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African Journal of Agricultural Research

Table of Contents: Volume 10 Number 36 3 September, 2015

ARTICLES

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Full Length Research Paper

Application of poultry manure and the effect on growth and performance of potted Moringa (*Moringa oleifera* Lam) plants raised for Urban dwellers' use

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The study was carried out in the University of Nigeria, Nsukka, Nigeria from 2010 to 2011 to assess the effect of poultry manure application on growth and performance of potted moringa (*Moringa oleifera* Lam) plants raised for urban dwellers' use. Application of five and 10 tonnes/ha of poultry manure to the soil medium ensured consistent increase in the plant height, stem girth, internode length, number of leaves and branches. The control treatment (0 tonne/ha) had the least values of all the morphological characteristics. The 10 tonnes/ha poultry manure treatment gave significant increase ($p < 0.05$) in the plant growth vigour in the first two months of growth with a decrease in the third month. The interaction effects of accession by poultry manure were significant ($p < 0.05$) on the days to initial flowering, days to 50% flowering and fresh leaf biomass production/plant but not significant ($p > 0.05$) on the days to 100% flowering, days to pod formation, number of pods/plant, number of seeds/pod, pod length and pod circumference. The 10 tonnes/ha poultry manure gave the highest values of all the yield traits in 2011 followed by five tonnes/ha and zero tonne/ha respectively. There were significant accession by manure interaction effects ($p < 0.05$) on all the yield traits except days to first and 50% flowering. There were lesser days to first, 50%, 100% flowering and pod formation and higher values of all the other yield traits in 2011 than in 2010.

Key words: Nigeria, potted Moringa plants, poultry manure, urban dwellers.

INTRODUCTION

Moringa oleifera is a small deciduous, fast growing and drought resistant plant. It is also a perennial tree which can reach a maximum height of 7-12 m and a diameter of 20-40 cm at chest height. The stem is normally straight and branches at a height of 1.5 to 3 m (Fuglie and Sreeja, 2011). It grows best in loamy or sandy loam soil and can tolerate poor but not water-logged soils. *M. oleifera* is a highly nutritious and medicinal plant with great agricultural, industrial and domestic uses (Moyo et al., 2011, Ndubuaku and Ndubuaku, 2011).

Cultivation of moringa plants in the urban cities has not been adequately exploited in Nigeria because of land limitations. This problem can sufficiently be resolved by raising moringa plants in pots like plastic buckets,

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earthenware pots, baskets etc. The nutrient status of the potting medium (soil) in the container should be relatively high to support the life of the plant for a long period and can be augmented periodically with split doses of poultry manure and NPK (Nitrogen:Phosphorus:Potassium) fertilizer (Annenber, 2010). Ede et al. (2015) worked on integrated nutrient management in moringa production and observed that weathered sawdust plus poultry manure medium gave the highest mean coefficient velocity of seedling emergence followed by 100% sawdust medium. The leaves of the potted moringa plants can be harvested periodically for use as the plant re-flushes fast, and pinching the terminal bud on the central leader stem is found to be necessary when it attains a height of 75 cm to promote the growth of many lateral branches and reduce the height of the tree (Amaglo, 2010). Potted moringa plants can be kept as outdoor plants and watered routinely especially during the dry season. Moringa trees can also be planted as hedge-row plants around houses, parks, schools, hospitals etc. Moringa plants respond well to irrigation and the yield can be doubled by drip irrigation as compared to rain fed crops (Oz, 2014).

MATERIALS AND METHODS

Fifty-four 30 L capacity black plastic buckets perforated at the base were used to raise the moringa plants in an un-shaded nursery in the Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka, Nigeria. Each bucket contained 25 kg of soil. Cured poultry manure was incorporated into the soil in the buckets at the rates of 0 tonne /ha, 5 tonnes /ha and 10 tonnes/ha, respectively before sowing the seeds. Three seeds were planted in each bucket. Two litres of water was added to each bucket of soil before planting and subsequently at three days intervals during the early periods of growth (between April and late May, 2010 when there was less rainfall). The water was just enough to sustain the growing seedlings in the buckets without leakage from the perforations below. However, the plants were rain-fed between June and November, 2010 (during regular rains). Controlled watering was continued from December, 2010 to May, 2011 before regular rains commenced again. The moringa seeds used for the experiment were collected from three locations in Nigeria namely; Nsukka (in the southeast), Ibadan (in the west) and Jos (in the north). The experimental design was a 3 × 3 factorial in completely randomized design with three replications. Morphological growth and yield attributes of the plants were monitored for two years; 2010 and 2011. Sowing of the seeds was done in April, 2010 and the records of the morphological growth and yields were taken till December, 2010 for the first phase of growth. The subsequent yield records were taken till September, 2011.

The morphological characteristics measured included the stem height (cm), stem girth (cm), number of leaves, number of branches, internode length and plant growth vigour. The parameters were recorded monthly. The root volume was determined at the ninth month of the study to avoid frequent destructive sampling.

Plant height was measured from the base of the plant to the tip of the terminal leaf bud with a meter rule. Stem diameter was measured five centimeters above ground level using micrometer screw gauge and converted to girth using the following formula:

$$G = D\pi$$

Where G is the stem girth, D is the stem diameter and π is a constant (π = 22/7).

The number of leaves and branches were determined by counting. The number of leaves /plant was determined by the number of nodes on the plant to include the abscised leaves. The internode length was measured with a meter rule between two adjacent nodes. The root volume was determined by immersing the root system into a calibrated cylinder with a known volume of water and recording the displacement (d). The formula is as follows:

Root Volume (RV) = Final water volume (FWV) – Initial water volume (IWV) = Displacement (d)

Thus, RV = FWV – IWV = d.

The plant growth vigour was determined by using the assessment indices for the growth of *M. oleifera* plant as shown in Table 1. The yield attributes measured included days to flowering which was recorded as the number of days from sowing to first flower production; days to 50 and 100% flowering recorded as the number of days from sowing to when 50 and 100% of the plants flowered, days to pod formation and setting, number of pods/plant, number of seeds per pod, pod length (cm) and pod circumference (cm). After the 2010's yield, the apical shoot was pruned from a height of 50 cm above the ground and all the leaves harvested (apart from the abscised leaves) to determine the fresh leaf biomass and prepare for 2011 yield.

Statistical data collected were analyzed using analysis of variance (ANOVA). Significant means were compared using Fisher's least significant difference (FLSD) at 5% probability according to Obi (2002). The statistical package used was Genstat 3.0 release 4.23 Discovery Edition (2008).

RESULTS

Application of five and 10 tonnes/ha of poultry manure to the soil medium ensured consistent increase in the plant height, stem girth, internode length, number of leaves and branches. The control treatment (0 tonne/ha) had the least values of all the morphological characteristics (Figures 1 to 6). The poultry manure levels showed significant differences ($p < 0.05$) in the plant height and stem girth all through the growth period (Figures 1 and 2), and in internode length at the second month (Figure 4), number of leaves and branches at the third month (Figures 3 and 5). The internode lenghts of the moringa accessions showed a decrease on the seventh month of planting and started inproving from the eighth month. The 10 tonnes/ha poultry manure treatment gave significant increase ($p < 0.05$) in the plant growth vigour in the first two months of growth with a decrease in the third month. The plants treated with five tonnes/ha poultry manure grew vigorously within the first three months after planting and decreased in their growth vigour on the fourh and sixth months. The control plants decreased consistently in their growth vigour from the fourth month after planting. However, there were significant differences ($p < 0.05$) in the effects of the poultry manure levels on plant growth vigour which reflected all through the growth period in 2010 (Figure 6). The 10 tonnes/ha poultry manure gave

Table 1. Plant vigour assessment indices for the growth of *Moringa oleifera* plant (Ede, 2014).

S/N	Description	Score
1	Vigorous growth evident, dark green coloured and fully unfurled leaves ≥ 8	5
2	Dark green, vigorous but fully unfurled leaves < 8	4
3	Pale green, vigorous but fully unfurled leaves <8	3
4	Moderate growth with chlorotic appearance and some necrotic spots	2
5	Retarded growth evident and plants generally poor	1
6	Dead plant	0

Note: No dead plants were recorded in the study.

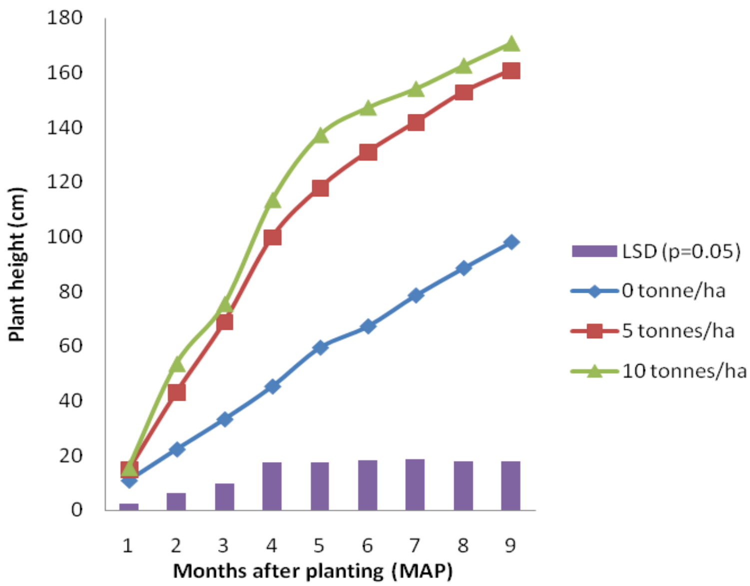

Figure 1. Plant height (cm) of the potted moringa plants in 2010.

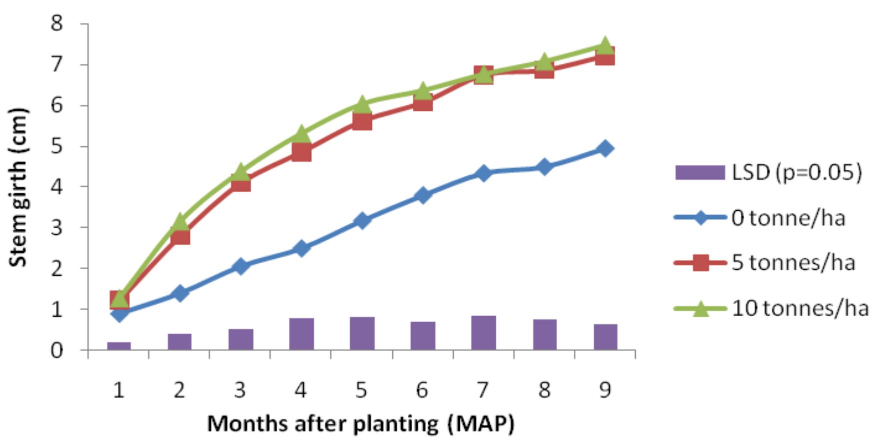

Figure 2. Stem girth (cm) of the potted moringa plants in 2010.

the highest values of the plant height, stem girth, plant growth vigour, number of leaves and branches at the ninth month of the plants growth in 2010. The internode length values obtained in the five and 10 tonnes/ha

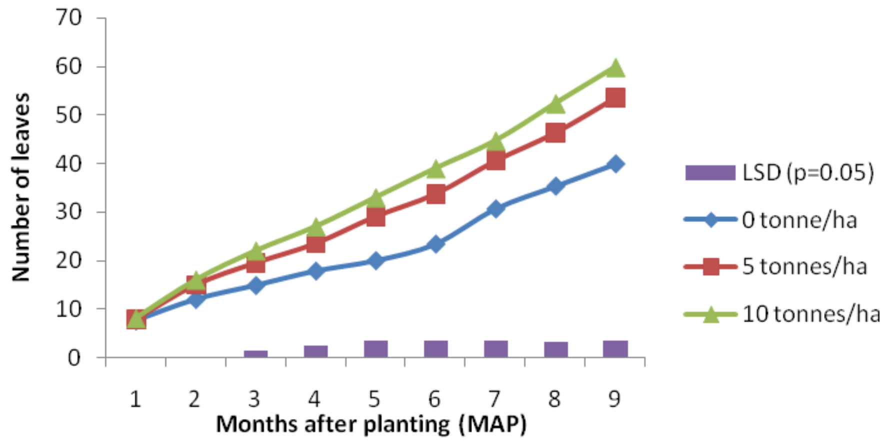

Figure 3. Number of leaves of the potted moringa plants in 2010.

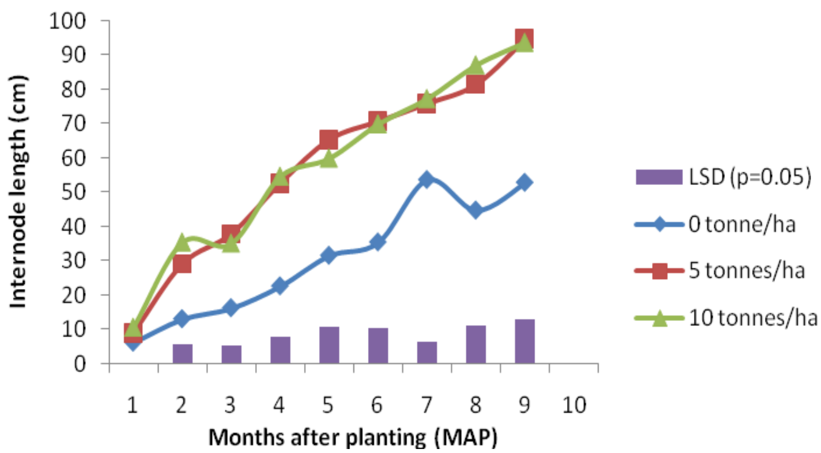

Figure 4. Internode length (cm) of the potted moringa plants in 2010.

Table 2. Main effect of the accessions on morphological growth parameters of *Moringa oleifera* plant nine months of study in plastic pots.

Accessions	PHT (cm)	SG (cm)	NL	INL (cm)	NB	GV
Nsukka	156.7	7.43	52.2	83.3	0.11	4.22
Jos	133.5	5.87	53.0	77.9	0.67	4.00
Ibadan	114.2	6.34	48.2	80.1	0.00	3.78
$LSD_{(0.05)}$	ns	ns	ns	ns	ns	ns

PHT = Plant height, SG = Stem girth, NL = Number of leaves, INL = Internode length, NB = Number of branches, GV = Growth vigour, ns = not significant.

manure treatments were statistically similar at the ninth month. Figures 1 to 6 show the average values of the morphological parameters of the three moringa accessions as influenced by the different rates of poultry manure.

There were no significant accession differences ($p > 0.05$) in the morphological traits at the ninth month of the plants growth in 2010 (Table 2). Table 3 shows the interaction effects of accessions and poultry manure levels on yield traits in 2010. There were significant differences ($p < 0.05$) in the days to initial, 50% flowering and fresh leaf biomass production/plant and none in the

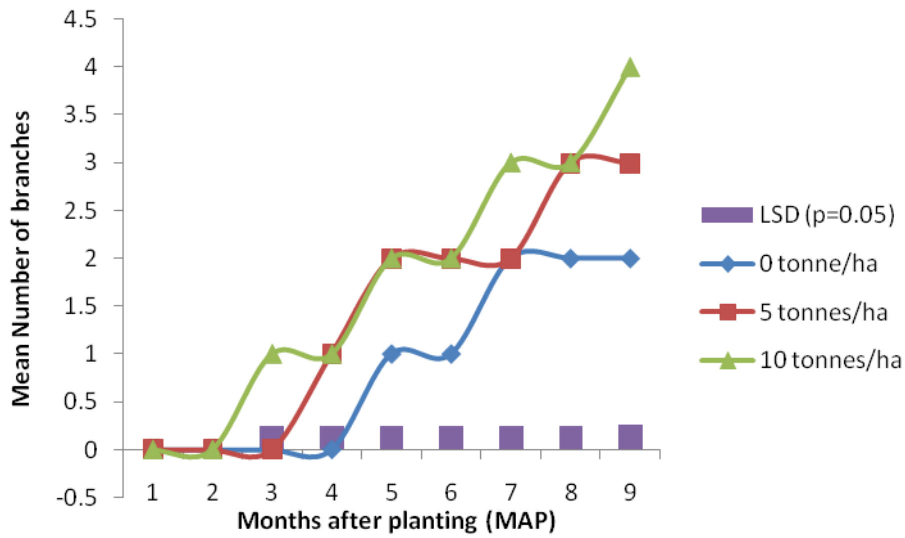

Figure 5. Mean number of branches of the potted moringa plants in 2010.

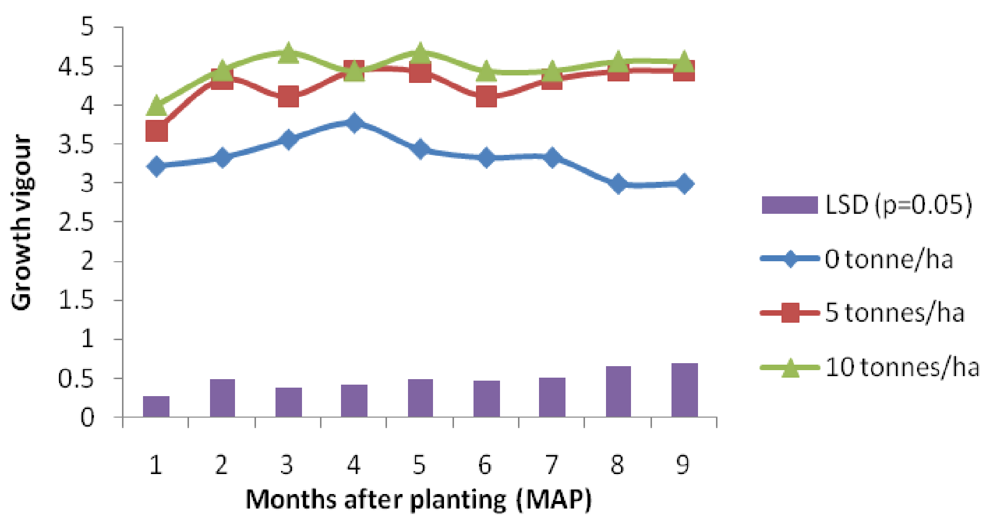

Figure 6. Growth vigour of the potted moringa plants in 2010.

days to 100% flowering, days to pod formation, number of pods/plant, number of seeds/pod, pod length and pod circumference. The five tonnes/ha poultry manure gave the least number of days to initial flowering in Nsukka and Ibadan accessions while the control (0 tonne/ha) gave the least number of days to 50% flowering in Nsukka and Jos accessions and to 100% flowering in all accessions. The Nsukka accession gave the highest leaf biomass production followed by Jos and Ibadan accessions respectively. The 10 tonnes/ha gave the least number of days to pod formation and the highest values of the number of pods/plant, number of seeds/pod, fresh leaf biomass, pod length and circumference. Table 4 shows the interaction effects of accessions by poultry manure on the yield traits in 2011. The 10 tonnes/ha poultry manure gave the highest number of days to first, 50% and 100% flowering as well as all the yield traits in 2011 followed by five tonnes/ha and zero tonne/ha respectively. The Nsukka accession flowered most promptly and thus, had the least number of days to initial flowering (85 days). There were significant accession by manure interaction effects ($p < 0.05$) on all the yield traits except days to first and 50% flowering. There were lesser days to first, 50%, 100% flowering and pod formation but higher values of all the other yield traits in 2011 than in 2010 as shown in Tables 3 and 4.

No records of the morphological growth characteristics were taken in 2011, because of the pruning of the shoot

Table 3. Interaction effects of accession by manure on yield of *Moringa oleifera* plant raised in plastic pots in 2010.

Accession	Manure	DF_1	DF_{50}	DF_{100}	DPF	PL (cm)	PC (cm)	NS	NP	FLB (kg)	NS	DPF
Nsukka	0	126.0	126.0	126.0	182	24.3	4.36	12	5	1.54	12.0	182.0
	5	85.0	140.0	158.7	198	40.7	5.75	14	7	2.66	14.0	198.0
	10	123.0	140.0	168.0	175	42.7	6.23	16	8	3.45	15.7	174.7
Jos	0	141.0	140.5	168.5	180	29.6	3.88	13	2	1.24	0.00	180.3
	5	140.0	149.3	177.3	185	33.2	4.67	15	4	2.27	15.0	185.0
	10	131.7	142.7	186.7	180	39.7	5.91	18	6	3.06	17.7	180.3
Ibadan	0	177.0	162.1	147.9	183	20.0	2.94	5	2	0.92	0.00	182.8
	5	132.0	196.0	196.0	198	22.0	3.11	8	3	1.85	4.7	198.0
	10	149.3	149.3	149.3	175	17.7	3.89	10	4	2.68	10.7	175.0
$LSD_{(0.05)}$		23.57	18.82	ns	ns	ns	ns	Ns	ns	0.64	ns	Ns

DF_1 = Days of first flowering, DF_{50} = Days of 50% flowering, DF_{100} = Days of 100% flowering, DPF = Days to pod formation, PL = Pod length, PC = Pod Circumference, NS = Number of seed/pod, NP = Number of pods/plant, FLB/pot = Fresh leaf biomass/plant, ns = not significant.

in December, 2010, to avoid inconsistencies in the results. Subsequent study will show the growth pattern of the plants from 2012 to date.

DISCUSSION

The higher values of the morphological and yield traits obtained with poultry manure application was a good indication that moringa plants could respond positively to manure application. Ndubuaku et al. (2014) observed that poultry manure increased the nutrient status of the soil and boost crop productivity. According to Ojeniyi et al. (2012), application of liquid agro-industrial by-products increased soil-plant nutrient supply by releasing structurally bound elements such as N, P and Ca in soil solution during decomposition thereby increasing crop growth and yield. Ede et al. (2015) integrated nutrient management encompassing poultry manure and other organic media in moringa production and obtained a positive response of moringa to the application of poultry manure. Topsoil and poultry manure mixtures can, therefore, constitute a suitable medium for potting moringa plants for urban dwellers' use. There was faster flowering and pod setting in 2011 than 2010 probably due to age of the plants. There was delayed pod setting in the plants raised in the poultry manure media in 2011. This could be due to competitive effects of vegetative growth which could have delayed the onset of reproductive growth such as flowering and pod formation in the poultry manure media. The 10 tonnes/ha poultry manure application gave the greatest values of both morphological and pod yield. However, the optimum level should be ascertained in subsequent trials.

Greater values of all the pod yield traits were obtained in the poultry manure media in 2011 than 2010 probably due to residual effects of the nutrients released from the manure and pruning of the shoot in 2010 which probably led to higher leaf density, higher plant productivity and yield. Manures are slow-release fertilizers and usually have residual effects on the crops in subsequent cropping seasons as earlier found by Ndubuaku et al. (2014). There was greater fresh leaf biomass in 2011 than 2010 as a result of pruning of the shoot done in December, 2010 which resulted into profuse branching and flushing (leaf production) in 2011. The differences exhibited by the accessions in the morphological and yield traits could be caused by environmental and not genotypic conditions. However, this can be confirmed in further studies.

The urban dwellers can, therefore, be afforded the privilege of growing moringa in plastic buckets or any other suitable containers with a mixture of topsoil and poultry manure. The nutrient status of the planting medium can be upgraded periodically with split doses of poultry manure and NPK fertilizer. The leaves can be harvested periodically

Table 4. Interaction effects of accession by manure on yield of *Moringa oleifera* plant raised in plastic pots in 2011.

Accession	Manure	DF_1	DF_{50}	DF_{100}	DPF	PL (cm)	PC (cm)	NS	NP	FLB (kg)	NS	DPF
Nsukka	0	81	85	97	135	20.6	3.84	12	3	1.45	12.0	182.0
	5	92	88	96	155	44.8	5.92	16	9	3.28	14.0	198.0
	10	105	92	105	163	48.6	7.20	18	11	4.66	15.7	174.7
Jos	0	83	85	88	140	18.4	3.56	11	2	1.18	0.00	180.3
	5	88	92	94	148	35.6	5.22	17	6	2.86	15.0	185.0
	10	90	96	102	153	41.0	6.72	19	7	3.40	17.7	180.3
Ibadan	0	80	82	87	147	15.8	2.14	9	2	0.85	0.00	182.8
	5	85	90	95	151	24.5	3.45	13	4	2.25	4.7	198.0
	10	92	93	105	158	20.2	4.20	16	6	3.06	10.7	175.0
$LSD_{(0.05)}$		ns	ns	5.02	6.85	5.67	2.26	3.11	3.0	1.62	ns	ns

DF_1 = Days of first flowering, DF_{50} = Days of 50% flowering, DF_{100} = Days of 100% flowering, DPF = Days to pod formation, PL = Pod length, PC = Pod Circumference, NS = Number of seed/pod, NP = Number of pods/plant, FLB/pot = Fresh leaf biomass/plant, ns = not significant.

for use as pruning of leaves in this study increased leaf biomass production and pod yield.

Conflict of Interest

There is no conflict of interest among the authors.

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Full Length Research Paper

Suitability of major agricultural land uses around Kibale National Park

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The current agriculture land uses around Kibale National Park forest have been expanding towards the forest area threatening its conservation efforts and this has contributed to crop raiding and destruction to the neighbouring agricultural communities. This study was conducted to identify and assess the suitability of major agricultural land uses around Kibale National Park. The major agricultural land uses included; Banana (Musa spp), Maize (*Zea mays*) and Tea production (*Camellia sinensis*) and these were identified using three randomly selected transects of 5 km per transect, and obtaining information using a structured questionnaire administered to 30 key farming households near Kibale National Park. Soil samples were also taken within the identified major agricultural land uses at the depths of 0-15 and 15-30 cm. Physical suitability of the major agricultural land-uses was assessed by matching their requirements with existing land qualities extracted from the Uganda soil memoires. Results showed that, western part of Kabarole District is highly suitable for tea production, the southern part is highly suitable for maize and the northern part is highly suitable for banana. The central part of Kabarole was found to be highly suitable for both banana and maize. The study finally recommended a buffer zone of 3.5 km from the national park planted with tea and eucalyptus around the national park boundary for crop defense separating agricultural communities and Kibale National Park since there not affected by animal raids and destruction.

Key words: Major agricultural land use, land qualities, land suitability.

INTRODUCTION

Agricultural land suitability assessment is a key process to ensure sustainable crop production in particular agro-ecological zone in relation to the environment (Elsheikh et al., 2012). Assessment of agricultural land suitability in this context is defined as the process of land performance when used for alternative purposes of agriculture to predict potential and limitations of the land for crop production (Pan and Pan, 2012; He et al., 2011; Mu, 2006). Agricultural communities neighbouring protected areas like forests and national parks often disproportionally accrue the costs and challenges of conservation, though they also benefit from the existence of a protected area. Protected areas which have for long served as centre pieces of conservation and priority areas of biological and cultural attachment have always been encroached and converted into agricultural land

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uses because their landscapes are often regarded highly suitable for agriculture production (Goldman et al., 2008; Howard et al., 2002). This has contributed to various migrations of animal population, crop raiding and destruction of the animal habitant leading to conflicts between the neighbouring communities and wildlife national park management authorities (UWA, 2009; Owoyesigire, 2007).

Oftenly, conflicts between communities and wildlife exist as people argue that, wild animals trespass and vandalize their crops on their lands (Laudati, 2010; Sifuna, 2005). On another hand, environment conservationists assert that the ever growing human population has encroached on wild life existence in search of highly suitable agricultural land, where crops are raided and damaged by animals (Sitati et al., 2005; Oerke et al., 1994). Such incidences contribute to approximately 86 and 88% of farmer's loss in Africa (Mackenzie and Ahabyona, 2012; Naughton-Treves, 1997; Newmark et al., 1994; Weber et al., 2007). This as a result makes communities retaliate by killing animals to destroy and destruct their habitat a commonly observed crisis in protected areas across sub Saharan Africa.

The surrounding communities in areas of Kibale National Park (KNP) in Uganda are experiencing crop raiding and destruction due to wild animal raids as a result of the past encroachment by agrarian communities. According to Mackenzie and Ahabyona (2012), five major wildlife species account for crop damage events namely: 85% baboons (*Papio cynocephalus*), 42% bush pigs (*Potamochoerus procus*), 69% red tail monkeys (Cercopithecus ascanius), 15% chimpanzees (*Pan troglodytes*), and 79% elephants (*Loxodonta africana*).

Crop farms are dominated by annual and perennials crops within a distance of 500 m of the forest boundary and 4 to 7% of the crops per season are destroyed though the distribution of damage was highly skewed during the study such that maize and cassava fields were on occasionally reported raided and completely destroyed. The farm communities have resorted to tree planting notably eucalyptus and pines as a mitigation strategy for crop raiding and income generation to compensate on the crop seasonal loss. Other various crop raiding defense mechanisms are namely; human guarding, fire, sounding drums, scare shooting by UWA rangers, elephant trenches, Dogs, Dung spread on maize and among others (Mackenzie and Ahabyona, 2012).

Factors towards expansion to protected areas like KNP are due to population pressure and the search for highly suitable lands are due to the decline in soil fertility, horizontal expansion of agricultural land including; forest conversion which has aggravated the tension between the surrounding community and the national park (Majaliwa et al., 2010; Chapman et.al, 2000; Bekunda and Woomer, 1996). This is threatening social and environmental benefits provided by the forest reserve (Banana and Sembajjwe, 2000; Brown and Lugo, 1990).

Since the late 1980s, declining soil fertility has been recognized as an important cause for low agricultural production in sub- Saharan Africa leading to land conversions (Sanchez et al., 2002; Palm et al., 2001; Henao and Baanante, 1999; van der Pol, 1992; Stoorvogel and Smaling, 1990). Primary causes of soil fertility decline include; poor land use practices, over-cultivation, inadequate land use allocation (poor land use planning and management). The declining soil fertility has contributed to conversion of forest lands as they are regarded highly suitable for agriculture production hence encroaching on the protected areas which conserve and act as habitats for wild animals.

Furthermore, over cultivation and continuous cultivation of land is responsible for continual loss and removal of s oil nutrients by the harvested produce every production season (O.S.Bello et al., 2010). Farming activities without proper management practices, such as replenishment of nutrients using organic matter applications, rainwater harvesting for moisture retention measures to reduce soil losses lead to land degradation of farmlands (Bodhankar et al., 2002; Krishna, l996). Poor agricultural land use practices affect the distribution and supply of soil nutrients by directly altering soil properties and influencing biological transformations in the rooting zone (Murty et al., 2002). For instance, the conversion of forest to crop land has been associated with reduction in organic matter content of the top soil (Singh and Singh, 1996; Ross, 1993), and subsequent decline in productivity. Since organic matter content is responsible for the productivity in soils (Sanchez et al., 2002; Palm et al., 2001). Islam and Weil (2000) reported an increase in bulk density and a reduction in porosity and aggregate stability following the conversion of forest lands to crop lands. Similar findings were reported by Motavalli and McConnell (1998) and Riezebos and Loerts (1998). Such changes in soil properties predispose the soil to soil erosion, a major soil degradation process in Uganda (Tukahirwa, 2003).

In an agricultural context, land suitability and evaluation is an assessment for a specified kind of land utilization form and the final result of agricultural evaluation is a map which partitions the landscapes into suitable and unsuitable land areas for a particular land-use of interest (Triantafilis et al., 2001). Land evaluation analysis determines whether the requirements of land-use are adequately met by the properties of land characteristics. The objective of land suitability potential evaluation is to predict the inherent capability of land in order to support the specific land use for long periods of time without deterioration (Bandyopadhyay et al., 2008).

This study was therefore, carried out to identify the major agricultural land uses and determine the overall suitability of identified agriculture land uses around KNP. This information is useful for agricultural planners and wildlife conservation authorities in order to reduce the conflicts between the communities, and KNP authorities.

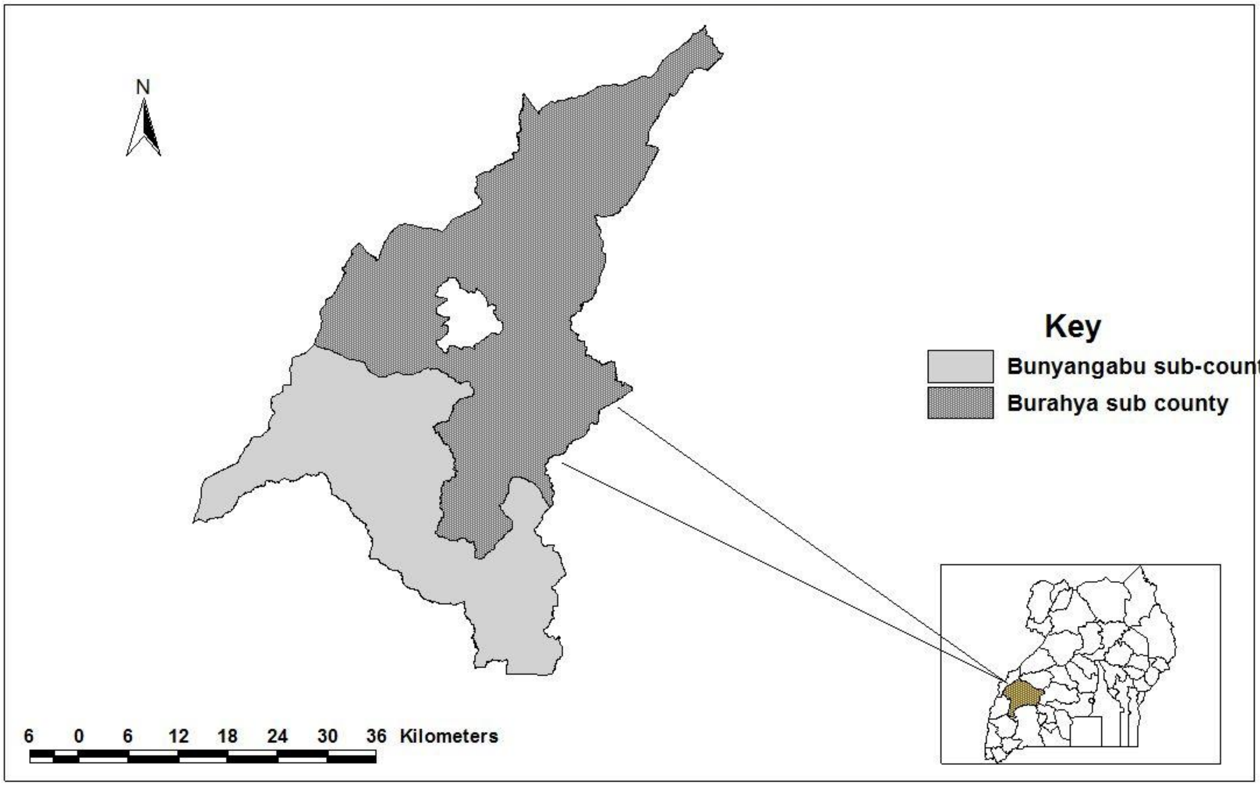

Figure 1. Map showing the study area.

Description of the study area

This study was conducted around Kibale National Park (KNP), located in western Uganda near the foothills of Rwenzori Mountains. It contains moist evergreen forest with a remnant of a transitional forest between savannah and mid-altitude tropical forest surrounded by a large agricultural population. It is also a home to one of the largest populations of chimpanzees in East Africa and to 12 other primate species (UWA, 2009; Plumptre et al., 2003; Chapman and Lambert, 2000). The population around KNP increased by more than 300% between 1959 and 1990 (Naughton Treves et al. 1998), and in 2006 the population density within 5 km of the park boundary was estimated to be over 260 individuals/km^2 (Hartter, 2010), ranging as high as 600 individuals/km^2 in some locales by 2009 (Mackenzie and Ahabyona, 2012).

The study was undertaken in three different locations, Kasenda sub-county, Busoro sub-county, in Burahya county and Buhesi Sub-county of Bunyangabu County, in Kabarole district (Figure 1). The three sub-county are located at coordinates 207631 m E, 71876 m N, and 201985 m E, 52118 m N, 191856 m E, 60231 m N (UTM Zone 36N, WGS 84 Spheroid) in western Uganda .

Currently, KNP is moist evergreen forest surrounded with tea plantation, small scale agriculture, and woodland plantations as the dominant land-use/cover in the area (Chapman and Lambert, 2000; Chapman et al., 2000).

Soils and climate of the study area

Generally, the soils are classified as Lixic Ferralsols. The area receives bimodal rainfall occurring from March to May and September to November. The mean annual rainfall in the region is 1750 mm whereas the mean daily minimum and maximum temperatures are 15.5 and 23.7°C respectively. (Majaliwa et al., 2009).

METHODOLOGY

Identification of major agricultural land uses

Major agricultural land uses were determined through field observations and conducting 30 Household interviews using a semi-structured questionnaire. The observation method was used to check for the accuracy of the information got from the interview method (Mulhall, 2003) as well as validation of the identified land uses, their location, pattern and spatial outlay. The interviews were also aimed at establishing the major agricultural land uses, their economic viability, sizes and yield output of crops grown around the park. Observations and interviews were carried out following three pre-determined transects of 5 km cutting across the different landscape positions [summit (>152 cm), back-slope (1465 to 1520 m), and valley (<1465 m)], on both Eastern and Western facing slopes, with an average inclination of 10°. A total of 30 household

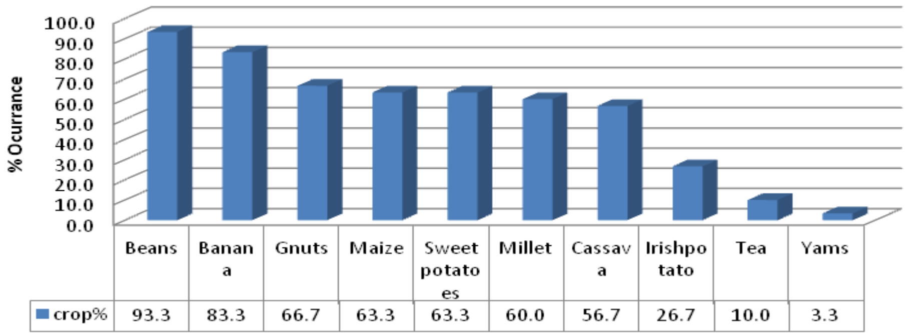

Figure 2. Different agricultural land uses around KNP.

heads were randomly selected and interviewed using a semi structured questionnaire developed by the research team during the study.

The data collected from interviews was entered, analyzed using SPSS version 17.0. The two data generated included; frequency and average plot sizes for the major agricultural land uses in the study area.

Determination of the overall suitability of major agricultural land uses around KNP

The overall land suitability was determined by matching the land-use requirements of the crops with land qualities. The requirements of the identified major agricultural land-uses were obtained from FAO framework (FAO, 1975). The land qualities were obtained from soil memoires and field soil data collected, with soil Information obtained from the different soil units of the western province soil memoire (Harrop, 1962). Composite soil samples were collected from the different identified land uses at different landscape positions (summit, back slope and valley), and soil depth was taken at 0-15 and 15-30 cm because it is the major agricultural layer for tillage and rooting zone (Tenywa, 1998).

Soil samples were collected, air dried, analysed for texture, pH, P, N, K, OM, and the bases following adhoc procedures (Okalebo et al., 2002). Soil pH was measured using pH meter (1: 2.5 soil: water), SOM and total N were determined using Walkley and Black method and Kjedhal method respectively. Soil texture was determined using Bouyoucos hydrometer method (1962) and their textural classes according to the FAO classification (FAO-UNESCO-ISRIC, 1990).

Matching the requirements with Land qualities was done in a GIS environment by use of multi-criteria analysis with Integrated Land and Water Information System (ILWIS v3.3) software. Kabarole soil map was used as a base map and key relevant land qualities and their characteristics were extracted from the soil memoires (Harrop, 1962) with the field soil data collected during the study. Attribute maps for the different land characteristics were created using ArcGIS software. Land qualities were inferred from the land characteristics with the following Land qualities; nutrient availability (P, K, pH, OC, % saturation), rooting condition (depth 0-30 cm), nutrient retention capacity (CEC), and moisture availability (Rainfall).

Suitability maps (with four classes: s1-Highly suitable, s2-Moderately suitable, s3-Marginally suitable and N-Unsuitable) for the three major agricultural land uses were generated by combining the different land qualities using map calculation function in ILWIS v3.3. The maps were normalized and sliced using factor rating values provided in the FAO framework (1975).

Two scenarios of possible distribution of the three major land uses were generated based on their suitability classes and labeled (scenario 1 and 2). Under scenario 1, only the highly suitable class was considered for the three major land uses by overlaying the three individual physical suitability maps (Banana, Maize and Tea). To consider the distance and conservation factors between KNP and farm communities, a buffer zone of 3.5 km was created using the spatial analyst in ArcGIS (3.1) software. The forest was clipped out from the land use map of Kabarole and exported as a shape file to ArcView-GIS, and the distance was determined. The “forest buffer map” was imported back to ILWIS, crossed and glued with the highly suitable generated map to form scenario 1 which included soil suitability, distance factor against animal raiding and conservation area of the forest.

Scenario 2 map was generated following the same procedure (scenario 1) though included moderately suitable class and the highly suitable class for each agriculture land use type.

RESULTS

Major agricultural land-uses around Kibale National Park

The major agricultural land uses around KNP identified included; perennial crops (Banana and tea) and other annual crops (beans, ground nuts, maize, sweet potatoes,millet, cassava, Irish potato and yams) as show n in Figure 2. Beans were generally intercropped (93%) with other crops like; maize, bananas and cassava. It was also observed that banana which is a major staple food, was grown around homesteads with other annual crops as indicated below;

The area covered by each agricultural land use type is shown in Figure 2. Tea is grown on large scale of land (140,000 ha), it is followed by maize (11, 309.5 ha), banana (11, 302.8 ha), groundnuts (8500 ha), beans (7,321 ha), cassava (7, 205.9 ha), sweet potatoes (5, 126.8 ha), yams (5, 000 ha), Irish potato (4, 218.8 ha), and millet (4, 027.8 ha).

Selected characteristics of the soils under the different agricultural land-use types are presented in Table 1. Generally banana was found on sandy clay soils, tea on

Table 1. Soil analysis results.

Soil properties	Agricultural land use			P<0.05
	Maize	Tea	Banana	
pH	4.75	6.0	5.82	
SOM (%)	6.82	7.52	7.95	Ns
Total N (%)	0.33	0.32	0.30	Ns
Av. P (ppm)	46.0	84.0	48.16	Ns
Ca (cmol(p+)/kg)	8.75 [a]	12.4 ±4.5[b]	11.52[a]	>0.001
Mg (cmol(p+)/kg)	2.48	5.38	4.30	Ns
K (cmol(p+)/kg)	0.83	2.00	1.74	Ns
Na (cmol(p+)/Kg)	0.40	0.10	0.04	Ns
Sand (%)	72.0	64.0	67.00	Ns
Clay (%)	28.0	26.5	24.00	Ns
Silt (%)	16.0	24.0	23.00	Ns
Textural class	Sandy loam	Sandy clay loam	Sandy clay	

sandy clay loam while maize on sandy loam. Although the soil under which the three crops were found were acidic, maize tended to be on strongly acidic soils compared to tea and banana. The organic matter and TN levels were very high for the soil under the three agricultural land-use types. The soil under banana and tea tended to have a relatively high amount of Ca, Mg, and K compared to the soils under maize.

Suitability of the major agricultural land use types in Kabarole district

In Figure 3a to c, the suitability of individual crops in Kabarole district. The central region of Kabarole is highly suitable for both maize and banana; with 67% maize-suitability area (122,427 ha) and 61% banana-suitable area (110,004 ha). Tea is marginally suitable to be grown in Kabarole District. The area which can be dedicated to tea with improved management represented 56% of the district area (100,000 ha). Only 15% (27,171 ha) is, the highly suitable for tea and is located in the south-western parts of Kabarole.

Figure 4a to c show the combined suitable area for all crops (Scenario 1), combined suitable and moderately suitable areas for all crops (scenario 2) and the current land-use map of the district. Scenario one represents the ideal distribution of agricultural land use types in different areas with in Kabarole district. The intercroping of Banana and Maize had the highest percentage (30%), Tea (25%) and Maize (10%) the highly suitable class. Under scenario two Banana, Maize and Tea occupied (75%) area coverage. By comparing the current distribution of the agricultural land-use with the distribution provided in scenarios 1 and 2 we observed the current agricultural land use distribution in Kabarole district is very close to that provided under scenario 2.

DISCUSSION

Agricultural land-use types in Kabarole district is dominated by Banana, tea and annual crops. This in line with findings by Nuwategeka et al. (2013). The dominance of banana is due to the fact that it is a highly valued food crop and is grown around the homesteads (Nkwiine et al., 2002) and it perform well on rich top soil layer (0-15 cm), moist cool climate and on deep fertile volcanic ash soils (Chapman and Lambert, 2000). The high frequency of occurrence of Beans (93.3%), ground nuts (66.7%) and maize (63.3%) was due to the fact that they are considered as sources of protein and carbohydrates (Eltayeb et al., 2011) by the community. Maize is also considered as one of key cash crop for communities. Beans and ground nuts are leguminous crops which improve soil fertility through Biological Nitrogen Fixers (BNF) and act as cover crops (Okito et al, 2004). Maize is grown in rotation with other crops (groundnuts and beans), which improve the soil fertility (Rossing et al., 2002). Maize is a gross feeder (exhausts), soil nutrient therefore intercropped with beans and ground nuts will reduce on nutrient losses and hence reducing the need to apply nitrogen fertilizer (Environmental issues-WWF, 2009; Okito et al., 2004). Ground nuts are grown due to their commercial value that is appealing to farmers since they fetch high market value (Emerging Markets Group, Ltd, 2008). Tea plantations seem to be immune to damage from animal raiding since few wild life species seem to directly go through the tea (Kirya, 2005). The tea is unpalatable to the wild animals that would be crop raiders originating from the park. However, elephants continue to use tea company road and path for passage to raid neighboring cultivated fields (Warner, 2008; Mugisha, 2002).

The similarity between scenario 2 and the current agricultural land-use spatial distribution is an evidence of

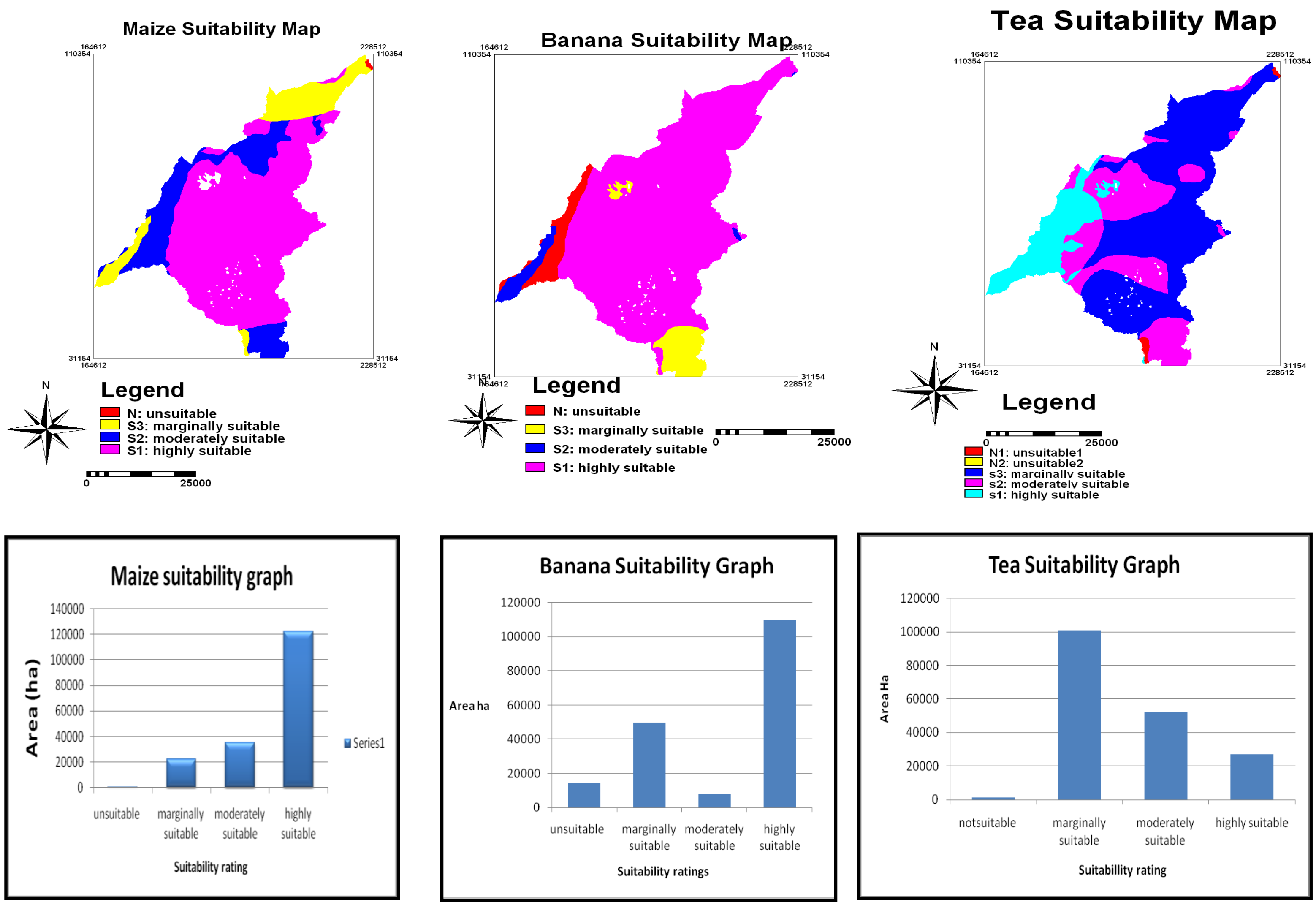

Figure 3. Suitability maps and bar graphs for major land uses.

the demographic pressure exerted on the existing natural resources. It is also explain the low resilience observed on certain soils of the district and need for more soils for the agricultural production (Bekunda and Woomer, 1996; Sseguya et al., 1999). Recent studies also demonstrate that declining yields observed on most banana plantations is largely explained by poor adoption of agronomic practices, poor soil

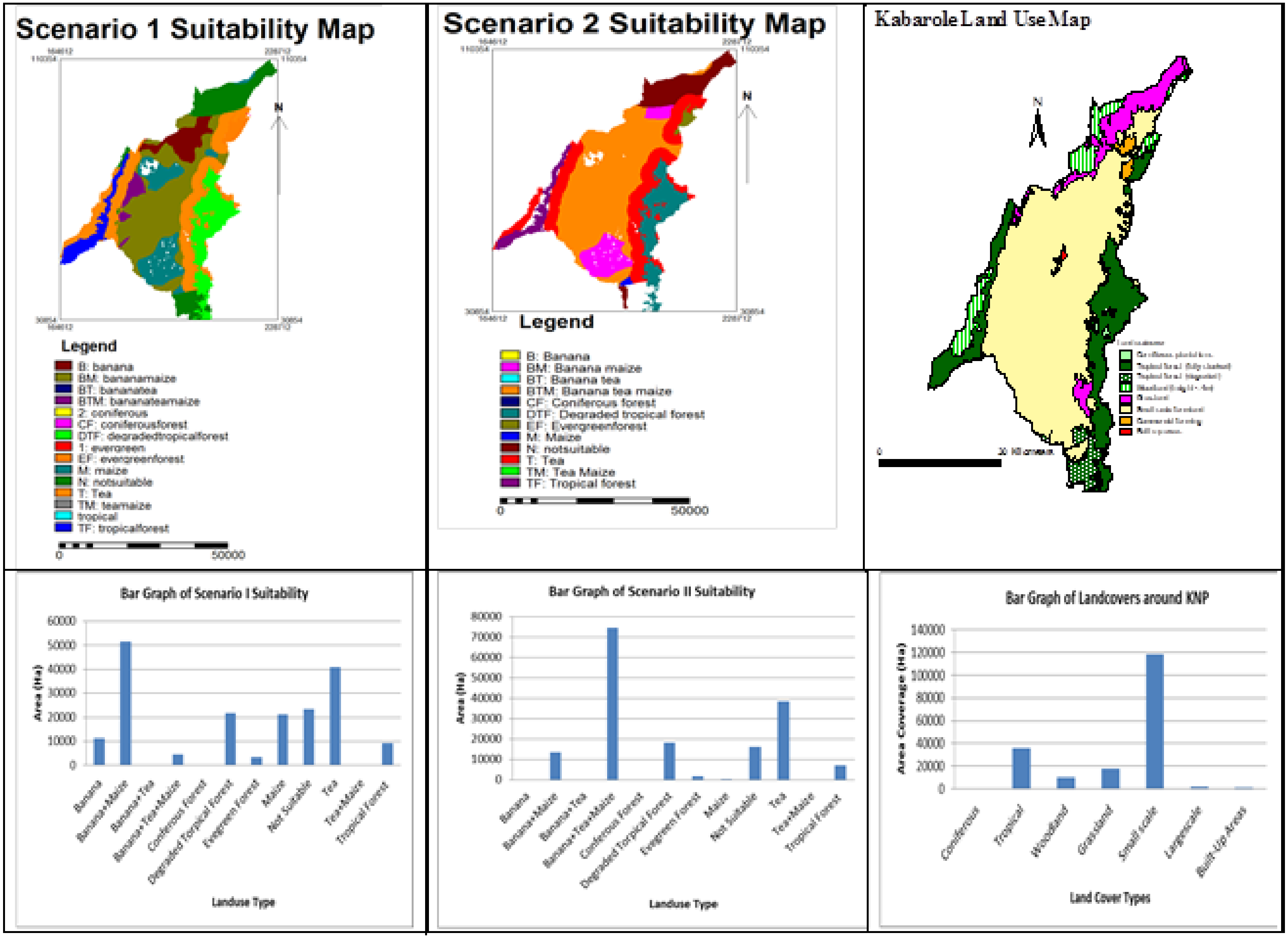

Figure 4. Suitability scenario and current land use maps and graphs.

organic matter management, mulching, weeding and diseases mainly Banana bacterial wilt, Sigatoka and inadequate extension services available to the farmers (Gold et al., 1999).

CONCLUSION AND RECOMMENDATIONS

The western part of Kabarole district is highly suitable for tea, the southern part is highly suitable for maize and the northern part is highly suitable for banana. The central part of Kaborole was found to be` highly suitable for banana and maize. There is a need therefore to grow crops according to scenario one (the highly suitable scenario) and improve crop management in the district. Scenario two (the current land use practices) should only be continued with external inputs like NPK fertile or compost manure. Tea should also be grown within a recommended buffer zone of 3.5 km with additional inputs, However efforts should be made to control pollution associated with pesticides and fertilizer application.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Rheological and physical properties of wormcast and termite mound soils in Nsukka subtropical area

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The study investigated variability of rheological and physical properties of wormcast (Wc) and termite mound (Tm) soils in relation to surrounding soils (Ss) in Nsukka tropical and subtropical areas. Rheological and physical properties, shear strength and compaction at different moisture contents were obtained. Relationships between rheological and other physical attributes were established. Consistency limits of Wc and Tm soils were significant. Mean shear strength (cohesion) of Wc and Tm were high. Mean shear strength of Wc was 85.09 kpa and 91.55 kpa for Tm soils), while Ss had 69.80 kpa. The maximum shear strength of compacted (15 blows) Wc and Tm was 188 and 190 kpa at 12 (db) moisture content, respectively. There was significant ($p<0.05$) positive relationship between gravimetric moisture content and clay. Sand had significant negative relationship with plasticity index. The study demonstrated the usefulness of characterizing Wc and Tm in relation to surrounding soil (Ss) and their relative efficacy in predicting soil behavior.

Key words: Rheology, physical property, termite mound, wormcast, tropical and subtropical area.

INTRODUCTION

Earthworms and termites are common biological agents that produce significant physico-chemical and rheological modifications to soil. Cast of earthworm is a digested material that is excreted back into the soil by different species of earthworm. Termite mound is a mixture of soil, organic debris or living plant tissues collected, often over extensive foraging areas, transported to their domain and subjected to intense degradation when it is digested by the termites (Ekundaye and Orhue, 2011). Reports have been made on detailed studies wormcast and Tm contribution to improve soil fertility and crop productivity (Debruyn and Conacher, 1997); improvement of soil porosity, soil nutrient availability and uptake by plants, minimizing production costs and maximizing yield and profit (Mba, 1978; Kang, 1978; Ariha, 1979; Lal, 1988; Frageria and Baligar, 2004; Semhi et al., 2008; Ekundayo and Orhue, 2011) and produce significant physical and chemical modifications especially clay mineral composition of these minerals (Jouquet et al., 2002).

In southeastern Nigeria, soils belong to different lithologies, hence variation in soil groups such as alluvial, coastal plain sands, false bedded sandstones, lower coal measures, shale and upper coal measures (Ndukwe et al., 2009). These soil groups vary in their particle size distribution and soil moisture retention characteristics.

There are rheological differences among Nigerian clays. Clay content, nature of clay, exchangeable cations and organic matter content of soils vary and these

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influence activity levels and plasticity of soils (Ndukwe et al., 2009). Clay has greater cohesion, plasticity and activity than other primary soil particles. Variation in hydraulic properties of soils including water flow characteristics of the pedosphere are determined by clay contents (Gerke et al., 2001). Thus, particle size of soils, soil moisture and plasticity determine stability of soils in response to loading forces from traffic, tillage and building foundations (Imhoff et al., 2004).

Differences in soil water content determine the strength of soils. Several reports have shown spatial heterogeneity in soil water behavior (Gerke et al., 2001), non-uniform water repellency in different soils under different vegetation (Dekker et al., 2001) and land use (Hallet et al., 2004). Published scientific study ascertaining the rheological properties and other physical characteristics of wormcast and termite mound as plant-soil systems is desired. Based on this, we investigated and characterized the dynamics and variability in rheological properties of soils formed under different species of earthworm and termites in relation to surrounding soil.

MATERIALS AND METHODS

Description of study area

The study was conducted in the laboratories of the Department of Soil Science and Land Resources Management, as well as Department of Mechanical Engineering, University of Nigeria, Nsukka. Earthworm cast and termite mound soils were collected from three locations: Nsukka, Ede Oballa and Orba. Nsukka and Ede Oballa are situated in Nsukka local government area (LGA), while Orba is in Udenu LGA. Nsukka LGA lies by latitude 6°51'24" and longitude 7°23'45"E with land area of 45.38 km^2. Udenu LGA lies between coordinates 6°55'N and 7°31'E with a total land area of 897 km^2 (http://www.nipost.gov.ng/postcode.aspx).

Rainfall is bi-modal; the rainy (April - October) and the dry season (November - March). There is usually a short break in August. Average annual rainfall is about 1600 mm. Average minimum and maximum temperature is 22 and 30°C, respectively. The relative humidity is rarely below 60% (Ezeaku and Egbemba, 2014). The geomorphology of the study areas is of the highlands stretching through the undulating hills to plain lands. The vegetation belongs to the semitropical rainforest type and complemented by typical grassy vegetation.

Field work

The study was conducted in 3 ha (300 m x 100 m) blocks in each location (Nsuuka, Ede Oballa and Orba). Each block of 1 ha consisted of four land use types: Cassava/yam cultivated field (LUT1), Fallow land (LUT2), Oil palm land (LUT3), and residential area (LUT4) with land area of 1250 m^2, 1250 m^2, 2500 m^2, and 5000 m^2, respectively. This delineation was done for the 3-ha blocks to avoid the overlap of land use systems.

In each 1 ha block a 3 m x 3 m grid was mapped out. Wormcasts and termite mound soils, taken as plant-soil systems, were collected through hand sampling in the grids replicated thrice to cover the 3 ha blocks. Earthworm species predominantly identified were *Eudrilus eugeriae* and *Agrotoreutus nyongii* (Mba, 1978; Fragoso and Lavelle, 1992), while the genera *Macrotermes* and *Odontotermes* species were identified in the termite mounds (Arihad, 1979). The number of earthworm casts and termite mounds in each land use type was recorded. The population count was done within the grid size (3 x 3 m) for the 3 ha block. The collected plant-soil systems were carefully put into plastic polyethylene bags, properly labelled and taken to the laboratory for air-drying. In each location, soils of the surrounding were collected at least 5 m away from each LUT at 0 to 30 cm depth, analysed and used for standardization. Core ring (inner volume of 96.6 cm^3) samples were taken at soil depth of 0 to 10 cm for soil physical determination.

In situ shear strength determination was done for all soil samples. It was measured with a shear vane tester (16 mm diameter vane) at depth 40, 80 and 120 mm on each sample and average strength was calculated. The corresponding moisture contents of the soil samples were obtained with a moisture meter.

Laboratory studies

The air-dried soil samples were taken to the laboratory and sieved through 2 mm size sieve to determine the followings: shear strength and compaction at different moisture contents using the shear vane tester. The soil samples were subjected to 5, 10 and 15 blows respectively with a standard proctor rammer (2.5 kg) at different moisture content level in a cylindrical metal mould. The mould was 100 mm in diameter and 120 mm height. A round wooden pad was placed on the soil before compaction to ensure uniform compaction. The shear vane was graduated in kilopascal (kpa). Measurements were taken at two depths: 40 and 80 mm respectively on each sample and the mean value of each set of two-depth reading was calculated and recorded.

Rheological (consistency) properties determined were plastic limit (PL), liquid limit (LL), shrinkage limit, plasticity index and coefficient of linear extensivity (COLE). Particle size distribution (fraction of sand, silt, and clay) was determined using hydrometer method (Gee and Or, 2002) with NaOH as dispersant. Bulk density (Bd) and linear extensibility were determined using Grossman and Keinch (2002) method. Gravimetric water content was determined.

Data analysis

Data on soil physical rheological properties were subjected to analysis of variance (ANOVA) using Genstat Discovery Edition 3, while significant variations in the means were determined using standard error difference ($SED_{0.05}$) and P-value. Relationships between physical and rheological properties were determined using Pearson correlation analysis.

RESULTS AND DISCUSSION

Distribution of earthworm casts and termite mounds

The population of wormcast and termite mounds (determined from population counts) in four land use types (LUT 1, 2, 3 and 4) across the three sites are presented in Figure 1. Total number of casts produced by *Agrotoreutus* spp was significantly higher ($p<0.05$) than that of *Eudrilus* spp by 34.7%. This suggests that the benefits derivable from the activities of *Agrotoreutus* spp could be more useful in improving soil productivity in tropical and subtropical area. This finding accords earlier

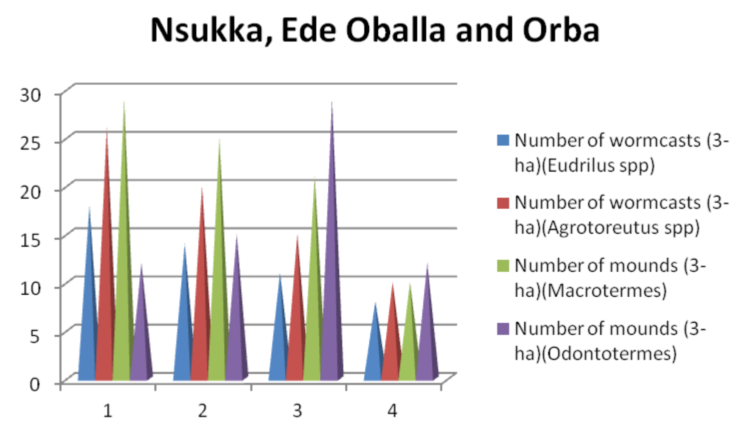

Figure 1. Number of wormcasts and termite mounds across the study location LUTs.

Table 1. Some physical properties of wormcast and termite mound soils in the 3-ha study sites.

Location	Sand gkg^{-1}	Siltgkg^{-1}	Clay gkg^{-1}	Silt + Clay gkg^{-1}	Texture	Bulk density gcm^{-3}	Φm gkg^{-1}
Wormcast (*Eudrilus* spp and *Agrotoreutus* spp)							
Nsukka	360	270	370	640	CL	1.38	243
Ede-Oballa	340	190	470	660	CL	1.42	320
Orba	320	240	440	680	CL	1.44	238
All location fallow	700	80	220	300	SCL	1.51	170
$SED_{0.05}$	51.6	32.1	43.8	57.1		0.03	45.2
P-value	<0.0001	<0.0001	<0.0001	<0.0001		n.s	<0.0001
Termite (*Macrotermes* and *Odontotermes spp*) mounds							
Nsukka	380	160	460	620	CL	1.38	244
Ede-Oballa	400	220	380	600	CL	1.42	321
Orba	340	220	420	640	CL	1.44	228
All location fallow	720	100	180	280	SCL	1.51	156
$SED_{0.05}$	54.2	28.8	46.1	47.2		0.03	55.1
P-value	<0.0001	<0.0001	<0.0001	<0.0001		n.s	<0.0001

Ks, soil saturated hydraulic conductivity; Bd, soil bulk density; TP, total porosity; NS, not significant; Nd, not determined; SL, sandy loam; SCL, sandy clay loam; LSD (P<0.05), least significant difference at 5% level of probability, $^{*}p<0.05$, $^{**}p<0.01$.

report (Karlen et al., 1997) that earthworm number is 'Level 1' indicator of a soil's ability to accommodate water entry for prolonged periods during high intensity rainfall and frequent irrigation events.Total number of *Macrotermes* spp mounds in LUT 1 & 2 across the study locations were significantly higher (p<0.05) than that of *Odontotermes* by 53.2%.

Soil physical properties of wormcasts and termite mounds

The percentage of finer particles in wormcasts and termite mounds could probably be the main effect on physico-chemical properties of soils. The statistical mean values of rheological and physical properties of wormcasts and termite mounds are presented in Table 1. The textural analysis showed that significantly higher clay contents were obtained in the wormcast (p<0.01) and termite mound soils (p<0.05) than the surrounding soils. The higher clay fractions that predominantly gave rise to clay loam texture could be associated to the preferential incorporation of clay by the species of earthworms and termites identified. This confirms earlier report by Kang (1978) and Debruyn and Conacher (1987) who reported significantly higher clay content in termite mounds than

Table 2. Rheological properties (Consistency limit) of wormcast and termite mound soils in the 3-ha study sites.

Location	Liquid limit	Plastic limit	Shrinkage limit	Plastic limit	COLE
		Wormcast (*Eudrilus spp* and *Agrotoreutus spp*)			
Nsukka	32.2	19.7	9.4	17.3	0.031
Ede-Oballa	33.7	20.3	10.3	17.0	0.035
Orba	38.3	18.1	8.4	12.8	0.033
All location fallow	18.6	12.1	4.2	5.4	0.009
$SED_{0.05}$	1.42	0.69	0.47	0.64	0.006
P-value	<0.0001	<0.085	<0.0001	<0.0001	<0.0003
		Termite (*Macrotermes* and *Odontotermes spp*) mounds			
Nsukka	42.5	28.7	28.1	18.0	0.035
Ede-Oballa	30.8	20.0	20.0	15.7	0.029
Orba	30.3	18.6	15.8	20.3	0.031
All location fallow	20.1	9.9	7.4	10.3	11.2
$SED_{0.05}$	1.37	1.36	0.33	0.69	0.004
P-value	<0.0001	<0.0001	<0.0001	0.0001	<0.0002

COLE = coefficient of linear extensivity, P= probability, SED= standard error deviation.

the surrounding soil.

Sandy clay loam obtained in surrounding soils could be attributed to nature of parent materials and high rainfall that could favor washing away and leaching of silt-sized and clay-sized fractions (Mbagwu, 1995; Lal, 1988, Franzluebbers, 2002).

Soil bulk density (Bd) value was significantly higher ($p<0.05$) in the surrounding soil (1.52 gcm^{-3}) when compared to the Wc and Tm soils. Soil Bd decreased by 13.2% in Wc and 5.3% in Tm relative to Ss and were statistically similar. Low Bd implies a positive productivity indicator as it helps in easing root penetration, and encourages downward movement of water, more soil water retention and availability for greater water use efficiency by crops (van Schaik et al., 2014). These results had been corroborated. Lavelle (1997), Francis and Fraser (1998) and Chan (2004) reported decreases in Bd and increases in moisture content.

The higher Bd in the surrounding soil may be associated to seasonal flooding of soils; resulting to continued wetting and drying of soils. There is also the possibility of soil surface crusting and crusting by compaction through raindrop impact and surface erosion. It is quite possible that the higher content of sand in the control soil (Table 1) was due to washing away of clay and silt sized-fractions by rainfall and runoff water and may have contributed to the higher bulk density obtained.

The *in situ* shear strength of Wc and Tm soils were found higher than that of Ss. Shear strength decreased with reduction in moisture content and increased as the gravimetric moisture content increased. Termite mound had highest mean shear strength of 90.36 kpa and could be associated to higher silt and clay content in it. Surrounding soil had the least shear strength of a total mean value of 56 kpa and may be due to presence of sandy soil mixed with clay soil.

Generally the shear strength of Wc and Tm soils increased with the number of Proctors' hammer blows and reached a maximum of about 15 blows in the moisture content range of about 12 to 14 (db). It was recorded that about 85% of the maximum shear strength was achieved at 10 blows. Greater shear strength in the Wc and Tm relative to Ss implies less susceptibility to compaction. The rheological properties of Wc and Tm relative to surrounding soils are shown in Table 2. Higher plasticity values were recorded in Wc and Tm across the locations than those of the Ss. Higher plasticity reflects higher clay contents and implies the possibility of higher activity in the forms which portend instability of soils especially under high engineering activity. Higher plasticity index (PI) values affected shrinkage behavior of soils as given by the coefficient of linear extensivity (Table 2). Atterberg limits (Liquid limit, plastic limit and plasticity index) had significant ($p<0.05$) positive relationship with gravimetric moisture content (Φm) and clay (Table 3). Coefficient of linear extensivity (COLE) had strong positive relationship with (Φm) and clay. These findings suggest possible use of these soil physical properties in predicting rheological properties in WC and Tm soils in the study locations. Sand content had significant ($p<0.05$) correlation with rheological properties, implying its efficacy in predicting soil behavior.

Strong relationship between soil moisture and PI ($r = 0.82$, $p<0.0001$) (Table 2) suggests that soil moisture is a major determinant of soil compressibility (McNabb and Boersma, 1996) among other factors such as bulk density (Imhoff et al., 2004) but the r-value (0.81) suggests that other undetermined factors could be

Table 3. Relationship between rheological properties (Consistency limit) and Some physical properties across wormcast and termite mound soils in the 3 ha study sites.

Factor correlated	Pearson correlation coefficient (r)	Significance p<0.09
COLE Vs Φm	0.83	<0.0001
COLE Vs clay	0.76	<0.001
COLE Vs **Bd**	0.48	<0.0001
COLE Vs sand	0.33	<0.001
LL Vs Bd	0.12	Ns
LL Vs sand	0.62	<0.0001
PL Vs Φm	0.70	<0.001
PL Vs clay	0.55	<0.001
PI Vs Φm	0.59	<0.001
PI Vs Bd	0.09	Ns
PI Vs sand	0.28	<0.001

COLE = coefficient of linear expansivity, Φm = gravimetric water content, Bd= bulk density, LL= liquid limit, PL= plastic limit, PI= plastic index, Ns= not significant.

influencing plasticity.

Conclusion

The results of this study show that clay contents in wormcast (Wc) and termite mound (Tm) soils, regarded as plant-soil system, were higher than the surrounding soils (Ss). Decreased soil bulk density and increased gravimetric water content were recorded in the plant-soil systems relative to Ss. Wormcast and termite mound soils had higher consistency limits and greater shear strength than the surrounding soils. Though the rheological and other physical properties varied with the Wc and Tm soils, they have shown their relative efficacy in predicting soil behavior. The study has demonstrated the usefulness of characterizing Wc and Tm in relation to surrounding soil.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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Full Length Research Paper

Weed dry mass accumulation in response to the application of NPK fertilizers in cassava crop

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Fertilizers can be used to change competition relationships between crop and weed by promoting crop species growth, since rival species has a differentiated response to nutrients. We aimed to evaluate weed dry mass accumulation when or not NPK (nitrogen, phosphorus and potassium) fertilizers were applied to cassava crop. Therefore, weed samples were collected every 35 days, from 35 to 525 days after cassava planting, by randomly throwing a 0.25 m^2 metal square (0.5 m × 0.5 m) within useful area of the plots with and without fertilization. After each thrown, it was removed weed shoot, separated by species, then counted and weighed to determine number of plants and shoot dry mass. Fertilization did not influenced the weed population during most part of the crop cycle; however, promoted greater weed dry mass accumulation in the second crop year, 350 days after planting. *Panicum maximum*, *Brachiaria plantaginea*, *Sida rhombifolia*, *Pavonia cancellata*, *Setaria parviflora* and *Cynodon dactylon*, which were the most abundant weeds, had different responses to fertilization. *P. maximum* and *B. plantaginea* have outstood because of their shoot dry mass increase of 41.59 and 36.25%, respectively, when comparing fertilized and non-fertilized treatments.

Key words: *Manihot esculenta*, weed community, biomass, fertilizers.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) has great importance for tropical regions, wherein it is one of the major sources of carbohydrates for needy communities. The tuberous roots are the most important part of the plant, being rich in starch, which is used for human and animal nutrition or as raw material for several industrial derivatives (Albuquerque et al., 2012). It plays a crucial role in job and income growth especially for small and medium farmers (Albuquerque et al., 2008), being among the most important food harvested in Brazil, and outnumbered only by soybean, wheat, rice and corn (IBGE, 2014).

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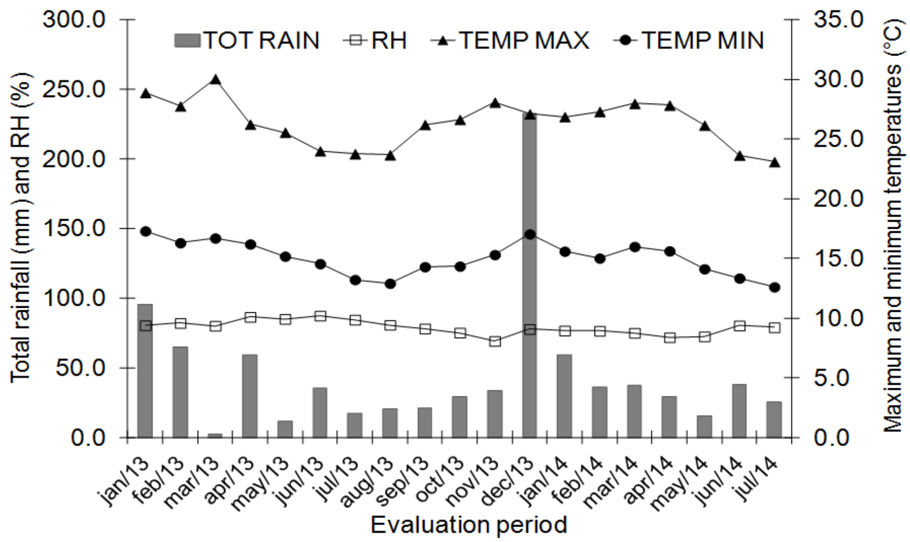

Figure 1. Monthly average of rainfall, air relative humidity and maximum and minimum temperature, in Vitória da Conquista-BA, Brazil. From January 2013 to July 2014. *Source: National Institute of Meteorology - INMET/ Vitória da Conquista, BA, Brazil, 2014.

On national scenario, Bahia is one of the main producing states, generating around 8.7% of the 21.22 million tons of national production. Cassava yield averages 13.91 tons ha^{-1} (IBGE, 2014). Vitória da Conquista city is a prominent micro region of cassava production, accounting for approximately 10% of the state production (IBGE, 2008). However, despite its importance, the root yield is considered low when compared with the crop production potential of up to about 90 t ha^{-1} of roots (Cock, 1979).

Cassava cultivation is largely concentrated on small farming, which is characterized by low use of feedstock. It occurs because of the cassava ability to be grown on low-fertility soils (Carvalho et al., 2007b). Nonetheless, it is also one of the reasons of the short Brazilian root production in the last decades. Addionally, Cardoso et al. (2013) complemented that such small production is due to low adoption of agricultural techniques, little productive varieties and/ or adapted the region, and mainly competition with weed.

Weed in cassava cultivation has been reported as one of the main factors affecting crop yield. According Albuquerque et al. (2008), root yield can be reduced by more than 90% in absence of weed control. This is due mainly to a slow initial growth of cassava plants, which facilitates weed species development, favoring the competition for water, light, nutrients, carbon dioxide and physical space (Azevêdo et al., 2000). In addition, cassava harvest can occur up to two years after planting, when roots are delivered to processing industry (Silva et al., 2012). Because of long cultivation and the soil partial covering by the plant, several weed infestations can occur within the planting area, what might increase crop yield losses (Johanns and Contiero, 2006).

Among resources liable to weed and crop competition, nutrient extraction and accumulation appear to be a crucial feature when studying the entire weed community in competition with intermediate cycle crops, such as cassava (Albuquerque et al., 2012).

Fertilizers can be used to alter competitive relationships between crop and weed, favoring crop plants by changing weed community composition and density, since the species have different responses to nutrient inputs (Armstrong et al., 1993). Even though a large amount of knowledge and technological advances on crop mineral nutrition are available, there is a lack of that regarding infesting communities, what impairs the understanding of interfering factors in competition for nutrients between weed and crop plants (Procópio et al., 2005).

Based on the above considerations, the current research aimed to assess weed dry mass accumulation in response to NPK fertilization in cassava crop.

MATERIALS AND METHODS

The study was carried at experimental area from the State University of Southwestern Bahia, campus in Vitória da Conquista – BA, Brazil, from January 2013 through July 2014. The campus is located at 14° 51'S latitude and 40° 50' W longitude, in average altitude of 941 m. According to Köppen classification, climate is *Cwa* type (high altitude tropical), with average annual rainfall of 717 mm. Figure 1 displays weather data of the assessed period related to rainfall, relative humidity, maximum and minimum temperature. Soil is classified as dystrophic Yellow Latosol (Oxisol) (EMBRAPA,

Table 1. Particle size and chemical analyses of topsoil (0-20 cm), belonging to a dystrophic Yellow (Oxisol) from the experimental area[1/]. Vitória da Conquista - BA, Brazil, UESB (2015).

Particle size analysis (dag kg^{-1})										
Clay		**Silt**		**Coarse sand**		**Fine sand**		**Texture**		
29		1		56		14		Sandy clay loam		
Chemical analysis										
pH	**P** [2/]	**K^+** [2/]	**H + Al** [3/]	**Al^{+3}** [4/]	**Ca^{+2}** [4/]	**Mg^{+2}** [4/]	**CEC $_{total}$**	**V**	**m**	**OM**
H_2O	...........mg dm^{-3}.........		………………………….$cmol_c$ dm^{-3}…………………………..					………..%.............		dag kg^{-1}
5.2	10.0	0.11	3.4	0.3	0.9	0.5	4.9	31	17	19.0

[1/]Data generated by the Laboratory of Soil Analyses from the UESB; by various extractants: [2/] Mehlich-1; [3/] 0.5 mol L^{-1} Ca (OAC) 2 at pH 7.0; [4/] mol L^{-1} KCl^1.

2006), whose main physical and chemical characteristics are shown in Table 1.

Soil tilling consisted of plowing, harrowing and grooving. Fertilization of treatments was based on soil analysis and recommendation for cassava crop proposed by Nogueira and Gomes (1999). It was applied 17.5 kg ha^{-1} P, directly into planting groove; and 70 kg ha^{-1} N and 25 kg ha^{-1} K as topdressing, sixty days after planting. In the second year, 60 Kg ha^{-1} N and 50 Kg ha^{-1} K were applied as topdressing at the beginning of rainy season (December 2013). For treatments without fertilizer application (control), it was considered soil natural fertility (Table 1).

Planting was manually performed in January 2013, using 'Caitité' variety with approximately 2 to 3 cm stem diameter, 20 cm length and seven buds. Plant spacing was 1.0 m between rows and 0.6 m between plants, totaling 16,666 plants ha^{-1}. Each plot consisted of four rows within an area of 8.4 m length per 4.0 m width, totaling 33.6 m^2. The plot useful area of 14.4 m^2 was composed of the two central lines, leaving a 0.6 m border at each edge.

The experiment was set up in an entirely randomized block design comprising two treatment groups evaluated with and without fertilizer application and three replications. In the first group, it was evaluated six growing periods of convivial between crop and weed, starting from planting time (DAP): 35, 70, 105, 140, 175 and 540 days (control with weed). After the interaction period, crop remained out of competition with weed by manual weeding. In the second group, we evaluated six growing periods of control, starting from planting time (DAP): 35, 70, 105, 140, 175 and 540 days (control without weed). After the end of each period, weed were left to emerge freely.

Weed evaluations were performed at 35, 70, 105, 140, 175, 210, 245, 280, 315, 350, 385, 420, 455, 490 and 525 days after cassava planting (DAP). During these evaluations, weed samples were collected for measurements. Samples were made by throwing randomly a metal square of 0.5 × 0.5 m (0.25 m^2) within the plot useful area. Thirty-six samplings were carried at each period, totaling 540 samplings.

Weeds from sampled areas were cut close to ground and then taken to the laboratory, where they were counted and separated by species, to determine their number and shoot dry mass, dried in a forced-air oven at 70°C, up to constant weight.

Unprocessed data underwent variance analysis; and the treatments were set in a 15x3 factorial scheme (fifteen evaluation periods and two fertilization conditions). The F test compared mean squares, and means were compared by the Scott-Knott test at 5% probability. Each species dry mass of fertilized and non-fertilized treatments were compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Fifty weed species were identified during inventories, which are distributed into 39 genera and 15 botanical families. Regarding species number, Malvaceae (14), Asteraceae (08), Poaceae (07) and Fabaceae (05) are the most notable and account for 68% of all species (Table 2). Otsubo et al. (2002), Albuquerque et al. (2008) and Guglieri et al. (2009), who have highlighted the same families as the richest in weed species for cassava fields, observed similar findings.

Weed community composition was considered heterogeneous when compared to results from Albuquerque et al. (2014), who evaluated 27 species distributed into 21 genera and 8 families in cassava plantations in Roraima savannah (Boa Vista – RR, Brazil). In addition, Huziwara et al. (2009) found 10 species belonging to nine genera and 9 families in cassava field located in Campos de Goytacazes – RJ, Brazil.

Cassava fertilization promoted an increase of 23% in weed dry mass accumulation, compared to non-fertilized treatments (Table 2). This results show that fertilization also promotes weed growth; thus, there are major losses of cassava root yield due to weed and crop competition. As reported by Cruz and Pelacani (1993), among the effects of weed presence within crops, shading promoted by fast growth species seems to be the most important one, that is, as long as shading area increases, cassava plant height increases and its leaf area decreases, without biomass accumulation. These authors also concluded that as cassava plants are less exposed to light, stem and leaf dry matter as well as root yield become compromised. Consequently, shading promotes formation delay and decreases the growth rate of tuberous roots.

Panicum maximum (31.88%), *Brachiaria plantaginea* (15.98%), *Sida rhombifolia* (12.03%), *Pavonia cancellata* (5.43%), *Setaria parviflora* (4.16%) and *Cynodon dactylon* (4.03%) were the weed species that had the highest dry mass accumulation. Regarding the total dry

Table 2. Weed species found in cassava field, organized by family, scientific name, Brazilian common name and dry mass of treatments with and without fertilization. Vitória da Conquista - BA, Brazil, UESB (2015).

Family/ species	Brazilian common name	Dry mass ($g ha^{-1}$)		
		F[1/]	NF[2/]	Total
Amaranthaceae				
Amaranthus retroflexus	Caruru-gigante	76.72^{A}*	8.43^{B}	85.15
Chenopodium carinatum	Anserina-rendada	105.98^{B}	358.95^{A}	464.94
Asteraceae				
Acanthospermum australe	Carrapicho-rasteiro	1296.60^{A}	1470.86^{A}	2767.46
Acanthospermum hispidum	Carrapicho-de-carneiro	11.74^{A}	2.15^{B}	13.89
Bidens pilosa	Picão-preto	28.11^{B}	52.58^{A}	80.69
Blainvillea rhomboidea	Picão-grande	521.15^{B}	1038.17^{A}	1559.32
Emilia fosbergii	Falsa-serralha	208.82^{A}	122.52^{B}	331.34
Eupatorium ballotifolium	Picão-roxo	1696.22^{A}	1827.67^{A}	3523.89
Siegesbeckia orientalis	Botão-de-ouro	2.15^{A}	2.48^{A}	4.63
Synedrellopsis grisebachii	Agrião-do-pasto	15.54^{A}	3.14^{B}	18.68
Boraginaceae				
Heliotropium indicum	Crista-de-galo	8.10^{A}	12.07^{A}	20.17
Brassicaceae				
Lepidium virginicum	Mentrusto	12.57^{A}	0.99^{B}	13.56
Commelinaceae				
Commelina benghalensis	Trapoeraba	997.17^{A}	7.27^{B}	1004.44
Euphorbiaceae				
Chamaesyce hyssopifolia	Burra-leiteira	20.83^{A}	7.94^{B}	28.77
Euphorbia prostrata	Quebra-pedra-rasteira	15.05^{A}	-	15.05
Fabaceae				
Aeschynomene denticulata	Angiquinho	17.03^{A}	9.09^{B}	26.12
Crotalaria incana	Chocalho-de-cascavel	-	76.22^{A}	76.22
Senna obtusifolia	Fedegoso	335.47^{A}	323.90^{A}	659.38
Stylosanthes viscosa	Vassourinha	-	1.98^{A}	1.98
Zornia reticulata	Alfafa-do-campo	-	60.68^{A}	60.68
Malvaceae				
Gaya pilosa	Guanxuma	-	126.98^{A}	126.98
Herissantia crispa	Mela-bode	-	9.42^{A}	9.42
Herissantia tiubae	Malva-de-bode	4.30^{A}	-	4.30
Malvastrum coromandelianum	Falsa-guanxuma	502.14^{A}	242.55^{B}	744.69
Pavonia cancellata	Malva-rasteira	2883.03^{A}	3272.91^{A}	6155.94
Pavonia sidifolia	Vassoura	297.45^{B}	928.22^{A}	1225.67
Sida carpinifolia	Malva-baixa	387.06^{A}	75.73^{B}	462.79
Sida cordifolia	Malva-branca	2395.61^{A}	1640.17^{A}	4035.78
Sida glaziovii	Guanxuma-branca	10.09^{A}	-	10.09
Sida rhombifolia	Guanxuma (vassourinha)	6372.37^{A}	7265.54^{A}	13637.90
Sida santaremnensis	Guanxuma	9.76^{A}	-	9.76
Sida spinosa	Guanxuma-de-espinho	886.72^{A}	892.51^{A}	1779.22
Sida urens	Guanxuma-dourada	190.14^{A}	41.00^{B}	231.15
Waltheria indica	Malva-branca	1.16^{B}	8.60^{A}	9.76
Molluginaceae				
Mollugo verticillata	Molugo	29.93^{A}	19.51^{A}	49.44

Table 2. (Contd).

Nyctaginaceae				
Boerhavia diffusa	Agarra-pinto	103.34^{A}	37.20^{B}	140.54
Passifloraceae				
Passiflora cincinnata	Maracujá-do-mato	30.09^{A}	-	30.09
Poaceae				
Brachiaria plantaginea	Capim-marmelada	11059.43^{A}	7049.60^{B}	18109.03
Cenchrus echinatus	Capim-carrapicho	1924.72^{A}	1480.62^{A}	3405.34
Cynodon dactylon	Grama-seda	3031.01^{A}	1540.97^{B}	4571.98
Digitaria horizontalis	Capim-colchão	29.43^{B}	108.13^{A}	137.56
Panicum maximum	Capim-colonião	22807.99^{A}	13321.11^{B}	36129.10
Rhynchelytrum repens	Capim-favorito	1813.78^{A}	917.80^{B}	2731.58
Setaria parviflora	Capim-rabo-de-raposa	1772.11^{B}	3019.27^{A}	4791.39
Portulacaceae				
Portulaca oleracea	Beldroega	1253.28^{A}	724.35^{B}	1977.63
Portulaca mucronata	Onze-horas	2.15^{B}	11.74^{A}	13.89
Rubiaceae				
Diodia teres	Mata-pasto	640.53^{A}	387.56^{B}	1028.08
Richardia scabra	Poaia-do-cerrado	155.92^{A}	175.10^{A}	331.01
Solanaceae				
Solanum americanum	Maria-pretinha	143.18^{A}	50.76^{B}	193.94
Solanum erianthum	Caiçara	1.32^{B}	458.65^{A}	459.98
	Total	**64107.28**	**49193.11**	**113300.39**

*Means followed by the same letter in the line do not differ from each other by the Tukey test at 5% probability. [1]/ and [2]/ Crop with and without fertilization, respectively.

mass accumulation for treatments with and without fertilizer application, these species comprised 74.69 and 71.99%, respectively (Table 2).

Concerning the responses of these species to fertilization, *P*. *maximum*, *B*. *plantaginea* and *C*. *dactylon* (Poaceae) had significant dry mass accumulation when fertilizer was applied, representing 41.59; 36.25 and 49.15%, respectively. Contrarily, *S. parviflora* had higher accumulation of dry mass in non-fertilized treatments, reducing this rate in 41.3% for fertilized ones. However, *S. rhombifolia* and *P. cancellata* (Malvaceae) had no significant results about fertilization, with slightly reduction of crop dry mass in treatments with fertilizer application (12.29 and 11.91%, respectively), although not differing from non-fertilized treatments (Table 2). Such results demonstrate that weed responses to fertilizers are variable with regards to shoot dry mass accumulation. According to Brighenti and Oliveira (2011), some weed species have greater efficiency to use fertilizers to grow faster, increasing the competition against crop.

With respect to number of plants, it was found a total number of 192,968 weed individuals per hectare, being 93,813 from fertilized treatments and 99,154 from non-fertilized ones (Figure 2); therefore, plant number did not differ from fertilized to unfertilized treatments.

Number of individuals has reduced considerably from 105 DAP on, having less than 10,000 individuals per hectare; the lowest number was observed starting from 455 DAP (Figure 2). Yet the highest number was checked at 35 DAP due to a lower growth of cassava plants, which favored weed emergency during this period. Biffe et al. (2010) reported similar results; the authors also noted that weed interference on cassava crop is major from 18 to 100 DAP. Thus, weed control strategies should be taken within this time, aiming to preserve the maximum root yield.

According to Lorenzi and Dias (1993), cassava has a slow initial growth, which combined with a wide row spacing results in low competitive ability against weed population. It is mainly related to soil shading, enabling weed emergence for a longer period, what explains the elevated number of weeds up to 105 DAP.

Cassava fertilization increased significantly the amount of weed individuals at 105 days after crop implementation, being around 3,000 more individuals than non-fertilized ones. While, at 70 and 385 DAP, non-fertilized treatments increased in 20.1 and 36% the number of weed individuals, respectively, if compared to fertilized ones. However, in the other evaluation periods,

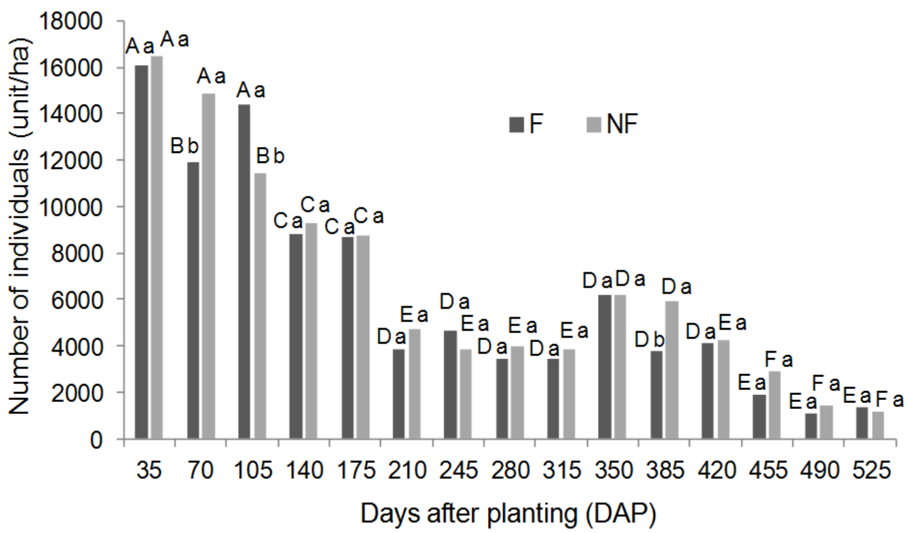

Figure 2. Number of weed individuals in cassava crop from 35 to 525 days after planting (DAP) of treatments with (F) and without (NF) fertilization. Vitória da Conquista – BA, Brazil, UESB (2015). *Columns followed by the same lowercase letter (fertilized treatments at each evaluation time) and uppercase letter (evaluation times in fertilized and non-fertilized treatments) do not differ from each other by the Scott-Knott test at 5% probability.

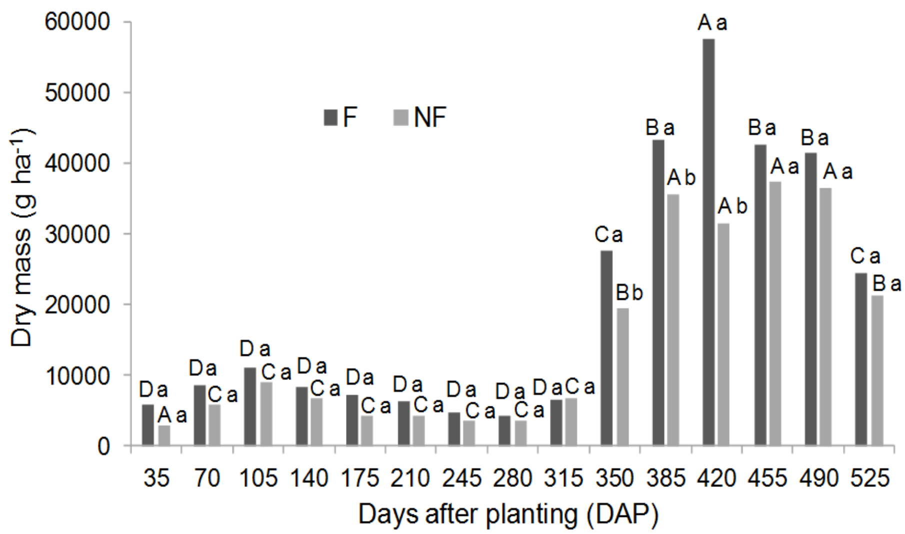

Figure 3. Weed shoot dry mass (g ha^{-1}) from 35 to 525 days after planting (DAP) of treatments with (F) and without (NF) fertilization. Vitória da Conquista – BA, Brazil, UESB (2015). *Columns followed by the same lowercase letter (fertilized treatments at each evaluation time) and uppercase letter (evaluation times in fertilized and non-fertilized treatments) do not differ from each other by the Scott-Knott test at 5% probability.

no differences have been found between fertilized and non-fertilized treatments (Figure 2).

Figure 3 shows the results of dry mass accumulation of weed shoot within cassava cultivation areas with and without fertilization. Dry mass accumulation was observed from 350 DAP on, in which fertilized treatments stood out. This treatment had a maximum buildup at 420 DAP, having 45% more weed dry mass compared to non-fertilized ones (Figure 3). Therefore, cassava fertilizations increased weed dry mass accumulation, notably during the second cropping year; time when nutrient competition might not be harmful to cassava plants, since they already have well-formed shoot and root.

According to Radosevich et al. (1996), as density and

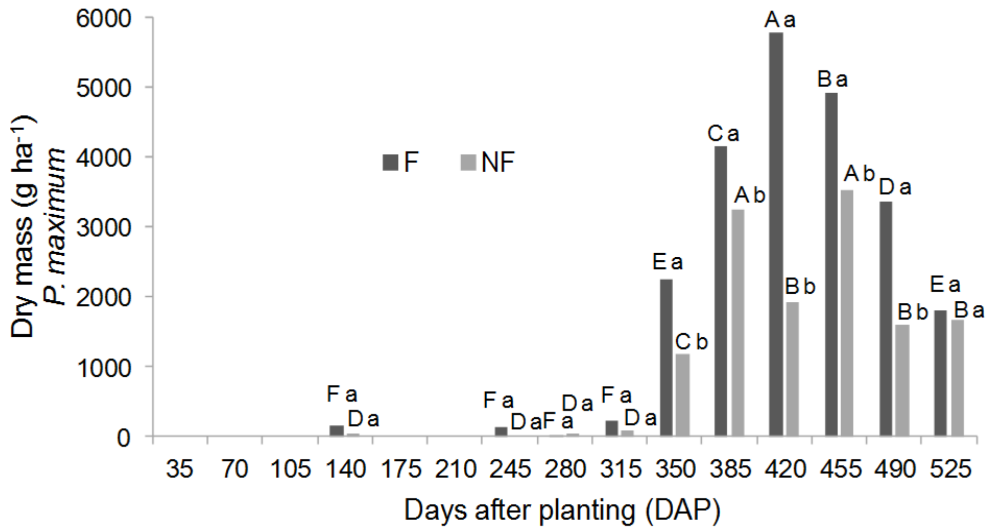

Figure 4. Shoot dry mass of *Panicum maximum* plants (g ha^{-1}) infesting cassava crop from 35 to 525 days after planting (DAP) of the treatments with (F) and without (NF) fertilization. Vitória da Conquista - BA, Brazil, UESB (2015). *Columns followed by the same lowercase letter (fertilized treatments at each evaluation time) and uppercase letter (evaluation times in fertilized and non-fertilized treatments) do not differ from each other by the Scott-Knott test at 5% probability.

development of weeds increase, especially at the beginning of the crop cycle, intraspecific and interspecific competition is enhanced; thus, higher and well-developed weed plants become dominant, while small and weak are suppressed or come to death. This scenario explains the reduction in weed individuals from 105 DAP, also the increase of weed shoot dry mass after 350 DAP (Figures 2 and 3).

Despite being less expensive, weed control during second year can be difficult, because crop shoot has already been formed, which makes it difficult to enter into the field (Peressin and Carvalho, 2002). In this case, between the two cycles, the crop is in physiological rest. Falling leaves and plant reduced metabolic activity characterize this phase and its duration is related to environmental conditions especially. Therefore, it is during this period that a new infestation starts, which was also observed in this study, mainly in fertilized treatments from 350 DAP. It is therefore necessary to control these plants, to avoid possible losses and to facilitate crop harvesting (Silva et al., 2012).

Evaluating cassava growth (Cacau UFV cultivar) and weeds due to phosphate fertilizing (0, 80, 800 and 4,000 kg ha^{-1} P_2O_5), Pereira et al. (2012) found that cassava showed greater shoot growth with increasing phosphorus availability; while weeds showed higher responses to lower phosphorus levels. Complementary effect was observed by Fidalski (1999), when evaluating cassava growing with NPK fertilization in sandy soils in Northwestern Paraná. This author noted that root production (Fibra cultivar) showed no response to nitrogen (0, 20, 40 and 60 kg ha^{-1} N) and potassium (0, 40, 80 and 120 kg ha^{-1} K_2O). On the other hand, Alves et al. (2012), evaluating NPK doses (10:28:20 commercial formula) on cassava yield in Moju – PA, Brazil; they concluded that Paulozinho variety responded linearly with increasing doses in sandy and low fertility soils.

In general, fertilization carried to favor cassava development against weed community, had also benefited shoot dry matter accumulation of these plants in the second year. Therefore, depending on the level of competition at this stage, the crop may be adversely affected. As stated by Procópio et al. (2005), depending on crop management, the application of nutrients can most benefit weeds to the detriment of the crop.

As aforementioned, *P. maximum*, *B. plantaginea* and *S. rhombifolia* rose to predominance at the experimental area, which have stood out along infesting community evaluations of fertilized and non-fertilized treatments (Figures 4 to 6).

P. maximum, popularly known as capim-colonião in Brazil, had elevated values of shoot dry mass, accounting for 35.57% of the total dry weed mass recorded in fertilized cultivation and 27.07% in the cultivation without fertilization (Table 2); showing a great competitive potential as function of its high production capacity of biomass compared to the other species. This weed occurrence was recorded in some periods of the first year; though reduced dry mass accumulation. However, from 350 DAP, which matched to the rainy season (Figure 1), there was a significant increase in the dry mass accumulation of *P. maximum* in fertilized treatments, reaching a maximum dry mass accumulation at 420 DAP of 66.5% compared to treatments without

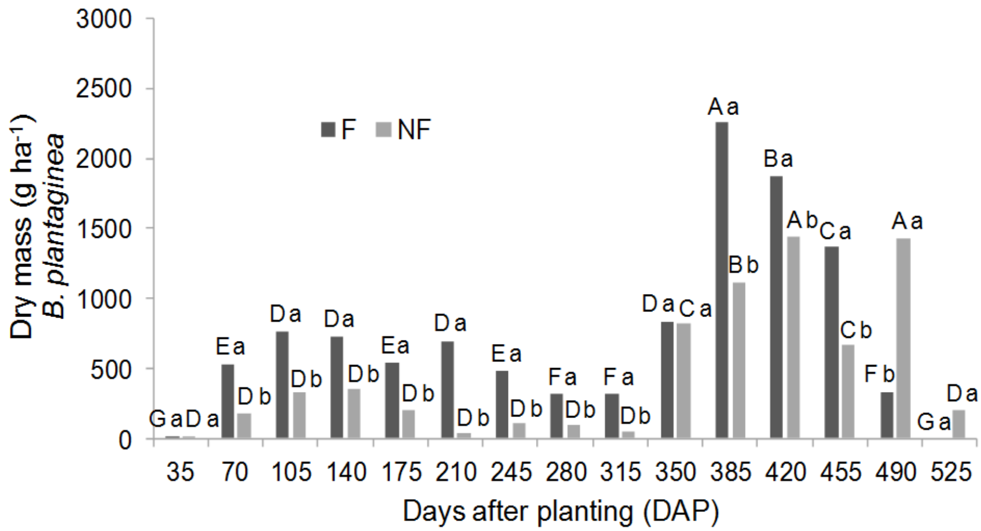

Figure 5. Shoot dry mass of *Brachiaria plantaginea* (g ha^{-1}) infesting cassava crop from 35 to 525 days after planting (DAP) of the treatments with (F) and without (NF) fertilization. Vitória da Conquista - BA, Brazil, UESB (2015). *Columns followed by the same lowercase letter (fertilized treatments at each evaluation time) and uppercase letter (evaluation times in fertilized and non-fertilized treatments) do not differ from each other by the Scott-Knott test at 5% probability.

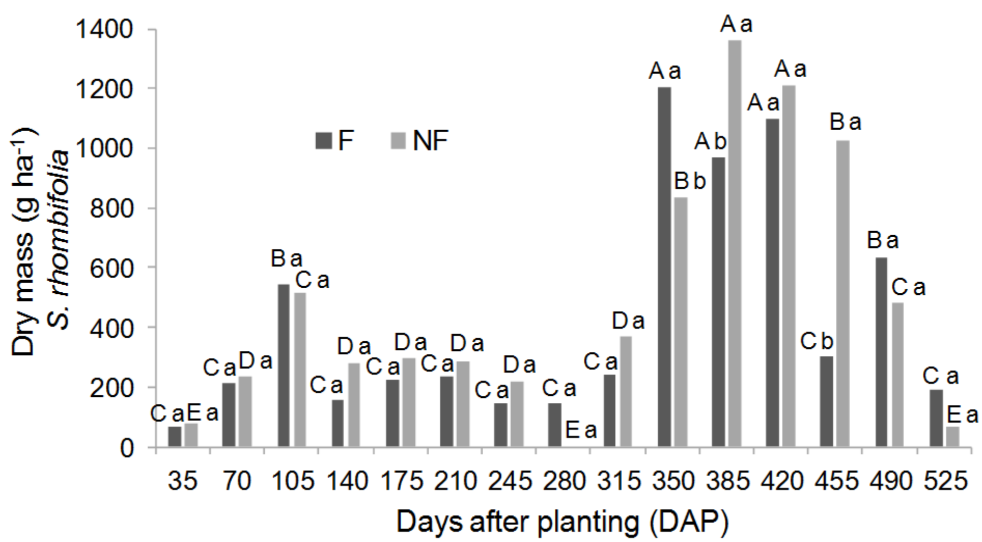

Figure 6. Shoot dry mass of *Sida rhombifolia* (g ha^{-1}) infesting cassava crop from 35 to 525 days after planting (DAP) of the treatments with (F) and without (NF) fertilization. Vitória da Conquista - BA, Brazil, UESB (2015). *Columns followed by the same lowercase letter (fertilized treatments at each evaluation time) and uppercase letter (evaluation times in fertilized and non-fertilized treatments) do not differ from each other by the Scott-Knott test at 5% probability.

fertilization (Figure 4).

Among the reasons of *P. maximum* dry mass accumulation, it can be listed its presence in neighboring areas, cassava defoliation at maturation, weed seed dispersal, crop fertilization and early rainy season. Such conditions certainly favored this weed establishment and development over the area, once it is quite demanding in light, fertility and soil moisture.

Since *P. maximum* is a fierce and competitive plant, it can generate significant losses during peak infestations in the first crop year. Cruz et al. (2010), who evaluated the effect of *P. maximum* on initial growth of eucalyptus clones, found that all studied clones showed negative influence of the coexistence with variations on leaf area, dry weight of leaves and stem. Moreover, some studies had proved *P. maximum* efficiency to produce shoot dry mass. For example, Ferreira et al. (2008), who assessed the effect of increasing phosphorus doses (P_2O_5) (30, 60, 90, 120 and 150 kg ha^{-1}) on *P. maximum* growth, checked a linear raise of shoot dry mass production up to the dose of 103 kg ha^{-1} de P_2O_5. Nevertheless, Lugão et al. (2003), studying nitrogen fertilization (0, 150, 300 and 450 kg/ ha/ year) efficiency on shoot dry mass accumulation of *P. maximum*, observed high rates with nitrogen use, having the greatest efficiency at 150 kg nitrogen/ ha /year.

Commonly known as capim-marmelada in Brazil, *B. plantaginea* demonstrated good adaptation and aggressiveness, being logged at all evaluations, representing 17.25% of total dry mass in fertilized treatments and 14.32% in non-fertilized ones (Table 2). This Poaceae, originally from Africa, has as main means of spread, the seeds, which are characterized by presenting primary dormancy during maturation stage (Lorenzi, 2008); thus, germination is distributed over time, which makes its control difficult (Kissmann, 1997).

The greatest dry mass values are checked for the second year between 350 and 490 DAP (Figure 5), matching local rainy season (Figure 1).

Cassava fertilization enhanced *B. plantaginea* production of shoot dry mass, especially from 385 to 455 DAP, increasing it more than 50% compared to non-fertilization. Nevertheless, at the end of the crop cycle, 490 and 525 DAP; dry mass values of the fertilized treatments decreased and represented 76.5 and 12.05% of those of unfertilized treatments, respectively (Figure 5). Weeds as *B. plantaginea*, at the final crop stage, could hamper cassava root harvest, in addition to the occurrence of venomous animals as much reported by Brazilian farmers, and mainly in weed infested areas (Albuquerque et al., 2014).

In a comparative study of dry mass accumulation and macronutrients with corn and *B. plantaginea*, Carvalho et al. (2007a) concluded that maximum competition for macronutrients occurs at 100 days after emergence (DAE), during which corn plants begin physiological maturity, which could cause serious damage to final production. Agostinetto et al. (2009), evaluating competitive ability of soybean against *B. plantaginea*, found antagonism between them without competitive dominance of one over the other, and in both, intraspecific competition was more important than interspecific. For the cassava crop, Aspiazú et al. (2010), evaluating the water use efficiency by cassava in competitive conditions with weeds, they found that *B. plantaginea* is very efficient in water use, primarily due to its C4 metabolism, and remains competitive even under temporary low water availability.

Poaceae family has a major space among the infesting plants; it is considered one of the most important in cassava crops. Pinotti et al. (2010) identified *B. decumbens* and *D. horizontalis* as the most important species in Pompéia – SP, Brazil. Albuquerque et al. (2014), still studying cassava crop in Roraima, observed the highest dry mass accumulation for *D. sanguinalis*, *B. brizantha*, *B. decumbens* and *B. humidicola*. According to Maciel et al. (2010), several species from Poaceae family are perennial and produce a great amount of seed, fact that enhance their spread and colonization at different environments.

S. rhombifolia, popularly known as guanxuma in Brazil, had widespread distribution within experimental area, being recorded in all evaluations; it accounted for 9.94% of accumulated total dry mass in fertilized cultivation and 14.76% in non-fertilized crop (Table 2). Such an occurrence could have been attributed to their high potential infestation, since it presents high seed yield and ease of dispersion.

Higher shoot dry mass accumulation was seen in the second year, starting from 350 days after cassava planting. It noteworthy to highlight non-fertilized treatments at 385 and 455 DAP, promoting an increase of 28 and 70% respectively, compared to the fertilized ones. However, in other evaluations, it was found no significant differences between cultivation with and without fertilization; demonstrating that, in general, dry mass accumulation of that weed do not depend on fertilizer supply (Figure 6).

S. rhombifolia is found in annual and perennial crops, being highly competitive because of its root system that can reach 50 cm depth (Lorenzi, 2008; Kissmann and Groth, 2000). There are reports showing that this plant is able to produce up to 28.2 thousand seeds m^{-2} in a single summer season infesting soybean crop (Fleck et al., 2003). Among “guanxuma” species found in Brazil, this is considered most widely spread and hard to be controlled at various farming environments (Constantin et al., 2007). It was also reported as infesting plant in cassava field by Azevêdo et al. (2000) and Albuquerque et al. (2008), besides corn (Macedo et al., 2003), sugarcane (Oliveira and Freitas, 2008) and soybean (Voll et al., 2005).

Concerning *S. rhombifolia* dry mass accumulation, Bianco et al. (2014) reported that N and K are the most required macronutrients; macronutrient accumulation daily rate is cumulative until 94 DAE; and periods of increased accumulation of dry mass and macronutrients occur after 122 DAE.

Although *S. rhombifolia* is present in all evaluations with a high number of individuals, *P. maximum* and *B. plantaginea* showed greater efficiency in using fertilizers and environmental resources, and therefore a greater accumulation of dry weight. Both species are widely exploited for local cattle raising, and, like other species from Poaceae family used in animal husbandry, they should be eliminated from cassava plantations, since they are perennials, produce large amounts of seeds, has high biomass production and ease to adapt to various environments.

Conclusions

Fertilizers applied into cassava crops did not influenced the number of individuals of the weed community during most part of the cassava cycle. *P. maximum*, *B. plantaginea*, *S. rhombifolia*, *P. cancellata*, *S. parviflora* and *C. dactylon* showed the highest accumulation of shoot dry mass, which also presented varied responses to NPK fertilization in cassava crop. Generally, cassava fertilization promoted weed dry mass accumulation during the second year of the cassava cycle, mainly from 350 days after planting on, and being more significant for the grasses *P. maximum* and *B. plantaginea*.

Conflict of Interest

The authors have not declared any conflict of interest.

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Characterization of rambutan plants by foliar aspects

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The present study was conducted to verify the possible distinct genotypes and material sexes of rambutanusing foliar aspects, enabling the recognition of plants in the early stages of development. Ten productive plants and ten male plants were selected, and they were named according to their arrangement in the orchard. They were evaluated for leaf and leaflet size (length and width in cm), leaflet area (cm^2), the number of leaflets, rachis length (cm), length gaps between the leaves (cm), and the color (L*, a*, b*, C* e H*) of the leaflets (superior and inferior surfaces). The data were subjected to an analysis of variance using an F test, and the means were compared using the Scott-Knott test ($p<0.05$). A cluster analysis of twenty genotypes was performed from the matrix of Euclidean distances. Based on the results obtained in this experiment, it can be concluded that the characteristics related to the color of the leaflets can be a leaf differential aspect in production plants as observed in the plants LB10_F, LB11_F and LB91_F. The multivariate analyses showed that there is low genetic distance between the studied plants; based on the foliar aspects analyzed, it was not possible to identify a discriminatory feature for all plants of the same sex.

Key words: Leaves, leaflets, *Nephelium lappaceum,* morphology.

INTRODUCTION

Fruits provide a means of plant reproduction and dispersal, and they are the hallmarks of the angiosperm lifestyle. The development of flowers and fruits has been attributed to the success of angiosperm evolution, as exemplified by a great diversity in species found around the world (Monfrote et al., 2014); among these species is the rambutan. Belonging to the family Sapindaceae, rambutan (*Nephelium lappaceum* L.) is native to Malaysia and Indonesia (Tindall, 1994), and it is grown throughout Southeast Asia, Australia, South America and Africa (Sousa et al., 1994).

According to Andrade et al. (2008), the largest Brazilian consumer market is the state of São Paulo, and producers have established cultivars through seedlings that originated from seeds with high genetic variability, but there is no information on the regional behavior. This behavior is because consumers purchase the fruit based on the type rather than the cultivar, as with mangos, apples, bananas and the like.

However, there is an obstacle in the implementation of this cultivation because the species has three types of plants: Male flowers, functionally female hermaphroditic

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flowers and hermaphroditic flowers producing some functional females and some functional males (Valmayor et al., 1970). However, this plant sexuality condition is only noticed as individuals enter maturity. This can be a great inconvenience because the males represent approximately fifty percent of the population originating from seeds; thus, four to five years could be wasted on male plants from planting to gender detection upon flowering. Thus, an alternative is to plant three seedlings per pit until the sex of the plants can be identified during flowering (Sacramento and Luna, 2004). However, the maintenance of three plants per pit requires time and costs.

Therefore, morphological characters can be used as signatures of identity varietal purity and genetic (Ambiel et al., 2008) and for several fruit trees; the distinction between plants can be performed based on foliar aspects differentiating them even before they flourish or blossom (GalánSaúco and Menini, 1989). This method has been used by several authors,such as Andrade and Martins (2007) in carambolasand Andrade et al. (2009) in rambutan cultivation.

Thus, this study aimed to verify the possible distinct genotypes and material sexes of rambutanbased on foliar aspects to enable the recognition of plants in the early stages of development.

MATERIALS AND METHODS

Selection and collection of materials

The work was conductedusing twelve-year-old plants in production from an orchard in the city of Taquaritinga, São Paulo, Brazil. The orchard is located atthe coordinates 21°26'45,5"south latitude and 48°37'57,4" west longitude atheighten elevation of 493 m.Under the Köeppen classification system, the climate is Aw and characterized as rainy tropical with dry winters.

The orchard was formed by seedlings from Bahia State, which resulted in great variability in the characteristics of these plants, including the leaf. The cultivar, at a spacing of 7 × 4 m, is drip irrigated whenever the drought exceeds thirty days and receives fertilizer N:P:K – 19:10:19 (1 kg $plant^{-1}$) during February and October. From a total of 288 plants, 148 productive and 140 male, 10 productive and 10 male plants were selected and named as follows according to the provision in the orchard: LA13, LA113, LB01, LB10, LB11, B62, LB87, LB91, LC13, LD120 – productive plants (F) and LA02, LA30, LA91, LA114, LB04, LB17, LB43, LC09, LD51 and LD92- male plants (M).At the time of collection the plants were in a vegetative stage. The female plants were chosen for their history of high productivity, low susceptibility to cold and presentation of a red inner bark, considered by the producer to be the most appropriate for the market.From each plant, five samples of ten leaves were obtained during the full stage of development throughout the periphery of the top, totaling 50 leaves per plant. One plant was collected per visit to the orchard, and the leaves were held in the early morning hours to avoid dehydration of the material. These leaves were placed in polystyrene boxes (Styrofoam) and taken immediately to the laboratory for evaluation.

The evaluations were performed in the Department of Plant Production, Faculty of Agriculture Sciences and Veterinary, Universidade Estadual Paulista (UNESP) Jaboticabal Campus, São Paulo State, for evaluations on leaf size (length (LL) and width (LW) in cm), leaflet area (LFA) (cm^2), leaflet number (NLF) and size of leaflets (length (LFL) and width (LFW) in cm), length of the rachis (LR) (cm), length of the intervals between the leaflets (IL) (cm), and upper (upp) and lower (low) color of the leaflets (L*, a*, b*, C* and H*).

The lengths and widths were measured with the aid of a graduated ruler using the measured length from base to tip and the width at the widest point of the leaves and leaflets. The area of the leaflets was obtained with the LI-3100 Area Meter. The color was measured at the upper and lower surface of each leaflet using the calorimeter Konica Minolta (Chroma Meter CR-400), and the values were expressed in the system CIELAB. The observed values were L*, C*, H*, a*,and b*, signifying brightness, which ranges from zero to 100 (black/white); saturation; Hue angle (0° is pure red; 90° is pure yellow; 180° pure green and 270° pure blue); intensity of red/green (+/-) and intensity of yellow/blue (+/-), respectively. Instrument calibration was performed using a white ceramic plate.

Statistical analyses

The experimental design was completely randomized, consisting of 20 plants (10 productive and 10 male) with 5 replicates of 10 leaves, resulting in 50 leaves per plant and totaling 1,000 leaves.The data were subjected to an analysis of variance using a F test, and the means were compared with aScott-Knott test ($p < 0.05$). A cluster analysis of 20 genotypes was performed from the matrix of Euclidean distances as dissimilarity measures according to the method of WARD. The importance of the characteristics for the study of divergence was obtained from the major component analysis assuming that the least important features were those with the higher eigenvector coefficients from the last major component until an associated eigenvalue of 0.7 was found (Cruz and Carneiro, 2003). Genetically different accessions were identified in the dendrogram from the average Euclidean distance between all pairs of genotypes. The analyses were performed with the statistical program GENES (Cruz, 2008), and the dendrogram was obtained by the program Statistica 7.0 (STATSOFT, 2007).The data of leaflets lenght (LFL) were transformed into log (x) for analysis purposes.

RESULTS AND DISCUSSION

The lengths and widths of the leaves, area, number, length and width of the leaflets, and lengths of the rachis were significantly different (Table 1), and these values coincide with the results of other researchers such as Tindall (1994) and Andrade et al. (2009). However, these differences are so minute that they cannot be used toappropriately differentiate a plant in practice.

Color of leaflets

In Table 2, it can be seen that asignificant difference was found for hue angle (H) at the bottom of the leaflets, indicating that there is low variability among the studied plants. Because of this, the use of multivariate techniques can help to quantify this dissimilarity (Cruz and Carneiro, 2003). Colorimetry has been used to characterize different color pigments such as anthocyanins (Montes et al., 2005), chlorophyll (Sinneckeret al., 2002) and

Table 1. Mean values per plant for the characteristics leaf length (LL) and leaf width (LW); leaflets area (LFA); number of leaflets (NLF); length (LFL) and width (LFW) of the leaflets; total length of the rachis (LR) and the intervals between leaflets (IL) between petioles in rambutan plants.

Plant	LL** (cm)	LW** (cm)	LFA** (cm^2)	NLF**	LFL** (cm)*	LFW** (cm)	LR** (cm)	IL**(cm)
LA13_F	29.12^a	25.74^c	44.01^b	6.40^a	10.74^b	5.59^a	16.42^a	2.92^a
LA113_F	28.43^a	30.12^a	42.90^b	6.04^b	11.90^a	5.05^b	13.43^b	2.85^a
LB01_F	22.84^c	24.53^c	37.67^c	5.74^c	10.06^b	4.89^c	9.94^d	2.36^b
LB10_F	22.80^c	21.58^d	24.88^d	5.06^c	9.79^b	4.67^c	10.75^d	2.59^b
LB11_F	20.19^d	16.96e	25.01^d	5.92^b	8.17^b	3.90^d	11.24^d	2.59^b
LB62_F	24.13^b	24.13^c	32.49^c	5.92^b	9.46^b	4.62^c	11.86^c	2.51^b
LB87_F	23.91^b	24.88^c	33.18^c	5.66^c	9.89^b	4.64^c	11.49^d	2.61^b
LB91_F	27.19^a	28.05^b	43.50^b	5.64^c	10.72^b	5.65^a	12.60^c	2.67^b
LC13_F	27.63^a	27.09^c	35.96^c	6.17^b	10.97^b	4.80^c	13.36^d	2.73^b
LD120_F	22.70^c	22.51^d	26.62^d	5.72^c	8.88^b	4.25^d	11.09^c	2.51^a
LA02_M	28.69^a	26.45^c	37.39^c	6.08^b	10.22^b	5.45^a	15.09^a	2.96^b
LA30_M	23.41^c	24.99^c	33.27^c	5.40^c	9.91^b	4.44^c	10.64^d	2.74^a
LA91_M	23.17^c	24.65^c	38.75^c	5.34^c	9.87^b	5.12^b	10.97^d	2.43^a
LA114_M	23.08^c	25.55^c	34.09^c	5.48^c	10.27^b	4.53^c	10.30^d	2.47^b
LB04_M	29.06^a	26.53^c	46.10^b	6.80^a	10.98^b	5.44^a	14.88^a	2.89^b
LB17_M	25.87^b	26.21^c	36.84^c	5.66^c	10.31^b	4.82^c	12.35^c	3.06^a
LB43_M	24.94^b	26.10^c	35.30^c	5.44^c	16.16^a	4.66^c	11.54^d	2.90^a
LC09_M	24.19^b	25.58^c	32.21^c	5.80^c	10.01^b	4.98^c	11.40^d	2.47^b
LD51_M	29.02^a	31.32^a	52.53^a	6.28^b	12.50^a	5.67^a	13.95^b	2.92^a
LD92_M	25.64^b	26.08^c	41.56^b	5.92^b	10.28^b	5.25^b	12.65^c	3.08^a
CV (%)	7.08	6.33	15.04	6.65	6.50	7.87	9.87	10.58

Means followed by the same letter do not differ in the test Scott-Knott ($p > 0.05$). *Original data** Significant ($p < 0.01$).

carotenoids (Meléndez-Martínez et al., 2003), and it has been widely used in studies related to food quality. It is the first criterion used in consumer acceptance of the product; therefore, it is an important attribute in the food industry (Batista, 1994). Its use in possibly differentiating between plants and sexes is still not very widespread, but it can become an important tool for studies in this direction as the presence or intensity of color in the leaves can be an advantage to plants that produce fruits or not, as is the case in the present study.

The highest average related to Luminosity (L) on the upper surface of the leaflets was obtained in the LB11_F productive plant and later in LB10_F, that is, they exhibited lighter colors than the other genotypes in study (Table 2). The highest average for the same plants along with the LB91_F and LC13_F genotypes, all productive,were repeated for Chromaticity (C), and the plants are brighter compared with the others. As for hue angle (H), on the upper surface these plants had the lowest values compared with the others.The male plants were observed to have the highest values of this angle; therefore, in the genotypes closer to pure green, no significant effect for the inferior surface was found.Negative values of the color parameter a* indicated the presence of the green component in the leaves studied, as expected because this component is more intense on the inferior surface of the leaflets. Positive values of b* characterized the presence of a yellow color in the leaflets, which was also higher on the underside. The LB10_F, LB11_F and LB91_F plants were selected as some of the best in the orchard and classified as highly productive, with agronomic and higher market characteristics.Producers register at least one out of the three as being cultivated. These same plants were the only ones with statistically higher means in all characteristics related to a lower color inthe leaflets; thus, we can infer that the plants exhibiting all color means (L *, C *, H *, a * and b *) on the inferior surface of the leaflets with statistically higher values, that is, all classified as "a," are likely to be productive. They should therefore be selected for the establishment of orchards because the description of the morphological characteristics is the usual methodology accepted from a legal point of view for patenting and registering varieties (Badenes et al., 1998).

Genetic divergence between plants

It is noted that the most similar plants (shortest distance) are LA30_M and LA114_M (1.72) and LB62_F and LB87_F (1.74), and the most divergent (longest distance) are LA113_F and LB11_F (11.21) (Table 3). However, it is very difficult to determine which plants are more or less

Table 2. Mean values per plant for characteristics related to color of the leaflets in the upper (L = brightness, C = saturation; H = hue angle; a = intensity red / green b = intensity of yellow / blue).

Plant	Upper					Lower				
	L **	C**	H**	a**	b**	L**	C**	H ^{ns}	a**	b**
LA13_F	32.24^{c}	15.11^{c}	124.16^{b}	-8.27^{b}	12.45^{c}	41.51^{b}	22.39^{b}	117.34^{a}	-9.96^{b}	19.42^{c}
LA113_F	31.20^{d}	13.64^{c}	124.98^{b}	-7.75^{b}	11.25^{c}	39.58^{c}	22.91^{b}	118.60^{a}	-10.92^{a}	20.05^{c}
LB01_F	30.38^{d}	14.88^{c}	127.68^{a}	-8.69^{b}	11.44^{c}	39.34^{c}	22.80^{b}	119.33^{a}	-11.23^{a}	19.86^{c}
LB10_F	34.99^{b}	19.43^{a}	122.64^{c}	-10.10^{a}	16.53^{a}	**43.61^{a}**	**27.68^{a}**	**117.31^{a}**	**-12.41^{a}**	**24.57^{a}**
LB11_F	36.33^{a}	21.29^{a}	121.03^{c}	-10.83^{a}	18.27^{a}	**43.27^{a}**	**28.02^{a}**	**116.51^{a}**	**-12.24^{a}**	**24.95^{a}**
LB62_F	31.10^{d}	15.65^{c}	127.04^{a}	-8.73^{b}	12.77^{c}	40.47^{b}	23.59^{b}	117.53^{a}	-10.43^{b}	20.45^{c}
LB87_F	31.40^{d}	15.11^{c}	127.41^{a}	-9.10^{b}	12.17^{c}	39.86^{c}	21.92^{b}	117.53^{a}	-10.10^{b}	19.27^{c}
LB91_F	33.87^{b}	19.16^{a}	123.75^{b}	-9.84^{a}	15.37^{b}	**43.45^{a}**	**26.56^{a}**	**116.70^{a}**	**-11.61^{a}**	**23.78^{a}**
LC13_F	33.88^{b}	19.59^{a}	123.71^{b}	-10.13^{a}	17.49^{a}	43.78^{a}	24.56^{b}	116.67^{a}	-11.01^{a}	21.95^{b}
LD120_F	32.53^{c}	17.16^{b}	124.74^{b}	-7.96^{b}	13.25^{c}	41.13^{b}	23.12^{b}	116.90^{a}	-9.00^{b}	20.98^{b}
LA02_M	30.86^{d}	16.64^{c}	128.29^{a}	-10.16^{a}	13.08^{c}	43.20^{a}	25.21^{b}	118.33^{a}	-11.92^{a}	22.17^{b}
LA30_M	30.64^{d}	14.86^{c}	127.82^{a}	-9.06^{b}	11.76^{c}	40.09^{c}	24.68^{b}	119.34^{a}	-12.08^{a}	21.35^{b}
LA91_M	30.93^{d}	15.28^{c}	126.23^{a}	-8.90^{b}	12.37^{c}	39.56^{c}	23.77^{b}	117.29^{a}	-11.10^{a}	20.97^{b}
LA114_M	31.86^{c}	15.27^{c}	126.87^{a}	-9.10^{b}	12.23^{c}	39.78^{c}	24.00^{b}	119.49^{a}	-11.80^{a}	20.88^{b}
LB04_M	32.72^{c}	17.52^{b}	128.33^{a}	-10.79^{a}	13.77^{c}	42.45^{a}	24.70^{b}	118.54^{a}	-11.81^{a}	21.66^{b}
LB17_M	32.41^{c}	17.58^{b}	125.18^{b}	-11.81^{a}	14.60^{b}	40.95^{b}	25.88^{a}	124.56^{a}	-9.78^{b}	19.70^{c}
LB43_M	33.98^{b}	18.52^{b}	120.71^{c}	-9.14^{a}	15.79^{b}	41.27^{b}	24.30^{b}	114.28^{a}	-10.23^{b}	22.26^{b}
LC09_M	32.21^{c}	17.19^{b}	126.85^{a}	-10.26^{a}	13.77^{c}	41.14^{b}	24.68^{b}	117.85^{a}	-11.75^{a}	21.70^{b}
LD51_M	32.67^{c}	18.31^{b}	125.96^{a}	-10.30^{a}	14.47^{b}	41.24^{b}	24.90^{b}	118.06^{a}	-11.55^{a}	21.51^{b}
LD92_M	30.88^{d}	15.43^{c}	128.52^{a}	-9.19^{b}	11.62^{c}	41.21^{b}	23.18^{b}	118.46^{a}	-11.04^{a}	20.36^{c}
CV (%)	3.67	12.11	1.32	14.45	13.66	2.50	9.83	3.36	9.77	6.50

Means followed by the same letter do not differ in the test Scott-Knott ($p > 0.05$) . ** Significant ($p < 0.01$) ^{ns} not significant.

Table 3. Euclidean distance (longer and shortest) between twenty rambutan plants for eighteen leaf traits.

Plant	Longer		Shortest	
LA13_F	10.829	(LB11_F)	3.599	(LA113_F)
LA113_F	11.216	(LB11_F)	3.450	(LD92_M)
LB01_F	9.800	(LB11_F)	2.100	(LA114_M)
LB10_F	9.344	(LA13_F)	3.882	(LB11_F)
LB11_F	11.216	(LA113_F)	3.882	(LB10_F)
LB62_F	8.458	(LB11_F)	1.742	(LB87_F)
LB87_F	9.467	(LB11_F)	1.742	(LB62_F)
LB91_F	7.337	(LB11_F)	3.281	(LC13_F)
LC13_F	7.035	(LB01_F)	3.281	(LB91_F)
LD120_F	8.023	(LD51_M)	2.868	(LB62_F)
LA02_M	9.132	(LB11_F)	2.749	(LB04_M)
LA30_M	8.651	(LB11_F)	1.720	(LA114_M)
LA91_M	8.920	(LB11_F)	2.109	(LB01_F)
LA114_M	8.441	(LB11_F)	1.720	(LA30_M)
LB04_M	9.339	(LB11_F)	2.749	(LA02_M)
LB17_M	9.130	(LB11_F)	5.360	(LD92_M)
LB43_M	8.290	(LB11_F)	4.995	(LC13_F)
LC09_M	7.174	(LB11_F)	2.628	(LA114_M)
LD51_M	10.097	(LB11_F)	2.966	(LB04_M)
LD92_M	9.946	(LB11_F)	3.394	(LA02_M)

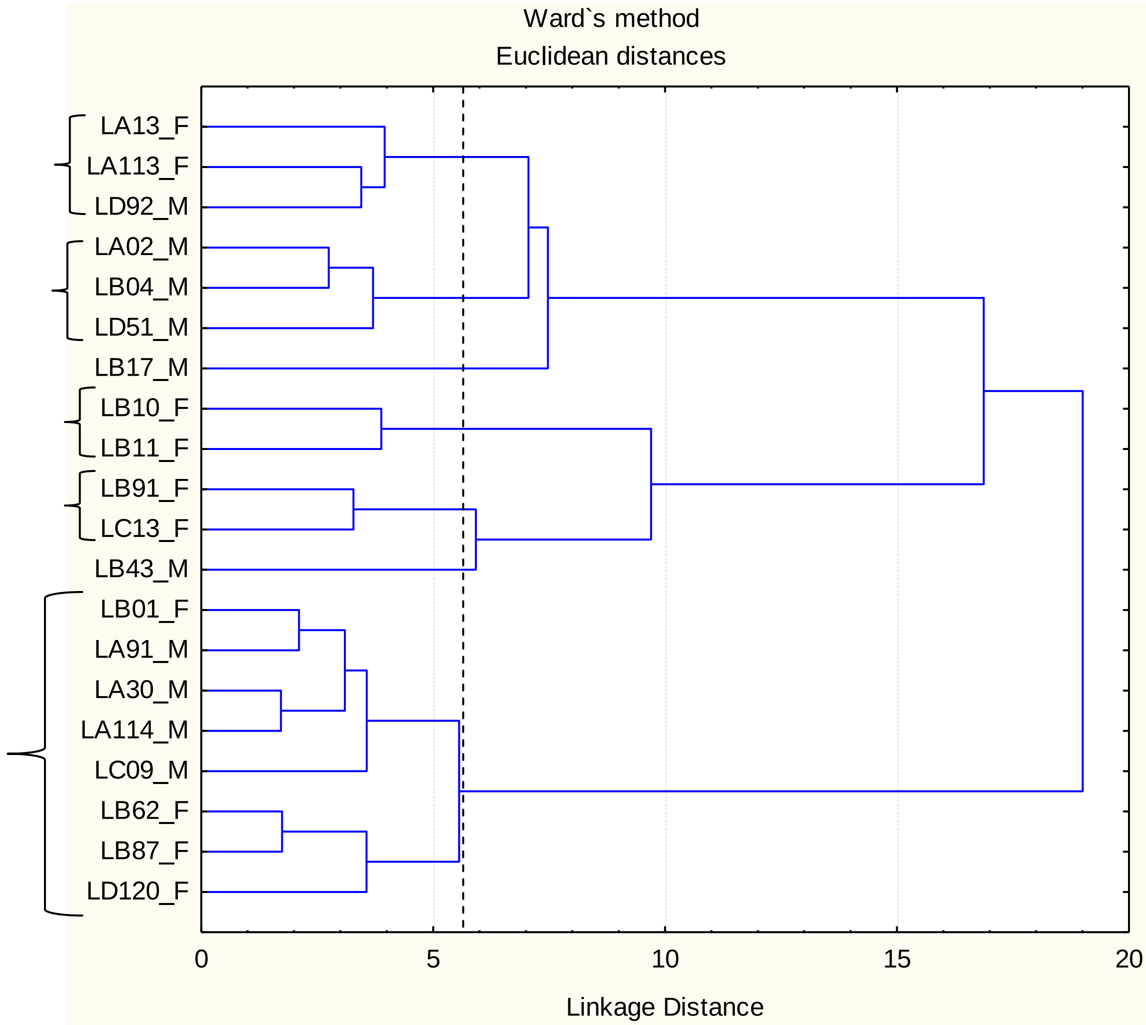

Figure 1. Dendrogram of genetic divergence (Ward Method - –Euclidian distance) among 20 rambutan obtained with a set of 18 leaf features.

similar using only the analysis of the distances between pairs; it is necessary to perform a cluster analysis (Paula, 2007). For the group promoted by the Ward method in relation to the 20 plants studied, the set of 18 features can be seen in Figure 1. Seven groups were formed from the cutting line drawn by estimating the average arithmetic complement line (5, 7) (Sokal and Rohlf, 1962). The first group was formed by a subgroup consisting of LA13_F, LA113_F and LD92_M materials; the second by LA02_M, LB04_M and LD51_M_M, the third by LB17_M plant, the fourth by LB10_F and LB11_F, the fifth by LB91_F and LC13_F, the sixth by LB43_M materials, and the seventh gathering several subgroups related to the other plants analyzed (LB01_F, LA91_M, LA30_M, LA114_M, LC09_M, LB62_F, LB87_F and LD120_F), with small genetic distances among them. The dissimilarity between some plants was very small, as observed in the formed groups; as observed by Andrade et al. (2009) in studies of the same species, despite not finding any similar material, the genetic distance between some plants was also small (less than 10%). In studies with 20 accesses of *Nephelium lappaceum* L. through 8 leaf features, Andrade et al. (2009) found two large groups by bringing together various subgroups with small distances. With 18 plants of the same species, Andrade et al. (2011) also found two large groups with great genetic variability when all of the variables were analyzed together. In this work, despite the small distance between some plants, therewere a large number of aspects evaluated together; therefore, the contribution of each variable must be verified by analyzing them separately toensure that the variables contribute differently to the observed results. Morphological characterization continues to be the first step for the description and classification of germplasm, and statistical methods such as principal components analysis (PCA) are useful tools for screening the accessions of a collection (Cantini et al., 1999; Badenes et al., 2000). The Principal Component

Table 4. Principal components (PC), estimates of eigenvalues, variance (Var) and cumulative variance (Cum. Variance) obtained from the correlation matrix between the characteristics: leaflet area (LFA, in m^2), leaf length (LL in cm), leaf width (LW in cm), number of leaflets (NLF), leaflet length (LFL in cm), leaflet width (LFW in cm), length of the rachis (LR in cm), length of the intervals between leaflets (IL in cm), coloring of the upper surface of the leaflet (L*_upp, C*_upp, H*_upp, a*_upp e b*_upp) and coloring on the lower surface (L*_low, C*_low, H*_low, a*_low e b*_low) evaluated in 20 rambutan plants (*Nephelium lappaceum* L).

Principal components	Eigen value	Var (%)	Cum. variance (%)	Elements of the eigenvectors associated to																	
				LFA	LL	LW	NLF	LFL	LFW	LR	IL	L*_upp	C*_upp	H*_upp	L*_low	C*_low	H*_low	a*_upp	b*_upp	a*_low	b*_low
PC1	6.82	37.9	37.9	-0.24	-0.2	-0.26	-0.13	-0.07	-0.2	-0.14	-0.1	0.33	0.33	-0.26	0.25	0.32	-0.13	-0.18	0.33	-0.14	0.34
PC2	4.92	27.34	65.23	0.29	0.37	0.24	0.28	0.2	0.31	0.37	0.32	0.15	0.19	-0.08	0.28	0.14	-0.03	-0.22	0.18	-0.08	0.12
PC3	2	11.1	76.34	0.01	-0.03	-0.06	0.05	-0.42	0.06	-0.03	0.02	-0.16	-0.02	0.41	0.05	0.25	0.5	-0.4	-0.09	-0.37	0.03
PC4	1.28	7.1	83.44	-0.19	0	-0.23	0.23	-0.2	-0.26	0.19	0.28	0.1	0.08	-0.12	0.04	-0.1	0.34	-0.17	0.12	0.59	-0.3
PC5	1.2	6.64	90.08	0.08	-0.05	0.28	-0.45	0.46	-0.06	-0.3	0.24	0.01	0.03	-0.16	-0.24	0.16	0.39	-0.26	0.06	0.07	-0.08
PC6	0.5	2.78	92.86	-0.29	0.06	-0.2	-0.33	0	-0.06	0.22	**0.62**	-0.14	-0.26	-0.03	0.22	0.13	-0.03	0.28	-0.23	-0.14	0.17
PC7	0.42	2.34	95.19	-0.02	-0.13	-0.16	0.47	0.4	**-0.48**	-0.04	0.26	0.01	-0.03	0.17	-0.24	-0.12	-0.14	-0.15	0.00	-0.37	-0.02
PC8	0.32	1.75	96.95	0.29	-0.01	-0.1	0.17	-0.18	-0.04	0.14	-0.05	0.28	-0.21	**-0.53**	-0.33	0.21	0.32	0.31	-0.09	-0.24	0.02
PC9	0.22	1.2	98.15	-0.37	0.38	**0.53**	0.09	-0.02	-0.45	0.02	-0.22	-0.03	-0.08	-0.02	0.2	0.00	0.22	0.22	0.13	-0.14	0.01
PC10	0.13	0.74	98.89	0.36	-0.14	0.3	0.12	-0.32	-0.22	**-0.37**	0.37	0.03	0.27	0.18	0.02	0.09	-0.07	0.31	-0.06	0.19	0.25
PC11	0.07	0.41	99.3	-0.12	0.06	-0.12	0.27	0.29	0.02	0.07	-0.22	-0.31	0.07	0.06	-0.14	**0.49**	0.1	0.07	-0.26	0.37	0.41
PC12	0.06	0.31	99.61	-0.04	-0.01	-0.22	0.17	0.31	0.21	-0.36	-0.05	0.33	0.04	0.11	**0.44**	-0.21	0.39	0.27	-0.23	-0.04	-0.01
PC13	0.04	0.22	99.83	-0.27	0.06	0.23	-0.05	-0.07	0.05	0.1	0.02	**0.67**	-0.1	0.23	-0.26	0.02	-0.15	-0.2	-0.41	0.12	0.14
PC14	0.02	0.09	99.92	**0.49**	0.28	-0.28	-0.34	0.11	-0.42	0.2	-0.2	0.19	-0.14	0.35	0.12	0.07	0.02	0.03	0.07	0.13	0.04
PC15	0.01	0.04	99.96	0.07	0.24	-0.05	0.18	-0.11	0.01	-0.48	0.05	-0.02	**-0.65**	-0.19	0.17	0.08	-0.19	-0.3	0.06	0.15	0.11
PC16	0	0.03	99.99	-0.15	0.03	-0.05	0.07	0.05	0.2	-0.12	0.08	0.17	-0.11	0.33	-0.14	**0.52**	-0.12	0.31	0.39	0	-0.45
PC17	0	0.01	100	0.15	**-0.47**	0.25	0.05	0.08	-0.17	0.16	-0.12	0.00	-0.12	-0.1	0.44	0.33	-0.13	-0.11	-0.34	0.02	-0.38
PC18	0	0	100	0.00	**-0.52**	0.18	0.05	0.09	0.11	0.25	-0.01	0.07	-0.41	0.18	0.04	-0.16	0.19	0.07	0.43	0.13	0.38

(PC) with major contributions to the analysis of plant diversity were PC1 (37.90%), PC2 (27.33%), PC3 (11.11%), PC4 (7.10%) and PC5 (6.64%); 90.08% of the divergence was explained by the first five components, and the first three components explained 76.34% of the divergence (Table 4). Thus, making the analysis of the last 13 eigenvectors, that is, after the last principal component to that in which the associated eigenvalue assumed value of 0.7 (Cruz and Carneiro, 2003), was identified in the study, the characters LL, C * _low, C * _upp, LA, L * _upp, L * _low, LR, LW, H * _upp, LFW and IL as possible disposal in future studies, by small contribution of phenotypic diversity (Dias et al., 1997; Sousa, 2003). Studying the morphological diversity of the rambutan plant, Andrade et al. (2009) observed that the length component of the leaflets had a greater influence (22.79%) and was of great importance in studies of divergence for the cultivar, as observed in this work. According to these authors, a lower influence was exerted by leaf width – LW (7.75%); in this study, the LW contributed only 1.2%. Morphological characterization has been used in different species, such as baru (Ferreira et al., 1998), guariroba (Nascente, 2003) and purple passion fruit (Meletti et al., 2005). This shows the importance of the use of visual and measurable characteristics in the differentiation of plants. Therefore, given the observed results, morphological characterization is of great importance. It consists of identifying each material using data to study the genetic variability of each sample (Ramos and Queiroz, 1999). This type of analysis is simpler and less costly (Ballve et al., 1997) but has limitations related to characteristics that have additive heritage, which are highly influenced by the environment, and cultivars with great phenotypic similarity (Oliveira et al., 2000), as observed in the rambutan leaves.

In the literature, no studies aimed at gender distinction from leaf characteristics were found. However, the results of this study indicated low genetic divergence between the materials when grouped by foliar aspects, rendering the visual distinction of materials difficult.

According to the results obtained, we can conclude that the productive plants LB10_F, LB11_F and LB91_F all had statistically higher means in all characteristics related to the lower color of the leaflets; in other words, the color of the leaflets can be a differential leaf aspect in productive plants.

Multivariate analyses indicate that there is low genetic divergence between the plants studied; based on the foliar aspects analyzed, it was not possible to identify a discriminatory feature for all plants of the same sex.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Growth performance and biochemical analysis of the genus *Spirulina* under different physical and chemical environmental factors

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***Spirulina* is useful to man in many aspects of life including health, food and cosmetics. In the present study, we aimed at investigating the optimum physical and chemical conditions that promote *Spirulina* mass production by varying a set of physical and chemical parameters, namely pH levels; Mg^{2+} ion concentration; nitrogen, phosphorous and carbon sources; salinity and different growing media. Temperature, light intensity, and light/dark cycle were maintained at 30°C±2, 4400 lx and 14:10 respectively throughout the study. At pH 9, the dry weight, protein, and chlorophyll *a* contents were 7.83±0.29 g/L, 1.34±0.12 mg/mL and 18.64±0.06 µg/mL respectively. At 0.8 mmol/L MgO, maximum dry weight, protein, and chlorophyll *a* contents were obtained. By using $NaNO_3$ as the source of nitrogen, the dry weight was 2.24±0.13 g/L while the protein and chlorophyll *a* contents were 3.24±0.30 mg/mL and 2.53±0.24 µg/mL respectively. The highest biomass, chlorophyll *a* and protein contents were obtained by using K_2HPO_4 as the source of phosphorous. When $NaHCO_3$ was used as the carbon source, the highest dry weight, protein and chlorophyll *a* yields were observed. Of the growing media used, Zarrouk's medium yielded the highest biomass protein and chlorophyll *a* contents. Furthermore, under different salinities, the optimal dry weight, protein and chlorophyll *a* yields were obtained at 2.5%. This study provides the basis for high biomass production of *Spirulina* which is a promising microalga endowed with many health benefits due to its high protein content.**

Key words: Biomass, chlorophyll *a*, protein, *Spirulina*.

INTRODUCTION

Spirulina platensis is a planktonic photosynthetic filamentous cyanobacterium that forms massive populations in tropical and subtropical water bodies that have high level of carbonate, bicarbonate and pH values

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Table 1. Composition of Zarrouk's medium (standard medium, SM).

Constituents	Composition (g/L)
$NaHCO_3$	18.0
$NaNO_3$	2.5
K_2HPO_4	0.5
K_2SO_4	1.0
NaCl	1.0
$CaCl_2.2H_2O$	0.04
Na_2EDTA	0.08
$MgSO_4.7H_2O$	0.2
$FeSO_4.7H_2O$	0.01
A_5 micronutrient sol.[a]	1 mL/L

[a]A_5 micronutrient solution consists of H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.222; $CuSO_4.5H_2O$, 0.079; $(NH_4)_6Mo_7O_{24}$ (g/L).

Table 2. Composition of ES medium (West and McBride version).

Component	Primary stock solution	Quantity (1 L)	Molar concentration in final medium
$NaNO_3$	-	3.85 g	4.53×10^{-4} mol/L
$Na_2\beta$-glycerophosphate.$5H_2O$	-	0.4 g	1.31×10^{-5} mol/L
Fe-EDTA solution	(see recipe below)	100 mL	-
Trace metal solution	(see recipe below)	20 mL	-
Thiamine (Vit. B1)	500 mg/L dH_2O	8.0 mL	1.19×10^{-7} mol/L
Biotin (Vit. H)	50 mg/L dH_2O	8.0 mL	1.78×10^{-8} mol/L
Cyanocobalomin (Vit. B12)	25 mg/L dH_2O	3.5 mL	6.46×10^{-10} mol/L

Fe-EDTA solution consists of $Na_2EDTA.2H_2O$, 6.00 g/L; Fe $(NH_4)_2(SO_4)_2.6H_2O$, 7.00 g/L. The trace metal solution consisted of $Na_2EDTA.2H_2O$, 2.548 g/L; H_3BO_3, 2.240 g/L; $MnSO_4.4H_2O$, 0.240 g/L; $ZnSO_4.4H_2O$, 0.044 g/L; $CoSO_4.7H_2O$, 0.010 g/L.

of up to 11. This cyanobacterium features morphological traits of the genus, that is, the arrangement of multicellular cylindrical trichomes in an open lift-hand helix along entire length of the filaments (Soni et al., 2012). The genus *Spirulina* has gained importance and international demand for its high phytonutrients value and pigments, which have applications in healthy foods, feeds, and therapeutics (Becker, 2007). It represents the second most important commercial microalgae (after Chlorella) for the production of biomass used as healthy food and animal feed (Sotiroudis et al., 2013). Its annual worldwide production in the year 2000 was estimated to be approximately 2000 tonnes (Spolaore et al., 2006). *Spirulina* has been used as food and nutritional supplements since antiquity (Falquet and Hurni, 2006). It is generally considered as a rich source of proteins, vitamins, essential amino acids, minerals, essential fatty acids such as linolenic acid and sulfolipids (Mendes et al., 2003). In addition to poly-unsaturated fatty acids, it also has 6 poly unsaturated fatty acids, phycocyanin and other phytochemicals (Chamorro et al., 2002). The present study reports the impact of different physical and chemical environment for mass production of *Spirulina*.

MATERIALS AND METHODS

Microorganism

Cyanobacterium *Spirulina* used in the present study was purchased from Collection Centre of Marine Microalgae, Chinese Academy of Sciences, China. It was grown and maintained in 2 L sterilized Erlenmeyer flasks containing 1 L Zarrouk's medium (Amala et al., 2013), chemical composition of which is shown in Table 1, at room temperature, pH 9 with continuous illumination using cool white fluorescent tubes whose light intensity was controlled and maintained at 2500 lx using the TES-1330A Digital Lux Meter (TES Electrical Electronic Corp, ISO 9001:2008). The experiment was done in triplicate. Agitation was done thrice a day manually. All the reagents used were of analytical grade and were obtained from Shoude Experimental Equipment Co., Ltd, Nanjing.

Culture media and growing conditions

Zarrouk's medium (Amala et al., 2013) was used as the standard control medium (SM) and its constituents are shown in Table 1. It was autoclaved (LDZX-75KBS Vertical Heating Pressure Steam Sterilizer (Shanghai Shenan Medical Instrument Factory) at 121°C for 15 min and incubation was done aseptically. ES medium (West and McBride Version) (West et al., 1999) was used as the growing medium in cultivation of *Spirulina* under different salinity levels and its constituents are shown in Table 2. The cyanobacterium *Spirulina* was grown under the following physical conditions: temperature 30 ±2°C, light intensity 4400 lx and light/dark cycle 14:10. Agitation

Table 3. Composition of CFTRI medium**.**

Ingredients	**g/L dH$_2$O**
$NaHCO_3$	4.5
K_2HPO_4	0.5
$NaNO_3$	1.5
K_2SO_4	1.0
NaCl	1.0
$MgSO_4.7H_2O$	1.2
$CaCl_2.2H_2O$	0.04
$FeSO_4.7H_2O$	0.01

Table 4. Composition of Bangladesh media No.3.

Ingredients	g/L dH$_2$O
$NaHCO_3$	2.0
Urea	0.05
NaCl	1.0
Gypsum	1.5

Table 5. Composition of BG-11 (Blue Green-11) medium**.**

Stock solutions	**g/L dH$_2$O**	
$NaNO_3$	15.0	
$K_2HPO_4.3H_2O$	4.0	
$MgSO_4.7H_2O$	7.5	
$CaCl_2.2H_2O$	3.6	
Citric acid	0.6	
Ferric ammonium citrate	0.6 (Autoclave to dissolve)	
EDTA	0.1	
Na_2CO_3	2.0	
Trace metal mixture	1 ml, g/L	
H_3BO_3	2.86	Add each constituent separately to ~800 mL of dH$_2$O and fully dissolve between each addition. Then make up to 1 L
$MnCl_2.4H_2O$	1.81	
$ZnSO_4.7H_2O$	0.222	
$Na_2MoO_4.2H_2O$	0.39	
$CuSO_4.5H_2O$	0.079	
$Co(NO_3)_2.6H_2O$	0.0494	

was done thrice a day and the experiments were done in triplicate. To achieve growth under different chemical conditions, the Zarrouk's medium was set at pH 7, 8, 9 (control), 10, 11 and 12; the MgO concentration was set at 0.0, 0.4, 0.8 (control), 1.2 and 1.6 mmol/L; for nitrogen source 2.5 g/L of $NaNO_3$ (standard), urea and NH_4Cl were used; for phosphorus source 0.5 g/L of K_2HPO_4 (standard), KH_2PO_4 and NaH_2PO_4 were used and for the carbon source 18 g/L of $NaHCO_3$ (standard), Na_2CO_3 and K_2CO_3 were used; salinities of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5% were used and finally CFTRI media, natural sea water, Bangladesh media No.3, BG-11, RaO's, OFERR, and Zarrouk's media (control) were used as the different growing media and their constituents are shown in Tables 3, 4, 5, 6, 7, and 1, respectively. All the sets of experiment were done in triplicate.

Cultivation

Spirulina was cultivated aseptically in the 250 mL sterilized Erlenmeyer flasks containing 150 mL of respective sterilized media. The temperature in the incubator was maintained at 30±2°C and illuminated with day-light fluorescent tubes with light intensity at 4400 lx under Light/dark cycle, 14:10. During the process of growth, the flasks were shaken manually 3 times per day. The experiments

Table 6. Composition of RaO's medium.

Ingredients	g/L dH_2O
$NaHCO_3$	15.0
K_2HPO_4	0.5
$NaNO_3$	2.5
K_2SO_4	0.6
NaCl	0.2
$MgSO_4.7H_2O$	0.04
$CaCl_2.2H_2O$	0.008
Fe-Ethylene diamine tetra acetate	0.2
Microelement solution	1 mL

The microelement solution consisted of H_3BO_3, 2.86 g/L; $MnCl_2.4H_2O$, 1.81 g/L; $ZnSO_4.7H_2O$, 0.222 g/L; $CuSO_4.5H_2O$, 0.079 g/L; $(NH_4)Mo_7O_{24}$, 0.02 g/L.

Table 7. Composition of OFERR medium.

Ingredients	g/L dH_2O
$NaHCO_3$	8.0
NaCl	5.0
Urea	0.2
K_2SO_4	0.5
$MgSO_4.7H_2O$	0.16
$FeSO_4.7H_2O$	0.05
H_3PO_4	0.052 mL

were run in triplicate.

Biomass yield

At 2-days intervals, 10 mL of homogenized algal suspension were filtered through a Whatman GB/T 1914 to 2007 filter paper that had been pre-combusted in an oven for 1 h at a constant temperature of 100°C and stored in a vacuum dessicator until use. The biomass yield (the dry weight (g/l)) was determined by the method described by Moheimani et al. (2013) as described above. The biomass yield of the samples under different salinity levels was then calculated through Optical density (OD) measurements using a spectrophotometer (SpectraMax M5 Microplate Reader, Molecular Devices, United States) and the growth rate (*K'*) was calculated using the equation below (Van Leeuwe et al., 1997):

$$K' = \ln (N_2 / N_1) / (t_2 - t_1) \quad (1)$$

Where N_1 and N_2 are biomass at time 1 (t_1) and time 2 (t_2) respectively.

Biochemical analysis

Chlorophyll *a* content was estimated according to Oncel et al. (2006), Soni et al. (2012) and Ritchie (2006 and 2008). The algal biomass was harvested through centrifugation and mixed with pure methanol, heated in 70°C water bath for 3 min for chlorophyll *a* extraction and later centrifuged at 3500 r/min for 5 min. The absorbencies of chlorophyll *a* containing supernatant were measured at 665 and 750 nm according to Oncel et al. (2006) and Soni et al. (2012). The algal biomass was harvested through filtration and then washed with distilled water and then frozen at -20°C until use for chlorophyll *a* extraction according to Ritchie (2006 and 2008),. Then later the frozen filter paper was crushed in a motor and pestle to homogenize the filter and algae. 5 to 10 mL of 100% ethanol was then added to the sample and centrifuged at 5000 r/min for 10 min. The absorbencies of chlorophyll *a* containing supernatant were measured at 630, 647, 664, and 691 nm using 100% ethanol as the blank. The protein content was determined by the Bicinchominic Acid (BCA) Protein Assay (Smith et al., 1985) using bovine serum albumin as the standard. 5 mL of homogenized algal suspension was centrifuged at 3500 r/min for 5 min and later mixed with 1X Phosphate Buffer Saline (PBS) (pH 7.4). The sample was then sonicatend in an Ultra Sonic Crasher Noise Isolating Chamber (Ningbo Scientz Biotechnology Co. Ltd.). The absorbencies of protein containing supernatant were measured at 562 nm using bovine serum albumin as a blank. For estimation, the following equations were used and expressed in mg/L per dry weight:

$$\text{Chl } a \text{ (mg/L)} = 13.9 (A_{665} – A_{750}) \quad (2)$$

$$\text{Chl } a \text{ (µg/mL)} = 0.0604 E_{630} – 4.5224 E_{647} + 13.2969 E_{664} – 1.7453 E_{691} \quad (3)$$

$$\text{Protein content (µg/mL)} = OD_1 / OD_2 \times 563 \times \text{dilution ratio} \quad (4)$$

Where OD_1 is the unknown sample, and OD_2 is the standard

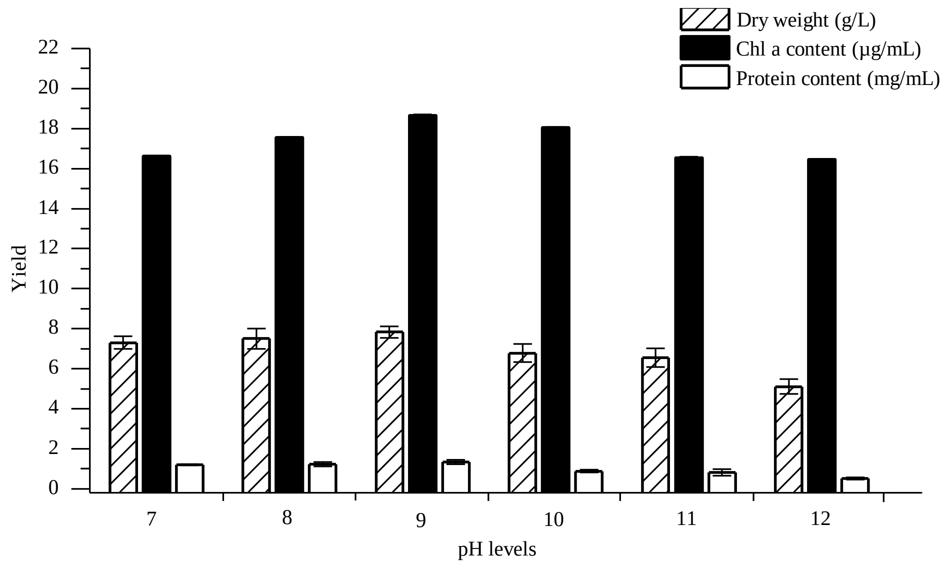

Figure 1. Cultivation of *Spirulina* under different pH levels

sample.

Statistical analysis

The data obtained from the experiment were subjected to the paired sample statistics (t-Test) using SPSS Software; version 14.0, 2007. The significance of the result was set at 5% level and compared based on the mean values by least significant difference.

RESULTS AND DISCUSSION

Effect of different pH levels on biomass production of *Spirulina*

pH is one of the main factors influencing the abundance of inorganic carbon, dissolved total carbon (DTC). When the pH is below 5, the majority of dissolved inorganic carbon (DIC) is CO_2, which is equal to HCO_3^- at pH 6.6 but at pH 8.3 almost all DIC is HCO_3^- (Ogbonda et al., 2007). Therefore, the pH should be controlled during cultivation to enhance the absorbability and utilization of CO_2 by microalgae. The pH can also directly affect the permeability of the cell and the hydronium forms of the inorganic salt, and indirectly influence the absorption of the inorganic salt. CO_2 in the culture is consumed by the microalgae during photosynthesis, thereby increasing the pH of the medium. Therefore, substances like hydrochloric acid and acetic acid have to be added to control the pH to stop it from increasing beyond the tolerance of the microalgae. Compared with hydrochloric acid, acetic acid has the advantage of not only adjusting the pH value but also acting as a carbon source to enhance the growth rate of microalgae (Junying et al., 2013). Culturing *Spirulina* in conical flask has its limitation in providing complete information related to growth, development and production of value added chemicals namely vitamins, amino acids, fatty acids, protein and polysaccharides both in quantity and quality and disposing of carbon (II) oxide which is one of the major causes of global warming (Bergman et al., 2013). Extensive research has been conducted on production of *Spirulina* at salt lakes in the tropical regions (Sassano et al., 2004). Physico-chemical profile of *Spirulina* involves describing the relationship between growth and the environmental factors especially irradiance flux, density and temperature (Sotiroudis et al., 2013), which are important in the evolution of micro algae and cyanobacteria for biomass production, as well as their general characterization. High alkalinity is mandatory for the growth of *Spirulina* and bicarbonate is used to maintain the high pH (Pandey et al., 2010; Jones et al., 1998). Sources of nutrition also affect the growth rate of cyanobacteria (Costa et al., 2001). The pH value of the culture medium combined with the dry cell weight may be an indirect method for determining the degree of cell growth of *Spirulina*. This is because the pH gradually rises as bicarbonate added to the culture medium is dissolved to produce CO_2, which releases OH^- during the cultivation of *Spirulina* (Moberg et al., 2012). The organism was adapted to six pH regimes (7, 8, 9, 10, 11 and 12) in flask culture, monitored and yield expressed in term of dry weight. The maximum bulk density of about 7.83±0.29 g/L was observed when the pH of the culture medium was maintained at 9.0 with medium volume 150 mL in a 250 mL flask as illustrated in Figure 1. The maximum bulk density was attained on the 25th day after the inoculation of culture in Zarrouk's medium. The increase in the yield of *Spirulina* could have been due to the availability of mire space, oxygen, and light to the

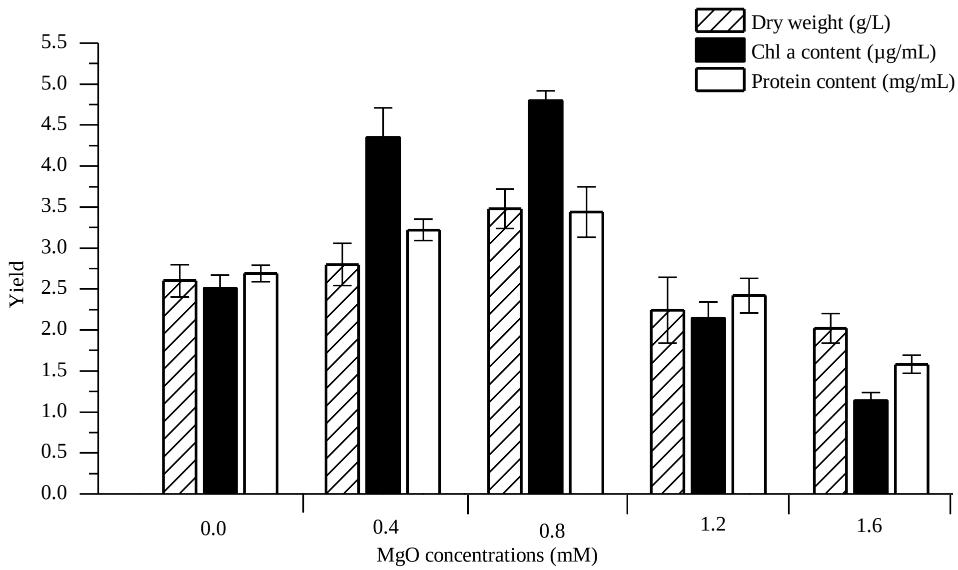

Figure 2. *Spirulina* mass production under different Mg^{2+} ions concentrations.

culture flask and also due to the different solubility of CO_2 and other mineral compounds affected by the different pH levels. Earlier results also demonstrated that optimum pH for maximum growth of *Spirulina* was 9 to 9.5 ranges (Pandey et al., 2010). *Spirulina* is considered an alkalophilic organism by nature (Jones et al., 1998). The chlorophyll *a* content and protein content were maximum at pH 9 (Figure 1). Chlorophyll *a* content was 1.34±0.12 mg/mL while the protein content was 18.64±0.06 µg/mL. Similar results have also been reported by various cyanobacteria researchers (Carvalho et al., 2002; Pandey et al., 2010).

Effect on different concentrations of Mg^{2+} ions to *Spirulina* biomass production

Magnesium oxide is known to hydrolyze in water to generate hydroxide (Shand, 2006) and thus resulting to increase in the pH level. In the present study, concentrations of 0.0, 0.4, 0.8 (control), 1.2 and 1.6 mmol/L of nano-MgO were added into the growing medium.0.8 mmol/L was set as the control measure since it is the amount in the Zarrouk's media (Amala et al., 2013). According to a study by Liu et al. (2012), nano-MgO is known to inhibit BOD and its toxicity results primarily from the increase in pH level following MgO hydrolysis thus the decrease in biomass yield in higher concentrations. In terms of biomass yield, highest biomass was obtained at 0.8 mmol/L (control) (3.48±0.24 g/L) and lowest at 1.6 mmol/L (2.02±0.18 g/L) respectively as illustrated in Figure 2. The low biomass yield at the highest concentration could be attributed to substrate toxicity. Wakte et al**.** (2011), studying metal ions toxicity on *Spirulina*, demonstrated a similar phenomenon.

Effect of different nitrogen, phosphorus, and carbon sources to biomass production of *Spirulina*

Many elements have to be provided for the growth of *Spirulina*, such as carbon (C), oxygen (O), hydrogen (H), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), sulfur (S), phosphorus (P) and trace elements with the major nutrients being carbon, oxygen, hydrogen, nitrogen, phosphorus, and potassium. The first three are obtained from water and air while the latter three have to be absorbed from the culture medium (Xin et al., 2010). Nitrogen is one of the essential elements for the growth, development, reproduction, and other physiological activities of *Spirulina*. The nitrogen source and concentration also affect the accumulation of lipid in *Spirulina*. Usually, ammonium salts, nitrates, and urea are used as nitrogen sources, but their absorption rates and utilization are different (Xin et al., 2010). This is because ammonia is directly used to synthesize amino acid while the other nitrogen sources have to be converted to ammonia to synthesize amino acid (Junying et al., 2013). It also has been found that microalgae grow well with urea and nitrate. In the present study, sources of nitrogen used included; $NaNO_3$ (it is used as the standard source of nitrogen), urea and NH_4CL of the same osmolatity (0.03 mol/L). Highest biomass recordings were observed in $NaNO_3$ nitrogen source (2.24±0.13 g/L) as illustrated in Table 8 while Chl *a* protein contents were 2.53±0.24 and 3.24±0.30 mg/mL respectively (Table 8). However, in urea and NH_4CL, there

Table 8. *Spirulina* cultivation under different nitrogen, phosphorus, and carbon sources.

Nutrient	Nutrient source (g/L)	Dry weight in g/L	Final pH of culture	Chl *a* content in µg/mL	Protein content in mg/mL
Nitrogen	2.5 $NaNO_3$ (std)	2.24±0.13	10	2.53±0.24	3.24±0.30
	2.5 Urea	N/D	10	N/D	N/D
	2.5 NH_4Cl	N/D	10	N/D	N/D
Phosphorus	0.5 K_2HPO_4(std)	2.08±0.17	10	2.52±0.21	2.93±0.06
	0.5 KH_2PO_4	1.85±0.16	10	1.44±0.13	2.30±0.07
	0.5 NaH_2PO_4	0.80±0.07	10	1.30±0.07	2.28±0.22
Carbon	18 $NaHCO_3$(std)	1.18±0.02	10	1.15±0.11	2.97±0.10
	18 Na_2CO_3	0.87±0.06	11	0.69±0.05	2.03±0.13
	18 K_2CO_3	0.75±0.03	11	0.46±0.04	1.50±0.08

were no detections of biomass increase as a result of a high concentration of NH_4^+ which is not suitable for growth as respiration of *Spirulina* is adversely affected by too high concentration of NH_4^+ ions (Chen et al., 2011). Thus, $NaNO_3$ is a better recommendation for nitrogen source.

Phosphorus is another essential element for the cultivation of *Spirulina*. Phosphate, hydrogen phosphate, and other phosphates, play an important role in the metabolic processes of microalgae, as well as the succession of phytoplankton in aquatic ecosystems. Phosphorus takes part in many metabolic processes, such as signal transduction, energy conversion and photosynthesis (Navarro et al., 2008). The metabolic mechanisms of P in the different forms are different in microalgae. Orthophosphate is most easily absorbed and significantly promotes the growth of microalgae (Navarro et al., 2008). According to the current work, sources of P used were K_2HPO_4 (it is used as the standard source of nitrogen), KH_2PO_4 and NaH_2PO_4 of the same osmolatity (0.003 mol/L). Optimal biomass yields were observed in K_2HPO_4 (2.08±0.17 g/L) as shown in Table 8. Chl *a* content (2.52±0.21 µg/mL) and protein content (2.93±0.06 mg/mL) are illustrated in Table 8. The lowest biomass concentration was observed when K_2HPO_4 was replaced by NaH_2PO_4 (0.80±0.07 g/L) as illustrated in Table 8 and this is due to the changing of the N:P ratio, as too high concentrations inhibited cell division of *Spirulina* thus the low biomass attained (Chu et al., 2013). Microalgae are phototrophic microorganisms, and they include prokaryotic photosynthetic bacteria, called cyanobacteria (Borges et al., 2013). These microorganisms have been investigated for their potential to enrich foods. The main nutrient required for *Spirulina* cultivation is carbon, because the cells contain about 50% (w/w) of this element. Thus, the carbon source is the most expensive component of *Spirulina* production. For autotrophic growth (which is more suitable for large-scale open cultivation), carbon can be provided as CO_2, carbonate or bicarbonate. If bicarbonate is used, it represents 60% of the cost of nutrients (Alava et al., 1997), which is one of the reasons for studying the effects of different carbon source and their concentrations and finding alternative sources of this nutrient, such as molasses (Andrade et al., 2008), residual CO_2 (Ferreira et al., 2012) and synthetic CO_2 (Rosa et al., 2011). The concentration of dissolved inorganic carbon in anaerobic effluent is lower than the amount indicated in the formulation of standard culture media for production of *Spirulina* biomass, such as Zarrouk medium (Amala et al., 2013). In the current work, sources of C used were $NaHCO_3$ (it is used as the standard source of nitrogen), Na_2CO_3 and K_2CO_3 of the same osmolatity (0.21 mol/L). Maximum yield was observed with $NaHCO_3$ carbon source (1.18±0.92 g/L) as shown in Table 8. Chl *a* and protein contents were 1.15±0.11 µg/mL and 2.97±0.10 mg/mL respectively while the lowest biomass concentration was observed when $NaHCO_3$ was replaced by K_2CO_3 (0.75±0.52 g/L) due to less CO_3^{2-} in the medium which are responsible for low alkalinity of the growing medium (Table 8).

Effect of different salinities in biomass production of *Spirulina*

Salinity is the presence of high levels of soluble salts in soils or waters. Management of salinity is important, as elevated salt levels can have detrimental effects on production and the environment (Rafiqul et al., 2003). *Spirulina* can readily adapt to the environment, and it can inhabit lake, creek, reservoir, or oceans, which have appropriate alkalinity, especially the southern ocean enjoying tropical climate. Additionally, seawater *Spirulina* performs better than freshwater *Spirulina* because of its excellent nutrition content.

In the present study artificial seawater medium, ES medium (West and Mcbride Version) (West et al., 1999), was used as the growing medium and its constituents are shown in Table 9. Its salinity levels were 1.0, 1.5, 2.0, 2.5

Table 9. Biomass production of *Spirulina* under different salinities.

Sample number	Salinity levels (%)	Dry weight in g/L	Chl *a* content in mg/L	Protein content in µg/mL
1	1.0	0.88±0.07	1.34±0.12	131.57±3.00
2	1.5	0.97±0.03	1.41±0.12	129.33±2.00
3	2.0	1.23±0.10	1.50±0.14	127.40±1.00
4	2.5 (control)	1.44±0.06	1.86±0.13	117.77±2.00
5	3.0	0.77±0.08	1.31±0.12	109.11±4.50
6	3.5	0.69±0.08	1.00±0.20	85.68±3.50

Table 10. Specific growth rate (*K'*) of the cell.

S/N	Salinity level (%)	Specific growth rate of the cell (µm/day)
1	1.00	0.17 ±0.01
2	1.50	0.18 ±0.01
3	2.00	0.19 ±0.01
4	2.50	0.22 ±0.01
5	3.00	0.13 ±0.01
6	3.50	0.12 ±0.02

(control), 3.0 and 3.5% as shown in Table 9. To reduce the cost of production of *Spirulina*, many methods have been attempted. The best method to date involved the use of nutrient-enriched seawater culture medium like the one used in this current study (Rafiqul et al., 2003). In the early stages of growth, there was an increase in biomass to 1.44±0.06 g/L (Table 9) and the specific growth rate (*K'*) of *Spirulina* (0.22±0.01 µm/d (Table 10) respectively in the 12^{th} day at 2.5% salinity. As the salinity increased beyond 2.5%, biomass and growth rate decreased though the range of salinity was not high enough to inhibit growth. The decrease in growth with increasing salinity has frequently been reported in the literature of Rosales et al. (2005); Shimamatsu (2004), Zeng et al. (1998) and (Kebede (1997). It is accompanied by a decrease in photosynthetic efficiency, phycobilin/Chl *a* ratio and PSII activity and an increase in carbohydrate metabolism (Shimamatsu, 2004; Warr et al., 1985). In the present study, chlorophyll *a* content was stimulated at lower salinities with the highest content being observed at 2.5% (1.86±0.13 mg/L) followed by significant decrease at high salinity levels (Table 9). This could be as a result of enhanced respiration that indicates that the response to salinity stress is an energy consuming process (Zeng and Vonshak, 1998), but the mechanisms involved have not yet been elucidated. It is probable that salinity stress affects light utilization and metabolism (particularly carbohydrates involved in osmoregulation) to counteract ionic and osmotic stresses (Kebede, 1997; Rosales et al., 2005). Protein content was also influenced by the salinity of the medium (Table 9). There was a drastic decrease in protein content with increase in salinity levels. However, protein content results in this study are somewhat contradictory to those reported earlier by some researchers. Most researchers have found similar results (Shimamatsu, 2004; Zeng et al., 1998) whereas some found an augmentation of protein contents with salinity increase (Rosales et al., 2005). However, strains and culture conditions were very different for all these studies so the results are difficult to compare. In this work, it could be suggested that stressed cells had a lower protein synthesis (at 3.5%; 85.68±3.50 µg/mL) capacity linked to the higher carbohydrate metabolism (Kebede,1997; Rosales et al., 2005). As protein content is a very important parameter for nutritional uses of *Spirulina*, the values have to be compared to the range of protein in *Spirulina* products found on the market, which is 50 to 65%. The maximal value near 50%, obtained for 1.0% is satisfactory for food applications.

Biomass cultivation of *Spirulina* using different liquid media

Large-scale production of Cyanobacterial biomass is essentially a complex process involving a large number of variables and for their successful growth; the environment needs to be conditioned to meet as many of the essential requirements of the organism. Among the several constraints to the multiplication of cyanobacteria physical, physiological and economic limitations are of major importance. In tropical countries, especially developing countries such as India, emphasis is placed more on the production costs (Larsdotter, 2006; Raoof et al., 2006). Culture of *Spirulina* in conical flask has its limitation in providing complete information related to

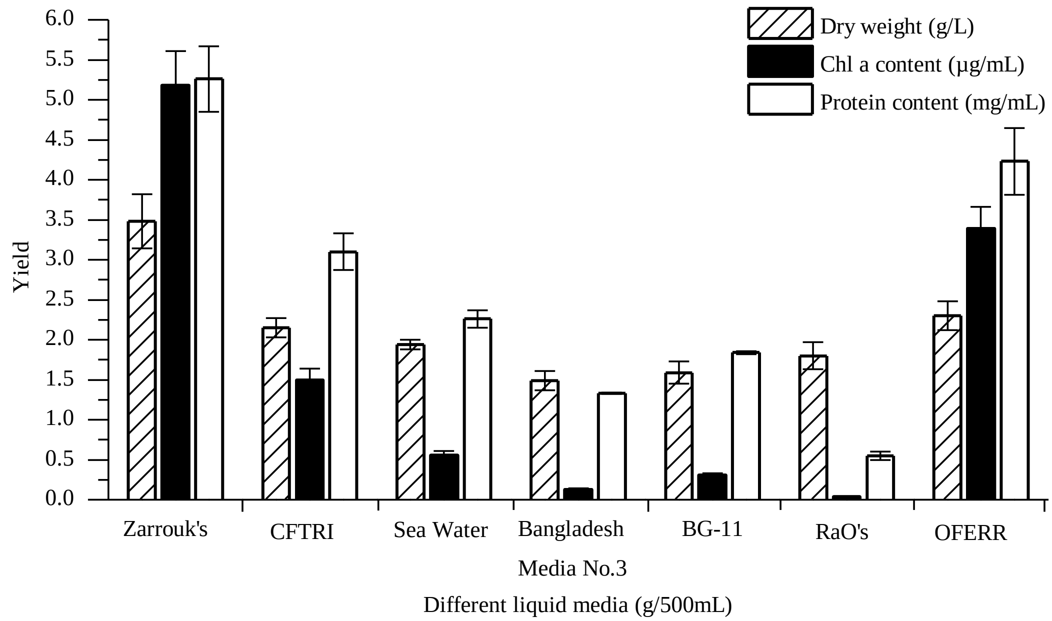

Figure 3. Biomass production of *Spirulina* using different liquid media.

growth, development and production of value added chemicals namely vitamins, amino acids, fatty acids, protein and polysaccharides both in quantity and quality and disposing of carbon dioxide one of the major causes of global warming (Bergman et al., 2013; Ferreira et al., 2004). Extensive research has been conducted on production of *Spirulina* living at salt lakes in the tropical regions (Sassano et al., 2004). In addition, sources of nutrition also affect the growth rate of *Spirulina* (Sotiroudis et al., 2013; Pandey et al., 2010). In the present study we investigated comparative growth rate of *Spirulina* on CFTRI medium (Pandey et al., 2010), Natural seawater, Bangladesh Media No.3 (Bharat et al., 2011), BG-11 medium (Ruiz et al., 2004), RaO's medium (Devanathan et al., 2013), OFERR medium (Devanathan et al., 2013) and Zarrouk's medium (Amala et al., 2013) as the control. The growth of *Spirulina in* flask culture was then monitored and expressed in terms of dry weight. Figure 3 shows that the specific dry weight of *Spirulina* was 3.48±0.34 g/L on Zarrouk's medium, 1.80±0.17 g/L on RaO's medium, 2.15±0.12 g/L on CFTRI medium, 2.30±0.18 g/L on OFERR medium, 1.49±0.12 g/L on Bangladesh medium No. 3, 1.94±0.06 g/L on natural sea water and 1.59±0.14 g/L on BG-11 medium respectively. The data shows that specific growth of *Spirulina is* higher on Zarrouk's medium. The chlorophyll *a* content of *Spirulina was* 5.18±0.43 µg/mL on Zarrouk's medium, 0.04±0.00 µg/mL on RaO's medium, 1.50±0.14 µg/mL on CFTRI medium, 3.39±0.27 µg/mL on OFERR medium, 0.13±0.01 µg/mL on Bangladesh medium No. 3, 0.56±0.05 µg/mL on natural sea water and 0.31±0.02 µg/mL on BG-11 medium respectively. The protein content of *Spirulina was* 5.26±0.41 mg/mL on Zarrouk medium, 0.55±0.05 mg/mL on RaO's medium, 3.10±0.23 mg/mL on CFTRI medium, 4.23±0.42 mg/mL on OFERR medium, 1.33±0.01 mg/mL on Bangladesh medium No. 3, 2.26±0.11 mg/mL on natural sea water and 1.84±0.02 mg/mL on BG-11 medium respectively (Figure 3). Similar studies were conducted by Hall et al. (2004), Bharat et al. (2011) and Bharat et al. (2011**).**

Conclusions

Standardization of *Spirulina in* different media was summarized and maximum growth noticed in Zarrouk's media. It is after the treatment of different pH that the best growth resulted at pH 9. Mg^{2+} ion concentration effect was important for *Spirulina* cultivation as it is known to hydrolyze in water to generate hydroxide and thus resulting to increase the pH level, which favors the growth of *Spirulina*. Biomass production of *Spirulina was* higher in media containing $NaNO_3$ as the source of nitrogen, K_2HPO_4 as the source of phosphorus and $NaHCO_3$ as the source of carbon. *Spirulina inoculated* in 2.5% salinity medium recorded the highest biomass yield. The result of this investigation shows that both the physical and chemical environmental factors are very important in biomass production and also chlorophyll *a* and protein biosynthesis in the *Spirulina* species.

Conflict of Interest

The authors have not declared any conflict of interest.

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Farmers' knowledge, perceptions and management practices of termites in the central rift valley of Ethiopia

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A high density of epigeal termite mounds is common in the Central Rift Valley of Ethiopia (CRVE). A survey of farmers' perceptions of termites was conducted in the CRVE using semi-structured questionnaires and in-depth interviews with farmers for documenting their knowledge about termites, identification of termite types, crops and crop growth stage susceptible to termites, perception of the role of termites, estimate of potential yield loss to the major crops, and termite management practices in field crops among others. A field survey was also conducted on maize and haricot beans on farmers' fields to assess termite damage. Farmers were knowledgeable about the existence of termites. However, they could not identify different types of termite (species). Farmers mentioned the existence of 19 different pre-harvest crop pests and 69% of them mentioned that termites are among the most important pests in their farming system after porcupine. Farmers considered termites as of no benefit to human nutrition and mound soil as fertilizer, but they acknowledged termites for the mound soil used in house construction. Maize and haricot beans were the major crops grown in the area and most of the farmers (87%) considered the crops as the most susceptible to termite damage and they estimated potential pre-harvest yield loss as 18.02 ± 2.67 and 10.58 ± 1.91 kg (mean ± SE) per hectare, respectively. Farmers complained about termite mounds as they reduce farmlands and stand as obstacle for ox cultivation. All the farmers reported that damage to the crops occurs from maturity stage onwards and this was also confirmed in the field survey. Lodging of maize plants recorded in the surveyed fields ranged between 3 and 33% which was mainly due to *Macrotermes* and *Microtermes*. Although the majority of farmers considered termites as pests, only few (9%) of them managed termites using cultural control practices such as removing lodged maize and prompt harvesting. The study has shown that farmers viewed termites as pests of crops at maturity stage.

Key words: Central Rift Valley, knowledge, maize, perception, termite, yield loss.

INTRODUCTION

A distinct dichotomy exits between the pest management literature that depicts termites as "pests" and the ecological literature demonstrating their crucial role in ecosystem services (Sileshi et al., 2009). Termites play a

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key role as decomposers of organic matter, nutrient cycling, and soil structure improvement in savannah as well as in subtropical and tropical ecosystems (Wood and Sands, 1978; Ackerman et al., 2009; Ayuke, 2010). Despite the potential beneficial role of termites, of the over 2800 described species, about 10% of these have been recorded as pests of crops, forestry, housing structures, and rangelands (Wood and Sands, 1978; Borer et al., 1981; Logan et al., 1990; Munthali et al., 1999; Culliney and Grace, 2000; Ackerman et al., 2009; Sileshi et al., 2009). However, not all timber, crops and trees are susceptible, and their resistance may vary with time or stage of growth (Pearce, 1997).

Susceptibility of crops and trees to termites is governed by several factors. In general, damage by termites is greater in rain-fed than irrigated crops, during dry periods or droughts than periods of regular rainfall, in lowland rather than highland areas, and in plants under stress (lack of moisture, disease or physical damage), rather than in healthy and vigorous plants. In particular, exotic crops and trees are more susceptible to termite attacks than indigenous crops (Logan et al., 1990; Pearce, 1997; UNEP, 2000).

Over 90% of the termite damage in agriculture, forestry and urban settings is attributed to members of the Macrotermitinae which build the large mounds that form the spectacular features of the African landscape (Sileshi et al., 2009; Adekayode and Ogunkoya, 2009; Abdurahman et al., 2010). The reputation of termites as pests is also associated with the presence of termitaria in crop fields and near trees (Sileshi et al., 2009; Abdurahman et al., 2010).

Despite the significant roles of termites in tropical ecosystems and the damage they cause mainly to crops and forestry is widely expressed in the scientific literature, yet less is known about farmers' perception and management of termites by small scale farmers (Ayuke, 2010). Farmers' perception is based on local experience and indigenous knowledge which can provide valuable insights not presently covered in scientific literature. Scientists can learn from farmers' knowledge about termites, their behavior, impact on crop production and their management under different agro-ecological conditions. Insights on farmers' perception may also provide scientists with some understanding on how research could be conducted so as to address the needs and constraints of farmers (Poubom et al., 2005; Sileshi et al., 2008; Ayuke, 2010).

The current philosophy in pest management is that if scientists have to work with farmers to improve crop protection and production, they should value farmers' indigenous technical knowledge systems (ethnoscience) and recognize farmers' constraints. Therefore, the first step towards the development of successful pest management strategies adapted to farmers' needs is an understanding of farmers' perceptions of the pests and their control methods. Farmers are, in general, good decision-makers; as such, their views should be considered when changes such as new technologies are to be introduced. The level of adoption of such technologies will therefore be high where farmers' perceptions are considered in their development (Poubom et al., 2005; Sileshi et al., 2008; Ayuke, 2010).

Though a high density of *Macrotermes* termite mounds making spectacular feature of the rural landscape of the Maki-Batu area was reported, no systematic studies exist on farmers' perceptions of termites and farmers-termite interactions. Therefore, the main objective of this study was to investigate and document farmers' indigenous knowledge and their perceptions of beneficial and detrimental roles of termites.

MATERIALS AND METHODS

Description of the study sites

The study was conducted at nine sites in five Kebele Administrations (KAs) in the three districts of East Shawa Zone of Oromia Regional State. Kebele is the lowest administrative level. Descriptions of the districts and the KAs are shown in Table 1.

Data collection methods and analyses

Farmers' perceptions of termites

Data were collected through semi-structured questionnaires and individual interviews held between July and December 2012. The questionnaires were first prepared in English and then translated into the local Afan Oromo language before administration. The questionnaires were administered to 51 household farmers in four KAs selected randomly from lists obtained from the Development Agents of the respective KA. The questionnaires had focused on identification of termite types, major crops grown in the area, farmers' perceptions of the benefits of termite mound soil and termites and detrimental roles of termites. A total of 32 farmers were purposively selected based on recommendation from Development Agents and interviewed individually on crops susceptible to termites, crop growth stage susceptible to termite damage and the average yield losses to the major crops grown in the area, termite management practices in field crops and to list different pests of standing cereal crops.

Farmers' crop yield loss estimate data of five major crops grown by farmers: maize, haricot bean, teff, wheat, barley, and sorghum were gathered carefully by reading the questionnaire to the respondents by the first author. The respondents were asked to estimate for an individual crop potential yield in kilograms they would harvest in the absence of any attack by termites per one *qarxii* of land which is equivalent to 50 m × 50 m (0.25 ha), followed by asking to estimate the quantity they would get in case of termite severe damage. The local people were knowledgeable about measurements in kilograms.

Farmers' yield loss estimate was then calculated as:

$$\text{Yield loss (\%)} = \frac{Y}{X} \times 100\%$$

Where X = Farmers' estimated potential yield without termite attack, and Y = Farmers' estimate yield loss due to termites.

Table 1. Some information about the Kebele Administrations in which the study sites were located.

District	Kebele Administrations	Coordinates*	Elevation (m.a.s.l.) *	Study sites
Bora	Tuqa Langano	8°16'N, 38°55E	1686	$Bora_1$
	Barta Sami	8˚14'N, 38˚53'E	1683	$Bora_2$
Dugda	Oda Boqota	8°10'N, 38°50'E	1666	$Dugda_1$ and $Dugda_2$
ATJK	Warja Washgula	7°56'N, 38°41'E	1652	$ATJK_1$ and $ATJK_2$
	Garbi Widana Boramo	7°53'N, 38°41'E	1650	$ATJK_3$

*Kebele Administrations' coordinates and elevations were recorded using GPS during the study period.

Termite damage and yield loss assessment on haricot beans and maize

Maize and haricot beans were selected for the study because they were identified by farmers as the most important major crops grown in the area and the most susceptible crops to termites. This was also confirmed by East Shawa Zone of Agricultural and Rural Development Office.

Haricot bean

Haricot bean (*Phaseolus vulgaris* L.), seeds were planted at three sites: Garbi Widana Primary School (GWPS), Warja Washgula Kebele Administration Farmers Training Center (WWKAFTC) and Oda Baqota Kebele Administration (OBKA) in the 2012 cropping season. Each site contained two blocks each divided into 4 plots measuring 3 m × 3 m. Seeds were planted on each 6 rows of each plot at a spacing of 40 cm between rows and 15 cm between plants. Two plots of each block were randomly treated with Diazinon 60%EC at field application rate of 2 l/ha as soil spray and the other two were left as untreated control. Two weeks after seedling emergence, the number of standing plants per plot was determined and termite damage to different plant parts was assessed in two weeks interval until harvest. At harvest, all plants in each plot were pulled out by hand, sufficiently dried and then threshed separately. The grains were weighed and the yields from the plots of the same treatment of each site were combined. The percentage yield loss was calculated for each treatment using the formula below as the difference between the treated and untreated plots.

$$\text{Yield loss (\%)} = \frac{(U - V)}{U} \times 100\%$$

Where U = mean grain yield of chemical treated plots and V = mean grain yield of untreated plots

Maize

Six plots of 3 m × 3 m were delineated at a certain distance-interval (depending on the size of the field) along the diagonal of the field on seven volunteer farmers' fields which had been cultivated by oxen in the traditional way and managed under farmers' conditions. Three weeks after emergence, the number of standing plants per sample plot was determined and plants in each plot were assessed for termite damage at two weeks interval until harvest.

Termite damage to crops is generally expressed as percentages of plants attacked or plant mortality, and degree of plant damage (UNEP, 2000). Thus, during each assessment the number of wilted, unhealthy looking, dead or lodged plants were checked for termite presence and damage. When present, termites were collected and preserved in 80% ethanol for later identification. When termites were not found, termite species were determined by damage symptoms. For damaged plants which were not cut either totally or partially at ground level, damage was assessed by pulling out the plants and checking the roots for the presence of termites or their damage symptoms. Plants were considered attacked when termites were seen feeding inside the root system or when root damage showed subterranean termite damage symptoms (Abdurahman, 1990). *Microtermes* species enter and consume the roots and continue their excavation into the stem, which can be excavated and packed with soil, while *Macrotermes* species cut the base of well-established plants (Abdurahman, 1990). Damage was assessed based on the method of Gudeta et al. (2005) by recording the number of lodged plants in each plot. At harvest the total numbers of plant lodged in all plots were combined and the mean percentage of lodged plants per site (field) was calculated.

Farmers' responses were summarized and analyzed as percentages using simple descriptive statistics. Farmers' estimate of percentage yield losses was analyzed using SPSS computer program version 17.0 for Windows.

RESULTS

Farmers' identification of pests of crops

Farmers mentioned 19 major different kinds of pest problem they faced in growing crops irrespective of pest ranks (Table 2). Without giving any hint about termites earlier in the questionnaires, the majority of the farmers considered porcupine (94%) followed by termites (69%) and foxes (56%) as the major pests of crops. Besides, warthogs, dogs and stem borers were also considered as important pests by some of the farmers and these were reported mainly as pests of maize. The major and devastating birds in the area were *Quelea* birds. Cutworms affected different types of crops at seedling stage. Farmers mentioned armyworms as occurring occasionally, but when they occur they cause economic damage on cereal crops. A farmer in Oda Boqota Kebele Administration (OBKA) considered aardvarks as pests for two reasons. First, aardvarks damage crops and cover them with soil, while digging the soil/mound to feed on termites. Second, they feed on maize which is an unusual recent phenomenon.

Table 2. Percentage of respondents' response to pests of standing cereal crops in Bora, Dugda, and Adami Tullu Jido Kombolcha districts of Central Rift Valley of Ethiopia (n = 32).

Pests of standing cereal crops	Percent (number)
Porcupines	94 (30)
Termites	69 (22)
Foxes	56 (18)
Stem borers	47 (15)
Dogs	34 (11)
Birds (mainly *Quelea*)	34 (11)
Cutworms	28 (10)
Armyworms	25 (8)
Warthogs	25 (8)
Fungi (diseases)	13 (4)
Shootfly	9 (3)
Rats	9 (3)
Domestic animals	9 (3)
Weeds	9 (3)
Mole rats	6 (2)
Antelopes	6(2)
Wild pigs	3 (1)
Aardvark	3 (1)
Grasshoppers	3 (1)

Number in the parenthesis indicate the number of respondents farmers.

Farmers' knowledge about the existence of termites and identification of termite types

All farmers were aware of the existence of termites and termite damage to crops and wooden construction. Farmers mentioned that termites were not uniformly distributed in their areas and that they were more abundant in relatively drier areas than wet areas and absent on vertisols. They also pointed out that the termite mounds were very old, no one knew the age of the mounds or when termites appeared in the area.

Farmers were not aware of the existence of different types (species) of termites, and they simply knew termites as a single entity. Even they were not aware of the existence of the minute species, *Microtermes*, which were common in their crop fields and also cause some damage. However, they recognize that a colony consisted of different castes which they could mention some of them using certain features as: the queen (*haadhoo* - the mother), soldiers (*diimtuu kan nama ciniintu* - the red-coloured which bites humans), and alates by flying behaviour (*roobaan jireettii* - the ones which appear during rain). All farmers reported the presence of epigeal mounds on their farmlands and that mounds were constructed by termites. Some also mentioned that termites could be found in non-moundy areas.

Farmers' perception of the benefits of termites

The utilization of mound soil and termites by the community is shown in Table 3. The majority of farmers mentioned that termite mound soil and termites are used for different purposes. About 90 and 78% of the respondents mentioned that mound soil was used for painting of walls of house and making mud bricks for house construction, respectively. Only very few (6%) farmers used termite mound as fertilizer and no one mentioned the use of termites as food for humans.

Farmers' perceptions of susceptibility of crops and crop growth stage susceptible to termite damage

Most of the farmers (78%) considered haricot beans and maize as the most susceptible crops to termites in the area (Table 4). The majority of respondents (99%) and all the participants (interviewees) mentioned that damage on maize plants (lodging) commences at maturity stage and continues until harvest and this was also confirmed by the surveys made on farmers' fields. Farmers reported that damage to haricot beans starts after physiological maturity and they associated damage to unpredictable cloudy weather which may be followed by rain. Farmers mentioned further that termites cause yield loss not by

Table 3. Percentages of respondents about the benefits rendered by termite mound soil and termites in the Central Rift Valley of Ethiopia (n = 51).

Use of	Variables	Percent (number)
Mound soil	Soil fertility/fertilizer	6 (3)
	Pottery making (traditional oven, pots, etc)	71 (36)
	Painting the wall of houses	90 (46)
	Making bricks to build houses	80 (41)
	Construction of stored products' structures	61 (31)
Termites	Food for humans	0 (0)
	Fishing bait*	51 (26)
	Chicken feed†	78 (40)

Number in the parenthesis indicate number of respondents; * Only those farmers who live around Lake Dambal use termites for fishing as baits or sell termites nesting in mounds found in their premises or farmlands to fishermen. †Chickens feed on alates only during alate flight; ‡Responses do not add up to 100% because multiple responses were possible.

Table 4. Crops reported by farmers as susceptible to termites and farmers' estimate of percentage yield losses of standing crops and harvested crops in the Central Rift Valley of Ethiopia.

Crop	% of respondents considered standing crops as susceptible	Yield loss (%)	
		Standing crops (mean ± SE)	Harvested crops (mean ± SE)
Maize	87	10.58 ± 1.91	6.78 ± 1.32
Haricot bean	87	18.02 ± 2.67	17.92 ± 3.54
Teff	39	4.67 ± 1.53	10.93 ± 2.15
Wheat	32	2.81 ± 0.96	4.49 ± 1.45
Barley	16	1.49 ± 0.66	1.49 ± 0.64
Sorghum†	0	0 ± 0.00	0.27 ± 0.27

*The figures are averages of several careful interviews by the researcher; †Sorghum was not mentioned by farmers in the list of susceptible standing crops to termites and this is in agreement with literature.

eating the seeds, but by eating the pods after which the seeds are scattered on the soil. This was also confirmed by observations made on farmers' fields (Plate 1). Under such conditions farmers even estimated high yield loss. Moreover, they reported that damage was highly localized even in the same field and some farmers could identify specific places where termites damage crops in their farmlands.

Farmers' perception of termites as pests and farmers' estimate of yield loss

The majority of respondents (94%) complained about the presence of termite mounds in their farmlands and thus they would like if these mounds are removed from their fields because of certain problems created by the mounds (Table 5). Only few respondents (6%) appreciated the presence of the mounds for their soil used in house construction, derive financial benefits from the sale of the soil, and soil in the perimeter of the mounds was more fertile than adjacent soil which increases production. Farmers also mentioned that termites damage crops and they were able to estimate yield loss to certain major crops (Table 4).

Crop damage and yield loss assessment

Yield loss assessment on haricot bean

No termite damage to standing haricot bean plants was recorded. The yield losses were very low and are shown in Table 6. Haricot beans planted at OBKA site were totally damaged by disease at pod formation stage and thus there are no results for the site.

Assessment of termite damage on maize on farmers' fields

Lodging of maize plants between 3 and 33% was

Plate 1. Haricot seeds scattered on the soil after the pods were damaged by termites on farmers' field.

Table 5. Farmers' perceptions of problems caused by termite mounds found on farmlands in the Central Rift Valley of Ethiopia (n = 51).

Variables	Percent (number)
Narrow farm land and decrease production	90 (34)
Used as nests for animals like rats, foxes, and hyenas which damage crops and/or attack domestic animals	3 (1)
Create obstacle for ox cultivation	21 (8)
Contain termites which eat crops	18 (7)
Source of weeds as weeds grow on mounds and then spread to adjacent area	3 (1)

*Responses do not add up to 100% because multiple responses were possible.

Table 6. Mean haricot bean yield loss due to termites at GWPS and WWKAFTC sites in the 2012 cropping season.

Site	Weight of seeds of treated plots (kg)	Weight of seeds of untreated plots (kg)	% loss (kg)
GWPS	9.006	8.949	0.632
WWKAFTC	9.577	9.563	0.146

GWPS = Garbi Widana Primary School; WWKAFTC = Warja Washgula Kebele Administration Farmers Training Center.

recorded (Table 7). *Macrotermes* were the most common termites responsible for the lodging which cut the plants at ground surface (Plate 2). *Microtermes* were also sampled from roots of some lodged plants. *Odontotermes* were found attacking plants rarely and *Amitermes* were also sampled from few lodged plants.

Farmers' termite management practices in field crops

Only few farmers used cultural management practices and no farmer used chemicals (Table 8). During the interviews, farmers noted that termites rarely damage standing crops on fields to which decomposed animal

Table 7. Mean percent of lodged maize plants and termite species responsible for the lodging at seven sites in the Central Rift Valley of Ethiopia in the 2013 cropping season.

Sites	Lodged plants		Termites caused lodging of plants
	Number	Percent	
$Bora_1$	15	8	*Macrotermes, Odontotermes* and *Microtermes*
$Bora_2$	9	7	*Macrotermes* and *Microtermes*
$Dugda_1$	24	15	*Macrotermes* and *Microtermes*
$Dugda_2$	12	7	*Macrotermes* and *Microtermes*
$ATJK_1$	12	10	*Macrotermes*
$ATJK_2$	4	3	*Macrotermes*
$ATJK_3$	51	33	*Macrotermes*, *Microtermes* and *Amitermes*

ATJK = Adami Tulu Jido Kombolcha.

Plate 2. Lodged maize plants by *Macrotermes* at $ATJK_3$ study site.

Table 8. Termite management practices mentioned by farmers to protect standing crops from termite damage (n = 32).

Indigenous management methods	Percent (number)
Wood ash	3 (1)
Wood ash + Cow dung + Mound destruction	3 (1)
Proper weeding	3 (1)
Collecting lodged maize	9 (3)
Prompt harvesting	19 (6)

dung was applied for soil fertility improvement. However, except collecting lodged maize plants, the use of these practices were not witnessed in the area applied for managing termites during the study period.

DISCUSSION

The inclusion of termites by the majority of the respondents (69%) in the list of pests of standing crops is indicative that termites are important pests in the area. Farmers were aware of the existence of termites for a long time in the area. Elders who were in their seventies, born and lived in the study area to date said that "*Akaakayyonni keenyallee akkanumatti argine jedhanii dubbatu.*" which means "Even our grandfathers said that they saw the mounds from their childhood as they are nowadays." The elders also mentioned that some mounds still existed which they knew during their childhood. Mr. Bariso Karu, a 76 year old indigenous elder, reported that termite mounds had existed in the area before his grandfather was born. Reconstructing the time, his grandfather was born in 1820, about 194 years ago. From this information, termite appearance in the area dates back at least about 200 years. However, to date, there is no evidence of the time termites appeared in the area before the aforementioned time. Termites appeared in western Wallaga (part of western Ethiopia) in about 1938 around a small town known as Qiltu Kara, and termite damage to crops was noticed in 1953 in the same area (Gauchan et al., 1998).

Farmers in the area did not have much knowledge on the presence of diverse species of termites. The lack of knowledge of termite diversity may be attributed to the absence of any benefit gained from termites as food and/or medicine contrary to elsewhere in some African countries. In those countries where termites are used as food, different termite species may be delicious and have better odor/taste than others and thus the people are able to identify termites based on such features.

Farmers of some African countries like Uganda, Kenya, Somalia, Zambia, Malawi and Ghana can identify different types of termites (species) in their areas by local names (Sileshi et al., 2009; Akutse et al., 2012). In Tororo district of Uganda, for instance, farmers identify a total of 14 species of termites in the local language and these are markedly consistent with scientific identifications. The farmers' identification of termites is based on a number of characteristics: (i) Mound building, (ii) Size of mound; (iii) Presence or absence of vents on mounds; (iv) Size, colour, odour and taste of alates (winged reproductives), soldiers or workers and (v) Seasonal and diurnal flight periods of alates (Nyeko and Olubayo, 2005).

In the current study farmers considered maize and haricot beans as the major crops grown in their area and also the most susceptible crops to termite damage. Among cereal crops, maize is the most often damaged by termites (UNEP, 2000). In western Kenya 97 and 3% of the farmers rate maize and sorghum as the most susceptible crops, respectively, whereas in eastern Zambia, all farmers rate maize as the most susceptible crop (Sileshi et al., 2009). Maize is susceptible because it was introduced recently into Africa by the Portuguese explorers in 1502 and has not been exposed to the range of termite life-history strategies of those species occurring in Africa (Ayuke, 2010). In parts of Africa, indigenous crops such as sorghum and pear millet, cowpea, arabica coffee and teff are more resistant to termite damage because of co-evolution and selection by farmers over many centuries (Pearce, 1997; Ayuke, 2010).

Over 98% of farmers reported that damage (lodging) to maize plants starts at maturity stage and continues until harvest and this was confirmed by the intensive field studies conducted on farmers' fields and both were in agreement with most literatures. Maize seedlings are rarely attacked by termites while plants are growing vigorously and lodging commences at physiological maturity (Gudeta et al., 2005). In Ghana farmers also reported that crops are most susceptible to termite attack at maturity stage and they attribute high termite damage in dry seasons mainly to the fact that several crops, especially maize, millet, groundnuts and sorghum, mature and are harvested in these months (Akutse et al., 2012). Several crops are most susceptible to termite attack at maturity (from tasselling/seed set to harvest) (Nyeko and Olubayo, 2005) and plants under stress, such as in drought conditions and those near ripening stage are most vulnerable to termite attack, and younger seedlings can contain repellent compounds such as phenols and cyanides (Pearce, 1997).

Lodging was the only damage symptom recorded caused mostly by *Macrotermes*, to a less extent by *Microtermes* and rarely by *Odontotermes*. *Amitermes* were also sampled from few lodged plants. Although *Microcerotermes* were recorded from farmlands in the area (Daniel and Emana, 2014), they were not sampled from damaged maize plants. Lodging is considered to be the most symptom of termite attack and may entail in further attack by rodents and fungi, in post-harvest decay and aflatoxin contamination (Gudeta et al., 2005, 2008; Ayuke, 2010). Prompt harvest of such crops may therefore reduce yield loss (Nyeko and Olubayo, 2005).

Besides, their perception of the commencement of termite damage to crops at maturity stage, farmers also viewed that damage was highly localized even in the same field and this was confirmed during the intensive field studies on maize on farmers' fields. The results imply that termite damage showed both spatial and temporal variation governed by certain factors. Abdurahman (1990) also noted that the extent of *Microtermes* spp. damage varies from location to location and from year to year and generally plants growing under poor agronomic conditions are more prone to termite damage than those growing under optimum conditions.

In contrary to the finding of the current study, Abdurahman (1990) recorded greater stand loss of maize due to *Macrotermes subhyalinus* at the vegetative growth stage (and more damage to the seedling stage) of the crop as compared to post-tasselling period in western

Wallaga and attributed this to the foraging behavior of the termites. Similarly, UNEP (2000) also reported that in Africa *Macrotermes* spp. cause damage to maize at seedling stage. The differences between these reports and the current study could be attributed to firstly, differences in termite pest species which attack crops at different stages and availability of wood litter and/or crop residues during the early crop growth stage from the previous cropping season which attract termites. There would be more crop residues during the early crop growth stage and these deplete gradually which coincide with crop maturity and then termites will shift to crop plants. Secondly, in the current study, the lack of crop damage during vigorous growth stage and damage incident during crop maturity stage can also be related to the foraging ability of termites governed by rainfall. The heavy rainfall during the vigorous plant vegetative growth stage, that is, rainy season, prevents termites from foraging because termites can hardly construct foraging tunnels and the flood suffocates the termites (the area is generally flat) and thus they stay in their nests. Therefore, during the rainy period the colony's food reserve will become less and thus workers start foraging to feed the hungry colony as soon as the rain decreases (the onset of dry season) and finally stops which coincides with crop maturity stage during which crops start facing moisture stress and become more susceptible to termite damage.

Farmers in the area mainly acknowledged termites for their mound soil used in house construction in making mud bricks and plastering of walls. In wooden wall houses, farmers used a combination of different wood species in building their houses and some of these woods are very sensitive to termite attacks resulting in a decreased lifespan. The replacement of damaged wood and rebuilding of new houses is uneconomical for the farmers and has serious impacts on natural vegetation as it demands additional use of wood. It is therefore of high importance to further spread the knowledge regarding the use of mound soil in making bricks as construction material in a sustainable approach for the future (Emana and Daniel, 2014). The high clay content, the chemical and physical properties and mineralogical composition of termite mound soils which are different from adjacent soils make them useful for making bricks for buildings and contribute a lot in constructions (Pearce, 1997; Akutse et al., 2012). Mound soil is also used for pots, plastering walls, making ovens and is spread for growing plants (Pearce, 1997).

In the current study, the local people did not use termites as food and even there were no available document on the use of termites as food in Ethiopia. However, in the country some local people in Benishangul Gumuz Zone eat swarming alates (Expert of Crop Protection of Agricultural and Rural Development Office of Asosa Zone; Manager of Asosa Plant Health Clinic, pers. comm.). In some African countries, however, some termite species in the subfamily Macrotermitinae are eaten and farmers have extensive knowledge of the value of termites in human nutrition. For instance, the use of termites as food was reported in Uganda (Nyeko and Olubayo, 2005), Namibia (Yamashina, 2010), Kenya (Ayuke, 2010), and Ghana (Akutse et al., 2012). In these countries, the local people can easily identify edible termites from those unsuitable for consumption (Sileshi et al., 2009).

Although farmers reported crops grow better around mounds and fields on which mound soil was spread during mound destruction and all of them had termite mounds in their fields, only few of them (6%) used mound soil as fertilizer contrary to reports elsewhere in Africa. In other parts of sub-Saharan Africa farmers have been seen to leave the mounds and plant crops around them or spread termite-modified soils on their fields (Sileshi et al., 2009; Ayuke, 2010; Akutse et al., 2012). Farmers' use of termite-modified soil in crop production has been documented in Uganda, Zambia, Zimbabwe, Tanzania, Niger, and Sierra Leone (Sileshi et al., 2009) and Laos (Shuichi et al., 2011). Farmers in Ghana attested that the old mounds of termites were source of nutrients for crops. Some vegetables especially pepper, garden eggs, tomato, and other crops such as corn, cassava are usually grown around the mounds. Farmers break the mounds and scatter it to cover more surfaces before the planting or sowing time (Akutse et al., 2012).

Although, farmers acknowledged termites especially for their mound soil in house construction, they considered termites as serious pests of wooden construction and crops, and other problems created by the occurrence of high mound density on the fields. In the interview made with a farmer in the current study about the benefits of termites, the farmer said "*akkamittiin diinni bu'aa qaba*?" which means "How can an enemy have benefits?"

The study has shown that the majority of the respondent farmers regarded termites as the worst pests because termites damage both pre- and post-harvest crops, wooden construction and furniture unlike any other organism. Besides they reported that the high density of termite mounds in their farmlands limits the area available for cultivation and create obstacle during ox-cultivation.

Farmers considered haricot beans and maize as the most susceptible crops and estimated yield loss to these crops before harvest as 18.02 ± 2.67 and 10.58 ± 1.91 (mean ± SE), respectively. According to the farmers, termites cause economic damage to these crops. In the field study 3 to 33% lodging of maize plants was recorded on farmers' fields. However, lodging of maize after senescence may not result in yield loss, if the number of cobs is less, sufficiently matured and all can be used as food by the family and picked daily before they are further attacked by termites and other animals. Generally, farmers considered lodged maize used as food as a loss or wastage because it is used without their intention and also not all is consumed immediately or stored for later use if it is not physiologically matured. It was observed that not all the lodged maize plants were further attacked by termites and other animals and these would add to the farmers' yield. Therefore, physiologically matured lodged plants may not necessarily result in considerable yield loss when they are collected daily for food and also as harvesting in the area is not mechanized. Abdurahman (1990) reported that, when harvesting is mechanized, collecting individual lodged maize from the ground during harvesting can make inconvenience and may demand additional labour to the farmer. Incomplete harvesting of lodged plants in commercial agriculture where the crop is mechanically harvested leads to high loss of yield (up to 100%) unless cobs are harvested by hand incurring added costs.

Contrary to the current study, from surveys they conducted in Maki-Batu area about 25 years ago, and report from local farmers' and area crop protection staff, Cowie and Wood (1989) and Abdurahman (1990) reported that there is no intensive crop damage even on highly susceptible crops such as maize and hot peppers despite the high density of *Macrotermes* mounds. The difference between these reports and the current study may imply that termites have achieved pest status over time. This is also evidenced by the majority (67%) of the respondents perceived that termite severity increased from time to time to standing crops and 52% of them considered termites as serious pests to especially susceptible crops like maize and haricot beans and thus control measure would be necessary. Similarly, Sileshi et al. (2009) reported that farmers in Uganda and Zambia perceive that termite problems are more serious now than in the past. They attribute the increasing severity of termite damage on trees and crops to the depletion of the usual termite food due to deforestation and overgrazing.

Although, farmers in this study considered termites as pests, they did not abandon growing any crop or have not left their lands. Contrary, in western Wallaga farmers abandoned farm fields or abandoned growing certain crops like field pea, faba bean, and chickpea and to some extent millet and migrated to other areas due to termite problems (Sanna, 1973; Abdurahman, 1990; EECMY-WS, 1997).

Whether termite pest problem in the area justifies control or not, detail crop yield loss assessment has to be carried out for a number of years at different sites. In line with this, Logan et al. (1990) reported that the first principle of control is to decide whether control is both desirable and economically feasible. For the latter, yield loss estimates are essential but have been rarely assessed in detail; damage levels may be only poor indicators of ultimate yield loss. For instance, in maize, compensatory growth by surviving plants following early season attack, harvest of cobs on the ground from plants lodged late in the season, and damage to vegetative parts occurring after cob formation will result collectively in over-estimates of yield loss if they are based only on attack or damage scores.

Although most of the farmers perceived termites as crop pests and farmers' average potential crop yield loss estimate, especially losses of maize and haricot beans sounds high, only few (9%) of them reported the use of few management practices. Of these, prompt harvesting of haricot beans and collecting of lodged maize cobs for family food were witnessed during the field study.

All the respondents reported that termites attack of haricot beans after senescence (towards harvest) and associated damage to incident of unpredictable cloudy weather which may be followed by rain. According to them, prompt harvesting of haricot beans and placing the harvested crop in a well protected place is the only means of termite management. However, if the crop is harvested before senescence and heaped, total crop loss results because of decay.

Conflict of Interest

The authors declared that they have no conflict of interest.

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