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Full Length Research Paper

Production of liquid fertilizer by fermented livestock liquid and application on crops

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The livestock liquid commonly contains humic acid, amino acids and minerals; hence, it can be used for the fertilization of crops. These studies were conducted to examine the use of livestock fermented liquid in producing liquid fertilizers and irrigation application on two kinds of crops (tomato and wax apple). The experiments were designed as common liquid fertilizer and diluted by groundwater (CF), fermented livestock liquid fertilizer and diluted by groundwater (LF), fermented livestock liquid and diluted by groundwater (LL), fermented livestock liquid fertilizer and diluted by effluent (LFE), and the treatment of no liquid fertilizer (CK). The results showed that the highest values for fruit weight, width and length were obtained by the treatment of LF, and the sugar degree value was registered by the treatment of LFE. Hence, the treatment of LF was superior compared to CF. Analysis of microbiology in LF, CF and LL showed that the microorganisms' species were most abundant in the LF compared to the other two samples. The main microorganisms were *Bacillus mycoides* and *Bacillus subtilis*. Anthracnose of mango was inhibited in specific isolates of the *B. subtilis* in LF. The results provided the primary information on reusing of livestock waste for crops.

Key words: Livestock liquid, effluent, tomato, wax apple

INTRODUCTION

In Taiwan, the main livestock farming was concentrated at Pingtung, Yunlin and Chianghua counties. The treatment of livestock waste was commonly provided by the three-stage system including solid-liquid separation, anaerobic and aerobic fermentation in the field area of livestock farming. On completion of the treatment, the effluent was drained to rivers. However, part of the river was still polluted occasionally. The biogas was produced after anaerobic fermentation of livestock waste (Chen et al., 2008; Weiland, 2010; Bao et al., 2019), and then the residual solid and liquid were used as fertilizer to farmland (Holm-Nielsen et al., 2009).

In addition to the influence on plant diseases and insects, nutrients absorption imbalance will influence plant growth. The 16 main kinds of nutrient elements are necessary for plants (Kholmanskiy et al., 2019). To sustainably obtain the balanced nutrition, fertilization is necessary.

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If the appropriate ingredients of liquid fertilizer were modified and supplied to crops, it will be rapidly adsorbed by crops and increase the quality of fruits. There are great amount of minerals, amino acids and humic acids in the livestock liquid that can promote the growth of plants (Richardson and Ternes, 2011); however, the negative influence of heavy metals and environmental hormones should be evaluated (Kelessidis and Stasinakis, 2012). This study was conducted to evaluate irrigated water that was replaced by livestock liquid to produce the liquid fertilizer. On the other hand, after application, the effect of different liquid fertilizers on tomato and wax apple were compared. In this experiment, circular agriculture and appropriate fertilization has been achieved.

MATERIALS AND METHODS

Experimental design and production of liquid fertilizer

This experiment was designed for the production of liquid organic fertilizers by livestock liquid. The materials used include: drainage water (or livestock liquid) (300 L), soybean powder (9 kg), rice bran (9 kg), phosphate rock powder (9 kg), seaweed powder (9 kg), molasses (18 kg) and microorganisms (3 kg). The hydrolysis of soybean powder will produce high polypeptide and amino acids and then offer abundant nitrogen fertilizer to plants (Kanu et al., 2009; Zhao et al., 2012). Phosphate rock powder (tricalcium phosphate) is commonly a kind of phosphorus fertilizer, or simply a phosphorus source in fertilizer (Xu et al., 2019). Vijayakumar et al. (2018) showed that application of a lower concentration (20%) of seaweed liquid fertilizer (SLF) will increase fresh weight, leaf area, protein, carbohydrate and lipids of *Capsicum annum* L. Hence, the seaweed was used to supply the potassium in liquid fertilizer. The productive methods are described as follows: At first, the 300 L plastic barrels were arranged, and the groundwater (or fermented livestock liquid) along with prepared materials were all put into the barrel. Thereafter, the liquid fertilizer was stirred by electric stirrer two times every day for 14 days. The stirring duration was 5 min every time. After 30 days, the crop experiments began. The experimental designs were described in Table 1. The tomato (CK, CF, LL, LF, LFE) and wax apple (CF, LF) experiments began from young fruit stage of the two kind of crops. The five irrigative times, the 1,500 L every time, and hence, the total liquid fertilizer amount was 7,500 L. The soil and leaves were sampled and analyzed before and after experiments. The quality of fruits was also estimated at the harvest stage.

Analysis of soils and plants

Pretreatment and analysis of soil

After taking back soil samples, these were air dried and grinded. Thereafter, the samples were shaken through a 2 mm sieve. Further, the soil was analyzed for the following indicators: (1) pH value: water : soil = 1:1, measured by a pH meter (McLean, 1982); (2) Organic matter content was measured by wet oxidation method described by Nelson and Sommer (1982); (3) calcium (Ca), magnesium (Mg), potassium (K) content by extraction of the Ca, Mg, K from the soil with 0.1 N HCl. The content of Ca, Mg and K was measured by inductively coupled plasma spectrometer (Jobin Yvon- 2000) (Baker and Suhr, 1982); (4) phosphorus (P) was measured by molybdenum blue method (Bray No.1) (Murphy and Riley, 1962); (5) iron (Fe) and manganum (Mn) microelements have been extracted from the soil by using 0.1 N HCl. Their contents were measured by an atomic absorbtion spectrometry (AAS) (Cope and Evans, 1985).

Analysis of leaves

Dust and chemical residuals on the leaves were cleaned by tap water; leaves were put into an oven (70-75°C), then after 2 to 3 days were grinded and put into a bottle (Chang, 1981). The leaves were resolved by concentrated sulphuric acid and analyzed by the following methods: (1) nitrogen (N) was measured by Kjeldahl method. (2) P was measured by molybdenum yellow method (Bray No.1). (3) K, Ca, Mg were measured by inductively coupled plasma spectrometer (Jobin Yvon-2000), and (4) Fe and Mn concentrations were measured by inductively coupled plasma spectrometer (Jobin Yvon-2000).

Analysis and application of microbiology in liquid fertilizer

Microbiological analysis in the different liquid fertilizers

After sampling 1 ml of liquid fertilizers, 9 ml distilled water was added and the mixture was shaken. Then, 1 ml mixture was sampled and 9 ml distilled water was added. After multiple dilutions, the liquid was spread on the surface of solid medium (potato dextrose agar (PDA). Three replicates were made; afterwards, the solid medium was placed to the growth chamber at a constant temperature (27°C). After apparently observing the microorganisms, the total amount of microorganisms was calculated averagely for three replications. The calculation formula employed was as below:

$$\text{Average amount of microorganisms} = \frac{\text{Average microorganisms of three replications}}{\text{Dilutive fold 10}} (\text{CFU/ml})$$

Where CFU = Colony forming units.

Test of microorganisms isolated from liquid fertilizer for the antibiosis of mango anthracnose

Anthracnose is a kind of important disease that affect mango (Liu et al., 2014). Some methods were found to effectively inhibit anthracnose (Jiao et al., 2018). At first, the processing groups were set as follow: The isolated microorganisms strain from liquid fertilizer was inoculated in the medium containing agar located at the 1/4 right district. The medium were cultured in the growth chamber at a temperature of 30°C for 1 day. Thereafter, the anthracnose strain was inoculated on the 1/4 left district. The medium was continuously placed in the growth chamber and the temperature was set at 30°C. On the other hand, the treatment of check (CK) was set in such a way that only inoculating anthracnose strain was found on the 1/4 left district of medium; there were no isolated microorganisms strain on the medium. When the hyphae of anthracnose strain in the CK treatment was spread to the 1/4 right district of medium, the inhibition zones of processing groups were investigated.

Statistical analysis

Windows SPSS 10.0 was applied to treat the data statistically for variables analysis. Duncan's multiple range tests were used in differentiating the variances. It was significantly different if $P<0.05$.

Table 1. The experimental treatments of livestock liquid fertilizer.

Treatment	Description
CK	The treatment without liquid fertilizer
CF	Common liquid fertilizer and diluted by groundwater for 400X
LL	Fermented livestock liquid and diluted by groundwater for 10X
LF	The liquid fertilizer was produced by livestock liquid and diluted by water for 400X
LFE	The liquid fertilizer was produced by livestock liquid and diluted by effluent for 400X

Table 2. The variation of soil properties in tomato garden before and after experiments.

Treatment	pH	[2]OM (%)	P	K	Ca	Mg	Fe	Mn	Cu	Zn
			mgkg^{-1}							
[1]B.E.	6.77[a3]	2.50[c]	128[a]	276[b]	3045a	401[b]	198[ab]	121[b]	10[a]	20[b]
CK	6.75[a]	2.45[c]	135[a]	266[b]	3334[a]	382[b]	244[a]	114[b]	14[a]	15[b]
CF	6.12[a]	3.60[b]	134[a]	345[b]	3202[a]	620[a]	162[b]	221[a]	7.9[a]	71[a]
LL	6.46[a]	4.31[a]	124[a]	370[ab]	3492[a]	661[a]	284[a]	246[a]	17[a]	22[b]
LF	6.15[a]	4.97[a]	160[a]	395[a]	3064[a]	559[a]	293[a]	245[a]	20[a]	50[a]
LFE	6.11[a]	3.99[b]	143[a]	383[ab]	3157[a]	560[a]	175[b]	241[a]	16[a]	33[a]

[1]B.E.: Before experiment; [2]OM: organic matter; [3]Values within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 3. The variation of nutrients in the leaves of tomato before and after experiments.

Treatment	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
	%			mg kg^{-1}					
[1]B.E.	2.35[a2]	0.356[a]	2.08[a]	63825[a]	3835[a]	230[a]	145b	1272a	110[a]
CK	2.25[a]	0.399[a]	2.03[a]	62990[a]	3800[a]	151[a]	148[b]	1325[a]	124[a]
CF	2.00[a]	0.400[a]	1.90[a]	66879[a]	4465[a]	168[a]	367[a]	1308[a]	120[a]
LL	2.21[a]	0.360[a]	2.19[a]	69150[a]	3943[a]	153[a]	371[a]	1263[a]	131[a]
LF	2.69[a]	0.391[a]	1.91[a]	65851[a]	4482[a]	150[a]	393[a]	1307[a]	131[a]
LFE	2.97[a]	0.389[a]	1.90[a]	67999[a]	4230[a]	159[a]	381[a]	1265[a]	120[a]

[1]B.E.: Before experiment; [2]Values within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

RESULTS AND DISCUSSION

Experimentation of liquid fertilizer by fermented livestock liquid for crops

Table 2 shows that the soil pH of tomato was 6.77 before experiment. However, it was reduced after experiment in all treatments. It was possible that the application of chemical fertilizer and fermented livestock liquid led to the reduction of pH. The reason why the application of LF was reduced to the lowest pH among all treatments may be stronger acidity in fermented livestock liquid (data not shown). The soil organic matter, phosphorus, potassium, calcium and magnesium were all higher in the entire treatments after experiments. In addition to supplementation of essential nutrients, either with increasing the available elements; however, the increase of microelements was limited. Table 3 shows that the nitrogen, phosphorus, calcium, magnesium, manganese, copper and zinc in the leaves of tomato were slightly higher after all treatments. Regarding the fruits quality of tomato, effects of the treatments of fermented stock liquid (LF and LFE) were higher than that of non-fermented (Table 4). The fruit weight, fruit width and fruit length were all excellent in the treatment of LF; however, soluble solid was highest in the LFE. In general, after the production of liquid fertilizer by fermented livestock liquid, it was then applied to tomato in a bid to increase its quality. Vijayakumar et al. (2018) showed that the usage of seaweed liquid fertilizer can increase the quality of *Capsicum annum* L.

In the wax apple comparative experiment, the focus

Table 4. The compared fruit quality of tomato at harvesting stage.

Treatment	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Soluble solids (°Brix)
CK	12.8[b1]	3.9[a]	2.3[a]	7.3[b]
CF	.13.8[ab]	4.1[a]	2.2[a]	7.8[a]
LL	14.7[a]	4.2[a]	2.4[a]	7.3[b]
LF	15.9[a]	4.2[a]	2.5[a]	7.5[ab]
LFE	12.9[b]	4.0[a]	2.3[a]	7.9[a]

[1]Values within columns within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 5. The variation of soil properties in wax apple orchard before and after experiments.

Treatment	pH	[2]OM (%)	P	K	Ca	Mg	Fe	Mn	Cu	Zn
			mg kg^{-1}							
[1]B.E.	6.76[a3]	1.51[a]	280[a]	350[a]	6109[a]	372[a]	128[a]	198[a]	6.9[a]	23[a]
CF	6.74[a]	1.74[a]	291[a]	361[a]	6115[a]	371[a]	120[a]	177[a]	6.8[a]	19[a]
LF	6.75[a]	1.76[a]	293[a]	359[a]	6130[a]	376[a]	130[a]	185[a]	7.1[a]	22[a]

[1]B. E.: Before experiment; [2]OM: organic matter; [3]Values within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 6. The variation of nutrients in the leaves of wax apple before and after experiments.

Treatment	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
	%			mg kg^{-1}					
[1]B.E.	1.82[a2]		1.72[a]	7332[a]	2979[a]	308[a]	41a	6a	18a
CF	1.85[a]	0.121[a]	1.72[a]	7312[a]	2888[a]	311[a]	45[a]	10[a]	20[a]
LF	1.87[a]	0.125[a]	1.74[a]	7318[a]	2797[a]	314[a]	44[a]	12[a]	22[a]

[1]B.E.: Before experiment; [2]Values within columns within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 7. The compared fruit quality of wax apple at harvesting stage.

Treatment	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Soluble solid (°Brix)
CF	101.1[a1]	6.1[a]	6.7[a]	8.5[b]
LF	107.4[a]	6.2[a]	6.8[a]	11.9[a]

[1]Values within columns within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

was only on the production and application of liquid fermented livestock fertilizer and common fertilizer (LF and CF). Table 5 shows that soil pH was reduced among different treatment. All nutrients were increased after treatments. The elements in the leaves of wax apple (Table 6), fruit weight, fruit width and fruit length along with soluble solid for LF were all higher than CF (Table 7). Lin and Chiu (2019) showed that the quality of wax apple was influenced by the absorption of abundant nutrients and water. In this experiment, we found that when the appropriate liquid fertilizer was irrigated, the quality of wax apple was increased.

The water can be replaced by fermented livestock liquid for the production of liquid fertilizer; thereafter, it can be applied to tomato after diluting 400 times by irrigation water of fermented livestock liquid during fruit-setting stage. The fermented livestock liquid was used in producing liquid fertilizer, and then diluted 400 times by irrigation water for wax apple at fruit-setting and fruit mature stages.

Table 8. The microorganisms' phases in different liquid fertilizer were analyzed.

Treatment	C-1	C-2	C-3	C-4
	CFU/ml			
LL	-	-	-	-
CF	-	-	-	-
LF	**230**	**310**	**190**	**47**

*C-1: Fungus, C-2: *Bacillus mycoides,* C-3 and C-4: *Bacillus subtilis.*

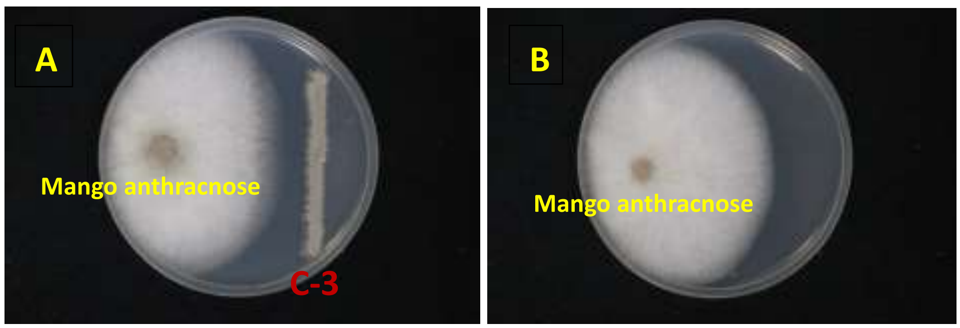

Figure 1. *Bacillus subtilis* (C3) strain screened from fermented livestock liquid fertilizer (LF) chosen for the antibiosis test of mango anthracnose (A: treatment, B: check).

Analysis and application of microorganisms in liquid fertilizer

In Table 8, it is shown that the microbiology phase in LF was most abundant among the treatments of LL, LF and CF; also, fungus and bacteria (*Bacillus mycoides* and *Bacillus subtilis)* were the main microorganisms. Additionally, the microorganisms were the most abundant in the treatment of WF. The C-3 was chosen for the inhibitive experiment of mango anthracnose. The result showed that anthracnose was inhibited by C-3 (Figure 1). Hence, the microbiology of fermented livestock liquid for the inhibition of pathogen can be an advanced research. Duan et al. (2019) reported that *B. subtilis* is a kind of microorganism that can inhibit some plant pathogens.

The results showed that the liquid fertilizers could be produced by livestock liquid, and the quality of tomato and wax apple could be promoted by appropriate dilution of specific liquid fertilizers.

Conclusion

The fermented livestock liquid contains some beneficial materials that can promote the growth of plants. In addition to its use in direct irrigation of crops, it can be regarded as the medium of liquid fertilizer products. From this experiment, the quality of tomato and wax apple were both increased when the fermented livestock liquid fertilizer (LF or LFE) was applied On the other hand, the microbial phase was abundant in the treatment of LF and provided an important clue for advanced research in the screening of beneficial microorganisms for fermented livestock liquid.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of combined salt and flooding stresses on the growth and physiological behavior of alfalfa (*Medicago sativa* L.)

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The present work focused on evaluation of the physiological and metabolic behavior of alfalfa (*Medicago sativa* L., var. Siriver), when faced with two important ecosystems constraints, namely salinity and flooding-associated hypoxia. After germination, seedlings in symbiosis with *Sinorhizobium meliloti* were grown on different salt concentrations (0, 20 and 50 mM NaCl). At four weeks post-germination, plants were submitted to a five-day long flooding treatment and then analyzed for various physiological (growth, biomass) and biochemical (proteins, chlorophylls, ions, proline) parameters. Our results showed that when *M. sativa* was cultured on moderate (20 mM NaCl) or average (50 mM NaCl) salt stress, the classical mechanisms of stress response (ions and proline accumulation) are set up and the growth (FW, DW, growth of aerial parts) was moderately affected by salt. In a situation of average salt stress (50 mM NaCl), the combined salinity and short-term flooding led to an early alteration of the photosynthetic machinery and a modification of ions and proline contents in the leaves.

Key words: Chlorophyll, hypoxia, legume, NaCl, proline, salinity.

INTRODUCTION

Agricultural soils in North Africa are subject to numerous environmental constraints such as salinity, drought during the dry season, flooding or waterlogging during the rainy season, and pollutions caused by industrial and mining discharges (Zahran, 1999). Soil reduced fertility is often due to the presence of salt, and the use of salted water supplies for irrigation resulting in decreased crop productivity (Glenn et al., 1999). Depending on the agricultural region, limitation of water reserves and irrigation practices lead to periodic irrigation of the fields. Thus, either during the rainy seasons or between two irrigation turns, agricultural areas may be flooded or waterlogged for several days, even weeks, at the time of vegetative growth of crops. Both flooding and

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waterlogging result in hypoxia due to the rapid consumption of oxygen in the rhizosphere and a near 10^4 reduction in diffusion of oxygen in water relative to air (Armstrong, 1980).

Alfalfa, or lucerne (*Medicago sativa* L.), is a forage legume cultivated around the world, and among others in North Africa (Bouton, 2012; Zahran, 1999). *M. sativa* is considered to be relatively tolerant to salinity (Bruning and Rozema, 2013; Zahran, 1999) and drought (Bouton, 2012), but sensitive to flooding-induced hypoxia (Striker and Colmer, 2017). Several studies have analyzed its behavior under either saline stress or flooding-induced hypoxia (Li et al., 2010; Rogers et al., 2008, 2009, 2011; Smethurst et al., 2005), but the effects of the double saline and flooding constraints on its growth have not been investigated. In the present work, we initiated the analysis of young *M. sativa* (var. Siriver) plant response to moderate/average salinity combined to short-term flooding treatment. This was accomplished through the measurement of biometric and physiological parameters of the aerial part to evaluate the effects of the combined constraint on forage production, as well as concentrations of mineral ions (Na^+, K^+, Cl^-) and proline, chosen as markers of salt-stress response.

MATERIALS AND METHODS

Ionic analysis of irrigation waters of *M. sativa* cultures

To carry out this study, the salinity of irrigation water of two agricultural soils in northern Tunisia, where alfalfa (*M. sativa* var siriver) is cultivated, was analyzed. The first site of Ghezala Mateur (37° 05' 02" North, 9° 32' 08" East) is located in the heart of an area of 5,300 ha from the Northwest of the town of Mateur to the South of Lake Ichkeul, and it includes the plain of Mateur and the perimeter of Ghezala, which is fed from the dam of Joumine. The second site of Oum Hani, in the suburb of Menzel Bourguiba (37° 09' 13" North, 9° 47' 09" East) is located in agricultural land close to the lakes of Ichkeul and Bizerte. Electrical conductivity and salinity of irrigation waters during the winter of 2014 were measured as in Bahri (1993). The averaged electrical conductivity (one measurement) and nitrate and NaCl concentrations (3 measurements) of the irrigation water were as follows: 1) water of the Ghezala well: 0.86 dS/m, 0.21 ± 0.04 mM NO_3^-, 19 ± 5 mM NaCl; 2) water of the Oum Hani well: 4.65 dS/m, 0.92 ± 0.03 mM NO_3^-, 52 ± 6 mM NaCl.

Based on nitrate and NaCl contents measured in irrigation waters, we chose to test the behavior of *M. sativa* seedlings under the following salt conditions: 1) a control condition (Ctrl0), with 0.2 mM KNO_3 (a low concentration of nitrate which allows a minimum supply of nitrogen without affecting the symbiotic process) in the absence of NaCl, 2) a T20 treatment, with 0.2 mM KNO_3 and 20 mM NaCl, representing the irrigation water characteristics of the Ghezala well, which corresponds to a moderate salt stress, and 3) a T50 treatment, with 0.9 mM KNO_3 and 50 mM NaCl, representing the characteristics of the irrigation water of the Oum Hani well, which corresponds to an average salt stress.

Plant material

Seeds of *M. sativa* (var. Siriver) were sterilized with sodium hypochlorite (2% active chloride) for 20 min, thoroughly rinsed, and imbibed in sterile distilled water for 3 h. Then, germination was carried out at 22°C in Petri dishes, lined with filter papers moistened with distilled water. Germination, identifiable by the emergence of radicles, took place in the dark after 5 days. The germinated seeds were transplanted in plastic pots (8 cm deep) filled with B5 sand (0.6-1.6 mm diameter), provided with adequate drainage device and then grown in culture room at 25°C, with a 16 h/8 h (day/night) photoperiod, and 60/80% (day/night) humidity. Plants were watered every 3 days, once with water, once with a mineral solution containing the following basic macro- and micro-nutrients: 2.5 mM $MgSO_4$, 0.5 mM K_2SO_4, 2 mM KH_2PO, 1 mM $CaCl_2$, 0.05 mM Fe-EDTA, 16 µM H_3BO_3, 5 µM $MnSO_4,4H_2O$, 1 µM $ZnSO_4,7H_2O$, 1 µM $CuSO_4,5H_2O$, 0.7 µM $NaMoO_4,2H_2O$, and 0.5 µM $CoCl_2,6H_2O$. In addition to the basic nutrients, either water or mineral solution also contained either 0.2 mM KNO_3 (Ctrl0), 0.2 mM KNO_3 and 20 mM NaCl (T20), or 0.9 mM KNO_3 and 50 mM NaCl (T50). Plants were inoculated 7 days post-imbibition with *Ensifer meliloti* 2011 (previously named *Sinorhizobium meliloti* 2011), the predominant microbial symbiotic partner (Frendo et al., 2005).

At 28 days post-imbibition (three-leaf vegetative stage approximately), the plants were either maintained under normoxia (N), or subjected to flooding (H) for 5 days. Flooding treatment was done by submerging pots containing plants to 1 cm above substrate level in the mineral solution supplemented, or not, with NaCl and KNO_3 to maintain a medium close to that of the irrigation waters of Ghezala and Oum Hani. At 33 days post-imbibition, the shoots of the plants were harvested, and either measured for biometric parameters or quickly frozen in liquid nitrogen and stored at -80°C until further analysis.

Vegetative growth analysis

The growth and biomass production of shoots was assessed by measuring the following parameters: height, fresh weight (FW), dry weight (DW) and water content, expressed as WC (%) = 100 (FW – DW)/FW, and leaf area (LA), that was measured using the MESURIM free access software: (http://acces.ens-lyon.fr/acces/logiciels/mesurim/telechargement). The dry weight is measured after drying the fresh material for 48 h at 70°C.

Protein measurements

Shoot samples (50 to 150 mg) were ground in a mortar and pestle in a buffer containing 25 mM Tris-HCl, pH 7.0, 0.5 mM β-mercaptoethanol, and 0.3% (p/v) polyvinylpolypyrrolidone. Homogenates were centrifuged for 15 min at 13400 g, and the clarified extracts in the supernatant were recovered. Soluble proteins were quantified on clarified extracts (Bradfor, 1976), using bovine serum albumin as standard.

Determination of chlorophylls

The extraction and determination of the concentration of leaf chlorophyll *a*, chlorophyll *b* and total chlorophyll were performed as in Arnon (1949), and calculations were according to the following formula:

Chlorophyll *a* = 12.7 (A663) - 2.69 (A645),
Chlorophyll *b* = 22.9 (A645) - 4.68 (A663),
Total chlorophyll = 20.2 (A645) + 8.02 (A663).

Determination of proline

Proline extraction and measurement was carried out according to the method of Bates et al. (1973), on alfalfa shoots previously dried in an oven at 60°C. The optical density was measured at 520 nm. The standard range was established based on pure proline.

Determination of mineral elements

Mineral elements were extracted using the technique of cold nitric extraction from dried plant material according to (Gauquelin et al., 1992). Potassium (K^+) and sodium (Na^+) were determined by flame emission spectrometry according to Morard and Gullo (1970). The determination of chloride (Cl^-) was done by coulometry (chloridometer Buchler-Cotlove) according to Morard and Gullo (1970).

Statistical analysis

Every parameter was analyzed in three independent experiments, and measurements were carried out on samples of five to nine plants per treatment. One-way ANOVA followed by a Tuckey test (http://www.statskingdom.com/index.html) was carried out to determine the effect of the treatments in the analyzed variables. Differences were considered to be significant at the threshold of $P < 0.05$.

RESULTS AND DISCUSSION

Vegetative growth analysis

The effects of saline and hypoxic constraints were first analyzed on the growth of *M. sativa* shoots (Figure 1). As compared to Ctrl 0 in normoxia, after 33 days of growth, the size of the shoots and the leaf area (LA) were not significantly affected by moderate salt treatment (T20), whereas they decreased by 33% and 65%, respectively, in the presence of 50 mM NaCl (T50) (Figure 1A and B). The fresh weight (FW) and dry weight (DW) masses of the shoots were not significantly modified by the salt treatment (Table 1), and the water contents (WC) remained constant (close to 80%) regardless of the NaCl content in the nutrient medium (Table 1). A similar pattern was maintained in shoot dry weight in response to salt stress in 3 month-old *M. sativa* (var sativa) plants subjected to varying concentrations of NaCl for 4 weeks (Rogers et al., 2008, 2009), which confirms that *M. sativa* is quite tolerant to salinity. The imposition of 5-day flooding stress did not result in a profound change in growth parameters (Figuure 1 and Table 1), which means that short-term hypoxia did not significantly affect the growth of *Medicago sativa* in the absence as well as in the presence of NaCl.

Considered together, these results show that the biomass production of *M. sativa* was not significantly affected by average salt stress, while the growth of aerial parts and LA already showed a moderate reduction. Such a pattern of maintaining biomass production, while aerial parts and LA are reduced, may be explained by an increase in leaf thickness and mass per area as already observed in *M. sativa* response to salinity (Smethurst et al., 2008). Interestingly, an increase in leaf mass area was also observed in *M. truncatula* response to hypoxia (El Msehli et al., 2016), and it may be thought that such response is reinforced in response to combined salinity and hypoxia stresses. Actually, the maintenance of water content revealed that *M. sativa* has a good ability to adjust its osmotic potential. The absence of additional deleterious effects on the growth parameter, when exposed to the combined treatments of salinity and flooding-induced hypoxia, indicated that, at least over a 5 day-long hypoxia period, *M. sativa* is able to cope with this double constraint. To go deeper into the physiological study of the effects of single and combined stress, we analyzed parameters related to shoot protein content and photosynthetic capacity on the one hand; and we measured the major ions accumulated under salt constraint (Na^+, K^+, Cl^-), as well as proline which is a salt stress response marker in plants (Tujeta, 2007), on the other hand.

Effect of salinity and hypoxia on protein and chlorophyll contents

The soluble protein content is a valuable indicator to evaluate the impact of stress on the overall functioning of cells and tissues. As shown in Table 1, shoot protein contents were not modified significantly by either saline treatment, or after 5 days of flooding, indicating that *M. sativa* tolerates the imposition of the combined constraint at least for short periods. Chlorophyll contents were also analyzed to evaluate the capacity of *M. sativa* shoots to maintain their photosynthesis under combined salinity and hypoxia. Table 1 shows that under normoxia chlorophyll contents (*a*, *b* and total) were unaffected by salinity treatments. Similarly, a 5-day long flooding treatment did not change significantly the global chlorophyll contents when compared to the other hypoxically treated plants. But, it should be noted that for plants combining both 50 mM NaCl and flooding treatments (T50, H), their chlorophyll contents were approximately 50% lower than that of 50 mM treated normoxic plants (T50, N). Decreased chlorophyll levels (Table 1), as well as reduced leaf area in plants treated with 50 mM NaCl (Figure 1B), indicate a decline in overall plant photosynthetic capacity. It has been reported that under salt stress, as well as under hypoxia, a close relationship exists between chlorophyll level and photosynthetic capacities (Netto et al., 2005; Striker and Colmer, 2017). Present data indicate, first, that the photosynthetic machinery is affected earlier than is the overall protein content, and second that each stress, considered separately, does not affect the photosynthetic machinery; as already observed in previous studies (El Msehli et al., 2016; Li et al., 2010). However, the combination of the two stresses clearly affects it and

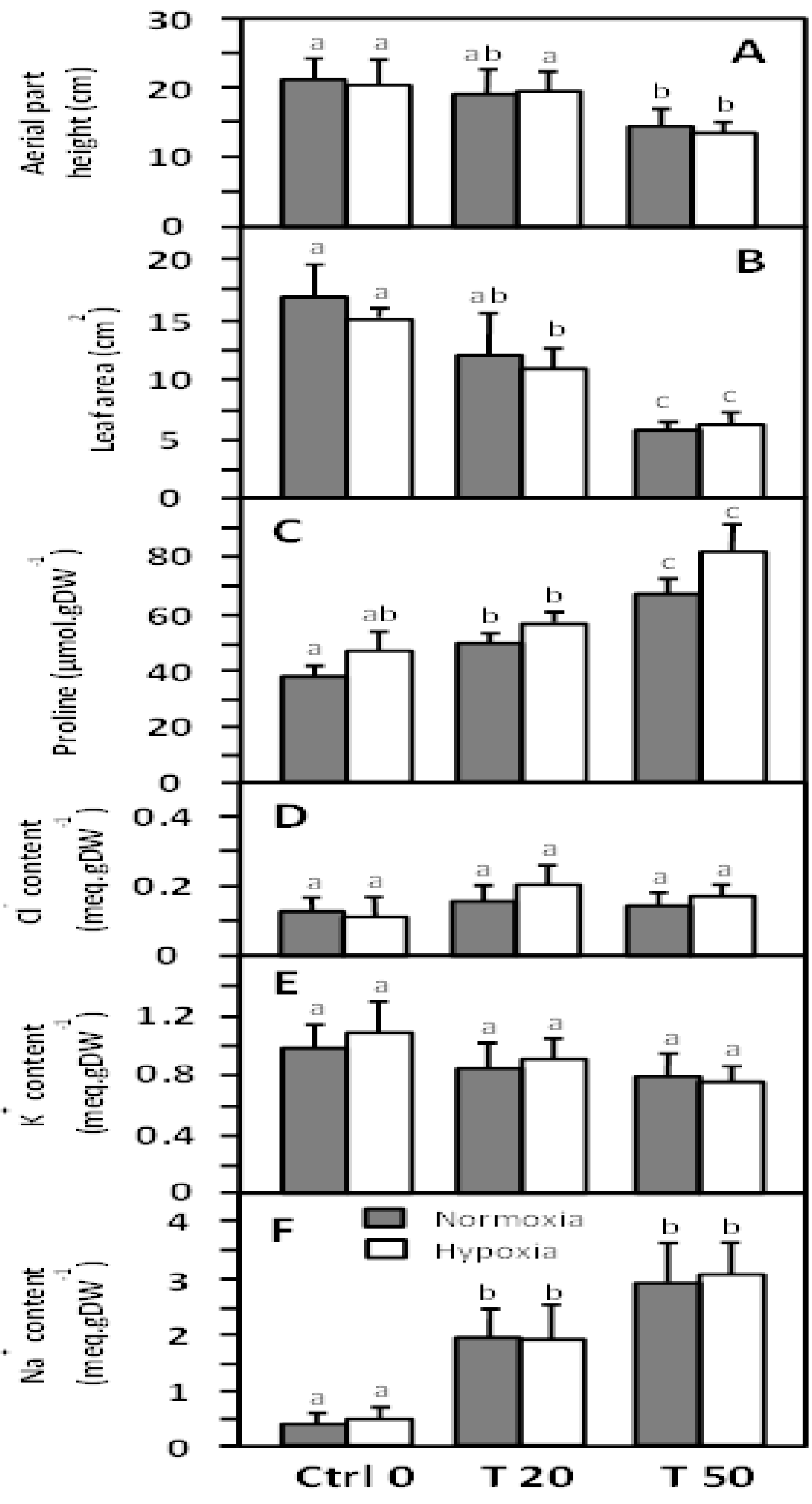

Figure 1. Effects of salinity and hypoxia on biometric parameters (height, leaf area), protein and metabolite contents (ions, proline) of *M. sativa* aerial parts. A, aerial part height; B, leaf area; C, proline content; D, Cl^- content; E, K^+ content; F, Na^+ content. Normoxic and hypoxic treatments were respectively figured as shaded and non-shaded bars. Treatments with 0, 20 and 50 Mm NaCl were respectively labeled Ctrl0, T20 and T50. The values are means ± SD of 3 independent experiments. Values followed by different letters are significantly different with P<0.05.

Table 1. Effects of salinity and hypoxia on dry and fresh weight, water content and chlorophylls of *M. sativa* plant shoots.

Variable	Treatment	NaCl		
		Ctrl0	T20	T50
Fresh weight (mg/plant)	N	288±79[a]	290±79[a]	255±68[a]
	H	274±84[a]	326±61[a]	325±89[a]
Dry weight (mg/plant)	N	52±15[a]	49±12[a]	44±14[a]
	H	51±18[a]	56±20[a]	51±16[a]
Water content (%)	N	82±3[a]	83±3[a]	83±7[a]
	H	81±3[a]	83±2[a]	84±2[a]
Protein content (mg/g FW)	N	10.5±1.8[a]	10.7±2.0[a]	9.7±1.3[a]
	H	9.6±1.5[a]	8.9±1.6[a]	8.8±2.1[a]
Chlorophyll a (mg/g FW)	N	0.36±0.06[a]	0.36±0.06[a]	0.41±0.10[a]
	H	0.30±0.09[ab]	0.24±0.08[ab]	0.23±0.05[b]
Chlorophyll b (mg/g FW)	N	0.29±0.09[a]	0.30±0.04[a]	0.45±0.14[a]
	H	0.22±0.07[ab]	0.28±0.07[a]	0.15±0.05[b]
Total Chlorophyll (mg/g FW)	N	0.65±0.15[ab]	0.65±0.09[a]	0.86±0.24[a]
	H	0.52±0.16[ab]	0.52±0.10[ab]	0.42±0.10[b]

Normoxic and hypoxic treatments were respectively labeled N and H. Treatments with 0, 20 and 50 mM NaCl were respectively labeled 0, 20 and 50. The values are means ± SD of 3 independent experiments. Values followed by different letters are significantly different with $P<0.05$.

impairs the capacity of *M. sativa* plants to maintain photosynthesis. In this situation, insufficient sugar production cannot cover the needs in maintaining the carbon skeleton for symbiotic nitrogen assimilation and plant growth, and compromises the plant capacity to cope with longer-lasting stress conditions.

Effect of salinity and hypoxia on mineral nutrition

The submission of *M. sativa* plants to increase salt stress in normoxia was accompanied by a significant increase in the Na^+ content in the leaves, from 0.43 meq.g^{-1} DW in Ctrl0 shoots to 2.0 and 2.95 meq. g^{-1} DW in T20 and T50 shoots, respectively (Figure 1F). The content of K^+, on the order of 1 meq. g^{-1} DW, did not change significantly as a function of salinity; while the Cl^- concentration remained close to 1.6-1.8 meq.vg^{-1} DW in T50 plants (Figure 1D and E). Such observations are comparable to the results obtained for other saline-treated varieties of *M. sativa* (Li et al., 2010; Rogers et al., 2008, 2009, 2011). However, in the present study, the Na^+/K^+ ratios measured in T20 and T50 plant shoots (2.3 and 3.7, respectively) are much higher than those measured in previous reports (Li et al., 2010; Rogers et al., 2009). This is likely due to the fact that in these reports *M. sativa* plants were first grown in the absence of NaCl for several weeks before being subjected to 2-4 weeks salt stress, whereas in our study plants were subjected to salinity from germination, as is the case in the field, and they have developed from the beginning a system of salt storage in the aerial parts. It also shows that *M. sativa* can store high amounts of NaCl in the leaves without significantly affecting plant growth. In comparison with normoxia treated plants, the application of flooding stress did not change the Na^+, K^+ and Cl^- contents in the shoots (Figure 1D, E and F), which means that ion transport and metabolism is not significantly altered by short-term flooding.

Effect of salinity and hypoxia on proline accumulation

As shown in Figure 1C, compared to normoxic treatment (Ctrl0), exposure to increasing concentrations of NaCl resulted in a significant and expected increase in leaf proline contents of 29 and 79%, in T20 and T50 shoots, respectively. Proline is a general marker of salt stress response in most plants (Mansour and Ali. 2017; Per et al., 2017), including legumes (Bruning and Rozema, 2013). Proline biosynthesis was expected to occur as a consequence of disturbance in cell homeostasis and to reflect damage in response to salt stress (Khalil et al.,2016). Either an external supply (Ehsanpour and Fatahian, 2003), or an increased cellular level (Campanelli et al., 2013) in proline, promotes salt tolerance in *Medicago* species. Moreover, salt tolerance was found to be enhanced in *Medicago truncatula* plants transformed with a gene for proline synthesis (de la Pena et al., 2010). As compared with normoxia-treated plants, the application of hypoxia led to a slight increase (8-24%) in proline contents after 5 days of treatment. Although the difference was not significant between N-50 and H-50 plants, such an increase is of interest, because proline accumulation has recently been observed in *Phaseolus vulgaris* response to hypoxia (Velasco et al., 2019). It was suggested to serve as a source of energy, nitrogen or carbon to reinitiate growth after the hypoxia stress period (Aloni and Rosenshtein, 1982). However, further study would deserve investigation to confirm this observation and to analyze the potential role of proline in the combined salinity-hypoxia stress response.

Conclusion

M. sativa is a legume crop known to be tolerant to salinity (Bruning and Rozema, 2013; Zahran, 1999) and drought stress (Bouton, 2012), and sensitive to waterlogging and hypoxia (Rogers, 1974; Striker and Colmer, 2017). In northern Africa, *M. sativa* is often grown in areas where, during the rainy season or after irrigation turn, cultivated fields are subject to the double stress of salinity and flooding. Our results showed that when *M. sativa* is cultured on moderate (20 mM) and medium (50 mM) NaCl concentrations, the classical mechanisms of stress response (mineral ion accumulation, proline synthesis) are set up and the growth (FW, DW, growth of aerial parts) is only moderately affected by salt. At low NaCl concentration, short-term hypoxia did not significantly affect growth. But, in a situation of average salt stress (that is, 50 mM NaCl), the combined stresses seemed to lead, starting from 5 days of hypoxia, to an early alteration of the photosynthetic machinery and a modification of the compartmentalization of ions between roots and leaves. A study of the combined effects of salt and hypoxia during a longer period of hypoxia is needed to evaluate more deeply the impact of combined stresses on growth and yield of *M. sativa*. However, as a first step, it is recommended to control crop irrigation regimes to avoid too long period of flooding and the risk to significantly impact final yields.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Promotion of rice growth and productivity as a result of seed inoculation with *Azospirillum brasilense*

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The present study refers to the results obtained in four experiments carried out in the 2015/2016 crop: Toledo/PR; Palotina/PR; Cascavel/PR; and São Miguel do Iguaçu/PR. The objective of the present study was to evaluate the agronomic efficiency of *Azospirillum brasilense* (pest strains Ab-V5 and Ab-V6) in four distinct rice culture regions (*Oryza sativa* L.). The tests were performed to meet the requirements of the Ministry of Agriculture, Livestock and Supply regarding the registration of the product. The experiments were conducted in a randomized block design with seven treatments and four replications, totaling 28 plots each. Rice cultivar IAC 201 was used in all four experiments. At stage R7, 10 plants were collected to measure morphobiometric variables and nitrogen content in grains (N grains). The results showed that in all evaluated sites the use of liquid inoculation with *A. brasilense* increased rice crop development as well as productivity. Thus, inoculation of rice seeds with *A. brasilense* strains Ab-V5 and Ab-V6 based inoculant in the liquid formulations increased the yield and yield components of rice crop in different regions. The inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6, using the liquid vehicle, presented higher agronomic efficiency for rice cultivation. The inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6 using the peat medium has good agronomic efficiency similar to the standard inoculant. These results were satisfactory contributing also to the sustainability in the agriculture.

Key words: Diazotrophic bacteria, plant growth promoting bacteria, nitrogen fertilization, seed inoculation, *Oryza sativa L*.

INTRODUCTION

Rice (*Oryza sativa* L.) is a cereal that is present daily in the diet of much of the world's population, being one of the most widespread crops in the world (Areias et al., 2006). Estimated world production for grain since 2017 is

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over 475 million tons, of which 8.3 million tons are produced in Brazil (USDA, 2017). In relation to the world ranking in recent years, Brazil is always among the top ten producers of *O. sativa* compared to Latin American countries (CONAB, 2016). These results are important because there is a small gradual reduction in the country over the same period of rice acreage. However, in this scenario, the state of Paraná maintains its prominence, as it does not present large production fluctuations, currently guaranteeing the 7th position in the national ranking of producers of this crop.

It is well known to the scientific community that there are several factors that can interfere with rice crop productivity, among them nitrogen fertilization plays an important role (Hernandes et al., 2010). Mainly due to the nutritional sources required for this crop which come from the mineralization of soil nitrogen (Vogel et al., 2013). Of all the chemical elements, nitrogen undoubtedly deserves prominence in the development of any crop, mainly because it directly influences the growth of plants thus enhancing their production (Malavolta and Moraes, 2007). In relation to rice cultivation, this chemical element may contribute to the increase in the number of tillers and, thus, also increase the number of plant panicles, besides promoting greater number of spikelet grenades and higher protein content in grains (Rojas et al., 2012). However, despite the importance of this mineral, its use for a good yield of *O. sativa* production is dependent on high nitrogen doses. However, their excess can cause problems caused by self-shading or bedding, factors that may also provide adequate conditions for the creation of favorable conditions for disease occurrence (Neves et al., 2004).

Since the early nineties, several researchers have directed their investigations to increase the efficiency of biological nitrogen fixation performed by plant growth promoting bacteria in Brazil (Dobereiner, 1990).

In the literature it is possible to observe different beneficial effects caused by a wide list of micro-organisms. Known results range from mineral solubilization, nutrient supply and / or plant phytohormones synthesis, to biological pathogen control (Babalola, 2010).

Some positive responses in the interaction between the use of *Azospirillum brasilense* inoculation in peat medium in two rice cultivars were observed, mainly for nitrogen (N) accumulation in plants and grains were verified by Guimarães et al. (2007). These authors emphasize that inoculation associated with balanced fertilization contribute significantly to the development and production of rice grains.

Considering the root system of the rice plant, Beutler et al. (2016) report that the interaction between nitrogen fertilization and bacterial inoculation provided greater development of root and shoot system.

Similarly Vogel et al. (2013), evaluating the agronomic performance of *A. brasilense* in rice, found that the use of *A. brasilense* associated with rice culture presents promising results regarding the plant morphological aspect and the increased grain yield and a sustainable means of production.

Other excellent results were described by Moura (2011) when they found that different water slides associated with inoculation of *A. brasilense* with Ab-v5 and Abv6 strains at a concentration of 200 mL, with different N rates offered in rice cover (0, 25, 50, 75 and 100 kg ha^{-1}), increased the number of tillers by approximately 11% compared to the treatment without inoculant.

Already according to Sabino et al. (2012), inoculation using strains of diazostrophic bacteria, associated with 50 kg ha^{-1} of nitrogen, provided the largest biomass accumulations in rice seedlings.However, there are few scientific investigations that have shown concrete scientific response on the agronomic performance of *A. brasilense* in relation to inoculation of these bacteria and rice cultivation (Vogel et al., 2013).

Objective

The objective of this study was to evaluate the agronomic efficiency under field conditions of *Azospirillum brasilense* inoculant (strains Ab-V5 and Ab-V6) in the liquid and peat formulation for rice crop.

MATERIALS AND METHODS

Experimental site

The study was conducted in four different locations: Toledo, Palotina, São Miguel do Iguaçu and Cascavel all in the state of Parana (Table 1). The tests were performed from October 2016 to February 2017. The geographical coordinates, altitude and soil type of the areas are also presented, according to EMBRAPA (2013).

Determination of soil and weather conditions while conducting the experimente

The chemical and physical characterizations of the soils of the experimental areas were carried out through material analysis from samples composed by ten subsamples in each experimental area; at a depth of 0 to 0.20 m. Results are presented in Table 2.

According to the Köppen and Geiger classification (1928), the São Miguel do Iguaçu region has a subtropical humid mesothermal regional climate with hot summers (average temperature of 20.0°C and 1755 mm of rainfall for the respective regions). As for the regions of Toledo, Palotina and Cascavel, they have hot and temperate climate with hot summers (average temperature of 19.4; 20.8 and 18.2°C temperature and 1483; 1508 and 1822 mm of rainfall for the regions). Regardless of the region, there is a tendency to concentrate rainfall and winters with infrequent frost. The meteorological data of the four experimental areas during the conduction of the experiments are presented in Figures 1A, B, C and D.

Regarding the correction of these soils, when necessary, it was performed 30 days before sowing; the elevation of the base saturation was performed according to Barbosa Filho et al., 1999. The basic fertilization was performed by nutrient distribution in

Table 1. Geographic site, type of soil and climate of the places where the experiments were carried out.

Coordinates	Level	Soil type	Weather
24°40'12"S 53°39'19'W	531	Dystrophic red latosol	Humid subtropical mesothermic
24°17'44"S 53°49'40"W	267	Eutrophic red latosol	Humid subtropical mesothermic
25°23'07"S 54°12'33'W	297	Eutrophic red nitosol	Humid subtropical mesothermic
25°01'57"S 53°28'55"W	721	Eutrophic red latosol	Humid subtropical mesothermic

Table 2. Chemical and particle size characteristics of the soil collected in the 0.0-0.2m layer from the Biogenesis Experimental Station, Research and Development Center, located in Toledo - PR, 2016.

L	Chemical characteristics											Granulometry		
	pH	V	P	MO	Ca^{2+}	Mg^{2+}	K^{+}	Al^{3+}	H+Al	SB	CTC	Clay	Silt	Sand
	$CaCl_2$			-%	mg dm^{-3}			g dm^{-3}		$cmol_c$ dm^{-3}			g kg^{-1}	
1	5.42	60.19	13.28	23.09	5.77	2.19	0.38	0.00	4.32	8.22	13.21	530	378	92
2	5.21	60.15	13.92	22.28	5.09	2.27	0.75	0.02	5.09	8,49	12.29	633	305	52
3	5.78	57.25	15.22	24.02	5.05	2.05	0.99	0.00	4.97	7.96	13.03	560	358	82
4	5.86	62.15	13.28	23.17	5.56	1.28	0.69	0.00	4.91	6.41	12.99	584	371	45

P, K, Micronutrients, Mehlich^{-1} Extractor; Al, Ca, Mg, 1 mol L^{-1} KCl Extractor; H + Al pH SMP (7.5); (pH) 0.01 mol L^{-1} $CaCl_2$ extractor. Analysis performed at the Agricultural and Environmental Chemistry Laboratory, Unioeste. *Campus* Marechal Cândido Rondon, PR.

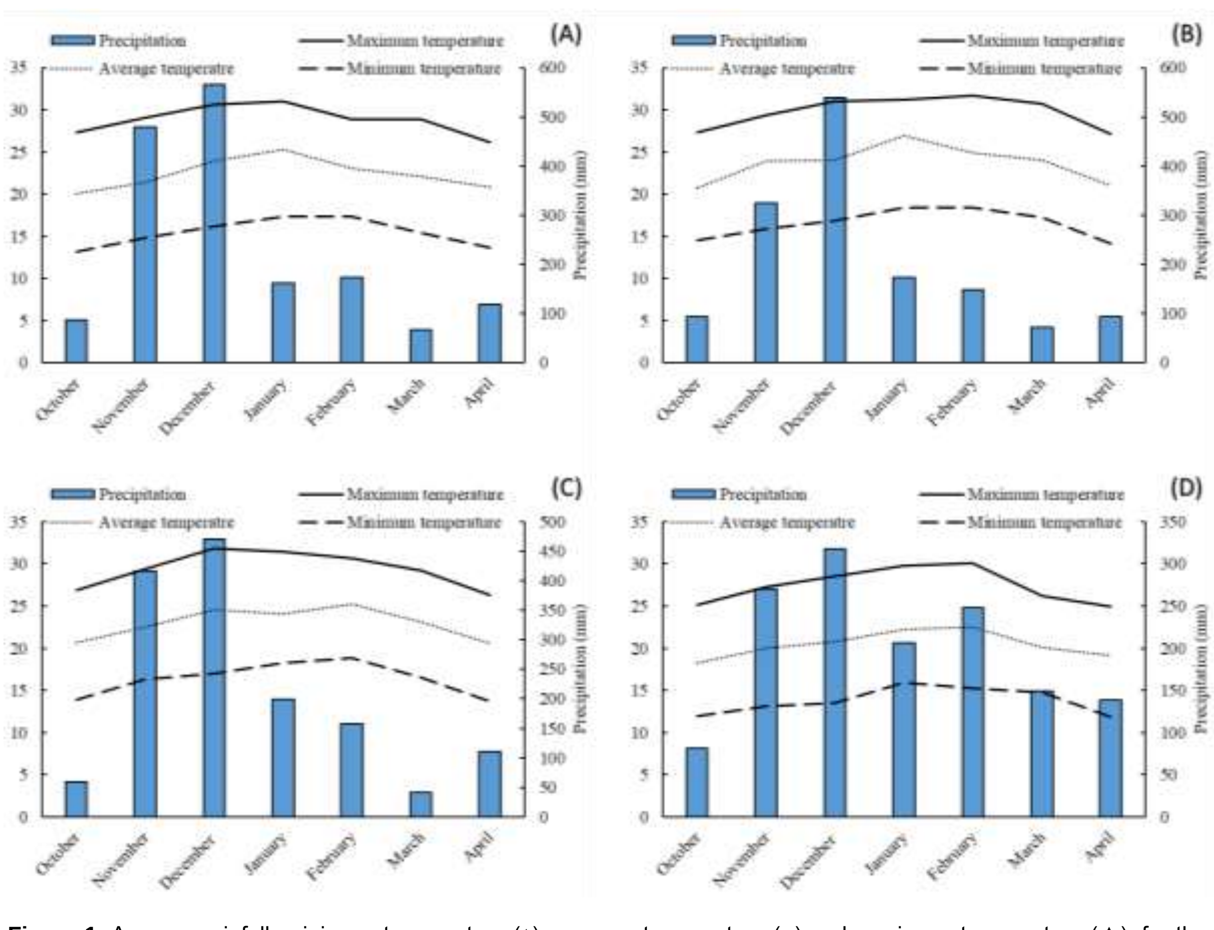

Figure 1. Average rainfall, minimum temperature (♦), average temperature (■) and maximum temperature (▲), for the different locations: A) Toledo - PR; B) Palotine - PR; C) São Miguel do Iguaçu - PR and D) Cascavel - PR, from October 2016 to February 2017.

furrows parallel to a depth of approximately 5 cm, simulating mechanical sowing.

Most likely number (MLN) of endophytic diazotrophic bacteria present in soils of experimental areas

The counting of diazotrophic microorganisms to determine the bacterial population in cell number per mL was performed by estimating the Most Probable Number (MPN) using the MacCrady table in semi-solid medium NFB, according to methodology described by Döbereiner et al. (1995).

The results of the counting of diazotrophic microorganisms in the experimental soils of the four experimental areas at the time of sowing presented population of: 1.6x 10^6 (Toledo - PR); 1.4x10^6 (Palotina - PR); 0.9x10^6 (São Miguel do Iguaçu - PR) and 1.1x10^7 (Cascavel - PR), colony-forming unit (CFU) g^{-1} of diazotrophic bacteria.

Identification of NITRO 1000 liquid and peaty rice inoculum samples used in the four experiments

According to the company, NITRO 1000 - Biological Inoculants, inoculants have the following characteristics: - Liquid rice product: Warranty: 2.0x10^8 CFU/mL *A. brasilense*, strains Ab-V5 and Ab-V6; Physical nature: liquid; Density: 1.0 g/mL; Target crop: Rice (*Oryza sativa* L.); Tested dosage for 25 kg of seed: 100 g; Lot: 400 1003 15; Manufacture: 09/05/2015 and Validity: 03/05/2016; - Liquid rice product: Warranty: 2,0x10^8 CFU/mL *Azospirillum brasilense*, strains Ab-V5 and Ab-V6; Physical Nature: Solid; Density: 1.0 g / mL; Target crop: Rice (*Oryza sativa* L.); Tested dosage for 25 kg of seed: 100 g; Lot: 410 10003 15; Manufacture: 08/28/2015 and Validity: 02/28/2016.

Identification of inoculant sample standard

In the four experiments, two standard inoculants duly registered with the Ministry of Agriculture, Livestock and Food Supply (MALFS) were used as references: - Liquid product containing *Azospirillum brasilense*: MASTERFIX L GRAMÍNEAS® commercial inoculant. The inoculant has the following characteristics: Warranty: 2.0x10^8 CFU/mL *A. brasilense*, strains Ab-V5 and Ab-V6; Physical nature: liquid; Density: 1.0 g/mL; Target crop: Rice (*Oryza sativa* L.); Test dosage used for 50 kg of seed: 100 mL; Lot: 0515843; Manufacture: 11/22/2015; Validity: 05/2016.

Quality control of tested inoculants

Inoculants from Nitro 1000 - Biological Inoculants and Standard inoculants from MASTERFIX L GRAMÍNEAS®, both used in the four experiments conducted in the different regions, were subjected to laboratory tests of concentration (Colony Forming Units), purity and characterization. The analyses followed official methods, according to Normative Instruction N° 30 of November 12, 2010 (MAPA).

The following results were obtained for the concentration of bacterial strains present in the inoculants: Liquid Standard Inoculant - MASTERFIX L GRAMÍNEAS®: 6.91x10^8 CFU mL^{-1} (Colony Forming Units); NITRO 1000 Rice Inoculant: Liquid: 4.79x10^8 CFU mL^{-1}; and NITRO 1000 Peat Inoculant: 6.12x10^8 CFU g^{-1}.

Implementation and experimental design of the four experiments

The experiments were implemented in a randomized block design with seven treatments and four replications, totaling 28 experimental plots. The treatments used were absence, presence or association between diazotrophic bacteria and nitrogen fertilization, as described below: T1, Control (absence of nitrogen fertilization and inoculation); T2, Nitrogen fertilization, 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3, Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4, Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gram 'Liquid' at the dose of 100 mL 25 kg^{-1} seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

For seed liquid inoculation aliquots of inoculants were removed according to the recommended calculations for each treatment, using a Micronam micropipette with a capacity of up to 10 mL. For the solid inoculation of the seeds, portions of inoculants were removed and measured on a Celtac model FA 2104N analytical digital scale, accurate to 0.0001 g.

Then, 2.0 kg of rice seeds were packed in high density plastic bags, where the liquid or solid inoculant was deposited directly on the seed mass according to the treatments. They were then agitated for approximately 1 min to evenly distribute the inoculant in the seeds. The seeds were placed on the bench in the shade for 2 h and then sowed.

According to the results of the chemical analysis of the soil and crop needs, basic fertilization was performed. Considering that the areas presented clay contents above 50% to 60% and P contents above 12 mg dm³ (Table 1) were classified as good and very good, requiring fertilization of 60 kg ha^{-1} of P_2O_5. For fertilization with K, the soils were classified as very good; values above 0.31 $cmol_c$ dm³, thus requiring the addition of 40 kg ha^{-1} of K_2O. For the distribution of fertilizers, a precision fertilizer seeder was used, equipped with an endless screw system, where at that moment the experimental area was furrowed.

Part of the urea nitrogen (100 kg ha^{-1} N) was manually applied to the sowing furrow for treatment with nitrogen fertilization. The remaining nitrogen, for the treatment that received nitrogen, was applied as a cover, at stage V3, as urea (100 kg ha^{-1} of N). The upland rice cultivar used in the four trials was IAC 201, with an average early cycle of 110 to 120 days.

Each experimental plot consisted of 10 sowing lines per 8 m in length, with 1 m spacing between plots. 0.5 m row spacing was used, with 170 plants m^{-1}, according to the cultivar specification, making an average population density of 40 kg of ha^{-1} plants. The useful experimental plot, considered for evaluations, consisted of the 6 central lines, disregarding 1 m at each end, resulting in a usable area of 48 m^2.

Prior to sowing, the seeds were treated using the fungicide Metalaxil - M + Fludioxonil (commercial product Maxim XL®) at a dose of 200 mL p.c. for 100 kg of seeds, still for disease prevention in the early stages of development, the insecticide Fipronil (commercial product Cinelli 250 FS ®) was also used, at a dosage of 150 mL of p. c. for 100 kg of seeds.

After germination, the plants were monitored for adequate development until ITreaching the S_1 stage, requiring herbicide application at the S_3 stage of the culture, with the application of Ricer (Penoxsulam) at a dose of 100 mL p.c. to 200 L ha^{-1}. The first application of fungicide treatments was performed at stage V_3 with ciproconazole (0.2 mL L^{-1}) + Picoxistrobin (0.2 mL L^{-1}) to 200 L ha^{-1}. When the plants reached the R_4 stage, the same fungicides were used, and the presence of panicle required insecticide application for insect control through the insecticide Alterne (Tebuconazole 0.75 L ha^{-1}) to 40 L ha^{-1}. One last fungicide application was carried out at the R_5 stage, with ciproconazole (0.2 mL L^{-1}) + picoxistrobin (0.2 mL L^{-1}), for 200 L ha^{-1}, at which time the insecticide Alterne was applied (Tebuconazole 0.75 L ha^{-1}) to 40 L ha^{-1}.

Evaluation of morphobiometric variables of rice plants

At the phenological stage, R_7, plant collections were performed to perform the evaluation of plant morphobiometric variables. For this, 10 plants with intact roots were collected in the second line of each experimental plot.

The plants were placed in plastic bags and immediately taken to the laboratory to measure the following variables: root dry mass (RDM); shoot dry mass (SDM); tillering number (TN); plant height (PH); 100 grain weight (W100); leaf nitrogen content (leaf N); and nitrogen content in grains (N grains). Plant height and stem diameter were measured with a graduated ruler and digital caliper, respectively. The dry mass variables of the different organs were obtained by weighing in a precision scale, after drying the vegetable materials in a forced air circulation oven at 65ºC, until it reached constant mass. To obtain the root dry mass and number and dry mass of nodules, the roots were washed in running water with the aid of sieves for soil removal and recovery of nodules for counting and determination of dry matter.

To determine the nitrogen content of the flag leaves, the leaves of the upper third of the plants of each useful plot were collected. The collected leaves were washed in running and distilled water and then placed in paper bags for drying in a forced air oven at 65°C for 72 h. After drying, the samples were ground and subjected to sulfuric digestion and afterwards distillation by vapor drag according to Tedesco et al. (1995) was done, then the leaf nitrogen content was determined. At the R8 phenological stage, when the plants were with the mature grains, useful plots of each treatment were harvested for subsequent determination of yield. To calculate the yield, the grain mass of the plot was corrected to 13% moisture in the humid base and the extrapolated values to kg ha^{-1}. The determination of the N content in the grains was performed with samples of the harvested grains of each plot, using the methodology already described for the determination of the N content in the leaves.

Statistical analysis

After being tabulated, the data were subjected to analysis of variance by the F test ($P \leq 0.05$) and the treatment means were compared by the Tukey test ($P \leq 0.05$). The statistical software SISVAR, version 5.3 (Ferreira, 2011) was used.

RESULTS

Experimental area in the city of Toledo/PR

By observing the results of the data obtained for the municipality of Toledo - PR, it is possible to verify a significant effect of the treatments employed for all variables tested according to Table 2 ($p \leq 0.05$) (Figure 1). In general, it can be seen in Table 3, that for all evaluated variables, superiority of treatment was obtained 40 kg ha^{-1} of N + inoculation with liquid inoculant based on *A. brasilense* at a dose of 100 mL of inoculant to 25 kg of rice seeds (T5). However, it is also possible to verify that the same treatment did not differ from 40 kg ha^{-1} N + inoculation with *A. brasilense* liquid inoculant at a dose of 150 mL inoculant for 25 kg of rice seeds for all variables tested (T6) (Table 3).

On the other hand, the lowest averages determined in this experiment occurred for all variables in the control treatment (T1), which was devoid of inoculation with *A. brasilense* and nitrogen fertilization (Table 3).

It was possible to verify, according to the tested conditions, that the inoculation of rice seeds with *A. brasilense* bacteria through the T5 liquid vehicle presented the greatest increase in the root and shoot system of 15.8 and 8.8%, respectively compare with the treatment that received the full nitrogen dose, that is 80 kg N ha^{-1} T3 (Table 3). These observed conditions reflected a gain of at least 4% in tillering and 4.6% in plant height, when also compared to the T3 treatment. On the other hand, seed inoculation with the solid vehicle (T7) also promoted gains for rice plants, exceeding by 6.4, 1.6, 2.5 and 2.3% the treatment with 80 kg N ha^{-1} (T3), in root, shoot, tiller number and plant height, respectively.

When observing the mass of 100 grains of rice for the Toledo/PR experiment, it is noted that only 40 kg ha^{-1} treatment of N + inoculation with liquid inoculant at a dose of 100 mL for 25 kg of rice seeds (T5) differed from the control treatment by 16.5%, and the others presented similar means. Similar behavior occurred for the variable N content in grains, where again the treatment with only T5 surpassed the control treatment (T1) by 18.8% and the treatment with 40 kg ha^{-1} N (T2) by 17.6%.

Likewise, the leaf N content was higher in T5 treatment, without differentiating from 40 kg ha^{-1} N + inoculation with liquid inoculant at 150 mL dose to 25 kg of rice seeds (T6). When the N content in the grains was observed, differences were observed only between 40 kg ha^{-1} of liquid inoculant N + at a dose of 100 mL to 25 kg of rice seeds, when compared to the control and applied only 40 kg ha^{-1} from N.

When evaluating the final yield, it was observed that the T5 treatment, 40 kg ha^{-1} of N + liquid inoculant 100 mL for 25 kg of rice seeds promoted the highest average, but without differentiating from 40 kg ha^{-1} of rice seeds N + liquid inoculant 150 mL for 25 kg of rice seeds, 40 kg ha^{-1} of N + Standard liquid inoculant 100 mL for 25 kg of rice seeds and 80 kg ha-1 of N ha^{-1} (Figure 2). The yield gain provided by T5 represented 13.96% compared to fertilization of 40 kg ha^{-1} N.

Experimental area in the city of Palotina/PR

When the experiment was conducted in Palotina - PR, the response patterns were conserved, with a significant effect of the treatments used for all variables ($p \leq 0.05$), and the T5 treatment promoted the highest average (Table 4). For RDM was observed superiority of 10.4% compared to fertilization with 80 kg ha^{-1} of N.

Table 3. Average biometric characteristics: root dry mass (RDM); shoot dry mass (SDM); tillering number (TN); plant height (PH); 100 grain weight (W100); leaf nitrogen (N leaf) and grain nitrogen (N grain) content of 10 IAC 201 rice plants cultivated under different nitrogen fertilization concentrations and different seed inoculation with *Azospirillum brasilense* strain Ab-V5 and Ab-V6 and nitrogen fertilization. Municipality of Toledo / PR.

Treatments	RDM	SDM	TN	PH	W100	N leaf	N grains
	g plant^{-1}			Cm	g	g kg^{-1}	
T1	24.33c	171.67^{c}	72.44^{c}	97.30^{c}	2.42^{b}	29.59^{d}	6.91^{b}
T2	27.15b	168.35^{c}	76.67^{bc}	99.16^{bc}	2.56^{ab}	30.46^{cd}	6.98^{b}
T3	27.45b	179.20^{bc}	79.71^{ab}	102.35^{abc}	2.50^{ab}	30.76^{bcd}	7.45^{ab}
T4	28.62b	181.82^{abc}	81.18^{ab}	103.57^{ab}	2.62^{ab}	31.57^{bc}	7.50^{ab}
T5	31.80a	194.95^{a}	84.44^{a}	108.32^{a}	2.82^{a}	33.55^{a}	8.21^{a}
T6	29.46ab	189.00^{ab}	82.17^{a}	105.20^{ab}	2.72^{ab}	32.50^{ab}	7.67^{ab}
T7	29.22b	182.17^{abc}	81.70^{ab}	104.72^{ab}	2.60^{ab}	31.64^{bc}	7.60^{ab}
CV(%)	13.74	13.59	14.72	12.60	15.65	12.50	17.06
SMD	2.47	15.20	5.06	6.26	0.34	1.83	1.23
F cal	19.27**	8.04**	13.69**	7.79**	3.50**	11.37**	2.95**

**Significant by the F test at 5% probability. Means followed by the same lower case letters in the column do not differ from each other by the Tukey test at 5% probability. Coefficient of variation (CV (%)); significant minimum difference (SMD). T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas® 'liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Grass Peat inoculant at a dose of 100 g 25 kg^{-1} seeds.

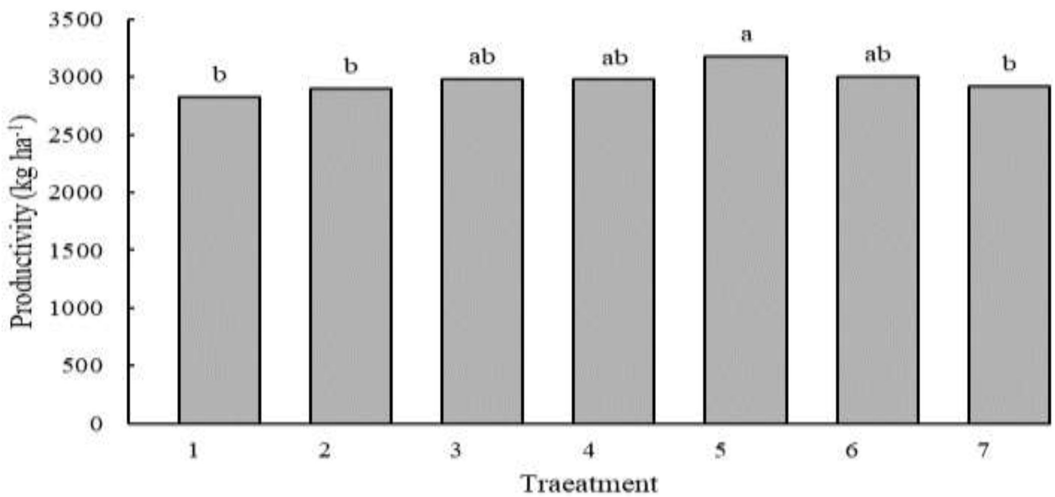

Figure 2. Productivity of cultivar IAC 201 rice plants cultivated on different forms of seed inoculation with diazotrophic bacteria, in the municipality of Toledo / PR. Means followed by the same lower case letters in the column do not differ from each other by the 5% Tukey test; CV = 15.07%; SDM = 243.49; and F cal = 4.73. T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Grass Peat inoculant at a dose of 100 g 25 kg^{-1} seeds.

Table 4. Average biometric characteristics: root dry mass (RDM); shoot dry mass (SDM); tillering number (TN); plant height (PH); 100 grain weight (W100); leaf nitrogen (N leaf) and grain nitrogen (N grain) content of 10 IAC 201 rice plants cultivated under different nitrogen fertilization concentrations and different seed inoculation with *Azospirillum brasilense* strain Ab-V5 and Ab-V6 and nitrogen fertilization. Municipality of Palotina – PR.

Treatments	RDM	SDM	TN	PH	W100	Nleaf	Ngrains
	$gplant^{-1}$			Cm	g	gkg^{-1}	
T1	23.40^{d}	167.82^{b}	71.26^{d}	95.60^{b}	2.42^{d}	29.58^{d}	6.78^{b}
T2	26.52^{c}	167.82^{b}	76.10^{cd}	100.38^{ab}	2.53^{bcd}	30.52^{cd}	7.29^{ab}
T3	27.06^{bc}	181.30^{a}	$78.59b^{c}$	101.68^{ab}	2.44^{cd}	30.60^{cd}	7.24^{ab}
T4	28.86^{bc}	181.65^{a}	81.70^{ab}	101.45^{ab}	2.68^{abc}	31.55^{bc}	7.72^{ab}
T5	31.86^{a}	189.17^{a}	83.92^{a}	105.71^{a}	2.88^{a}	33.47^{a}	8.17^{a}
T6	29.55^{cb}	190.05^{a}	82.37^{ab}	103.05^{a}	2.74^{ab}	32.55^{ab}	7.80^{a}
T7	28.83^{bc}	180.07^{a}	82.35^{ab}	102.29^{a}	2.62^{abcd}	32.01^{bcd}	7.55^{ab}
CV(%)	14.47	9.84	15.83	17.66	14.26	12.51	15.65
SMD	2.92	11.91	5.24	6.30	0.26	1.84	0.98
Fcal	18.29**	12.45**	19.96**	5.20**	8.94**	11.44**	4.56**

** significant by the F test at 5% probability. Means followed by the same lower case letters in the column do not differ from each other by the Tukey test at 5% probability. Coefficient of variation (CV (%)); significant minimum difference (SMD). T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha $^{-1}$ N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg $^{-1}$ seeds.

However, for SDM, TN, PH and W100, this effect was not observed and similar averages were found between the two treatments. For these variables, it is noteworthy that treatments T6 and T4 produced similar or higher averages for some variables when compared to treatment, with 80 kg N ha^{-1} (T3) or 40 kg N ha^{-1} (T2) (Table 4).

The quantified contents of N leaf in rice plants cultivar IAC 201 were higher when using T5, without differentiating from treatment T6, these treatments had superiority, respectively 6% and 3.1% compared to the treatment that received complete fertilization 80 kg N ha^{-1}. In turn, the N content quantified in the cultivar IAC 201 rice grains was similar for practically all treatments, except for T5 and T6 that exceeded the T1 control treatment (Table 4).

The final rice yield obtained in the city of Palotina/PR showed that the treatment 40 kg ha^{-1} of N + peat inoculant promoted the highest average, but without differentiating from 40 kg ha^{-1} of N + liquid inoculant and 40 kg ha^{-1} N + standard inoculant (Figure 3). The yield gain provided by 40 kg ha^{-1} N + peat inoculant represented 10.97% and 11.46% compared to fertilization of 40 kg ha^{-1} N and 80 kg ha^{-1} N, respectively.

Experimental area in the city of Cascavel/PR

For the experiment conducted in Cascavel/PR, the response patterns observed were similar for rice cultivated in Toledo/PR and Palotina/PR. These response patterns were maintained in order to observe a significant effect of the treatments used for all studied variables ($p \leq 0.05$), and for all variables the T5 treatment promoted the highest mean, except for the SDM, which was the same followed by T6 treatment (Table 5).

For the tiller number (TN) variable in the same table, it was noted that the statistical differences observed for the treatments were smaller. The control treatments T1 and T2 presented the lowest values for this variable compared to the other treatments tested (Table 5).

The RDM variable obtained by rice plants cultivar IAC 201 was higher in inoculant treatments associated with mineral fertilization of 40 kg N ha^{-1} at a dose of 100 mL for 25 kg of rice seeds (Table 5). It is also noted in the same table, that through treatments T4, T6 and T7, it was possible to determine higher values of 14.6%; 5.4 and 8.9% against fertilization with 80 kg ha^{-1} of N (T3). This response tendency with higher averages in plants inoculated and fertilized with N were obtained in SDM, TN, W100 and N leaf (Table 5).

For the variable height of rice plants (PH), the use of 100 mL liquid inoculant for 25 kg of rice seeds associated with fertilization of plants with 40 kg N ha^{-1} produced higher plants with values higher than 5, 6% compared to80 kg N ha^{-1}; in turn the N content in the grains was similar among all treatments, with mineral nitrogen and/

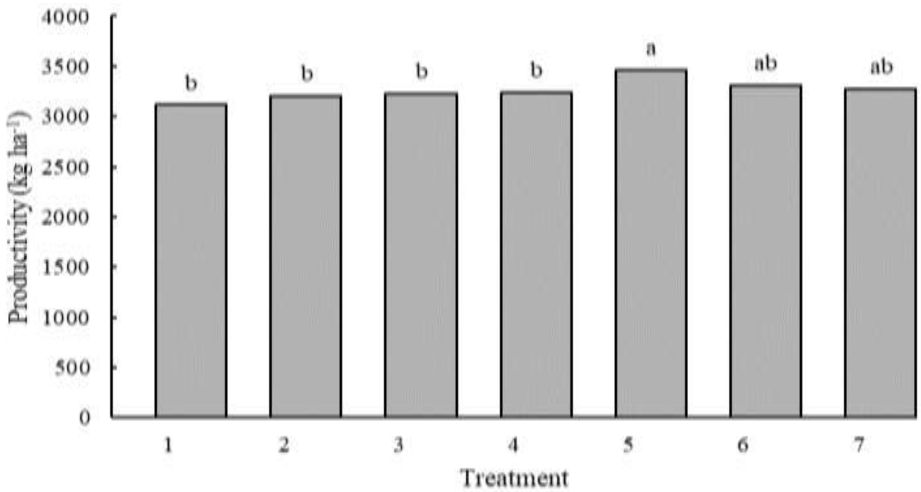

Figure 3. Productivity of rice cultivar IAC 201 cultivated on different forms of seed inoculation with diazotrophic bacteria, in Palotina/PR. Means followed by the same lower case letters in the column do not differ from each other by the 5% Tukey test; CV = 15.07%; SMD = 243.49; and F cal = 4.73. T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

Table 5. Average biometric characteristics: dry root mass (DRM); shoot dry mass (SDM); macollage number (MN); plant height (PH); 100 grain weight (W100); leaf nitrogen content (N leaf) and grain nitrogen content (N grains) of 10 IAC 201 rice plants cultivated under different nitrogen fertilization concentrations and different seed inoculations with the *Azospirillum brasilense* Ab-V5 and Ab-V6 strain and fertilization nitrogenous. Municipality of Cascavel/PR.

Treatments	DRM	SDM	MN	PH	W100	Nleaf	Ngrains
	$gplant^{-1}$			cm	g	gkg^{-1}	
T1	24.06^{d}	173.25^{b}	73.73^{b}	102.02^{e}	2.69^{a}	30.82^{d}	7.27^{b}
T2	26.19^{cd}	173.42^{b}	77.57^{b}	103.80^{de}	2.73^{ab}	32.21^{cd}	7.92^{ab}
T3	26.43^{cd}	184.10^{ab}	82.12^{a}	104.70^{cd}	2.74^{ab}	32.04^{cd}	8.42^{a}
T4	28.98^{bc}	182.52^{ab}	84.64^{a}	105.42^{cd}	2.84^{ab}	33.01^{bc}	7.70^{ab}
T5	33.21^{a}	192.32^{a}	84.47^{a}	111.32^{a}	2.99^{a}	34.97^{a}	8.38^{a}
T6	31.50^{ab}	186.02^{a}	84.37^{a}	108.27^{b}	2.93^{ab}	33.65^{ab}	8.09^{ab}
T7	30.51^{ab}	182.87^{ab}	84.70^{a}	106.75^{bc}	2.89^{ab}	34.08^{ab}	8.05^{ab}
CV(%)	14.27	12.77	12.37	20.84	14.15	11.76	15.20
SMD	2.86	11.76	4.53	2.07	0.27	1.35	0.96
Fcal	28.61**	7.31**	21.23**	47.85**	3.75**	23.40**	3.68**

**Significant by the F test at 5% probability. Means followed by the same lower case letters in the column do not differ from each other by the Tukey test at 5% probability. Coefficient of variation (CV (%)); significant minimum difference (SMD). T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

Table 6. Average biometric characteristics: root dry mass (MSR); shoot dry mass (MSPA); tillering number (NP); plant height (ALT); 100 grain weight (M100); leaf nitrogen (N leaf) and grain nitrogen (N grain) content of 10 IAC 201 rice plants cultivated under different nitrogen fertilization concentrations and different seed inoculation with *Azospirillum brasilense* strain Ab-V5 and Ab-V6 and nitrogen fertilization. Municipality of São Miguel do Iguaçu / PR.

Treatments	RDM	SDM	NP	PH	w100	Nleaf	Ngrains
	$gplant^{-1}$			cm	g	gkg^{-1}	
T1	24.15^{e}	167.47^{c}	71.62^{d}	101.43^{c}	2.56^{c}	29.98^{d}	7.22^{b}
T2	26.22^{de}	168.17^{bc}	76.36^{c}	101.11^{c}	2.63^{c}	30.94^{cd}	7.88^{ab}
T3	27.33^{cd}	183.40^{ab}	81.22^{b}	102.54^{bc}	2.64^{bc}	31.69^{bcd}	8.77^{ab}
T4	29.22^{bc}	181.65^{abc}	82.30^{ab}	102.71^{bc}	2.81^{ab}	32.75^{abc}	7.99^{ab}
T5	32.61^{a}	189.00^{a}	83.98^{a}	106.59^{a}	2.94^{a}	34.10^{a}	8.53^{a}
T6	30.75^{ab}	184.87^{a}	83.19^{ab}	104.46^{ab}	2.89^{a}	33.44^{ab}	8.14^{ab}
T7	29.37^{bc}	180.77^{abc}	83.18^{ab}	102.81^{bc}	2.87^{a}	33.00^{ab}	8.05^{ab}
CV(%)	14.11	9.65	11.12	11.11	15.76	12.61	9.35
SMD	2.73	15.29	2.09	2.66	0.17	1.96	0.99
Fcal	23.67**	6.45**	104.09**	10.95**	15.24**	10.09**	3.39**

**Significant by the F test at 5% probability. Means followed by the same lower case letters in the column do not differ from each other by the Tukey test at 5% probability. Coefficient of variation (CV (%)); significant minimum difference (SMD). T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

or inoculation, being observed lower average in the control treatment against peat and liquid addition inoculants, 40 kg N ha^{-1}.

The ultimate productivity, again demonstrated that T5 treatment promoted the highest mean, but without differentiating from T6 and T7 treatment (Figure 4). The yield gain provided by the treatment that received 40 kg ha^{-1} of N + liquid inoculant with 100 mL for 25 kg of rice seeds, represented 6 and 8% compared to fertilization of 80 kg ha^{-1} of N and 40 kg. ha^{-1} of N, respectively.

Experimental area in the city of São Miguel do Iguaçu/PR

Finally, in the experiment conducted in São Miguel do Iguaçu/PR, significant effects were obtained from the treatments used for all variables (p≤0.05), and in all variables the T5 treatment promoted the highest average, except for SDM, where this treatment was followed by treatment with 40 kg N ha^{-1} + liquid inoculant and 150 mL for 25 kg rice seeds (Table 6).

For the city of São Miguel do Iguaçu/PR, the RDM variable of rice plants presented higher values for T5 treatment, surpassing by 11.6% fertilization with 80 kg ha^{-1} of N. The same response tendency was observed with higher averages in plants inoculated and fertilized with N for the variables SDM, MN, W100 and N leaf (Table 6).

For the variable height of rice plants (PH) the use of 100 mL liquid inoculant for 25 kg of rice seeds associated with fertilization of plants with 40 kg N ha^{-1} (T5) produced higher plants, higher than by 3.7% compared to 80 kg N ha^{-1}. In turn, the N content obtained in rice grains presented similar values among all treatments, with mineral nitrogen and / or inoculation, being observed only lower average for the control treatment compared to T5 treatment.

When observing the productivity in the municipality of São Miguel do Iguaçu/PR, they maintained the standards of Toledo/PR, Palotina/PR and Cascavel/PR, confirming the stability of the treatments employed and the agronomic efficiency of the inoculants *A. brasilense* with greater emphasis on what it used as a liquid vehicle at a dose of 100 mL for 25 kg of rice seeds (T5).

The T5 treatment promoted the highest average, however without differentiating from the T4 and T7 treatments (Figure 5). The yield gain provided by T5 treatment represented 8.9 and 4.9% compared to 40 kg ha^{-1} N and 80 kg ha^{-1} N fertilization, respectively.

DISCUSSION

The bacteria of the genus *Azospirillum* interact with rice plants naturally. In this sense, it is shown in studies developed in the state of Santa Catarina that the association between the rice plant and the *Azospirillum* bacteria occur naturally, both in roots and stalks, and

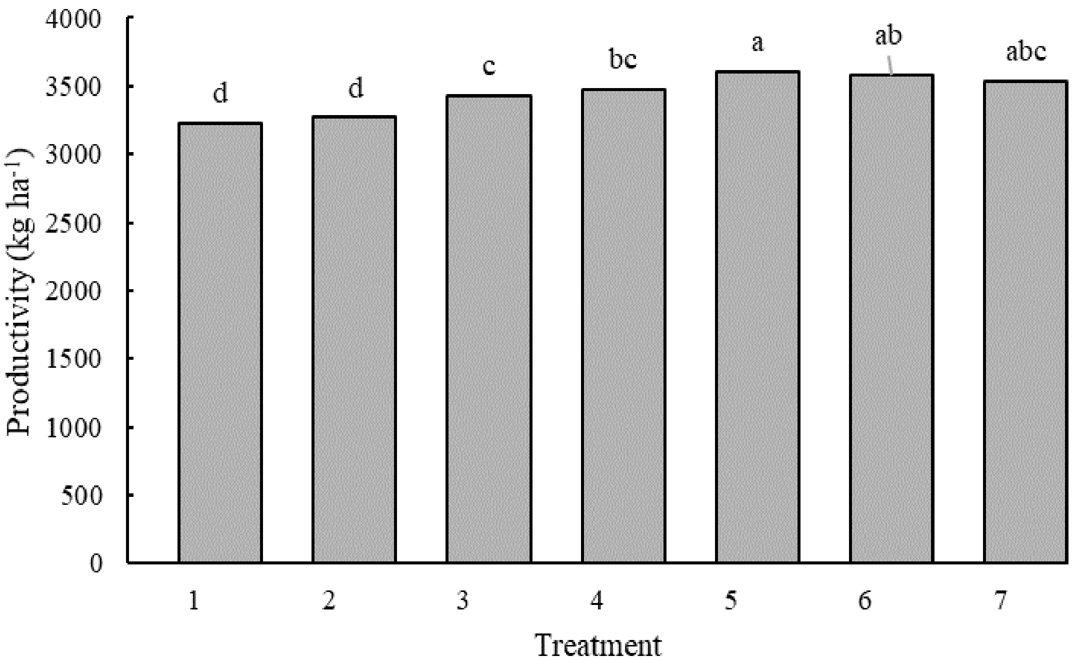

Figure 4. Productivity of rice cultivar IAC 201 cultivated on different forms of seed inoculation with diazotrophic bacteria in the municipality of Cascavel/PR. Means followed by the same lower case letters in the column do not differ from each other by the 5% Tukey test; CV = 20.97%; SMD = 109.84; and F cal = 39.49. T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

62.9% of the bacteria associated with roots belonging to species *A. Amazonian* (Cardoso et al., 2010). Similarly, isolates of the genus *Azospirillum* were found associated with rice cultivation (Perin et al., 2003).

In this sense, the use of this technology brings benefits to the sustainable system of agricultural production. In their study on the agronomic performance of *A. brasilense* in rice (Vogel et al., 2013), they concluded that the use of *A. brasilense* associated with rice cultivation presents promising results in relation to the sustainable production environment. This highlights a contribution in relation to the morphological aspect of the plant and increased grain yield.

The greater root and shoot development obtained in plants inoculated with *A. brasilense* is explained by the growth promoting capacity of the bacteria. It interacts with plant release growth promoting factors such as plant hormones (Perrig et al., 2007), which act as growth promoters by stimulating root and shoot development. In this sense, increments of both variables are shown when rice cultivars were studied (Guimarães and Baldani, 2013). In a study of the interaction between nitrogen fertilization and bacterial inoculation, a greater development of rice plants in both shoots and roots was observed (Beutler et al., 2016).

The increase in the number of tillers reflects the greater development of the plant in relation to the root system and shoots, since plants with higher root development have a higher capacity for water and nutrient absorption, especially N, allowing the plant to emit a larger number of plants. Tillers, in agreement with these results, showed that the inoculation of *A. brasilense* promoted gains in tiller number of 11%, which resulted in higher number of panicles (data not evaluated in this study) (Moura, 2011).

For plant height the results differ from the literature whereas Kuss et al. (2007) report that the use of *A. brasilense* does not present significant results in rice plant height; Goes (2012) found better results in plant height similar to that found in the present study. It is believed that part of this lack of results is linked to the genotypes used and the cultivation conditions, because the plant height varies according to each cultivation

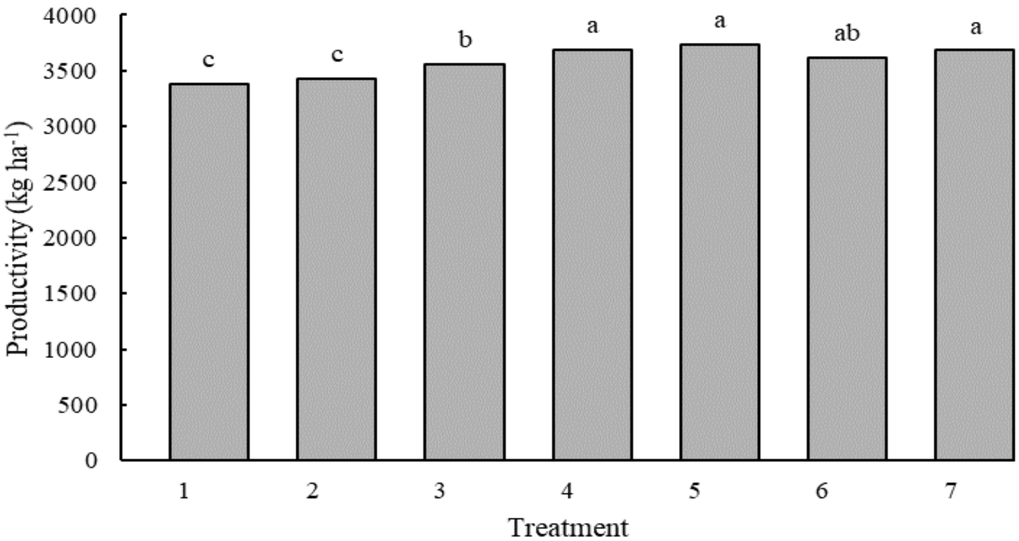

Figure 5. Productivity of rice cultivar IAC 201 cultivated on different forms of seed inoculation with diazotrophic bacteria, in São Miguel do Iguaçu/PR. Means followed by the same lower case letters in the column do not differ from each other by the 5% Tukey test; CV = 19.72%; SMD = 103.81; and F cal = 37.39. T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

region.

Under the conditions tested in these four experiments, the higher plant height was a reflection of the higher crop development in the aerial part, which due to the higher presence of tillers caused it to grow in height to seek greater light exposure, but without reducing its productivity. It increases productivity.

It should also be noted that increases in plant height are not always positive from the production point of view, as they favor plant lodging; but in the present study no plant lodging was observed during the entire conduction period. Higher height development favored the increase in productivity.

Inoculated and fertilized plants with 40 kg ha^{-1} of N promoted greater N accumulation in the leaves allowing better plant nutrition and consequently greater nutrient availability for grain filling. Similar results for M100 grains are shown in a study with rice inoculation with *A. brasilense* (Gitti et al., 2012).

Gaibor et al. (2017) demonstrated that the use of *Azospirillum* application promoted higher phosphorus accumulation in plants. However, they did not observe that this increase promoted grain mass gain, being only reported increases in productivity where the association of *Azospirillum* with 140 kg N ha^{-1} promoted productivity of 5236,5 kg ha^{-1}, surpassing 791 kg ha^{-1} and 478 kg ha^{-1}, inoculation with *Azotobacter* and *Bacillus* sp., respectively. Garcia et al. (2015) demonstrated 19% gains in upland rice crop yield when inoculated with *A. brasilense* via seeds, sowing furrow or leaf spraying.

However, divergent results of the present study are shown by Gitti et al. (2012 and Beutler et al. (2016) who did not find differences between the yield of rice inoculated or not with *A. brasilense*.

The best results observed in the liquid inoculant that always promoted higher averages, even when not differing from the others, may be associated with its greater capacity to protect bacterial cells. However, the peat inoculant deserves attention especially when compared to a study with *Bradyrhizobium japonicum* inoculant. Because it has been shown that peat inoculant can provide greater protection when treating seeds with fungicides and insecticides (Vieira Neto et al., 2008), they cite cell protection as the main effect for the results. For inoculants based on *A. brasilense* it is also shown that peat provided greater survival, and it was reported that for 110 days, the concentration of $2x10^8$ CFU per gram was maintained (Ferreira et al., 2010).

Thus, the agronomic efficiency of *A. brasilense* strains Ab-V5 and Ab-V6 based inoculants for rice cultivation is demonstrated, and the liquid carrier is a good option for rice cultivation. However, the peat inoculant also shows how option, as it resembles the standard inoculant in all characteristics and locations evaluated.

Conclusion

Inoculation of rice seeds with A. *brasilense* strains Ab-V5 and Ab-V6 based inoculant increases the yield and yield components of the rice crop. The inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6 using the liquid carrier showed higher agronomic efficiency for rice cultivation. The inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6 using the peat medium has good agronomic efficiency similar to the standard inoculant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Severity and prevalence of the destructive fall armyworm on maize in Uganda: A case of Bulambuli District

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Maize kernel contains a large quantity of carbohydrates, proteins, vitamins, oils, fats and competes favorably with root and tuber crops as a rich energy source. In Uganda, the per capita consumption ranges from 28 to 125 kg per annum. However, the yields remain low, fluctuating around 1.5 tons per hectare. Although some losses to maize production occur through the post-harvest period, pre-harvest factors such as biotic and abiotic constraints significantly affect its production. The most important biotic constraints include pests and diseases. Among the pests, Lepidopterans such as the fall armyworm (FAW) (*Spodoptera frugiperda* JE Smith) has become an important pest of maize during the early growing stages of the crop. Yet there is no information on the destructive levels of this pest in maize fields and this hinders management options for this pest. To determine the incidence, severity and prevalence of the FAW which may be responsible for low yields and poor maize quality, a survey was carried out in Bulambuli district in Uganda. Forty fields were sampled in Bwinkhonge Sub County to determine the level of damage caused by this pest. The severity of damage on leaves (df = 9, $\chi2$ = 87.66, P = 0.000*) ears (df = 9, $\chi2$ = 299.2, P = 0.000*) and kernels (df = 7, $\chi2$ = 19.9, P = 0.005*) was high and significantly different in two parishes surveyed.

Key words: Larvae, incidence, pests, damage.

INTRODUCTION

Maize (*Zea mays* L.) also known as corn, zea, silk maize, makka, barajovar (Kumar and Jhariya, 2013) belongs to the class Liliopsida. Maize is believed to have originated from central Mexico about 7000 years ago from a wild grass, and was transformed by Native Americans into a better source of food. Maize is one of the three most widely cultivated crops in the world (Abdel-rhman, 2015). The USA, China and Brazil contribute 63% of the global maize production. Other major producing countries include; Mexico, Argentina, India, Ukraine, Indonesia,

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France, Canada and South Africa (http://www.fao.org/3/BS183E/bs183e.pdf). In Uganda, an an average of 1.5 tons per hectare is produced annually making it the third most important cereal crop after sorghum and millet in terms of area under cultivation, production and human consumption (Daly et al., 2016).

Maize kernel contains a large quantity of carbohydrates, proteins, vitamins, oils and fats and competes favorably with root and tuber crops as a rich energy source (Rouf Shah et al., 2016). Maize is used in the local brewery industry, and is eaten when still fresh on cobs either as cooked or roasted. The flour is used to prepare a local paste called ‘porridge’ and ‘posho’ whose demand is on the increase in hotels and restaurants in several urban centers including Kampala City (Agona et al., 1999). Maize is also used in the manufacture of feeds for livestock and is now a key export to surrounding countries like Kenya, Sudan (Rouf Shah et al., 2016).

Per capita maize consumption ranges from 28 to 125 kg per annum in Uganda. However, the yields remain low, fluctuating around 1.5 tons per hectare (Daly et al., 2016). Improving the productivity of maize-based farming could significantly reduce hunger and enhance food security (Stevens and Madani, 2016).

Although losses to maize production occur throughout the post-harvest period, pre-harvest factors of maize production such as biotic and abiotic constraints affect maize production (Pandey et al., 2017). Among abiotic factors, soil nutrients and moisture are the most important while key biotic constraints include pests and diseases. Among the pests, stem borers such as (African maize stalk borer), American grasshoppers *(Schistocerca americana*), and lepidopterans are important pests of cereals including maize (Masika et al., 2017a). Lepidopterans like the fall armyworm (FAW) (*Spodoptera frugiperda* JE Smith) are important pest of maize during the early growing stages of the crop (Otim et al., 2018).

Invasion of the FAW was first reported in Africa in September 2016. Since then, it has become resident in most Sub Saharan countries where severe damage in maize fields is observed (Goergen et al., 2016). By the end of 2017, about 38 countries in Africa were infested with the FAW. The suitable climate in Africa favors the growth of main and alternative hosts of the FAW; therefore, they do not need to migrate within the continent (Hailu et al., 2018). Furthermore, according to Hailu et al. (2018), the rapid spread of FAW poses unprecedented threat to food security, incomes and livelihoods in the country, most especially among smallholder farmers grappling with the parasitic weed Striga. The FAW was first detected in Uganda in 2016 and caused serious devastation to the crop (http://www.fao.org/uganda/news/detail-events/en/c/1149243/). Towards the end of 2018, the pest sprung and farmers do not have a solution on how to manage them (http://www.fao.org/uganda/news/detail-events/en/c/1149243/). The eastern region particularly Bugisu and Sebei sub-regions are key maize producing areas in Uganda (FAO, 2014); where about 75 to 95% of the household production is sold to earn money. Towards the end of 2018, we inadvertently observed in various farms in Bwikhonge Sub County, Bulambuli District in eastern Uganda sporadic incidences of the FAW. Consequently, we followed up with systematic studies to assess the incidence, prevalence and severity of FAW damage in maize fields in Bwikhonge Sub County at the onset of the rain season which also coincides with the main maize growing season in the area. There is currently no information on incidence, severity and prevalence of this pest in maize fields in Uganda, let alone in Bwikhonge Sub County which may be hindering any management options for this pest.

MATERIALS AND METHODS

Study area

The study was carried out in Bwikhonge Sub County, Bulambuli District located in the Mt Elgon region. It is bordered by Nakapiripirit district in the north, Kapchorwa district to the east, Sironko district to the south and Katakwi district to the west. Bulambuli is located approximately 32 km (20 miles) by road, north east of Mbale at coordinates (0122N, 3409E) consisting of nine sub counties, 35 parishes and 455 villages with a total land area of 651.8 km^2 and an approximate population of 125,400 people. The Sub County has parishes such as Buwenkanda Parish, Buwabwala, Buwekanda and Buyaka parish. The major economic activity is agriculture including animal husbandry and crop farming. The crops grown include; millet, sorghum, bananas, beans with maize being the major crop (https://www.ugandainvest.go.ug/wp-content/uploads/2019/06/Elgon-Investment-Profile.pdf).

The study area is located in the Mt Elgon region and receives approximately a bimodal rainfall pattern with the periods between March/April and October/November being the wettest. The dry period is from December to February while the period between July to August receives less intense rainfall. The mean annual rainfall varies from 1200 at low altitudes to 1400 and 1800 mm at mid altitudes and at high altitudes respectively (Liebig, 2016). The mean annual temperatures also range from 23 to 21°C and 18°C in the low, mid and high altitudes (Hijmans et al., 2005).

Sampling and data collections

The survey was carried out in two parishes in the Bwikhonge Sub County namely; Buwabwala and Buwekanda parishes as these are the ones where extensive maize cultivation is being carried out. Data was collected on prevalence, incidence and severity of FAW damage on the maize shoot, ears, and kernels. During the survey, we collected data from a total of forty farmers, twenty from every parish. From each farmer, we collected data from one field measuring at least one acre and we skipped at least two fields before sampling the next field.

Incidence of FAW damage on maize in Bulambuli

FAW incidence, defined as the extent of infestation on maize plants in the field was calculated by expressing the number of infested plants as a percentage of the total number of plants in the field. It was recorded and estimated as percentage infection, whereby 1-20% = low incidence; 21-49% = moderate incidence; and 50 - 100% = high incidence (Nono-Womdim et al., 1996).

Table 1. Incidence of FAW damage on maize in Bwikhonge Sub County.

Incidence category	Number of fields in Buwabwala parish	Number of fields in Buwekanda Parish	Total number of fields in the Sub county
No incidence	1 (5.0%)	7 (35.0%)	8 (20.0%)
Low (1-20%)	3 (15.0%)	3 (15.0%)	6 (15.0%)
Moderate (21-49%)	4 (20.0%)	5 (25.0%)	9 (22.5%)
High (50-100%)	12 (60.0%	5 (25.0%)	17 (42.5)
Total	20	20	40

Numbers (and percentages in the parentheses) of maize farms showing the level of damage of the FAW in the study parishes.

Prevalence of FAW on maize in Bulamuli

Prevalence of the FAW was determined at parish level according to Masika et al. (2017b). In this case, twenty farmers' fields were surveyed and the fields which were observed to have maize plants damaged by the fall armyworm were expressed as a percentage of all farms sampled in that parish. Afterwards, prevalence was determined for the whole Sub County. Assessment for the presence of FAW larva was done by counting the number of larva on the infested plant including on the kernels and ears. This was done by careful observation and opening of the plants with leaf damage caused by FAW larvae and also those plants with FAW frass in the whorl and on the ears (Baudron et al., 2019).

Severity of infestation of FAW on maize in Bulambuli

In the field, sampling for severity of leaf damage was visually rated on 20 randomly selected plants from 5 different locations in the field on a scale of (0-9) representing the different levels of damage, according to Davis and Walliams (1992). Similarly, sampling severity of ear damage was visually rated at the same time that 10 randomly selected ears from 20 randomly selected plants from 5 different locations in the field were inspected.

Data analysis

Data were analyzed using Stata Corp, 4905 Lakeway Drive to output frequencies, tables and pie charts for descriptive statistics while categorical data (severity data) were analyzed using non parametric chi-square analysis.

RESULTS

Incidence of FAW on maize

Generally, for all the forty fields sampled in the Sub County, FAW incidence was high, where more fields showed a high incidence, followed by those with moderate incidence, those which showed no FAW damage and then those with low incidence. In individual parishes, Buwabwala had more fields with a high FAW incidence as compared to those from Buwekanda (Table 1 and Figure 1).

Prevalence of the FAW larvae on maize

In Bwikhonge Sub County, FAW larvae were so prevalent when infested plants were carefully inspected. Buwabwala parish had the highest percentage prevalence of FAW larvae as compared to Buwekanda parish. Three hundred and fifty-four FAW larvae were collected from the twenty fields surveyed in Buwabwala parish. In Buwekanda parish, 196 FAW larvae were collected from plants which were inspected in the twenty fields surveyed. The inspection and collection of the FAW larvae was done together with the farmers of the fields as one of the methods of managing the destructive FAW pest most especially in maize fields with young and still growing maize plants.

Severity of FAW damage on maize in Bwikhonge Sub County

A total of 800 maize plants were analyzed for severity of FAW damage on leaves in the Sub County, where 400 plants were from Buwabwala parish and 400 also from Buwekanda parish. Generally there was a high FAW damage on maize leaves in the Sub County. Of the total number of plants inspected for FAW damage on leaves in Buwabwala parish, only 160 (40.0%) plants had leaves with no FAW damage while in Buwekanda parish, a total of 272 (68.0%) maize plants had no FAW damage on the leaves. The remaining plants had leaves damaged at different severity levels according to Davis and Walliams (1992). The differences in severity of FAW damage on the leaves in Buwabwala and Buwekanda parishes were statistically significantly (df = 9, $\chi 2$ = 87.66, P = 0.000*) (Table 2 and Figure 2).

A total of 800 maize plants were selected and their ears were inspected for FAW damage in the Sub County. Fifty per cent (400) maize plants were from Buwabwala and 50% (400) were from Buwekanda parish. A total of 223 (55.8%) maize plants from Buwabwala had ears which did not have FAW damage and frass while from Buwekanda Parish, 380 (95.0%) maize plants did not have FAW damage and frass on their ears. Only twenty

Figure 1. A-Inspecting FAW damage on maize plant, B-Field infested with FAW, C- Maize tassel destroyed by FAW and D- Plant leaves drying because of extensive FAW damage.

maize plants from Buwekanda parish had ears damaged by the FAW and 177 plants from Buwabwala had ears damaged by the FAW. The damage on the ears had different severity scores as described by Davis and Walliams (1992). The severity of FAW damage on the ears in the two parishes were different and these differences were statistically significant (df = 9, $\chi 2$ = 299.2, P = 0.000*) (Table 2 and Figure 2).

A total of 800 maize plants were selected and their ears inspected for FAW damage on their kernels in Bwikhonge Sub County. 400 maize plants were from Buwabwala and 400 also from Buwekanda. Of the total number of maize ears analyzed, 383 (97.8%) plants did not have any FAW damage on their kernels in Buwabwala parish while 398 (99.5%) plants from Buwekanda did not have kernels damaged by the FAW. The severity of FAW damage on the kernels was also scored according to Davis and Walliams (1992). The damaged kernels showed different severity scores and the differences in severity of FAW damage on the kernels were statistically significant (df = 7, $\chi 2$ = 19.9, P = 0.005*) (Figure 3 and Table 2).

DISCUSSION

Incidence of fall armyworm damage in maize fields

There was a high incidence of FAW in Bwikhonge Sub County. The Sub County is located next to River Sipi that never dries even in the dry season and also next to a permanent swamp in Buryalo. These features provide constant humid conditions coupled with cultivation of maize, the primary host of the FAW all year round, and facilitate proliferation of the pest throughout the year as the pest life cycle is never broken (Prasanna et al., 2018).

Prevalence of fall armyworm in maize farms in Bwikhonge Sub-county

Prevalence of falls armyworm larvae

There was a high prevalence of the FAW with over 198 larvae collected on 800 maize plants during the survey.

Table 2. Chi square analysis for severity of FAW damage on plant leaves, ears and kernels in Bwikhonge Sub County.

Severity	Severity on plant leaves			Severity	Severity on plant ears			Severity	Severity on kernels		
	Buwekanda	Buwabwala	Total		Buwekanda	Buwabwala	Total		Buwabwala	Buwekanda	Total
0	68.0 (272)	40.0 (160)	54.0 (432)	1	95.0 (380)	55.8 (223)	75.4 (603)	0	97.8 (383)	99.5 (398)	97.6 (781)
1	8.8 (35)	14.5 (58)	11.6 (93)	2	0.3 (1)	25.8 (103)	1.3 (104)	1	0.3 (1)	0.0 (0)	0.1 (1)
2	2.5 (10)	11.3 (45)	6.9 (55)	3	1.3 (5)	5.0 (20)	3.1 (25)	2	1.0 (4)	0.0 (0)	0.5 (4)
3	1.3 (5)	7.3 (29)	4.3 (34)	4	0.3 (1)	6.8 (27)	3.5 (28)	3	0.8 (3)	0.5 (2)	0.6 (5)
4	5.3 (21)	5.5 (22)	5.4 (43)	5	0.5 (2)	1.8 (7)	1.1 (9)	4	1 (4)	0.0 (0)	0.5 (4)
5	3.8 (15)	3.3 (13)	3.5 (28)	6	0.3 (1)	1.3 (5)	0.8 (6)	5	0.5 (2)	0.0 (0)	0.3 (2)
6	3.0 (12)	4.8 (19)	3.9 (31)	7	0.8 (3)	3.0 (12)	2.1 (15)	6	0.3 (1)	0.0 (0)	0.1 (1)
7	1.3 (5)	3.5 (14)	2.4 (19)	8	0.3 (1)	0.5 (2)	0.4 (3)	7	0.5 (2)	0.0 (0)	0.3 (2)
8	2.3 (9)	3.8 (15)	3.0 (24)	9	0.5 (2)	0.3 (1)	0.4 (3)	Total	400	400	800
9	4.0 (16)	6.3 (25)	5.1 (41)	Total	400	400	800	**df = 7, χ2 = 19.9, P = 0.005***			
Total	400	400	800	**df = 9, χ2 = 299.2, P = 0.000***							
df = 9, χ2 = 87.66, P = 0.000*											

Percentages (and numbers in the parentheses) of plant leaves, ears and kernels surveyed for severity of FAW damage in Bulambuli District. *Statistically significant.

The FAW was also present throughout the growth stages of maize in the two surveyed parishes. This may be attributed to presence of maize plant residues in the swamps of River Sipi which result in no breakage of pest life cycle, hence high prevalence of FAW which is supported by what has been reported by Prasanna et al. (2018). Furthermore, the high prevalence may be mainly due to lack of proper management strategies of the pest because it is a new invader in the region and proper integrated pest management options are yet to be designed (Thierfelder et al., 2018). Moreover, the cultural methods which have been reported to reduce FAW infestation (Midega et al., 2018; Hailu et al., 2018) are not yet known by the local farmers. Similarly, there is little empirical evidence to guide farmers on how to effectively control the FAW through agronomic management practices in Africa (Baudron et al., 2019).

Infestation by the larvae showed that at the time of the survey, the larvae incidence of 0.88 was not too high to cause economic loss which occurs at a mean infestation of 3 or more fall armyworms per plant in 20 damaged plants/100 (Davis and Walliams, 1992). However, any increase in infestation would lead to economic loss. Since the survey was carried out early in the season when most of the maize in the fields was silking, a follow up survey after one month would result in a higher mean infestation since the FAW takes 30 days to complete its life cycle (FAO and CABI, 2019) and according to the biology of FAW, it does not diapause, meaning that there was a continuous population buildup and several generations can overlap within a single crop cycle when suitable conditions prevail, hence, there may be no need for the pest to migrate from Bulamuli to other areas since it enjoys favourable conditions throughout the year (FAO and CABI, 2019).

Although efforts have been made by the farmers to apply pesticides to manage the FAW, potential health issues to the environment and the population arising from hazardous chemicals and long term exposure to pesticide crop residues in consumed produce and in the environment raises unresolved issues (Harrison et al., 2019). Furthermore, some of the pesticides being used may not be the right ones, their doses and concentrations may not be optimal and may be applied at a wrong age of plant growth which was frequently observed in farmers' fields. This renders the chemicals ineffective, inconsistent and unsatisfactory to control the pests in a given local context (Midega et al., 2018). Most chemicals used are broad spectrum chemicals which may end up killing what would be the natural enemies that control the FAW population (Harrison et al., 2019). Integrated pest management options including CABI`S plant-wise advice to small scale farmers to use hand picking and destroying egg masses and larvae and putting sand mixed with lime or ash in the leaf funnels to kill the FAW larvae may help to reduce its prevalence. This is one of the

Figure *2.* A-FAW larvae on one of the plants surveyed in Buwekanda, B- Larva on one of the plants surveyed for FAW damage in Buwabwala, C- Maize plant ear destroyed by FAW larva in Buwekanda and D-Maize plant ear damaged by FAW larva in Buwabwala.

methods we employed as we surveyed the farmers' fields and collected the larvae in determination of the incidence. This is supported by Grzywacz et al. (2014), who asserts that low-cost options of pest control which build on indigenous knowledge and ecological options are more relevant to smallholder farmers.

Severity of fall armyworm larva in maize farms in Bwikhonge Sub County

Generally, there was a high severity level of FAW damage on maize leaves and ears which where statistically significant between the two parishes (Table 2). The high levels of damage caused by the FAW of 32% in Buwekanda and 60% in Buwabwala is almost in the same range with what has been reported by previous studies in Ethiopia, Kenya and Zimbabwe (Baudron et al*.,* 2019; Kumela et al., 2018). The severity of FAW damage on maize kernels was low (Table 2) which could be attributed to the early silking stage at which most of the maize in the fields were during the survey. During this time, most of the maize fields had not yet reached milking stage for the kernels to be damaged. However, the significant damage caused by the FAW on maize leaves and ears has great potential to further spread and damage the kernels which may lead to economic loss as the FAW has been reported to be the second most dangerous pest in agriculture causing $39 to $297 million every year (Sparks, 1986). The high severity of the FAW are similar to what has been reported by Aguirre et al. (2016). Continuous cultivation throughout the year and warm humid conditions which promote population buildup allowing several generations to overlap within a single

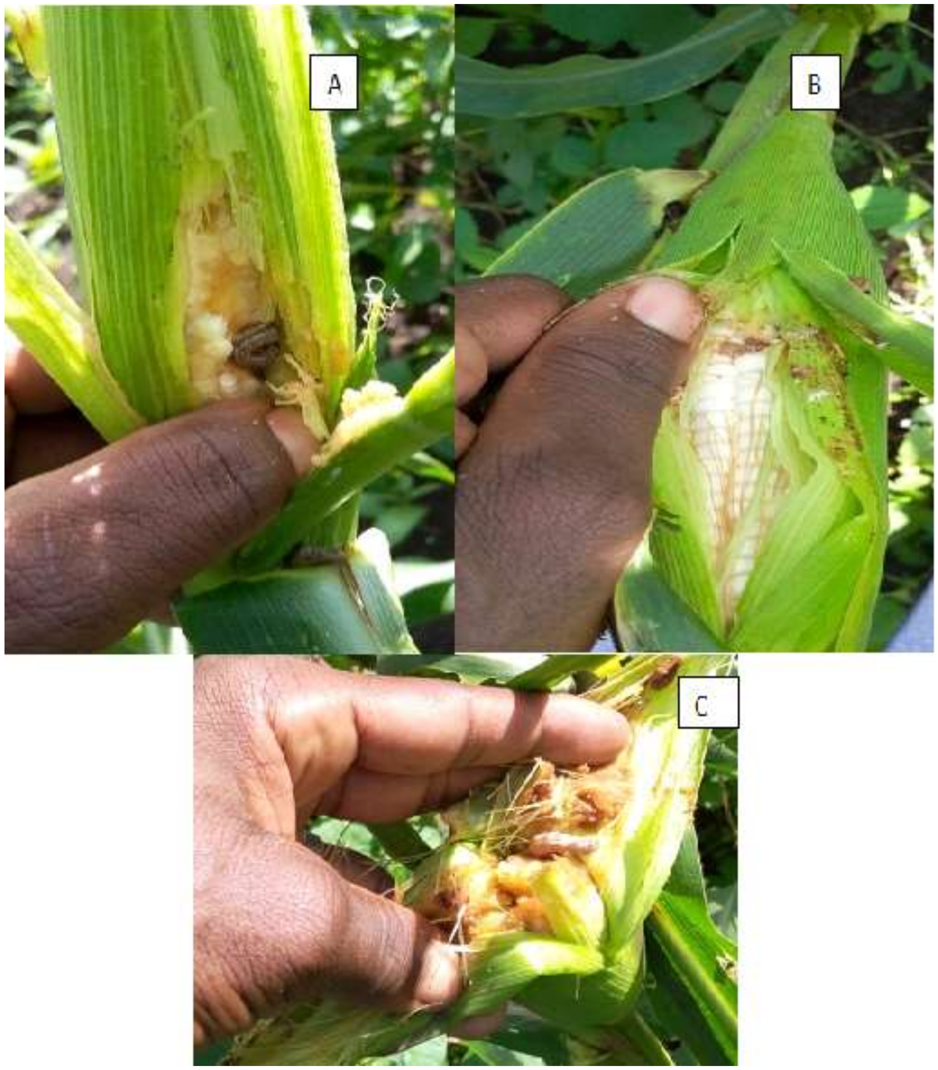

Figure 3. A, B-Maize kernels destroyed by FAW in Buwabwala and C-maize kernels destroyed by FAW in Buwekanda.

crop cycle with suitable prevailing conditions should be responsible for the high severity observed in maize farms in Bulambuli (FAO and CABI, 2019).

CONCLUSION AND RECOMMENDATIONS

The study showed that there was a high incidence of FAW in Bwikhonge Sub County which was attributed to the humid conditions that are provided by the surrounding water bodies and facilitate FAW buildup and overlap within a single crop cycle. High population buildup will lead to economic loss when proper management options are not designed. Therefore, there is need to design collaborative research incorporating indigenous knowledge of pest control, biological control and plant breeding options to develop integrated pest management strategies for the pest.

Surveying the FAW severity in all the agro-ecologies in Uganda across the different generations and growth stages of the plant is important to give an idea of the level of damage and economic losses that the pest is inflicting on the smallholder farmers.

There is need to model weather conditions with the population dynamics of FAW and desert locust to give an idea of the factors perpetuating the proliferation of insect pests in Africa. For example, the looming invasion of the Eastern part of Africa with the desert locust is attributed to the extreme weather conditions where 2019 started with a dry spell and ended with an extremely wet season ever which is just speculative.

There is need to evaluate the chemical being used to control the FAW as most of the broad spectrum chemicals being used may be dangerous to the natural enemies of these pests or may leave residues in the food and cause damage to the environment and human life.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Potential of reduced agricultural lime application rates to increase yield and profitability of maize through microdosing in central Malawi - A short note

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Maize (*Zea mays* L.) is a staple food crop in Malawi, with average yields of 1.8 -2.2 t ha^{-1} compared to potential yields of 5 to 10 t ha^{-1}. In some areas, soil acidity is a serious constraint in crop production. The current recommendation is to apply 2.0 t ha^{-1} of lime as pre-plant broadcast and incorporated into soils with pH_W < 5.5. A pilot study was conducted during 2015/2016 season at Bunda Campus (14° 35 S'; 33° 50 E') to evaluate the response of reduced lime application rates of 0, 100, 250 and 500 kg ha^{-1} applied by point placement or dollop method (mirodosing) in comparison to recommendation pre-plant broadcast application. A uniform fertilizer application of 69:23:0+4S was made on all plots. Gross Margin (GM) analysis, Value Cost Ratio (VCR) and Benefit-Cost Ratio (BCR) were undertaken. A gross Margin analysis was done using prevailing input/out values of 2019 at exchange rate of Malawi Kwacha, MK 745 = 1USD. Results showed that the 'no lime' control (4.01 t ha^{-1}) gave the lowest grain yield and was significantly (P<0.05) out-yielded by the rest of the treatments. The highest yield of 5.75 t ha^{-1} was observed from the 500 kg ha^{-1} application, while the recommended control of 2t/ha yielded 4.90 t ha^{-1}. The BCR was >1.0 for all treatments at two price scenarios of US$0.201 kg^{-1} and US$0.268 kg^{-1} grain, but >2.0 only for 100, 250 and 500 kg ha^{-1} lime application rates. VCR ranged from 4.4 to 10.4, except for 2 t ha^{-1} treatment which had 0.78 and 1.05 at US$0.201 and US$0.268 kg^{-1} grain price, respectively. The results demonstrated that there is potential in reduced lime application rates to increase yield of maize by microdosing of agricultural lime. This study has provided a solid basis for wider evaluation of the concepts for subsequent rolling out to famers.

Key words: Dolomitic lime, soil acidity, microdose applications, aglime profitability.

INTRODUCTION

In Malawi, maize (*Zea mays* L.) is the staple food crop that dominates the cropping systems. Average yields in the period, 2005 to 2012, ranged between 0.81 and 2.65 t ha^{-1} (GoM, 2012). Poor soil fertility is one of the major reasons for low yields (Kumwenda et al., 1997; ICRISAT/MAI, 2000; Blackie and Mann, 2005; MoAFS, 2012). Other constraints include recurrent droughts, poor management, foliar diseases, stalk borers, termites and

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the parasitic weeds species *Striga* (MoAFS, 2012; Kabambe et al., 2008).

For some parts of the country, especially in high rainfall upland areas, aluminium toxicity due to low pH is a major constraint. Chilimba and Saka (1998) reported that over 40% of the soils in Malawi belong to soil orders Oxisols and Ultisols (US Taxonomy), which are highly weathered and strongly acid with a pH (in water) of < 5.5. Many reports confirm occurrence pH levels below this critical minimum level (Maida, 1985; Chilimba and Nkosi, 2014; Snapp, 1998; Kabambe et al., 2013). There is a current recommendation to apply 2 ha^{-1} agricultural limes as pre-plant broadcast incorporation for soils with pH_w (H_20) < 5.5 (Chilimba, 2005; Chilimba and Nkosi, 2014; Chilimba et al., 2013).

Aluminium toxicity is considered as the most important plant-growth limiting factors in many acid soils, particularly those with pH below 5.0 to 5.5. Tully et al. (2015) reported that up to 24% of Sub-Saharan Africa is under Aluminium toxicity while 8.49 are subject to high P fixation. When acid soil are limed, exchangeable Al^{3+} and hydrohyaluminium cations such as $Al(OH)_2^+$ are converted to insoluble $Al(OH)_3$; resulting in removal of the Al_3^+ from cation exchange competition (Marschner, 2011; McCauley et al., 2009). Availability of P and K are improved with liming. Excess aluminium interferes with cell division in plant roots, fixes phosphorus into less available forms, decreases root respiration, and interferes with uptake, transport, and use of Ca, Mg and P (Fairhurst, 2012; Goulding, 2016). In Cameroon, Yamoah et al. (1996) reported that application of lime and green manure improved stand count, root and stem weights and yields of maize, bean and potato in an acid soil and that high P application was unnecessary when lime was applied. Onwonga et al. (2008) reported that lime application increased soil pH within two months of application and that organic amendments had similar effects in Kenya. The et al. (2012) reported that continuous cultivation of maize resulted in a soil acidity in Cameroon, falling from 4.89 to 4.38 and drops in soil availability of Ca and K. There is potential to reduce the lime requirements and increase yield through microdosing of lime application (The et al., 2012; Kisinyo et al., 2015; One Acre Fund, 2016), including application by point placement or dollop method (One Acre Fund, 2016). Malawi has large deposits of limestone and dolomite. An explorative study was therefore conducted to compare the effectiveness of applying reduced rates of agricultural lime on maize growth and yield at Bunda Campus of Lilongwe University of Agriculture and Natural Resources, central Malawi in 2015-2016 season.

MATERIALS AND METHODS

Experimental design, treatment descriptions and general crop and management

A field trial was conducted utilizing a randomized complete block to evaluate seven treatments and four replicates (Table 1); which were designed to compare the recommended application of lime at 2.0 t ha^{-1} to reduce rates applied as point placements (dollop method), hereafter referred to as microdosing.

The maize variety DK8053, with a maturity period of 140 days, was used in the study. The maize was planted on ridges spaced at 75 cm apart with stations at 60 cm, 2 seeds per station, giving a target plant population of 44,444 plants/ha. Gross plot sizes were 5 ridges x 0.75 cm x 5.3 m long (19.875 m^2), while the net plot comprised three middle ridges excluding end of ridge plants (10.575). The compound fertilizer 23:21:0+4S was applied at 100 kg/ha for basal dressing, one week after planting. The remaining 46 kg/ha N was applied as top dressing at 4 weeks after planting in the form of urea. Ridge preparation, lime application and planting were done in mid-December, 2015. The fields were kept free of weeds by weeding with a hand-hoe. The field preparations and fertilizer, lime application and planting were done in mid-December 2015. Figure 1 shows daily cumulative rainfall (mm) recorded at the site. In general, the season was considered good for maize production.

Study site

A field experiment was conducted at Lilongwe University of Agriculture and Natural Resources – Bunda campus, at the student Crop and Soil Sciences research farm in the 2015/2016 cropping season (November 2015 to April 2016). The farm is 1159 masl at 14°35' S and 33°50' E with an annual rainfall of 1031 mm. The soil types vary from clay loam to sandy loam (Jones and Kanyama, 1977). The specific site for the study had mean pHw of 5.1 (SD 0.22, n=10).

Data recording and analysis

The data recorded in this report were maize plant height, grain yield and stover weight. Grain yield and stover were determined by harvesting all plants from net plot area. For stover, 6 plants were randomly sampled from the net plot area and oven dried to determine percent moisture content of stover, which was used to adjust field weight to dry weight. Grain moisture content was determined using grain moisture meter and yield adjusted to 12.5% storage moisture. Plant height (from base to center of leaf whorl or flag leaf) was determined from an average of 6 plants randomly selected from the net plot. Data were subjected to analysis of variance procedure using Genstat 18th Edition (VSN International, Memel, Hemstead, UK) and mean separation by LSD at ≤ 0.05 probability.

Determination of gross margins (GM), value cost ratio (VCR) and benefit-cost-ratio (BCR)

Gross margins (GM) were determined as gross income from sales minus production costs that vary. The costs that vary mainly consist of labor (land preparation, ridging, planting, fertilizer application, harvesting shelling, cleaning and packaging). These are basic components described by several authors (Dzanja, 2008; Ngulube et al., 2001; Takane, 2008). Dzanja (2008) estimated the total labor requirement to be 139 mandays for maize and these were used in the calculations. However, Takane (2008) estimated labor requirement for maize to be 176 mandays. Another factor considered as a variable cost was packaging, which comprised a new sack for each 50 kg of harvest. Tables 2 and 3 show the total costs for each of the five rates of lime application. The costs of inputs and labor use were those of the October 2019 in Malawi. At this time the exchange rate of the Malawi Kwacha to US $ was 1: 745. The labor was valued based on the minimum wage rate of

Table 1. List of treatments in the lime rate and microdosing study.

Treatment number	Description Kg ha^{-1} lime	Treatment details
1	No lime	$NP_2O_5K_2OS$ 23:21:04. Fertilizer applied by dollop or point placement method one week after emergence. No agri lime application
2	100 as microdose	$NP_2O_5K_2OS$ 23:21:04 applied as in 1. Plus 100 kg lime applied in dollop on one side of maize station
3	250 as microdose	$NP_2O_5K_2OS$ 23:21:04, applied as in 1, plus 250 kg/ha lime applied in dollop on one side of maize station
4	500 as microdose	$NP_2O_5K_2OS$ 23:21:04, applied as in 1, plus 500 kg/ha lime applied in dollop on one side of maize station
5	2000 kg/ha lime, band	$NP_2O_5K_2OS$ 23:21:04, applied as in 1, plus 2000 kg lime applied in band made by opening the ridge to 15cm then covering up

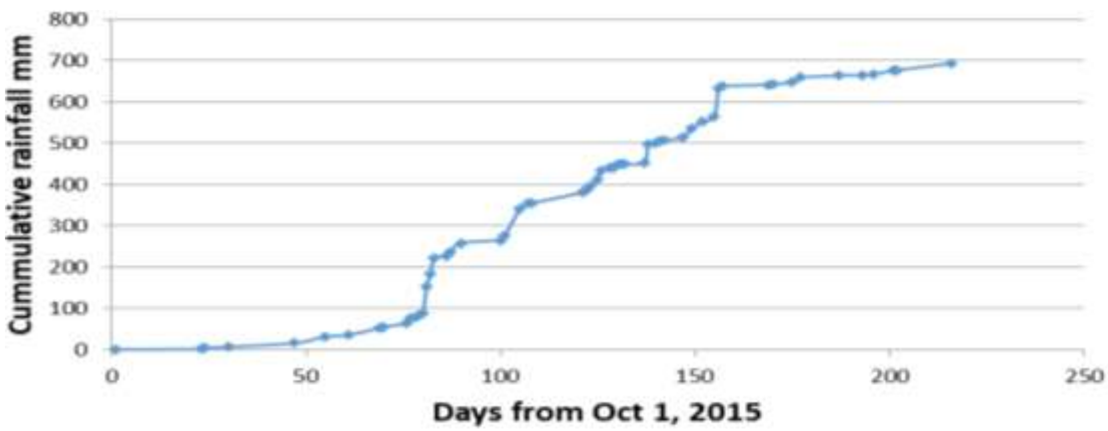

Figure 1. Daily cummulative rainfall (mm) for Bunda College, 2015/2016 season.

Table 2. Variable costs (US $) for the different lime fertilizer rates used in the gross margin calculations for maize (cf 1 US$= Malawi Kwacha, MK, 745).

Input type	Fertilizer package kg ha^{-1} NPKS and inputs costs in MK ha^{-1}				
	69:21:0+4S	69:21:0:4 and 100 kg ha^{-1} lime	23:21:0:4 250 kg ha^{-1} lime	23:21:0:4 500 kg ha^{-1} lime	23:21:0:4 2000 kg ha^{-1} lime
Seed 25 kg/ha	37.58	35.57	35.58	35.58	37.58
Labour at US$1.29manday	356.39	356.39	356.39	356.39	356.39
Fertilizer cost	128.86	128.86	128.86	128.86	128.86
Lime at US $ 8.05 for100 kg +30% transport mark up	0	10.47	26.17	52.34	161.07
Labour Lime application	0	2.58	6.46	12.91	51.65
Bagging	16.10	18.52	19.32	23.15	19.53
Total variable costs*	538.94	554.41	574.79	611.29	755.08

US$ 1.29 for time (October 2019). Calculations were made for two output price scenarios of US$0.201 kg^{-1} and US$ 0.268 kg^{-1} as realistic average prices in the August-October 2019 period. The evaluation also involved calculation of Value Cost Ratio (VCR) and Benefit Cost Ratio (BCR). The VCR was calculated as incremental value of maize divided by treatment/coast associated with implementing the treatment (Kihara et al., 2015). Specifically the costs were those associated with lime purchase and application (Table 2). Benefit–cost ratio was determined by dividing gross income of a treatment by total variable cost. There was no discounting of costs, as the comparison is for one-season data rather than a long- duration project (Ajayi, 2009). The economic calculations are presented in US $ to facilitate 'universal" communication.

Table 3. Effect of lime application rate on maize plant height (cm) 2 to 10 weeks after planting

Treatment	Plant height (cm) x weeks after planting				
	2	4	6	8	10
0 kg/ha lime	18.9	33.6	92.9	130.1[a]*	143.2[a]
100 kg/ha lime, microdose	18.6	34.6	93.8	133.2[a]	148.5[ab]
250 kg/ha lime, microdose	18.1	34.8	96.6	135.3[ab]	155.6[b]
500 kg/ha lime, microdose	18.7	35.1	95.9	151.5[bc]	167.5[c]
2000 kg/ha lime, banding	19.5	34.4	95.5	147.1[c]	160.2[c]
Mean	18.8	34.5	94.9	139.5	155.0
F Prob	0.72	0.84	0.67	<0.001	<0.001

*means denoted by same letter are not significantly different at P≤ 0.05.

Table 4. Effect of treatments on maize grain and stover yield (kg/ha).

	Grain yield kg ha^{-1}	Stover weight kg ha^{-1}
0 kg/ha lime	4012[a]*	7459
100 kg/ha lime, microdose	4601[b]	7884
250 kg/ha lime, microdose	4847[b]	8742
500 kg/ha lime, microdose	5751[c]	9476
2000 kg/ha lime, banding	5064[b]	9080
Mean	4896	8610
F Prob	0.016	0.18

*means denoted by same letter are not significantly different at P≤ 0.05.

RESULTS AND DISCUSSION

Agronomic results

There were no significant treatment differences in plant heights at 2, 4 and 6 weeks after planting. Significant differences were observed at 8 and 10 weeks after planting (Table 3). In both cases 500 kg ha^{-1} microdose treatment gave tallest plants, which were significantly higher than no-lime control and 100 kg ha^{-1} at 8 weeks only but similar to the rest of the treatments including commended 2 t ha^{-1} treatment. For grain yield (Table 4), the 500 kg ha^{-1} lime microdose gave the highest yield which was also significantly higher than all other treatments treatment. There were no significant treatment differences on stover yield.

The increased growth and yield due to lime application are in agreement with local literature (Kabambe et al., 2013; Mtonga, 2013) and regional literature (Kisiyo, 2015; The, 2012) and is agreement with recommendation to apply lime for soils with pHw <5.5 (Chilimba and Nkosi, 2014). It of interest to note the significant yield increase to the lower lime rates as applied by point placement. Rates of 100-250 kg ha^{-1} are substantially lower and would be relatively feasible for smallholder farmers compared to 2 t ha^{-1} currently recommended. The et al. (2012) reported that application of lime at the rate of 250 kg ha^{-1} increased maize grain yield in some varieties of maize, but not others. However, agricultural lime applied at high rates to a large volume of soil has advantage of residual effects which may last for 2-3 seasons. In Malawi, Kabambe et al. (2012) confirmed residual effects of 2 t ha^{-1} after one season with maize and common beans. There are different ways which can be employed in the integrated management of acid soil in order to reduce amount and cost of lime while improving crop productivity. These include use of tolerant varieties (The et al., 2012), amendments with organic inputs including wood ash, compost, and legume rotations (Kabambe et al., 2012; Onyonga et al., 2008; Yamoah, 1996; Goulding, 2016) and agroforestry (De Pauw, 1994). Farmers may also opt for crop species with greater acid resistance (De Pauw, 1994; Goulding, 1989). The yields obtained in this study are higher than average smallholder farmer yields (MoAFS, 2012). On farm yields from research plots with fertilizer application normally fall in the range 2.5-3.5 t ha^{-1} (Ngwira et al., 2012; Kabambe et al., 2013, 2018). The results obtained are explorative in nature and mainly serve to provide a basis for wider on farm verification on the both the rates and method of application before recommendations to farmers.

Gross margins, value cost ration and benefit-cost ratio

The results on Gross Margins (GM) (Table 5) show that all treatments had positive GM, the highest coming at

Table 5. Value cost ratio (VCR).

Treatment	Costs, benefits and VCR in MK at two price scenarios				
	Lime cost, application and bagging	Increment benefit US \$ 0.201 kg^{-1}	Increment benefit US \$ 0.268 kg^{-1}	VCR US \$0.201 kg^{-1}	VCR US \$ 0.268 kg^{-1}
No lime	0	-	-	Na	Na
100 kg ha^{-1} lime	15	121	161	7.8	10.4
250 kg ha^{-1} lime	36	161	215	4.5	6.0
500 kg ha^{-1} lime	72	351	470	4.9	6.5
2000 kg ha^{-1} lime	216	170	228	0.78	1.05

500 kg ha^{-1} lime and lowest with the 2 t ha^{-1} lime treatment using both maize product prices. The results are in line with yield results. In a similar GM analysis Kabambe et al. (2018) also reported that GM of maize was positive for yields and prices of 4,000 kg ha^{-1} and US\$0.27 kg^{-1} respectively. Recently, Ngwira et al. (2020) reported GMs of US\$1000-1800 from on-farm maize trails in Malawi.

Conclusion

The results consistently demonstrate that 500 kg ha^{-1} lime as micro dose resulted in better maize growth and yield compared to no lime treatment and 100 kg ha^{-1} rate. However, both growth and grain yields were similar with the other treatments. The results suggest potential to reduce application rates and increase yield of maize through microdosing of agricultural lime and provide a solid basis for wider evaluation of the concepts for subsequent rolling out to famers. Profitability was clearly related to yield and the associated costs of production. These results are from an explorative study. As the concept of microdosing, as defined here, is the reduction of application rates and the precise point placement. It is recommended that it should be evaluated along with other integrated soil management options that are proven to ameliorate soil acidity and improve yields.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Role of leaf rolling on agronomic performances of durum wheat subjected to water stress

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In arid and semi-arid climates characterized by low rainfall with a great annual and inter-annual irregularity, drought can occur at any time inducing large losses in crop yield. Leaf rolling is one of the adaptive morphological responses to the water deficit observed in a number of species including cereals. It reduces the leaf area exposed to sunlight and transpiration. The aim of the present work was to characterize the agronomical impact of water stress on a set of 16 Moroccan durum wheat varieties and to examine the possible correlations between leaf rolling and agronomic performances of these varieties. Experiments were conducted during three cropping seasons on a soil with a clay-loam texture at Tamellalet, in Marrakech region. The water stress was applied for one week to the studied varieties at tillering, and the degree of leaf rolling was determined at the end of the stress period. Strong differences between the varieties in the degree of leaf rolling upon water stress and in the ability to counteract drought were observed. Varieties displaying high leaf rolling showed less reduction in the yield components (number of ears per plant, number of grains per ear and weight of grains). The strong correlation observed between the degree of leaf rolling and maintaining of agronomic performance suggests that leaf rolling can be a criterion for water stress tolerance in durum wheat. This trait could thus be used as a morphological marker of tolerance to water stress.

Key words: Durum wheat, water stress, leaf rolling, agronomic traits, grain yield, drought tolerance, Morocco.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. subsp. durum) accounts for about 10% of the global wheat production (Kantety et al., 2005). Its cultivation is concentrated in latitudes corresponding mostly to the North America, the Middle East, Australia and especially the Mediterranean Basin. The aforementioned represent around 60% of its total growing area. In the southern Mediterranean countries, it occupies a key place in agricultural production. In Morocco for instance, out of a total of 8.7 million hectares cultivated annually, 5.3 million hectares

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are devoted to cereals, and durum wheat cultivation (1.1 to 1.3 million ha) ranks third after bread wheat and barley (Belaid et al., 2003; MAPM, 2014).

Durum wheat is mainly grown under rainfed conditions (e.g., cultivated in “Bour” at 81% in Morocco) (MAPMDREF, 2016). Its cultivation under arid and semi-arid conditions is thus expected to face water deficit or drought (Chennafi et al., 2006), and its annual production is therefore highly dependent on unpredictable seasonal rainfalls and temperatures (Anderson, 2010; Royo et al., 2010). In the Mediterranean region, losses in durum wheat yield due to water deficit vary from 10 to 80% depending on the year (Nachit et al., 1998).

In durum wheat as in other crops, the comparison between optimal yields and mean yields observed in the field generally reveals considerable differences, especially in traditional farming systems. These differences are explained by the influence of agronomic, genetic and climatic factors (Ricroch et al., 2011). Water stress can be quantified as the ratio between the amount of water required for optimal growth of the plant and that available in its environment (Laberche, 2004). In wheat, the moisture deficit in the soil affects the three main components of yield, namely the number of ears, the number of grains per ear and the weight of 1000 grains (Assem et al., 2006). The effect on these components, and therefore on yield, depends on the stage at which the deficit occurs (Mongensen et al., 1985; Debaeke et al., 1996). For instance, drought, at the beginning of cultivation cycle is known to affect emergence and tillering and, at end of the cycle, to affect the filling of the grains (Fisher, 1973; Watts and El Mourid, 1988; Kobata, 1992).

Maintenance of the major physiological functions (photosynthesis, transpiration, growth) under water shortage represents an important challenge (Passioura, 1996; Tardieu, 2003, 2005; Amigues et al., 2006). In addition to the maintenance of growth of leaves and reproductive organs, delayed leaf senescence, by keeping the photosynthetic capacity, helps to feed the reproductive organs. This strategy can allow high yields but also increases the risk of total yield loss. It is favorable in conditions of moderate water deficit but may prove to have detrimental consequences in the case of more severe water deficit (Amigues et al., 2006). Tolerance to water deficit may therefore be considered rather as the ability of a genotype to produce an acceptable yield under conditions of water deficit according to the scenario of the constraint (degree, developmental stage, duration) (Yokota et al., 2006; Hamon, 2007; Tardieu and Tuberosa, 2010).

In cereals, leaf rolling is a characteristic response to water deficit (O’Toole and Cruz, 1980; Kadioglu et al., 2012). It is believed to occur when the evaporative demand is no longer balanced by water uptake by the roots. This movement of the limb is indeed caused by the loss of turgor of bulliform cells, which are large epidermal cells of the upper epidermis (Begg et al., 1980; Willmer, 1983; Jane and Chiang, 1991). The degree of leaf rolling is often used as a marker of intensity of drought stress in cereal cultures (Riboldi et al., 2016), but leaf rolling can also be considered as a mechanism of avoidance of dehydration (Belhassen et al., 1995; Amokrane et al., 2002). It contributes, by decreasing the leaf surface exposed to sunlight, to the reduction of transpiration and to increase water use efficiency in water stressed conditions (O’Toole and Cruz, 1979; Monneveux and Belhassen, 1996). Furthermore, it allows the plant, by limiting the direct illumination of leaf surface, to avoid overheating of leaf tissues, harmful to cellular metabolism and is considered to play a significant role in the resistance to high temperatures and end-of-cycle water deficit (Ortiz et al., 1991). In rice, leaf rolling was reported to improve photosynthetic efficiency and to delay leaf senescence (Richards et al., 2002; Richards et al., 2004; Zhang et al., 2009). The available photosynthetic surface of rolled leaf is however reduced, which has a negative impact on plant growth (Li et al., 2016a).

The objective of this study was to characterize 16 durum wheat varieties for their leaf rolling ability under drought stress and their agronomic performance in order to investigate the relationship between leaf rolling and tolerance or sensitivity to water stress applied at tillering .

MATERIALS AND METHODS

Plant material and experimental site

The study concerns 16 varieties of durum wheat (*Triticum turgidum* L. var. *durum*) provided by the National Institute of Agronomic Research of Settat (Morroco): Kyperounda (“2777"), Amjad, Anouar, Irden, Isly, Jawhar, Korifla, Marjana, Marouane, Massa, Oum Rabia, Sebou, Tomouh, Vitron, Waha and Yassmine. These varieties displayed diverse ranges of agronomic adaptation, a number of them being adapted to semi- arid areas (Taghouti et al., 2010; Nsarellah et al., 2011; Zarkti et al., 2012). The field experimentation was conducted at Tamellalet, CMV 408, Marrakech region (31 "81'N, 7" 50'W), during three consecutive cropping seasons: 2013-2014, 2014-2015 and 2015-2016. The Supplementary Table 1 represents more information on durum wheat varieties studied.

Climatic conditions

The monthly precipitations recorded over the three crop years, quite variable from one year to another, are given in Supplementary Table 2. The rainfall received from sowing to maturity was 186 mm, 171 mm, and 95 mm for the first, the second and the third years, respectively. The end-of-cycle period of culture at the field trial site coincided with low rainfall associated with high temperatures. Irrigation water was added to overcome the water deficit during the whole cultivation cycle, to meet the needs of the crop, fully or only partially when water stress was applied.

Field trial stress application

After the preparation of the soil (tillage, then leveling of the plot and

Table 1. Water to be delivered by precipitation and irrigation to control and stressed parcels.

Month		Dec	Jan	Feb	March	April	May	Total	Water deficit of the week
Mean ET0 in the Haouz (mm)*		60	60	65	93	112	152	542	
wheat Kc**		0.35	0.4	0.6	0.8	1.1	1.1	4.35	
ETM (mm)=ET0*Kc: the quantity in mm / 30 days		21	24	39	74	123	167	448	
m^3 equivalent for 16 m^2		0.336	0.384	0.624	1.184	1.968	2.672	7.168	
Water to be received:									
	1st year	0.336	0.384	0.624	1.184	1.968	2.672	4.496	
Control parcel (m^3)	2nd year	0	0.384	0.624	1.184	1.968	2.672	6.832	
	3rd year	0	0.384	0.624	1.184	1.968	2.672	6.832	
	1st year	0.336	0.288	0.624	1.184	1.968	2.672	4.4	(24/31)*7=5,4mm
Stressed parcels 1 (m^3)	2nd year	0	0.384	0.468	1.184	1.968	2.672	6.676	(39/28)*7=9,75mm
	3rd year	0	0.384	0.468	1.184	1.968	2.672	6.676	(39/28)*7=9,75mm
	1st year	0.336	0.288	0.624	1.184	1.968		4.4	(24/31)*7=5,4mm
Stressed parcels 2 (m^3)	2nd year	0	0.384	0.468	1.184	1.968	2.672	6.676	(39/28)*7=9,75mm
	3rd year	0	0.384	0.468	1.184	1.968	2.672	6.676	(39/28)*7=9,75mm

*ET0: Evapotranspiration of reference crop, from IAV of Agadir publication towards ORMVA technicians of the Haouz (2001); **Kc : crop coefficient.

limitation of the three subplots of 16 m^2 each) with a clay-loam soil, it was proceeded to the mineral fertilization consisting of a triple super phosphate feed (TSP-46%) as a baseline fertilizer applied before sowing (with a dose of 130 g / 16 m^2) and ammonitrate 33% as a cover fertilizer (with a dose of 220 g / N). 16 $m^{2)}$ applied to tillering. The application of fertilizers was done on the fly.

A fungal treatment (product offered by the INRA of Settat) was applied on March 20th for the second and the third year, then during the first year this product was applied late at the stage of the run. Fertilization during the first, second and third years of study took place on 30/11/2013, 29/12/2014 and 30/12/2015, respectively. Before the sem-grains durum wheat, the three subplots were first irrigated to saturation of the soil. After 24 h, the water was brought in daily to reach the lost ETM (Table 1).

Seeding was carried out the first year on 01/12/2013 and the two other years in early January (01/01/2014 for the second year and 01/01/2015 for the third year). The test was carried out under two irrigation treatments. The first treatment (T1) consisted of normal irrigation (control) providing volumes of water just to compensate for the ETM. The second treatment (T2) consisted of a week of water stress, applied 46 days after the semi, during tillering: the first year of irrigation was stopped on 16/01/2014, and 16 / 02/2014 for the second year and 16/02/2015 for the third year. The total water amounts received by the two irrigation treatments are given in Table 2.

The trial was set up on a plot of 48 m2 (Supplementary Figure 1) divided into 3 subplots of 16 m^2. One subplot was used for the control treatment (T1). The water stress treatment (T2) was applied to the two other subplots. Each subplot received the 16 varieties studied. Each variety was sown on one line with 30 seeds on the line (Supplementary Figure 1). The arrangement of these varieties at each subplot was organized according to a completely randomized experimental setup. Sowing was done in December in the first year and in January in the following two years. The period of stress application coincided with the second week of January in first year, and the second week of February in second and third years. The water deficit on the two plots under water stress during the week was as follows:

First year

*1st plot: 24 mm/31 days * 7days = 5.4 mm which represents the water deficit of the stress week.
* 2nd plot: 24 mm/31 days * 7 days = 5.4 mm which represents the water deficit of the stress week.

Second year

* 1st plot: 39 mm/28 days * 7 = 9.75 mm which represents the water deficit of the stress week?

* 2nd plot: 39/28 * 7 = 9.75 mm which represents the water deficit of the stress week.

Third year
* 1st plot: 39 mm/28 days * 7 = 9.75 mm which represents the water deficit of the stress week?
* 2nd plot: 39 mm/28 days * 7 = 9.75 mm which represents the water deficit of the stress week?
The total water amounts received with the two irrigation treatments are given in Table 1.

Table 2. Data on the different vegetative stages of durum wheat.

Year	Semi date	Early emergence	Early tillering	Early run	Beginning of heading	Maturity
1	01/12/2013	12/12/2013	21/12/2013	04/02/2014	24/02/2014	02/04/2014
2	01/01/2014	10/01/2014	19/01/2014	04/03/2014	24/04/2014	31/05/2014
3	01/01/2015	12/01/2015	22/01/2015	07/03/2015	28/04/2015	03/06/2015

Leaf rolling determination

In our experiment, the degree of winding of durum wheat varieties studied was evaluated according to the indices used at INRA to estimate the winding and the type of adaptation of each of the varieties having manifested a winding under the supervision of Dr N. Nasser Elhaq (Research director at INRA Settat Genetic improvement and plant genetic resources unit Specialized: Genetic improvement of cereals). There are other methods for estimating leaf roll in cereals, for example in rice, Leaf Roll Index (LRI) has been calculated at flag leaf level with the following formula: LRI = (Lw-Ln) / Lw (Li et al., 2016). Lw was the largest flag leaf width in extending leaf blade, Ln was the natural distance from flag leaf margins at the same Lw measurement area.

One week after the water stress application, the flag leaf rolled from noon to 4 pm (at the daytime of peak temperature), and beyond 4 pm, unrolled. The degree of leaf rolling was evaluated from noon to 2 pm on the 7th day of water stress, based on the indices established by the INRA of Settat (N. Nsarellah (Research director at INRA Settat Genetic Improvement and Plant Genetic Resources Unit and Specialized: Genetic improvement of cereals) 8 different rolling degrees from absence of rolling to a leaf rolled all over its length displaying a thorn shape (Table 4 and Supplementary Figure 2). The flag leaf is the most sensitive leaf to water stress, when the soil starts to dehydrate; this leaf rolls on itself and just 30 min after hydration of the soil the leaf begins to unfold. Its presence is obligatory for growth and normal production in wheat, because it feeds the ear (Table 4).

Agronomic parameter measurements

(i) The number of tillers per plant was counted in each plant.
(ii) The height of the plant at maturity was measured from the visible base to the average top of the ears.
(iii) The number of ears per plant was counted in each plant.
(iv) The number of grains per ear was counted in each ear in the different varieties.
(v) The weight of 1000 grains (WTG) was determined per variety using a balance, with 1000 grains counted.
(vi)The grain yield (in g) per plant was calculated by multiplying the number of ears per plant by the number of grains per ear and by 10^{-3} times the weight of 1000 grains.

Statistical analyses

Statistical analysis of the data was performed using the Statistical Product and Service Solutions, SPSS software version 20.0, an IBM product since 2009 (Hejase and Hejase, 2013: 58; https://www-01.ibm.com /support/docview.wss?uid=swg24031901). For each irrigation treatment, we performed variance analysis to determine the effect of the year, the variety and the irrigation treatment and that of their interaction. When the effect of the variety was significant, we performed the Newman and Keuls test to identify groups of homogeneous varieties. The Newam and Keuls test is a multiple comparison procedure that allows sample means significantly different from each other to be identified. This procedure is often used as a post-hoc test whenever an analysis of variance (ANOVA) revealed a significant difference between three or more sample means.

RESULTS

Yield variability of the different durum wheat varieties in control and water stress conditions

The grain yield of all sixteen varieties of durum wheat was determined at the end of each three cropping seasons by examining the number of ears per plant, the number of grains per ear and the weight of 1000 grains (Figure 1; Supplementary Table 5) in twenty plants randomly chosen per variety.

Under normal irrigation conditions (T1 treatment), the mean grain yield over the three crop years varied between varieties by a factor of 2.3 (from 11.5 g per plant in Anouar to 26.5 g per plant in 2777). Only moderate conservation of the grain yield varietal distribution was observed between years ($0.43 < R < 0.65$; Figure 1A). However, the range of grain yield variation between varieties was very similar on each of the three crop years (factor of 2.45 to 2.9; Figure 1A). The variety showing the highest yield was consistently 2777, while that showing the lowest one varied (Jawhar, Tomouh or Waha).

In plants subjected to the one-week water stress at tillering (T2 treatment), the grain yield decreased in all varieties (Supplementary Table 5). The extent of decrease depended on the variety. This led to a strong extension in the range of grain yield variation between varieties as compared to the control treatment. In the water stressed plants, the mean grain yield over the different campaigns ranged within a factor of 25 between varieties (from 0.9 g/plant in Marjana to 22.5 g/plant in2777). Thus, the range of grain yield variation between varieties in water stressed plants increased by a factor of 5 to 11 as compared to that in the control plants on the three crop years (Figure 1B). A stronger conservation of yield between years ($0.57 < R < 0.89$; Figure 1B) than for plants grown under full irrigation was observed. It shouldbe noted that four varieties susceptible to rust, 2777, Isly, Marjana and Yassmine, did not produce any grain in the first year in the water stressed parcels due to fungal infection. Only results of the two last years concerning grain yield under water stress were taken into account in the analyses for these varieties.

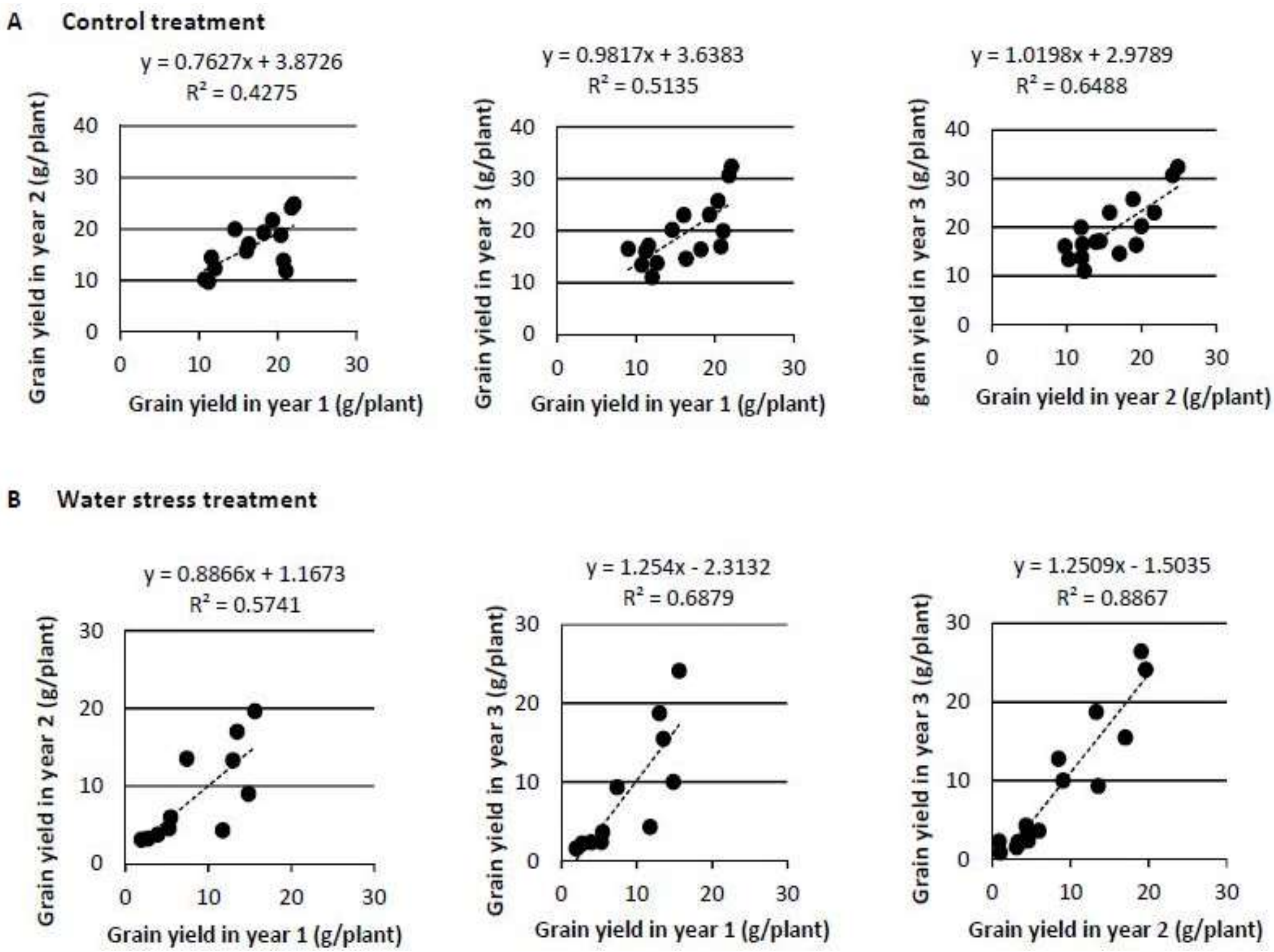

Figure 1. Grains yield of the 16 durum wheat varieties under control conditions (A) or subjected to water stress treatment (B). The grains yield was determined for each variety in plants grown in control conditions (A) or subjected to water stress treatment (B), using mean values of the number of ears per plant, the number of grains per ear and the grains weight, determined for each variety and treatment on 20 plants randomly chosen. Comparison between year 1 and year 2 (left), year 1 and year 3 (middle) and year 2 and year 3 (right).

Similar conclusions as those relative to the grain yield could be drawn when analysing the different yield components (the number of ears per plant, the number of grains per ear and the weight of 1000 grains), and the number of tillers and height of plants at maturity (Table 3). The variance analysis of examined agronomic parameters and yield indicated both highly significant inter-varietal and inter-annual variations (Tables 3 and 6). The variability between varieties was higher in water stressed parcels than in control ones for all the agronomic parameters. In water stressed parcels, the variability between varieties was also higher than the variability between years, except for the weight of grains. The variability between years was the highest in control conditions, except for the number of grains per ear and the weight of grains (Table 3). The parameters showing the highest variability between varieties were the height of plants at maturity and the number of grains per ear in both control and water stress conditions (Table 3).

Two varieties, Irden and 2777, showed the best performance both in control conditions and when subjected to water stress (Supplementary Table 3): Irden for the mean number of ears over the three years (9.9 in control conditions, and 11% less in water stress), and2777 for the mean values of other yield parameters (10.7 tillers in control conditions and 9% less under water stress, 61 grains per ear in control conditions, and 5% less under water stress, 46 g per 1000 grains, and 8% less under water stress). 2777 also showed the tallest pants at maturity (103 cm as mean height in control conditions and 5% less under water stress).

The varieties showing the worst performances varied

Table 3. Variance analysis (ANOVA) of agronomic parameters in control (T1) and water stressed (T2) cultures.

Parameter*	Source: Between years			Between varieties			Combined effect (year x variety)		
	df	F-value	P (α=5%)	df	F-value	P (α=5%)	df	F-value	P (α=5%)
Well-watered parcel (T1)									
Number of tillers per plant	2	266.253	<0.001	15	44.801	<0.001	30	24.607	<0.001
Height of plants at maturity	2	327.845	<0.001	15	225.093	<0.001	30	43.327	<0.001
Number of ears per plant	2	238.373	<0.001	15	53.143	<0.001	30	23.447	<0.001
Number of grains per ear	2	37.956	<0.001	15	281.234	<0.001	30	41.456	<0.001
Weight of 1000 grains	2	13.805	<0.001	15	6.822	<0.001	29	3.506	<0.001
Water stressed parcels (T2)									
Number of tillers per plant	2	91.856	<0.001	15	369.216	<0.001	30	42.718	<0.001
Height of plants at maturity	2	85.694	<0.001	15	853.708	<0.001	30	38.241	<0.001
Number of ears per plant	2	53.544	<0.001	15	322.496	<0.001	30	35.759	<0.001
Number of grains per ear	2	669.269	<0.001	15	1103.024	<0.001	30	264.808	<0.001
Weight of 1000 grains	2	262.050	<0.001	15	192.341	<0.001	29	87.451	<0.001

*df = degree of freedom, F-value = Fischer ratio, P = probability.

on the other hand according to the agronomic parameter in control conditions, but Marjana was the worst for all parameters when plants were subjected to water stress (Supplementary Tables 3 to 7): the lowest mean number of tillers over the three years, in control conditions (8.15) was displayed by Waha, and under water stress (53% less) by Marjana, the lowest mean number of ears in control conditions (7.1) and under water stress (52% less) were both found in Marjana, the lowest mean number of grains per ear, in control conditions (40.5) was displayed by Anouar, and under water stress (53% less) by Marjana, and the lowest mean weight of 1000 grains, in control conditions (36 g) occurred in Jawhar, and under water stress (58% less) in Marjana. In addition, the smallest plants at maturity, in control conditions (71 cm mean height) were displayed by Tomouh, and under water stress (48% less) by Marjana.

From agronomic performances measured in the control and water stressed parcels, the performance decrease due to water stress (or conversely preservation under water stress) was also examined for all selected agronomic parameters (Supplementary Figure 3). An important variability in the sensitivity to water stress was observed within the panel of wheat varieties (mean grain yield decrease under water stress from 21 to 93%). Slight differences were noticed for the different agronomic parameters: the weight of grains showed the largest range of variability in stress sensitivity between species (mean decrease under water stress varying by a factor of 8.3), and the number of ears per plant showed the lowest range of variability (mean decrease under water stress varying by a factor of 5). Analysis of inter-annual variation indicated that the preservation of agronomic performances under water stress was more strongly reproducible (slope of inter-annual correlation between 1.02 and 1.07, $0.79 < R < 0.89$ for grain yield; Supplementary Figure 3) than the agronomic performances in control conditions or under water stress (Figure 1 and Table 5). Thus, the sixteen varieties of durum wheat of our study displayed a large range of variability in their agronomic performance, especially when subjected to the one-week water stress. Low inter-annual variability was noticed when the decrease in performance due to water stress was considered.

Effect of water stress on leaf rolling in the different durum wheat varieties

Plants of the water-stressed parcels displayed flag leaf rolling at the end of the water stress treatment during the hottest hours of the day, in contrast to those of the well-watered control parcel where no leaf rolling was observed at that time. A large variability among the 16 durum wheat varieties in the degree of leaf rolling was noticed (Figure 2). Indeed, observed rolling in the different varieties ranged from concerning at most the very tip (mean score of the variety between 0 and 1, e.g., in Marjana and Waha) to reaching at least two third of the leaf (mean score between 6 and 7, e.g., in Irden and 2777). Thus, the rolling behavior in the different varieties almost ranged from absence of rolling (score 0) to maximal degree (score 7), all the rolling degrees being represented. The same range of variation among varieties was observed in each of the three crop years (Table 4 and Figure 2A-C).

Most of the studied varieties showed little inter-annual variations in their degree of leaf rolling following the water stress treatment (Figure 2D). Amjad, Irden, Oum Rabia, Tomouh and Vitron, for instance, maintained the same

Table 4. Leaf rolling index.

Rolling class	Rolling index	Description
No rolling	0	Absence of rolling
	1	The tip of the leaf rolls up
Weak rolling	2	A quarter of the leaf rolls up
	3	One third of the leaf rolls up
Medium rolling	4	Half of the leaf rolls up
	5	More than half of the leaf rolls up
High rolling	6	Two third of the leaf rolls up
	7	Thorn shape of leaf fully rolled

Table 5. Grain yield in control conditions and in case of water stress in durum wheat varieties studied.

	Yield control				Yield stress		
	Y1	Y2	Y3		Y1	Y2	Y3
2777	30,356895	29,44682	38,4478545	32,7505232		25,779264	34,422328
Amjad	26,3008424	27,4332175	28,203751	27,3126036	23,12386	21,7600535	24,525743
Sebou	27,553653	20,3063395	20,164889	22,6749605	20,785234	13,083834	13,1870465
Vitron	25,085586	29,74033	29,377455	28,0677903	17,6184465	22,811722	23,4837515
Irden	31,4400595	35,810755	40,350417	35,8670772	25,688186	30,3891495	35,375484
Yassmine	23,4497815	23,5332675	27,719531	24,90086		12,0627955	16,783031
korifla	21,022584	24,1561476	20,478958	21,8858965	11,210721	19,1771	12,6077085
Marouane	29,3426855	16,788875	20,00176	22,0444402	19,7521525	5,06861	4,135872
Isly	18,526273	22,43526	22,145231	21,035588		4,81052	3,420162
Massa	25,253107	22,898212	19,868142	22,6731537	7,244058	4,75215	3,2837805
Oum rabia	20,4252375	19,6140565	17,356197	19,1318303	6,2803195	4,4084845	3,087328
Anouar	15,0064275	15,260097	15,421576	15,2293668	3,999351	3,718512	1,8161525
Tomouh	14,248202	14,188638	16,63162	15,02282	6,076832	3,436372	2,752547
Waha	17,152797	16,2869025	14,3515595	15,9304197	2,5892985	3,2894645	1,7174455
Marjana	13,9232915	14,193675	13,645991	13,9209858		1,273194	1,2759825
Jahwar	13,5480875	16,685339	19,742334	16,6585868	2,282578	0,9451155	4,137845

degree of rolling over the three years of study. In varieties where the rolling behavior varied with the crop year, the observed score was generally shifted by less than 1 (that is to adjacent indices in plus or minus directions in the classification scale) from the previous year's value. For instance, the variety Massa, a weak-rolling variety, was given a mean rolling score of 3 (one third of the leaf rolled) during the first two years, while in the third year its rolled leaf area decreased a bit and its mean rolling score became close to 2 (one fourth of the leaf rolled). Only 3 out of the 16 varieties showed inter-annual variations of rolling score higher than 1 unit (Figure 2A-D). These three varieties, Sebou, Isly and Jawhar, corresponded to high, medium and weak-rolling ones, respectively. Sebou and Isly displayed a higher rolling score by 1.5-2 during the first year as compared to the two other years, and Jawhar, a higher rolling score by 2.5 in the third year as compared to the first ones.

A ranking of the 16 varieties according to their mean rolling score over the three years is shown in Figure 2E. Two varieties, Marjana and Waha, could be sorted in the "no rolling" class (mean score <1; Table 3). Four varieties, Jawhar, Tomouh, Anouar, Massa, corresponded to the weak rolling class (1<mean score<3). Four varieties, Oum Rabia, Marouane, Isly, Yassmine, showed medium rolling (3<mean score<5), and the six last ones, Korifla, Sebou, Amjad, Vitron, 2777 and Irden, displayed high rolling (mean score >5).

Correlations between leaf rolling under water stress and preservation of agronomic performance

Possible correlation between the grain yield and the extent of leaf rolling under water stress in the different wheat varieties was examined (Figure 3). A highly

Table 6. Variance analysis (ANOVA) of yield in control (T1) and water stressed (T2) cultures.

Parameter*	Source: between years			Between varieties			Combined effect (year x variety)		
	df	F-value	P (α=5%)	df	F-value	P (α=5%)	df	F-value	P (α=5%)
Control yield	2	194,01	<0.001	15	234,44	<0.001	30	32,68	<0.001
Yield stress	2	148,12	<0.001	15	1399,19	<0.001	30	201,42	<0.001

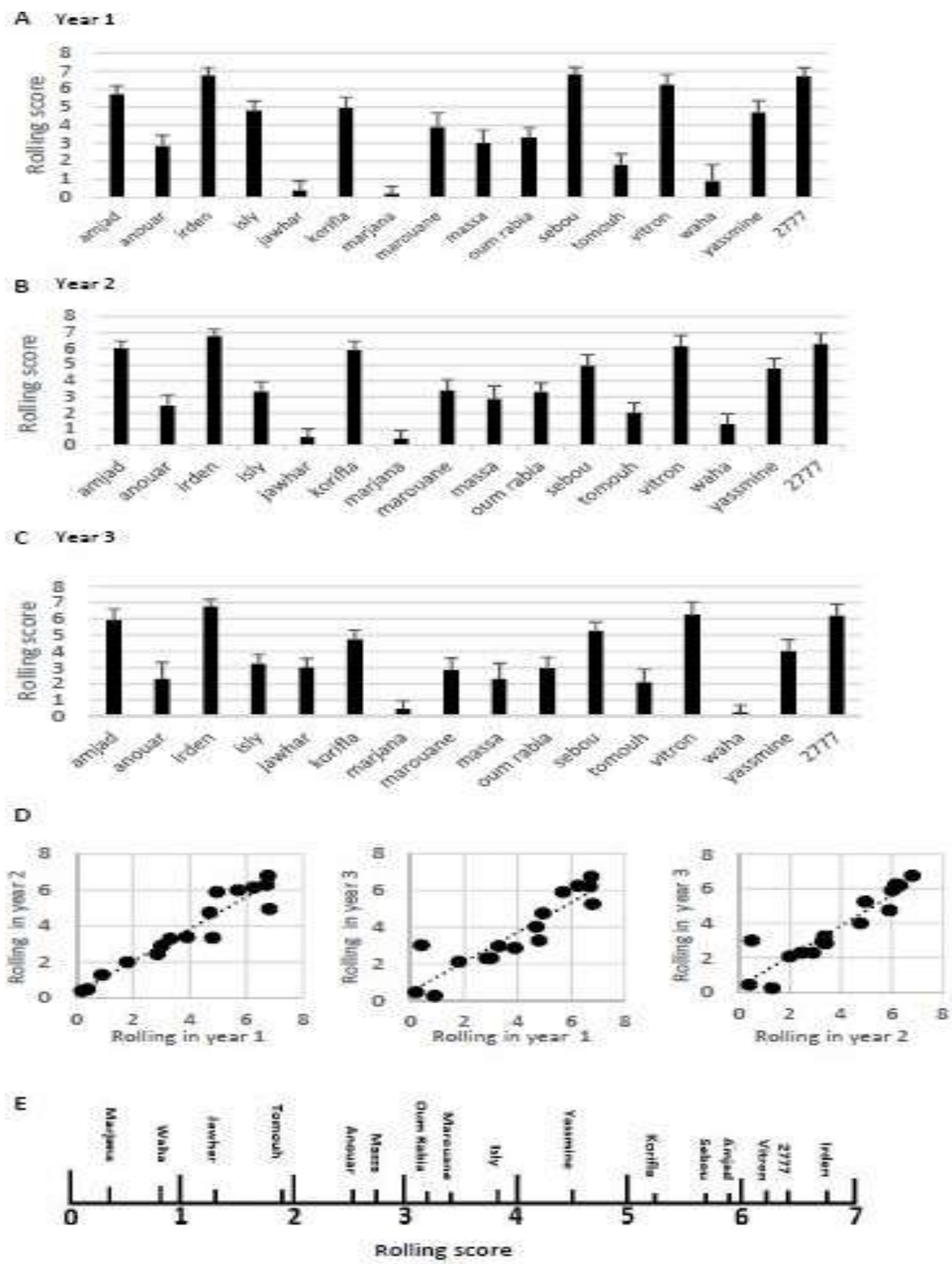

Figure 2. Leaf rolling index of 16 varieties of durum wheat subjected to water stress, over three crop years. Variation of flag leaf rolling among 16 varieties of durum wheat subjected to water stress in the first (A), second (B) and third (C) crop year. The rolling index was determined on 20 plants per variety randomly chosen. Data are means ± SE. In panel D, variation among year of the leaf rolling index of the 16 varieties (D). Comparison between year 1 and year 2 (left), year 1 and year 3 (middle), year 2 and year 3 (right). In panel E, ranking of the 16 varieties of durum wheat according to their mean leaf rolling index over the 3 years.

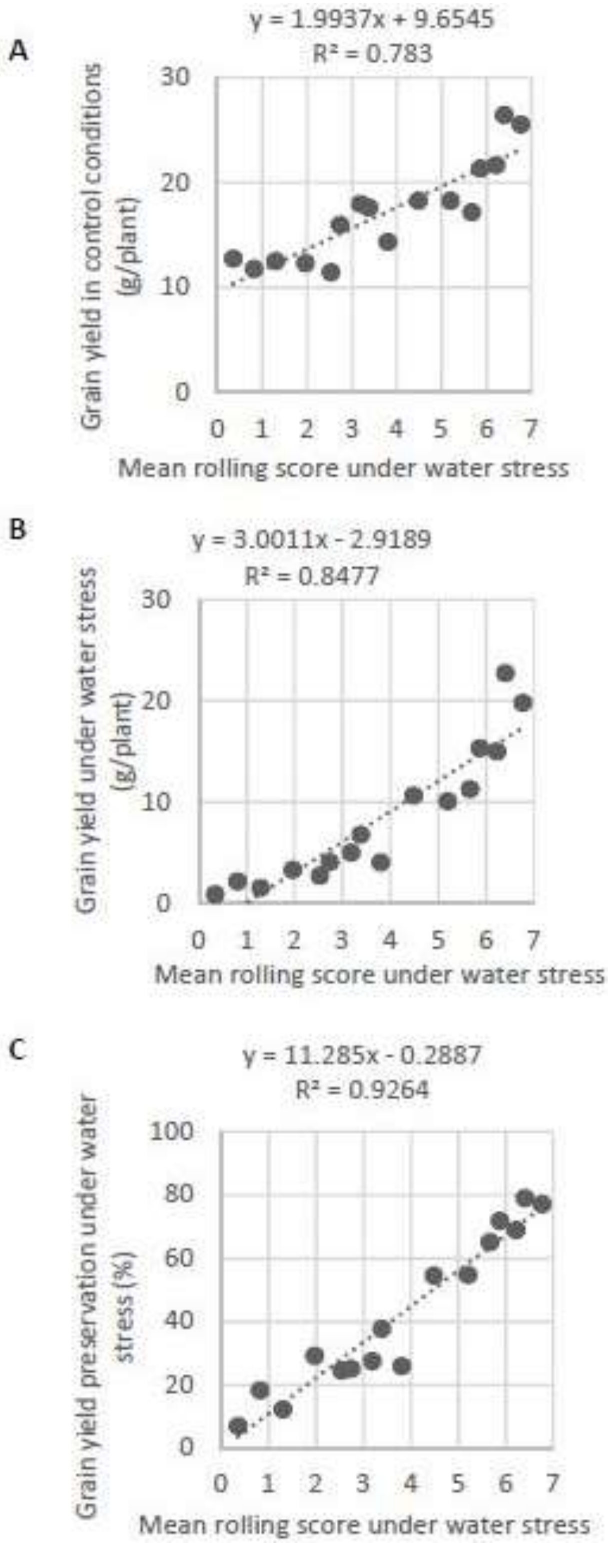

Figure 3. Correlation analysis between the grain yield of the 16 durum wheat varieties and the rolling index under water stress. The grain yield was determined each year for each variety in control (A) or water stress (B) conditions as in Fig. 1 and was averaged over the three campaigns. In panel C, correlation between the mean rolling index under water stress over the three campaigns and the grain yield preservation under water stress.

significant positive correlation (R= 0.93) was noted between the mean level of preservation of grain yield under water stress and the average leaf rolling score over the three campaigns (Figure 3C). The grain yield under water stress showed weaker correlation with the leaf rolling score (R = 0.85; Figure 3B) than the grain yield preservation under water stress. A positive correlation was also noted between the grain yield in well-irrigated plants and the leaf rolling score, but the strength of this correlation was the weakest (R = 0.78; Figure 3A).

The mean levels of preservation over the three campaigns of each of the grain yield parameters were found to be positively correlated with the mean leaf rolling score under water stress (Figure 4C-E). The preservation of the number of grains per ear and the number of ears per plant, as well as that of other agronomic parameters like the number of tillers and the height of plant at maturity showed strong correlations with the mean rolling score (0.89 <R <0.93; Figure 4A-D). Only the preservation of the weight of grains showed weaker correlation with leaf rolling (R = 0.74; Figure 4E). A separate analysis of the three cropping campaigns confirmed the weaker correlation with leaf rolling for the preservation of grain weight in the two last years (Supplementary Figure 4). On the other hand, the lowest correlation with leaf rolling in the first year was found for the number of ears (Supplementary Figure 4).

DISCUSSION

The experimentation was conducted on a set of durum wheat varieties maintained at the National Gene Bank of Morocco (INRA, Settat). This set gathered varieties originating from different world areas, although essentially Mediterranean ones (and mostly Morocco), and were issued from breeding programs at work at different periods (http://wheatatlas.org/varieties) over the 20th century (oldest variety: Kyperounda “2777" released in 1956 in Cyprus; most recent varieties: Irden and Marouane released in 2003 by INRA Mococco). Most of the selected varieties have been released in the late 1980s and the 1990s, as a result of programs in which the objective of productivity increase and yield stabilization between years was associated with a reduction of the plant cycle duration and plant size (Nsarellah et al., 2011; Taghouti et al., 2017). These objectives led to varieties with strongly homogenized plant development and, owing to the reduction in plant cycle and biomass, tended to improve the tolerance to drought. A disparity in drought adaptation however remained in the durum wheat cultivars released during that period (Nsarellah et al., 2011), a specific objective of tolerance to abiotic stresses having been introduced as one of the most recent steps in breeding programs with released varieties more systematically tolerant to drought only since the beginning of the 21th century (Nsarellah

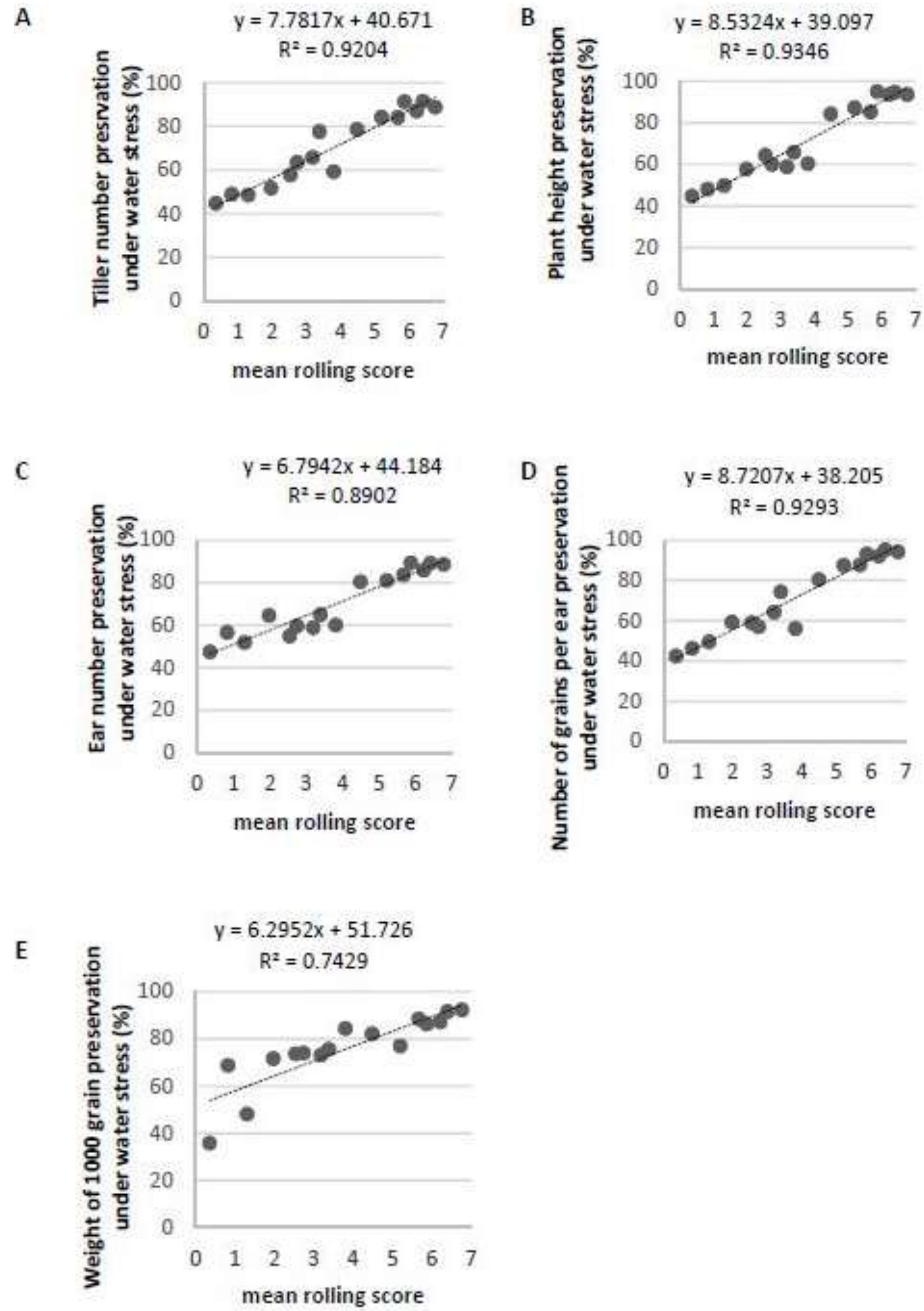

Figure 4. Correlation between the mean leaf rolling index and the maintaining of agronomical performances upon water stress within the panel of the 16 durum wheat varieties. The rolling indices correspond to those of Fig. 2D averaged over the three experimental campaigns. The preservation under water stress of the number of tillers (A), the plant height at maturity (B), the number of ears (C), grains per ear (D), and weight of 1000 grains (E), was determined by comparison with plants grown in parallel on the well-watered control parcel. In each experimental campaign, values of the different agronomical parameters were determined on twenty plants randomly chosen in each variety and each irrigation regime, and were averaged. Shown data correspond to the average over the three campaigns of mean values of preservation of performance under water stress determined each year.

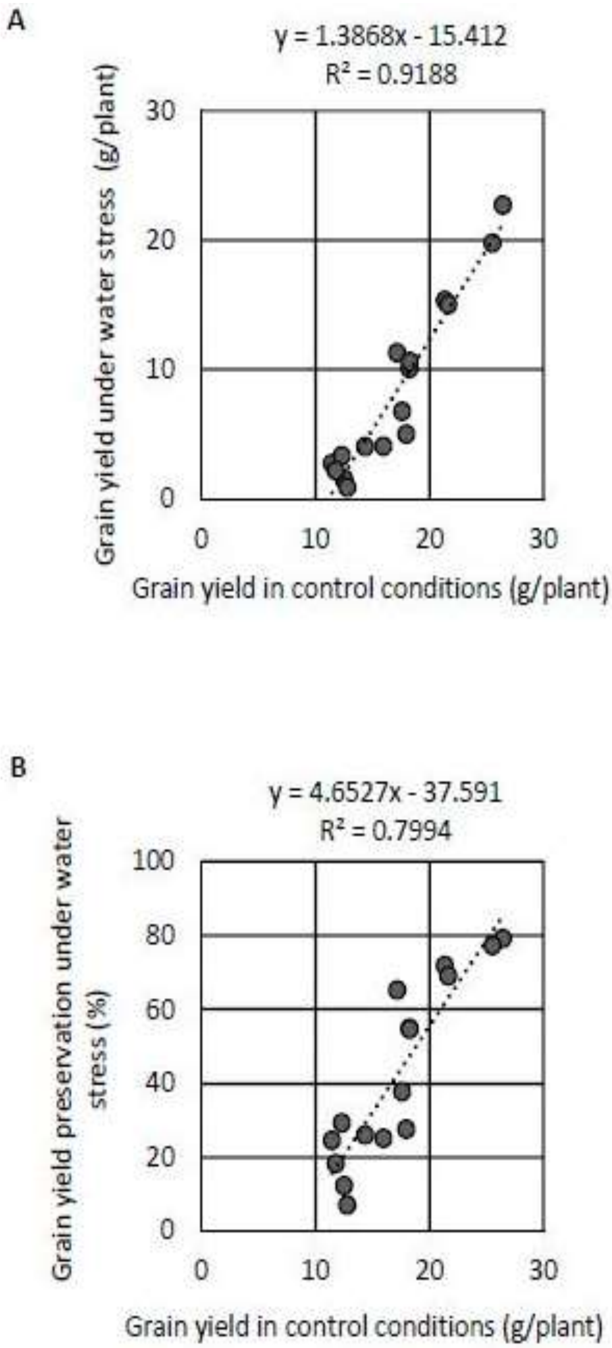

Figure 5. Analysis of correlation between the grain yield of the 16 durum wheat varieties in control and water stress conditions. Grain yield in control conditions was correlated to grain yield in water stress conditions in (A) and to grain yield preservation under water stress conditions in (B). Grain yield was determined as in Figure 3.

et al., 2011). The selected panel of 16 varieties of the present study displayed in our trial a large range of sensitivity to water stress with mean grain yield preservation over the three campaigns varying by a factor of 12 between varieties (Figure 1 and Supplementary Figure 3). The grain yield under water stress in our panel of varieties appeared strongly correlated to that in control conditions (Figure 5; R = 0.92). This is in line with the assumption that selection for high yield over the years tend to associate characters of general dependability, the yield improvement being then based on the ability of the plant to overcome varying small or bigger stresses (Duvick, 2005; Tardieu, 2012).

The application of the one-week water stress widely affected the agronomic performances of the durum wheat varieties studied (Supplementary Table 3). All studied parameters (number of tillers, plant height, number of ears, number of grains, weight of grains) were affected, although slight variations in the level of sensitivity between the different parameters were noticed (e.g., decrease under water stress between 4.5 and 10% for the different parameters in the tolerant variety 2777, between 51 and 64% in the sensitive variety Marjana). The parameters that were the most affected (e.g., number of ears in 2777, number of grain per ear in Isly, and weight of grains in Marjana) depended on varieties. The water stress was applied at tillering. Grain yield is set up all over the plant cycle. At tillering is set up the number of tillers. Moreover, from the middle of tillering when the apex makes its floral transition and elaborates sketches of spikelets, the fertility of the ears starts being developed. Thus, a stress at tillering is expected to be able to affect different parameters of yield in relation to ear number and features. All the varieties from our panel were early or semi-early varieties, except 2777 which was classified as semi-late variety. A significant difference in the stage of embryonic ear development upon drought stress application in 2777 as compared to the other varieties may explain at least in part the low sensitivity of this variety to the applied water stress. In the other varieties, the quite similar cycles make it unlikely that differences in the developmental stage upon drought stress application would explain the strong differences observed between varieties in terms of subsequent agronomic performances. The observed strong differences between varieties in the sensitivity to the applied drought stress may denote differences in efficiency in water absorption of the water available for the crop or in its use (Royo et al., 2014). The response of the studied varieties to the imposed water stress appeared strongly linked to the ability of these varieties to manifest leaf rolling under water stress conditions (Figures 3 and 4). Leaf rolling in cereals is known as an adaptive response to water deficit in leaf tissues (O'Toole and Cruz, 1980; Kadioglu et al., 2012). Leaf rolling is believed to occur when the evaporative demand is no longer balanced by water uptake by the roots and the extent of rolling is often used as a marker of intensity of drought stress in cereal cultures (Riboldi et al., 2016). When leaf tissue hydration is restored, the leaf unrolls. Upon rolling, by decreasing the leaf surface exposed to sunlight, the water loss by transpiration is reduced (O'Toole and Cruz, 1979). This also allows the plant, by limiting the direct illumination of leaf surface, to limit the heating of leaf tissues, harmful to cellular metabolism. In rice, it was as such, reported to improve photosynthetic efficiency and to delay leaf senescence (Richards et al., 2002; Richards et al., 2004; Zhang et al., 2009). It however reduces the available photosynthetic surface, which negatively affects the plant growth (Li et al., 2016 b).

Leaf shape (angle, width/area), which influences light capture and gas exchange capacity, belongs to the agronomic traits highly considered for potential in grain yield improvement (Yuan, 1997; Wang and Li, 2005; Moon et al*.,* 2011). Leaf rolling, which controls the photosynthetic surface and the water status of the leaf, is one of the traits commonly considered in modern programs of cereal selection (Turner, 1982; Richards et al., 2002; Hu et al., 2009). In rice, a number of QTL of leaf rolling have been mapped (Price et al., 1997, Singh and Mackill 2008, Zhang et al., 2016). Little assessment of the impact of leaf rolling on productivity under water stress conditions is yet available in other cereals (wheat, sorghum, etc.) (Peleg et al., 2009; Bogale et al., 2011). However, for example, five leaf-rolling QTLs co-localizing with QTLs associated with productivity in a population of durum wheat crossed with emmer wheat were recently reported (Peleg et al., 2009).

In the studied panel of durum wheat varieties, leaf rolling positively correlated with the maintaining of growth (height at maturity) and with that of the grain yield parameters (Figures 3 and 4). Only the preservation of grain weight showed lower correlation with leaf rolling index (Figure 4, Supplementary Figure 4), which is in agreement with the period of application of the drought stress which induced the leaf rolling at early stage of plant and ear development. The strong positive correlation observed between the leaf rolling index and the maintaining of grain yield in plants subjected to water stress, may be explained by better preservation of the water in leaf tissues when rolling is stronger, thanks to the reduction of the surface of heated tissue and transpiration. This would help maintaining photosynthetic activity and metabolism during the critical period of water stress, allowing to preserve agronomic performance. Epidermal bull-shaped cells (so-called bulliform cells) are involved in the regulation of leaf rolling (Itoh et al., 2005; Li et al., 2010; Zou et al., 2011; Xiang et al., 2012). According to Willmer (1983), these cells serve as water storage. The loss of turgor pressure in these cells due to the lack of water causes a winding of the leaves. During drought, loss of moisture due to vacuoles causes bulliform cells to roll the leaves of many grass species as the two edges of the leaf fold together (Hsiao et al. 1984, Moulia 1994, Price et al 1997). Once enough water is available, these cells expand and the leaves unfold again. Folded leaves provide less sun exposure and, as a result, they receive less heat, which reduces transpiration and helps retain the remaining water in the plant. In addition, they also play a role in the development of developing leaves (Moore and Clarke, 1998). Thus, bulliform cells can be considered as a primary target in molecular selection, to modulate leaf rolling and unwinding in response to alternating wet and dry periods.

During water deficit, it may be possible that the water, coming from bulliform cells, contributes to the maintenance of the mesophyll cell functioning, thus allowing them to store photosynthetic assimilates (Bois et al., 1987). This could explain that varieties displaying strong rolling maintain at best agronomic performance. On the other hand, in varieties with low or no rolling under stress conditions, a decrease in transpiration via stomatal closure would cause tissue heating and slow down photosynthesis. In this case, both the water stress, the reduction of the photosynthetic capacity of the plant during this period of tillers developments and formation of the ears will penalize the yield. Based on the present results, leaf rolling is concluded to be one of the morphological parameters that can be used as powerful indicator of strong productive performance in semi-arid conditions, for pertinent selection of adapted wheat varieties.

Conclusion

Leaf rolling belongs to the traits commonly considered in modern programs of cereal selection for drought tolerance. Very few, and contradictory, results however exist on its impact on yield. Here, a water stress was applied at tillering and resulted in a decrease in height and number of stems and a reduction in the yield components in the sixteen varieties of durum wheat studied, thus affecting the final grain yield. These negative impacts of the water stress were much reduced in the varieties with strong rolling behavior. This provides evidence that leaf rolling can mitigate the effects of water deficit.

The agronomic parameters studied in our sixteen varieties of durum wheat revealed a significant variability according to the rolling group. The high-rolling varieties (Amjad, Vitron, 2777, and Irden) showed the best performances in terms of yield (number of ears per plant, number of grains per ear, and weight of grains) in both water stress and control conditions. This suggests that these varieties with high leaf rolling behavior are likely to display a general tolerance to environmental conditions. These varieties are thus promising for cultivation in arid and semi-arid zones. In these areas, high temperatures may also be a limiting factor in durum wheat production, which justifies the choice of both high-rolling and short-cycle varieties that escape drought and high temperatures at the end of the cycle (Karrou, 2003).

Future physiological and genetical investigations will allow to better understand the mechanisms underpinning leaf rolling and their contribution to drought resistance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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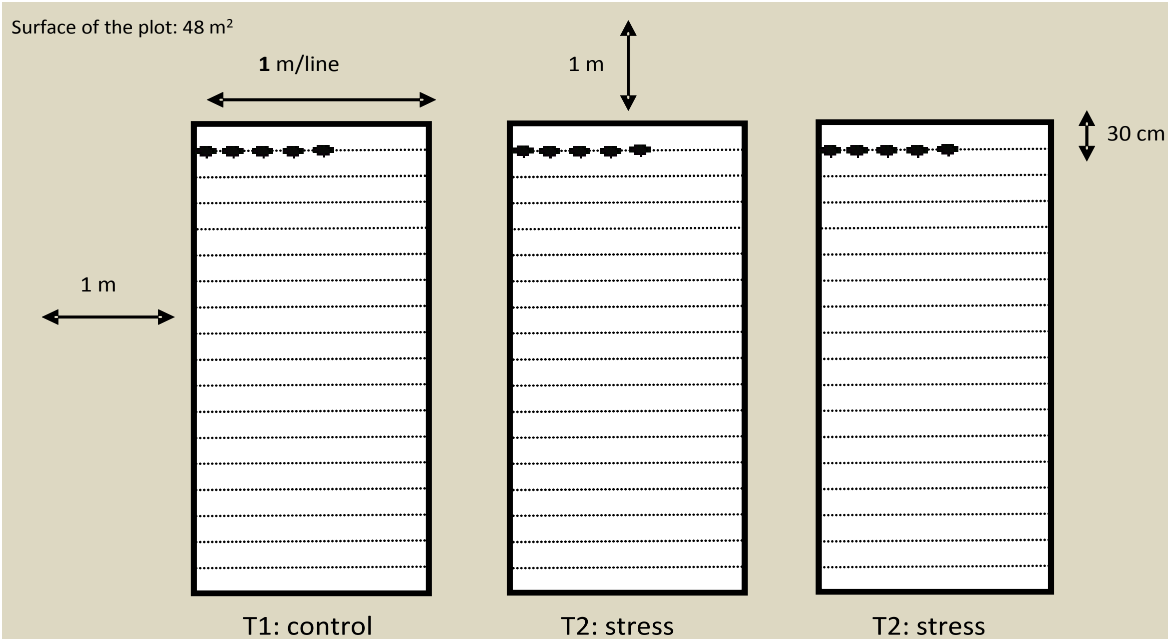
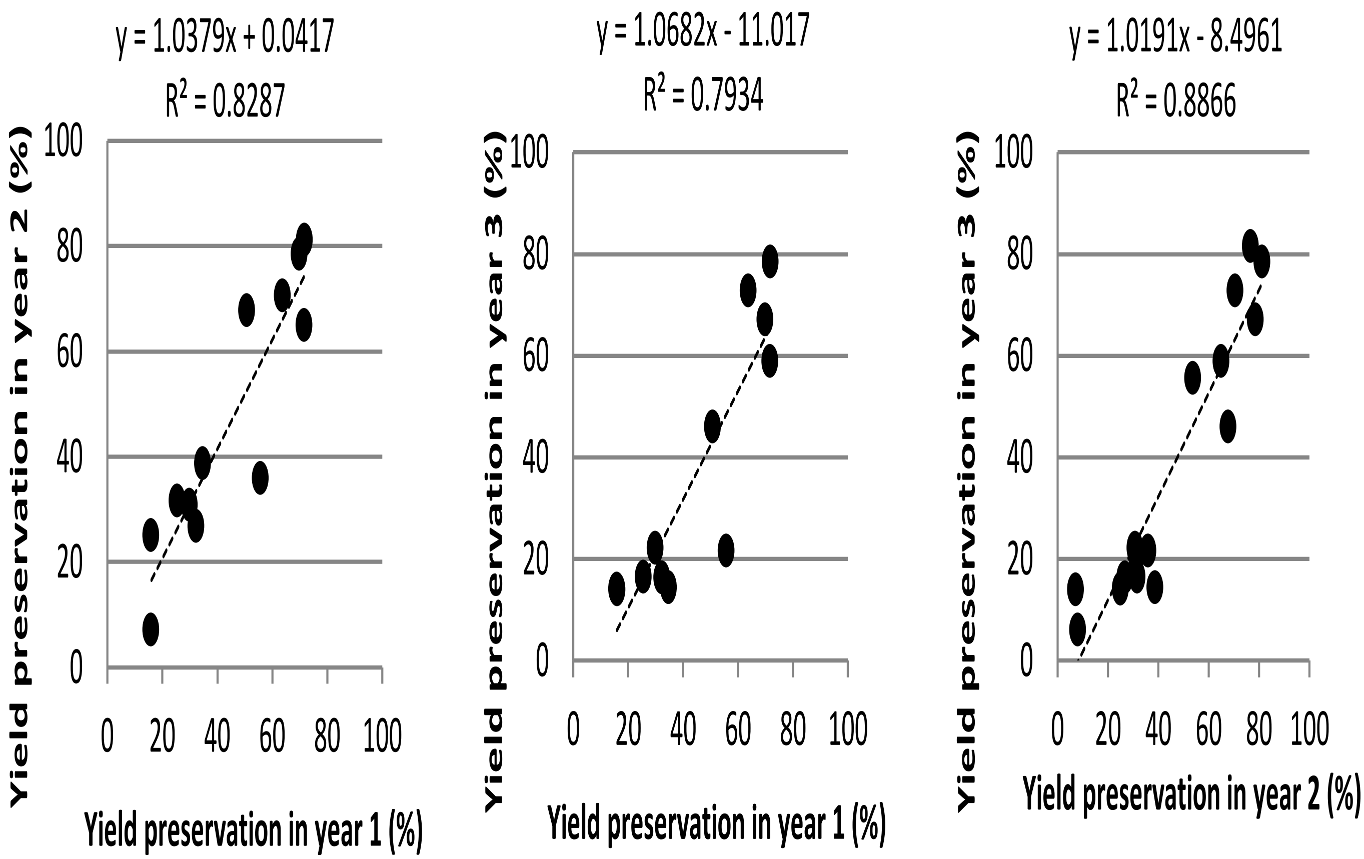
SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure 1. Scheme of the experimental plot. Sixteen varieties of durum wheat (Kyperounda ("2777"), Amjad, Anouar, Irden, Isly, Jawhar, Korifla, Marjana, Marouane, Massa, Oum Rabia, Sebou, Tomouh, Vitron, Waha and Yassmine) were sown on three identical parcels of 5.5 m^2 at Tamellalet (Marrakech region, CMV 408, Morroco), each parcel receiving all varieties. Each variety occupied one line, with random position in the parcel. Seeds were sown every 3.45 cm with 30 seeds per line, the lines being spaced by 30 cm. One parcel was watered all over the culture (T1 treatment: control) to fully compensate the mean evapotranspiration of the culture (ETM). In the two other parcels, plants were subjected to 1 week of water stress at tillering stage (T2 treatment: stress) with ETM compensated at only 75%, and besides this stress were watered like in the control parcel.

Supplementary Figure 2. Representative picture of the different leaf rolling classes. Flag leaf rolling level was assessed at the end of the water stress period, between 12 and 14 p.m.

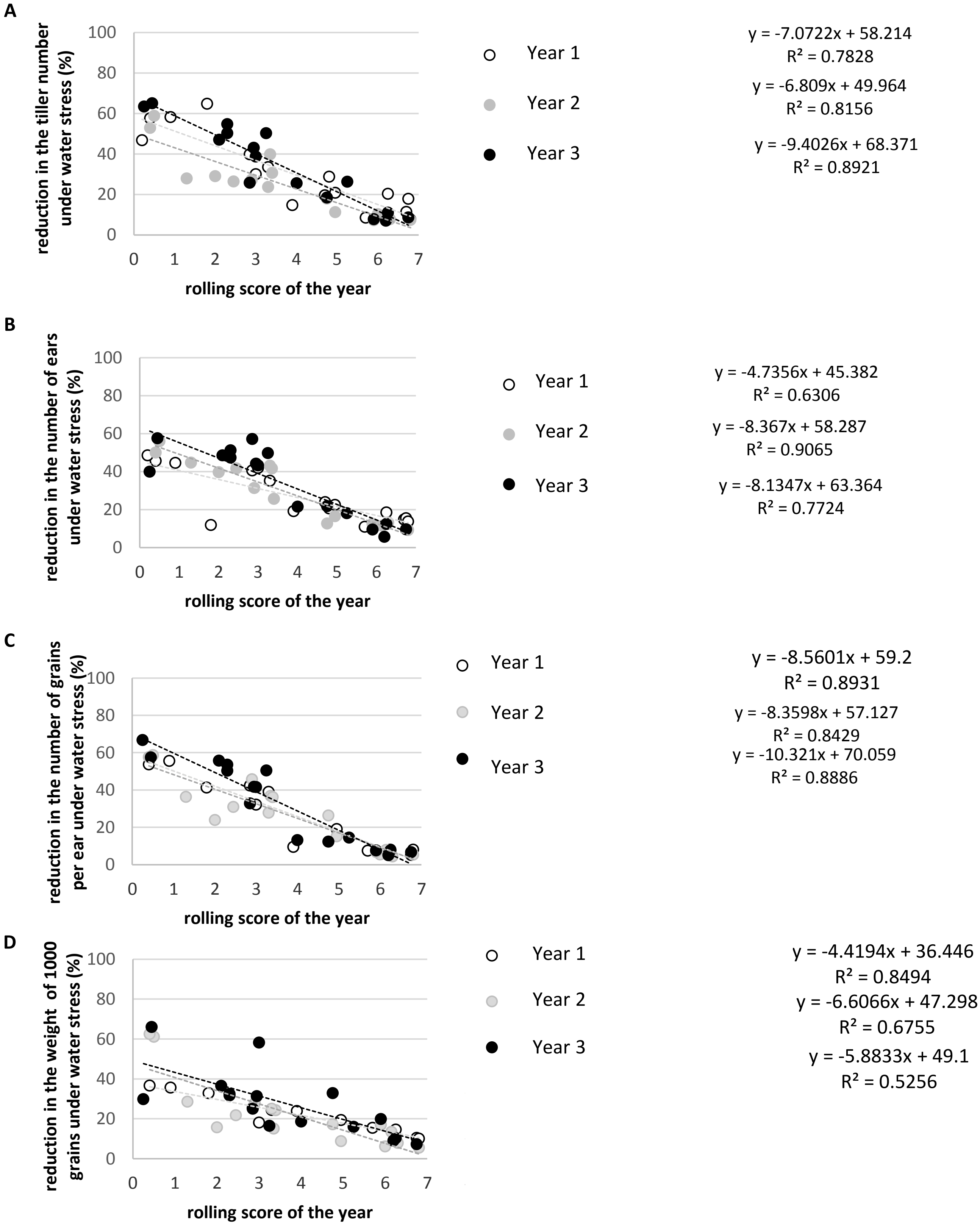


Supplementary Figure 3. Grain yield preservation of the 16 durum wheat varieties under water stress. The percentage of grain yield preservation under water stress was determined for each variety as 100 - (yield in control conditions - yield in water stress) / yield in control conditions. Comparison between year 1 and year 2 (left), year 1 and year 3 (middle), and year 2 and year 3 (right). Grain yield data used for grain yield preservation calculation are those shown in Figure 1, determined using mean values of the number of ears per plant, the number of grains per ear and the grain weight, measured for each variety and treatment on 20 plants randomly chosen.

Supplementary Table 1. Climatic data recorded at the experimental station during the three crop years 2013-2014 to 2015-2016.

Crop year	2013-2014			2014-2015			2015-2016		
	Precipitation (mm)	T max (°C)	T min (°C)	Precipitation (mm)	T max (°C)	T min (°C)	Precipitation (mm)	T max (°C)	T min (°C)
December	20.8	19.1	4.8						
January	66.2*	17.5	4.7	20.5	16.9	3.6	8.6	21.8	5.0
February	17.0	17.5	4.8	10.5	15.7	4.1	31.8	19.5	4.5
March	37.0	22.0	7.7	80.6	22.9	8.6	42.2	20.5	5.5
April	30.7	27.5	11.7	7.4	25.4	11.9	5.6	24.3	8.8
May	1.8	31.6	16.5	18.5	32.0	15	6.8	28.6	12.6
June	12.6	33	15.0	33.5	33.0	17.7	0	34.4	15.6

*Main precipitations as storm after water stress application to the experimental culture.



Supplementary Figure 4. Correlation between the leaf rolling score specific to each year and the maintaining of agronomical performances upon water stress within the panel of the 16 durum wheat varieties. The rolling indices correspond to those of Figure 2A-C. The water stress-induced reduction in the number of stems (A), ears (B), grains (C), and weight of 1000 grains (D), was determined by comparison with plants grown in parallel on the well-watered control parcel. Data on twenty plants randomly chosen in each variety and each irrigation regime were averaged.

Supplementary Table 2. Mean variation over three years in the tiller number per plant in wheat varieties grown under control conditions (T1) or subjected to water stress (T2).

Variety	Year 1			Year 2			Year 3			Mean		
	T1	T2	% reduction	T1	T2	% reduction	T1	T2	% reduction	T1	T2	% reduction
2777	9.20^{cde}±0.951	8.15^{f}±0.99	11.40^{ab}	10.90^{cde}±0.91	10.05i±0.95	7.60^{a}	12.05h±0.76	11.20h±0.83	6.80^{a}	10.7^{a}±1.41	9.80^{f}±1.51	8.55^{a}±2.30
Amjad	9.95^{def}±0.826	9.10g±0.97	8.55^{a}	9.25^{def}±0.85	8.30g±0.92	9.80^{a}	11.55gh±0.94	10.55gh±1.14	8.30^{a}	10.25^{a}±1.22	9.31^{ef}±1.16	8.78^{a}±0.98
Anouar	7.15^{a}±1.089	4.30^{b}±0.81	39.9^{d}	7.20^{a}±0.89	5.30^{cd}±0.57	25.35^{c}	10.40^{de}±1.57	4.70^{c}±0.66	53.60^{e}	8.23^{a}±1.85	4.76^{abcd}±0.50	39.16^{bcde}±14.20
Irden	10.05^{ef}±0.945	8.25^{f}±0.86	17.90^{a}	10.10^{ef}±0.64	9.35h±0.67	7.25^{a}	12.05h±0.89	11.00h±1.16	8.50^{a}	10.71^{a}±1.13	9.53^{f}±1.40	10.91^{ab}±5.72
Isly	7.80^{ab}±1.005	5.55^{c}±0.61	27.35^{c}	10.15^{ab}±0.99	6.10^{e}±0.72	39.00^{d}	11.05^{ef}±1.15	5.50^{d}±0.61	49.70^{de}	9.66^{a}±1.66	5.71^{abcde}±0.32	38.68^{bcde}±10.70
Jawhar	10.15^{ef}±1.23	4.30^{b}±0.74	57.00^{f}	8.15^{ef}±0.75	3.35^{a}±0.59	58.50^{e}	9.80^{cd}±1.01	6.00^{d}±0.97	38.00^{c}	9.36^{a}±1.06	4.55^{abc}±1.32	51.16^{e}±11.24
Korifla	8.15^{b}±1.23	6.45^{d}±0.52	19.15^{b}	8.25^{b}±0.72	7.65^{f}±0.81	6.85^{a}	10.55^{def}±1.09	8.60^{f}±0.88	17.95^{b}	8.95^{a}±1.38	7.56^{bcdef}±1.10	14.65^{ab}±7.26
Marjana	7.90^{ab}±1.021	4.20^{b}±0.77	46.30^{e}	8.70^{ab}±0.87	4.10^{b}±0.55	52.50^{e}	9.15^{bc}±0.87	3.20^{a}±0.52	64.70^{f}	8.58^{a}±0.65	3.83^{ab}±0.55	54.5^{e}±9.26
Marouane	10.85^{f}±1.09	9.25g±1.02	14.30^{ab}	6.35^{f}±0.99	4.40^{b}±0.50	29.05^{c}	8.90^{b}±0.78	6.60^{e}±0.99	25.55^{b}	10.2^{a}±2.21	6.73^{bcdef}±2.40	22.96^{abcd}±8.19
Massa	9.00^{c}±0.98	6.30^{d}±0.74	29.35^{c}	10.25^{c}±0.97	7.45^{f}±1.09	26.85^{c}	11.05^{ef}±1.32	5.50^{d}±0.76	49.55^{de}	9.68^{a}±1.00	6.41^{abcdef}±0.95	35.25^{abcde}±12.51
Oum.rabia	8.95^{c}±1.06	5.95^{cd}±0.89	32.85^{cd}	9.30^{c}±1.13	7.10^{f}±0.55	22.50^{bc}	11.95gh±1.19	6.80^{e}±1.10	42.20^{cd}	9.95^{a}±1.65	6.61^{bcdef}±0.56	32.51^{abcde}±9.70
Sebou	7.20^{a}±1.06	6.10^{cd}±0.86	14.55^{ab}	8.40^{a}±1.23	7.45^{f}±0.83	9.95^{a}	9.50^{bc}±1.05	7.00^{e}±0.97	25.05^{b}	7.96^{a}±1.15	6.85^{bcdef}±0.66	16.51^{ab}±7.76
Tomouh	10.10^{ef}±1.45	3.55^{a}±0.61	64.35^{f}	8.10^{ef}±0.72	5.75^{de}±0.72	27.95^{c}	7.75^{a}±0.85	4.10^{b}±0.72	46.40^{de}	9.31^{a}±1.25	4.46^{abc}±1.15	46.23^{cde}±17.95
Vitron	8.10^{b}±1.17	6.45^{d}±0.69	18.90^{b}	9.40^{b}±0.82	8.55g±0.76	8.60^{a}	11.35^{f}gh±0.81	10.10g±0.85	10.80^{a}	9.18^{a}±1.66	8.36^{def}±1.86	12.76^{ab}±6.08
Waha	9.10^{cd}±1.12	3.80^{ab}±0.77	57.90^{f}	7.15^{cd}±0.81	5.15^{c}±0.67	27.00^{c}	8.20^{a}±0.83	3.00^{a}±0.45	63.00^{f}	8.8^{a}±0.95	3.98^{a}±1.11	49.3^{de}±19.11
Yassmine	9.20^{cde}±1.11	7.40^{e}±0.51	18.35^{b}	8.90^{cde}±1.07	7.30^{f}±0.73	16.90^{ab}	11.95gh±1.05	8.90^{f}±0.91	24.90^{b}	10.11^{a}±1.71	7.86^{cdef}±0.89	20.05^{abc}±3.95

*Same letter indicates no difference between varieties with significance level α=5%.

Supplementary Table 3. Mean variation over three years in the plant height at maturity in wheat varieties grown under control conditions (T1) or subjected to water stress (T2).

Variety	Year 1			Year 2			Year 3			Mean		
	T1	T2	% reduction	T1	T2	%reduction	T1	T2	%reduction	T1	T2	%reduction
2777	100.45^{h}±1.53	97.35^{g}±3.57	3.15^{a}	105.90j±1.77	97.40k±4.46	8.00^{a}	102.50i±6.76	97.75k±5.98	4.60^{ab}	102.95^{c}±2.75	97.5^{d}±0.21	5.2^{a}±2.55
^{A}mj^{a}d	90.45^{g}±1.79	86.05i±4.07	5.00^{a}	92.35^{g}±1.56	86.20i±4.07	6.65^{a}	96.75^{gh}±4.49	93.30j±4.96	3.55^{a}	93.18^{abc}±3.231	88.51^{bcd}±4.14	5.06^{a}±1.55
^{A}nou^{a}r	60.70^{a}±1.21	45.30^{d}±8.25	25.20^{c}	87.20^{f}±1.19	58.45^{e}±4.05	33.00^{d}	84.85^{cde}±10.36	46.00^{e}±5.68	44.75^{e}	77.58^{ab}±14.66	49.91^{a}±7.39	34.31^{bc}±9.84
Irden	100.60^{h}±1.14	94.50^{g}±4.23	5.90^{a}	100.65i±1.22	93.35j±4.06	7.30^{a}	93.95^{fg}±6.96	88.25i±8.46	6.30^{ab}	98.4^{bc}±3.85	92.03^{cd}±3.32	6.5^{a}±0.72
Isly	80.50^{e}±1.43	56.05^{e}±6.83	30.20^{d}	87.20^{f}±1.50	63.60^{f}±6.31	27.20^{c}	91.10^{ef}±7.01	36.85^{bc}±5.02	59.35^{gh}	86.26^{abc}±5.36	52.16^{a}±13.79	38.91^{d}±17.75
J^{a}wh^{a}r	70.90^{c}±3.81	31.65^{a}±2.53	54.85^{f}	75.00^{c}±2.24	36.45^{a}±3.88	51.30^{g}	69.80^{a}±8.09	39.00^{cd}±4.41	43.60^{e}	71.9^{a}±2.74	35.7^{a}±3.73	49.91^{d}±5.75
Korifl^{a}	79.80^{e}±1.05	61.95^{f}±7.14	22.45^{c}	82.00^{e}±1.21	76.90^{h}±3.47	6.20^{a}	89.35^{def}±5.33	80.50^{h}±7.11	9.90^{bc}	83.716^{abc}±5.00	73.11^{bc}±9.83	12.85^{ab}±8.51
M^{a}rj^{a}n^{a}	76.45^{d}±1.57	35.65^{b}±5.31	53.25^{f}	80.05^{d}±1.93	41.25^{b}±5.84	48.30^{fg}	89.15^{def}±5.85	32.70^{b}±7.14	62.90^{h}	81.88^{abc}±6.54	36.53^{a}±4.34	54.81^{d}±7.42
M^{a}rou^{a}ne	85.20^{f}±1.10	66.90^{g}±9.08	21.25^{c}	67.55^{a}±1.57	40.10^{b}±4.67	40.50^{e}	87.20^{cde}±4.43	50.70^{f}±4.24	41.60^{e}	79.98^{ab}±10.81	52.56^{a}±13.49	34.45^{bc}±11.44
M^{a}ss^{a}	77.60^{d}±1.14	52.80^{e}±5.39	31.75^{d}	83.20^{e}±2.19	55.40^{d}±8.45	33.45^{d}	90.55^{def}±5.92	42.55^{de}±3.20	52.80^{f}	83.78^{abc}±6.49	50.25^{a}±6.79	39.33^{d}±11.69
Oum.r^{a}bi^{a}	70.90^{c}±1.02	45.80^{d}±5.36	35.05^{de}	100.25i±1.77	68.55^{g}±3.91	31.80^{d}	94.70^{fg}±5.05	41.85^{de}±6.90	55.70^{fg}	88.61^{abc}±15.59	52.06^{a}±14.41	40.85^{d}±12.96
Sebou	79.85^{e}±1.38	73.50^{h}±5.25	7.90^{a}	81.30^{de}±1.26	68.85^{g}±5.09	15.00^{b}	100.00^{h}i±0	79.95^{h}±7.62	20.15^{d}	87.05^{abc}±11.23	74.1^{bc}±5.57	14.35^{ab}±6.15
Tomouh	66.75^{b}±6.31	40.60^{c}±3.76	38.40^{e}	70.85^{b}±1.87	48.00^{c}±5.87	32.25^{d}	75.05^{b}±12.01	34.05^{b}±6.48	53.30^{f}	70.88^{a}±4.15	40.88^{a}±6.97	41.31^{d}±10.82
Vitron	90.50^{g}±2.03	82.85i±6.07	8.35^{a}	94.15^{h}±1.72	88.15i±2.73	6.20^{a}	89.90^{def}±5.11	85.65i±6.18	4.70^{ab}	91.51^{abc}±2.30	85.55^{bcd}±2.65	6.41^{a}±1.83

Supplementary Table 3. Contd.

W^{a}h^{a}	66.45^{b}±6.42	40.90^{c}±4.98	37.90^{e}	83.25^{e}±7.99	45.20^{c}±3.47	45.15^{f}	82.35^{c}±6.48	25.40^{a}±5.23	68.95i	77.35^{ab}±9.45	37.16^{a}±10.41	50.66^{d}±16.24
Y^{a}ssmine	81.50^{e}±1.76	68.05^{g}±5.92	16.30^{b}	87.50^{f}±1.10	71.70^{g}±3.90	17.90^{b}	84.30^{cd}±8.00	73.35^{g}±7.86	13.00^{c}	84.43^{abc}±3.00	71.03^{b}±2.71	15.73^{ab}±2.49

*Same letter indicates no difference between varieties with significance level α=5%.

Supplementary Table 4. Mean variation over three years in the number of ears per plant in wheat varieties grown under control conditions (T1) or subjected to water stress (T2).

Variety	Year 1			Year 2			Year 3			Mean		
	T1	T2	% reduction	T1	T2	% reduction	T1	T2	% reduction	T1	T2	% reduction
2777	8.45^{ef}±0.99	7.20^{fgh}±0.89	14.45^{ab}	9.10^{de}±0.85	7.90^{f}±1.02	13.00^{a}	11.40^{f}±0.59	10.75i±0.63	5.50^{a}	9.65^{a}±1.55	8.61^{e}±1.88	10.98^{a}±4.80
Amjad	7.75^{cde}±0.85	6.90^{efg}±1.11	10.90^{a}	8.30^{cd}±0.65	7.35^{e}±0.87	11.10^{a}	10.60^{e}±0.82	9.60^{h}±0.82	9.20^{a}	8.88^{a}±1.51	7.95^{e}±1.67	10.40^{a}±1.04
Anouar	6.65^{ab}±0.58	3.95^{a}±0.60	40.10^{cd}	7.20^{b}±1.39	4.20^{b}±0.41	39.25^{c}	8.80^{c}±0.69	4.30^{bc}±0.92	50.60^{ef}	7.55^{a}±1.11	4.15^{ab}±0.18	43.31^{d}±6.32
Irden	9.10^{f}±1.41	7.70^{h}±1.12	14.65^{ab}	9.25^{e}±0.71	8.40^{f}±0.75	8.80^{a}	11.40^{f}±0.68	10.30i±0.86	9.35^{a}	9.91^{a}±1.28	8.80^{e}±1.34	10.93^{a}±3.23
Isly	6.30^{a}±0.73	5.00^{bc}±0.56	19.60^{ab}	10.20^{f}±1.00	5.95^{d}±0.75	41.05^{c}	10.15^{e}±0.58	5.10^{d}±0.71	49.70^{e}	8.88^{a}±2.23	5.35^{abcd}±0.52	36.78^{bcd}±15.49
Jawhar	8.65^{ef}±1.22	4.70^{bc}±0.47	44.85^{d}	7.60^{bc}±1.04	3.35^{a}±0.58	55.10^{d}	8.10^{b}±0.85	4.60^{cd}±0.68	42.45^{de}	8.11^{a}±0.52	4.21^{ab}±0.75	47.46^{d}±6.71
Korifla	7.35^{bcd}±1.03	5.70^{d}±0.57	20.95^{ab}	8.40^{de}±0.68	7.35^{e}±0.58	11.90^{a}	8.95^{cd}±0.51	7.00^{e}±0.72	21.40^{c}	8.23^{a}±0.81	6.68^{bcde}±0.86	18.08^{abc}±5.35
Marjana	7.10^{bc}±1.02	3.65^{a}±0.74	47.20^{d}	6.20^{a}±1.05	3.10^{a}±0.64	48.00^{cd}	8.00^{b}±0.64	3.40^{a}±0.50	57.15^{f}	7.10^{a}±0.9	3.38^{a}±0.27	50.78^{d}±5.52
Marouane	9.15^{f}±1.18	7.40^{gh}±1.14	18.25^{ab}	6.25^{a}±0.91	4.65^{b}±0.74	23.95^{b}	9.10^{cd}±0.85	3.90^{ab}±0.30	57.05^{f}	8.16^{a}±1.66	5.31^{abcd}±1.84	33.08^{abcd}±20.95
Massa	8.35^{ef}±0.87	4.85^{bc}±0.58	41.60^{cd}	9.25^{e}±0.96	6.35^{d}±0.48	30.60^{b}	9.50^{d}±0.76	5.00^{d}±0.64	46.90^{e}	9.03^{a}±0.60	5.40^{abcd}±0.82	39.70^{cd}±8.31
Oum.rabia	8.10^{de}±0.71	5.25^{cd}±0.63	35.05^{c}	9.05^{de}±1.23	5.15^{c}±0.87	42.25^{c}	8.95^{cd}±0.82	5.00^{d}±0.85	43.50^{de}	8.70^{a}±0.52	5.13^{abcd}±0.12	40.26^{cd}±4.56
Sebou	8.05^{de}±0.68	6.95^{efg}±0.82	13.25^{ab}	7.25^{b}±1.20	6.05^{d}±0.60	15.00^{a}	9.10^{cd}±0.64	7.45^{e}±0.99	17.80^{bc}	8.13^{a}±0.92	6.81^{bcde}±0.70	15.35^{ab}±2.29
Tomouh	6.35^{a}±0.48	5.60^{d}±0.68	11.50^{a}	7.05^{b}±0.94	4.25^{b}±0.55	38.75^{c}	8.75^{c}±1.01	4.50^{cd}±0.51	48.05^{e}	7.38^{a}±1.23	4.78^{abc}±0.71	32.76^{abcd}±18.99
Vitron	8.10^{de}±1.33	6.60^{ef}±0.82	17.00^{ab}	9.05^{de}±0.60	8.00^{f}±0.56	11.20^{a}	10.05^{e}±0.75	8.80^{g}±0.89	12.20^{ab}	9.06^{a}±0.97	7.80^{de}±1.11	13.46^{ab}±3.10
Waha	8.20^{de}±1.00	4.55^{b}±0.68	43.80^{cd}	7.50^{b}±0.88	4.15^{b}±0.93	43.10^{c}	6.00^{a}±0.56	3.60^{a}±0.50	39.50^{d}	7.23^{a}±1.12	4.10^{ab}±0.47	42.13^{cd}±2.30
Yassmine	8.35^{ef}±0.87	6.35^{e}±0.58	22.90^{b}	8.30^{cd}±0.57	7.25^{e}±0.71	12.30^{a}.90	10.20^{e}±0.76	8.00^{f}±0.85	20.90^{c}	8.95^{a}±1.08	7.20^{cde}±0.82	18.70^{abc}±5.63

*Same letter indicates no difference between varieties with significance level α=5%.

Supplementary Table 5. Mean variation over three years in the number of grains per ear in wheat varieties grown under control conditions (T1) or subjected to water stress (T2).

Variety	Year 1			Year 2			Year 3			Mean		
	T1	T2	%reduction	T1	T2	%reduction	T1	T2	%reduction	T1	T2	%reduction
2777	62.10^{j}±1.20	n.a..	n.a..	65.00^{f}±2.40	62.20^{j}±2.01	4.30^{a}	56.95^{fg}±2.23	54.10^{j}±2.19	4.85^{a}	61.35^{f}±4.07	58.15^{abc}±5.72	4.57^{a}±0.39
Amjad	60.60^{i}±1.04	56.15^{j}±4.24	7.40^{a}	60.70^{de}±1.17	57.40^{hi}±1.90	5.40^{a}	54.00^{ef}±2.61	49.90^{i}±3.69	7.55^{abc}	58.43^{def}±3.83	54.48^{c}±4.01	6.78^{a}±1.20
Anouar	42.70^{c}±1.30	24.60^{c}±	42.15^{d}	41.20^{ab}±1.43	28.50^{d}±4.87	30.75^{cd}	38.05^{a}±3.56	18.90^{bc}±1.11	49.80^{fg}	40.65^{a}±2.37	24.00^{abc}±4.82	40.90^{a}±9.58
Irden	60.95^{i}±1.31	57.80^{j}±3.84	5.20^{a}	61.45^{e}±1.31	58.20^{i}±2.64	5.30^{a}	58.55^{g}±3.60	54.70^{j}±4.90	6.45^{ab}	60.31^{ef}±1.55	56.90^{c}±1.91	5.65^{a}±0.69
Isly	49.35^{e}±2.05	n.a..	n.a..	39.95^{a}±1.79	25.25^{c}±6.23	36.60^{d}	42.80^{bc}±9.63	21.20^{c}±3.28	47.90^{f}	44.03^{abc}±4.81	23.22^{ab}±2.86	40.31^{a}±3.71

Supplementary Table 5. Contd.

Jawhar	35.70^{a}±0.92	16.55^{b}±1.14	53.65^{e}	40.80^{ab}±1.54	16.90^{a}±1.94	58.40^{f}	51.50^{e}±3.17	30.10^{e}±2.10	41.15^{e}	42.66^{ab}±8.06	21.18^{abc}±7.72	51.06^{a}±8.91
Korifla	50.50^{f}±1.19	40.90^{g}±3.59	18.90^{b}	58.85^{d}±1.38	54.90^{h}±3.56	6.80^{a}	50.90^{e}±2.82	44.65^{g}±2.97	12.25^{bc}	53.41^{bcdef}±4.70	46.81^{abc}±7.24	12.65^{a}±6.05
Marjana	45.40^{d}±1.27	n.a..	n.a..	49.30^{c}±6.13	20.95^{b}±2.52	56.40^{f}	39.45^{ab}±5.48	16.80^{ab}±2.30	56.60^{h}	44.71^{abc}±4.96	18.87^{a}±2.93	56.5^{a}±0.14
Marouane	57.85^{h}±1.59	52.40^{i}±1.23	9.30^{a}	50.90^{c}±5.38	32.50^{e}±2.50	35.25^{d}	52.70^{e}±2.53	35.40^{f}±5.11	32.55^{d}	53.81^{bcdef}±3.60	40.10^{abc}±10.75	25.70^{a}±14.26
Massa	54.15^{g}±1.56	36.80^{f}±	31.80^{c}	50.55^{c}±1.46	27.45^{cd}±4.65	45.65^{e}	40.85^{ab}±3.13	19.00^{bc}±3.14	53.00^{fgh}	48.51^{abcde}±6.87	27.75^{abc}±8.90	43.48^{a}±10.76
Oum.rabia	51.25^{f}±1.25	31.30^{e}±	38.85^{d}	51.50^{c}±1.46	37.20^{f}±4.06	27.65^{c}	41.30^{ab}±2.47	24.10^{d}±3.25	41.50^{e}	48.01^{abcd}±5.81	30.86^{abc}±6.56	36.00^{a}±7.35
Sebou	57.45^{h}±2.96	53.00^{i}±3.19	7.65^{a}	50.10^{c}±1.44	42.55^{g}±3.63	15.05^{b}	52.80^{e}±4.69	45.20^{g}±3.34	13.90^{c}	53.45^{bcdef}±3.71	46.91^{abc}±5.43	12.20^{a}±3.98
Tomouh	45.15^{d}±1.95	26.55^{d}±2.50	41.05^{d}	42.80^{b}±4.85	32.60^{e}±2.13	23.15^{c}	45.15^{cd}±3.95	20.00^{c}±2.02	55.30^{gh}	44.36^{abc}±1.35	26.38^{abc}±6.30	39.83^{a}±16.10
Vitron	51.60^{f}±1.81	47.50^{h}±4.05	8.00^{a}	59.20^{d}±1.54	54.55^{h}±3.15	7.85^{a}	58.65^{g}±3.92	54.25^{j}±2.84	7.35^{abc}	56.48^{cdef}±4.23	52.10^{bc}±3.98	7.73^{a}±0.34
Waha	38.90^{b}±1.41	17.30^{b}±1.55	55.45^{e}	40.45^{a}±1.50	25.80^{cd}±7.25	35.95^{d}	47.45^{d}±3.64	15.80^{a}±1.70	66.50^{i}	42.26^{ab}±4.55	19.63^{abc}±5.39	52.63^{a}±15.46
Yassmine	54.10^{g}±	n.^{a}..	n.^{a}..	50.90^{c}±1.55	37.55^{f}±4.54	26.00^{c}	54.75^{ef}±6.34	47.55^{h}±4.53	12.55^{bc}	53.25^{bcdef}±2.06	42.55^{abc}±5.0	19.27^{a}±9.51

*Mean on years 2 and 3 only
n.a..: no grain in T2 plants due to fungal infection
Same letter indicates no difference between varieties with significance level α=5%.

Supplementary Table 6. Mean variation over three years in the weight of 1000 grains in wheat varieties grown under control conditions (T1) or subjected to water.

Variety	Year 1			Year 2			Year 3			Mean		
	T1	T2	% reduction	T1	T2	% reduction	T1	T2	% reduction	T1	T2	% de reduction
2777	42.00^{bc}±3.28	n.^{a}..	n.^{a}..	42.00^{ef}±1.71	38.67^{f}±1.59	8.00^{ab}	50.00^{c}±1.86	45.33^{f}±3.54	9.00^{ab}	44.66^{a}±6.08	42^{ab}±4.7093	8.5^{a}±0.70
Amjad	41.33^{bc}±9.05	34.67g±2.05	14.00^{a}	43.00^{f}±3.53	40.33^{f}±2.15	6.00^{a}	40.33^{ab}±11.38	32.00^{d}±2.94	18.33^{abc}	41.55^{a}±2.30	35.66^{ab}±2.64	12.77^{a}±4.59
Anouar	37.66^{b}±6.59	28.00^{de}±2.31	24.33^{abc}	34.67^{ab}±2.12	27.33^{bc}±1.81	21.67^{bcd}	40.00^{ab}±4.15	27.33^{bc}±3.57	31.67^{cd}	37.44^{a}±3.78	27.55^{ab}±4.50	25.89^{a}±7.01
Irden	39.00^{bc}±0.28	35.00g±3.53	10.33^{a}	42.33^{ef}±3.48	40.00^{f}±2.60	5.66^{a}	46.00^{bc}±6.06	42.67^{f}±3.28	6.67^{a}	42.44^{a}±3.78	39.22^{b}±3.46	7.55^{a}±0.91
Isly	37.33^{b}±9.81	n.^{a}..	n.^{a}..	35.67^{abc}±0.60	30.00^{bcd}±1.39	15.00^{abcd}	39.33^{ab}±3.06	33.00^{d}±2.52	16.33^{abc}	37.44^{a}±4.5	36.5^{ab}±4.0	15.66^{a}±0.94
Jawhar	29.00^{a}±0.06	18.33^{b}±0.84	36.67^{c}	38.67^{bcdef}±2.24	15.00^{a}±2.90	61.33^{e}	39.67^{ab}±0.94	16.67^{a}±3.64	58.00^{e}	35.78^{a}±5.03	16.66^{ab}±2.64	52^{a}±7.87
Korifla	39.33^{bc}±1.90	31.67^{f}g±4.87	19.00^{abc}	40.33^{cdef}±1.53	33.67^{de}±1.41	17.00^{abcd}	44.67^{abc}±1.14	29.67^{bcd}±1.40	33.00^{cd}	41.44^{a}±6.50	31.67^{ab}±11.67	23^{a}±15.74
Marjana	39.33^{bc}±3.76	n.^{a}..	n.^{a}..	39.33^{bcdef}±5.15	14.67^{a}±0.03	62.00^{e}	43.33^{abc}±3.36	15.00^{a}±2.53	66.00^{e}	40.66^{a}±1.73	14.83^{a}±0.23	64^{a}±2.82
Marouane	39.67^{bc}±6.12	30.00^{ef}±3.15	23.67^{abc}	37.67^{bcdef}±2.01	28.67^{bc}±3.82	24.00^{cd}	41.33^{ab}±0.77	31.33^{cd}±1.59	25.00^{bcd}	39.55^{a}±7.37	30^{ab}±7.21	24.22^{a}±6.98
Massa	36.00^{ab}±0.88	29.33^{ef}±1.11	18.00^{ab}	36.33^{abcd}±2.65	26.33^{b}±3.32	28.00^{d}	37.33^{ab}±3.32	25.33^{b}±1.64	32.00^{cd}	36.55^{a}±3.60	26.99^{ab}±5.03	26^{a}±7.39
Oum.rabia	44.00^{bc}±0.88	33.33g±3.88	24.33^{abc}	41.33^{def}±0.97	31.33^{cde}±2.12	25.00^{cd}	44.00^{abc}±2.94	30.33^{cd}±3.63	31.00^{cd}	43.11^{a}±4.04	31.66^{ab}±2.08	26.77^{a}±5.63
Sebou	44.66^{bc}±2.24	40.33h±1.07	9.67^{a}	38.00^{bcdef}±3.10	34.67^{e}±1.41	8.67^{ab}	35.33^{a}±3.54	29.67^{bcd}±0.64	15.67^{abc}	39.33^{a}±9.29	34.89^{ab}±7.23	11.33^{a}±1.77
Tomouh	39.33^{bc}±1.20	26.00^{cd}±1.48	32.67^{bc}	32.33^{a}±2.52	27.33^{bc}±0.56	15.33^{abcd}	40.67^{ab}±1.44	25.67^{b}±0.95	36.67^{d}	37.44^{a}±4.58	26.33^{ab}±8.50	28.22^{a}±13.61
Vitron	48.67^{c}±4.00	41.67h±0.57	14.67^{a}	35.00^{abc}±1.69	30.33^{bcd}±0.32	13.33^{abc}	43.67^{abc}±3.22	39.33^{e}±2.39	10.00^{ab}	42.44^{a}±5	37.11^{ab}±3.60	12.66^{a}±2.53
Waha	38.00^{b}±3.76	24.33^{c}±1.54	35.33^{bc}	40.33^{cdef}±0.92	29.00^{bc}±2.03	28.33^{d}	38.33^{ab}±3.37	27.00^{bc}±1.63	29.67^{cd}	38.88^{a}±2.30	26.77^{ab}±2.08	31.11^{a}±2.05
Yassmine	35.33^{ab}±3.04	n.^{a}..	n.^{a}..	37.33^{bcde}±2.51	30.67^{bcd}±1.34	17.00^{abcd}	41.00^{ab}±8.57	33.67^{d}±0.95	17.00^{abc}	37.88^{a}±3.05	32.17^{ab}±2.12	17.0^{a}±0

*Mean on years 2 and 3 only; n.a..: no grain in T2 plants due to fungal infection; Same letter indicates no difference between varieties with significance level α=5%.

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Fertilizer-nitrogen use optimization for Tef in South Wollo Zone of Ethiopia

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Tef [Eragrotis tef] is a major staple cereal crop in Ethiopia but yields are low due to inadequate nutrient supply and other constraints. A field study was conducted in 2014 and 2015 in Jamma district of South Wollo Zone of Amhara Region to determine the economic optimum rate of fertilizer-N for tef. Fertilizer-N rates of 0, 23, 46, 69, 92, 115 and 138 kg ha^{-1} were evaluated. The randomized complete block design with three replications was used. The fertilizer-N rate means fit a quadratic yield response function with R^2=0.25**. The maximum mean grain and straw yields were recorded with 138 kg N ha^{-1} which was not statistically different from the 92 and 115 kg ha^{-1} N rates. The net economic returns to N were optimized with the 115 and 92 kg ha^{-1} N rates with respective net benefits of Ethiopian Birr 30508 and 28971 ha^{-1} and with marginal rate returns of 236 and 288%, respectively. The highest value to cost ratio (VCR) and highest benefit to cost ratio (BCR) of 10.9 and 5.2, respectively were obtained with 46 kg N ha^{-1}. Using the yield response function determined, the profit-maximizing optimal rate of N was found to be 117 kg N ha^{-1}. Therefore, for financially constrained farmers 46 kg N ha^{-1} is recommended, while for farmers without financial constraints 92 to 117 kg N ha^{-1} can be recommended for economic optimum return from tef production on the Vertisols in Jamma District and similar agroecologies.

Key words: Economic optimum, Jamma district, nitrogen fertilizer, yields response of tef.

INTRODUCTION

Tef [*Eragrostis tef (Zucc.)]* is the most important cereal crop in Ethiopia covering 30% of the area and 20% of the total cereal production (CSA, 2015). It is an excellent source of essential amino acids, including lysine which is commonly deficient in cereal grain (Doris, 2002). It is gluten-free (Spaenij et al., 2005). However, tef has the lowest productivity as compared to the other cereal crops as the national average yield is meager, ranging between 1.28 and 1.58 t ha^{-1} (CSA 2015).

Vertisols account for 23% of the cropland in Ethiopia and are the dominant soil type in the highlands where Jamma District of South Wollo Zone is located (Kamara and Haque, 1988). These soils are characterized by very low water infiltration rate or low saturated hydraulic conductivity and, therefore, are susceptible to waterlogging under high intensity rainfall conditions. Tef is the most important food crop in Jamma District (Getachew, 1991). However, tef yield in the District is

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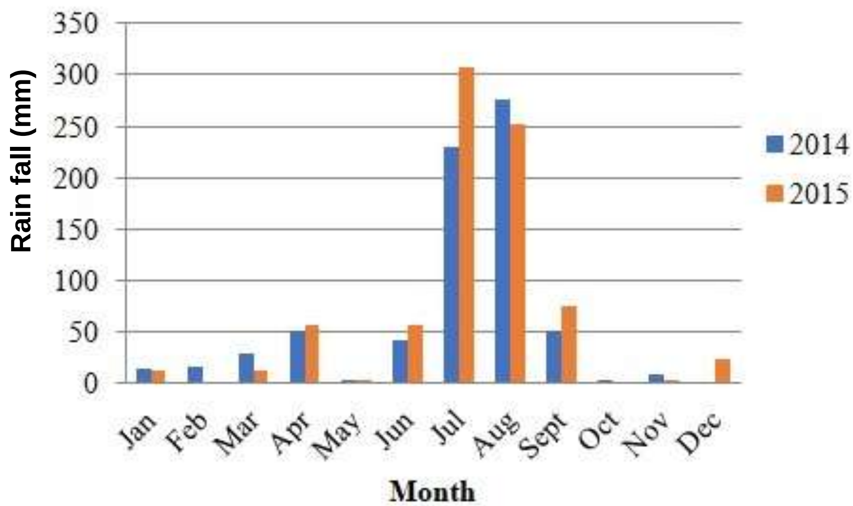

Figure 1. Monthly rainfall distribution of Jamma District in 2014 and 2015.

below the national average, at least partly due to inadequate supply of plant nutrients (Stoorvogel and Smaling, 1990; Asnakew, 1994; Fassil and Charles, 2009; Tulema et al., 2005). In addition, the Ethiopia highland Vertisols tend to exhibit low total N mainly due to leaching and denitrification (Tekalign et al., 1988) limiting cereal production.

The current blanket fertilizer recommendation for tef and other cereal crops in Jamma District is to apply 64 kg N and 46 kg P_2O_5 ha^{-1} (NFIU, 1992). Therefore, refinement of the previous fertilizer recommendations is essential from the basis of dynamic property of soils as a result of different natural and man-made factors. However, nutrient fertilization recommendations must be based on crop yield optimization and profit maximization. Fertilization recommendations are usually based on results from field crop nutrient response trials which were lacking for Jamma District. The recent soil fertility assessment map produced by Ethiopian Soil Information System (EthioSIS) showed that in addition to N and P, K, S, B and Zn deficiencies are widespread in Ethiopian soils (EthioSIS 2014). Therefore, this research was conducted to develop a yield response curve of tef to fertilizer-N under adequate supply of all nutrients and determine the economically optimum N rate for tef.

MATERIALS AND METHODS

Description of the study area

The study was carried out in 2014 and 2015 main cropping seasons in Jamma District of South Wollo Zone of the Amhara Region in Ethiopia. The district is situated within the geographical boundaries of 10° 06' 24'' - 10° 35' 45'' N latitudes and 39° 04' 04'' - 39° 23' 03'' E longitudes and altitudinal ranges of 1428 - 2752 m above sea level (masl). The district receives a mean annual rainfall of 1130 mm rainfall with approximately 60% falling in July and August (Figure 1); with 10.3°C mean minimum temperature; and 21.6°C mean maximum temperature. The cropping season is from June to November. The soil type for the study sites is Pellic Vertisol with some of the physico-chemical properties mentioned in Table 1. The previous crop produced on the study sites had been wheat.

Treatments and experimental design

The treatments comprised fertilizer-N at rates of 0, 23, 46, 69, 92, 115 and 138 kg ha^{-1} with uniform application of 69/80/30/2/1 kg ha^{-1} $P_2O_5/K_2O/S/Zn/B$. In addition, there was a zero fertilizer treatment and the recommended 69/46 kg ha^{-1} N/P_2O_5. The randomized complete block design (RCBD) with three replications was used on each of three representative farmers' fields in 2014 and 2015. The gross plot size was 3 x 3 m and 2 x 2 m was the harvested area for data collection.

Experimental materials and procedures

Tef variety with local name *dega tef - DZ675* was planted in rows of 20 cm spacing at a seed rate of 10 kg ha^{-1} on flat fine seed beds. Phosphorus, K and S fertilizers were band-applied at basal as triple super phosphate (TSP), muriate of potash (KCl) and calcium sulfate ($CaSO_4$) straight fertilizers, respectively. Fertilizer-N was applied as urea with 50% at planting and 50% at 45 days after planting. The micronutrients Zn as zinc sulphate ($ZnSO_4$) and B as borax ($Na_2B_4O_7.5H_2O$), each weighed in plot level, each dissolved in 16 L volume tap water in a knapsack sprayer were sprayed at foliage 45 and 60 days after planting, respectively.

Data collection

Fresh biomass yield was measured by weighing the total above ground biomass of the entire harvestable area. The dry biomass weight was obtained by drying a sample of plants, with seed panicles, in an oven at 105°C for 12 h and adjusting the fresh biomass weight into dry weight basis. Grain yield was determined from harvested grain weight and straw yield was calculated by subtracting the grain weight from the dry biomass weight. Harvest index was obtained by dividing the grain yield by the dry biomass

Table 1. Range of physico-chemical properties of surface soil (0-30 cm) of the study sites.

Soil property*	Value	Rating**
pH (H_2O)	6.5-6.8	Slightly acidic to neutral
Organic matter (OM) (%)	1.36-1.75	Low
Total N (TN) (%)	0.10-0.11	Low
Olsen extractable P (mg kg^{-1} soil)	3.08-5.20	Low
Exchangeable Ca ($cmol_C$ kg^{-1})	30.6-46.3	Very high
Exchangeable Mg ($cmol_C$ kg^{-1})	9.9-12.9	Very high
Exchangeable K ($cmol_C$ kg^{-1})	0.6-0.7	High
Cation exchange capacity (CEC) ($cmol_C$ kg^{-1})	52.0-61.7	Very high
Percent acid saturation (PAS) %	82.5-97.6	Very weakly leached
Sand %	16.3-17.5	
Silt %	20.0-21.3	
Clay %	62.5	
Textural class	Clay	

The soil analysis was conducted at the Ethiopian National Soil Research Center.
*Source: Abebe et al. (2013). **Ratings are based on pH (Jones, 2003), OM and TN (Tekalign, 1991), available P (Cottenie, 1980), exchangeable Ca, Mg and K (FAO, 2006), CEC and PAS (Hazelton and Murphy, 2007).

weight.

Data analysis

The collected data were subjected to analysis of variance (GLM procedure) using SAS software version 9.00 (SAS, 2004). The mixed model procedure was used for the combined analysis over the testing sites with treatments as a fixed variable and with site and replication as random variables. Treatment means separation was done with Duncan's Multiple Range test (DMRT) at P ≤0.05. Simple non-linear regression analysis was run using SAS to determine the goodness of fit of the yield response curve to N. The farm-gate prices of Ethiopian Birr (ETB) 20, 2 and 23.9 per kg for variable factors; tef grain, tef straw and N (derived from cost of urea, ETB 11.0 kg^{-1}), respectively, were used for partial budget analysis following the CIMMYT procedure (CIMMYT, 1988). The other factors were constant as they were applied uniform to all treatments. The mean grain and straw yields used in the partial budget analysis were adjusted to 90% of the measured yield.

RESULTS AND DISCUSSION

Yield response of Tef to N fertilizer

A highly significant (P≤ 0.01) grain and straw yield response to N was found at all testing sites in 2014 (Table 2). There was no significant difference in the response to fertilizer-N across testing sites. The grain yield was increased from 0.6 - 0.8 t ha^{-1} to 1.3 - 1.4 t ha^{-1} while the straw yield was increased from 1.6 - 1.8 t ha^{-1} to 2.7 - 3.4 t ha^{-1} by applying N_{115}. The significant yield response of tef to N was attributed to the low indigenous soil N supply (Table 1; Tekalign 1991). The results agree with other reports of tef response to fertilizer-N especially in the highland Vertisols (NFIU, 1993; Tekalign et al., 2001; Minale et al., 2004). The combined analysis of the yield data pooled over the testing sites in 2014 indicated that N had significant (P≤0.05) effect on the yield of tef with a mean maximum grain yield of 1.2 t ha^{-1} and mean maximum straw yield of 2.9 t ha^{-1} with N_{115} and N_{138} which were statistically at par with N_{69} and N_{92} (Table 4). However, treatments did not affect the harvest index (HI).

Tef grain yield was increased with fertilizer-N at only one of the three sites in 2015 (Table 3). The lack of response for the two sites was attributed to these being newly cultivated fields which were previously range land had better soil fertility. Fertilizer-N application decreased grain yield at Site 6. However, straw yield was increased at all testing sites in 2015. This result is supported by Alkamper (1973) and Legesse (2004) who concluded that straw compared with grain yield is more responsive to N while P encourages good tef grain production. The mean grain yield of 1.9 t ha^{-1} in 2015 was above the then national average yield (CSA, 2015). Grain yield was not affected by N rate in the pooled analysis in 2015 (Table 4). The non-significant grain yield response to N and significant treatment by site interaction effect in 2015 was due to the non-significant grain yield response to N at two of the testing sites. The highest mean straw yield was 7.8 t ha^{-1} with N_{155} which was statistically at par with the straw yield from N_{92} and N_{138}. The HI declined with increased N rate in 2015 due to relatively great straw compared with grain yield response to N rate.

On average across the two years, grain yield increased from 0.7 to 1.5 t ha^{-1} and straw yield increased from and 1.8 to 4.0 t ha^{-1} due to fertilizer-N application, but HI was not affected (Table 4). The average grain yield increase due to fertilizer-N of 0.8 t ha^{-1} was higher than the mean yield increase from 52 sites in Amhara Region of 0.59 t ha^{-1} (Wakene and Yifru, 2013). The mean grain and straw yields were not significantly increased with N rate > 92 kg ha^{-1}.

Table 2. Mean grain and straw yields of tef obtained in the three testing sites as affected by fertilizer treatments in 2014.

Treatment* (kg N ha^{-1})	Grain yield (kg ha^{-1})			Straw yield (kg ha^{-1})		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
No fertilizer	642.8^{b}	603.7^{c}	827.0^{d}	1632.2^{d}	1813.0^{cd}	1839.7^{b}
0	633.9^{b}	708.8^{bc}	898.6^{cd}	1699.4^{d}	1874.5^{cd}	1768.1^{b}
23	960.1^{ab}	704.3^{bc}	1041.6^{bcd}	2123.3^{cd}	1629.0^{d}	1858.4^{b}
46	1050.3^{ab}	948.2^{ab}	1063.1^{bc}	2366.4^{bcd}	2051.8^{cd}	2603.6^{a}
69	1087.5^{ab}	920.3^{ab}	1163.4^{ab}	2745.8^{abc}	2246.3^{bc}	2336.6^{a}
92	1397.7^{a}	1004.8^{a}	1133.9^{ab}	2727.3^{abc}	2745.2^{ab}	2491.1^{a}
115	1390.8^{a}	1049.1^{a}	1309.8^{a}	3442.6^{a}	2700.9^{ab}	2590.3^{a}
138	1497.6^{a}	1062.2^{a}	1186.3^{ab}	3169.1^{ab}	3104.5^{a}	2605.3^{a}
69/46 N/P_2O_5	1235.5^{a}	878.0^{abc}	1145.3^{ab}	2681.2^{abc}	2038.7^{cd}	2338.0^{a}
Mean	1099.6	875.5	1085.4	2509.7	2244.9	2270.1
CV (%)	26.5	17.3	11.3	17.4	13.8	11.3
SEM	292.0	151.5	122.5	435.7	310.7	256.8

Treatment means within a column followed by the same letter are not significantly different at $p > 0.05$. The N rate treatments had a uniform band application of 69/80/30 P_2O_5/K_2O/S kg ha^{-1} and a uniform foliar application of 2/1 Zn/B kg ha^{-1}.

Table 3. Mean grain and straw yields of tef obtained from the three testing sites as affected by application of N in 2015

Treatment* (kg N ha^{-1})	Grain yield (kg ha^{-1})			Straw yield (kg ha^{-1})		
	Site 4	Site 5	Site 6	Site 4	Site 5	Site 6
No fertilizer	683.3^{d}	2152.5	1765.0^{ab}	2407.5^{de}	3098^{e}	4151.7^{e}
0	725.0^{d}	2243.3	1858.3^{ab}	1775.0^{e}	4668^{cde}	4308.3^{e}
23	1318.3^{c}	2015.0	1881.7^{ab}	3848.3^{cd}	3318de	6285.0^{bcd}
46	1841.7^{b}	1935.0	2066.7^{a}	5491.7^{abc}	5732^{bcd}	5766.7^{d}
69	2203.3^{ab}	2189.9	1531.7^{bc}	5713.3^{abc}	5977^{bc}	7468.3^{ab}
92	2228.3^{ab}	2053.3	1546.7^{bc}	6771.7^{ab}	7613^{ab}	7370.0^{abc}
115	2346.7^{a}	2038.3	1686.7^{ab}	6903.3^{ab}	8128^{ab}	8313.3^{a}
138	2356.7^{a}	1970.0	1260.0^{c}	7560.0^{a}	8863^{a}	5906.7^{cd}
69/46 N/P_2O_5	1861.7^{b}	2495.0	1826.7^{ab}	4888.3^{bc}	6672^{abc}	7173.3^{abcd}
Mean	1729.4	2120.2	1713.7	5141.1	6177.6	6304.8
CV (%)	14.1	17.6	12.1	20.9	21.1	12.8
SEM	243.9	372.4	207.4	1078.5	1303.8	806.0

*Treatment means within a column followed by the same letter are not significantly different at $p > 0.05$. The N rate treatments had a uniform band application of 69/80/30 P_2O_5/K_2O/S kg ha^{-1} and a uniform foliar application of 2/1 Zn/B kg ha^{-1}.

Minale et al. (2004) and Alemayehu et al. (2007) recommended 60-80 kg N ha^{-1} with 18-20 kg P_2O_5 ha^{-1} for maximum tef production on Vertisols in Northwest Ethiopia. Application of 80 kg N ha^{-1} plus 80 kg ha^{-1} P_2O_5 with tef row spacing of 10 cm gave the highest tef yield (Kumela and Thomas, 2016) while Fissehaye et al. (2009) reported maximized tef grain yield with 69 kg N ha^{-1} on Vertisols. In contradiction to these findings, Miller (2009) and Nosberg et al. (2009) stated that tef requires only 32 to 46 kg N ha^{-1} and that excessive N rate results in increased lodging.

Wakene and Yifru (2013) showed that optimal N rates for tef in Ethiopia varied from 15-90 kg ha^{-1} due to diversity in agro-ecology, climate and soil type and recommended 15-60 kg ha^{-1} N for Nitisols, Luvisols, Fluvisols and Andosols, but 60-90 kg ha^{-1} N for Vertisols. However, N fertilization for tef could be either omitted or reduced by half when the precursor crops are legumes (Tekalign et al., 2001; Alemayehu et al., 2010) or oil crops (Abedenna et al., 2006; Alemayehu et al., 2010).

Yield response function analysis

The mean grain yield response to N rate over two years fit a quadratic function. The adjusted $R^2 = 0.25^{**}$ when the

Table 4. Mean grain and straw yields (kg ha^{-1}) and harvest index pooled over testing sites in 2014 and 2015 and pooled over two experimental years.

Treatment* (kg N ha^{-1})	2014			2015			Pooled over years		
	GY	SY	HI	GY	SY	HI	GY	SY	HI
No fertilizer	691.2^{e}	1761.6^{c}	0.28	1456.3	3352.1^{d}	0.31^{a}	721.6^{e}	1950.3^{de}	0.27
0	747.1^{de}	1780.7^{c}	0.29	1608.9	3448.1^{d}	0.31^{ab}	741.6^{e}	1779.3^{e}	0.29
23	902.0^{cd}	1870.2^{c}	0.32	1738.3	4483.9^{d}	0.29^{abc}	975.1^{d}	2371.4^{d}	0.30
46	1020.5^{bc}	2340.6^{b}	0.30	1947.8	5663.3^{c}	0.26^{bcd}	1273.9^{c}	3203.4^{bc}	0.30
69	1057.1^{abc}	2442.9^{b}	0.30	1975.0	6386.1^{bc}	0.24^{def}	1343.6^{bc}	3260.5^{bc}	0.30
92	1178.8^{ab}	2654.5^{ab}	0.31	1942.8	7251.7^{ab}	0.21^{def}	1441.2^{ab}	3683.8^{ab}	0.29
115	1249.9^{a}	2911.3^{a}	0.30	2023.9	7781.7^{a}	0.21^{ef}	1539.4^{a}	3893.9^{a}	0.30
138	1248.7^{a}	2959.6^{a}	0.30	1862.2	7443.3^{ab}	0.20^{f}	1544.0^{a}	4091.4^{a}	0.29
69/46 N/P_2O_5	1086.3^{abc}	2352.6^{b}	0.32	2061.1	6244.4^{bc}	0.26^{cde}	1290.4^{bc}	3087.2^{c}	0.30
Mean	1020.2	2341.6	0.30	1851.1	5876.2	0.25	1213.3	3067.8	0.29
CV (%)	19.5	15.4	11.4	14.9	19.7	18.9	14.8	17.9	10.49
SEM	198.9	360.3	0.03	276.2	1157.8	0.05	179.5	551.5	0.03
Trt*Site	ns	ns	ns	**	*	*	**	**	ns

GY = Grain yield; SY = Straw yield; HI = Harvest index; Treatment means within a column followed by the same letter are no significantly different at p > 0.05. * and **- significant at 0.05 and 0.01 probability levels, respectively. ns = non-significant at p > 0.05.

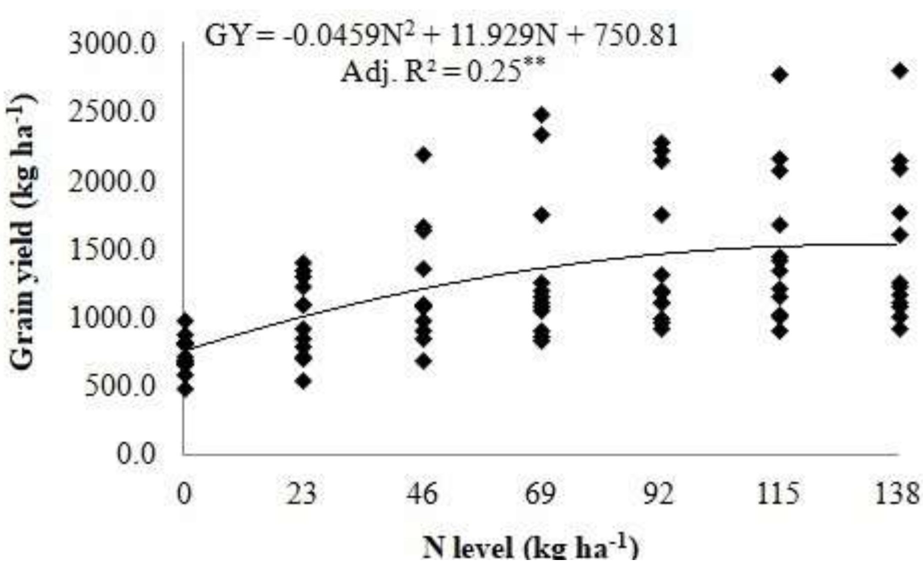

Figure 2. The quadratic response curve of the plot yield data to fertilizer-N in the six sites in 2014and 2015 on Vertisols in Jamma District of Ethiopia.

analysis was with plot data but 0.988 where the regression analysis was with overall N rate means (Figures 2 and 3). This generally agrees with tef responses reported for the central highlands of Ethiopia with Vertisols (Tekalign et al., 2001; Minale et al., 2004). Others have reported good fits of quadratic yield response to N fertilizer including for potato (Gilles et al., 2000), wheat (Cassman and Plant, 1992; Zhejun et al., 2014) and rice (Cassman and Plant 1992; Dobermann et al., 1992).

Economic analysis

The partial budget analysis showed that the highest net economic return of Ethiopian Birr (ETB) 30508 per ha with MRR of 236% was estimated from N_{115} followed by the net economic return of 28971 ETB per ha and MRR of 288% from N_{92} (Table 5). The dominance analysis showed that application of N_{138} was dominated and thus was excluded from the analysis. The sensitivity analysis with the assumption of increasing fertilizer price and labor

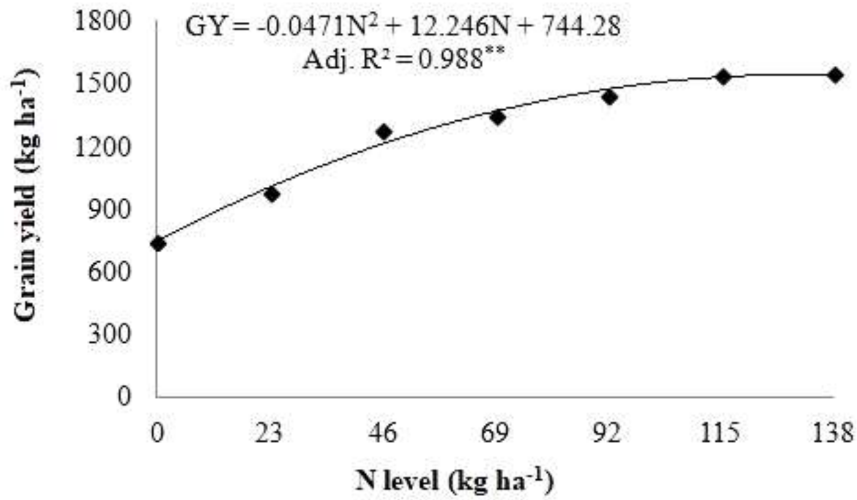

Figure 3. The quadratic response curve of the mean grain yield to N pooled over six sites in 2014and 2015 on Vertisols in Jamma District of Ethiopia.

Table 5. Partial budget analysis for overall mean grain and straw yields with grain value of 20 Ethiopia birr (ETB), straw value of 2, fertilizer-N cost of 23.9 for scenario 1 and grain value of 17 ETB, straw value of 1.7 ETB and fertilizer-N cost of 27.5 ETB for scenario

N	AGY	ASY	GR	LC	FC	TVC	NR	MRR (%)	VCR	BCR
					Scenario 1					
0	667	1601	16542	0	0	0	16542	-	-	-
23	878	2128	21816	1000	550	1550	20266	240	9.6	3.4
46	1147	2816	28572	1200	1099	2299	26273	801	10.9	5.2
69	1209	2935	30050	1300	1649	2949	27101	127	8.2	4.6
92	1297	3315	32570	1400	2199	3599	28971	288	7.3	4.5
115	1386	3518	34756	1500	2749	4249	30508	236	6.6	4.3
					Scenario 2					
0	667	1601	14060.7	0	0	0	14061	-	-	-
23	878	2128	18543.6	1150	632	1782	16761	152	7.1	1.5
46	1147	2816	24286.2	1380	1264	2644	21642	566	8.1	2.9
69	1209	2935	25542.5	1495	1896	3391	22151	68	6.1	2.4
92	1297	3315	27684.5	1610	2529	4139	23546	187	5.4	2.3
115	1386	3518	29542.6	1725	3161	4886	24657	149	4.9	2.2

AGY=Adjusted grain yield (kg ha^{-1}); ASY=Adjusted straw yield (kg ha^{-1}); BCR=Benefit to Cost Ratio; FC=Fertilizer-N Cost (TEB); GR=Gross Returns (ETB); LC=Labor Cost (ETB); MRR=Marginal Rate of Return; N=N level (kg N ha^{-1}); NR=Net Return (ETB); TVC=Total Variable Cost (ETB); VCR=Value to Cost Ratio.

cost by 15% while decreasing grain and straw yields' price by 15% also showed that the highest net economic returns were obtained from application of N_{115} and N_{92}. However, the highest value to cost ratio (VCR) and highest benefit to cost ratio (BCR) of 10.9 and 5.2, respectively, in the first scenario and 8.1 and 2.9, respectively, in the second scenario were obtained from application of N_{46} (Table 5). Using the yield response function determined, the profit-maximizing optimal rate of N, calculated by setting the first derivative of the response function that is, $GY = -0.0459N^2 + 11.929N + 750.81$ to the price ratio of the fertilizer-N to the grain price, that is, (ETB 23.9/ETB 20) was found to be $23.9/20 = -0.0918N + 11.929$; N = 117 kg ha^{-1} for scenario 1, while in the case of scenario 2, $27.5/17 = -0.0918N + 11.929$; N = 112 kg ha^{-1}. Therefore, for the financially constrained farmers seeking maximum profits with least fertilizer costs, application of 46 kg N ha^{-1} is recommended. However, for the farmers without financial constraints seeking for maximum net economic returns, application of 92-117 kg N ha^{-1} is recommended. This result is supported by previous studies by Tekalign et al.

(1996) and Wakene and Yifru (2013) who recommended application of 60-90 kg N ha^{-1} on Vertisols in the highlands of Ethiopia.

Conclusion

The result revealed that quadratic response curve was the best fitting curve for the mean grain yield response of tef to application of N. The highest net economic returns and highest marginal rate of returns were obtained with 115 and 92 kg N ha^{-1}. However, the highest VCR and highest BCR were found with 46 kg N ha^{-1}. The profit-maximizing optimal rate of N using the yield response function determined in the study was found to be 117 kg N ha^{-1} in scenario 1 and 112 kg N ha^{-1} in scenario 2. However, taking the limited resource endowment of small holder farmers in the Jamma District in to consideration, 46 kg N ha^{-1} is recommended for financially constrained farmers seeking maximum profits with least fertilizer costs, while 92-117 kg N ha^{-1} is recommended for farmers without financial constraints seeking maximum net economic returns with higher investment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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Perception and uptake of aquaculture technologies in Kogi state, central Nigeria: imperative for Improved Management practices for sustainable aquaculture development

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The drivers of perception and adoption of aquaculture innovations were studied. Data obtained from 300 aquaculture operators, were analyzed using Heckman Probit sample selection model. Results revealed that perception and adoption of aquaculture innovations were high. Education (a=0.281), extension contact (a=0.149), experience (a=0.021), gender (a=0.440) and information source (a=0.145) increased the likelihood of positive perception of innovations, while age (a=-0.456), primary occupation (a=-1.54) and distance to urban center (a=-0.55) were negatively signed. Technical know-how (b=0.116), "other income" (b=1.17-e06), education (b=0.115) and gender (b=0.11) were drivers of adoption. Access to credit (b=-0.074), age (b=-0.095), pond size (b=-0.094) and Information source (b=-0.05) were negatively related to adoption. Adoption of innovation will rely on policies involving on these relationships. In doing these, attention should be paid to variables with conflicting influences on perception and adoption. Electronic sources may be employed in introducing an innovation, followed by personal contacts with experienced fish farmers. Furthermore, assisting fish farmers to increase incomes from other sources might be a better funding alternative for innovation adoption than credit. Provision of practically oriented education will elicit adoption. Provision of productive resources and reduced domestic burdens on female folks will increase their likelihood of innovation perception and adoption.

Key words: Drivers, perception, adoption, aquaculture, innovation, Heckman Probit.

INTRODUCTION

World apparent yearly fish per capita consumption increased steadily from 9.9 kg in the 1960s to 16.4 kg in 2005 (FAO, 2008). Recent data from FAO (2018) show that from 2011 to 2016, the figure had increased from 18.5 to 20.3 kg. The Nigerian per capita fish consumption has remained far below the world average. Per capita fish consumption in Nigeria declined from 13 kg per capita in the 1980s through 9.68 kg in 2007 to about 8 kg in 2009

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(Amire, 2010), before increasing to 11.0 kg in 2016. Data from Central Bank of Nigeria indicate that Nigeria's Annual fish production from 1960 to 2016 was at an average of 3.6 kg per capita. In contrast, global production of fish has stabilized at an average of 9.0 to 10 kg of fish per capita for 5 decades (FAO, 2001). According to FAO records, between 2011 and 2016, world per capita production rose from 22 to 24 kg. Nigeria's per capita production for the same period staggered between 5.3 and 6.1 kg.

Nigerian consumption of this commodity continues to remain at levels below production fueling huge loss of foreign exchange. In 2016 only 30% of her demand of 2.7 mmt was met by local production. Part of balance was imported at an estimated cost of 125 billion naira (408,496,732 USD). This is in contrast to some other African countries like Cote-d'voire, Ghana and Egypt that produce 33, 50 and 70.8% of their demand (Proshare, 2016, World fish center, 2016). Table 1 shows that Nigeria trails some selected countries in per capita consumption of fish.

While world per capita aquaculture production trended progressively from about 9 to about 11 kg between 2011 and 2016, Nigeria's per capita aquaculture production remained below 2 kg during the same period (FAO, 2018). In addition to low level of consumption, and loss of foreign exchange (which negatively impacts development in other sectors), low production level have also constrained the ability of the sector to generate income through the marketing chains. To elicit development, fish production has to greatly improve. Improvement in fish production is also necessary to guarantee future supply of animal protein.

According to USAID SPARE (undated), the estimated increase in world consumption of fish from 2017 to 2030, implies that the world will need 1500 to 160 million tons of aquatic food. Of this, the capacity of capture aquatic food is limited to 100 million tons, in which case aquaculture will need to provide a good portion of the supply. This is especially as livestock production as well as capture fisheries systems are reaching their productive limits (USAID SPARE, undated). Thus aside its role in making up for the inability of the capture fish, aquaculture will have to assume a prominent role as animal protein source. This role is enlivened by the advantage it enjoys over capture fish and livestock. For instance, Aquaculture is relatively more efficient mass producer of animal proteins than an array of animal protein sources (Costa-Pierce, 2002). For example, production efficiencies of edible mass for aquaculture range from 2.5 to 4.5 kg dry feed/kg edible mass compared with 3.0 to 17.4 kg for conventional terrestrial animal production systems (USAID SPARE, undated).

In Nigeria, the role of aquaculture as key supplier of fish is gradually becoming prominent as the capture fish has also been declining. Data on Nigerian fish production indicate that the aquaculture sector had steeper slope. Evident from the data also is the fact that total supply, which had overlapped with capture fish supply all along – up till the late 1990s, (owing to the negligible contribution of aquaculture) had begun to deviate from capture fishery owing to increased contribution from aquaculture. Consequently, the gap between capture fish and aquaculture supplies is being bridged. Point is, in recent times, aquaculture has begun to assume a prominent role.

Per capita Aquaculture production in Nigeria has been trending positively since 1960 at an average of 6.1%. Aquaculture contribution to total fish supply, though on the increase as well, has been low, providing only about 12.56% on the average with capture fish accounting for the bulk of fish protein supply**.** In the last 2 decades, aquaculture has accounted for up to 18.1% of total fish production in the country. More recent data from FAO, CBN and NBS show that between 2011 and 2015, contribution from aquaculture averaged 28.2%. This is an improvement from the contribution of 4.8% between 1960 and 2007. This recent improvement owes much to the support provided for aquaculture by the recent civilian administrations. The increased production may also have stemmed from supply responses to increased economic growth under these administrations (Onuche et al., 2015a). Within the last few years however, the steady growth enjoyed under the last 20 years has been waning. In fact by 2016, a negative growth rate had been recorded after the subsector experienced a decline from 317mmt in 2015 to 307 mmt (FAO, 2018). This follows from the recession in the economy, beginning 2016. Generally however, aquaculture holds great promise.

The potentiality of this subsector however needs focused policy attention to achieve sustainability in production. FAO (2018) data indicate that despite recording a generally encouraging growth and outperforming artisanal fisheries in terms of growth rate in recent years; its growth rate has declined. Conscious sustained efforts are therefore required to enable it provide the bulk of fish supply in the future. Such efforts will have to rely on sustained production and cost efficiency (Ekunwe and Emokaro, 2009; Ogundari, 2008; Awoyemi and Adekanye, 2005). But to attain increased efficiency levels in production and costs, improved production techniques and materials are important.

Nigeria's aquaculture resources are enormous and can provide up to 2.5 mmt (FAO, 2006, Federal Department of fisheries (FDF), 2008), that is, about 93% of Nigeria's 2016 fish needs. But capacity utilization has been very poor, providing only 28% of total supply in recent time; although, second to Egypt in Africa, Nigeria's production (based on 2016 estimate) of 307 mmt is about just 22.4% of Egypt's 1371 mmt.

Aquaculture in Nigeria is basically small scaled-ranging from homestead concrete ponds of 25 to 40 m^2 to small earthen ponds of 0.02 to 0.2 ha (FAO**,** 2005). Compared to southern Nigeria, the volume of fish farming

Table 1. Per capita fish consumption in selected countries.

Country	Per capita consumption (kg)	Year of estimate
China	48.3	2016
Europe	27.0	2016
Korea	78.5	2016
Nigeria	11.0	2016
Malaysia	58.6	2016
Egypt	23.5	2014
Cote d'voire	13.5	2016

Source: Authors' compilation from miscellaneous data sources.

in central and northern Nigeria is low. Fish farming in Kogi state is particularly nascent. Aquaculture in the state is basically on a homestead mono culture basis, except for a handful of commercial fish farms. Production takes place 2 to 3 times yearly and it is characterized by high production cost and low profit (Ogbe et al., 2018). According to FAO (2007), a 2004 data indicated that Kogi state had 32 (1.2% of the number of) fish farms across Nigeria. Edo state in the south had as high as 420 fish farms while Borno state in the north had just two. Fingerlings are sometimes sourced from the wild while feeds are most locally sources although foreign feeds are more reliable. Technological investment in the subsector (except for a few farms) is thus generally poor. Farmed fish species in Nigeria at present include, Catfish, Tilapia and Carp. Prominent among these three is Catfish (*Clarias gariepinus* or *Heterobrancus longifilis*) due to its higher market value which is two to three times that of tilapia (Olagunju et al., 2007). Faster growth rate, adaptability to changes in production conditions and its wide acceptance are other reasons why catfish production has received the most attention. While growth in the subsector has been generally heartwarming in the last 2 decades (albeit with some discouraging trend in recent times), the gap needed to be covered by aquaculture remains enormous. Reducing this gap has to be through conscious proactive policy formulation and implementation investment in the subsector. These investments have to be made in the areas of technologies and techniques. Proven innovations exist across the globe but the level of investment in adoption in Nigeria leaves much to be desired. Poor management practices leading to technical inefficiency identified for crop agriculture (Kolawole and Ojo, 2007, Ogundari, 2008; Iheke and Nwaru, 2015) also persist in aquaculture (Tsue, 2010; Ekunwe and Emokaro, 2009). The level of technology in the production process has remained the bane of the Nigerian agricultural sector, aquaculture inclusive. This is has constrained the sustainability of aquaculture (Ogbe et al., 2018).

Sustainable aquaculture production will guarantee food sufficiency, provide employment opportunities and reduce the pressure of on our foreign reserves. Hence, there is the need to develop the subsector. Efforts to boost production in the past have been basically focused on provision of equipment, materials and credit. These have not elicited much result. Sustainable aquaculture will have to be built on an improved management and application of improved technologies. But how do aquaculture practitioners perceive and adopt improved management skills and technology? To adopt a technology, a farmer needs to have perceived the innovation, that is, perception precedes uptake of innovation (Asrat and Simane, 2018) based on a need to be met (Damanpour and Schneider, 2006), e.g. a better alternative. And if a farmer knows that an innovation is more profitable than the current alternative, he makes effort to adopt it. Adopting innovations is also dependent on some socio-cultural factors (Asrat and Simane, 2018). For instance, a positive response, of increased production may be intended but could be hampered by lack of resources (Muchapondwa, 2009, Onuche et al., 2015a). In fact, Alomia-Himojosa et al. (2018) reported that adoption decisions in Nepal were hampered by labour scarcity, cultural preferences and poor access to inputs.

Adoption could be planned (where government policies and programmes push for implementing a project) or autonomous (at individual small scale level). Country wise agricultural adoption has not been successful in the Nigerian agriculture. This is largely because the Macro-Meso-Micro agricultural economic framework is not completely unified (Akinyosoye, 2005). Although these tiers have similar aspirations in agricultural development, the federal, States and local governments are not in concert as regards approaches to agricultural development. Hence, innovation adoption has been largely limited to the autonomous domain.

Individual farm level autonomous adoption of innovation requires the succinct understanding of the drivers of innovation adoption. This will help engender sustainable and productive policy targets. As Asrat and Simane noted in 2018, an empirical understanding of the perception and adoption of new techniques in aquaculture, and their determinants provides clear information and hence, better insight into how policies can be adjusted to help address the challenges of sustainable development (Boston University, 2018). Furthermore, acquaintances with elements of innovation adoption will scale up developmental process (Mottaleb, 2018) since this will lead to improved uptake of other innovations (Wisdom et al., 2014). The understanding of the drivers of adoption will provide encouragement for innovators and further engender development.

Certain low level improved technologies have been introduced in Nigerian aquaculture subsector in the past few years. These include fingerlings from certified hatcheries as against fingerlings in the wild, pelleted floating feed as against unpelleted and pelleted sinking feed, use of drugs/chemicals in fish ponds to reduce pollution and use of mobile ponds. Fingerlings from certified Hatcheries have advantages of higher growth rate and productivity over fingerling sourced in the wild. Similarly, floating pelleted feeds enjoy the advantage of eliciting low level of pollution as the level of non-floating feeds are usually visible, allowing the farmer to adjust the feeding frequency. Finally, the use of some drugs (antibiotics) has been shown to reduce the level of water pollution, thereby reducing the frequency and, in essence, the cost of changing water. The fourth is mobile ponds which has the advantage of flexibility in location. These innovations are cost saving and production increasing measures. They are usually disseminated through personal contacts and a skeletal extension delivery system. They are also cheaper and easy to adopt relative to other innovations. We concentrate on these cheap, easy-to-use and easy-to-adopt technologies since the area is resource poor (Kogi state is one of the poorest state of the federation and is plagued perennial issue of nonpayment of staff salaries) and aquaculture is an up an coming venture there. Other (more expensive/more technical) innovations relevant to fish farming include: Automated feeders, Aquaponics (a combination of aquaculture and hydroponic), water re-circulatory system, aeration and cage culture.

Works on adoption in agriculture have shown various drivers of adoption. Wandji et al. (2012) applied univariate dichotomous Logit to data on farmers' perception and adoption of new aquaculture technologies in the west high lands of Cameroon. Ainembabazi (2014) investigated the role of farming experience on the adoption of agricultural technologies by small holder farmers in Uganda. Mudombi (2015) investigated the adoption of improved sweet potato in Wedza community of Zimbabwe while Alomia-Hinojosa et al. (2018) explored farmer perceptions of agricultural innovations for maize legume intensification in Nepal. Deresa et al. (2011) have also studied the perception of and adoption to climate change by farmers in the Nile basin of Ethiopia. Guteta and Abegaz (2015) studied factors influencing on scaling up of agro forestry based spatial land use integration for soil fertility management in southwestern Ethiopia. Asrat and Simane (2018) investigated the perception of climate change and adaption strategies in dry lowland and wet lowland areas of Dabus watershed of North-West Ethiopia, using the Heckman sample selection model.

Empirical reports from primary efficiency studies have relayed the management level of aquaculture in the country and the attendant impact on production. These studies have not treated how aquaculture practitioners perceive and adopt innovations. This study focused on the perception and adoption of innovation by aquaculture practitioners in Kogi State, Central Nigeria. The following questions guided the study: How do aquaculture practitioners perceive new technologies and management skills? What is the level of uptake of these innovations? And what factors influence their perceptions and uptake? Thus the objectives of the study were to: (1) analyse the perception and adoption of the innovations and (2) determine the drivers of perception and uptake of the innovations in Kogi State.

MATERIALS AND METHODS

Study area

Kogi State lies between latitude 6°30'N and 8°48'N and line of longitude 5°23'E and 7°48'E in central Nigeria (Figure 1). It consists of twenty one government areas. Kogi state consists of a timbered grassland region bisected by the southward-flowing Niger River. The confluence of Benue and Niger is found within the capital town. The Benue watercourse, a significant tributary of the Niger, forms a part of the state's northeastern border. The total land area of the state is 28,313.5 km^2 and consists of a wide stretch of arable land for farming, good grazing ground for livestock and large bodies of water for fishing (Encyclopedia Britannica). Agriculture is the mainstay of the economy and provides employment for the majority of the population who are involved with the production of yams, cassava, rice, sorghum, beans, maize, pea nutty and cotton. Homestead husbandry, aquaculture and fisheries activities also are undertaken within the space. Data from www.citypopulation.de (2017) indicate that the population of the state increased from 3.3 million in 2006 to about 4.5 million in 2016. Kogi climate is marked by 2 distinct seasons: The wet season (mid-April to October) and the dry season that spans through (November to mid-April).

Sample and data

The sample for this study was generated from the register of Aquaculture Association of Kogi State. All known practicing aquaculturist (307) in 2018 were interviewed. It was considered in the study that since only 307 of the less than 500 registered practitioners in the area were in operation when the survey was conducted, sampling will further decrease the number of respondents. Hence, total enumeration was embarked on. Data on socio-economic characteristics, perception and adoption of innovation were generated using FGD and structured questionnaire.

Following Asrat and Simane (2018), the use of any 2 or more of the identified 4 common innovations as an adoption was noted in this study. In this case, a fish farmer who adopted at least 2 of the 4 innovations scored 1 in the Probit model. Adoption of one or none of the adoptions scored 0. This method was also applied to the data on perception. In order to analyse perception, the respondents were asked if they felt that the innovations were likely to have positive impact on their productivity (and by implication profit). Perception was treated as a dichotomous variable: An individual either perceives an item - as being useful or does not. In this study, a

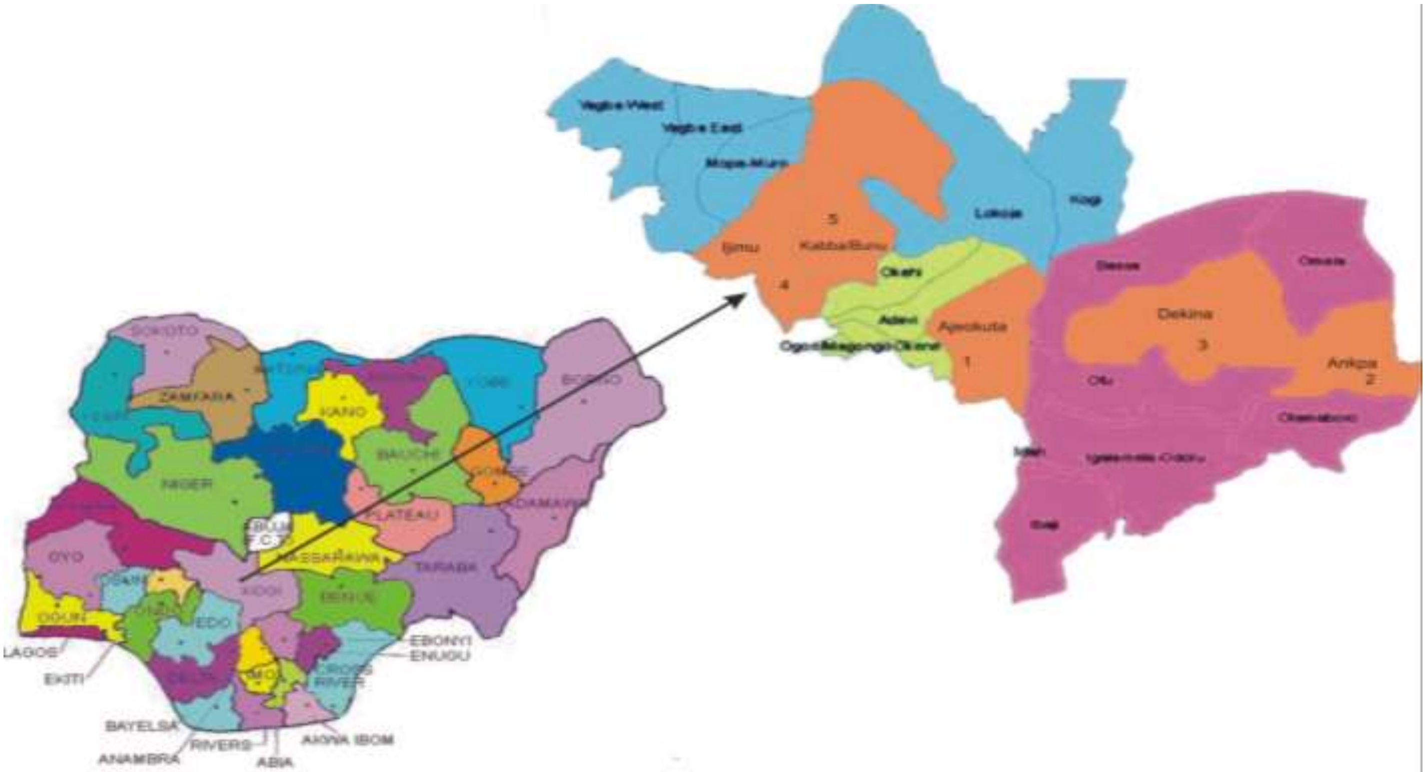

Figure 1. Map of Nigeria showing Kogi state (enlarged).

positive response implies perception (1), otherwise zero. The scores generated were used as the dependent dichotomous variable of the ith respondent in a double Probit model (Heckman's sample selection model). Seven copies of the questionnaire were either not returned or not valid for data extraction. Hence, analysis was restricted to 300 copies.

Method of data analysis

Descriptive statistics and the Heckman sample selection model were employed in the analysis of the data. While the descriptive were used to analyse socioeconomic variables, perception and adoption, the Heckman sample selection model was used to determine factors influencing perception and adoption. The working of the Heckman sample selection model is based on Heckman (1976) as quoted by Asrat and Simane (2018), where sample selection is deemed important in the event that the farmer's adoption decision involves more than one step. First the farmer needs to perceive that the innovation is of superior advantage to his present method/tool. In this case, two-step regressions, such as Heckman's sample selection, are appropriate to correct for selection bias generated during the decision making processes. The model relies on the assumption that due to the farmer's utility/profit maximizing behaviour, he will only adopt an innovation if it is perceived that the derivable utility profit is significantly greater than his prevailing alternative (Asrat and Simane, 2018).

It is possible that the second sample (that is, a sub- sample of the first) which consists only of the fish farmers that adopted the innovation is non-random and necessarily different form the first (which include those who did not perceive the innovation as well as those who did). This leads to sample selection bias (Deresa et al., 2011) which has to be corrected. This correction is undertaken by the use of the Heckman's maximum likelihood procedure (Asrat and Simane, 2018).

Heckman's sample selection model is given by:

$$y_i=bQ_j + u_{1j} \quad (1)$$

In which case, we observe only the binary outcome in the probit model as:

$$y_i^{prob}=(y>0) \quad (2)$$

The dependent variable (assumed to be influenced by Q) is observed only if j is observed in the selection equation given as:

$$y_i^{select}= aX_j + u_{2j}>0 \quad (3)$$

Where y_i^{select} is the model which concerns whether a fish farmer has perceived an innovation or not, X_i is the vector of independent variables assumed to have influence on perception and, a and b are regression coefficients. Equation 3 represents the first stage of the Heckman's two-step model. u_1 and u_2 are error terms assumed to have the standard econometric properties of random distribution, null mean and unity variance.

Equation 1 is the outcome model otherwise noted as $Y_{outcome}$, which analyses whether the aquaculturist adopted the innovation ($Y_{outcome}$=1) or not ($Y_{outcome}$=0), and is dependent on Equation 3, the perception model where Y_{select} = 1 if the ith farmer perceived the innovation and Y_{select}=0, otherwise. Explicitly, the equations are given as:

Outcomemodel:$Y_{outcome}$= b_0+$b_1$techknowhow +$b_2$accesscredit+$b_3$costofadopt+$b_4$otherincom+$b_5$edu+$b_6$extcontact+$b_7$age+$b_8$ponds+$b_9$gen

Table 2. Descriptive statistics of key demographic variables of fish farmers in Kogi State.

Variable	Mean	Std. Dev.	Minimum	Maximum
Monthly income	26275	17172.09	1200	70000
Number of pond	2.27	1.08	1	5
Extension advise (contact)	1.02	1.66398	1	3
Experience	4.3	3.34	2	15
Family size	6.7	4.6	2	12
Age (years)	51.24	19.56	21.2	75
Education (years of schooling)	15.3	9.5	5	23
Gender				
Male	277(92.3%)			
Female	23(7.7%)			

Source: Authors' computation from field survey data, 2018.

der+ b_{10}primaryoccup+ b_{11}exp + b_{12}distan+ b_{13}inforsource +u_1 (4)

Selection model Y_{select}=a_0+$a_1$edu+$a_2$extcontact+$a_3$age+$a_4$gender+a_5 primaryoccu+$a_6$exp+$a_7$distan + $a_8$inforsource + u_2 (5)

Where: Techknowhow=Technical know-how (1 if positive, 0 otherwise); Accesscredit=access to credit (1 if positive, 0 otherwise); Costofadopt= cost of adoption ('000 Naira); Otherincom= other income i.e income from sources other than fish farming ('000 Naira); Edu= education (years of formal education); Extcontact= extension contact (number of contacts with extension personnel for advice); Age= age of fish farmers (years); Ponds =pond size (square meter); Gender= gender of fish farmer (male =1, female=2); Primaryoccup= primary occupation; Exp= experience in fish farming in years; Distan= distance from fish farm to urban center (km); Inforsource: sources of information (1 interpersonal, 2=extension worker, 3=prints, 4= electronic); The interdependence of error terms, u_1 and u_2 (that is $\rho \neq 0$), leads to biased estimates from the standard Probit technique (Asrat and Simane, 2017). As such the Heckman Probit will provide consistent and asymptotically efficient estimates for all the parameters in the model.

RESULTS AND DISCUSSION

Table 2 presents the summary of key demographic variables. The mean average income suggests high level of income poverty in the area. This reflects also on the size of the ponds which relays the small scale nature of the venture in the area. Extension contact was very low. This low level of extension contact could impact perception and adoption and greater number of extension contact will elicit favourable disposition to the adoption of innovations. The table also shows that the fish farmers in Kogi state were moderately educated (average years of education = 15.3). The population of fish farmers in the area is an ageing one with a mean of 51 years. Average household size is about the national household size and extension visits to fish farmers in the area is very low. The venture is also male dominated in the area. Ogbe et al. (2018) have documented the socioeconomic and production characteristics of catfish farmers in Kogi state.

Table 3 presents the perception and adoption of aquaculture innovations in Kogi state. The use of pelleted feed recorded the highest adoption level followed by use of fingerlings from certified hatcheries. Use of mobile pond is the least adopted. Accordingly, the use of pelleted feed was the most adopted while the use of mobile ponds was the least adopted. This is probably due to the relatively higher cost implication. Aggregate data indicate that more than half of the fish farmers in the area perceived these innovations while only 38% was recorded for adoption. This buttresses the point that although perception precedes adoption, it does not necessarily translate to adoption.

Result of the Heckman sample selection Probit model is presented in Table 4. The null hypothesis of no dependence of error terms is rejected since the rho (0.58) is statistically greater that zero (Wald $\chi 2$ =4.16, p<0.05). That is, there is the presence of sample selection problem. This supports the suitability of the procedure. Furthermore, the likelihood function of the Heckman Probit model was significant (Wald $\chi 2$ = 132.16, P < 0·0001), implying that it has a strong explanatory power. Result of the selection model indicate that education (a=0.281), extension contact (a=0.149), experience (a=0.021), gender (a=0.440) and information source *(a=0.145)* increased likelihood of positive perception of the identified innovative practices. Education improves understanding of a concept and thus enhances correct perception. Niles and Mueller (2016) submitted that effective adaptation requires knowledge (education) and understanding. Extension contact on its own part provides knowledge and is also key to understanding and perceiving innovations. Extension contact frequency was found to be positive in influencing the uptake of aquaculture innovation in Cameroon by Wandji et al. (2012) and Alomia-Hinojosa et al. (2018). As Tripathi and Mishra (2017) summed the connect between education and extension contact in their submission that right perception is dependent on knowledge and ease of access to information, and that knowledge depends on the educational attainment and experience of the person.

Table 3. Perception and adoption of aquaculture innovation in Kogi state.

Innovation	*Perception	*Adoption
Patronage of certified hatcheries	139 (46.3%)	81(27.0%)
Use Pelleted feed	147(49.0%)	98(32.7%)
Use of drugs for water treatment	96(32.0%)	65 (21.7%)
Mobile ponds	65(21.7%)	25(8.3%)
Aggregate*	171 (57%)	114(38%)

Source: Authors' computation from field survey data, 2018. *Multiple responses were analysed.

Table 4. Heckman Probit selection model for the uptake of aquaculture innovations.

Explanatory variable	Outcome model				Selection model			
	Regression		Marginal effect		Regression		Marginal effect	
	b	Z	b*	Z	A	Z	a*	Z
Technknowhow	0.116**	2.28	0.116**	2.28				
Credit access	-0.074*	-3.95	-0.074*	-3.95				
Costofadopt	-0.030	-0.76	-0.030	-0.76				
Otherincom	1.17-e06*	7.18	1.17-e06*	7.18				
Education	0.115*	4.51	0.115*	4.51	0.281*	4.18	.0764*	4.49
Extension advice	0.010	0.55	0.010	0.55	0.149**	2.26	.0404*	2.33
Age	-0.095**	-2.25	-0.095**	-2.25	-0.456*	-3.88	-0.124*	-4.08
Pond size	-0.094*	-3.12	-0.094*	-3.12				
Gender	0.110*	2.88	0.110*	2.88	0.440*	3.41	0.119*	3.62
Pri occupation	-0.049	-1.54	-0.049	-1.54	-0.152**	-2.12	-0.042**	-2.16
Experience	0.0004	0.15	0.0004	0.15	0.021*	2.51	0.006*	2.58
Distance	-0.022	-0.55	-0.022	-0.55	-0.484*	-3.61	-0.132*	-3.83
Inforsource	-0.050*	-2.88	-0.050*	-2.88	0.145*	2.48	0.040*	2.54
Constant	-0.012	-0.05			-1.323**	-2.08		
Observation	300							
Censored	129							
Unsensored	171							
Wald chi-square (zero slopes)	132.16 (P < 0·0001)							
Wald chi square (independent equations)	4.16 (P <0.05)							
Rho: 0.597, lambda: 0 .191, Sigma: 0.329								

^{a*}*and* ^{b*} marginal effects of regression coefficients a and b respectively. Source: Authors' computation from field survey data of 2018.

Furthermore, experience with particular techniques may enhance the attention poor farmers give to understanding new methods. Experience was found to exert a positive on adoption by Ainembabazi (2014) in the study of the role of farming experience on adoption of innovation among small holder farmers in Uganda. Again, that the likelihood of perception seems to be higher with male folks may not be unrelated to the disadvantaged position of the female folks. Finally, perhaps, electronic sources have greater appeals in and thus, their higher likelihood for enhancing perception.

Results on perception also indicated that age (a=-0.456), primary occupation (a=-1.54) and distance to urban center (a = -0.55) were negatively related to perception of the innovation. Age has generally been found to diminish to agricultural prospects. Furthermore, if primary occupation was something other than fish farming, the likelihood of non-perception increases. Having an aquaculture firm as the primary occupation implies commercial orientation. Wandji et al. (2012) reported a positive relationship between commercial orientation and innovation adoption. The negative relationship may probably be due to lesser attention an operator gives to the fish farm when it is not the primary venture. This could be because income from these sources may be sufficient for them they may not need to any serious improvement on their fish farms. Finally, distance from urban center implies rural settings. Such settings are less likely to have good information flow, regular extension visits, and poor markets for products. These may diminish any interest in further developing the aquaculture.

Results of the outcome model also presented in Table 4 indicate that Technical know-how (b=0.116), Other income (b=1.17-e06), Education (b=0.115) and gender (b=0.11) had significantly positive influence on aquaculture innovation adoption in the study area. The finding on Technical know-how implies that the ability of a fish farmer to use a particular innovation plays significant role in the farmer's decision to adopt the innovation. Analysis of marginal effect of Technical know-how on the adoption of aquaculture innovation revealed that a unit increase in the level of technical know-how increases adoption by 11.6%. Mudombi (2015) in a study of improved sweet potato adoption in Wedza community of Zimbabwe found that technical training plays a positive in the adoption of the innovation. On farm extension trials which promotes the acquisition of technical know-how was also found by Alomia-Hinojosa et al. (2018) to be positively related to adoption of maize legume intensification in Nepal.

A 1000 naira increase in “other incomes” increases the likelihood of aquaculture innovation adoption in the area by a minimal 0.017%. The implication is that fish farmers are likely to commit some little part of their incomes to adoption of aquaculture innovation. In Asrat and Simane (2018), while results from dry lowland area disagree with this finding, findings from wet low land areas are in support of the positive influence of other income sources. Perhaps aquaculture participants in the area see additional “other income” as opportunity for adoption of innovation so as to elicit sustainable development in their aquaculture venture, instead of perceiving it as an improvement in income which is capable of making them less dependent on aquaculture

Further marginal effect estimates indicate that adoption of aquaculture innovation increased by 11.5% for an additional year of formal education. A higher level of education confers a greater reasoning and comprehension capability on an individual and also makes for easier understanding of accessed information. The finding in this study is in tandem with those found elsewhere. Wandji et al. (2012) found a positive influence of education by in a study of aquaculture innovation uptake in west high lands of Cameroon. Asrat and Simane (2018) also found a similar result in a study of perception and adoption of climate change mitigation strategies in Dabus region of Ethiopia

Male folks have a higher likelihood of adoption aquaculture innovation than female folks in the area. Guteta and Abegaz (2015) and Asrat and Simane (2018) also reported similar results. These studies argued that women are so encumbered with a plethora of chores that they hardly find time to investment in innovations. Moreover, since they are less likely to have access to productive resources, it is usually difficult for them to invest in adoption of innovation. This scenario plays out in the lager part of underdeveloped African countries, including central Nigeria.

Conversely, access to credit (b=-0.074), age (b=-0.095), pond size (b=-0.094) and information source (b=-0.05) negatively influenced adoption. Since fish farmers in the area may be willing to invest some of their income from other sources in adoption of innovation, it would have been easy to conclude that access to credit will increase their adoption of aquaculture innovation. But this is not so. The likelihood of innovation adoption by fish farmers in the study area decreased by as much as 7.4% for an addition increase in access to credit. It is possible that fish farmers in the area will want to invest accessed credit in some other ventures in a bid to diversify income bases.

Age, as in the case the selection model, showed a negative influence on adoption. Marginal effect analysis indicate that the likelihood of adopting aquaculture innovation the area decreased by 9.55 for any 1 year increase in age. This disagrees with the finding of Asrat and Simane (2018). Many ageing aquaculture practitioner in the area are civil servants or retirees (Onuche et al., 2015b) and may not see the need to adopt innovation due to their guaranteed income or due to the vigour associated with knowledge seeking and practical demonstration of innovations.

Increase in ponds size by a square meter reduced the likelihood of adoption 9.4%. This implies that smaller aquaculture farms are more likely to adopt an innovation. This may not be unrelated to the fact that sizes of agricultural enterprises have financial implications that may hinder adoption. Deresa et al. (2011) reported similar findings. A reduction in likelihood of adoption was observed when the information source is less sophisticated print and electronic, implying preference for personal contact at the adoption stage.

CONCLUSION AND POLICY RECOMMENDATIONS

This investigation was undertaken to explore the drivers of the perception and adoption of some aquaculture innovations in Kogi State, central Nigeria. The goal was to identify those factors that evoke perception and adoption, for policy engendering in order to achieve sustainable aquaculture production. Findings might also be useful in promoting other aquaculture innovations, including those of other sectors. The study found that adoption of aquaculture innovation in the area was high and could be enhanced by embarking on policy frameworks built around the identified perception and adoption drivers in the area. Education, extension contact, experience, gender, and information source were identified as the drivers of perception. The drivers of adoption on the hand include Technical know-how, “other income", education and gender. Policy mixes will have to tinker with certain variables or related variables that have conflicting influences in perception and adoption, in addition to the identified drivers. For instance, the study noted that while electronic sources enhanced perception, adoption relied more on personal contact with innovators and extension personnel, indicating the need to combine the two categories of information sources at different points. Also, proper understanding of the how to finance adoption is vital as we found that credit did not drive adoption but fish farmers were willing to commit a part of income from other sources to adoption. Thus attention may be better focused on assisting participants increase their incomes from other sources rather than granting them access to credit. Closely related to this was the fact that primary occupation other than aquaculture exerted negative influence but fish farmers were inclined to committing a little part of their incomes from those occupations to adopting innovations. Furthermore, although extension contact positively influenced perception, it was not significant in influencing adoption. The low intensify of extension activity in the area may not have been impactful enough in inspiring adoption. Thus, while extension contact may be viable in eliciting perception, more contacts will be required to reduce adoption. Higher intensity of extension contacts should also be implemented to provide training on "technical know-how" to elicit higher levels of adoption. Policy engendering must also consider the issue of gender, as it was noted that the female folks were disadvantaged in perceiving and adopting innovation

From the foregoing, the following recommendations are proffered. First, emphasis may be place more on electronic sources during the introduction stage of the innovation while more practical oriented information sources like the personal contacts should be exploited thereafter. Second, assisting aquaculture practitioners increase their incomes from other sources through increased wages/pension, increased credit for expansion, or price adjustment might be a better alternative to funding adoption than providing them with credit for aquaculture. Next, increased impact of extension delivery system will be achieved by paying more attention to practical training to enhance the acquisition of requisite techniques that will assist in improving the likelihood of adopting aquaculture innovations. Finally, a case was made for gender consideration in policy mixes as the female folks appear disadvantage in perceiving and adopting aquaculture innovation in the area. Special arrangements like more access to productive resources and reduction of their domestic burdens will improve on their chances of perceiving and adopting innovations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of pre-rice cassava/legume intercrops and weed management practices on weed dynamics and yield of low land rice in Badeggi, Nigeria

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The effects of cassava/legume intercrop-rice relay and weed management practices on weed infestation, growth and yield of rice were investigated at Badeggi, Southern Guinea Savanna of Nigeria in 2011 to 2013 cropping seasons. The treatments consisted of factorial combination of cassava (IIT 427) intercrop with: Mucuna or Velvet bean [*Mucuna pruriens* (L.) DC.], Cowpea [*Vigna unguiculata* (L.) Walp.], Soybean [*Glycine max* (L.) Merr.], Hyacinth bean [*Lablab purpureus* (L.) Sweet.] and Porcupine Jointvetch (*Aeschynomene histrix* Poir.) and weed management practices: (i) application of propanil at 1.44 kg a.i ha^{-1} plus 2,4-D at 0.80 kg a.i ha^{-1} (Orizo Plus®) at the rate of 2.24 kg a.i ha^{-1} at three weeks after transplanting (WAT) rice followed by hoeing at 6 WAT, (ii) two hoeing at 3 and 6 WAT, (iii) one hoeing at 3 WAT, and (iv) weedy check with sole cassava and natural fallow as control laid in a split plot arranged in a randomized complete block with three replications. Across cassava/legumes intercrops, cassava/mucuna had lower weed density and dry matter, cassava/*Aeschynomene* and cassava/cowpea produced comparable taller rice plants, more rice panicles and paddy yield, and cassava/*Aeschynomene* produced greater number of rice tillers. Irrespective of the weed management practices, two hoeing at 3 and 6 WAT gave better weed control, taller rice plants, greater number of tillers and panicles, and higher paddy yield comparable to application of Orizo Plus® at 3 WAT followed by one hoeing at 6 WAT. This study suggests that sustainable weed control with *Mucuna* intercrop and rice productivity with *Aeschynomene* and cowpea intercrops can be achieved with two hoeing at 3 and 6 WAT or application of Orizo Plus® at 3 WAT followed by hoeing at 6 WAT in this agro-ecology of Nigeria.

Key words: Intercrops, legumes, *Oryza sativa* L., paddy yield, weed suppression.

INTRODUCTION

Weeds are a major biotic constraint to increased rice production worldwide. Its occurrence is a constant component of the ecosystem in comparison with the epidemic nature of other pests which makes farmers

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unaware of the significant losses they incur from their infestation (Ismaila et al., 2015). Farmers can spend over US $400 ha^{-1}, or 20% of their production costs to control weeds during the growing cycle (Islam et al., 2005). Weeds can cause serious yield reduction in rice production worldwide. Losses caused by weeds vary from one country to another, depending on the predominant weed flora and the control methods practised by the farmers (Mishra et al., 2016). The extent of loss varies depending upon cultural methods, rice cultivars, weed species and the density and duration of competition. For example, uncontrolled weed growth is reported to have caused 28 to 74% yield losses in transplanted lowland rice, 28 to 89% in direct-seeded lowland rice, and 48 to 100% in upland ecosystems (Rodenburg and Johnson, 2009). The potential yield loss from weed is less in transplanted rice than in dry-seeded rice (Joshi et al*.,* 2013).

However, improved weed control has been estimated to increase rice yields by 15 to 23%, depending on production system in the ecosystem (Rodenburg and Johnson, 2009). It is rare, however, for farmers not to undertake some form of weed control and therefore losses on farmer' fields are likely to be less depending on the control measures adopted. Common weed management practices in rice-based cropping systems include soil tillage, clearance by fire, hand- or hoe-weeding, herbicides, flooding, fallow and crop rotations, and these are often used in combinations (Rodenburg and Johnson, 2009). Good cultural practices cannot be underestimated in their importance to weed management. Most, if not all of these cultural methods should be a necessary part of crop management procedures in controlling weeds (Gbanguba et al., 2011)**.**

The management of weeds requires integrated strategies to be successful. The combination of direct weed control methods, such as herbicides or hand weeding, with indirect methods such as land preparation, flooding and competitive crops, can suppress weed growth. The optimum combination of weed control methods will depend on the farming system, economic conditions, and farmers' resource and knowledge base (Johnson, 2009). During the off-season, rain fed rice lands are typically fallowed (Gbanguba et al., 2014). The straw and fallow weed vegetation are subjected to grazing by livestock. In a minor fraction of the area with conducive residual soil water-holding capacity, and/or a high ground water table, upland crops, including legumes, are grown in the post-rice season. This practice is most common in well-drained rice lands. In this case, upland crops are grown prior to rice during the dry-to-wet season transition period. Very short duration crops are advantageous to permit maturity before the soil becomes waterlogged. Rotating crops with different planting dates and growth periods, contrasting competitive characteristics and dissimilar management practices can be used to disrupt the regeneration niche of different weed species in rice field. For example, Liebman and Davis (2000) reported that *Bromus tectorum* (L.) density remained relatively stable when winter wheat (*Triticum aestivum* L.) was rotated with oilseed rape (*Brassica napus* L.), whereas the density of the weed increased rapidly when wheat was grown continuously. Also, Liebman and Davis (2000) mentioned that *Setaria faberi* Herrm. seedling density tended to be greatest in continuous maize, intermediate in a two year maize/soybean rotation and lowest in a three year maize/soybean/winter wheat rotation. A well-planned crop rotation system can help producers avoid many of the problems associated with weeds, particularly perennial weeds (Mohler, 2012). Crop rotation is an effective practice for controlling weeds because it affects weed growth and reproduction negatively and in turn reduces weed density (Sims et al., 2018). In a previous study, Filizadeh et al*.* (2007) found that rice yields in rotation with soybean were higher by 17 to 21% compared with continuous rice. Anders et al. (2004) reported higher yield of rice in rice grown after soybean than in rice wheat rotation. Toomsan et al*.* (2000) recorded 50% higher rice yields in rice followed cover crop green mixtures than rice in bare fallow rotation. The grain yield of rice preceded by a legume fallow had been reported to be on average 0.2 kg ha^{-1} or 30% greater than that preceded by a natural weedy fallow control (Gbanguba et al., 2014).

In Nigeria, cassava/legume intercropping preceding rice production is a common practice among farmers in the Southern Guinea agro-ecology. But it is not known, how this practice under varying weed control methods affects weed growth, growth and yield of rice in this region. Thus, this study was aimed at evaluating the effect of one year rotation of pre-rice cropping with cassava/legume intercrops and weed management practices on weed suppression, growth and yield of low land rice.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in 2011 to 2013 growing seasons at the lowland experimental field of the National Cereals Research Institute, Badeggi (latitude 09° 45′N, longitude 06° 70′E, elevation 70.57 m above sea level). The area is located in the Southern Guinea Savanna zone of Nigeria with mean annual rainfall of 2066.3, 1163.6 and 899.7 mm distributed between April and October in 2011, 2012 and 2013, respectively. The average maximum and minimum air temperature was 30 to 38°C and 14 to 26°C, respectively. The soil texture of the experimental site was sandy clay. All the soils were moderately acidic with a pH (H_20) around 5.24.

Treatments and experimental design

The treatments were a factorial combination of Cassava (IIT 427) intercrop with Mucuna or Velvet bean [*Mucuna pruriens* (L.) DC.], Cowpea [*Vigna unguiculata* (L.) Walp.], Soybean [*Glycine max* (L.)

Table 1. Influence of cassava/legume intercrops on some soil nutrient status.

Cassava/Legume intercrop	Organic carbon (g kg^{-1})			Total nitrogen (g kg^{-1})			Available phosphorus (mg kg^{-1})		
	2011	2012	2013	2011	2012	2013	2011	2012	2013
Cassava/Mucuna	2.8^b	2.9^b	3.1^a	0.14	0.16^a	0.20^a	30.8^a	32.1^a	32.4^b
Cassava/Cowpea	3.2^a	3.1^a	3.2^a	0.18	0.18^a	0.20^a	31.3^a	31.6^a	34.1^a
Cassava/Soybean	3.2^a	3.1^a	3.1^a	0.14	0.14^b	0.20^a	30.8^a	31.1^a	32.4^b
Cassava/*Lablab*	2.9^b	2.9^b	2.7^b	0.12	0.13^b	0.10^a	29.7^a	30.5^b	31.9^b
Cassava/*Aeschynomene*	3.3^a	3.2^a	3.3^a	0.18	0.18^a	0.20^a	31.4^a	32.0^a	32.7^b
Sole Cassava	2.2^c	2.1^c	2.2^c	0.10	0.08^c	0.05^b	20.5^c	21.8^c	23.3d
Natural Fallow	2.0^c	2.0^c	2.1^c	0.10	0.04^c	0.04^b	28.4^b	29.1^b	29.2^c
SE±	0.04	0.10	0.02	0.40	0.01	0.04	0.30	0.50	0.90

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT).

Merr.], Hyacinth bean [*Lablab purpureus* (L.) Sweet.] and Porcupine Jointvetch (*Aeschynomene histrix* Poir.) legumes, sole cassava and natural fallow. The weed management practices were (i) application of propanil at 1.44 kg a.i. ha^{-1} plus 2, 4 D at 0.80 kg a.i ha^{-1} (Orizo plus^R)) at the rate of 2.24 kg a.i ha^{-1} at 3 weeks after transplanting (WAT) followed by hand weeding at 6 WAT, (ii) two hand weedings at 3 and 6 WAT, (iii) one hand weeding at 3 WAT, and (iv) weedy check. Cassava/Legume intercrop was assigned to the main plot, while weed management practices were assigned to the sub plot. The trial was laid out in a split plot arranged in a randomized complete block with three replications. Main plot size was 12.5 × 5 m and sub plot size was 3 × 5 m replicated three times.

Cultural practices

The experiment was initiated by intercropping of cassava with the legumes in January in 2011, 2012 and 2013 on manually prepared raised beds (2.5 m × 0.5 m × 0.75 m) using residual moisture. Beds were spaced 0.5 m. Cassava (IIT 427) was planted on the top side of the bed in two rows at intra-row spacing of 0.5 m (ten stands per bed) and legumes were planted by the side of the beds at intra-row spacing of 0.25 m, except for soybean (TGX 1019EN) which was drilled at 5 cm intra row immediately the beds were constructed. Cassava cutting and soybean varieties were sourced from the International Institute of Tropical Agriculture, Ibadan. Among the other legumes, *Aeschynomene histrix* was sourced from the International Institute of Tropical Agriculture, Kubwa-Abuja, and cowpea (IAR 48) and *Mucuna* seeds were obtained from the Institute for Agricultural Research, Samaru-Zaria. The cassava/legume cropping lasted till August when cassava was harvested. Superimposition of rice (Faro 52) variety obtained from seed unit of National Cereals Research Institute, Badeggi, on the plots previously cropped with cassava/legume intercrops were levelled for rice cultivation on 6, 5 and 9th August of 2011, 2012 and 2013, respectively. Rice was transplanted at rate of two seedlings per hill at a spacing of 20 cm × 20 cm. Weeding was carried out as per the treatments. Orizo Plus® was applied with a CP3 knapsack sprayer using a spray volume of 250 L ha^{-1} at 206 kPa. Fertilizer application was done by broadcasting in split application of NPK 40: 60: 60 as basal, while N 40 kg ha^{-1} was applied as top dressing. Harvesting was achieved by using sickle. Drying, threshing and winnowing were manually carried out.

Data collection

Before the superimposition of the rice seedling, soil samples were taken from three randomly selected spots from each cassava/legume intercrop plot, bulked and used to determine organic carbon, total nitrogen, and available phosphorous. The method of Walkley and Black (Anderson and Ingram, 1993) was used to determine the organic carbon. Total nitrogen was determined by the macro Kjeldahl method (Jackson, 1962). Available phosphorous was determined by the Olsen method (Okalebo et al., 2002).

Weed samples were collected from 1 m^{-2} quadrant randomly placed in each plot in each year at 9 WAT for determination of weed density, dry weight and control efficiency. All the weed species in each quadrat were counted and clipped above the soil surface; oven dried at 70°C to a constant weight for weed dry weight determination. Rice plant height, tiller and panicle numbers were determined by collecting rice samples from the 1 m^2 quadrat used for weed sampling at 9 WAT. Rice grain yield was obtained from tillers harvested from 2 × 4 m net plot. Tillers were harvested at physiological maturity and manually threshed and winnowed. Grains obtained from the tillers were measured and converted to kg ha^{-1}. Data collected were subjected to analysis of variance (ANOVA) and differences between means were separated using Duncan Multiple Range Test (DMRT) at P≤ 0.05 using M-Stat-C software (M-Stat-C Version 1.3).

RESULTS

Some soil nutrient status after cassava/legume intercrop

A significant effect of cassava/legume intercrop was observed on organic carbon, total nitrogen and available phosphorous in this study (Table). Cassava/Cowpea, ca ssava/soybean and cassava/*Aeschynomene* intercrops in each year and cassava/*Mucuna* intercrop in 2013 added more organic carbon to the soil than all other treatments. Total nitrogen was the highest and comparable in cassava/*Mucuna*, cassava/cowpea and cassava/*Aeschynomene* intercrops in 2012 and 2013, and cassava/soybean or *Lablab* intercrops in 2013 than all other treatments. In terms of available phosphorous, cassava/cowpea intercrops significantly contributed more of this nutrient, which was comparable to cassava/*Mucuna*, cassava/soybean and cassava/*Aeschynomene* intercrops in 2011 and 2012, and cassava/*Lablab* intercrop in 2011 only.

Table 2. Effects of pre-rice cassava/legume intercropping and weed management practices on weed density in rice at 9 WAT.

Treatment	Weed density (no^{-2})		
	2011	2012	2013
Cassava/legume intercrop (I)			
Cassava/*Mucuna*	71.0^{f}	139^{d}	129^{f}
Cassava/Cowpea	102.5^{e}	171^{c}	156^{e}
Cassava/Soybean	123.3^{d}	188^{c}	169^{cd}
Cassava/*Lablab*	137.4^{c}	200^{b}	178^{c}
Cassava/*Aeschynomene*	126.4^{d}	188^{c}	152de
Sole cassava	165.0^{b}	224^{b}	218^{b}
Natural fallow	`199.8^{a}	325^{a}	377^{a}
SE±	2.9	6.1	4.4
Weed management practices (W)			
Orizo Plus fb hoeing at 6 WAT	170^{c}	172^{c}	172^{c}
Two hoeing at 3 and 6 WAT	165^{c}	168^{c}	152^{c}
One hoeing at 3 WAT	223^{b}	225^{b}	210^{b}
Weedy check	266^{a}	229^{a}	246^{a}
SE±	2.2	4.6	3.3
Interaction			
I × W	*	*	*

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT). *Significant at 5% level of probability. fb: follow by; WAT: weeks after transplanting.

Weed density

Rice grown after cassava/*Mucuna* intercrop produced the least weed density compared with other intercrops in each year of the study (Table 2). Furthermore, weed density produced in rice after cassava/legume intercrops and natural fallow ranged from 29.5 to 47.2%. Rice grown after natural fallow consistently had the highest weed density followed by sole cassava.

On weed management practices, application of Orizo Plus fb hoeing at 6 WAT and two hoeing at 3 and 6 WAT produced similar lower weed densities in 2011, 2012 and 2013 rainy seasons (Table 2). Weedy check accounted for the highest weed density in both years of the study which ranged between 30.0 and 38.0% over Orizo Plus fb hoeing at 6 WAT, and two hoeing at 3 and 6 WAT.

A significant ($p \leq 0.05$) interaction between cassava/legume intercrops and weed management practices was recorded for weed density in each year in this study (Table 3). In 2011, weed density was significantly ($p \leq 0.05$) lowest in cassava/*Mucuna* intercrop in combination with each of the weed management options. Furthermore, in 2012 and 2013, cassava/*Mucuna* in combination with Orizo Plus fb hoeing at 6 WAT and two hoes weeding at 3 and 6 WAT caused similar significant decrease in weed density. Similarly, reduction of weed density by cassava/*Mucuna* intercrop in combination with one hoe weeding at 3 WAT was comparable to that recorded in cassava/cowpea intercrop in 2012 and 2013. Irrespective of the weed management practice, the highest weed density was observed in natural fallow which were similar for each weed management practice in the three years of the study.

Weed dry matter

Table 4 shows that the lowest weed dry matter was obtained in rice grown after cassava/*Mucun*a intercrop compared with others throughout the period of study. Weed dry matter production in rice grown after sole cassava and natural fallow was found to be 16.7 to 56.9% and 31.2 to 64.5% more than that of the intercrops, respectively.

Considering weed management practices effects, application of Orizo Plus fb hoe weeding at 6 WAT and hoe weeding at 3 and 6 WAT had similar lowest weed dry matter, than the other treatments in the study period (Table 4).

Irrespective of the weed management practice, maximum weed dry matter was recorded in combination natural fallow with each of the weed management option

Table 3. Interaction between cassava/legume intercrops and weeds management practice on weed density at 9 WAT.

Treatment	Orizo Plus fb hoeing at 6 WAT	Two hoeing at 3 and 6 WAT	One hoeing at 3 WAT	Weedy check
		2011 Rainy season		
Cassava/*Mucuna*	111.0^{q}	111.0^{q}	178.0^{jkl}	223.0^{f}
Cassava/Cowpea	139.0^{op}	139.0^{op}	197.0^{ghi}	240.0^{e}
Cassava/Soybean	160.0^{mn}	160.0^{mn}	204.0^{gh}	246.0^{de}
Cassava/*Lablab*	173.0^{j-m}	173.0^{j-m}	209.0^{fg}	262.0^{cd}
Cassava/*Aeschynomene*	166.0^{l-n}	166.0^{lmn}	203.0^{gh}	246.0^{de}
Sole cassava	187.0^{hij}	187.0^{hi}	251.0^{de}	270.0^{c}
Natural fallow	258.0^{cd}	258.0^{cd}	322.0^{b}	375.0^{a}
SE±	3.82			
		2012 Rainy season		
Cassava/*Mucuna*	111.0^{op}	98.0^{p}	159.0^{j-m}	221.0^{fgh}
Cassava/Cowpea	240.0^{def}	134.0^{n}	184.0^{ijk}	221.0^{fgh}
Cassava/Soybean	174.0^{ijk}	166.0^{i-m}	270.0^{cd}	248.0^{ef}
Cassava/*Lablab*	158.0^{k-n}	150.0^{lmn}	187.0^{h-k}	227.0^{efg}
Cassava/*Aeschynomene*	158.0^{k-n}	150.0^{lmn}	187.0^{h-k}	227.0^{efg}
Sole cassava	195.0^{ghi}	193.0^{g-j}	246.0^{def}	264.0^{cd}
Natural fallow	264.0^{cd}	282.0^{c}	337.0^{b}	426.0^{a}
SE±	4.6			
		2013 Rainy season		
Cassava/*Mucuna*	100.0^{q}	96.0^{q}	163.3^{j-n}	187.0^{k-o}
Cassava/Cowpea	133.0^{op}	129.0^{p}	165.0^{j-m}	198.0^{fgh}
Cassava/Soybean	150.0^{l-o}	145.0^{mno}	173.0^{h-l}	210.0^{efg}
Cassava/*Lablab*	161.0^{k-n}	155.0^{k-n}	179.0^{h-k}	219.0^{ef}
Cassava/*Aeschynomene*	143.0^{mno}	139.0^{nop}	172.0^{i-l}	197.0^{f-i}
Sole cassava	191.0^{ghi}	187.0^{ghij}	234.0^{f}	259.0^{d}
Natural fallow	327.0^{c}	315.0^{c}	384.0^{b}	484.0^{a}
SE±	3.3			

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT).

in each year in this study. The lowest weed dry matter was obtained from cassava/*Mucuna* intercrop irrespective of the weed management practice in each year of study (Table 5). Contrarily, optimum weed dry matter was produced in natural fallow.

Rice plant height

Table 6 shows that rice plant height differed between cassava/legume intercrops, such that cassava/*Mucuna*, cassava/cowpea and cassava/*Aeschynomene* in 2011 and 2012 produced similarly taller plants. The natural fallow plots had the shortest plants.

Two hoe weeded plots consistently produced taller plants (Table 6). Similar taller plants were observed in plots with application of Orizo Plus fb hoeing at 6 WAT, and one hoeing at 3 WAT in 2011 only.

There was significant interaction between cassava/ legume intercrop and weed management practices on rice plant height in 2012 and 2013 (Table 7). The use of cassava/cowpea intercrop in combination with the weed management practices generally had taller rice plants in 2012. Similarly, in 2013, irrespective of the weed management practice, rice plant height was tallest under cassava/cowpea, and cassava/*Aeschynomene*.

Rice tiller number

Table 8 shows that more tiller were recorded in cassava/*Aeschynomene* in 2011, 2012 and 2013 rainy seasons, which was at par with cassava/*Mucuna* in 2011 and 2012. It was observed that greater number of tillers

Table 4. Effect of pre-rice cassava/legume intercropping and weed management practices on weed dry matter in rice at 9 WAT.

Treatment	Weed dry matter (g^{-2})		
	2011	2012	2013
Cassava/Legume Intercrop (I)			
Cassava/*Mucuna*	155[f]	139[d]	129[f]
Cassava/Cowpea	177[e]	171[c]	156[e]
Cassava/Soybean	190[d]	188[c]	169[cd]
Cassava/*Lablab*	202[c]	200[b]	178[c]
Cassava/*Aeschynomene*	192[d]	188[c]	152[de]
Sole cassava	223[b]	224[b]	218[b]
Natural fallow	302[a]	325[a]	377[a]
SE±	1.9	6.1	4.4
Weed management practices (W)			
Orizo Plus fb hoeing at 6 WAT	80.9[c]	172[c]	172[c]
Two hoeing at 3 and 6 WAT	81.8[c]	168[c]	152[c]
One hoeing at 3 WAT	137.4[b]	225[b]	210[b]
Weedy check	223.6[a]	229[a]	246[a]
SE±	1.4	4.6	3.3
Interaction			
I × W	*	*	*

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT). *Significant at 5% level of probability. fb: follow by; WAT: weeks after transplanting.

was recorded in two hoe weeded plots in the three years of investigation (Table 8).

The interaction between pre-rice cropping of cassava/ legume intercrops and weed management practices on rice tiller per stand was significant in each year of study (Table 9). The use of cassava/*Mucuna* and cassava/*Aeschynomene* with each weed management practice had greater number of tillers per stand in 2011. In 2012, the use of cassava/*Aeschynomene* in combination with each of the weed management practice, had higher number of tillers per stand than other treatment combinations. In 2013, the use of cassava/ *Aeschynomene* under each weed management practice generally produced greater number of rice tillers per stand than other combinations.

Rice panicle count

More rice panicles were recorded in cassava/cowpea and *Aeschynomene* intercrops throughout the period of study and cassava/*Mucuna* plots in 2011 and 2012 only (Table 10). In contrast, natural fallow consistently gave the lowest number of panicles in each year of the study.

Weed management with two hoeing at 3 and 6 WAT in each year of the study, and application of Orizo Plus fb hoeing at 6 WAT in 2012 and 2013 only, resulted in greater number of panicles (Table 10).

The interaction between pre-rice cassava/legume intercrop and weed management practice showed that rice panicle count were least in cassava/*Lablab* intercrops irrespective of the weed management practice in 2011 and 2012, and in addition to cassava/soybean in 2013 (Table 11). Rice panicle number was consistently the lowest in the natural fallow treatment.

Rice paddy yield

Rice paddy yield was significantly (P≤0.05) higher in cassava/Cowpea and cassava/*Aeschynomene* intercrop throughout the study and in 2012 and 2013, respectively (Table 12). Rice yield was consistently significantly lower in the natural fallow treatment.

In terms of weed management, two hoes weeding had the greatest paddy yield followed by use of Orizo Plus and one hoe weeding (Table 12).

DISCUSSION

The high organic carbon added to the soil by cassava with cowpea, soybean and *A. histrix* Poir., was due to the high rate of growth and the bushiness of the legumes

Table 5. Interaction between cassava/legume intercrops and weeds management practice on weed dry matter at 9 WAT.

Treatment	Orizo Plus fb hoeing at 6 WAT	Two hoeing at 3 and 6 WAT	One hoeing at 3 WAT	Weedy check
		2011 Rainy season		
Cassava/*Mucuna*	42.2^{n}	42.2^{n}	81.9^{kl}	117.6^{h}
Cassava/Cowpea	59.1^{m}	58.4^{m}	97.2^{ij}	195.4^{d}
Cassava/Soybean	75.6^{l}	74.3^{l}	127.4^{gh}	216.1^{c}
Cassava/*Lablab*	88.1^{jk}	87.8^{jk}	147.8^{f}	225.8^{c}
Cassava/*Aeschynomene*	75.9^{l}	75.1^{l}	135.7^{g}	218.8^{c}
Sole cassava	102.1^{i}	99.4^{i}	$171.^{e1}$	287.3^{b}
Natural fallow	129.9^{g}	129.1^{g}	200.3^{d}	339.2^{a}
SE±	3.9			
		2012 Rainy season		
Cassava/*Mucuna*	34.7^{t}	32.2^{t}	55.2^{rs}	109.3^{lm}
Cassava/Cowpea	53.6^{rs}	52.0^{s}	78.5^{n}	184.1^{g}
Cassava/Soybean	67.1^{pq}	60.2^{qr}	121.8^{k}	197.8^{f}
Cassava/*Lablab*	76.7^{no}	70.3^{op}	130.8^{j}	224.3^{d}
Cassava/*Aeschynomene*	65.2^{pq}	60.2^{qr}	108.1^{m}	190.0^{g}
Sole cassava	115.8^{kl}	113.0^{lm}	211.1^{e}	311.4^{b}
Natural fallow	148.6^{h}	140.2^{i}	243.3^{c}	390.3^{a}
SE±	0.9			
		2013 Rainy season		
Cassava/*Mucuna*	32.2^{p}	29.2^{p}	50.1^{no}	104.4^{i}
Cassava/cowpea	54.0^{mno}	49.2^{o}	74.7^{k}	183.5^{e}
Cassava/soybean	62.4^{lm}	58.6^{mn}	115.5^{gh}	191.8^{e}
Cassava/*Lablab*	73.9^{k}	69.3^{kl}	122.6^{g}	217.4^{d}
Cassava/*Aeschynomene*	59.1^{mn}	55.9^{mno}	86.5^{j}	165.8^{f}
Sole cassava	113.2^{hi}	112.0hi	214.3^{d}	315.4^{b}
Natural fallow	164.5^{f}	160.1^{f}	272.0^{c}	467.5^{a}
SE±	1.2			

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT).

compared with other species. These translated into high leaf litter production and its subsequent decomposition. In a previous study, Osundare (2015) reported a significant increase in soil carbon content in *Centrosema pubescens* (Bentham) Kuntze; planted fallow compared to continuous maize cultivation.

The increase in soil total nitrogen in all cassava/legume intercrops, was probably due to greater addition of Nitrogen (N) as a result of leaf litter decomposition and atmospheric fixation. This finding is in agreement with the work of Matata et al*.* (2017) who reported high total nitrogen content in the biomass of *M. pruriens* (L.) DC., and *Canavalia ensiformis* (L.) DC., cropping systems relative to no fertilizer application.

In the present study, the intercropped legumes generally contributed more phosphorus than natural fallow. However, the highest addition of available phosphorus by cowpea intercrop in each year in this study could be tied to the high organic carbon added to the soil. In a previous study, Matata et al. (2017) reported that soil organic matter enhances the plant nutrients supply compared to treatments without crop residues.

The result of the present study demonstrated that in terms of weed density reduction, cassava/*Mucuna* intercrop was best. This observation corroborated with the findings of Liebman and Davis (2000) who reported that cover crops can suppress weed establishment and growth. This in turn reduced the number of weed seeds and vegetative propagules that could infest succeeding crops. The ability of cassava/*Mucuna* intercrop to effectively suppress weed growth (weed dry matter produced) the most, might be attributed to the allelopathic effect of the intercrop residues. These residues might have hindered subsequent weed seed germination and growth. This is consistent with the findings of Mhlanga et al. (2015) who noted that effectiveness of intercrops or

Table 6. Effect of pre-rice cassava/legume intercropping and weed management practices on rice plant height at 9 WAT.

Treatment	Rice plant height (cm)		
	2011	2012	2013
Cassava/Legume Intercrop (I)			
Cassava/*Mucuna*	57.5[a]	63.4[a]	70.3[b]
Cassava/cowpea	57.7[a]	64.0[a]	72.2[a]
Cassava/soybean	57.6[a]	61.7[b]	68.5[c]
Cassava/*Lablab*	52.3[b]	61.1[b]	67.4[c]
Cassava/*Aeschynomene*	56.3[a]	64.0[a]	71.4[ab]
Sole cassava	50.9[bc]	53.3[c]	54.0[d]
Natural fallow	47.5[c]	49.5[d]	48.7[e]
SE±	1.3	0.6	0.5
Weed management practices (W)			
Orizo Plus fb hoeing at 6 WAT	59.8[a]	65.9[b]	71.9[b]
Two hoeing at 3 and 6 WAT	61.8[a]	77.3[a]	77.9[a]
One hoeing at 3 WAT	61.8[a]	63.7[c]	69.0[c]
Weedy check	33.9[b]	36.9[d]	39.8[d]
SE±	1.0	0.4	0.4
Interaction			
I × W	NS	*	*

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT). *Significant at 5% level of probability. fb: follow by; NS: not significant; WAT: weeks after transplanting.

Table 7. Interaction effect of cassava/legume intercrop and weed management practices on rice plant height at 9 WAT in 2012-2013 rainy season.

Treatment	Orizo Plus fb hoeing at 6 WAT	Two hoeing at 3 and 6 WAT	One hoeing at 3WAT	Weedy check
		2012 Rainy season		
Cassava/*Mucuna*	70.0[cd]	77.3[a]	70.0[cd]	77.3[a]
Cassava/Cowpea	71.1[ab]	77.8[a]	71.1[ab]	77.8[a]
Cassava/Soybean	67.3[de]	76.5[ab]	67.3[de]	76.5[ab]
Cassava/*Lablab*	65.3[ef]	74.5[ab]	65.3[ef]	74.5[ab]
Cassava/*Aeschynomene*	70.0[cd]	78.0[a]	70.0[cd]	78.0[a]
Sole cassava	40.7[j]	59.8[gh]	40.7[j]	59.8[gh]
Natural fallow	57.6[hi]	55.5[hi]	57.6[hi]	55.5[hi]
SE±	0.43		0.43	
		2013 Rainy season		
Cassava/*Mucuna*	104.1[c]	111.9[b]	104.1[c]	111.9[b]
Cassava/Cowpea	113.1[b]	118.4[a]	113.1[b]	118.4[a]
Cassava/Soybean	98.5[de]	106.7[c]	98.5[de]	106.7[c]
Cassava/*Lablab*	96.6[e]	100.2[d]	96.6[e]	100.2[d]
Cassava/*Aeschynomene*	112.5[b]	118.1[a]	112.5[b]	118.1[a]
Sole cassava	78.0[hi]	80.7[gh]	78.0[hi]	80.7[gh]
Natural fallow	66.4[k]	67.7[k]	66.4[k]	67.7[k]
SE±	1.23		1.23	

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT). fb: Follow by.

Table 8. Effect of cassava/legume intercrop and weed management practices on rice tiller per stand.

Treatment	Number of rice tillers per stand		
	2011	**2012**	**2013**
Cassava/Legume Intercrop (I)			
Cassava/*Mucuna*	19.0[a]	25.0[a]	27.0[d]
Cassava/Cowpea	18.0[b]	23.0[b]	32.0[b]
Cassava/Soybean	16.0[c]	22.0[c]	28.0[c]
Cassava/*Lablab*	15.0[d]	21.0[d]	28.0[c]
Cassava/*Aeschynomene*	20.0[a]	26.0[a]	34.0[a]
Sole cassava	12.5[e]	14.5[e]	18.0[e]
Natural fallow	9.3[f]	9.0[f]	7.0[f]
SE±	0.2	0.3	0.2
Weed management practices (W)			
Orizo Plus fb hoeing at 6 WAT	18.0[b]	24.0[b]	30.0[b]
Two hoeing at 3 and 6 WAT	22.0[a]	27.0[a]	31.0[a]
One hoeing at 3 WAT	14.0[c]	16.0[c]	24.0[c]
Weedy check	9.0[d]	11.0[c]	15.0[d]
SE±	0.1	0.2	0.2
Interaction			
I × W	*	*	*

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT). *Significant at 5% level of probability. fb: follow by; WAT: weeks after transplanting.

smother crops may in part depend on their allelopathic ability. The decomposition of products of organic mulches and cover crops residues may continue to prove toxic to weeds in subsequent crops (Silva and Rezende, 2016).

Application of Orizo Plus fb hoeing at 6 WAT and two hoeing at 3 and 6 WAT effectively reduced weed growth, in terms of weed density and dry matter. Weeds were adequately controlled thereby reducing their quantity of soil seed shedding for the succeeding year's infestation. The present results are consistent with the findings of Hasanuzzam et al. (2007) who reported that application of pre-emergence herbicide followed by one hoeing can effectively reduce weed growth in rice production. Also, Ansari et al. (2018) observed an effective reduction in weed growth when hoe weeding was carried out twice at 20 and 45 DAS in rice production.

The significant interaction between cassava/legume intercrop and weed management practices for weed density and dry matter produced suggest that reduction in weed growth among the cassava/legume intercrops responded differently to weed management practice for these parameters. The best reduction in weed growth (density and biomass) from cassava/*Mucuna* in combination with application of Orizo Plus fb hoeing at 6 WAT and two hoeing at 3 and 6 WAT would be attributable to the shading effect and competitive stress produced by the canopy of the *Mucuna* legume. This legume produced high biomass which reduced weed seed germination in preceding cropping, and reduced weed seed number in subsequent rice. This result is in agreement with Choudhary et al. (2014) who noted that the main factor enhancing weed suppression in an intercrop system is the shading effect by the crop canopy.

In this study, cassava/cowpea and cassava/*Aeschynomene* intercrop gave taller rice plants than all the other intercrop practices. These intercrops also gave comparable taller rice plants in some years with cassava/*Mucuna* and cassava/soybean intercrops than all others. This observation might be an indication of greater addition of some plant nutrients by the legumes in the intercrops especially N, which in turn enhanced rice growth. This result agrees with the findings of Morteza et al. (2008) who observed variation in rice height planted after different legumes.

It was also obvious that two hoeing at 3 and 6 WAT, gave taller rice plants than the other weed management treatments, though comparable to application of Orizo Plus fb hoeing at 6 WAT, and one hoeing at 3 WAT. The improvement in rice growth in terms of the increased height suggest the effectiveness of these weed management treatments in reducing weed-crop competition, and providing condition for better resource availability which ultimately enhanced rice growth (Khaliq et al., 2013).

The interactions between cassava/legume intercrops and weed management practice on plant height revealed

Table 9. Interaction effect of cassava/legume intercrop and weed management practices on rice tiller/stand in 2011-2013 rainy seasons.

Treatment	Orizo Plus fb hoeing at 6 WAT	Two hoeing at 3 and 6 WAT	One hoeing at 3 WAT	Weedy check
		2011 Rainy season		
Cassava/*Mucuna*	23.0[cd]	26.0[a]	18.0f[g]	11.0[klm]
Cassava/Cowpea	21.0[e]	24.0[abc]	16.0[h]	9.0[no]
Cassava/Soybean	19.0[fg]	24.0[bcd]	14.0[i]	9.0[no]
Cassava/*Lablab*	18.0f[g]	23.0[c]	12.0[jk]	9.0[no]
Cassava/*Aeschynomene*	23.0[cd]	25.0[ab]	19.0[fg]	11.0[kl]
Sole cassava	14.0[ij]	18.0[fg]	10.0[lmn]	7.0[pq]
Natural fallow	10.0[lmn]	12.0[k]	8.0[op]	6.0[q]
SE±	0.53			
		2012 Rainy season		
Cassava/*Mucuna*	28.0[ef]	31.0[bc]	20.0[g]	14.0[lm]
Cassava/Cowpea	28.0[ef]	34.0[a]	19.0[gh]	13.0[mno]
Cassava/Soybean	28.0[ef]	30.0[cd]	18.0[hi]	12.0[op]
Cassava/*Lablab*	27.0[f]	29.0[de]	17.0[ij]	17.0[ij]
Cassava/*Aeschynomene*	32.0[b]	35.0[a]	20.0[g]	16.0[jk]
Sole cassava	15.0[kl]	20.0[g]	14.0[lmn]	9.0q
Natural fallow	11.0[p]	12.0[no]	7.0[r]	5.0[s]
SE±	0.22			
		2013 Rainy season		
Cassava/*Mucuna*	33.0[d]	35.0[c]	25.0[f]	15.0[jk]
Cassava/Cowpea	39.0[b]	40.0[ab]	33.0[d]	17.0[i]
Cassava/Soybean	34.0[cd]	35.0[c]	27.0[e]	17.0[i]
Cassava/*Lablab*	34.0[cd]	35.0[c]	27.0[e]	17.0[i]
Cassava/*Aeschynomene*	41.0[ab]	42.0[a]	33.0[d]	19.0[h]
Sole cassava	22.0[g]	22.0[g]	15.0[k]	13.0[l]
Natural fallow	9.0[m]	9.0[m]	7.0[n]	5.0[o]
SE±	0.52			

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT). fb: Follow by.

that cassava/*Mucuna*, cassava/cowpea, cassava/*Aeschynomene* in combination with two hoeing at 3 and 6 WAT resulted in producing taller rice plants. This could be attributed to efficient weed control observed, which might have supported the uptake of essential nutrients by the rice plant and translated into enhanced vegetative growth (Nadeem et al*.,* 2011).

Cassava/*Aeschynomene* intercrop gave greater number of rice tillers per stand than all the other intercrops, but compared with cassava/*Mucuna* intercrop. This intercrop practice was able to provide season long weed control, which in turn provided favourable condition for enhanced crop growth and production of yield attributes of rice. This finding is in agreement with the work of Anders et al*.* (2004) who observed higher rice yield in rice grown after soybean than in rice-wheat rotation.

The practice of two hoeing at 3 and 6 WAT gave greater number of rice tillers, suggesting that this treatment gave efficient weed control, which provided good crop yield attributes. Kolo and Umaru (2012) and Hakim et al. (2013) also observed the production of more rice tillers in weed free plots that received two or three hoe weedings.

The significant interaction between cassava/legume intercrop on number of rice tillers per stand affirmed that the combined use of cassava/*Mucuna*, cassava/*Aeschynomene* intercrops with two hoeing at 3 and 6 WAT probably gave rise to better weed control and soil nutrient availability and utilization.

The greater number of rice panicles per plant produced by two hoeing at 3 and 6 WAT than the other weed management treatments, though comparable to application of Orizo Plus fb hoeing at 6 WAT was due to efficient weed control. This provided conditions for better

Table 10. Effect of cassava/legume intercrop and weed management practices on number of rice panicle.

Treatment	Number of rice panicle (m^{-2})		
	2011	2012	2013
Cassava/Legume Intercrop (I)			
Cassava/*Mucuna*	273.0[a]	291.0[a]	307.0[b]
Cassava/Cowpea	273.0[a]	297.0[a]	326[a]
Cassava/Soybean	268.0[b]	276.0[b]	298.0[b]
Cassava/*Lablab*	225.0[c]	250.0[c]	298.0[b]
Cassava/*Aeschynomene*	272.0[a]	298.1[a]	327.0[a]
Sole cassava	193.0[d]	180.0[d]	188.0[c]
Natural fallow	118.0[e]	116.0[e]	144.0[d]
SE±	1.1	4.5	4.5
Weed management practices (W)			
Orizo Plus fb hoeing at 6 WAT	299.0[b]	313.0[a]	341.0[a]
Two hoeing at 3 and 6 WAT	305.0[a]	319.0[a]	344.0[a]
One hoeing at 3 WAT	218.0c	231.0[b]	259.0[b]
Weedy check	105.0d	113.0[c]	114.0[c]
SE±	0.8	3.4	3.4
Interaction			
I × W	*	*	*

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT). *Significant at 5% level of probability. fb: follow by; WAT: weeks after transplanting.

Table 11. Interaction between cassava/legume intercrops and weed management practices on number of rice panicle (m^{-2}) in 2011-2013 rainy seasons.

Treatment	Orizo Plus Two fb hoeing at 6 WAT	Herbicide + hand weeding	One hand weeding	Weedy check
		2011 Rainy season		
Cassava/*Mucuna*	354.0[ab]	357.0[a]	262.0[ef]	120.0[l]
Cassava/Cowpea	353.0[ab]	358.0[a]	260.0[ef]	120.0[l]
Cassava/Soybean	348.0[ab]	353.0[b]	256,0[fg]	117.0[l]
Cassava/*Lablab*	298.0[d]	308.0[c]	194.0[i]	100.0[n]
Cassava/*Aeschynomene*	349.0[b]	354.0[ab]	263.0[e]	121.0[l]
Sole cassava	243.0[h]	252.0[g]	186.0[j]	93.0[o]
Natural fallow	150.0[k]	155.0[k]	107.0m	61.0[p]
SE±	2.24			
		2012 Rainy season		
Cassava/*Mucuna*	371.0[ab]	376.0[ab]	286.0[d]	132.0[hi]
Cassava/Cowpea	378.0[ab]	385.0[ab]	291.0[d]	134.0[hi]
Cassava/Soybean	361.0[bc]	368.0[ab]	254.0[e]	120.0[ij]
Cassava/*Lablab*	340.0[c]	340.0[c]	208.0[fg]	113.0[ij]
Cassava/*Aeschynomene*	379.0[ab]	388.0[a]	289.0[d]	135.0[hi]
Sole cassava	214.0[f]	222.0[f]	188.0[g]	98.0[j]
Natural fallow	149.0[h]	152.0[h]	103.0[j]	60.0[k]
SE±	3.44			

Table 11. contd.

		2013 Rainy season		
Cassava/*Mucuna*	399.0^{cd}	400.0^{bcd}	295.0^{e}	134.0^{h-k}
Cassava/Cowpea	420.0^{abc}	425.0^{ab}	318.0^{e}	141.0^{hij}
Cassava/Soybean	386.0^{d}	390.0^{d}	296.0^{e}	122.0^{jkl}
Cassava/*Lablab*	385.0^{d}	389.0^{d}	296.0^{e}	122.0^{jkl}
Cassava/*Aeschynomene*	425.0^{ab}	427.0^{a}	314.0^{e}	225.0^{f}
Sole cassava	226.0^{f}	144.0^{hij}	194.0^{g}	110.0^{kl}
Natural fallow	147.0^{hi}	151.0^{h}	99.0^{l}	62.0^{m}
SE±	9.03			

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT).

Table 12. Effect of cassava/legume intercrop and weed management practices on rice paddy yield.

Treatment	Rice paddy yield (kg ha^{-1})		
	2011	**2012**	**2013**
Cassava/Legume intercrop (I)			
Cassava/*Mucuna*	2733.3^{b}	4565.6^{b}	4684.0^{b}
Cassava/Cowpea	2933.3^{a}	4836.9^{a}	5039.6^{a}
Cassava/Soybean	2200.0^{c}	3963.5^{c}	4329.5^{c}
Cassava/*Lablab*	2066.7^{d}	3558.6^{d}	3821.7^{d}
Cassava/*Aeschynomene*	2800.0^{b}	4718.5^{a}	5000.0^{a}
Sole cassava	1466.7^{e}	2576.5^{e}	2670.0^{e}
Natural fallow	1096.7^{f}	1042.5^{f}	1005.0^{f}
SE±	66.7	49.2	77.1
Weed management practices (W)			
Orizo Plus fb hoeing at 6 WAT	2733.3^{b}	4921.2^{b}	5196.8^{a}
Two hoeing at 3 and 6 WAT	3200.0^{a}	5219.8^{a}	5330.9^{a}
One hoeing at 3 WAT	1666.7^{c}	2732.9^{c}	2848.8^{b}
Weedy check	866.7^{d}	852.1^{d}	843.3^{c}
SE±	20.0	37.2	58.3
Interaction			
I × W	NS	NS	NS

Means followed by the same letter (s) within the same column are not significantly different at 5% level of probability (DMRT). NS: Not significant; fb: follow by; WAT: weeks after transplanting.

crop yield. The present result is consistent with previous studies in which plots weeded twice at 15 and 30 DAS, increased number of rice panicles, and comparable to plots given bispyribac-sodium or ethoxysulfuron fb manual weeding at 30 DAS (Ihsan et al*.,* 2014).

The interaction between cassava/legume intercrop and weed management practice on number of rice panicle revealed that cassava/*Mucun*a, cassava/cowpea, cassava/*Aeschynom*e intercrops with two hoeing at 3 and 6 WAT produced similar highest number of rice panicles per unit area. This could be attributed to the greater number of tillers produced per stand, which might have produced more panicles per unit area (Maite et al., 2015). The greater number of rice panicles per plant from cassava/cowpea and cassava/*Aeschynomene* intercrops compared with cassava/*Mucuna* intercrops could be attributed to effective weed growth reduction, which translated into enhanced crop yield attributes. Mobasser et al. (2007) also observed that greater number of panicles m^{-2} gave higher grain yield.

In this study, cassava/cowpea intercrop gave the highest paddy yield than the other intercrop practices, though comparable to cassava/*Aeschynomene*. These intercrop practices gave taller rice plants and more rice panicles, thereby provided conditions for enhanced rice growth and yield.

Expectedly, paddy yield of rice was more in plots given two hoeing at 3 and 6 WAT, because it gave the best weed control (reduced weed density and biomass), produced taller plants, greater number of tillers and panicles per stand which translated into enhanced paddy yield of rice. Conversely, the highest paddy yield recorded with two hoeing at 3 and 6 WAT was comparable with application of Orizo Plus fb hoeing at 6 WAT. This may be attributed to efficient weed control which suggests reduced nutrients depletion by the weeds, which in turn enhanced rice growth, yield and yield attributes. Similar results have been reported in a previous study in Pakistan in which paddy yield of rice was increased with two hoeing at 15 and 30 DAS, which was comparable to application of bispyribac sodium or ethoxysulfuron ethyl followed by one manual weeding at 30 DAS (Ihsan et al., 2014).

Conclusion

Based on the results of the present investigation, it can be concluded that in terms of weed management, the best treatments were cassava/*Mucuna* intercrop under two hoeing at 3 and 6 WAT, or Orizo Plus fb hoeing at 6 WAT. Increased growth and yield of rice can be realized in this agro ecology with cassava/cowpea, cassava/ *Aeschynomene* intercrop in combination with two hoeing at 3 and 6 WAT or with application of Orizo Plus fb hoeing at 6 WAT.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Analysing chicken meat production comparative advantage of South Africa

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The study examined the relative efficiency of producing chicken meat in South Africa, in 2017, and its comparative advantage in chicken meat production in Southern African Development Community (SADC). The study used the Policy Analysis Matrix (PAM) to calculate the net financial and economic profitability, Nominal Protection Coefficient of output (NPCO), Nominal Protection Coefficient of input (NPCI), Effective Protection Co-efficient (EPC), Private Cost Ratio (PCR) and Domestic Resource Cost (DRC). Data used in this study was taken from the statistics collected by the Department of Agriculture, Forestry and Fisheries (DAFF) and South African Poultry Association (SAPA). The findings, based on the indicators of NPCI, EPC and PCR conclude that the existing government policy environment tends to protect the interest of the chicken meat producers at the production level. The DRC results indicated that South Africa had comparative advantage of producing chicken meat in 2017.

Key words: Domestic resource cost, policy analysis matrix, private cost ratio.

INTRODUCTION

The South African gross value of agricultural products was projected to be over R 246 billion in 2016. A bigger share came from the animal products sector. The sector contributed 46.9% (R 117 billion), 30% was from horticultural products and 23.1% was contributed by field crops ((Department of Agriculture Fisheries and Forestry) (DAFF), 2017).

The South African broiler industry contributed R37 billion, which formed 16.5% of the overall agricultural production output in the financial year 2014/15 (Joubert, 2017). About 108,000 indirect and direct jobs in South Africa are provided by the chicken meat production industry and related value chain industries (DAFF, 2016). The chicken meat value chain integrates into other value chain industries, for example, maize production since maize is used as feed to chicken (Joubert, 2017).

Chicken meat end products are categorised into five categories, namely live, fresh, frozen, offal and Individually Quick Frozen (IQF) chicken meat. Blood and feathers are the only non-edible parts of the chicken products (Lubinga et al., 2018). The non-edible parts are taken to rendering plants for further processing. The industry is highly competitive with a few commercial chicken meat producers and a large number of small scale chicken meat producers (Lubinga et al., 2018).

Poultry meat is believed to be a cheap source of

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protein as opposed to livestock products. Other people are also of the view that poultry meat is a healthy substitute of protein as compared to red meat. In 2016, over 935 million chickens were slaughtered in South Africa. The number of chickens slaughtered is 3.1% less than those slaughtered in 2015 (DAFF, 2017).

The production of white meat has increased in South Africa in previous years. Per capita consumption has increased too. In 2001, only 869,000 tons of white meat was produced. The quantity has since increased in 2016 to 1,704,000 tons. During the same period, total consumption has also increased to 2,200,000 tons from 938,000 tons. Per capita consumption also shows a positive growth to 40.04 kg in 2017 from 21.48 kg in 2001. The per capita consumption growth of white meat is more than what was recorded for red meat in the same period. Per capita consumption for red meat increased from a total of 18.96 kg in 2001 to only 27.74 kg in 2017 (DAFF, 2017).

Louw et al. (2011) identified the quality, consistency and cost of feed as the main contributing input, a major challenge to South African chicken meat producers. However, the price of soybean is the main factor underlying the general costs of feed. This influences the competitiveness of South African chicken meat producers. In terms of ratio, soybean cake is roughly around 18% of the total weight of the chicken feed ration. Both the US and Brazil are net exporters of soybean cake while South Africa is a net importer (DAFF, 2017).

The South African local crushing industry has started to increase the production volume of soybean cake locally. This suggests that whereas the price of soybean cake trades at export parity levels in Brazil and the US, the South African price of soybean cake trades at import parity levels (Schmidhuber, 2008).

The South African chicken meat industry is an important subsector within South African agriculture. It is the single largest contributor to total gross agricultural production. The industry provides an affordable source of protein, thus making it an important contributor to the country's food security. As a strategic sector of the economy, the flow of chicken imports into South Africa recently has threatened the sustainability of the sector and its ability to compete on the international markets (Bureau for Food and Agricultural Policy (BFAP), 2016).

Chicken imports into South Africa have increased by an annual average of more than 10% since 2001 (Joubert, 2017), which has been one of the critical factors underpinning questions related to competitiveness. An increase in imports would suggest that there is a need for expansion of local production if producers were able to compete more successfully with imported products (Joubert, 2017).

In 2016, it is estimated that broiler, hatchery and rearing industries employed 14,250 people, the processing sector employed a total of 27,122 people, and the broiler distribution industries employed 5,975 people. The grand total of employment within the broiler industry is 47,347 employees. These jobs are threatened by an influx of chicken meat imports which are cheaper than the locally produced chicken meat (SAPA, 2017).

The industry's application by the South African Poultry Association (SAPA) for an increase in the import tariff protection was granted in 2013. According to Davids et al. (2013), the amount of imports that come into South Africa duty-free, restricts the impact of the tariffs on the local production prices. This raises a question regarding the comparative advantage of South African chicken meat production.

Van Rooyen et al. (1999) explained a comparative advantage as to how can a country benefit from production and trade through the most optimum and efficient use of the available resources. Competitive advantage shows how industries optimise opportunities in an environment to create and continue with a sustainable business, which results in a sustainable industry (Van Rooyen et al., 1999).

This study, therefore, attempted to suggest a possible solution to job losses in the poultry industry, in South Africa by doing a comparison on the competitive advantage of chicken meat production in South Africa against its SADC counterparts. Such information is missing in the SADC trade and the availability of this information would be of help for policy makers when it comes to designing a policy that can protect the interest of chicken farmers in South Africa. On the basis of this, the objectives of the current study are to:

i) Analyse the comparative advantage of chicken meat production in South Africa.
ii) Analyse the competitiveness of chicken production in South Africa.
iii) Assess the effect of government policies on chicken production in South Africa.

METHODOLOGY

Study area

The study was conducted in South Africa, which is the southernmost country in Africa. It is bounded to the south by 2,798 km of coastline of Southern Africa stretching along the South Atlantic and Indian Oceans to the north by the neighbouring countries of Namibia, Botswana and Zimbabwe and to the east and northeast by Mozambique and Swaziland. South Africa is the largest country in Southern Africa and the 25th-largest country in the world by land area and, with close to 56 million people (Wikipedia, 2019).

Data collection

The study used secondary data which was obtained from the South African Poultry Association, Department of Agriculture, Fisheries and Forestry (DAFF) and also from own calculations where necessary. The data collected included input requirements, market

Table 1. PAM framework.

Parameter	Revenues	Production costs		Profits
		Tradable inputs	Domestic factors	
Private price	A	B	C	D
Social price	E	F	G	H
Divergence	I	J	K	L

Source: Monke and Pearson (1989).

prices for inputs and outputs, transportation cost, returns and subsidy from chicken meat production in South Africa for the year 2017.

Data analysis

Different approaches have led to the measurement of comparative advantage in agriculture being developed. In the past, attempts to measure comparative advantage in agricultural production directly using economic models were practised by many researchers. They used to capture the interaction of national resources, production technology, product demand, and government interventions in measuring the comparative advantage. Some of the developed models were built to answer specific questions about agricultural production. Those models required a large investment in data collection and analysis. As a result, those models were appropriate primarily for academic research or high-stakes investment decisions and policy choices (Masters and Winter-Nelson, 1995).

The Policy Analysis Matrix (PAM), developed by Monke and Pearson (1989) is one of the approaches developed in a systematic way. It includes all data needed to calculate the Producer's Subsidy Equivalent (PSE), Net Social Profits (NSP), Domestic Resource Costs (DRC), and the Social Cost Benefits (SCB) (Monke and Pearson, 1989).

The Policy Analysis Matrix (PAM)

The PAM approach is based on the estimation of budgets using market prices and social opportunity costs. Benefits, costs and profits are determined in a systematic way: firstly, using budgets derived through market prices, and secondly, using social opportunity costs. Inputs are sub-divided into tradable and domestic. Table 1 presents the PAM approach. Matrix entries A, B, and C are the sum of products of market prices and quantities. Entries E, F, and G use the same quantities but are valued at social opportunity costs or shadow prices. The bottom row is the difference between the other two rows. The last column is the benefits minus costs. Thus, the PAM is a double-entry accounting system of identities, with no behavioural equations. The behavioural content of the PAM has embodied in the shadow prices used and in the interpretation of the matrix (Monke and Pearson, 1989).

The Policy Analysis Matrix (PAM) is computational framework, delivered by Monke and Person (1989) and augmented by Masters and Winter-Nelson (1995) for measuring input use efficiency, comparative advantage among commodities and the degree of government interventions (Joubert, 2017).

The study used Policy Analysis Matrix (PAM) to analyse the competitiveness and comparative advantage and the effect of government policies on chicken production in South Africa. The PAM is a Matrix of two accounting identities; one set defining profitability and the other defining the difference between private and social values of a commodity system. The framework of the PAM is shown in Table 1.

The approach used in Policy Analysis Matrix begins with the calculation of existing levels of private (actual market) and social (efficiency) revenues, costs, and profits. This calculation reveals the extent to which actual profits are generated by policy transfers rather than by underlying economic efficiency. A PAM contains two cost columns, one for tradable inputs and the other for domestic factors. Production costs included feeds and medicine as tradable inputs while domestic factors included labour (Monke and Pearson, 1989).

The following ratios were calculated from the Policy Analysis Matrix:

Private Cost Ratio (PCR)

The Private Cost Ratio (PCR) is used in measuring competitiveness. It shows the private efficiency of the producers and is an indication of how much one can afford to pay domestic factors (including a normal return to capital) and still remain competitive. The PCR will be used to achieve the second objective (Monke and Pearson, 1989).

PCR= (Private domestic factors)/ (Private revenues-Private tradable inputs) = C/(A-B).

When PCR>1, it indicates that the resource cost is greater than the value added and thus, it is not profitable to process the commodity.
If PCR<1, it indicates that the value added is greater than the resource cost thus, it is profitable. If PCR=1, it indicates the breakeven point.

Social profitability

The social profitability is a measure of comparative advantage and efficiency because inputs and outputs are valued in prices that reflect scarcity values. It is the difference between revenue and costs of domestic factors and tradable inputs prices at social opportunity cost (social values). Social values provide a benchmark policy environment for comparison as these were considered those that would hypothetically occur in free market without policy intervention (Monke and Pearson, 1989). The social profitability will be used to achieve the first objective.

Social profit (H) = Social revenues – Social tradable inputs – Social domestic factors = E-F-G

When (H>0), it indicates that the system uses scarce resources efficiently and the commodity has a static comparative advantage.
If (H<0), it indicates that the sector cannot sustain its current output without assistance from the government, with a resulting waste.

Domestic Resource Cost (DRC)

The Domestic Resource Cost (DRC) is a measure of relative efficiency of domestic processing by comparing the opportunity cost of domestic processing to the value generated by the product. The ratio can be used to compare different economic activities in terms of social cost of domestic resource employed in earning or saving a unit of foreign exchange. The DRC will complement the study in achieving the first objective. The relationship between DRC and comparative advantage is straight forward and expressed as:

DRC= (Social domestic factors)/ (Social revenues-Social tradable costs) = G/ (E-F)

If DRC<1, the chicken meat production in South Africa enjoys a comparative advantage as compared to its SADC counterparts. If DRC>1, it signifies that the country has a disadvantage in the production of chicken meat as compared to its SADC counterparts. When DRC = 1, the economy neither gains, nor saves foreign exchange through domestic processing (Monke and Pearson, 1989).

Divergence/Policy transfer

The measurement of divergence and transfer effect of policies is carried out in the third (bottom) row of the Policy Analysis Matrix. The divergence between the observed private (actual market) price and the estimated social (efficiency) price must be explained by the effects of policy or by the existence of market failures. Distorting policies that lead to inefficient use of resources enhances the stated divergence (Monke and Pearson, 1989).

SRP= (Divegence profits)/ (Social revenues) = L/E

Effective Protection Coefficient (EPC)

The EPC measures the value-added in market prices, about the value-added in economic prices, that is, it measures the degree of policy transfer from product market-output and tradable-input policies.

EPC = (Market Income – Tradable Market Expenditure)/ (Economic Income – Tradable Economic Expenditure)

If the EPC is higher than one (>1), it indicates that the market profit is higher than it would have been if no commodity policies had been in place. Thus, it indicates that policies are in place that increase profits artificially (Monke and Pearson, 1989). The EPC will be used for the third objective.

Nominal Protection Coefficient for outputs (NPCo)

An NPCo greater than one (>1) shows that policies have increased the market price to levels higher than the economic price. Thus, if the domestic price is constantly higher than the economic or shadow price (international price), it indicates that policies on the domestic market because prices being paid by domestic consumers to be higher than they would have paid in the absence of such policies. Hence, a NPCo greater than one (>1) indicates that consumers are indirectly taxed (Monke and Pearson, 1989).

Nominal Protection Coefficient for inputs (NPCi)

The NPC on tradable inputs (NPCi), defined as Market Tradable Inputs/Economic Tradable Inputs, shows the degree of tradable-input transfer. A NPC on inputs of greater than 1 (>1) shows that policies are increasing input costs more than the world prices (Monke and Pearson, 1989).

The Policy Analysis Matrix was used to calculate income (revenue), profits and cost items at the farm level to produce 1 tonne of chicken meat in South Africa in 2017. In the undistorted market, the efficient values of inputs and output are meant to lead to the highest possible levels of national income. Social profits are derived from the difference between social or economic revenues and costs. This measures economic efficiency. New investments and technologies reduced the social costs, increased social profits and therefore improved the effectiveness of production (Monke and Pearson, 1989).

It is important to understand the grouping of economic/social profitability's of agricultural systems to correctly measure the economic efficiency (Monke and Pearson, 1989).

Market/private income was calculated as an average income per tonne per year. This represents the average income for the year 2017. The income was derived from several enterprise budgets compiled in South Africa. Values of the second row are computed by adjusting the individual components of the first row, using economic prices. As proxy for the economic prices, world market prices adjusted to their import and export parity price are used. Opportunity costs are used to estimate the domestic factors of production.

Social values are calculated, in the case of exported goods in F.O.B. (Free On Board) prices and import goods in C.I.F (Cost, Insurance, Freight) prices. This is necessary in order to validate that the social prices are out of policy interventions and in the assumption of competitive markets for inputs and outputs.

In the second row, outputs (E) are valued at C.I.F. prices, inputs (F) are valued according to F.O.B. prices and international prices are used since the products are traded at world prices.

RESULTS AND DISCUSSION

The results are presented in Tables 2 and 3.

Market/Private profitability

In 2017, the South African chicken meat production shows a market/private profit of R19921.05 per tonne. Private profitability was calculated as follow: R 34830- R 12392.32 - R 2516.63 = R 19921.05 in 2017. The industry shows profitability.

Private Cost Ratio (PCR)

The South African chicken meat production industry in 2017 shows a PCR with the figure of 0.11. The value is smaller than one (>1), which indicates that the industry adds value, therefore, has a competitive advantage.

Economic/social profitability

The South African chicken meat production industry's economic profit for 2017 is R 678.48 per tonne. This indicates high margins, which occurs as a result of the output prices of final products keeping up with the cost to produce the final product.

Table 2. Policy Analysis Matrix (PAM) results.

Parameter	Revenue (R)	Cost (R)		Profit (R)
		Tradable inputs (R)	Non-tradable inputs (R)	
Market/private prices	34830	12392.32	2516.63	19921.05
Economic prices	23675.92	20982.44	2015	678.48
Effect of divergences and efficient policy	11154.08	- 8590.12	501.63	19242.57

Source: Own calculation (2017).

Table 3. Ratio indicators for comparison.

Private Cost Ratio (PCR = $\frac{C}{A-B}$)	0.11
Social profit (E – F – G)	678.48
Domestic Resource Cost Ratio (DRC =$\frac{G}{E-F}$)	0.75
Nominal Protection Coefficient on Tradable Outputs (NPCO= $\frac{A}{E}$)	1.47
Nominal Protection Coefficient on Tradable Inputs (NPCI= $\frac{B}{F}$)	0.59
Effective Protection Coefficient (EPC= $\frac{(A-B)}{(E-F)}$	8.33
Profitability Coefficient (PC= $\frac{D}{H}$)	29.36

Source: Own calculation (2017).

Domestic Resource Cost ratio (DRC)

The DRC for the South African broiler industry is 0.75, which is less than 1, and indicates that South Africa has got a comparative advantage as compared to other SADC countries. Tsakok (1990) argues that the level of comparative advantage is greater if the DRC ratio is close to zero.

Policy transfer

Nominal Protection Coefficient for outputs (NPCo)

The NPC of output is 1.47, which is greater than 1 (>1), and indicates that the private price of chicken meat in South Africa is greater than the SADC price and that policies have caused domestic output price of the poultry industry in South Africa to be approximately 47% higher than the SADC price (Mahlanza et al., 2003).

Nominal Protection Coefficient for inputs (NPCi)

An NPC of inputs is 0.59, which is less than 1 (<1) and indicates that the inputs are subsidized.

Effective Protection Coefficient (EPC)

The EPC measure for the South African chicken meat production in 2017 is 8.33. When an EPC is greater than one (>1), it shows that profits are higher than they would be when there is no commodity policy in a country. It further means that the net impact of government policy influences the product markets using outputs prices on the price of inputs (Yao, 1997).

Profitability Coefficient (PC= D/H)

The results show a profitability coefficient of 29.36. When the profitability coefficient is greater than 1 (>1), it indicates that the current policy implications are an incentive to chicken meat production in South Africa in 2017.

Conclusion

By applying the Policy Analysis Matrix (PAM) approach to analyse the comparative advantage of the South African chicken meat production, the study showed that the chicken meat in 2017 had a comparative and competitive advantage. Moreover, it was found out that that government policy had positive impacts on the chicken meat producers. It even enhanced the competitiveness of the South African chicken meat in 2017. Since PAM analysis could not capture the potential changes in prices and productivity, the results of scenarios are subject to changes in market conditions. The three hypotheses were completely rejected based on the reasons provided below. The study has recommended to government and chicken meat producers to increase the production of local chicken meat.

The hypothesis that South Africa does not have a comparative advantage in chicken meat production is rejected based on the explanation given below.

Indicators of protection and comparative advantage were used in the analysis. In analysing the relationship between world prices and domestic prices for output and inputs, the NPC was used to see the extent of protection in the sector. From the analysis, the NPC on output was found to be 1.47, indicating that domestic broiler meat prices are 47% higher than the SADC prices. On inputs used, the research indicated an NPCI of 0.59, implying that domestic prices are 41% higher than social prices. The comparative advantage of an agricultural system is indicated by the value of the Domestic Resources Cost Ratio (DRC). Based on the DRC of 0.75, the result indicates that chicken production in South Africa has a comparative advantage.

The hypothesis that South Africa's chicken meat production industry is not competitive is rejected based on the following explanation.

The determination of profit received by chicken meat producers in South Africa is a straightforward and important initial result of the PAM approach. The results indicate if the farmers are currently competitive. The competitiveness of the South African chicken meat production is measured by the private profitability (D) or Private Cost Ratio (PCR). The PCR of the South African chicken meat production in 2017 is 0.11. This result indicates that South African chicken meat production is profitable and thus competitive.

The hypothesis that Government's policy interventions do not affect chicken meat production in South Africa is rejected based on the explanation given below.

Regarding the total effects of government intervention in the output of chicken meat production in South Africa and tradable input markets, the study estimates the value of EPC= 8.33. The EPC is greater than 1, implying that the overall impact of the existing policy results in a net positive incentive to produce chicken meat in South Africa.

Recommendations

To the government

Increase chicken meat exports: The newly merged Department of Agriculture, Land Reform and Rural Development (DALRRD) should prioritize the support of chicken meat producers in South Africa since it is productive; to save the jobs this industry is currently offering and creating new jobs. The Department of Trade and Industry and Economic Development should facilitate talks with SADC counterparts to increase exports to the region taking advantage of the Free Trade Areas (FTA) policy that allows zero tariffs on intra exports.

The emergency of transboundary diseases: Broiler production is always under threat from diseases such as Newcastle disease and, more recently, Highly Pathogenic Avian Influenza (HPAI). Vaccines need to be made available to contain the threat. These measures depend on the strength of the veterinary service, which needs restoration.

Monopolistic behaviour in broiler production: The DALRRD should put in place policies to increase the number of participants in the broiler supply chain through expansion of broiler producer base, focusing on smallholder producers through provision of suitable financing, improved production and supply of day-old chicks, promotion of full utilization of production capacity at the newly resettled farms, training of new entrants into the industry, production of self-help production manuals and improvement of broiler extension services.

Currently, in South Africa there are few commercial companies that produce chicken meat in the value chain. This may result in the establishment of cartels that extract monopoly rents from society. Therefore, there is a serious need to promote the manufacture of appropriate technology or promote the existing technological efficiency for small to medium commercial broiler enterprises. Formal marketing systems in rural areas can help the industry to develop hygienic slaughter facilities, improve feeding systems and farmer training and the promotion of the use of green energy like solar in rural area. The DALRRD should speed up the development of Agri-Hubs to supply these functions.

To producers

Increase production: The chicken meat production in South Africa needs to be expanded to enhance the industry. The main target should be on smallholder farmers. They should get access to the following services: suitable financing, improved production and supply of day-old chicks, promotion of full utilization of production capacity at the newly resettled farms, training of new entrants into the industry, production of self-help production manuals and improvement of broiler extension services.

Policy implications

The results of the study indicated that the country has a comparative advantage in broiler production as indicated by the DRC. This implies that it is economical to produce locally and save foreign currency from importing broiler products. The NPCs for both output and inputs show that prices of tradables are higher on the domestic markets than on the world market. Therefore, the distortions in the domestic economy are making production costs expensive. On the other hand, the domestic prices for outputs are higher than the world prices and therefore attract imports. Domestic consumers are therefore paying more than what they ought to be paying. There is therefore a need for policies that will protect the local production, like tariffs or subsidies.

Areas of further research

The PAM is a partial equilibrium model which does not capture the linkages between broiler and other products that are either compliments or substitutes to broiler meat such as beef, pork and non-meat foods like vegetables. The analysis is only applicable at sectoral and commodity levels and therefore does not provide a complete picture of the economy wide effects following policy changes. A study on the linkages between broiler and these will help determine policy options in the face of broiler meat supply to the SADC region.

Limitations

Historical data is used in PAM and therefore, does not take into consideration the recent changes. PAM is constructed for a given period of time to trace the evolution of policy effects.

When the Domestic Resource Coefficient (DRC) is calculated in the PAM, it does not take into account the costs of domestic factors and that can lead to understating the social profitability of activities that make intensive use of domestic resources.

Shadow prices are complex and difficult to calculate. It is very important that the researchers do the calculations correctly.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Impacts of landuses [old home garden (HG), oilpalm plantation (OP), fallow arable field (FA), grass lawn (GL)] on physico-chemical properties of an ultisol on a flat in the Coastal plains sand of Imo State, Nigeria were evaluated. Landuse significantly (at 5%) influenced the properties sand, organic matter (OM) content, soil reaction (pH), exchangeable cations Ca, Mg, K, exchangeable acidity (Al+H), effective cation exchange capacity (ECEC), base saturation (BS); while available phosphorous (av.P), Ca, Mg, K, Al+H, varied most within and among the management units. Properties varied least in HG, but most in FA and GL. Strong soil acidity and associated problems of nutrient (e.g. Ca, Mg, K) deficiencies, nutrient imbalance, low ECEC were the main limiting factors. The ratio Ca+Mg/Al+H, among other indices OM, Ca+Mg, Al+H, ESP, captured the soils' degradation best. On its basis, the soils' qualities were in the order, HG > OP > FA > GL. Management should aim at meeting the conditions of HG and that should include liming to correct nutrient imbalance as it had not been mitigated by long fallow. Indices identified should be considered in developing quantitative models for estimating soil quality in the location of study. Soil property variability as influenced by land fragmentation in the area needs further investigation using geostatistical technique.

Key words: Management, Coastal plains sand, indices of soil quality, soil degradation, soil productivity.

INTRODUCTION

The land of the Coastal plains sand of Imo State, Nigeria is densely populated hence the fragmentation, intensification of landuse and a general lack of virgin forests in the area. The soils are degraded and the degradation is attributed to unsustainability of landuse (Asadu et al., 2008; FDALR, 1999; Lal, 1986; Ogunkunle, 2015; Smalling et al., 1996).

The main rural landuses/land utilization types in Lorji, Aboh-Mbaise L.G.A. of Imo State are the arable cultivation, which include home gardens and oilpalm plantations (Lagemann, 1981; Uzozie, 1975). The grass lawns of schools in the area represent yet another landuse type which is often neglected.

Assessment of soil quality is considered invaluable in determining the sustainability of land management systems (Karlen et al., 1997). Soil quality is differentiated into inherent and dynamic components. While the inherent is determined by soil forming factors (which

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effects are assessed looking at the entire soil profile), the dynamic focuses on the surface 20 to 30 cm, describing the status or condition of a specific soil due to relatively recent landuse or management decision (Ogunkunle, 2015). According to Rodrigo-Comino et al. (2019), the major topsoil quality indicators are soil texture, soil organic carbon, bulk density and the heavy metals (Fe, Mn, Zn, Cu). On the other hand, Adaikwu and Ali (2013) have noted the suitable indicators for crop production to be soil pH, organic matter (OM) content, total nitrogen, available phosphorus, exchangeable cations, cation exch. capacity (cec), saturated hydraulic conductivity, and base saturation. Oti (2002) recommended silt/clay, fine sand/coarse sand, Ca/Mg, Ca+Mg, Ca+Mg/Al+H, OC and Al-Saturation as reliable indices of erosion of tropical ultisols. It is also recommended that the evaluation of soil quality should incorporate spatial and temporal variation of functions, which should be done in relation to environment and landuse (Idowu, 2003; Ogunkunle, 2015; Young, 1998).

While there had been studies comparing soils under forests (some of which were virgin) with those of arable cultivation to determine the effect of the later on soils of the area (Akamigbo, 1999; Asadu and Bosah, 2003; Nnaji et al., 2002), the same cannot be said of the other landuses recorded in the area of study. And how varied management in a small area will impact the soils was also not investigated.

So, the aim of the study was to determine the long term effect of landuses, oilpalm plantation, arable cultivation, home garden, grass lawn, on physico-chemical properties of soils on a plain in Lorji. With landuse being the only differentiating factor in the sampled area, the specific objectives of the study were to determine:

(1) Intra- and inter-management units' variations of soils' physico-chemical properties;
(2) Statuses of parameters under each management unit;
(3) Most important soil quality parameters indicating soil degradation in the location;
(4) Management practice best suited to soils.

MATERIALS AND METHODS

Study area

The location of study is Eziala village, Lorji Nwe-Ekeukwu autonomous community, Aboh-Mbaise Local Government Area, Imo State Nigeria. The place is between Lat. 5°24^{I} to 23.2^{II}N, and Long. 7°16^{I} to 56.9^{II}E. The geology is dominantly Coastal plains sand. It is in the humid tropics with 3 dry months each having less than 60 mm rainfall a month. Total annual rainfall is 2,250 to 2500 mm. Temperatures are high, changing only slightly during the year, with mean daily temperatures of about 27°C (Oti, 2002). Location belongs to the low land rainforest dominated by Oilpalms (*Elaeis guineensis*) and pennisetum grass species (Igbozurike, 1975). The area of the Coastal plains sand in Imo State, Nigeria is about 3,401.29 km^2 (340,129 ha), 61% of which is made up of flat land having not more than 0.5% slope. By the latest (2006) census figures as recorded in the Federal Government of Nigeria Gazette of 2007, the location has an average population density of 1,206 persons/km^2, while it is 1,055 persons/km^2 for Aboh-Mbaise Local Government Area of the state. The soils are classified as Typic Kandiudult (USDA, Soil Taxonomy)/Haplic Ferralsols (FAO/Unesco) (Ukaegbu, 2014). Oti (2002) describes the soils as acidic, having low CEC, low base saturation, low fertility status and multinutrient deficiencies.

Altitude of site of study is 83 m above sea level. Figure 1 shows location of study.

Experimental design

Four landuses were used for the study. These were an old home garden site (HG), oilpalm plantation (OP), fallow arable field (FA), and grass lawn (GL). The different landuses were contiguous. Site of study is a flatland that had been subjected to the same landuses for over 30 years. The oilpalm plantation was sandwiched by the home garden and arable field, while the grass lawn was beside them, separated by a pathway. This layout is illustrated by Figure 2.

The home garden had assorted tree crops that were irregularly spaced, and these included Oilbean (*Pentaclethra macrophylla*), Ogbono (*Irvingia gabonensis*), Bitter kola (*Garcinia kola*), Kola (*Cola nitida*), Rose Apple (*Eugenia jambes*), Local Pea (*Dacryodes edulis*), Oilpalm (*Elaeis guineensis*), and Mango (*Mangifera indica*). The home garden had been subjected to cultivation in the past, with the cultivation involving bush clearing with cutlass, burning of trash, making of mounds with hoe, planting of crops such as yam, cassava, maize and vegetables (e.g. *telfaria*) on the mounds, as well as applying farm yard manure which included ash from the kitchen. The cultivation was on a yearly basis. It was a form of traditional agroforestry practice. However, the plot had been fallow (uncultivated) for six years by the time of the experiment.

The oilpalm plantation plot was planted to oilpalms, with the normal spacing of 9 m in triangular pattern (Komolafe et al., 1979) observed. At the time of the experiment, the plantation was 38 years old. The plot was intercropped with cassava and maize at interval of three years. Cultivation involved clearing of the bush (that is, undergrowths) with cutlass, burning of trash, making of mounds with hoes, planting of crops on mounds, and application of fertilizers (mainly, N, P, K, 15:15:15). At the time of the experiment the plot had not been cultivated for six years, and thus had the oilpalm trees with undergrowths.

The arable field was cultivated mainly to cassava and maize, to which were applied N, P, K, 15:15:15. The cultivation was at interval of three years and also involved slashing with cutlass, mound making with hoes, application of fertilizer (NPK, 15:15:15). At the time of the experiment, the plot was in its first year of fallow.

The grasslawn was made up of mainly Bahama grass and was slashed periodically. At the time of the experiment, the lawn was well kept with low grasses. This plot had not been cultivated for over 30 years.

With the exception of the grasslawn managed by a school, the rest of the landuses (plots) were farmer-managed.

Each of the landuses was taken as a treatment. The experiment was of a randomized complete block design (RCBD).

Sampling

An area of 600 m^2 (20 m × 30 m) was mapped out from each management unit and taken as a plot. Total area mapped out from all landuses was thus 2,400 m^2. Each of the plots was further subdivided into four equal subplots (150 m^2 = 10 m × 15 m) as illustrated in the FA section of Figure 2, for the purpose of sampling. From each subplot, four surface (0-30 cm) samples were systematically collected with the Auger. This meant 16 of such

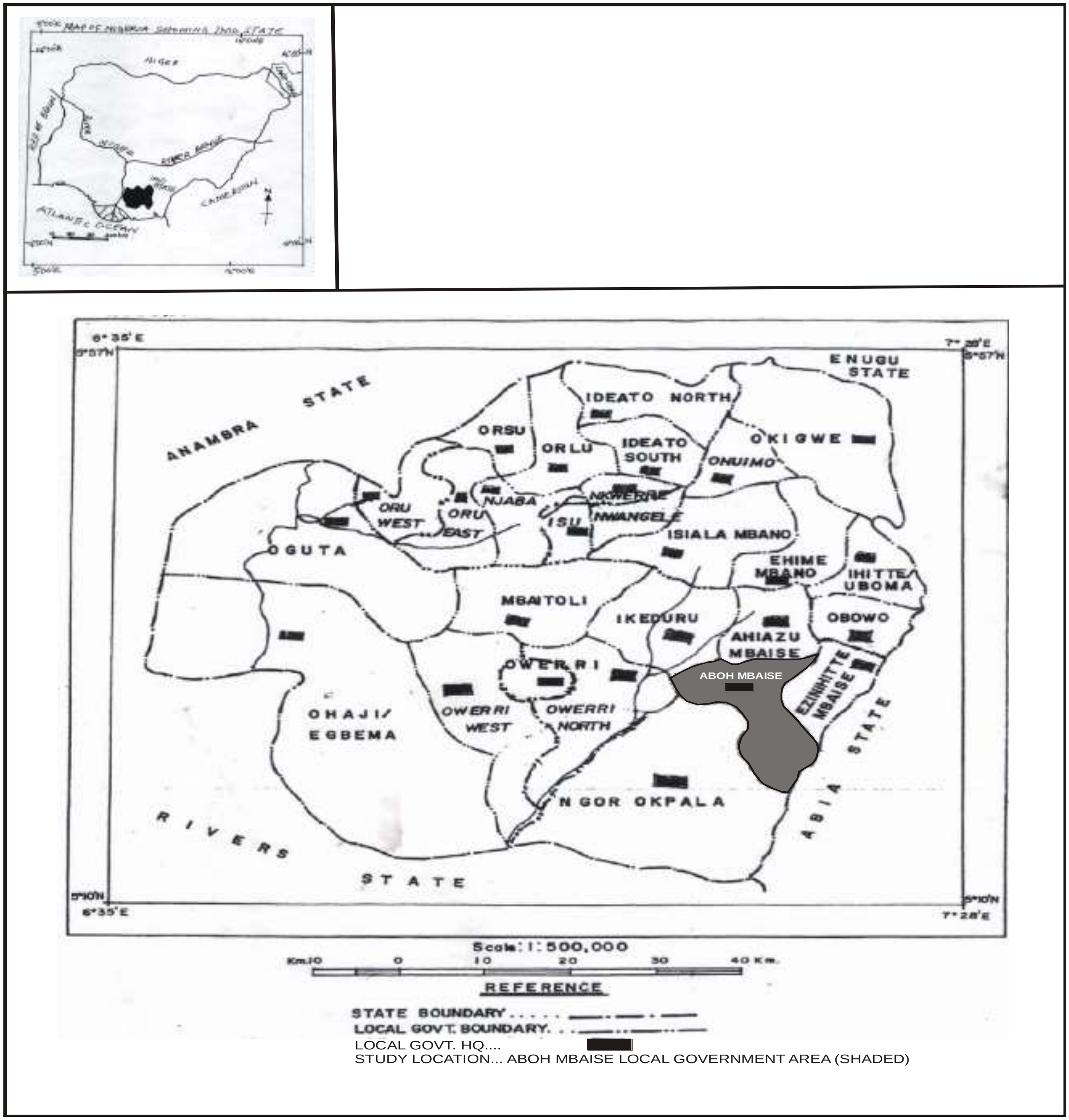

Figure 1. Map of Imo state showing local government areas.

samples were got from each plot, while a total of 64 samples were taken from all plots at this stage of sampling. The four samples from each subplot were then bulked, with the result that each treatment had four bulk samples. Each of the samples thus represented a replicate. A total of 16 bulked samples were therefore collected and used for the study. The depth of sampling represents the average for flat surface soils of the area (Oti, 2002; Ukaegbu, 2014).

Sampling was done once and in the month of June, during the rains when leaching process was at its peak. Sampling was not repeated in the next year as plots under the management of farmers were cultivated.

Laboratory analyses

The soil samples were air-dried and sieved with a 2-mm sieve. The particle size analysis was by the method of Bouyoucos (1951). Soil reaction (pH) was determined using the glass electrode meter at a

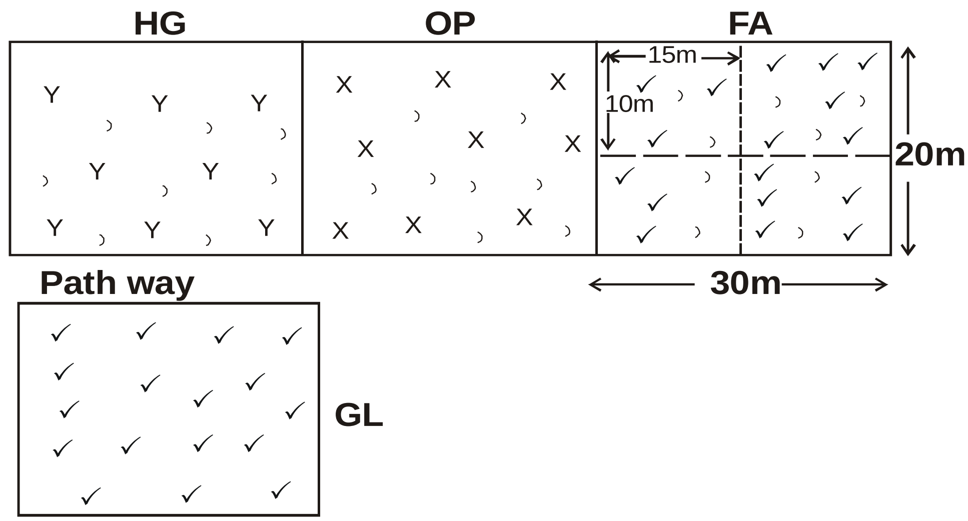

Figure 2. Diagram illustrating layout of fields.

soil: water ratio of 1:2.5. Organic carbon was determined using oxidation method modified by Nelson and Sommers (1990). Organic matter was then got by multiplying the value of organic carbon with 1.724. Available phosphorus was determined using Bray II method of Bray and Kurtz (1998). Exchangeable bases were extracted with neutral normal ammonium acetate solution. Ca^{2+} and Mg^{2+} in the extract were determined by EDTA titration, while K^+ and Na^+ were got by flame photometer. Exchangeable acidity was determined by the method of Mclean (1965). Effective cation exchange capacity (ECEC) was calculated as the summation of total exchangeable bases and exchange acidity. Base saturation was got by expressing total exchangeable bases (TEB) as percentage of ECEC.

Statistical analyses

Spatial variability of each parameter within each management unit and over the whole area sampled was determined using coefficient of variation (CV), given by expressing standard deviation as a percentage of mean of parameter. The CVs got were rated based on the rating scale of Wilding et al. (1994): CV (%) ≤15%, low; >15 ≤35%, moderate; >35%, high.

The CV values were plotted in the form of frequency distribution graph for each management unit and entire field.

Data of each parameter in the various management units were subjected to Analysis of Variance (ANOVA), with the means for the various units separated by LSD at 5% significance level. Relationships of some indices of soil degradation used in soil evaluation were determined using Pearson's Product Moment Correlation Model at 5% significance level.

Qualities of soils under different management units were rated on the basis of parameters to determine their usefulness as indices of soil quality.

RESULTS

Variability of soil properties

The variability of soil properties expressed as CV is shown in Table 1, with the attached small letters representing variability groupings according to Wilding et al. (1994).

Parameters with low variation against the management units were: sand, silt, pH, OM, Ca, K, Na, ECEC, BS (HG); sand, clay, pH, OM, ECEC, BS (OP); sand, silt, clay, pH, OM, ECEC, BS (FA); sand, pH, OM, K, Al+H, BS (GL); sand, pH (Entire field). Those with moderate variation were: clay, Mg, Al+H, (HG); silt, av.P, Ca, Mg, K, Al+H (OP); Ca, Mg, K, Na (FA); silt, clay, av.P, Na, ECEC (GL); silt, clay, OM, K, Na, ECEC, BS (Entire field). Those with high variation were: av.P (HG); av.P, Al+H (FA); Ca, Mg (GL); av.P, Ca, Mg, Al+H (Entire field), and none for unit OP.

The line graphs represented by Figure 3a, b, c, d, and e show the distributions of CV values to vary as management and land area. While the parameters whose CVs constitute the peaks of frequency curves vary with management in most cases, those that constitute the lowest points are sand and pH in virtually all the cases. Distributions tend to be normal in HG but skewed in the others.

The analysis of variance (ANOVA) as shown by the capital letters in Table 1 indicated the parameters sand, OM, pH, Ca, Mg, K, Al+H, ECEC, and BS to be significantly influenced by management.

Rating of parameters

The parameters are rated following the ratings of Enwezor et al. (1989) and Chude et al. (2011). The average pH value for plot A is moderately acid, while the others are strongly acid. The OM contents were generally high for soils of the area. Exchangeable Ca was very low for plot D, but only low for the other units. Exchangeable Mg was high (2.63 Cmol kg^{-1}) for plot A, but moderate for

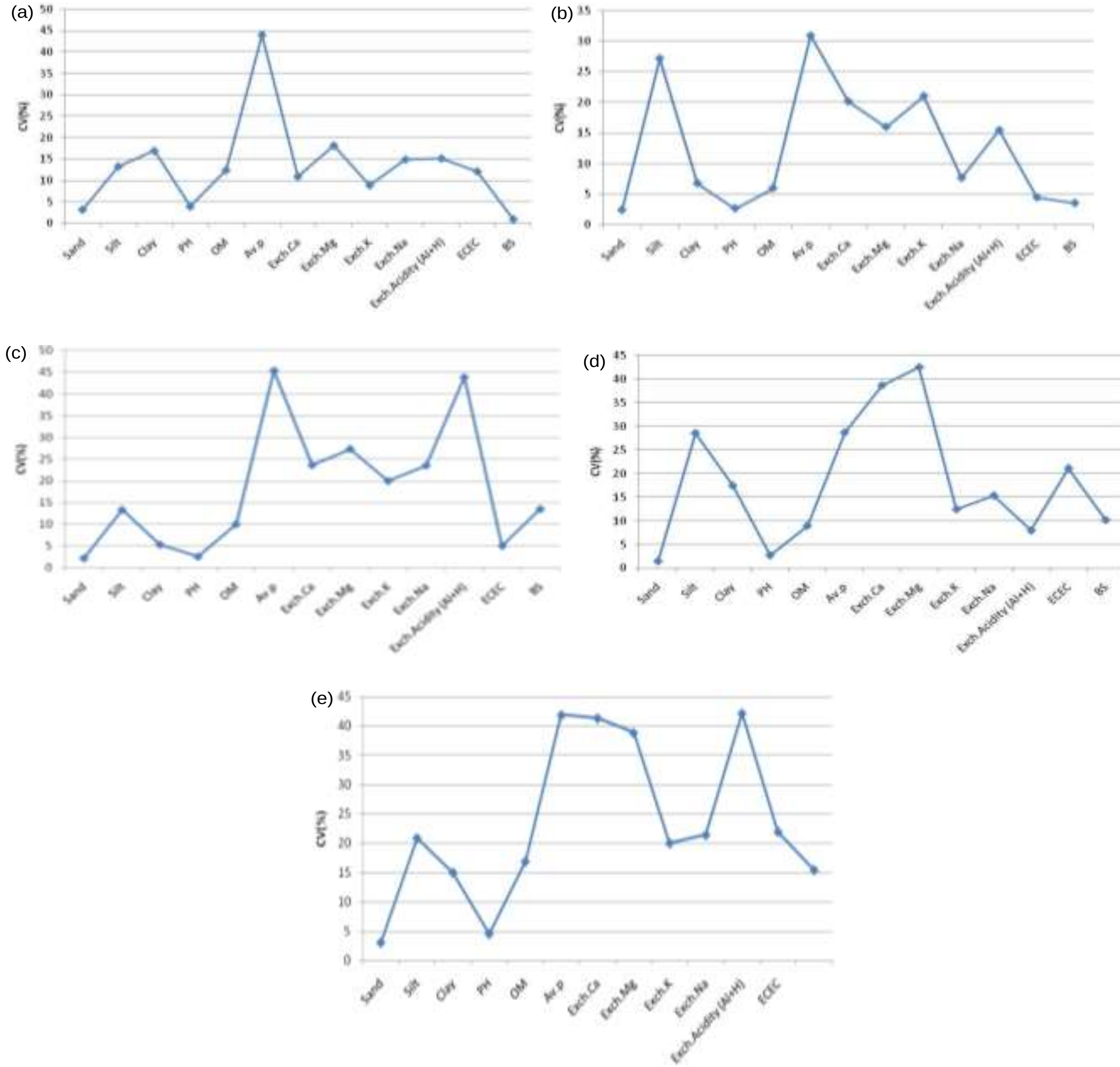

Figure 3. Frequency distribution of CVs of parameters in (a) Home garden (HG), (b) Oil palm plantation (OP), (c) Arable field (FA), (d) GrassLawn (GL) and (e) entire field.

the rest, with plot D having the least value of 1.13 Cmol kg^{-1}. Exchangeable K was low for plot A (0.25 Cmol Kg^{-1}), but very low for the rest, with the least value of 0.16 Cmol kg^{-1} got in plot D. Exchangeable Na was low for plots A, B and D (0.25 - 0.26 Cmol kg^{-1}), but moderate for plot C which value was 0.34 Cmol kg^{-1}. Base saturation was very high for plot A (92.29%), but high for the rest with the least value of 63.48% got in plot D. Available-P was high for all the plots. Exchange acidity for plot A was the least at an average value of 0.63 Cmol kg^{-1}, but highest for plot D which had a value of 1.75 Cmol kg^{-1}. Effective cation exchange capacity (ECEC) was low for plots A and C (8.12 and 6.31 cmol kg^{-1} respectively) but very low for plots B and D which had values 5.8 cmol kg^{-1} and 4.92 cmol kg^{-1}, respectively, indicating dominance of 1:1 type of clay. From the ratings it can be seen that the soils, particularly of plots B, C and D, are limited mainly by strong soil acidity, low ecec, as well as low contents of Ca and K.

Nutrient balance

Availability of nutrients for plant uptake does not depend

Table 1. Variations in soil properties within and between plots and entire field.

Soil property	Plot A (HG)			Plot B (OP)			Plot C (FA)			Plot D (GL)			Entire Field		
	$\bar{X}$	S	CV (%)	$\bar{X}$	S	CV (%)	$\bar{X}$	S	CV (%)	$\bar{X}$	S	CV (%)	$\bar{X}$	S	CV (%)
Sand (%)	77.3^{AB}	2.52	3.26^{a}	79.3^{A}	1.92	2.42^{a}	73.8^{B}	1.63	2.21^{a}	76.8^{AB}	1.15	1.5^{a}	76.8	2.31	3.0^{a}
Silt (%)	7.5^{A}	1.00	13.33^{a}	6.0^{A}	1.63	27.17^{b}	7.5^{A}	1.0	13.33^{a}	7.0^{A}	2.0	28.57^{b}	7.0	1.46	20.87^{b}
Clay (%)	15.2^{A}	2.58	16.97^{b}	14.7^{A}	1.0	6.8^{a}	18.7^{A}	1.0	5.35^{a}	16.2^{A}	2.83	17.47^{b}	16.2	2.42	14.95^{b}
pH	5.48^{A}	0.22	4.01^{a}	5.2^{B}	0.14	2.69^{a}	5.03^{B}	0.13	2.58^{a}	$5..05^{B}$	0.14	2.77^{a}	5.19	0.23	4.5^{a}
O.M (%)	5.68^{A}	0.71	12.49^{a}	4.18^{B}	0.25	5.98^{a}	5.08^{A}	0.51	10.04^{a}	4.12^{B}	0.37	8.98^{a}	4.77	0.80	16.86^{b}
Av. P ($mgkg^{-1}$)	48.38^{A}	21.29	44.01^{c}	34.63^{A}	10.70	30.9^{b}	49.75^{A}	22.55	45.33^{c}	32.25^{A}	9.26	28.71^{b}	41.25	17.28	41.89^{c}
Exch. Ca (cmol kg^{-1})	4.38^{A}	0.48	10.96^{a}	2.38^{BC}	0.48	20.17^{b}	2.75^{B}	0.65	23.64^{b}	1.63^{C}	0.63	38.65^{c}	2.78	1.15	41.37^{c}
Exch. Mg (cmol kg^{-1})	2.63^{A}	0.48	18.20^{b}	1.75^{B}	0.28	16.0^{b}	1.5^{B}	0.41	27.33^{b}	1.13^{B}	0.48	42.48^{c}	1.75	0.68	38.86^{c}
Exch. K (cmol kg^{-1})	0.25^{A}	0.02	8.96^{a}	0.19^{AB}	0.04	21.05^{b}	0.2^{AB}	0.05	20.0^{b}	0.16^{B}	0.02	12.5^{a}	0.20	0.04	20.0^{b}
Exch. Na (cmol kg^{-1})	0.25^{A}	0.04	14.96^{a}	0.26^{A}	0.02	7.69^{a}	0.34^{A}	0.08	23.53^{b}	0.26^{A}	0.04	15.38^{b}	0.28	0.06	21.43^{b}
Exch. Acidity (cmol kg^{-1})	0.63^{B}	0.10	15.22^{b}	1.23^{A}	0.19	15.45^{b}	1.53^{A}	0.67	43.79^{c}	1.75^{A}	0.14	8.0^{a}	1.28	0.54	42.19^{c}
ECEC (cmol kg^{-1})	8.12^{A}	0.99	12.19^{a}	5.8^{B}	0.26	4.48^{a}	6.31^{B}	0.32	5.07^{a}	4.92^{B}	1.04	21.14^{b}	6.29	1.38	21.94^{b}
BS (%)	92.29^{A}	0.95	1.03^{a}	78.91^{B}	2.81	3.56^{a}	76.03^{B}	10.28	13.52^{a}	63.48^{C}	6.48	10.21^{a}	77.68	11.96	15.40^{b}

The small letters represent variability groupings following the rating of Wilding et al. (1994). thus: a = cv < 15%; b = cv 15-35%; c = cv >35%. The capital letters represent LSD groupings. Means with different letters are significantly different ($p<0.05$). The small letters are read vertically while the capital letters are read horizontally across the plots comparing their means.

Table 2. Soil nutrient balance for the plots.

Landuse	Ca/Mg	Mg/K	K/Mg	Ca/TEB	% (K/TEB)
HG	1.67	10.52	0.10	0.58	3.33
OP	1.36	9.21	0.11	0.32	2.53
FA	1.83	7.50	0.13	0.37	2.66
GL	1.44	7.06	0.14	0.22	2.13

entirely on their absolute values in soils, but also on their ratios/balance. Table 2 shows average values of the ratios Ca/Mg, Mg/K, K/Mg, Ca/TEB, and % (K/TEB) for the various management units.

These ratios are noted to influence the availability of nutrients (Ahukaemere et al., 2014; Msanya et al., 2016; Udo et al., 2009). Table 2 shows plot C to have the highest value of 1.83 for Ca/Mg ratio, while plot B had the least value of 1.36. Msanya et al. (2001) record that Ca/Mg ratio of 2 to 4 are considered favourable for most crops. Udo et al. (2009) record that for Ca:Mg ratio > 5:1, there is possibility of Mg and (with high pH) P inhibition; 3:1 – 5:1 is normal range; while < 3:1 signifies possibility of P inhibition and Ca deficiency. Similarly, Landon (1984) notes Ca/Mg ratio that is less than 3 to result in unavailability of Ca and P. Observed values of the ratio are less than the optimal range indicating possible unavailability of Ca and P. This could be worse for plots B(OP) and D(GL) with the lowest values. Mg/K ratio ranges from 10.52 for plot A to 7.06 for plot D. Landon (1991) records Mg/K ratio of 1 to 4 as optimal for nutrient uptake by plants. Observed values are above the optimal thus signifying imbalance. On the other hand, K/Mg ratio ranges from 0.10 for HG to 0.14 for GL unit. Ahukaemere

Table 3. Average values of parameters as indices of soil degradation for the landuses.

Landuse	Ca+Mg	Ca/Mg	Ca+Mg/Al+H	ESP	Silt/Clay
HG	7.01	1.67	11.13	3.08	0.49
OP	4.13	1.36	3.36	4.48	0.41
FA	4.25	1.83	2.78	5.39	0.40
GL	2.76	1.44	1.58	5.28	0.43
CV (%)	**33.98**	**11.78**	**79.84**	**20.0**	**8.14**

et al. (2014) note a K/Mg ratio of 3:1 or little wider as favourable for plants; while Udo et al. (2009) record ratio greater than 2:1 could affect Mg uptake. However, the low K/Mg ratio recorded indicates need for liming (Ahukaemere et al., 2014). Ca/TEB ratio varied from 0.22 (GL) to 0.58 (HG). According to Landon (1991), Ca/TEB ratio of less than 0.5 is favourable for the uptake of other cations particularly Mg and/or K. The ratios observed may be indicating availability of Mg and K, except perhaps for plot A. According to Uwingabire et al. (2016) and Uwitonze et al. (2016), % (K/TEB) for topsoil above 2% is considered favourable for most tropical crops. The observed values range from 2.13% for plot D, to 3.33% for plot A, which are therefore conducive for plant growth.

Evaluation by other indices of soil degradation

The factors silt/clay, fine sand/coarse sand, Ca/Mg, Ca+Mg, Ca+Mg/Al+H, OC and Al-saturation had been recommended by Oti (2002) as reliable predictors for identification and delineation of erosion classes for the tropical ultisols. Some of these parameters have been used in evaluating the soils as indicated by Table 3.

The values for Ca+Mg ranged from 2.76 (GL) to 7.01 (HG). Ca/Mg ratio varied from 1.36 (OP) to 1.83 (FA). The ratio Ca+Mg/Al+H had values ranging from 1.58 (GL) to 11.13 (HG). ESP varied from 3.08 (GH) to 5.39 (FA). Silt/clay ratio had values ranging from 0.40 (FA) to 0.49 (HG). These were however, average values for the management units. The coefficients of variation (CV) of these values, among the management units (as shown by Table 3) were 33.98, 11.78, 79.84, 20.0, and 8.14% for the indices Ca+Mg, Ca/Mg, Ca+Mg/Al+H, ESP, silt/clay, respectively. This means Ca/Mg and silt/clay ratios have varied lowly among the management units, while the others varied moderately to highly. Those that varied relatively highly are better differentiators of the management units.

Using the parameters, soils of management units can be rated on the basis of that of the home garden (HG) with best quality or, in the reverse order that of grass lawn (GL) with worst quality. This can be shown using the indices: OM, Ca+Mg, Al+H, Ca+Mg/Al+H. On the basis of OM, the value of 5.68% for HG is taken as 100%. Relative to this, the contents for the other units are: 73.59% (OP); 89.44% (FA); 72.54% (GL). So, the extent to which the other landuses are degraded, in terms of OM are: 26.41% (OP); 10.56% (FA); 27.46% (GL). Using Ca+Mg as basis for evaluation, the value of 7.01 for HG is the standard, taken as 100%. Then the values for the other units, expressed as percentage of that of HG are: 58.91% (OP); 60.63% (FA); 39.37% (GL). So, the extent to which the units are degraded by this index are: 41.09% (OP); 39.37% (FA); 60.63% (GL). Using the ratio Ca+Mg/Al+H as basis for evaluation and taking the value of 11.13 for HG as standard, then the values for the other units, OP, FA, GL are, 30.19, 24.98, 14.20% respectively. So, by this index the extent to which the units OP, FA, GL are degraded are: 69.81, 75.02, and 85.80%, respectively.

In reverse order, the value of Al+H which is highest in the GL (1.75 cmol/kg) can be used as standard. So, as percentage of the GL value, the other units are: 36% (HG); 70.29% (OP), 87.43% (FA). Because this index is in the negative, subtracting these values from 100 gives the actual quality of each of the units thus: 64% (HG); 29.71% (OP), 12.57% (FA).

How these indices of soil quality or degradation relate among themselves is shown in Table 4. The ratio Ca+Mg/Al+H has significant ($p<0.5$) positive correlation with Ca+Mg and OM, with correlation coefficients (r) of 0.97 and 0.82, respectively; but significant negative correlation with esp and exchangeable acidity with correlation coefficients of -0.94 and -0.96, respectively. ESP and exch. acidity had significant positive correlation ($r = 0.97$) with each other. Organic matter has correlated with most of the indices. It has significant positive correlation ($r = 0.78$ to 0.88) with Ca+Mg, Ca/Mg, Ca+Mg/Al+H, but negative correlation (though not significant) with ESP and exch. acidity. This shows OM to influence the indices much. The higher the values of OM, Ca+Mg, and Ca+Mg/Al+H, the greater the quality of soils; while the higher the values of ESP and exch. acidity, the more degraded the soils.

DISCUSSION

Spatial variability of soil properties in any field is due to soil forming factors, including management (Beckett and Webster, 1971; Igbal et al., 2005). In the present study, only management varied while the other factors were constant. Variation in management included differences

Table 4. Relationships among the indices of soil degradation.

Correlation factor	Correlation coefficient (r)
Ca+Mg VS Ca/Mg	0.55^{NS}
Ca+Mg VS Ca+Mg/Al+H	0.97**
Ca+Mg VS Silt/Clay	0
Ca+Mg VS ESP	-0.37^{NS}
Ca+Mg VS OM	0.88**
Ca+Mg VS Exch. Acidity	-0.33^{NS}
Ca/Mg VS Ca+Mg/Al+H	0.30^{NS}
Ca/Mg VS Silt/Clay	0
Ca/Mg VS ESP	0
Ca/Mg VS OM	0.78**
Ca/Mg VS Exch. Acidity	-0.5^{NS}
Ca+Mg/Al+H VS Silt/Clay	0
Ca+Mg/Al+H VS ESP	-0.94**
Ca+Mg/Al+H VS OM	0.82**
Ca+Mg/Al+H VS Exch. Acidity	-0.96**
Silt/Clay VS ESP	0
Silt/Clay VS OM	0
Silt/Clay VS Exch. Acidity	0
ESP VS OM	-0.63^{NS}
ESP VS Exch. Acidity	0.97**
OM VS Exch. Acidity	-0.17^{NS}

DF = 6; P<0.05; Table r = 0.707.

in crops, soil amelioration/fertilizer application, and in tilling. The influence of management on variability of properties has manifested in various ways.

Available-P varied most in plots A, B, C, with the highest CV of 45.33% recorded in plot C and the least (28.71%) recorded in plot D which had not been fertilized. While tilling/cultivation homogenizes the soils' particle sizes, it also brings up finer particles (Akamigbo, 1999; Dilkova et al., 1998) hence the sand separate was significantly lower in plot C at 73.8% than the rest. Generally, the particle sizes had least variation in plot C. The high within-field variation of some of the properties in plot C is attributed to the fact of the plot being the most recently cultivated. Beckett and Webster (1971) reported that variability rises in recently fertilized fields but reduces with fallow. Burning of trash might have contributed to the high variability of exch. acidity (Al+H) in plot C. The biggest within field CV for exch. acidity of 43.79% was recorded in plot C. while the least (8.0%) was recorded in plot D. Fraser and Scott (2011) as well as Nye and Greenland (1964) recorded the burning of timber and trash to not only increase soil pH but also to contribute to the spatial variability of soil acidity. Relatively high values of OM and ECEC in plot C might have arisen from fertilizer application. Ellerbrock and Gerk (2016) found all types of fertilizer to increase CEC and SOM in soils.

Most parameters: OM, pH, av.P, exchangeable bases, BS, ECEC; had their biggest values in plot A (HG). This is attributed to the greater addition of organic matter in the plot as had also been recorded for fallow forests in the area (Akamigbo, 1999; Asadu and Bosah, 2003; Nnaji et al., 2002). Trees and shrubs with their deep roots have been noted by Young (1998) to mobilize bases from soil substrate which help counteract soil acidity, increase nitrogen content and produce enough biomass to maintain soil humus content. The other management practices lack these qualities. Residual effect of soil amendments/organic manuring of the HG unit might as well have contributed to the quality of the soils. Application of woodash as well as animal manures to the acid ultisols of southeast Nigeria has been noted to significantly reduce the soils acidity (Ano and Ubochi, 2007; Nottidge et al., 2006). Such amendments have been found to persist in soils seven years after application (Wuest and Reardon, 2016). This further explains some of the contrasts with earlier studies. Nnaji et al. (2002) found soil pH, BS and av.P to be significantly lower in forest soil, while OC, TN, total exch. acidity and cec were higher relative to the cultivated. With the exception of exch. acidity which was significantly lower in plot A, the other chemical properties were higher in the plot than in the others. The relatively low variability of soil properties in HG unit (plot A) agrees with Ghartey et al. (2012) who found natural fallows to decrease soil variability.

Generally, variations of properties within management units were less than those over the entire field. The reasons, according to Beckett and Webster (1971) include the fact that variances increase with size of area sampled; the between-field component of variance for properties much affected by management tends to be greater than that of more durable properties; the bulking of samples masks the within field component of variance. However, properties have varied on disparate scales. Sand and pH had low variation within management units and entire field, indicating macrouniformity. On the other hand, OM, ECEC, BS generally had low variation within management units but moderate variation over the entire field. The other properties, particularly av.P, Ca, Mg, exch. acidity varied highly within field and over the entire field. These later properties can be said to be microvariable and most influenced by management. Zheng et al. (2009) noted strong spatial dependency of soil properties to be related to structural intrinsic factors such as texture, parent material and mineralogy, while weak spatial dependency is related to random extrinsic factors such as plowing, fertilization and other soil management practices.

The rate of variation of parameters within a small area of 2,400 m^2 in the study is attributable to land fragmentation and accompanying differences in management. The limit of 33% (CV) set by Akamigbo (1986) to signify homogeneity within a map unit in the area of study has been exceeded in the small area sampled. Soil sampling in the area for fertility survey should therefore be at high intensity.

By rating, the soils particularly of plots B, C and D are limited mainly by strong soil acidity, low ECEC, low contents of Ca and K. This is supported by low Ca/Mg and K/Mg ratios, as well as high Mg/K ratio signifying nutrient imbalance, the potential to limit availability of Ca, Mg, and P, while indicating the need for liming. Relating soils of units OP, FA, and GL to that of HG further showed the parameters OM, Ca+Mg, Ca/Mg, Al+H, Ca+Mg/Al+H, ESP as indices of degradation. The parameters that have varied more proved to be better indices of degradation. Among these indices, the ratio Ca+Mg/Al+H varied most as shown in Table 3 and captured the degradation most. The soil qualities as quantified by the index (Ca+Mg/Al+H) are in the order HG>OP>FA>GL. The other management practices accentuate soil acidification more than the HG unit. Though OM had not captured the full extent of degradation, it had significant positive correlation with the positive indices Ca/Mg, Ca+Mg, Ca+Mg/Al+H, thus showing its significance on the soils' qualities. These characteristics identified as indices of soil quality can be used to develop quantitative models for estimating soil quality in the area (Brevik et al., 2016). The soils, particularly of OP and FA, can be improved by adding organic manure, liming and planting of legumes. The soil conditions of HG unit should generally guide soil management in the area. In the absence of virgin forests, soils of old home garden sites that are fallow for not more than seven (7) years can be used as standards to measure the extent of degradation of soils under other landuses.

Conclusion

Landuse has influenced physico-chemical properties of the soils. Strong soil acidity and associated problems of nutrient deficiencies are the main soil fertility constraints, which have been captured best by the ratio Ca+Mg/Al+H. Based on the index the order of qualities of the soils of the management units were HG>OP>FA>GL. Management should aim at meeting the standards of the home garden site. Study has shown organic manuring and liming as critical for the soils management.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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Comparison of laboratory methods in predicting the lime requirement of acid soil in Wombera District, North western Ethiopia

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Soil acidity is one of the major constraints of crop production in the Ethiopian highlands where precipitation is high enough to leach basic cations leaving the soil acidic. Liming is the major practice used to ameliorate the problem of soil acidity. Currently farmers use Ag lime in their fields to reduce soil acidity. However, the suitability of the existing methods of lime requirement (LR) has not yet being determined. Shoemaker-McLean-Pratt (SMP) single buffer, Adams-Evans buffer, Modified Mehlich buffer, titration with single addition of Ca $(OH)_2$, and exchangeable aluminum methods were evaluated using the $CaCO_3$ moist incubation method. The result revealed that the LR estimated by the buffer methods and titration highly correlated with the incubation LR since the correlation coefficient(r) was ≥ 0.98. The modified Mehlich buffer was better in predicting the LR on average, for target pH values of 5.5, 6.0, 6.5, 6.8 and 7.0 as r = 0.99 and as the standard error of estimate ($S_{y.x}$) 0.57, the minimum among the buffer methods. The modified Mehlich buffer should be calibrated further with the reference $CaCO_3$ incubation using a set of soils and a calibration equation to calculate the lime requirement.

Key words: Acidity, lime requirement, calibration, buffer methods, incubation.

INTRODUCTION

One of the major constraints of crop production in many regions of the world is soil acidity. Soil acidification or decrease in soil pH is primarily a natural process that is accelerated by human activities in crop production practices, as they use acid bearing fertilizers such as those containing nitrogen (Anderson et al., 2013). Soil acidity directly and indirectly influences the growth of plants; it affects the availability of plant nutrients, level of phytotoxic elements, and microbial activity and is a serious limitation to crop production in many regions of the world (Pagani and Mallarino, 2012).

Acid soils are found in large areas in both tropics and temperate regions. In Ethiopia, it is estimated that around 43% of the total cultivated land is affected by soil acidity (Yirga et al., 2019). Out of this percent, about 28.1% is dominated by strong acid soils (pH 4.1-5.5) (Yirga et al., 2019), and such soils are less fertile due to toxicity of Aluminum and Manganese and deficiencies of calcium, magnesium, phosphorus and molybdenum (Havlin et al. 2005). To correct this, acid soils are usually limed.

Soil pH referred to as the concentration of hydrogen ion in soil solution is necessary to determine whether to lime

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a soil or not. However, it is necessary to measure the buffer capacity of the amount of lime needed by the soil (Ketterings et al., 2010). Lime requirement is the amount of lime required to increase the pH of soil from an acidic condition to a value that is considered optimum for the desired use of the soil (Sims, 1996).

Various methods have been developed to estimate the LR of acidic soils: Soil-lime incubation, direct titration, use of buffer solution and exchangeable acidity/Al extracted with unbuffered salt. All the methods have their own strength and weakness. The soil-lime incubation with $CaCO_3$ is the ideal method to determine the LR of acid soils as it simulates field liming and is conducted in greenhouses or laboratories (Sims 1996). The method is commonly used to calibrate other methods (Hoskins and Erich, 2008; Shoemaker et al., 1961). However, it takes several weeks to complete the reaction between soil and lime applied and thus is not used for routine analysis (McLean, 1982). Buffer pH methods have been developed for a quicker assessment of LR. The Shoemaker-McLean-Pratt (SMP) buffer method (Shoemaker et al., 1961), Adams-Evans (AE) buffer method (Adams and Evans, 1962), and the modified Mehlich (MM) (Hoskins and Erich, 2008) are some of the commonly used buffer methods. All the methods relate the pH of the soil that is allowed to react with the buffer to the LR of a soil population.

According to McLean et al. (1966), the SMP buffer method is accurate and well adapted to soils requiring > 4480 kg/ha, having pH <5.8, soil organic matter (OM) < 10% and having appreciable quantities of soluble aluminum. However, for soils with low LR (<4480 kg/ha), high pH (pH>5.8), and high organic matter (>10%), the results have been inaccurate (McLean et al., 1966). In comparing SMP with other buffer solutions, it is revealed that it is applicable to a broad group of soils having a wide range in LR values (McLean et al., 1977). The Adams and Evans (A-E) buffer for LR determination was developed to measure the LR of low activity clays and course- textured soils that are dominated by kaolinite and sesquioxide soil minerals (Adams and Evans, 1962).

Modified Mehlich buffer was developed by Hoskins and Erich (2008) to simulate the original buffer except barium chloride is replaced by calcium chloride in the buffer to remove the protocols for hazardous waste disposal due to the presence of barium. The authors reported that the modified Mehlich (MM) buffer is better than SMP during routine usage; it eliminates hazardous waste and has fewer adverse effects on electrode degradation.

Soil-base titrations procedure entails titrating (equilibrating) a soil suspension with a basic solution such as calcium hydroxide [$Ca(OH)_2$] to estimate the amount of lime required (Liu et al., 2005; Barouchas et al., 2013). The direct titration procedures initially developed by Dunn (1943) using multiple rates of 0.002 M $Ca(OH)_2$ took 4-days and were found to be too time-consuming for use in routine soil testing. However, 3-day incubation with a $Ca(OH)_2$ is a widely accepted reference method (McConnell et al., 1990; Owusu-Bennoah et al., 1995). However, the titration method was adapted and used as a routine soil testing procedure for LR determination; it became an alternative to the buffer method (Liu et al., 2005).

Calculation of the LR based on the amount of exchangeable aluminum extracted using unbuffered salt is used around the world. It has been suggested that the amount of lime sufficient to neutralize the exchangeable aluminum is adequate to avoid possible aluminum toxicity which is described as the main yield limiting factor in highly weathered acid mineral soils (Kamprath, 1970; Van Lierop, 1990). Accordingly, liming such soils containing sufficiently high level of aluminum to pH 5.5 can significantly increase crop yield (Van Lierop, 1990). The method is popular in areas where crop production is limited by highly acidic aluminous soils and the availability of limestone is limited (Sims, 1996). However, routine analysis of exchangeable Al for LR estimation is a challenge due to time and cost (Ketterings et al., 2010).

Soil testing laboratories in Ethiopia mainly use the SMP single buffer or the classical exchangeable acidity/Al method as a routine soil testing method to determine LR. Nevertheless, data on the suitability of these methods in predicting the LR of soils of Ethiopia in general and Wombera District in particular are lacking. With this, it is important to identify accurate and rapid LR determination method that better suits routine analysis. Therefore, this study aims to meet the following objectives: (1) To compare the LR determination methods (SMP, Adams-Evans, Modified Mehlich, $Ca(OH)_2$ titration, exchangeable Al) with the reference (greenhouse incubation) method; (2) To select the method that correlates better with the incubation LR for different target pH values.

MATERIALS AND METHODS

Description of the soil sampling site

Wombera District (Wereda) is located in Metekel Zone of the Benishangul Gumuz Regional State, North Western Ethiopia. The district is located within 9°56´24.33´´ and 11°8´17.09´´ north latitude and 35°9´3.49´´and 35°55´35.51´´east longitude. The agroecological zone (AEZ) of the area belongs to the tepid sub-humid mid highlands (Ministry of Agriculture, 2005). The mean annual rainfall of the area is 1550 mm. The average maximum and minimum temperatures are 27 and 12°C, respectively. According to BCEOM (1998), the soil class of the area is Rhodic Nitisols. The topography of the area is undulating or hilly. Mixed farming system is practiced by the local society of the study area that involves animal husbandry and crop production. The major crops grown are :tef (*Eragrostis* tef), niger seed (*Guizotia abyssinica*).Other crops grown are wheat (*Triticum aestivum* L.), barley (*Hordeumvulgare* L.), and potato (*Solanum tuberosum* L.) (Figure 1).

Soil sampling

Prior to soil sampling, farm plots having severe soil acidity problem

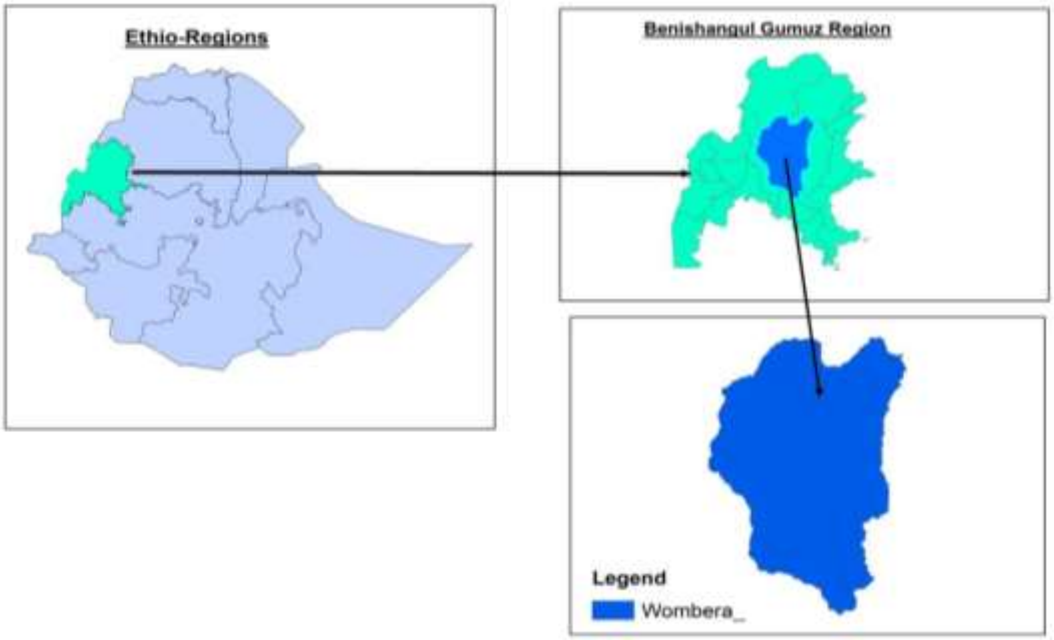

Figure 1. Location Map of Wombera Wereda.

(low pH) were screened from those that have less or no soil acidity problem. Following the selection of the farm plots, soil samples were collected from 0-20 cm depth at random to make one composite sample and a handheld auger was used to collect the samples. The composite soil sample was taken to the National Soil Testing Center where physicochemical analysis and incubation experiment was conducted. The composite soil sample was air dried ground and sieved to pass through a 2 mm sieve for physicochemical analysis and soil-lime incubation experiment. For total nitrogen and organic carbon determination, the soil was further pulverized and passed through 0.5 mm sieve.

Physicochemical analysis

The soil physicochemical properties were measured prior to incubation and lime requirement determination. Soil particle size analysis was determined following Bouyoucos hydrometer (Bouyoucos, 1951). Soil pH in water (pH_w) and pH KCl were determined in a 1:2.5 suspension according to Van Reeuwijk (2002). Soil organic carbon (OC) was determined by the chromate acid oxidation method (Walkley and Black, 1934) and soil OM was calculated by multiplying percent OC by a factor of 1.724. Cation exchange capacity (CEC) was measured using the ammonium acetate method. Exchangeable acidity was determined by saturating the samples with 1 M KCl solution and titrated with 0.02 M NaOH as described by Rowell (1994). The exchangeable Al was determined from the same extract by applying 1 M NaF which formed a complex with Al and released NaOH; and then the NaOH was back titrated with a standard solution of 0.02 M HCl.

Lime requirement methods

Incubation method

Three kilograms of soil was incubated in a greenhouse for a period of one month with different rates of analytical grade $CaCO_3$ including no liming (control). The rate of $CaCO_3$ to achieve target pH of 5.5 was first determined based on the soil-buffer pH measured and the corresponding LR value obtained from the adapted SMP table (Van Lierop, 1990). The rate of $CaCO_3$ was converted from tons/ha to gm/kg assuming the soil bulk density to be 1.4 gcm^{-3} and incorporation depth to be 20 cm.

The amounts of $CaCO_3$ added in the incubation experiment were: 11.4, 17.1, 22.8, 28.5, 34.2 and 39.9 gm. Each of the lime rates were replicated three times. The various rates of $CaCO_3$ were first mixed thoroughly with the dry prepared soil using a spatula. A polyethylene pot was used in the incubation experiment (Figure 2). The mixtures were incubated while maintaining the field capacity. The temperature of the greenhouse ranged from 25-27°C. After the incubation period, the mixtures were air dried and the pH was determined in a 1:2.5 (soil-water) suspension.

SMP single buffer method

The SMP buffer solution preparation and measurement of soil-buffer pH were done according to Watson and Brown (1998). The LR corresponding to the soil-buffer pH value for different target pH values was obtained from the table prepared by Shoemaker et al. (1961).

Adams-Evans buffer method

The Adams-Evans buffer solution preparation, buffer pH measurement procedure, and determination of the LR were made as described by Sims (1996).

Modified Mehlich buffer Method

The buffer solution preparation and determination of the LR in ton/ha were done using equations adapted from Sikora and Moore (2014).

Figure 2. Greenhouse incubation experiment.

Single addition of Ca $(OH)_2$ method

Soil pH measurements and titration were performed in 1:1 soil-water ratio. The soil $Ca(OH)_2$ mixture was shaken for 5 min on an end to end shaker and left to stand for 25 min before taking the soil pH measurement (Liu et al., 2005). Successive 3 ml aliquots of 0.022 M Ca $(OH)_2$ were added and pH was measured until it reached 7.30. All determination was made in triplicate. Calculation of the titration slope and determination of the LR for different target pH were determined using the formula described by Liu et al. (2005) as follows.

The slopes of the titration curve were estimated using consecutive two pH readings [before and after the addition of 3 ml of Ca $(OH)_2$], and the LR was calculated using the equation;

$$LR = \frac{((Target\,pH - initial\ \ pH))}{b} \times \mathrm{f} \quad (1)$$

Where b is the slope of the relationship of pH vs. $Ca(OH)_2$ added and f is the factor.

The calculation of the titration slopes for the different target pH values were determined using a series of conversion equations adapted from Liu et al. (2005).

Exchangeable aluminum method

The exchangeable Al determined withthe unbuffered salt was used to estimate the LR that would possibly increase the pH of the soil to 5.5. Accordingly, the LR for 20 cm soil depth and bulk density of 1.4 gcm^{-3} was calculated by converting meq/100 g soil of exchangeable Al to kg/ha as illustrated in Equation 2.

$$LR = \frac{2.8 \times 10^6\ \ kg\ soil}{ha} \times \frac{meqAl}{100\,g\ soil} \times \frac{0.05\,gCaCO\,3}{meq} \quad (2)$$

Data analysis

Duncan's mean separation test was conducted to determine the completion of the incubation period using SAS/STAT User's Guide (2008). Linear regression and correlation analysis were generated using Microsoft Excel (Microsoft Corporation, 2007).

RESULTS AND DISCUSSION

Physicochemical characteristics of the soil

The textural class of the soil is clay as illustrated by the high clay content (48%).The pH H_2O and pH KCl were 5.2 and 4.14 respectively. The percent of the organic carbon content (5.00) and CEC (29.52 meq/100 g) of the soil are rated medium and high according to Landon (1991). The high clay content of the soil could be the reason for the high CEC value. The delta pH value of the soil was negative (-1.06), indicating that the soil had a net negative charge (CEC) in the clay colloid (Table 1).

Lime incubation study

The amount of $CaCO_3$ applied in the incubation study resulted in an increase in soil pH value of at least 6.37 (Figure 3). Accordingly, the incubation study can be used to calibrate other methods. The decrease in pH of the control (no treatment) soil (5.15) from the pre-incubation pH level (5.2) could have been due to nitrification effect, which is noted as one of the main sources of soil acidity. The quantity of lime added versus pH response was plotted and fitted by non-linear (polynomial) regression. The lime requirement for the target pH values (5.5, 6.0, 6.5, 6.8 and 7.0) was calculated using the equation. The amount of lime required for the corresponding pH values was 2.72, 7.18, 12.52, 16.47, and 19.68 tons of lime per hectare. The graph clearly indicates that there was a gradual increase in the pH of the soil, as lime application increased up to pH 7.4 from where, it started to decrease. Similar results were reported by Shoemaker et al. (1961) and Hoskins and Erich (2008).

Table 1. Physicochemical properties of the soil.

Sand (%)	Silt (%)	Clay (%)	OC (%)	pHW	pH KCl	∆pH	EC (dsm^{-1})	CEC (meq/100 g)	Ex. Acidity (meq/100 g)	Ex. Al (meq/100 g)
16	36	48	5.00	5.20	4.14	-1.06	0.079	29.52	2.33	2.19

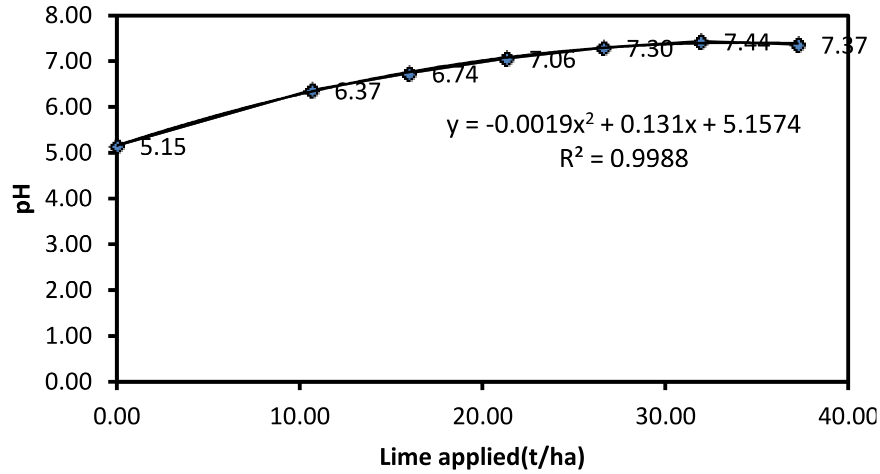

Figure 3. Lime response curve of the incubation experiment.

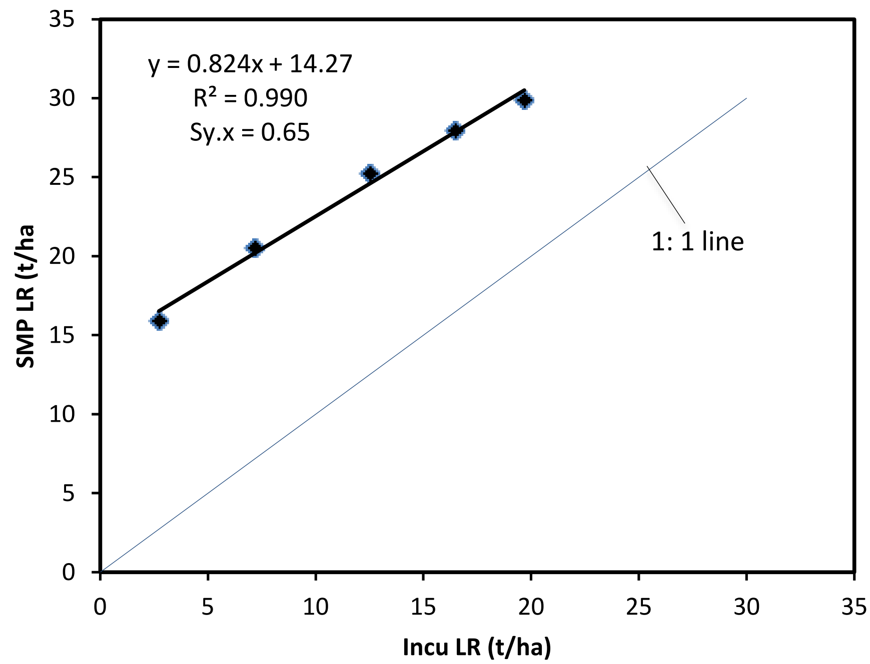

Figure 4. SMP versus Incubation LR in tons/ha.

SMP buffer indicated vs. calcium carbonate incubation measured LR

The LR estimated using the SMP buffer pH value and incubation highly correlated ($r^2 = 0.99$). Nevertheless, the SMP overestimated the LR for all target pH values as indicated in Figure 4. The t-statistics in the regression analysis revealed that the slope of the regression equation was significantly different from one *(p*= 0.00041), and the intercept was also significantly different

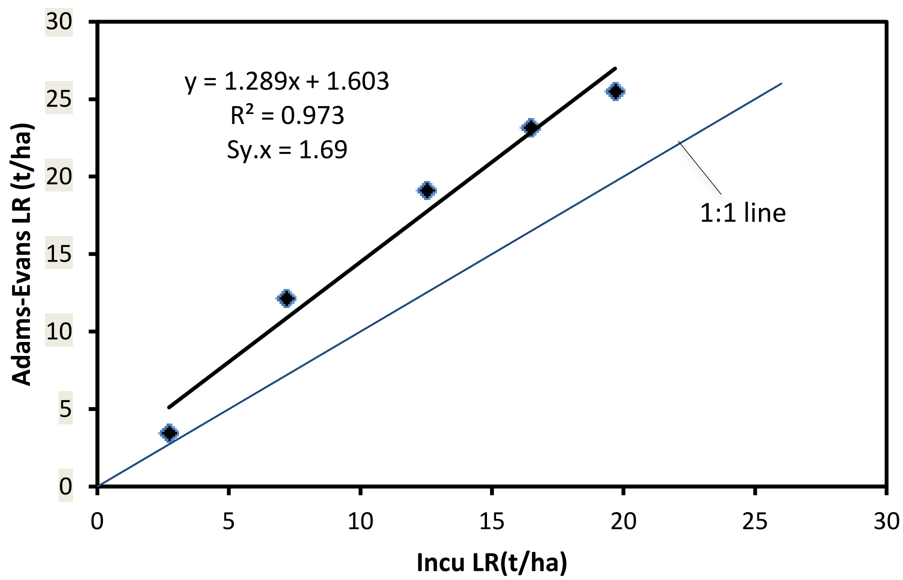

Figure 5. Incubation LR versus Adams-Evans estimated LR.

from zero (p = 0.0001). This indicates that, the SMP overestimated the LR in both the low and high LR values. Machacha (2005) found that the SMP single buffer method showed a high correlation (r= 0.80) between the LR predicted by the buffer method compared to the incubation LR; though it overestimated the actual LR for target pH of 6.5.

Adams evans buffer estimated LR vs. incubation LR

The Adams-Evans buffer estimated LR was also highly correlated with the incubation LR, as indicated by the high correlation (r^2= 0.97) value (Figure 5). The t-statistics calculated in the regression analysis indicated that the slope of the regression equation (1.289) was significantly different from one (p=0.00187). However, the intercept (1.603) was not significantly different from zero (p = 0.398). This implies that the Adams-Evans buffer LR was relatively close to the incubation LR at low LR values (Figure 5). Nevertheless, as the LR value increases, the Adams-Evans estimated LR departs from the incubation LR. The standard error of estimates for the Adams-Evans was higher than the SMP. Thus, the regression equation poorly explains the variability in LR as compared to the SMP.

Modified Mehlich (MM) buffer estimated LR vs. incubation LR

Similarly, the modified Mehlich buffer estimated LR was highly correlated with the incubation LR (r^2 = 0.989). In contrast to the SMP and Adams-Evans, the modified Mehlich (MM) narrowly underestimated the LR for target pH of 6.5, 6.8 and 7.0 and slightly overestimated the LR for target pH of 5.5 (Figures 6 and 7). For target pH of 6.0, the incubation and modified Mehlich LR are similar. The standard error of estimates for the modified Mehlich (Sy.x=0.57) was relatively lower than the SMP and Adams-Evans (Figure 6). Accordingly, the regression equation for the modified Mehlich better explains the variability in LR than the two methods. Hoskins and Erich (2008) also found that the modified Mehlich predicted the LR better than the SMP for the target pH of 6.0.6.5 and 7.0. Wolf et al. (2008) reported that the modified Mehlich was better than SMP in predicting the LR for target pH of 6.5 and 7.0.

Titration curve of the pH vs. $Ca(OH)_2$ added

The plot of soil pH as a function of $Ca(OH)_2$ added was linear (Figure 7). It shows an increase in pH of soil with the addition of $Ca(OH)_2$. Similar result was reported by Barouchas et al. (2013).

LR calculated using Single Addition of $Ca(OH)_2$ vs. Incubation LR

As compared to the incubation, the calcium hydroxide titration greatly underestimated the LR for both the low and high LR values (Figure 8). The slope of the regression equation clearly showed that it measured about 11.6% of the incubation LR on average for the target pH values. The finding is similar to what Godsey et al. (2007) found. The authors reported that titration

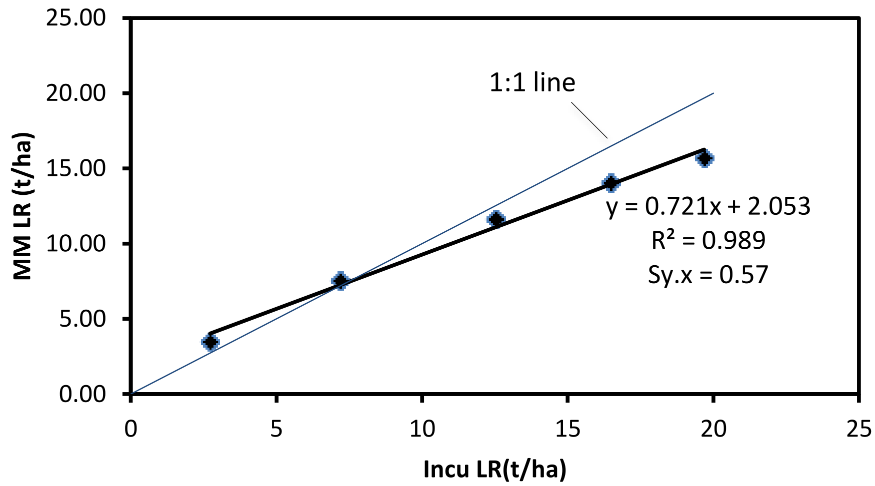

Figure 6. Modified Mehlich versus incubation LR in tons/ha.

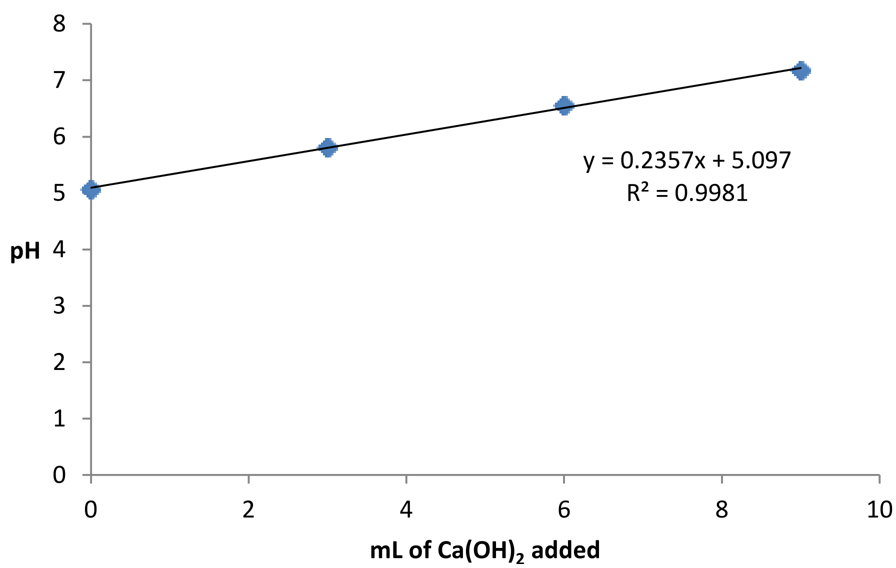

Figure 7. Titration curve displaying the amount of added Ca $(OH)_2$ vs. pH.

underestimated the 60-day incubation LR and measured only 45% of it. In contrast, Liu et al. (2004) found that titration with $Ca(OH)_2$ measured 80% of the LR from a 3-day incubation. The disagreement in the findings could be the high clay and CEC content of the soil used in this study which would give the high buffering capacity to the soil compared to the soil used in a 3-day incubation period.

The exchangeable aluminum method for LR estimation of LR

Based on the exchangeable Al, the LR was calculated using Equation 2; it is about 3.066 kg/ha or 3.07 ton/ha, and is the equivalent lime amount required to neutralize the exchangeable Al. According to Kamprath (1970), liming equivalent amount of exchangeable Al resulted in the neutralization of most of the exchangeable Al of the acid soils and raised their pH near 5.5. If we compare the exchangeable Al equivalent LR (3.07 ton/ha) with the incubation LR (2.72 ton/ha) assuming the 3.07 ton/ha would bring the soil pH to 5.5 from the initial pH of 5.2, it is a little bit higher than the incubation LR, though the difference is small (0.25 tons/ha). For target pH of 6.0, 6.5, 6.8 and 7.0, comparing the method with the incubation or other LR methods is impossible as the LR is only based on neutralizing the exchangeable Al.

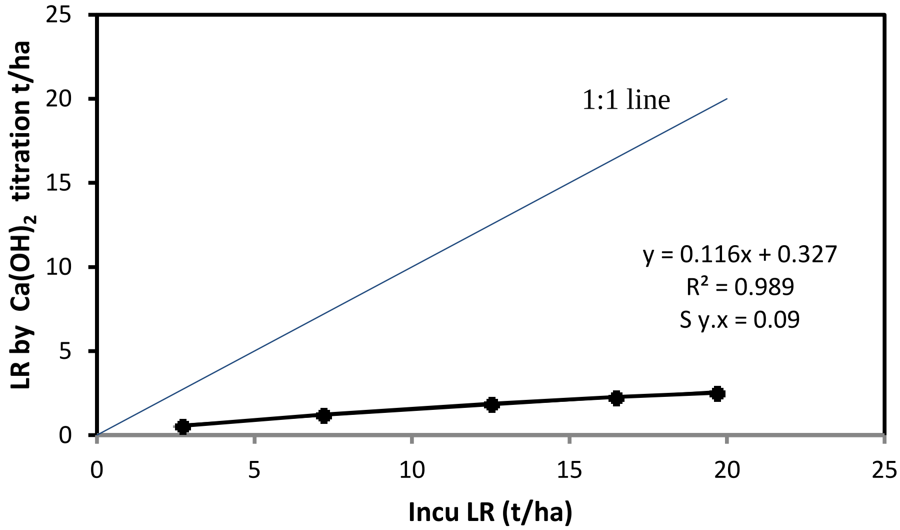

Figure 8. Relationship between the LR estimated by Ca $(OH)_2$ and incubation.

Conclusion

All the methods compared with the incubation LR except the exchangeable acidity method showed a high correlation as the r^2 values illustrated. Of the methods compared, the Modified Mehlich buffer was found to be closer in estimating the LR to the reference, incubation LR as Figure 6 depicts. However, further calibration of the method using a set of soils would be necessary to derive a calibrated equation to calculate LR.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of different rates of filter cake against bruchids (*Zabrotes subfasciatus* (Boheman) and *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) on common bean and cowpea

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Storage of common bean and cowpea is limited due to different species of bruchids. *Zabrotes subfasciatus* and *Callosobruchus maculatus* are the most important species of bruchids attacking stored common beans and cowpeas, causing yield losses reaching up to 38-100%. Two experiments, one on the effect of different rates of Filter Cake against *Z. subfasciatus* on common bean, and the other experiment on the effect of different rates of Filter Cake against *C. maculatus* on cowpea were conducted at Melkassa in 2015, 2016 and 2017. Half liter capacity transparent plastic bags were used for the experiment. About 0-12 h old ten unsexed *Z. subfasciatus* adults were introduced to common bean seeds. For cowpea experiment, 0-12 h old 10 *C. maculatus* were used with similar procedures with that of common bean. The experiment was laid out in a Completely Randomized Design. Different crops and insect related data were collected. The results obtained demonstrated that significant differences (P<0.05) were observed among the treated and un treated seeds. The rate of mortality was significantly (P<0.05) higher in all the rates of filter cake when compared to the untreated check 3 days after treatment application. Percent weight loss was nil in all the treatments on common bean and cowpea, while it was above 10% in the untreated check. The germination percentage was not impaired by the treatments. From the current result, it can be concluded that Filter Cake can be used for the control of *Z. subfasciatus* and *C. maculatus*.

Key words: *Callosobruchus maculatus,* common bean, cowpea, filter cake, mung bean, *Zabrotes subfasciatus.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculate*) (L.) Walp are among the most important food and cash crops in Eastern and Southern Africa (Abate and Ampofo, 1996; Schmale et al., 2002; Aliyu and Wachap, 2014; Bhattarai and Mehlenbacher, 2017; Boukar et al., 2016)). Pre-harvest and post-harvest

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damage by insect pests are the major limiting factors of common bean and cowpea (Wright et al., 1989). Stored beans and peas suffer heavy losses in terms of both quality and quantity mostly by bean bruchids (Negasi, 1994; Abate and Ampofo, 1996). *Zabrotes subfasciatus* (Boheman) and *Callosobruchus maculatus* (Fabricius) are the most important species of bruchids attacking stored beans and peas, causing yield losses ranging from 38-100% (Schoonhoven and Cardona, 1986; Adane and Abraham, 1995; Abraham et al., 1994).

Bruchids infestation results in quantity, quality and viability losses (Adane and Abraham, 1995). The degree of losses depends on the storage period and storage conditions. Ahmed (2017a) reported an average grain loss of 60% within 3-6 months of storage period due to bruchids on common beans. Losses on cowpea reach 100% in 3 months' time in the storage which shares the same species of bruchids, *C. maculatus* (Emana et al., 2003; Swella and Mushobozy, 2007). A number of pest control methods which range from cultural control methods (use of inert materials, mixing with small seeded grains for storage, etc.), use of different botanicals, use of tolerant/resistant varieties and use of insecticides among others were tried (Strong and Suber, 1968; Abraham, 2003; Emana et al., 2018; Mulatwa et al., 2017). However, the level of control arrived at could not keep the pest below economic injury level. The use of filter cake on *Sitophilus zeamais* (Motsch) was effective (Abraham, 2003). Hence, the objective of the current experiment is to see the efficacy of different rates of filter cake on two species of bruchids attacking common bean and cowpea under laboratory condition.

MATERIALS AND METHODS

Description of the study area

Melkasa Research Centre is found at 130 km away from Addis Ababa to the East. It is located on the road side of Asella at an elevation of 1500 m above level and coordinates of 8°24'N and 39°21'E. The experiment was conducted at 40-50% relative humidity and a mean daily temperature of 28±0.5°C.

Experimental procedures

Common bean and cowpea seeds used for the experiment were obtained from Melkassa Lowland Pulse Research and multiplied in the center to obtain sufficient amount of seeds required for the experiment. The treatments (T1-T8) include different rates of filter cake (T_1 = 0.03% w/w, T_2 = 0.05% w/w, T_3 = 0.0.08% w/w, T_4 = 0. 09% w/w, T_5 = 0.188% w/w, T_6 = 0.37 % w/w, T_7 = 0.75%w/w, and T8=Untreated check). Filter cake was obtained from Awash Melkassa Aluminium Sulphate and Sulphuric Acid Factory.

Adult bruchids were collected from stores holding common bean and cowpea to establish cultures of *Z. subfasciatus and C. maculatus* of similar age (0-12 h) bruchids for the experiment. A susceptible variety, Aregene was used for mass rearing of bruchids under an average room temperature of 28°C and a relative humidity of 50%. The experiment was intentionally set between March and May as these months were found to be the pick infestation periods of bruchids which is aligned with high temperatures of the months. One hundred and fifty-gram seeds were put in 250 cm^3 capacity glass jars with brass screen lids that permit ventilation. Adult bruchids were introduced in each jar at the ratio of one bruchid to 15 g of seeds, which was equivalent to 10 unsexed adult bruchids to each jar. Treatments were applied accordingly and the experiments (1 common bean and 1 cowpea experiments) were laid out in a completely randomized design (CRD) in three replications.

Data collection

Dead bruchids were counted at the 3rd and 7th days after infestation. At the 7th day, both dead and live bruchids were counted and removed and the grains were kept under the same conditions for emergence of F_1 progenies. The F_1 progenies were counted and removed each day until emergence ceased. Additional data were collected on number of adult bruchid mortality, number and weight of damaged and undamaged grains. Percent weight losses were calculated using count and weigh method.

$$\text{\% Weight loss} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u (N_d + N_u)} \times 100$$

Where, Wu= weight of undamaged seed, Nu=Number of undamaged seed, Wd= weight of damaged grains, Nd= Number of damaged seed.

Germination of seeds was tested by randomly taking one hundred seeds from each jar and placing them on moist filter paper in a Petri dish for five days. Then seeds germinated against non-germinated seeds were counted.

Statistical analysis

All data were subjected to statistical analyses using SAS version 9.0 computer software. Mean separations were done using Student-Newman-Keuls (SNK) Range Test.

RESULTS AND DISCUSSION

Mean adult mortality of *Z. subfasciatus* 3 days after treatment application (ATA), mean number of F1 progeny of Z. *subfasciatus*, percent weight loss and percent seed germination 90 days (ATA) in common bean in 2015, 2016 and 2017 are shown in Tables 1 to 3. All rates of filter cake significantly (P<0.05) resulted in almost 100% adult *Z. subfasciatus* mortality 3 days ATA. No F1 progeny was recorded in all filter cake treatments, but 26 mean F1 progeny was recorded in the untreated check. With respect to percent weight loss there was no significant differences among the filter cake treatments. However, all rates of filter cake significantly different from the untreated check on which economic losses (10.32%) were recorded. All common bean seeds treated with different rates of filter cake germinated 90 ATA which was significantly (P<0.05) different from the untreated check in which 15% germination reduction was recorded (Table 1).

Mean adult mortality of *Z. subfasciatus* 3 days after

Table 1. Effect of different rates of filter cake on the adult mortality of *Z. subfasciatus* 3 days after treatment application (ATA), mean number of F1 progeny, percent weight loss and percent germination 90 days ATA in common bean, 2015.

Different rates of filter cake	Adult mortality 3 days ATA	Number of F1 progeny	Percent weight loss 90 days ATA	Percent seed germination 90 days ATA
0.03	9.50±0.1[a]	0.00±0.00	0.03±0.01[a]	100.00±3.4[a]
0.05	9.50±0.1[a]	0.00±0.00	0.17±0.01[a]	100.00±3.4[a]
0.08	9.30±0.1[a]	0.00±0.00	0.06±0.01[a]	100.00±3.4[a]
0.09	10.00±0.3[a]	0.00±0.00	0.00±0.01[a]	100.00±3.4[a]
0.188	10.00±0.3[a]	0.00±0.00	0.00±0.01[a]	100.00±3.4[a]
0.37	10.00±0.3[a]	0.00±0.00	0.01±0.01[a]	100.00±3.4[a]
0.75	10.00±0.3[a]	0.00±0.00	0.00±0.01[a]	100.00±3.4[a]
Untreated check	0.00±0.00[b]	26.00±1.65	10.32±0.01[b]	85.00±2.8[b]

Means (±se) followed by the same letter (s) within a column are not significantly different from each other at 5%, Student-Newman-Keul`s Range Test.

Table 2. Effect of different rates of Filter Cake on the adult mortality of *Z. subfasciatus* 3 days after treatment application (ATA), mean number of F1 progeny, percent weight loss and percent germination 90 days ATA in common bean, 2016.

Different rates of filter cake	Adult mortality 3 days ATA	Number of F1 progeny	Percent weight loss 90 days ATA	Percent seed germination 90 days ATA
0.03	10.00±0.1[a]	0.00±0.00	0.00±0.00[a]	100.00±3.4[a]
0.05	10.00±0.1[a]	0.00±0.00	0.00±0.00[a]	100.00±3.4[a]
0.08	10.00±0.1[a]	0.00±0.00	0.00±0.00[a]	100.00±3.4[a]
0.09	10.00±0.1[a]	0.00±0.00	0.00±0.00[a]	100.00±3.4[a]
0.188	10.00±0.1[a]	0.00±0.00	0.00±0.00[a]	100.00±3.4[a]
0.37	10.00±0.1[a]	0.00±0.00	0.00±0.00[a]	100.00±3.4[a]
0.75	10.00±0.1[a]	0.00±0.00	0.00±0.00[a]	100.00±3.4[a]
Untreated check	0.00±0.00[b]	25.00±2.54	11.33±0.1[b]	80.00±2.6[b]

Means (±se) followed by the same letter (s) within a column are not significantly different from each other at 5% level, Student-Newman-Keul`s Range Test.

treatment application (ATA), percent weight loss and percent seed germination 90 days (ATA) in common bean in 2016 are shown in Table 2. In terms of mean adult mortality, percent weight loss and percent germination, similar results with that of 2015 recorded such that all rates of filter cake resulted in 100% adult mortality 3 days ATA, no effect on germination and no weight loss 90 days ATA. About 20% germination reduction was recorded in the untreated check. There was no F1 progeny recorded in all Filter Cake treatments, but 25 mean F1 progenies were recorded in the untreated check.

Table 3 shows the results of 2017. Results obtained were similar to the results of 2015 and 2016 except the figure that changed. All filter cake treatments significantly killed *Z. subfasciatus* 3 days ATA. However, no adult mortality was recorded 3 ATA in the untreated control. No F1 progeny was recorded in all filter cake treatments, but 35 mean F1 progeny was recorded in the untreated check. Percent weight loss 90 days ATA was insignificant and/or absent, but it was 17.6% in the untreated check. Percent seed germination was 100% 90 days ATA, but it was 76.5% in the untreated check.

The effect of different rates of filter cake on the management of *C. maculatus* on cowpea in 2015, 2016 and 2017 are shown in Tables 4 to 6. *C. maculatues* mean adult mortality was 100% in all filter cake treatments, but 0% in the untreated check 3 days ATA in cowpea in 2015. Number of F1 progeny emerged from all the treatments of filter cake, but 22 mean number of progenies emerged from the untreated check which was significantly ($P<0.05$) different from the treated seeds of cowpea with filter cake. Percent weight loss was not recorded in all filter cake treatments, but significant ($P<0.05$) economic loss of 12.43% was recorded in the untreated check. Cowpea seeds treated with filter cake germinated by 100%, 90 days ATA, but 24% germination reduction were recorded in the untreated check (Table 4). Results obtained in 2016 (Table 5) and 2017 (Table 6) were similar to results of 2015 in terms of *C. maculatus* adult mortality, number of F1 progeny, percent weight loss and percent seed germination 90 days ATA; adult mortality was 100% (10/10 dead), number of F1 progeny and percent weight loss were nil and percent seed

Table 3. Effect of different rates of Filter Cake on the adult mortality of *Z. subfasciatus* 3 days after treatment application (ATA), mean number of F1 progeny, percent weight loss and percent germination 90 days ATA in common bean, 2017.

Different rates of filter cake	Adult mortality 3 days ATA	Number of F1 progeny	Percent weight loss 90 days ATA	Percent seed germination 90 days ATA
0.03	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.05	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.08	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.09	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.188	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.37	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.75	0.00±0.00[b]	25.00±2.54[b]	13.50±0.4[b]	71±1.5[a]

Means (±se) followed by the same letter (s) within a column are not significantly different from each other at 5% level, Student-Newman-Keul`s Range Test.

Table 4. Effect of different rates of Filter Cake on the adult mortality of *C. maculatus* 3 days after treatment application (ATA), mean number of F1 progeny, percent weight loss and percent germination 90 days ATA in cowpea, 2015.

Different rates of filter cake	Adult mortality 3 days ATA	Number of F1 progeny	Percent weight loss 90 days ATA	Percent seed germination 90 days ATA
0.03	10±0.[1a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.05	10±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.08	10±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.09	10±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.188	10±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.37	10±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.75	10±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
Control	0±0.0[b]	22.00±2.21[b]	12.43±0.3[b]	76±2.7[b]

Means (±se) followed by the same letter (s) within a column are not significantly different from each other at 5%, Student-Newman-Keul`s Range Test.

Table 5. Effect of different rates of Filter Cake on the adult mortality of *C. maculatus* 3 days after treatment application (ATA), mean number of F1 progeny, percent weight loss and percent germination 90 days ATA in cowpea, 2016.

Different rates of filter cake	Adult mortality 3 days ATA	Number of F1 progeny	Percent weight loss 90 days ATA	Percent seed germination 90 days ATA
0.03	10.00±0.1[a]	0.00±0.00[a]	0.00±0.00[a]	100±3.5[a]
0.05	10.00±0.1[a]	0.00±0.00[a]	0.00±0.00[a]	100±3.5[a]
0.08	10.00±0.1[a]	0.00±0.00[a]	0.00±0.00[a]	100±3.5[a]
0.09	10.00±0.1[a]	0.00±0.00[a]	0.00±0.00[a]	100±3.5[a]
0.188	10.00±0.1[a]	0.00±0.00[a]	0.00±0.00[a]	100±3.5[a]
0.37	10.00±0.1[a]	0.00±0.00[a]	0.00±0.00[a]	100±3.5[a]
0.75	10.00±0.1[a]	0.00±0.00[a]	0.00±0.00[a]	100±3.5[a]
Control	0.00±0.00[b]	26.00±1.31[b]	26.35±1.35[b]	75±2.42[b]

Means (±se) followed by the same letter (s) within a column are not significantly different from each other at 5%, Student-Newman-Keul`s Range Test.

germination was 100%.

From the results obtained, it can be inferred that different rates of filter cake can effectively control the two species of bruchids: *Z. subfasciatus* and *C. maculatus*. Filter cake is an industry waste product which may not be used by the industry except in rare cases; but if the material can be used for the management of important insect pests like these ones it can cause up to 100%

Table 6. Effect of different rates of filter cake on the adult mortality of *C. maculatus* 3 days after treatment application (ATA), mean number of F1progeny, percent weight loss and percent germination 90 days ATA in cowpea, 2017.

Different rates of filter cake	Adult mortality 3 days ATA	Number of F1 progeny	Percent weight loss 90 days ATA	Percent seed germination 90 days ATA
0.03	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.05	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.08	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.09	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.188	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.37	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
0.75	10.00±0.1[a]	0.00±0.00[a]	0.00±0.0[a]	100±3.5[a]
Control	0.00±0.0[b]	32.00±2.22[b]	14.38±0.5[b]	72±1.9[b]

Means (±se) followed by the same letter (s) within a column are not significantly different from each other at 5%, Student-Newman-Keul`s Range Test.

losses in 6 to 9 months storage periods. In addition to its efficacy, materials like filter cake have no and/or little damage to the non-target organisms including those natural enemies associated to stored grain insect pests. Materials like filter cake can be used to protect grains that can be stored as seed materials for the next planting season though it can be used to protect seeds that can be consumed by human beings and animals. A number of authors reported the efficacy of filter cake in the management of stored grain insect pests which is in line with the current findings (Abraham, 2003; Ahmed, 2017a, b; Mulatwa et al., 2017; Emana et al., 2018). There is no single bullet to control any insect pests like *Z. subfasciatus* and *C. maculatus*, but looking for alternatives which can be pulled together in the form integrated pest management the current fashion of pest management (Emana et al., 2018). In this regard the use of filter cake at the minimum rate is of paramount importance.

Conclusion

In the current study, filter cake at the minimum rate is found to be effective against *Z. subfasciatus* and *C. maculatus* on common bean and cowpea. Hence, farmers who are growing common bean and cowpea in Ethiopia can use filter cake for the management of pest as an option of integrated pest management.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Comparison of yield performance and rice quality between direct-seeded and hand-transplanted rice under different nitrogen rates in Eastern China

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In order to clarify whether the shift from hand-transplanting seedlings to direct seeding will bring negative effects to rice production and to find optimal nitrogen management for direct-seeded rice in Eastern China, research has been conducted in Quzhou City, in the Zhejiang Province of China in the year 2017 and 2018. One *indica* inbred rice variety, "Zhongjiazao-17" was planted by two different rice establishment methods (direct seeding and hand-transplantation), and five different nitrogen application rates were set as experimental treatments (0, 120 and 180 kg ha^{-1} in 2017 and 0, 165 and 195 kg ha^{-1} in 2018). The grain yield, economic profit, and rice quality were compared between direct-seeded rice and hand-transplanted rice under different nitrogen rates. Our results indicate that the direct-seeded rice showed no obvious disadvantages in grain yield compared to the hand-transplanted rice, but improved economic profit significantly. The highest grain yield and production profit were achieved at 180 kg N ha^{-1} in direct-seeded rice while the hand-transplanted rice achieved highest grain yield and profit at 165 kg ha^{-1}. The direct-seeded rice showed lower physical quality but higher cooking and tasting quality than the hand-transplanted rice. Increasing the nitrogen rate improved the physical quality but decreased cooking and tasting quality in both the direct-seeded rice and hand-transplanted rice. Hence, direct-seeded rice has the potential to be promoted in Eastern China, and 180 kg ha^{-1} is the optimal nitrogen rate. In addition, applying sufficient nitrogen at panicle initiation is necessary to increase the grain yield of direct-seeded rice.

Key words: Direct seeding, hand-transplantation, grain yield, nitrogen management, physical quality, cooking and tasting quality.

INTRODUCTION

China is one of the largest rice-producing countries in the world, which accounts for more than 30% of the world's rice output (Liu et al., 2019); about 65% of Chinese people consume rice as their stable food (Huang et al.,

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2014). In China, the traditional and dominant method of rice cultivation is hand-transplantation (Kumar and Ladha, 2011; Peng et al., 2009). Since the 1990s, the economic growth has been rapid in China, and many labourers have transferred from rural areas to cities, particularly in the Zhejiang Province, which is located in Yangtze River Delta economic zone in Eastern China. This phenomenon has caused the labor costs for crop production to rise significantly. In order to reduce labour costs and maintain profit margins, many farmers in China have chosen to directly-seed rice instead of using the traditional hand-transplantation method (Liu et al., 2014; Zhang et al., 2018; Ladha et al., 2009), as direct-seeded rice is considered to be a more labour- and resource-effective rice cultivation method compared to traditional hand-transplantation (Sun et al., 2015). As a result, the area of direct-seeded rice continues to increase with each passing year (Mao et al., 2018; Wang, 2015).

Apart from reduced costs of rice planting, rapid increases in the planting area of direct-seeded rice poses negative consequences to rice production systems, such as enhancing the weed-, insect-, and pest-derived damages, increasing the risk of lodging and reducing total grain yield (Akbar et al., 2011; Jat et al., 2019). Numerous studies have been conducted to compare the yield performance of direct-seeded rice and transplanted rice, but the results varied greatly among these studies (Kumar and Ladha, 2011; Farooq et al., 2011). Thus, more studies are needed to investigate the differences of yield performance and other physiological characteristics between direct-seeded rice and transplanted rice to maintain food security.

Nitrogen fertilizer is essential for vigorous rice growth and high yields, and chemical nitrogen fertilizers are extensively used to increase rice production (Cui et al., 2020). However, overuse of nitrogen fertilizer has become a very common phenomenon in rice-producing countries, especially in China (Peng et al., 2010), which has brought negative effects to grain yield and caused serious environmental problems. Numerous studies have sought to improve nitrogen management to reduce total nitrogen use and improve nitrogen use efficiency for hand-transplanted rice (Peng et al., 2010; Li et al., 2014; Chen et al., 2014). However, direct-seeded rice requires different nitrogen management because of different growth characteristics compared to transplanted rice. For example, direct-seeded rice usually has shorter crop duration than transplanted rice (Farooq et al., 2006) because hand-transplanted rice will be injured after transplanting, delaying progression (Tuong et al., 2000). Nevertheless, several studies have indicated that the method and amount of nitrogen loss is also different in direct-seeded rice compared to hand-transplanted rice (Li et al., 2015; Zhang et al., 2018). Thus, studies need to be conducted to investigate the optimal nitrogen fertilization rate and nitrogen management practices for direct-seeded rice, particularly in regions with suboptimal agricultural practices such as China.

As the living standard continues to improve and demand for high-quality rice in China increases, improving rice quality has become a new focus for rice research (Zeng et al., 2019; Peng et al., 2009). The rapid shift from traditional hand-transplanting of rice to direct seeding continues in China; more attention should be concentrated on the quality of direct-seeded rice. It has been established that nitrogen is an important factor that affects rice quality, thus a reasonable nitrogen usage amount and optimal nitrogen management could further increase rice quality as well as improve the head rice rate, reduce the percentage of grain chalkiness, and so on (Hao et al., 2007; Zhu et al, 2017; Zhou et al., 2015). However, most of these works investigated nitrogen-dependent effects in the transplanted rice system, and studies focused on the impacts of nitrogen input and management practices compared to the quality of direct-seeded rice are limited.

In this work, we conducted a series of field experiments in the early season of 2017 and 2018 in Zhejiang province of China. The objectives of this study are: 1) To compare the grain yield, yield components, and rice quality between direct-seeded rice and hand-transplanted rice under different nitrogen application rates to investigate the effects on rice production in Zhejiang Province, Eastern China; and 2) to elucidate the optimum nitrogen rate and nitrogen management for direct-seeded rice.

MATERIALS AND METHODS

Experiment site description

The experiments were conducted in a field located in the Lianhua Township, Quzhou City, Zhejiang Province, China (29°05′N, 119°01′E) in 2017 and 2018. The climate of the township is typically sub-tropical. The soil type in the area is sandy loam with 161 mg kg^{-1} available N, 19.0 mg kg^{-1} available P, 209 mg kg^{-1} available K, 45.5 g kg^{-1} organic C, and soil pH of 6.55.

Experimental design and treatment

The experiments were organized in a split-plot design with three replications. Main plots consisted of two different rice establishment methods: direct seeding (DS) or hand-transplantation (HT); sub plots consisted of three different nitrogen treatments: N0, N120, and N180 in 2017, and N0, N165, and N195 in 2018. "N0," "N120," "N165," "N180," and "N195" refer to the nitrogen application rates of 0, 120, 165, 180, and 195 kg ha^{-1}, respectively, and the detailed information is presented in Table 1. The area of each sub plot was 27 m^2 (6 m × 4.5 m) and there were approximately 1620 and 1500 seedlings in the sub plot of hand-transplanted rice in 2017 and 2018, respectively. Also, 162 g of seeds were used in the sub plot (27 m^2) of direct-seeded rice in each of the two years.

One commercial inbred *indica* rice variety, Zhongjiazao-17, was used as the experimental plant material. This rice variety has a life cycle duration (105 days) of good yield performance, and has been widely used in the early season of the Zhejiang Province. The direct-seeded rice was sown in each experiment plot on April 1, 2017 and March 30, 2018, respectively. The seedbeds were

Table 1. Seeding density and nutrient management of the three nitrogen treatments under different rice establish methods.

Year	Treatment	Density (m^{-2})	Nitrogen ($kg\ ha^{-1}$)	Potassium ($kg\ ha^{-1}$)	Phosphorus ($kg\ ha^{-1}$)
2017	N0	DS [a] 6 g seeds HT: 30 hills (20 cm×16.5 cm, 2 seedlings $hill^{-1}$)	0	90 (90-0-0)[b]	60 (60-0-0)
	N120	DS: 6 g seeds HT: 30 hills (20 cm×16.5 cm, 2 seedlings $hill^{-1}$)	120 (48-36-36)	90 (90-0-0)	60 (60-0-0)
	N180	DS: 6 g seeds HT: 30 hills (20 cm×16.5 cm, 2 seedlings $hill^{-1}$)	180 (72-54-54)	90 (90-0-0)	60 (60-0-0)
2018	N0	DS: 6 g seeds HT: 18.5 hills (30 cm×16.5 cm, 3 seedlings per $hill^{-1}$)	0	90 (90-0-0)	60 (60-0-0)
	N165	DS: 6 g seeds HT: 18.5 hills (30 cm×16.5 cm, 3 seedlings per $hill^{-1}$)	165 (66-49.5-49.5)	90 (90-0-0)	60 (60-0-0)
	N195	DS: 6 g seeds HT: 18.5 hills (30 cm×16.5 cm, 3 seedlings per $hill^{-1}$)	195 (78-58.5-58.5)	90 (90-0-0)	60 (60-0-0)

[a]DS and HT represent direct seeding, hand-transplantation, respectively.
[b]The numbers in the parentheses represents the split rates of the fertilizer application at basal, mid-tillering, panicle initiation, respectively.

prepared for the seedlings of transplanted rice, and compound fertilizer ($N:P_2O_5:K_2O$=15:15:15) was applied to the seedbed at a rate of 150 kg ha^{-1}. The hand-transplanted rice was sown on the same day as the direct-seeded rice in each of the two years and the transplanting dates were May 6th and May 3rd in 2017 and 2018, respectively.

Soils were mechanically ploughed and harrowed before sowing (for direct-seeded rice) and transplanting (for hand-transplanted rice). The basal fertilizer was applied to each experimental plot one day before sowing and transplanting. Pesticides and herbicide were used to prevent proliferation of insects, diseases, and weeds. The water management practices of direct-seeded and transplanted rice were followed as adopted by the local farmers, respectively.

Measurement and data analysis

The plant samples were harvested at maturity in each year. The sample area was 0.2 m^2 (0.4 m×0.5 m) for direct-seeded rice and 0.3 m^2 (9 plants) for hand-transplanted rice in each plot in 2017, while in 2018 the sample area was 0.25 m^2 (0.5 m×0.5 m) for direct-seeded rice and 0.3 m^2 (6 plants) for hand-transplanted rice in 2018. Each sample was separated into straw and panicles, then the panicles were threshed by hand; the spikelets were separated into filled and unfilled populations by submerging in tap water. In 2017, a subsample of 50 g of filled spikelets was taken in each plot, and in 2018, three subsamples of 30 g filled spikelets were taken in each plot, respectively. The subsample of filled spikelets and all of the unfilled spikelets were counted to calculate spikelets per panicle, grain weight, and filled grain percentage. The grain yield was determined from a 5 m^2 area in each plot and grain was adjusted to the standard moisture content of 0.14 H_2O g^{-1} fresh weight. All plant materials were kept in an oven at 75℃ for three days and weighed to determine aerial biomass accumulation.

Rice quality was measured in 2018. The filled grain samples from each of the plots were kept for approximately 3 months under ventilated conditions for standby application. The filled grains were mechanically milled (SLJM-2009, Shanghai Shalun, China), then the milled rice was weighed and manually separated into head rice and chalky grains. The milled rice rate, head rice rate, and the percentage of grain chalkiness were calculated by the following formulas:

Milled rice rate = Milled rice weight / Filled grain weight
Head rice rate = Head rice weight / Milled rice weight
Percentage of grain chalkiness = Chalky rice number / Head rice number

A rapid visco-analyzer (RVA), RVA-TECMASTER (Perten Instruments, Sweden), was used to measure the rice starch viscosity characteristics, and the parameters of RVA spectrum include: peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown (BD), setback (SB), peak time (PT), and pasting temperature (Pat). All data were subjected to statistical testing via analysis of variance (ANOVA) using Statistic 8 software, and the means were compared by the least significant difference (LSD) test at a level of 0.05.

RESULTS

Grain yield and yield components

We found increases in grain yield as nitrogen rate increased in both the direct-seeded rice and hand-transplanted rice in 2017 (Table 2). The N180 treatment resulted in the highest grain yield, which was 6.38 t ha^{-1} for the direct-seeded rice and 5.71 t ha^{-1} for the hand-transplanted rice. The difference was not statistically significant between N120 and N180, but the grain yield of N0 was significantly lower than that of the N120 and N180 treatments. In 2018, grain yield was higher in the N195 treatment compared to the other treatments in direct-seeded rice which was 6.15 t $ha^{-1;}$ while the N165 treatment achieved the highest grain yield in hand-transplanted rice with a yield of 6.07 t ha^{-1}, but the difference between N165 and N195 was not significant. The N0 treatment also showed significantly lower grain

Table 2. Grain yield and yield components among different nitrogen treatments for direct-seeded rice and hand-transplanted rice.

Year	Methods	Treatment	Grain yield (t ha^{-1})	Spikelets number. (m^{-2})	Panicle number (m^{-2})	Spikelets per panicle	Percentage of filled grians (%)	Grain weight (mg)
	DS[a]	N0	2.86^{bb}	21263^{b}	248.3^{b}	85.8^{a}	79.1^{a}	25.4^{b}
		N120	5.60^{a}	26674^{ab}	273.3^{ab}	99.6^{a}	71.6^{ab}	26.9^{a}
		N180	6.38^{a}	38102^{a}	366.7^{a}	102.3^{a}	46.5^{b}	27.2^{a}
		Mean	4.95^{A}	28680^{A}	296.1^{A}	96.1^{A}	65.8^{B}	26.5^{A}
		CV(%)	37.4	30	21.1	9.2	26	3.6
2017	HT	N0	3.84^{b}	12230^{b}	113.3^{b}	107.0^{a}	88.9^{a}	25.5^{a}
		N120	5.67^{a}	17442^{ab}	176.7^{a}	123.2^{a}	81.0^{a}	25.7^{a}
		N180	5.71^{a}	22503^{a}	177.8^{a}	98.6^{a}	75.5^{a}	25.9^{a}
		Mean	5.07^{A}	17242^{B}	155.9^{B}	109.6^{A}	81.8^{A}	25.7^{B}
		CV(%)	21	29.5	23.6	11.4	8.2	0.8
		DS/HT	0.98	1.66	1.89	0.88	0.8	1.03
	DS	N0	4.29^{b}	23581^{a}	198.6^{a}	122.1^{a}	74.9^{a}	26.5^{a}
		N165	6.07^{a}	28809^{a}	229.3^{a}	126.4^{a}	71.8^{a}	26.9^{a}
		H195	6.14^{a}	28992^{a}	210.7^{a}	139.0^{a}	76.5^{a}	26.7^{a}
		Mean	5.50^{A}	27123^{A}	212.9^{A}	129.2^{A}	74.4^{A}	26.7^{A}
		CV(%)	19.1	11.3	7.3	6.8	3.2	0.75
2018	HT	N0	3.70^{b}	19323^{b}	143.3^{b}	134.7^{a}	76.7^{a}	27.0^{a}
		N165	6.23^{a}	27271^{ab}	184.4^{a}	140.9^{a}	70.4^{a}	26.3^{a}
		N195`	6.00^{a}	29431^{a}	210.0^{a}	147.3^{a}	70.2^{a}	25.9^{a}
		Mean	5.31^{A}	25342^{A}	179.2^{A}	141.0^{A}	72.4^{A}	26.4^{A}
		CV(%)	26.3	21	18.8	4.5	5.1	2.1
		DS/HT	1.04	1.07	1.19	0.92	1.03	1.01

[a]DS, HT, represent direct seeding, hand-transplantation, respectively.
[b]Within a column in each method, means followed by the same letters are not significantly different according to LSD (0.05).Low-case and upper-case letters indicate comparisons among three different nitrogen treatments and between two rice establish methods.

yield than the other treatments in both of the direct-seeded rice and hand-transplanted rice in 2018. The average grain yield of direct-seeded rice and the hand-transplanted rice was not significantly different in either of the two years.

Compared to the hand-transplanted rice, direct-seeded rice had a higher average number of spikelets and panicles, and increased grain weight, but a lower number of spikelets per panicle, although the difference was only significant in 2017 (Table 2). Nitrogen supplementation significantly affected spikelet number and panicle number in both the direct-seeded rice and hand-transplanted rice. The highest spikelet number and panicle number was observed in the N180 treatment in 2017 and in the N195 treatment in 2018, with the N0 treatment resulting in significantly lower spikelet number and panicle number compared to the other treatments (Table 2). The number of spikelets per panicle also increased as nitrogen supplementation rate increased but the difference was not significant among the three nitrogen treatments. No consistent difference was observed in grain weight among the different nitrogen treatments in both the direct-seeded rice and hand-transplanted rice.

Economic profit

Table 3 summarizes the estimation of total income, labor and agricultural material input, and profit among the three nitrogen treatments for direct-seeded rice and hand-transplanted rice. The total income ranged from 2537.0 to 2890.3 \$ ha^{-1} and the final profit ranged from 913.9 to 1228.5 \$ ha^{-1} in direct-seeded rice with the N180 treatment, which achieved the highest income and profit. As for hand-transplanted rice, the total income and final profit ranged from 2568.4 to 2817.8 \$ ha^{-1} and 651.9 to 827.3 \$ ha^{-1}, respectively, with the highest total income and profit being observed in the N165 treatment. The mean profit of direct-seeded rice was 1098.0 \$ ha^{-1} which was significantly higher than that of hand-transplanted rice, and the main reason was that the direct seeding of rice reduced the labor cost substantially.

Rice physical, cooking, and tasting quality

The physical qualities of rice are presented in Table 4. The milled rice rate ranged from 59.3 to 69.7% in

Table 3. Estimation of the labor costs, input, and production profit in different treatments in direct-seeded rice and hand-transplanted rice.

Year	Methods	Treatment	Grain yield (t ha^{-1})	Income[a] ($ ha^{-1})	Labor cost ($ ha^{-1})	Fertilizer + seeds cost ($ ha^{-1})	Rest input ($ ha^{-1})	Profit ($ ha^{-1})
2017	DS[b]	N120	5.6	2537	931.8	395.9	295.4	913.9
2018	DS	N165	6.07	2750.5	931.8	424.6	295.4	1098.7
2017	DS	N180	6.38	2890.3	931.8	434.6	295.4	1228.5
2018	DS	N195	6.14	2822.3	931.8	444.4	295.4	1150.7
		Mean	6.0^{Ac}	2750.0^{A}	931.8^{B}	424.8^{A}	295.4^{A}	1098.0^{A}
		CV(%)	5.4	5.6	0	4.9	0	12.1
2017	HT	N120	5.67	2568.4	1500	314.2	102.3	651.9
2018	HT	N165	6.23	2817.8	1500	343.2	102.3	827.3
2017	HT	N180	5.71	2586.8	1500	352.9	102.3	631.6
2018	HT	N195	6	2718.4	1500	362.7	102.3	753.4
		Mean	5.9^{A}	2672.8^{A}	1500.0^{A}	343.2^{B}	102.3^{B}	716.5^{B}
		CV(%)	4.5	4.4	0	6.1	0	12.8
		DS/HT	1.02	1.03	0.62	1.24	2.89	1.53

[a] The estimation of income, labor cost, fertilizer and seeds cost, rest input were based on the local early rice price, the average price for a labor a day, and the average fertilizer, seed and rest local price in 2017 and 2018. The income, costs and profit were converted in US dollar with an exchange rate of RMB against the US dollar 6.6 in 2018.
[b] DS, HT, represent direct seeding, hand-transplantation, respectively.
[c]Within a column in each method, means followed by the same letters are not significantly different according to LSD (0.05).

Table 4. Physical qualities among different nitrogen treatments in direct-seeded rice and hand-transplanted rice.

Methods	Treatment	Milled rice rate (%)	Head rice rate (%)	Percentage of grain chalkiness (%)
DS[a]	N0	59.3^{ab}	55.8^{a}	97.0^{a}
	N165	64.0^{a}	57.6^{a}	98.3^{a}
	N195	69.7^{a}	58.0^{a}	96.3^{a}
	Mean	64.4^{A}	57.1^{B}	97.2^{A}
	CV(%)	8.1	2	1
HT	N0	67.7^{a}	58.0^{b}	98.0^{a}
	N165	71.3^{a}	63.2^{ab}	98.7^{a}
	N195	67.4^{a}	71.2^{a}	98.0^{a}
	Mean	68.8^{A}	64.1^{A}	98.2^{A}
	CV(%)	3.1	10.4	0.41
	DS/HT	0.93	0.89	0.99

[a]DS, HT, represent direct seeding, hand-transplantation, respectively.
[b] Within a column in each method, means followed by the same letters are not significantly different according to LSD (0.05).Low-case and upper-case letters indicate comparisons among three different nitrogen treatments and between two rice establish methods.

direct-seeded rice and ranged from 67.4 to 71.3% in hand-transplanted rice. The treatments of N195 and N165 achieved the highest milled rice rated in direct-seeded rice and hand-transplanted rice, respectively. However, the differences were not significant among the three treatments in both the direct-seeded rice and hand-transplanted rice. The head rice rate ranged from 55.8 to 58.0% in direct-seeded rice and ranged from 58.0 to 71.2% in hand-transplanted rice. The highest head rice rate was observed in the N195 and N165 treatments in direct-seeded rice and hand-transplanted rice, respectively. The percentage of grain chalkiness was greater than 90% in all of the treatments and no significant differences were observed among different treatments for

Table 5. RVA values among different nitrogen treatments in direct-seeded rice and hand-transplanted rice.

Methods	Treat	PV (cP)[a]	TV(cP)	FV (cP)	BD(cP)	SB (cP)	PT(min)	Pat(℃)
DS[b]	N0	3698.7[ac]	2739.0[a]	4887.3[a]	959.7[a]	1188.7[a]	5.9[a]	81.2[a]
	N165	3620.0[a]	2786.0[a]	4927.5[a]	834.0[a]	1307.5[a]	6.0[a]	81.0[a]
	N195	3356.0[a]	2597.3[a]	4651.3[a]	758.6[a]	1295.3[a]	5.9[a]	81.4[a]
	Mean	3558.2[A]	2707.4[A]	4822.0[A]	850.8[A]	1263.8[B]	5.9[A]	81.2[A]
	CV(%)	5	3.6	3	11.9	5.2	1	0.2
HT	N0	3552.7[a]	2739.3[a]	4867.7[a]	813.3[a]	1315.0[b]	5.9[a]	81.7[a]
	N165	3318.3[a]	2684.3[a]	4791.3[a]	634.0[a]	1473.0[a]	6.0[a]	82.5[a]
	N195	3319.7[a]	2662.7[a]	4748.7[a]	657.0[a]	1429.0[ab]	6.0[a]	82.5[a]
	Mean	3396.9[A]	2695.4[A]	4802.6[A]	701.4[B]	1405.7[A]	6.0[A]	82.2[A]
	CV(%)	4	1.5	1.2	13.9	5.8	1	0.6
	DS/HT	1.04	1	1	1.21	0.9	0.99	0.99

both the direct-seeded rice and hand-transplanted rice. The hand-transplanted rice showed higher average milled rice rate and higher head rice rate than the direct-seeded rice, but only the difference in head rice rate was significant.

The RVA spectrum of rice in 2018 is shown in Table 5. Our results indicate that the value of PV, TV, and FV decreased with an increase in nitrogen rate while BD and SB increased with an increase in nitrogen rate. Furthermore, the value of PT and Pat exhibited little change among the different nitrogen treatments. However, most of the parameters of the rice RVA spectrum were not significantly different among the three nitrogen treatments, and the only exception was in SB of hand-transplanted rice. Nevertheless, the average value of each of the parameters of the rice RVA spectrum between direct-seeded rice and hand-transplanted rice were not significantly different with the exception of BD and SB where the BD of direct-seeded rice was significantly higher than that of hand-transplanted rice and the SB was significantly lower for direct-seeded rice than that of hand-transplanted rice.

DISCUSSION

Direct seeding is considered as a labour cost-saving method in rice production and has been accepted by an increasing number of farmers in China in recent decades. However, the growth characteristics and the yield performance of direct-seeded rice need to be evaluated in order to maintain the food security of China. In our study, we compared grain yield, yield components, economic profit and quality of direct-seeded rice and hand-transplanted rice in eastern China. The results revealed that direct-seeded rice presented no obvious disadvantage in yield performance while improving economic benefits significantly when compared to hand-transplanted rice, thereby indicating that direct-seeded rice has the potential to gain popularity and be adopted as a cost-effective method of cultivation. However, cultivation technologies need to be improved to support the growth characteristics of direct-seeded rice accordingly.

Nitrogen is one of the most important nutrients for rice (Ladha et al., 2016), and many studies have been performed to investigate the optimal nitrogen fertilization rate for direct-seeded rice, but results have varied greatly (Mahajan et al., 2011; Mahajan and Timsina, 2011; Pan et al., 2017). In our study, we found that the hand-transplanted rice achieved the highest grain yield (Table 2) and production profit (Table 3) with the N165 treatment in the year 2018, and the yield components were also at a high level with this treatment. Thus, we propose 165 kg N ha^{-1} as a reasonable nitrogen rate for hand-transplanted rice in eastern China. In contrast from hand-transplanted rice, the direct-seeded rice reached the highest grain yield with the N180 treatment in 2017, and the rice yield components and the production profit of rice were also higher than the other treatments. Therefore, we think the nitrogen rate for the direct-seeded rice in eastern China should be higher than for that of hand-transplanted rice; perhaps 180 kg N ha^{-1} is suitable for the direct-seeded rice.

The increase in rice yield was always associated with improvement in yield components, especially sink size (Wu et al., 2013; Zhang et al., 2013; Shi et al., 2017). The sink size is determined by the panicle number, the spikelets per panicle, and the grain weight at physiological maturity (San-oh et al., 2004; Huang et al., 2019). In our study, we found that the grain yield was positively correlated with spikelet number per m^2, panicle number per m^2, and spikelets per panicles in both the direct-seeded rice and hand-transplanted rice (Figure 1), which was consistent with the previously mentioned studies. However, the grain yield of hand-transplanted rice showed a tighter relationship with panicle number per m^2 than the direct-seeded rice did, while hand-transplanted rice showed a less robust relationship between spikelets per panicle than the direct-seeded rice did. These results

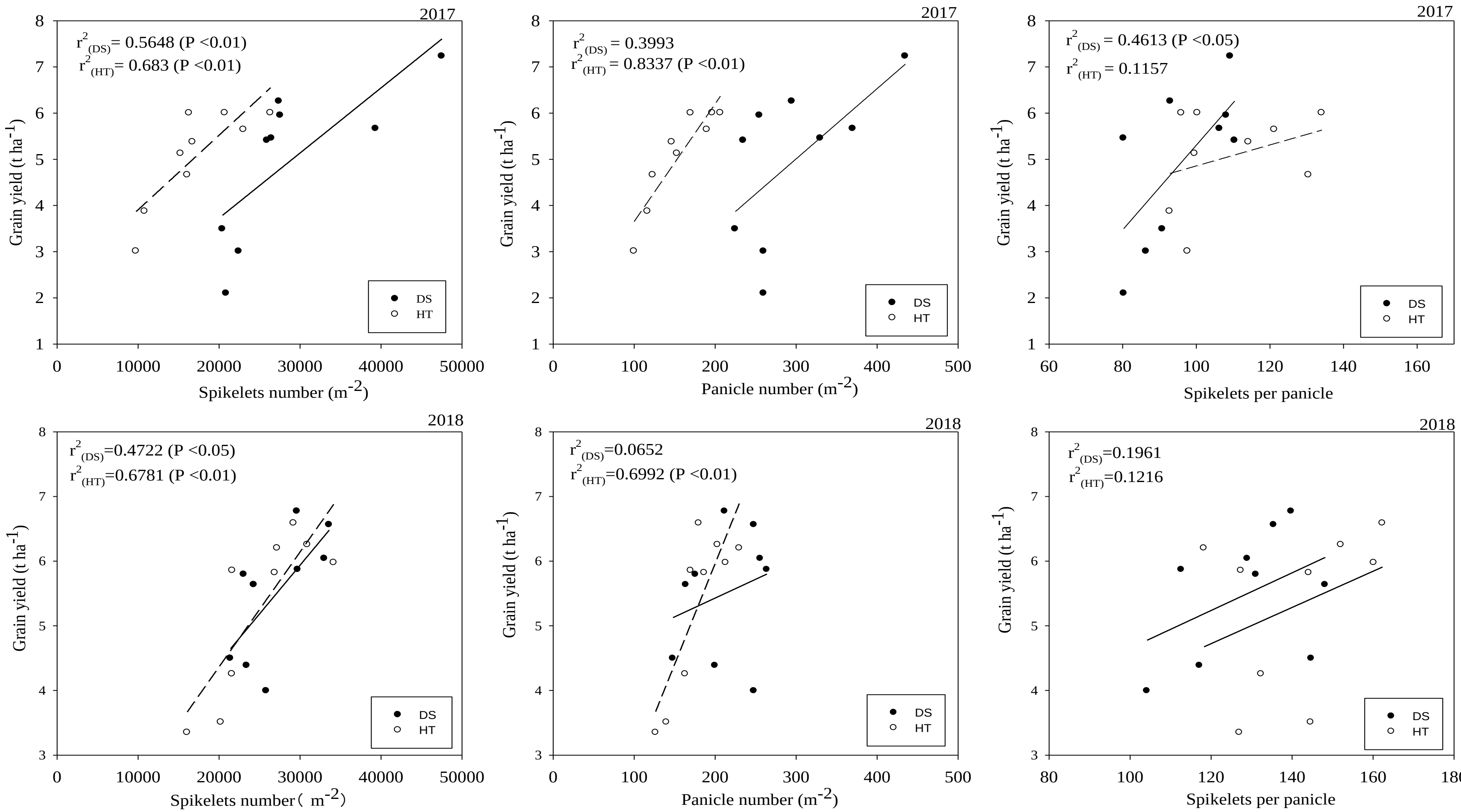

Figure 1. The relationship of grain yield with spikelet number per m^2, panicle number and spikelets per panicle in direct-seeded rice and hand-transplanted rice. DS, HT represent direct-seeding, hand-transplantation, respectively.

indicate that increasing panicle size is the most effective way to enhance yield performance of direct-seeded rice and that panicle number is important for improving the grain yield of hand-transplanted rice. Numerous studies indicated that sink size is closely related to nitrogen uptake in rice (Tadahiko, 2011; Fu et al., 2019; Chu et al., 2019). As our results indicated that grain yield was enhanced more effectively by increasing the panicle number in hand-transplanted rice, and by increasing spikelets per panicle in direct-seeded rice, it follows that more nitrogen should be applied at the tillering stage to increase the panicle number for hand-transplanted rice because the panicle number is positively related with the tiller number (Ao et al., 2010). Likewise, providing sufficient nitrogen at the panicle initiation stage perhaps is important to increase the spikelets number per panicle and grain yield in direct-seeded rice.

The physical quality of direct-seeded rice and hand-transplanted rice has been compared in our research, and we found that the average milled rice rate and the head rice rate of direct-seeded rice was lower than that of hand-transplanted rice. This result indicates that promoting direct-seeded rice cultivation will perhaps bring some risks that might decrease rice physical quality. Many studies found that increasing nitrogen use amounts could improve rice physical quality (Wei et al., 2018, Zhu et al., 2017). In our research, we found that compared to the treatment of N0, the N165 and N195 treatments showed higher milled rice rates and head rice rates in both the direct-seeded rice and hand-transplanted rice although most differences were not significant, which was consistent with the conclusions of the previous studies (Table 4). However, the milled rice rate and head rice rate was highest with the N195 treatment in direct-seeded rice, while the hand-transplanted rice achieved the highest milled rice rate with the N165 treatment, so perhaps the direct-seeded rice should have more nitrogen applied to improve rice physical quality.

Rice RVA values have been used to reflect the rice cooking and tasting quality (Tong et al., 2014), and it has been observed that rice with high cooking and tasting quality has high BD values and lower SB values (Tang et al., 2019). From the results of our study, we found that the direct-seeded rice showed higher cooking and tasting quality than the hand-transplanted rice because the average value of BD in direct-seeded rice was higher than that of the hand-transplanted rice and the average SB value was lower in the direct-seeded rice compared to the hand-transplanted rice (Table 5). Nevertheless, we found that nitrogen will bring negative effects to rice cooking and tasting quality and is likely a result of the decrease in BD and increase in SB relative to the N0 treatment in both the direct-seeded rice and hand-transplanted rice, although most of the differences were not significant.

Conclusion

Generally speaking, our research found that the direct-seeded rice has the potential to be promoted as an effective form of rice cultivation in eastern China because it showed no obvious disadvantages in yield performance when compared to hand-transplanted rice while improving economic profit margins and rice tasting quality significantly. We determined the optimal nitrogen application rate for direct-seeded rice to be 180 kg N ha^{-1}, which was higher than that of hand-transplanted rice. Additionally, the grain yield of direct-seeded rice had a more positive correlation with the number of spikelets per panicle than hand-transplanted rice. Therefore, we suggest that more nitrogen should be applied at panicle initiation to promote panicle formation in direct-seeded rice. Finally, we found that although increasing the nitrogen rate could increase rice physical quality, the tasting quality was reduced concurrently, and further studies are needed to understand how to resolve this issue.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of food types of *Galleria mellonella* L. (Lepidoptera: Pyralidae) on biological aspects and life table of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae)

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In the present work, laboratory and field strains of the greater wax moth, *Galleria mellonella* L. (Lepdoptera: Pyralidae) were reared on two food types (artificial diet for laboratory strain and wax frames for field strain) to study some biological aspects of the developmental stages of braconid wasp, *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) the parasitoid of *G. mellonella*. Results of total developmental period, percentages of adults' emergence and longevity of females of the parasitoid are different when reared on two strains of *G. mellonella*. Total developmental period of the immature stages was shorter on field strain (47.26 days) than that reared on laboratory strain of *G. mellonella* recording 57.5 days. In contrast, no significant differences were observed between the longevity of adult females reared on the two strains, where the longevity period was 25.33 and 26.3 days in laboratory and field strains, respectively. Also, life cycle of immature stages, longevity of adults and reproductive parameters of the parasitoid *A. galleriae* were determined. The fecundity of *A. galleria* decreased when reared on laboratory strain of *G. mellonella;* it was 140 progeny/females and increased to 177.3 progeny/females when *A. galleria* was reared on *G. mellonella* field strain. On the other hand, the sex ratio of the parasitoid and longevity are different between the two strains. Results of the life tables for *G. mellonella* and the parasitoid, *A. galleria* strains, showed shortage in generation periods for that reared on the field strain. The present study was conducted to explore the effect of food types on some biological parameters of *G. mellonella* and its parasitoid, *A. galleria*.

Key words: Parasitoid, greater wax moth, biology, life table, host preference.

INTRODUCTION

The greater wax moth, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) is a severe pest of field-based honeybee colonies and is stored in combs causing high losses to honey bee colonies in different countries. This

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pest has received more attention as a model organism for toxicological and biological control investigations. Wax is an important product for the honeybee, which is used in some medicines, dental as well as cosmetic industries. Wax contains many nutrients, such as pollen and honey bee, and is therefore attacked by *G. mellonella*. The larvae of this insect feed on wax combs stored especially in the dark. They cause damage to active combs and severe economic damage. It is important for beekeepers to worry about controlling this serious pest, because it is very destructive and weakens honey bee colonies. Many studies have been performed to find ways to control this dangerous insect pest, especially biological control agents (Hanumanthaswamy and Rajagopal, 2017; Kwadha et al., 2017). The rearing of *G. mellonella* larvae was studied by some authors. Kwadha et al. (2017) showed that diet quality affects larva development. Abiotic factors such as temperature and relative humidity affect the entire life cycle. It has been shown that temperature averages of 29 and 33°C are optimum for development and 29 to 33% relative humidity (RH) appears to be appropriate for survival. El-Gohary et al. (2018) and Vanzyl and Malan (2015) investigated the suitability of different suggested artificial diets for mass rearing multiplication of the greater wax moth, *G. mellonella* L. using entomopathogenic nematodes as well as many parasitoids for insect biological control purposes.

The braconid wasp, *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) is a solitary endoparasitoid and effective parasitoids on the greater and lesser wax moths, *G. mellonella* and *Achroia greissella.* This parasitoid attacks the early-instars larvae of wax moths G. mellonella and emerges to spin its cocoon and pupate well before the host larvae reach full size (Abo Abdalla and Gadelhak, 2016). Hegazi et al. (2017) reported that *A. galleriae* may be host specific for *Galleria mellonella;* 4th and 5th instars are the preferred hosts. The female lays its eggs singly inside the caterpillar, and the life cycle takes 16 to 22 days. *A. galleriae* parasitoid and its host larvae, *G. mellonella* live in near-continuous darkness in the beehives or wax comb stores. These species are with no known diapauses in Egypt. It has been shown that *A. galleriae* has the characteristics of a biological control agent. The present study was designed to investigate the effect of food types on some biological parameters of *G. mellonella* and its parasitoid, *A. galleria*.

MATERIALS AND METHODS

Field strain of *G. mellonella* (reared on old wax comb)

Stock cultures of greater wax moth, *G. mellonella* (L.) were established from larvae. Adults were collected from beekeeping of Faculty of Agriculture, Menoufia Governorate in June 2018 and reared on old wax comb which was collected from the same location and kept at 32± 1°C and 65 - 70% RH; it was reared for several generations. Ten newly emerged females and males were used for each replicate; there were three replicates. Males and females were kept in glass jar (2 kg) and covered by cloth of muslin under the previously mentioned rearing conditions, then kept in 18 h dark and 6 h light. The glass jars were examined daily until eggs were laid; and hatching was observed and collected. Incubation period of eggs, larval and pupal duration, and adults' longevity were estimated.

Laboratory strain of *G. mellonella* reared on artificial diet

The susceptible laboratory strain of the wax moth, *G. mellonella* (L.) was reared for several generations on an artificial diet that included wheat corn, glycerin, soya flour, milk powder, inactive dry yeast and honeybee wax. It was done in a glass jar, under the laboratory conditions at 32±1°C and 65-70% R.H. at Economic Entomology Department, Faculty of Agriculture, Menoufia Governorate**.**

Rearing of *A. galleriae*

Stock cultures of the parasitoid, *A. galleriae* were established from pupae and adults were collected from beekeeping of the Faculty of Agriculture at Menoufia Governorate during June 2018. They were reared on field strain of *G. mellonella* for several generations.

Laboratory colonies of *A. galleriae* were reared on early larval instars of laboratory and field strains of *G. mellonella*. Different stages (from 2^{nd} to 4^{th} instars larvae) of *G. mellonella* (field and laboratory strain) were exposed to parasitoid which reared at 25±1°C and 65 - 70% RH. Ten newly emerged females of the parasitoid were used for each replicate in a glass cage under previous rearing condition. Adults of *A. galleriae* were fed on 30% honey solution and kept at the same conditions with host larvae of *G. mellonella*. Life cycle from eggs to pupae and longevity of newly emerged adult female and male *A. galleriae* for laboratory and field strain of *G. mellonella* host was estimated in the same rearing conditions with the host species. All experiments were repeated three times.

Statistical analysis

All experiments contained three replicates. The results were analyzed by one – way analysis of variance (ANOVA) using COSTAT statistical software (Cohort Software, Berkeley). When the ANOVA statistics were significant (P < 0.01), means were compared by the Duncan's multiple range test. Life table parameters were calculated according to Birch (1948) using Life 48 basic computer program (Abou-Setta et al., 1986) and Euler-Lotka equation:

$$^{\infty}\Sigma_0\ e^{-rm}\ Lx\ mx\ dx = 1,$$

Where: (X) Age in days, (Lx) Age specific survival rates, (Mx) Female fecundity, ($R_\circ$) Net reproductive rate($R_\circ$= Σ lx*mx), (T)Generation time (T = (Σ lx* mx*x) / $R_\circ$), (rm) Intrinsic rate of increase (rm= log $R_\circ$ / T), (λ)Finite rates of increase (λ = exp. rm) and (Dt) Doubling time (Dt = log(2)/rm).

RESULTS AND DISCUSSION

Effect of food types on *G. mellonella* development

Data in Table 1 show the effect of food types on different stages of *G. mellonella*. The data show that there are no significant effects of food kind on egg incubation period of

Table 1. Effect of food types on life duration of different stages of laboratory and field strains of *G. mellonella*.

Strain	Incubation period/egg	Larval stage	Pupal stage	Total immature stages	Adult stage	
		Duration	Duration time/days		Longevity female	Emergence %
Laboratory	8.0±0.1[a]	37.10±1.4[a]	12.41±1.7[a]	57.5±2.3[a]	25.33±2.8[a]	83.0±1.25[b]
Field	7.3±0.2[a]	29.66 ±1.3[b]	10.3±1.3[b]	47.26±2.8[b]	26.3±0.66[a]	94.0±2.81[a]

Means followed by same letter in column are not significantly different at 5% level.

Table 2. Effect of food types on life table parameters of *G. mellonella.*

Parameter	*G. mellonella* (field strain)	*G.mellonella* (laboratory strain)
Survival to maturity	0.90	0.82
Sex ratio (females/total)	0.67	0.51
The net reproductive rate (R_o)	49.4	31.57
Mean generation time (T)	57.4	68.4
The intrinsic rate of increase (r_m)	0.07	0.05
The finite rate of increase (λ)	1.07	1.05
Time of population doubling (DT)	10.5	14.7

both laboratory and field strains, but larval and pupal periods were significantly affected by the food type. In the laboratory strains were fed on artificial diet; the larval and pupal periods were prolonged than in field strain fed on honeycomb. These periods were 37.10 days/larvae, 12.41 days /pupa, 29.66 days/larvae and 10.3 days/ pupa for laboratory and field strains, respectively.

Also, the total immature stages period of *G. mellonella* laboratory strain highly elongated to 57.5 days (from eggs to pupae), compared to 47.26 days in field strain. In addition, adult emergence% was also significantly affected by the food type where it was 83 and 93% for laboratory and field strain respectively. No significant effect occurred in female longevity, which was 25.33 and 26.3 days /female for laboratory and field strain, respectively.

Htet and Ueno (2019) reported that the impacts of the larval diet were in relation to protein and cholesterol. However, the present study showed the influence of larval diets on the development and survival of all stages of G. mellonella. Also larvae obtain nutrients from honey, pupal skins, pollen, wax, and other impurities found in the beeswax; beeswax contains a lot of important elements for growth and development such as protein and vitamins.

Effect of food types on life table of *G. mellonella*

Results obtained on life table parameters are presented in Table 2 and Figures 1 and 2 Tested food types affected survival rates of immature stages to adult stage. Survival rates as 0.90 and 0.82 were obtained when larvae of *G. mellonella* fed on honeybee comb (field strain) and artificial diet (laboratory strain), respectively.

Sex ratio was also affected by tested food kind. Sex ratios (female / total) as 0.67 and 0.51 were obtained for field strain and laboratory strain larvae of *G. mellonella*, respectively. The net reproductive rate (R_o) varied according to the used food kind. Respective record of 49.4 females/ female was recorded for field strain compared to 31.57 females/ female was recorded for laboratory strain larvae of *G. mellonella*.

Accordingly, it seems that feeding on natural food of honey bee comb is the most favorable food kind to field strain larvae of *G. mellonella* for reproduction; showing the highest value of net reproductive rate. From the mean generation time (T), evident differences were observed between the two food types. This period decreased with the feeding on natural food of honey bee comb in field strain of *G. mellonella*, but increase with the feeding on artificial diet in laboratory strain; the average values were 57.4 and 68.4 days, respectively.

Associating with the two tested food kind, the intrinsic rates of increase (^rm) were in respective 0.07 and 0.05 for field and laboratory strains. When these were converted into finite rates of increase (λ), the population of *G. mellonella* had the capacity to increase by the respective values 1.07 and 1.05 times/female/day when *G. mellonella* larvae were reared on honey bee comb and artificial diet; they had the highest capacity to increase in field strain. Population doubling time (DT) was estimated to be 10.5 and 14.7 days for field and laboratory strains, respectively. Obtained results indicated that wax comb

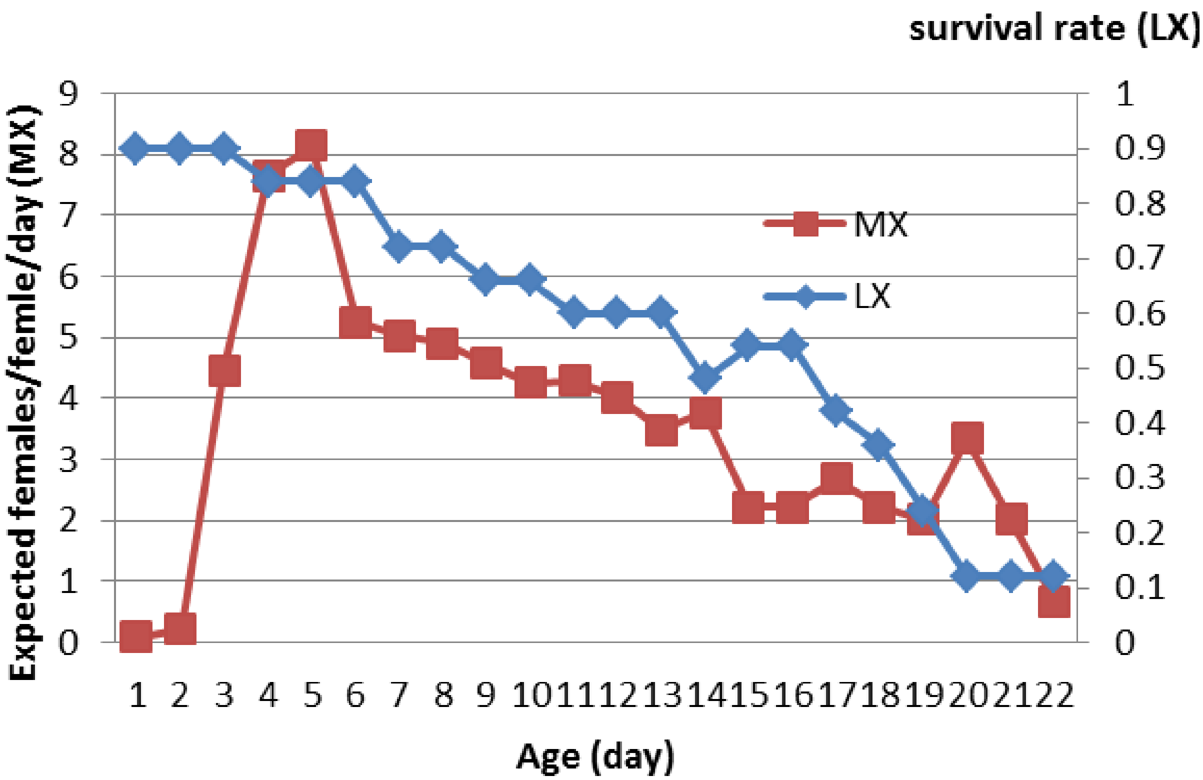

Figure 1. Age- specific fecundity and survival rate of *G. mellonella* field strain feeding on old wax comb (natural food). LX: Survival rate; MX: Female fecundity.

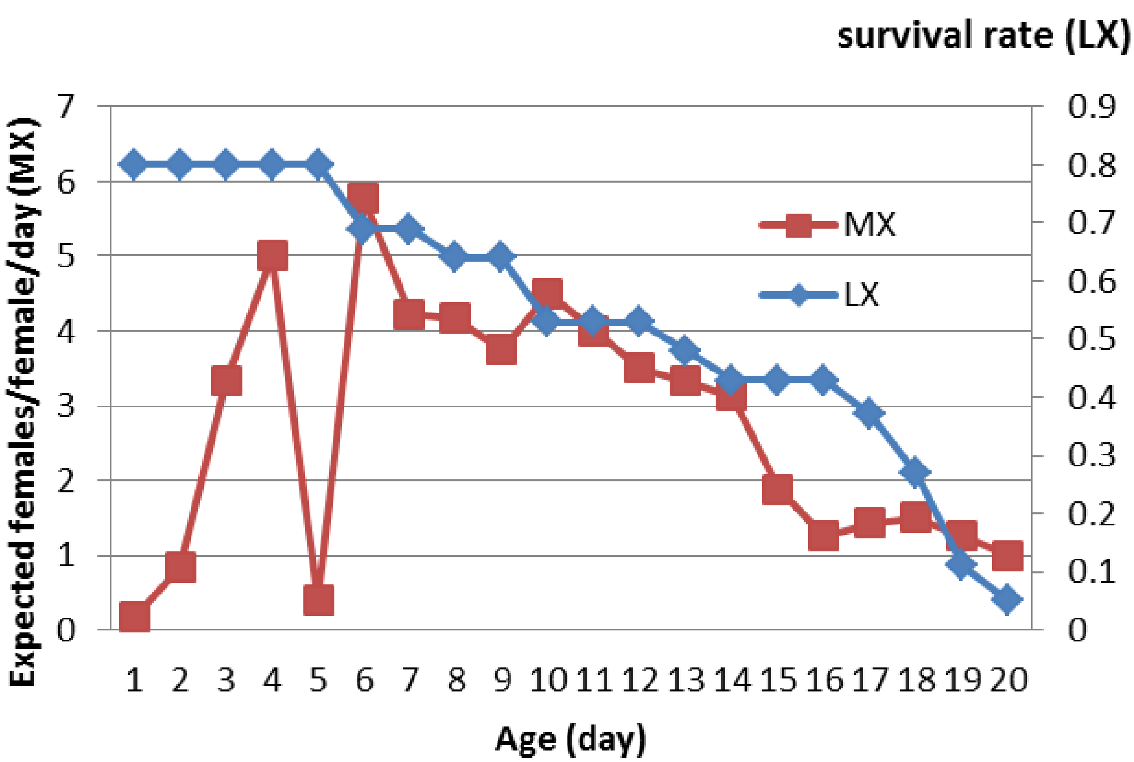

Figure 2. Age- specific fecundity and survival rate of *G. mellonella* laboratory strain fed on artificial diet. Where LX: Survival rate and MX: Female fecundity.

Table 3. Rearing of *A. galleriae* on laboratory and field strains of *Galleria mellonella.*

Strain	% Parasitism	Eggs to pupal stage duration (days)		Pupal duration (day)	Pupal dead%	Adult emergence %
		pupation%	Duration			
Laboratory	66.0±2.6[b]	78.6[b]	14.33±0.3[a]	6.3±0.5[a]	13.4[a]	87.6±2.3[b]
Field	86.66±2.4[a]	89.3[a]	13.36±0.88[a]	5.4±0.32[a]	7.7[b]	92.3±1.6[a]

Means followed by same letter in column are not significantly different at 5% level.

Table 4. Sex ratio, fecundity and longevity of parasitoid *A. galleriae* reared on laboratory and field strain of *G. mellonella.*

Strain	Sex ratio	Fecundity	Longevity	
			♂	♀
Laboratory	48.66±1.1[b]	141.0±3.2[b]	28.3±0.9[a]	30.0±0.4[a]
Field	50.33±0.68[a]	177.3±2.6[a]	28.6±0.5[a]	31.3±0.9[a]

*****Means followed by same letter in column are not significantly different at 5% level.

was more favorable for feeding *G. mellonella* larvae. These results agree with Abedelsalam et al. (2014); they studied the effects of five natural diet materials on the biology of the greater wax moth (Tables 1 and 2).

Effect of host food kind types on the biology of the parasitoid *A. galleriae*

Data in Table 3 show the significant difference in the percentages of parasitism between laboratory and field strains of *A. galleriae* reared on laboratory and field strains of *G. mellonella*; the percentages of parasitism were 66 and 86.66%**,** respectively. Duration of immature stages from eggs to pupae was significantly affected by type of host strain. Duration of immature stages of *A. galleriae* reared on laboratory strain of *G. mellonella* was longer than which reared on field strain. These durations were 20.63 days and 18.76 days**,** respectively (Figures 1 and 2).

Also data presented in Table 3 summarize the effect of type of host strain on percentages of pupation and adults' emergence of *A. galleriae*. It is clearly obvious that pupation percentages and adults' emergence of *A. galleriae* reared on field strain of *G. mellonella* significantly increased (89.3% for pupation percentage and 92.3% for adults emergence); while these percentages significantly decreased when *A. galleriae* was reared on laboratory strain of *G. mellonella* (78.6% for pupation percentage and 87.6% for adults' emergence). The fecundity (Total number of progeny produced by a single mated female), sex ratio and time longevity in days of *A .galleria* reared on either *G. mellonella* laboratory and field strain are given in Table 4. The fecundity of *A. galleria* reared on *G. mellonella* field strain was slightly higher than *A. galleria* reared on *G. mellonella* laboratory strain. There was significant difference between field strain (177.3 progeny/female) and 144.0 progeny/female on laboratory strain.

The sex ratio of *A*. *galleria* obtained from strains reared on *G. mellonella* laboratory and field strains were 50.33 female /female when *A. galleria* was reared on *G. mellonella* field strain compared to 48.66 on *G. mellonella* laboratory strain. Also longevity of female and male were significantly affected by host kind strain; it was 25.9 days/female and 30 days/male in laboratory strain while it was 28.4 days/female and 27.3 days/male in field strain. Present results agree with Hegazi et al. (2019) who found that the period from egg to adult for both sexes of *A. galleriae* changed according to the diet ingredients of the host on which the parasitoid completed its immature stages of development.

Effect of food types on life table parameters of parasitoid *A. galleriae*

Results obtained from life table parameters are presented in Table 5 and Figures 3 and 4.Tested food types affected survival rates of immature stages to adult stage. Low survival rate of 0.41 was obtained when parasitoid feeds on laboratory strain larvae of *G*. *mellonella* while 0.82 was obtained when parasitoid feeds on field strain larvae of *G*. *mellonella*.

Sex ratio (female / total) of 0.53 and 0.48 was obtained when parasitoid feeds on field and laboratory strain larvae of *G*. *mellonella*, respectively. The net reproductive rate (R_o) varied according to the food types used. (R_o) was 5.31 and 1.82 females/female when parasitoid fed on field and laboratory strain larvae of *G*. *mellonella,* respectively. The results showed that feeding on field strain larvae of *G*. *mellonella* is the most favorable food

Table 5. Effect of food types on life table parameters of parasitoid *A. galleria.*

Parameter	Feeding on field strain larvae of *G. mellonella*	Feeding on laboratory strain larvae of *G. mellonella*
Survival to maturity	0.82	0.41
Sex ratio (females/total)	0.52	0.48
The net reproductive rate (R_o)	5.31	1.82
Mean generation time (T)	28.0	29.7
The intrinsic rate of increase (r_m)	0.05	0.04
The finite rate of increase (λ)	1.05	1.04
Time of population doubling (DT)	13.83	14.33

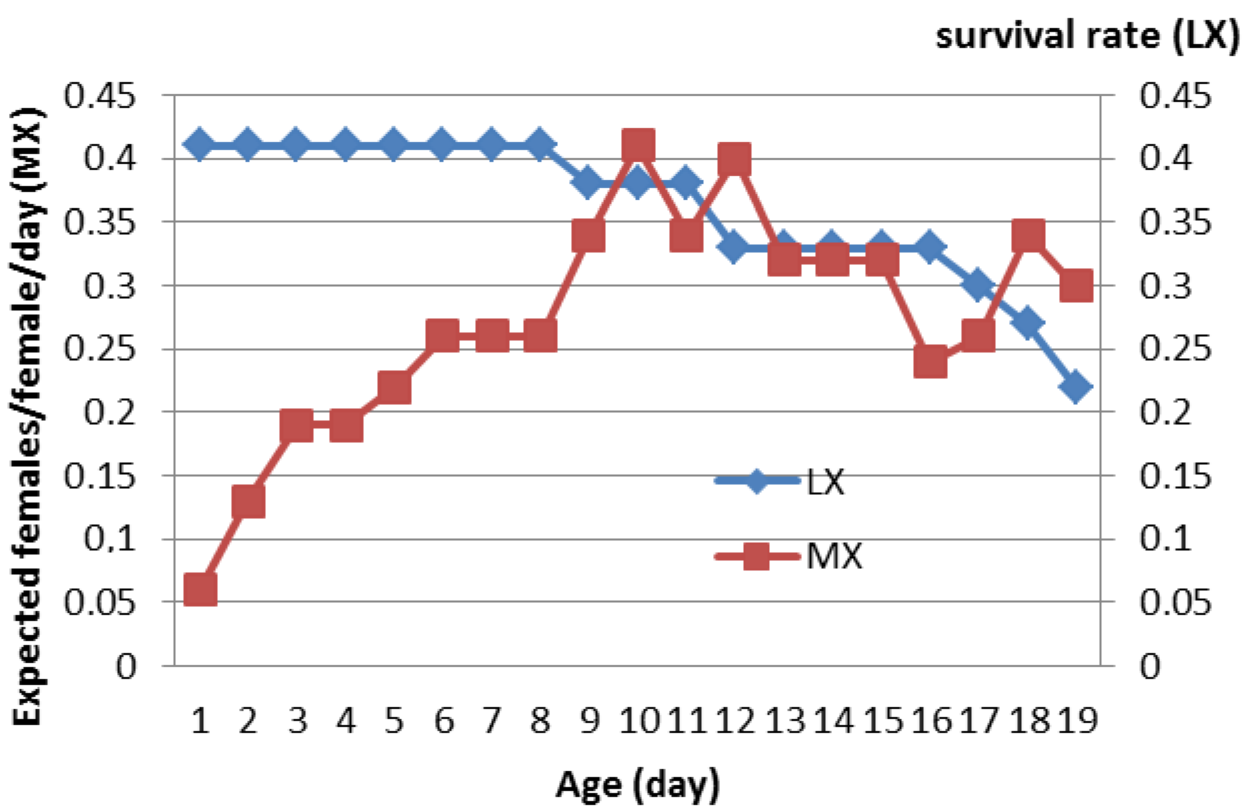

Figure 3. Age- specific fecundity and survival rate of *A. galleria* fed on laboratory strain of *G. mellonella*. Where LX: Survival rate and MX: Female fecundity.

kind for reproduction of parasitoid; it has the highest value of net reproductive rate.

Evident differences were observed in the mean generation time (T) between the two food types. This period decreased as *G. mellonella* fed on field strain, but increased with the feeding on laboratory strain; the average values were 28.0 and 29.7 days, respectively. The intrinsic rates of increase (^{r}m) were 0.05 and 0.04 for field and laboratory strains, respectively. When these were converted into finite rates of increase (λ), the population capacity of *G. mellonella* increased. The values were 1.05 and 1.04 times/female/day when *G. mellonella* larvae were reared on wax comb and artificial diet, respectively; showing the highest capacity to increase in field strain. Population doubling times (DT) were 13.83 and 14.33 days for field and laboratory strains, respectively. Results obtained indicated that wax comb was more favorable for feeding *G. mellonella* larvae. We note from the previous results that the feeding of the host (*G. mellonella* larvae) also affects the feeding of the parasitoid and therefore some biological aspects of this parasitoid. Also the nutrition type promotes the growth

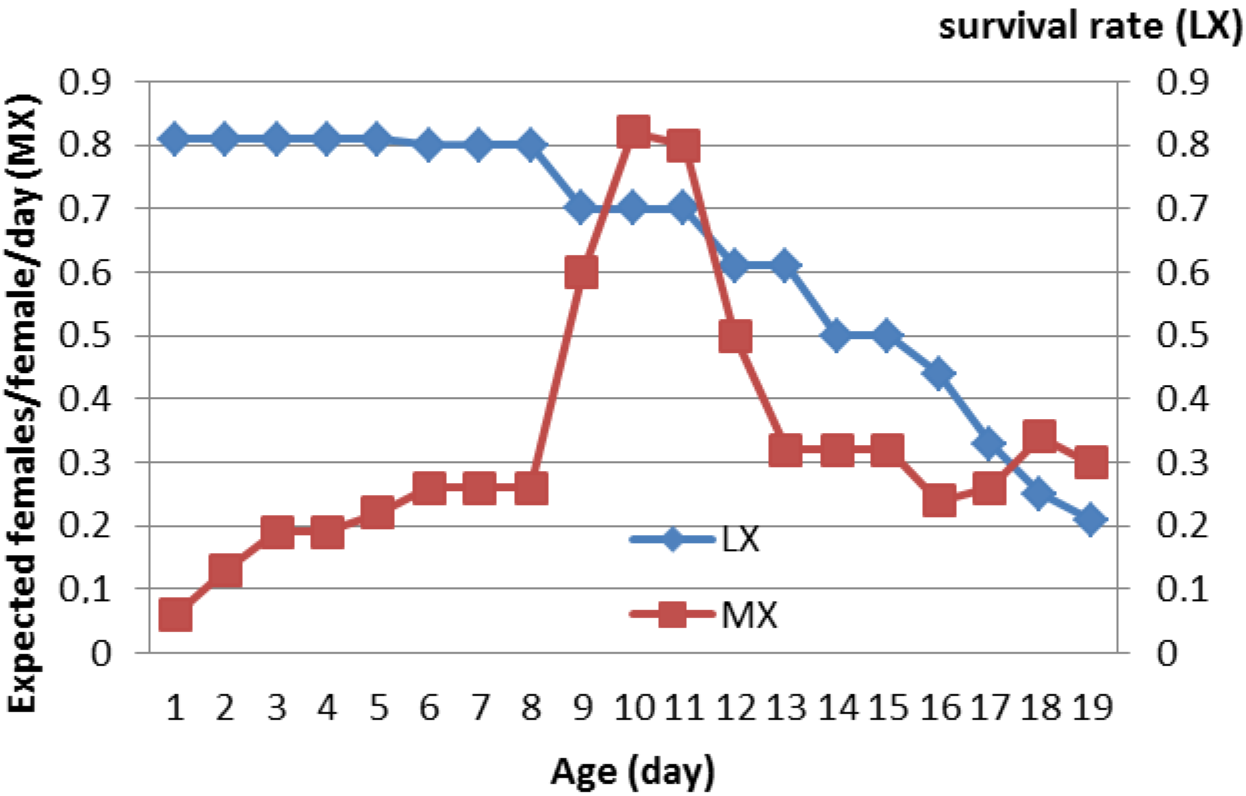

Figure 4. Age- specific fecundity and survival rate of *A. galleria* fed on field strain of *G. mellonella*. Where LX: Survival rate and MX: Female fecundity.

of both host and its parasitoid, where we find that nutrition of the old wax containing abundant quantities of good food ingredients promoted the growth of the host positively. This reflected on the sexual ratio. The net reproductive rate (Ro) reduced mean generation time and time of population doubling (DT) for its parasitoid.

These results agree with Adly and Marzouk (2019) who studied the biology of parasitoid *Bracon hebetor* Say. (Hymenoptera: Braconidae), on *G. mellonella* in laboratory, honeybee colonies, and stored wax combs. In addition, the results confirm those of Abo Abdalla and Gadelhak (2016) and Farag et al (2015) who studied the effects of natural food additives on the protein content of adult and immature stages of *A. galleriae;* it was shown that fecundity, sex ratio, progeny and parasitization ability vary according to parasitoid age. In addition, it has been shown that fecundity and sex ratio are affected by nutritional factors of host species .They explained that the differences in the fecundity, female sex ratio and longevity are affected by feeding on different host diets.

Conclusion

Generally, the positively differences in all biological parameters in rearing of the parasitoid *A. galleriae* on laboratory and field strain of *G. mellonella*, may be related to the host nutrition of field strain and laboratory strain of *G. mellonella*. This relation was also found in behavior of parasitism in host insects. These effects include development, sex ration and longevity adults of parasitoid *G. mellonela* larvae feed on the old wax. Perhaps the components of the old wax, its pollen and its high protein contents may have a positive effect on the growth of the developmental stages of both wax larvae, and the parasitoids. These conclusions can be used in laboratory education and mass rearing of both *G. mellonela* and its parasitoid, *A. galleriae* that feeds on them.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Extent and pattern of genetic diversity for pheno-agro-morphological traits in Ethiopian improved and selected farmers' varieties of Tef (*Eragrostis tef* (Zucc.) Trotter)

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Forty-nine tef, *Eragrostis tef* (Zucc.) Trotter, genotypes comprising 36 improved and 10 farmers' varieties, and, three elite breeding lines were field evaluated using 7 × 7 simple lattice design at two contrasting locations (Debre Zeit and Alem Tena) in central Ethiopia during the main cropping season. The objective was to assess the extent and pattern of genetic diversity for 23 quantitative pheno-agro-morphological traits. Differences among the varieties were significant for most of the traits except lodging index, thousand seed weight, plant weight and grain yield per plant at each location. Similarity, the pooled analysis over locations showed significant genotype effect for most of the traits except number of total and fertile tillers, lodging index, plant weight and grain yield per plant. The varieties were grouped into seven clusters of different sizes. Improved varieties resulting from direct selection and hybridization were clustered together while local varieties mostly remained solitary. Principal component analysis depicted cumulative effects of a number of traits resulted in the differentiation of the varieties into clusters. Genetic distances among most of the clusters were significant such that crosses between parents selected out of them are expected to generate desirable genetic recombination. Hence, incorporation of farmers' varieties in the breeding program would be imperative for broadening the genetic base of the released varieties. Moreover, future research strategies on germplasm collection, conservation, rejuvenation, characterization, selection and incorporation must be given due emphasis in the tef breeding program.

Key words: Clustering, genetic diversity, improved varieties, farmers' varieties, pheno-agro-morphological traits, principal component analysis, tef.

INTRODUCTION

Tef, *Eragrostis tef* (Zucc.) Trotter, underutilized crop, is the staple food in Ethiopia and Eritrea. Ethiopia is the centre of origin and diversity of tef (Vavilov, 1951). In Ethiopia, tef occupies almost one-third 30% of the total area under cereals and represents about 20% of cereal production with average yield of 1.75 t/ha (CSA, 2018).

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The national tef production increased from 5.047 million tons in 2017 to 5.156 million tons in 2018, an increase of 5.24% in a year (CSA, 2018). A number of wild forms are also known to exist and from the total of 350 *Eragrostis* species 44 of them are found in the country (Phillips, 1995), and 14 (26%) are said to be endemic (Cufodontis, 1974). For effective breeding and germplasm collection and conservation scheme, it is primarily essential to know the extent and pattern of genetic diversity (Demissie and Bjørnstad, 1997). Cognizant of this fact, various genetic diversity studies have been conducted and revealed tremendous amount of phenotypic variation in tef varieties (Jifar et al., 2011); cultivars (Tefera et al., 1990) and landraces (Assefa 2001a, 2002a, 2003b; Kefyalew et al., 2000; Adnew et al., 2005; Plaza-Wüthrich et al., 2013). These local genetic resources are invaluable in the breeding system as they provide premium genes of different traits to improve the quality and productivity of tef. On top of that, farmers' seeds are generally considered by agronomists as resources with limited potential, with a share of responsibility for the low productivity of traditional agricultural systems (Vernooy, 2003). This is due to the widespread adoption of traditional tef varieties with low production potential. Undeniably, in traditional agriculture, local varieties constitute the bulk of the plant material used (Missihoun et al., 2012). While improved tef varieties have better agronomic performance, they are not well adopted and thus little cultivated by the growers due to their unavailability (Plaza-Wüthrich et al., 2016), requisite for specific inputs and their organoleptic qualities which still fall sort of meeting users' needs.

Nowadays, agriculture needs to be focused not only on selecting good performing varieties but also to the diversification of cultivars in seed system (Baco et al., 2007). The study was made to get information about the tef improvement efforts so far done and their contribution towards maintaining genetic diversity. Therefore, the objective of this study were to assess the pattern and extent of genetic diversity among the Ethiopian local and improved tef varieties and to identify the major morphological traits having relative contribution to the overall variation.

MATERIALS AND METHODS

Plant materials

The experimental materials comprised 49 tef genotypes. These including 36 improved varieties which were released by federal and regional agricultural research centres of Ethiopia until the year 2016, three elite lines of which one was introduced from University of Bern, and 10 farmers' varieties widely grown in different areas, and commonly used as local checks in national tef yield trials in the country (Table 1).

Study sites

The field experiment was carried out at two contrasting locations (Debre Zeit and Alem Tena) in the central highlands of Ethiopia during the 2014 main season. Debre Zeit is located 47 km and Alem Tena 109 km in southeast of Addis Ababa. The two locations represent two different agro-ecologies of the country. Debre Zeit shows relatively constant rainfall with about 73% of the annual total of 832 mm of rainfall received during the main growing season from June to September. The experimental field at this site is characterized by heavy black soil described as very fine Montmorillonitic Typic Pellustert or Pellic Vertisol (Tsegaye, 1992) with very high moisture retention capacity. In contrast, Alem Tena often receives more than half of the annual total of 500 mm rainfall in only two months (July and August). The poor rainfall is distribution coupled with relatively high temperature and the experimental field at this site is characterized by very light sandy soil (Andosols) with low moisture holding capacity. The weather conditions during the growing season were favorable and the experiments were received sufficient amount of rainfall for normal growth of tef crop at each of the test sites. The geographical coordinates, climatic and soil-related data of the two locations during the growing period have been summarized in Table 2.

Experimental design and management

The field experiment was conducted using 7×7 simple lattice designs with two replications. Each plot (1 m × 1 m) consisted of five rows of 1m length with an inter-row spacing of 0.2 m. The distances were 1 m both between plots and incomplete blocks, and 1.5 m between replications. The varieties were allotted to plots at random within each replication. As per the research recommendations of 15 kg ha^{-1}, 1.5 g $plot^{-1}$ of seeds was hand broadcasted along the surface of each row. The experiment was planted at Alem Tena (light soil) and Debre Zeit (black soil) in the middle and end of July 2014, respectively. Fertilizers used were 40 kgN and 60 kg P_2O_5 per hectare for light soil at Alem Tena, and 60 kg N and 60 kg P_2O_5 per hectare for black soil at Debre Zeit. DAP was applied all at planting, while urea was applied two weeks after sowing and top dressed at tillering stage. Hand weeding was made three times during the crop growth stage.

Data collection

Data for the 23 quantitative pheno-agro-morphological traits were recorded on plot and individual plant basis. The following traits were taken on plot basis:

(1) Days to heading (DTH): The number of days from sowing up to the emergence of the tips of the panicles from the flag leaf sheath in 50% of the plot stands.
(2) Days to maturity (DTM): The number of days from sowing up to 50% of the plants in the plot reaching physiological maturity stage (as evidenced by eye-ball judgment of the plant stands when the colour of the vegetative parts changed from green to colour of straw).
(3) Grain filling period (GFP): Number of days from 50% heading to 50% maturity of the stands in each plot obtained by subtracting the former from the latter.
(4) Lodging index (LOGI): It is measured according to the method of Caldicott and Nuttall (1979). The method was taken as the product sum of the lodging degree taken on a 0-5 scale (0 being erect plant and 5 completely lodged) and the lodging severity as percent of the stand.
(5) Thousand seed weight (TSW): The weight of thousand kernels in milligram sampled from the entire plot.
(6) Biomass yield (BY): Above ground total (shoot plus grain) biomass in gram for the entire plot.
(7) Grain yield (GY): The weight of seeds harvested in gram from

Table 1. Description of plant materials used in the experiment.

S/N	Varieties name	Origin	S/N	Varieties name	Origin
1	Asgori	DZARC	26	Genete	SARC
2	Magna	DZARC	27	Zobel	SARC
3	Enatite	DZARC	28	Mechare	SARC
4	Wellenkomi	DZARC	29	Laketch	SARC
5	Menagesha	DZARC	30	Guduru	BARC
6	Melko	DZARC	31	Kena	BARC
7	Tsedey	DZARC	32	Ajora	Areka ARC
8	Gibe	DZARC	33	Gemechis	MARC
9	Dukem	DZARC	34	Kora	DZARC
10	Ziquala	DZARC	35	Werekiyu	SARC
11	Gerado	DZARC	36	Dagim	DZARC
12	Koye	DZARC	37	Elite lines (RIL–129A/DZ–Cr–387 X Kaye Murri)	DZARC
13	Key Tena	DZARC	38	Elite lines (RIL–27 /DZ–Cr–387X DZ–Cr–37)	DZARC
14	Gimbichu	DZARC	39	Elite lines (GA–10–3)	University of Bern
15	Dega Tef	DZARC	40	Local check	DebreZeit
16	Amarach	DZARC	41	Local check	Minjar
17	Quncho	DZARC	42	Local check	AlemTena
18	Simada	DZARC	43	Local check	Holetta
19	Boset	DZARC	44	Local check	AdadiMariam
20	Ambo Toke	HARC	45	Local check	Adet
21	Holetta Key	HARC	46	Local check	Motta
22	Yilmana	AARC	47	Local check	Enewary
23	Dima	AARC	48	Local check	Melkassa
24	Etsub	AARC	49	Local check	Sirinka
25	Gola	SARC			

*DZARC= DebreZeit Agricultural Research Centre; HARC= Holetta Agricultural Research Centre; AARC=Adet Agricultural Research Centre; SARC= Sirinka Agricultural Research Centre; BARC= Bako Agricultural Research Centre; Areka ARC =Areka Agricultural Research Centre; MARC=Melkasa Agricultural Research Centre.

each plot.
(8) Harvest index (HI): It is the ratio of grain yield to shoot biomass sampled from the entire plot expressed in percent.
(9) Economic growth rate (EGR): It is the ratio of grain yield to grain filling period considered from the entire plot expressed in percent.

The following traits were taken on individual plant basis, and these observations were made on five random samples of plants from the central row of each plot, and the averages of the five plants were used for analysis.

(1) Plant height (PLH): The length from the base of the stem of the main tiller to the tip of the main shoot panicle at maturity recorded as the average of five plants per plot and measured in centimeter.
(2) Panicle length (PaL): The length from the base of the main shoot panicle where the first branch emerges to the tip of the panicle at maturity recorded as the average of five plants per plot and measured in centimeter.
(3) Culm length (CL): The length of the main shoot culm from the ground level to the point of emergence of the panicle branches at maturity recorded as the average on five of plants per plot and measured in centimeter.
(4) Peduncle length (PDL): The length from the last culm node to the base of the panicle recorded as the average on five plants per plot and measured in centimeter.
(5) Number of total tiller per plant ($NTTP^{-1}$): It is recorded as the number of all tillers produced per plant assessed as the mean of five random plants per plot.
(6) Number of fertile tillers per plant ($NFTP^{-1}$): It is recorded as the number of panicles bearing (fertile) tillers per plant assessed as the mean of five random plants per plot.
(7) Numbers of primary branches per main shoot panicle ($NPBMPa^{-1}$): The average number of primary branches that emerged from the rachis of the main panicle.
(8) Number of spikelets per main shoot panicle ($NSpPa^{-1}$): It is the average number of spikelet's on the main shoot panicle of five plants measured in millimeter.
(9) First basal culm internode diameter (FBCD): The girth of the stem of the first internode from the ground level taken using caliper and recorded as the average of five plants measured in millimeter.
(10) Second basal culm internode diameter (SBCD): The girth of the stem of the second culm internode from ground level taken using caliper and recorded as the average of five plants measured in millimeter.
(11) Main shoot panicle weight (MPaW): The average weight of the main panicle of five plants at harvest measured in gram.
(12) Main shoot panicle seed weight (MPaSw): The average weight of the seeds harvested from the main panicle of five plants measured in gram.
(13) Plant weight (PW): The average weight of single plant including

Table 2. Geographical coordinates weather data and soil physio–chemical properties of the test locations.

Parameter	Trail sites	
	DebreZeit	**AlemTena**
Latitude	8°44' N	8°20' N
Longitude	38°58' E	39°E
Altitude (m.a.s.l)	1860	1575
Rain fall (mm)	832	500
Soil type	Vertisols	Light soil
Maximum mean daily temperature (°C)	24.3	29.8
Minimum mean daily temperature (°C)	8.9	8.0
Soil physico–chemical properties		
Clay (%)	65	50
Silt (%)	29	18
Sand (%)	6	32
Organic carbon (%)	2.6	1.4
Nitrogen (%)	0.1	0.1
Carbon/Nitrogen ratio	26	14
Phosphorus (ppm)	41	3.9
pH (1:1 H_2O)	6.97	7.4
CEC (meq/100 g soil)	42.7	25

tillers harvested at the level of the ground of five plants measured in gram.

(14) Grain yield per plant (Gy P^{-1}): The average weight of seeds for a single plant including all tillers yield of five plants measured in gram.

Data analyses

Analysis of variance (ANOVA)

For each trait analysis of variance was made first for individual location, and eventually upon getting positive results from tests of homogeneity of variances using the method F–max of Hartley (1950), a combined analysis of variance was made across the two test locations. For the analysis of variance, appropriate models suitable for the experimental design were employed (Gomez and Gomez, 1984) using SAS software version 9.00 (SAS Institute, 2002). This was used to get estimates of the variances for the different sources of error for subsequent analysis.

Multivariate analyses

For all multivariate such as cluster, distance and principal component analysis mean records on all traits were pre-standardized to means of zero and variances of unity to avoid bias due to the differences in measurement scales (Manly, 1986).

Cluster analyses

Agglomerative hierarchical cluster analysis was done by average linkage method using the mean data of varieties. The number of clusters was determined based on Pseudo –F and –t^2statistics using SAS software version 9.00 (SAS Institute, 2002) and the dendrogram was constructed based on the average linkage and Euclidean distance as a measure of dissimilarity (the distance) technique using the MINITAB14 statistical package.

Distance analyses

Genetic distances between clusters as the standardized mean data were calculated using Mahalanobis's D^2 statistics (Mahalanobis, 1936). The D^2 values obtained for pairs of clusters were considered as the calculated values of Chi–square (X^2) and tested for significance both at 1 and 5% probability levels against the tabulated value of X^2 for 'P' degree of freedom, where P is the number of characters considered (Singh and Chaudhary, 1985).

Principal component analysis

Principal components (PC) analyses were employed to identify the main traits accounting much of the total variation among the varieties. In this analysis, only PCs with eigen values greater than one is considered as important.

RESULTS AND DISCUSSION

Variation in pheno–agro–morphological features

The analysis of variance for each location showed significant variation (p<0.01) among the varieties for all traits except lodging index, thousand seed weight, plant weight and grain yield per plant at both locations. The combined analysis of variance over locations depicted significant genotype variation for all traits except number of total and fertile tillers, lodging index, plant weight and grain yield per plant (Table 3), indicating that these traits could be genetically operated in order to improve the

Table 3. Mean square, significance and CV% of quantitative pheno–agro–morphological traits of tef genotypes.

Trait	Mean square (CV %)			
	AlemTena	DebreZeit	Pooled	G x L
DTH	22.77**(2.51)	17.80**(2.86)	38.63**(2.72)	2.48*(2.72)
DTM	4.51**(1.32)	34.38**(1.4)	21.58**(1.32)	22.19**(1.32)
GFP	10.29**(2.81)	34.8**(4.52)	27.1**(4.27)	19.77**(4.27)
PLH	165.75**(5.28)	201.22**(6.65)	292.96**(6.06)	79.16**(6.06)
PaL	39.83^{ns} (11.45)	29.45**(6.14)	54.89**(9.53)	15.35^{ns}(9.53)
CL	88.28**(8.65)	100.54**(10.47)	137.60**(9.67)	53.98^{ns}(9.67)
PDL	12.57**(11.96)	6.06 ^{ns}(10.58)	11.33**(11.85)	6.99*(11.85)
NTTP^{-1}	5.96 ^{ns}(23.59)	20.84*(27.49)	13.68^{ns}(29.67)	16.91**(29.67)
NFTP^{-1}	2.57^{ns}(25.27)	18.74*(29.52)	10.61^{ns}(32.61)	14.07**(32.61)
NPBMPa^{-1}	41.22^{ns}(19.25)	13.15*(9.17)	33.97**(15.57)	18.82^{ns}(15.57)
NSpPa^{-1}	2687.49*(2.70)	27582.63**(12.69)	38813.23**(16.94)	13094.72*(16.94)
LOGI	36.83^{ns}(7.91)	43.86^{ns}(11.17)	48.40^{ns}(9.5)	32.89^{ns}(9.5)
FBCD	0.003^{ns}(30.69)	0.003**(14.95)	0.004**(23.35)	0.002^{ns}(23.35)
SBCD	0.006**(29.71)	0.004**(16.79)	0.005**(22.71)	0.006**(22.71)
TSW	0.005^{ns}(19.52)	0.006^{ns}(19.65)	0.006*(19.55)	0.005^{ns}(19.55)
MPaW	0.64**(24.84)	0.16*(21.00)	0.53**(25.66)	0.302**(25.66)
MPaSw	0.12**(32.80)	0.09*(25.36)	0.12**(31.2)	0.113**(31.2)
PW	0.15^{ns}(15.02)	0.64^{ns}(21.07)	0.39^{ns}(21.78)	0.541^{ns} (21.78)
Gy P^{-1}	0.09^{ns}(27.82)	0.32^{ns}(26.03)	0.22^{ns}(29.00)	0.240^{ns} (29.00)
BY	138428.62**(11.05)	181146.77**(20.71)	220200.40**(15.72)	64489.80**(15.72)
GY	3153.62*(20.13)	8463.36**(27.75)	6897.40**(25.96)	5891.13**(25.96)
HI	14.34**(20.19)	17.81**(16.17)	22.71**(18.11)	11.09**(18.11)
EGR	15983.1^{ns}(20.60)	26283.46**(28.47)	27661.33**(25.15)	19011.94*(25.15)

^{Ns}, *, ** indicates non–significant, significant and highly significant differences, respectively.

yield of tef and these results are parallel with the previous reports of Chanyalew et al. (2009). Likewise, the genotype x location (G x L) interactions were also significant for most of the traits apart from panicle length, culm length, primary branch per main shoot panicle, lodging index, first basal culm diameter, thousand seed weight, plant weight and plant seed weight (Table 3). The main cause of differences instability between genotypes is the occurrence of substantial genotype x location interactions which is due to both genetic and non–genetic effects, showing the importance of testing the varieties in place and time.

Assembling pattern

The genotypes were grouped into seven diversity classes (Table 4 and Figure 1), based on the trait considered different members in the same clusters were supposed to be more similar to each other than those in other clusters. The first cluster had the largest number 59% of genotypes resulting from direct selection, hybridization and farmer's varieties but dominated by varieties resulting from direct selection. These varieties tended to form a sub–group as well. The second cluster comprised eight of the test tef genotypes originated from direct selection and hybridization but dominated by hybridization. These clusters generally contained highest performance in most of desirable traits. Hence, it directs that hybridization might be good approach for accumulating of the disable trait in a single variety. The fourth cluster comprised six of the test tef varieties originating from direct selection, hybridization and local varieties in equal proportion. Though, in this cluster varieties resulting from inter–specific hybridization and elite line resulting from TILLING were included. It also constituted the best yielding varieties with highest harvest index and economic growth rate. Local varieties remain solitary and form their own distinct group in cluster three, five, six and seven. Cluster three and seven encompassed inferior varieties for almost all of the traits. Furthermore, from the agronomic view point the solitary lines appear very fascinating in various aspects (Assefa et al., 2003b). Similar to this finding. the current study revealed interesting features of un–grouped lines (cluster five, six and seven), such as cluster five and six constituted longest grain filling period and peduncle length, highest total and fertile tiller with highest single plant and seed weight this ultimately result the highest lodging index record and this is factual in practical

Table 4. Grouping of 49 tef genotypes into different classes using mean of 23 response traits.

Cluster	Number of genotype	Genotypes include in this cluster	Source
C_1	29	Asgori, Enatit, Wellenkomi,Werekiyu, Mechare, Gola, Yilmana, Koye, Gerado, Ajora, Ambo Toke, Zobel, Kena, Genete, Key Tena, Dega Tef, Magna, Gimbichu	Direct selection
		Tseday, Elite (RIL–27), Elite (RIL–129A), Menagesha, Laketch, Ziquala, Gibe, Gemechis.	Hybridization (intra specific)
		Sirinka, Minjar, Motta.	Local variety
C_2	8	Etsub, Guduru, Dukem.	Direct selection
		Melko,Quncho, Kora, Dagim, Amarach.	Hybridization (intra specific)
C_3	3	Melkasa, AdadiMariam, Adet.	Local variety
C_4	6	Simada	Hybridization (inter specific)
		Boset	Hybridization (intra specific)
		Holetta Key, Dima	Direct selection
		Elite(GA–10–3)	TILLING
		Alemtena	Local variety
C_5	1	DebreZeit	Local variety
C_6	1	Holetta	Local variety
C_7	1	Enewary	Local variety

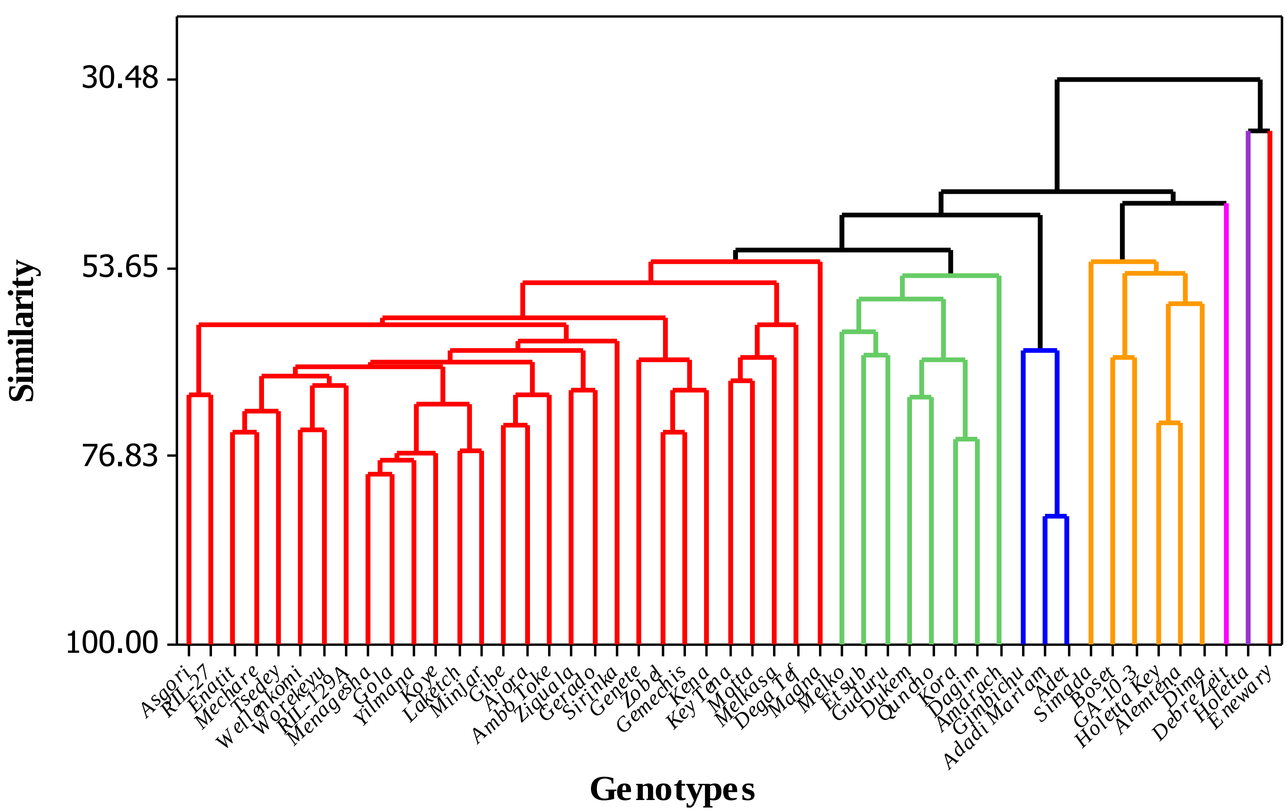

Figure 1. Dendrogram showing relationships among 49 tef genotypes based on average linkage and Euclidean distance using the mean of 23 quantitative pheno-agro-morphological traits.

Table 5. Clusters mean for 23quantitative pheno–agro–morphological traits in 49 tef genotypes.

Traits	Clusters							Grand mean
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	
DTH	45.65	50.08^H	48.45	42.53	40.26	41.50	40.22^L	44.10
DTM	94.61	96.50	95.21	91.64^L	92.92	97.03^H	91.67	94.23
GFP	48.75	46.61^L	46.99	47.43	52.97	55.63^H	51.41	49.97
PLH	108.24	117.89^H	101.62	93.88	99.29	94.89	80.54^L	99.48
PaL	42.97	46.48^H	42.93	36.17	37.02	40.54	31.80^L	39.70
CL	65.27	71.41^H	58.69	57.71	62.27	54.35	48.74^L	59.78
PDL	18.38	16.87	16.84^L	17.41	20.05^H	19.73	19.64	18.42
$NTTP^{-1}$	10.53	8.76	8.02^L	11.72	12.43	16.10^H	9.16	10.96
$NFTP^{-1}$	8.49	7.11	6.04	9.13	10.48	13.35^H	7.00^L	8.80
$NPBMPa^{-1}$	28.50	30.73^H	29.36	23.87	22.24	21.92	21.45^L	25.44
$NSpPa^{-1}$	539.23	647.25^H	513.13	419.71	380.32	348.00	234.84^L	440.4
LOGI	82.74	80.14^L	81.00	87.30	89.47	93.99^H	83.59	85.46
FBCD	0.19	0.23^H	0.19	0.18	0.16	0.16	0.14^L	0.18
SBCD	0.19	0.23	0.18	0.16	0.34^H	0.13^L	0.15	0.20
TSW	0.31	0.32	0.27	0.31	0.42^H	0.26	0.24^L	0.30
MPaW	1.53	2.05^H	1.16	1.15	1.15	0.70	0.39^L	1.16
MPaSw	0.67	0.90^H	0.55	0.57	0.41	0.46	0.28^L	0.55
PW	12.88	11.94	7.16	10.57	11.36	12.90^H	6.33^L	10.45
Gy P^{-1}	3.36	2.74	0.95^L	2.98	2.04	3.44^H	1.76	2.47
BY	746.39	972.48^H	734.23	578.61	791.32	526.69	307.36^L	665.3
GY	205.58	217.59	115.12^L	241.98^H	207.86	159.20	120.08	181.1
HI	13.23	12.14	8.41^L	16.88^H	13.42	10.99	13.78	12.69
EGR	430.37	472.30	265.61	516.71^H	400.55	312.61	252.44^L	378.7

^{L}, Lowest value and ^{H}, Highest value.

circumstance. While cluster seven depicted inferior performance in most of the trait (Table 5).

The result showed that, in most cases the varieties originating from direct selection and hybridization clustered together while local varieties remained distinct and ungrouped. This may be attributed to an exchange of genetic materials between the two breeding approaches. The local varieties grouped separately due probably to the presence of distinct farmer varieties in different areas. The current cluster analysis indicated that the diversity presented in tef genotypes cannot be reduced into a few numbers of groups as was done in earlier studies (Assefa et al., 2003b; Jifar et al., 2015).

Magnitude of genetic diversity

The pairwise generalized squared distances (D^2) among the seven clusters is showed in Table 6, members in clusters with non-significant distance were assumed to have more close relationship with each other than they are with those in significantly distant clusters. The genetic distance out of 21 pairs, the genetic divergences between 20 pairs were highly significant at p<0.01. The maximum distance was found between clusters seven and five with D^2=589 followed by cluster six and five with D^2=543 which was presumably due to the distinct nature of the solitary grouped farmers' varieties. The minimum and non–significant distances were found between cluster two and one D^2=30, representing close relationship among the improved varieties included hybridization and direct selection. In view of that, the high inter–cluster distance values obtained in this study is largely due to the inclusion of the local varieties. It's recognized that, geographically and environmentally isolated genotype were dominantly found in the growers hand and plays a prominent role to maintain genetic diversity through providing prospects for selection of desirable agronomic traits and for hybridization (Teshome et al., 1997).

Parents for hybridization could be selected on the basis of maximum inter-cluster distance for isolating useful recombinants in the segregating generations (Singh, 1990; Wallace and Yan, 1998; Chahal and Gosal, 2002). It is anticipated that relatively better genetic recombination and broad-spectrum of variability in segregating progenies were obtained from crosses among chosen varieties from these clusters. Moreover, to increase the yield limitation caused by narrow genetic base (Chandel and Joshi, 1983) and to develop varieties with broad genetic base (Chandel and Joshi, 1983; Singh, 1990; Keneni et al., 1997) crosses among parents with high inter–parental diversity might have a significant

Table 6. Pair–wise generalized square distance (D^2) between seven clusters constructed from 49 tef genotypes.

Cluster	C_1	C_2	C_3	C_4	C_5	C_6	C_7
C_1	0	30^{ns}	74**	41**	362**	191**	182**
C_2		0	89**	115**	426**	289.**	308**
C_3			0	81**	536**	260**	143**
C_4				0	404**	172**	93**
C_5					0	543**	589**
C_6						0	269**
C_7							0

^{ns}, * and ** non–significant, significant, highly significant respectively.

Table 7. Eigen vectors, eigen values and percentage of total variance explained by the first six principal components (PC) for 23 response traits in 49 tef genotypes.

Traits	Eigen vectors					
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
DTH	0.28	-0.18	0.07	0.26	-0.07	0.15
DTM	0.15	-0.05	0.11	-0.22	0.46	-0.03
GFP	-0.12	0.09	0.04	-0.44	0.46	0.04
PLH	0.34	0.04	0.04	-0.03	0.16	0.10
PaL	0.27	-0.06	0.03	0.01	0.38	0.02
CL	0.29	0.13	0.03	-0.04	-0.03	0.29
PDL	-0.04	0.24	0.09	-0.29	0.07	0.06
$NTTP^{-1}$	-0.14	0.29	0.28	0.28	0.09	0.16
$NFTP^{-1}$	-0.11	0.30	0.21	0.35	0.13	0.28
$NPBMPa^{-1}$	0.26	-0.15	0.11	0.16	0.09	-0.01
$NSpPa^{-1}$	0.29	-0.01	-0.03	0.06	0.18	-0.19
LOGI	-0.20	0.11	-0.20	0.16	0.33	0.07
FBCD	0.24	0.13	0.16	-0.20	-0.25	0.01
SBCD	0.19	0.17	0.15	-0.27	-0.26	0.14
TSW	0.14	0.27	0.02	-0.27	-0.21	0.28
MPaW	0.32	0.01	-0.03	0.14	-0.10	-0.09
MPaSw	0.23	-0.01	-0.16	0.20	0.00	-0.26
PW	0.08	0.32	0.34	0.17	0.03	-0.18
Gy P^{-1}	0.04	0.34	0.29	0.13	0.08	-0.33
BY	0.28	-0.01	-0.21	0.03	0.16	0.23
GY	0.44	0.11	0.19	0.02	0.04	0.09
HI	-0.09	0.35	-0.31	0.06	-0.02	-0.16
EGR	0.10	0.30	-0.43	0.08	-0.04	0.03
Parameter						
Eigen value	7.36	3.15	2.93	1.91	1.48	1.32
% of total variance	30.65	13.12	12.19	7.94	6.16	5.52
% of cumulative total variance	30.65	43.77	55.96	63.91	70.06	75.58

contribution. Although, the selection of parents must also consider the distinct plus of each cluster and each variety within a cluster depending on the specific purposes of hybridization as described by Singh (1990) and Chahal and Gosal (2002). Ideal level of desired traits for yield, biotic and abiotic stress and quality factors must be other criteria for parent selection beside genetic diversity (Wallace and Yan, 1998).

Principal component analysis

The first six principal components explained about 76% of the total variation among 49 tef varieties evaluated for 23 quantitative pheno-agro–morphological traits (Table 7). The first principal component alone accounted for about 31% of the total variation which is similar to the findings of Assefa et al. (2001a). Clustering within each principal component is highly influenced by the traits with larger absolute values closer to unity than those with lower absolute values closer to zero (Chahal and Gosal, 2002). Consequently, the differentiation of varieties into different clusters was articulated by the cumulative effects of each trait since most traits alone contributed small effects (±0.04-0.44) to the total variation. Nevertheless, the total diversity is relatively influenced by the traits which have comparatively greater weight in PC1.

Additionally, the presence genetic diversity among the varieties revealed that the entire variation cannot be explained in terms of few PCs. In the current study, 76% of the variation among 49 varieties with a greater number of PCs from the previous other studies of Assefa et al. (1999) who reported that about 71-74% of the variation in 320 tef germplasm lines was explained by five PCs. On the other hand, only five PCs were reported to have explained about 81% of the gross variation in 1080 (Assefa et al., 2001a) and 60 (Assefa et al., 2003b) tef germplasm lines.

To sum up, the results depicted that there was high genetic diversity in the local tef varieties and this implies that farmer's seeds have substantial quantitative pheno–agro–morphological trait diversity which can be exploited in the genetic improvement of tef. However, the genetic architecture of improved varieties resulted from hybridization and direct selection showed low genetic diversity among them. Several possible reasons could be given for the genetic similarity among the improved varieties from the two breeding techniques. This included gene flow, use of few parents in the breeding program and utilization of improved variety as a gene donor material. It is suggested that depending on the specific breeding aim, selection of parents for hybridization need to encompass local varieties with discrete desirable traits and genetic diversity. In line with this, high gene recombination would be expected from accessions drawn from significantly distant clusters. Ethiopia is the ace wealthiest country in terms of genetic diversity of tef but this opportunity has not been copiously exploited by the previous breeding efforts. Therefore, in the efforts towards developing farmers and consumers preferred tef variety it is required to perform multiple crossing with distinct parents from different origin. Additionally, further research activities are required to assess the genetic diversity among landraces across the country and point out the desirable traits and incorporate the peak line in the breeding program.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Natural parasitism of hymenoptera (insect) on bruquids associated with *Fabaceae* seeds in Northern Sinaloa, Mexico

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With the purpose of identifying the parasitic hymenoptera species associated with the diversity of bruchidae over Fabaceae, in 2017 seeds of 25 plant species were collected from the municipalities of Ahome, El Fuerte, Choix and Guasave (Sinaloa, Mexico). From 68,344 seeds, 19,396 adult bruquids were obtained, distributed in nine species: *Acanthoscelides desmanthi*, *Callosobruchus maculatus*, *Merobruchus insolitus*, *Mimosestes mimosae*, *Mimosestes nubigens*, *Microsternus ulkei*, *Stator limbatus*, *S. pruininus* and *Zabrotes subfaciatus*. Of the bruchids emerged 4,775 parasitoids, grouped in families Eulophidae (3,159), Braconidae (692), Pteromalidae (443), Eurytomidae (304), Bethylidae (134), Eupelmidae (40), and Trichogrammatidae (3), in 17 species; out of them, *Horismenus depressus*, *Triaspis* sp. and *Eurytoma* sp. had a presence in the four municipalities; 77.84% of the parasitoids was associated with *M. mimosae* (1,352), *A. desmanthi* (898), *M. ulkei* (753), and *M. insolitus* (714). The predominant species was *H. depressus*, with 60.63% of the population. In Ahome the highest parasitism was obtained (40.14%).

Key words: Bruchidae, *Fabaceae*, biological control, parasitism, *horismenus depressus*.

INTRODUCTION

The diversity of plant species included in the *Fabaceae* family are important as timber resources, coal production and human and animal feed; besides that species like *Prosopis juliflora* (Sw.) DC. 1825, with drought resistance, prevents soil erosion (Aguado and Suarez, 2006).

In countries such as the United States of North America, native legumes are a desirable component as plant cover, although seed predation by insects can substantially reduce the cultivation of these species (Boe and Johnson, 2017). Of little known biology, the insects known as brúquidos, weevils or seed beetles (Coleoptera: Bruchidae) represent one of the main regulatory factors of the fabaceae populations (Yus-Ramos et al., 2008). These insects feed mainly on seeds (Johnson, 1989; Luna-Cozar et al., 2002; Romero and Johnson, 2004), where they pass their pupa state; and once they emerge as adults, oviposit various plant seeds belonging to 33 botanical families, with preference over *Fabaceae* and *Convolvulaceae* (Luna-Cozar et al., 2002). Oviposition occurs in the remaining pods of the previous season, which have not fallen. This results in high levels of brúquidos during the following season (Saiz, 1993). These insects, considered as important pests in beans under field and storage conditions (Aebi et al., 2008), are distributed in most continents, for which case, most species are found in tropical regions of Asia, Africa, Central and South America (Ramírez et al., 2013). At present, there are about 1,700 species of turquids reported. They are distributed in 66 genera, of which, of the 20 genera and 324 species present in Mexico, 90 species correspond to the state of Sinaloa (Lugo-García et al., 2015). Due to their sperm eating habits (Romero, 2002), these insects play an important role in the population regulation of wild plants, with damage to seeds that range between 50 and 100% (Romero and Johnson, 2000). In the particular case of beans, estimated damages have been attributed to 35% in Mexico and Central America, 13% in Brazil and 7.4% in Colombia (Van Schoonhoven and Cardona, 1986). The biological control of pests is related to the use of natural enemies, parasitoids and predators to combat pest insects, which, although not as effective and spectacular as chemical control, is very effective when used opportunely (Gómez and Vargas, 2014), with parasitoids and predators determining the regulation of their host populations in natural ecosystems (Hastings and Godfray, 1999). In the last decade, several successful biological control programs based on parasitoids on noctuid larvae, diamond back palomilla, among others (Godfray, 1994) have been documented. Although this information is relevant, this group of hymenoptera, as well as their biological and economic importance, has been little studied in Northern Sinaloa, with little information on the diversity of families in natural and agricultural ecosystems. Therefore, the objective is to identify the parasitic Hymenoptera species associated with the diversity of Bruchidae over fabaceae in four municipalities in Northern Sinaloa, Mexico.

MATERIALS AND METHODS

For the study, the diversity of natural vegetation of fabáceas established in the north of Sinaloa was considered, with thorny scrub and coastal dunes over the municipalities of Ahome (located between the geographical coordinates of 26°02´00´´ N and 109°01´00´´ W) and Guasave (25° 45´27´´ N and 108° 49´17´´ W); as well as spiny forest in the municipalities of El Fuerte (26°27´00.5´´ N and 108°35´20.1´´ W) and Choix (26°53´10.5´´ N and 108°23´13.8´´ W) (Figure 1). The locations considered in the study have altitudes ranging from 0 to 115 m, with slopes that vary from slight to steep. The climate is very dry (BW) and dry (BS), with rainfall between 326-607 mm annually; the average temperatures range between 23.5 and 25.6°C, and has monthly extremes of 4.0 and 40.7°C.

In order to obtain bruquids associated with *Fabaceae*, in accordance with the phenological stage of fruiting of the diversity of plant species, nuts (pods) from the diversity of established plant species were collected during the months of February to December 2017 in the four municipalities. The fruits were deposited in Kraft paper bags model S-13236, individually labeled with data such as location, date, species of fabaceous, etc. The collected material was transferred and conserved in the laboratory of the Entomological Collection of the Valle del Fuerte, of the Autonomous University of Sinaloa (CEVF-UAS). Here after the seeds were separated and deposited in plastic containers of 16.5 × 13 cm, covered with thin tulle cloth to allow air circulation and to prevent the escape of insects, maintained at temperatures of 23°C were checked every third day until adult emergencies were obtained. They were deposited in plastic bottles of 200 ml with 70% ethanol. For the morphological identification of the insects, the genitalia of the males were extracted, which were processed according to what was considered by authors such as Kingsolver (1970) and Kingsolver and Whitehead (1974). Also, the interpretation of genital structures was based on the nomenclature proposed by Romero and Johnson (1999). The material studied was deposited in the CEVF-UAS facilities.

To identify the emerging parasitoids, they were separated and placed in 5 ml polypropylene tubes with 70% ethanol; they were dehydrated with ethanol at different doses of purity (80, 90 and 100%, respectively) for a period 30 min. A subsequent treatment with amyl acetate was carried out to clean and accommodate its wings, antennae and legs for assembly. When the insects were quantified, representative samples of similar morphological characteristics were mounted on entomological pins and were morphologically identified with the help of a stereoscopic microscope, using identification keys of various authors, with corroboration of some species by specialists.

RESULTS

In the period 68,344 seeds were collected (Ahome 20,708, El Fuerte 24,346, Choix 9,860 and Guasave 13,430), from 25 species of *Fabaceae*. From these seeds

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Figure 1. Mexico map showing the sampling area for bruchids in the state of Sinaloa.

Table 1. Monthly comparative of adult bruquids emerged from fabaceas seed in northern Sinaloa, Mexico.

Municipality/Month	E	F	M	A	M	J	J	A	S	O	N	D	Total
Ahome	24	23	53	138	42	394	325	472	693	236	182	48	2,630
El Fuerte	76	56	79	989	578	1,134	964	889	981	1,093	867	83	7,789
Choix	25	19	31	27	111	149	328	476	379	501	322	78	2,446
Guasave	43	36	58	61	248	204	1,188	2,134	967	693	476	423	6,531
Total	**168**	**134**	**221**	**1,215**	**979**	**1,881**	**2,805**	**3,971**	**3,020**	**2,523**	**1,847**	**632**	**19,396**

Table 2. Monthly emergency battle of adult parasitoids over inmature bruquids in northern Sinaloa, Mexico.

Municipality/Month	E	F	M	A	M	J	J	A	S	O	N	D	Total
Ahome	13	58	57	133	190	245	212	223	324	87	136	86	1,764
El Fuerte	5	18	17	46	124	166	69	92	207	389	122	42	1,297
Choix	0	0	0	2	6	28	43	176	192	182	65	56	750
Guasave	0	2	8	4	47	123	127	181	96	168	162	46	964
Total	**18**	**78**	**82**	**185**	**367**	**562**	**451**	**672**	**819**	**826**	**485**	**230**	**4,775**

emerged 19,396 specimens of adult weevils (Table 1). The relative dominance of emergencies by municipality corresponded to Ahome (7,789), Guasave (6,531), Ahome (2,630) and Choix (2,446), equivalent to 40.16, 33.67, 13.55 and 12.51%, respectively. On a monthly level, the period between the months of July to October represented 63.51% of emergencies.

In relation to the emergencies of parasitoids from the brúquidos present in the seeds collected in each of the municipalities (Table 2), the dominance in municipalities regarding the number of specimens corresponded to Ahome, El Fuerte, Guasave and Choix, with 36.94,

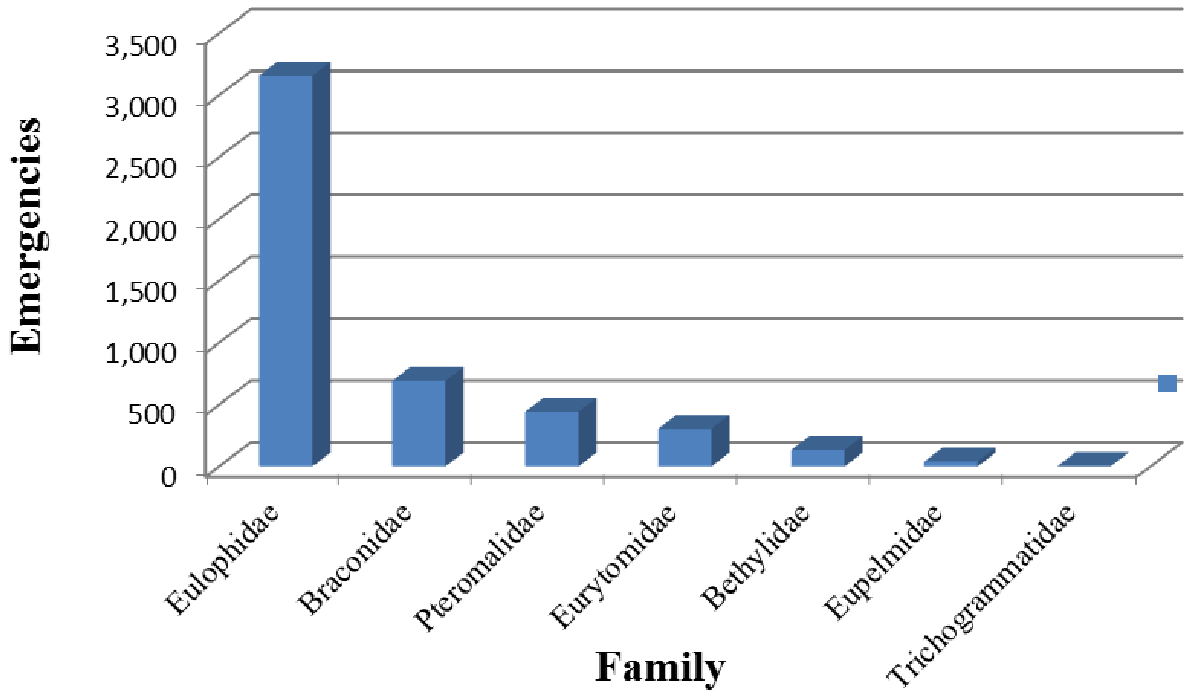

Figure 2. Distribution of parasitoid emergencies by family, associated with bruquids on fabaceae in northern Sinaloa, Mexico.

27.16, 20.19 and 15.71%, respectively. In turn, the highest amount of parasitoids obtained on a monthly level corresponded to the period from May to November, with 4,182 emergencies, equivalent to 87.58% of the population. All parasitic hymenoptera specimens were grouped into two superfamilies: 1. Ichnemunoidea, represented by the Braconidae family (692 specimens: 14.49%) and 2. Chalcidoidea, for the families Eulophidae, Pteromalidae, Eurytomidae, Bethylidae, Eupelmidae and Trichogrammatidae, with 3,159, 443, 304, 134, 40 and 3 emergencies, corresponding to 66.16, 9.27, 6.37, 2.81, 0.84 and 0.06%, respectively (Figure 2).

The emerged parasitoids were obtained from nine species of Bruchidae as prey: *Acanthoscelides desmanthi* Johnson, *Callosobruchus maculatus* (Fabricius), *Merobruchus insolitus* (Sharp), *Mimosestes mimosae* (Fabricius, 1781), *M. nubigens* (Motschulsky, 1874), *M. ulkei* (Horn, 1873), *Stator limbatus* (Horn, 1873), *S. pruininus* (Horn, 1873) and *Zabrotes subfaciatus* (Boheman, 1833) (Table 3). The greatest parasitoid emergencies were obtained from the *M. mimosae* (1,352), *A. desmanthi* (898), *M. ulkei* (753) and *M. insolitus* (714) bruquids, equivalent to 77.84% of the population. From the diversity of Hymenoptera, *Horismenus depressus* (Eulophidae) and *Bruchophagus mexicanus* (Eurytomidae) were identified at the species level, at the genus level at *Heterosphilus* sp. (two species) (Braconidae), *Triaspis* sp. (Braconidae), *Eurytoma* sp. (Eurytomidae), and at the family level four species of Braconidae, three of Pteromalidae, two of Bethylidae, one of Eupelmidae and one of Trichogrammatidae, for a total of 17 species. The species *H. depressus*, *Triaspis* sp. and *Eurytoma* sp. were distributed in the four municipalities. Also, the species with the greatest diversity of Bruchidae species attacked were *H. depressus* (8) and *Eurytoma* sp. (5). Finally, the predominant species in the study was *H. depressus* with 2,895 specimens, equivalent to 60.63% of emergencies.

DISCUSSION

Boe and Johnson (2017) reported the presence *Acanthoscelides fraterculus* and *Bruchophagus mexicanus* living together in mature pods of *Astralagus plattensis* in northwestern Dakota, USA, with destruction of 44% of the seeds obtained in a sample of 10 pods, by these insects. Aebi et al. (2008) point to weevils of the genus *Zabrotes* as responsible for large economic losses on beans in the field and storage in Mexico and Central America.

Noyes (2013) indicates the presence of 62 *Horismenus* species parasitizing larvae belonging to the orders Lepidoptera, Coleoptera and Hymenoptera, considered

Table 3. Diversity of parasitoids emerged on fabaceas bruquids in northern Sinaloa, Mexico.

Bruchidae species	Parasitoid species	Specimens by municipality[1]			
		Ahome	El Fuerte	Choix	Guasave
Acanthoscelides desmanthi (Johnson, 1977)	*Horismenus depressus* (Gahan, 1930)	158	260	0	0
	Heterospilus sp. 1 (Haliday,1833)	0	67	0	0
	Heterospilus sp. 2	0	34	0	0
	Braconidae specie 3 (striated) *	3	1	0	0
	Pteromalidae specie 2 *	0	154	177	0
Callosobruchus maculatus (Fabricius)	*Bruchophagus mexicanus* (Ashmead, 1894)	0	15	4	0
	Eurytoma sp. Illiger, 1807	8	9	0	0
	Eupelmidae Walker, 1833	1	7	0	0
	Pteromalidae specie 2*	0	27	0	0
Merobruchus insolitus (Sharp, 1885)	*H. depressus*	199	185	0	167
	Triaspis sp. Haliday	3	2	0	0
	Braconidae specie 4*	1	6	0	0
	Eurytoma sp.	5	12	0	0
	Bethylidae Haliday specie 1*	69	1	0	53
	Bethylidae Haliday specie 2*	8	3	0	0
Mimosestes mimosae (Fabricius, 1781)	*H. depresus*	20	183	212	12
	Triaspis sp.	165	1	140	3
	Braconidae specie 1*	48	0	89	0
	Pteromalidae specie 1*	16	0	12	0
	Pteromalidae specie 2*	35	8	20	1
	Pteromalidae specie 3*	82	17	38	72
	Eurytoma sp.	44	6	119	2
	Eupelmidae specie 1*	2	0	5	0
Mimosestes nubigens (Motschulsky, 1874)	*H. depressus*	10	0	0	8
	Heterospilus sp.1	2	0	0	5
	Heterospilus sp. 2	1	0	0	3
	Pteromalidae specie 2*	48	0	0	34
	Eurytoma sp.	4	0	0	6
Mimosestes ulkei (Horn, 1873)	*H. depressus*	303	46	0	305
	Triaspis sp.	43	0	0	56
Stator limbatus (Horn, 1873)	*H. depressus*	128	78	0	23
Stator pruininus (Horn, 1873)	*H. depressus*	88	139	127	0
	Heterospilus sp. 1	0	6	0	0
	Braconidae spp.	4	0	0	0
	Pteromalidae specie 1*	0	9	0	0
	Pteromalidae specie 2*	0	6	8	0
	Eurytoma sp.	22	0	0	0
	Eupelmidae specie 1*	0	12	13	0
	Trichogrammatidae specie 1*	0	3	0	0
Zabrotes subfaciatus (Boheman)	*H. depressus*	244	0	0	0
Total		**1,764**	**1,297**	**964**	**750**

1= number of specimens,* unidentified.

primary and secondary hosts. They are distributed in 14 countries of the neotropical region over 10 angiosperm families. Hansson et al. (2004) identified 131 specimens of four *Horismenus* species: *H. butcheri* Hansson Aebi & Benrey, *H. depressus* Gahan, *H. missouriensis* (Ashmead) and *H. productus*, attacking the bruquids *Acanthoscelides obtectus* and *A*. *obvelatus* Bridwell associated with *Phaseolus coccineus* subsp. *coccineus* and *P. vulgaris* var. *mexicanus* Delgado. Likewise, these authors indicate an emergency of 59.1% of parasitoids (*Horismenus* spp.) from *P. coccineus* seeds infested with *Acanthoscelides* sp. and *Zabrotes* sp. This situation is similar to that obtained in this study, with 57.97% of *H. depressus* emergencies on the *A. desmanthi* and *Z. subfaciatus* in Ahome, El Fuerte and Choix.

In this study, there was a low level of parasitism by braconids on *A. desmanthi* and *C. maculatus* (Ahome and El Fuerte); it was significant on *M. mimosae* (Ahome and Choix) and low on *S. pruininus* (Ahome). In this regard, Campan et al. (2005) studied the influence of three populations of the braconid *Stenocorse bruchivora* (Crawford) on *Z. subfasciatus* in wild and cultivated seeds of *P. vulgaris* of the states of Puebla, Estado de Mexico and Morelos. They did not find any differences between populations and there was a greater parasitism (60%) in wild seeds than in the cultivated ones. This is attributed to both the quality of the host plant as the genetic variation between populations is the crucial factor that determines the nature and evolution of the interaction between parasitoids and their prey.

In Burkina Faso (USA), Sanon et al. (1998) recorded efficient control of *C. maculatus*, responsible for large losses in *V. unguiculata*, through flood releases of *Dinarmus basalis* (Pteromalidae). This is similar to what happened to species of this family in this study, by virtue of having obtained a 100% parasitism on the bruquid in the municipality of El Fuerte, as well as 97.79% parasitism on *A. desmanthi* in Choix; in Ahome and Guasave it registered 73.84 and 62.96% parasitism on *M. nubigens*.

Although in the study the presence of parasitoids belonging to Eupelmidae was only 40 specimens associated with the species of *A. desmanthi*, *M. mimosae* and *S. pruininus*, in the particular case of species of the genus *Eupelmus* in the Palearctic region, Noyes (2013) points to the orders Coleoptera, Diptera, Hemiptera, Hymenoptera Lepidoptera and Orthoptera. Brúquidos on which they develop like primary or secondary ectoparasitoides of larvae or pupae inside protect habitats like buds, seeds, fruits or gills (Gibson, 1995).

The highest total parasitism percentage was presented by the municipality of Ahome (40.14%), followed by Choix (23.46%), El Fuerte (14.27%) and Guasave (12.86%). The high percentage of parasitism in the first three municipalities may have been due to its great richness in *Fabaceae* species. This is in line with Molina-Ochoa et al. (2004), who argue that the distribution and rates of natural parasitism may be related to the diversity of habitats with the proximity of forests. It is similar to that obtained in Guasave, by virtue of the fact that the sampled *Fabaceae* are located on the banks of agricultural plots. This could cause the parasitoid population to decrease due to the application of insecticides in agricultural crops. This also agrees with Hajek (2004), considering that insecticides significantly reduce the populations of existing natural enemies in crops.

Conclusion

From 68,344 seeds, 19,396 adult bruquids were obtained, and distributed in nine species: *Acanthoscelides desmanthi*, *Callosobruchus maculatus*, *Merobruchus insolitus*, *Mimosestes mimosae*, *M. nubigens*, *M. ulkei*, *Stator limbatus*, *S. pruininus* and *Zabrotes subfaciatus*. The 4,775 adult parasitoids obtained were grouped into seven families: Eulophidae (3,159), Braconidae (692), Pteromalidae (443), Eurytomidae (304), Bethylidae (134), Eupelmidae (40), and Trichogrammatidae (3), distributed in 17 species, of which, *Horismenus depressus*, *Triaspis* sp. and *Eurytoma* sp are present in the four municipalities. 77.84% of the population showed an association with bruquids *M. mimosae* (1,352), *A. desmanthi* (898), *M. ulkei* (753), and *M. insolitus* (714). The predominant species in the study was *H. depressus* (2,895 specimens: 60.63% of the population); also, with a general average parasitism of 19.75%, the greatest impacts corresponded to the municipalities of Ahome and Choix, with 40.14 and 23.46%, respectively.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Water use efficiency and fodder yield of maize (*Zea mays*) and wheat (*Triticum aestivum*) under hydroponic condition as affected by sources of water and days to harvest

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This study was conducted at Soilless Culture Unit, Azemor Agribiz Limited, Ibadan to evaluate the effects of sources of water (fish hatchery wastewater (FHW), borehole water (BW) and nutrient solution (NS)) and days to harvest (8, 10 and 12 days after sowing (DAS)) of two forage crops (maize (*Zea mays*) and wheat (*Triticum aestivum*)) for green fodder production and water use efficiency under hydroponic conditions. The experiment was conducted under average room temperatures of 25.27^{o} and 27.92^{o}C and humidity of 82.8% and 64% in the morning and evening, respectively. The results showed that the pH of the irrigation water samples ranged from 6.20 to 6.70 while the nitrogen and P contents varied from 0.01 - 0.02 %. Maize crop used more water efficiently on the 10 DAS using NS and wheat at 8 DAS using BW, while total water use (lt/kg fresh matter) was lowest on the 10th DAS using NS and BW to produce maize and wheat fodder, respectively. Generally both crops used less than two liters of water to produce 1 kg green fodder. A fresh yield (t ha-1) of 60.42 was recorded in maize using NS as against 58.38 recorded in wheat using BW. Dry matter yield (t ha^{-1}), DM% of fresh fodder and green fodder (kg tray $^{-1}$) were highest using NS in maize but not for wheat using NS. In conclusion maize crop can be considered the better choice for production of hydroponic green fodder with better water efficiency and yields using nutrient solution.

Key words: Hydroponic, days to harvest, fish hatchery wastewater, borehole water and nutrient solution, maize, wheat

INTRODUCTION

Green fodder which forms an essential component and natural diet for ruminant livestock is of low quality and quantity during the dry season, leading to overall poor performance of ruminants. Consequently, for improving livestock products, quality green fodder should be fed more often to animals (Dung et al., 2010). The natural

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pasture supplies most of the feed resources for ruminants. As the gap between the demand and supply of green fodder for ruminant livestock increases, an alternative complementing fodder production method that would enhance fodder and livestock production is hydroponics.

A hydroponic system can be used for green fodder production of many forage crops in a clean environment free from chemical substances including but not limited to insecticide, herbicides, fungicides, and artificial growth enhancers (Tudor et al., 2003; Al-Hashmi, 2008). The technique guarantees high fodder yield, year round production and least water demand (Cuddeford, 1989; Al-Karaki, 2011). Unlike conventional fodder production system that use run-to-waste irrigation practices, recirculation system is used in the hydroponic fodder production, therefore decreasing the waste of water. Tudor et al. (2003) reported that hydroponic fodder production needs only about 2-3% of that water used under field conditions to produce the same amount of fodder. Under this system, fodder can be produced within a short growth period 7 to 10 days, on a small piece of land. Fodder produced is of a high quality, higher in proteins, fiber, vitamins, and minerals. These aforementioned exceptional characteristics of hydroponic system, in addition to others make it one of the most important techniques currently in use for green forage production in many countries especially in arid and semiarid regions of the world. Therefore, the objectives of this study were to evaluate fodder yield and water use efficiency of maize (*Z. mays*) and wheat (*T. aestivum*) under hydroponic condition as affected by sources of water and days to harvest.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in the Hydroponic Fodder Unit of Azemor Agribiz Ltd, KM 15, Ibadan-Ife Expressway, Ibadan, Nigeria located on latitude 7.38°N and longitude 3.95°E. Ibadan has a tropical wet and dry climate, with a lengthy wet season and relatively constant temperatures throughout the course of the year. The wet season runs from March through October, with rainfall peak in August. This peak nearly divides the wet season into two different wet seasons. November to February forms the dry season in the city, during which Ibadan experiences the typical West African harmattan wind. The mean total rainfall for Ibadan is 1420.06 mm, falling in approximately 109 days. There are two peaks for rainfall, June and September. The mean minimum and maximum temperatures were 21.42 and 26.46°C, respectively, while the relative humidity was 74.55% (Wikipedia, 2017).

The hydroponic system

The hydroponic fodder system consists of an enclosure made of planks covered with mesh net (2 mm) and mosquito net to prevent rodents and insects, respectively as well as allowing cross ventilation. The length, breadth and height of the house were 930, 960 and 240 cm, respectively. The roof was made of white translucent polyethylene materials which only permit the light penetration and not the sun rays thereby providing the needed light for photosynthetic activities as well as reducing the heat absorption thus supporting enabling conditions for seed germination, growth and development. The house has a dwarf wall of 1.5 m made of bricks, plastered with strong concrete floor.

The house consist of sixteen units of metal shelves (coated with aluminum to prevent rusting) each 140 cm in length, a width of 92 cm, and a height of 220 cm (Plate 1). Each shelf was divided into five layers at 30 cm interval, each layer accommodated five trays; thus each shelf contained 25 trays making a total of 400 trays in the hydroponic system. Each of the trays made of aluminum material was 108.3 cm in length, a width of 26 cm, and a depth of 5 cm. The trays were perforated at the bottom to allow for dropping of water from the upper trays to the lower ones. This did not only reduce stress during watering but also prevented waterlogging. The 400 trays can produce approximately 2,400 kg of green fodder per growth cycle (8 – 10 days), depending on crop, variety, and growth conditions with a minimum of 6 kg seed per tray. By estimation, a total of 60,000 kg of green fodder was attainable per year at 25 growth cycle/year. The experiment was conducted under absolute ambient climatic conditions.

Plant material

Two fodder crops were evaluated in this trial: Maize (*Z. mays*) and wheat (*T. aestivum*). Seeds of these crops were obtained from ACE Feed Mill in Ibadan. The seeds were subjected to germination (by placing the seeds on moist cotton-wool) test to check for their viability before sowing.

Irrigation water

Three sources of irrigation water were used in this trial. These were: fish hatchery wastewater (FHW), nutrient solution (NS) and borehole water (BW). Fish hatchery wastewater was sourced from the main hatchery located beside the hydroponic building. Nutrient solution was constituted using 'BIC Concept' hydroponic liquid solution A and B at 1 and 2.5 mlL^{-1}, respectively. Borehole water was obtained from the main water source of the farm. The borehole water is safe for drinking and used for the fish culture without any recorded detrimental effect, hence it can be concluded to be free from harmful minerals.

Treatment of seeds before planting

Seeds of maize and wheat were cleaned from debris and other foreign materials and weighed. The seeds were sterilized by soaking for 30 min in a 20% sodium hypochlorite solution (household bleach) to control the formation of mould. Planting trays were also cleaned and disinfected. The seeds were thoroughly washed from residues of the bleach and re-soaked overnight (about 12 h) in borehole water before sowing.

Seed sowing and irrigation

Trial seeds of the two crops were sown in the planting trays, which were lined with aluminum sheets and perforated at the bottom to allow for drainage of excess water during irrigation. The seeding rate used in this experiment was 2 kg seed $tray^{-1}$. This rate was based on the size and weight of the seeds. The trays were labeled accordingly and stacked on the shelves. Treatments meant for the same irrigation water (but of different days to harvest) were randomly stacked together on the same shelf. Trays were irrigated

Plate 1. The shelves and the trays used for hydroponic fodder production.

manually with experimental water (allotted to each treatment) twice daily (7.00 am and 7.00 pm) at a fixed rate of 600 mL $tray^{-1}$ day^{-1} which was enough to keep the seeds/seedlings moist.

Nutrient composition of the irrigation water

Water from the three sources was analyzed for the type and quantity of the nutrient present in each of them. In addition, the pH of the water was measured daily and recorded.

Experimental design and procedure

This experiment involved two fodder crop species namely: Maize (*Z. mays*), and wheat (*T. aestivum*), three sources of irrigation water: fish hatchery wastewater (FHW), nutrient solution (NS) and borehole water (BW); and three days to harvest: 8, 10 and 12 days after sowing (DAS). The experiment was therefore a 2 x 3 x 3 factorial. The treatments in trays were randomly arranged on the shelf and well labeled. Each treatment was in five replicates; hence 90 aluminum trays were used in this trial.

Data collection

The experiment was terminated at 8, 10 and 12 DAS when the fodder was ready for harvest. At harvest, the entire mat of the fodder (comprising the root and the green leaves) was lifted and removed from the tray. At each harvest, fresh yield from each tray was weighed in kg and used to extrapolate for the fresh yield in tons per hectare. A representative fresh fodder sub-sample (300 g) from each tray was taken for the evaluation of the parameters listed below:

Total green and dead fodder yields: The representative green fodder sub-sample (300 g) taken from each tray was separated into the dead/yellow (if any) and green fodder. Each unit was weighed, recorded, and packaged separately in labeled appropriaten envelopes. The summation of the two sub-units amounted to the total biomass yield per tray/treatment.

$$\%\ \text{Green fodder proportion} = \frac{\text{fresh fodder weight}}{\text{weight of sub} - \text{sample}} \times 100$$

$$\%\ \text{Dead fodder proportion} = \frac{\text{dry fodder weight}}{\text{weight of sub} - \text{sample}} \times 100$$

Total water use and water use efficiency: Total quantities of water added and drained from each tray throughout the course of experiment were recorded daily to compute the total volume of water use and water use efficiency. The total volume of water used by plants (liters/tray) was computed according to the equation:

Total volume of water used = Total volume of water added in irrigation − Total volume of water drained from the trays

At each watering, a known quantity of water was used to irrigate the seeds. The water drained from each tray was collected in an empty tray placed directly under each planting tray, measured, and recorded to compute for total water use and water use efficiency.

Water use efficiency (WUE) was computed according to the equation:

$$\boldsymbol{WUE} = \frac{\text{Total green fodder produced (kg/tray)}}{\text{total water used (liter/tray)}}$$

Statistical analysis

All data were subjected to two-way analysis of variance (ANOVA) in a Randomized Complete Block Design (RCBD) using statistical package SAS, 2012 version 9.4 while significant means were separated and compared using Duncan's Multiple Range Test(Duncan, 1955) at 5% level of significance.

Experimental model

$Y_{ij} = \mu + T_i + G_j + (TG)_{ij} + \sum_{ij}$

Table 1. pH and mineral composition of different sources of irrigation water.

Parameter	Borehole water	Nutrient solution	Fish hatchery wastewater
pH	6.40	6.20	6.70
% N	0.01	0.02	0.01
P (mg kg^{-1})	0.01	0.01	0.02
K (mg kg^{-1})	23.50	558.43	60.13
Ca (mg kg^{-1})	6.40	230.46	8.50
Mg (mg kg^{-1})	14.07	18.50	19.10
Mn (mg kg^{-1})	0.07	3.00	0.17
Fe (mg kg^{-1})	6.56	12.90	7.50
Cu (mg kg^{-1})	0.56	0.34	1.32
Zn (mg kg^{-1})	3.91	2.00	0.25

μ = Population mean; T_i = Main effects of the water source (A); G_j = Main effects of the days to harvest (B); $(TG)_{ij}$ = A x B interaction effect; $\sum_{ij}$ = Random error

RESULTS

The pH and mineral composition of the three sources of irrigation water at the beginning of the trial are shown in Table 1. The pH of the water samples ranged from 6.20 to 6.70 while the nitrogen and P contents varied from 0.01 - 0.02%. Nutrient solution (NS) had higher composition of K, Ca, Mn and Fe than the other sources of water.

The effects of sources of water and days to harvesting on water use efficiency (WUE) in producing a unit of a kilogram of fresh maize fodder was highest (P < 0.05) from fodder irrigated with NS and harvested at 10 DAS (18.09 kg/lt). Similar WUE values were observed from fodder harvested at 8 and 10 DAS with borehole water (Table 2). Total water used (lt/kg fresh matter) was lowest at 10 DAS with NS. The results showed that total water used to produce 1 kg green fodder and quantity of water used per tray was not affected by the sources of water and day to harvest (P > 0.05). The effects of the two factors on WUE in producing a kilogram of green fodder were at the maximum (P < 0.05) level 10 DAS using borehole water. This was similar to the value recorded using FHW and harvested 8 DAS (Table 3). In addition, the least total water use (0.89 lt./kg fresh matter) was recorded from treatment with borehole water harvested 10 DAS. Nevertheless, the values were similar for the water treatments as the days to harvest increased.

Irrigating maize with NS and harvesting at 10 DAS had the highest fresh yield (60.42 t ha^{-1}), dry matter yield (39.68 t ha^{-1}) and green fodder (4.66 kg $tray^{-1}$) (Table 4). The least dry matter yields (32.93 – 34.33 t ha^{-1}) were recorded from fodder irrigated with NS (12 DAS), borehole water (12 DAS) and FHW (8, 10 DAS). The highest percentage DM of fresh fodder was recorded from fodder irrigated with NS harvested at 12 DAS and borehole water harvested at 10 and 12 DAS. The least percentage DM of fresh matter was from fodder irrigated with NS, harvested at 8 DAS (31.11%), borehole water, harvested at 8 DAS (31.74%) and using FHW harvested at 12 DAS (31.67%).

Effects of sources of water and days to harvest on the fresh yields, DMY and proportion of green fodder of wheat are shown in Table 5. The highest (P<0.05) fresh yield was recorded from fodder treated with borehole water and harvested at 10 DAS (58.38 kg/ha). This was not significantly different from yield obtained using FHW and harvested 8 DAS (58.33 kg/ha). The least fresh yield (44.57 kg/ha) was recorded 12 DAS using NS as the source of water. The highest dry matter yields were from fodders treated with FHW and harvested 8 and 12 DAS, and using borehole water but harvested 10 DAS. The least yield was from fodder treated with NS and harvested at 12 DAS.

DISCUSSION

The pH of the three sources of water used for producing fodder from maize and wheat seeds indicated that they were close to neutral with a range of 6.20 – 6.70. The higher proportions of Ca, K, Mg, Mn and Fe in nutrient solution (NS) than in borehole water and fish hatchery wastewater (FHW) was a clear indication that NS was synonymous to a liquid fertilizer, hence it contained minerals for plant growth and development. Marschner (1995) proposed that in hydroponic systems, plant productivity was closely related to nutrient uptake and the pH regulation. The pH of the nutrient solution and borehole water used in this study were within the range of 5.5 and 6.5 recommended by Resh (2004) for the development of crops.

Water is very important for seed germination and seedling growth as it is essential for enzyme activation, reserve storage breakdown and translocation (Copeland and Mcdonald, 1995). Water use efficiency (WUE) was highest 10 DAS for the production of maize fodder with

Table 2. Effects of sources of water and day to harvest on water utilization of maize fodder.

Parameter	Nutrient solution			Borehole water			Fish Hatchery wastewater			SEM
	8	10	12	8	10	12	8	10	12	
Water use efficiency (kg fresh matter/lt.)	14.19[b]	18.09[a]	14.51[b]	15.57[ab]	16.22[ab]	14.60[b]	13.74[b]	14.91[b]	14.66[b]	5.74
Total water use (lt./kg fresh matter)	1.17[b]	1.07[c]	1.13[b]	1.21[a]	1.26[a]	1.13[b]	1.14[b]	1.16[b]	1.14[b]	0.01
Total water use (liter/tray)	3.70	3.34	3.50	3.50	3.40	3.50	3.66	3.45	3.60	0.10

a,b,c: means on the same row with different superscripts are significantly varied (P < 0.05); SEM = Standard error of mean.

Table 3. Effects of sources of water and days to harvest on water utilization of wheat fodder.

Parameter	Nutrient solution			Borehole water			Fish hatchery wastewater			SEM
	8	10	12	8	10	12	8	10	12	
Water use efficiency (kg fresh matter/lt.)	13.48[bc]	12.54[cd]	11.43[d]	12.74[cd]	15.40[a]	13.21[bc]	15.35[a]	12.88[cd]	14.43[ab]	1.06
Total water use (lt./kg fresh matter)	1.05[bc]	1.19[a]	1.11[ab]	0.99[cd]	0.89[d]	1.03[bc]	0.91[d]	1.00[cd]	1.12[ab]	0.01
Total water use (liter/tray)	3.96[ab]	3.88[ab]	3.90[ab]	3.98[ab]	3.79[b]	3.88[ab]	3.80[b]	3.83[b]	3.82[b]	0.02

a,b,c,d: means on the same row with different superscripts are significantly varied (P < 0.05); SEM = Standard error of mean.

Table 4. Effects of sources of water and days to harvest on the fresh and dry matter yields and proportions of green and dead maize fodder.

Parameter	Nutrient solution			Borehole water			Fish hatchery wastewater			SEM
	8	10	12	8	10	12	8	10	12	
Fresh yield (t ha^{-1})	52.55[b]	60.42[a]	50.78[b]	54.49[b]	55.14[b]	51.10[b]	50.28[b]	51.43[b]	52.77[b]	16.51
Dry matter yield (t ha^{-1})	36.20[b]	39.68[a]	32.93[c]	36.93[b]	35.83[b]	33.95[b]	33.22[b]	34.33[b]	35.91[b]	2.20
DM% of fresh fodder	31.11[b]	33.89[ab]	35.11[a]	31.74[b]	35.21[a]	34.10[a]	33.86[ab]	33.20[ab]	31.67[b]	7.17
Green fodder (kg $tray^{-1}$)	4.08[ab]	4.66[a]	3.94[b]	4.20[b]	4.29[ab]	4.00[b]	3.90[b]	3.99[b]	4.08[b]	6.10
Green fodder proportion (%)	100	100	98.7	100	100	99	100	100	99.1	22.01
Dead fodder proportion (%)	0	0	1.3	0	0	.01	0	0	0.9	0.00

a,b,c: means on the same row with different superscripts are significantly varied (P < 0.05); SEM = Standard error of mean.

Table 5. Effects of sources of water and days to harvest on the fresh and dry matter yields and proportions of green and dead wheat fodder.

Parameter	Nutrient solution			Borehole water			Fish hatchery wastewater			SEM
	8	10	12	8	10	12	8	10	12	
Fresh yield (t ha^{-1})	53.38[bc]	48.66[cd]	44.57[d]	50.71[bc]	58.38[a]	51.25[bc]	58.33[a]	49.33[cd]	55.12[ab]	14.11
Dry matter yield (t ha^{-1})	36.70[b]	32.25[c]	28.85[d]	34.06[b]	37.06[a]	33.25[c]	37.28[a]	32.89[c]	37.64[a]	2.54
DM % of fresh fodder	1.14[abc]	3.78[cd]	3.46[d]	3.92[bc]	4.51[a]	3.98[bc]	4.52[a]	3.82[cd]	4.28[ab]	0.08
Green fodder (kg $tray^{-1}$)	100.00	100.00	98.70	100.00	100.00	99.00	100.00	100.00	99.10	2.12
Proportion of green fodder (%)	0.00	0.00	1.30	0.00	0.00	0.01	0.00	0.00	0.90	0.00

a,b,c,d: means on the same row with different superscripts are significantly varied (P < 0.05); SEM = Standard error of mean.

NS and at 8 DAS for wheat fodder using borehole water. At these sources of water and days to harvest, maize and wheat used a total of 1.07 and 0.89 lt/kg fresh yield, respectively. Both maize and wheat seeds used 1.0 – 1.2 L of irrigation water to produce one kilogram of fresh fodder which was lower than 1.80, 1.55 and 1.58 L reported for wheat, barley, and cowpea by Ghazi et al. (2012). The higher quantity of water required by maize than wheat to produce same fresh fodder could be as a result of the bigger size of maize which necessitated the need for more water (solution) for the needed metabolic activities and seed softening during germination and development. This report agreed with the findings of Al-Karaki and Al-Momani (2011) that producing green fodders under hydroponic conditions is a highly efficient process in terms of water saving compared to field production of green fodders. Thus, this technique will reduce the cost of irrigation of fodder and thereby, increasing profit maximization, as well as promoting fodder production in the dry season and regions where water is scarce. Total water use (lt/kg fresh matter) was lowest on the 10th DAS using NS and borehole water to produce maize and wheat fodder, respectively. This indicated that the peak of water utilization was at 10 DAS. Up till this day, the crops used less water for metabolic activities.

The use of NS for production of fodder was optimal ($P < 0.05$) for maize but not for wheat despite the higher nutrient composition in it than borehole water and FHW. Dry matter yields of wheat and maize when harvested 8 and 10 DAS, respectively were higher than at other period. However, according to Jeton (2016), the maximum fresh yield of 60.02 and 58.22 t ha^{-1} recorded from maize and wheat fodders, respectively will be enough to feed 13 -15 cattle daily with an average body weight of 300 – 400 kg and 140 -150 sheep with an average body weight of 25 -35 kg. The fresh yield of 58 - 60 t ha^{-1} recorded in this study was similar with the result of Al-Karaki (2011) who recorded fresh yield of 56.0 t ha^{-1} from barley grown hydroponically under irrigation with borehole water. A fresh yield of 8-10 kg from one kg of locally grown maize seeds in 7-10 days was reported from farmers producing hydroponics maize fodder under low-cost greenhouses devices in India (Naik et al., 2013). AI-Karaki and Al-Hashimi (2012) recorded average green forage yields of 217, 200, 194, 145 and 131 t ha^{-1} for one production cycle (8 days), for cowpea, barley, alfalfa, sorghum and wheat, respectively. Genotypic variations in forage yields have been reported for different cereal and legume crops (Ansar et al., 2010). Factors that can influence the fresh yield and DM content of the hydroponics fodder included the type of crops, days to harvesting, degree of drainage of free water prior to weighing, type and quality of seed, seed rate, seed treatment, water quality, pH, irrigation frequencies, nutrient solution used, light, growing period, temperature, humidity, clean and hygienic condition of the greenhouse. Downward trend in maize and wheat fodder yields was observed after day 10, signifying cessation in growth and development. Day 10 seems to be the best day to harvest maize fodder under hydroponic conditions. This could not be inferred in this study with respect to production of wheat fodder through hydroponics.

Conclusion

(i) Under hydroponic system, production of green fodder (44-60 t/ha) can be achieved within 8 to 12 days from seeds of maize and wheat using nutrient solution, borehole water and fish hatchery wastewater as sources of water.
(ii) Less fish hatchery wastewater and nutrient solution was used to produce 1 kg of fresh maize and wheat fodder, respectively. Both crops required less than two liters of water to produce 1 kg of green fodder, hence production of fodder from hydroponic system requires small quantity of water, and will support fodder production where and when there is water scarcity.
(iii) Fodder yields were averagely higher when irrigated with nutrient solution in maize and when irrigated with borehole water and fish hatchery wastewater in wheat at 10 to 12 days after sowing. Hence, 10 DAS is considered the best time to harvest fodder from both crops under hydroponic conditions using NS for maize, and borehole water or FHW for wheat.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Gender differences in agri-marketing farmer organizations in Uganda and Malawi: Implications for R4D delivery mechanisms

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Although farmer organizations are acknowledged to link their members to markets, enhance their business skills and enable access to services, there is need for evidence that ascertains if all members benefit equitably. This paper examines benefit distribution and sources of gender differences in mixed-sex collective marketing farmer organizations in Uganda and Malawi. Through a cross-sectional survey, key informant interviews and gender segregated focus group discussions, data were pooled from 10 farmer organizations and 492 individual members. An independent samples t-test revealed that in both countries, men benefited more than women. In Uganda, significant differences existed in skills training, planning meetings, business networks, and impromptu general meetings. In Malawi, significant differences manifested in extension training and decisions regarding investment and marketing. In both countries, men were significantly more dissatisfied with the collective marketing initiatives than women, which resulted in side selling in Malawi. On the other hand, women were significantly more satisfied than men with the collective marketing and, in Malawi, they sold significantly higher quantities through their organizations than men. We conclude that collective marketing farmer organizations connect women members to markets but neither address gender inequalities nor stimulate women empowerment. We propose strategies that can improve gender responsiveness in mixed farmer organizations.

Key words: Farmer organizations, gender differences, benefit distribution, smallholders, Uganda, Malawi.

INTRODUCTION

Farmer organizations (FOs) have been advanced by several governments and other development stakeholders as a means for alleviating market access challenges that impede smallholder farmers from competing in agricultural markets (Mojo et al., 2017; Wanyama et al., 2008; World Bank, 2007). This is because through collective action, FOs can enable smallholder farmers to strengthen their bargaining power and participate in profitable value chains, as well as exploit economies of scale to transact business with big buyers of their outputs

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or suppliers of the inputs they need (Hilhorst and Wennink, 2010; Penrose-Buckley, 2007; Shepherd, 2007). Where extension service is limited or lacking, FOs such as cooperatives and farmer associations have been promoted as an efficient approach for providing support services e.g. extension advice, market information, credit and market outlets to farmers (Chagwiza et al., 2016; Otieno, 2019; Bijman and Wollni, 2008; Shepherd, 2007). In developed nations, such as United States, Canada, and Japan, agricultural cooperatives have become a powerful economic force shouldering marketing, supply, credit, insurance, as well as handling international businesses (Aref, 2011; Birchall, 2004). In Africa and other developing countries, cooperatives have been appreciated for creation of employment, poverty reduction, facilitating technology adoption (Bayan, 2018; Chagwiza et al., 2016), extending social protection, and representation of interests of the majority poor who have no voice (Develtere et al., 2008; Mrema, 2008). Other analysts find that cooperatives provide more inclusive trade relations and value chains and offer opportunities for gender mainstreaming and women empowerment (ICA, 2016; Thangata, 2016), helping poor women to lift themselves out of poverty and have their voices heard collectively (ILO, 2014). However, in all cases, authors recommend deliberate actions to increase and incentivize women participation in cooperative activities, leadership and decision making and enabling active engagement.

Despite the growing literature on the potential benefits that FOs give to their members, not enough studies have been conducted to understand whether the distribution of the benefits to members is fair enough to guard against empowering a few while using others as ladders of their success; thus, widening the poverty gap. The documented successes of FO as a mechanism for collective marketing have often not disaggregated results to indicate whether all members accessed the specific benefits that were generated by the FOs (Berdegué et al., 2008; Chirwa et al., 2005; Coulter, 2007; Kyazze, 2010; Ikwera and Twongyirwe, 2019; ILO, 2012; Shiferaw et al., 2009; Thangata, 2016; Wanyama, 2012; Mrema, 2008). Disaggregated information is particularly important for studies of mixed sex FOs, where women and men face unequal constraints that put women at a disadvantage when negotiating with men (Kaaria et al., 2016). Women are likely to join with the burden of triple roles, have low mobility and education relative to men, have limited access to and control over productive resources, and are generally expected to behave in a certain way by society (FAO, 2011). Thus, it is critical that disaggregated information on distribution of benefits among FO members differentiated by sex be made available to inform the design of strategies for addressing gender equity gaps.

This paper examines the distribution of benefits of participation in collective marketing among members of FOs and asks whether these FOs work equitably for men and women members in the context of Uganda and Malawi. The paper contributes towards filling the gaps in literature on sex-disaggregated evidence in the context of FOs in different ways. First, we analyse the sex-disaggregated composition and governance of collective marketing FOs, which are of a mix of area cooperative enterprises (ACEs), also referred to as secondary cooperatives, and farmer associations at second-tier level. We focus on the sex of the members and occupants of leadership position as these may influence the nature and distribution of benefits. Then, we investigate the nature and extent of benefits that accrue to members and test whether there is equal distribution of those benefits between men and women. From the analysis, we draw implications for the potential of farmer organizations to deliver on gender equality and women empowerment as postulated by literature (ICA, 2016; ILO, 2015).

Historical perspectives of farmer organizations in Uganda and Malawi

In both Uganda and Malawi, FOs have been embraced and promoted as a means for rural development for over four decades. In Malawi, FOs started as farmer clubs in 1978 through the Ministry of Agricultural for purposes of facilitating farmer access to agricultural credit and extension advice in the country (Kishindo, 1988). These clubs have since evolved into a hierarchy of zonal/regional and national level apex organizations (Chirwa et al., 2005; Coulter, 2007; Diaz, 2004; Mapila et al., 2010a).

In Uganda, FOs were started way back in 1913 as lobby pressure groups that mobilized to fight against the exploitation of European and Asian traders and get high returns from their marketed produce (Msemakweli, 2012; UNDP, 2016). At the time, the colonial government had enforced a policy that required smallholder farmers to produce cotton and coffee while the European and Asian business persons were required to process and market the products. Unlike in Malawi, cooperatives in Uganda served as a channel through which the government controlled marketing of coffee and cotton, which were important crops for foreign exchange earnings for the government of the time, while economic development and integrating the poor farmers into the economy became secondary. These cooperatives were linked to the market through the marketing board that handled most of the export marketing activities and provided credit in form of inputs to support production (Msemakweli, 2012).

Like many other countries across Africa, cooperatives in Uganda and Malawi also suffered from poor performance due to governments' interference that used them as platforms for rewarding political royalties with employment, thus running down their performance and reputation (Futures Agriculture, 2014). During this era, rules and regulations that came to shape the way cooperative members interacted were gender blind as women were only represented at the level of household headship while most women in agriculture living in male headed households were represented by their husbands.

After market liberalization that started in the 1990s, FOs underwent reforms that brought about changes in how FOs are organized and managed internally. They have since taken up multiple functions such as advocating for policy change (FUM, 2010), and serve as platforms for extension service delivery, mobilization of financial resources, agro-processing and collective marketing, as an avenue for linking members to markets (Biteete, 2019; Thangata, 2016).

The management of FOs in the post liberalization period has been highly decentralized in nature, which allows them to form their rules and procedures that provide a framework of incentives that shape their economic, political, social organization and behaviour (Dorward et al., 2009). According to the new institutional economics theory, this enhances the supervision of contracts and generates a stream of benefits to members that includes reduced transaction costs, risk, as well as overcoming problems of weak enforcement of laws and low community level social capital (Dorward et al., 2009). However, as argued in the Futures Agriculture (2014), organizational rules and procedures that shape incentives to members who cooperate are functions of internal and external factors mediated by gender aspects. Internally, power relations and group characteristics influence bargaining and decision-making processes, with more powerful members likely to influence the outcome of the interactions in their favour as the weak lose out (Kaaria et al., 2016).

Gender inclusion in the organization of FOs

The diversification of functions combined with decentralised managerial structures enabled participation of other social groups that were originally excluded from cooperatives during the pre-liberalisation era. In particular, social inclusion in farmer organization in the two countries shifted in favour of women. For example, FOs formed under the National Agricultural Advisory Services (NAADS) in Uganda, the dominant advocate for the group approach, were either constituted by more female members than males (ILO and ICA, 2014) or there were no differences (Benin et al., 2007; Davis et al., 2007). Different strategies have been promoted to enhance women participation in FOs. In the context where gender inequalities are deeply entrenched and socio-cultural norms prohibitive, the strategy has been to promote women only FOs (Carr and Hartl, 2008; Hilhorst and Wennink, 2010; Quisumbing and Pandolfelli, 2009). In case of collective marketing where the primary objective is linking farmers to new markets, women-only FOs are viewed as less successful in terms of market search, access to new information and negotiating with buyers (Barham and Chitemi, 2008; Gotschi et al., 2009; Katungi et al., 2008), which necessitates presence of men members in the organization to fill in the gaps. For these reasons, sex mixed FOs have tended to dominate where the interest is to link smallholder farmers to globalized markets through formalized FOs. Kaaria et al. (2016) highlight additional strategies that have been used to increase women participation in FOs. They include basing membership on individuals rather than the household; aligning entrance requirements to what women can control; reducing membership fees for women and getting more women on the executive boards. Authors caution that these strategies serve to bring women into a mixed organization but do not automatically guarantee their active continuous engagement.

In both Uganda and Malawi, women continue to be under-represented at political, policy and decision-making levels in mixed FOs. This is partly due to cultures that assign leadership and decision-making roles to men and organizational rules that attach high preferences on literacy skills as eligibility criteria for leadership (Mapila et al., 2010a), but discriminate against less educated members, majority of whom are women (IFPRI, 2005b; Mapila and Makina, 2012; Sinyolo and Mudhara, 2018).

Despite the pre-entry differences in capabilities, all FOs in this study adopted the voluntary and open membership cooperative principle: all persons who can use the FO services and are willing to accept the responsibilities of membership are free to join. Thus, men and women are subjected to the same criteria that qualify them as members - payment of membership and annual subscriptions fees and willingness to market a proportion of their produce through their FOs. The amounts of membership and subscription fees are democratically agreed upon and payable by all members. The members are also bound by a common set of rules that structure their behaviour, prescribe the rules of business conduct and a leadership code (Ampaire et al., 2013b). In mixed sex FOs, the leadership code stipulates the proportion of men and women that constitute leadership committees, among other things. The quota system, which is adopted from government macro policies as a blue print, is adhered to for women representation on executive leadership committees but not necessarily on sub-committees.

There is evidence that FOs can enable women to break through the community safety concerns or socio-cultural norms and access markets, increase their assets and gain control over decision-making processes that affect their lives (Hilhorst and Wennink, 2010; Penrose-Buckley, 2007). However, it has also been shown that the success of FOs in providing benefits to women members, particularly in mixed sex FOs, does not come freely but requires design and implementation of measures that address the constraining gender norms (Kaaria et al., 2016; Lecoutere, 2017; Quisumbing and Pandolfelli, 2009; Rubin et al., 2009). For example, there is evidence that successful agriculture production depends on whether one has access to enabling assets such as natural, physical, human, financial, and political capital (Meinzen-Dick et al., 2013). As such, the capacity of women to produce and improve livelihoods is constrained by lack of or limited access to and control over assets such as land, inputs, credit, extension services (Ampaire et al., 2013a; Lodhia, 2009; Rubin et al., 2009). Because women join FOs with such constraints, there is always need for FOs to put in place mechanisms that can help women overcome these constraints to not only be members of FOs, but beneficiaries of the benefits that come with it.

MATERIALS AND METHODS

Data sources and collection methods

The data used in the study were obtained from both secondary and primary sources to enable triangulation of information during analysis. In Uganda, secondary data was obtained from documents provided by Uganda Cooperative Alliance (UCA), the apex body coordinating cooperatives in the country, who also recommended a list of ACEs to work with, from which a final sample was purposively selected based on the target commodity and ease of access. Additional documents were downloaded from the ministry of trade, industry and cooperatives website. At ACE level, transaction records were obtained from the executive, who also provided an updated list of member grower cooperative societies (GCSs). Membership records were obtained from the leaders of the randomly sampled GCSs. In Malawi, the beans programme at Kitedze research institute, in collaboration with NGO partners, sampled the five target farmer organizations. Leaders of the sampled associations provided business records and lists of their member clubs. Club leaders provided lists of their respective members.

Data from primary sources were gathered via a mixed methods approach combining gender segregated focus group discussions (FGDs), key informant interviews and a survey of individual FO members selected from primary cooperatives or clubs. Sampled FOs include five ACEs in Uganda, which were constituted by GCS and five second-tier farmer associations in Malawi that were constituted by clubs. The ACEs and associations were purposively selected to include those that prioritized bean production[1] as one of the priority commodities and collectively marketed the produce through the FOs at least once. Confirmation was made with the leadership of the organizations to ascertain that the FOs were still active and involved in collective marketing before the study commenced. A total of 22 GCSs and 26 clubs were sampled from Uganda and Malawi respectively. For each of the selected GCS/club, a full list of individual members was obtained from the leaders and stratified proportionate sampling method applied based on the composition of men and women in the total membership.

In Uganda, two FOs were selected from the eastern region, two from the central and one from western Uganda. Most FOs sold bean grain to local markets and only one FO supplied bean seed to a seed processing company through contract farming. Table 1 indicates that the socio-economic context of the study areas in Uganda can be distinguished in terms of socio-cultural dynamics - access to services and markets, and gender. In general, male dominance existed in all sampled communities although it is moderated by social networking behaviours. A total of 23 FGD constituting 184 farmers; five for top executive and 18 for members aggregated from 22 primary cooperatives in five ACEs were organized and conducted. The membership in secondary cooperatives in Uganda was spatially spread, demanding that we conduct more FGDs to take care of variability from 23 FGDs.

In Malawi, the study was conducted in Ntchisi, Dowa and Nkhotakota districts of the central region that is majorly matrilineal (Berge et al., 2014). This matrilineal lineage system offers the primary land rights to women which are passed on from mother to daughter (Kishindo, 2010a). The social economic-context of Malawi in which the FOs is embedded varies in terms of socio-cultural dynamics, type of collective businesses and support from service providers (Table 2). Three out of five FOs sampled were registered under the grain legume association (GALA) umbrella, one association was supported by World Vision and one cooperative was registered by the ministry of agriculture and supported by Total Land Care. In all cases, beans were an additional collective enterprise to soybean, groundnuts, and maize although a random list of crops grown by the sampled households put beans in second position to maize. A total of 124 farmers were met in 15 FGDs, five for top executives and 10 for members selected from 26 clubs.

FGDs were administered by the principal researcher both in Uganda and Malawi, with support from translators and a voice recorder. Information gathered from FGDs was intended to understand FO history, activities, mode of establishment, membership, how farmers are linked to markets, markets supplied, sales, leadership capacity, governance structures, physical assets owned, access to other market support institutions such as credit, market information and extension services. They also made suggestions on how women participation and access to benefits could be improved. FO members were also asked if they received any benefits from their organizations and responses were captured as yes/no. Those whose response was yes were required to list benefits received (Figure 1). The interviewer went ahead to probe for the specific benefits (Table 3) using a pre-scribed list that was generated from responses by the FO leaders. For decision makingand meetings, respondents listed decisions and meetings they were involved in the previous 12 months (Table 4).

Information from FGD was complemented by primary data collected from key informants and a formal survey of FO members. A total of 12 key informants were purposively selected to include ministry officials, extension staff and local leaders in both Uganda and Malawi. Key informants highlighted the history and relevancy of FOs at national and local levels, described the socio-economic and cultural context, and ascertained activities of the sampled FOs. For the survey, we adopted the recommendation by Sekaran (2003) for an appropriate sample size larger than 30 and less than 500 and sampled a minimum of 30 members per GCS/club depending on membership size. A total of 492 individual FO members (303 men and 189 women) from both Uganda (245; 167 men and 78 women) and Malawi (247; 136 men and 111 women) were interviewed in the cross-sectional survey. A team of trained enumerators with good knowledge of English and the local language conducted individual interviews using a pretested questionnaire administered through face-to-face interviews. The individual interviews elicited information on demographic characteristics of members, such as gender, age, position in household, marital status and education level. Information was also gathered on size of land holding, distance from residence to the bulking centre, commodities produced and those marketed, non-farm sources of income, housing structure, physical assets owned, participation in decision-making processes and meetings, access to and type of benefits generated by FOs and perceptions of satisfaction with FO performance. Only variables relevant to collective marketing were analysed for this paper.

[1] The beans enterprise provided sufficient population across the different FOs from which we could sample. Also, the project that funded this study was focused on beans. This enabled us to get data that not only meet the study objectives but also contributes to the project.

Table 1. Characterization of the sampled FOs in Uganda (July 2011).

FO	Year started	Membership at data collection			Reason for starting	Location	Collective enterprises	Services given to members	Gender inclusion strategies	Socio-economic context
		M	W	Y						
U1	1981	19	16	1	-To protect land -Pool savings for credit -Improve access to services	Lwengo district, Central region	Bean seed Starting coffee	-Providing credit -Savings accounts -Access to inputs -Provision of market information -Quality assurance	None	RPO membership constituted by historical Nyankore/Kiga migrants, high social networking behaviour, moderate male dominance, beans traditionally important part of diet but currently is a major source of income. Access to improved varieties from seed processor. Ready market but low price margins. RPO originally formed to overcome common land ownership problems.
U2	1952	330	94	0	-Collective marketing -Pool savings for credit -Improve access to services	Rakai district Central region	Coffee Bean seed	-Providing credit -Savings accounts -Access to inputs -Provision of market information -Agricutural extension -Coffee processing	None Household head model	RPO members mainly Ganda, low social networking behaviour, high male dominance, beans moderately important for food security but currently an alternative source of income after the coffee wilt incidence. Access to improved varieties from seed processor. Ready market but low price margins. RPO formed in colonial times for coffee marketing & coffee enterprise still predominates.
U3	2002	449	286	106	-Collective marketing -Improve access to services	Manafwa district Eastern region	Coffee Maize Bean grain	-Savings accounts -Access to inputs -Provision of market information -Agricultural extension -Grading coffee -Transporting produce	Women constitute 1/3 of the executive committee	RPO members mainly Gisu, to a bigger extent an elite community, low male dominance, moderate social networking behaviour, beans important for food security but also a 3rd priority market enterprise for RPO. Lack of access to improved varieties. Supply local markets.
U4	2001	1784	820	516	-Collective marketing -Value addition	Manafwa district Eastern region	Coffee Bananas Bean grain	-Providing credit -Savings accounts -Access to inputs -Provision of market information -Agricultural extension -Grading coffee -Transporting produce	Women constitute 1/3 of the executive committee	RPO members mainly Gisu. RPO management has evident capacity issues, moderate male dominance, and moderate social networking behaviour, beans important for food security but becoming important for cash due to coffee and banana wilt epidemics. Unlimited access to improved varieties from across the Kenya border, high influx of traders from Kenya. Supply local markets.
U5	2003	1962	942	639	-Collective marketing -Value addition -Collective voice	Ntungamo district South western region	Bean grain	-Savings accounts -Access to inputs -Paying cash upon delivery -Provision of market information -Agricultural extension -Transporting produce -Warehouse receipt system	Women constitute 1/3 of the executive committee	RPO members mainly Nyankore. High male dominance, high social networking behaviour, beans traditionally important part of diet but has become a major source of income. Limited access to improved varieties. Inconsistent market availability. In some instances, women leaders were left out of important decisions.

Names of FOs in Uganda have been withheld for confidentiality reasons as pledged to respondents.

Table 2. Characterization of the sampled FOs in Malawi (February 2012).

FO	Year started	Membership at data collection			Reason for starting	Location	Collective enterprises	Services given to members	Gender inclusion strategies	Socio-economic context
		M	W	Y						
M1	2009	15	48	7	-Collective marketing -Bulk purchase of inputs -access agricultural extension	Ntchisi district Central region	Beans Soybeans	-Provision of market information -Access to inputs -Agricultural extension	Women constitute 75% of leaders on the executive committee	RPO mainly dominated by women. Beans and soybeans were regarded as female crops until recently when men are also producing legumes due to the collapse of tobacco market in 2010/11. Good access to improved varieties through GALA who also provide an output market for the grain legumes.
M2	2010	38	129	-	-Collective marketing -Pool savings for credit -Improve access to services	Ntchisi district Central region	Beans Soybeans	-Provision of market information -Access to inputs -Agric. extension	Women constitute 30% of leaders on the executive committee	Seed production is the main enterprise for the RPO. Women are very active in decision making processes and the chairperson is a woman. Members of the RPO are encouraged to join the village savings schemes to enable them access agricultural inputs easily. The savings schemes are not run by the RPO but are separate initiatives available in the same community
M3	2003	898	502	-	-Collective marketing -Improve access to services -Quality assurance	Dowa district Central region	Maize G/nuts Beans Livestock	Access to inputs	Women constitute 30% of the executive committee	The area experienced acute food shortages, school dropouts, outbreaks of diseases & domestic violence before World Vision started working in the area. Several interventions were then implemented to address these issues until the area attained food security, improved health and sanitation and reduced school dropouts. Farmers started realizing food surpluses and marketing became very important. Apart from crops, the RPO also deals in dairy farming.
M4	2001	40	18	-	Collective marketing	Nkhotakota district, Central region	Beans	-Agricultural extension -Access to inputs -Quality assurance	20% women representation in the executive as committee members	Main enterprise for the RPO is beans which are normally grown in winter along rivers. Beans are mainly grown for commercial purposes as seed. At data collection, women were not physically represented on the executive committee despite the regulations on paper.
M5	2007	54	25	-	-Collective marketing -Pool savings for credit	Ntchisi district, Central region	Macadamia Beans Soybeans	-Access to credit -Agricultural extension -Quality assurance	Women constitute 40% of leaders on the executive committee	The main enterprise for the RPO is Macadamia production. Members are only those with macadamia trees. Since the trees take time to start bearing fruits, the cooperative has considered other enterprises like beans and soybeans. Men dominate in most decision making.

Names of FOs in Malawi have been withheld for confidentiality reasons as pledged to respondents. Most FOs in Malawi did not segregate youth as a category of members.

RESULTS

Characteristics of sampled FOs

Tables 1 and 2 indicate that most sampled FOs in both countries were started purposely for collective marketing reasons. Additional roles such as value addition, bulk purchase of inputs, pooling savings for credit, provision of market information, transportation of produce and improving member access to services were added to strengthen collective marketing.

In Uganda, two out of five FOs had beans as

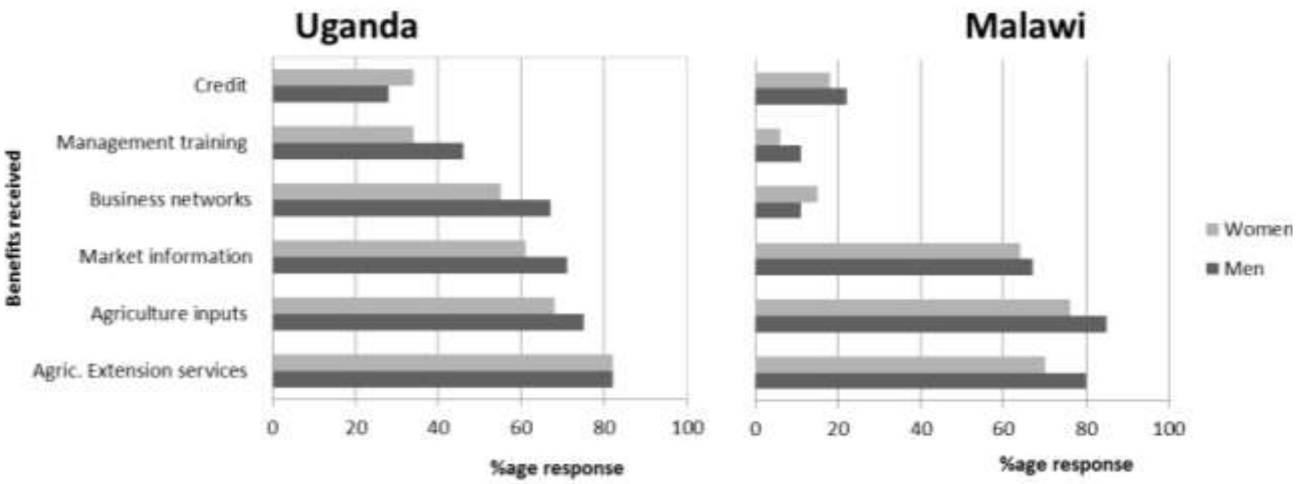

Figure 1. Benefits received by men and women FO members. Percent responses are based on surveyed male and female respondents that received benefits. Proportions have been calculated as a fraction of the separate male and female samples per country.

Table 3. FO member characteristics.

Uganda (N=245)	**Male (167)**		**Female (78)**		**All**		**P-value**
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age (# of years)	47.68	15.27	45.59	12.06	47.01	14.34	0.289
Education (years of formal schooling)	7.39	3.33	6.51	2.86	7.13	3.21	0.066
Land size (acre)	5.22	11.94	3.97	3.70	4.82	10.09	0.370
Household size (# of people)	7.713	3.978	7.500	3.345	7.645	3.782	0.683
Distance from home to bulking center (km)	2.28	2.45	1.95	1.64	2.18	2.22	0.286
Years in association	11.77	13.52	8.54	9.42	10.74	12.44	0.058
Malawi (N=247)	**Male (136)**		**Female (111)**		**All**		**P-value**
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age (# of years)	45.58	14.38	42.22	12.17	44.07	13.51	0.051
Education (years of schooling)	6.60	2.85	5.34	3.06	6.04	3.00	0.001
Land size (acre)	6.91	6.81	4.62	2.95	5.88	5.54	0.001
Household size (# of people)	7.03	3.18	6.42	2.42	6.76	2.87	0.099
Distance from home to bulking center (km)	2.43	2.71	2.19	2.31	2.32	2.53	0.464
Years in association	3.76	2.69	3.14	2.43	3.48	2.59	0.059

the priority enterprise while three ranked beans as a second or third priority crop. In Malawi, three out of five produced beans as the priority enterprise while two ranked beans in second or third position. However, beans remained a major enterprise due to availability of good local markets and its importance for both food security and income. At the time of data collection in Malawi, men were increasingly taking on the bean enterprise as a substitute for the 'staggering' tobacco enterprise. In both countries, we neither found statistically significant differences associated with other enterprises nor between bean seed and bean grain.

FOs in Uganda provided more services to members than those in Malawi (Tables 1 and 2). Probably this is because the FOs in Uganda have existed longer than those in Malawi. Observations and key informant interviews indicated that Malawi FOs had not received sufficient support regarding institutional building although all of them had received external support at the start.

Analysis of FO composition

The FO composition is analysed with respect to selected demographic and socioeconomic factors that have been reported to be important in decisions regarding participation in agricultural marketing by smallholder farmers since these FO were primarily initiated to enhance market access. Marketing through FOs offsets issues of high transaction costs associated with long distances from the market and constraints emanating from lack of marketing experience and market information. This means that individual farmers experiencing these constraints are likely to be attracted to FO but there might be differences between men and women.

Table 3 presents key statistics of FO members in both Malawi and Uganda and gives a statistical comparison of selected characteristics between men and women members of the studied FOs in each country. Overall, results show that in both countries, the FO is composed of members' representative of a typical farmer in each context. A typical member is in his/her middle age (that is above 45 years) with few years of formal education. The average distance from home to the bulking centres is 2-2.4 km, which demonstrates that, members in FOs are recruited from close neighbourhoods.

Results reveal greater similarities between men and women members in Uganda FOs. In this context, both men and women were of similar age, come from households that have comparable size of land holding and location with respect to the bulking centres. This means that both men and women had to travel same distance to centralized aggregation centres from where the FO leaders transported the produce. Comparable land sizes do not mean that women own land but suggest right of access to use (Kabahinda, 2017), which is a very important factor that determines women's commitment to collective action through cooperatives (Selhausen, 2016). However, men were of higher education than women and had longer experience in FOs than women - thus at an advantage when dealing with challenges that come with collective marketing (Barham and Chitemi, 2008).

The story was slightly different in Malawi, with results showing more differences between men and women members of FOs that favour men over women when it comes to negotiations (Mapila et al., 2010a). In addition to possessing higher education and the tenure of membership in FOs, men in Malawi were on average older than women. Similarly, men were from households with relatively larger land holdings compared with women and reported significantly larger household sizes.

Governance of farmer organizations in Uganda and Malawi

Here, we examine the structures of leadership and decision-making processes.

Leadership in FOs

In both countries, FOs are generally governed through democratically elected leadership committees. At the second-tier level, the executive committee is constituted by representatives from the member primary groups or clubs. Each organization is managed through the constitution that mandates 30% of the positions on the leadership committee to be held by women. This representation was adopted from government legal provisions as a blue print. Most FOs have additional sub-committees e.g. for marketing, procurement, monitoring), which offer additional leadership opportunities. These committees are small and tend to be technical thus do not often observe the 30% representation. The FOs are also managed through a set of written rules and regulations, enshrined in their constitutions. Other than the 30 percent women representation, the organization constitutions did not have in place any strategy that provide for gender inclusion or active participation of women.

FO members in Uganda were asked why women participation in leadership is limited and 22% of responses pointed to women's multiple roles in the household, 18% argued that the FO constitutions are already predetermined that women participation is limited to 30 and 18% pointed to women being shy and lacking confidence, while 15% blamed it on women's low education levels. In Malawi, some of the FO leaders, especially men, neither appreciated the importance of involving women in leadership nor ensuring that they actively engage. In one FO, which had no woman representative on the executive committee (except on paper), the chairperson wondered why any attention should be paid to women. 'Rural women in Malawi are shy and lack confidence to take up leadership roles', he affirmed his stand. In one ACE in Uganda, a female treasurer explained that male leaders would in some cases not invite her for a meeting if they felt that she would not support their decision.

Decision making

Decisions in FOs are made as a consensus through meetings that are constituted differently and are held at different levels. At both the second tier and GCS/club levels, respective executive committees make day-to-day management decisions, based on the mandate laid out in the constitution. In other instances, e.g. abrupt requests from apex organizations that need backing by members and election of leaders, all members meet to decide. More technical decisions such as planning, problem solving, and marketing are made by the executive or assigned sub-committees.

Table 4 compares men and women member participation in FO meetings and decision making, which are key indicators of democratic governance.

Results show that the participation of men and women in meetings is comparable in Uganda. In Malawi, significant differences between women and men

Table 4. Sex disaggregated participation in FO meetings and decision making.

Variable	Uganda				Malawi			
	Men		Women		Men		Women	
Type of meeting attended	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**
Executive/Board meeting	33	20.1	18	23.1	16	11.8	7	6.3
Planning meetings	55	33.3	22	28.2	16	**11.8****	5	4.5
Marketing meetings	63	38.2	22	28.2	22	16.2	18	16.2
Problem-solving meetings	77	46.7	29	37.7	10	7.4	4	3.6
Annual General Meeting	157	94.6	74	96.1	36	26.5	27	24.3
Technical training	130	79.3	55	70.5	24	**17.6*****	7	6.3
Special general meetings	93	56.7	44	56.4	19	**14.0*****	4	3.6
Type of decision taken								
Election of leaders	112	67.5	52	66.7	62	45.6	51	45.9
Inclusion of new members	86	51.8	42	53.8	17	12.5	7	6.3
Investment in new building/equipment/land	23	**13.9****	4	5.1	6	4.4	4	3.6
Buyer to whom produce is sold	69	41.6	28	35.9	22	**16.2****	8	7.2
Timing when produce should be sold	86	51.8	32	41.0	16	11.8	19	7.1
Price given to members for their produce	76	46.1	36	46.2	17	12.5	18	16.2
How benefits are shared	70	42.4	29	37.2	3	2.2	2	1.8
Selecting the marketable enterprise	61	37.4	24	31.6	36	**26.5****	17	15.3

Percent response has been calculated as a fraction of the total numbers of men and women that attended specific meetings or participated in making specific decisions. Asterisks *, **, *** denote significant at 10, 5, and 1% confidence levels.

participation exist regarding planning meetings, technical training and special general meetings that are often not previously planned for but rather are conducted impromptu to address specific issues that come up. Women's limited participation in planning meetings points to low representation of women in the leadership since these meetings are mainly attended by leaders. Their low education levels constrain attendance of trainings while inability to attend impromptu meetings signifies women's limited mobility. These findings agree with previous research by Kaaria et al. (2016). The observations show that FOs are not building in mechanisms to enable equitable participation of both men and women, which is a pre-requisite to equitable access to benefits.

Between countries, both men and women in Uganda were involved in making most decisions with a few exceptions. The bivariate analysis shows that significantly fewer Ugandan women participated in making investment decisions while in Malawi, there were limited investment decisions made either by men or women. Significantly fewer women in Malawi were involved in making marketing decisions such as selecting the marketable enterprise and deciding who the buyer of their produce would be. Inability to involve women in marketing decisions reinforces a disempowered status quo where women's voices are not heard, their priorities are not considered on the FO agenda, and their confidence to undertake marketing roles remains low.

Sex-disaggregated access to benefits generated by FOs

In organizing for collective marketing, FOs strategize to benefit members in two main ways. They provide markets for members' produce in response to their primary objective. This is made possible through searching for market information e.g. prices that help in determining suitable buyers. Secondly, FOs provide other services or attract development stakeholders that provide the services that FO members would not be able to access as individuals. Such services include extension advice, value addition, grading and transportation of products to the market, and mobilizing savings that translate into low interest financial loans for members (Tables 1 and 2). The services offered by FOs and partner service providers are supposedly accessible to all members, who perceive these services as benefits that accrue to them because they are members. In the survey, individual members of FOs were asked to specify the benefits that they had received from the FOs they subscribe to and Figure 1 presents the individual responses provided by men and women.

In both countries, most men and women FO members received agricultural extension services, agricultural inputs, and market information from their FOs. On average, a lower %age of women, compared to men respondents, accessed these benefits except in the case of credit and agriculture extension in Uganda. More Ugandan men and women FO members, compared to Malawians, developed personalized business networks from the connections initiated by the FOs. In general, fewer farmers in both Uganda and Malawi accessed credit and participated in management training. We tested these observations to see if any significant differences exist between men and women and results are reported in Table 5.

In Uganda, men had significant access to the management training compared to women. We analysed this further to find out the level of participation in the management training among FO leaders and members and found that out of 39 leaders that obtained training in Uganda, 32 were men and only seven were women and the difference was statistically significant (p=0.0001). This represents 15% of the total leaders that benefited from training which is far below the 30 % mandatory representation, which is evidence that women benefited less. In Malawi, five men and zero women leaders had attended the training, and the difference was statistically significant (p=0.084). Focused discussions with leaders revealed that the design and delivery of the management training was marred by inequalities. First, few women sit on the executive thus only those few can be trained. Second, the training is residential in centralized locations meaning that most women would be constrained from attending by either spouses or cultural norms (Kaaria et al., 2016). Third, the topics (group leadership, financial management, book keeping) are technical requiring literacy skills that women may not have, and the mode of instruction requires participants to be able to read and write. We conclude that in both countries, training of leaders does not only favour men but also reinforces existing gender inequalities.

Similarly, the training of ordinary members benefits men more than women. In Uganda, out of 61 ordinary members that received extension training, 42 were men and 19 were women. In Malawi, training on good agronomic practices, quality management and record keeping favoured men. Significant differences also existed in developing business networks in Uganda and access to extension training in Malawi. Women's limitation to forge business networks point to restricted mobility (Pandolfelli et al., 2008) and socio-cultural norms that restrict women's interaction spaces (Kaaria et al., 2016). The limited extension training by Malawian women could be the effect of the extension delivery which follows a unitary household model where the household head is invited to attend the training or receive inputs, among other things.

In both countries, members used FOs in addition to other market outlets. On average, 48 and 49% of marketed bean was sold through FOs in Malawi and Uganda respectively, which reflects the extent of member satisfaction with FOs. Women were significantly satisfied with FO performance in both Uganda and Malawi and they marketed a significantly bigger proportion of their marketable products through the FOs in Malawi. On the other hand, men were significantly dissatisfied with FO performance in both Uganda and Malawi. This is evidenced by the fact that men sold significantly lower proportions of their marketed produce though FOs in Malawi. About 23% of Ugandan respondents mentioned that the dissatisfaction stemmed from delayed payments that discouraged some members from collective marketing. In some ACEs, this dissatisfaction had resulted in factions in which some primary cooperatives started own bulking initiatives disputing the principle of selling collectively at secondary cooperative level. FO members were asked to list the constraints they faced in bulking. In Malawi, 36% of respondents said they lacked alternative sources of income to deal with emergencies, 19% said they harvested small quantities and 10 % were constrained by FO quality requirements.

DISCUSSION

FO composition and governance

Results from the study reveal significant difference between men and women members of FOs in Uganda and Malawi. In both countries, the results suggest that men had opportunities that equip them as better negotiators than women. Men had stayed in the FO longer, had better education and benefited from training, which makes them better equipped with skills and knowledge that women lack. This provides an advantage to women: as men pull new information into the group consistent with findings in Katungi et al. (2008) that men participated in information exchange as suppliers of information while women were likely to participate as recipients of information. This implies that FOs are beneficial in helping women to overcome marketing challenges that often constrain them individually e.g. small volumes of production, limited mobility and business connections, and low negotiation power (Ampaire et al., 2013b; Gotchi et al., 2009; Katungi et al., 2008). As a matter of fact, women demonstrated better satisfaction with the performance of FO collective marketing initiatives than their male counterparts because according to them, FO offers better prices than they would have obtained if they handled marketing outside the FO.

However, possession of better education and experience by men means that they can easily dominate leadership positions and decision-making processes in the group to the disadvantage of women if no deliberate interventions are designed and implemented. In fact, most of FO were structurally gender-blind and they select leaders based on criteria that does do not sufficiently consider gender gaps. As a consequent, women were disproportionately underrepresented at higher positions of

Table 5. Men and women differences in access to FO generated benefits.

UGANDA	Male (167)		Female (78)		All (N=245)		
Type of benefit	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P-value**
Benefit extension training	0.79	0.41	0.71	0.46	0.76	0.43	0.135
Benefited from inputs	0.75	0.44	0.68	0.47	0.73	0.45	0.327
Benefited from credit	0.28	0.45	0.34	0.48	0.30	0.46	0.345
Benefit market information	0.71	0.46	0.61	0.49	0.67	0.47	0.123
Benefit from management training	0.46	0.50	0.34	0.48	0.42	0.49	**0.088**
Business networks	0.67	0.47	0.55	0.50	0.63	0.48	**0.086**
Quantity of beans sold	325.22	399.20	301.00	429.08	317.48	408.26	0.465
Quantity of beans sold through fos	132.43	216.96	132.26	262.27	132.37	231.86	0.529
Quantity of beans sold outside FO	192.79	353.51	168.74	355.09	185.10	353.46	0.494
Proportion of beans sold through FO	0.49	0.45	0.50	0.45	0.49	0.45	0.831
Satisfied with the FO performance	0.58	0.495	0.76	0.43	0.64	0.48	**0.008**
Dissatisfied with FO performance	0.35	0.48	0.19	0.40	0.30	0.46	**0.010**
MALAWI	**Male (136)**		**Female (111)**		**All (N=247)**		
Type of benefit	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P-value**
Benefit extension training	0.18	0.38	0.06	0.24	0.13	0.33	**0.007**
Credit as benefit	0.22	0.42	0.18	0.391	0.207	0.41	0.521
Benefited from inputs	0.85	0.36	0.76	0.43	0.81	0.40	0.137
Benefit market information	0.67	0.47	0.64	0.48	0.66	0.48	0.660
Benefit from management training	0.11	0.32	0.06	0.24	0.09	0.29	0.241
Business networks	0.11	0.32	0.15	0.36	0.13	0.34	0.478
Quantity of beans sold	191.52	189.70	219.50	226.54	203.59	206.15	0.408
Quantity of beans sold through FO	96.09	169.46	158.76	227.27	123.12	198.25	**0.053**
Quantity of beans sold outside FO	95.43	155.86	60.74	131.64	80.46	146.46	0.148
Proportion of beans sold through FO	0.41	0.48	0.57	0.49	0.48	0.49	**0.044**
Satisfied with FO performance	0.71	0.46	0.82	0.34	0.76	0.43	**0.038**
Dissatisfied with FO performance	0.18	0.39	0.07	0.26	0.13	0.34	**0.010**

The quantity of beans sold and all proportions indicated in both countries have been calculated from members that actually sold beans. In Uganda (men=166; women=78; total=244). In Malawi (men=87; women=66; total=153).

leadership. Coupled with low technical capacities, women participated less in extension advisory, planning and decision-making meetings. These observations suggest that women may not have sufficient space to voice all their concerns and articulate their priorities on the FO agenda. Thus, they are unlikely to influence changes that address the limitations they come with into the FOs.

The quota system for women representation in leadership

Results have indicated that FOs in both countries Have adopted the quota system, but as a blue print from the government. Whereas the quota system serves as an entry opportunity to uplift the status of women, it is not sufficient for addressing all gender inequalities in collective marketing initiatives. Hence, there is need for other guidelines to ensure active participation of the few women representatives. However, none of the sampled FO had such guidelines for gender considerations. In the short term, the sub-committees offer additional opportunities for expanding women representation in the leadership if qualifications can be adjusted to admit more women. In the long term, FOs need to develop structures that enable gender equity. For example, Manchón and Macleod (2010) find that the National Federation of Cooperatives in Nicaragua increased women representation on the board from one to three and instituted a women's commission responsible for decision making and co-ownership of land to be able to overcome gender disparities.

Distribution of benefits

Training in management, extension and business networks

Results revealed unequal distribution of access to training in management between men and women leaders of FOs. For example, in Uganda, more men than women trained in management and developed business networks than women while in Malawi, more men than women accessed extension training. The limited training in management and extension pushes the women plight further to the edge since they already have low education levels. Yet women's access to education, training and information has been found to be one of the key factors that influence women participation in producer organizations (Kaaria et al., 2016; Selhausen, 2016), and constitute important elements of empowerment (Meinzen-Dick et al., 2019). Thus, the inequalities do not only constrain women's participation but also impend their empowerment. This calls for changes in the extension delivery mechanisms to integrate gender responsive approaches such as the gender action and learning system approach.

Access to income from beans and credit

From FGDs we found that utilization of income generated from the sale of produce varied according to cultural values upheld by different communities. For example, in western Uganda, utilization of income from bean sales was jointly agreed in the household. However, in male dominant cultures of Eastern Uganda, income from beans was handed over by men to their wives for safe custody, but the latter had no authority to spend without the husband's permission - meaning that the husbands control the use of income. In both Uganda and Malawi, income generated by women from beans was mainly spent on household needs. In contrast, income generated by men was used for investments. With men controlling incomes, it implies that women may be shouldering the burden of feeding and attending to the details of household needs without income from beans. Based on findings of other studies (Blackden et al., 2006; IFPRI, 2005b), women's loss of control over income from beans also might lead to food and nutrition insecurity at household level since beans are such an important crop for nutrition security. Yet as Figure 1 indicates, few women have access to credit from FOs that they would use to bridge the gap. Further analysis indicated that only 24% of men and 20% of women in Malawi have just enough saving to meet their regular consumption needs. As some studies have indicated, lack of control over income, lack of access to credit and associated decision-making, signal to the fact that FOs in the study areas have not sufficiently contributed to empowering women to overcome the inequalities that constrain them from accessing markets (Sraboni et al., 2013).

CONCLUSION AND RECOMMENDATIONS

The paper sought to examine the distribution of benefits of members of FOs to find out whether men and women were equitably benefiting from being members of these organizations. Findings of the study have indicated that there are gender differences in the distribution of benefits; with men more likely than women to scoop higher benefits. The inequalities stem from the fact that men and women join the FO with differences in key factors such education, access to and control of production resources, which influence access to benefits. Despite these differences FOs do not build in mechanisms to mitigate barriers faced by some members to ensure equitable participation and access to opportunities by all members. The rules governing recruitment of members, selection of leaders and access to benefits treat members as a homogenous group. This results in women being underrepresented in the leadership, planning and decision-making processes. This means that increasing the number of women members in FOs neither guarantees them access to accruing benefits nor empowers them. Deliberate strategies need to be put in place to address contextual causes of gender inequalities. Based on the findings of the study, we recommend strategies that can redress the situation.

Implications of research for development delivery mechanisms

The study findings have demonstrated that FOs enable women to sell at better prices and access information via social network with men. However, there are still gaps to be closed. It is important that FOs increase women involvement in planning and decision-making processes and increase women access to skills training and extension service. The increased numbers of women in activities should be supplemented with guidelines that specify affirmative actions. FO constitutions can include statements that stipulate minimum numbers of women that attend extension and management training; and set a quorum that must be present before a meeting can take place or a decision can be made. Such guidelines can be developed by FO members with support from extension service providers and the apex organizations the FOs are affiliated to. However, service providers supporting these FOs should first create awareness and educate both men and women on the benefits of joint decision-making at both household and FO levels. At coordination level, umbrella organizations to which FOs are affiliated need to revisit the policies that guide formation and operation of FOs to make gender analysis and inclusion a priority in the FO business.

Increasing women's participation in FO leadership

FOs need to interpret the quota system as a minimum requirement but not a sufficient condition for women representation on leadership committees. Women representation should be increased beyond 30% on executive committees and should be extended to sub-committees, and their roles should be diversified beyond the treasury. There is also need for targeted capacity building to enhance women skills in management, decision making, networking and negotiation. In addition, FOs should modify requirements for women leaders from emphasizing literacy skills to include other capacities such as ability to speak in public. Writing skills should be mandatory only for secretaries and those responsible for record keeping but not others.

The need to improve women's access to credit

The findings made clear the important need for women to access credit. In some communities in Malawi, there were village saving schemes and FO members were encouraged to join. In Uganda, there are SACCOs affiliated to each ACE plus other merry-go-rounds.

However, women save small amounts, yet the amount of loans requested is often based on amounts available in saving. The small amounts accessible by women cannot be used for meaningful investment in agriculture due to competing demands such as children school fees, medical services and other domestic needs. FOs need to engage with private sector financial institutions to demand financial packages that are suitable for the different categories of members. Some ACEs in Uganda have been successful in accessing such loans from microfinance institutions as associations and these loans are distributed to members based on ability and size of business. Deductions for loan recovery are made by FO leaders every after sale and farmers receive balances. If gender responsive financial packages are negotiated and integrated in such initiatives, they can benefit women.

Implementing gender responsive extension delivery mechanisms

The current extension delivery mechanisms are largely gender blind. Gender is often understood as the numbers of men and women participating in an activity. There is need to move beyond representation to critically analyse and address issues that constrain women, men and youths from actively engaging in FOs. In Malawi, there is need to change extension service delivery from the unitary household model to the collective bargaining model to take care of competing interests and preferences in a household. Such shifts demand that extension service providers have gender analysis skills and technical capacity to educate the farmers and FO leaders about the need for gender responsiveness in day-to-day activities. There is apparent need for service providers and FOs to strengthen gender sensitization and community education about the importance of equitable involvement of men and women. An example that education of farmers is important is the Tikorane Project in Chiseu community, Kasungu district. This community went through the Enabling Rural Innovations (ERI) training in 2003/2004 (See a detailed description of the ERI approach in Kaaria et al. (2007). This community had deeply entrenched cultural barriers that kept women in the background e.g. they were never allowed to speak in public, were never believed to be leaders, and would not question husbands on anything including marketing produce and how the income was spent. After the ERI exposure, farmers responded by forming a bean seed producing association that is led by 50 % men and 50 % women representation and is guided by other gender responsive rules. Women are treated as equal decision makers both at the FO and household levels and are given extra time to voice their concerns in association meetings. At the time of data collection for this study, men still held the responsibility of marketing the bean seed, but women were very engaged and committed throughout the process. The women FGD revealed that the decision to sell at household level is joint, they receive the payment in the absence of their husbands, and money accruing from sales is spent as agreed by spouses. This has kept the women very motivated to invest energies in the marketable enterprise; they were involved in securing planting seed, collective spraying of member fields, calculating production costs, setting selling prices, and preparing produce for the market when buyers give notification. This example shows that institutionalizing gender norms in mixed FOs works for both men and women even amidst deeply rooted gender biased socio-cultural norms.

Limitations of the study and future research

The study focuses on analyzing benefits that accrue to members but does not consider other contextual factors that influence how farmers organize. Further research should analyze the contexts to come up with new innovations that can enrich gender inclusion in FOs. In addition, the study was based on bivariate analysis and was unable to establish casual relationships between the contextual factors with access to benefits, which is important to inform selection and design of interventions.

Although the lessons from this study are important in terms of informing research for development initiatives, the sampled FOs are not necessarily a representative sample of all FOs in the study countries. Since a variety of development agencies have undertaken supporting FOs, a more representative picture could be obtained by looking at a wide range of FOs across a diversity of enterprises. Innovations about what works for gender inclusion and women empowerment within the confines of FOs can better be reached by analyzing the different strategies that have been employed by different FOs and what worked well in which contexts. Future studies could focus on that.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Response of wild smooth-head catfish (*Clarias liocephalus*) fingerlings reared in earthen ponds

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***Clarias liocephalus*, an important small fish in the diet of rural households, is threatened by wetland degradation and overfishing for use as live bait. This study aimed at establishing the survival, condition, growth rate and feed utilization indices of *C. liocephalus* wild fingerlings reared outside the wetland environment through a feeding experiment. Fingerlings were fed with an isocaloric feed with four levels of crude protein for eleven weeks. Results showed that *C. liocephalus* could endure wide ranges of water temperatures, low levels of dissolved oxygen and could efficiently utilize artificial feeds. The 35% crude protein diet was the best utilized with a feed conversion ratio of 4.18. The mean specific growth rate was 2.2 to 2.5%, which is comparable to that of other reared Clariidae. Fish condition was best with the 30 and 35% diets and mean survival was 46.44% (±3.159SE) and not significantly different (p<0.05), for the four diets. This new information is useful as reference in recommending the species for aquaculture. Rearing *C. liocephalus* could also reduce rural malnutrition and fish-protein deficiency especially in rural poor communities. Rearing trials for longer periods and measurement of other key production indices required in aquaculture of *C. liocephalus* were recommended.**

Key words: Wetland habitats, micronutrients, hapas, fish feeds.

INTRODUCTION

Today, national development plans are focusing on ensuring economic and food security for the ever increasing human population, projected to hit 8.6 billion by 2030, alongside strategies to manage the apparent degradation of terrestrial and aquatic ecosystem resources (Bierbaum and Cowie, 2018). Fisheries and aquaculture constitute a substantial sector in agricultural development for economic and nutritional purposes and its contribution in the alleviation of nutritional and economic insecurity is upheld from global to local perspectives (FAO, IFAD, UNICEF, WFP, WHO, 2019). Fish provide an essential protein-rich component to the

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human diet especially in communities with predominantly carbohydrate-based diets common in developing countries (Thilsted, 2012). As a rich source of multiple micronutrients including zinc and iron, fish have the capacity of meeting the micronutrient deficiencies predominant among the vulnerable groups in sub-Saharan Africa (Phillips et al., 2015).

As captured fisheries become unsustainable due to over-fishing, habitat loss and aquatic environmental pollution, aquaculture is increasingly being considered as the alternative to the development and improvement of fisheries resources and revitalization of the ecosystems. Investment in environmentally sustainable aquaculture production presents opportunities for food and economic securities particularly for developing countries (World Bank, 2013).

Uganda's per capita fish consumption reported to be about 6 kg person^{-1} year^{-1} (MAAIF, 2004; NARO, 2017), is still well below the WHO-FAO recommended level of 17.5 kg person^{-1} year^{-1}. The slight increase, despite the greatly reduced wild fish stocks, could partly be attributed to the growing aquaculture industry, now at about 100,000 tonnes anum^{-1} (NARO, 2017). However, the high price tag on fish in Uganda today dictates that only the economically stable communities can access it. According to Uganda's Demographic Health Survey (UDSH) report of 2016, the indicators of malnourishment that include stunting and iron deficiency are specifically high among the rural poor communities (Uganda Demographic and Health Survey [UDSH], 2017). It is worth noting that the human population in Uganda is now at >39 million and 76% of this population lives in rural areas as indicated in the 2018 statistical abstract (Uganda Bureau of Statistics [UBOS], 2018). It is possible that a large proportion of the Ugandan rural population could be nutritionally deficient in essential proteins and micronutrients partly provided through adequate fish consumption.

Evaluating the potential of some locally available small fish like *Clarias liocephalus*, which may not be as expensive to rear but nutritionally competent, could make a significant contribution towards addressing the problem of fish-food deficiency and indirectly reduce malnutrition and poverty in rural poor households. Since most of the local small fish species are not domesticated (Kumar, 2010) and their natural habitats, especially wetlands, are threatened (Chapman et al., 2001; Ministry of Water and Environment, 2018), it is increasingly becoming necessary to promote the rearing of such fish, at least from the perspective of conservation. The motivation behind this study was to test whether the Clariidae catfish *C. liocephalus,* a small wild wetland fish species, could survive and grow outside its natural environment thriving on an artificial diet. The study hypothesized that *C. liocephalus* could grow and survive under culture conditions and that it could offer parallel nutritional attributes like other established cultured fishes especially the commonly cultured species of tilapias and *Clarias gariepinus*. The choice for *C. liocephalus* was based on the fact that it is highly acceptable in the diet of local people in the study area; seems to tolerate a wide range of environmental conditions and that its survival in the wild is threatened by habitat loss and the unregulated live-bait market (Ajangale, 2007; Yatuha et al., 2012). *C. liocephalus* exhibits some basic qualities required in a potential aquaculture fish species like being a generalist feeder and possessing an air-breathing accessory organ to boost survival in environments with low dissolved oxygen levels (Yatuha, 2015); however, its production indices which are important for fish farmers, are not yet adequately defined and its response outside its natural wetland environment is poorly documented. A feeding experiment was mounted to define the diet at which *C. liocephalus* would attain maximum growth response and nutrient utilization. The study specifically evaluated the effect of dietary crude protein level on growth performance, feed utilization, survival and general condition of wild *C. liocephalus* fingerlings kept under pond conditions for eleven weeks. Determining the minimum feed needed to meet the species protein requirements and achieve maximum growth is important because there is an economic advantage in identifying and feeding fish at an optimal rate, that is, at the lowest feed conversion ratio (FCR) and highest specific growth rate (SGR) point (De Silva and Anderson, 1995; Kim and Lee, 2009). Knowledge of the growth rate and proximate body composition of a species for a given diet level provides a useful guideline in selecting a diet that is both nutritionally adequate and most affordable. Principal factors that determine fish growth and body composition are important when considering the role of fish as a source of nutrition (Ahmed et al., 2010).

MATERIALS AND METHODS

About 2500 *C. liocephalus* fingerlings of mean wet weight of 2.83 g (±0.003SE) were fished from Kigambira wetland, found in Lake Mburo National Park Uganda, using local basket traps. The wetland is a constituent of the Rwizi-Rufuha wetland system that drains the southwestern part of the Lake Victoria basin. The fingerlings were allowed to acclimate in a collection tank for seven days and were fed on a 30% crude protein (CP) general juvenile *Clarias* diet (Jauncey et al., 2007).

An isocaloric complete commercial floating diet with 4 levels of crude protein (CP) graded as low protein (25% CP), medium protein (control: 30% CP), high protein (35% CP) and very high protein (44% CP) was sourced and its proximate composition established (Table 1) before they were used for the experimental feeding. Each of the test diets was replicated four times giving a total of 16 units. The feed pellet size for each of the four diets was graded to 3±0.5 mm, which is 30% of the average gape size of *C. liocephalus* fingerlings (Yatuha, 2015).

The experimental fish were reared in a semi-intensive setting in an earthen fish pond which was selected and prepared from a set of other fish ponds at Mbarara Zonal Agricultural Research and Development Institute (MBAZARDI) near Mbarara University of Science and Technology in Mbarara municipality, Southwestern

Table 1. Proximate composition (%) of the test diets used in *C. liocephalus* feeding experiment.

Diet	Diet code	Dry matter (g)	Ash (g)	Crude fat (g)	Crude fibre (g)	Crude protein (g)
25% CP	1	27.78±0.026SE	6.20± 0.039SE	8.79± 0.013SE	3.44± 0.008SE	24.99± 0.024SE
30% CP	2	33.38±0.005SE	6.93± 0.060SE	14.69± 0.102SE	3.38± 0.024SE	30.20± 0.017SE
35% CP	3	38.15±0.044SE	7.35± 0.066SE	20.56± 0.186SE	3.15± 0.006SE	34.62± 0.040SE
44% CP	4	47.82±0.112SE	6.33± 0.083SE	28.87±0.023SE	3.64± 0.086SE	43.71± 0.021SE

Figure 1. Set up of experimental units in the pond for the feeding experiment of *C. liocephalus*.

Uganda. Mbarara municipality is at altitude 1,432 masl; 0.6167° S, 30.6568° E; average annual temperature 25°C and rainfall of 1125 mm. The pond was fitted with 16 nylon hapas of size 1.73 m^2 (1.2 m × 1.2 m × 1.2 m) and a mesh size of 1 mm. The hapas were arranged at a depth of 1.3 m in a line at a distance of 1.5 m between any two hapas (Figure 1).

At the start of the experiment, after the fish had not been fed for 24 h to enable them to empty their stomachs, one thousand six hundred (1 600) fish with an average weight of 2.83 g (±0.003SE) body weight were selected, visually identified, individually weighed and measured for total length and randomly assigned into the 16 hapas (experimental units) at a stocking rate of 100 fingerlings per hapa. The four diets in their four replicates were randomly assigned to the 16 stocked experimental hapas. A general commercial *Clarias* feed formula (30% CP) was used as a control having been a standard catfish diet for a long time (Legendre, 1986; Robinson et al., 2001). Since there were no data on the nutrient requirements of juvenile *C. liocephalus*, it was acceptable to adopt from closely related species (Kaushik, 2000).

The experimental fish were fed at 5% body weight, regarded as apparent satiation (Jauncey, 1982) two times a day for 11 weeks and records of daily feed intake were kept. The duration of the feeding experiment was deemed adequate to get the required results since results of growth performance in *Clarias* fingerling feeding experiments have been realized from experiments of six weeks (Solomon and Okomoda, 2012) or eight weeks (Odulate et al., 2014; Adebisi and Ologhoba, 1998). About 10% of the pond water was exchanged for fresh water once every three days, from a common reservoir that supplied the other ponds on the fish farm. To prevent clogging by algae and left over feeds, the hapas were washed weekly during the time of weekly measurement of the fish.

Data collection and analysis

Three water quality parameters were recorded in this study. The general pond water temperature was measured daily between 6:00 and 7:00 am in the morning and between 12:00 and 1:00 pm in the afternoon. The dissolved oxygen (DO) and pH of the pond water were measured twice a week between 10:00 and 12:00 h. A digital thermometer, a pH meter (pH meter model HANNA HI98129) and DO meter (Oxy meter model YSI 550A) were used for measuring the said water quality parameters.

At weekly intervals, all the fish in each hapa were removed and counted to establish the survival. Fifty percent (50%) of the fish were randomly sampled and individually weighed and measured for total length.

The mean weight gain (WG) was established by subtracting the mean initial weight (g) from the final mean weight.

WG = Final mean weight - Initial mean weight and the percent weight gain (WG %) = [Final mean weight (g) - Initial mean body weight g) / Initial body weight] × 100 (1)

The Feed Conversion Ratio (FCR) was expressed as the proportion of dry feed fed per unit live weight gain of fish calculated as:

FCR = Dry weight of feed (g) / Wet weight gain by fish (g) (2)

The Specific Growth Rate (SGR), that is, the weight gained by fish per day was calculated as:

$$SRG = [Ln (W_2) - Ln (W_1) \times 100] / T_2 - T_1 \quad (3)$$

where W_2 = Weight of fish at time T_2, W_1 = Weight of fish at time T_1, Ln = Natural log.

Survival (%) was calculated as follows:

Survival = (Initial number at start of experiment - Number at end of experiment) × 100 (4)

Condition factor: Fulton's Condition Factor (Froese, 2006) was calculated thus:

$$K = (100W) / L3 \quad (5)$$

where K=Condition factor, L=Standard length (cm) and W=Wet weight (g).

Data were analyzed using SPSS Inc.17 (IBM Corp. Chicago, USA) and Minitab Inc. 14 statistical software. Relationships in the datasets were subjected to correlation-regression analyses and variations to ANOVA followed by Tukey HSD test (or its non-parametric Kruskal Wallis Test) at a significance threshold of 0.05.

RESULTS

The overall mean dissolved oxygen of water in the experimental pond was 1.39 mg l^{-1}, indicating a general poor oxygen circulation in the experimental units. Mean pH was 6.65, well within the range of freshwater fishes and the mean morning and afternoon temperatures were

Table 2. Mean dissolved oxygen (mg/L) pH and temperature (°C) in the experimental pond water through the experimental period.

Week	Mean DO Mg/L	pH	Temperature (mean and SE)	
			Morning	Afternoon
1	2.17±0.58	6.11±0.28	19.72±0.456	27.59±0.525
2	1.35±0.22	6.32±0.71	20.81±0.998	28.22±0.872
3	1.27±0.24	6.51±0.19	19.33±0.235	27.33±1.269
4	0.77±0.17	6.81±0.06	21.76±0.495	29.38±0.416
5	1.59±0.12	7.03±0.11	20.53±0.461	28.69±0.779
6	1.69±0.50	6.65±0.35	18.96±0.460	26.55±0.599
7	1.05±0.12	6.8±0.00	19.52±0.367	26.72±0.330
8	1.09±0.15	6.77±0.75	19.81±0.481	27.12±0.355
9	1.19±0.08	6.72±0.02	18.22±0.236	27.38±0.505
10	1.40±0.10	7.07±0.00	17.35±0.109	27.75±0.512
11	1.5±0.24	7.14±0.14	18.57±0.370	28.30±0.336

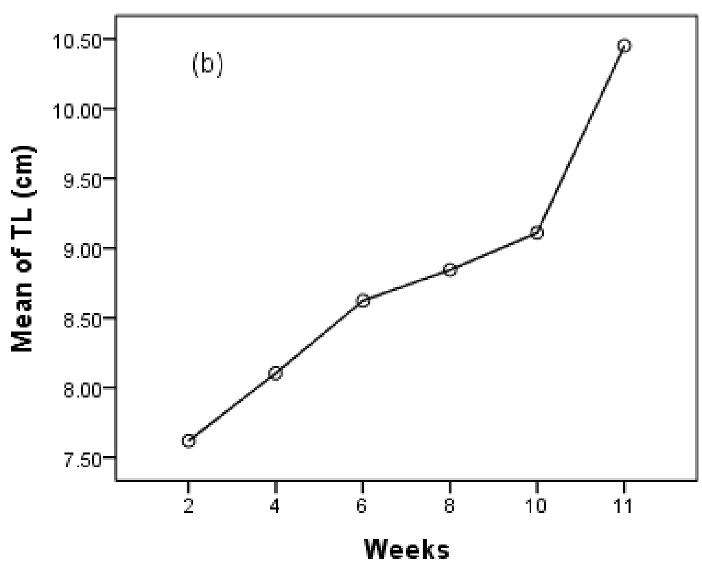

Figure 2. Fish body weight increment (a) and total length increment (b) in *C. liocephalus* experimental fish over the 11 weeks period of experimental feeding.

19.36°C ±0.153SE and 27.53°C±0.162SE, respectively. The results for DO, pH and temperature over the experimental period are summarized in Table 2.

The overall growth response of *C. liocephalus* fingerlings in terms of mean weight gain and length increment showed a positive trend especially after the 5th week of the feeding experiment (Figure 2). While there was no significant difference in weight and length between treatment groups at the start of the feeding experiment (Kruskal Wallis Test P=0.122, n=1600); the difference became significant (P<0.01) by the end of the experiment (Kruskal Wallis Test P=0.001 and 0.000 for weight and TL, respectively). Fish fed on the diet of 30% CP registered the highest weight gain and had the heaviest individual fish by the end of the experiment (20.7 g) while that of 25% CP had the lowest weight gain and had the lightest individual fish that weighed only 2.2 g at the end of the experiment (Table 3).

The mean feed conversion ratio (FCR) of the fish fed on the four test diets over the experimental period was 5.01, 4.18, 4.53 and 4.85, respectively (Table 3). It was noted that FCR values were high in the first weeks of the experiment and steadily improved with time (Figure 3). There was a significant difference in FCR between the first and last weeks of the experiment period (FCR 23.7, p<0.001). Specific growth rate was the highest in the 35%

Table 3. Growth response parameters of *C. liocephalus* fed on four different diets over 11 experimental weeks (±SE).

Parameter	Treatment			
	D1 (25% CP)	D2 (30% CP)	D3 (35% CP)	D4 (44%CP)
Initial mean weight (g)	3.14±0.06	3.09±0.07	2.98±0.06	3.21±0.06
Final mean weight (g)	8.52±0.36	9.40±0.31	9.71±0.27	8.49±0.25
Final minimum weight (g)	2.2	2.2	3.7	2.8
Final maximum weight (g)	14.6	20.7	19.7	17.0
Initial mean TL (cm)	7.58±0.05	7.59±0.05	7.45±0.06	7.56±0.06
Final mean TL (cm)	10.14±0.16	10.30±0.12	10.36±0.10	10.31±0.10
Initial mean Feed Conversion ratio (fcr)	5.74±0.05	6.77±0.21	7.46±0.22	7.38±0.20
Final Mean Feed Conversion ratio (fcr)	3.32±0.10	3.18±0.06	2..98±0.04	3.48±0.05
Condition (K)	1.04±.0053[a]	1.06±.0047[b]	1.05±.0052[b]	1.01±.0040[a]
Specific growth rate (SGR)	2.10	3.03	3.10	2.81
Survival (%)	43	46	49	47
Total feed fed (g)	312	451	474	457

*Mean values in the same row with different superscript are significantly different (ANOVA, P<0.05).

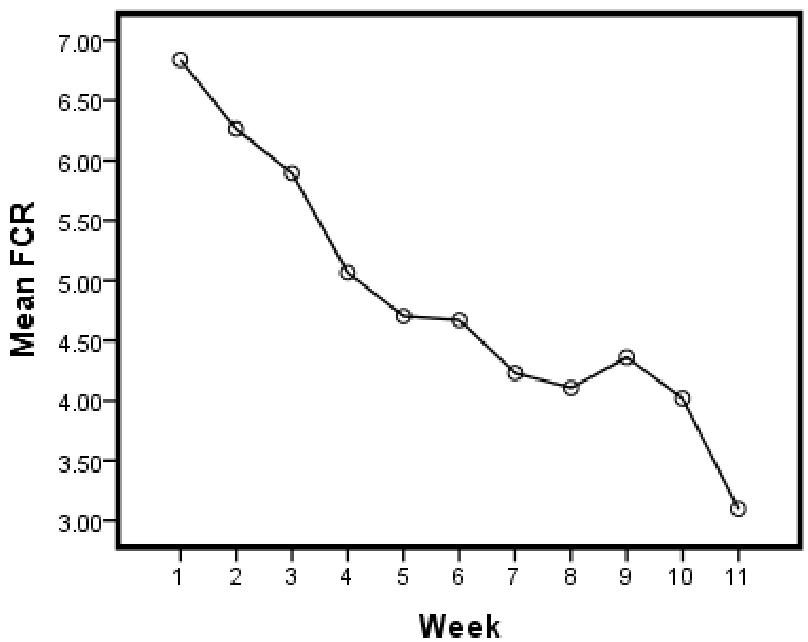

Figure 3. FCR trends of *C. liocephalus* fingerlings fed on 4 test diets over 11 weeks.

diet and the lowest in the 25% CP diet (Table 3).

Percent survival was 46.44% ±3.159SE for all the four treatment diets. The individual means for diets 1 to 4 were 43, 46, 49 and 47%, respectively. Percent mortality was the highest in the first four weeks, but dropped to almost 0% from week 5 to the end of the experiment (Figure 4).

The condition of fish fed on Diet 1 (25% CP) and 4 (40% CP) was poor and significantly different from fish fed on Diets 2 (30% CP) and 3 (35% CP).

DISCUSSION

This study intended to establish whether *C. liocephalus*, a wild wetland small catfish, could survive in an artificial environment, utilize an artificial diet to grow in weight and length, maintain good condition and adequately convert the feed into fish flesh. The over 40% overall survival is a clear indication that *C. liocephalus* can survive in an artificial environment and on artificial diet and the fact that some of the experimental fish greatly increased in body

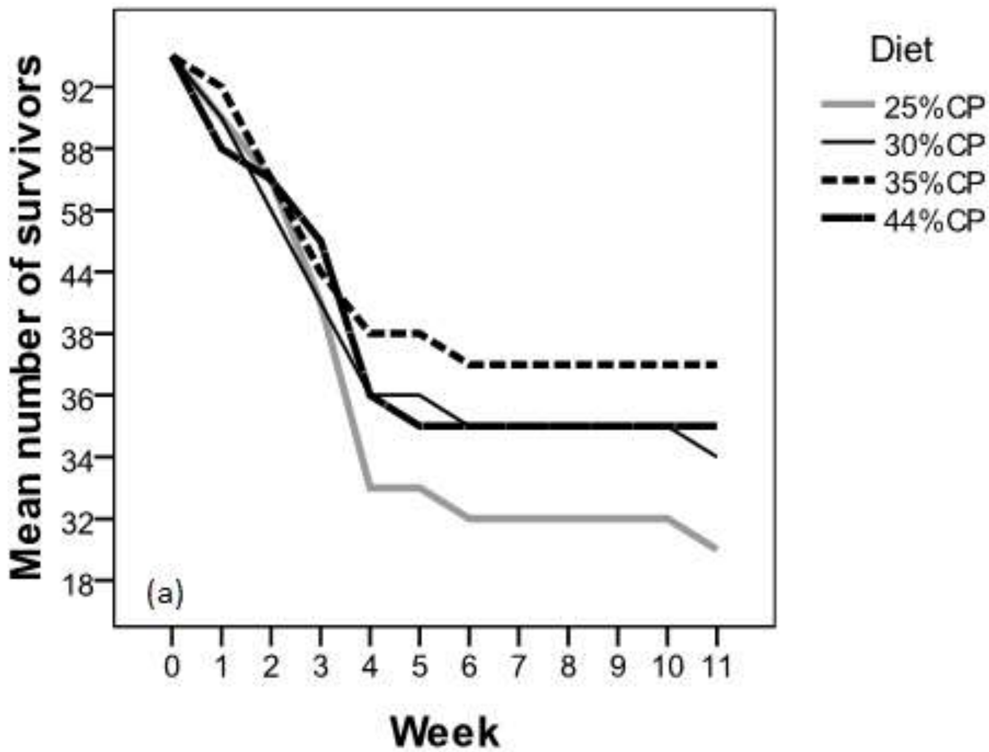

Figure 4. Survival trends of *C. liocephalus* fingerlings fed on 4 test diets over 11 weeks

mass (from 5.5 g maximum at beginning to 20.7 g maximum at end of experiment), is a positive indicator that *C. liocephalus* could attain table size in a relatively short period. However, there was size disparity within the stocked fish in all the treatments although similar sized fish were stocked at the beginning of the feeding experiment. Size disparity is a common phenomenon in catfish stocks during the initial stages of development (De Graaf and Janssen, 1996). Since size-grading was not done during the course of the experiment, size disparity could be partly explained by the nature of the fish species. Persistence of stunted individuals throughout the study period could be attributed to poor seed quality because the stocked juveniles were randomly picked from the wild. Besides feed quality, seed quality is known to be a very important factor in fish production (De Graaf and Janssen, 1996; Pouomogne, 2008). Stunted growth could also be attributed to starvation because of competition with larger individuals. Since feeding was done only twice a day, it is possible that less competitive individuals could have starved. It has been reported that when frequently fed, fish yielded more because dominant individuals become less aggressive in such circumstances (Aderolu et al., 2010). Feeding three times a day is known to be the most efficient frequency for effective growth and nutrient utilization for juveniles and fingerlings of *C. gariepinus* (Aderolu et al., 2010).

The feed conversion ratio (FCR) for the 4 treatments was generally higher than expected given the typical 1.4 to 2.5 recorded in catfish production experiments (Robinson et al., 2001). However, a consistent reduction in FCR from high figures in the first weeks and low figures in the last weeks was noted. This is an indication that as the fish grew and became used to the feed, utilization improved. If the experiment was to run for more time (up to normal harvest time), the results suggest that the FCR would have dropped to acceptable levels of two and below. The FCR could also have been influenced by the low DO levels since the water in the experimental pond was not on a flow through design and therefore poorly aerated. The oxygen levels were below the required minimum most of the time during the experiment (overall mean of 1.39 mg/l), and this could have compromised the quality of the water. Low DO levels have significant effects on fish growth as well as food conversion (Chang and Ouyang, 1988). In poorly aerated waters, catfish spend a lot of energy to obtain atmospheric oxygen and this stresses the fish and lowers its appetite and feed utilization (De Graaff and Janssen, 1996). Lack of flow-through water system and short duration of experiment have been implicated for poor values of FCR in other fish feeding experiments (Mwangamilo and Jiddawi, 2003). The SGR of 2.2 to 2.5%, is comparable to that reported in other Clariidae reared within an almost similar experiment duration (Akinwande et al., 2009; Solomon and Taruwa, 2011).

However, SGR in *C. liocephalus* was low compared to *C. gariepinus* (5.7%) fed on a diet of maggots (Otubusin and Ifili, 2000). The difference could lie in the source of feed, duration of the experiment, seed quality and other conditions in the experimental environment.

In the first four weeks of the experiment, mortality was the highest and dropped to almost 0% in the last weeks. The high mortalities could be attributed to handling stress as well as high stocking density. One hundred fingerlings in a 1 m^3 hapa in a non-recirculatory water system could have been stressful and a likely cause of mortality and high survival after the stock fell by almost 50%. High mortalities in the first weeks of feeding experiments have been reported in other catfish (Akinwande et al., 2009).

The findings of this study indicate that *C. liocephalus* has a number of attributes that could make it a suitable choice for subsistence aquaculture in rural settings. Subsistence aquaculture has the potential to contribute to most of the relevant sustainable development goals (SDGs). This is due to the family level of operations, where work is well distributed, meaningful and empowering. While there is no direct impact on poverty, it does provide a regular supply of high quality protein, sparing income for other food and living expenses. It is also environmentally efficient, especially when integrated into other farming activities. It can make households and communities more resilient to economic or environmental shocks.

CONCLUSIONS AND RECOMMENDATIONS

From the findings of this study, we conclude that *C. liocephalus* can grow and survive outside its natural wetland environment. The species can endure low levels of DO and a wide range of temperature variations and register a specific growth rate that is comparable to that reported in other reared Clariidae. The species exhibited a key aquaculture advantage in its ability to survive both in hypoxic and hyperoxic water environments that are typical of fish pond waters in a tropical rural setting. Unlike some obligate air-breathers like *Protopterus* species, which die if deprived of atmospheric oxygen, *C. liocephalus* can survive under stress of both atmospheric and dissolved oxygen. The survival of *C. liocephalus* in turbid water ponds at low dissolved oxygen levels is a positive indicator that the species has some desirable qualities for survival in the prospects of climate change, where predicted droughts will likely cause deterioration of water quality. That *C. liocephalus* can withstand a wide range of water quality parameters is an opportune coping strategy to provide nutrition and revenue to rural communities in the face of global demand for aquatic food. Since the species has the required traits to be raised in an artificial environment, further rearing trials for longer periods and measurement of the rest of the production indices required in aquaculture fish species are recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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