39 mg kg^{-1}) and Mg (127 mg kg^{-1}) and (115 mg kg^{-1}). Mg content in both TC and CP was not significantly different from those of FS and BS at 10 to 30%. Total organic carbon was significantly higher in TC but not different from that of BS at 30%. Potassium and Mg were significantly higher in BS at 10 to 30%. Mg content significantly reduced in BS at 40 to 60%, as Na content became significantly ($p < 0.05$) higher in the substrate. Manganese level was significantly ($p <$

Table 3. Macro-element characteristics of the substrate's mixtures (BS: FS).

Substrates (v/v)	ToN (mg g^{-1})	ToP (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	C (mg g^{-1})	C:N
Recommended ^{x}	**0.1^x**	**70^x**	**700^x**	**1000^x**	**700^x**	**-**	**-**	**-**
FS	4.3±0.8^e	69.1±8.8^e	132.5±3.3^e	21.9±1.5^b	131.1±9.7^a	62.9±5.6^f	91.7±9.4^c	21.3±1.6^a
BS 10%	5.9±0.9^{de}	83.0±9.7^{bcde}	412.3±1.9^{ab}	24.0±2.9^b	126.1±3.9^{ab}	254.8±9.1^c	115.0±3.6^{ab}	19.7±2.7^{ab}
BS 20%	7.4±0.3^{cde}	90.3±6.9^{bcd}	419.9±14.0^{ab}	22.8±1.8^b	117.7±7.7^{ab}	242.1±16.1^c	114.4±5.5^{ab}	15.4±1.2^{bc}
BS 30%	12.9±1.4^{ab}	101.0±2.8^{ab}	427.8±6.2^a	29.5±1.7^b	119.1±3.5^{ab}	252.8±8.2^c	122.1±6.4^a	9.6±1.4^{dc}
BS 40%	9.6±0.6^{bcd}	95.9±1.7^{abc}	422.4±5.7^{ab}	27.0±5.7^b	113.8±7.3^b	343.3±13.2^b	122.0±3.5^a	12.7±0.6^{cde}
BS 50%	8.9±1.5^{bcd}	79.3±6.8^{cde}	403.7±7.8^b	28.5±1.6^b	47.7±3.0^c	349.8±7.7^{ab}	127.9±5.6^a	14.7±2.^{8bcd}
(BS) 60%	10.5±1.8^{bc}	70.3±4.4^{de}	403.5±15.5^b	27.5±2.0^b	37.2±2.8^c	376.3±12.1^a	129.6±5.7^a	12.5±1.7^{cde}
Tea C (TC)	16.3±3.0^a	116.1±11.5^a	369.6±7.3^c	43.5±5.0^a	126.6±5.8^{ab}	114.8±3.4^e	120.5±7.9^a	7.6±1.4^e
C P	9.2±0.9^{bcd}	33.8±2.0^f	344.1±2.5^d	38.5±5.9^a	114.6±7.9^{ab}	164.4±7.0^d	99.6±5.8^{bc}	10.8±1.1^{cde}

Means ± standard error in a column followed by letter are not significantly different according to Tukey's HSD test ($p<0.05$).^{X} Recommended rates for plant nutrient content in tomato.
Source: Sainju et al. (2003).

Table 4. Micro-element characteristics of the substrates.

Substrates (v/v)	Mn (mg kg^{-1}) (20)^y	Fe(mg kg^{-1})	Zn(mg kg^{-1})
Forest soil (control)	69.6±8.0^c	27.0±1.9^f	4.7±0.3^d
Biosolid 10%	530.4±13.9^a	2490.0±29.3^a	47.4±1.2^a
Biosolid 20%	524.8±7.4^a	2473.9±8.8^a	44.0±2.3^a
Biosolid 30%	539.4±31.9^a	2479.1±18.6^a	44.0±1.1^a
Biosolid 40%	553.9±24.7^a	2471.5±34.6^a	45.9±0.9^a
Biosolid 50%	551.9±11.3^a	1184.1±4.9^b	24.4±2.1^b
Biosolid 60%	544.8±18.3^a	852.5±6.5^c	25.4±0.9^b
Tea compost	167.0±18.5^b	207.4±6.7^d	21.9±2.6^b
Coco peat	29.8±1.2^c	114.1±9.9^e	16.4±2.2^c

Means ± standard error in a column followed by letter are not significantly different according to Tukey's HSD test ($p<0.05$). ^{y} Recommended value of nutrient in substrate for tomato production.
Source: Sainju et al. (2003).

Zn content were significantly ($p < 0.05$) higher in BS from 10 to 40% (Table 4).

Heavy metals

Copper (Cu) content varied from 4.4 to 14 mg kg^{-1} and was significantly ($p < 0.05$) higher in all the substrates except FS and CP (Table 5). Likewise, Cd content varied from 0.0023 to 0.0128 mg kg^{-1} and was significantly ($p < 0.05$) higher in all the substrates except FS. Lead (Pb) content varied from 2.1 to 109.6 mg kg^{-1} but was significantly ($p < 0.05$) higher in FS and lower in the rest of the substrates.

Physico-chemical properties of the substrates

Physical properties of the substrates showed

Table 5. Heavy metal characteristics of the substrates**.**

Substrates (v/v)	Cu (mg kg^{-1}) (100)^{z}	Cd (mg kg^{-1}) (1)	Pb (mg kg^{-1}) (150)
Forest soil (control)	4.4±0.5^{b}	0.0023±0.1^{b}	109.6±9.0^{a}
Biosolid 10%	12.2±0.6^{a}	0.0128±0.8^{a}	2.8±0.9^{c}
Biosolid 20%	12.7±1.0^{a}	0.0115±0.4^{a}	2.1±0.4^{c}
Biosolid 30%	10.3±0.9^{a}	0.0127±0.6^{a}	5.1±0.4^{c}
Biosolid 40%	12.7±0.9^{a}	0.0122±0.7^{a}	3.1±0.5^{c}
Biosolid 50%	13.1±0.9^{a}	0.0122±0.4^{a}	6.0±0.8^{c}
Biosolid 60%	13.3±0.9^{a}	0.0122±0.7^{a}	2.5±0.5^{c}
Tea compost (Control)	14.0±0.2^{a}	0.0122±0.7^{a}	20.1±4.1^{b}
Coco peat (Control)	6.5±0.3^{b}	0.0121±1.6^{a}	4.3±1.0^{c}

Means ± standard error in a column followed by the same letter are not significantly different according to Tukey's HSD test *(p<0.05).* *Maximum ceiling values of heavy metals for agricultural land application.
Source: NSW EPA (2000).

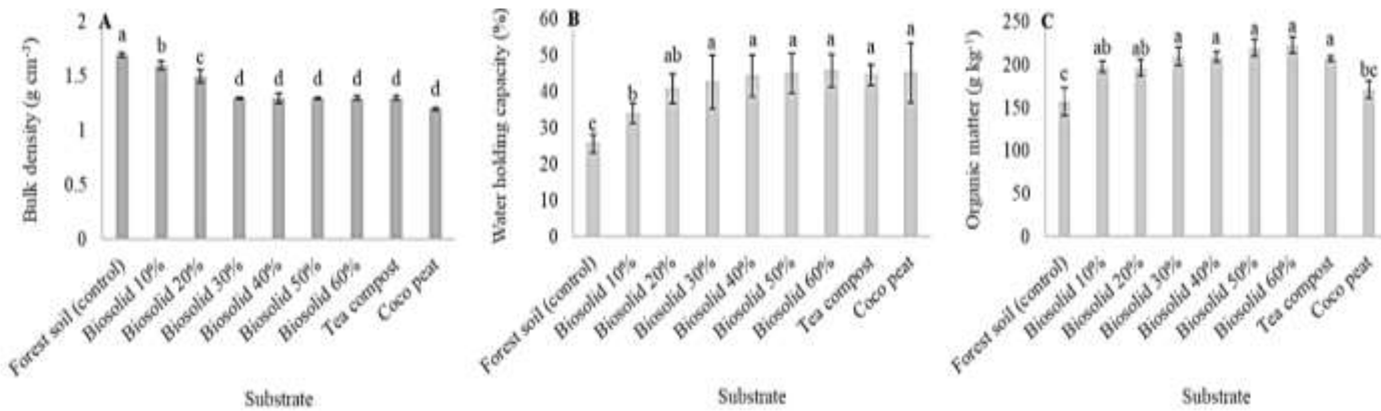

Figure 3. A- Bulk density, B - Water holding capacity and C- Organic matter characteristics of different growing media. Means ± standard deviation followed by the same letter are not significantly different according to Tukey's HSD test *(p<0.05).*

significant differences on bulk density (BD), water holding capacity and organic matter contents (Figure 3). Bulk density varied from 1.2 to 1.7 g cm^{-3} and was significantly ($p < 0.05$) higher in FS, followed by BS at 10% and lower in the rest of the substrate. Water holding capacity varied from 26 to 46% but was significantly ($p < 0.05$) higher in the all substrates except forest FS and BS at 10%. Organic matter content was higher in the all the media except FC and CP. Electrical conductivity varied from 2.6 to 5.4 µSm^{-1} but registered higher in BS 40 to 60%. Forest soil registered a higher pH but was not significantly ($p < 0.05$) different from the two commercial substrates TV and CP. However, BS recorded significantly ($p < 0.05$) lower pH than the other substrates with a reducing trend from BS at 20 to 60% (Figure 4).

Biological properties of biosolids

Selected bacteria of economic importance: Salmonella sp., Escherichia coli and Staphylococcus were observed in the substrates (Table 6). Total viable counts (TVC) showed a rising trend from BS at 10 to 20% followed by a downward trend up to BS at 100%. The presence of microbes was evident in the substrates at different dilution levels. Forest soil recorded a TVC of 42×10-4; tea compost had moderate growth while coco peat had no growth (NG). For the colony forming units (CFU), FS and BS at 10 to 40% had too numerous to Count (TNTC); however above BS 40%, it was possible to numerate them at dilution level 10^{-7}. Regardless of the rates of BS tested, the targeted microbes *Salmonella* sp., *E. coli* and *Staphylococcus* were absent on the dry BS substrates tested. Further confirmation test done on the pond for the presence or absence of targeted microbes of the seven ponds indicated the presence of *Salmonella* sp*.* and *Staphylococcus* sp*.,* at various stages except in pond 6 and 7, which had dry BS sample (Table 7). However, *E. coli* persisted on pond 6 and 7.

DISCUSSION

The utilization of biosolids in agriculture has gained popularity as a source of waste disposal. Analysis of biosolids alone for the macro- and micro-elements in

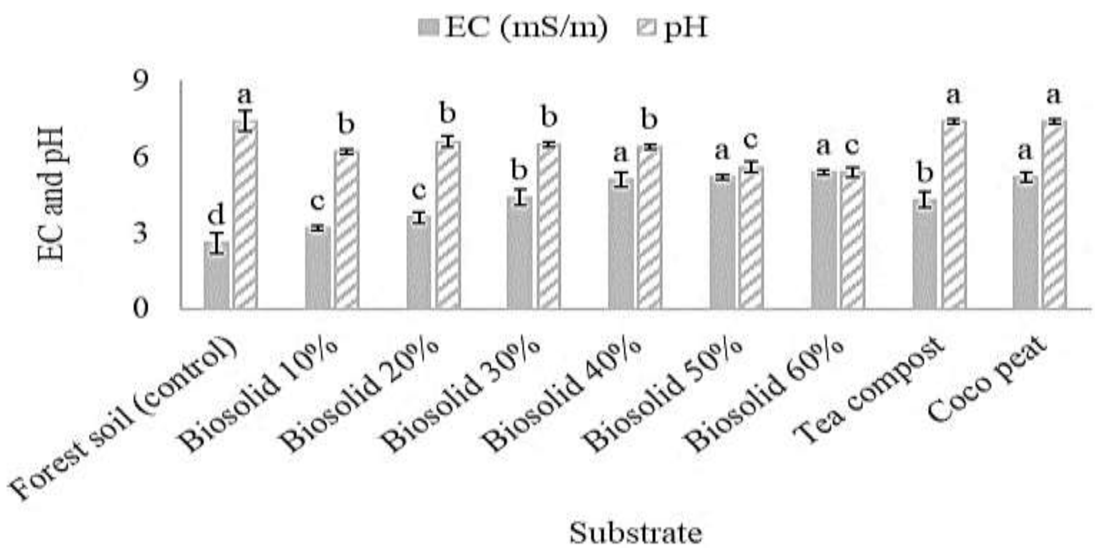

Figure 4. Electrical conductivity and pH on different type of substrates. Means ± standard deviation followed by the same letter are not significantly different according to Tukey's HSD test *($p<0.05$).*

Table 6. Numeration of *Salmonella* sp., *Escherichia coli* and *Staphylococcus* sp.in the substrates.

Substrates	TVC (PCA)	CFU (MAC)	*E. coli* (EMB)	*Salmonella sp.* (BPA)	*Staphylococcus sp.* (BPA)
Forest soil (FS)	42×10^{-4}	TNTC	-ve	-ve	-ve
Biosolids 10%	50×10^{-7}	TNTC	-ve	-ve	-ve
Biosolids 20%	96×10^{-7}	TNTC	-ve	-ve	-ve
Biosolids 30%	72×10^{-7}	TNTC	-ve	-ve	-ve
Biosolids 40%	52×10^{-7}	TNTC	-ve	-ve	-ve
Biosolids 50%	22×10^{-7}	13×10^{-7}	-ve	-ve	-ve
Biosolids 60%	11×10^{-7}	6.5×10^{-7}	-ve	-ve	-ve
Biosolids 100%	5×10^{-7}	6.5×10^{-7}	-ve	-ve	-ve
Tea compost	58×10^{-7}	6.5×10^{-7}	-ve	-ve	-ve
Coco peat	N/G	N/G	-ve	-ve	-ve

TVC- Total Viable Count, CFU- Colony-Forming Units, PCA- Plate Count Agar, EMB- Eosin Methylene Blue (*E. coli*), MacConkey Agar, BPA- Bairvd Parker Agar (*Staphylococcus sp.*), BPA- Bairvd Parker Agar (*Salmonella sp.*). TNTC- Too Numerous to Count, VFC- Very Few Colonies, N/G- No Growth, -ve= absent, +ve = Present, ++ve =highly present, +++ve - Very highly present.

Table 7. Numeration of bacterial total viable counts (TVC) and colony forming units (CFU) in Egerton University ponds.

Pond type	TVC (PCA)	CFU (MAC)	*E. coli (EMB)*	*Salmonella* sp.	*Staphylococcus* sp.
POND 1	TNTC	TNTC	+++ve	++++ve	+++ve
POND 2	TNTC	TNTC	+++ve	+++ve	+++ve
POND 3	TNTC	TNTC	+++ve	+++ve	+++ve
POND 4	TNTC	TNTC	+++ve	+++ve	+++ve
POND 5	TNTC	TNTC	+++ve	+++ve	+ve
POND 6	TNTC	TNTC	++ve	.ve	-ve
POND 7	TNTC	VFC	+ve	-ve	-ve

POND 1- Effluent, POND 2 -Second pond (water), POND 3-Third pond (water), POND 4- Fourth pond (water), POND 5- Fifth pond (water), POND 6 -Sixth pond (water), POND 7-Seventh pond (dewatered biosolid). TVC- Total Viable Count, CFU- Colony Forming Units, PCA- Plate Count Agar, EMB- Eosin Methylene Blue (*E. coli*), MacConkey Agar (MAC), BPA- Bairvd Parker Agar (*Staphylococcus sp.*), BPA- Bairvd Parker Agar (*Salmonella sp.*). TNTC- Too Numerous to Count, VFC- Very Few Colonies, N/G- No Growth, -ve= absent, +ve = Present, ++ve =highly present, +++ ve – Very highly present.

comparison to other substrates revealed the significant presence of organic nitrogen (N), molybdenum (Mo) and to lesser extent, phosphorus (P), potassium (K) calcium (Ca), sulphur (S), magnesium (Mg), iron (Fe), manganese (Mn) and boron (B). On the other hand, forest soil (FS) as a natural substrate significantly had higher in Fe, Mn and B. This study suggests that dewatered biosolids (BS) would be a better source for slow release nitrogen and molybdenum. However, if BS is combined with FS their ability as substrate would possibly be enhanced to provide five elements fore-mention. Total organic carbon was significantly higher and has a big role in plants, fostering healthier and more productive growth of the plants through photosynthesis process. Amending soil with organic carbon not only facilitates healthier plant life, but also helps the substrate to drain well with enhanced bulk density, prevents water pollution and besides, it is beneficial to useful microbes, insects and eliminates the need for using synthetic fertilizers. Additionally, availability of C and N in organic form, in BS has benefits to crop since the plant nutrients are released over entire period of crop production. The organic nitrogen present in BS is an essential macro nutrient for the synthesis of amino acids, the building blocks of proteins, and also a major part of the chlorophyll molecule necessary for photosynthesis. Even though BS did not show significance in ToP, K and Mn compared to other substrates tested, they were within maximum recommended values of plant nutrient in the substrate according to Sainju et al. (2003). Among the macro-elements of importance in plant nutrition, K however, was critically lower in the BS. This was probably leached out in the process of BS formation since it is in ionic form and therefore deficient. Pakhnenkoa et al. (2009) earlier reported the same on the availability of K in biosolids used in agriculture. These results are in agreement with report by Kirchmann et al. (2016) who also reported on organic form of N and P with only 10% of each that may be available. In another study, Paz-Ferreiro et al. (2018) reported similar findings, that N availability is partially controlled by type of treatment process sludge undergo and that agricultural value of biosolids will also depend on the mineralization rate of the organic N pool. In terms of its functions in plant, significant level of molybdenum (Mo) in BS observed in this study is indicative of the potential of the substrate in providing this trace element for crop development. Molybdenum is a necessary component of two major enzymes in plants, nitrate reductase and nitrogenase, which are required for normal assimilation of N in plant function (Silva and Uchida, 2000). The result also showed forest soil (FS) as a better source of Iron, Manganese and Boron. These are important trace elements essential in plant nutrition. When compared to maximum recommended values of plant nutrient in the soil or substrate suggested by Sainju et al. (2003), these elements are found to be sufficient for crop productivity in the three substrates tested: FS, BS and TC (Commercial substrate). Considering the significant presence of trace elements, Fe, Mn and B in FS indicated the potential of the substrate in combination with BS. This combination would possibly contain Fe, Mn, B and Mo which are equally essential in plant functions and crop production. Even though these elements are very important in plant physiology and development, they may not be found at instant in synthetic fertilizer (Zamann et al., 2002), unless supplemented by other means to crops. Boron was significantly available in FS. Its role in the synthesis of one of the bases for RNA formation particularly in cellular activities has been reported by Silva and Uchida (2000).

Combination of BS with FS to form substrates at different rates in the range of 0 to 60% revealed a new trend in plant nutrient availability, showing complex variations of important plant nutrients in the substrates. Significantly higher Mg observed in FS in a reducing trend to BS 60% was an indication of FS being the source and the donor in the substrate complex mixes. This study shows significant presence of Mg in FS and BS rates from 10 to 30% of the substrate. The reducing trend of Mg in the substrate mixes with the declining of the pH of the substrate observed indicated that the element is pH dependent. Studies have shown that Mg as reported by Sainju et al. (2003). This leaves a deficit of 580 mg kg^{-1} to be supplemented or the liming of the substrate to pH can make the element available. Magnesium tends to become unavailable as pH decreases. Calcium followed similar trend reducing in the substrate with increasing BS rates and this may allude to the pH as a factor in the substrate. Similarly, increase of EC with Na in the substrate probably was also a factor influencing the presence of macro element like Mg. Magnesium is normally available within a pH range of 6.0 to 8.5. As the rate of BS increased from 40% and above, the pH of the substrate was reducing and this was a limiting factor that probably caused significant reduction of Mg and Ca. The results of this study is consistent with Sullivan et al. (2015) who reported that depending on the process of producing BS, the pH may be acidic to alkaline, and therefore adjusting the pH by liming the substrate would be necessary to get the right substrate with biosolids. This is also in agreement with Ingram et al. (2016) on the effect of soil pH and nutrient availability. In this work, decrease in Mg with increased EC observed is associated with higher Na and subsequently higher salinity in BS at 50 and 60%. This is in line with observation made by Mtshali et al. (2014) that high Na concentration is associated with elevated EC of soils amended with sludge, with or without lime. The deficiency of K^+ which may necessitate the supplement of the elements in the substrate to support plant growth has also been reported by Paz-Ferreiro et al. (2018).

The C:N ratio is normally used as a growing media index to represent its stability, as it has been known that a C:N ratio of 15 permits plants N uptake without leaching as nitrate, and any C:N ratios above 15 represent values within which nitrogen is immobilized according to Dresboll and Magid (2006). The results indicates that FS without BS had significantly higher C:N ration of above 15 at rates of BS 10 and 20% and this was a sign of better chemical property of the substrate for crop production. From BS 30% and below, the C:N ration was within require range for nutrients availability. The Influence of BS in the substrate reduced the C:N ratio, which was a positive effect on nutritional quality. This concurred with Rawat et al. (2013) observation on BS waste as a source of high mineral elements, important in crop production.

The significance in BS combination with FS (BS 10 to 60%) observed was indicative of a better substrate that provides the three Mn, Fe and Zn. Plants use the element in Mn^{2+} or Mn^{3+} form and primarily functions as part of the plant enzyme system, activating several metabolic functions (Silva and Uchida, 2000). Like Manganese trace element, Fe is essential in the heme enzyme system in plant metabolism (photosynthesis and respiration). Iron is also part of protein ferredoxin and is required in nitrate and sulfate reductions, essential in the synthesis and maintenance of chlorophyll in plants and strongly associated with protein metabolism. All these physiological function may contribute to the crop growth and development, hence higher yield. The results on substrate analysis also revealed metals (Zn, Cu, Mo, and Ni), which are essential for plant growth and crop development available in both FS and BS combined, especially at BS 30%. On the elements essential for plant growth, Copper was significantly high in BS 30% and TC (commercial substrate), indicating the potential of substrate BS in plant growth at the same rate. In particular, Cu is essential in several plant enzyme systems involved in photosynthesis and is part of the chloroplast protein plastocyanin, which forms part of the electron transport chain and also plays a role in the synthesis stability of chlorophyll and other plant pigments (Sainju et al., 2003).

Heavy metal concentration indicated that, even though Cu and Cd were high in BS waste, they were found to be below the permissible limits of Standards (NSW EPA, (2000). Thus, the BS in this study at rate of BS 30% and below were observed to be suitable for use in agricultural purposes. This is in line with Miezah et al. (2015) who reported that availability of heavy metals is less in a more compost form of sludge as it has more humic acid, thus binds more metals and decreases their availability. Similarly, this work is in agreement with recommendation by Naveen et al. (2017) on quality of good biosolids; fully decomposed, and low in heavy metal and salt contents. The present study revealed the concentration of heavy metals below EPA 2000 standards. Further, these results are also in agreement with the work of Mohammad et al. (2011), where heavy metal concentration did not exceed the permissible level, on wastewater (which forms biosolids) for tomato production. However, the concentration of heavy metals in BS normally depends on the source and processes involved on production as reported by Paz-Ferreiro et al. (2018), and since BS are produced from different source, they are unique and comprehensive test is necessary before use in crop production systems.

Trend in EC level with increase in BS rates was observed and this was an indicator of higher salt concentration in the substrate, where Na was higher in BS 40 to 60%. This also confirms a report by Mtshali et al. (2014) that BS normally have higher Na concentration. EC is an important parameter to determine the substrate quality as high salt concentration can inhibit the plant growth (Fathi et al., 2014). Higher EC would result in reverse osmosis which would be detrimental to crop physiology and development leading to salt stress. On pH, a study has shown that sewage sludge may vary between slightly acidic to neutral and alkaline ranges depending on the degree of treatment and application of sludge conditioners (Yilmaz, and Temizgul, 2012). Moreno et al. (1997) earlier reported, confirms our result that application of BS may reduce the pH of soils due to humic acid release and may also increase the EC of soils or substrate. In line with our study, Youssef and Eissa (2017) reported that soil pH was decreased due to the production of organic acids during the mineralization of organic manure. However, acidic soils (Forest) have been observed to increase in pH following sludge amendment as earlier reported by Parkpain et al. (2000) and Wong et al. (2001). In addition, pH of soils or substrate may increase due to the exchangeable Ca and other cations present in sewage sludge such as Na (Tsadilas et al., 1995).

The physical properties of the substrate are the most important parameters related to plant performance. In this work, combination of FS and BS at 20 to 60% played a vital part in the structure adjustment, decrease in the BD, increased water holding capacity and soil organic matter. Bulk density (BD) was enhanced by FS as indicated in these results, while water holding capacity and organic matter contents were influenced by BS presence at any level, indicating that the essence of organic matter in the BS. Reduction observed in BD did not only make the substrate lighter, but also creates large pores that play an important role in roots growth, gas and water to penetrate into the substrate, and in any growing media, aeration is positively related to air-filled porosity (Wallah, 2007). As have been reported by other authors in various studies, BS has higher level organic matter of which 60 - 90% are biodegradable (Mami and Peyvast, 2010). This study has demonstrated that water holding capacity is an important parameter in a substrate because it dictates how frequent crops can be irrigated in a given period. Similarly, the increasing trend of water holding capacity of the substrate with increased organic matter indicates the important property of organic matter in water retention for plant use in favour of biosolids application. In line with this study, Pascual et al. (2018) reported that easily available water in a substrate should range from 20 to 30%. These results were found to be with the ideal water holding capacity of 40–65% that corresponds with water retention of 25–30% (Abad et al., 2001). These results also concur with Naveen et al. (2017) that BS increases the buffering capacity of soil and also improve water holding capacity of soil.

On the biological properties of the substrate, main focus was on the presence and or absence of selected bacteria of economic importance; *Salmonella* sp., *E. coli* and *Staphylococcus* sp. The presence of microbes in the soil or substrate depends on the physic-chemical condition of the substrates. These results confirm that pH may dictate the presence and absence of microbes. There are microbes that may survive in acidic soils while others are neutral. The other factor that determines the population, survival and mobilization would be moisture and EC of the media. In this study, these factors are proposed to be determinants of the presence and absence of *Salmonella* sp., *E. coli* and *Staphylococcus* sp. The results of this work can be explained from the soluble salt concentration in the BS substrates rates. At BS 50% and above, the EC was at its highest in the substrate, which was also reflected by higher Na. This was an indication of increased osmotic pressure (salt stress), that would possibly make the substrate draw water out of the cells of microorganism, hence death in the media or reduced chance of survival. This observation is in agreement with Andronov et al. (2012), that salt stress can reduce microbial activities, biomass and community structures in soil or substrate as in this case. Results are also in agreement with the observation of Yan et al. (2015) on the reduction of soil microbes with the influence of soluble salts in the substrate. Overall, making a better substrate for crop production requires balancing of the biosolids amount applied in soil mixes.

On enumeration of specific bacteria, *E. coli* was present and persisted even in the dewatered samples in 6^{th} and 7^{th} pond. This observation was in agreement with Arthurson (2008) findings on persistence of *E. coli*, which was the most resistant pathogen in whole process of sludge treatment. The reduction of *Salmonella* sp., *E. coli* and *Staphylococcus* sp. may indicate that in the process of solarisation, storage and further decomposition was a possible elimination process for the targeted pathogens including the stubborn *E. coli* which persisted in the 7^{th} pond. Our general observation in this study was also in line with an earlier report by De´portes et al. (1998), that there was a decrease in faecal contamination indicators and disappearance of faecal pathogens when BS were stored before use over a period of one year.

Conclusion

The present study evaluated the physico-chemical and biological characteristics of biosolids to determine its suitability for crop production as a substrate. The results indicated that application of biosolids increases organic matter and may possibly reduce mineral fertilizers with addition of elements such as potassium, magnesium and calcium. However, addition of biosolids beyond 30% may increase soil EC and decrease pH. Biosolids contain efficient amount of organic matter, ToN, which can improve soil physico-chemical and biological properties of the substrate for crop production, while heavy metals were within the allowable range. This study recommends use of BS as a substrate mixture with soil (forest soil), to the limit of 30% (v/v) and below for crop production. Liming may be a good option for adjusting the pH of biosolids substrate. It is thus recommended that more studies be conducted on various test crops as potted substrate and comprehensive analysis be carried out on plant response as well as biosafety of these agricultural food products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Behavior of nursing *Apis mellifera* after application of entomopathogenic fungi to control *Varroa destructor*

Jaime E. Araya[1*], Josefina Mas[2] and Francisco Zuazúa[2]

[1] Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile.
[2]Escuela de Agronomía, Facultad de Ciencias, Universidad Mayor, Camino La Pirámide 5750, Huechuraba, Santiago, Chile.

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To study the possible use of fungi to control *Varroa destructor* Anderson & Trueman, a parasitic mite of *Apis mellifera* L., without effects on its pollinator's behavior, the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) were applied at 30ºC in the dark, at 10^8 conidia/mL, a concentration known previously for its pathogenicity on *V. destructor*. Both fungi did not affect diverse parameters of the normal behavior of nursing honey bees in the laboratory, including the duration of walking periods, antennae tapping, resting, feeding, drinking, communication with the antennae (touching other bee), and grooming, as observed using a PC with the Observer® software. These results indicate that it would be possible to use both fungi against the mite, without any side effects on the bee. However, as pathogenicity varies greatly in diverse strains, further studies are recommended.

Key words: *Beauveria bassiana*, hives, honey bees, *Metarhizium anisopliae*, observer, varroasis.

INTRODUCTION

Severe infestation by *Varroa destructor*, the main parasitic mite of the honey bee can lead to reduction in bee population, survival and overall hive productivity, and if not controlled, leads to the collapse of the colony (Sima, 2013). This mite feeds the haemolymph and debilitates the larvae, pupae, and adults (Sammataro et al., 2000; Chandler et al., 2001; Kanga et al., 2003; Márquez et al., 2003), which also emerge deformed (De Jong, 1997; Shaw et al., 2002). Although the bees remove the infested larvae and pupae, the hive weakens, and as the mite density increases for the larvae available, *V. destructor* can affect severely and rapidly a colony, and often eliminate it in the fall (Meikle et al., 2007). *Varroa* prefers young to mature bees, probably these last have a greater concentration of geraniol, that strongly repels the mite (Hoppe and Ritter, 1989), and commonly there are females of the parasite on foraging bees, that disperse them (phoresis), while serving as short term hosts (Meikle et al., 2007). The mite bores soft parts between the abdominal sclerites or behind the neck of the bee, and feeds its haemolymph. When arriving to an active colony, the parasite gets off and looks for rearing cells with 3rd stage larvae (Meikle et al., 2007), it enters them before they are sealed and hides in them (Donzé and Guerin, 1997), it sticks to the larval abdomen while this weaves its cocoon, and begin to feed from it when the prepupa develops (Meikle et al., 2007).

*Corresponding author. E-mail: jaimearaya@yahoo.com.

The traditional control of the mite with chemicals leaves residues that contaminate the honey and wax (Shaw et al., 2002; Kanga et al., 2003, Sima, 2013), it achieves insufficient results, and their repeated use has developed resistance (Gerson et al., 1991; Milani, 1995; Colin et al., 1997; Neira et al., 2003). Plant oils are also used (Sammataro et al., 1998; Menn, 1999; Chandler et al., 2001), with varied efficacy and a narrow range of selective doses (Kanga et al., 2003). They are also complex compounds that may cause undesired effects on honeybees and beekeepers (Schaller and Korting, 1995), and could also contaminate the hive products (Sammataro et al., 2000). Other control possibility is the use of pathogenic fungi that do not affect *A. mellifera*. Among them stand *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, species that act on a wide range of arthropods (Poinar and Thomas, 1984; Khetan, 2001), including *V. destructor* (Kanga et al., 2002, 2003; Sterk et al., 2002; Meikle et al., 2007). Although the hymenopterans can be susceptible, the social species have behavior mechanisms that avoid infection or minimize their effects (Lord, 2000; Aumeier, 2001). In *A. mellifera*, the nursing bees remove dead and weak juveniles (Büchler et al., 1992 (1993); Boecking et al., 1993], a very variable hygienic conduct (Büchler, 1994) that reduces varroasis in untreated hives, that require then less chemical treatments (Spivak, 1996).

According to Hamiduzzaman et al. (2012), entomopathogenic fungi could reduce varroa mite damage to honey bee brood by both infecting the parasite and preventing varroa-associated suppression of honey bee immunity.

In laboratory bioassays by Ramírez and Gerding (no publication date), *M. anisopliae* strain Qu-M845 has obtained 98% control of *Varroa*, and 67% in field trials when applied in the fall on and between the combs. Sprays in the spring increased 50% the fall of the mite from the level in untreated hives. Further, two *B. bassiana* isolates caused varroa mortality (20 to 30%) after 6 days of having been treated (Rodríguez et al., 2009).

Meikle et al. (2007) found that *B. bassiana* had no effect on colony weight, adult bee weight or honey production.

With the hypothesis that the use of isolates of *B. bassiana* and *M. anisopliae* does not affect the normal conduct of nursing bees, a study was done to compare the conduct of healthy nursing bees with others exposed to isolates of both fungi, in order to evaluate their possible use for control of *V. destructor*.

MATERIALS AND METHODS

In this study, developed in the spring of 2006 in the Technological Centre of Biological Control of INIA in Quilamapu, Chillán, Chile, 1-day old healthy bees were used, selected at sunset from healthy hives in glass cages, and incubated a night at 35ºC to stimulate emergence from the cells. From these bees, ten nursing workers were selected at random, that were set in a glass cage with an 8 × 8 cm comb piece with larvae, cut with a 90% alcohol disinfected knife to avoid rotting dead larvae at the borders.

To obtain the inoculum, the fungi *B. bassiana* strain Qu-B303 and *M. anisopliae* strain Qu-M845, from the INIA >800 isolate collection at Quilamapu, Chillán, Chile (Rodríguez and Gerding, 2005), were inoculated onto Petri dishes with PDA and incubated at 25ºC until sporulation. The conidia were collected from the surface of these dishes, and suspensions were made on sterile distilled water with 1% Tween 80 moisturizer at 0.1%, and homogenized with a magnetic stirrer. The concentration of conidia in each solution, prepared in 10 mL solution of 30% honey in distilled water, was verified in 10^8 conidia mL^{-1}, that according to Kanga et al. (2003) kill *V. destructor*, using a BOECO Neubauer counting chamber (Neubauer, Germany).

To evaluate the effect of the fungi on the nursing bees, 2 mL were poured of each spore solution on chicken water providers with small sponge pieces to allow drinking and avoid the bees from drowning. The bees were provided also with 3 cm diameter 1:1 pollen granules with honey to feed them. As control for comparisons bees unexposed to the fungi were used. Ten nursing bees were used for each treatment in 23.5 x 19.5 x 18.5 cm glass cages with red light and the corresponding drinking water provider, and were set in the dark at 30ºC, in a completely random experiment design, with 5 replicates. At 18 h from the beginning of the bioassay a bee was selected at random from each cage for observation under red light (not visible for the bees) during 3 min, during which conduct parameters were registered using the Observer® software, that allows to collect, analyze, and present observations measurements of behavior through activities, postures, gestures, expressions, movements, and interactions, both in humans and animals (Suazo et al., 2003). The conduct parameters evaluated were the time spent walking, antennal tapping, feeding, drinking, resting, communication (repeated movements of the antennae in front of another bee), and grooming. To facilitate observations, each activity was registered in a PC with the Observer program using separate keys. The records were made of 15 bees per treatment during 9 days, period which they behave as nursing bees (Lesser, 2004). The results obtained graphs for each activity during the bioassay at 18, 42, 66, 90, 114, 138, 162, 186, and 192 h exposure to the treatments were normalized and subjected to analysis of Variance and Dunnett tests.

RESULTS

The results of exposing the nursing bees to *M. anisopliae* are presented in Figure 1, where the *V. destructor* mites killed by the fungus are easily identified.

The results obtained when registering each conduct parameter are presented in Table 1, where no significant differences occurred between the treatments in all parameters, thus indicating just small variation throughout the exposure bioassay, but importantly, no effects on nursing honey bees.

Walking

The results presented in Table 1 varied numerically from 25 to 175 s.

Antennal tapping

The results in Table 1 neither presented significant differences between the control and the treatments with

Figure 1. *Varroa destructor* (shiny bright dark red center spot) infected and killed by *Metarhizium anisopliae*.

both fungi throughout the bioassay. Antennal tapping was very variable (0-33 s), but insufficient to cause statistical differences.

Duration of feeding

As in the previous parameters, the results of the feeding period in Table 1 were not significantly different between the control and the exposure treatments with both fungi, but presented wide numerical variations (0-99 sec).

Duration of drinking

Again, the results of the time spent drinking in Table 1 varied only numerically between the control and the exposure treatments with both fungi (0-24 s).

Duration of resting periods

The same as for the displacements and antennal tapping, the results of the resting time in Table 1 were not significantly different but presented wide numerical variation differences (0-66 s) between the control and the exposure treatments with both fungi.

Duration of antennal communication activities (touching)

The mean registered values in Table 1 were also not significantly different between the control and the exposure treatments with both fungi, although they varied numerically from 0 to 48 s.

Duration of grooming

These activities, estimated as very important for the sanitary condition of the hive (Büchler et al., 1992 (1993); Boecking et al., 1993), were again not significantly different between the control and the exposure treatments with both fungi, but varied greatly during our bioassay (0-55 s).

Despite the lack of statistical differences in all conduct parameter between the treatments, the P values of the results are presented at 18, 96 and 192 h exposure in Table 2.

DISCUSSION

The absence of significant differences in the conduct parameters of nursing bees exposed to both fungi indicate that it is possible to use *B. bassiana* or *M. anisopliae* in treatments against *V. jacobsoni*. The honeybee has a series of behavior characteristics that protect it of fungal infections, such as a hard and non-permeable cuticle, gut pH and peripheral membrane, that together with the tracheal system form an effective mechanical and physiological barrier (Gliñski and Buczek, 2003). The honeybee is characterized by the rapid detection and removal of sick and dead individuals, and the detailed cleansing of the hive by nursing workers (Flores et al., 1998). For example, this behavior is important in the resistance of the bees chalkbrood an aspergillosis, caused by the fungi

Table 1. Mean duration (s) of conduct parameters of nursing honeybees exposed to *Beauveria bassiana* (*Bb*) or *Metarhizium anisopliae* (*Ma*) and a water control for 18 up to 198 h.

Exposure (h)	Bb	Ma	Water	Bb	Ma	Water	Bb	Ma	Water	Bb	Ma	Water	Bb	Ma	Water	Bb	Ma	Water	Bb	Ma	Water
	Walking			Antennal tapping			Feeding			Drinking			Resting			Antennal touching			Grooming		
18	52	45	37	23	33	33	42	19	24	0	0	0	5	14	13	26	8	24	13	22	21
30	95	71	84	4	18	14	31	27	19	0	0	0	3	32	3	11	13	25	25	10	19
42	73	122	94	8	2	22	13	18	0	2	0	0	2	2	2	19	35	4	55	0	44
54	75	143	111	3	17	7	24	0	0	0	0	0	5	18	48	32	4	48	12	5	49
66	130	146	160	6	18	8	13	0	0	3	3	0	6	22	0	9	6	12	12	2	0
78	125	134	175	3	3	2	24	0	0	24	0	0	11	3	24	13	12	7	2	3	7
90	115	168	115	1	1	3	15	16	25	2	0	0	6	9	27	14	5	15	21	0	3
102	160	122	99	16	0	2	5	24	16	0	0	0	3	8	23	4	9	31	1	8	30
114	113	130	133	0	2	4	50	23	16	2	3	6	0	0	4	2	18	12	3	0	0
126	117	128	135	0	3	0	37	52	5	3	8	7	0	24	0	13	3	9	1	34	13
138	112	55	152	1	12	6	67	16	0	0	0	0	31	6	0	0	10	10	0	0	0
150	103	133	160	4	10	0	23	68	55	8	0	0	0	22	0	38	0	12	23	3	12
162	25	71	73	28	0	0	38	82	78	0	0	0	60	0	17	3	17	3	10	0	3
174	44	111	72	0	4	1	99	76	76	8	0	0	12	0	6	3	0	11	16	0	11
186	46	149	172	1	1	0	53	24	0	4	2	9	66	8	0	0	0	1	6	0	0
198	98	107	99	0	12	2	24	24	24	0	0	0	12	7	12	11	13	30	24	2	28
Means	93.5	114.7	116.9	6.1	8.5	6.5	34.9	29.3	21	3.5	1	1.4	13.8	10.9	10.4	10.7	9.1	14.4	13.9	4.2	13.7

Ascosphaera apis (Maasen ex Clausen) Olive & Spiltoir, and *Aspergillus flavus* Link, respectively; the workers retire with their mandibles the mummified larvae and carry them out of the hive (Gliñski and Buczek, 2003).

Our results agree with Lord (2000), who pointed that although many hymenopterans are susceptible to *B. bassiana*, their behavior including mechanisms of grooming, hygiene care of the nest, secretion of antimicrobial compounds and temperature regulation, avoid or minimize infection by pathogens, which is in line with the results of Flores et al. (1998), and Ibrahim and Spivak (2006) for bacteria, fungi, and arthropods (like *V. destructor*). This mite is highly susceptible to *B. bassiana* and *M. anisopliae* at a concentration of 10^8 conidia/mL and temperature similar to that reached in the hives of *A. mellifera*. The fungi during their reproductive phase do not infect *V. destructor* into the operculate cells of *A. mellifera*, but this occurs when the mites emerge to keep feeding from a newly emerged bee, which is not affected by the microorganisms (Kanga et al., 2002). The death of the mite caused by the fungi is due to the mechanical destruction of its tissues, the loss of water, and the effect of toxins (Chandler et al., 2001).

Kanga et al. (2002) indicated that the effects of *B. bassiana* and *M. anisopliae* on honeybees and the environment of the hive require more investigation, because these fungi present a high potential as a biological control alternative against *V. destructor*, as they do not affect the normal behavior of the bees. In field studies with *B. bassiana* in southern France, Meikle et al. (2007) observed a significant increase in the percentage of infection on *V. destructor* that increased also in untreated hives suggesting certain conidial movement between colonies, probably derived from flying workers.

Sima (2013) determined in laboratory bioassays the pathogenicity of nine Canadian isolates of fungi of the

Table 2. P values of the results of nursing honeybee conduct parameters, obtained at 18, 96, and 192 h exposure to *Beauveria bassiana* or *Metarhizium anisopliae*.

Parameter	**Exposure (h)**		
	18	96	192
Beauveria bassiana			
Displacement (walking)	0.05	0.084	0.975
Antennal tapping	0.317	0.374	0.498
Resting time	0.367	0.408	0.966
Feeding time	0.283	0.487	0.666
Drinking time	0.463	0.233	0.458
Communication with the antennae	0.469	0.231	0.364
Grooming	0.531	0.279	0.315
Metarhizium anisopliae			
Displacement (walking)	0.952	0.906	0.476
Antennal tapping	0.952	0.51	0.845
Resting time	0.882	0.61	0.427
Feeding time	0.889	0.725	0.922
Drinking time	0.579	0.45	0.392
Communication with the antennae	0.358	0.52	0.312
Grooming	0.91	0.687	0.847

genera *Metarhizium*, *Beauveria*, and *Clonostachys* on the parasitic mite *V. destructor*. All isolates were pathogenic to *V. destructor* with *M. anisopliae* UAMH9198, *C. rosea* UAMH 9161 and *B. bassiana* GHA being the most pathogenic within each species, with LC_{50} values of 1.6×10^5, 9.6×10^6, and 5.4×10^6 conidia/mL, respectively. *M. anisopliae* and *B. bassiana* affected brood and adult honey bee survivorship and their immune responses; however, they affected the bees, with LC^{50s} of 3.70×10^6 and 2.62×10^5 conidia/mL, respectively. Combined treatments of fungi and thymol caused significantly greater mite mortality than single fungal treatments, which obtained ≤61% control levels.

Conclusion

Exposure to *B. bassiana* Qu-B303 or *M. anisopliae* Qu-M845 at 10 conidia/mL at 30ºC in darkness did not affect the normal behavior of nursing *A. mellifera* bees, thus the use of both fungi strains for control of *V. jacobsoni* should continue to be studied, including other best strains in further research.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The performance of selected commercial organic fertilizers on the growth and yield of bush beans in Central Uganda

Tugume Esau, Byalebeka John and Mwine Julius

Faculty of Agriculture, African Centre of Excellence in Agro-Ecology and Livelihood Systems (ACALISE), Uganda Martyrs University-Nkozi, Uganda.

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To assess the quality of commercial organic fertilizers in Uganda market, a quick survey was conducted to establish the organic fertilizers being sold in Container village market in Kampala Capital City. Different products were found being sold as organic fertilizers. Four of these fertilizers are two solid (Biochar and Fertiplus) and two liquid (Biogrow and Digrow) selected for a field study. Field trials to assess the performance of the fertilizers and local farmyard manure on bush beans *(Phaseolus vulgaris*) were conducted at two locations (Kabanyolo and Nkozi) for two successive seasons in 2017 and 2018 using a randomized complete block design in four replications. Forty days after planting, fresh and dry weights were estimated while biomass and grain yield were determined at harvest. The results showed inconsistencies. For example, although Biochar was found to significantly (P=0.043) increase the mean fresh weight of bush beans in season 1 (2017) at both sites (Kabanyoro and Nkozi), this was not the case in season 2 (2018). Similar performance was found with the other treatments. The mean biomass did not increase significantly (P>0.05) in season 1 (2017) and season 2 (2018) at both locations. The results in season 2 (2018) showed no significant (P>0.05) increase in the mean yield. These results were attributed to the extreme weather conditions experienced in both seasons (severe drought during season 1 and heavy rains that led to prolonged vegetative growth during season 2). These field trials should be repeated under controlled environment to minimize the weather effect.

Key words: Organic fertilizers, plant growth, yield.

INTRODUCTION

Uganda is considered as one of the countries with highest soil nutrient depletion rates in the world; it has the lowest rates of annual inorganic fertilizer application – only 1.8 kg per ha (Namazzi, 2008). Consequently, most crops grown in the country produce only a small fraction of their potential yield (Tadele et al., 2017; Nabbumba and Bahigwa, 2003). This very low use of inorganic fertilizers is mostly attributed to the very high cost of these inorganic fertilizers and also to lack of knowledge by the majority smallholder farmers on the benefits of

*Corresponding author. E-mail: tugumeesau@gmail.com Tel: +256779499788.

using inorganic fertilizers (Okoboi and Barungi, 2012). According to Namazzi (2008), the World Bank calculated that the value of replacing these depleted soil nutrients could be 20 percent of average rural Uganda household income. A recent survey conducted in the Container Village market in Kampala where most traders and farmers buy agro-inputs (Tugume, 2018) revealed that 95 percent of the agro-input shops visited in the village were stocking / selling both organic and inorganic fertilizers. It also revealed that the fertilizer market was dominated (82%) by liquid fertilizers which were organic fertilizers. High market dominance of these liquid organic fertilizers was that they are quite cheap compared to the solid inorganic fertilizers as one or two liters of the liquid organic fertilizers can cover an acre of crops through foliar application compared to 100 kg or more of solid inorganic fertilizers such as urea or NPK to cover same acre of crops. So smallholder farmers in Uganda have persistently shown no interest in adopting the use of inorganic fertilizers; they are increasingly being forced by circumstances to invest in use of both local manures and imported organic fertilizers to enhance soil productivity (Sheahana and Barrett, 2017; Nalubwama et al*.,* 2011; Kidd et al., 2001; Elzakker and Leijdens, 2000). Therefore, there is increasing demand for organic fertilizers in the country to improve crop productivity and farm returns to investment.

Currently there are many products on the agro input market in Uganda that are promoted and marketed as organic fertilizers and plant growth promoters. Unfortunately most of these products are not locally tested for their effectiveness as fertilizers. Therefore their ability to improve crop productivity is speculative. Government policies on the procurement and use of fertilizers are important determinants of the intensity of fertilizer use in a country and directly affect the profitability of fertilizer use by smallholder farmers (Bayite et al*.,* 2011). In Uganda, there has been limited policy regulation to guarantee the marketing and use of quality fertilizers, particularly organic fertilizers (MAAIF, 2016). Generally there is no evidence that shows that the products on the market are genuine organic fertilizers with the right and recommended quantity and quality of nutrients required to support proper crop growth and increase production in agriculture.

Bush beans however are cultivated and produced in all districts in Uganda but commonly in central, eastern and western parts of Uganda (FAO, 2017). They are often cultivated by smallholder farmers on wide range of soils (Tugume, 2018; Sibiko, 2012). Farmers grow both local varieties like yellow, kanyebwa and improved varieties of NABE and K series. The NABE series is more tolerant to droughts, diseases and therefore adapted to face unreliable rains and rainfall variability induced by climate change (FAO, 2017). The study used NABE 17 variety that is tolerant to diseases and also commonly grown by farmers with high acceptability in the market. Bush beans are also selected due to their positive response to Nitrogen nutrient fertilizers at vegetative stage whichincreases the plant yield (Henson and Bliss, 1991). Therefore the purpose of this study was to assess the performance of a few selected commercial organic fertilizers on the market in Uganda in increasing the growth and yield of bush beans that are commonly cultivated by smallholder farmers in most parts of the country.

MATERIALS AND METHODS

Location of the study

The study was conducted at Kabanyolo near Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), located 17 km north of Kampala and at Equator Valley Farm of Uganda Martyrs University near Nkozi Trading Centre located about 80 km south west of Kampala on the Kampala-Masaka highway. Both sites are located in the Lake Victoria Crescent agroecological zone in central Uganda (Figure 1). They both receive an average annual precipitation of about 1200 mm and two dry periods in June to July and December to February. The monthly average temperature in season one from March to June is 22°C for both locations; 20 and 21.3°C from September to December for both Kabanyolo and Nkozi sites (Mibulo and Kigundu, 2018; Mpigi District local Government; 2015). Both sites have sandy clay to sandy clay loam soils with 54-76% sand, 4 - 14% silt, and 20- 42% clay (Table 1).

This study included six treatments consisting of four commercial organic fertilizers (Biochar, DIgrow, Fertiplus, Biogrow), local farmyard manure and a control for two consecutive seasons using a randomized complete block design in four replicates. Plot size was 4m by 3m and beans were spaced 50cm by 20 cm with two seeds planted in each hole.

Samples of the selected organic fertilizers, soils from the experimental fields at Kabanyolo and Equator Valley Farm in Nkozi and local farmyard manure were collected and taken for physical and chemical analysis by Soil, Plant and Water Analytical Laboratory of the Department of Agricultural and Environmental sciences at Makerere University to establish their nutrient contents. These samples were analyzed using routine procedures outlined by Okalebo et al. (2002) and other standard operating procedures (SOPs) that are internationally recommended.

Experimental set up and management

Fertilizer application

The rates, method and time of application of the organic fertilizers were determined according to the manufacturers' recommendations (Table 1). The liquid fertilizers, Biogrow and DIgrow, were applied at vegetative stage, three weeks after planting, when nitrogen is sufficiently needed by beans for shoot development and at flowering 6 weeks for high pod development (Henson and Bliss, 1991).

Planting

Experimental plots were planted with bush bean *(Phaseolus vulgaris),* variety NABE 17, and an improved variety tolerant to drought stress and are early maturing at 58-70 days. Planting was done at the first week of October 2017 in the first season and in the second week of March 2018 in second season. The seed rate was two seeds per hole at a spacing

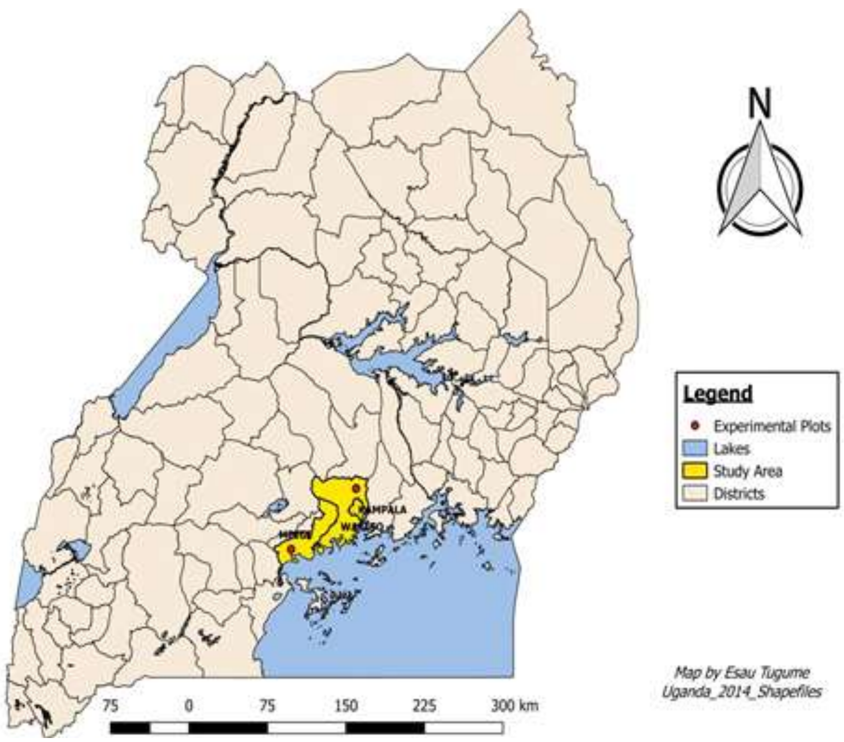

Figure 1. Map of Uganda showing the location of the experimental sites.

Table 1. Organic fertilizers used in the study, their rates, method and time of application.

Fertilizer type	Recommended rate of application	Stage of application
Biochar (solid pellets)	1500 Kg ha^{-1}	At planting using broadcasting and incorporation into the seedbed
Fertiplus (solid pellets)	1500 Kg ha^{-1}	At planting using broadcasting and incorporation into the seedbed
Farmyard manure (solid)	5000 Kg ha^{-1}	At planting using broadcasting and incorporation into the seedbed
DIgrow (liquid)	1 L ha^{-1}	Foliar application carried out at 3 weeks after planting and 1 week after onset of flowering
Biogrow (liquid)	500 ml ha^{-1}	Foliar application carried out at 3 weeks after planting and 1 week after onset of flowering

of 50 by 20 cm.

Weeding

First weeding was done three weeks after planting; before application of liquid organic fertilizers and the second weeding was done in the sixth week after planting.

Data collection

Plant fresh and dry weight

At the onset of flowering (40 days after planting), a representative sample of five bean plants was randomly selected and uprooted from each plot in the experimental field and immediately weighed using an electronic weighing balance to record plant fresh weight at that stage. The five bean plants uprooted from each plot were then put in well-labeled paper bags and dried under shade for a period of two weeks then measured using the same electronic weighing scale.

Harvest and yield measurement

Harvesting was done at 10 weeks after planting when all bean plants and pods were completely dry. Hand pulling was used to uproot the bean plants from the soil and all the plants from each plot were carefully packed in a large well labeled polythene bag (sack) and weighed to obtain the biomass yield from each plot. Bean plants were then dried for five days before threshing to obtain the grain yield from each plot. Finally, the bean grain from each plot was weighed using an electronic spring balance. This was done following one of the methods of measuring crop yield outlined in FAO (2018).

Table 2. Laboratory analysis results of the organic fertilizers and local farmyard manure used in the study.

Fertilizer	pH	O. M. (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
Biochar	-	-	3.02	0.34	0.82	2.52	0.83	0.63
Biogrow	4.63	-	0.34	0.31	1.47	2.52	0.87	0.50
DIgrow	6.60	-	1.23	2.31	1.85	2.21	0.77	0.63
Fertiplus	-	-	3.25	3.40	1.83	3.57	1.21	0.84
Kabanyolo manure	-	12.08	1.40	1.90	1.05	2.63	0.88	0.68
Nkozi manure	-	14.29	1.26	2.20	1.05	3.15	1.08	0.63

The sign (-) refer that this parameter not analyzed; the obtained results carried by laboratory Analysis at Soil, Plant and Water Analytical Laboratory of Makerere University.

Table 3. Soil sample analysis results for two seasons at Kabanyolo and Nkozi.

Soil parameter	Kabanyolo		Nkoz1	
	1st season field	2nd season field	1st sesoan field	2nd season field
pH	6.54	6.89	5.47	6.40
OM (%age)	3.51	3.51	3.22	2.63
N (%age)	0.20	0.21	0.18	0.16
P (mg/kg)	2.56	4.13	16.00	16.73
K (Cmoles/kg)	0.27	0.41	0.27	0.68
Ca (Cmoles/kg)	7.69	7.88	6.66	7.93
Mg(Cmoles/kg)	2.10	2.24	1.87	2.13
Na (Cmoles/ kg)	0.23	0.21	0.28	0.37
Textual %ages				
Sand	54.00	56.00	76.00	68.00
Clay	42.00	30.00	20.00	26.00
Silt	4.00	14.00	4.00	6.00
Textual characterization	Sandy clay	Sandy clay loam	Sandy loam	Sandy clay loam

Statistical data analysis

The experimental design was randomized complete block design with four replicates. The obtained data were submitted to analysis of variance according to GenStat 14 Edition package. Differences among treatment means were determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

Laboratory analysis results

The results of the laboratory analysis of the organic fertilizers and local farmyard manures used in the study are presented in Table 2, while those of soil samples collected from the experimental fields at Kabanyolo and Equator Valley Farm in Nkozi are presented in Table 3. The results in Table 2 show that these materials had good contents of the major plant nutrients Beside the analysis of the experiment soil as presented in Table 3 indicate that it was sandy and had low content of organic matter, therefore, the selected organic fertilizers and manures were expected to have significant effects on bean growth and yield on these soils.

Effect of selected organic fertilizers and local manure on the plant fresh weight of bush beans

Table 4 shows results of plant fresh weight mean at 40 days after planting. These results show significant difference (P=0.043) between the fertilizers and control treated plots in season 1 (2017) at Kabanyolo. Fertiplus and Biochar significantly increased the fresh weight of the bean plants (143.5 and 135.0 g) as compared with the control and other treatments. Biogrow fertilizer did not significantly differ from the control in increasing mean vegetative fresh weight of bush beans (*P. vulgaris*).

At Nkozi in season 1 (2017), application of organic fertilizers did not result in any significant increase in plant fresh weight (P=0.140). However, amendment of soil with Biochar produced a slightly higher increase in plant fresh weight (131.25 g) compared to the control (73.00 g) and

Table 4. Effect of selected organic fertilizers and manure on mean plant fresh weight mean (g) at 40 days after planting.

Treatment	1st season		2nd season	
	Kabanyolo	Nkozi	Kabanyolo	Nkozi
Control	92.20^{a}	73.00^{a}	481.8^{a}	311.0^{a}
Biogrow	94.80^{a}	80.75^{a}	522.8^{a}	314.5^{a}
DIgrow	118.0^{ab}	90.50^{ab}	531.0^{a}	322.5^{a}
Cow dung	131.5^{ab}	102.25^{ab}	533.0^{a}	343.8^{ab}
Fertiplus	135.0^{b}	102.25^{ab}	534.8^{a}	361.0^{ab}
Biochar	143.5^{b}	131.25^{b}	570.5^{a}	449.5^{b}
e.s.e	12.35	14.58	52.0	38.0
P.Value	0.043	0.140	0.906	0.158

Means with similar letters in each column are not significantly different at P≥0.05. Means were separated by Duncan's multiple range tests. e.s.e is the estimated standard error of mean.

other treatments. In season 2 (in 2018), there was no significant increase in plant fresh biomass at both sites but Biochar again performed better than other treatments. Therefore Biochar consistently performed better than the other treatments in both seasons and across the two locations. This may be attributed to its nutrient composition especially nitrogen and its ability to release nutrients into the soil for plant uptake that consequently facilitated plant growth. Relatedly Islam et al. (2016) reported that vermicompost significantly increased the plant height of long and bush beans. Similar results were reported by Singh and Chauhan (2009) in bush beans. Pulak (2014) also found that organic growth promoters significantly increased the plant growth of chick peas with respect to height and branching. Such insignificant increase in plant fresh weight mean concur with findings of Karambu (2013), who reported that soya bean plant height was insignificantly affected by farm yard and poultry manure. Shehata and Helaly (2010) also reported that pod length in bush bean (*P. vulgaris*) was not significantly different when treated with vermicompost and ordinary compost. Insignificant increase in fresh weight can be attributed to slight differences in the nutrient composition of organic fertilizers as well as slow mineralization of cow dung that affect nutrient uptake by plants (Otieno et al., 2007).

The differences in mean plant fresh weight of bush beans under similar treatments from different locations and seasons could be attributed to climatic factors such as rainfall distribution and soil properties. In season 1 (2017) little rain distributed at the start of the growing season affected the physiological processes and consequently affected the plant growth. It is well known that rainfall increases soil moisture availability which affects mineralization of soil nutrients which could have affected plant nutrient absorption. This argument agree with Vanlauwe et al. (2002) who noted that variability in climatic factors such as rainfall and temperature make the synchrony between nutrient release from tree litter and crop nutrient uptake become difficult goal to achieve in practical terms. Jonasson et al. (2004) also reported that insufficient moisture or warming in soils limit the response of crop to N nutrient which hinders plant physiological activities. In season 2, rainfall was adequately distributed in Kabanyolo and Nkozi sites which could have led to leaching of important organic nutrients available in organic fertilizers and leading to insignificant increase in the mean of plant fresh weight of bush beans. Rainfall distribution could have affected positively the soil nutrient mineralization process resulting from available organic matter and nitrogen which lead to equal distribution and nutrient availability for plant uptake across all treatments, consequently leading to high insignificant increase in plant fresh weight.

Effect of selected commercial organic fertilizers and local manures on plant dry weight of bush beans *(P. vulgaris)*

Results presented in Table 5 indicate no significant increase in the mean value of vegetative dry weight of bush beans in plots treated with fertilizers and control (P=0.06) in season 1(2018) at Kabanyolo site. There was an observed significant difference in increase of plant dry weight mean in plots treated with Biochar (29.75 g) and Cow dung (27.75 g) compared to other fertilizers and control (17.00 g) treatment. At Nkozi site, there was no significant difference in increase of mean weight of bush beans (P=0.140) in season 1(2017) (Table 5). Mean value of plant dry weight in bush beans differed significantly in plots treated with Biogrow (28.75 g) as compared with control treatment (17.50 g).

Data results of season 2(2018) as presented in Table 5 also showed no significant difference in dry weight as affected by fertilizer and control (P=0.906, P=0.158) in both locations. The effect of different organic fertilizers on the mean vegetative fresh weight was found not significant as compared with control. The effect of organic fertilizers in increasing plant dry weight may be

Table 5. Effect of selected commercial organic fertilizers and local manures on the plant dry weight.

Location	1st season		2nd season	
	Kabanyolo	Nkozi	Kabanyolo	Nkozi
Treatment	**Mean dry weight (g)**	**Mean dry weight (g)**	**Mean dry weight (g)**	**Mean dry weight (g)**
Control	17.00[a]	17.50[a]	63.25[a]	49.00[a]
Biogrow	21.75[ab]	28.75[b]	63.25[a]	51.50[a]
DIgrow	22.25[ab]	23.75[ab]	71.75[a]	45.75[a]
Fertiplus	25.25[ab]	23.00[ab]	63.00[a]	47.00[a]
Cow dung	27.75[b]	17.25[a]	64.00[a]	58.25[a]
Biochar	29.75[b]	19.25[ab]	68.50[a]	52.00[a]
e.s.e	2.8	3.4	6.1	4.6
P.Value	0.062	0.183	0.618	0.468

Means with similar letters in each column are not significantly different at P≥0.05. Means were separated by Duncan's multiple range tests. e.s.e is the estimated standard error of mean.

attributed to the high rain fall distribution that facilitated vegetative growth leading high shoot formation with high water content. The water content could have been lost in evaporation and transpiration processes leading to also equal loss of water content in the bush bean shot system during drying process. The results are also in agreement with Islam et al. (2016) who recorded that vermin compost was found to be not significantly different from control in increasing mean dry biomass plant height of yard long beans. A contrasting finding confirmed non-significant effect on plant height as a result of spray of varied concentration of panchagavya foliar application on green gram (Somasundaram et al*.,* 2003). However, the results contradicted with Valdez et al*.* (2011) who reported that significant increase was observed in total shoot, root and pod biomass of legumes grown under vermin compost.

Effect of selected commercial organic fertilizers and local manure on the plant biomass of bush beans *(P.vulgaris)*

Figures 2 and 3 show mean biomass yield in season 1(2017) and 2(2018), respectively. No significant difference was recorded in season 1, (P=0.55 and P=0.22) and season 2 (P=0.6 and P=0.07) in mean biomass yield of bush beans at Kabanyolo and Nkozi. However, fertilizers Biogrow (0.1715 kg) were significantly different from other fertilizers and control in increasing the mean biomass yield of bush beans in season 1(2018) at Nkozi. Similarly Biochar (0.256 kg) was significantly higher than the control in season 2 (2018) at Nkozi. Fertilizer treatments produced higher mean biomass yield of bush beans in both season in Nkozi than in Kabanyolo.

The lack of response in bean biomass yield to the application of organic fertilizers and local manure could be attributed to severe drought which seriously affected the field trials during the first season and excessive rainfall during the second season that resulted into high vegetative growth especially since soils had relatively adequate nitrogen and organic matter content. This can also be attributed to high rainfall distribution that tends to encourage high mineralization of applied organic fertilizers and stored soil nutrients thereby increasing nutrient uptake and exponential shoot development forming large plant canopy that affect flowering and pod formation.

The results concur with Islam et al*.* (2016) who reported no significant increase in the mean dry pod weight of long beans in plots treated with vermin compost compared to control. Shehata et al. (2011) also reported that almost similar trend was observed when vermin compost did not significantly increase the pod length of bush beans compared to compost treated plots. In contradictory view, results of this study disagree with Lalljee (2006) findings who reported that in vermin compost treatment, pod dry weight of the legumes was significantly higher than in plants from compost and farmer's practice treatments.

Effect of commercial organic fertilizers on the grain weight mean of bush beans

Results presented in Table 6 show the grain yield mean of bush beans (in Kgha^{-1}) for season two. No grain yield was recorded in season one because field trials in both locations were severely affected by drought.

In season 2(2018), no significant increase in grain yield weight mean was observed in both locations. All fertilizer treated plots did not significantly differ from the control in increasing grain yield of bush beans. Season two, grain yield was most likely positively affected by the adequate rainfall distribution that influenced mineralization of soil

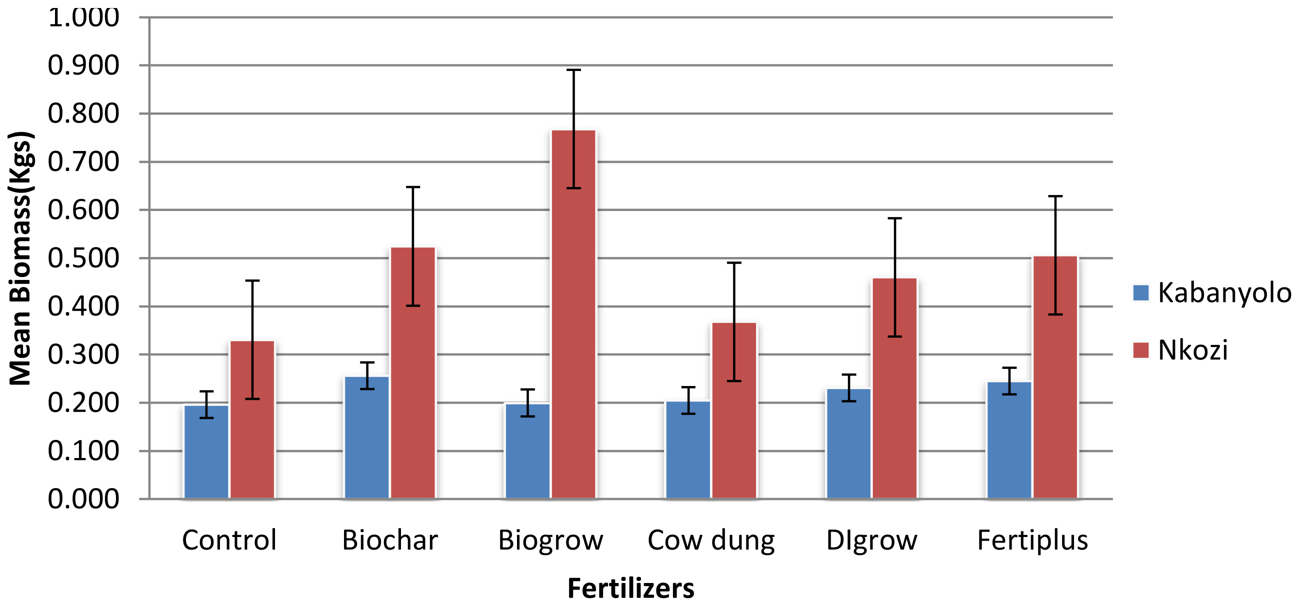

Figure 2. Yield biomass mean at Kabanyolo and Nkozi in season 1 (2017).
Source: Field experimental data, season 1(2017).

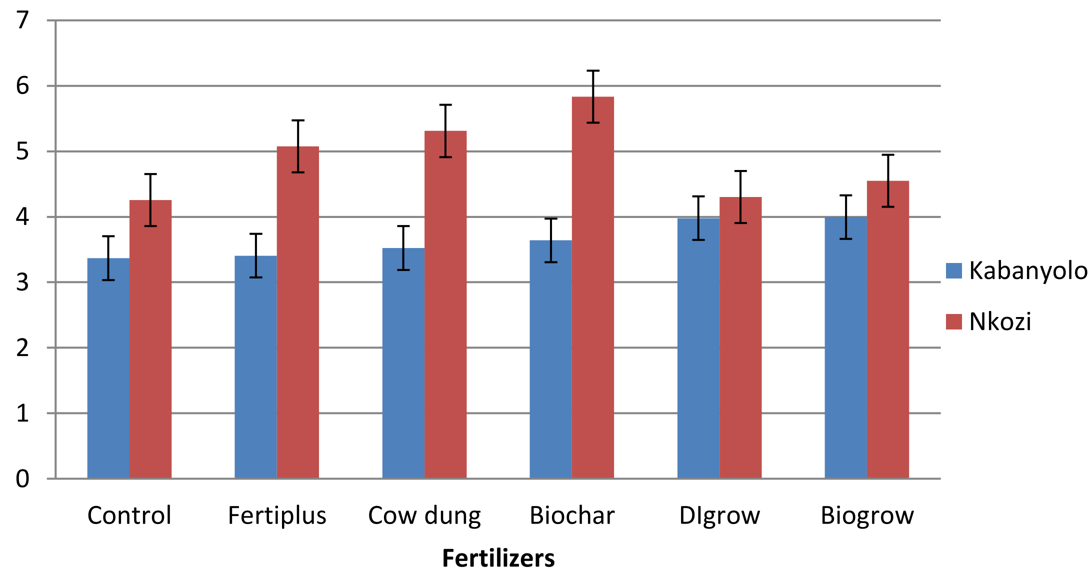

Figure 3. Yield biomass mean at Kabanyolo and Nkozi in season 2 (2018).
Source: Field experimental data, season 2 (2018).

nutrients leading to high nutrient plant nutrient uptake. This could have encouraged high photosynthetic rates facilitating exponential development of growth and yield parameters. Karambu (2013) reported closely related finding that different soil amendments did not differ significantly from each other and control in increasing mean stover yield of TGX Variety of Soybean. Since all soil samples from the experimental plots were found containing nitrogen levels (Table 3), high rain fall distribution could have facilitated quick nutrient release and absorption by plants leading to increase physiological development of bush beans in season 2, 2018. Organic fertilizer laboratory results in Table 2 also confirm that some organic fertilizers were deficient of major macro nutrients which could have led to insignificant effect on the grain yield of bush beans

Table 6. Effect organic fertilizers on grain yield mean of bush beans.

Location	Season 2, 2018	
	Kabanyolo	Nkozi
Fertilizers	Mean grain weight ($Kg^{-1}ha$)	Mean grain weight ($Kg^{-1}ha$)
Control	1565[a]	1406[a]
Cow dung	1581[a]	1525[a]
DIgrow	1637[a]	1450[a]
Biogrow	1607[a]	1623[a]
Fertiplus	1570[a]	1629[a]
Biochar	1584[a]	1804[a]
e.s.e	107.89	144.39
P.Value	0.997	0.45

Means with similar letters in each column are not significantly different at P≥0.05; Means were separated by Duncan's multiple range test. e.s.e is the estimated standard error of mean.

compared to control treatments.

Conclusion

The main aim of this study is to assess the quality of selected commercial organic fertilizers on the market in comparison with local farm yard manure.

(i) The nutrient content reported on the labels were varying from laboratory reports which justifies the current problem of adulteration and marketing of counterfeit agro input products in Ugandan markets.
(ii) Results also showed that local manure had higher nutrient content compared to some of the organic fertilizer brands like Biogrow. Therefore, farmers can use locally available farm yard manure which is less costly and cheap to access than Biogrow fertilizers.
(iii) The performance of the selected organic fertilizers was not consistent in season 1(2017) and 2(2018) in both locations and therefore need to be tested in more confined environment to control of extreme weather factors for more subsequent seasons to have conclusive results on their effects on growth and yield of bush beans.

Recommendation

(i) There is need to conduct regular laboratory tests on organic fertilizers sold in the market to check the nutrient composition reported on the product labels by manufacturers
(ii) It is recommended that these field trials should be repeated under controlled environment to minimize the extreme weather effect.
(iii) Establishing different application levels and frequency regimes would also guarantee an opportunity to measure the extent of the effect at different rates and frequencies. This will also help to establish what are the sufficient quantities needed to be applied to obtain significant production output.
(iv) The future studies should focus on exploring the effect of organic fertilizers on the growth and yield parameters of bush bean (P. vulgaris) or other crops grown under greenhouse or controlled environment and sterilized growing medium.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The role of soil nutrient ratios in coffee quality: Their influence on bean size and cup quality in the natural coffee forest ecosystems of Ethiopia

Abebe Yadessa[1,2,4,5]*, Juergen Burkhardt[2], Endashaw Bekele[3], Kitessa Hundera[4] and Heiner Goldbach[2]

[1]Center for Development Studies, University of Bonn, Walter-Flex-Str. 3, D-53113, Bonn, Germany.
[2]Institute of Plant Nutrition, University of Bonn, Karlrobert-Kreiten-Str. 13, D-53115, Bonn, Germany.
[3]College of Natural and Computational Sciences, Addis Ababa University, P. O. Box 28513, Addis Ababa, Ethiopia.
[4]Department of Horticulture and Plant Sciences, Jimma University, P. O. Box 307, Jimma, Ethiopia.
[5]College of Agriculture and Natural Resources, Wollega University, P. O. Box 395, Nekemte, Ethiopia.

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Nutrients are essential for plant growth and development, and soil nutrient ratios play key roles in coffee quality. The objective of this study was to investigate the influence of soil nutrient ratios on the quality of wild Arabica coffee in Ethiopia. Results revealed that the balance between the soil nutrients was well correlated with coffee quality attributes. The balance between magnesium and calcium (Mg:Ca) and the balance between nitrogen and phosphorus (N:P) were found to be very important factors for bean size. Increase in Ca relative to Mg increased bean size, and vice versa. The higher the concentration of available P in relation to soil organic carbon (P:C) or total N (P:N), the better the cup quality of the coffee, and vice versa. The Mg:K ratio, P:N ratio, P:C ratio and P:Zn ratio were very important factors for cup quality. Although the ratio between Mg and K was important for cup quality, it was not apparent for bean size. The ratio between Ca and Mg was of no or little importance for coffee cup quality as opposed to that of bean size. Therefore, coffee growers should make careful decisions depending on the demands of the buyers/consumers and environmental requirements.

Key words: Arabica coffee, bean size, cup quality, coffee forest, nutrient ratios.

INTRODUCTION

Mineral nutrients are required for normal plant growth and development. Mineral nutrition refers to the supply, availability, absorption, translocation, and utilization of inorganically formed elements for growth and development of crop plants (Fageria, 2009). Next to water, nutrients are the environmental factor that most strongly constrains terrestrial productivity (Lambers et al., 2008). Plants differ in their requirement for nutrients and in their capacity to acquire nutrients from the soil (Koerselman and Meuleman, 1996; Lambers et al., 2008; Martins et al., 2015). Generally, plants require 17 essential nutrients for optimal growth and development

*Corresponding author. E-mail: ay.tarfa@yahoo.com.

(Barker and Pilbeam, 2007; Fageria, 2009). These nutrients are essential because they have specific metabolic functions in plants (Hopkins and Hüner, 2009). Macronutrients are required in large quantities and associated with their role in making up the bulk of the carbohydrates, proteins, and lipids of plant cells, whereas micronutrients are required in small amounts and mostly participate in the enzyme activation process of the plant (Barker and Pilbeam, 2007; Fageria, 2009).

Generally, mineral nutrients have many functions in plants; they are constituents of plant tissues, catalysts in various reactions, osmotic regulators, regulators of membrane permeability, etc. (Taiz and Zeiger, 2002; Roy et al., 2006; Pallardy, 2008; Clemente et al., 2018). Many enzymes are active only in the presence of ions such as Mg^{2+}, Mn^{2+}, Ca^{2+}, and K^{+} and these are known as metal activators (Pallardy, 2008). Each essential element thus has a role to play in the biochemistry and physiology of the plant (Hopkins and Hüner, 2009). The outstanding feature of life is the capability of living cells to take up substances from the environment and use these materials for the synthesis of their own cellular components or as an energy source (Mengel and Kirkby, 2001). Mineral nutrients are essential for plant growth and development through the incorporation of these mineral nutrients into organic substances such as pigments, enzyme cofactors, lipids, nucleic acids, and amino acids (Taiz and Zeiger, 2002). And hence nutrients influence the chemical composition and the sensory quality of plant products (Wiesler, 2012; Melke and Ittana, 2015). Nutrients are accumulated by the fruit during its development, and coffee fruits/beans are strong sinks for minerals and carbohydrates (Covre et al., 2016), which affect it quality. Coffee quality is the result of the presence of volatile constituents, caffeine, proteins, amino acids, fatty acids, phenolic compounds, and the action of enzymes on some of these constituents producing compounds affecting coffee quality (Clemente et al., 2015).

Plant growth is limited by the essential element that is most limiting (least available) when all other elements are present in adequate quantities (Liebig's Law of the Minimum). Once its supply is improved, the next limiting nutrient controls plant growth (Roy et al., 2006). Plants exhibit several mechanisms that can increase the supply of the most limiting resource (Chapin III et al., 2002). Thus, integrated plant nutrient management strives to ensure that plants have adequate but not excessive supplies of all essential elements (Alley and Vanlauwe, 2009), which is a prerequisite for product quality. The more the nutrient levels depart from the optimum, the more costly it will be to provide the correct nutrition (Willson, 1985a).

Any nutrient present in less than the optimal balance is likely to limit growth (Chapin III et al., 2002). Generally, coffee plants receiving a balanced nutrition, in which the required elements are supplied in appropriate amounts, are capable of producing quality beans. Coffee plant prospers well in slightly acid soils with a pH of 5.5-6.5 (Mitchell, 1988; Snoeck and Lambot, 2004), where most nutrients are usually more available to plants. When the pH level is less than 4.0, the levels of aluminium and manganese can be high, and this requires liming to correct the toxicity effects (Snoeck and Lambot, 2004).

Coffee is a major agricultural commodity in the world, and its production is economically important to several tropical countries, including Ethiopia, Brazil, Vietnam, Colombia, Indonesia, Mexico and Kenya, among others (Hein and Gatzweiler, 2006). Coffee is the most cultivated and consumed beverage in the world, yielding approximately 90 billion dollars per annum and involving about 500 million people from cultivation to final consumption (DaMatta, 2004). Nutrients are required for both vegetative growth of coffee trees and production of high quality beans and hence nutrient imbalances can affect coffee quality (Njoroge, 1998). Nitrogen and potassium are the two dominant nutrients required for coffee, K being more important in fruit development and N for vegetative growth. Phosphorus is essential for root, flower bud and fruit development, and it plays an important role in energy storage and transfer in crop plants (Fageria, 2009). Calcium, magnesium and other major and micro nutrients are essential for a balanced nutrition of the coffee plant although the required quantities are usually small to minimal in coffee (Willson, 1985b; Mitchell, 1988). When plants are grown without adequate essential nutrients, characteristic deficiency symptoms result (Nagao et al., 1986).

Inadequate supply of an essential element results in a nutritional disorder, and nutritional disorders occur because nutrients have key roles in plant metabolism. Nutrients serve as components of organic compounds, in energy storage, in plant structures, as enzyme cofactors, and in electron transfer reactions. Insufficient supply of an essential element leads to metabolic disorders (Taiz and Zeiger, 2002; Pallardy, 2008; Clemente et al., 2018), indicating that the necessary nutrients should be available at a reasonable amount and in appropriate relative proportions. Since there is usually interaction between nutrients, there should be a balance between soil minerals that determines a soil's productivity, and this should be determined by research. And little is known about the influence of soil on coffee quality in general and the influence of soil nutrient ratios (nutrient balance) in particular. The present study is the first report in its kind. The influence of soil properties (nutrient amounts) on coffee quality in the natural coffee forest ecosystem was previously reported by Yadessa et al. (2008), but the influence of soil nutrient ratios (nutrient balances) was not documented although both are equally important for plant nutrition and coffee quality. Plants require nutrients in balanced amounts (Roy et al., 2006; Hall, 2008), and nutrient interactions are very important aspect in mineral nutrition of plants (Clark and Baligar, 2000; Fageria,

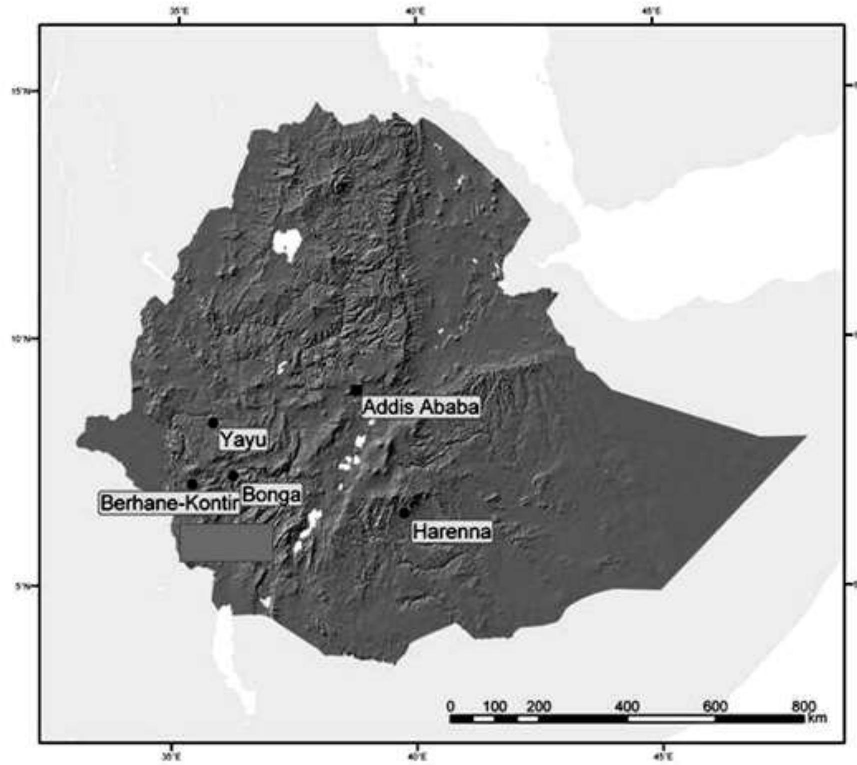


Figure 1. A map of Ethiopia showing the geographical location of the study sites.

2009). Proper coffee nutrition is thus required since it affects coffee quality and the overall productivity of the crop (Melke and Ittana, 2015). It is hypothesized that soil nutrient ratios (nutrient balances) in coffee plots from the natural coffee forest ecosystems are distinct since these Afromontane rainforests are the origin of Arabica coffee. The objective of this study was to assess the influence of soil nutrient ratios (the balance of soil nutrients) on wild Arabica coffee quality in the natural coffee forest ecosystems of southwest and southeast Ethiopia. Research information on soil nutrient ratios from the natural coffee forests of Ethiopia as a home of wild Arabica coffee (*Coffea arabica* L.) with distinct environmental conditions can be used as a guideline for simulating suitable soil nutrient ratios for future commercial production and quality improvement (e.g. fertilizer requirements, site selection, etc.) for Arabica coffee in other parts of Ethiopia or elsewhere.

MATERIALS AND METHODS

The study sites

The study was conducted in the natural coffee forests of southwest and southeast Ethiopia, which harbour the wild populations of *C. arabica* L. (Senbeta, 2006). The specific research sites are Berhane-Kontir or Sheko (in Bench-Maji zone), Bonga (in Kaffa zone), and Yayu (in Illubabor zone) in the SW coffee forests, and Harenna (in Bale zone) in the SE coffee forests of Ethiopia. Sheko, Bonga and Yayu are located west of the Great Rift Valley System, whereas Harenna is located east of the Great Rift Valley System (Figure 1).

The Yayu natural coffee forest is located in the Yayo district, Illubabor Zone of Oromia Regional State in the southwest Ethiopia. Yayu has got its name from the word Yayo, the name of the Oromo sub-clan living in the Illubabor Zone. The soils of the area are red or brownish Ferrisols derived from volcanic parent material (Tafesse, 1996). The total annual rainfall is about 1900 mm with mean temperature of 19.7°C (minimum temperature 7.6°C, maximum temperature 34.7°C) and relative humidity of 80.9% (Kufa, 2006).

The Berhane-Kontir natural coffee forest is also called Sheko forest. It is located in the Sheko district, Bench-Maji zone in the South Nations, Nationalities and Peoples Regional State, and hence the name Sheko forest. It represents the transition between the Afromontane moist forest and the lowland dry forest, located west of the Great Rift Valley (Senbeta, 2006). The total annual rainfall is about 2100 mm with mean temperature of 20.3°C (minimum temperature 13.8°C, maximum temperature 31.4°C) and relative humidity of 68.9% (Kufa, 2006).

The Bonga natural coffee forest is located in Kaffa Zone of the Southern Nations, Nationalities and Peoples Regional State in the southwest Ethiopia. Bonga has got its name from Bonga, the king of Kaffa Kingdom. Nitisols are the most dominant soils in southwestern Ethiopia, prevailing mainly in coffee and tea growing areas such as the Bonga region (Schmitt, 2006). The total annual rainfall is about 1700 mm with mean temperature of 18.2°C (minimum value of 8.7°C, maximum value of 29.9°C) and relative humidity of 80.4% (Kufa, 2006).

The Harenna natural coffee forest is located in Bale Zone of the Oromia Regional State in the south-eastern part of the country. It is

Figure 2. Coffee cherry collecting and processing activities in the natural coffee forests of Ethiopia.
Source: photo by Abebe Yadessa.

a part of Bale Mountains, and the Bale Mountains include the northern plains, bush and woods, the Sannate Plateau, and the southern Harenna forest. The area is known for its floral and faunal diversity and endemicity (Friis, 1986; Hillan, 1988). It is located east of the Great Rift Valley System. The total annual rainfall is about 950 mm with mean temperature of 22.2°C (minimum temperature 10.4°C, maximum temperature 34.4°C) and relative humidity of 63.2% (Kufa, 2006).

The coffee soils in the southwestern areas are highly weathered and originate from volcanic rock. These soils are deep and well drained, have a pH of 5-6, and have medium to high contents of most of the essential elements except nitrogen and phosphorus (Dubale and Mikiru, 1994). Phosphorus is generally low in the coffee soils of Ethiopia (Höfner and Schmitz, 1984; Schmitt, 2006). In its natural habitat where wild Arabica coffee grows, the soils are acidic to slightly acidic and have low available phosphorus (Senbeta, 2006; Muleta et al., 2007). The soils in the southeast are more sandy and less weathered (Yimer et al., 2006), as compared to the clay dominated and highly weathered soils in the southwest (Dubale and Mikiru, 1994). In these natural coffee forests of Ethiopia, wild populations of *C. arabica* occur across wide ranges of geographical locations, topographic features and soil characteristics (Senbeta, 2006). Coffee is the major means of making livelihood for the local community in the study areas.

Sampling procedures and coffee cherry sampling

Before starting coffee cherry sampling, during site selection preliminary information from the local people and key informants were collected to assess their perceptions on what local factors might affect coffee quality. Transects were laid out systematically along the toposequence of the study sites. Forty one samples from Sheko, 19 from Bonga, 34 from Yayu and 20 from Harenna were studied. Moreover, the level of forest management was assessed, rated from 0 to 2, where 0 (no or little management) stands for relatively undisturbed forest, 2 (high management intensity) stands for semi forest (disturbed coffee forest), and 1 (medium management intensity) stands for management intensity in between the two. In semi-forest coffee system, farmers slash weeds, lianas and cut competing shrubs and trees (Senbeta and Denich, 2006).

Coffee cherry harvesting and processing

Cherries were harvested at full maturity, which is usually during peak harvesting period. Coffee cherries matured and harvested first in Berhane-Kontir (Sheko), followed by Bonga and Harenna, and lastly in Yayu according to their maturity order in the field. Red cherries were hand-picked from the coffee trees in the forest and all the samples were then dry processed. The dried cherries were manually depulped and the beans were made ready for different analyses as shown in Figure 2.

Measurement of coffee bean characteristics and cup tasting

Bean size distribution of wild Arabica coffee beans collected from the natural coffee forests was determined by conventional screen analysis; perforated plate screens of different sizes (screen 18, screen 17, screen 16, screen 15 and screen 14) were used, with respective hole diameter of 7.14, 6.75, 6.35, 5.95 and 5.55 mm.

Table 1. Correlation coefficients between soil nutrient ratios versus bean size distribution of wild Arabica coffee from the natural coffee forests of Ethiopia.

Soil nutrient ratios	Proportion of bean retained on different screens§					
	SC 18+	**SC 17**	**SC 16**	**SC 15**	**SC 14**	**SC14-**
C:N	-0.049	-0.037	0.119	0.03	-0.057	-0.027
P:C	0.005	-0.113	-0.101	0.052	0.153	0.158
P:N	0.013	-0.121	-0.088	0.055	0.144	0.153
N:P	-0.095	0.295**	0.329**	-0.207*	-0.365**	-0.370**
N:K	0.068	0.092	-0.044	0.001	-0.087	-0.114
Mg:K	-0.05	0.058	-0.056	0.059	-0.021	-0.056
Ca:K	0.116	0.208*	0.009	-0.122	-0.203*	-0.201*
Mg:Ca	-0.317**	-0.324**	-0.056	0.339**	0.321**	0.299**
P:Zn	0.014	-0.106	-0.054	0.052	0.118	0.101
Silt:Clay	0.080	0.222*	0.009	-0.189*	-0.138	-0.173
Silt:Sand	-0.188*	-0.251**	-0.016	0.235*	0.232*	0.184
Clay:Sand	*-0.199**	*-0.266***	*-0.022*	*0.244***	*0.247***	*0.211**

§ Screen 18+ denotes the proportion of beans retained on screen 18 and above; screen 14- means those beans passed through screen 14 but retained on screen size below 14; and for others it is just the proportion of beans retained on the respective screens; e.g. screen 17 denotes the proportion of beans retained on screen 17 (diameter 17/64th of an inch).

The size of the screen hole is usually specified in 1/64 inch, and the screen hole diameter (in mm) is equivalent to screen number multiplied by 1/64 inch (Feria-Morales, 2002; Wintgens, 2004). Weight fractions retained on each sieve were recorded as described in Muschler (2001), and then converted into percentage basis. Bean size is evaluated either by grading on sieves or by calculating the average weight of 100 beans (Eskes and Leroy, 2004).

Bean length, width and thickness representing the major, intermediate and minor axes respectively, were measured by using digital caliper. Bean shape index was determined as a ratio of bean length to bean width (Montagnon and Bouharmont, 1996). Cup tasting was conducted at the Coffee Quality Inspection and Auction Center in Addis Ababa, Ethiopia by a panel of five experienced cup tasters (three from Ethiopia, two from Germany). The major coffee quality attributes (fragrance, aroma, acidity, body, flavour, aftertaste and overall quality) were assessed using the beverage quality denominations ranging from 1 to 10, corresponding to the total absence (or presence) of the criterion in the coffee, respectively.

Soil sampling and analysis

Soil samples (0-20 cm) were collected from each plot. Five samples were collected per plot and then bulked to obtain a composite sample, and finally one representative sample was taken from the bulk per plot as described in Yadessa et al. (2001, 2009). Soil samples were analyzed for chemical and physical properties following the standard procedures. Soil texture was determined by the Boucoucos hydrometer method (Day, 1965); soil pH by pH meter in a 1:2.5 (v/v) soil: water suspension; organic carbon (O.C.) by the wet oxidation method (Walkley and Black, 1934); available P following the procedures of Bray and Kurtz (1945); and total N by the Kjeldahl method (Jackson, 1958). Cation exchange capacity (CEC) was analyzed after extraction with 1 N ammonium acetate at pH 7 (ammonium acetate method). Micro-nutrients were extracted following the method of Lindsay and Norvell (1978) and the concentrations in the extract were determined using atomic absorption photometer.

Data analysis

Correlation and regression analyses were used to assess the relationships between soil nutrient ratios and coffee quality attributes in the natural coffee forests of Ethiopia. Analysis of variance (ANOVA) was used to assess the variation in soil nutrient ratios and coffee cup quality between the differently managed coffee forest systems. Principal component analysis (PCA) was used to explore the interrelationships between soil nutrient ratios, sensory and bean characteristics. PCA is a data reduction technique whereby new composite variables (or components) are constructed as linear combinations of the original independent variables, which are uncorrelated and usually the first few components capture or explain most of the variation in the entire original data set (Jolliffe, 2002). The statistical analysis was performed using SPSS, version 17 (SPSS, 2008).

RESULTS

The correlation between soil nutrient ratios and coffee bean size distribution is presented in Table 1. The data on soil nutrient ratios are shown in Supplementary data 2. Results showed that the balance between some soil nutrients (soil nutrient ratios) significantly correlated with bean size distribution of wild Arabica coffee. The concentration of magnesium relative to calcium (Mg:Ca) and also the concentration of nitrogen relative to phosphorus (N:P) were found to be very important factors for bean size. There was a positive correlation between bean size and Ca; that is, increase in Ca relative to Mg increased bean size, and vice versa. Regarding soil texture, increasing the proportion of clay in relation to sand (clay:sand) decreased the bean size, as opposed to the case in cup quality. This means there is a positive relationship between soil particle size and coffee bean

Table 2. Correlation coefficients between soil nutrient ratios versus bean weight and shape of wild Arabica coffee from the natural coffee forests of Ethiopia.

Variable	100 BWt	BL	BW	BT	L:W	L:T	W:T
C:N	-0.010	0.025	-0.224*	0.045	0.137	-0.010	-0.158
P:C	-0.252*	-0.394**	0.116	0.011	-0.440**	-0.326**	0.051
P:N	-0.251*	-0.391**	0.111	-0.005	-0.435**	-0.311**	0.063
N:P	0.402**	0.509**	-0.022	-0.038	0.487**	0.435**	0.010
N:K	0.352**	0.362**	0.111	0.001	0.281**	0.296**	0.056
Mg:K	0.302**	0.373**	0.097	-0.028	0.301**	0.324**	0.076
Ca:K	0.399**	0.472**	0.195*	0.004	0.341**	0.383**	0.097
Mg:Ca	-0.437**	-0.359**	-0.114	-0.198*	-0.284**	-0.137	0.127
P:Zn	-0.130	-0.349**	0.057	0.063	-0.366**	-0.328**	-0.028
Silt:Clay	0.268**	0.103	0.079	0.170	0.066	-0.045	-0.117
Silt:Sand	-0.479**	-0.482**	0.013	-0.203*	-0.469**	-0.236*	0.189
Clay:Sand	-0.505**	-0.467**	0.024	-0.257**	-0.463**	-0.187	0.242*

BWt= Bean weight; BL=bean length; BW=bean width; BT=bean thickness; L:W=bean length:bean width ratio (bean shape index).

Table 3. Correlation coefficients between soil nutrient ratios and cup quality traits of wild Arabica coffee from the natural coffee forests of Ethiopia.

Variable	Fragrance	Aroma	Acidity	Flavor	Body	Aftertaste	Overall
C:N	-0.038	-0.076	-0.052	-0.052	-0.038	-0.100	-0.048
P:N	0.237*	0.265**	0.122	0.275**	0.209*	0.317**	0.255**
P:C	0.242*	0.263**	0.111	0.266**	0.204*	0.309**	0.236*
N:P	-0.142	-0.101	0.061	-0.03	-0.03	-0.091	-0.059
N:K	-0.121	-0.238*	-0.060	-0.194	-0.177	-0.247*	-0.169
Mg:K	-0.165	-0.256**	-0.08	-0.237*	-0.186	-0.294**	-0.217*
Ca:K	-0.141	-0.217*	-0.033	-0.155	-0.179	-0225*	-0.135
Ca:Mg	-0.152	-0.17	0.017	-0.044	-0.05	-0.085	-0.007
P:Zn	0.161	0.213*	0.105	0.218*	0.200*	0.225*	0.219*
Silt:Clay	0.045	0.130	0.193	0.073	0.107	0.039	0.122
Silt:Sand	0.279**	0.334**	0.098	0.222*	0.154	0.294**	0.199*
Clay:Sand	0.235*	0.272**	0.043	0.198*	0.107	0.265**	0.148

*, **Correlations are significant at 0.05 and 0.01 level of significance.

size in the natural coffee forest ecosystems. But the ratio between Mg and K (Mg:K), which was important for cup quality (Table 3), was not found to be important for bean size (Table 1). Increasing the concentration of soil total N relative to soil available P, increased bean size, and vice versa. Furthermore, increasing the concentration of soil Ca relative to Ma, increased bean size, and vice versa, indicating the importance of interaction between Ca and Mg for bean size. As indicated in Table 2, changes in soil nutrient ratios led to changes in bean weight, bean length and bean shape. Increase in the concentration of soil Ca with respect to Mg led to increase in bean weight, bean length and bean length-to-width ratio (bean shape index). Similarly, increase in the concentration of soil total N relative to available P or K led to increase in bean weight, bean length, bean length-to-width ratio, and bean length-to-thickness ratio. To the contrary, increase in the concentration of P relative to N or C led to decrease in bean weight, bean length, bean length-to-width ratio, and bean length-to-thickness ratio. This means increase in Ca relative to Mg or increase in N relative to P enhanced the development of elongated coffee beans (beans with higher shape index), whereas increase in P relative to N or C favoured the development of more rounded beans (beans had lower shape index).The balance (ratio) between the different soil nutrients also matters for cup quality shown in Table 3. The higher the concentration of available P in relation to soil organic matter or total N (P:C or P:N), the better the cup quality of the coffee, and vice versa. The relative concentrations of Mg and K (Mg:K), P and N (P:N), P and C (P:C), and P and Zn (P:Zn) were very important factors for cup quality. There was a positive relationship between P:N, P:C and P:Zn ratios versus cup quality traits, whereas an inverse

Table 4. Coffee cup quality traits and soil nutrient ratios as influenced by the level of forest management (forest coffee vs. semi-forest coffee).

Production system	Cup quality traits						
	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall
Forest Coffee	5.54	5.22	5.48	4.75	5.53	4.64	5.20
Semiforest Coffee	5.99	5.85	5.99	5.55	6.04	5.36	6.00
P value	0.011	0.004	0.014	0.001	0.024	0.004	0.001
	Soil nutrient ratios						
	P:N	P:C	Mg:K	Silt:sand			
Forest Coffee	17.15	1.69	9.66	0.95			
Semiforest Coffee	75.37	8.61	4.92	1.44			
P value	0.017	0.016	0.015	0.029			

Medium and high level of forest management (scales 1 and 2 in the Materials and Methods section) pooled to form the semiforest coffee, while little level of management (scale 0) alone form forest coffee system.

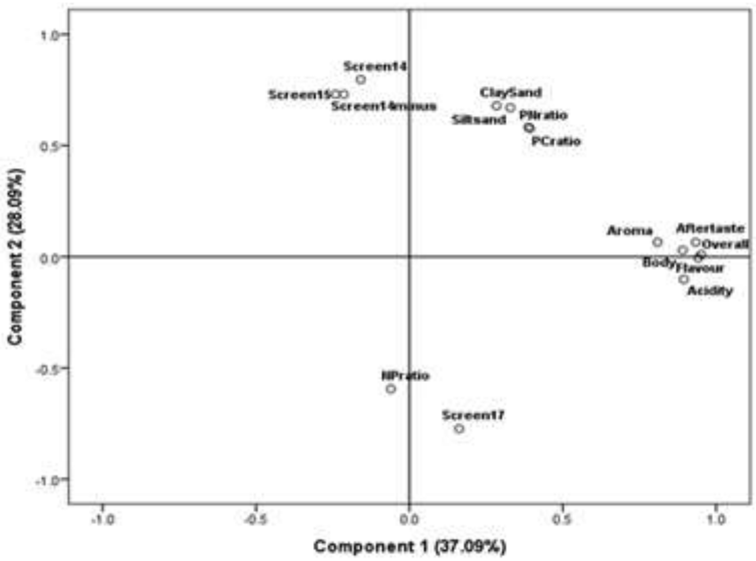
relationship between Mg:K and N:K ratios versus cup quality traits. Although most cup quality traits were significantly correlated with available P and P:N ratio, none of them was significantly correlated with N and N:P ratio. This is interesting and surprising, highlighting the importance of interaction between N and P for cup quality in the natural habitat of wild Arabica coffee, indicating that the more limiting nutrient is more important. Similarly, the relative proportion of clay and sand (clay:sand) was also important for coffee quality. But the ratio between Ca and Mg was of no or little importance for coffee cup quality as opposed to the case of bean physical quality (bean size). Positive relationship between silt:clay ratio versus proportion of bold beans, but negative relationship between silt:clay ratio versus proportion of medium beans shows that beans from less weathered soils (that is, younger soils) are bolder in size and vice versa, since silt: clay ratio and stage of weathering are inversely related (Thompson and Troeh, 1985; FAO, 2001).

Apart from this, results revealed that some soil nutrient ratios were significantly influenced by forest management, indicating that forest disturbance also influence nutrient balance in the natural coffee forest ecosystem (Table 4). The proportion of available P in relation to total N (P:N ratio), the proportion of P in relation soil organic matter or organic carbon (P:C), and the proportion of Mg in relation to K (Mg:K) were significantly different between the forest coffee and semi-forest coffee production systems. This means P:N and P:C ratios were significantly higher under the semi-forest coffee production system (managed forest) than under that of the forest coffee production system (less disturbed forest), but Mg:K ratio was higher under the latter than the former. This could be the probable reason for quality difference between coffees from semi-forest coffee and forest coffee production systems noticed in the present findings (Table 4). Cup quality was better under semi-forest coffee production system (moderately managed) than under forest coffee production system (little managed). As mentioned earlier, the balance between the nutrients or cations found to be very important for cup quality. These ratios also significantly differed across the forest management practices (Table 4), which might be the probable reason for significant difference in cup quality of wild Arabica coffee across the different traditional forest management practices in the natural coffee forest ecosystems of Ethiopia.

As shown in component plot (Figure 3) based on the first two axes, sensory characteristics were more correlated with the first axis (explaining about 37.09% of the variance), whereas bean characteristics were more correlated with the second axis (explaining about 28.09% of the variance). Both axes together explained about 65% of the total variance in the data set. Among soil nutrient ratios, P:N and P:C ratios were more correlated with higher proportion of smaller beans, whereas N:P ratio was more related to higher proportion of bold beans. In PCA plot, N:P ratio was almost perpendicular to cup quality traits, indicating that N:P ratio is not correlated with cup quality but well correlated bean size. Available P, clay, potassium and zinc contributed positively to the cup quality of coffee (that is, they promoted the production of coffees with better aroma, flavour and acidity) (Yadessa et al., 2008), whereas organic matter, total N, Mn and sand content contributed more to bean size (promoted the development of bolder beans) (Supplementary data 3). Plants from nutrient-rich sites tend to produce more biomass per unit nutrient in the plant, whereas plants from nutrient poor sites tend to keep the nutrients they have acquired for a longer time (Lambers et al., 2008), and soils from SE are more nutrient rich as compared to those from SW (Supplementary data 1).

DISCUSSION

The present study demonstrated that soil nutrient ratios



	Component	
	1	2
Screen17	0.162	**-0.773**
Screen15	-0.241	**0.731**
Screen14	-0.159	**0.797**
Screen14minus	-0.214	**0.73**
PCratio	-0.388	**0.584**
PNratio	0.393	**0.576**
NPratio	-0.06	**-0.594**
Siltsand	0.329	**0.67**
ClaySand	0.284	**0.679**
Aroma	**0.809**	0.065
Acidity	**0.894**	-0.102
Flavour	**0.951**	0.011
Body	**0.891**	0.029
Aftertaste	**0.934**	0.065
Overall	**0.942**	-0.005

Figure 3. Component plot in rotated space (left) and rotated component matrix (right) based on soil nutrient ratios, cup quality and bean size; KMO = 0.790, Bartlett's test of sphericity is significant (Chi square = 2130.88, degrees of freedom =105, P=0.000); extraction method: principal components; rotation method: varimax with Kaiser normalization.

(nutrient balances) are important factors for coffee quality. On one hand, the concentration of magnesium relative to calcium (Mg:Ca) was very important for bean physical quality; that is, Mg:Ca was positively correlated with bean size and weight. On the other hand, the concentration of magnesium relative to potassium (Mg:K) and the concentration of phosphorus relative to nitrogen (P:N) were very important for cup quality, the former being negatively correlated with cup quality, whereas the latter positively correlated with cup quality. This shows that higher concentration of Ca in relation to Mg enhances bean physical quality, while higher K in relation to Mg and higher P in relation to N improve cup quality. Coffee quality is lowered when the balance between the base cations move away from the optimum. Njoroge (1998) also reported that a balance of nutrients in the soil is important for better bean quality. According to Snoeck and Lambot (2004), the optimum K:Ca:Mg ratio is approximately 6:76:18% of the sum of the exchangeable bases, which is comparable to 6:74:19% in the present study. In the present findings, for instance, increase of Mg in relation to K (Mg:K) in the coffee soils was associated with a drop in beverage quality (cup quality) as presented in Table 3. This means, on average, Mg is about three times higher than K and Ca is about four times higher than Mg. These nutrients (Ca, Mg and K) are strongly antagonistic to each other, and excess concentration of one element inhibits the uptake of the other (Nguyen et al., 2017). For example, a study in Ethiopia by Laekemariam et al. (2018) showed that K deficiency due to antagonistic effects of Mg was about 54%.

The present study revealed that most cup quality traits were significantly correlated with P:N ratio, but none of them was significantly correlated with N:P ratio (Table 3). This is interesting, and this difference in response could be due to the different functions N and P have in the plant system. A study by De Groot et al. (2003) also showed that N concentration in plant tissue is sensitive to P limitation, but P concentration in the plant tissue is not sensitivity of N limitation, which supports the present finding.

A study in Ethiopia by Mintesinot et al. (2015) showed that coffee quality attributes increased with increase in the levels of soil Mg, but decreased with the increase in the levels of soil total N, although the authors did not mention about the nutrient ratios. A study in Tanzania by Kilambo et al. (2015) reported positive correlation between cup quality and some soil parameters (Ca, Mg, and K), and they also reported that soils with excessive calcium and potassium produce coffees with hard and bitter tasting liquor without mentioning about nutrient balance. In the present study, Ca:K ratio is negatively correlated with coffee aroma (Table 3). A study by Yadessa et al. (2008) revealed that higher levels of soil Mg, Mn and Zn were associated with improved coffee aroma in Ethiopia. A study in Uganda by Ngugi et al. (2016) showed that Mn and Zn were important elements in the determination of organoleptic cup quality in Robusta coffee. A study in Brazil by Clemente et al. (2015) showed that the relative proportion of nitrogen and potassium (N:K ratio) was found to be important factor in cup quality, which is in contrast with the findings of the present study (Table 3). And a study by Nguyen et al. (2017) reported better fruit qualities of pummelo (*Citrus maxima* Merr.) associated for higher soil K:Ca, K:Mg and Ca:Mg ratios in Thailand.

Increasing the supply of only one nutrient stimulates growth, which in turn can induce a deficiency of the other by dilution. Optimal ratios between nutrients in plants are often as important as absolute concentrations (Römheld, 2012). The balance between nutrients is therefore essential for coffee quality; otherwise, the imbalance between them will create undesirable antagonistic effects (Snoeck and Lambot, 2004), which in turn leads to poor quality coffee. Nutrient imbalances (Nojorge, 1998) and deficiencies in nutrients lead to lower quality coffees (Feria-Morales, 1990 cited in Feria-Morales, 2002). For instance, the balance between K, Mg and Ca is very important for coffee quality because K is antagonistic to Mg and Ca (Snoeck and Lambot, 2004). Higher Mg:K ratio leads to a drop in cup quality, and higher Mg:Ca ratio leads to poor bean physical quality, and vice versa. Therefore, plants need proper supply of all macronutrients and micronutrients in a balanced ratio throughout their growth, and the basics of balanced crop nutrition are governed by Liebig's law of the minimum (Roy et al., 2006), which is not exception to coffee plant growth and its quality. Since interactions usually occur between nutrients in nature, no nutrient act alone and the uptake of one nutrient is affected by the other, as also reported by Nguyen et al. (2017) and Laekemariam et al. (2018).

Plant growth is limited by the essential element that is most limiting (least available) when all other elements are present in adequate quantities (Alley and Vanlauwe, 2009). Any nutrient present in less than the optimal balance is likely to limit growth, so plants invest preferentially in absorption of the nutrients that most strongly limit growth, which also holds true for coffee plant. As a result, nutrients that accumulate in excess of plant requirements are absorbed more slowly (Chapin III et al., 2002). Optimal coffee quality is thus directly dependent on a correct ion balance in the soil (Snoeck and Lambot, 2004). This shows that both the availability and the balance between the nutrients in the soil are highly important for coffee quality.

The balance between the different soil nutrients, especially the balance between cations of different valency (e.g. between monovalents and bivalents) matters for cup quality rather than differences within the same valency number. The relative proportion between Mg and K was the most important factor in this regard; it was inversely correlated with most of the organoleptic properties of coffee assessed except for acidity. The ratio between Ca and Mg was of no or little importance for coffee cup quality. The ratio between the cations is very important for coffee because K is antagonistic to Mg and Ca (Snoeck and Lambot, 2004). High concentration of K will often cause Mg deficiency (Purseglove, 1968). A study by Oruko (1977), cited in Njoroge (1985) reported that excessive levels of K and Ca are believed to impair the quality of coffee beans, mainly as a result of imbalance with Mg. According to Willson (1985b), potassium and magnesium are antagonistic; that is, high levels of K in the soil or high K application can cause magnesium deficiency, and high Ca levels in the soil can restrict potassium uptake. A study by Laekemariam et al. (2018) showed that K availability depends on exchangeable K and relative amounts of other cations, and hence soil exchangeable K values alone may not adequately indicate K availability in areas where soil exchangeable Mg concentration is relatively high enough to compete with exchangeable K and cause K deficiency.

Interactions between nutrients occur when the supply of one nutrient affects the absorption, distribution or function of another nutrient. Interactions between ions can occur due to the formation of precipitates or complexes, which are generally most marked when the interacting ions have very different chemical properties (Robson and Pitman, 1983). This is in agreement with the present findings where Mg:K ratio (both with different valences) was much important for coffee cup quality. Thus, both deficiencies of essential nutrients and nutrient imbalances can affect coffee quality. Coffee quality is the resultant of the chemical constituents of coffee, and the action of enzymes on some of these constituents producing compounds affecting coffee quality (Clemente et al., 2015), which is related to soil characteristics where the coffee grows.

In short, increasing the concentration of a nutrient element where it is more limiting is essential for improving coffee quality, which is in line with the basics of plant nutrition (Roy et al., 2006). Increase in soil Zn concentration at Sheko, for instance, did not increase cup quality, but increase in soil Zn concentration increased cup quality at Yayu (Figure 4). This is because Zn might be excess at Sheko natural coffee forest, but it might be deficient at Yayu natural coffee forest (Supplementary data 1). A study in the Los Santos region of Costa Rica by Castro-Tanzia et al. (2012) also showed that where N, P, K and Mg are abundantly added through inorganic fertilizers, Ca has become the most limiting nutrient for coffee production, but cup quality improved when CaO was applied as a fertilizer. A study in Tanzania by Kilambo et al. (2015) showed that soils with adequate P, K, Clay-loam and silt positively influenced the cup taste.

Ethiopia holds a unique position in the world as *C. arabica* L. has its primary centre of diversity (Melke and Ittana, 2015). And the present study on the effect of soil nutrient ratios on coffee quality has wider importance since the natural coffee forests of Ethiopia is a birthplace of wild Arabica coffee, and the information obtained from this study can be used as a model for simulating suitable soil conditions such as nutrient balances for improving coffee quality and for expanding commercial production

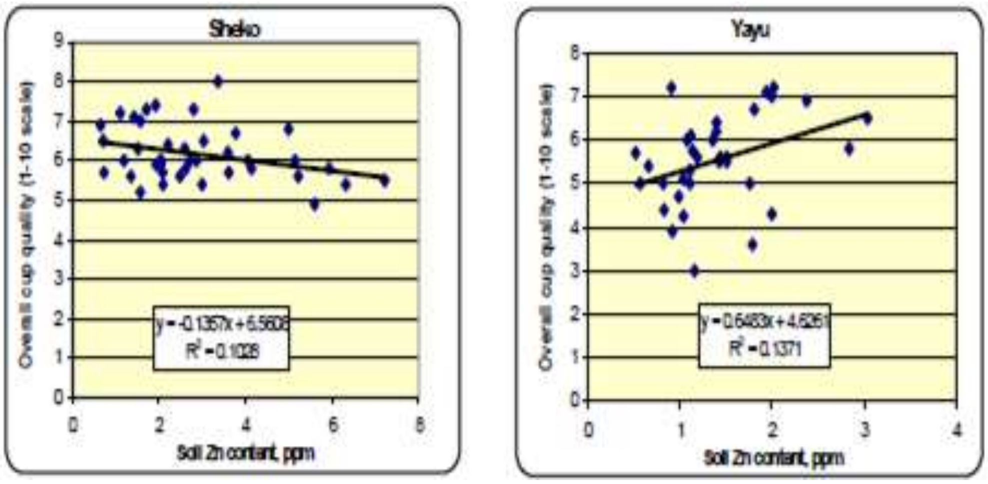


Figure 4. Overall cup quality of wild Arabica coffee as influenced by soil Zn content in Sheko and Yayu natural coffee forests in SW Ethiopia. Source: Yadessa et al. (2008).

of Arabica coffee in other parts of the country or elsewhere.

Conclusions

This study demonstrated that the balance (ratio) between the different soil nutrients matters for coffee quality. The concentration of magnesium relative to calcium (Mg:Ca) and the concentration of nitrogen relative to phosphorus (N:P) were found to be very important factors for bean size. Increase in Ca relative to Mg increased bean size, and vice versa. The higher the concentration of available P in relation to soil organic matter or total N, the better the cup quality of the coffee, and vice versa. The Mg:K ratio, P:N ratio, P:C ratio and P:Zn ratio were very important factors for cup quality. But the ratio between Mg and K, which was important for cup quality, was not important for bean size. The ratio between Ca and Mg was of no or little importance for coffee cup quality as opposed to the case of bean size. As pertaining to the soil texture, increasing the proportion of clay in relation to sand (clay:sand) decreased the bean size, as opposed to the case in cup quality (positive correlation between cup quality and clay). But the ratio between Mg and K, which was important for cup quality, was not found to be important for bean size (bean physical quality).

Generally, soil properties important for cup quality (P, silt, P:N, Mg:K, etc.) were not so important for bean size, whereas soil properties important for bean size (OM, Mn, pH, sand, N:P, Mg:Ca, etc.) are not so important for cup quality. Therefore, coffee growers should make trade-offs between cup quality and bean size depending on the prevailing conditions (consumers' demands, plant responses, environmental conditions, etc.), which could also be researchable issues in the future. In light of the present findings, further studies on the influence of soil nutrient ratios of coffee soils on coffee quality should be conducted based on detailed nutrient inputs and budgets in the future since this is the first paper reporting the role of soil nutrient ratios on coffee quality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary data 1. Mean values (±standard deviation) for the soil parameters from the four natural coffee forests in the SW and SE Ethiopia (n=111 samples).

Statistic	SW Soils			SE soils	P value
	B. Kontir (n=41)	Bonga (n=16)	Yayu (n=34)	Harenna (n=20)	
SOM (% DM)	4.64±1.34^{c}	6.52±1.25^{b}	7.21±2.20^{b}	8.49±1.00^{a}	0.000
Total N (% DM	0.32±0.07^{c}	0.41±0.05^{b}	0.41±0.13^{b}	0.52±0.005^{a}	0.000
Avail. P (ppm)	39.99±34.48^{a}	3.44±7.52^{b}	11.22±12.56^{b}	1.94±2.09^{b}	0.000
Na (meq/100 g)	0.05±0.06^{c}	0.10±0.06^{b}	0.04±0.02	0.16±0.07^{a}	0.000
K (meq/100 g)	1.23±0.68^{a}	1.34±0.80^{a}	1.07±0.74^{a}	0.56±0.40^{b}	0.002
Ca (meq/100 g)	11.88±4.87^{bc}	9.40±3.52^{c}	13.15±5.74^{b}	19.18±3.89^{a}	0.000
Mg (meq/100 g)	3.70±1.77	2.91±1.09	3.04±1.56	3.73±0.58	NS
CEC (meq/100 g)	29.08±7.39^{b}	34.96±5.05^{b}	32.22±12.33^{b}	43.77±4.69^{a}	0.000
BS (%)	56.58±12.57^{a}	39.01±13.68^{b}	53.89±11.83^{a}	54.44±10.23^{a}	0.000
pH	5.90±0.24^{b}	5.47±0.43^{c}	5.82±0.22^{b}	6.42±0.18^{a}	0.000
Sand (% DM)	20.18±9.07^{c}	29.13±6.37^{b}	43.82±11.14^{a}	46.70±5.92^{a}	0.000
Silt (% DM)	37.76±4.76^{a}	34.57±3.37^{a}	28.88±7.76^{b}	27.86±2.70^{b}	0.000
Clay (% DM)	42.06±8.02^{a}	36.31±5.49^{b}	27.30±4.69^{c}	25.44±5.95^{c}	0.000
Fe (ppm)	57.39±34.98^{b}	246.36±313.99^{a}	50.93±40.78^{b}	82.61±50.44^{b}	0.000
Mn (ppm)	136.91±45.96^{ab}	212.10±158.79^{b}	66.29±28.11^{b}	738.74±179.06^{a}	0.000
Zn (ppm)	2.97±1.72^{a}	3.26±01.85^{a}	1.41±0.60^{b}	2.38±0.55^{ab}	0.000

Means followed by similar letters within a raw are not significantly different by Tukey's Honestly significant test. DM = dry matter, BS=base saturation, SOM = soil organic matter. 1 ppm=1 mg/kg (solid substance); in terms of percents, 1 ppm equals 0.0001%.

Supplementary data 2. Summary data for soil nutrient ratios across the selected study sites.

Site	Statistic	C:N	P:C	P:N	N:P	N:K	Mg:K	Ca:K	Mg:Ca	P:Zn	Silt:Clay	Silt:Sand	Clay:Sand
Sheko (n=41)	Min.	11.38	0.62	8.02	0.002	0.08	1.29	3.87	0.14	0.50	0.56	0.67	0.54
	Max.	21.64	29.84	484.88	0.1246	3.13	13.60	77.73	0.76	124.89	1.71	4.10	4.97
	Mean	14.34^{b}	9.22^{a}	132.92^{a}	0.03^{c}	0.48^{b}	3.98^{ab}	13.78^{b}	0.32^{a}	16.40^{a}	0.94^{b}	2.19^{a}	2.52^{a}
Bonga (n=16)	Min.	11.29	0.08	1.41	0.01	0.13	0.82	4.33	0.14	0.10	0.63	0.73	0.65
	Max.	21.45	4.98	95.34	0.708	2.24	11.26	33.44	0.44	4.99	1.37	1.92	2.42
	Mean	15.92^{ab}	0.55^{b}	9.61^{b}	0.32^{b}	0.55^{b}	3.02^{b}	9.64^{b}	0.30^{ab}	1.02^{b}	0.97^{ab}	1.25^{b}	1.34^{b}
Yayu (n=34)	Min.	8.10	0.12	1.58	0.007	0.17	1.23	5.93	0.12	0.90	0.73	0.25	0.31
	Max.	40.89	6.13	147.88	0.63	7.60	65.0	168.67	0.54	69.76	1.73	1.91	1.64
	Mean	18.48^{a}	1.49^{b}	28.63^{b}	0.11^{c}	0.93^{ab}	6.52^{ab}	25.61^{b}	0.24^{bc}	8.99^{ab}	1.06^{ab}	0.76^{c}	0.70^{c}
Harenna (n=20)	Min.	14.45	0.06	0.93	0.067	0.34	2.79	12.86	0.11	0.24	0.64	0.42	0.28
	Max.	19.00	0.89	14.87	1.0769	3.44	22.13	134.06	0.31	3.18	1.85	1.02	1.55
	Mean	16.48^{ab}	0.23^{b}	3.79^{b}	0.49^{a}	1.34^{a}	9.30^{a}	49.46^{a}	0.20^{c}	0.80^{b}	1.15^{a}	0.61^{c}	0.57^{c}
P value		0.000	0.000	0.000	0.000	0.013	0.027	0.000	0.000	0.000	0.011	0.000	0.000

Means followed by similar letters within a column (across sites) are not significantly different by Tukey's Honestly significant test. Min.= minimum; Max. = maximum; Units of measurements for the elements as in Supplementary data 1.

Supplementary data 3. Pearson correlation matrix showing the relationships between bean characteristics and soil properties in the natural coffee forests of Ethiopia.

Variable	SC18+	SC17	SC 16	SC 15	SC 14	SC 14-	100 BW	BL	BW	BT
OM	-0.067	0.210*	0.218*	-0.152	-0.238*	-0.257**	0.312**	0.242*	-0.17	0.054
Total N	0.007	0.286**	0.129	-0.204*	-0.240*	-0.290**	0.402**	0.223*	-0.031	0.093
Available P	0.010	-0.120	-0.087	0.055	0.148	0.150	-0.255*	-0.278**	0.100	-0.003
Na	0.087	0.466**	0.185	-0.415**	-0.428**	-0.345**	0.187	0.438**	0.146	-0.131
K	-0.052	-0.262**	-0.112	0.200*	0.264**	0.230 *	-0.241*	-0.214*	-0.115	0.106
Ca	0.121	0.333**	0.079	-0.314**	-0.269**	-0.243*	0.341**	0.151	0.124	0.059
Mg	-0.109	0.024	0.058	-0.026	0.007	-0.004	0.043	-0.008	0.026	0.006
CEC	-0.091	0.166	0.267**	-0.163	-0.208*	-0.229 *	0.206*	0.186	-0.085	-0.042
pH	0.169	0.481**	0.060	-0.422**	-0.378**	-0.321**	0.316**	0.121	0.397**	-0.137
PBS	0.196*	0.171	-0.159	-0.173	-0.059	-0.026	0.179	-0.026	0.229*	0.183
Sand	0.247**	0.290**	-0.075	-0.264**	-0.220*	-0.164	0.497**	0.245*	0.049	0.238*
Silt	-0.194*	-0.192*	0.081	0.182	0.147	0.077	-0.343**	-0.253**	-0.033	-0.114
Clay	-0.235*	-0.302**	0.056	0.269**	0.227*	0.194*	-0.507**	-0.187	-0.050	-0.278**
Fe	-0.074	-0.089	0.144	0.049	0.002	-0.019	-0.088	-0.057	-0.212*	-0.067
Mn	0.076	0.606**	0.276**	-0.519**	-0.553**	-0.502 **	0.418**	0.363**	0.205*	-0.161
Zn	0.041	0.040	0.098	-0.072	-0.101	-0.065	-0.193	0.050	0.063	-0.164

OM = Organic matter; CEC = cation exchange capacity; PBS = percent base saturation; SC18+ = proportion of beans retained on screen 18 and above; SC17 = proportion of beans retained on screen 17; SC 16 = proportion of beans retained on screen 16; SC15 = proportion of beans retained on screen 15; SC14= proportion of beans retained on screen 14; SC14- = proportion of beans that passed through screen 14 (those retained on screens below 14); 100 BW = weight of 100 beans; BL = bean length; BW = bean weight, BT = bean thickness.

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Livelihood diversification and it's determinants on rice farming households in Ogun State, Nigeria.

O. J. Afodu[1*], C. A. Afolami[2], O. E. Akinboye[1], L. C. Ndubuisi-Ogbonna[1], T. A. Ayo-Bello[1], B. A. Shobo[1] and D.M. Ogunnowo[1]

[1]Department of Agriculture and Industrial Technology, School of Science and Technology, Babcock University, Ogun State, Nigeria.
[2]Department of Agricultural Economics, College of Agricultural Management and Rural Development, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

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This study assesses the nexus between livelihood diversification, technology adoption and food security status among rice farm households in the Ogun State. A multistage sampling technique was used to select two Agricultural Development Programme zones (Ikenne and Abeokuta zones) in the state. Purposive selection of two blocks per zone based on the concentration of rice farmers was done. Six farming cells were randomly selected from each block making a total of twenty-four (24) farming cells, seven rice farmers were randomly selected from each farming community giving a sample size of 168 rice farmers. A well-structured questionnaire was used to collect data. Out of the 168-questionnaire administered, 158 of it was gotten and used for the study. The data were analyzed using descriptive statistics, Simpson index for livelihood diversification and logit regression model. From the results, the age group 36 – 45 years is the modal category with 32.9% which was followed by 26 – 35 years with 27.9 percent. A large percentage (32.9) of the farmers had no formal education, 40.5 percent had school certificate, 20.3 percent had primary education, while 6.3 percent had tertiary education. Most farmers in the study area had extension agent at least once in every two months. The coefficients of age and education were found to be significant with the age carrying negative sign. It was concluded that rice farmers education in the study area was one of the major factors needed to improve their skills on other form of livelihood in order to enhance their well-being. It was recommended that young people should be encouraged to diversify their livelihood. The farmers should be educated on ways to diversify their livelihood. Credit facilities should be made available for the farm household either by the government or private parastatals to enhance farming activities. Production assets of the rice farmers in the study area should be improved on.

Key words: Rice farmers, livelihood, diversification, determinants, logit regression.

INTRODUCTION

Agriculture is the main source of livelihood in Nigeria, especially in the rural areas and is plagued with various problems (Adepoju and Obayelu, 2013). As a result, most of the rural households are poor and are beginning to diversify their livelihoods into off and non-farming activities as a relevant source of income. The farming

*Corresponding author. E-mail: afoduo@babcock.edu.ng.

sector employs about two-thirds of the country's total labour force and provides a livelihood for about 90 percent of the rural population (IFAD, 2009).

Agriculture as a livelihood activity is associated with immense risks and uncertainties which exposes the farming households to low standard of living, poverty and thereby decreasing their food security status. These risks and uncertainties associated with agricultural industry have led farming households to source for alternative sources of livelihood thereby diversifying their livelihood.

Livelihood diversification has received much attention from researchers and policy-makers in the past decades, with high hopes that promoting it can offer a pathway for poverty reduction and economic growth in sub-Saharan Africa (SSA) (World Bank, 2007). The term diversification refers to processes taking place at different levels of the economy, which are usually, but not always directly linked (Start, 2001). Firstly, diversification of the rural economy refers to a sectoral shift of rural activities away from farming to non-farming activities, associated with the expansion of the rural non-farming economy (Start, 2001); normally as part of a broader process of structural transformation (Timmer, 2009). Secondly, individual or household diversification refers to income strategies of rural individuals or households in which they increase their number of activities, regardless of the sector or location. Livelihood diversification is an active social process of individual or household diversification, involving the maintenance and continuous adaptation of a highly diverse portfolio of activities over time in order to secure survival and improve standards of living (Ellis, 2000b).

A number of studies have confirmed the inability of agriculture to fully support livelihood security (Samal, 2006; Shukla and Shukla, 2007; Shylendra, 2002; Unni, 1996). The following set of studies focuses on the issues related with livelihood diversification and highlights that livelihood diversification is the norm among rural households, and different income-generating activities offer alternative pathways out of poverty for households as well as a mechanism for managing risks in an uncertain environment (Davis et al., 2010; Dercon and Krishnan 1996; Ellis, 1998; Ellis and Biggs, 2001; Jodha, Asokan and Ryan, 1977; Nair and Menon, 2007; Papola, 2005 among others).

Various studies have shown that while most rural households are involved in agricultural activities such as livestock, crop, or fish production as their main source of livelihood, they also engage in other income generating activities to augment their main source of income (Adepoju and Obayelu, 2013). Non-farming local activities include all economic activities in rural areas except agriculture, livestock, fishing and hunting. It includes all off-farming activities, processing, marketing, manufacturing, wage and causal local employment in the rural villages (Agu, 2013).

Rice (oryza sativa) as a crop has received widespread attention from International and regional bodies due to its importance. It is a preferred food in urban centers of many countries including Nigeria (Igbokwe, 2001) and in institutions, because of the relative ease of preparation in catering for large numbers of people (Akande, 2002). In Nigeria, its importance is seen in the fact that it is accepted amongst all cultures (Okeke et al., 2008; Onimawo, 2012), and is normally preferably prepared in social functions. The major rice ecosystems in Nigeria are lowland upland rain-fed, lowland rain-fed, upland rain-fed and supplementation of precipitation by irrigated production systems which together account for 97% of rice produced in Nigeria (Daramola, 2005). Rice is processed simply by removal of husk and bran. Fat and protein content are low (Erhabor and Ojogho, 2011), so it can store well in a hot and damp climate. It has been noted that rice is the leading food in parts of the world with high population density and in areas where dietary levels are not adequate (Bouman et al., 2007; Huke, 1976). In terms of consumption in Nigeria, rice is the fourth most important staple crop after rising from a fifth position in the 1960's (Akande, 2002; Cadoni and Angelucci, 2013; Osifo, 1971). It is thus not surprising to note that rice production in Nigeria has been increasing over the decades. Despite the increase in it production, the demand for rice still exceeds the supply.

METHODOLOGY

Study area

This study was carried out in Ogun State, southwest of Nigeria. Ogun State lies between latitude 6° 54' 35.4" N of the equator and longitude 3° 15' 30.11" E of the Greenwich meridian (Tawan, 2006). Ogun State is made up of four Agricultural Development Programme zones, namely; Ilaro zone, Ikenne zone, Abeokuta zone and Ijebu ode zone. The state has a land area of 16,980 sq. km, a population of 3,751,140 people (National Population Commission, 2006). The state has twenty local government areas, and the vegetation is evergreen forests and savanna. The major crops grown in the state are cocoa, oil palm, rice, cassava, cotton and vegetables.

Sampling technique

A multistage random sampling technique was used for this study. The first stage involved the random selection of two Agricultural Development Programme zones (Ikenne and Abeokuta). The second stage random selection of two local government areas per zone based on the concentration of rice farmers. Thirdly, six farming communities were randomly selected from each local government area making a total of twenty-four (24) farming community. Lastly, seven rice farmers were randomly selected from each farming community giving a sample size of 168 rice farmers.

Sources and types of data

Primary data was used for this study. Data collected were households' demographic and socioeconomic characteristics such as age, educational level, marital status, sex, income, household size, as well as access to credit, and also, livelihood diversification strategies were collected through a cross-sectional survey of rice farmers in the study area with the use of a well-structured questionnaire.

Methods of data analysis

The analytical tools employed in this study were descriptive and inferential statistics. The descriptive statistical tools used were frequency, percentages, Simpson index for livelihood diversification and means, while Tobit regression model was used to capture determinants of rice farmers' livelihood.

Estimating the degree of income diversification (Simpsons Index of Diversity)

The Simpsons Index of Diversity (SID) was used in this study to estimate the degree of income diversification among rice farmers in Ogun State. The SID takes into consideration both the number of income sources as well as how evenly the distributions of the income between the different sources are (Minot et al., 2006; Joshi et al., 2003). This reason justifies the choice of the SID as applied in this study over other measures of diversification such as the Herfindahl, Shannon etc. The SID ranges between zero (0) and one (1). Thus, 0 denotes specialization and 1 the extremity of diversification. The more the SID value is closer to one, the more diversified the household is.

$$SID = 1 - \sum_{i}^{N} (P_i)^2 \tag{1}$$

SID = Simpsons Index of Diversity, n = number of income sources, Pi = proportion of income coming from the source i, the value of SID ranges from zero (0) to one (1); however, if there is only one source of income, Pi = 1, then SID = 0.

The SID model is expressed as:

$$SID = 1 - \sum_{i-l}^{8} \left(\left(\frac{fci}{thi}\right)^2 + \left(\frac{cci}{thi}\right)^2 + \left(\frac{nri}{thi}\right)^2 + \left(\frac{livsti}{thi}\right)^2 + \left(\frac{fwi}{thi}\right)^2 + \left(\frac{nfwi}{thi}\right)^2 + \left(\frac{sei}{thi}\right)^2 + \left(\frac{rei}{thi}\right)^2 \right) \tag{2}$$

Where: fci = food crops income, cci = cash crops income, nri = natural resource income, Livsti = livestock income, fwi = farm wage income, nfwi = non-farm wage income, sei = self-employment income, rei = remittance income, othersi = other income sources. SID = Simpson Index of Diversification (always falls between 0 and 1). N = number of farming households. The value of the index is zero when there is a complete specialization and approaches one as the level of diversification increases.

To determine factors influencing decision of livelihood diversification

The Tobit regression model was used to identify the factors which determine rice farming household engagement in livelihood diversification using SID. Schwarze and Zeller (2005), Babatunde and Qaim (2009) and Davendra et al. (2005) used this method to analyse the determinants of income diversification. The presence of zeros in the dependent variable, SID for some respondents (thus showing no diversification) demands the use of the censored (Tobit) regression model.

The general formulation for model specification is given as:

$$y_i^* = x_i \beta + \varepsilon_i \tag{3}$$

$$y_i = 0 \text{ if } y_i^* \leq 0 \tag{4}$$

$$y_i = y_i^* \text{ if } y_i^* > 0 \tag{5}$$

Where y_i^* is a censored variable of the SID, β is a parameter vector to be estimated, x is a vector of explanatory variables and ε_i is the error term.

Determinants of income diversification:

$$SID = \beta_0 + \beta_1 age + \beta_2 sex + \beta_3 numyrsedu + \beta_4 marstatus + \beta_5 hhs + \beta_6 accelectric + \beta_7 distmkt + \beta_8 tfarsize + \beta_9 extvisit + \beta_{10} prodassets + \beta_{11} acccredit + \varepsilon_i \tag{6}$$

SID = Simpsons Index of Diversification, ε_i = random term.

Table 1 shows the various livelihood diversification variables, their meaning, their sources and the a priori expectation of the various variables.

RESULTS AND DISCUSSION

Socio-economic characteristics of rice farming households

Table 2 shows the socioeconomic characteristics of the rice farming households in the study area. The age group 36 – 45 years is the modal category with 32.9% which was followed by 26 – 35 years with 27.9 percent. The least was the age group above 56 years which had 13.3 percent of the respondents. These imply that majority of the rice farmers in the study area are still in their active age. Some 60.8 percent of the farmers were male while 39.2 percent of them were female. This is in accordance with the work by Babalola et al. (2011), which opined that male are more involved in farming work compared to their female counterpart. This may be due to labour intensiveness of farm work. Majority (52.5%) of the farmers had household size of between 5 – 8, 31 percent had household size of 1 – 4, while households with 9 persons and above had the least being 16% of the respondents.

Education is an investment in human capital which may raise the qualities of skills of a man, narrow his

Table 1. Description of the variables specified in the livelihood diversification model.

Variable acronym	Variable meaning	Type of measure	A priori expectation with respect to livelihood diversification	Source
Marital status (Marstat)	Whether respondent is married or not married	Dummy variable (married = 1, otherwise 0)	+	Oni et al. (2011)
Age of household head (Age)	Age of the household head	In year	±	Oni et al. (2011)
Educational status (Edu)	Educational level of household head	Number of year of formal education	+	Sultana and Kiani (2011)
Household size (Hhsz)	Number of adults and children who are resident member	Number	+	Oni et al. (2011), Adebayo (2012), Shaikh (2007)
Sex	Sex of the household head	Dummy (male=, otherwise =0)	±	Babatunde et al. (2007)
Access to electricity (accelectric)	-	Dummy variable (having access = 1, otherwise 0)	+	-
Remittances (Rem)	Cash received from migrant members of family, friends and other groups	Amount in naira	+	Babatunde et al. (2007) Bamire (2010), Sultana and Kiani (2011)
Access to credit (Acrd)	Privilege of getting credit for household food consumption	Dummy variable (having access = 1, otherwise 0)	+	Arene and Anyaeji (2010)
Access to market (distmkt)	It is expected that households that have poor access to market are less diverse in income sources.	Dummy variable (having access = 1, otherwise 0)	±	Hoddinott and Yohannes 2002)
Agricultural land holding (Land)	Size of agricultural land held by household head	ha	+	Pankomera et al (2009), Bemire (2009)
Productive assets (prodassets)	Productive assets are those that are used as inputs into production processes.	Naira	+	-

information gaps and increase his allocative efficiency that leads to more productive performance. A large percentage (32.9) of the farmers had no formal education, 40.5 percent had primary school education, 20.3 percent had secondary education while 6.3 percent had tertiary education.

Livelihood activities engage in apart from rice farming

Out of the 158 rice farming households engaged in two or more livelihoods, the most preferred activity is livestock production (32.9%), followed by other food crops (17.7%) (Table 3). Other activities undertaken to complement rice farming include cash crops (15.2%), natural resources such as fishing (12.7%), agricultural wage (10.8%), non-agricultural wage (8.9%) and others (1.8%). It was observed that most of the rice farmers keep some livestock in abides to diversify their livelihood. 8.9 percent of the rice farming households earn income from non-agricultural employments. This finding is in line with the findings of Warren (2002) perspective on rural

Table 2. Distribution of respondents by socio-economic characteristics.

Characteristic		Frequency	Percent	Mean	Min.	Max.	Std.
Age (in years)	26 – 35	44	27.8	-	-	-	-
	36 – 45	52	32.9	-	-	-	-
	46 – 55	41	25.9	-	-	-	-
	56 and above	21	13.3	-	-	-	-
	Total	158	100.0	43.43	28.0	61.0	9.63
Sex	Male	96	60.8	-	-	-	-
	Female	62	39.2	-	-	-	-
	Total	158	100.0	-	-	-	-
Household size (in numbers)	1 – 4	49	31.0	-	-	-	-
	5 – 8	83	52.5	-	-	-	-
	>9	26	16.5	-	-	-	-
	Total	158	100.0	6.28	1.0	12.0	2.61
Educational status (in years)	No primary education	52	32.9	-	-	-	-
	Primary education	32	20.3	-	-	-	-
	Secondary education	64	40.5	-	-	-	-
	Tertiary education	10	6.3	-	-	-	-
	Total	158	100.0	6.73	0.0	18.0	5.80

Source: Field survey (2019).

Table 3. Distribution of respondents by livelihood activities engage in apart from rice farming.

Activity	Frequency	Percent
Livestock keeping	52	32.9
Other food crops	28	17.7
Cash crops	24	15.2
Natural resources	20	12.7
Agricultural wage	17	10.8
Non-agricultural wage	14	8.9
Others	3	1.8
Total	158	100.0

Source: Field survey (2019).

diversification alternatives.

The result of the Tobit regression estimates of the factors influencing livelihood diversification (SID) is presented in Table 4.

Table 4 show Age was found to be negative and significant at 5% probability level. The negative sign indicates that as the rice farm household grows older, the less diversified their livelihood. This is in line with the findings of Bernard et al. (2014) in Ghana which finds that as heads of farm households increases in age, the less they diversify their income sources. This is because they lack the physical strength and financial resources to add on to their farming or non-farming activities, since a majority of these activities are found to be labour intensive.

The coefficient of educational status of the farmers was found to be positive and significant at 5% level of probability which implies that the more educated the rice farming households are, the more they diversify their livelihood. This is in accordance with the work of Yunez-naude and Taylor (2001) which opined that having some educational level of attainment facilitates entry into high paying jobs such as teaching, produce purchasing clerks, masters of transport stations, lottery vending as well as improving farmers understanding of farming practices and related issues.

The result from the regression table also revealed that the coefficient of farm size was found to be negative but

Table 4. Factors influencing livelihood diversification (SID) of rice farm households in the Ogun State.

Variable	Coefficient	Std. error	t-value
(Constant)	0.985***	0.212	4.65
Age	-0.068**	0.029	2.34
Sex	0.002	0.002	-
Hhs	-0.006	0.006	-
Edu	0.0035**	0.0012	2.92
Farm size	-0.025***	0.006	4.17
Remittance	0.084**	0.035	2.40
Access to cre	0.036***	0.010	3.60
Marital	-0.013	0.015	-
Access to elec.	-0.018	0.019	-
Ext. Visit	0.003	0.007	-
Dist to mkt	-0.009**	0.004	2.25
Prod. Asset	2.956**	1.22	2.42

Source: Field survey (2019). *** significant at 1%, **significant at 5%, *significant at 10%. Number of observations = 158. Pseudo R^2 = 0.681. df = 12.

significant at 1% probability level. This implies that a unit increase in farm size will lead to 3% decrease in livelihood diversification.

Access to credit facility was positive and significant at 5% probability level implying that a unit increase in access to credit will lead to an increase in livelihood diversification.

Distance to market was observed to be negative and significant, which means that the closer the rice farmers are to the market the more diversified their livelihood.

The Tobit regression results show that remittance was found to have positive and significantly affected rice farming household's livelihood diversification strategy at 5% level of significance. If other factors are held constant, a unit increase in remittance will increase the opportunity of the rice farming households to diversify their livelihood by 8.4%. Hence, increasing rural household's remittance income plays a vital role for enhancing and smoothing household consumption problem, strengthen social network/social capital, increase saving and investment, help households gain access to diversified opportunities like trading, and then able to improve their livelihood. The result of this study is consistent with the findings obtained by Gebru et al. (2012) and Mohammed and Tolossa (2016).

Productive asset was found to be positive and significant. The ownership of such assets therefore facilitates entry of the farmers into businesses (farming and non-farming) thereby diversifying their livelihood. This finding is similar to that of Babatunde and Qaim (2009).

SUMMARY

The determinants of income diversification strategies pursued by farming households in the study area were the age, sex, household size, extension visits, education, remittance, farm size, marital status, access to credit, access to electricity, value of productive assets owned and distance to market. The study observed that the older household heads were less diversified in the sources of livelihood they pursue. This study revealed that efforts should be made to build the capacity of the youth to engage in farming and livelihood diversification by the government and other parastatals to enable youth en masse income for investment and also to sustain the farm industry and also diversify their livelihood.

The infrastructure status (such as road and electricity) of the farm economy in the study area should be improved. This may limit entry barriers into both farming and non-farming activities to enable households put their full capabilities into use.

Education was found to be one of the key determinants for livelihood diversification; this is because when the rice farmers are educated, they will be exposed to opportunities outside the rice farming activities.

The distance to market was another factor affecting the rice farming household's ability to diversify, since the closer the rice farmers are to the market the more diversified their livelihood.

Rice farming household's remittance income plays a vital role for enhancing and smoothing household consumption problem, strengthen social network/social capital, increase saving and investment, help households gain access to diversified opportunities like trading, and then able to improve their livelihood.

Conclusions

The study revealed that income from non-farming activities such as self-employment in non-agricultural activities play a huge role in the livelihood diversification of the rice farmers. The rice farmers education in the study area was one of the major factors needed to improve their skills on other form of livelihood in order to enhance their well-being.

RECOMMENDATION

The following recommendations were made from the study:

i. Young people should be encouraged to diversify their livelihood.
ii. The farmers should be educated on ways to diversify their livelihood
iii. Credit facilities should be made available for the farm household either by the government or private parastatals to enhance farming activities.
iv. Production assets of the rice farmers in the study area should be improved on.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of bio-stimulants on the yield of cucumber fruits and on nutrient content

Maria Ługowska

Department of Agricultural Ecology University of Natural Sciences and Humanities, Prusa 14, 08-110 Siedlce, Poland.

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The aim of the experiment was to assess the effect of bio-stimulants of different composition (Asahi SL - based on three nitrophenol compounds, naturally occurring in plants, as active ingredients; Optisil – mineral growth stimulant containing 24 g L^{-1} of Fe and 200 g L^{-1} of SiO_2; Kelpak SL – manufactured from *Ecklonia maxima* and containing auxins and cytokinins) on the content of macronutrients and micronutrients in cucumber fruits (*Cucumis sativus* L.) and on the fruit yield of the Akord F1 variety. With three replications, the field experiment was conducted in a completely randomised design on a private farm in Wólka Leśna in Poland (N 52°11'59", E 22°24'30"). The experiment was carried out in three growing seasons between 2015 and 2017. Three types of growth regulators and a cucumber variety were used as experimental factors. During the experiment, it was found that in response to bio-stimulants, fruit protein content and the yield significantly increased relative to control. The highest yield was noted after application of the bio-stimulant containing Fe and SiO_2. Additionally, the above products applied to cucumber plants significantly increased magnesium content in the fruits but decreased the amount of sodium. Similarly, higher amounts of iron, manganese, and boron in relation to control were recorded, and in the case of Optysil the content of Zn and Co also increased.

Key words: *Cucumis sativus* L., growth stimulators, macronutrients, micronutrients, protein.

INTRODUCTION

The highly competitive food market of the present times has forced farmers to find new crop growing methods that will allow them to obtain marketable yields of the highest quality. Increasingly, safety of the offered food and care for the natural environment are becoming a priority (Mikiciuk and Dobromilska, 2014; Wierzbowska et al., 2015). On the other hand, manufacturers and farmers must ensure that the technologies used in food production will make use of yield potential of plants in a maximum way. Therefore, due to the pressure exerted on food quality, farmers progressively apply new farming methods. Now, substances with bio-stimulating effects are used growingly around the world, which is an opportunity for the development of sustainable methods of agricultural production in the future (Calvo et al., 2014; López-Bucioa et al., 2015; Chagas Junior et al., 2019).

Increasingly, higher and better quality yields and reduced use of mineral fertilizers and chemical plant protection products are considered a primary issue (Owen et al., 2015; Filipczak et al., 2016). Biological

E-mail: maria.lugowska@uph.edu.pl

Table 1. Soil chemical properties before the experiment (data provided by OSCHR).

Element	Concentration	Rating
Concentration of available macronutrients (g kg^{-1})		
P_2O_5	0.224	Very high
K_2O	0.165	High
Mg	0.056	High
Concentration of available micronutrients (mg kg^{-1})		
B	0.21	Low
Mn	147.0	Medium
Cu	1.3	Low
Zn	5.3	Medium
Fe	1030	Medium

products are extremely effective in plant protection as they are eco-friendly to plants, soil environment, and human health. Substances with bio-stimulating effects increase the resistance of plants to environmental stresses, provide nutrients to plants, and trigger physical changes in the soil (Babuška, 2004; Schwarz et al., 2010; Paradikovic et al., 2011; Zarzecka and Gugała, 2012). Moreover, the literature increasingly points out to an increase in nutrient content in crops after the use of bio-stimulants of different compositions (Wierzbowska et al., 2016; Carillo et al., 2019; Chagas Junior et al., 2019). With their higher demand for nutrients, new crop varieties require even greater intensification of alternative methods of crop growing and plant protection (Bulgari et al., 2015). Their use has beneficial effect on the quality and amount of crop yields, maintaining the full safety of food products at the same time. The literature usually deals with the effect of different types of stimulants on initial plant growth, biomass growth, and on the yield (Boehme et al., 2005; Azizi and Mahmoudabadi, 2013; Musale et al., 2018; Chagas Junior et al., 2019). On the other hand, studies on the effects of growth stimulants on the chemical composition of edible parts of plants can rarely be found, which is why the aim of the experiment was to determine the effect of three bio-stimulating products with various active ingredients on macronutrient and micronutrient content in cucumber fruits of the Akord F1 variety and on their yield.

MATERIALS AND METHODS

The field experiment was conducted in three growing seasons between 2015 and 2017. It was set up on a private farm in Wólka Leśna in Poland (N 52°11'59" E 22°24'30") at the beginning of May, 2015, with a random design, three replications, and a plot area of 40 m^2. The soil on which the experiment was conducted was moderately heavy and its chemical and physical properties were determined by the OSCHR (Regional Chemical and Agricultural Station) in Warsaw. It was slightly acidic with the pH in 1 N KCl of 6.49. The concentration of macronutrients and micronutrients in the soil before the start of the experiment is presented in Table 1.

Mineral N was applied in split doses at 120 kg ha^{-1} in the form of ammonium nitrate. Half the dose was added before sowing, mixing the fertilizer with the soil, while the rest was divided into two portions and used as top dressing. The first was applied when plants had 3-4 leaves and the other at the beginning of flowering. K and P were not added to the soil due to their high content there. In the first year, the crop preceding cucumber was sweet clover, which was ploughed into the soil in the previous autumn; in the second year it was soybean and spring wheat in the third. Meteorological conditions during the experiment are given in Table 2.

Experimental factors

1. Kelpak SL, Asahi SL, and Optysil bio-stimulants in doses recommended by the manufacturers;
2. Control (without bio-stimulants);
3. Akord F1, a cucumber variety.

Characteristics of bio-stimulants

Asahi SL is a bio-stimulant with three active substances of the nitrophenol group naturally occurring in plants. It contains sodium para-nitrophenolate (0.3%), sodium ortho-nitrophenolate (0.2%), and sodium 5-nitroguaiacolate (0.1%). Optysil is a mineral growth stimulant that contains 24 g L^{-1} of Fe and 200 g L^{-1} of silicon dioxide SiO_2. Kelpak SL is produced from *Ecklonia maxima* (a seaweed species growing in the southern oceans), and in its composition contains auxins and cytokinins (11 and 0.03 mg L^{-1}). The use of bio-stimulants during the growing season of the cucumber and its doses are given in Table 3.

Cucumber seeds were planted directly into the soil on 26/05 in the first year, on 23/05 in the second year, and on 23/05 in the third year. In 2015, cucumber were harvested on 21/07 for the first time and on 27/08 for the last time; in 2016 on 14/07 for the first time and on 15/08 for the last time; and in 2017 on 17/07 for the first time and on 18/08 for the last time.

The content of ash, dry matter, macronutrients, protein, and micronutrients in fresh cucumber fruits was determined. Chemical analyses of the content of the aforementioned components were performed in the OSCHR (the Regional Chemical and Agricultural Station) in Warsaw. In each growing season, individual bio-stimulants did not affect the content of dry matter, ash, protein,

Table 2. Meteorological conditions during the research (data provided by the Meteorological Station in Siedlce, Poland).

Year	2015	2016	2017	2015	2016	2017
Month	Temperature (°C)			Precipitation (mm)		
01	0.7	-4.2	-4.7	51.4	26.6	11.4
02	0.5	2.9	-1.7	0.7	56.2	34.2
03	4.8	3.3	5.6	53.1	46.4	36.4
04	8.2	8.9	7	30	50.2	81.6
05*	12.3	14.6	13.6	100.2	35.5	45.6
06*	16.5	18.1	17.6	43.3	55.6	59.9
07*	18.7	19	18.1	62.6	126.8	72.1
08*	21	17.9	18.8	11.9	58.2	52.6
09	14.5	14.4	13.7	77.1	15.4	112.3
10	6.5	6.8	9.2	39	161.2	90
11	4.7	2.4	4.2	42.2	39.4	46.4
12	3.7	0.4	1.9	16.5	46.5	27.6
Mean total	9.3	8.7	8.6	528	718	670

*Cucumber growing season.

Table 3. Doses and application times of bio-stimulants.

Bio-stimulant	Characteristics
Kelpak SL	The first treatment at the 2nd-4th leaf stage (BBCH* 12-14), the next two at intervals of 14 days, all treatments with a single dose of 2 L ha^{-1}).
Asahi SL	The first treatment at the stage of the second true leaf on the main stem (BBCH 12-14), with three treatments at intervals of 7 days, each treatment with a single dose of 0.6 L ha^{-1}.
Optysil	5 treatments: 1- the stage of 3-5 true leaves on the main stem (BBCH 13-15); 2 – the stage of 6-9 leaves on the main stem (BBCH16-19); 3 - the stage of 1-2 flower initials with elongated ovary visible on the main stem (BBCH 51-52), 4 - the stage of 6-7 flower initials with elongated ovary visible on the main stem (BBCH 51-52); 5 - after the first harvest (BBCH 71), all treatments with a single dose of 0.5 L ha^{-1}

* The *BBCH*-scale is used to identify development stages of plants.
Source: Adamczewski and Matysak (2005).

macronutrients and micronutrients in cucumber fruits in a statistically significant way. For this reason, the average values for the effect of Kelpak SL, Asahi SL, Optisil and for control for the whole period of 2015 to 2017 were provided. Single factor analysis of variance for multiple groups was used to analyse the results, and a detailed comparison of means was made using Tukey's test at $p \leq 0.05$. Calculations were carried out with the statistical software Statistica 12.

RESULTS AND DISCUSSION

Application of bio-stimulants to cucumber plants of the Akord F1 variety resulted in a large variation in protein content, with statistically significant differences between all kinds of treatment (Table 4).

The highest content, 25.2% higher than in control fruits, was noted on plots with the bio-stimulant produced from *E. maxima* and containing cytokinins and auxins (Kelpak SL) as well as the lowest on the control plot. A similar statistically significant effect of this stimulant on protein content in grass was reported by Ciepiela and Godlewska (2014). Additionally, Zodape et al. (2010) confirmed the effect of seaweed extract on the growth of protein content in bean plants. In the present experiment, dry matter content in plants treated with Kelpak SL, based on *E. maxima*, increased by 15.5% in relation to the control plot. However, cucumber fruits did not respond in a statistically significant way with a higher amount of dry matter to the other bio-stimulants. According to the studies of Gawlik-Wolska et al. (2010), the application of bio-stimulants based on sea algae enriched with P and K and a mineral bio-stimulant to tomato plants did not affect the increase of this parameter in relation to control. Similar results were obtained by Maciejewski et al. (2007)

Table 4. Effects of bio-stimulants on selected parameter content in cucumber fruits and on the yield.

Treatment	Dry matter [%]	Ash [%]	Protein [%]	Yield [kg ha^{-1}]
Asahi SL	3.30^{AB}*	0.636^{AB}	1.17^{B}	18350.0^{B}
Kelpak SL	3.50^{A}	0.630^{AB}	1.24^{A}	17850.0^{B}
Control	3.03^{B}	0.616^{B}	0.99^{D}	16000.0^{C}
Optysil	3.20^{AB}*	0.643^{A}	1.10^{C}	20250.0^{A}
Year	**Growing season effect****			
2015	3.20^{B}	0.63^{A}	1.13^{AB}	17950.0^{B}
2016	3.33^{A}	0.63^{A}	1.14^{A}	19275.0^{A}
2017	3.25^{AB}	0.63^{A}	1.09^{A}	16800.0^{C}

* - the means in columns marked with the same letters do not differ significantly at P = 0.05; **The above means represent results of the experiment carried out between 2015 and 2017.

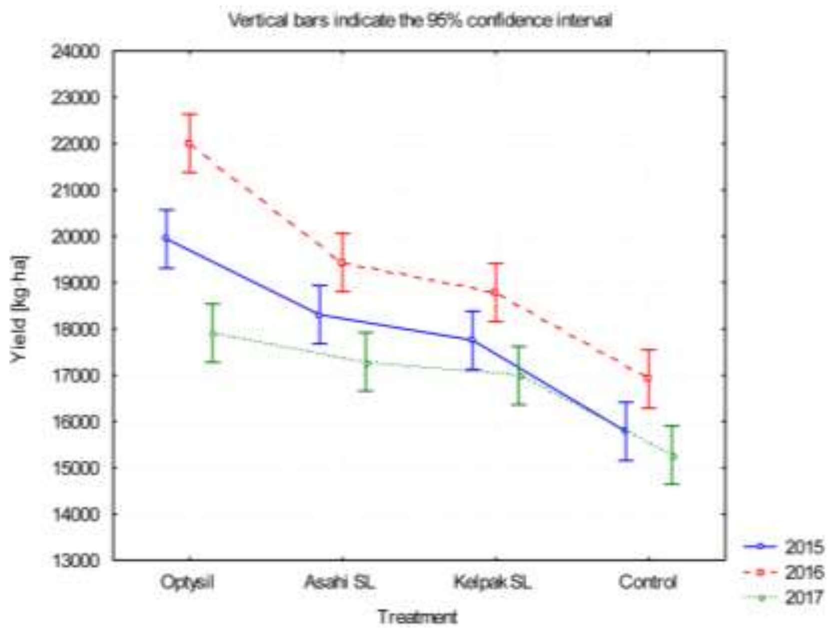

Figure 1. The yield of cucumber fruits throughout the experiment.

and Sawicka and Mikos-Bielak (2008), who after using Asahi SL did not record an increase in dry matter content in potato tubers. In this regard, Łyszkowska and Gajc-Wolska (2008) as well as Gawlik and Gołębiowska (2008) point out that this content is dependent not only on applied bio-stimulants but also on the species of plants.

The bio-stimulants significantly affected the yield of cucumber fruits. In all three growing periods, the lowest yields were obtained on the control plot. Plants treated with Asahi SL and Kelpak SL had their fruit yields at a similar level, significantly higher than from the control plot (Table 4). An increase in potato tuber yields by 14.2 and 24.7% was also recorded by Wierzbowska et al. (2015) after application of the Kielpak SL bio-stimulant. Compared with the effects of other bio-stimulants, Optysil significantly increased the yield of cucumber fruits in 2015 and 2016. In 2017, the fruit yield of plants treated with Optysil was significantly higher than from the control and higher than from plots treated with the other bio-stimulants. The increase in the fruit yield after applying the biostimulant based on Fe and SiO_2 (Optysil) was 26.6% higher than on the control plot. Cucumber fruits growing on the control and on plots where Asahi SL and SL Kelpak SL were applied had the highest yield in 2016, while in 2017 it was significantly the lowest. A similar trend occurred in the case of Optysil, except that the yield in different growing seasons varied significantly from each other (Figure 1).

A significant increase in the yield from individual plots in 2016 was related to the high amount of rainfall and its

Table 5. Effects of bio-stimulants on macronutrient concentration in cucumber fruits, average of three growing seasons (mg 100 g^{-1} DM).

Treatment	Ca	Mg	K	Na	P
Asahi SL	15.9^{AB}*	20.7^{AB}	298.0^{A}	0.85^{C}	39.6^{A}
Kelpak SL	15.9^{AB}*	21.3^{A}	291.2^{AB}	0.91^{B}	37.9^{BC}
Control	14.6^{B}	19.0^{C}	284.4^{AB}	1.15^{A}	36.9^{AB}
Optysil	16.3^{A}	20.0^{B}	294.6^{B}	0.68^{D}	38.6^{C}
Year			**Growing season effect**		
2015	15.2^{B}	19.6^{B}	280.4^{B}	0.84^{C}	38.1^{AB}
2016	16.5^{A}	20.6^{A}	302.2^{A}	0.97^{A}	39.1^{A}
2017	15.2^{B}	20.6^{A}	289.5^{B}	0.89^{B}	37.6^{B}

* - the means in columns marked with the same letters do not differ significantly at P = 0.05.

favourable distribution. In various growing seasons, the amount of rainfall varied (Table 2). In 2016, during the most critical time for the growth and yield of cucumber, from May to August, precipitation was 276.1 mm and was definitely higher than during the other growing seasons. According to Lipiński (2016), the demand of cucumber plants for water during a whole growing season ranges from 400 to 450 mm. Fruits of the Akord F1 cucumber variety were characterised by varying levels of macronutrients. Content of magnesium and sodium was significantly higher in plants treated with bio-stimulants (Table 5).

The highest amounts of magnesium were noted in the fruits treated with Kelpak SL, a bio-stimulant which in its composition contains auxins and cytokinins. It was higher by 12.1% in comparison with the fruits collected from the control plot (Table 5). In the case of Na, the applied products contributed to a reduction in the content of this element in the fruits, which was lower by 69.1% relative to control in plants treated with the mineral bio-stimulant containing Fe and SiO_2 in its composition. This bio-stimulant at the same time increased Ca content in the fruits by 11.6%, and the two others increased the content of this element by 8.9% relative to control. The highest content of K and P was noted in plants treated with the bio-stimulant based on substances from the group of nitrophenols. The content of the above elements increased in comparison with the control plot, respectively, by 4.8 and 7.3% (Table 5). Higher content of K (by 12%) and P (by 15%) in fruits of tomato after applying Asahi SL was noted by Ambroszczyk et al. (2016). An increase in macronutrient content in cucumber leaves after bio-stimulant application was also reported by El-Nemr et al. (2012).

There were significant differences in the content of trace elements between fruits collected from the control and those treated with Optysil, the bio-stimulant containing Fe and SiO_2 (Table 6). The highest increase in the content, by 21.6%, 20.0%, and 13.7%, respectively, was observed in the case of Fe, Cu, and B. The bio-stimulant containing substances from the nitrophenol group (Asahi SL) increased the content of Mn by 13.3% in comparison with cucumber fruits harvested from the control plot. Kelpak SL, the product based on marine algae, increased the content of Zn and Cu by 3.7 and 8.6%; however, it was not a significant difference compared with the control plot. The stimulating effect of Kelpak SL on the content of those elements was confirmed by Godlewska and Ciepiela (2016), who recorded a 10.3% increase in Zn content in grass, with a 9.1% increase in Cu content. Similarly, using an extract of red algae Zodape et al. (2009) obtained a 4.9% increase in Zn content in wheat grains.

An increase in Mn, Zn, Co, and Fe content in potato tubers after application of a substance based on brown algae was also reported by Głosek-Sobieraj et al. (2018). In the present experiment, there was a significant difference in the amounts of these trace elements in cucumber fruits between plants treated with Asahi SL and plants treated with Kelpak SL. Weather conditions, both the temperature and the quantity and distribution of rainfall, did not affect the content of Fe, Zn, Mn and B in cucumber fruits. However, Co content was not the same in different growing seasons, and the highest concentration of this element was reported in the year with the largest amount of rainfall (Tables 2 and 6). This was confirmed by research of Gugała et al. (2016), who recorded the highest content of Co in potatoes during a growing season with high precipitation.

Conclusion

The studies were designed to determine the effects of three bio-stimulants on ash, dry matter, protein, macronutrient, and micronutrient content in cucumber fruits of the Akord F1 variety. The products used in the experiment significantly increased protein content in

Table 6. Effects of bio-stimulants on micronutrient concentration in cucumber fruits (w mg kg^{-1} DM)

Treatment	Fe	Zn	Cu	Mn	B
Ashasi SL	1.98^{B}	2.53^{AB}	0.37^{AB}	0.68^{A}	0.76^{B}
Kelpak SL	1.94^{B}	2.52^{AB}	0.38^{AB}	0.66^{A}	0.78^{B}
Control	1.85^{C}	2.43^{B}	0.35^{B}	0.60^{C}	0.73^{C}
Optysil	2.25^{A}	2.54^{A}	0.42^{A}	0.63^{B}	0.83^{A}
Year			**Growing season effect**		
2015	1.99^{A}	2.50^{A}	0.37^{B}	0.65^{A}	0.77^{A}
2016	2.03^{A}	2.53^{A}	0.39^{A}	0.65^{A}	0.77^{A}
2017	1.99^{A}	2.48^{A}	0.38^{AB}	0.64^{A}	0.78^{A}

* - the means in columns marked with the same letters do not differ significantly at P = 0.05.

cucumber fruits. The highest content was observed after the use of the bio-stimulant produced from marine algae. In addition, all products used in the experiment significantly increased the yield. The highest yield was recorded after the use of the stimulator based on Fe and SiO_2, in the growing period with the highest precipitation. The bio-stimulants used in the experiment significantly lowered Na content in relation to control. In contrast, the highest Mg content was recorded after the use of the seaweed extract, with the increase significantly different from the effects of other treatments. In addition, the bio-stimulants significantly increased Fe, Mn, and B content in cucumber fruits in relation to control, and in the case of Optysila, the content of Zn and Cu as well.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Community based participatory forest resources management practices in Chilimo forest, Dendi District, West Shewa Zone, Oromia Regional State, Ethiopia

Dereje Mengist[1] and Mulugeta Alemu[2*]

[1]Life and Earth Sciences Institute (Including Health and Agriculture), Pan-African University, Ibadan, Oyo State, Nigeria.
[2]Department of Plant Biology and Biodiversity Management, College of Natural Science, Addis Ababa University, Addis Ababa, Ethiopia.

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This research has made an endeavor to analyze the practices of community based participatory forest management and its impacts on the incomes of the forest user groups and the forest cover of Chilimo forest, Dendi District, Ethiopia. 380 households were selected from seven peasant association proportionally and simple random sampling was adopted to choose the sample households from each peasant association. Both descriptive and inferential statistics were used to analyze and interprete the data. The income of forest user groups and the Chilimo forest cover were enhanced as a result of community based participatory forest management. Forest revenue and the introduction of some agricultural activities are attributed to the income improvement of the forest user groups. The statistical test result showed that there is a significant income difference (U=10078.5, P=0.00) between forest user group and non-forest groups. The magnitude of land use in general and forest cover change in particular was drastically changed between 1990 and 2010 at Chilimo forest. A significant forest cover change variation (P=0.00) within 1900-2010 has been observed. Plantation of seedlings and protection of existing trees are the major factor for the regeneration of the forest cover. A better outcome of participatory forest management can be achieved if the government supports the forest user groups to ensure its sustainability and expand the forest user group income generating activities into the whole cooperatives.

Key words: Community based participatory forest management, income, forest user groups, Chilimo, forest cover change.

INTRODUCTION

International agencies and organizations have jointly consented to cooperate in the reduction of Greenhouse Gas Emissions (GGE) from different anthropogenic activities. Hence, various climate friendly initiatives, treaties and conventions have been ratified in the last couple of decades. The Kyoto Protocol was the breakthrough in this regard. It was signed with an ambition to reduce the GHG of industrialized nations by 5%. It was assumed that the developed nation should contribute to 20% reduction in the overall emission of the

GHG. The most recent one in light of the higher ambition to limit temperature rise by 1.5 ^{0}C and net zero emissions by 2050 was signed at Paris in 2015 (FAO, 2015). The report also highlighted that the forest sector is among the top priority which offers some of the most effective methods for achieving this.

The Ethiopian Government is carrying out different policy measures and programs in order to lessen deforestation and reduce greenhouse gas emissions from deforestation and forest degradation. The forestry sector has been identified as one of the fast-track implementation pillars for achieving high CO_2 sequestration. According to May et al. (2011), the government is fully committed to the Reduction Emissions from Deforestation and Forest Degradation (REDD+) program as an integral part of the national Climate Resilient Green Economy (CRGE) strategy. Another important milestone by the government is its endorsement of the country's first forest policy and proclamation in 2007, with a set of incentives encouraging private sector and community participation in forestry activities (UN-REDD, 2011). Participatory Forest Management (PFM), an approach that promotes the local people engagement has recently become the very remarkable technique of forest management. PFM gives the communities sense of local ownership and right to manage forests in a sustainable manner. This study was conducted in an area where the local people are carrying out participatory forest management practice through forming Forest User Groups.

In the past, the study area (Chilimo Forest) was under state control and the forest was exposed to a wide range of forest degradation by local residents despite its recognition as one of the National Forest Priority Areas (NFPA). In 1996, Farm Africa, an international NGO introduced the concept of participatory forest management as first pilot project in Chilimo forest (Michelle, 2016). Local communities in Chilimo were structured into forest users groups (FUGs) cooperatives by government and NGOs and signed an agreement with the District Agricultural and Rural Development Office (DARDO) to manage the forest. The FUG, together with representatives of the forestry department of the district office and representatives of the NGO have elected 22 members from 12 FUGs that serve as the executive committee, control committee, development committee, saving and credit committee and forest protection committee (Mohammed and Inoue, 2012). Once again, during the commencement of PFM practice Farm Africa has given the local people an economic incentive to sustainably manage and protect forests by helping them set up forest-friendly businesses such as sustainable timber production.

Despite the work on attitudes of PFM users (Gobeze et al., 2009), socio economic impacts of PFM (Yemiru et al., 2010); PFM impacts on forest cover (Lawry et al., 2015); practice of CBFM in Ethiopia (Winberg, 2010; Wondimagegn and Kaba, 2013) that were conducted in Ethiopia and works on the challenge of PFM (Kassa et al., 2009; Deressa, 2014) comparative study on successful and failed PFM (Mohammed and Inoue, 2012); devolved forest governance that was particularly conducted on Chilimo forest, to my knowledge there have been no studies in Ethiopia in general and in Dendi District in particular that was conducted to explore the incomes difference between FUG and non-FUGs. Through examining the participatory forest management practice in the Chilimo PFM project, this study aimed to fill the gaps in the literature of income difference between forest user group and non- forest user groups as well as to contribute to the already existing literature on participatory forest management in Ethiopia and the study area in particular.

Study objectives

1. To analyze the pattern of participatory forest resource management practice in Chilimo forest area
2. To analyze the impact of participatory forest resource management activities on the incomes of the community in the Chilimo forest area
3. To examine the impact of participatory forest resource management practice on the forest cover changes of Chilimo forest area.

LITERATURE REVIEW

Participatory forest management

The terms such as community forestry, social forestry, joint forest management, and village forestry are used in different countries to indicate participatory forest management. However, Participatory Forest Management is used more often to comprise the other terms. According to FAO (2015) participatory forestry management is a processes and mechanisms which enable people with a direct involvement in forest resources as part of decision-making in all aspects

*Corresponding author. E-mail: mulugetaalex44@gmail.com.

offorest management, including policy formulation process. Drigo et al. (2013) mentioned the following different terms that can used interchangeable with participatory forest management. For instance, Community Forests (Nepal, Mexico, Thailand, Gambia, Uganda, Namibia, Cameroon, Guinea, Nigeria, Senegal, Ethiopia, Chad, South Africa, Sudan, Togo, Burkina Faso), Village Forest (Malawi, Mali, Benin), Social Forestry (Philippines, India), Village Forest (Malawi, Mali), and Joint Forest Management (India).

All these terms, however have the same objective of managing forest through the participation of the local people. According to Gilmour et al. (2004), the common principle of community based forest management (CBFM) is to involve local stakeholders in developing a process for the management of forests. Community Based Forest Management (CBFM) can be defined as collective forest management involving several families or communities for commercial purposes.

Global overview of community based participatory forest management

Community based participatory forest management practice in Ethiopia is a recent phenomenon as compared to other nations. For instance in Nepal, the beginning of official forestry has been recorded back to the 1950s (Gilmour et al., 2004). Philippines has also officially adopted community based forest management in 1995 as its strategy for sustainable forest management to improve the upland communities' socioeconomic condition, decentralize and devolve forest and forestland management (Gregorio et al., 2015). Similarly, in Latin America, Brazil has a good experience as a number of CBFM projects have emerged in the 1990s in the country (Drigo et al., 2013). In Africa, Tanzania has been managing over 500 village forest reserves and 1,000 clan owned forests since 1996 (Gilmour et al., 2004). Further, Benin, Cameroon, Burkina Faso, Zimbabwe, Congo have been practicing Community Based Forest Management since the last two decades.

Studies undertaken by different authors (Pokharel et al., 2007; Gobeze et al., 2009; Yemiru et al., 2010) on community-based forest management in Ethiopia indicated that the system has been established in some parts of Ethiopia. These studies witnessed that participatory forest management has brought a significant change in the social assets of the local communities as well as in the management of the forest. However, Pokharel and Nurse (2004) stated that in the Philippines community forestry has reduced PFM non-user groups access to resources, with consequent negative impacts on their livelihoods.

Significance of community based forest management practice

It is obvious that community based forest management practice is becoming a means to improve the livelihood of the community through increasing their income. A study in Nepal showed that the average FUG fund size of about 8,000 in 1996 has increased to 13,000 Nepalese rupees due to CBFM activities during the project lifetime (Pokharel and Nurse, 2004). Another study conducted by Gilmour et al. (2004) in Terai districts of Nepal showed that the local income from community based forest management practice amounted to almost 747 million Rupees. This is mainly due to implementation of different income generating activities under PFM. For instance, according to Gobeze et al. (2009) the annual income generated per household from wild coffee and honey was ETB 179 and ETB 127, respectively before the introduction of participatory forest management. These levels rose to ETB 582 and ETB394 respectively after the implementation of participatory forest management. It is evident that CBFM is important in shifting the communities' livelihood from dependence on forest products to diversified livelihood. The study conducted in Chilimo forest has also shown that the introduction of PFM increased agricultural income of FUGs (Kassa et al., 2009). Further, improved income and livelihood diversification have given the locals the commitment and sense of ownership to manage their forest sustainably. A study in Dendi, Ethiopia stated that the driving factor for better forest protection and forest regeneration in the area was the enhancement of the financial assets of the project members (Getacher et al., 2012).

In addition to this, participatory forest management implementation reportedly enhance the forest cover due to the limitations on forest resource extraction and the community desire for timber harvesting and charcoal production was reported to have ceased completely or decreased greatly (Winberg, 2011). Kassa et al. (2009) also observed that prior to participatory forest management, the Chilimo forest in Ethiopia was protected by the government and people have been exploiting the forest resources through illegal cutting and pit sawing. But, after the PFM implementation in Chilimo forest, the natural forest has recovered. Similarly, in Adaba-Dodolla, total stem density (a measure of forest growth) of four selectedspecies had higher density under participatory management as compared with forests that had not adopted this type of management (Lemenih et al., 2015).The study from Bonga also shows a healthy vegetation structure, with higher seedling, sapling and mature trees in PFM forests than in adjacent non-PFM forests (Gobeze et al., 2009).Once again another study conducted in Nepal showed that canopy cover of community forests increased from 11 to 23% in the

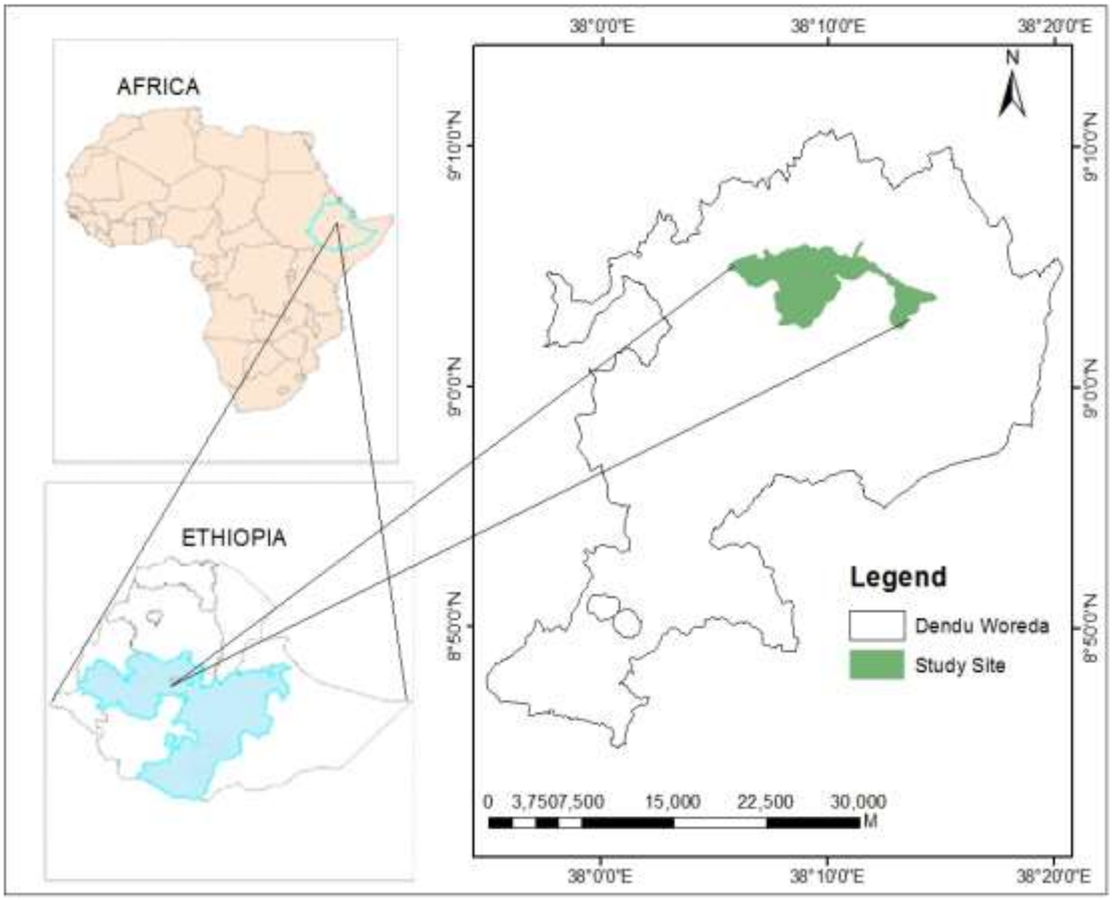

Figure 1. Map of the study area Country map of Ethiopia, showing geographic location of study area.
Source: Authors data obtained from satellite image.

Dhaulagiri hills due to the forest user group activity between 1996 and 2001 (Pokharel and Nurse, 2004).

MATERIALS AND METHODS

Description of the study area

The study was conducted in Dendi District of the Oromiya Regional State. Dendi District is one of the eighteen districts of West Showa Zone, as shown in Figure 1.

The district capital, Ghinchi is located at seventy-five kilometers West of Addis Ababa on the Addis Ababa-Naqamte road. The district has a total area of 109,729 m^2 with an altitudinal range from 2000-3200 m.a.s.l (Mohammed and Inoue, 2012). The population of the district is 209,554. It has 48 rural peasant associations and 7 urban and semi-urban peasant association, out of which 5 towns like Ginchi, Olankomi, Asgori and Bodda Asgori have municipal status (Deressa, 2014). The district is endowed with natural flora and fauna species which can attract tourists and researchers. Among these tourist destination sites, Chilimo Forest is one of the 58 National Forest Priority Areas of Ethiopia. Chilimo forest, the center of which is located at coordinates of 9° 5′ north latitude and 38° 10′ east longitude, is one of the very few remaining dry afromontane forests found in Ethiopia. The main species in the canopy layers are Junipers procera, *Podocarpus falcatus, Prunus africana, Olea europapaea subspecies cuspidata, Hagenia abyssinica, Apodytes dimidiata, Ficusspp, Erythrina brucei, and Croton macrosytachus* (Deressa, 2014). According to the locals, Chilimo was named by the then Emperor Minilik. “Chilimo” means dark in the local language, describing the once dense natural forest.

Data source and data collection

Both primary and secondary data sources were used for the accomplishment of this study. Primary data were collected from questionnaire, key informant interview, and observation. The questionnaire was validated and tested in the field before using it. The source of secondary data included published and unpublished materials, that is, books, journals, project reports, and maps. Further, online sources such as United States Geological Survey database (USGS) site were also used in order to get the imagery data of the study area and related information about forest cover

Table 1. Sample population of the study.

Peasant Associations (PAs)	Total number of the household	% from total household	Share of PAs from 380 household
GareArera	1150	15.5	59
Galessa	1028	13.8	53
DanoEjersa Gibe	1134	15.3	58
Tanko	936	12.6	48
YubdoLagaBatu	830	11.2	42
Galessa Kota Geshar	1122	15.1	58
QabaBareda	1200	16.2	62
Total population	7400	100	380

Source: Authorcomputed based on Dendi District Administration Office.

change. In order to collect data, the researcher used questionnaire, key informant interview, and observation methods.

Sample and sampling procedure

A multi-stage sampling procedure that involves, purposive and simple random sampling was used in this study. The first stage of sample choice was the selection of the peasant associations purposively. Then, 380 households were chosen proportionally from each peasant association. Again, simple random sampling was used to choose an equal number of households from two groups i.e. forest user groups and non-forest user groups.

There are seven peasant associations which surround the Chilimo Forest area. According to Dendi District Administration Office, a total household of 7400 live inside and around the seven peasant associations, out of which, 1439 households are participating in the 12 FUG cooperatives of participatory forest management project. Whereas, there are 5961 households who are living in the seven peasant associations who are not participating in forest management project scheme. All the 12 forest user groups are located in the seven peasant association. Hence, these peasant associations were selected purposively.

The sample selection method employed the following simplified formula provided by Yamane (1967) to determine the required sample size at 95% confidence level and with 5% level of error.

$$n= \frac{N}{1+\mathrm{N(e)2}} \quad (1)$$

Where; ‘n’ = is sample size, ‘N’= is the population size (total households) and ‘e’= margin error.

Using the above formula a total 380 households were selected from the two separate independent groups. The households from each peasant association were selected proportionally according to the total sample size of the household (Table 1). Again an equal number of forest user and non-forest user group were selected from each peasant association using simple random techniques. The reason for selecting two separate groups was to make a comparison and analyze to what extent the PFM has created an income difference between the two groups.

Research design

The study aimed at exploring the patterns of participatory forest management practice, the impact of participatory forest management practice on the incomes of the FUGs, and the impact of PFM on the forest cover in Chilimo forest area. In order to accomplish this, both categories of qualitative and quantitative research approaches were used. The data were analyzed and interpreted in the form of frequency, percentage, tables and charts while qualitative approach was used in describing and portraying accurately participatory forest management practice in the study area.

Methods of data analysis

Data collected through structured interview schedule were coded and processed using SPSS Version 20 software. In order to analyze the data, both qualitative and quantitative data analysis techniques were employed.

The descriptive statistics such as frequencies, percentages, cross tabulation, measures of central tendency, standard deviation, standard error of mean, minimum and maximum were employed in presenting and summarizing the quantitative data. On the other hand, the qualitative data were analyzed based on describing and portraying accurately the participatory forest management practice, incomes of the community, and the forest cover changes in the study area. The qualitative data include the key informant’s data that were collected from Farm Africa, Forest User Group Committee, and the District Agricultural office.

The strength and direction of the relationship between the different selected independent variables and the dependent variable (income) were examined using correlation and multiple regression analyses. Correlation between independent variables and between dependent and independent variables were used in order to measure the strength of the relationship between variables. Correlations were also used to check the collinearity problem among variables. Once again, multiple regression was used to analyze the relationships between the independent variables (family size, age, education, marital status) with the dependent variable (income). The aim of regression analysis was to see whether these variables have an impact on the income level of the households. Among the independent variables participation in PFM and education level were again categorized using dummy variables to allow the regression look at directionality by comparing two sides, rather than expecting each unit to correspond with some kind of increase. A dummy variable is a variable that assumes only a finite number of values (such as 0 or 1) for the purpose of identifying the different categories of a qualitative variable (Eyisi, 2016). Hence, each categorized variables (non-participant and participant under participation and no education, primary education, and secondary education under education status) were again coded as 0 and 1 numerical value. While, the other variables were used as continuous variables.

Mann-Whitney U test was used to investigate the income difference between the forest user groups and non-forest user groups. This test was chosen because of the non-normality distribution nature of the data. Further, according to Eyisi (2016) the data meet the following assumptions; the data available for analysis have been independently and randomly drawn from their respective populations, the measurement scale is at least ordinal, and the variable of interest (income) is continuous.

Moreover, Erdas Imagine Software and Arc GIS 10.3 were employed to analyze the spatial forest cover changes of the study area. Erdas Imagine is an image processing software package that allows users to process geospatial and other imagery as well as vector data. All the three images (1990, 2000 and 2011) data downloaded from Land Sat Enhanced Thematic Mapper Plus (ETM +) were analyzed using Erdas Imagine software and ARC GIS software. Also, one-way analysis of variance (ANOVA) was used to test the significant variation of the forest cover and the significant variation between three land uses.

RESULTS AND DISCUSSION

Patterns of participatory forest management practice

Organization and selection of forest user groups

Decentralized forest resource management in Ethiopia was initiated in the 1990s with the collaboration of international non-governmental organizations to mitigate natural resource degradation and to support the livelihood of the local people (Mohammed and Inoue, 2012). Chilimo and Bonga PFM sites were the first participatory forest management pilot project (Stephanie, 2016).

The district agricultural office experts reported that there are twelve FUG cooperatives under Chilimo forest that are taking part in participatory forest management practice. However, the PFM was first established through forming the forest user groups and later the forest user groups restructured in to forest user group cooperatives.

The Dendi agriculture experts also added that in Chilimo each FUG cooperative has their own elected executive committees which comprised the head of FUG, vice head, secretary, and cashier. These committees are responsible for a particular activity in the management of the forest. For instance, the head of FUG is responsible to receive appeals from non-members when unforeseen events such as fire happen in their residential areas. Apart from the executive committee, each cooperative has forest management committee comprising the head, vice head, and secretary. The main task of this committee is to organize and coordinate members in the management of the Chilimo forest.

Unlike other PFM sites of Ethiopia such as Adaba-Dodola where criteria such as forest carrying capacity are used to choose FUG members, the condition used to be members of the Chilimo forest user groups cooperatives depends on the following criteria. These criteria include the interest of an individual, the distance between the forest and the residential area of the households, awareness about PFM, availability during the establishment of PFM in the area, independence from family members, age limit, and membership fee.

Deressa (2014) noted that membership fee in Chilimo PFM was not the requirement to join FUG. It became a criterion after the FUGs were merged into FUG cooperatives. Although the fee is marginal, in some FUGs a certain period is given for poor members of the community to pay their membership fee.

Among all the FUG selection criteria used in Chilimo PFM, the distance between the forest and residents house is the main factor that determines one's membership application for a particular FUGs cooperatives. The household survey also examined the non-forest user group reasons for their exclusion from FUGs membership and the result revealed that 35% of the non-FUG members were excluded from membership due to their residence out of the forest periphery (Table 2). The FUG committees also stated that if a member is residing very far or out of the periphery, it would be difficult to keep the forest and easily take part in the participatory forest management activities. Due to these, they do not choose members who are living very far from the forest area. Lack of interest by the households is the second major factor for the exclusion of non-FUGs from membership. However, some of the non-FUGs admitted that they feel sad for missing the opportunity and they would consider the options to join the cooperative in the future.

Role of forest user groups in the forest

The forest user groups in the Chilimo forest often carry out one of the following activities simultaneously on weekly bases. These activities include nursery plantation, protection of existing trees, protection of regenerating trees, and protection of harvestable trees. The household survey shows that 87% of the FUGs carry out protection of regenerating trees, whereas 86% take part in the protection of the harvestable tree. Other activities, that is, nursery plantation and protection of existing trees are practiced by 22.1 and 62.6% of the respondents respectively. These activities are determined by variables such as education level, age, and land size of the forest user group. The statistical test result showed that education level has significant (x^2=18.4, p = 0.001) effect on the plantation activities of the forest user groups. Hence, members who are educated (members who have

Table 2. Non-FUG reasons for the exclusion from FUG membership.

Reasons for exclusion	N	%
Lack of interest	53	27.9
Residing very far from the forest	66	34.7
Lack of awareness	16	8.4
Financial problem to pay a membership fee	12	6.3
Dependence on parents	13	6.8
Unavailability in the PFM area	10	5.3
Age	2	1.1
Refusal by the committee	18	9.5
Total	190	100.0

Source: Household survey data (2017).

Table 3. The Effect of Education on the PFM activities.

PFM activities		Education Level			χ2 /P-value
		No education	Primary education	Secondary education	
Carry out plantation in the FUG	Yes	49	54	16	18.4/ 0.000
	No	12	51	8	
Total				190	
Protection of existing tree in the FUG	Yes	55	64	14	8.85/ 0.012
	No	11	36	10	
Total				190	
Protection of regenerating tree in the FUG	Yes	62	85	20	3.52/ 0.172
	No	4	15	4	
Total				190	

Source: Household survey data (2017).

completed primary and secondary education) plant more seedling than un-educated (non-members who have no education). Once again, the result indicated that education level has significant (x^2=8.85, p = 0.012) effect on the protection of existing trees (Table 3). This implies that the educated (household who have completed primary and secondary education) members have more awareness about the importance of forest conservation. Therefore, they were able to take part in guarding and patrolling of the forest as compared to un-educated members.

The landholding capacity of forest user group is highly associated with the participatory forest management practices. The statistical test indicated that land size of the households has asignificant effect on the seedling plantation(x^2=10.53, p=0.005) and protection of existing trees (x^2=8.76, p=0.02). Unlike the case of Chhetri (2005) where the participation of the respondents in forest management activities tended to be enhanced with the decrease in the land ownership of an individual, in Chilimo the forest user groups who own large hectares of land were enormously participating in the PFM activities. The forest user groups with large land size belong to the weathier member. Hence, they did not devote their time to other income generating activities, rather they invest their time in the PFM activities (Table 4).

The FUG cooperative members can decide how many seedlings to be planted per member. However, Leaders have significant involvement in all three decisions of when, where and what to plant, but final decisions are made by the majority of the forest user groups (Mohammed and Inoue, 2013).

These activities contributed highly to the increment of Chilimo forest cover. The reduction of forest extraction

Table 4. The effect of land size on the PFM activities.

PFM activities		Land size (hectares) <1.5	1.6-3	>3	χ2 /P-value
Carry out plantation in the FUG	Yes	37	57	25	10.53/0.005
	No	39	22	10	
Total				190	
Protection of existing tree in the FUG	Yes	45	58	30	8.76/0.02
	No	31	21	5	
Total				190	
Protection of regenerating tree in the FUG	Yes	63	72	32	
	No	13	7	3	2.97/0.23
Total				190	

Source: Household survey data (2017).

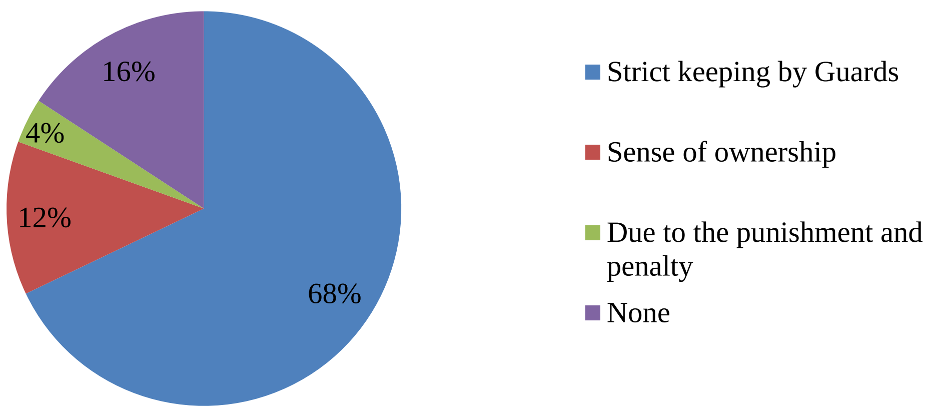

Figure 2. Forest user group reasons for reduction of cutting tree in the forest.
Source: Household survey data, 2017.

and cutting off trees by FUGs is also another factor for regeneration of Chilimo forest cover. These are confirmed by the household survey as 84% of the FUG replied that they have reduced cutting trees after the introduction of PFM in the forest. The strict keeping by guards is the major reason of FUGs for reduction of cutting the tree.

The forest user groups keep the forest as guards in accordance with their schedule in the forest management plan and agreement. The Chilimo FUG chairman reported that the executive committees have delineated the Chilimo forest into three blocks and in each block, the committee deploys four members per day to keep the forest (Figure 2). Each member will be assigned to keep the forest once in a week. It is the responsibility of each member to take part in guarding and patrolling. However, female-headed households only guard nearby planted area against animal trampling (Mohammed and Inoue, 2013). Similarly, a study in Nepal stated that despite their involvement in PFM decision-making activities, women rarely take part in patrolling and guarding activities (Agarwal, 2001). Though in most Nepal and India PFM sites patrolling and guarding are typically the responsibility of male participants.

Impact of participatory forest management on the incomes of the community

Income of forest user groups before PFM and after PFM

All the key informants agreed that participatory forest management practice has improved the incomes of forest user groups. The household survey result shows that the average annual income of FUG's has increased from 3828 ETB to 8952 ETB after the introduction of PFM practice. The introduction of participatory forest management in Bonga, Ethiopia has also increased annual incomes of the household by 8.446% (Gobeze et al., 2009). Also, in Nepal, the average FUG fund size of about 8,000 in 1996 has risen to 13,000 Nepalese Rupee in 2000 (Pokharel and Nurse, 2004).

According to the forest user group committees, seedling sale for different government and non-governmental organization have contributed to the increment in the incomes of forest user group. The committees also noted that sale of logs and seeds have helped the Chilimo of forest user groups to generate and improve their incomes. Pokharel and Nurse (2004) also indicated that in Nepal the forest user group fund was generated from the sale of forest products.

Further, the forest revenue from the sale of timber also contributed to the increment in the incomes of the FUGs. In order to sell the timber products, forest expert team comprising the concerned organization should first conduct a forest assessment and decide on the timber products to be sold in each cooperative. Consequently, the cooperative in collaboration with the district agriculture office announces a tender in Ethiopian Adiss Zemen Magazine. Also, as in the case of Mohammed and Inoue (2013) the plantation timber may be sold through bidding system to local wealthy merchants from the capital city Addis Ababa.

Accordingly, 70% the revenue from the sale will be distributed to the cooperative while 30% will be handed over to the government. Again, the FUG share of revenue will be distributed for different activities. Hence, 45% will be circulated for forest user group members equally, 20% for forest development activities, 30% will be deposited into the cooperatives account for contingency, and the remaining 5% will be invested for development activities such as irrigation, and other infrastructure development.

Similarly, the incomes generated from the sale of timber in other PFM sites were invested in different activities. For instance, about 36% of the income from community forests in Terai, Nepal was spent by the forest user groups on community development activities such as the building of schools, roads and drinking water facilities (Gilmour et al., 2004). However, in order to take the appropriate share of the 45% dividend, the FUGs must follow the rule and regulation stated under the forest management plan. This includes participating in meetings regularly and keeping the forest according to their schedule. A member who is taking part in all of these PFM activities will not get an equal share from the dividend.

The district agriculture office reported that the 70% of dividend which the cooperative gets from the sale of forest products such as trees have also contributed to the improvement in the incomes. The FUGs revenue from timber plantation has shown a drastic increase over time. For instance, before 2007 Chilimo and Mesalemiya FUG cooperatives were able to earn only 392,500 ETB but, both forest user group cooperatives were able to earn 5,086,391 ETB on approximately four occasions after 2007 (Mohammed and Inoue, 2013). Moreover, the introduction of some agricultural activities such as honey production, home garden crop production raised the incomes of FUG as confirmed by our transect walk. However, such agricultural activities are limited to some FUG cooperatives.

Similarly, in other PFM sites of Ethiopia, the incomes of FUGs from agricultural and other non-wood activities have improved following the introduction of PFM. For instance, in Bonga PFM site the annual income generated per household from wild coffee and honey has increased from 179 ETB and 127 ETB to 582 ETB and 394 ETB, respectively after the establishment of PFM (Gobeze et al., 2009). Likewise, a case study in Bale eco-region, Ethiopia discovered that following the implementation of PFM project, the Birbirsa FUG cooperatives total revenue from forest coffee has increased from 153,000 ETB to 1, 200, 000 ETB within two years. Gobeze et al. (2009) stated that the increase in the income of Chilimo FUGs comes partly from increased social and marketing services provided to members by the cooperative, and partly through the extension service and support of Farm Africa for vegetable farming.

Income difference between FUG and non-FUG

The community source of income varies among forest user groups and non-forest user groups. Apart from access to forest products such as dead tree and leaf, the forest user groups have more diversified means of income such as crop production, livestock production, coffee production, honey production, and timber production. Whereas, the non-forest user groups have limited means of livelihood to generate income. Their livelihood is dependent on crop and livestock production. Before the implementation of participatory forest management, they were exploiting the forest for their daily basic needs. However, currently their access to the forest resource is limited due to the strict procedure and regulation.

The household survey result showed that there is an income difference among the incomes of forest user group and non-forest user groups. The Mann-Whitney U statistical test result also indicated that there is significant income difference (U=10078.5, P=.001) between forest user groups and non-forest groups (Tables 5 and 6).

The U test showed that the mean rank of FUG and Non-FUG is 148.5 and 233 Ethiopian Birr respectively.

Table 5. Statistical test shows the income difference between FUG and Non-FUG.

Parameter	Value
Mann-Whitney U	10078.500
Wilcoxon W	28223.500
Z	-7.453
Asymp. Sig. (2-tailed)	0.000

Source: Household survey data (2017).

Table 6. Mean of income ranks for non-forest user group and forest user groups.

Group	N	Mean Rank	Sum of ranks
Non-FUG	190	148.54	28223.50
FUG	190	232.46	44166.50
Total		380	

Source: Household survey data (2017).

This indicated that forest user groups earn a higher income than non-forest user groups.

As it was discussed earlier PFM activities which were carried out by the members have contributed to the income difference between two groups. The non- forest user group cooperatives are not allowed to use forest products since the establishment of PFM in Chilimo forest. While the FUGs generate an income from the sale of other forest products in addition to 70% of the revenue that cooperatives get from the sale of the forest. According to Kassa et al. (2009) particularly those people who are considered very poor are allowed to collect the wood twice a week and they can sell fuelwood. However, they have to pay a monthly fixed fee to the cooperative. It is the responsibility of the cooperative executive committees to decide who is allowed to engage in minor income-generating activities such as sale of fuelwood in the district and the nearby town (Mohammed and Inoue, 2013). They are also responsible to make final decisions regarding who should receive logs and other wood products for subsistence uses (Kassa et al., 2009).

The forest resources benefited the FUGs to use forest wood for cooking/heating and construction purposes, and fodder/pastures for livestock (Getacher et al., 2012). This again has reduced the extra cost that the members would spend to fulfill the above-mentioned subsistence costs. Additionally, the FUG forest management by-law clearly states that when the FUGs house and fence are burned the committee provides a tree for house construction for the members based on different criteria (Deressa, 2014). However, the non-FUGs obviously spend money during such unforeseen events.

Factors affecting the income of forest user and non-forest user groups

There is a significant relationship (r=0.24, p=0.00) between participation in the PFM and the land size of the household. Further, age is significantly related to primary education level (r=-0.226, p=0.00) and family size of the households (r=0.265, p=0.00). The annual income of the household is positively related with land size (r=0.93, p=0.00), participation in PFM (r=0.26, p=0.00), secondary education (r=0.15, p=0.04), and family size of the household (0.26, p= p=0.00). In contrast, there is a negative correlation (r= -0.1, p=0.04) between primary education and annual income of the households. Generally, it can be concluded that there is no problem of collinearity among the variables.

The multiple regressions result indicated that among household-specific characteristics included only the size of land holding and participation of the community in participatory forest management activities are statistically significant in influencing the income of the community. As a result, a one-hectare increase in the land size of a household increased the annual income of a household by more than 7762 ETB, and households who participated in PFM activities earn more 599 ETB annual incomes than non-forest user groups. Further, the regression result showed that households who have completed primary education earn less 393 ETB annual income than households who have no education; while households who completed secondary education earn more 579 ETB annual income than households who have no education. Similarly, a household with one higher level of age, and

Table 7. Regression Result Shows Factors Affecting the Incomes of the Households.

Parameter	B	Sig.	Collinearity Statistics	
			Tolerance	VIF
Constant	-7778.87	0		
Primary Education	-393.556	0.212	0.819	1.221
Secondary Education	579.09	0.307	0.82	1.22
Participation in PFM	599.258	0.045*	0.912	1.096
Land size	7762.003	0.000*	0.874	1.144
Age of the respondent	4.653	0.719	0.737	1.356
Family sizes of the respondent	28.593	0.695	0.749	1.336
R^2	0.865			
Adjusted R^2	0.863			

*Denotes the significance of their corresponding coefficient estimates at 5%.
Source: Household survey data (2017).

family size earn more annual incomes of 5 and 28 ETB respectively than their respective lower variables. The factor affecting income is shown in Table 7.

Chilimo forest cover change

The status of Chilimo forest between 1990 and 2000

According to the Dendi district agriculture experts between 1970 to early 1980's the socialist government (DERG Regime) has replaced the vast agricultural lands that were owned by the feudal members and nobility with huge State-owned plantations for political and economic purposes. The then regime has established a very centralized and strong forestry institution which was capable of demarcating and administering all the forested land. The local peoples stated that intensive forest development activities had been conducted including various plantations activities. The chairman of Chilimo FUG also added that the Chilimo forest cover at the end of 1970's was estimated to be 12,000 ha.

However, administration of forest resource management activities by the central government and ignorance of the local community in the management of Chilimo forest has created a gap between government and the local residents. Hence, the residents were logging and exploiting the forest for different basic needs. It is understood from the discussion with district agriculture that the regime's decision to place armed guards around the forest boundaries to prevent communities logging has failed to prevent clearance of the forest for logging, firewood, and agriculture. Consequently, prior to PFM introduction, the locals were misusing the forest resources through illegal cutting and pit sawing and firewood sales were rampant (Kassa et al., 2009).

Further, following the transition of power from the socialist Derg to Ethiopian People's Revolutionary Democratic Front (EPRDF) in 1991, the government priority has shifted from forest development to agricultural intensification. Hence, the huge state-owned forest was converted into agricultural land for crop and livestock production. As a result, the forest has been degraded through time and the forest cover was registered to be 3292 hectares in 1990. The Satellite image also showed that the agriculture land in Chilimo has been expanded from 510.9 ha in 1990 to 1526.9 ha in 2000. In comparison to the previous regime, EPDRF has ensured the participation of local communities, NGOs and other community-based organization in the management of the forest. One such peculiar example was the introduction of community-based forest practice in Ethiopia. This has marked the beginning of PFM practice in Chilimo forest in 1996. However, according to Farm Africa Experts, the local communities did not understand the benefits of joint forest management at early stages of PFM practice. Further, lack of awareness by members has made it difficult to introduce the PFM practice easily. During early stages of the PFM in Chilimo forest, the management and conservation of forest were assumed the sole responsibility of forest guards and residents around the forest. Hence, the communities were exploiting the Chilimo forest for their daily use Similarly, Gobeze et al. (2009) stated that in Bonga, Ethiopia the burden of protecting the forest against outsider were handled by few community members. In addition to these, Mr, Abera, head of Chilimo Forest has indicated that mass forest degradation in the worebeo site has accounted for the reduction of the Chilimo forest cover. The forest cover details are shown in Table 8, Figures 3 and 4.

The forest cover of Chilimo forest between 2000-2010

The forest cover of Chilimo has started regeneration after

Table 8. The Area Coverage of Chilimoby Year (1990-2010).

Types of land use	Area in hectares (ha)		
	1990	2000	2010
Forest	3291.754815	2527.420274	2907.072896
Cultivated Land	510.916464	1526.900722	636.167028
Grass Land	851.96384	600.431631	1110.93271

Source: Data obtained from satellite image.

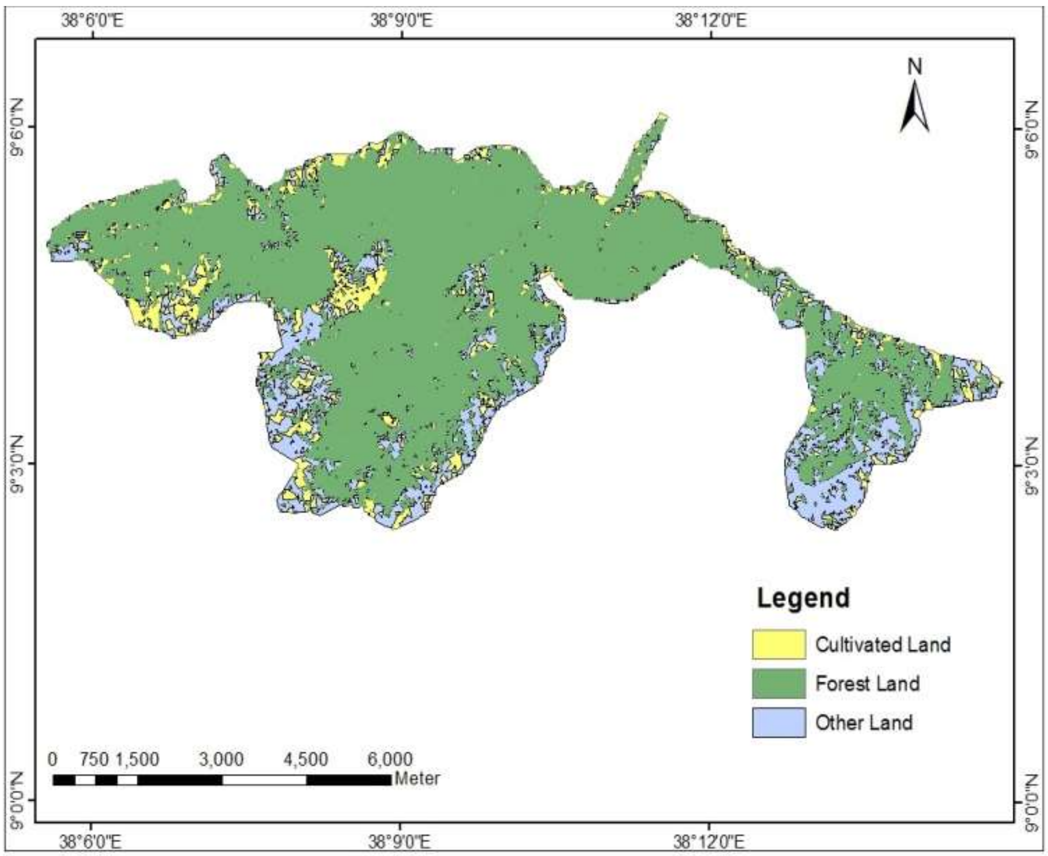

Figure 3. Chilimo Forest Map of 1990.
Source: Computed by author from data obtained from satellite image.

2000 where the FUG has a fully developed sense of ownership and start practicing the PFM very well as a result of improved awareness by the members. The district development agents explained that the increasing change in the forest cover is a result of PFM activities which were carried out by the FUGs. A study in Nepal also revealed that following plantation, protection of denuded hills, and other forest management activities the PFM forest cover showed improvement (Pokharel and Nurse, 2004). Further, A Case Study from Bayombong, Philippines indicated that because of strict forest protection policies, there was a relative increase in area of closed canopy, natural forest, open canopy forest and plantations 2010 compared to 1989 (Hashiguchi et al., 2016).

Farm Africa supported the FUGs by providing training to plant a variety of trees and enabling them to produce more seedlings from their community-managed nursery. These include fast-growing eucalyptus alongside slower-growing and higher-value grevillearobusta, pine and

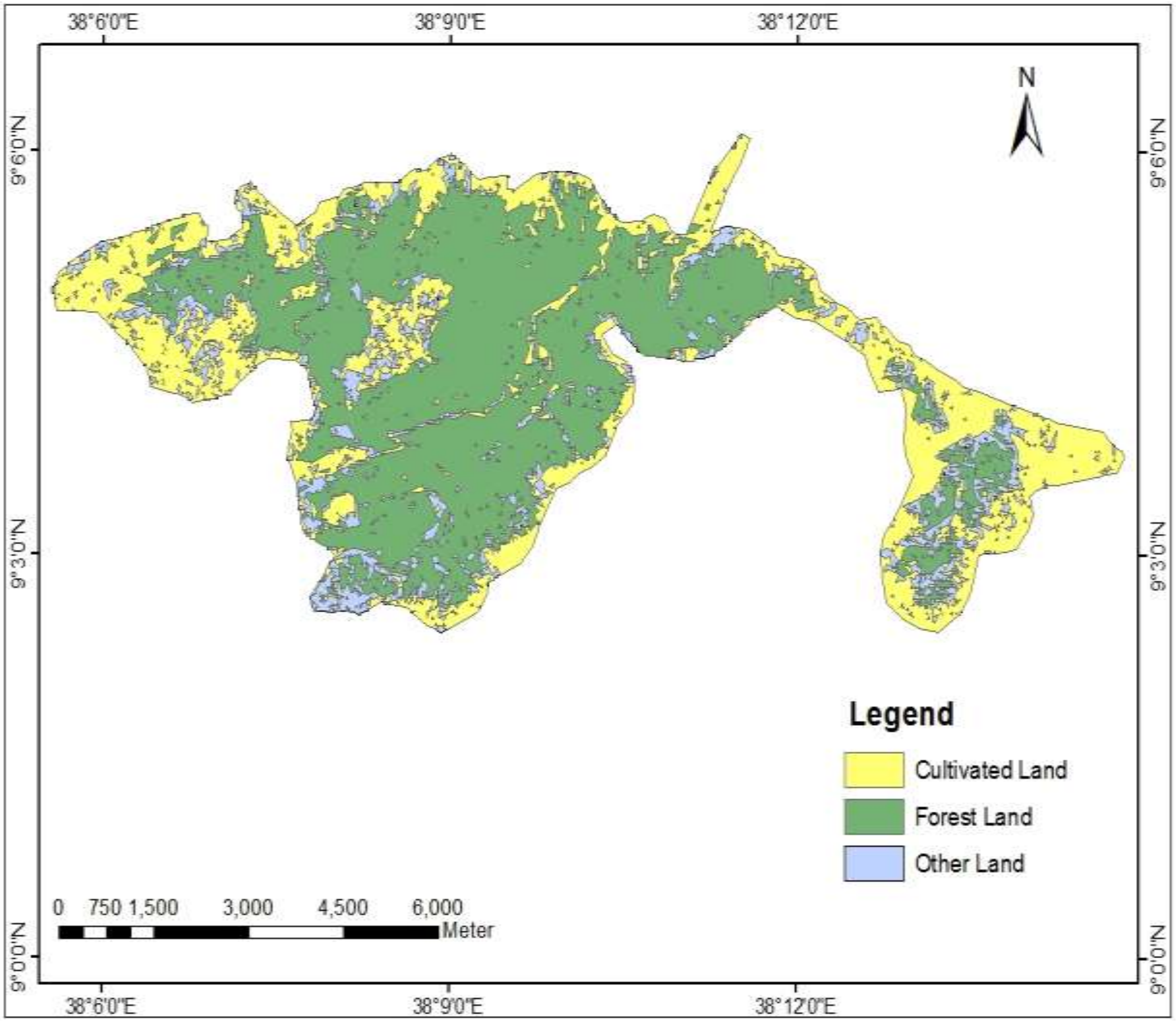

Figure 4. Chilimo Forest Map of 2000.
Source: Computed by author from data obtained from satellite image.

cypress. For instance, in Mesalemiya and Chilimo FUG cooperatives a total of 21,000 seedlings (covering an area of approximately 6.6 ha) and 53,000 seedlings (approximately 21.2 ha) were planted respectively (Mohammed and Inoue, 2013). The species were selected by the local community on the basis of either species-site matching or based on local knowledge. The common species planted were *Grevillearobusta, Cupressuslusitanica*, and *Eucalyptus globules*. In addition to these, some of the forest types were regenerating themselves. Further, the key informant's interview with the Dendi District expert revealed that strict keeping by guards also contributes highly to the improvement of the Chilimo forest cover. Hence, logging and deforestation of the forest have gradually reduced (Figure 5).

The forest cover of Chilimo forest between 1990-2010

Overall, the forest cover of Chilimo has gradually reduced within two decades. The forest cover which used to be 3291.7 hectares in 1990 is found to be 2907 hectare by the end of 2010. This is the result of mass destruction in the early 1990s during the regime changes. Unlike the 1980s where forest management was centralized and highly managed by the government, in the 1990s the concept of state- community joint forest management gives the local forest user open access to the forest. Hence, a tremendous amount of forest has been degraded in the 1990s. The introduction of PFM in the mid-1990s has resulted in improvement in the forest cover. However, it needs time to bounce the forest cover

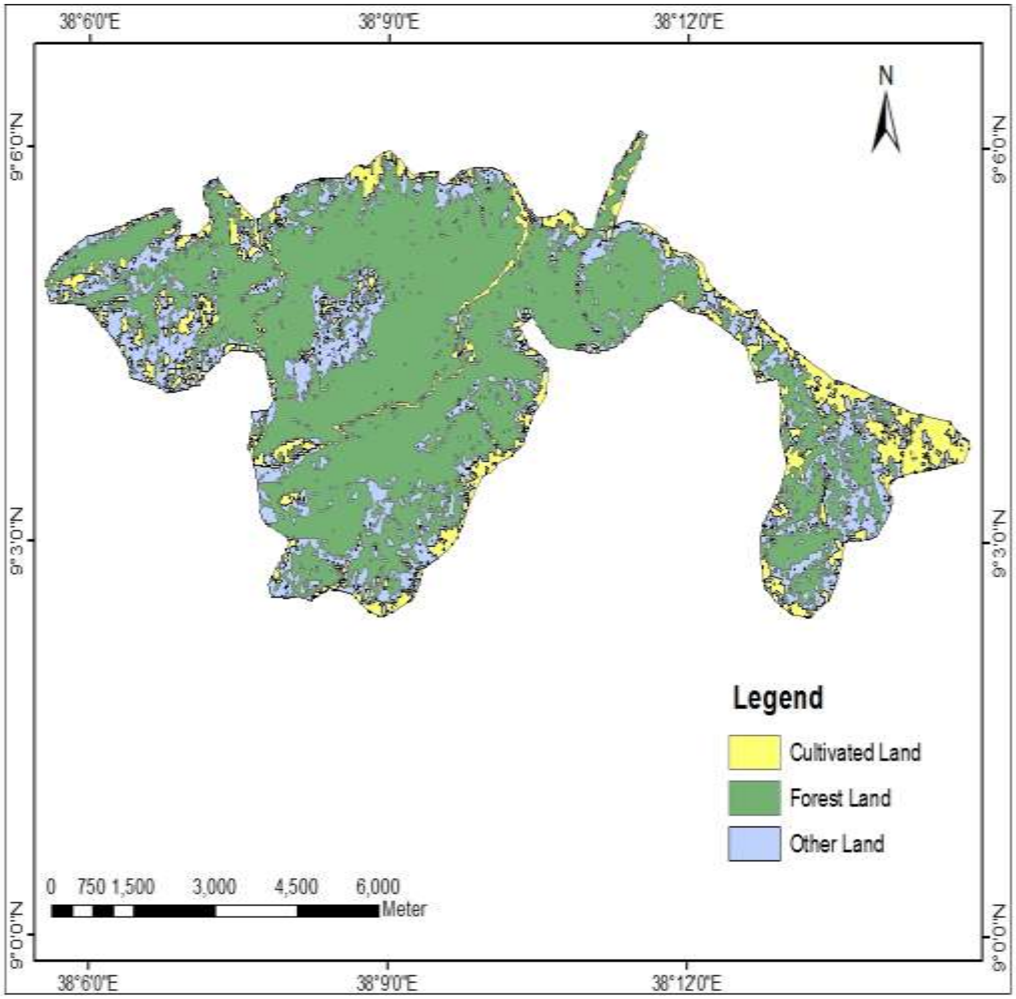

Figure 5. Chilimo Forest Map of 2010.
Source: Computed by author from data obtained from satellite image.

to its prior status.

Conclusion

Participatory forest management practice in Chilimo forest was initiated / undertaken in 1996 by an international NGO called Farm Africa. Twelve FUG cooperatives took part in participatory forest management practice activities and each FUG cooperative has their own democratically elected executive committees. In addition to this, each cooperative has its own forest management plan and signed an agreement with the district agriculture office on the management and use of the forest. The FUG members regularly carry out activities such as nursery plantation, protection of existing trees, protection of regenerating trees, and protection of harvestable trees.

The average income of forest user groups has increased from 3828 ETB to 8952 ETB following the implementation participatory forest management practice in Chilimo forest. Introduction of some agricultural activities such as honey production, home garden crop production and income generation from the sale of timber mainly contributed to the increase in the income of forest user groups. Further, the finding showed that there is an income difference between the incomes of forest user group and non-forest user groups. The Mann-Whitney statistical test result showed that there is significant income difference ($p=0.00$) between forest user groups and non-forest groups. The income from the sale of timber and the implementation of PFM activities are attributed to the income difference among two groups.

Additionally, the FUG cooperatives can use forest products such as timber for house construction, logs for fences, wood for coffins, and poles for house construction which again reduced their additional expenditure for these subsistence needs.

The magnitude of land use in general and forest cover change, in particular, was drastically changed between 1990 and 2010 at Chilimo forest. The ANOVA result also revealed that there is significant variation (p=0.00) in the forest cover within 1990-2010. Particularly, the decline of forest cover and expansion of both cultivated land and grassland were observed. The areal extent of Chilimo forest cover has been fluctuating from time to time. The empirical findings indicated that about 3291.75 ha of forest in 1990 declined to 2527.42 ha in 2000. But, this figure was increased to 2907.07 ha in the year 2010. The forest cover regeneration is the result of participatory forest management establishment in 1996. Among others, plantation of seedlings, and protection of existing trees are the major factors of the regeneration of the forest cover.

Recommendations

(1) Further research to be undertaken for introducing more interventions which can generate more income to forest user groups
(2) In order to fully prevent illegal logging, the district government officials should act timely and take necessary actions that can be a lesson for other community members.
(3) Measures should be undertaken by local government to expand PFM activities into more cooperatives
(4) Capacity building of participating forest user groups in order to manage the forest sustainably and improve local ecosystem

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Additive main effects and multiplicative interaction (AMMI) and genotype main effect and genotype by environment interaction (GGE) biplot analysis of large white bean (*Phaseolus vulgaris* L.) genotypes across environments in Ethiopia

Abel Moges Firew*, Berhanu Amsalu and Dagmawit Tsegaye

Ethiopian Institute of Agricultural Research, Melkassa Agricultural Research Center, Ethiopia.

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Undertaking a multi-environment trial prior to releasing a high yielding and stable varieties for a specific environment is a major step in plant breeding. Therefore, the objective of this work was to study the effect of Genotype × Environment Interaction (GEI) and evaluate the adaptability and stability of sixteen large white common bean genotypes. Additive main effects and multiplicative interaction (AMMI) and genotype main effect and genotype by environment interaction (GGE) biplot models were used. The experimental design was 4 × 4 triple lattice across environments. AMMI analysis of variance showed environments that explained the greater proportion (72.42%) of the total variation, followed by GEI (10.75%) and genotype (2.32%). This indicates the possibility of selecting stable genotypes. AMMI biplot analysis revealed that the first and second interaction axes captured 42.62 and 26.77% of the total variation due to GEI. GGE model showed that the nine environments used for the study belonged to two mega-environments. AMMI stability value (ASV), AMMI and GGE biplot identified one common genotype, G14 (SAA 2) that was the overall best in performance in relation to yield and stability. This suggests that for reliability and optimum result it is better to combine the result of two or more analytical tools for yield and stability in recommendation genotype for verification and release.

Key words: Biplot, genotype × environment interaction (GEI), grain yield, stability, additive main effects and multiplicative interaction (AMMI), AMMI stability value (ASV).

INTRODUCTION

The common bean (*Phaseolus vulgaris* L., 2n = 2x = 22) is one of the main cash crop and cheap protein sources in most lowland and mid-altitude areas of Ethiopia. Currently, common bean occupies 18.8% of the total area cultivated by pulses in Ethiopia, and contributes to 17.2% of the total pulse production in the country (CSA, 2017). It

*Corresponding author. E-mail: abvan23@gmail.com.

is cultivated primarily for dry seed and green pods (as snap beans). There are wide ranges of common bean types grown in Ethiopia including mottled, red, white and black varieties (Ali et al., 2006). The most commercial varieties are red and white color beans and these are becoming the most commonly grown types with increasing market demand (Ferris and Kaganzi, 2008). The white beans are grown for export to the canning industry and other types are mainly for households' food for national and regional markets (Yayis et al., 2011). Despite its importance, progress on large white bean genetic improvement is not well utilized. The development of high yielding cultivars with wide adaptability is the ultimate aim of plant breeders. However, attaining this goal is made more complicated by genotype and environment interaction (Gauch and Zobel, 1996).

Genotype × Environment Interaction (GEI), which is the differential response of cultivars to environmental changes, is an important factor determining the performance of cultivars (Crossa et al., 1990; Vargas et al., 1999). In order to exploit the existing variability and develop new high yielding cultivars, common bean improvement efforts under diverse environmental conditions are needed. The improved common bean genotypes are evaluated in multi-environment trial to test their performance across environments and to select the best genotypes in specific environments.

Different methods of statistical analysis are applied to understand GEI. The Analysis of Variance (ANOVA) is an additive model that describes the main effects effectively; it determines if GEI is a significant source of variation or not and estimates the proportion of contribution. It does not give an insight into the patterns of genotypes or environment that give rise to interaction (Samonte et al., 2005). Therefore, to see the details of interaction and their interpretation the combined data will be analyzed using additive main effects and multiplicative interaction (AMMI) and GGE models proposed by Gauch (1992) and Yan et al. (2000).

The AMMI model is a hybrid analysis that incorporates both the additive and multiplicative components of the two way data structure. AMMI biplot analysis is considered to be an effective tool to diagnose GEI patterns graphically (Mukherjee et al., 2013). The model separates the additive variance from the multiplicative variance and then applies Principal Component Analysis (PCA) to the interaction portion to a new set of coordinate axes that explains in more detail the interaction pattern and the estimation accomplished using the least squares principle (Thillainathan and Fernandez, 2001). The GGE biplot analysis is another method which integrates the genotype and the GEI effect in the evaluation of cultivars (Yan et al., 2000). GGE biplot is done using singular value decomposition to break the data matrix into component matrices. Therefore, the objectives of this study were to assess the yield performance and stability of large white bean genotypes evaluated in a multi-environmental condition and discovers high yielding and stable candidate varieties for possible release using AMMI and GGE models.

MATERIALS AND METHODS

Description of experimental sites

Field experiments were conducted at seven representative bean growing areas of Ethiopia in 2014-2016. The locations were namely Melkassa, Meiso, Pawe, Arsinegelle, Goffa, Jimma and Alemtena. Each year and location was treated as a separate environment, making 9 test environments. Descriptions of the locations are presented in Table 1.

Experimental materials

The fifteen large white common bean genotypes used in the study were obtained from CIAT – Uganda and one nationally released variety "Batu" was used as a standard check (Table 2).

Experimental design and analysis

The experiment was laid down in a 4 × 4 triple lattice across location. Each plot consisted of six rows of 4 m long with 0.4 m spacing between rows and 0.1 m between plants. Two seeds per hill were used, within 10 days after emergence; seedlings were thinned to one per hill. Fertilizer was applied to each plot at the rate of 18 kg N and 46 kg P_2O_5 ha^{-1} in the form of di-ammonium phosphate (DAP) at planting. Other agronomic practices were treated as non-experimental variables and applied uniformly to the entire experimental area. For data analysis, grain yield measured from the middle 4 rows of each plot was converted into kg ha^{-1} at 12.5% grain moisture content. Separation of the additive main effect was done using Duncan's Multiple Range Test (DMRT).

AMMI analysis uses ANOVA and PCA for estimating stability and GEI (Gauch, 1992). The AMMI model used for stability analysis is as follows:

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_{k=1}^{n} \lambda_n \gamma_{gn} \delta_{en} + \theta_{ge} + E_{ge}$$

Where: Y_{ge} = the mean yield of genotype g in environment e, μ = the grand mean, α_g= the deviation of the genotype mean from the grand mean, β_e= the deviation of the environment mean from the grand mean, λ_n= the singular value for the IPCA n, n = the number of PCA axis retained in the model, γ_{gn}= the PCA score of a genotype for PCA axis n, δ_{en}= the environmental PCA score for PCA axis n, θ_{ge}= the AMMI residual and E_{ge}= the residuals.

The AMMI stability value (ASV) as described by Purchase et al. (2000) was calculated as follows:

$$\text{ASV} = \sqrt{[\frac{SS\ IPCA1}{SS\ IPCA2} \times IPCA1\ Score]^2 + (IPCA2\ score)^2}$$

Where, ASV= AMMI stability value; SS= sum of square; IPCA1 and IPCA2= the first and the second interaction principal component axes, respectively.

Table 1. Description of the experimental sites.

Location	Soil type	Altitude (m.a.s.l)	Latitude	Longitude	Annual average		
					Min T (°C)	Max T (°C)	Rainfall (mm)
Alemtena	Andosols	1610	8° 18'N	38° 57'E	12.9	29.8	728
Arsinegelle	Nitosols	1890	7° 35'N	38° 65'E	13.8	23.3	807
Goffa	Acrisol	1284	6°19'N	36° 55'E	14.8	351	1020
Jimma	Eutric nitosols	1753	7°41'N	36° 48'E	11.5	26.2	1584
Melkassa	Andosols	1550	8°30'N	39° 21'E	14.0	33.0	763
Meiso	Vertisol	1332	9° 28'N	38° 08'E	14.9	28.2	787
Pawe	Nitosol	1120	11° 18'N	36° 32'E	15.9	33.0	1587

Where, m.a.s.l = meters above sea level, E=east, N=north, Min=minimum, Max=maximum, T=temperature, °c= degree centigrade, mm=millimeter

Table 2. Description of 16 large white bean genotypes used for a study.

Genotype name	Genotype code	Seed size and color
SAB 794	G1	Large white
SAA 15	G2	Large white
SAB 797	G3	Large white
SAA 8	G4	Large white
SAB 791	G5	Large white
SAA 10	G6	Large white
SAB 793	G7	Large white
SAA 7	G8	Large white
DAB 553	G9	Large white
SAA 1	G10	Large white
DAB 551	G11	Large white
SAA 18	G12	Large white
SAA 9	G13	Large white
SAA 2	G14	Large white
DAB 562	G15	Large white
Batu (check)	G16	Large white

GGE biplot analysis

Singular value decomposition (SVD) of the first two principal components was used to fit the GGE biplot model (Yan, 2002).

$$Y_{ij} - \mu - \beta_j = \xi_{i1}\eta_{j1} + \lambda_2\xi_{i2}\eta_{j2} + \varepsilon_{ij}$$

Where, Y_{ij} is the trait mean of genotype i in environment j, μ is the grand mean, β_j is the main effect of environment j, $\mu + \beta_j$ being the mean yield across all genotypes in environment j, λ_1 and λ_2 are the singular values (SV) for the first and second principal components (PC1 and PC2), respectively, ξ_{i1} and ξ_{i2} are eigenvectors of genotype i for PC1 and PC2, respectively, η_{j1} and η_{j2} are eigenvectors of j for PC1 and PC2, respectively, ε_{ij} is the residual associated with genotype i in environment j.

RESULTS AND DISCUSSION

AMMI analysis of variance for grain yield of the 16 large white common bean genotypes tested across 9 environments is presented in Table 3. The analysis showed that grain yield was significantly (p≤ 0.01) affected by environment, genotype and GEI. Of the total variance of grain yield, environment accounted for 72.42%, whereas genotype and GEI effects accounted

Table 3. AMMI analysis of variance for grain yield (kg ha^{-1}) of 16 large white common bean genotypes at 9 environments.

Source of variation	Degrees of freedom	Sum of Square	Mean square	F-values	Total Variation explained (%)	G × E explained (%)
Total	431	319095837	740362			
Treatments	143	272798936	1907685		85.49	
Genotypes	15	7408773	493918	4.67**	2.32	
Environments	8	231087201	28885900	29.34**	72.42	
Replications (E)	18	17722460	984581	9.30**	5.55	
Interactions	120	34302962	285858	2.70**	10.75	
IPCA 1	22	14618347	664470	6.28**		42.62
IPCA 2	20	9182739	459137	4.34**		26.77
IPCA 3	18	4556565	253142	2.39**		13.28
Pooled error	270	28574441	105831		8.95	

** Significant at 1% level of probability.

2.32 and 10.75% of the total variation, respectively (Table 3). The highly significant environmental effect and its high variance component could be attributed to the large difference between the test locations in altitude, daily temperature and a difference in both amount and distribution of rainfall. Previous reports on common bean in Ethiopia also indicated that environmental effects accounted for the largest part of the total variation (Firew, 2003; Asfaw et al., 2008; Zeleke et al., 2016).

The amount of variance contributed by GEI was 4 times larger than that contributed by genotype main effect. This result indicated that there was a noticeable GEI effect present in large white common bean multi-environment data, leading a substantial difference in genotypic responses across the test environments. The genotypes average grain yield across environments ranged from the lowest 1546.4 kg ha^{-1} for G12 to the highest 2035.1 kg ha^{-1} for G14 (Table 4). Genotypes give differential yield ranking across environments revealed that GEI effect was a crossover type (Matus-Cadiz et al., 2003; Kaya et al., 2006). The averaged environmental grain yield across genotypes ranged from the lowest 647.5 kg ha^{-1} at ME14 to the highest at 2910.5 kg ha^{-1} at JM16 (Table 4).

AMMI biplot analysis

The application of AMMI model for partitioning the GEI (Table 3) reveals the first three terms were significant and explained 82.67% of the GEI. In the study, the first and second multiplicative axis terms explained 42.62 and 26.77% of GEI sum of squares (SS), respectively. The adequacy of the multiplicative terms containing the real structure of GEI was inspected by estimating the amount of noise present in the interaction from the pooled error and comparing it with the sum of squares retained in the consecutive AMMI models (Voltas et al., 2002). Accordingly, the interaction contained about 120 × 105831 = 12699720 noise SS (27.02%), and 120 × 285858 = 34302960 pattern SS (72.98%). This last percentage was larger than that retained by the first two multiplicative terms that together accounted for 69.39% of GEI SS. Moreover, the first two terms had SS greater than that of genotypes and were highly significant ($p<0.01$). Hence, the AMMI with two interaction principal component axes was the best predictive model, which is in harmony with Zobel et al. (1988) and Annicchiarico (2002). Further AMMI axes captured mostly noise and therefore did not help to predict validation of observation.

In the AMMI1 biplot (Figure 1), the abscissa represents the main effects and its ordinate represents IPC1 scores. The horizontal dotted line showed the interaction PC1 score of zero and the vertical dotted lines indicated the mean of genotype effect. It thus provides a means of simultaneously visualizing both mean performance and stability of genotypes. Genotypes with IPC1 scores close to zero expressed general adaptation whereas the larger scores depict more specific adaptation to environments with IPC1 scores of the same sign (Ebdon and Gauch, 2002). Accordingly, genotypes G14 and G13 with mean yields greater than the overall mean and low IPC1 scores had a combination of high yield and stability performance. Check variety G16, G1, G2, G4 and G5 were similar to G14 and G13 in the main effect but tend to contribute more to GEI. Genotype G14 (SAA 2) was superior to the check variety G16 (Batu) with respect to yield and stability performance. MK16 and PW14 relatively were most stable environments than others for growing of widely and specifically adapted large white bean genotypes due to low interaction effect. JM16 and AN15 exhibited high grain yield performance with farthest IPCA values from zero. Therefore, they were highly interactive environments and suitable for specifically adapted genotypes.

According to AMMI2 biplot (Figure 2), the distance from biplot origin are indicative of the amount of interaction that was exhibited by genotypes over environments or vice versa. As genotypes located near the biplot origin

Table 4. Mean grain yield (kg ha^{-1}) of 16 large white common bean genotypes evaluated over 9 environments.

Genotype	Environments									
	MK14	**ME14**	**PW14**	**AN15**	**GF16**	**JM16**	**MK16**	**PW16**	**AT14**	**Mean**
SAB 794	2659.4^{a}	389.2^{e}	1733.0^{de}	1923.6^{cde}	853.7^{abcd}	3783.8^{a}	2622.2^{ab}	1064.2^{b}	1415.8^{d}	1827.2^{cdef}
SAA 15	2649.4^{a}	790.0^{abc}	2240.4^{bcd}	1858.7^{cdef}	1042.9^{a}	3149.6^{abc}	2427.3^{ab}	1213.3^{b}	1440.6^{cd}	1868.0^{abcd}
SAB 797	2157.9^{ab}	390.8^{e}	1815.9^{cde}	1314.6^{fgh}	782.2^{bcde}	3281.7^{ab}	2264.9^{ab}	1378.6^{ab}	1557.8^{abcd}	1660.5^{fgh}
SAA 8	2308.8^{ab}	399.5^{e}	1622.6^{e}	2840.8^{a}	927.5^{abc}	3020.0^{abc}	2497.7^{ab}	1305.6^{ab}	1753.4^{abcd}	1852.9^{bcde}
SAB 791	1881.6^{b}	807.6^{ab}	2057.5^{bcde}	2652.4^{ab}	582.3^{e}	3300.4^{ab}	2599.4^{ab}	1150.8^{b}	1897.6^{abc}	1881.1^{abc}
SAA 10	2619.8^{a}	806.8^{ab}	1998.2^{cde}	1741.3^{cdef}	718.7^{cde}	2953.0^{bc}	2162.3^{ab}	911.6^{b}	1385.8^{d}	1699.7^{defgh}
SAB 793	1888.4^{b}	675.6^{bcde}	1978.3^{cde}	2122.1^{bcd}	639.0^{de}	2450.0^{cd}	2653.8^{ab}	921.0^{b}	1913.5^{ab}	1693.5^{efgh}
SAA 7	2271.9^{ab}	736.2^{abcd}	2099.8^{bcde}	1959.1^{cde}	666.1^{de}	2365.8^{cd}	2361.4^{ab}	1275.1^{ab}	2005.8^{a}	1749.0^{cdefg}
DAB 553	2580.5^{a}	488.5^{cde}	1693.8^{e}	1107.6^{gh}	988.7^{ab}	2838.4^{bcd}	2447.4^{ab}	1109.7^{b}	1501.5^{bcd}	1639.6^{gh}
SAA 1	2449.2^{ab}	817.4^{ab}	2505.5^{ab}	1633.8^{cdefg}	780.2^{bcde}	2665.1^{bcd}	2384.9^{ab}	1301.4^{ab}	969.7^{e}	1723.0^{cdefg}
DAB 551	2426.2^{ab}	580.0^{bcde}	2036.1^{bcde}	1443.3^{efgh}	912.8^{abc}	2387.5^{cd}	2348.6^{ab}	1307.1^{ab}	1600.6^{abcd}	1671.3^{fgh}
SAA 18	2166.4^{ab}	471.2^{de}	1887.8^{cde}	931.3^{h}	860.2^{abcd}	3053.5^{abc}	2011.4^{ab}	949.6^{b}	1586.1^{abcd}	1546.4^{h}
SAA 9	2625.1^{a}	709.1^{babcd}	1998.1^{cde}	2042.5^{cde}	1026.6^{a}	3287.1^{ab}	2773.8^{ab}	957.8^{b}	1481.2^{bcd}	1877.9^{abc}
SAA 2	2384.9^{ab}	995.7^{a}	2252.6^{bc}	2193.8^{bc}	727.9^{cde}	3324.3^{ab}	3073.8^{a}	1718.6^{a}	1644.1^{abcd}	2035.1^{a}
DAB 562	2341.5^{ab}	436.3^{de}	2010.2^{bcde}	1539.0^{defg}	751.1^{cde}	2539.5^{bcd}	2218.7^{ab}	1149.8^{b}	1759.2^{abcd}	1638.4^{gh}
Batu(ch)	2403.3^{ab}	865.7^{ab}	2906.5^{a}	2775.5^{a}	755.1^{cde}	2168.8^{d}	3043.7^{a}	1280.5^{ab}	1551.1^{abcd}	1972.3^{ab}
Mean	**2363.4^{b}**	647.5^{h}	2052.3^{c}	1880.0^{d}	813.4^{g}	2910.5^{a}	2493.2^{b}	1187.2f	1591.5^{e}	
CV%	**12.98**	**24.7**	**12.6**	**16.7**	**14.42**	**13.78**	**19.79**	**20.24**	**14.78**	

Abbreviations: ME14 = Meiso 2014; AN15 = Arsinegelle 2015; GF16 = Goffa 2016; JM16 = Jimma 2016; AT14 = Alemtena 2016; MK14 = Melkassa 2014; MK16 = Melkassa 2016; PW14 = Pawe 2014; PW16 = Pawe 2016. Means followed by similar letters are not significantly different at the 0.05 probability level based on DMRT; underline values are highest and lowest means of genotypes yield across location and highest and lowest means of environmental grain yield across genotypes

are less responsive than the vertex genotypes indicating general adaptability to all growing environments (Voltas et al., 2002). Based on these, G14, G2, G6 and G13 relatively scattered close to the origin expressed genotypes have minimal interaction and more adapted to all growing environments. G4, G5, G16 and G1 scattered away from the origin in the biplot indicating that these genotypes were more sensitive to environmental effects. The biplot showed JM16, AN15 and PW14 with longer vectors which indicated very interactive and discriminated the difference among genotypes more than other environments with shorter vectors.

AMMI stability value (ASV)

The AMMI model does not make provision for a quantitative stability measure. Such a measure is crucial in order to quantify and rank genotypes according to their trait stability. In the ASV method, genotypes with least ASV score are the most stable (Purchase et al., 2000). Accordingly, G14, G13, G8 and G15 were most stable genotypes and G 12, G 16 and G 7 were unstable (Table 5). The ASV parameter has been used as an auxiliary criterion to define more stable genotypes in common bean (Tadele et al., 2018) and other crops such as wheat (Farshadfar et al., 2011).

GGE biplot analysis

Mega environment of trial environment

The PC1 and PC2 score of GGE biplot were used

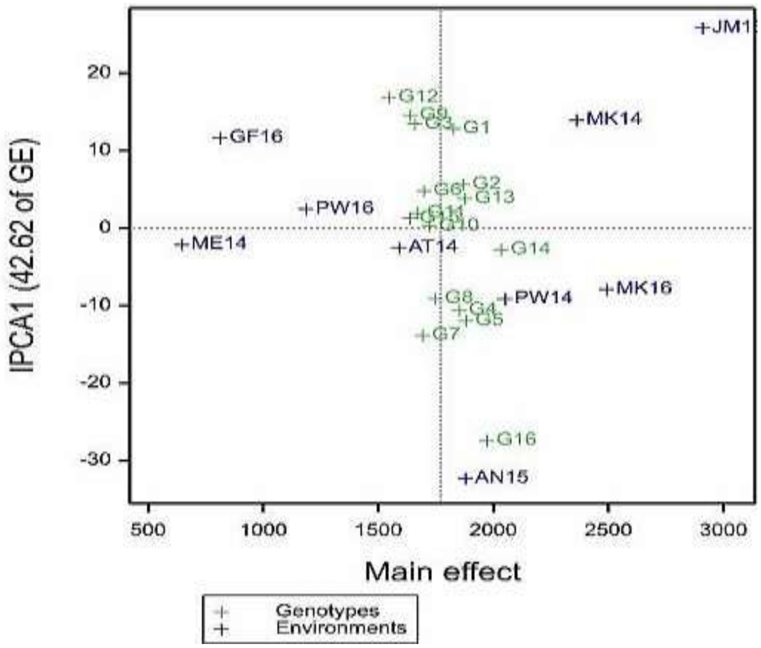

Figure 1. AMMI1 biplot showing the mean (main effect) vs. stability (IPCA1) view of both genotype and environment on grain yield.

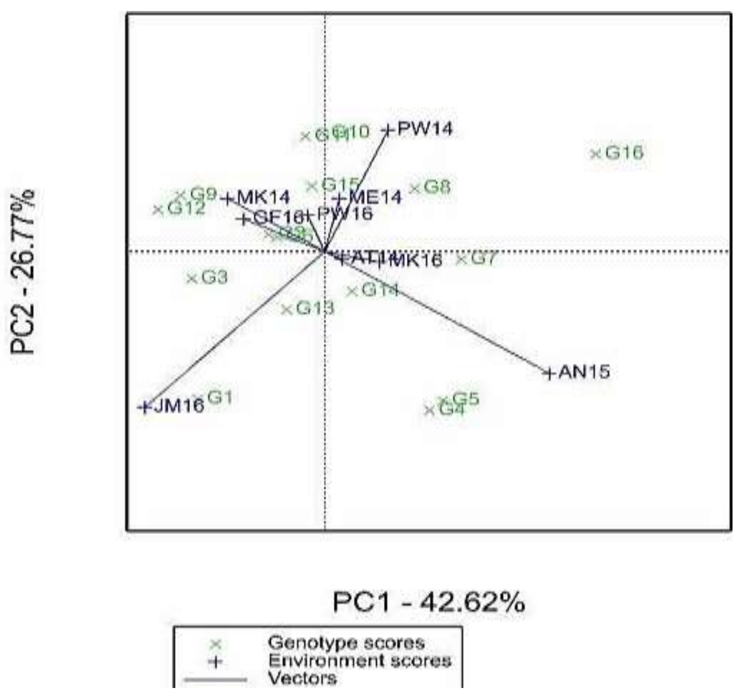

Figure 2. AMMI2 biplot of the first interaction principal component axis (IPCA1) vs. the second principal component axis (IPCA2) for grain yield.

to estimate the patterns of environments as shown in Figure 3. Environment PC1 and PC2 scores had positive and negative scores indicating that there was a difference in ranking for yield performance among genotypes across environments leading to a crossover GEI. Visualization of the which-won-where pattern of Multi-environment Trial (MET) data is important for studying the possible existence of different mega environments in a region

Table 5. Performance and stability of 16 large white common bean genotypes based on mean grain yield (kg/ha), IPCA1, IPCA2 scores and AMMI stability value (ASV).

Genotype	Mean yield	Yield rank	IPCA1	IPCA2	ASV	ASV rank
SAB 794	1827.2^{cdef}	7	12.84648	-17.4192	13.48026	7
SAA 15	1868.0^{abcd}	5	5.65917	2.01629	9.692995	6
SAB 797	1660.5^{fgh}	13	13.4516	-3.20938	27.35149	13
SAA 8	1852.9^{bcde}	6	-10.585	-18.7757	20.38855	11
SAB 791	1881.1^{abc}	3	-11.9597	-17.6203	20.18803	10
SAA 10	1699.7^{defgh}	10	4.83298	1.69072	8.344297	5
SAB 793	1693.5^{efgh}	11	-13.8308	-0.93001	53.3451	16
SAA 7	1749.0^{cdefg}	8	-9.08284	7.45041	6.713061	3
DAB 553	1639.6^{gh}	14	14.57859	6.62643	22.6164	12
SAA 1	1723.0^{cdefg}	9	0.28519	13.97456	13.97462	9
DAB 551	1671.3^{fgh}	12	1.96159	13.59659	13.61699	8
SAA 18	1546.4^{h}	16	16.87828	4.96994	31.49859	14
SAA 9	1877.9^{abc}	4	3.84634	-6.86665	6.234089	2
SAA 2	2035.1^{a}	1	-2.76676	-4.70369	5.160177	1
DAB 562	1638.4^{gh}	15	1.30246	7.7078	7.726373	4
Batu(ch)	1972.3^{ab}	2	-27.4176	11.49213	40.75994	15

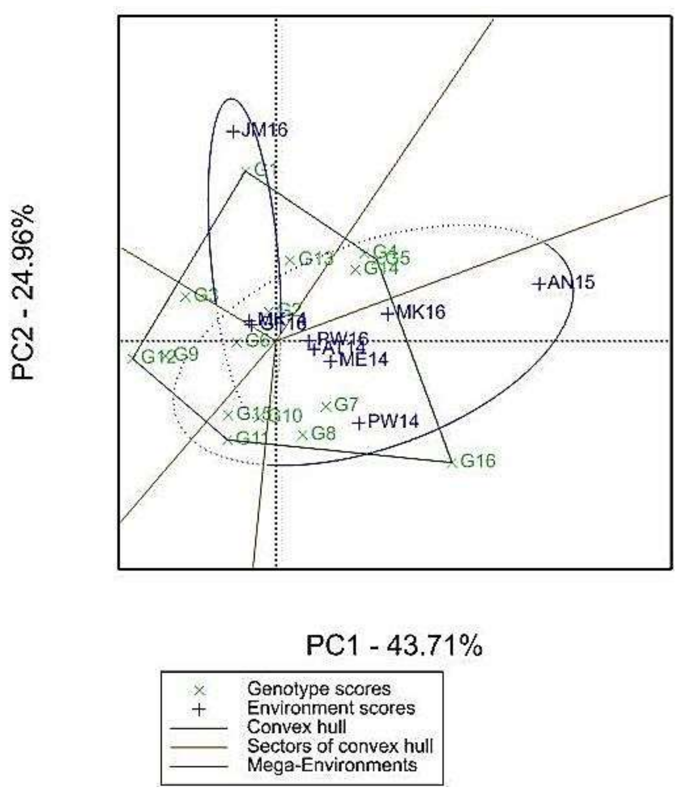

Figure 3. GGE biplot identification of winning genotypes and their related mega-environments.

(Gauch and Zobel, 1997; Yan et al., 2001). The polygon is formed by connecting the markers of the genotypes that are further away from the biplot origin, such that all other genotypes are contained in the polygon. Genotypes located at the vertices of the polygon performed either the best or the poorest in one or more locations since

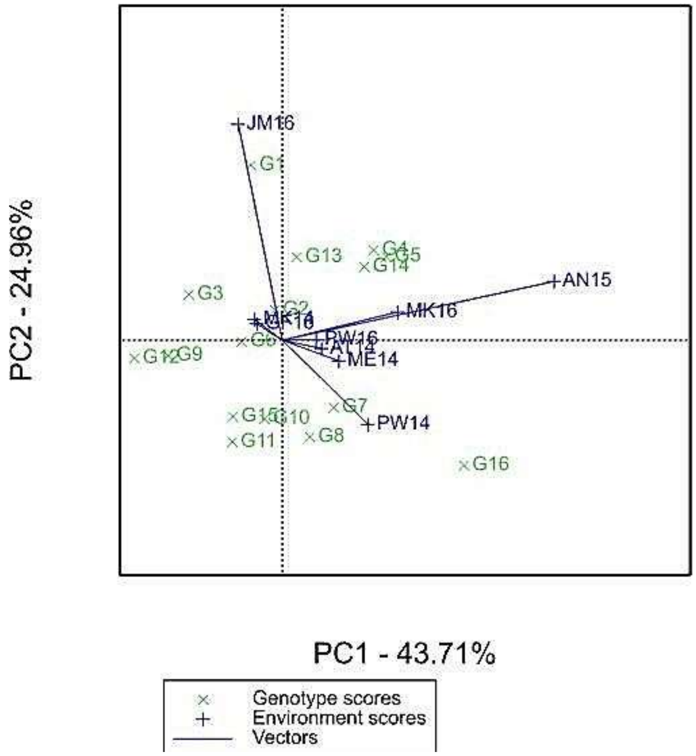

Figure 4. GGE biplot based on environment-focused scaling for environments.

they had the longest distance from the origin of biplot.

In the which-won-where view of the GGE biplot (Figure 3) based on the data in Table 4, the nine environments fell in two sectors with different winning genotypes. Sector 1 (Mega environment-1) consists of ME14, AN15, AT14, MK16, PW14 and PW16, or there are six environments that have good yielding capacity for genotypes G7, G8 and G16. The mega-environment-2 represents JM16, GF16 and MK14 are environments that are suitable for genotypes G2 and G13.

Relationship among test environments

To visualize the relationship between environments, lines are drawn to connect test environments to the biplot origin known as environment vectors. The cosine of the angle between the vectors of two environments is used to approximate the correlation between them (Yan, 2002). Based on the angle of environment vectors, the nine environments are separated into two groups (Figure 4). Group one includes MK16, PW16, AN15, ME14, PW14 and AT14 shows an angle less than 90°, which means these environments, are positively correlated. Group two involves JM16, MK14 and GF16, and the presence of obtuse angle from group one environments, they correlate negatively. The presence of close association among test environments suggests that the same information about genotypes could be obtained from few test locations, and hence by dropping one or two environments from each group can reduce cost of multi-location replicated trials (Tukamuhabwa et al., 2012).

Performance and stability of the genotypes

The yield and stability of genotypes were evaluated by using so-called average environment coordinates (AEC) method (Yan 2001, 2002). In this method, the average principal components will be used in all environments and it is presented with a circle, as shown in (Figure 5). The average ordinate environment (AOE) defined by the line which is perpendicular to the average environment axis (AEA) line and pass through the origin. This line divides the genotypes into those with a higher yield than average and into those lower than average (Naheif et al., 2013). Thus, G4, G5, G14 and G16 had the highest mean yield and G12 and G9 were the lowest. The non-arrowed line is AEC; it points to greater variability (poorer stability) in either direction. Thus, G1 and G16 were highly unstable genotypes, whereas G4, G5 and G14 were highly stable.

Ranking genotypes relative to ideal genotypes

The ideal genotype should have the highest mean

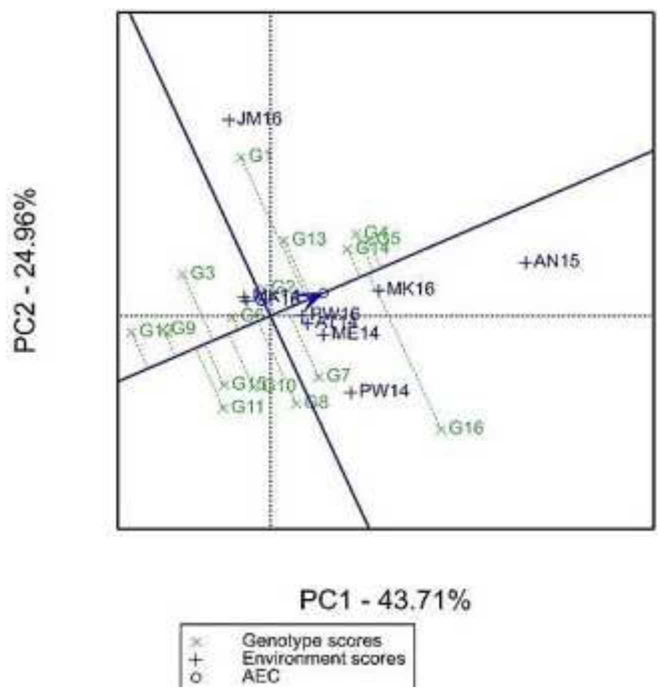

Figure 5. GGE biplot based on environment-focused scaling for mean performance and stability of the genotypes

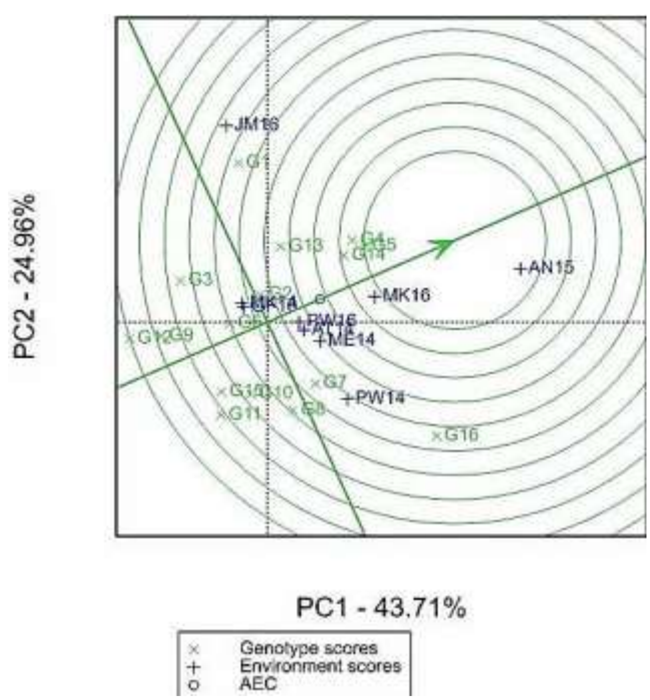

Figure 6. GGE biplot based on genotype-focused comparison of the genotype with ideal genotype.

performance and be absolutely stable (Yan and Kang, 2003), which represented by the small circle an arrow pointing to it (Figure 6). Such an ideal genotype is defined by having the greatest vector length of the high yielding genotype and with zero GEI. Concentric circles were drawn to help visualize the distance between each genotype and the ideal genotype; a genotype is more desirable if it is located closer to the ideal genotype

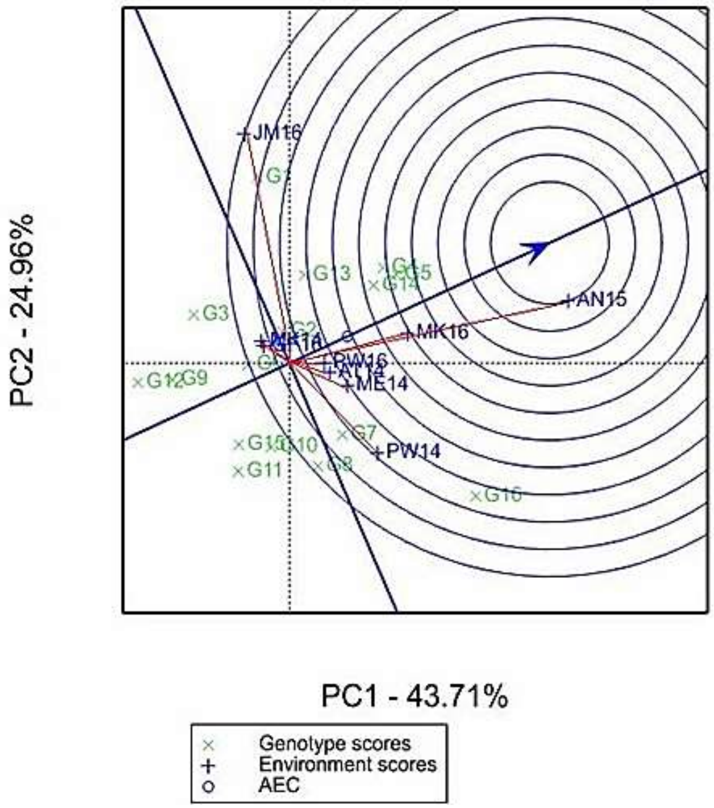

Figure 7. GGE biplot based on environment focused scaling for comparison of the environment with ideal environment.

(Mitrovic et al., 2012), so genotype G5 which fell into the center concentric circles was ideal in terms of high yielding ability and stability. In addition, G4 and G14, located on the next consecutive concentric circle, may be regarded as desirable genotypes.

Discriminating ability and representativeness

The concentric circle on the biplot help to visualize the length of environment vectors, which is proportional to the standard deviation within the respective environments (Yan and Tinker, 2006) (Figure 7). Therefore, among the nine environments AN15 and JM14 were most discriminating (informative) and GF16, MK14, PW16 and AT14 are least discriminating. The average environment (represented by the small circle at the end of the arrow) has the average coordinates of all the environments, and AEA is the line that passes through the average environment and the biplot origin (Yan and Tinker, 2006). A test environment that has a smaller angle with the AEA is more representative of other test environments. Thus, AN15 is the most representative whereas JM16, GF16 and MK14 are least representative. Test environments that are both discriminating and representative (e.g., AN15) are good test environment for selecting generally adapted genotypes.

Conclusion

Genotype × Environment Interaction (GEI) has been an important and challenging issue for plant breeders to select superior and adaptable cultivars for growing environments. Both yield and stability should be considered simultaneously to reduce the effect of GEI and to make a selection of genotypes more precise. The present study indicated that the large white common bean yield was liable to significant fluctuation with changes in the growing environments followed by the GEI and genotypic effect. AMMI analysis revealed that the high yielding genotypes SAA 2 and SAA 9 were top ranked in most environments and found the most stable across environments. According to GGE biplot, genotypes SAA 8, SAB 791 and SAA 2 were exhibited high yield and stable performance. By both models best performing genotype SAA 2 selected as stable genotype. Generally, the current study clearly demonstrates that the application of AMMI and GGE biplot facilitated the visual comparison and identification of superior and stable genotype, thereby supporting decisions of large white bean genotype recommended for the bean growing areas of Ethiopia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Consumers' awareness of the presence of pathogenic bacteria and pesticide residues on tomatoes sold in Nairobi

J. H. Nguetti[1*], J. K. Imungi[1], M. W. Okoth[1], E. S. Mitema[2], W. F. Mbacham[4] and J. Wang'ombe[3]

[1]Department of Food Science, Nutrition and Technology, Faculty of Agriculture, College of Agriculture and Veterinary Sciences, University of Nairobi, Kenya.
[2]Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi, Kenya.
[3]Department of Health Economics, School of Public Health, College of Health Sciences, University of Nairobi, Kenya.
[4]Department of Health Economics, Policy and Management, Catholic University of Cameroon, Bamenda, Cameroon.

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Tomato consumed worldwide for its vitamins and bioactive elements can harbor postharvest bacteria and pesticide residues. A cross-sectional survey using a semi-structured questionnaire was done in 101 households in Kangemi assessing consumers' awareness on pesticide residues and bacterial presence on tomatoes sold in Nairobi. Questionnaire was administered in Kangemi during weekends, systematic random sampling was applied during household recruitment. Data analyses used SPSS; analytical tools included means, standard deviation, binomial test and bivariate correlation. Male (64.86±0.48) had better awareness on pesticides on tomato (p=0.037) and consumers of 36 to 53 years old were more knowledgeable (58.29±0.34) than others. Awareness with education level was significant at 95% level of confidence (p=0.044); 86% respondents were more conversant with pathogens than with pesticides and 97% knew that pesticides were used in farms (p= 0.0001). About 91% indicated that pesticides are dangerous for health and 74% related pesticides in farms to their presence on tomatoes in markets (p= 0.0001). However, 74% believed that washing provides tomatoes without pesticides (p= 0.0001) while 65% mentioned that pesticides can be present on tomato eaten as salad (p= 0.004). Consumers' knowledge was insufficient on tomato with pesticides; this can be improved through information, communication and education.

Key words: Health, household, farms, knowledge, marital status, vegetable

INTRODUCTION

Tomato is widely cultivated and consumed worldwide for its peculiar virtues providing vitamins and bioactive elements (Kariathi et al., 2017). Dias (2012) encourage quotidian intake of vegetables and state that, tomatoes

*Corresponding author. E-mail: jhnguetti@gmail.com.

can provide phenolic acid, ascorbic acid and phytochemical compounds protecting against free radical and tumor cells in human body. Viuda-Martos et al. (2014) noticed that, the daily intake of 25 g of the processed tomato is hypolipidemic (decreases the level of lipids in blood). Dias (2012) reported that low consumption of vegetables such as tomato contributes to 11% of stroke and 31% of ischemia heart diseases in the world. Oguntibeju et al. (2013) on the other hand state that frequent consumption of vegetables helps to manage glucose in blood and reduces the incidence of diabetes type-2 and cancers (oropharynx, oesophagus, stomach, colon, rectum, lung cancer, prostate cancer). With such value, the vegetable is currently much diversified phenotypically and genotypically (Vijee et al., 2016). Its efficacy for health protection is being reinforced and the purple tomato or "Indigo Rose" for instance is a new variety with high concentration of lycopene (Scott, 2012). Other improvements are targeting the quality and taste of the crop (Ric et al., 2011).

It has been found that in spite of all these health benefits fresh tomatoes can harbor pesticide residues (Hammad et al., 2017) and pathogens (Liu et al., 2018). Pesticides can be found on tomatoes (Elpiniki and Amvrazi, 2011; Kariathi et al., 2016) as they are even misused in farms (Mutai et al., 2015; Kumari and Basavaraja, 2018). Similarly, potential bacterial pathogens can find themselves on tomatoes through diverse routes including running polluted water from rains, adulterated water from showers, wrongly treated and untreated manure, soil reservoir, wounds from pests and pesticides, irrigation with unprocessed sewage (Sheppard, 1998; Heaton and Jones, 2007; Orozco et al., 2008; Gu et al., 2013; Farakos and Frank, 2014). Kithure et al. (2014) studied the seasonal levels of vegetables with pesticide residues in Makuyu-Kenya and detected higher contamination of deltamethrin in dry period than in wet season. Pierangeli et al. (2014) analyzed bacterial contamination of fresh produce from open air markets and supermarkets in the Philippines and found presence of *Escherichia coli* and *Salmonella* species.

It is in light of the aforementioned that a study was conducted to assess consumers' awareness on the quality of fresh tomato consumed in Nairobi. Specifically, the study aimed to assess consumers' awareness on potential contamination of fresh tomatoes with pathogenic bacteria and pesticide residues.

METHODOLOGY

Study design

A cross sectional study assessing consumers' awareness on exposure to foodborne bacteria and pesticide residues on tomato was done in Kangemi suburb of Nairobi. A total of 101 households were randomly selected and interviewed. A semi-structured questionnaire on fresh tomatoes sold in Nairobi was designed for the collection of data from households. The questionnaire was pretested in Kawangware to confirm precision, appropriate answers and was reviewed after observations of the first interview.

Study setting

The study was undertaken in Nairobi region, the capital city of Kenya located in the South-East end of the Kenyan's heartland agriculture. It is the regional headquarters for international organizations and the city produces more than half of the GDP of the country. Kangemi slum located in the outskirt of the city and in a small valley on Waiyaki way was purposively selected and covered by the survey.

Kangemi is neighboring in the south with Kawangware and four middle class areas, Loresho and Kibagare in the north; Mountain View in east and Westlands in west. The low-income settlement is within coordinates' 1°16'17.4" S 36°44'36.4"; covers an area of 0.87 km^2 and is located at around 10 km from the central business of the metropolis. The population of the area is more than 100,000 inhabitants with a density of 22,243 dwellers/km^2 (Cherunya et al., 2015). Kangemi is a multi-ethnic area with a strong informal activity including street food ready-to-eat as "Kachumbari" reflecting the need to feed the growing population (Mwau, 2009; Oyunga-Ogubi et al., 2009).

Study tool

A semi-structured questionnaire was designed for data collection from tomato consumers

Target population

The lower class population was targeted assuming that, they are less informed on pesticide residues and bacteria presences in fresh tomatoes sold in markets compared to the middle and high classes.

Sampling procedure

A preliminary field visit of reconnaissance was done in Kangemi on both side of Wayaki way to decide on how to cover the area during data collection. Six enumerators, all Master's students were recruited and trained to administer the questionnaire in a face to face interview. They were divided in two groups to cover Kangemi on both side of Wayaki Way. The questionnaire was administered during weekends as described by Okello et al. (2015). A systematic random sampling was applied during household recruitment for interview. The head of the household was always requested to be the respondent. In case of absence of the head of the house, the wife was requested for interview or an appointed person by the household was responding to the questions. Consent and voluntary participation were always obtained from the respondents after introduction of the aim of study. The enumerators collected data from 101 elders of each household or from the person appointed by the household.

Data collection

The questionnaire targeted the sociodemographic (gender, age, level of education and marital status) characteristics of respondents. The socio demographic helped to know whether households were mostly males or females; if they were mostly old people, middle age or young; if awareness of tomato contamination is known by old, middle age or young people. As well, it was also to know the contribution of education on the issue and whether marital status

Table 1. The percentage of various demographic characteristics on gender, age, marital status and education.

Demographic characteristics	Variable	Frequency (N)	Percentage
Gender	Male	32	31.7
	Female	69	68.3
Age	18 to 25	19	18.8
	26 to 35	54	53.5
	36 to 53	28	27.7
Marital status	Married	61	60.4
	Single	37	36.6
Level of education	Never attended school	4	4
	Primary school	23	22.8
	Secondary school	43	42.6
	Tertiary level	17	16.8
	University level	9	8.9

was an important factor enhancing the awareness of respondents. The questionnaire also sought to get the overall knowledge of respondents on specific questions on enteric bacteria and pesticide residues. Some correlations on pesticide residues were assessed in order to measure the level of the perception of consumers on potential diseases able to be obtained by fresh tomato consumption.

Data analysis

The Statistical Package of Social Sciences (IBM SPSS Statistics 20) software was used for data analysis. Answers of each questionnaire were entered into the software. Descriptive statistics were used to generate the sociodemographic characteristics of household for their awareness on exposure to pathogens and pesticides residues in fresh tomato marketed in Nairobi. Respondents' knowledge on pesticide residues and pathogens were coded and differentiated as right or wrong answer and scores one for good answer and nil for wrong ones was allocated. Same procedure was applied on knowledge of potential diseases related to pathogens and pesticides contamination of tomato. Right answers were calculated and converted into percentages. As well, the standard deviation to measure the dispersion was also obtained. The binomial test was applied to assess frequencies of respondent on some specific questions of tomatoes contamination. The bivariate correlation using the Pearson coefficient two-tailed test was used to measure the degree of linkages between pesticide variables. The mean differences were calculated at 95% level of significance. Fisher's exact test was used to examine the significance of pesticides variables' association.

RESULTS

Socio demographic characteristics of the population studied

The survey conducted in Nairobi among 101 respondents showed more females (69/101) participation and majority of respondents (59/101) were between 26 and 35 years old. Very few participants (4/101) had not attended any school and more than half of interviewees were married. This participation has allowed measuring the reasons why some households have designated people or why some people stood by themselves to take the interview by gender, education and marital status. During the interviews, some participants were silent on some demographic characteristics as marital status and level of education. The percentage of the sociodemographic characteristic was obtained as shown in Table 1.

Consumers' general awareness on pesticides and bacteria on tomato and relations with other findings around contaminated crops

The overall assessment of enteric bacteria and pesticides residues on tomatoes showed levels of significance. About 74% of consumers knew that, pesticides use in farms can be present in tomato sold in markets, but majority (74%) believed that it is safe to eat fresh tomato from markets after washing. As well, 95% supported that, washing tomato before eating in salad prevents from any diseases. For some consumers, washing stands as the critical control point preventing from any disease infection. An overall knowledge of consumers reflecting pesticides and bacteria knowledge was designed as shown in Table 2

Expected awareness of consumers on pesticide residues using correlations between variables

Some variables of pesticide were correlated to measure their levels of linkages as shown in Table 3. In order to

Table 2. Percentage or frequency of food safety knowledge among respondents.

Food safety questions	Responses	N	Frequencies (%)	p-value
Pesticides				
1. Can fresh tomato cause any disease to someone?	Yes No	47 48	49 51	1
2. Do you know pesticides?	Yes No	79 22	78 22	0
3. Pests and diseases of tomatoes in the farms?	Yes No	95 6	94 6	0
4. Pesticides are made to protect tomatoes in farms?	Yes No	96 3	97 3	0
5. Farmers use pesticides to protect tomatoes in farms?	Yes No	99 2	98 2	0
6. Pesticides are dangerous for humans' health?	Yes No	87 9	91 9	0
7. Pesticides can cause diseases to consumers?	Yes No	48 50	49 51	0.92
8. Pesticides used in tomato farms can be present on tomato sold in markets?	Yes No	73 25	74 26	0
9. Pesticides used in tomato farms can be present on tomato eaten in salad?	Yes No	65 35	65 35	0.004
10. Pesticides used in tomato farms can be present in tomato cooked at home?	Yes No	46 52	47 53	0.641
11. Pesticides used in tomato farms can be dangerous for consumers' health?	Yes No	81 18	82 18	0
Enteric bacteria				
12. Do you know pathogens?	Yes No	80 21	79 21	0
13. Pathogens can be found on the surface of tomato?	Yes No	77 13	86 14	0
14. Pathogens can be found inside tomato?	Yes No	53 38	58 42	0.142
15. It is safe to eat raw tomato from farms or markets after simple washing?	Yes No	73 25	74 25	0
16. Tomato eaten in salad can affect human health?	Yes No	50 49	51 49	1
17. Tomato cooked in food can affect human health?	Yes	35	35	0.005

Table 2. Contd.

	No	64	65	
18. Tomato washing before eating in salad prevents from any disease?	Yes No	95 5	95 5	0

understand the validity and evidence of reliability (Goodwin and Leech, 2006), a correlation among pesticide variables was applied. The aim was to see whether variables used to assess knowledge on pesticides in this survey were adequate for evaluating consumers. As well, it was to see whether, a holder of a knowledge may also possess the understanding of the correlating other knowledge and thus, be able to respond adequately to the one with which it correlates.

Consumers' awareness on potential diseases related to pathogens and pesticide residues in tomato

About 49% of respondents knew that fresh tomato can cause any diseases to consumers. They pointed that raw tomato can cause diseases as cancers, stomachache and amebiasis. However, for illnesses related to pathogens, 24% pointed stomachache, diarrhea 19% and amebiasis 4%. For sicknesses related to pesticide residues, they indicated stomachache (32%) and cancer (11%) (Table 4).

Influence of sociodemographic characteristics on awareness of pesticide residues and bacterial organisms in tomato

Knowledge on pesticide residues and pathogens presences was assessed based on socio demographic characteristics as shown in Table 4. Males (64.86±0.48) had better knowledge than female (50.77±0.36); married people (60.16±0.31) had better understanding than single (39.75±0.43) and the level of education was an important factor among the respondents ($p < 0.05$) (Table 5).

DISCUSSION

Consumers' awareness by sociodemographic characteristics participation

Consumers awareness by gender

The study had a participation of 68.3% females and 31.7% males. Strong participation of females in this study holds on the fact that, cooking in Africa is naturally a duty destined to women. They were more expected to provide best answers due to the natural social rank given by the society between men and women in households. Pambo (2013) accordingly reminds that, females are mostly implicated because they are the designers of nutrition schedule and are responsible of food preparation in homes. Although this natural set up, socioeconomic development and its challenges have raised the matter of gender equality. From this reality, participation of men in this study may be justified by number of reasons obliging them to be fully or partially implicated in cooking. The reasons might include unavailability of the female committed to duties generating income, gender equality or good relationship in a couple and obligation to help or contribute in cooking at any time when the need arises. For such reasons, it was useful to have them in the survey.

The present observation is in concurrence with the work of Maschkowski et al. (2010) in Germany who reported 82% female participants in the study of parents' contribution in fruits and vegetables consumption in families. This finding also agrees with the study of Pambo (2013) in Kenya who reported 54.9% of female participation against 45.1% of male in his study of consumers' awareness on fortified sugar.

Consumers' awareness by marital status

About 60% respondents were married and 37% were single. Marital status can contribute to understand the awareness of vegetables' contamination and can improve the couples' knowledge through worries as wellbeing, diseases' prevention and family protection. Ambrožič et al. (2016) in Slovenia observed that, women have strong knowledge on viral presence on food than men because they care for their homes and families. Similar report came from Tomaszewska et al. (2018) in a survey that covered Poland and Thailand. Another study from Thailand by Kanang (2012) stated that, men in Bangkok care a lot compared to women on the quality of food purchase for family consumption. Both studies pointed food quality desired by parents in households no matter the sex when it comes to food provision for families. This behavior is mostly found among married people with children. They request for organic diet to avoid contaminated food with pesticide residues (Davies and Titterington, 1995; Kanang, 2012) to prevent foodborne infections and related disabilities adjusted life years (DALYs). It may be under such consideration that,

Table 1. Correlations between variables of pesticides.

Correlation	Can fresh tomato transmit any disease to someone?	Pesticides are dangerous for humans' health	Pesticides use in tomatoes farms can be dangerous for consumers' health	Pesticides use in tomatoes farms can be present on tomato in markets	Pesticides used in tomatoes farms can be present on tomato eaten in salad	Pesticides can transmit diseases to tomatoes consumers
Can fresh tomato transmit any disease to someone?	1	0.328** 0.002	0.487** (0.000)	0.331** (0.001)	0.399** (0.000)	0.217** (0.037)
Pesticides are dangerous for humans' health	0.328** (0.02)	1	0.394** (0.000)	0.257** (0.013)	0.208** (0.43)	0.185 (0.075)
Pesticides use in tomatoes farms can be dangerous for consumers' health	0.487** (0.000)	0.394** (0.000)	1	0.410** (0.000)	0.364** (0.000)	0.260* (0.010)
Pesticides use in tomatoes farms can be present on tomato in markets	0.331** (0.001)	0.257** (0.013)	0.410** (0.000)	1	0.705** (0.000)	0.094 (0.362)
Pesticides used in tomatoes farms can be present on tomato eaten in salad	0.399** (0.000)	0.208* (0.043)	0.364** (0.000)	0.705** (0.000)	1	0.091 (0.371)
Pesticides can transmit diseases to tomato's consumers	0.217* (0.037)	0.185 (0.075)	0.260* (0.010)	0.094 (0.362)	0.091 (0.371)	1

**Correlation is significant at the 0.01level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

studies point anxiety and worry of parents generated by their psychological attachment to families while looking for food of houses in markets (Maschkowski et al., 2010; Srinivasan et al., 2015). This attitude was also illustrated by Tanja (2015) in Finland who stated the structural dynamism of consumers and decision making when it comes to buying food. He stressed that, parents buy goods for the family to satisfy the needs as good health under personal, social and psychological factors. Pambo (2013) in Kenya supported the idea especially when he argues that, married people are more aware of food quality and that, households with children care more about the quality of food consumed. This finding also corroborates the work done in Turkey by Erdem et al. (2015) who found that, marital status had an influence on awareness of Halal among respondents.

Consumers' awareness by education

About 96% respondents had been to school including primary education, secondary, tertiary and university level. Studies showed that, understanding the worries of food safety requires a great level of education. Respondents with high level of education might be more curious, sensitive, more informed, open minded and interested on such topics. That is probably why Kanang (2012) in Thailand insists that, learned consumers in markets go for organic food for instance. It may be under such thought that, Hassan and Dimassi (2014) in Lebanon decided to assess knowledge on food safety among universities' students. They found that, knowledge grows with the level of education and showed that, the higher the level of education, better the awareness. Kimenju et al. (2005) in Kenya got similar finding in their study of Genetically Modified (GM) foods and said that, consumers' awareness increases with education.

This survey relates with the study in Turkey by Erdem et al. (2015) on consumers' perception and awareness in consumption of Halal. They got respondents with similar education (5% never attended school, 18% primary level and 47% secondary school) and affirmed that, consumers' knowledge is bound to education. The study also concurs with the survey done in Poland and Thailand by Tomaszewska et al. (2018) who observed that correct answers in their study were frequently given by educated participants.

Table 4. Safety perception of tomato consumption.

Variable	Answer	Frequency (N)	Proportion (%)
Diseases related to pathogens contamination			
Can fresh tomato transmit any diseases?	Yes	47	49
	No	48	51
Dysentery	Yes	4	4
	No	97	96
Diarrhea	Yes	19	18.8
	No	82	81.2
Stomachache	Yes	24	23.8
	No	77	76.2
Cancer	Yes	2	2
	No	99	98
Diseases related to pesticide residues contamination			
Can fresh tomato transmit any diseases?	Yes	48	49
	No	50	51
Stomachache	Yes	32	31.7
	No	69	68.3
Cancer	Yes	11	10.9
	No	90	89.1
Headache	Yes	4	4
	No	97	96
Nausea	Yes	2	2
	No	99	98

Consumers' general awareness of pesticides and pathogens on tomato and the need of consensus on vegetables harboring pesticide residues

Consumers have good notions on pesticides use n tomato farms and its potential presence on tomatoes sold in markets. Through this knowledge, they are somewhat alerted by the potential threat of pesticide residues on fresh tomatoes. This might help them to observe adequate practice of washing before cooking to reduce the levels of presence during consumption. Though little is known in Kenya on vegetables domestically consumed; some results are available. Mutai et al. (2015) reported that, vegetables in Kenyan markets contain organophosphates and pyrethroids at 42%.

The level of knowledge of pesticides found in this study corroborates the work of Bempah et al. (2010) in Ghana. He assessed consumers' knowledge and found that, 70% knew fruits and vegetables contamination with pesticide residues. The finding also agrees with the study of Kumari and Basavaraja (2018) in India who realized that, 55% of consumers were aware of pesticides use in vegetables farming.

Regarding consumers' knowledge and the safety of tomato ready to eat, 65% say that, pesticides used in tomato farms can be present on tomato eaten in salad ($p = 0.004$). Also, 74% indicated washing as a practice providing safe tomatoes for consumption ($p = 0.0001$). As well, 95% of participants disclosed that, washing fresh tomato before eating in salad prevents from disease transmission ($p = 0.0001$). This stand point can neither be accepted nor rejected. It is believable that, right information can be given if only pesticides' levels compared to MRLs are given in fresh tomatoes ready-to-eat.

This finding is contrary to that of Kumari and Basavaraja (2018) in India who found that, consumers have no trust on washing practices as a mean providing safe vegetable for consumption and they are restrained on vegetables to buy in markets. They argue that, consumers look for vegetables free of pesticides residues and are willing to spend more on organic crops for health

Table 5. Awareness of pesticides residue and bacterial pathogen contamination of tomatoes among various demographic variables.

Demographic variable		n	Knowledge of tomato with pesticides		Knowledge of tomato with pathogens	
			Mean (scores ± SD)	P-value	Mean (scores ± SD)	P-value
Gender	Male	32	64.86±0.48	0.037*	55.78±0.43	0.083
	Female	69	50.77±0.36		42.01±0.39	
Age	18-25	19	49.58±0.45	0.707	32.03±0.25	0.646
	26-35	54	57.89±0.23		47.79±0.34	
	36-53	28	58.29±0.34		49.67±0.54	
Marital status	Married	56	60.16±0.31	0.005*	67.16±0.39	0.26
	Single	37	39.75±0.43		45.58±0.41	
Level of education	Never attended school	2	24.17±0.26	0.044*	28.60±0.45	0.068
	Primary	28	35.88±0.65		34.83±0.74	
	Secondary	47	58.67±0.23		55.72±0.35	
	Tertiary	15	71.58±0.45		68.49±0.25	
	University	9	72.19±0.43		71.50±0.33	
Grand Mean			53.64±0.38		49.93±0.40	

*****Percentage difference significant at 0.05 level.

protection. Even their study pointed that, 30% of respondents mentioned long term infection due to consumption of vegetables with chemical residues regardless of levels. This view is supported by Kanang (2012) in Thailand who reported that, consumers in Thailand have adopted consumption of sustainable food and are more attached to green diet or produces free of pesticide residues.

Consumers' awareness seems influenced by the knowledge of washing raw produce for pathogens' reduction to acceptable levels of consumption (Sumonsiri and Barringer, 2014). In fact, of the 26% (25/101) stating that washing cannot provide safe crop to eat, only 3% (3/101) believe the crop can still contain pesticides residues, 2% (2/101) pointed that it can contain heavy metals and 15% (15/101) designated the presence of pathogens and 5% (5/101) remained silent on the question. Even, respondents with tertiary (16.8%) and university (8.9%) levels of education were not able to cover properly the point of chemical residues on raw tomatoes. This shows that, consumers mostly think of pathogens when cleaning tomatoes from markets and have less understanding on potential pesticide residues presence on the surface of tomato. It is believable that, knowledge on pathogens is well rooted among tomato consumers compared to pesticide residues from farms.

Consumers might have gained knowledge on pathogens through formal education, episodes of sicknesses related to pathogens, information received during diagnoses in health centers, costs of burden (expenditures for treatment for instance), DAILYs and cooking practices transferred by parents from childhood to adulthood. Consumers could not do better than this when studies on pesticides in Kenya focus on vegetables for export (Mutuku et al., 2014; Mutai et al., 2015) neglecting those consumed locally. It can be assumed that, knowledge on pathogens has been built with progress in science assorted by ways of preventing microbial infection.

Consumers might have either chosen these answers out of any knowledge or, they might be influenced by studies surrounding pesticides use in farms and related critics on side effects. By pointing pesticides as dangerous for human health and that they can be found in tomatoes marketed, they have probably learned from surveys on farmers and pesticides use in Kenya (Nyakundi et al., 2010; Mutuku et al., 2014) and worldwide (Nunifant, 2011; Huynh, 2014; Jamali et al., 2014; Paiboon and Tikampom, 2014; Kariathi et al., 2016). Similarly, respondents might be aware of pesticide multiple residues presence on vegetables demonstrated in South Africa, Sudan, Kuwait and in the European Union (Mutengwe et al., 2016, Hammad et al., 2017; Jallow et al., 2017; EFSA, 2017). Some interviewees might be aware of reports on chemical misuse in tomatoes farms (Latif et al., 2011; Firas, 2015; Mutai et al., 2015; Kamuri and Basavaraja, 2018). By indicating pesticides presence on tomato eaten in salad, they probably knew debates on MRLs adopted for chemical control in farms (FAO, 2009; Elpiniki and Amvrazi, 2011; Latif et al., 2011; Hammad et al., 2017) as well as the EU audit and evaluation outcomes on pesticide residues in Kenyans' fresh crops held in 2013 (European Commission, 2014). In the meantime, establishment of both Pest Control Products Board (PCPB, 2010) and Kenya Plant Health Inspectorate Service (KEPHIS, 2013) by the Kenyan government are some indicators or indices of consumers' knowledge. Lastly, when consumers indicate the safeness of tomatoes after washing, they might be influenced by studies depicting multiple residues on tomatoes ready to eat but pointing them as harmless for human health (Mohammed and Boateng, 2016). Though these probabilities, consumers need updates.

Consumers cannot imagine infiltration of synthetic chemicals in tissues of fresh vegetables (Kariathi et al., 2016). They could not also imagine that, pesticides residues on surface cannot be easily removed after washing. From these points, their knowledge is limited and their awareness insufficient. They think pesticide residues are like pathogens which can be reduced to acceptable levels for consumption through washing (Sumonsiri and Barringer, 2014). Washing chemical residues on surfaces of tomatoes seems ineffective. Studies depict surfactants or adjuvants in agrochemicals as containing oil and other water insoluble agents (Castro et al., 2013). Such oily and water insoluble components reduce the value of simple washing for the reduction of pesticide residues to acceptable levels. Thus, this increases exposure and potential health risks of consumers. This point corroborates the work of Bempah et al. (2010) in Ghana who analyzed health risk of chemical residues on tomato and recommended consumers' health protection through constant investigation. Analyses surrounding pesticides use for crops protection and potential contamination of consumers seem to have generated two thoughts; those rejecting potential human's infection versus those arguing human exposure and infection. One of the main argument standing between both is washing the crop before consumption.

Washing fresh tomato and its inability to transmit diseases to consumers has been demonstrated by number of studies as providing safe vegetables for consumption. Perez et al. (2016) in Mexico agreed with washing of vegetable as a practice preventing any human health infection with pesticides residues because those on the surface are usually removed. Their finding concurs with Akomea-Frempong et al. (2017) in Ghana who found multiple pesticide residues and molecules above MRLs in vegetables ready to eat and concluded that, consumers are not at risk of pesticides related diseases if they wash their crops with running water.

Studies supporting positive effects of washing contaminated vegetables with pesticides residues have been contradicted by a number of researches. Andersson et al. (2014) in France argued that, illnesses generated by pesticides do not manifest instantly after few hours or few days; they appear at long term. This view found support from Kamuri and Basavaraja (2018) in India who analyzed consumers' awareness of pesticides contamination in vegetables. They found respondents pointing health infection by pesticide residues on long term when consuming contaminated vegetables. This implies that, illnesses generated by chemical residues appear long after consumption of contaminated diet. For that reason, consumers or health practitioners cannot trace back the causes of ailments as reported by Ames et al. (1993) in their mutagenesis and carcinogenesis studies. They argued specifically that, effect of synthetic chemical injury is related to human system defense which is also influenced at its turn by previous history of exposure to synthetic chemicals.

Elpiniki and Amvrazi (2011) in Greece also contradicted the safety of contaminated produce even after washing with running water. The researcher insisted that, rinsability of vegetables is not bound to solubility and removability of pesticides on crops. He even added with support from Kiriathi et al. (2016) in Tanzania that, pesticides can infiltrate the flesh of crops and washing will not change their concentrations. This position found support in Brazil by Graziela et al. (2015) who studied effects of washing on contaminated tomatoes with pesticides. They studied the rinsability of tomatoes contaminated with multi residues in households by application of 3 (three) washings on each sample. They concluded after using different solvents (water, sodium bicarbonate 10% and vinegar cleaner 10%) that, all molecules could not be removed. These findings are calling for a need of consensus on pesticide residues on vegetables.

Expected awareness of consumers on pesticides residues using correlations between variables

The bivariate correlation using Pearson coefficients two-tailed test between pesticides variables revealed positive associations at 95 and 99% levels of significance. For instance, a weak positive connection (r=0.364; p= 0.0001) was found between pesticides use in farm can be dangerous to consumers' health and the presence of these pesticides in tomatoes eaten in salad. In the same line, a moderate association (r= 0.410; p= 0.0001) exist between pesticides use in tomatoes farms can be dangerous for consumers' health and, pesticides use in tomatoes farms can be present on tomatoes sold in markets. In addition, a strong correlation (r= 0.705; p= 0.0001) was found between pesticide use in tomatoes' farms can be present in markets and their presence on tomatoes eaten in salad. These positive correlations show that, a holder of one knowledge probably has the understanding of the other. This justifies why those questions were well answered. Though this understanding, consumers might still have some missing information. No significant correlation between: pesticides residues can be on tomatoes eaten in salad and pesticides can transmit diseases to tomatoes' consumers was noted (r= 0.091; p= 0.371). Therefore, respondents could not provide better answers to this association. In this case, interviewees with one of this knowledge might not link to the other. As such, they cannot improve washing practices inherited in households from childhood to adulthood. With this missing skill, respondents might not improve the culture of washing raw tomatoes to reduce more and more chemical residues before consumption in salad. This constitutes a gap to fill in order to increase consumers' awareness and see them starting using running tap water or its equivalent (Akomea-Frempong et al., 2017), detergent (Abou-Arab, 1999), sodium bicarbonate 10% and vinegar cleaner 10% (Graziela et al., 2015) to clean the crop before consumption. This might contribute to prevent human infection with chemical residues at the time when pesticide residues in vegetables are potential sources for systemic poisoning of consumers (Asiedu, 2013). This study corroborates the work done by Tomaszewska et al. (2018) in Thailand and Poland who found that, high positive correlation goes along with correct answers among Poland respondents.

Consumers' knowledge on potential diseases related to pathogens and pesticide residues in fresh tomatoes

With only 10% pointing cancer as a potential disease related to pesticides residues, consumers have poor knowledge. A number of studies have pointed pesticides use in farms as potential cause of cancer diseases (Ridgway et al., 1978; Pratibha et al., 2015). Perhaps, little official communication has been shared on the topic with consumers and maybe, the knowledge is not yet included in school programs at primary and secondary levels to educate young generations on the issue. Undoubtedly, information is still mostly shared within scientists' communities. Few participants responded to this question which probably was embarrassing to them. But, they seemed more conversant with diseases of pathogens. Insertion of this topic in education program will probably improve consumers' knowledge as reminded by Kanang (2012). He revealed that, educated consumers distinguish organic and potentially contaminated food and show preferences for sustainable diet due to related knowledge (avoiding high levels of chemical residues, standard and labelling, level of safety from label).

This study showed that, pathogens and pesticides are well-known by consumers. However, consumers seem to have more knowledge on pathogens compared to pesticides. This might be from the fact that, pathogens actions in humans are usually sudden, can manifest immediately after few hours or days following food consumption. On contrary, pesticide residues related action on health is not an instant or short term process rather; it is a long term procedure (Andersson et al., 2014). This might be the reason why European stakeholders rank pathogens as the priority to worry about on fresh produce compared to pesticides (Boxstael et al., 2013).

Also, consumers might be much conversant with pathogens because for a long time, capacities of African populations were built on pathogens. Their capacities were built through information, education and communications on personal hygiene and cooking of food. Pathogens have been taught in schools' programs since primary level. This strategy has been strong enough to inform and educate the populations on the threat. As well, microorganisms are always diagnosed in health centers and this has contributed to build the capacities and knowledge on the issue. Contrary to germs, pesticides use for crops' protection was recently adopted by the FAO for use in farms in the nineties to address the issue of food security (Shaw, 2007). Maybe, the topic is not yet developed enough to share its understanding with laypersons.

Influence of gender, age, education and marital status on awareness of pesticide residues in raw tomato

Gender consideration has shown a significant difference ($p= 0.037$) in knowledge of use of pesticides in tomatoes farms. Males had good knowledge on the topic compared to females. This knowledge tended to increase with age though there is no significant difference ($p= 0.707$) among age intervals. Marital status was an important factor on food safety among consumers ($p= 0.005$) at 95% level of confidence. Married people had better knowledge on both pesticide residues and pathogens compared to single who were slightly less aware of the concern. The level of education of participants in understanding the concern of tomato contamination with pesticides was an important factor and was statistically significant ($p= 0.044$). Answers of awareness came from respondents with higher level of education.

Both variables age and education relate with the work of Ambrožič et al. (2016) in Slovenia who observed that, knowledge of consumers on food safety increases with age. According to contaminants in food, their survey on foodborne viruses reveals that, consumers with higher level of education were much aware of viral food safety than those with low educated. The present study on marital status agrees with the work done by Pambo (2013) in Kenya and Erdem et al. (2015) in Turkey. These researchers found that, marital status was an important factor of food safety in food consumption in households.

Influence of gender, age, education and marital status on awareness of pathogens presence in raw tomatoes

Contrary to consumers' knowledge on pesticides use in farms, there was no significant difference on awareness of tomato contamination with pathogenic bacteria on gender (p= 0.083), age (0.646), marital status (0.26) and level of education (0.068). However, respondent of 36 to 53 years old had better knowledge followed by respondents of 26 to 35 years old and lastly the youngest 18 to 25 years old. Consumers with university level had better understanding followed by tertiary, secondary, primary and those who have never attended school. Although the level of education seemed important, scores obtained for awareness of bacterial load in fresh tomatoes was not significant (p= 0.068). This may be justified by the usual practice of washing raw tomatoes before consumption. This does not require any experience, degree or a higher understanding because the habit is rooted within the society and is transferred through generations. High score recorded by respondents of 36 to 53 years old may hold on the fact that, many of them are responsible and probably have families and children. As such, they are used to cooking and have knowledge for providing safe food in tables to protect the house dwellers from foodborne ailments. The middle age 26 to 35 years old may be on the same track as elders (36-53 years old) and they might have started building their life experience on safe food and health. The earlier age 18 to 25 years are respondents who have just left the age of teenagers, thus they have less experience in food contamination generally and specifically, vegetables and human's health infection. At this age, some might either start living alone due to studies or job opportunities or, are still in parents' houses. Although they were taught on pathogens in schools, they are not yet concerned on vegetables contamination as their experience in diseases and potential health infection may still be low. In other meaning, they are still not much concerned of where and how they might get infected with food consumed. In one case or another, they are mostly bound to social media (Facebook, Twitter, and WhatsApp) hardly promoting scientific knowledge or raising awareness on issues as this one. From the youngest to the oldest age, the knowledge is acquired progressively and consumers become fully aware with time and experience.

This finding is consistent with the work on consumers' awareness done in Kenya by Pambo (2013) who observed that, consumers' awareness on fortified sugar comes with experience and level of education. The study also corroborates the finding of Malavi et al. (2017) who assessed the practices of food handlers in Kenya and came to similar conclusion.

Conclusion

The study established that, consumers were aware of contamination of fresh tomatoes with pathogens than with pesticides residues. Knowledge on contamination is related to age, level of education, marital status. Consumers knew that pesticides are dangerous to human health and washing reduces their presence on tomatoes. Though respondents knew that, pesticides are dangerous for humans' health and can even be present in freshly prepared salad, they were not able to realize that, pesticide residues presence in salad might be a threat to humans' health. They were convinced that, washing with plain water reduces pesticide residues in raw tomatoes and makes it safe for consumption. This deficiency should be improved through studies on washing contaminated vegetables as well as education, information and communication with consumers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of vermicompost on growth, quality and economic return of garlic (*Allium sativum L.*) at Haramaya District, Eastern Ethiopia

Fikru Tamiru Kenea[1*] and Fikreyohannes Gedamu[2]

[1]College of Agriculture and Natural Resource Management, Dilla University, P. O. Box 419, Dilla, Ethiopia.
[2]College of Agriculture and Environmental Sciences, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia.

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Garlic (*Allium sativum* L.) is an important vegetable crop in Ethiopia. The yield of the crop is often constrained by low and unbalanced nutrient supply in the soil. This study was undertaken to assess effect of vermicompost (VC) on growth, quality and economic return of garlic variety Chelenko I during 2016 main rainy season in Haramaya University main campus, Ethiopia. The treatment consisted of four levels of vermicompost (0, 2.5, 5 and 7.5 tons ha^{-1}) laid out in a randomized complete block design in three replication. Data were collected and analyzed on days to emergency, plant growth, quality, and economic return of garlic. Results revealed early emergency (8.07 days) with application of 7.5 tons ha^{-1} of vermicompost while late emergency (10.13 days) was at nil application of vermicompost. Significant ($P<0.05$) maximum leaf area (37.05 cm^2), leaf area index (1.36) and biomass (61.54 g) were recorded with application of 7.5 tons ha^{-1}. Besides to this, maximum total dry matter (32.71%) and total soluble solid (12.72 Brix°) were also recorded at the rate of 7.5 tons ha^{-1}. Due to application of 7.5 tons ha^{-1} fertilizer, the economic analysis showed the highest net benefit cost of 431,188 ETB ha^{-1} and marginal rate of return (168.87%) with incurred highest total variable cost of 79,350 ETB ha^{-1}. Thus, it can be reasonably generalized that on short time basis, the application of high amounts of VC fertilizers can result in higher economic return than the low dose of VC fertilizer. Therefore, it can be concluded that, the maximum growth and quality and economic return of garlic was obtained with application of 7.5 tons of VC ha^{-1} fertilizer as it gave the highest net benefit cost. However, since the experiment was done only once and at one location, similar experiments should be carried out using additional higher rates of VC fertilizer over several seasons and locations to make a conclusive recommendation.

Key words: Chelenko I, economic return, marginal rate return, net benefit, total soluble solids.

INTRODUCTION

Garlic (*Allium sativum* L., 2n=16) belongs to the Alliaceae family, the same family as onions, shallots and leek (Allen, 2009; Hussena et al., 2014). According to Brewester (2008) cultivated garlic is a species of monocot, bulb- forming biennual grown as annual. Its relatives include onions and shallots (*Allium cepa*) and leeks (*Allium ampeloprasum*). It is the second most widely used cultivated *Allium* after onion (Rubatzky and

*Corresponding author. E-mail: fikr1.kenea@gmail.com.

Yamaguchi, 1997; Hamma et al., 2013; Hassan, 2015). Garlic is primarily grown for its cloves, which are used mostly as food flavoring condiments due to groups of sulphur containing compounds, allin and allicin (Messiaen and Rouamba, 2004). It is widely cultivated spice crops used for food and medicinal purposes (Diriba et al., 2013). Green tops are eaten fresh and cooked especially in tropical areas and consumption of immature bulbs for salad use is also popular (Block, 2010).

In Ethiopia, the acreage of garlic cultivation decreased from 16411.19 ha in 2013/2014 to 9257.71 ha in 2014/2015 with a total production of about 1590935.75 and 934868.73 tons of bulbs with the productivity of 9.7 and 10.1 tons ha^{-1}, respectively. Though acreage of garlic, production and productivity were not indicated in Eastern Hararghe, about 27190 farmers produced garlic (CSA, 2015). The yield of recently released garlic variety, Chelenko I, gave 9.3 t ha^{-1} on research field was appreciated and selected for Eastern and Western Hararghe (Tewdros et al., 2014), though its productivity is less than national productivity.

The economic importance of the garlic crop has increased considerably in the entire world in recent years. Despite its importance, growing garlic is faced by various problems during growth period (Shafeek et al., 2015). In Ethiopia, major production constraints include lack of proper planting material particularly shortage of improved varieties, imbalanced fertilizer use, lack of irrigation facilities, lack of proper disease and insect pest management and other agronomic practices, lower soil fertility status in many soil types, and lack of proper marketing facilities (Getachew and Asfaw, 2000; Mohamed et al., 2014) all which considerably reduce yield. Among the primary macronutrients, N, P and K are the most commonly reported deficient plant nutrients in most Ethiopian soils (Berga et al., 1994; Yohannes, 1994). Sub-optimal levels of these nutrients in the soil adversely affect the yield, quality and storability of bulbs of garlic crops (Gubb and Tavis, 2002).

Garlic is heavy feeder and most of the *Allium* species have low nutrient extraction capacity than most crop plants because of their shallow and un-branched root system. Therefore, adequate nutrient supply is essential for healthy crop growth and for attaining higher yield in sustainable way (Jones et al., 2011; Cantwell et al., 2006). Optimum application of fertilizers to garlic crop is important for improving growth, yield and marketable bulb proportions as well as bulb quality (Diriba et al., 2013). Organic inputs are often proposed as alternatives to mineral fertilizers. Organic fertilizers like wood ash, poultry manure, and fermented slurry were reported to produce garlic having low moisture content, high pungency, and higher mineral composition (Babatunde et al., 2009). However, the traditional organic inputs such as crop residues, and animal manures cannot meet crop nutrient demand over large areas because of the limited quantities available, the low nutrient content of the materials, and the high labour demands for processing and application (Pratap et al., 2011). Therefore, the application of bio-fertilizers like VC has been recognized as an effective means for improving soil aggregation, structure and fertility, increasing microbial diversity and populations, improving the moisture-holding capacity of soils, increasing the soil Cation Exchange Capacity (CEC) and increasing crop yields (Hargreaves et al., 2008). They also reported that municipal soil waste compost can also reduce the volume of the waste, kill pathogens that may be present, decrease germination of weeds in agricultural fields, and destroy malodorous compounds.

Vermicompost (VC) is slow releasing organic manure which has most of the macro as well as micronutrients in chelated form and fulfills the nutrient requirement of plants for longer period. Vermicompost helps in reducing C: N ratio, increased humic acid content and provide nutrient in the readily available form to the plants such as nitrate, available phosphorus, soluble potassium, calcium and magnesium (Talashilkar et al., 1999). Besides, Alemu et al. (2014) reported that increased levels of VC from 0 to 5 t VC ha^{-1} increased soil fertility with increased economic return.

Application of VC is crucial to increase productivity of plants with reduced pollution of environment. Possibilities to enhance garlic productivity in the Eastern Hararghe, Ethiopia have been the domain of investigation of recent years though nationally expected yield is not achieved yet. Farmers around the study area, Haramaya produce the local varieties of garlic crop in homesteads. However, recently Haramaya University has released new garlic variety Chelenko I (Tewdros et al., 2014). Varieties may also differ in their response to source and rate of applied fertilizers (Zhou et al., 2005). Moreover, no work has been done on effect of VC on the performance of garlic in the area. Therefore, the study was initiated to assess the effect of VC on growth, quality and economic return of garlic.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Haramaya University main campus during the main crop growing season (August-December) of 2016. Its altitude is about 2006 m above sea level, 9°24''N latitude and 42°03''E longitude. The site has a bimodal rainfall and is representative of a sub-humid and mid-altitude agro-climatic zone. The short rainy season extends from March to April whereas the long rainy season extends from June to October. The mean annual rainfall and temperature are 790 mm and 17°C, respectively (Belay et al., 1998; Simret et al., 2014). The minimum and maximum temperatures are 3.8 and 25°C, respectively (Tekalign and Hammes, 2005). However, in this crop growing season, from May to December 2016, the total annual rainfall was 566.1 mm, mean maximum and minimum temperature were 24.04 and 13.14°C, respectively. The soil of the experimental site is a well-drained deep alluvial (Tamire, 1975) with sandy loam texture

Table 1. Physical and chemical properties of the soil of the experimental site at Haramaya, Eastern Ethiopia.

Soil property	Value	Rating	Reference
Sand (%)	61	-	-
Clay (%)	23	-	-
Silt (%)	16	-	-
Textural class	Sandy clay loam	-	Moodie et al. (1954) and Rowell (1994)
pH 1: 2.5 (H_2O)	7.4	Slightly alkaline	Jones (2003)
OC	1.48	Low	Tekalign (1991)
OM (%)	2.55	Low	Tekalign (1991)
Total N (%)	0.18	Medium or moderate	Berhanu (1980) and Hazelton and Murphy (2007)
Available P (mg kg^{-1})	5.58	Low	Hazelton and Murphy (2007)
Exchangeable K(Cmolc kg^{-1})	0.32	Medium	Hazelton and Murphy (2007)
CEC (cmol (+) kg^{-1})	18.61	Medium	Landon (1991)

OC, Organic carbon; OM, organic matter; CEC, cation exchange capacity.

(Simret et al., 2014).

Experimental materials

Garlic variety Chelenko I was used which was released in 2014 for mid to high altitude garlic growing areas of eastern and western Hararghe Zones by Haramaya University. Its yield is stable over seasons and locations in the eastern highlands of the country. It is well adapted with productivity of 9.3 t ha^{-1} and moderately susceptible to garlic rust in Eastern Ethiopia. It takes about 132 days to mature (Tewodros et al., 2014).

Treatments and experimental design

The treatments consisted of four rates of vermicompost (VC: 0, 2.5, 5.0 and 7.5 t ha^{-1}). The experiment was laid out in randomized complete block design with three replications.

Experimental procedures and crop management

Experimental field was ploughed by a tractor. The plots were leveled and the ridges of about 20 cm height were prepared. The gross plot size was 2.0 m × 1.5 m. In between blocks and plots, 0.75 and 0.5 m space was left, respectively. Vermicompost (VC) was applied about two weeks before planting to randomly assigned treatments to each plot. Healthy and uniform medium-sized cloves of 1.5 to 2.50 g (Fikreyohannes et al., 2008), were selected and planting was done on 11 August 2016 at the depth of 3 to 4 cm. The cloves were planted on the ridge at a spacing of 30 cm between rows and 10 cm between plants. Thus, there were five rows in each plot and 20 plants in a row. The outer most one row on each side of a plot and 20 cm on both ends of each row were considered as border. Thus, the net plot size was 0.9 m × 1.8 m. Mancozeb was applied at the rate of 3.5 kg ha^{-1} mixing with water in a ratio of 2 gm L^{-1} (Anonymous, n.d.). This was done after a month from planting when fungus (garlic rust) symptom appeared on garlic leaf. All other recommended cultural practices to produce the crop such as weeding, harrowing and watering were applied uniformly and regularly to the entire plots throughout the experiment time as per the recommendation of Debre Zeit Agricultural Research Centre (Getachew and Asfaw, 2000). When 70% of the plants showed neck fall (Getachew and Asfaw, 2000; EARO, 2004), harvesting of bulbs was done on starting from 16 December 2016.

Vermicompost and soil sample nutrient analysis

Vermicompost sample, made from *Lantana camara, Partinium hystrophorous* and farmyard manure, was analyzed before applying to the soil. Samples were taken randomly from the entire bag. It was broken into small crumbs and prepared for determining chemical properties. The sample was air-dried and sieved through a 2 mm sieve. Its EC and pH were determined from the filtered suspension of 1:2.5 soils to water ratio using a glass electrode attached to a digital EC meter and pH meter (Jones, 2003). Sample was analyzed for electrical conductivity (EC), total N, available P, exchangeable K, organic matter and organic carbon. Total N was determined using the Kjeldhal method (Jackson, 1958). Available P was determined by extraction with 0.5 M sodium bicarbonate ($NaHCO_3$) according to the methods of Olsen et al. (1954). Exchangeable potassium was determined with a flame photometer after extraction with 0.5 ammonium-acetate according to Hesse (1971). Organic carbon of soil was determined by the Walkley-Black method (1934).

In similar way, soil sampling was done before planting. The samples were taken randomly using an auger in a zigzag pattern from the entire experimental field. Before planting, ten soil samples were taken from the top soil layer to a depth of 20 cm and composited in a bucket to represent the site. The soil was broken into small crumbs and thoroughly mixed. From this mixture, a composite sample weighing 1 kg was filled into a plastic bag. The chemical content of the soil was determined using similar procedures used for the VC as it was developed for the soil. Soil texture was determined by Bouyocous hydrometer method (Moodie et al., 1954).

Data collection and measurements

Days of emergency

Days of emergency was determined by counting when about 50% of the plants emerged.

Growth parameter

Leaf area was determined from 10 randomly taken plants from the central rows using the formula:

LA = LL × LW × 0.733

where LA = mean leaf area of the plant, LL = leaf length, LW = maximum leaf width, and 0.733 = conversion factor for leaf area.

Leaf area index was determined using the value of the leaf area divided by the area of the land occupied by the plants according to Watson (1958) using the following formula:

Leaf area index (LAI) = Lam × N/A

LA = LL × LW × 0.733

where Lam = mean leaf area of the plant, LL = leaf length, LW = maximum leaf width, 0.733 = conversion factor for leaf area, A=the area (cm^2) occupied by one plant in the cropping area, and N= number of leaves on the plant

Total fresh biomass yield (g/plant) was determined by taking the total weight of five randomly sampled plants from the four central rows of fresh bulbs, leaves, stems and roots using a sensitive balance.

Quality parameters

Total soluble solid: This was measured by taking 100 g juice and diluting it in 50% of distilled water, and then refractometer was used for evaluation.

Total dry matter (%): The average dry matter weight (g) of total biomass after curing was measured by drying 10 randomly sampled plants in an oven with a forced hot air circulation at 70°C until a constant weight was obtained. The percent of bulb dry matter calculated by taking the ratio of the dry weight to the fresh weight of the sampled plants and multiplying it by 100.

% BDM = (Weight of total dry matter / Total fresh weight) × 100

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using SAS software version 9.0 and the means separated by using Turkey's Method at 0.05 level of significant if treatments were found significant.

Partial economic return analysis

The partial budget analysis as described by CIMMYT (1988) was done to determine the economic feasibility of the garlic production using the prevailing market prices for inputs at planting and for the outputs at the time of crop harvest. It was calculated by taking into account the additional input and labor cost involved due to additional input and the gross benefits obtained from garlic production. Average yield was adjusted downward by 10% to reflect the difference between the experimental yield and the yield farmers could obtain under their management practices as described by CIMMYT (1988). The field price of garlic was calculated as (sale price minus the costs of harvesting, cleaning, bagging and transportation). The net benefit was calculated as the difference between the gross field benefit (ETB ha^{-1}) and the total variable costs (ETB ha^{-1}).

Marginal rate of return (MRR %)

MRR was calculated by dividing change in net benefit or gross benefit by change in cost which was the measure of increasing in return by increasing input. This means by subtracting gross benefit of nil from gross benefit of each treatment and divided by the total variable cost of each treatment and multiplying each value by 100%.

$$\text{Marginal rate of return (\%)} = \frac{\text{Change in net benefit}}{\text{change in total cost}} \times 100$$

RESULTS AND DISCUSSION

Physical and chemical properties of the soil

The result of laboratory analysis of selected physical and chemical properties of soils of the experimental area is shown in Table 1. The textural class of the soil was sandy clay loam based on the soil textural triangle of the International Society of Soil Science system (Moodie et al., 1954; Rowell, 1994). The pH of the experimental soil was 7.4 which is slighty alkaline on the basis of pH limit (7.4 to 7.8) described by Jones (2003). The pH is in the range of 6.5 to 7.5 favorable for garlic production (Bachmann, 2001).

The organic matter (OM) of the experimental soil was 2.55%. According to Tekalign (1991), OM ranging from 0.86 to 2.59 is low, hence the soil might respond to the applied VC, as its organic matter content was low.

As per the rating (0.12 to 0.25%) described by Berhanu (1980), the total N content of the soil (0.18%) was medium. This value showed that the crop might respond to the applied VC (Table 1) due to increased soil fertility with application of fertilizers. According to the rating (5 to 9 mg P kg^{-1}) suggested by Cottenie (1980), the available P of the soil was low (Table 1). This may be because of low percent of OM content of the soil (Table 1) which is also in agreement with the suggestion of Clark et al. (1998) who indicated that soil OM influences P availability to crops directly by contributing to P pool. However, Toung et al. (2000) reported that P response is likely in soils that have less than 20 mg kg^{-1} extractable P. The cation exchange capacity (CEC) of the experimental soil was 18.61 (cmol (+) kg^{-1}). This value was medium according to the rating (15 to 25) suggested by Landon (1991). This indicated that the soil of the experimental site might respond to the different rates of VC. Hazelton and Murphy (2007) categorized exchangeable soil potassium contents of 0.3 to 0.7 Cmolc kg as medium. In accordance with this category, the exchangeable soil potassium content of the experimental soil is in medium category. This indicates external application of mineral and/or organic fertilizers containing potassium is important for enhancing the fertility of the crop and yield of the crop.

Vermicompost chemical properties

Chemical analysis of VC is indicated in Table 2. Its component was with EC: 8.83 msm^{-1}, pH: 7.25, total N was 0.56%, 25.82 ppm of available P, exchangeable K

Table 2. Chemical properties of vermicompost.

No. of VC	Chemical properties						
	Total N (%)	**Available P (mg kg^{-1})**	**Exchangeable K [Cmol(+)kg^{-1}**	**OM (%)**	**OC (%)**	**pH**	**EC (msm^{-1})**
Value	0.56	25.82	23.69	15.39	8.92	7.25	8.83
Rating	Very high	Moderate	Very high	Very high	Very high	Nuetral	Very high

OC, Organic carbon; OM, organic matter; VC, vermicompost; EC, electric conductivity.
Source: Hazelton and Murphy (2007).

Table 3. Effects of application of vermicompost on days to emergency, fresh biomass, total dry matter, leaf area , leaf area index, and total soluble solid of garlic.

Factor	Treatment	Days to emergency (days)	FB (g)	Total dry matter (%)	Leaf area (cm^2)	LAI	TSS (Brix°)
VC (t ha^{-1})	0	10.13^c	56.19^c	27.01^c	30.32^c	0.87^c	11.92^d
	2.5	9.00^b	59.05^b	29.18^b	32.37^{bc}	1.03^b	12.27^c
	5	8.67^b	60.08^{ab}	31.37^a	33.99^b	1.12^b	12.45^b
	7.5	8.07^a	61.54^a	32.71^a	37.05^a	1.36^a	12.72^a
LSD (0.05)		0.50	2.29	1.50	2.48	0.09	0.08
CV %		5.76	3.96	5.09	7.57	8.79	0.67

was 23.69 Cmol(+)kg^{-1} VC, 15.39% of OM and OC was 8.92%. These VC increases soil fertility without polluting the soil, as well as the quantity and quality of crops. Moreover, beneficial effects of VC on plant growth under water deficit conditions may be due to better aeration to the plant roots, increasing amount of readily available water, induction of N, P and K exchange thereby resulting in better growth of the plants. Application of bio-fertilizers such as VC have been recognized as an effective means for improving soil aggregation, structure and fertility, increasing microbial diversity and populations, improving the moisture-holding capacity of soils, increasing the soil cation exchange capacity and increasing crop yields (Hargreaves et al., 2008).

Days to emergency

Vermicompost (VC) application significantly influenced days to emergence. With the increase in the rates of VC application, the number of days required by the garlic sprouts to emerge above the soil surface was decreased. This means that plants that were not treated with the VC emerged from the soil later than plants that were treated with the VC. Thus, increasing the rate of vermicompost from nil to 7.5 t ha^{-1} hastened the emergence of garlic sprouts from the soil by 20.35%. The hastened duration for emergence due to the increased application of the VC may be attributed to the influence of nutrients released from vermicompost on root initiation and development which might lead to early shoot emergence. Similarly, Atiye et al. (2000) found that seedlings emergence of tomato, cabbage, and radish was much faster in higher rates of vermicompost than in nil application. Juan et al. (2006) also observed that the use of vermicompost as a substrate produced an earlier shoot emergence and earlier start of bulbification. This corresponds to increase the total soluble carbohydrates and a subsequent modification in the non-structural carbohydrate distribution patterns, and hence a modification in the pattern of fructan (scorodose) metabolism. The author therefore concluded that scorodose accumulation is directly related to the harvest index and is shown as greater yield and bulb quality.

Growth parameters

Fresh biomass

Analysis of variance showed that application of VC had very highly significant effects (0.001) on fresh biomass. With increased VC from 0 to 7.5 t ha^{-1}, fresh biomass increased by 9.5% over control (Table 3). Earthworm castings (worm manure) are rich in microbial activity and plant growth regulators, and fortified with pest repellence attributes as well (Suparno et al., 2013) that increase total fresh biomass. An important feature of VC is that, during the processing of the various organic wastes by earthworms, many of the nutrients that it contains are changed to forms that are more readily taken by plants such as nitrate or ammonium nitrite, available phosphorous and soluble potassium, calcium and magnesium (Suthar and Singh, 2008). In line with this result, application of vermicompost significantly influenced fresh biomass yield (Alemu et al., 2016). The author also observed that fresh biomass yield increased by 4.71% as the vermicompost was increased from 0 to 5 t ha^{-1}.

Leaf area

Vermicompost (VC) fertilization significantly (P<0.01) affected the mean leaf area per plant of garlic. The highest mean leaf area per plant (37.05 cm^2) was achieved at 7.5 t VC ha^{-1} while the lowest was obtained from the unfertilized plots (Table 3). Therefore, increasing VC resulted in increased leaf area of garlic.

Leaf area index

Leaf area index was significantly affected by application of vermicompost (P < 0.001). Vermicompost supplement at a rate of 7.5 t ha^{-1} increased leaf area index of garlic by 56.32% compared to control (Table 3). The increase in leaf area index in response to increasing rate of vermicompost may be ascribed to the availability of optimum nutrients contained in manure that led to high leaf area index through facilitated vegetative growth. This result is in line with that of Mehdi et al. (2012) who reported that it significantly increased all the growth attributes such as plant height, stem diameter, number of leaves, and leaf area index in response to applied municipal solid waste and vermicompost under well-watered, moderate and severe stress conditions. Alemu et al. (2014) also reported increased leaf area index of garlic with increased rate of VC application.

Quality parameters

Total dry matter

Bulb dry matter percent was significantly influenced by vermicompost (P < 0.001). Garlic bulb dry matter percent was increased by 21.10% due to vermicompost application at 7.5 t ha^{-1} rate over the control (Table 3). This result is supported by Juan et al. (2006) who showed that vermicompost increased the bulb dry weight due to the accumulation of non-structural carbohydrates whose distribution patterns change, thus favouring the metabolism of fructan precursors and accumulating as scorodose. The author further explained that such reserve substance (scorodose) accumulation in the vermicompost treatment represented by scorodose polysaccharide, occurs for a longer period due to the earlier start of bulbing. This response translates into a 2-fold increase of the bulbs dry weight, increased size and therefore, higher quality and yield at harvest. Similarly, Fenwik and Hanley (1985) reported that, in garlic, the fructan polysaccharide is the scorode which accounts for 53% of garlic dry matter. Alemu et al. (2014) also reported that the garlic bulb dry matter percent was increased by 8.13% due to vermicompost application at 5 t ha^{-1} rate over the control.

Total soluble solid

Total soluble solid was significantly influenced by vermicompost (P < 0.001). Application of 7.5 t VC ha^{-1} increased TSS by 6.7% compared to control (Table 3). It might be due to more accumulation of reserve substances in the bulbs. This result is supported by the findings of Alemu et al. (2014) who found a higher fruit density and more TSS in tomato due to application of vermicompost as compared to the treatment to which vermicompost was not applied. Alemu et al. (2014) also reported that application of 5 t VC ha^{-1} increased TSS by 11.04% compared to control.

Economic return analysis

As indicated in Table 4, economic analysis was done for main effects as it shows significant effect on marketable bulb yields. The variable cost considered was VC fertilizer cost with its application cost as well as extended days for gardener (60 ETB per (day + night)) of each treatment over control was considered. Even though there is no variable cost (the costs of fertilizer requirements and application were not included) in absolute control or nil application of VC fertilizer, the lowest benefit cost was obtained. On the other hand, treatment with application of VC is economically sound full than over control. Alemu et al. (2016) pointed out that the highest net benefit with the highest total variable cost and the lowest was with no variable cost or the area which was with nil application of VC for garlic crop. The study undertaken on two soils types by Diriba et al. (2015) also showed that the growth, yield and economic potential of garlic were increased in response to fertilizer application.

Maximum net benefit (364250 ETB ha^{-1}) was obtained with application of 7.5 t VC ha^{-1} fertilizer while the least net benefit cost (309600 ETB ha^{-1}) was obtained with unfertilized. Verma et al. (2013) also reported that combined application of 5.0 t VC ha^{-1} and 60 kg S ha^{-1} was superior with respect to net returns of garlic. As to this finding, it is profitable to cultivate garlic with application of 7.5 t VC ha^{-1}. Alemu et al. (2016) also reported that net benefit of 163532 ETB ha^{-1} with application of 5 t VC ha^{-1}. Marginal rate of return percent

Table 4. Partial budget analysis of the economic performance of garlic under VC fertilization.

Factor	Treatments	Average yield (t ha^{-1})	Adjusted yield (t ha^{-1})	Gross benefit (ETB ha^{-1})	Total variable cost (ETB ha^{-1})	Net benefit (ETB ha^{-1})	%MRR
	0	8.6	7.74	309600	0	309600	-
VC	2.5	10.1	9.09	363600	26450	337150	204.36
(t ha^{-1})	5	11.34	10.21	408400	52900	355500	186.77
	7.5	12.32	11.09	443600	79350	364250	168.87

Price of vermicompost = 10.00 ETB kg^{-1} + application cost of 1390.00 ETB per 2.5 t ha^{-1} or 2780.00 ETB per 5 t ha^{-1} + 4170.00 ETB per 7.5 t ha^{-1}. Garlic selling price = 40.00 ETB kg^{-1}.
Source: Garlic selling price at Haramaya District (2017).

was reduced with increased rate of VC. Maximum marginal rate of return percent (204.36%) was obtained with application of 2.5 t VC ha^{-1} while the least (168.87%) was recorded with application of maximum levels of VC (7.5 t ha^{-1}). This was due to highly increased variable cost with increased rate of VC.

Conclusion

The analysis of variance indicated that effect of VC show significant effects on days to emergency, leaf area, leaf area index, fresh biomass, total dry matter percent and bulb quality. The highest net benefit was recorded with application of the highest rate of VC fertilizer (7.5 t ha^{-1}) while the highest marginal rate of return was obtained with application of 2.5 t VC ha^{-1} fertilizer application. The early emergency (8.07 days) was recorded with application of maximum rate of VC (7.5 t ha^{-1}) while late emergency (10.13 days) was with nil application of VC.

Growth parameters such as leaf area, leaf area index and fresh biomass are significantly influenced by the applied VC fertilizers. Maximum leaf area (37.05 cm^2) was recorded with application of maximum rate of VC (7.5 t ha^{-1}) fertilizer. The highest leaf area index (1.36) and fresh biomass (61.54 g) were recorded from 7.5 t VC ha^{-1} application. Total dry matter and soluble solid traits showed significant differences in response to the application of VC fertilizer. Maximum total dry matter (32.71%) and total soluble solid (12.72 Brix°) were recorded at the rate of 7.5 t VC ha^{-1}.

The economic analysis showed the highest net benefit cost of 431188 ETB ha^{-1} with incurred highest total variable cost of 79350 ETB ha^{-1} with application of 7.5 t VC ha^{-1} fertilizer. The least net benefit cost of 309600 ETB ha^{-1} was obtained with nil application of VC fertilizer. The highest marginal rate of return (204.36%) with application of 2.5 t VC ha^{-1} and the least (168.87%) with application of 7.5 t VC ha^{-1} was recorded. Thus, it can be reasonably generalized that on short time basis, the application of high amounts of VC fertilizers can result in higher economic return than the low dose of VC fertilizer. However, the results of the experiment have revealed that growth, quality and economic return did not reach the optimum since they all significantly increased in response to the application of VC fertilizer. Therefore, there is a possibility that significantly more growth characters, quality and economic return of the garlic could have been obtained if the rates of the VC fertilizers had been increased. Therefore, from the results of this study, it can be concluded that, the maximum growth and quality and economic return of garlic was obtained with application of 7.5 t VC ha^{-1} fertilizer as it gave the highest net benefit cost. However, since the experiment was done only once and at one location, similar experiments should be carried out using additional higher rates of VC fertilizer over several seasons and locations to make a conclusive recommendation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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