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Full Length Research Paper

Integrated management of *Meloidogyne incognita* in tomato (*Solanum lycopersicum*) through botanical and intercropping

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Root-knot nematode, *Meloidogyne incognita*, is the major limiting factor in tomato production in many regions of the world, including Ethiopia. Hence, there is a need for development of root-knot nematode management methods that are cheap and environmentally friendly. A greenhouse experiment was conducted with an objective to evaluate the integrated effect of botanicals and intercropping against *M. incognita* on tomato. The experiment was laid out in a 2×3×3 factorial completely randomized design (CRD) with four replications. About 30 ml of lantana leaf extract (at 5% v/v concentration) was applied one week before transplanting of convert to days tomato cv Moneymaker seedlings into 20 cm diameter size pot containing 2 kg sterilized soil. Seeds of mustard and garlic were sown directly into the plastic pots on either side of the tomato seedlings. *M. incognita* was inoculated at the rate of 1000 and 2000 second-stage juveniles (J2) per pot one week after transplanting. A control without any nematode treatment was also maintained. The results revealed that combination of lantana leaf extract and tomato-mustard intercropping at both nematode inoculum levels proved to be the most effective treatment that reduced the soil and root population of the nematode. Application of lantana leaf extract alone and in combination at both inoculation levels showed superiority on tomato growth characteristics. Hence, this ecofriendly approach could be incorporated into integrated nematode management in tomato. However, further research is needed to evaluate their efficacies under field conditions.

Key words: Egg-mass, gall, garlic, juvenile, *Lantana camara*, mustard, management.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most popular vegetables worldwide, owing to its high nutritive value and diversified use. Tomato is rich in minerals, vitamins, essential amino acids, sugars and dietary fibers.

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Ripped tomato fruit has high nutritive value and it is a good source of vitamins and minerals (USDA, 2005). Red tomato contains lycopene, an anti-oxidant that may contribute to protection against carcinogenic substances and which is known to prevent prostate cancer and improves the skin's ability to guard against harmful ultraviolet radiation (Rao and Rao, 2007). As it is a relatively short duration crop and gives a high yield, it is economically attractive. Area under world tomato cultivation is ever increasing. Over 170 million tons of fresh tomato was produced in the world in 2017 (FAOSTAT, 2018). In Ethiopia, tomato is an important cash crop to farmers and a widely cultivated vegetable crop both under irrigation and rain fed throughout the year (Lemma, 2002). Jiregna et al. (2012) reported as tomato ranking 8th in annual national production.

The quest for increased production is often constrained by biotic and abiotic factors. Plant parasitic nematodes (PPN) constitute one of the biotic factors negatively influencing increased tomato production. The latest statistics showed that the estimated losses induced by PPN worth US$ 157 billion worldwide (Singh et al., 2015). There are over 4,100 species of PPN described to date (Decraemer and Hunt, 2009). Of these nematode species, the most economically important PPN genus is root-knot nematode (RKN) (*Meloidogyne* species) (Jones et al., 2013). This genus alone consists of more than 101 described species until August 2015 (Seid et al., 2015). The genus consists of extremely polyphagous nematode groups because it has more than 3000 host species, including vegetables, fruit trees, oil, fiber, grains and leguminous crops, next to weeds that are considered secondary hosts to nematodes (Khalil, 2013). These nematode species parasitize almost every species of vascular plant (Jones et al., 2013). The most well-known species of RKN include *Meloidogyne incognita, Meloidogyne javanica*, *Meloidogyne arenaria* and *Meloidogyne hapla*, which are responsible for high economic losses to varied crops. Average crop yield losses due to nematode are estimated to be in the neighborhood of 25% with damage in individual fields even reaching up to 100% (Sasser et al., 1982; Seid et al., 2015). RKN species (*M. incognita, M. javanica, M. arenaria, Meloidogyne ethiopica* and *M. hapla*) have also been reported to occur in Ethiopia (Stewart and Dagnachew, 1967; Seid et al., 2017). Among them *M. incognita* is the most widespread species (Wondirad and Kifle, 2000). The occurrence of *M. incognita* had been reported on tomato in the eastern part of the country, particularly in eastern Hararghe where many vegetable crops were attacked by this RKN (Solomon, 1987; Tadele and Mengistu, 2000; Seid et al., 2017). The RKN, particularly *M. incognita*, is the major problem in tomato cultivation in the central and western parts, too (Mandefro and Mekete, 2002; Seid et al., 2017).

Prevention of the nematode can be done with planting material, seeds, or soil, crop rotation, intercropping, growing nematode resistant varieties or rootstocks, and through nematicides (Ploeg, 2008). Even though, nematicides are effective in nematode management, however, due to high costs, and the hazards they pose on human as well as on non-target organisms (Nagaraju et al., 2010) made an urge to search for better alternative management strategy.

In spite of wider distribution of the RKN on many crops in Ethiopia, very few work have been done on the management of this nematode species in the country. So far, no efforts have been made to exploit the integrated management of *M. incognita* in tomato using botanicals and intercropping. Hence, the present study was initiated to evaluate the integrated effects of lantana leaf extract and intercropping of tomato with mustard and garlic on *M. incognita* at different inoculation levels under greenhouse conditions.

MATERIALS AND METHODS

Experimental site

A pot culture experiment was conducted at Haramaya University greenhouse, East Hararghe Zone, Oromia Region, Ethiopia at 9°26'N latitude, 42°3' E longitudes. The altitude varies from 1980 to 2000 m above sea level (m.a.s.l.). The mean annual precipitation is 780 mm and the mean annual maximum and minimum temperatures are 23.4 and 8.25°C, respectively. Plants were watered daily and maintained at 23 ± 2°C (12 h day) with 60 to 65% relative humidity for the entire experimental period in a greenhouse at Rarree Research Station of Haramaya University.

Experimental materials

Tomato cv. Moneymaker from (Melkassa Agriuclutral Research Center), garlic (*Allium sativum*) and mustard (*Brassica carinata*) from Debre-Zeit Agricultural Research Center from vegetable research group were obtained, respectively. Leaves of lantana (*Lantana camara)* were collected from the nearby surroundings of Haramaya University. The *M. incognita* population was collected from Jittu farm, Hawassa and identified molecularly by Seid et al. (2017) at Ghent University.

Maintenance of pure culture of *M. incognita*

Pure culture of the *M. incognita* population used in this experiment was raised from a single egg-mass and maintained for ten weeks on Moneymaker at Haramaya University greenhouse. Infected plants were uprooted and roots were gently washed in water to remove the adhering soil particles. Further, the roots were cut into 2 cm pieces and left in a modified Baermann funnel to get fresh second-stage juveniles (J2) (< 24 h old). About 1 ml of *M. incognita* J2 suspension was pipetted into a counting dish and the population was enumerated under a stereomicroscope. Counting was repeated three times to record the mean nematode count.

Preparation of plant extracts

Leaves of lantana (*L. camara*) were collected from the trees grown in the surroundings of Haramaya University campus and used to

prepare the extract. Shade-dried leaves were powdered in an electric blender and 20 g of the botanical powder was soaked in 100 ml distilled sterile water for 24 h in 500 ml flasks. Twenty-four hours after soaking, the materials were filtered through cheese cloth (Wondimeneh et al., 2013) and the filtrates were used for the subsequent experiment.

Treatments and experimental design

There were a total of 18 treatment combinations with three levels of inocula of *M. incognita* (0, 1000 and 2000 J2/pot), including an uninoculated control. Three different cropping patterns viz., tomato with mustard (TM), tomato with garlic (TG) and pure tomato cropping (PT), two levels of the botanicals and untreated control were maintained. Seeds of tomato cv. Moneymaker were sown and raised in a sterilized soil with 1:2:3 proportions of sand, compost and clay, respectively, in a 2 kg capacity pots. About 30 ml of botanical extract (at 5% v/v concentration) was applied one week before transplanting of the seedlings. Seeds of mustard (*B. carinata*) and garlic (*A. sativum*) were planted directly into pots containing sterilized soil and four-week-old-seedlings of tomato cv. Moneymaker were transplanted into pots. The antagonistic plants (mustard and garlic) were planted on either side of the tomato seedlings in line with the treatments. *M. incognita* suspension was inoculated at the rate of 1000 and 2000 J2/pot one week after transplanting. An untreated control was also maintained. Inoculation was done around the root-zone of the tomato seedlings by making three holes and they were covered after inoculation. The experiment was laid out in a factorial completely randomized design (CRD) with four replications. All agronomic practices were maintained as recommended to tomato until termination of the experiment.

Data collection

The following nematode and plant related data parameters were collected sixty days after nematode inoculation (DAI).

Nematode related parameters

Number of galls per root system: After cutting the top parts of the plants, all the pots were turned upside down with care, to discharge the soil and the roots were uprooted and rinsed with tap water to remove adhering soil particles. Number of galls per root system was counted manually aided with hand lens.

Number of egg-masses per root system: Roots containing egg-masses were soaked in a solution of Phloxine B (15 mg 100 ml^{-1} in tap water) for 15 to 20 min and then the roots were rinsed in tap water to remove residual stain. The egg-masses were stained pink to red and observed and counted under stereomicroscope (Coyne et al., 2014).

Number of eggs per egg-mass: Ten egg-masses per plant were randomly taken using forceps. The egg-masses were shaken with 5% sodium hypochlorite (NaOCl) in stopper flasks for 2 min (Hussery and Barker, 1973). The numbers of eggs were counted under stereomicroscope.

Final nematode population density per pot (Pf): Soil and root samples from the pots were collected in marked plastic bags and were brought to the laboratory and nematode extraction was made using the modification of Baermann funnel (Southy, 1970). The *Pf* of *M. incognita* was counted by transferring the suspension to nematode counting dish.

Reproduction factor (RF): It was obtained from the ratio of *Pf to Pi* (initial population density).

Plant related parameters

Plant height: Plant height was measured in centimeters from the soil line to the tip of the tomato stem in each pot.

Root length: Root length was measured in centimeters from soil line to the tip of fibrous root after the adhering soil was washed using tap water.

Number of leaves per plant: Number of leaves per plant was counted in each pot.

Fresh shoot weight (g): The tomato plant was cut at the crown level in each pot and the fresh shoot weight was measured in gram using electronic balance soon after cutting.

Dry shoot weight (g): The shoots were put in paper bag and brought to laboratory just after taking the fresh weight and kept in an oven at 105°C for 24 h, and allowed to come to room temperature and the dry shoot weight was measured in gram using an electronic balance.

Data analysis

The data were subjected to the standard analysis of variance (ANOVA) procedures using Gens tat 15th edition statistical software package. The differences among treatment means were separated using Fisher's protected LSD test at 5% level of probability.

RESULTS

Nematode-related parameters

Number of galls per root system

The result revealed that the main factor botanical, intercropping and inocula level, as well as all possible two factors and three factors interactions had highly significant ($p \leq 0.01$) influence on number of galls produced per root system of tomato (Table 1). The highest (997.2) mean number of galls was observed in 2000 J2 inoculated, un-treated and tomato sole cropping treatment. The lowest (177.5) mean number of galls was recorded for 1000 J2 inoculated, treated and tomato-mustard intercropping treatment. Larger-sized galls were observed on 2000 J2 inoculated treatment than on 1000 J2 inoculation level to all cropping patterns and for both botanical treated and untreated tomato plants. Both tomato-garlic and tomato-mustard cropping patterns when added together with lantana leaf extracts recorded significant reduction in the number of galls per root compared to the treatment where either botanical or intercropping was used alone. The result of this investigation thus clearly showed the significant performance of the cumulative effect by botanical and intercropping.

Table 1. Interactions effect of botanical and intercropping at different inocula levels of second-stage juveniles (J2) of *Meloidogyne incognita* on number of galls per root system, number of egg-masses per root system, number of eggs per egg-mass and final population density per pot at HU, Rarree greenhouse.

Botanical	Inocula (J2s)	PT	TG	TM
		Intercropping on number of galls		
	0.00	0.0^{k}	0.0^{k}	0.0^{k}
T	1000	282.8^{h}	277.0^{h}	177.5^{j}
	2000	529.8^{d}	500.5^{e}	263.3^{l}
	0.00	0.0^{k}	0.0^{k}	0.0^{k}
UT	1000	459.0^{f}	452.5^{f}	299.0^{g}
	2000	997.2^{a}	976.8^{b}	658.5^{c}
LSD (5%)		11.89		
CV (%)		2.6		
		Intercropping on number of egg-masses		
	0.00	0.00^{l}	0.00^{l}	0.00^{l}
T	1000	253.5^{fg}	260.0^{f}	161.0^{h}
	2000	705.2^{b}	706.2^{b}	251.8^{g}
	0.00	0.00^{l}	0.00^{l}	0.00^{l}
UT	1000	404.2^{d}	403.5^{d}	290.0^{e}
	2000	842.2^{a}	844.2^{a}	609.2^{c}
LSD (5%)		6.67		
CV (%)		1.5		
		Intercropping on number of eggs per egg-mass		
	0.00	0.00^{j}	0.00^{j}	0.00^{j}
T	1000	996.8^{f}	975.5^{g}	859.2^{i}
	2000	1108.0^{c}	1115.3^{c}	968.0^{g}
	0.00	0.00^{j}	0.00^{j}	0.00^{j}
UT	1000	1035.5^{e}	1032.0^{e}	901.7^{h}
	2000	1156.8^{a}	1138.0^{b}	1043.5^{d}
LSD (5%)		7.87		
CV (%)		8		
		Intercropping on final population density per pot		
	0.00	0.00^{m}	0.00^{m}	0.00^{m}
T	1000	2003^{g}	1503^{i}	1003^{l}
	2000	3078^{b}	2068^{d}	1061^{k}
	0.00	0.00^{m}	0.00^{m}	0.00^{m}
UT	1000	2056^{e}	1516^{h}	1088^{j}
	2000	4016^{a}	3034^{c}	2017^{f}
LSD (5%)		4.94		
CV (%)		3		
		Intercropping on reproduction factor		
	0.00	0.0000^{l}	0.0000^{l}	0.0000^{l}
T	1000	2.0030^{c}	1.5030^{f}	1.0032^{j}
	2000	1.5391^{d}	1.0340^{h}	0.5306^{k}
	0.00	0.0000^{l}	0.0000^{l}	0.0000^{l}
UT	1000	2.0560^{a}	1.5162^{e}	1.0875^{g}
	2000	2.0079^{b}	1.5171^{e}	1.0086^{i}

LSD (5%)	0.003
CV (%)	2

Means followed by the same letter(s) within a row and column in each character are not significantly different at 5% level of significance. LSD (5%) = least significant difference; CV (%) = coefficient of variation, J2 = second stage juveniles of Meloidogyne incognita, T = treated with leaves extract of lantana at 5% concentration, UT = untreated treatment with lantana leaves extract, PT = pure tomato plant cropping, TG = tomato plant intercropped with garlic, TM = tomato plant intercropped with mustard.

Number of egg-masses per root system

The main factor botanical, intercropping and inocula levels, as well as all possible two factors and three factors interactions had highly significant ($p \leq 0.01$) influence on the number of egg-masses per root system. The combined effect of botanical and intercropping to all inoculation levels showed significant suppression of egg-masses per root system (Table 1). The maximum (842.2) mean number of egg-masses was observed in 2000 J2 inoculated, un-treated and tomato sole cropping treatment. The lowest (161.0) mean number of egg-masses was recorded for 1000 J2 inoculated treated and tomato mustard intercropping treatment. There was no significant difference in the number of egg-masses in tomato sole cropping and tomato-garlic intercropping in all inoculation levels of J2s of *M. incognita.* Combined effect of botanical and tomato-mustard intercropping was found to be the most effective in reducing the number of egg-masses compared to all other treatments.

Number of eggs per egg mass

The result showed that the main factor botanical, intercropping and inocula, as well as all possible two factors and three factors interactions had highly significant ($p \leq 0.01$) influence on the number of eggs per egg-mass (Table 1). Significant reduction in fecundity was observed when plants were treated using lantana leaf extract and intercropping of tomato with garlic and mustard alone. But combination of lantana leaf extract and tomato-mustard intercropping treatment resulted in a maximum (859.2) decline in eggs of *M. incognita.* Tomato alone without botanical treatment and without intercrops but inoculated 2000 J2 of *M. incognita* caused the highest (1156.8) number of eggs egg-mass^{-1} as compared to other treatments.

Final population density per pot

The obtained result revealed that the main factor botanical, intercropping and inocula, as well as all possible two factors and three factors interactions had a significant ($p \leq 0.01$) influence on nematode population (Table 1). Maximum (1003) reduction of nematode population occurred in soil treated with botanical, 1000 J2 inoculated and tomato-mustard intercropped treatment and the highest (4016) number of nematode population was recorded due to 2000 J2 inoculated, un-treated and tomato sole cropping treatment.

Reproduction factor (RF)

The main factor botanical, intercropping and inocula as well as all possible two factors and three factors interactions had highly significant ($p \leq 0.01$) influence on reproduction factor (Table 1). Tomato plants inoculated with 1000 J2 *M. incognita* without any treatment showed the highest (2.0560) reproduction rate. Application of botanical and intercropping with garlic and mustard either alone or in combination showed a significant reduction in reproduction of nematode but the reduction was more pronounced in tomato plant intercropped with mustard and treated with leaf extract of lantana (0.5306).

Plant parameters

Plant height

The main factors botanical, intercropping, inocula levels, and interactions effect of botanical and inocula as well as three factors interactions (botanical × intercropping × inocula) had highly significant ($p \leq 0.01$) influence on tomato plant height. However, the remaining two factors interactions (botanical × intercrop and intercropping × inocula) did not significantly affect plant height (Table 2). The tallest (73.75 cm) plant was observed in un-inoculated, botanical treated and tomato sole cropping treatment. The shortest (39.75 cm) plant height was recorded for 2000 J2 inoculated, un-treated and tomato-mustard intercropping treatment. The reduction of plant height might be due to the root damage of the plant by the *M. incognita* population used.

Root length

The experimental result revealed that all the main factors (botanical, intercropping and inocula) and the interactions effect of botanical and inocula as well as inocula and intercropping had highly significant ($p \leq 0.01$) influence on root length of tomato (Table 3). The longest (18.62

Table 2. Interaction effects of botanical, intercropping and inocula levels of second-stage juveniles (J2s) of *Meloidogyne incognita* on plant height of tomato at HU, Rarre greenhouse.

Botanical	Inocula (J2s)	Plant height (cm) in intercropping system		
		PT	TG	TM
T	0.00	73.75[a]	65.00[c]	51.75[e]
	1000	68.00[bc]	68b[c]	48.50[e]
	2000	56.5[d]	57.25[d]	36.75[g]
UT	0.00	70.75[ab]	70.5[ab]	49.00[e]
	1000	60.5[d]	51.25[e]	42.75[f]
	2000	49.75[e]	49.50[e]	39.75[fg]
LSD (5%)		4.07		
CV (%)		5.1		

Means followed by the same letter(s) within row and column in each character are not significantly different at 5% level of significance. LSD (5%) = Least significant difference; CV (%) = Coefficient of variation, J2 = Second stage juveniles of *Meloidogyne incognita,* T = treated with leaf extract of lantana at 5% concentration, UT = untreated treatment with lantana leaf extract, PT = pure tomato plant cropping, TG = tomato plant intercropped with garlic, TM = tomato plant intercropped with mustard.

Table 3. Interaction effects of inocula levels of second-stage juvenile (J2) of *Meloidogyne incognita with* botanical and intercropping on root length of tomato at HU, Rarre greenhouse.

Root length (cm)	Inocula		
Botanical	**0.00 J2**	**1000 J2**	**2000 J2**
T	15.79 [a]	13.33[b]	12.21[b]
UT	16.62[a]	8.83[c]	5.96[d]
LSD (5%)		0.96	
Intercropping	**0.00 J2**	**1000J2**	**2000 J2**
PT	18.62[a]	11.88 [bc]	10.00[bcd]
TG	18.12[a]	12.25[b]	9.94 [bcd]
TM	11.88[bc]	9.12[cd]	7.31[d]
LSD (5%)		1.18	
CV (%)		9.7	

Means followed by the same letter(s) within row and column in each character are not significantly different at 5% level of significance. LSD (5%) = least significant difference; CV (%) = coefficient of variation, J2 = second stage juveniles of *Meloidogyne incognita,* T = treated with leaves of lantana at 5% concentration, UT = untreated treatment with lantana leaves extract, PT = pure tomato plant sole cropping, TG = tomato plant intercropped with garlic, TM = tomato plant intercropped with mustard.

cm) root length was recorded on the un-inoculated tomato planted alone treatment, while the shortest (5.96 cm) length was recorded in un-treated, 2000 J2 inoculated treatment. The interaction effects of botanical and inocula and also inocula and intercropping had significant influence on root length. No significant difference was detected between 1000 J2 and 2000 J2 inoculation level treatments on root length but in un-treated pots raising inocula levels of *M. incognita* reduced the tomato root length. Generally, it was observed that the effect of nematode was more pronounced in roots than on shoots on *M. incognita* inoculated un-treated pots. There were no remarkable difference between tomato sole cropping and tomato-garlic intercropping at 1000 J2 and 2000 J2 inoculation level.

Number of leaves

The highest (444.5) number of leaves per plant was recorded on un-inoculated, tomato sole cropping treatment. The smallest (153.4) number of leaves was recorded on 2000J2 of *M. incognita* inoculated tomato-mustard intercropped treatment. Leaf number was significantly influenced by the interaction effects of intercropping and inocula levels (Table 4). Raising

Table 4. Interaction effects of intercropping and inocula levels of second-stage juvenile (J2) of *Meloidogyne incognita* on leaf number of tomato at HU, Rarre greenhouse.

Intercropping	Number of leaves		
	Inocula (0.00 J2)	Inocula (1000 J2)	Inocula (2000 J2)
PT	444.5[a]	320.7[b]	207.4[d]
TG	411.5[a]	257.6[c]	183.6[de]
TM	250.1[c]	207.7[d]	153.4[e]
LSD (5%)		28.32	
CV (%)		10.4	

Means followed by the same letter(s) within a row and column in each character are not significantly different at 5% level of significance. LSD (5%) = least significant difference; CV (%) = coefficient of variation, J2 = second stage juveniles of *Meloidogyne incognita,* PT = pure tomato plant cropping, TG = tomato plant intercropped with garlic, TM = tomato plant intercropped with mustard.

Table 5. Interaction effects of intercropping and inocula levels of second-stage juvenile of *Meloidogyne incognita* on fresh shoot weight of tomato at HU, Rarre greenhouse.

Intercropping	Fresh shoot weight (g)		
	Inocula (0.00 J2)	Inocula (1000 J2)	Inocula (2000 J2)
PT	295.8[a]	256.6[bc]	218.3[d]
TG	261.8[b]	247.6[bc]	231.5[cd]
TM	161.5[e]	208.1[d]	175.9[e]
LSD (5%)		22.24	
CV (%)		9.7	

Means followed by the same letter(s) within row and column in each character are not significantly different at 5% level of significance. LSD (5%) = least significant difference; CV (%) = coefficient of variation, J2 = second stage juveniles of *Meloidogyne incognita,* PT = pure tomato plant cropping, TG = tomato plant intercropped with garlic, TM = tomato plant intercropped with mustard.

inocula levels of J2 of *M. incognita* in all cropping patterns reduced tomato leaf numbers.

Fresh shoot weight

The main factors intercropping, inocula and interaction effect of inoculums and intercropping had highly significant ($p \leq 0.01$) influence on fresh shoot weight of tomato. However, the remaining possible two factors and three factors interactions did not significantly affect tomato fresh shoot weight (Table 5). Among the treatments, the highest (295.8 g) and the lowest (175.9 g) fresh shoot weight were recorded from un-inoculated tomato sole cropped treatment and 2000 J2 inoculated tomato plant intercropped with mustard, respectively. Interaction effects of inocula levels and intercropping showed significant effect on fresh shoot weight. Increasing inocula levels of second-stage juveniles of *M. incognita* in all cropping patterns reduced fresh shoot weight of tomato. There was no significant difference between tomato sole cropping and tomato-garlic intercropping at 1000 J2 of *M. incognita* and 2000 J2 of *M. incognita* level.

Dry shoot weight

The inocula levels and intercropping had highly significant ($p \leq 0.01$) influence on dry shoot weight of tomato plants. However, dry shoot weight was not significantly influenced by botanical; the interaction effect of all the possible two and three factors interactions (Table 6). Tomato sole cropping resulted in mean dry shoot weight (66.1 g) at par with tomato-garlic intercropping but both exceeded the tomato-mustard intercropping. Dry shoot weights of plants inoculated with 2000 J2 of *M. incognita* showed minimum mean value (58.3 g) than 1000 J2 of *M. incognita* inoculated and un-inoculated control.

DISCUSSION

The results of this study showed that raising the inoculation levels of *M. incognita* recorded significant reduction on growth characteristics of tomato and significant increase on nematode related parameters. This might be due root infection that resulted in stunting

Table 6. Effect of inocula of second-stage juveniles of *Meloidogyne incognita* and intercropping on dry shoot weight of tomato at HU, Rarre greenhouse.

Inoculum	Dry shoot weight (g)
0.00 J2	66.9[a]
1000 J2	63.3[b]
2000 J2	58.3[c]
LSD (5%)	1.39
Intercropping	
PT	66.1[a]
TG	66.1[a]
TM	57.08[b]
LSD (5%)	1.39
CV (%)	3.8

Means within a row in each treatment and character with similar letter(s) are not significantly different at 5% level of significance, LSD (5%) = least significant difference, CV (%) = coefficient of variation, J2 = second stage juveniles of *Meloidogyne incognita,* PT = pure tomato plant cropping, TG = tomato plant intercropped with garlic, TM = tomato plant intercropped with mustard.

action of *M. incognita* and distortion of the plant roots and nematode colonization of vascular bundles that reduce supply of nutrients to plants. Sikora and Fernandez (2005) reported that the galls on the root system might disturb important root functions like uptake and transport of water and nutrients.

Application of botanical (at 5% v/v concentration) had significant improvement on tomato growth and recorded suppressive effect on galling intensity and nematode population when used alone and in combination with other treatments. This might be due to the nematicidal activity of aqueous leaf extract of *L. camara* against *M. incognita* as indicated by the reduced galling and egg-masses production. Udo et al. (2014) also reported that nematostatic properties of *L. camara* leaf extract were attributed to poor coordination and orientation of infective juveniles towards the plant roots. Among the different treatments, combination of leaf extract of lantana and tomato-mustard intercropping was the most effective in reducing galling intensity and nematode population followed by combination of leaf extract of lantana and tomato-garlic intercropping. Untreated control recorded the highest galling intensity and nematode population, thereby showing poor tomato growth. Addition of botanical to soil leads to a better environment for the growth of the roots. This enhances the utilization of soil nutrients, as a consequence, nematode damage might have been markedly reduced (Abubakar et al., 2004).

Plants are important sources of naturally occurring compounds, many of which have nematicidal properties, like alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls (Chitwood, 2002). Mustard, African marigold, asparagus, castor, and sesame have been found to reduce population of RKN and may be grown as commercial crops, cover crops, or established in mixed planting with other crops for the management of root-knot nematodes (Bridge, 1996). Use of such antagonistic plants as green manures improves the fertility of the soil too. Certain nematode-toxic chemicals, which kill nematodes, are released during decomposition of the plant materials. Green manures increase the saprophagus bacteria, fungi, and other microorganisms in the soil, which aid in reducing RKN population (Barker et al., 1985). Halbrendt (1996) suggested that plant compounds elicit nematode behavior, such as attraction or repulsion from roots. Naturally occurring plant chemicals for nematode management have advantages being eco-friendly and posing no hazardous effect on produce over the current use of synthetic chemicals.

The efficient management of PPN requires the carefully integrated combination of several methods. Although each individual method of management has a limited use, together, they help in reducing the nematode populations more efficiently. With the ongoing progress in research, a public desire for methods of managing/reducing plant pests in ways that are cheap, easily available, eco-friendly and do not pollute or otherwise degrade the environment, has increased concomitantly. The integrated pest management provides a working methodology for pest management in sustainable agricultural systems. One such method employed for maintaining the populations of PPN below the economic threshold level, is the mixed cropping practice, sometimes also referred to as intercropping, which is a form of multiple-cropping system using host and non-host

crops at the same place and time (Rodriguez-Kabana and Canuilla, 1992).

It has been reported by several workers that different cropping sequences reduced the populations of some harmful PPN to the levels that do not cause economic losses (Idowua and Fawole, 1989; Haider et al., 2001). Haider et al. (2004) reported that the intercropping two rows of yellow mustard sarson (*Brassica campestris* var. *toria* / *B. campestris* var. *sarson*) with sugarcane, recorded the highest reduction (23.7%) in nematode populations followed by sugarcane + one row of yellow mustard at the time of harvest of intercrops. This sequence showed prolonged effect of toxicity as evidenced by 21% reduction in nematode population from initial density level at the time of harvest of sugarcane. Sugarcane and yellow mustard intercropping system exhibited the highest cane yield. Similar results of inclusion of mustard, a poor host for several nematodes, in different cropping sequences for reducing nematode populations have been reported by several other workers (Singh and Sitaramaiah, 1993; Kumar and Khanna (2006). Intercropping mustard could attribute the decrease in nematode populations by intercropping mustard to the presence of 2-propenyl isothiocynate in mustard having nematicidal activity as reported by Kowalska and Sonalinska (2001). However, no report was found in this type of three factors study to explain the result in relation to others' finding. Since this experiment was carried out under controlled (greenhouse) conditions, further study is required under farmer's field conditions to prove the consistency of the results obtained and improve their application technologies. Moreover, intercrops might have competitive effects on crops for essential growth factors, which may, in turn, affect the yield of crops; thus further trials geared towards using mustard and garlic as a short term fallow plants would give a more conclusive result in their use for management of *M. incognita*.

Conclusion

The present study was conducted to evaluate the integrated effect of botanical and intercropping against different inoculation levels of root-knot nematode, *M. incognita* on tomato cv. Moneymaker. The result of this study showed that raising the inoculation levels of second-stage juveniles of *M. incognita* in all treatments showed a significant reduction on growth characterstics of tomato and had significant increase on nematode related parameters. Application of botanical had significant improvement on tomato growth and showed suppressive effect on galling intensity and nematode population when used alone and in combination with other treatments. Among the treatments, the combination of leaf extract of lantana and tomato-mustard intercropping was the most effective in reducing galling intensity and nematode population; also, the combination of leaf extract of lantana and tomato-garlic intercropping was found the next effective treatment in reducing galling intensity and nematode population. Untreated inoculated tomato-sole cropping treatment was recorded the highest galling intensity and nematode population, thereby showing poor tomato growth. In conclusion, although this study was carried out in the greenhouse, it has clearly indicated that integrated use of botanicals and intercropping could be used as an alternative method for the management of root-knot nematode in tomato production. However, further research is needed to evaluate their efficacies under field conditions and improve their application technologies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vertical versus horizontal expansion of food security crops in Sudan: A causality analysis of 1961-2017

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This research used FAO statistical data to test the causality between production, area and yield for Sudan's three major food crops; sorghum, wheat and millet. Results indicated a sizable gap in yield between Sudan and some selected top producing countries for the selected crops. Two-way causality was observed from production to area and vice versa for sorghum crops, accentuating horizontal expansion, while the lack of causality observed from yield to output omitted the impact of vertical expansion. The non existence of any causality for wheat crops indicates the exclusion of both vertical and horizontal expansion, a result that could be explained by the unsuitability of the Sudanese climate for wheat growth. Causality results for the millet crop suggest the absence of causality between production, area and yield in all directions, which can be attributed to low yield, which is itself due to the lack of recommended technical packages required for enhanced production. The research recommends emphasis on vertical expansion to develop plans for sustainable agriculture in Sudan. Further recommendations focus on upgrading the efficiency of current agricultural production systems through the application of appropriate technological packages. Regarding the wheat crop, the study recommends in-depth integrated research on comparative advantage, developing heat-tolerant varieties and the economic feasibility of growing wheat in Sudan.

Key words: Cereals, climate change, yield gap, technological packages, sustainable agriculture, final prediction error.

INTRODUCTION

FAO (2006) defined the multidimensional nature of food security captured by availability, accessibility, food use and stability. Twenty-five of the 39 countries experiencing serious food insecurity thus requiring foreign support to overcome itare in Africa (Sudan being one of them) and 11 are in Asia. To overcome food insecurity, (FAO, 2006) emphasized rural development productivity enhancements, including improved food production by small-scale farmers.

According to FAO (2018), Africa experiences severe food insecurity; around 27.4% of the population suffered food insecurity in 2016, a rate nearly four times greater

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than any other region. Further, Africa especially sub-Saharan Africa is one of the regions where food insecurity is increasing.

Knox et al. (2012)adopted an organized review and meta-analysis of data in 52 original published articles to assess the anticipated impacts of climate change on the harvest of major crops in South Asia and Africa. The anticipated mean changeby the 2050s in the harvest of all crops, in both regions, is 8%. Across Africa, mean yield changes of 17% for wheat, 5% for maize, 15% for sorghum and 10% for millet are projected. Hence, signal of vigorous impact of climate change on crop harvest in Africa and South Asia is for wheat, maize, sorghum and millet, with an anticipated negative impact on food security.

The sources of growth in production of cereals in the state of Uttar Pradesh in India were investigated by (Sharadet al., 2018), adopting a methodology based on the dynamic nature of time and the regionalization of production, area and harvest of main cereal crops. The results indicated positive progress in the production, area and harvest of wheat, rice and maize, while other crops exhibited a mixed trend. Through the study period, variability was recognized as the highest in production, followed by harvest and area.Total production of cereals was caused by an increase in area, and its interference with other elements, thus emphasizing horizontal expansion (focusing on increases in area).Based on Sanders et al. (2019), sorghum maintains a vital role for food security in Sub-Saharan Africa. However, despite considerable research following the extreme African drought of 1968–1973, sorghum technological packages in Sub-Saharan Africa's farmers' fields were only slowly applied. The situation of sorghum was reviewed globally by Sanders et al. (2019), in the U.S. and in Sub-Saharan Africa during the period 2007-2017.The results of a 12-year program in the Sahel region of West Africa to host innovative sorghum technology were identified in Mali. The program identified innovative technologies that were provided to some farmers' associations. The Mali program was then combined with two agencies to enhance the pilot program. The pilot scheme confirmed that harvests with modest fertilization, new varieties and improved cultural practices could be increased between 50%and 100%, assuring vertical expansion (focusing on increases in yield).

Applying a world market model with short- to long-run yield response adopting available scientific research outcomes and data (Thompson et al., 2019) approximated yield elasticities that permit agricultural commodity and food policy analysis. Results highlighted substantial differences in quantity and price effects, depending on the yield elasticities. Moreover, results demonstrated the necessity of identifying yield responses to prices when evaluating impacts on food security in the face of population growth, climate change and other long-run pressures.

Liu et al. (2008) evaluated under nutrition at the national level for Sub-Saharan African (SSA) countries in order to locate regions of greatest challenge. The influence of climate change on the production of six main crops namely cassava, wheat, maize, sorghum, millet and rice was examined with a GIS-based environmental policy integrated climate model (GEPIC) with the same spatial resolution.

Upcoming hunger hotspots are estimated in the framework of predicted climate, economic, social and biophysical changes. The results indicated that some regions in Nigeria, Sudan and Angola where a large number of people currently suffer under nutrition might be able to increase their food security status through increasing purchasing power. In the near future, some regions in SSA will suffer from a low capacity to import food along with lower per capita calorie availability. Special attention should therefore be paid to these hotspot regions, Sudan being one of them, with the intention of meeting hunger alleviation goals in SSA.

Aiming to measure the impacts of temperature increase on wheat harvest, Asseng et al. (2017) constructed a grain yield–temperature response function combined with a quantification of model uncertainty using a multi-model ensemble from two irrigated spring wheat areas (Sudan and India) and applied it to irrigated spring wheat regions around the world. Wheat-growing regions with great harvest reductions as a result of increased temperatures corresponded with great poverty headcounts in southern Pakistan and southern India, indicating that these areas are forthcoming food insecurity hotspots.

The relative harvest reductions are higher in low-yielding atmospheres (for example high temperature areas in southern India, southern Pakistan and wheat-growing regions in Sudan). Farmers in the aforementioned regions are expected to be hit hardest by increasing temperatures. While Sudan could possibly produce more wheat provided irrigation is available, wheat harvests would be low owing to high temperatures, with additional temperature increases further restricting wheat production.

Sorghum, millet and wheat are considered staple food grains in Sudan. Sorghum is among the food grains spread geographically throughout Sudan. In the Northern State of Sudan, wheat is the major food grain, followed by sorghum. In eastern and central Sudan, sorghum is more dominant, while millet is the main food grain in western Sudan, followed by sorghum (Abdalla, 2016).

Mahran (2000)employed the ordinary least squares (OLS) method of analysis to evaluate the achievements of the national development strategies of medium-term plans and programs during the period 1970/1971-1992/1993. In particular, Mahran examined achievements as they relate to meeting the objective of national self-sufficiency in food through vertical and horizontal

expansions infood production. An exponential function was used for estimating the trends in area, production and productivity for the major staple food crops sorghum, wheat and millet, applying annual time series data during the period 1970-1995. Results indicated that vertical expansion alone does not increase output. Hence, policies should emphasize enhancing agricultural productivity through the production of new varieties and assured application of technical packages. Further, the research emphasized the importance of increased productivity to food security as a mean of paving the way for industrial growth.

The impact of meteorological drivers on crop yields and the effects of herbicide application on farm productivity were examined (Fahmiet al., 2017)at two locations in Sudan, namely El Dali and El Mazmum, for ten successive years, from 2001-2010. Analysis of time series annual and monthly precipitation and yields of sorghum, millet and sesame were undertaken using the Mann-Kendall test and Sen's slope estimator methodologies. Results indicated that variation in crop yields is caused mainly by inter-annual variations in precipitation and insufficient agricultural practices.

Based on Reynolds et al. (2016), wheat, rice, sorghum, millet and maize provide more than half of globally needed food calories. To preserve global food security constrained by the climate change challenge, there is an increased necessity to utilize prevailing genetic variability and evolving cultivars with higher genetic harvest potential. Hence, the prospect of sharing knowledge between researchers and recognizing priority traits for further research could enhance breeding effects and help to detect the genetic focus that regulates adaptation. A globally harmonized path to crop phenotyping and modeling, combined with operative sharing of knowledge, data and facilities, will enhance cost effectiveness and help to implement genetic benefits forall staple crops, resulting in a higher yield of food security crops.

Using descriptive and regression analysis, Elmulthumet al. (2011) provided some insightful forecasts concerning food security for the period 2009-2020, assuming exponential growth over time. Results proved self-sufficiency in cereals of less than 100% during the period 1986-2009, while forecasts for the production and consumption of cereals indicated that food insecurity would persist during the period 2009-2020. Research findings recommend the adoption of clear and sound agricultural policies to ensure the accessibility and availability of food crops at all times. Thus, agricultural strategies could encourage producers of food crops to boost food crop harvests.

Emphasizing the importance of food security for Sudan, the present research aimed to examine the causality between production, area and yield for the major staple food crops in Sudan: sorghum, wheat and millet.

METHODOLOGY

Based on Granger (1969), testing the causality between two variables, for example Y and X, involves estimating the following regression equations:

$$Y_t = \sigma + \sum_{i=1}^{n} \mu_i Y_{t-i} + \sum_{j=1}^{h} \pi_j X_{t-j} + £_t \quad (1)$$

$$X_t = \alpha + \sum_{i=1}^{k} \emptyset_i Y_{t-i} + \sum_{j=1}^{q} \partial_j X_{t-j} + €_t \quad (2)$$

Where,$£_t$ and $€_t$ denote white-noise errors, n, h, k and q denote the number of lagged variables in undertaken regressions. Granger methodology is based on calculating the ordinary least square estimates of regression parameters in the above equations and applying the Wald F statistical test of joint statistical significance. For detecting the existence and direction of causality, three cases are distinguished. First, unidirectional causality implicates two cases: causality from X to Y and vice versa. Causality from X to Y is proved if the coefficients of the lagged X variable in equation 1 differ significantly from zero ($\sum \pi_j \neq 0$), while the coefficients of the lagged Y variable in equation 2 are not statistically different from zero ($\sum \emptyset_i = 0$)). Unidirectional causality from Y to X occurs when the calculated coefficients of the lagged X variable in equation 1 are not significantly different from zero as a group$\sum \pi_j = 0$), while the calculated coefficients of the lagged Y variable in equation 2 are significantly different from zero ($\sum \emptyset_i \neq 0$)). Bilateral causality occurs if the coefficients of the lagged Y and X variables are significantly different from zero in both estimated regression equations. More properly, causality from X to Y co-occurs with causality from Y to X when the hypotheses of ($\sum \pi_j \neq 0$)and ($\sum \emptyset_i \neq 0$) are statistically accepted for equations 1 and 2. Independence of the two variables is advocated when the coefficients of the lagged X and Y variables are not significantly different from zero, accepting null hypotheses of $\sum \pi_j = 0$)and and$\sum \emptyset_i = 0$). It has been a common exercise in causality research to adopts Granger (1969) methodology to select the lag order on an ad hoc basis and to use the same lag order in all regressions. According to (Thornton and Batten 1985) and Hsiao (1979, 1981), the Granger procedure may give rise to misleading results. Based on the above (Hsiao 1979), (Hsiao 1981) proposed an alternative methodology combining the Granger (1969) test of causality and the final prediction error measure developed by Akaike (1969). Based on Akaike (1969), the final prediction error (FPE) statistic is a minimum for the optimum lag for the model and has solved the identical problem of determining the correct order of an autoregressive model for the data.

The proposed methodology has the advantage of allowing the data to define the optimum lag order for each variable. Based on Hsiao's (1979, 1980) methodology adopted by (Mahran, 2003), the Y variable is first assumed as the only output of the system. A series of auto-regressive regressions on Y variable starting from one lag, and adding one more lag in succeeding regressions were run. For each of the succeeding auto-regressive regressions, the final prediction error is estimated using the following equation:

$$FPE(h) = \frac{(T+h+1)}{(T-h-1)*((\frac{RSS(h)}{T})} \quad (3)$$

Where, T denotes the number of observations and RSS(h) the residual sum of squares with h lags. The optimum lag number h* is the one matching the autoregressive equation with the least FPE (h*). The equation with the least FPE (h*) is then regressed with lagged values of X, adding one more lag in each regression. The final prediction error is then calculated for each regression using the following formula:

$$FPE(h*,m) = \frac{(T+h++m+1)}{(T-h--m-1)*((\frac{RSS(h*,m)}{T})} \quad (4)$$

The optimum lag order (m*) from these regressions is defined as the one which leads to the lowest FPR (h*,m*). Hence, testing for causality comprises a comparison between FPE(h*) and FPE (h*,m*). The test is now straightforward using the following criteria:

FPR (h*, m*) < FPR (h*) X Granger causes Y
FPR (h*, m*) > FPR (h*) X does not Granger cause Y

To test whether Y Granger causes X, or vice versa, the above methodology is repeated using X as a controlled variable and Y as the manipulated variable.

Akaike whose final prediction error (FPE) statistic is a minimum for the optimum length model, solved the identical problem of determining the correct order of an autoregressive model for the data. Since causality tests require stationarity of data, the Dickey-Fuller test was used to test the null hypothesis that the autoregressive model has a unit root(Cheung and Lai, 1995).

RESULTS AND DISCUSSION

This section starts with some descriptive statistics, comparing the yield of selected food crops in Sudan to the yield of the same crops in some of the top producing countriesincluding Nigeria, Egypt and Indiafor one decade. Comparisons indicated a recognized gap, where the yield of sorghum, wheat and millet in Sudan was 52, 33, and 30% ofNigeria, Egypt and India, respectively (Table 1).

Results of the Dickey-Fuller test proved the non-stationarity of the data for the three selected food crops.To determine the lag structure of all variables, one-dimensional autoregressive regressions were estimated using an upper limit of five lags for each variable. The estimated final prediction error for the results for sorghum, wheat and millet are reported in Tables 2, 4 and 6, respectively. The next step was to fix the number of lags in the controlled variables determined in the first step and regress with lagged manipulated variables added successively to determine the final prediction error of bivariate regressions. Results of the final prediction error for the bivariate regressions for sorghum, wheat and millet are reported in Tables 3, 5 and 7, respectively.

All estimated autoregressive and bivariate equations were significant, as indicated by F-statistic. In addition, Durbin-Watson statistics suggested the absence of autocorrelation for autoregressive and bivariate estimated equations.

In view of the results for the sorghum crop shown in Tables 2 and 3, a two-way causality from production to area and vice versa is acknowledged. Further, results indicated that production Granger-causes yield while yield does not Granger-cause production. The above results indicate that the increase in production is influenced by the increase in area, leading to a further increase in area, hence emphasizing horizontal expansion for Sudan's dominant food crop. In addition, the large fluctuation in the output of the sorghum crop may contribute to an unforeseen impact of yield on output, excluding the impact of vertical expansion. The yield of sorghum is around 50% of the yield of one of the top producing countries, Nigeria (Table 1). The above results could be further explained by large areas cultivated for the sorghum crop, the main staple food crop in Sudan, together with conventional ways of crop production, in which the majority is grown byadopting low technical packages in rain-fed areas. The results in relation to sorghum are in line with(Mahran, 2003) regarding causality from production to area; however, the other causality results contradict the results of (Mahran, 2003). This contradiction could be explained by the length of time series and the dependence of policy makers on increasingly large areas for the required level of sorghum production to meet increasing demand.

Regarding the wheat crop, the results shown in Tables 4 and 5 proved the nonexistence of causality between production and area grown in any direction. The results also point to the absence of causality between production and yield in both directions. Results in relation to wheat may be explained by the unsuitability of environmental conditions for the planting of wheat in those areas where the crop is grown. Thus, according to policy makers, neither vertical nor horizontal expansion will pay off or motivate farmers to grow wheat. A sizable gap in the yield of wheat was observed when comparing the yield of Sudan to the yield of Egypt, one of the top ten wheat producing countries. The above results are in line with (Asseng et al., 2017), who argued that, despite the potential to grow more wheat in Sudan assuming the availability of irrigation water, crop yield would be low owing to high temperatures, with forthcoming rises in temperature limiting further production. Hence, farmers are expected to be hard hit by these increased temperatures.

Causality results for millet, reported in Tables 6 and 7, suggest the absence of causality between production and area in all directions. Reasons for this could be attributed to observed negative growth of production and area for the millet crop for most years of the study period (calculated from FAO statistics). In addition, the majority of the population does not consume millet, which is widely grown and consumed by the local population in western Sudan.

Results also indicated the nonexistence of causality for yield and production of millet in either direction, a result that could be explained by the low yield of millet compared to India, one of the top ten millet producing countries, where the millet yield was only 30% of Indian yield during the last decade (Table 1). The above results could also be attributed tothe problems facing women as the main producers of food crops in western Sudan (where the majority of millet is grown and consumed),

Table 1. Yield of selected crops relative to high yield countries (hg/ha).

Sudan as % of highest yield	Country	Highest yield average 2008-2017	Sudan average 2008-2017	Crop
30	India	11218	3357.8	Millet
52	Nigeria	11838	6180.4	Sorghum
33	Egypt	64205	21326	Wheat

Source: authors' calculations based on FAO statistics.

Table 2. Final prediction error of one-dimensional autoregressive processes for area (A), production (P) and yield (Y) of sorghum.

Final prediction error (FPE) of controlled variable			
h	A	P	Y
1	4.96079E-07	4.04101E-06	4.50472E-06
2	3.62588E-07*	1.48751E-06	2.09627E-06
3	3.67046E-07	1.23406E-06*	1.95255E-06
4	3.96381E-07	1.3E-06	1.8178E-06*
5	4.31087E-07	6.14083E-05	2.07833E-06

Source: Authors' calculations; asterisks denote the minimum FPE of autoregressive process; h denotes the lag number.

Table 3. Sorghum causality results.

Controlled variable	Manipulated variable	FPE (h*)	FPE (h*,n*)	Causality results
A (2)	P(3)	3.62588E-07	3.1763E-07	Production causes area
P(3)	A (2)	1.23406E-06	7.22573E-07	Area causes production
P(3)	Y (1)	1.23406E-06*	1.29884E-06	Yield does not cause production
Y(4)	P(1)	1.8178E-06*	4.32247E-08	Production causes yield

Source: Author's calculation. Figures in brackets are the optimum lag orders of variables.

Table 4. Final prediction error of one-dimensional autoregressive processes for area (A), production (P) and yield (Y) of wheat.

Final prediction error (FPE) of controlled variables			
h	A	P	Y
1	0.000164*	3.64E-05*	6.129E-08
2	0.000181	3.89E-05	5.59103E-08
3	0.000199	4.16E-05	5.69077E-08
4	0.000227	4.47E-05	5.19165E-08*
5	0.000242	4.56E-05	5.87051E-08

Source: Authors' calculations; asterisks denote the minimum FPE of autoregressive process; h denotes the lag number.

as indicated by (Ibnouf 2011). The main problem, however, is a lack of the full package of enhanced production methods, including upgraded seeds, fertilizers, recent farming methods, pesticides, credit services,

Table 5. Wheat causality results.

Controlled variable	Manipulated variable	FPE (h*)	FPE (h*,n*)	Causality results
A (1)	P (1)	0.000164*	0.000168	Production does not cause area
P(1)	A (1)	3.64E-05*	3.77E-05	Area does not cause production
P(1)	Y (1)	3.64E-05*	3.75687E-05	Yield does not cause production
Y(4)	P (3)	5.19165E-08*	5.77E-08	Production does not cause yield

Source: author's calculation. Figures in brackets are the optimum lag orders of variables.

Table 6. Final prediction error of one-dimensional autoregressive processes for area (A), production (P) and yield (Y) of millet.

Final prediction error (FPE) of controlled variables			
h	**A**	**P**	**Y**
1	7.31E-08	1.79E-06*	1.74E-08*
2	6.89E-08	9.93E-07	2.02E-08
3	6.23E-08*	7.05E-07	2.51E-08
4	6.9E-08	7.42E-07	3.11E-08
5	7.59E-08	6.47E-07	3.17E-08

Source: Authors' calculations; asterisks denote the minimum FPE of autoregressive process; h denotes the lag number.

Table 7. Millet causality results.

Controlled variable	Manipulated variable	FPE (h*)	FPE (h*,n*)	Causality results
A (3)	P (1)	6.23E-08*	3.39451E-06	Production does not cause area
P(1)	A (3)	1.79E-06*	3.97E-05	Area does not cause production
P(1)	Y (4)	1.79E-06*	7.86E-05	Yield does not cause production
Y(1)	P (3)	1.74E-08*	1.07E-06	Production does not cause yield

Source: author's calculation. Figures in brackets are the optimum lag orders of variables.

proper technologies and marketing services.

Conclusion

The present research results indicate an emphasis on horizontal expansion for the major staple food crop in Sudan, while neither horizontal nor vertical expansion is proved for either millet or wheat. The main policy lesson derived from the above causality results concerning food crops in Sudanespecially sorghum and milletis to focus more on vertical expansion in developing strategies for sustainable agricultural development. The most economical and practical method to attaining a large increase in yield lies in improving the efficiency of the current agricultural economyadvances in the quality of inputs together with the application of recent technological packages. Regarding the wheat crop, the study recommends further comprehensive research on comparative advantage, developing heat-tolerant varieties and the economic viability of growing wheat in Sudan.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Assessment of physicochemical properties of Besease wetland soils, Ghana

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The shallow and erodible soils of low fertility uplands have led to farmers extending their cultivable areas to wetlands for optimal crop production since these systems have the potential for exploitation in the dry season. To ensure its sustainable use, the physicochemical and the hydrological characteristics of the valley bottom should be ascertained. Studies were conducted to assess the suitability of wetlands for crop production by analysing the physicochemical properties of Besease wetland soils. Soil samples were collected from specific sites and profile pits for physical and chemical analysis in the laboratory. Field experiments were also conducted for soil physical properties. Soil textural analysis revealed that the average texture of the Besease inland valley was sandy loam with the distribution of sand, silt and clay as 55.42, 35.04 and 9.50%, respectively. Bulk density and moisture content on the field increased with depth in all profiles. Results of hydraulic conductivity using the mini disk infiltrometer ranged from 2 to 88.3 cm/day. The infiltration rate on the studied wetland ranged from 0.02 to 0.78 cm/min. The pH, OC, TN and CEC of the soil profile distribution for site P11-P14 obtained ranged from 6.9-4.6, 4.69-0.19%, 0.2-0.01%, 9- 2.6 meq/100 g down the horizon respectively. The study unraveled a sustained plant nutrient availability and elongation of water level ponding which will result in increased water storage under rice cultivation in the studied wetland.

Key words: Wetlands, physicochemical, crop production, nutrient, water storage.

INTRODUCTION

Production of crops in Ghana has generally been restricted to upland farms, which constitute about 70% of the country's total land area (Masoud et al., 2013). In Ghana, rice production is mostly confided to the inland valleys (e.g. wetlands) forming about 12% of the total land area. Inland valleys in Ghana have been documented to exhibit high potential for rice production, especially at the small-scale level, due to their suitability in terms of physical, chemical and biological properties (Annan-Afful et al., 2005; Nakamura et al., 2016). Wetlands have been endowed with specific structural and functional attribute performing major ecological roles in the biosphere

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(Mahajan, 1991; Reddy et al., 2013; Alam et al., 2018). The quality of soil in wetland is of vital concern for mankind since it is directly linked with human welfare (Sultana et al., 2017). In Ghana, there exist fragmented areas of inland valleys, especially in the semi-deciduous agroecological zone, which has been left unattended to or underutilised. Crop production in upland areas within sub-Saharan Africa have been faced with increased limitations chiefly from erratic rainfall patterns and poor soil fertility. These limitations have led to increased awareness and development of inland valleys for sustainable crop production (Andriesse et al., 1993). Ejisu-Besease, located in the semi-deciduous agroecological zone of Ghana, is endowed with the presence of the Oda River with which sustenance small-scale farmers usually crop in wetlands around the river boundaries as an alternate source of irrigation. Several initiatives by the Rice Sector Support Project (RSSP), Council for Scientific and Industrial Research (CSIR) and the Government of Ghana (GoG) have revealed the important role of wetlands, which can be used for crop production during the dry season to ensure food security (Atta-Darkwa et al., 2016).

The people of Besease normally use the swamp land for rice cultivation. During high rainfall intensities, the runoff and overflow of the river run over the cultivated area causing it to be flooded. The flood damages the crops, stays on the field for few weeks leading to subsequent leaching of fertiliser applied to the field. The behaviour of the catchment (alternating flooding and receding of water in the wetland) will have ramifications on the soils fertility. Therefore, understanding the spatial and temporal characteristics and variability of wetland soil physical and chemical properties is key for proper utilization and management of agricultural soils.

The cultivation of crops (e.g. rice, maize, millet) in any given environment encompasses a complex interaction between the environment, soil parameters and nutrient dynamics (Ololade et al., 2010). The physical properties of soil according to Mamun et al. (2011), determines the availability of oxygen, the mobility of water into or through soils and the ease of root penetration. The chemical properties of soils include Soil pH, cation exchange capacity, mineral solubility and availability. Delgado and Gomez (2016) posited that soils offer support and act as a reservoir of water and nutrients. Lack of agricultural inputs, continuous cultivation practice, uncontrolled drainage, coupled with environmental factors aggravates the degradation of soil physicochemical properties (Habtamu, 2011). Therefore, a decline in the fertility of soil and the weakening of soil strength would lead to low crop productivity and threatens food security. For wetlands which can support year round cultivation, there is the need to investigate its soil quality in an effort to stabilize and sustain agricultural productivity of the soils. This study sought to analyse the physico-chemical properties of Besease wetland soils and apply the necessary management options to sustain agricultural crop production.

Study area

The study was conducted in the Besease inland valley which has a total land area of about 72 hectares (Figure 1). Besease, a farming community found in the Semi-deciduous agro-ecological zone of Ghana, is located in the Ejisu Municipal District of the Ashanti Region in Ghana. The area geographically lies between latitude 1º 15` N and 1º 45` N and longitude 6º15` W and 7º 00` W. The area is marked by a bimodal rainfall pattern, thus the rainy season and the dry season. The two distinct seasons are conditioned by the Inter Tropical Convergence Zone (ITCZ). Typically, the major rainy season starts from mid-March to July, followed by a short dry spell, which is then followed by the minor rainy season and begins from September to mid-November. The main dry season proceeds after the minor rainy season and begins from mid-November to mid-March (MoFA, 2018). The Besease inland valley records a mean l rainfall of 1450 mm per annum, with a mean annual temperature ranging from 24 °C to 29 °C and an evapotranspiration (ETo) rate of 1230 mm per year.

According to Kankam-Yeboah et al. (1997), Besease is seasonally drained by the Oda River with a basin extending for about 143 km^2. Soil around the Besease in Ejisu is described as the Offon soil series. This soil series is generally grey to light brownish grey, which are poorly drained alluvial sands and clays. Specifically, the soils are Orthi-ferric Acrisol, Eutric Fluvisol, Gleyic Arenosols, Eutric Gleysols and Dystri-Haplic Nitisol. The Besease aquifer is composed of heterogeneous sequence of layers which is dominated by sand, clayey sand and silts. The valley bottom is developed by small holder farmers who cultivate rice in the wet season and also grow vegetables like cabbage, lettuce, sweet pepper, cauliflower, cucumber and okra and other cereals like maize in the dry season when the water table is low. Internal drainage in the catchment areas of the Besease wetlands are very slow, exhibiting rapid permeability and moderate moisture holding capabilities. Dominant grasses and tree species in the area includes *Chromolaeve ordorata, Imperata cylindrical, Mimosa pigra, Ceiba patendra, Centrosema pubescens, Raphia hookeri (Raphia palm), Alstonia boonei,* and *Malotus oppositifolius*.

MATERIALS AND METHODS

Soil physical properties

Sample collection

Soil samples were collected as described by Tuffour et al. (2019) with core samplers of height 10 cm to an average depth of 100 cm from the field at site P1-P2, P7-P8, P11-P14, and P13-P4 (Figure 1)

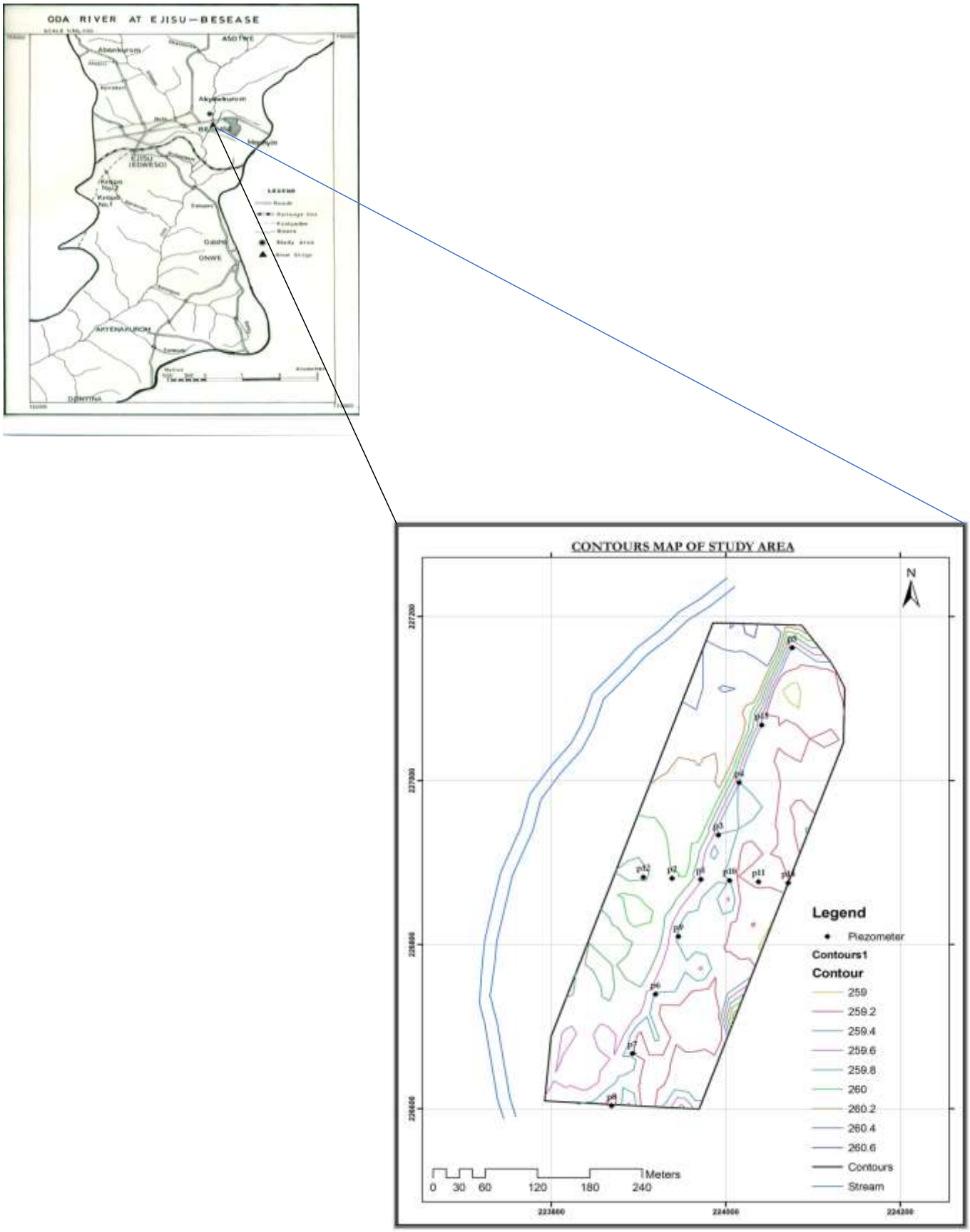

Figure 1. Map of the study area.

and were used for the determination of bulk density and unsaturated hydraulic conductivity. Disturbed soil samples were also taken and air dried, ground and passed through the 2 mm sieve to obtain the soil fractions for the determination of soil physicochemical properties.

Field methods

In the determination of unsaturated hydraulic conductivity, the mini disk infiltrometer was used. The infiltrometer filled with water was positioned to touch with the soil surface at time zero. The volume was recorded at regular time intervals of 30 s as the water infiltrated into the soil at a suction rate of 2 cm which is suitable for most soils. Excel was used to calculate the slope of the curve of the cumulative infiltration versus the square root of time from the infiltrated volume of water recorded. Zhang (1997) formulated an equation for determining the hydraulic conductivity of soil. Infiltration is calculated using the equation: $I = C_1 t + C_2\sqrt{t}$ Where, C_1 (ms^{-1}) and C_2 ($ms^{-½}$), and C_1 is related to hydraulic conductivity and it is the soil sorptivity. The hydraulic conductivity of the soil (*k*) was then computed from:$k = \frac{C_1}{A}$ Where, C_1 is the slope of the curve of the cumulative infiltration versus the square root of time, and A is a value relating the van Genucthen parameters for a given soil type to the suction rate and radius of the infiltrometer disk.

Double ring infiltrometers, consisting of two concentric rings, were used to measure the infiltration rate. The rate of fall of the water level in the inner cylinder was measured at 2, 3, 5, 10, 15, 20, 30, 45 and 60 min and at 30 min intervals thereafter. This was done to obtain a steady-state infiltration rate.

Laboratory methods

The bulk density was determined by using the core sampler method. That was calculated by dividing the oven dried soil mass at 105°C for 24 h (Black, 1965) by the internal volume of the cylinder that was used to collect the sample. The total porosity was then calculated from the bulk density using the equation: Porosity = (1-ρ_b/ρ_s) × 100…(1). Where, ρ_b [g/cm^3] is bulk density and ρ_s is particle density (2.65 g/cm^3). The saturated hydraulic conductivity (K_{sat}) measurements were made on core samples with a length of 10.0 cm and diameter of 8.3 cm using the falling head method developed by Klute and Dirksen (1986). The saturated hydraulic conductivity of the soil samples was calculated by the equation:$K_{sat} = \left(\frac{AL}{A_t}\right) ln\left(\frac{H_1}{H_2}\right)$..(2). Where, K_{sat} is the hydraulic conductivity (LT^{-1}), A is the cross-sectional area of the sample, H_1/H_2 is the difference in the hydraulic head between the up gradient end of the sample and the down gradient end, L is the length of the sample or the distance over which the head is lost, and t is time. The bouyoucos hydrometer method was sued to determine soil texture after deflocculating soil with sodium hexametaphosphate.

Soil pH was determined by using a pH meter (H1 9017 Microprocessor) in a soil-water ratio of 1:1. Carbon content and total nitrogen (TN) concentration at the study site was done using the Walkley and Black method (Nelson and Sommers, 1982) and Kjeldahl digestion and distillation procedure as described by Soil Laboratory Staff (1984). Available phosphorus (P) was determined using a spectronic 21 D spectrophotometer. Exchangeable cations (calcium (Ca), magnesium (Mg), potassium (K), sodium (Na)) were determined using the ammonium acetate (1.0 *M* NH4OAc) (Black, 1986). Effective cation exchange capacity (ECEC) was determined by the summation of exchangeable base (Ca^{2+}, Mg^{2+}, K^+ and Na^+) and exchangeable acidity (H^+ and Al^{3+}). Electrical conductivity of the study site was also determined using the electrical conductivity meter and probe.

RESULTS AND DISCUSSION

Dynamics of soil physical properties

Soil texture has been regarded as a very stable soil physical property with little or no change from external factors (Msanya et al., 2003). It typically influences soil erodibility, water holding capacity, texture, cation exchange capacity, and soil penetrability. Characteristically, the textures of the soils at the Besease Wetlands contrasted distinctly from silt loam to sand among the various soil profiles (Table 1). The average soil texture was loamy sand (73.6% sand, 22.22% silt, 4.15% clay) in the profile pit P1-P2, loam (51.57% sand, 35.70% silt, 12.70% clay) in the profile pit P11-P14, loam (37.80% sand, 47.16% silt, 15.0% clay) in the profile pit P7-P8, and sandy loam (58.64% sand, 35.07% silt, 6.12% clay) in the profile pit P4-P13 respectively. As evident from the Table 1, the average texture of the Besease inland valley was sandy loam with the distribution of sand, silt and clay as 55.42 %, 35.04 % and 9.50 % respectively. From our findings, the coarser nature of the soil exhibited at site P1-P2 controls variations in cation exchange capacity, increased soil permeability and water leaching, hence leading to less water storage. These varied soil textures exhibited at the Besease floodplains could be due to the nature of the parent material which formed the soil above it. Beasease in the Ejisu Municipal is underlaid with complex associations of pre-Cambrian igneous (Birimian formation) and metamorphic rocks. Smyth and Montgomery (1962) reported that parent material (rocks) exhibits various minerology and texture ranging from pegmatite to fine grained schist, and from acid quartzite to basic rocks consisting largely of amphibolites. In their studies on soil texture, Hekstra and Andriesse (1983) observed correlations between rocks and soil texture and they concluded that metamorphic rocks generally tend to generate fairly fine-textured soils, whilst soils formed from granitic parent materials are relatively coarser in nature.

In all the soil profiles, soil bulk density generally increased with increase in soil depth (Figure 2), with its associated decrease in soil porosity. Bulk density and the moisture content on the field increased with depth in all profiles as shown from Figures 2 and 3. As observed from Figure 2, profile pits P7 – P8 and P11 – P14 recorded soil bulk density values ranging from 1.1 g cm-1 to 1.9 g cm-1 within the depths of 0 – 100 cm. However, profile pits P1 – P2 and P13 – P4 generally recorded bulk densities value beyond 2.0 g cm-1 when soil depth increased after 70 – 80 cm. The increase in soil bulk density with increasing soil depth in the various soil profiles could be indicative of dense packing of soil particles (compaction), and thus impact on soil porosity and root permeability. Landon (1991) observed that increase is soil bulk density significantly influence soil aeration and porosity, affecting root establishment, and ultimately impact on nutrient uptake and crop yields. Low

Table 1. Particle size analysis for the Besease Inland valley bottom site.

Profile pit	Depth of soil (cm)	% Sand	% Silt	% Clay	Texture
P11-P14	0-10	31.58	56.42	12	Silt Loam
	20-30	17.54	60.46	22	Silt Loam
	20-30	38.32	44.58	16.8	Loam
	30-40	51.94	36.06	12	Loam
	40-50	45.58	36.22	18.2	Loam
	50-60	63.9	24.1	12	Sandy Loam
	60-70	76.38	17.42	6.2	Loamy Sand
	70-80	87.32	10.28	2.4	Sandy
P1-P2	0-10	63.04	34.96	2	Sandy Loam
	20-30	62.06	35.74	2.2	Sandy Loam
	20-30	63.34	31.66	5	Sandy Loam
	30-40	63.92	29.88	6.2	Sandy Loam
	40-50	61.76	31.84	6.4	Sandy Loam
	50-60	60.14	31.86	8	Sandy Loam
	60-70	59.34	32.66	8	Sandy Loam
	70-80	65.6	28.2	6.2	Sandy Loam
	80-90	80.58	17.42	2	Loamy Sand
	90-100	87.58	10.42	2	Sand
	100-110	95.12	2.88	2	Sand
	110-120	96.69	1.04	2	Sand
	120-130	97.76	0.24	2	Sand
P7-P8	0-10	17.2	69.2	13.6	Silt Loam
	20-30	15.84	65.96	18.2	Silt Loam
	20-30	21.02	60.98	18	Silt Loam
	30-40	27.16	50.74	22.1	Silt Loam
	40-50	31.38	45.62	23	Loam
	50-60	34.96	47.84	17.2	Silt Loam
	60-70	40.82	42.18	17	Loam
	70-80	45.68	40.42	13.9	Loam
	80-90	59.64	35.36	5	Sandy Loam
	90-100	84.74	13.26	2	Loamy Sand
P4-P13	0-10	65.22	32.78	2	Sandy Loam
	20-30	64.18	31.82	4	Sandy Loam
	20-30	59.96	34.04	4	Sandy Loam
	30-40	57.04	37.16	5.8	Sandy Loam
	40-50	56.34	34.46	9.2	Sandy Loam
	50-60	55.74	35.86	8.4	Sandy Loam
	60-70	56.84	34.76	8.4	Sandy Loam
	70-80	56.84	34.76	8.4	Sandy Loam
	80-90	57.38	33.82	8.8	Sandy Loam
	90-100	54.38	39.82	5.8	Sandy Loam
	100-110	54.68	39.52	5.8	Sandy Loam
	110-120	60.76	33.44	5.8	Sandy Loam
	120-130	62.96	33.84	3.2	Sandy Loam

soil compaction observed in the top layers of our study could be linked to presence of organic matter at this layer, low soil strength, and less mechanical manipulation by tillage implements (Gachene and Kimaru, 2003). Thus, the only restrictive conditions in such layers may arise from the depth of the underlying water table, which

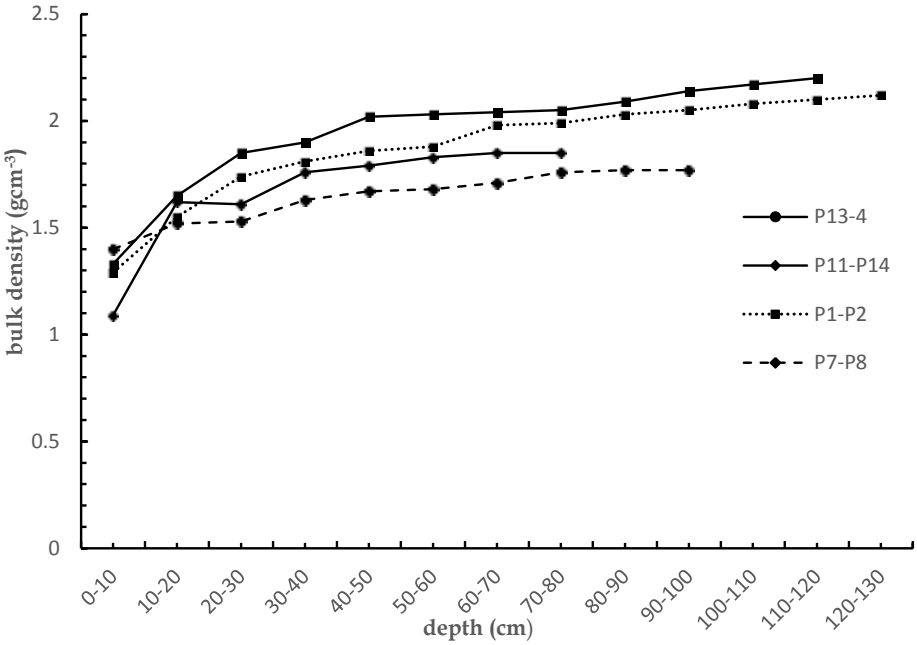

Figure 2. Relationship between bulk density and depth of the various profiles.

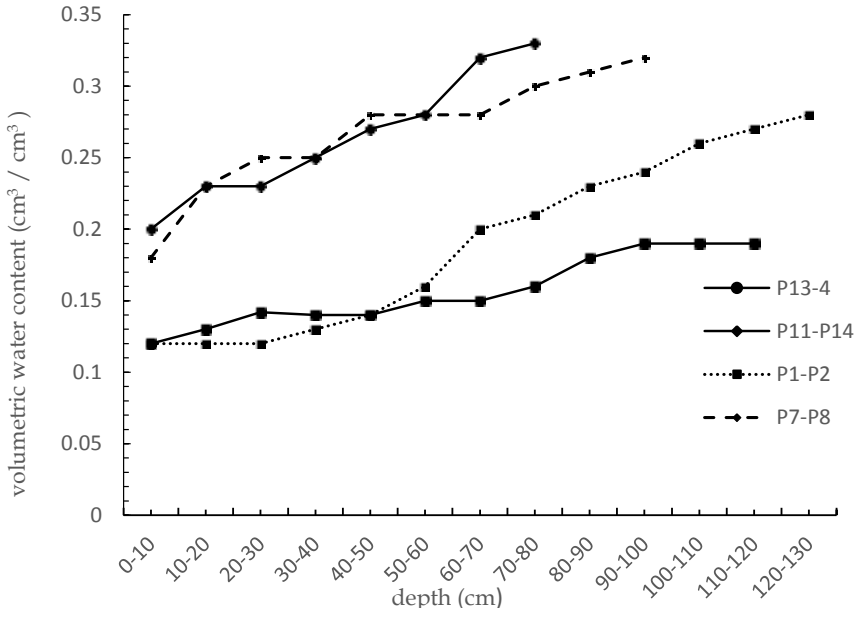

Figure 3. Relationship between volumetric water content and depth of the various profiles.

may come up due to capillary action, or varies between the dry and wet seasons. Porosity, an indirect measure of soil compaction was, however, higher in the surface layers, which decreased further down the soil profile. It could be observed from Figure 4 that, profile pit P7 – P8 generally recorded the highest porosity with increasing soil depth whilst profile pit P13 – P4 recorded the least values of soil porosity. This implies that the horizontal movement of moisture would be favoured as opposed to the vertical movement of soil moisture. This conclusion could further be explained from Figure 4 which demonstrated that, a fine textured soil with improved soil aggregation and high in soil OM exhibits high soil macro porosity than a compact and massive soil. Understanding

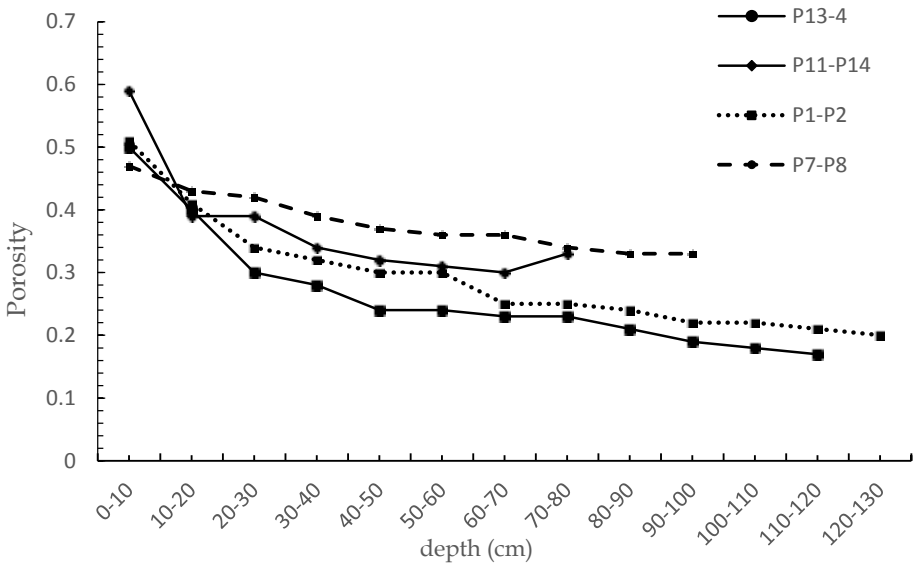

Figure 4. Relationship between porosity and depth of the various profiles.

the mechanisms that control the rate of water infiltration and percolation through the soil system are of great importance as they influence runoff and subsequent excess overland flow.

Hydraulic and infiltration properties of soils

Saturated hydraulic conductivity (K_{sat}), has been denoted as the degree of the soil to transmitted water under saturated conditions which has been subjected to a hydraulic gradient. A unit volume of water passing through a unit cross sectional area of soil in inland valley bottoms reflect differences in hydraulic properties of soils because as fluid flow increases, inter-aggregate pores reduce the possibility of obtaining equilibrated pore water pressure profiles. Hence, macropore continuity and the more tortuous pore system found at the western portion of the valley bottom where fauna activity and high root density dominated enable preferential flow particularly at saturation, thereby giving high conductivity values (Table 2 to 4).

Although macropores make up a relatively small fraction of a soil's total porosity (Watson and Luxmoore, 1986), they can have a disproportionate effect on the soil's infiltration properties. For example, German and Beven (1981) demonstrated that small amounts of macropores could increase saturated hydraulic conductivity by more than an order of magnitude in soils with low-to-moderate matrix conductivity. The vertical K_{sat} measured in Besease sites were not the same for each depth of sample collected. Conductivity tests revealed that K_{sat} varied spatially within the site, and that each layer possesses different conductivity values. For instance, over the first 20 cm of depth, K_{sat} ranged from 6.02-4.9 cm/day and in the lower depth of 70 cm was 0.002 cm/day (Table 3). The vertical flow direction within layers was likely to be different, because layers show marked differences in vertical hydraulic conductivity. Particle size also affects conductivity of soils. Soils constituted by clay can have different infiltration characteristics depending on the amount of aggregation present. The presence of clay mostly indicates a low K_{sat}, but may on the other hand be subjected to cracks and macropores (in comparison to a coarser grained soil), and thus give rise to higher K_{sat}. Profile P11-P14 pit had higher clay content than the other pit sampled which experienced a lower K_{sat} (Table 1). Such a site undergoes longevity in the hydro-period which also lowers hydraulic conductivity. Soils with high clay content subjected to decreasing water content govern the conditions for crack formation. The cracks form a network of macropores which will be of great importance for water infiltration (Vogel et al., 2005). The flow in the unsaturated soil at the study sites is more complicated than flow through a continuously saturated pore space. Within unsaturated soils, macropores are filled with air leaving the finer pores to accommodate water movement. Therefore, gravity does not dictate the movement of water through the soil but rather differences in matric potential. Sobieraj et al. (2004) attributed the differences in K_{sat} to microbial processes, especially in cases with clay rich soils at shallow depth. They also suggest that the classical theory of K_{sat} being mostly influenced by particle size is only true for soils consisting of more than 80% sand. Topography and slope greatly influence the microclimatic properties in the soil, and hence also the physical properties (Casanova et al., 2000). Fine textured soils are often found at the bottom of slopes, and have small water intake and large runoff potential (Casanova et al., 2000).

Table 2. Spatial saturated hydraulic conductivity of the site from falling head method.

Location	Depth of soil	
	10 cm	20 cm
	K (cm/d)	
P1-P2	6.021	4.896
P6-P9	4.885	1.438
P7-P8	0.198	0.365
P3-P4	5.502	5.178
P5-P13	1.091	0.155
P11-14	0.107	0.101
NU P6-P7	0.320	1.033
NRP4-P13	1.346	1.123
NUP4-P13	0.463	0.465

NR- Near river, UP- Near Upland.

Table 3. Spatial saturated hydraulic conductivity of the site from falling head method.

Depth (cm)	K_{sat} (cm/d) for P1-P2 Profile
10	6.021
20	4.896
30	0.463
40	0.877
50	0.620
60	0.107
70	0.002
80	0.119

Table 4. Hydraulic conductivity of the site using the mini disc infiltrometer.

Location	Hydraulic conductivity (cm/d)
NU P7-P8	88.3
NRP4-P13	22.0
NR P7-P8	2.20
P1-P2	66.3
P7-P8	5.44
P3-P1	44.2
P6-P7	66.3
P11-P14	2.00
P6-P9	66.3
P10-P11	54.5
P4-P13	18.1
P1-P9	16.7

NR- Near river,NU- Near Upland.

The process of erosion should be greater at higher slopes and thus give rise to a deposition of finer particles at gentle slopes (Casanova et al., 2000). Therefore, profile pit at P1-P2 at a higher elevation showed a high K_{sat}.

The results from Figures 5 to 8 show a plot of cumulative infiltration against the square root of time. The coefficients of correlation, R^2 ranges from 0.9915 to 0.9982 for hydraulic conductivity experiment from sites P1-P2, P6-P9, NR P6-P7 and NRP13-P4. Values of R^2 range from 0 to 1 and as the value approaches 1, the better the relationship between variables. The correlation coefficient obtained meant a very strong positive relationship between cumulative infiltration and square root of time.

Infiltration characteristics of Besease Inland valley bottom

From our results, the rate of infiltration in the Besease valley bottom varied remarkedly under gravity and capillary forces. From the study, the floodplains at Besease showed a high infiltration rate which declined gradually over time (Figures 9 to 11). The higher infiltration

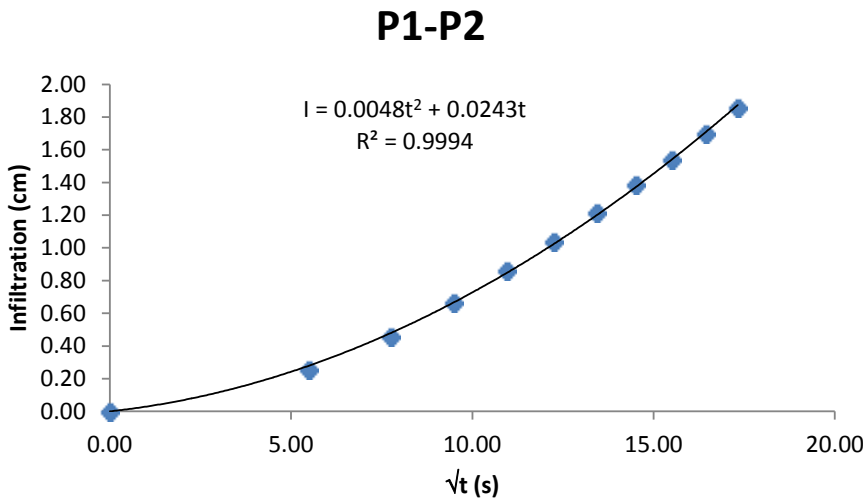

Figure 5. Cumulative infiltration versus the square root of time for P1-P2.

rate at the Besease inland valley bottom may be associated with capillary forces acting on the water, as well as the effect of gravity. Variations in infiltration rates are facilitated by extensive root system and animals burrowing in the soil, inadequate prewetting, and soil disturbance by the infiltration ring. The infiltration rate on the studied floodplain ranged from 0.02-0.78 cm/min (Table 5). The average infiltration rate for the entire population was 0.28 cm/min. Site P1-P2 with high percent sand fraction had the highest infiltration rate of 0.78cm/min. Site P11-P14 and site P8-P7 at lower elevation with low percent sand and moderate clay content (Figures 9 to 11) exhibited a low infiltration rate of 0.02 cm/min and 0.06 cm/min respectively. This shows that water level ponding could elongate, which could result in increased water storage under rice cultivation in the floodplain at Besease.

Characteristics of the soil chemical properties

The soil pH was higher at site P11-P14 followed by P4-P3, P7-P8 and P1-P2 (Figure 12). The OM content of the soils was highest (6.38%) in the P7-P8 area. A relatively higher value was recorded at P11-P14 (4.69%) and the lowest values were observed at P4-P13 and P1-P2 (Figure 13). Also, the highest level of total nitrogen was recorded at P7-P8 followed by P11-P14, P4-P13 and P1-P2. Site P11-P14 had the highest eCEC which was slightly higher than that of P1-P2 (Fig 17). This was followed by P7-P8 and P4-P13 in decreasing order. Again, the electrical conductivity (EC) was higher at site P11-P14 followed by P4-P3, P1-P2 and P7-P8 (Figure 14). The sodium absorption ratio (SAR) also varied in the wetland for which 0.376 mg/l was observed at P7-P8, as the highest. P11-P14, P4-P13 and P1-P2 followed in decreasing order (Figure 14). The soil profile distribution for the site from the top 10 cm to the bottom 80 cm horizon showed that pH, total nitrogen and organic matter decreased slightly with depth (Table 6). However, the exchangeable cations decreased with depth and there was a slight change at the 40 cm depth and continued to decrease again except Ca and Mg which showed some variations from high to low and vice versa from the 40 cm to the bottom 80 cm.

Dynamics of soil chemical properties

The valley system exhibits a slightly acidic to a moderate acidity and this was also replicated in the profile pit P11-P14 ranging from slightly acid in the topsoil to moderately acid in the bottom horizon. A lower soil pH may be due to addition of inorganic fertilizers (e.g. urea), loss of organic matter through erosion, and the leaching of basic cations as a result of seasonal flooding of the wetland (Nakamura et al., 2016; Agbeshie and Adjei, 2019). For rice production, the results obtained is suitable, considering that, upon reduction of the (top) soil following submergence, the pH tends to change towards neutral (pH 6.5-7.0). Most plant nutrients are most readily available for uptake by roots in a slightly- acid to near-neutral environment (IRRI, 1978). The high organic matter content and total nitrogen in the surface layers of P7-P8 and P11-P14 were attributed to concentration of vegetation litter and that decomposition processes are usually slow in hydromorphic soils. However, in waterlogging conditions as it is always experienced in valley systems, it reduces N, availability due to low mineralization rates and the risk of denitrification under alternating wet and dry conditions (Annan-Afful et al, 2005). These loss mechanisms act most severely in strongly alternating wet and dry environments such as the Besease Wetlands. During the soil drying phase,

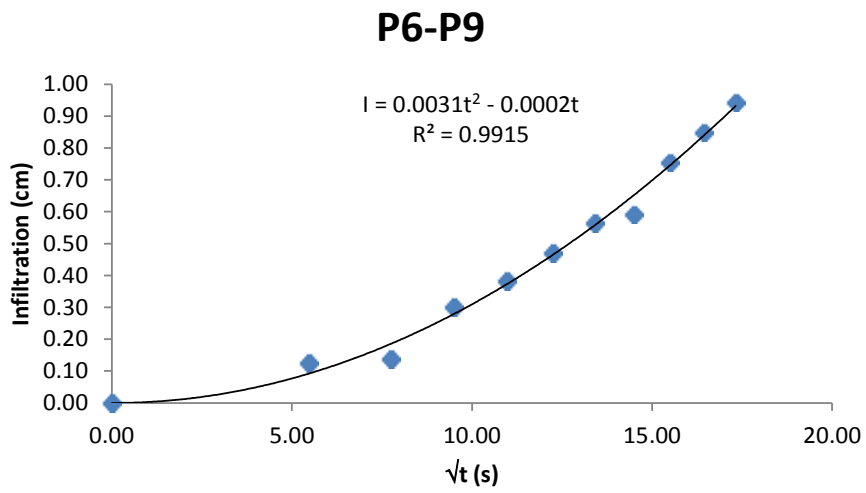

Figure 6. Cumulative infiltration versus the square root of time for P6-P9.

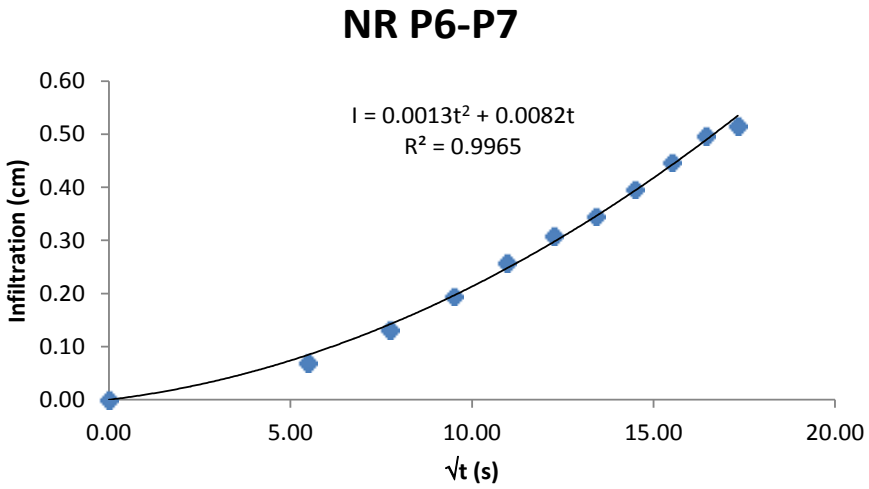

Figure 7. Cumulative Infiltration Versus the Square Root of Time for P6-P7.

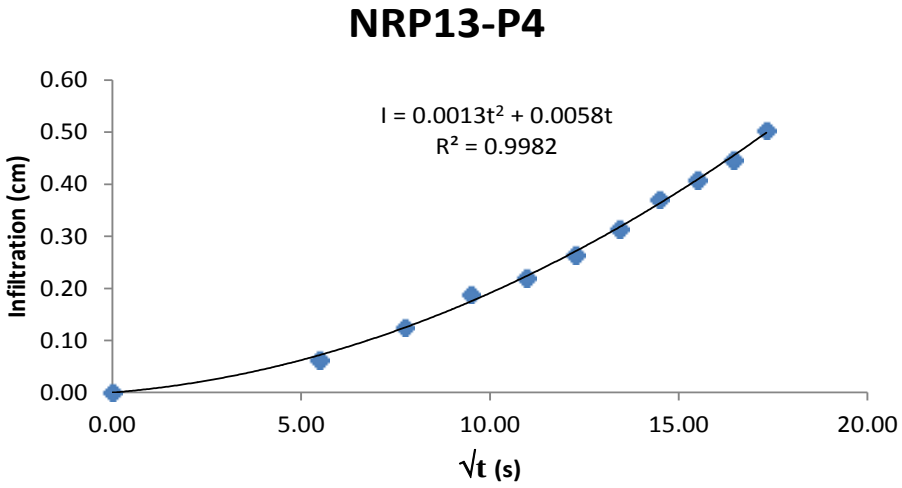

Figure 8. Cumulative Infiltration versus the Square Root of Time for P13-P4.

Table 5. Infiltration rates of the Besease Wetland Site.

Site	Infiltration capacity (cm/min)
P1-P2	0.78
P1-P9	0.63
P6-P9	0.05
P7-P8	0.06
P4-P13	0.15
P11-P14	0.02

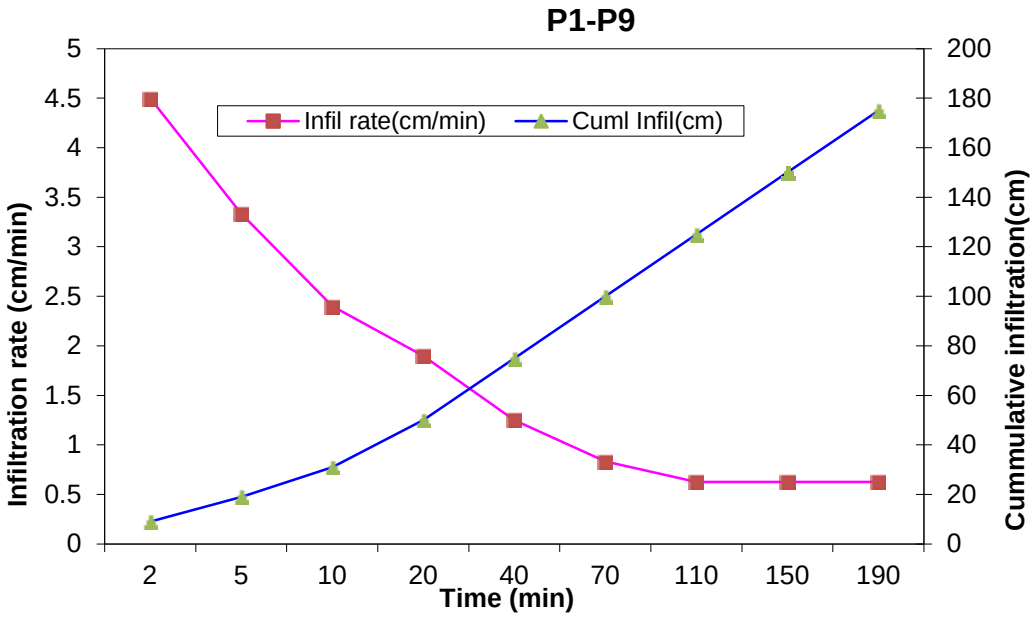

Figure 9. Infiltration in Besease Wetland Site P1-P9.

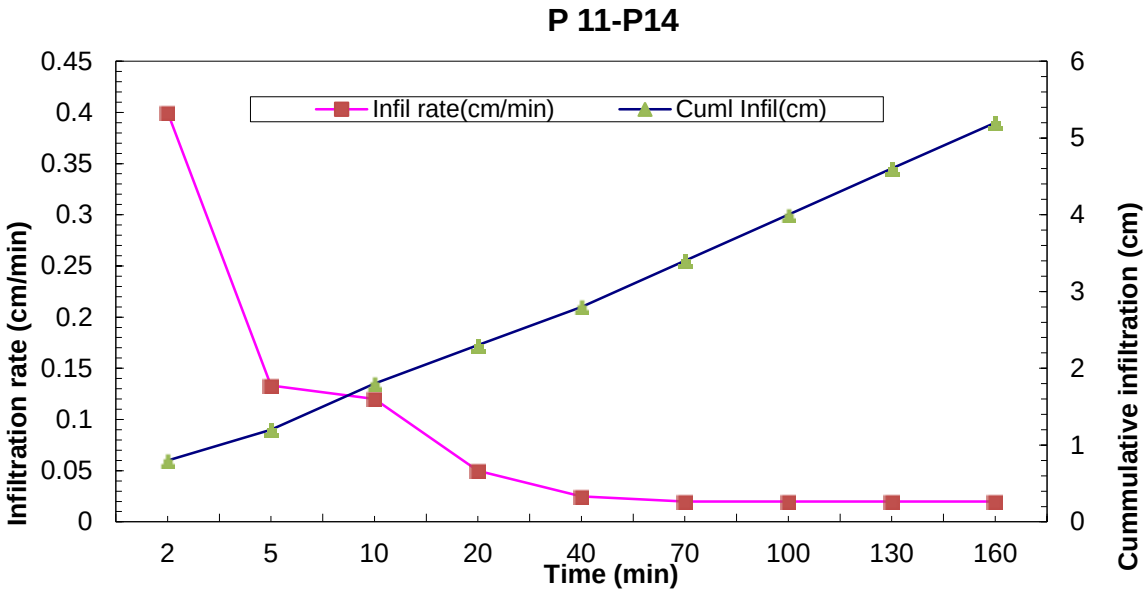

Figure 10. Infiltration in Besease Wetland Site P11-P14.

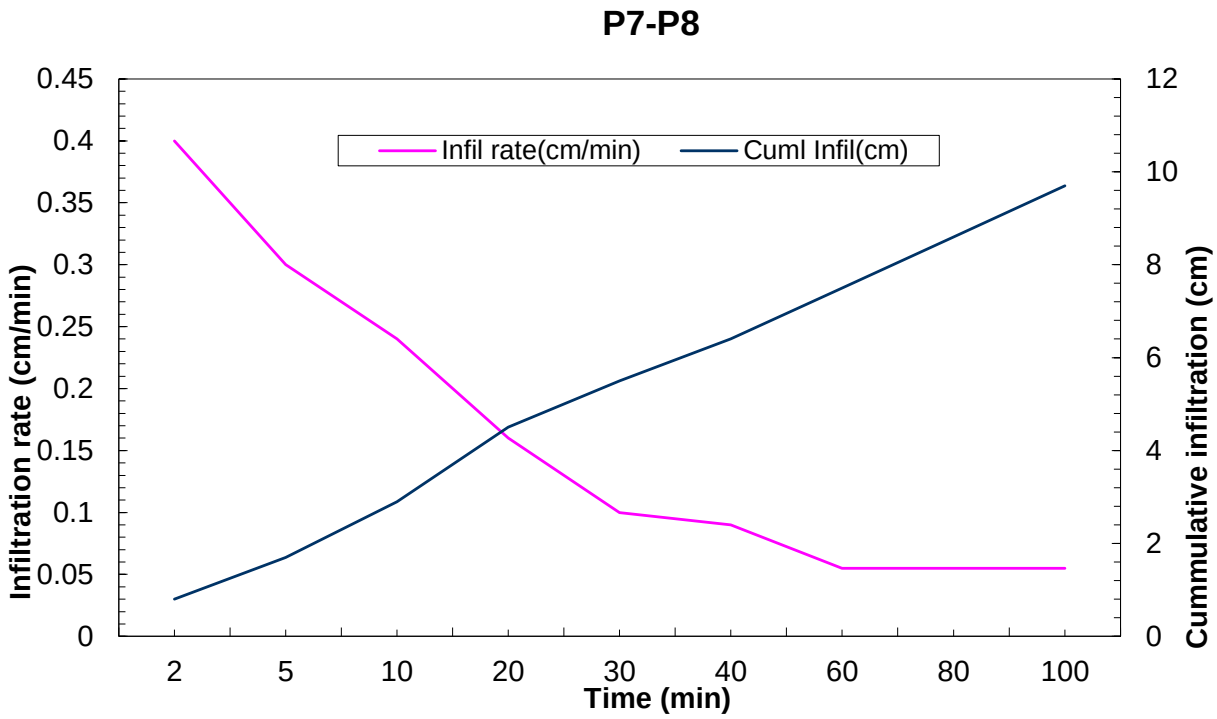

Figure 11. Infiltration in Besease Wetland Site P7-P8.

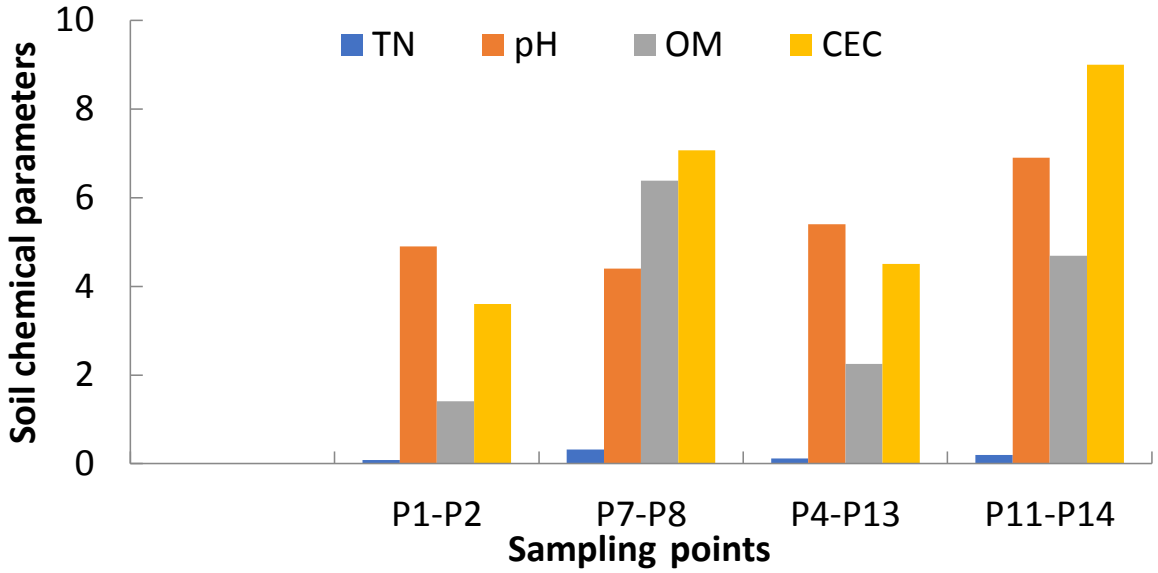

Figure 12. Soil pH, Organic Matter (OM), Total Nitrogen (TN) and ECEC of the Wetland.

reduced forms of N, particularly NH_4^+, are nitrified to NO_3^- (Sanchez, 1976). After soil flooding, NO_3^- may be lost by leaching or by denitrification to N gasses. To ameliorate the losses of N, efficient use of fertiliser application must be employed. The higher eCEC at site P11-P14 and P7-P8 was as a result of higher clay content (Table 1) and organic matter coupled with sedimentation and less leaching. The higher EC observed at P11-P14 may be due to possible groundwater discharge and evaporation associated with the area. The SAR observed from the four sampling points (Figure 14) shows the valley systems suitability for crop production.

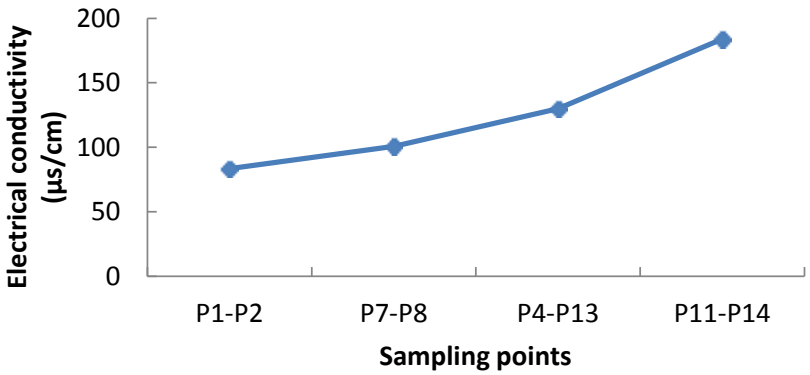

Figure 13. Electrical conductivity for the different sampling points.

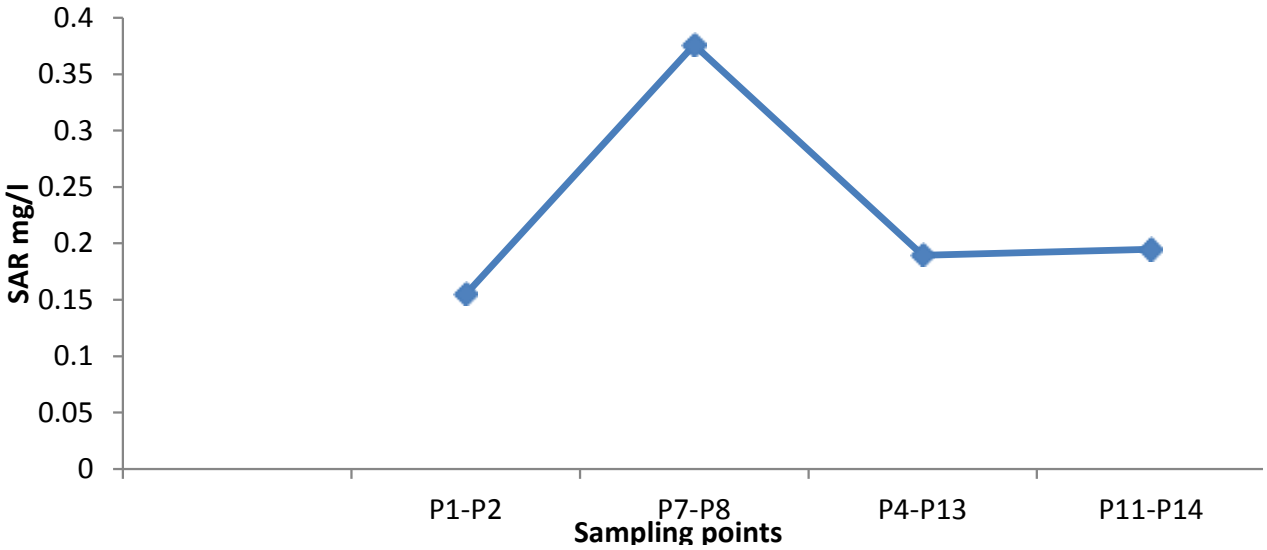

Figure 14. SAR for the different sampling points.

Table 6. Chemical properties of soils.

Horizon	pH	Org. C %	Total N %	Org. M %	Exchangeable cations me/100 g				C.E.C me/100 g
					Ca	Mg	K	Na	
0-10	6.9	2.72	0.2	4.69	4.81	3.20	0.50	0.39	9.00
10-20	6.5	0.60	0.05	1.03	2.94	1.87	0.45	0.24	5.85
20-30	5.6	0.41	0.03	0.70	1.87	1.34	0.28	0.18	4.22
30-40	5.3	0.21	0.03	0.36	1.34	0.94	0.24	0.15	3.32
+40-50	5.5	0.18	0.03	0.31	1.60	1.20	0.31	0.15	3.76
50-60	4.7	0.14	0.01	0.25	0.80	0.27	0.20	0.10	2.27
60-70	5.1	0.11	0.01	0.19	1.07	0.53	0.15	0.08	2.58
70-80	4.6	0.11	0.01	0.19	0.80	0.53	0.15	0.07	2.60

Conclusion

The assessment of wetlands fertility and water holding capacity is a prerequisite for the development of wetlands for crop production. The saturated hydraulic conductivity was high at the soil profile pit P1-P2 which was at a

higher elevation. However, a lower saturated hydraulic conductivity which was experienced at profile pit P11-P14 at a lower elevation with characteristic fine textured soils indicated a small water intake. Also possible elongation of water level ponding at P7-P8 and P11-P14 with low infiltration rates of 0.06 cm/min and 0.02 cm/min showed an increase in water storage that is ideal for rice production. The higher EC observed at P11-P14 may be due to possible groundwater discharge and evaporation associated with the area. The SAR observed from the four sampling points showed the valley system suitability for crop production. There is the possibility of sustained plant nutrient and elongation of water level ponding which will result in an increased water storage under rice cultivation in the studied wetland.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

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New microchondrometer to measure hectoliter weight in small samples of wheat

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The hectoliter weight or test weight is an important wheat quality parameter for international trade and is traditionally evaluated on devices with a volume of 250, 500, 1000 or 1100 ml. At the experimental level, especially in crop improvement and in greenhouse studies, the amount of grain is often insufficient to determine hectoliter weight. The present work aimed to evaluate the feasibility of using a new 15.30 ml microchondrometer to evaluate hectoliter weight. The testing process was carried out in two steps: (i) To evaluate the need to compress wheat grains inside the microchondrometer cylinder with weights of 0, 4.4, 8.8 and 13.2 kg, and (ii) To verify the effect of different piston weights (4.0, 9.52, 17.56, 28.44 and 31.69 g). A comparison of four compression treatments and five piston weights between 250 ml and 15.30 ml chondrometers were performed by Spearman's correlation coefficient and t-test. The results showed a highly significant correlation coefficient (r=0.99) between the two apparatus and lack of significance for compression and piston weights. The 15.30 ml microchondrometer, in addition to allowing better characterization in small grain samples, will also help to discard unwanted genotypes early in the selection process.

Key words: Plant breeding, grain density, genotype screening.

INTRODUCTION

Test weight or hectoliter weight, a physical quality parameter commonly used in the cereals, is an estimate of bulk density (g cm^{-3}) and the most used indicator by the milling industry. Any damage caused by weathering, shriveled or immature grains as well as rain-induced field sprouting tends to reduce test weight (Donelson et al., 2002). Besides genetic differences among varieties, other stress factors such as, nutrient deficiencies, high temperature during grain filling, plant lodging, insect damage, or adverse weather events like frost and hail also affect test weight negatively (Isleib, 2012). According to Ilker et al. (2009), a safety-first selection index may be effective in selecting superior wheat genotypes especially for test weight, which is one of the physical quality parameters important in determining flour yield in wheat. The hectoliter weight has also been positively correlated with grain yield (Iqbal et al., 2016) but greatly influenced by the environment (Joshi et al., 2018).

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Besides cereals, where test weight is most frequently used, it may also be practiced in the field crops like, millets, small millets, pulses, oilseeds, fiber, fodder and green manure crops, where higher values generally fetch a better price and provide a better quality of grains (Deivasigamani and Swaminathan, 2018).

The test weight has been accepted as a measure of the physical quality of wheat and other cereals in the international trade due to its simple and expeditious measurements. All else being equal, a high-test weight variety is likely to produce more flour.Hence, this trait is used as an indicator for the evaluation of milling quality. High-quality wheat is generally above 76 kg hl^{-1}, while a value below this limit implies wheat of low quality (Protic et al*.,* 2007).

The devices and volumes for hectoliter determination vary among grain-producing and/or trading countries. According to Manley et al. (2009), two types of devices are being used at present: i) those equipped with a funnel which provides uniform packing in a 500-ml (South Africa and Canada) or 1100-ml (USA) measuring cups and ii) chondrometer of 500 ml (Australia and the United Kingdom) or 1000 ml volume (France and Germany). For experimental purposes, a 250-ml chondrometer has been an option to measure test weight (Stagnari et al., 2008; Durazzo et al., 2015; Botelho et al., 2018).

In studies on nutrition investigations, preharvest quality surveys, crop breeding, and wheat grown in nutrient cultures, there are limitations on the volume of the grain produced, which in turn impedes measuring the hectoliter weight (Harris and Sibbitt, 1942).

The need to develop a method for determining the hectoliter weight in small samples was stressed by Aamodt and Torrie (1934). To overcome it, a 25 cc graduated cylinder, cut off at the 4 cc point, was used to measure the grain from individual plants. Similarly, Harris and Sibbitt (1941) described a procedure for determining test weight in which 4-ml and 16-ml measures were used. The glass measures were made from standard graduated cylinders by cutting off the lower end at the proper height. The discrepancies between the values of the 4 cc micro and standard methods observed in relation to Aamodt and Torrie (1934) were attributed to differences of the technique in making the micro determinations (Harris and Sibbitt, 1941).

Three decades later, Ghaderi et al. (1971) developed another micro-test weight procedure using a small (47 ml) glass jar to evaluate fifty-nine cultivars and advanced lines of soft winter wheat. They reported a high correlation (r=0.982) between their values and the standard test weight, suggesting a micro-test to be a reliable predictor.

Using a glass 100-cm^3 graduated cylinder (approximate height: 248 mm, sub-divisions: 1 mL), Donelson et al. (2002) also evaluated the hectoliter weight in 20 and 40 g wheat samples. They observed a relationship between specific gravity and linear weight test unless the samples were severely shriveled. While both 20 and 40 g samples produced satisfactory data, those of 40 g were statistically superior.

More recently, Stepochkina and Stepochkin (2015) evaluated wheat grains in a small cylindrical container (diameter 1.8 cm, height 1.125 cm) of 2.86 cm^3 volume. On comparison with standard 0.25 Lchondrometer, they found a correlation coefficient of 0.98.

As the methodologies described above are based on the use of different volumes of the glass-graduated cylinders, no standard equipment has been made till date to determine hectoliter weight in small samples. The objective of this work was to evaluate the feasibility of using a new 15.30 ml microchondrometerto achieve this goal in small wheat samples.

MATERIALS AND METHODS

The 15.30 ml microchondrometer, which requires about 20 g of wheat grains, was designed and manufactured based on a 250-ml chondrometer of the DalleMolle® company. The specifications of 250 ml and 15.30 mlchondrometers were respectively: total height (cm): 39.00 and 18.60; total weight (g): 949.32 and 639.60; external diameter (mm): 56.18 and 28.49; cutter bar (g): 70.55 and 16.43; piston volume (cm^3): 61.58 and 3.59.

In order to evaluate the efficiency of the new equipment, two experiments were conducted in 2016 and 2017, at the Instituto Agronômico do Parana (IAPAR), Londrina, Brazil, to compare the data between the microchondrometer and commercial 250-ml chondrometer. The hectoliter weight was evaluated by weighing the grains that filled the cylinder on a digital scale (Marte® AS2000; 0.01 g). The weights thus obtained were multiplied by 6.5359 for the 15.30 ml microchondrometerand 0.4 for the 250-ml chromometer.

The first experiment was conducted to evaluate the need to compress the wheat grains inside the microchondrometer cylinder, while the second wasdesigned to verify the effect of the piston weights. Approximately 800 g of wheat grain samples used in both experiments were obtained from experimental plots, farmers' fields and samples submitted to forced sprouting in a humid chamber.

The compression experiment consisted of four treatments (0; 4.4; 8.8; and 13.2 kg weights) in the microchondrometer, using 28.44 g piston. With the exception of the check (0 kg), the treatments were applied to the upper part of the samples placed inside the microchondrometer. The weights were applied by means of an iron structure, using steel cable, pulley and lever, and released on top of the grain samples with a wooden rod (18.05 mm in diameter and 13.70 cm in length) (Figure 1). Fifty wheat grain samples were evaluated by microchondrometer, in three replications, for two years and the results were compared with the data from commercial 250-ml chondrometer.

Additional evaluations were performed to verify the effect of the microchondrometer piston weights on the hectoliter weight. Five different piston weights (4.0, 9.52, 17.56, 28.44, 31.69 g) were used, with the same volume, made of plastic, aluminum, bronze with durepoxy, steel and bronze, respectively. Sixty wheat grain samples were evaluated, in three replications, for two years and the results were compared with the data from commercial 250-ml chondrometer (Figure 2).

The mean values (three replications) offour compression treatments and five piston weights were compared between two chondrometers using the Spearman´s correlation coefficient and the Student's t-test by the Microsoft Excel 2013 software and SAS package (SAS, 2001).

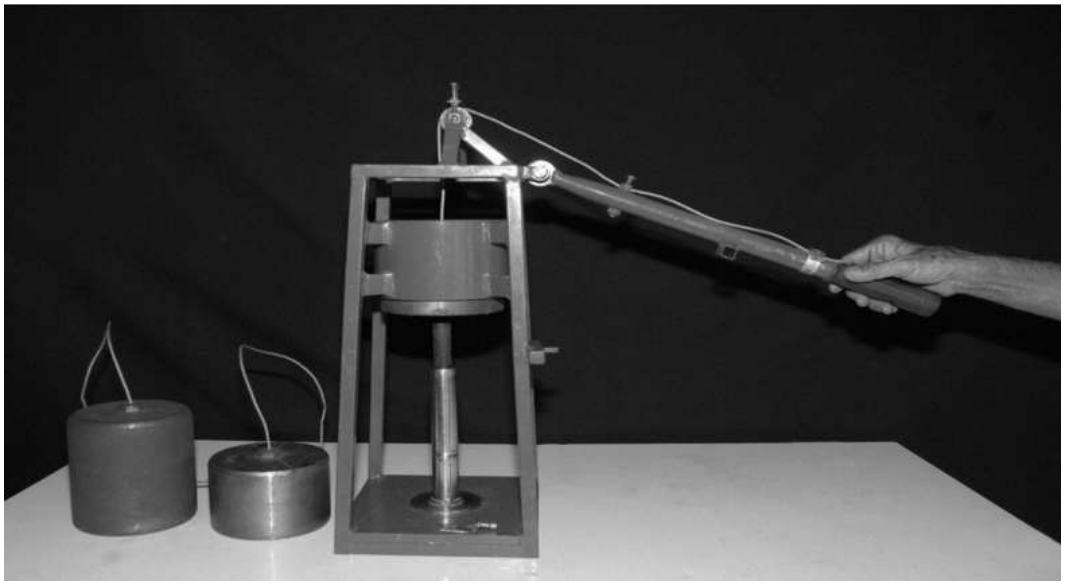

Figure 1. Compression treatment: from left to right: 13.2 and 8.8 kg weights; iron structure with 4.4 kg weight inside, lever for compression control, wooden stick to compress wheat grains, 15.30 ml microchondrometer with cutter bar blade in front.

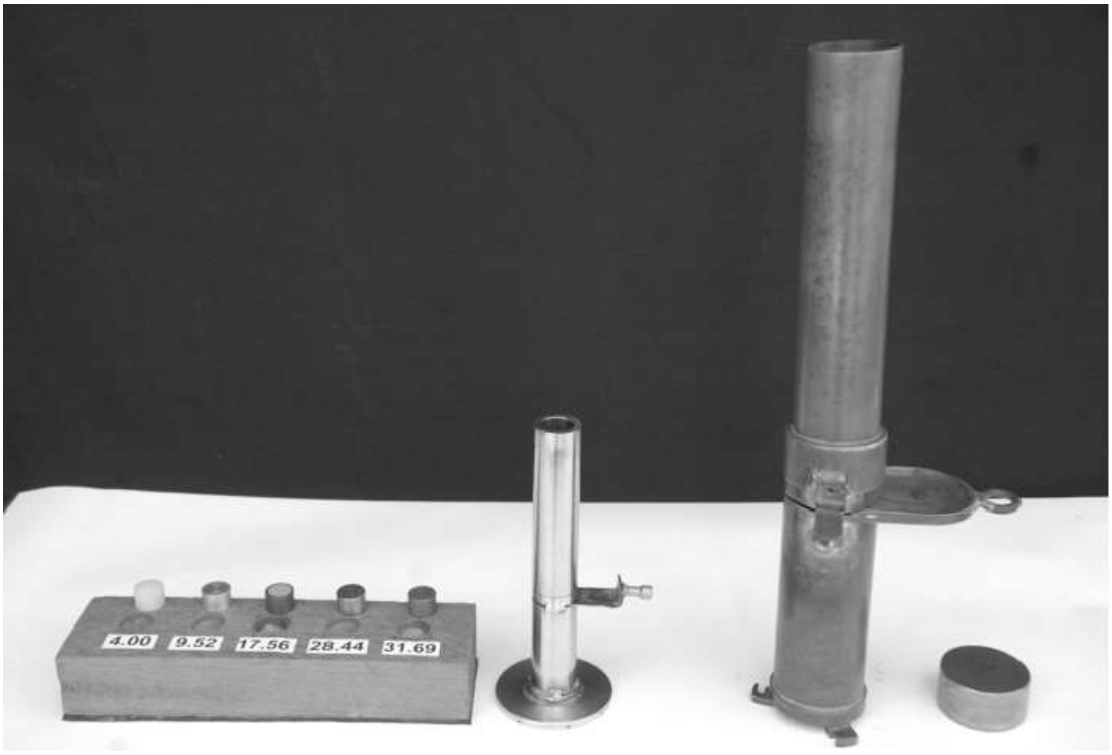

Figure 2. From left to right: Five pistons weight used in the 15.30 ml microchondrometer and the 250 ml chondrometer with its piston.

RESULTS

The wheat grain samples used in the two experiments represented a wide range and contrasting values of hectoliter weight. In the compression experiment, the values obtained with the commercial 250-ml chondrometer ranged from 53.66 to 86.64 kg hl^{-1} in the first year and from 62.78 to 84.54 kg hl^{-1} in the second

Table 1. Mean, standard deviation (SD), sum, maximum (max) and minimum (min) hectoliter weight values of 50 wheat samples measured by commercial and microchondrometer using different compressions over two years.

Chondrometer			Hectoliter weight (kg/hl)				
Year	Volume (ml)	Compression (kg)	Mean	SD	Sum	Min	Max
A	250	0	72.93	8.06	3646	53.66	86.64
A	15.3	0	73.11	8.06	3656	52.76	86.60
A	15.3	4.4	76.59	6.03	3830	62.64	89.63
A	15.3	8.8	77.35	5.90	3868	63.76	88.48
A	15.3	13.2	78.48	5.26	3924	66.54	89.84
B	250	0	72.99	5.73	3650	62.78	84.54
B	15.3	0	73.27	5.89	3663	62.16	85.02
B	15.3	4.4	76.89	4.98	3844	67.21	87.76
B	15.3	8.8	77.73	5.03	3886	68.37	89.46
B	15.3	13.2	77.44	4.87	3872	68.51	87.40

Table 2. Mean, standard deviation (SD), sum, minimum (min) and maximum (max)hectoliter weight values of 60 wheat samples measured by commercial chondrometer and microchondrometer using different piston weights over two years.

Year	Chondrometer		Hectoliter weight (kg/hl)				
	Volume (ml)	Piston (g)	Mean	SD	Sum	Min	Max
A	250	115.87	74.59	6.95	4475	59.42	85.44
A	15.3	4.00	74.55	7.37	4473	58.55	86.17
A	15.3	9.52	74.76	7.20	4485	58.90	85.84
A	15.3	17.56	74.65	7.04	4479	58.92	85.64
A	15.3	28.44	74.80	7.17	4488	59.59	85.66
A	15.3	31.69	74.83	7.23	4490	57.84	85.74
B	250	115.87	73.50	5.55	4410	61.46	84.54
B	15.3	4.00	74.33	5.92	4460	62.02	86.58
B	15.3	9.52	74.30	5.76	4458	62.31	85.15
B	15.3	17.56	74.04	5.78	4442	61.42	84.17
B	15.3	28.44	74.05	5.92	4443	62.16	86.02
B	15.3	31.69	73.88	5.95	4433	61.72	86.60

year (Table 1).

In the experiment to evaluate pistons weight, the values ranged from 59.42 to 85.44 kg hl^{-1} in the first year, and from 61.46 to 84.54 kg hl^{-1} in the second year (Table 2).

The compression experiment was conducted to evaluate grain compaction, especially those of sprouted kernels with radicle. The results of the four compression treatments for each of the 250-ml chondrometer and the 15.30 ml microchondrometer are presented in Table 3. The comparison by Student's t-test demonstrates no significant difference ($p>0.05$) between the instruments without compression.However, there were significant differences between the instruments for compression weight of 4.4 kg ($p<0.05$), and for 8.8 and 13.2 kg ($p<0.01$).

In terms of predictability of test weight by the microchondrometer, the treatments without compression demonstrated highly significant ($p<0.001$) correlation values, with $r=0.9969$ and $r=0.9954$, for the first and second year, respectively. However, an increase in the compression weight reduced the level of correlation between the two instruments for the remaining three treatments (4.4; 8.8 and 13.2 kg). These values, highly significant ($p<0.001$), were 0.9969, 0.9875, 0.9242 and 0.9954, 0.9691, 0.9699.

The results of the second experiment to compare five same-volume microchondrometer pistons, made of plastic, aluminum, bronze with durepoxy adherent, steel and bronze (representing4.0, 9.52, 17.56, 28.44, and 31.69 g respectively), with 250-ml commercial chondrometer showed no significant differences ($p>0.05$) by t-test for each piston weight (Table 4).

Table 3. Hectoliter weight, standard deviation and t-test significance of 50 wheat grain samples measured by 250 ml and 15.30 ml chondrometers using different compressions over two years.

Year	Chondrometer			Hectoliter weight comparison		
	250 ml Hectoliter weight (kg/hl)	15.30 ml		250 ml vs. 15.30 ml		
		Compression (kg)	Hectoliter weight (kg/hl)	t-test	Probability	Significance
A	72.93	0	73.12	0.12	0.9084	ns
A	72.93	4.4	76.59	2.57	0.0117	*
A	72.93	8.8	77.35	3.13	0.0023	**
A	72.93	13.2	78.48	4.08	<0.0001	**
B	72.99	0	73.27	-0.23	0.8162	ns
B	72.99	4.4	76.89	-3.62	0.0005	*
B	72.99	8.8	77.73	-4.39	<0.0001	**
B	72.99	13.2	77.44	-4.18	<0.0001	**

*, ** Significantly different at 5 and 1% level, respectively, ns = non-significant. Comparison in each row.

Table 4. Hectoliter weight, standard deviation and t-test significance of 60 wheat grain samples measured by the 250 ml and 15.30 ml chondrometers using different piston weights over two years.

Year	Chondrometer			Hectoliter weight comparison		
	250 ml	15.30ml		250 ml × 15.30 ml		
	Hectoliter weight (kg/hl)	Piston (g)	Hectoliter weight (kg/hl)	t-test	Probability	Significance
A	74.59	4.00	74.55	0.03	0.979	ns*
A	74.59	9.52	74.76	-0.13	0.895	ns
A	74.59	17.56	74.65	-0.05	0.961	ns
A	74.59	28.44	74.80	-0.17	0.869	ns
A	74.59	31.69	74.83	-0.19	0.850	ns
B	73.5	4.00	74.34	-0.79	0.429	ns
B	73.5	9.52	74.30	-0.77	0.441	ns
B	73.5	17.56	74.04	-0.52	0.606	ns
B	73.5	28.44	74.05	-0.52	0.606	ns
B	73.5	31.69	73.88	-0.36	0.723	ns

*ns = non-significant. Comparison in each row by t-test.

In addition, no significant differences ($p>0.05$) were observed when the primary steel piston of 28.44 g was compared with other piston weights (4.0; 9.52; 17.56; 31.69 g). The correlation between the 250-ml chondrometer with 15.30 ml chondrometers for 4.00, 9.52, 17.56, 28.44 and 31.69 g pistons were highly significant ($p<0.001$), with values of 0.9948, 0.9963, 0.9959, 0.9953, 0.9967 and 0.9869, 0.9878, 0.9872, 0.9922,0.9909 for the first and second year, respectively.

DISCUSSION

It is important to point out that the predictability values seen in the compression experiment are significantly higher than those of Aamodt and Torrie (1934), who obtained a positive correlation of 0.947 for 184 samples of spring wheat and 0.834 for 59 samples of winter wheat. As mentioned earlier, they used a 4-ml micro test weight tube, obtained by cutting down a 25-ml graduated cylinder and multiplied the values by 20 to get a close approximation of the test weight in pounds per bushel, as determined by the standard apparatus. They concluded that the differences were very small and insignificant for all practical purposes.

Similarly, Ghaderi et al. (1971), who used a small glass jar (47 ml. capacity) to evaluate fifty-nine soft wheat samples, achieved a correlation of 0.982 with the standard

test weight, considering it a reliable prediction. They stressed on the use of the most rapid procedure to measure test weight in a breeding and selection program since the correlation between test weight and kernel packing efficiency was highly significant (r=0.961).

Using the 100-cm^3 glass graduated cylinder, Donelson et al. (2002) reported that 40 g samples produced better statistical relationships than 20 g samples. Also, they found a linear relationship between specific gravity and the test weight, except for the samples that were severely shriveled. In other words, when grains are not well formed or have problems in their shape, size and weight, the graduated cylinder has limitations to measure hectoliter weight.

In the present study, which represents a wider range of test weight values, the correlation coefficients are higher than those obtained by Aamodt and Torrie (1934), Ghaderi et al. (1971) and Stepochkina and Stepochkin (2015). Also, the 15.30 ml device is easier to be operated than that built by Taylor (1965) and more efficient than the one usedby Donelsen et al. (2002). These results not only vouch to the usefulness of our microchondrometer for small research samples but also confirm its excellent predictability for measuring test weight in wheat. It is relevant to point out that two years of comparison between the 15.30 ml microchondrometer and commercial 250 ml chondrometer, yielded no significant difference between the two instruments. Despite its smaller diameter and volume, the 15.30 ml microchondrometer has proven to be a reliable device to evaluate test weight under a wide range of conditions.Well-formed grains or severely shriveled and germinated grains have all resulted in providing consistent test weight values with high precision and without extra work to compress samples. The piston weights did not affect the results of the hectoliter weight in the microchondrometer. We believe it to be a very important result and conclusion because it widens the range of materials which can be used in its manufacture.

The wide variation of hectoliter weight measured with the microchondrometer, using a piston of 28.44 g, ranged from 59.59 to 85.66 kg hl^{-1} in the first year and from 62.16 to 86.02 kg hl^{-1} in the second year. Such ranges of measurements guarantee that this new device can be used efficiently to evaluate test weight for all purposes where the sample size is small.

It may be pointed out that this new microchondrometer does not aim to replace the standard 250-ml chondrometer. It was built to facilitate the measurement of the hectoliter weight in research experiments carried out in the greenhouse and individual plants which is not done otherwise due to lack of adequate equipment. As a result, important information regarding plant health, quality of grain filling and grinding are lost.

According to Protic et al. (2007), the test weight varies from 60 to 84kg hl^{-1} and values below 76 kg hl^{-1} are classified as those of low quality. The Brazilian standard norms establish the following minimum values of hectoliter weight for different purposes: wheat for grinding and other purposes, type 1 (78 kg hl^{-1}); type 2 (75 kg hl^{-1}) and type 3 (72 kg hl^{-1}). A test weight value of less than 72 kg hl^{-1} is considered to mistype, unacceptable for the industry and to be commercialized for feed purposes (Brasil, 2010).

Based on two-years of data, we are confident that the new microchondrometer of 15.30 ml, which requires approximately 20 g of wheat sample, can be used for a wide range of grain conditions to determine hectoliter weight. It is also an excellent option in researches conducted in greenhouse, individual plants, segregating populations, nutritional treatments, disease assessment and germinated samples, where the grain volume is a limiting factor to use commercial 250-ml chondrometer. Besides agronomic characteristics such as productivity, resistance to biotic and abiotic stresses and grain quality traits, the use of the microchondrometer to measure hectoliter weight in breeding populations may set a good benchmark for elimination of genotypes that do not meet the minimum requirements of the market.

Conclusion

There was no need to compress grain inside the microchondrometer and the different piston weights didnot influence the hectoliter weight measurements. The highly significant correlation coefficient (r=0.99) between the 250 ml and 15.30 ml devices showed that the new 15.30 ml microchondrometer is an excellent alternative for hectoliter weightassessmentin small wheat samples.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Lime requirements for bean production on two contrasting soils of Lake Victoria Crescent agro-ecological zone

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In East Africa, research has indicated that N, P and soil acidity are the major production constraints to common bean production. The optimum pH for bean production in tropical soils ranges from 5.8 to 6.5. But in Uganda, 23% of beans are grown in soils with pH below 5.0. Research conducted on common bean production is mainly about the major nutrients and information about lime requirements to address soil acidity in different soils is patchy. A study was carried out to determine the lime requirements for *Phaseolus vulgaris* L. production in Cambisols and Umbrisols and this was based on their low soil pH and Ca levels. The lime requirement was determined using titration method and titration curves for each soil type established by titrating 30 g soil in 60 mL 0.01 *M* $CaCl_2$ (1:2) with 3 mL 0.022 *M*$Ca(OH)_2$ per addition. Results indicate that to raise pH from 5.02 to 6.5, the Cambisol ("Limyufumyufu") requires 6.1 tonnes of $Ca(OH)_2$ per hectare, while the Umbrisol("Luyinjayinja") requires 5.4 tonnes of $Ca(OH)_2$ per hectare to raise pH from 5.26 to 6.5. There is need to address soil acidity in Cambisol and Umbrisol through liming using the lime requirement equations determined in this study. In order to provide growers and farmers with more options for such acid soils,however, plant breeding programs should select or develop germplasm tolerant to Al toxicity and/ or low soil available phosphorus as well.

Key words: *Phaseolus vulgaris* L., titration, Al toxicity, Cambisol, Umbrisol.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) isestimated to be the second most important source of dietary protein and the third most important source of calories (FAOSTAT, 2012). Common bean is considered a low status food,

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often referred to as “meat of the poor” (Katungi, 2009) with the annual per capita consumption being higher among low-income people who cannot afford animal protein (Beebe et al*.,* 2013).

Uganda’s bean production is common in the central, eastern and western regions (Sibikoet al*.,* 2013). In the Lake Victoria Crescent agroecological zone, beans are mainly grown on three soil types locally classified as: ‘Liddugavu’’ (Phaoezem, Hapludoll), ‘‘Limyufumyufu’’ (Cambisol, Kandiudalf) and ‘Luyinjayinja’’ (Umbrisol, Hapludoll) (Tenywaet al., 2014). The latter two soils are locally known as having the “lunnyo” condition, which according to local, indigenous knowledge suggests multiple factors limit bean (*P. vulgaris*, L.) production (Fungo et al., 2010).

Smallholder farmers encounter multiple constraints such as pests and diseases (Beebe et al*.,* 2013), low labour productivity and unreliable climatic conditions (Birachi et al*.*, 2012). Soil related constraints account for about 30% of the widely acknowledged ‘yield gap’ (Folmer et al*.,* 1998; Kapkiyai et al*.,* 1999) that threatens food security.

Among soil related constraints, low extractable phosphorus, nitrogen, and high soil acidity associated with aluminium and manganese toxicity (Lunze et al*.,* 2012) are the major soil fertility problems associated with the “lunnyo” soils. Soil pH strongly influences the availability of nutrients in the soil, the activities of soil microorganisms, plant growth and yield (Anderson et al*.,* 2013). The optimum pH for bean production in tropical soils ranges from 5.8 to 6.5 (Edmeades et al*.,* 2012). However, most of the soils in Sub Saharan Africa are acidic and possess high phosphorus-fixing capacities (Nziguheba, 2007).

The major options for improving soil fertility include use of wood ash, crop residues and manures, but they vary widely in quantity and quality (Ebanyat, 2009). Inorganic fertilizers are highly nutrient concentrated, but at times they give no yield response when applied where soil acidity is severe (Fageria and Baligar, 2008).The lime requirement to address soil acidity in different soils is not known. Therefore, this study was conducted to determine the lime requirements for bean production in the extensive local soils “Limyufumyufu” and “Luyinjayinja”.

MATERIALS AND METHODS

Study soil collection and preparation

The two soils known to have the “lunnyo” condition were collected from farmers’ fields in two representative communities (Mukungwe and Lwankoni) in Masaka district located in Central Uganda at 31.7361°E latitude and 0.34111°S longitude). These soils were selected for study based on a series of farmer meetings from three communities, which indicated that these two soils are important, farmer-recognized soil series for common bean production. In addition, the farmers indicated that bean production on the two soils was problematic and therefore were considered to have the “lunnyo” condition or characteristic. Soil analysis results (Table 1).

indicate that, indeed, the two soils are low in soil pH and the Limyufumyufu is low in soil Ca. KCl-extractable Al, a measure of level of toxic aluminium, was high in the Limyufumyufu suggesting another probable reason that Al-sensitive bean (*P. vulgaris*, L.) was known by farmers to grow poorly in this soil. The two soil types(Limyufumyufu and Luyinjayinja) classified in the FAO Mapping Legend as Cambisol and Umbrisol, respectively). They are classified in the US Soil Taxonomy as “TypicKandiudalfs” and “TypicHapludolls”, which, for the former suggests highly weathered and the latter, less weathered status, respectively.Soil samples were obtained in a zig-zag pattern at ten locations within each field, from a depth of 0 to 15 cm.

A composite sample of about 25 kg soil was obtained from an area of approximately 50 m x 100 m for each soil. Soil was takenfor laboratory analyses. Soil samples were air dried in a dust free area, and crushed with a mortar and pestle to pass a 2-mm sieve.

Laboratory analyses for soil samples

Total soil organic carbon was determined by dry combustion method using total organic carbon analyzer(American Society for Testing and Materials, 1994).Extractable P was determined using the Olsen method (Kuo, 1996). Exchangeable K^+, and Na^+ were determined using a flamephotometer, while Ca^{2+} and Mg^{2+} were determined using an atomic absorbance spectrometer. The exchangeable cations were extracted from the samples by shaking for 16 to 24 h with 100 ml 2 MNaClfor 0.5 to 2.5 cmol/kg of exchangeable cations (Clark, 1965). Soil pH was measured in a 1:2.5 soil to water ratio using a pH electrode. The Kjeldahl method was used to determine total N (Bremner, 1965). Micronutrients were extracted in the Mehlich 3 extractant solution (Mehlich, 1984). The micronutrients Cu, Mn and Zn were measured by atomic absorption, while boron was measured using a colorimetric method (Berger and Truog 1939).Soil texture was determined using the hydrometer method (Bouyoucos, 1936).

Titration method of determining the lime requirement in the laboratory

The lime requirementwas determined in the laboratory at Makerere University using titration method as follows:The pH meter was calibrated with standard pH 4.00 and 7.00 buffers before each titration, soil pH measurements and titrations were performed in a soil/0.01 M $CaCl_2$ (1:2) suspension while being stirred. Titration curves for each soil type were established by titrating 30 g soil in 60 mL 0.01 M $CaCl_2$ (1:2) with 3 mL 0.022 M $Ca(OH)_2$ per addition (Kisselet al*.,* 2010; Barouchas et al*.,* 2013). The time interval to achieve an ion-exchange balance between 0.022 M $Ca(OH)_2$ additions was 30 min based on the research results of Liu et al*.* (2004) and Weaver et al*.* (2004). The soil suspension was stirred continuously by a magnetic stirrer during titration and the pH was measured at the end of each time interval. Increments (3-mL aliquots) of 0.022 M $Ca(OH)_2$ were added until the pH reached 6.5. All determinations were performed in triplicate. After each measurement, the electrodeswere rinsed with distilled water to avoid cross contamination from sample to sample. For the evaluation of the Al^{3+} in the extracts of the 1 mol L^{-1}KCl solution, 1:10 (v/v) soil/solution ratio (McLean, 1965), Titrimetric method(standard method), according to the routine methodology adapted from McLean (1965) was used. Primarily, the exchangeable acidity (Al^{3+} + H^+ tit) is determined by titration of 25 mL KCl extract with 0.025 mol L^{-1}NaOH, using 1 g L^{-1} phenolphthalein as an indicator (titration from colorless to pink). Then, the concentrationof $Al^{3+}$was obtained by back-titration of the same KCl extract, previously used, after the acidification with a drop of HCl and addition of 40 g L^{-1}NaF, with 0.025 mol L^{-1}HCl (titration from pink to colorless).

Table 1. Initial physico-chemical properties of the two selected soils used in the LRS.

Soil measurement	Units	Limyufumyufu	Luyinjayinja
pH (H_2O)		5.02	5.26
pH ($CaCl_2$)		3.52	4.56
OM	%	2.35	2.26
K	cmol/kg	1.5	1.5
Ca	cmol/kg	4.8	7.5
Mg	cmol/kg	4.2	8.9
Na	cmol/kg	1.48	1.06
Mn	mg/kg	153	110
Fe	mg/kg	149	151
EC(S)	uS/cm	43	20
Al	mg/kg	1410	1250
C.E.C	cmol/kg	12.3	8.13
Exch.Al	cmol/kg	1.2	0.8
Sand	%	52	51
Silt	%	36	35
Clay	%	14	12
Textural Class	USDA	SCL	SCL

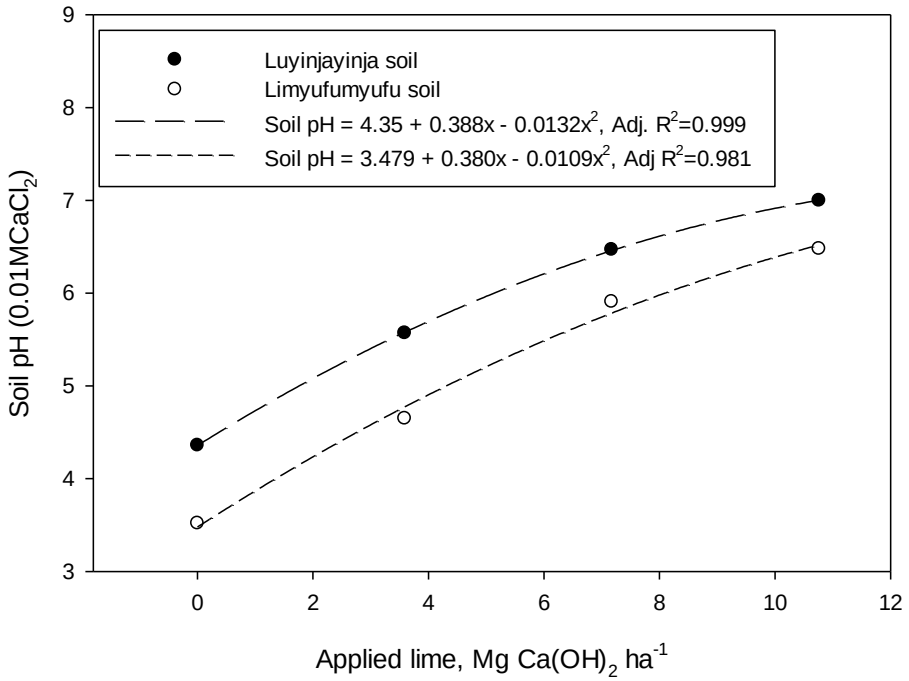

Figure 1. Soil pH (0.01M $CaCl_2$) resulting from additions of $Ca(OH)_2$.

Statistical analysis

Determining the pH resulting from lime ($Ca(OH)_2$) application

The first step in the incubation procedure was to determine the soil pH that resulted from the increasing amounts of added $Ca(OH)_2$. This was determined by plotting the pH resulting from the application of $Ca(OH)_2$ in the $CaCl_2$ suspension on the y-axis and the amounts of added $Ca(OH)_2$ on the x-axis (Figure 1).

Determining the amount of $Ca(OH)_2$ needed to attain a specified soil pH

The incubation data were then re-plotted as the amount of $Ca(OH)_2$ on the y-axis and the soil pH on the x-axis to properly predict the

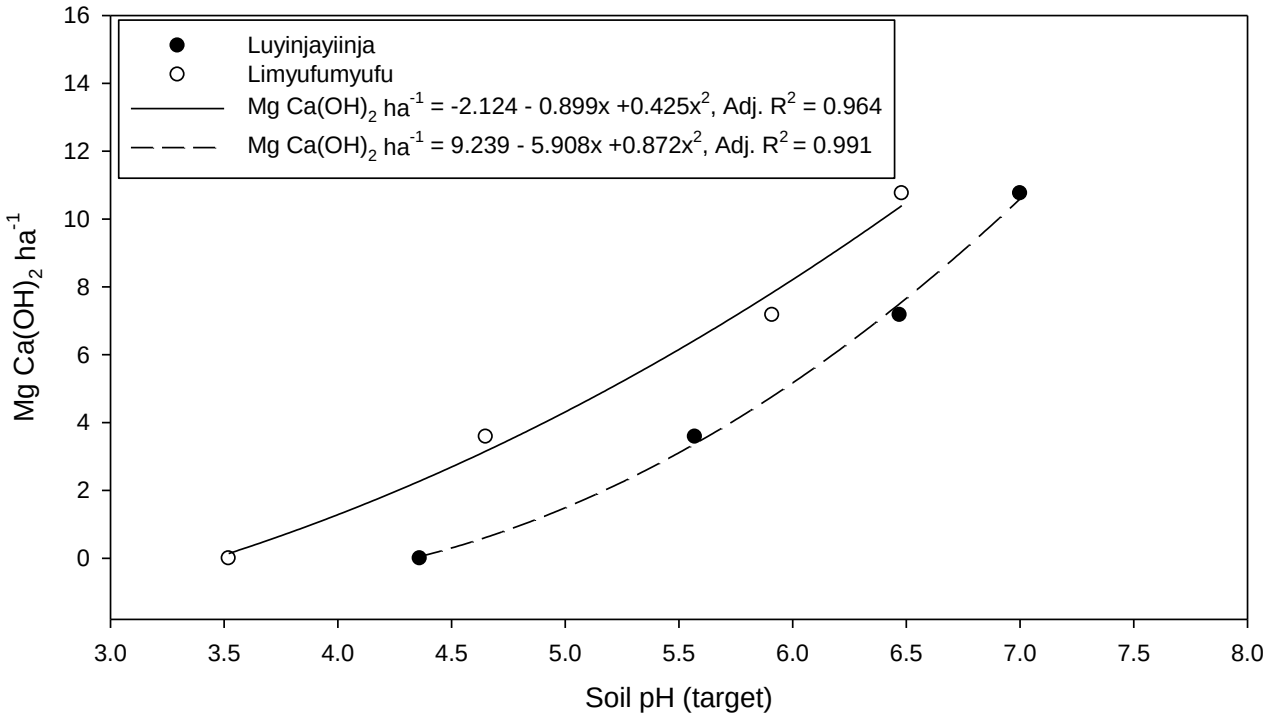

Figure 2. Estimated lime ($Ca(OH)_2$ requirements needed to attain specified soil pH levels.

Table 2. Lime requirements estimated for two soils for various target soil pH's depending on the intended crop.

Soil	Target soil pH (water)				
	Original water pH	**5.0**	**5.5**	**6.0**	**6.5**
Luyinjayinja	5.26	-	0.83	2.89	5.39
Limyufumyufu	5.02	0	1.77	3.83	6.11
Luyinjayinja	Liu (2005) single	-	0.71	2.19	3.68
Limyufumyufu	Liu (2005) single	-	1.42	2.90	4.39

amounts of $Ca(OH)_2$ needed to attain a specific soil pH (in $CaCl_2$) suspension. This step is sometimes called a "Calibration" and differs from simply re-arranging the regression equation from the plot of soil pH on the y-axis and the amounts of $Ca(OH)_2$ on the x-axis as described above. This "calibration" step is needed because a regression equation cannot be simply re-arranged as if it were a standard algebraic equation. For example, one cannot simply re-arrange a regression equation in the above paragraph to predict the amounts of $Ca(OH)_2$ needed for a specific soil pH. After plotting the amounts of $Ca(OH)_2$ on the y-axis and soil pH on the x-axis, a regression equation was fitted to the data to enable the prediction of the amounts of $Ca(OH)_2$ needed to attain a specified soil pH (Figure 2). The estimate of lime requirement is then the difference in the amounts of $Ca(OH)_2$ between the two predictions and is given in Table 2. This replotting and fitting a regression equation to the data is required because a regression equation is a prediction of the dependent variable assuming no errors in the predictor variable (Gelman and Hill, 2009).Various target soil pH values were then selected as possible target values (5.0, 5.5, 6.0, and 6.5, Edmeades et al*.,* 2012). The corresponding amounts of $Ca(OH)_2$ required based on the laboratory incubations for the two soils were then tabulated (Table 2) based on target crop pH requirements after the time allocated for the neutralization reaction to occur.

RESULTS AND DISCUSSION

Soil pH increase with applied $Ca(OH)_2$

The change in soil pH that occurred with lime application is shown in Figure 1. The amount of $Ca(OH)_2$ needed to increase soil pH increases in a nonlinear manner as shown by the quadratic curves that were fit to the data.This nonlinear response to lime applications probably reflects the presence of additional buffering compounds on the soil surfaces that result in smaller increases in pH as higher pH's are obtained. This is not unusual in highly buffered variable charge soils in which the CEC increases as soil pH increases (Uehara and Gilman, 1981).

In order to obtain estimates of lime requirement to attain various target soil pH's, the data shown in Figure 1 are replotted in order to obtain regression estimates of the amount of lime needed to attain various target pH's. We note that it is not valid to take the regression equations fit in Figure 1 and re-arrange them to estimate lime requirement. This occurs because regression equations are fit on the assumptions that there are no errors in the predictor variable and thus regression equations are not the same as a typical algebraic equation with which re-arrangement is valid. Figure 2 shows the replotted data and again illustrate that as the target soil pH increases the amount of lime needed further increases, resulting in the curvilinear relationship between lime applied and the resultant soil pH. It is important to note that this highly buffered behavior of the Ugandan soils differs from the soils in the Liu et al.(2004, 2005) and the Barouchas (2013) papers where the increase in soil pH was linear for the lower levels of applied lime. Consequently, the recommendation of Liu(2005) to use a one-point incubation curve to determine lime requirement does not hold for these two Ugandan soils. Consequently, these data suggest that a lime incubation curve needs to be determined for each of these soil groups, which then can be used to estimate lime requirements to attain various target pH's depending on the desired crop. This is illustrated in Table 2.

For comparison, we have also calculated the lime requirement using Liu et al. (2005)'s recommendation single lime addition method (Table 2) rows 3 and 4. As expected, the lime requirement estimates from single addition underestimate the lime requirement obtained from an incubation curve that spans the range of soil pH 5.0 to 6.5.

Estimates of lime requirement

The practical determination of lime requirements for these soils is not concluded with these estimates; rather, it is only the beginning of practical estimates. These are only estimates of lime requirement based on controlled laboratory conditions. Practical estimates need field verification under realistic conditions of use of a locally available liming material, whose chemical quality relative to the 100% Calcium Carbonate Equivalency of $Ca(OH)_2$ must be determined. In addition, the physical quality of the agricultural limestone must also be determined, since lime that does not pass a 0.15-mm sieve lower possibility of dissolving and thus of lower effectiveness. In addition to these variables that strongly affect lime reactivity, the soil and crop management practices such as amounts and form of nitrogen also strongly affect soil pH in practical situations. Examples abound of the strongly acidifying effects of ammoniacal fertilizers on lowering soil pH (Chao et al*.,*2014). Other factors will affect how frequently soil pH should be monitored. As suggested above, if the lime contains some larger particles these will require a longer time to react, thus affecting the effective residual effectiveness of the limestone. Consequently, soil pH should be monitored over time to ensure crop growth is not limited.

Physico-chemical properties of the two soils used in the LRS

The two soil types were sandy clay loam textural class and they were quite acidic. The Limyufumyufu had a pH of 5.02 while the Luyinjayinjainitial pH was 5.26 (Table 1). Considering the critical levels of K, Mg and Ca (1.15, 3.12 and 0.68 $cmol_c/kg$, respectively), these nutrients were above the critical levels as indicated in Table 1. The Limyufumyufu soil had 33.9% higher Cation Exchange Capacity (CEC) compared to Luyinjayinja. The Cambisol had a much higher exchangeable Aluminium content, which may have resulted in sharply reduced bean growth.

Physico-chemical properties of the selected soils used in the LRS

The initial pH of the two soil types was below the critical level for bean growth which ranges from 5.8 to 6.5 (Table 1) (Edmeades et al*.,* 2012). The availability of phosphorus is also influenced by the pH, which is readily available at a pH (H_2O) of 6 to 7 (Plessis et al*.,* 2002). This is inagreement with the nutrient omission study results, which indicate that phosphorus is the most limiting nutrient in the three soils where beans are mainly grown in Lake Victoria Crescent (Kyomuhendo et al*.,* 2018).

Soil pH in 0.01 M$CaCl_2$ were depressed at all levels of $Ca(OH)_2$ addition in all soils, a common effect due to displacement of Al^{3+} and H^+ from increased soil solution of Ca^{2+}, and due to elimination of the junction potential effect (Bloom, 2000). Fageria andBaligar (2008) noted that Effective Cation Exchange Capacity (ECEC) is an important parameter for predicting fertility behavior of agricultural soils, and in their study, ECEC increased significantly ($P < 0.01$) with increasing pH in aquadratic, nonlinear response.

At low pH values, Al^{3+} is the predominant exchangeable cation on clay minerals. As the pH is raised, the Al^{3+} hydrolyzes, freeing the exchange sites for Ca^{2+}, and results in an increase in the ECEC (Kisinyo et al*.,* 2013). This may be one of the reasons the Limyufumyufu soil requires more lime to achieve suitable pH levels for *P. vulgaris* bean.

Titration curves for the two soil types used in the lime requirement study (LRS)

Cambisol had a higher lime requirement than Umbrisol

(Figure 1). This can be attributed to the differences in the initial pH where Cambisol was more acidic than Umbrisol (Table 1). According to Edmeades et al. (2012), the initial pH of the soil is the major factor determining the quantity of lime required to raise pH either to 5.8 or 6.5, a range of pH for bean production.

The higher lime requirement for Cambisol than Umbrisol can be attributed to differences in terms of exchangeable cations where by Ca^{2+}, Mg^{2+}, and K^{+} contents were lower in Cambisol than Umbrisol (Table 1). This is in agreement with results by Fageria and Baligar (2008) who reported that soils with high fertility in terms of exchangeable Ca^{2+}, Mg^{2+}, and K^{+} require less lime than do those with lower soil fertility. When Ca^{2+}, Mg^{2+}, and K^{+} contents are higher, a lower lime rate is required, because of higher levels of these basic cations in the soil, meaning relatively higher base saturation and higher pH than with lower levels of these cations (Fageria and Baligar, 2008).

Anderson et al. (2013) reported that in soils with a negligible orno exchangeable A1, the pH did not change by liming meaning that most of the base added had been consumed by deprotonation of hydroxyl groups of organic matter and on clay mineral surfaces (Guadalix and Pardo, 1994).

Conclusions

As indicated above, these laboratory incubations are only relatively quick estimates of lime requirement. Clearly, longer term studies with locally available liming materials that typically vary in Calcium Carbonate Effectiveness (%CCE) and also vary in particle size analysis need to be conducted. Furthermore, local crop management practices such as rates and types of nitrogen fertilizers need to be assessed in field studies.

Nonetheless, these results suggest that major growth limitations due to soil acidity merit further study and field testing in order to more fully utilize such acid soils with food production potential.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

The response of pigs to diets containing varying levels of cocoa placenta meal (CPM) supplemented with an exogenous enzyme complex

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A nineteen-week experiment was conducted to establish the effects of an enzyme supplementation on growth performance, economics of production, carcass components and blood profile of pigs fed diets containing different levels of cocoa placenta meal (CPM). Twenty-five Large White grower pigs with mean initial live weight of 15.4 kg were randomly allocated to five treatments: T1 (0% CPM), T2 (5% CPM), T3 (10% CPM), T4 (15% CPM) and T5 (20% CPM) in a randomized complete block design (RCBD). Diet T1 had no enzyme but diets T2, T3, T4 and T5 contained 35 g enzyme per 100 kg feed. Each treatment had five pigs and each pig served as a replicate. Feed and water were provided *ad-libitum*. Pigs were slaughtered upon the attainment of a live weight of 70 ± 2.5 kg for carcass studies. Blood samples were collected during slaughtering. Feed cost (€ per kg) was inversely proportional to the inclusion level of the CPM + enzyme. Pigs on the T1 and T2 diets utilized their feed more efficiently ($p < 0.05$) than those on the T3, T4 and T5. However, no differences ($p > 0.05$) were observed in the variations of the feed cost per kg gain values recorded. The CPM + enzyme inclusion resulted in decreased values ($p < 0.05$) for backfat thickness. There were no dietary ($p > 0.05$) effects on the blood profile. Dietary inclusion levels up to 20% CPM + enzyme can be fed to growing-finishing pigs without any detrimental effects on most of the growth performance and carcass criteria.

Key words: Agro-industrial by-product, blood profile, carcass, growth performance.

INTRODUCTION

Much consideration has been drawn to the use of cheaper and less demanded alternatives such as Agro-Industrial by-products and non-conventional feed resources (NCFRs) in the feeding of livestock (Obirikorang et al., 2015) as a result of high cost of conventional feed ingredients. Agro-industrial by-products such as dried brewers spent grains (DBSG), cocoa pod husk (CPH), rice bran, and other NCFRs have been evaluated in

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Ghana as potential feed ingredients for non-ruminant farm animals (Atuahene et al., 2000; Donkoh et al., 2013; Nortey et al., 2015). Moreover, some research done on these products have proved that their use in animals' diets often reduce feed cost (Okai, 1998). Yet, there are other prospective by-products which have not been adequately studied. One of such is the cocoa placenta, a by-product of cocoa bean production.

Cocoa placenta is the slender, fibrous, rope-like tissue which holds the seeds (beans) in position inside the cocoa pod and also supplies nutrients to the cocoa seeds during the developmental stage of the cocoa fruit. The cocoa placenta accounts for about 3% of the cocoa fruit (Atiemo, 2015), which on the average weighs 400 g and therefore a large quantity is produced during the fermentation process of the cocoa beans but are eventually removed and discarded haphazardly during sun drying of the fermented beans. Atiemo (2015) reported that 30,966 metric tons of cocoa placenta is produced annually in Ghana. This can be a nuisance as it invites a lot of houseflies, blocks drain and also pollutes water bodies via run off when scattered around the cocoa drying sites in the communities.

One major challenge apart from the presence of theobromine in cocoa by-products is the high crude fibre content or non-starch polysaccharides (NSP). Choct (2004) indicated that NSP are poorly digested by monogastric animals such as pigs because they do not produce enzymes that are capable of digesting these fibre components. According to Bedford (2000), exogenous enzymes can be used to address the problems of some anti-nutritional factors and high fibre levels that limit feed value, thereby leading to a more economic and efficient utilization of AIBP. This enzyme complex intended to increase the bioavailability of carbohydrates, proteins and fats in the diets of pigs and poultry. It has been suggested that it improves digestibility of feed ingredients and FCR. There is a dearth of information on its usefulness in monogastric diets in Ghana. Therefore, the objective of this study was to determine the growth performance, carcass traits and blood profile of grower-finisher pigs fed diets containing varying levels of CPM (0-20%) supplemented with an enzyme complex.

MATERIALS AND METHODS

Study location and duration of the experiment

The study's location was the Livestock Section of the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The feeding trial lasted for 19 weeks.

Source and processing of feed ingredients

The wet cocoa placenta (WCP) were gathered from cocoa farmers inKukuom in the Asunafo South District of the Ahafo Region and were sun dried on a raffia palm mat on a platform for 5-8 days, depending on the intensity of the sunshine and the humidity. The dried cocoa placenta (DCP) was ground in a hammer mill to produce Cocoa Placenta Meal (CPM) whilst the other ingredients were obtained from Rakeb Company Limited, Kumasi. Those that required grinding e.g. maize, were handled in the same way as the DCP

Proximate composition of CPM

Proximate analysis of the CPM was carried out using standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2002). The nutrient compositions of other ingredients were obtained from the NRC (1998).

Experimental animals, diets and design

Twenty-five Large White grower pigs (15 entire males and 10 gilts) with an overall mean initial live weight of 15.4 kg were selected and randomly allotted to five isonitrogenous (17.0% CP) dietary treatments (Table 1) that is, 0% CPM, 5% CPM, 10% CPM, 15% CPM and 20% CPM replacing equal amounts of maize. Adjustments of the wheat bran and soya bean meal levels were made to obtain the crude protein (CP) level desired and all diets contained the same level of fishmeal (5%). The allocations of the pigs were based on sex and live weight in a Randomised Complete Block Design. Each treatment had three boars and two gilts and each pig represented a replicate. A kg of the exogenous enzyme complex contains- Cellulase, 100,000,000 U; Xylanase DS: 5,000,000 U; Beta-glucanase: 70,000 U; Amylase: 300,000 U; Pectinase: 70,000 U; Phytase: 1,450,000 IU; Protease: 3,000,000 U; Lipase: 10,000 U; Arabinase, Alpha galactosidase and Hemicellulase.

Management of pigs

Prior to the commencement of the experiment, all the pigs were tagged and treated with Tectin (Ivermectin) Inj. They were housed in a scrubbed and disinfected welded mesh, individual concrete-floored cages (that is, 160×66×104 cm), constructed within an aluminium-roofed building. Each cage was provided with a 43×12×10 cm concrete water trough. Shallow feeding troughs measuring 46×23×13 cm were used during the first two weeks and they were replaced with deeper and heavier troughs measuring 54×24×27 cm (depth of 11cm at the feeding end) from the third week onwards. Feed and water were provided without restriction throughout the study period.

Parameters measured

Growth performance and economics of production

Weekly feed intake and weight gain were measured and used to calculate the daily feed intake, daily weight gain and feed conversion ratio (FCR). Total feed intake and weight gain were alsocalculated. Cost per kg of each diet was computed by using the open market prices to estimate the cost of all ingredients used in the study. The cost of collecting, transporting and processing of CPM were estimated and added to the cost of the CPM diets. Inaddition, the cost of the inclusion (¢/kg) of enzyme was added to the CPM diets. Feed cost per kg gain for each diet was obtained by multiplying the cost per kg feed by the FCR.

Table 1. Composition (%) of the experimental diets.

Ingredients (%)	0% CPM	5% CPM$^+$	10% CPM$^+$	15% CPM$^+$	20% CPM$^+$
CPM	0	5	10	15	20
Maize	60	55	50	45	40
Soya bean meal	15.5	13.8	12.1	10.5	8.7
Wheat bran	18.5	20.2	21.9	23.5	25.3
Fishmeal	5	5	5	5	5
Dicalcium phosphate	0.25	0.25	0.25	0.25	0.25
Vit-min. premix$^{\#}$	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25
Oyster shells	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**
Calculated composition (%)					
CP	17.0	17.0	17.1	17.0	17.0
CF	3.68	4.48	5.28	6.07	6.87
DE (MJ/kg)	15.9	15.5	15.1	14.7	14.3
Calcium	0.51	0.52	0.54	0.57	0.56
Phosphorus	0.74	0.73	0.87	0.92	0.98

+ The enzyme was added at the rate of 35 g/100 kg to each of the CPM diets.#Vit-min. premix per 100 kg diet: Vitamin A (8×105U.I); Vitamin D3 (1.5×104U.I); Vitamin E (250 mg); Vitamin K (100 mg); Vitamin B2 (2×102 mg); Vitamin B12 (0.5 mg); Folic acid (50 mg); Nicotinic acid (8x102 mg); Calcium panthotenate (200 mg); Choline (5×103 mg). Trace elements: Mg (5×103 mg); Zn (4×103 mg); Cu (4.5×102 mg); Co (10 mg); I (100 mg); Se (10 mg). Antioxidants: Butylatedhydroxytoluene (1×103 mg). Carrier: Calcium carbonate q.s.p (0.25 kg).

Carcass and internal organs measurement

Four animals (two males and two females) from each dietary treatment were slaughtered for carcass evaluation, upon attaining the targeted weight of 70 ± 2.5 kg after the weekly weighing. Carcass parameters considered on the day of slaughter were; dressed weight, dressing percentage and weights of viscera, respiratory tract, full GIT, empty GIT, empty stomach, liver, spleen, heart, kidneys, trotters and head. After chilling the carcasses at 5°C overnight, the parameters measured were: Chilled dressed weight, carcass length, meanback fat thickness, P_2, loin eye area and weights of leaf fat, fillet, belly, loin, shoulder and thigh. An 8cm-length of the ileum, obtained between the caecum and the small intestine was taken for histological processing and microscopic observations for the villi count, height, width and villi area using the standard procedures outlined by Baker and Silverton (1976).

Haematological and serum biochemical studies

Two samples of blood were taken from each pig using heparinized vacutainer (Venoject, lithium heparin, Terumo Europe, Leuven, Belgium) and sterilized micro tubes.The first sample from each pig was subsequentlyanalysed for haematological parameters whilst the serum obtained from the other sample was used for biochemical studies (Tiezt,1995).

***In vitro*digestion**

An *in vitro* trial was conducted on the test ingredient (raw CPM) and the five diets (that is, 0% CPM, 5% CMP$^+$, 10% CPM$^+$, 15% CPM$^+$ and 20% CPM$^+$) to mimic the digestion process in the pig so as to ascertain the effect of the enzyme. Parameters measured were sugar levels and the viscosities of the diets.

Statistical analysis

All data collected were subjected to the analysis of variance procedure of the GenStat Statistical Package Version 11.1 (2009) and differences were deemed significant at $p < 0.05$.

Ethical statement

Protocols used were in this study were approved by the Animal Ethics Committee of Kwame Nkrumah University of Science and Technology, Kumasi.

RESULTS AND DISCUSSION

Nutrient composition of the dried cocoa placenta (CPM)

Proximate composition of CPM (Table 2) showed highervalues for most components than that reported byBoatenget al. (2016) except the NFE and ME values. Boatenget al. (2016) reported NFE and ME values of 63.17% and 3006.91 (kcal/kg) respectively on as-fed basis. Torres-Morenoet al. (2015) attributed variations of proximate values of cocoa beans and African LocustBean Pulp (ALBP) to varietal differences, geographicallocation, type of soils, maturity of fruit at harvest, method used in drying, processing and duration of storage period. The cocoa placenta used by Boatenget al. (2016) was obtained from the Plantations Section of the Department of Crop and Soil Sciences, KNUST, Kumasi, Ghana.

The CP value of the CPM obtained in this study is

Table 2. Proximate composition of the dried cocoa placenta meal (CPM).

Proximate composition (%)	As-fed (%)	Dry matter (%)
Moisture	14.3	-
CP	16.0	18.6
CF	19.0	22.1
EE	3.02	3.52
Ash	9.98	11.6
NFE	37.8	44.1
ME (MJ/kg)$^{\beta}$	9.04	10.5

$^{\beta}$Metabolizable energy was calculated using Pauzenga (1985) equation (that is, ME = 37 × % CP + 81.8 × % EE + 35 × % NFE)

higher than the levels of CP in most of the conventional energy feed ingredients such as maize (8.3%) which is usually used in the diets of pigs in Ghana. It is worth mentioning that, except cocoa bean meal (23.2% CP), the CPM (Table 2) contained more CP than cocoa pod husk (8.4%) and cocoa bean shell (16.7%) in percentage dry matter terms (European Food Safety Authority, 2008). The CF value of 19.0% for the CPM is higher than the CF values of most agro-industrial by-products (AIBPs) reported by Rhule (2015) {that is, cassava peel (13.7 ± 0.23%), cocoa expeller cake (8.57 ± 0.22%), coconut chaff (13.8 ± 1.86%), copra cake (13.9 ± 0.65%), pineapple waste (14.7 ± 2.89%), pito mash (12.4 ± 2.84%) and brewer's spent grains (16.1 ± 1.29%)}. Therefore, the inclusion of the enzyme was to help degrade the high fibre in the CPM diets in order to release the nutrients that were bound in the fibre to the pigs. As a result of the fibrous nature of most AIBP, several research studies using fibre-degrading exogenous enzymes have been undertaken in Ghana (Alemaworet al., 2009;Nortey et al., 2015).

Growth performance of pigs

There were no differences (p = 0.77) in the average daily feed intake among the different dietary treatments although the 20% CPM^{+} diet recorded the least value. There was a trend of decreasing daily weight gain (p = 0.02) with increasing levels of the CPM. The 0% CPM and 20% CPM^{+} diets recorded the highest and lowest average daily weight gain (ADWG) value respectively. Boateng et al. (2016) obtained a divergent result of the ADWG (p > 0.05) when rats were fed diets containing varying levels of CPM plus XZYME^{TM}. The least ADFI and ADWG recorded by pigs on the 20% CPM^{+} diet had an impact on the duration or number of days spent to reach the slaughter weight of 70±2.5kg because pigs on the 20% CPM^{+}diet spent noticeably (p = 0.04) more days (116 days) compared to those on the 0% CPM (84 days) (Table 3).

Contrarily, Tengan et al. (2012) observed similar duration (p > 0.05) with varying levels of ALBP at the highest inclusion level (20%), where the pigs took 100 days compared with 105 days (no ALBP) to reach the target weight. The FCR values obtained implied that pigs on 0% CPM diet utilized their feed more efficiently (p = 0.001) than those on the 10, 15 and the 20% CPM^{+} diets although the FCR was similar to the 5% CPM^{+} diet. This study confirms the statement made by Whittemore et al. (2003), that feed efficiency is directly affected by growth rate and feed intake and in all cases of feeding high CF diets, feed conversion efficiency decreased, with the decreases being more pronounced in young pigs. The feed cost decreased with increasing level of dietary CPM inclusion even with the addition of the enzyme. This implied that, the feed cost (GH₵ per kg) was inversely proportional to the inclusion level of the CPM^{+} diets. However, there were no differences (p = 0.1) in the variations of the feed cost per kg gain values recorded among the dietary treatments and the variations did not follow any particular trend.

Carcass characteristics

All the absolute and relative fat parameters studied (that is,backfat thickness, P_2 measurement and leaf fat) recorded substantial differences (p < 0.05) between the control diet (0% CPM) and the CPM^{+} diets except the mean back fat thickness which was similar (p > 0.05) withthe 5% CPM^{+} diet. The CPM^{+} inclusion in the diets resulted in decreased values of the fat parameters. For example, the P_2 fat measurement was inversely proportional to the levels of CPM^{+} in the diets. It may beinferred that pigs fed the CPM^{+} diets converted their feed more into lean meat rather than fat deposits as a result oftheir high fibre levels but lower energy concentrations (Table 1). Amoah et al. (2017) observed a similar trend when they studied the performance of pigs at different phases of growth on sun-dried brewers spent grain (DBSG)-based diets. Specifically, the diets with high and low metabolizable energy values (that is, 25% DBSG and30% DBSG) recorded lower values for fat parameters (that is, back fat thickness, leaf fat and P_2 measurement). There were dietary influences (p < 0.05)

Table 3. Growth performance of the experimental pigs.

Parameter (kg)	0% CPM	5% CPM$^+$	10% CPM$^+$	15% CPM$^+$	20% CPM$^+$	SEM	p- value
Initial weight	15.5	15.3	15.4	15.6	15.4	0.05	1.00
Final weight	70.0	69.3	69.7	69.5	70.2	0.16	0.91
Duration of trial, days	84.0^{c}	92.4^{bc}	107^{ab}	105^{abc}	116^{a}	5.66	0.04
Daily feed intake	1.87	1.72	1.75	1.72	1.64	0.04	0.77
Daily weight gain	0.65^{a}	0.60^{ab}	0.51^{bc}	0.52^{bc}	0.48^{c}	0.03	0.02
Feed conversion ratio	2.87^{bc}	2.84^{c}	3.33^{a}	3.34^{a}	3.41^{a}	0.12	0.001
Feed cost, €	0.26	0.24	0.23	0.22	0.20	0.01	-
Feed cost/kg gain, €	0.74	0.70	0.77	0.75	0.68	0.02	0.10

a, b, c- Means on the same row bearing different superscripts are significantly different ($p < 0.05$).

Table 4. Absolute and relative carcass characteristics of the experimental pigs.

Parameter	0% CPM	5% CPM$^+$	10% CPM$^+$	15% CPM$^+$	20% CPM$^+$	SEM	p-value
Absolute (kg)							
Warm carcass wt.	51.8	51.1	49.0	50.0	48.6	0.61	0.33
Chilled carcass wt.	44.4	43.2	41.6	41.7	40.7	0.66	0.09
Dressing percentage, %	73.9	73.8	70.9	71.8	69.6	0.83	0.15
Loin eye area, cm^2	32.7	31.4	33.5	32.3	34.5	0.53	0.18
Mean backfat thickness, cm	2.58^{a}	2.50^{a}	1.58^{b}	1.62^{b}	1.50^{b}	0.24	0.001
P_2 measurement, cm	1.94^{a}	1.66^{b}	1.16^{b}	0.72^{c}	0.69^{c}	0.25	0.003
Leaf fat	0.64^{a}	0.47^{b}	0.34^{c}	0.33^{c}	0.34^{c}	0.06	0.002
Head	5.10	5.10	4.94	4.94	5.05	0.04	0.96
Trotters	0.96	1.04	1.07	1.07	1.08	0.02	0.46
Thigh	6.95	6.54	6.90	6.90	6.85	0.07	0.60
Loin	5.80	5.66	5.69	5.69	5.84	0.04	1.00
Fillet	0.41	0.41	0.41	0.41	0.40	0.002	1.00
Viscera	12.1^{b}	12.2^{b}	12.3^{b}	12.3^{b}	14.6^{a}	0.48	0.02
Full GIT	8.56^{b}	8.15^{b}	8.50^{b}	8.50^{b}	11.3^{a}	0.58	0.01
Empty GIT	2.94	3.17	2.84	2.84	3.04	0.06	0.40
Empty stomach	0.55^{d}	0.67^{b}	0.60^{c}	0.60^{b}	0.68^{a}	0.02	0.001
Relative (%)							
Leaf fat	0.91^{a}	0.67^{b}	0.49^{c}	0.47^{c}	0.49^{c}	0.08	0.002
Head	7.27	7.37	7.16	7.09	7.23	0.05	0.96
Trotters	1.36	1.50	1.55	1.53	1.55	0.04	0.39
Thigh	9.91	9.45	10.0	9.93	9.80	0.10	0.59
Loin	8.25	8.17	8.23	8.18	8.36	0.03	1.00
Fillet	0.58	0.60	0.60	0.59	0.57	0.006	1.00
Viscera	17.2^{b}	17.6^{b}	17.8^{b}	17.7^{b}	20.8^{a}	0.65	0.01
Full GIT	12.2^{b}	11.8^{b}	12.3^{b}	12.2^{b}	16.1^{a}	0.80	0.01
Empty GIT	4.19	4.57	4.12	4.09	4.36	0.09	0.38
Empty stomach	0.78^{c}	0.96^{a}	0.87^{b}	0.87^{b}	0.97^{a}	0.03	0.001

a, b, c - Means on the same row bearing different superscripts are significantly different ($p<0.05$).

on the absolute and relative weights of viscera, full GIT and empty stomach of the experimental pigs. Pigs on the 20% CPM$^+$ dietary treatment obtained the highest absolute and relative viscera, full GIT and empty stomach weights compared to the rest of the dietary treatments. According to Jørgensenet al. (1996), pigs adapt to diets with increased fibre content by increasing gut volume and weight (Table 4).

Table 5. Villi parameters of the experimental pigs.

Parameter	0% CPM	5% CPM$^+$	10% CPM$^+$	15% CPM$^+$	20% CPM$^+$	SEM	p-value
Villi count, mm^2	1190	1476	1512	1602	1602	75.8	0.27
Villi height, µm	1003	1237	1291	1362	1362	66.3	0.32
Villi width, µm	187	239	221	239	239	10.1	0.76
Villi area, µm^2	586287	948108	940540	1017699	1056279	83717	0.42

Table 6. Haematological and serum biochemical parameters of the experimental pigs.

Parameter	0% CPM	5% CPM$^+$	10% CPM$^+$	15% CPM$^+$	20% CPM$^+$	SEM	p-value
Haematological assay*							
HCT, %	42.9	46.3	43.8	49.4	45.7	1.13	0.67
HGB, g/dL	13.4	13.9	13.7	14.3	13.6	0.15	0.93
RBC, 10^6/ml	6.98	8.01	7.30	7.73	7.68	0.18	0.72
LYM, 10^3 /µl	12.8	12.6	12.9	10.9	11.9	0.37	0.95
MCH, pg	19.2	17.6	18.7	18.6	17.8	0.30	0.25
MCHC, g/dL	31.3	30.4	31.2	39.1	29.8	1.71	0.12
MCV, µm^3	61.4	57.9	60.0	63.9	59.7	1.00	0.25
MON, 10^3 /µl	2.90	0.51	0.43	0.33	0.60	0.49	0.58
NEU, 10^3 /ml	5.72	9.41	11.6	15.5	8.85	1.62	0.22
PLT, 10^3 /ml	234	278	203	247	301	17.1	0.73
WBC, 10^3/ m	19.7	24.3	25.8	27.6	22.4	1.37	0.43
BAS, 10^3 /Ml	0.24	0.23	0.33	0.19	0.13	0.03	0.24
Serum biochemical assay							
Total cholesterol, mmol/L	2.28	2.21	2.34	2.12	2.52	0.07	0.23
Globulin, g/l	31.1	35.2	33.1	35.5	36.2	0.94	0.65
Total BIL, umol /L	2.90	2.90	2.90	2.58	2.86	0.06	0.69
Total protein, g/Dl	64.4	71.9	67.9	70.4	65.3	1.44	0.68
Triglycerides, mmol/L	0.69	0.54	0.83	0.67	0.84	0.06	0.42
Albumin, g/L	33.2	36.8	36.9	34.9	33.4	0.80	0.88

*HCT- Haematocrit, PCV- Packed Cell Volume, HGB- Haemoglobin, RBC- Red Blood Cell, LYM- Lymphocytes, MCH- Mean Cell Haemoglobin, MCHC- Mean Cell Haemoglobin Concentration, MCV- Mean Cell Volume, MON- Monocytes, NEU- Neutrophils, PLT- Platelets, WBC- White Blood Cell and BAS- Basophils.

The 20% CPM$^+$ diet had the highest crude fibre percentage (Table 1) and that could have accounted for the highest weights of the viscera, full GIT and empty stomach. Pigs that were fed a diet containing 30% distillers dried grains with solubles showed increased visceral organ mass relative to the control fed pigs (Agyekumet al., 2012).

Some villi parameters

Montagne et al. (2003) had reported that, an increase in villi to crypt ratio leads to an increase in surface area for greater digestion and absorption of available nutrients to occur. However, the villi count, height, width and surface area measurements showed no dietary effects ($p > 0.05$) among the dietary treatments studied (Table 5). However, there were trends with the villi counts and heights; that is, as the inclusion rates of the CPM$^+$ increased the mean values increased.

Haematological and Serum Biochemical Studies

There were no dietary effects ($p > 0.05$) on all the haematological and serum biochemical parameters (Table 6) considered in the experiment. It is worth stating that, all the mean values obtained across the different dietary treatments fell within the physiological ranges for Large White pigs raised in Ghana as reported by Okai et al. (1995). It can be deduced that the haematological and serum biochemical values obtained attest to the fact that

Table 7. Total sugars and viscosity of raw CPM and the dietary treatments.

Parameter	Raw CPM	0% CPM	5% CPM+	10% CPM+	15% CPM+	20% CPM+	SEM	p-value
Sugar, mg/L	5450[f]	13865[a]	10546[b]	9554[c]	9248[d]	9228[e]	1103	0.001
Viscosity, mm^2/s	1.27[a]	1.09[d]	1.13[cd]	1.13[cd]	1.15[bc]	1.17[b]	0.03	0.001

a, b, c, d e f- Means on a row with different superscripts are significantly different ($p < 0.05$).

CPM plus enzyme-an exogenous enzyme complex, had no adverse effects on the physiology of the experimental pigs.

In vitro digestibility

Dietary effects ($p < 0.05$) were found in the mean values of the total sugar and viscosity measurements (Table 7). The total sugar decreased clearly ($p < 0.05$) as the inclusion level of CPM+ increased. This trend could probably be attributed to the inability of the supplemented enzyme to satisfactorily degrade the cell structures of the CPM to release sugars (energy) bound in its cells (McDonald et al., 2010). It has been reported that soluble fibre increases digest a viscosity and thereby slowdown the diffusion of the substrate and enzymes in the porcine small intestine, which hampers nutrient digestion and absorption (Wenk, 2001). The highest viscosity values recorded by the 15 and 20% CPM+ diets (Table 7) could mean that the high crude fibre values recorded (Table 1) may contain some soluble fibre components which led to increase in the viscosity values. It could also mean that there were important ($p < 0.05$) enzymatic degradation on soluble fibres of the CPM+ diets since the viscosity values seemed to have increased with an increasing in CPM inclusion.

Conclusions

The growth rate and daily weight gain parameters of growing-finishing pigs were substantially lowered when CPM-based diets at 10-20% inclusion levels plus enzyme were fed. However, feed cost per kg body weight gain, haematological parameters and serum profiles were not affected by the inclusion of CPM+ in the diets. All fat parameters considered were clearly lowered with the CPM-based diets containing 10-20%. In summary, the CPM+ diets were not only cheaper but also resulted in leaner carcasses though such pigs took considerably longer to reach the market weight.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Initiation of callus from different genotypes of *Sorghum bicolor* L. Moench

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The study aimed to examine three genotypes of sorghum for their response in tissue culture using Murashige and Skoog (MS) medium supplemented with auxins (2,4-dichlorophenoxy acetic acid (2,4-D), naphthalene acetic acid (NAA)) and cytokinins (Kinetin, 6-benzyle amino purine (BAP)) at different concentrations. The cultures were initiated from different explants (seed, embryo and hypocotyl). The response of explants varied with the genotype. Callus culture were initiated successfully on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l Kinetin from G4 (L58) seedling and embryo explants. G2 (94) hypocotyl explants gave callus on media supplemented with 2,4-D only. G5 (2) explants failed to initiate callus but bulging from the embryo explants was observed on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l Kinetin. The initiated calluses differed morphologically and in their growth rates.

Key words: *Sorghum bicolor,* callus formation, embryo, Kinetin, 2,4-dichlorophenoxy acetic acid (2,4-D).

INTRODUCTION

Successful application of plant biotechnology for plant improvement requires the development of an efficient plant regeneration system from cultured cells or tissues. Many authors have stated that callus derived from monocots is more difficult to regenerate *in vitro* when compared with that from dicots (Bahieldin et al., 2000; Pola et al., 2007).

Sorghum is a monocotyledon, member of Poaceae, it has been considered as one of the difficult plant species to manipulate through tissue culture (Harshavardhan et al., 2002; Kishore et al., 2006; Gupta et al., 2006; Maheswari et al., 2006).

In sorghum, plant regeneration (*via* callus) has been described by many researchers using various explants (Thomas et al., 1977; Ma et al., 1987) such as, immature inflorescences (Elkonin et al., 1995), mature embryo (Kresovich et al., 1986), and mesophyll derived protoplasts (Sairam et al., 1999); also somatic embryogenesis was achieved from shoot tip explants of sorghum (Seetharama et al., 2000). Efficient regeneration in sorghum tissue culture was also reported by many researchers (Mishra and Khurana, 2003; Pola and Mani, 2006; Kishore et al., 2006). However, the rate of plant regeneration per explant is not sufficiently high to be of practical application (Pola et al., 2007).

The main objective of the study was to examine three genotypes of sorghum for their response in tissue culture using Murashige and Skoog medium supplemented with

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different auxins and cytokinins at different concentrations

MATERIALS AND METHODS

Three lines of sorghum (*Sorghum bicolor*), namely 94, L58 and 2 referred to by G2, G4 and G5, respectively were used to find out the most suitable genotype for maximum callus production. Seeds of these cultivars were obtained from the Agronomy Department, Faculty of Agriculture, University of Khartoum. Harvests of 2009/2010 were used.

Seeds, embryos, hypocotyls, epicotyls and leaves were chosen as explants for the study. Seeds of three selected genotypes G2, G4 and G5 were surface sterilized with 70% (v/v) ethanol for 1 min and then immersed for 15 min in a 2.5% (v/v) sodium hypochlorite solution or "Clorox" in a presence of few drops of a liquid detergent. Then, the seeds were rinsed 3 to 4 times with sterile distilled water.

Sterile seeds were grown in moistened filter paper as well as in solidified MS basal medium. One-day-old embryos were separated and cultured in MS basal medium supplemented with different components in order to initiate callus, 7-day-old hypocotyls and leaves were selected from the *in vitro* raised seedlings and used as explant for callus initiation.

The media contained MS basal mineral nutrients (Murashige and Skoog, 1962) supplemented with 3% sucrose were used as media for culture growth. For callus initiation the basal MS media were manipulated with auxins {indole- butyric acid (IBA); naphthalene acetic acid (NAA); 2,4- dichlorophenoxy acetic acid (2,4-D)}, and cytokinins {6-benzyle amino purine (BAP), and Kinetin}, in different concentrations and combinations in a range from 1 to 12.5 mg/l have been used in all experiments. L-Ascorbic acid at 100 mg/l was added as an anti-oxidant agent. The pH of the media was adjusted to 5.7. For preparation of semi solid media, 0.8% agar was used as the gelling agent.

The sterilization of media and glassware was carried out in an autoclave at 121°C, (15 lb/in^2) pressure for 15 min before dispensing in culture vessels.

Callus multiplication and maintenance

For callus multiplication and maintenance the MS medium was supplemented with 2 mg/l 2,4-D and 0.5 mg/ml Kinetin in the presence of 3% sucrose, 0.8% agar and 100 mg/l L-ascorbic acid. Also, 2 mg/l 2,4-D was used without Kinetin in the presence of other supplements for the same purpose.

RESULTS AND DISSCUSION

In the preliminary experiment, it was found that three out of five genotypes of *S. bicolor* studied for salinity tolerant G2, G4 and G5 were efficient for callus induction when different explants were used. For callus initiation, healthy explants of mature seeds, embryos and segments of hypocotyls and leaves were used as the source materials.

Callus induction

Sorghum mature embryos from the three genotypes were removed under aseptic conditions and transferred aseptically to MS medium supplemented with 3% sucrose, 2 mg/l 2,4-D, 0.5 mg/l Kinetin and the medium was solidified with 0.8% agar.

After 34 days, G2 cultured embryos failed to show tissue proliferation and failed to form callus (Figure 1a). On the other hand G4 embryos proliferated rapidly forming a prominent callus within 34 days (Figure 1b). G5 embryos bulging appeared in certain parts of the embryo tissue (Figure 1c).

When whole grain of *S. bicolor* from G4 were used for callus initiation on the same medium used for the mature embryo, callus initiation on the surface of seedling was achieved from all seedlings grown under aseptic conditions.

Callus growth and morphology

The growth rate of the initiated callus was followed for a period of 90 days (Figure 2a to e).

Low levels of 2,4-D have been the most commonly used auxin for callus induction in the cereals. Lu et al. (1983), Hagio (1994), Bi et al. (2007) and Pola et al. (2008) reported that cereals in general require 2,4-D to initiate callus culture and its higher concentrations have been found to be less effective in the formation of embryogenic callus.

Pola et al. (2008) reported that 2 mg/l 2,4-D was the optimum concentration to obtain high frequency of embryogenic callus in sorghum.

A similar trend was observed in the present study. These results revealed that a combination of auxins with cytokinin boosted the embryogenic callus formation.

Previous reports by Maheswari et al. (2006), Gupta et al. (2006), Pola and Mani (2006) and Pola et al. (2008) showed that auxin and cytokinin combination will improve embryogenic callus induction. Gupta et al. (2006) suggest that, to overcome the genotypic limitation of plant regeneration from callus in sorghum, the callus induction medium must be supplemented with strong cytokinin like kinetin with 2,4-D.

When whole grains of *S. bicolor* G2 were grown on MS medium supplemented with 1 mg/l NAA plus 2 mg/l BAP, the explant showed dark purple exudation diffused to the medium. However, when that of G4 supplemented with 1 mg/l NAA plus 2 mg/l BAP were used, brown exudate was produced (Figure 3a and b).

G5 seedlings cultured on the same medium gave abnormal seedlings, showing tissue proliferation in the hypocotyl region (Figure 3c). No exudate was released to the medium.

These results agree with those reported with Pola et al. (2008) who reported that callus initiation was accompanied by the exudation of dark brown and purple colour pigment leading to the brown colorization of the medium around their bases and these cultures underwent necrosis.

To control these phenolic secretions, 0.1% L-ascorbic

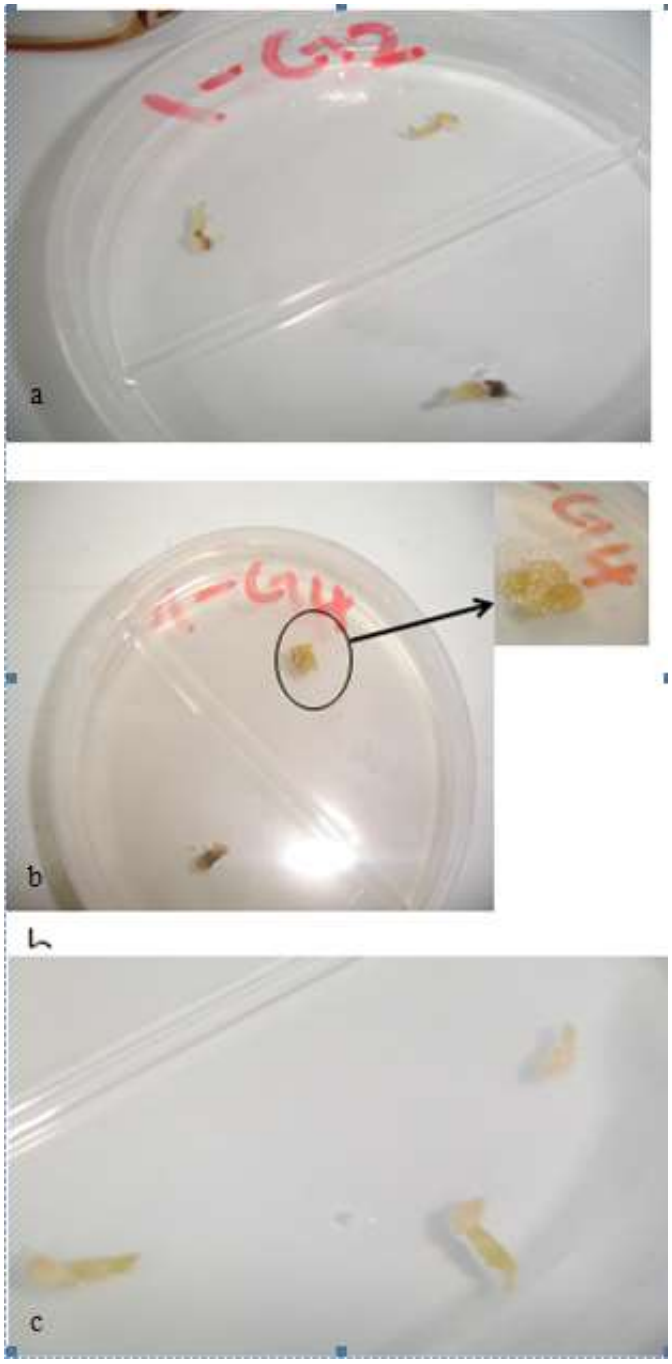

Figure 1. Mature embryo explants grown on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l kinetin for a period of 34 days. (a) G2 no callus initiation (×1), (b) G4 callus initiation (×3/4), (c) G5 bulging for embryo tissue (×1.5).

acid was used in culture media. Frequent subculturing to fresh media with same composition was also tested to overcome the problem.

Segments from seedlings grown under aseptic conditions were also used as explant for callus initiation. Hypocotyl and leaf segments were inoculated to MS medium supplemented with 2 mg/l 2,4-D only. Explants from G2 initiated callus at the cut end of the hypocotyl segments (Figure 4), while leaf explants failed to initiate callus.

The cultures response was greatly influenced by the genotype in all types of explants. Genotypes effects on callusing ability from sorghum were reported previously by Cai and Butler (1990).

Calli formed from all the explants, usually appear in two types based on their colour and quality, that is, friability or compactness. These two types were similar to the embryogenic and non-embryogenic types of callus described earlier in sorghum by Cai and Butler (1990) and more recently by Pola et al. (2008). The embryonic callus appeared yellow, comparatively more compact and morphogenic in nature. At the same time the non-embryogenic callus were unorganized, friable, soft, loosely packed and pale yellow or dull creamy in colour (Figure 2).

General embryogenic callus showing globular structure was visible on the 34th day after inoculation. Changes in the callus morphology were observed in embryogenic and non- embryogenic callus by increasing the number of subcultures. Formation of globular compact or loose friable callus was observed in all callus initiated from the different genotypes, irrespective of their auxin type or concentration. Pola et al. (2008) reported that callus induction frequencies ranged from 40 to 84%. In this study, callus induction frequency was the highest in G4, that is, 100% on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l Kinetin (Figure 2).

The total callus area was estimated in all types of calluses. Of the three genotypes used in this study, genotypic differences were observed with respect to total callus amount. The total area of the callus was highest in genotype G4 and minimum in G5 (Figure 1).

For the study of growth rate of the callus, daily measurements were recorded for all initiated calluses. In general, maximum growth rate, in term of increase of callus area was observed in G4 (Figure 2).

Therefore, among the three genotypes studies, the most suitable genotype to produce maximum embryogenic callus was that of G4. The G4 genotype, also illustrated higher value in term of periodicity of embryogenic callus, quantity of embryogenic callus and growth rate. While genotypes G2 and G5 showed lower rate for most of the characters. These genotypic differences were also observed by Hagio (1994), Gupta et al. (2006), Jogeswar et al. (2007) and Pola et al. (2008) in sorghum.

In the present study, the use of mature embryo tissue provides high embryogenic callus induction frequency, while mature explant failed to show such efficient response. In fact, this was the most critical factor for obtaining large amount of callus tissues which may lead to the formation of large number of somatic embryos from mature embryos.

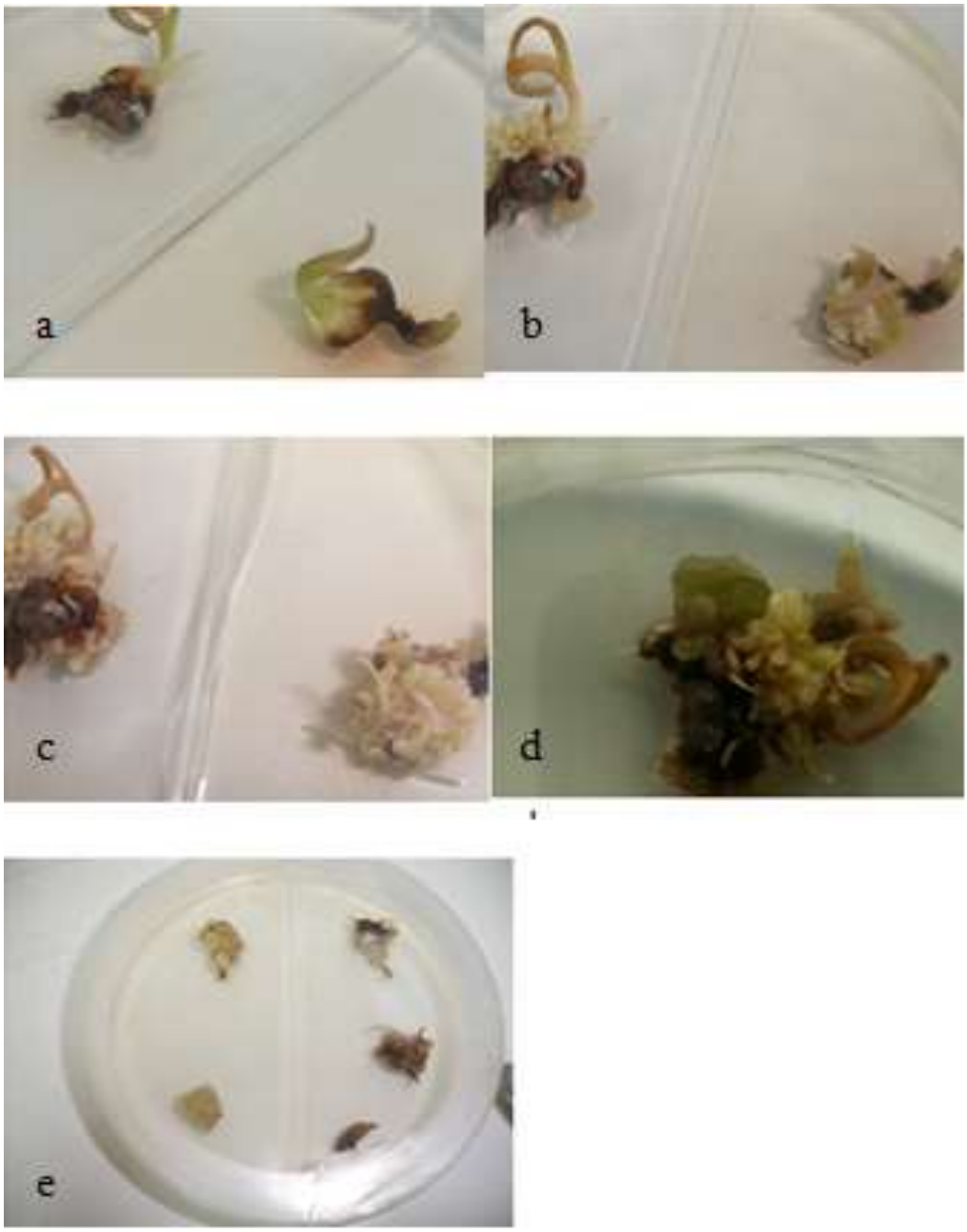

Figure 2. *S. bicolor* G4 callus initiation from sterile seedlings on MS medium with 2 mg/l 2,4-D and 0.5 mg/l kinetin at different stages of development. (a) 27 days after inoculation (×1), (b) 54 days after inoculation (×1), (c) 79 days after inoculation (×1), (d) 84 days after inoculation (×1.5), (e) 90 days after inoculation (×3/4).

Bhojwani and Razdan (1996) reported that, the ability to form large number of somatic embryos from immature embryos is especially true for cereals. Rathus et al. (2004) showed that the physiological stage of source material (explant) used for callus initiation was found to be critical. Gupta et al. (2004) also reported that immature embryos size influenced callus formation and plant regeneration in sorghum.

The callus culture incubated under diffused light underwent necrosis due to the phenolic exudation (Figure 3). These results agree with those reported by Pola et al. (2008).

Recently, many researches are published dealing with *in vitro S. bicolor* tissue culture and regeneration (Pola et

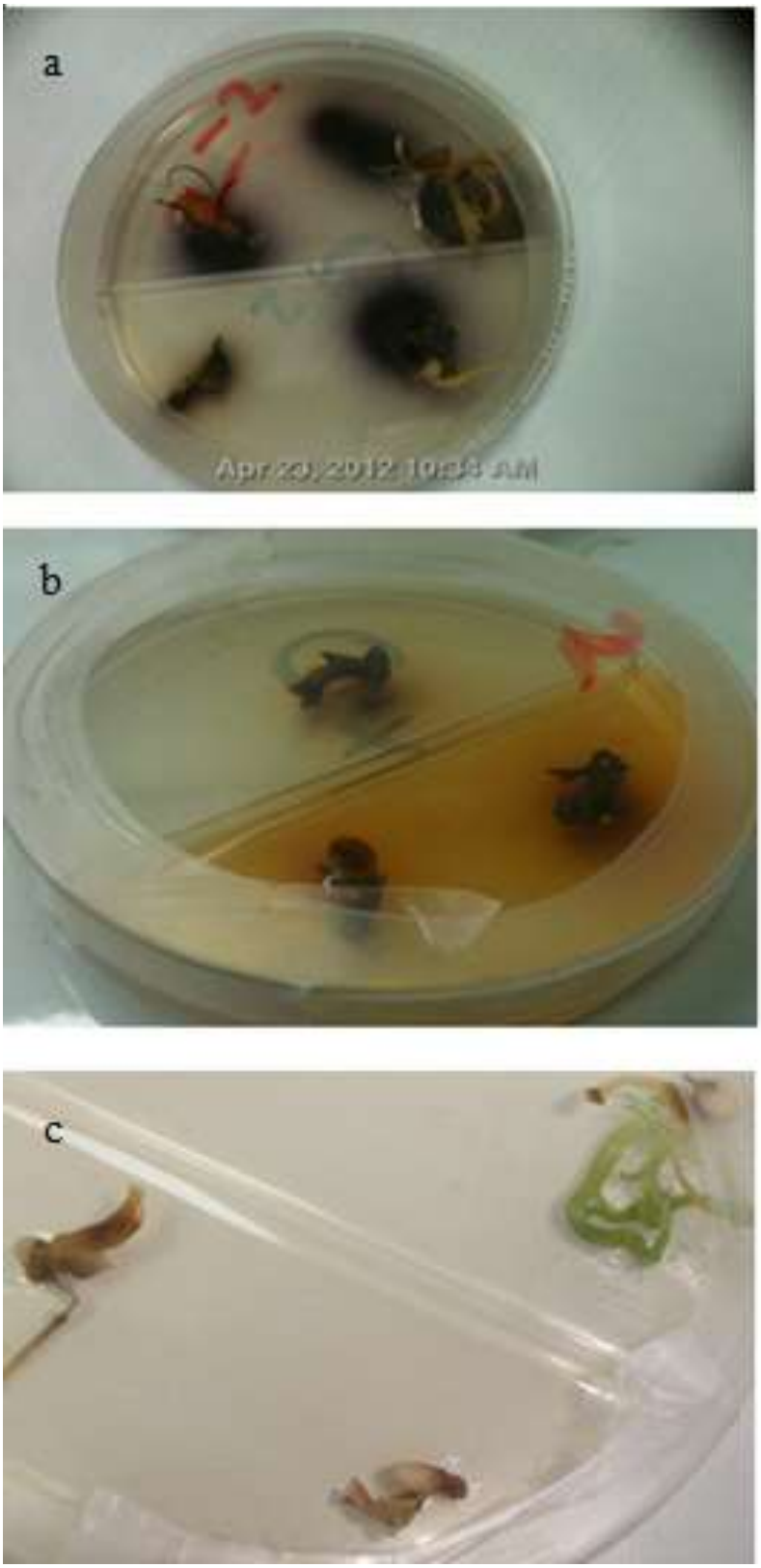

Figure 3. *S. bicolor* seeds grown on MS medium supplemented with 1 mg/l NAA and 2 mg/l BAP. (a) G2, dark purple exudates around the germinating seeds (×1/2). (b) G4 brown exudates on the culture medium (×1). (c) G5 no pigment exudation, growth of seedlings in abnormal and showing tissue proliferation in the hypocotyl region (×1.25).

Figure 4. *S. bicolor* G2 callus initiation from hypocotyl and maintained on MS medium supplemented with 2 mg/l 2,4-D (×2).

al., 2007, 2008).

In most of these studies, they concentrated on the choice of explant and regeneration using various growth regulators.

Conclusion

The study revealed that the amount of the callus formed was genotype and type of explant dependent in the concentration of 2,4-D used. These differences in callusing ability of the different genotypes need further study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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