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Full Length Research Paper

Quality of Soil in the function of biological fertilization and plant covering

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The yield of soybean was influenced by the chemical and microbiological properties of the soil, which was favored by management techniques that promote improvement in soil quality. The objective of this study was to evaluate soybean yield, chemical and microbiological quality of the soil as a function of biological fertilization and soil cover conditions. The experiment was conducted in both crops seasons 2015/16 and 2016/17 with experimental design in randomized blocks, with the agronomic characteristics of the soybean analyzed as a function of the factors of biological fertilization and soil cover conditions, as well as the chemical and microbiological indicators of the soil, besides the two factors mentioned earlier, fragment of forest. It was observed higher height of plants, pods with three grains, leaf area and yield of soybean cultivated on *Crotalaria ochroleuca*, followed by *Pennisetum glaucum*. Biological fertilizer, *P. glaucum* and *C. ochroleuca* promoted increases in the fertility of soil cultivated with soybean, mainly potassium, and contributed to reduce carbon losses, indicated by lower basal respiration and soil metabolic quotient. Based on the results, we conclude that the soybean presented highest grain yield with *C. ochroleuca*, and the biological fertilization and cover plants increased the chemical and microbiological quality of the soil. These techniques can be implemented in the soybean farming system.

Key words: Fertility of soil, microbiological quality, *Glycine max*, yield, management.

INTRODUCTION

The soybean (*Glycine max* (L.) Merrill) was one of the most economically important crops of Brazilian agriculture, on a world scale, Brazil is the second largest producer of this oilseed, with production and average yield in the 2016/2017 crop season of 113,930.2 Mg and of 3,362.0 kg ha^{-1}, respectively (Companhia Nacional de Abastecimento, 2017). In order to meet the productive demands of the national and international market, it was necessary to adopt techniques that contribute to increase the quality of the soil, and that allow adequate development of the crop, since, correct managements increased the levels of soil fertility, consequently,

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obtaining higher production (Sousa and Lobato, 2004).

Inadequate soil using produces negative effects on its chemical and microbiological properties and may lead to imbalances in pedogenetic formation processes, intensifying losses of soil, organic matter, organic carbon and soil nutrients (Ebeling et al., 2011; Cunha et al., 2012). The microorganisms are directly linked to the process of organic matter decomposition, nutrient cycling and energy flow. The microbial biomass of the soil was constituted basically by fungi and bacteria, and were considered very sensitive the changes caused in the environment (Trannin et al., 2007; Fidelis et al., 2016; Goenster et al., 2017).

Changes in the dynamics of organic carbon, degradation of organic matter, changes in fertility, among others, were monitored from soil quality indicators. Among the most used microbiological properties were basal respiration, microbial biomass carbon, soil metabolic and microbial quotient (Jenkinson and Powlson, 1976; Vance et al., 1987; Anderson and Domsch, 1993; Matias et al., 2009). For the chemical properties, were applied some indicators that represent macro and micronutrients, pH, hydrogen and aluminum (Ebeling et al., 2011; Cunha et al., 2012).

As a practice of agricultural management, the introduction of plant covering increased the production of plant residues, and thus contribute to the increase of microbial activity, fixation and cycling of nutrients in the soil. Among the most used cover crops in the farming system, we mention millet (*Pennisetum* sp.) and Crotalaria (*Crotalaria* sp.), both with high potential of dry mass production and nutrient cycling (Pacheco et al., 2011; Cunha et al., 2012; Rossetti et al., 2012; Cherubin et al., 2015).

With the decomposition process, the minerals fixed in the plant residues were released into the soil and/or fixed by the microorganisms, which after the dead minerals were mineralized again, contributing to the aggregation of the particles and increase soil fertility (Carneiro et al., 2008; Gomide et al., 2011; Vezzani and Mielniczuk, 2011).

The microorganisms benefited from the presence of diversified organic residues in the soil, due to the utilization of the substrate for increased microbial biomass. Studies has indicated a reduction of the biodiversity of the ecological relations in the soil. Thus, biological fertilizer contributed to the multiplication and development of microbial populations, since it was composed of water, organic manure, organic compound enriched with macro and micronutrients, enzymes and vitamins (Medeiros and Lopes, 2006; Muñoz et al., 2017). In the search for sustainability in farming systems, the application of biological fertilizer and cover crops, minimize the impacts caused by farming practices on the chemical and microbiological properties of the soil, having a better vegetative development and increases in crop yield (Saeed et al., 2015).

Therefore, the objective of this study was to evaluate the soybean yield, the chemical and microbiological quality of the soil as a function of biological fertilization and soil cover conditions.

MATERIALS AND METHODS

Study site and sampling design

The experiment was conducted for two years - crops seasons 2015/2016 and 2016/2017, in the experimental field of the State University of Mato Grosso, Tangara da Serra - MT, at the geographic coordinates 14°39'53"S and 57°25'46"W. A forest fragment located geographically at 14°39'07"S and 57°25'21"W was considered as control area. The soil of both sites was classified as Dystrophic RED LATOSOL (Oxisol) (Embrapa, 2013).

For the chemical and microbiological characteristics of the soil, a randomized complete block design was used in a double factorial with additional control (2 x 3 + 1), with two conditions of biological fertilizer (with and without), three soil cover conditions (millet (*Pennisetum glaucum* LR Br.); crotalaria (*Crotalaria ochroleuca* G. Don.); clean fallow) and a control in the forest fragment with four replications. The biological fertilizer and soil cover for the vegetative and productive characteristics of the soybean crop was considered. The plots were constituted from five meters long by five meters wide.

Before the implantation of this experiment, the field was cultivated with *Gossypium hirsutum* for five consecutive years in the conventional farming (two soil plows). Chemical analysis of the soil in the layer from 0 to 0.20 m depth in the month of July of 2015, with the results (Table 1) was applied to 1,300 kg ha^{-1} of dolomitic limestone for soil correction (Sousa and Lobato, 2004). Monitoring of climatic data during the development of the study in an automatic meteorological station (Figure 1) was carried.

The sowing of millet (Cv. ADR 500 - 25 kg ha^{-1}) and crotalaria (Cv. Common - 15 kg ha^{-1}) was carried out on October 3 of the years 2015 and 2016, and incorporated with leveling grid closed, being dried at 50 and 65 days after sowing of millet and crotalaria, respectively (Pacheco et al., 2013).

The seeding in straw of the soybean was performed on December 20, 2015 and 2016, using cultivar 98Y30, with spacing between 0.5m lines and density of 14 plants per meter. As the useful area of the plot, the four central lines were considered, disregarding 0.5 m at each end as edge effect. In both crop season, the fertilization was performed with 280 kg ha^{-1} of P_2O_5 and 50 kg ha^{-1} of K_2O. At 30 and 50 days after sowing (DAS) was applied 50 + 50 kg ha^{-1} of K_2O, respectively. The sources of nutrients were Mono-ammonium phosphate (52% P_2O_5 + 9% N) and Potassium Chloride (60% K_2O).

Leaf fertilization with micronutrients was performed 30 DAS (Sousa and Lobato, 2004). The biological fertilizer was prepared in 100 L drum in the proportion of 20 L of water, 4 L of bovine manure and 1 kg of biological compound enriched with minerals, 30 days before its application. The mineral characteristics of the organic fertilizer was presented in Table 1, with microorganisms responsible for aerobic and anaerobic fermentation, such as bacteria and fungi. The biological fertilizer was filtered and applied 24 h after sowing the soybean, in the dose of 150 l ha^{-1} (Medeiros and Lopes, 2006).

Agronomic characteristics of soybean

In relation to the vegetative characteristics of soybean, was considered the leaf area measured at the phenological stage R_1 (Adami et al., 2008). The plant height, height of insertion of the first

Table 1. Result of soil chemical analysis of the layer 0 to 0.20 m performed in July 2015, and mineral quality of biological fertilizer.

					Soil chemical quality						
pH	**P**	**K**	**Ca**	**Mg**	**Al**	**H**	**Al + H**	**T**	**V**	**OM**	
$CaCl_2$	**mg dm^{-3}**		-------------------------------		**cmol$_c$ dm^{-3}**	---------------------			**%**	**g dm^{-3}**	
5.52	1.03	0.20	2.17	1.37	0.00	4.33	4.33	8.07	46.34	32.67	
Ca	Mg	K	Zn	Cu	Fe	Mn	B	S	Clay	Sand	Silt
------------- % -----------				-------------------------	**mg dm^{-3}**	--------------------			------------	**g kg^{-1}**	-------
26.93	17.10	2.47	1.33	3.53	43.67	30.60	0.28	13.13	544	86	370
					Mineral quality of biological fertilizer						
pH	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Zn**	**Cu**	**Fe**	**B**	**OM**
H_2O		-----------------------	**g L^{-1}**	------------------		----------------------	**mg L^{-1}**	------------------			**%**
6.2	0.71	0.3	0.02	0.06	0.01	0.04	4.7	2.8	175	14.4	5.2

Potential hydrogen (pH), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), hydrogen (H), cation exchange capacity (T), base saturation (V), organic matter (OM), calcium saturation (% Ca), magnesium saturation (% Mg), potassium saturation (% K), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), boron (B), sulfur (S) and nitrogen (N).

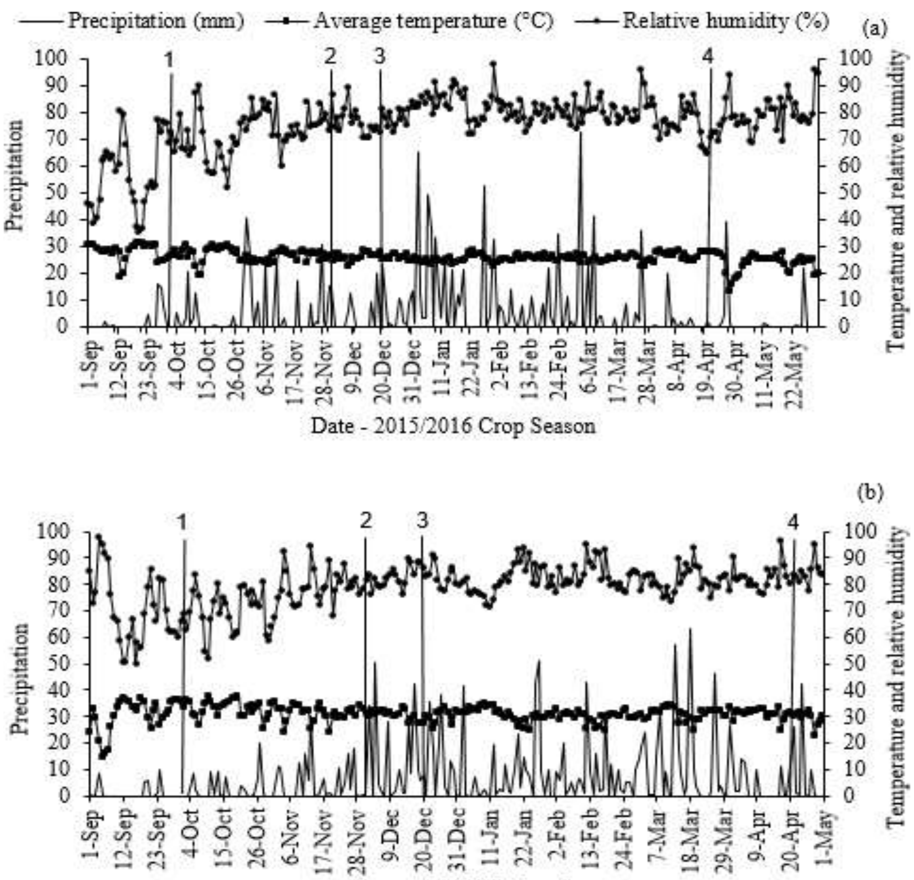

Figure 1. Accumulated precipitation (mm^{-1}), average temperature (T ºC) and relative air humidity (%) of the experimental area in the 2015-2016 (a) and 2016-2017 (b) crop seasons. 1 - sowing of millet and crotalaria; 2 - desiccation of plants; 3 - soybean sowing; 4 - soybean harvesting, soil sampling and evaluation of the soil chemical and microbiological quality.

pod and number of nodes of plant were considered. For the productive characteristics, it was considered the number of pods with one, two, three and four grains. At harvesting, all the plants present in the plot area were evaluated, with the grain moisture corrected to 13%. Mass of one thousand grains and the yield in kg ha^{-1} was determined (Souza et al., 2010).

Soil chemical characteristics

The soil quality was determined in each plot after the physiological maturity of the soybean, and in the forest fragment. Soil was collected in the space between soybean sowing lines at five points per plot. The chemical characteristics were determined in the 0 to 0.20 m depth layer, considering organic matter (OM), hydrogenation potential (pH), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), cation exchange capacity (T) and potassium saturation (% K) (Embrapa, 2011).

Soil microbiological characteristics

The soil microbial biomass carbon by the fumigation-extraction method (Vance *et al*., 1987), basal respiration of the soil obtained by the quantification of CO_2 released after the soil microbiological analysis, from 0 to 0.10 m depth (Jenkinson and Powlson, 1976), a microbial quotient determined by the relationship between soil microbial biomass carbon and total organic carbon, the metabolic quotient obtained by the relationship between basal respiration of the soil and the carbon of the microbial biomass of the soil were evaluated (Anderson and Domsch, 1993).

Statistical analyses

Shapiro Wilk normality tests and homogeneity by the Bartlett test was performed. The chemical and microbiological characteristics of the soil were submitted to the Dunnett test ($p \leq 0.05$), considering an additional plot in the forest fragment. Soil chemical and microbiological indicators, vegetative and productive characteristics of soybean were compared by Tukey's test ($p \leq 0.05$), using ASSISTAT software (Silva and Azevedo, 2016).

RESULTS

Agronomic characteristics of soybean

The vegetative and productive characteristics of the soybean crop in the 2015/16 crop season did not present significant differences in relation to the biological fertilization and soil cover, except the number of pods with three grains, which was higher in the soil maintained in clean fallow. Different behavior occurred in the 2016/17 crop season. When the biological fertilizer was applied, the soybean had higher insertion height of the first pod, the soil cover increased the plant height, leaf area and grain yield (Table 2). In the 2016/17 crop season, with soil cover there was a considerable influence on soybean grain yield, when grown on plant remains of millet and crotalaria presented increases of 10 and 16% yield, respectively, in relation to the soil in fallow clean. In this crop season, there were increases of 27, 31 and 17% of the yield of soybean cultivated on millet, crotalaria and soil in clean fallow, respectively, in relation to the 2015/16 crop season (Table 2). The increase in soybean yield reveals the importance of the practices proposed in this study, because in two years of management with soil cover increased grain yield in relation to the clean fallow (Table 2). In addition, the higher precipitation recorded in the 2016/17 crop season may also has contributed to a better productive development of soybean in relation to the 2015/16 crop season (Figure 1).

Soil chemical characteristics

The deposition of vegetable material in the soil provided conditions for the no-tillage of soybean, protection of the soil, organic matter production and fertility increments. Significant effects of the biological fertilizer and plant covering on soil chemical properties in both crops seasons was observed (Tables 3 and 4).

Highest concentration of potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg) and pH of the soil cultivated with soybean in the 2015/16 and 2016/17 crops seasons was observed. In the cultivated field with soil correction, mineral and biological fertilization presented higher chemical quality than the soil of the forest fragment, which presented lower fertility, but higher levels of organic matter in the soil (Tables 3 and 4).

The organic material was important for the quality of the chemical and microbiological properties of the soil. The soil of the forest fragment presented 27 and 31% more organic matter than the cultivated field with soybeans in the 2015/16 and 2016/17 crop seasons, respectively (Tables 3 and 4). The presence of organic matter was important for the maintenance of the productive capacity of the soil, therefore, there is need for the production of straw of millet and crotalaria, or other plant covering, on the agricultural soil.

There was also an increase of concentration of the K in the soil with application of the biological fertilizer and the use of millet in the 2015/16 crop season. The response in the 2016/17 crop season only for biological fertilization (Tables 3 and 4) was repeated. This is due to the ability of the millet to cycle a large quantity of K in the dry matter, which was also present in the mineral composition of the biological fertilizer, later mineralized in the soil through the organic matter decomposition process.

The soil of the forest fragment showed a higher cation exchange capacity, but with a higher acidity on the soil in the 2016/17 crop season (Table 4). The lowest P levels occurred in the soil with use of biological fertilizer, behavior not observed in the previous crop season. With crotalaria followed by millet, the concentration of P was higher than the clean fallow, which may be related to the nutrient cycling promoted by these plants covering.

Increased fertility of soil - mainly of the K and P, higher levels of organic matter in the soil, residues of millet and crotalaria and higher precipitation volume (Figure 1) were characteristics that contributed to the increases in soybean yield in 2016/17 crop season (Table 2).

Table 2. Soybean crop responses to biological fertilization (BF) and plant covering (COV) in the 2015/16 and 2016/2017 crop seasons.

2015/2016 crop season										
BF	**PH**	**HIFP**	**NN**	**P1**	**P2**	**P3**	**P4**	**LA**	**1000-GW**	**GY**
	------- cm -------			**-------------------- un --------------------**				**cm²**	**g**	**kg ha⁻¹**
With	66.3	16.5	13.8	11.2	22.3	20.0	0.4	1450.3	140.5	3020.3
Without	65.4	15.9	14.1	10.9	24.5	24.0	0.5	1720.1	137.9	3039.8
COV										
Millet	66.1	16.2	13.7	10.9	21.5	18.1^{b}	0.3	1637.7	141.1	3028.9
Crotalaria	68.2	16.6	14.1	10.6	22.5	20.8^{ab}	0.5	1613.8	141.0	3080.2
Clear Fallow	63.3	15.7	14.1	11.6	26.3	27.1^{a}	0.6	1503.9	135.6	2981.0
CV (%)	6.0	13.6	5.5	18.7	16.17	25.5	68.1	26.4	4.4	10.0
2016/2017 crop season										
BF										
With	67.7	18.8^{a}	14.4	8.5	17.0	14.6	0.2^{b}	1981.7	176.2	3890.6
Without	67.8	16.3^{b}	14.7	9.3	19.2	17.2	0.4^{a}	2114.8	173.4	3688.6
COV										
Millet	68.9^{a}	18.6	14.7	8.2	16.6	15.1	0.2	2114.9^{ab}	176.2	3833.3^{ab}
Crotalaria	69.9^{a}	17.0	14.9	10.3	18.8	16.3	0.3	2260.0^{a}	173.5	4047.0^{a}
Clear fallow	64.4^{b}	17.0	14.1	8.0	18.9	16.4	0.3	1769.9^{b}	175.2	3488.5^{b}
CV (%)	5.0	9.4	4.5	29.5	24.4	21.4	77.5	14.7	5.1	10.5

Means followed by the same letters in the column do not differ statistically by the Tukey's test (p≤0.05). Plant height (PH), height insertion of the first pod (HIFP), number of nodes (NN), pods with 1, 2, 3 and 4 grains (P), leaf area (LA), 1,000-grain weight (1000-GW) and grain yield (GY).

Table 3. Chemical quality of soil layer 0 to 0.20 m in the 2015/16 crop season as a function of biological fertilization (BF) and plant covering (COV), and control in the forest fragment.

		Dunnett's test (p≤0,05)									
BF	**COV**	**OM**	**pH**	**P**	**K**	**K**	**Ca**	**Mg**	**H+Al**	**T**	**K**
		g kg⁻¹	**CaCl₂**	**---- mg dm⁻³ ----**		**---------------- cmolc dm⁻³ --------------**					**Percentage**
	Millet	22.8^{b}	6.5^{a}	5.3^{a}	195.5^{a}	0.5^{a}	4.2^{a}	2.3^{a}	1.6^{b}	8.6^{a}	7.1^{a}
With	Crotalaria	25.5^{a}	6.6^{a}	3.6^{a}	147.6^{a}	0.4^{a}	4.2^{a}	2.1^{a}	1.4^{b}	8.1^{b}	5.4^{a}
	Clear fallow	26.8^{a}	6.4^{a}	3.4^{b}	97.7^{b}	0.2^{b}	4.3^{a}	2.1^{a}	1.6^{b}	8.3^{b}	3.6^{b}
	Millet	21.5^{b}	6.6^{a}	4.5^{a}	157.4^{a}	0.4^{a}	3.7^{a}	2.1^{a}	1.4^{b}	7.7^{b}	5.7^{a}
Without	Crotalaria	23.8^{b}	6.6^{a}	3.9^{a}	124.1^{a}	0.3^{a}	4.3^{a}	2.3^{a}	1.4^{b}	8.3^{b}	4.5^{a}
	Clear fallow	24.2^{b}	6.7^{a}	4.1^{a}	85.0^{b}	0.2^{b}	2.8^{a}	2.3^{a}	1.4^{b}	7.8^{b}	3.1^{b}
Forest fragment		30.6^{a}	5.0^{b}	0.7^{b}	55.7^{b}	0.1^{b}	2.3^{b}	1.1^{b}	3.3^{a}	6.9^{b}	2.0^{b}
FB		**Tukey's test (p≤0.05)**									
With		25.1	6.5^{b}	4.1	146.9^{a}	0.4^{a}	4.2	2.2	1.5	8.3	5.4^{a}
Without		23.2	6.6^{a}	4.2	122.2^{b}	0.3^{b}	4.0	2.2	1.4	7.9	4.5^{b}
COV											
Millet		22.1	6.5	4.9	176.4^{a}	0.5^{a}	4.0	2.2	1.5	8.1	6.4^{a}
Crotalaria		24.6	6.6	3.7	135.9^{b}	0.3^{b}	4.2	2.2	1.4	8.2	5.0^{b}
Clear fallow		25.5	6.6	3.8	91.4^{c}	0.2^{c}	4.1	2.2	1.5	8.0	3.3^{c}
CV (%)		10.6	2.3	39.5	20.3	20.3	16.1	19.8	11.1	10.1	20.3

Means followed by the same letter in the column do not differ statistically by the Dunnett's test (p≤ 0.05) and Tukey's test (p≤0.05). Organic matter (OM), potential hydrogen (pH) phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), hydrogen + aluminum (H + Al), cation exchange capacity (T), and potassium saturation (%K) at pH 7.

Table 4. Chemical quality of soil layer 0 to 0.20 m in the 2016/17 crop season as a function of biological fertilization (BF) and plant covering (COV), and control in the forest fragment.

BF	COV	Dunnett's test ($p \leq 0,05$)									
		OM	pH	P	K	K	Ca	Mg	H+Al	T	K
		g kg^{-1}	$CaCl_2$	------ mg dm^{-1} -----		---------------- $cmol_c$ dm^{-3} ------------					Percentage
With	Millet	30.7^{b}	5.8^{a}	2.7^{b}	202.0^{a}	0.5^{a}	4.2^{a}	1.4^{b}	1.7^{b}	7.9^{b}	7.4^{a}
	Crotalaria	29.3^{b}	5.9^{a}	4.2^{a}	194.2^{a}	0.5^{a}	4.4^{a}	1.4^{b}	1.1^{b}	7.4^{b}	7.1^{a}
	Clear fallow	30.7^{b}	6.1^{a}	2.8^{b}	152.5^{a}	0.4^{a}	4.5^{a}	1.3^{b}	1.3^{b}	7.5^{b}	5.6^{a}
Without	Millet	30.3^{b}	5.9^{a}	7.3^{a}	157.7^{a}	0.4^{a}	4.7^{a}	1.3^{b}	1.6^{b}	8.0^{b}	5.8^{a}
	Crotalaria	29.0^{b}	5.9^{a}	9.2^{a}	155.1^{a}	0.4^{a}	4.4^{a}	1.2^{b}	1.7^{b}	7.7^{b}	5.7^{a}
	Clear fallow	30.0^{b}	6.1^{a}	3.5^{b}	122.5^{a}	0.3^{a}	4.4^{a}	1.5^{a}	1.6^{b}	7.8^{b}	4.5^{a}
Forest fragment		39.3^{a}	5.0^{b}	0.7^{b}	45.6^{b}	0.1^{b}	3.7^{b}	1.2^{b}	4.3^{a}	9.3^{a}	1.7^{b}
BF		Tukey's test ($p \leq 0.05$)									
With		30.2	5.9	3.2^{b}	182.9^{a}	0.5^{a}	4.4	1.4	1.4	7.6	6.7^{a}
Without		29.8	6.0	6.6^{a}	145.1^{b}	0.4^{b}	4.5	1.3	1.6	7.8	5.3^{b}
COV											
Millet		30.5	5.8^{b}	5.0^{ab}	179.7	0.5	4.4	1.4	1.6	7.9	6.6
Crotalaria		29.2	5.9^{b}	6.7^{a}	174.6	0.4	4.4	1.3	1.4	7.6	6.4
Clear fallow		30.3	6.1^{a}	3.2^{b}	137.5	0.3	4.5	1.4	1.4	7.6	5.0
CV (%)		4.7	1.2	39.16	22.9	22.9	4.4	10.2	23.2	6.6	22.9

Means followed by the same letter in the column do not differ statistically by the Dunnett's test ($p \leq 0.05$) and Tukey's test ($p \leq 0.05$). Organic matter (OM), potential hydrogen (pH) phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), hydrogen + aluminum (H + Al), cation exchange capacity (T), and potassium saturation (%K) at pH 7.

Soil microbiological characteristics

There was a lower microbiological indicators quality of the soil in the cultivated field with soybeans in both the 2015/16 and 2016/17 crop seasons, compared to the soil of the forest fragment, where was observed greater total organic carbon, microbial biomass carbon, microbial quotient and basal respiration (Table 5). There were no observed effects of the biological fertilizer on the microbiological properties of the soil in the crop season 2015/16. The implementation of the management proposed in this study in the 2016/17 crop season showed higher carbon fixation efficiency, lower soil CO_2 losses and soil metabolic quotient by microbial biomass (Table 5). The climatic conditions of this last crop season may have favored the development of microbial biomass in relation to the 2015/16 crop season, as there was frequent rainfall before soil collection for analysis (Figure 1). The mean values of total organic carbon, microbial biomass carbon, microbial quotient, basal respiration and metabolic quotient of soil cultivated with soybean increased (25, 45, 18, 117 and 47%, respectively) in the 2016/17 crop season in relation to the 2015/16 crop season. However, the metabolic quotient indicates that the microbial biomass is under stress condition (Table 5). The management with biological fertilizer and plant covering reduced the impacts resulting from agricultural practices in the soil, and the microbiological indicators were sensitive tools to monitor such modifications. The results observed in the area cultivated with soybean with application of the biological fertilizer and plant residues remaining of millet and crotalaria, had lower soil metabolic quotient in the both crop season, when compared to the absence of biological fertilizer and clear fallow (Table 5).

DISCUSSION

Agronomic characteristics of soybean

In this study, there were no effects of the biological fertilization on the grain yield of soybean crop. However, in other crops such as *C. sativus*, the biofertilizer or the combination of biofertilizer with mineral fertilizer promoted an increase in the number of fruits per plant, fruit weight, fruit size and yield per green house/kg (Saeed et al., 2015). Increase in soybean yield when cultivated on plant

Table 5. Microbiological quality of the soil layer 0.00 to 0.20 m in the 2015/16 and 2016/17 soybeans crop seasons as a function of biological fertilization (BF) and plant covering (COV), using a forest fragment as control.

BF	COV	Dunnett's test ($p \leq 0.05$)									
		2015/2016 crop season					2016/2017 crop season				
		TOC	SMBC	*q*MIC	C-CO_2	*q*CO_2	TOC	SMBC	*q*MIC	C-CO_2	*q*CO_2
With	Millet	13.2^b	102.6^b	0.7^b	0.2^b	1.4	17.8^b	135.2^b	0.6^b	0.4	2.7^b
	Crotalaria	14.8^a	104.7^b	0.7^b	0.3^b	2.9	17.0^b	129.2^b	0.6^b	0.4	3.2^a
	Clear fallow	15.6a	95.6^b	0.6^b	0.2^b	2.7	17.8^b	157.4^b	0.9^b	0.6	4.0^a
Without	Millet	12.5^b	87.3^b	0.7^b	0.2^b	2.6	17.6^b	151.8^b	0.9^b	0.6	4.1^a
	Crotalaria	13.8^b	91.2^b	0.7^b	0.3^b	2.6	16.8^b	131.4^b	0.8^b	0.4	3.2^a
	Clear fallow	14.0^b	85.3^b	0.7^b	0.3^b	3.3	17.4^b	118.2^b	0.7^b	0.6	5.7^a
Forest fragment		17.7^a	439.3^a	2.6^a	0.8^a	1.9	21.4^a	324.7^a	1.5^a	0.5	1.5^b
BF		Tukey's test ($p \leq 0.05$)									
With		14.5	101.0	0.7	0.2	2.4	17.5	140.6	0.8	0.5	3.3^b
Without		13.4	88.0	0.7	0.2	2.8	17.3	133.8	0.8	0.6	4.3^a
COV											
Millet		12.8	95.0	0.7	0.2	2.0^b	17.7	143.5	0.8	0.5^{ab}	3.4^b
Crotalaria		14.3	98.0	0.7	0.3	2.8^{ab}	16.9	130.3	0.8	0.4^b	3.2^b
Clear fallow		14.8	90.4	0.6	0.3	3.0^a	17.6	137.8	0.8	0.6^a	4.9^a
CV (%)		10.6	13.0	15.4	18.3	24.3	7.75	24.8	27.2	25.4	18.^{6}

Means followed by some letter in column not differ statistically for Dunnett's test ($p \leq 0,05$) and Tukey's test ($p \leq 0,05$). Total organic carbon (TOC – g kg^{-1}), soil microbial biomass carbon (SMBC – mg C microbial kg^{-1} solo), microbial quotient (qMIC – %), soil basal respiration (C-CO_2 – mg de C-CO_2 kg^{-1} soil $hour^{-1}$) and metabolic quotient (qCO_2 – mg C-CO_2 g^{-1} SMBC h^{-1}).

residues of millet and crotalaria in the 2016/17 crop season was recorded. In the soybean cultivated in soil with plant covering and low water availability, greater grain yield of soybean occured, because the dry matter of *Avena strigosa*, *Vicia sativa* and *Raphanus sativus* deposited on the soil contributed to the maintenance of moisture (Debiasi et al., 2010). On the other hand, whit *Brachiaria ruziziensis, Pennisetum glaucum, Cajanus cajan* and *Cenchrus echinatus* and uniform distribution of rainfall throughout the crop cycle, did not record effects of plant covering on soybean grain yield (Pacheco et al., 2011; 2013).

Soil chemical characteristics

There was an increase of K concentration in the soil with millet. Among all the coverages tested, the millet was the one with the highest concentration of K remaining in the dry matter in relation to the other cover plants, being 58 kg ha^{-1} of K, besides contributing 70 kg ha^{-1} of N and 12 kg ha^{-1} of P (Pacheco et al., 2011).

The fertility of soil of the Cerrado Region was low naturally, with the forest fragment analyzed in this study. It was necessary to adopt management that increases the concentration of nutrients in the soil of the areas submitted to agricultural crops (Lopes et al., 2013). In this way, the needs of the crop were made available and the nutrients of the soil were replenished (Sousa and Lobato, 2004).

In the present study, the organic matter was lower in soil cultivated with soybean in relation to the forest fragment. Cherubin et al. (2015) also observed losses of organic material in agricultural areas in relation to the natural environment. The organic matter was an important indicator of soil changes in microbiological quality. The loss of organic material was intensified in the conventional farming system in relation to no-tillage, which was considered a soil conservation practice that allows the accumulation of vegetable dry matter (Vezzani and Mielniczuk, 2011).

The efficiency of the biological fertilizer depends on the presence of organic matter in the soil, because the introduction of decomposing microorganisms, mainly bacteria and fungi, use it as a substrate for its growth. These biological agents benefit the soil by decomposing organic matter, promoting the cycling of nutrients and improving the efficiency in the use of mineral fertilization, used to supply the nutritional needs of crops (Medeiros and Lopes, 2006).

Soil microbiological characteristics

The soil microbial biomass carbon was lower in the cultivated field compared to the forest fragment. In the municipality of Primavera do Leste - MT, a reduction in the area used for agricultural practices in relation to native vegetation was noticed (Matsuoka et al., 2003). The soil microbial biomass carbon positively correlates with the total organic carbon, this justifies the greater soil microbial biomass of the forest fragment observed in this study (Lisboa et al., 2012; Lopes et al., 2013).

Therefore, the accumulation of organic matter in the cultivated areas resulting from management techniques such as the addition of liquid residues of pigs in the farming system contributed to increase the carbon levels of soil microbial biomass (Cherubin et al., 2015). In a study testing different forms of soil cover, the carbon of microbial biomass was larger using straw mulch in relation to plastic mulching of the soil (Muñoz et al., 2017).

Soil cover plants are important for soil management, where millet has been used in no-tillage systems due to high dry matter production. This characteristic paved way to increase carbon levels in soil cultivated for four years in no-tillage. From the implantation of this cultivation system, accumulation of carbon in the soil and increases in the carbon of the microbial biomass was recorded, in relation to the conventional system made with a rotation and without presence of straw on the soil (Matias et al., 2009).

To obtain reference values of the microbiological quality of farming field, Mendes et al. (2015) elaborated different levels of interpretation of soil quality bioindicators of soybean and maize crops. Considering the minimum value of 205 mg C kg^{-1} of soil based on the organic matter content proposed by the authors mentioned earlier, the soil microbial biomass carbon of the present study was found to be low in the 2015/16 and 2016/17 crop seasons (Mendes et al., 2015).

The microbial quotient was formed by the relationship between the soil microbial biomass carbon and the total organic carbon, and indicates the percentage of soil carbon immobilized by microorganisms with highest and lowest values indicating carbon gain and loss in the soil microbial biomass, respectively. Considering that 2.20% was considered an equilibrium level for this indicator, the values observed in the soybean cultivated soil in this study were lower, indicating low carbon fixation in the biomass (Jenkinson and Ladd, 1981).

The study reported values of microbial quotient higher than 1 in fields submitted to different farming systems, being higher in no-tillage system. Higher values of microbial quotients indicated that soil organic matter is more available to be processed and transformed by soil microbial biomass (Matias et al., 2009). Soil basal respiration, soil microbial biomass carbon and microbial quotient had the lowest values in the soybean cultivated soil in relation to the forest fragment. A similar relationship between bioindicators was observed by Cunha et al. (2012). Soils submitted to conventional tillage presented higher basal respiration in relation to no-tillage (Lisboa et al., 2012). Greater respiration of the soil was submitted for mechanical scarification (Cherubin et al., 2015). The release of CO_2 by respiration was related to the increase in the metabolic activity of microbial biomass, which is usually caused by stress conditions in the soil. The rapid carbon consumption allows greater release of nutrients to plants in the short term, but in the long term, indicates low efficiency of fixation in the biomass, generating losses of carbon in the form of CO_2 by microbial respiration (Cunha et al., 2012). The basal respiration of the soil was usually lower in natural areas or in equilibrium, and according to the classification levels of the bioindicators, all values of basal respiration observed in this study were low (Mendes et al., 2015).

The study also observed a low metabolic quotient with biological fertilizer, millet and crotalaria. The highest metabolic quotient, as observed in the soil without biological fertilizer and clean fallow, indicate unbalanced areas, which is due to the accelerated metabolic process of the microorganisms of the soil. The fast process of organic matter decomposition increased the losses of carbon in the form of CO_2 by soil microbial biomass (Cunha et al., 2012).

The quality of plant residues also influences the carbon fixation. The metabolic quotient was higher in soil with straw of *P. glaucum*, *C. spectabilis* and in fallow area. This indicates that the straw of these plants may have caused some type of stress to the microorganisms, which consume more energy, reducing the carbon fixation in the soil and increasing the losses by soil respiration (Carneiro et al., 2008). With low values of soil metabolic quotient, the biomass has greater efficiency in the fixation of carbon in the microbial cell, thus having less energy expenditure. With soils that present stress conditions, the microorganisms tend to spend more energy to survive, so the values of the metabolic quotient are higher (Gomide et al., 2011; Fidelis et al., 2016).The microorganisms were important for the functioning of the soil and its interactions with the root system of the crops, and can also influence the soil chemical attributes (Mendes et al., 2015). Works involving soil microorganisms require a longer evaluation time to obtain reliable results (Cherubin et al., 2015).

The soil quality indicators were tools used to monitor agricultural soils (Gomide et al., 2011). Based on the results of this study, it was recommended to make use of biological fertilization and plant covering, since this management improved the chemical and microbiological quality of soil cultivated with soybean, besides the significant effects on the vegetative development and the grain yield of the soybean crop.

CONCLUSIONS

The grain yield of the soybean was higher when cultivated in the plant residues of crotalaria in the 2016/2017 crop season. Effects of the biological fertilization on the agronomic performance of the soybean crop were not observed. The application of the biological fertilizer, the use of millet and crotalaria increased the fertility and microbiological quality of the soil in the 2015/16 and 2016/17 crop seasons.

CONFLICT OF INTERESTS

The authors had not declared any conflict of interests.

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Full Length Research Paper

A survey of the insect pests and farmers' practices in the cropping of yellow pepper *Capsicum annuum* Linnaeus in Enugu State of Eastern Nigeria

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Yellow pepper (*Capsicum annuum* L.) is the second market gardening crop after tomato, and subsequently, a major source of income to farmers in Nigeria. Pests and diseases reduce yields and quality of marketable fruits. A study was conducted in Ibagwa-Agu, Lejja, Edem, Alor-Uno and Eha-Alumona agro-based communities of Nsukka Local Government Area to assess the economic implications of insect infestation and control strategies on yellow pepper yield. Data on insect pests was collected using the modified Whittaker sampling techniques from July to September 2015, while farming practices and economic consequences of pest infestation were monitored using questionnaires in December 2015. A total of 2,279 insects comprising 10 pests and 2 predators were collected. *Myzus persicae* Sulzer (27.6% of total collections), *Bemisia tabaci* Genn. (21.1%), *Aphis gossypii* Glov. (14.1%) and *Zonocerus variegatus* Gestro(7.99%) were the four prominent insect pests, while Ladybird beetle larva of *Harmoni axyridis* (2.3%) and praying mantis *Stagmonantis crolina* (1.4) were the main predators encountered. Mean percentage of plants attacked and yield losses were significantly (P <0.05) lower in communities with a high percentage of farmers utilizing cultural control methods. A higher percentage of the farmers were females. Farmers in Edem, Eha-Alumona, Lejja, Alor-Uno and Ibagwa-Agu in Nsukka Local Government Area of Enugu State therefore experience enormous economic loss in yellow pepper cultivation from the infestation of insect pests. Communities with a high percentage of farmers practicing cultural control methods had fewer plants attacked, while the predators were also more abundant. Further research is needed to ascertain the efficacy of these cultural methods.

Key words: Yellow pepper, Insect pests, Yield reduction, Farmers' cropping practices, Control measures, Gender perception.

INTRODUCTION

Yellow pepper (*Capsicum annuum* L.) is a spicy fruit, consumed extensively at world level (Dias et al., 2013). It has both nutritive and medicinal values. Some health-related phytochemical compounds found in yellow peppers are important in preventing chronic diseases such as cancer, asthma, coughs, sore throats, toothache, diabetes and cardiovascular diseases (El-Ghoraba et al., 2013; Wahyuni et al., 2013). *C. annuum* also has antioxidant, anti-mutagenesis, hypocholesterolemic and immunosuppressive properties (El-Ghoraba et al., 2013), as well as inhibits bacterial growth and platelet agglomeration (Wahyuni et al., 2013). Consequently, yellow pepper is in high demand both by the general public and pharmaceutical companies (Nwankiti, 1981; Denton and Swarug, 2007).

In Nigeria, yellow pepper is the second market gardening crop after tomato (Assogba-Komlan et al., 2009), and it is a major income source for farmers. The Nsukka Area in Enugu State, eastern Nigeria, is especially important in the cultivation of yellow pepper as it has the environmental conditions suitable for its cultivation. Yellow pepper cultivation is the major and sometimes the only agricultural activity of rural women in the State. Yellow pepper production in Nigeria has been facing many biotic and environmental constraints. Prominent among such constrains are pests and diseases which reduce yields and quality of marketable fruits (Echezona and Nganwuchu, 2006) In the tropics, particularly in Nigeria, some insect pests are directly associated with yellow pepper damage and yield losses, while others are important as vectors of diseases (Khan et al., 2009; Segnou et al., 2013; Zhani et al., 2013).

Information on the insect pests and farmers' practices in the cultivation of yellow pepper in Nsukka area is lacking.

This survey was conducted in some major yellow pepper producing communities in Nsukka Area to assess the status of yellow pepper production with a view to address production problems related to insect pests. The survey had the objective of (i) identifying through questionnaire, technologies which influence insect spread and damage and (ii) identifying through field sampling major yellow pepper insect pests and their spread.

MATERIALS AND METHODS

Study area

The Nsukka Local Government Area (6°5' 24" N and 7°23'45"E) is located in the northern part of Enugu State in south-eastern Nigeria. The study was conducted between June and July, and December (2015) in five agro-based communities: Ibagwa-Agu, Lejja, Edem, Alor-Uno, and Eha-Amluona, chosen based on the following criteria: (i) relative importance of the yellow pepper crops in the communities, and (ii) its level of production. The average annual temperature falls between 27 and 28°C and average annual rainfall is about 1600 mm. The natural vegetation of the area is a derived savannah type and the trees found are usually drought resistant (Ugwu, 1964). Inhabitants in the Nsukka area have agriculture as their main source of income, being yellow pepper the main crop with economic importance.

Ethical consideration

Consent was obtained from the University of Nigeria ethical committee. The respondents were acquainted with the purpose of the study and assurances of confidentiality and anonymity were given to them.

Data collection

Insect collection: Whittaker sampling protocol

Twenty (20) yellow pepper farms were chosen each from the five agro-based communities, making a total of 100 farms, using systematic random selection. A total of 310 m^2 per farm were sampled using the modified Whittaker sampling protocol (Whittaker, 1972). Whittaker plots (20x50 m) were centrally outlined with wooden pegs, threaded with rope, at each farm. Nested in each plot were ten 0.5x2 m subplots systematically spaced along the inside border, two 2x5 m subplots in alternate corners, and a 5x20 m subplot in the center of the plot. Fifty plants were sampled randomly from the mapped out area in each of the sampled farmlands, 30, 10 and 10 from the 5x20, two of the 2x5 m and ten 0.5x2 m subplots respectively. Handpicking and insect sweep nets were used to collect the insects. Collection was biweekly and at the morning and evening hours when the insects were less active.

Observations were made on leaves, stems and fruits for insects and insect damage. Percentages of plants damaged by insects per farm were calculated. Insects' samples were identified in the field and the laboratory with a hand lens and a microscope, using picture vouchers and specimens in the museum in the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Insects with mean percentage abundance of above 6% were regarded as prominent.

Knowledge/attitude of farmers and socio-economic impacts of insect pests of *C. annuum:* Descriptive survey method

The study population consisted of the twenty farmers whose farms were sampled for insect pests and an additional twenty randomly selected, in each community, making it a total of 200 respondents. The farmers' knowledge on insect pests of yellow pepper, yield losses from the insects and farming practices used to control them, were investigated using descriptive survey method, involving interview questions administered as semi-structured questionnaire. The interview questions were coined from 4 research questions: 1.Is yellow fever cultivation the major source of income amongst the farmers?; 2. What is the level of knowledge of the farmers of insect pests of pepper?; 3. What are the economic impacts of insect pests on *Capsicum annuum* on farmers in Ibagwa-Agu, Lejja, Edem, Alor-Uno and Eha-Alumona agro based communities of Nsukka Local Government Area? ; 4. What are the insect pest control methods practiced in Ibagwa-Agu, Lejja, Edem, Alor-Uno and Eha-Alumona agro based communities of Nsukka Local Government Area?

Nine interview questions were coined from the four

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Table 1. No of farmers' using yellow pepper cultivating as their major source of income in the agro-based communities.

Age range (years)	No of farmers	Sex		Edem		Lejja		Eha-Alumona		Alor uno		Ibagwa Agu	
		Male	Female	Male	female	male	female	male	Female	Male	Female	Male	Female
21-25	12	8(11.77%)	4(3.03%)	4(22.20%	-	-	-	2(14.20%)	-	2(16.60%)	-	-	4(13.30%)
26-30	22	10(14.71%)	12(9.09%)	4(22.2%)	2(9.0%)	2(14.2%)	-	-	2(7.6%)	2(16.60%)	4(14.2%)	2(16.6%)	2(15%)
31-35	28	10(14.71%)	18(13.6%)	2(11.1%)	6(27.2%)	2(14.2%)	2(7.6%)	2(14.2%)	4(15.3%)	2(16.60%)	2(7.1%)	2(16.6%)	4(13.30%)
36-40	46	12(17.65%)	34(25.7%)	4(22.29%)	8(36.3%)	2(14.2%)	2(7.6%)	4(28.5%)	6(23.07%)	-	12(42.8%)	2(16.6%)	620%)
41-45	48	12(17.65%)	36(27.20%)	2(11.1%)	6(27.2%)	4(28.5%)	2(7.6%)	2(14.2%)	8(30.7%)	2(16.60%)	8(28.5%)	2(16.6%)	2(15%)
46-50	28	8(11.75%)	20(15.1%)	2(11.1%)		-	12(46.1%)	4(28.5%)	6(2.07%)	-	2(7.1%)	2(16.6%)	6(20%)
51-60	12	6(8.87%)	6(4.5%)	-		4(28.5%)	6(23.07%)	-	-	2(16.60%)	-	-	4(13.30%)
< 60	4	2(2.94%)	2(1.5%)	-		-	2(7.6%)	-	-	2(16.60%)	-	-	2(15%)
Total	200	68(34%)	132(66%)	18(45%()	22(55%)	14(35%)	26(65%)	14(35%)	26(65%)	12(30%)	28(70%)	10(25%)	30(75%)

research questions: 1. Is yellow pepper cultivation your major source of income?; 2. How long have you cultivated yellow pepper?; 3. Do you utilize manual or mechanized farming method?; 4 What is your annual income from yellow pepper cultivation?; 5 Are you aware that some insects destroy yellow pepper?; 6. Can you identify at least one pest of yellow pepper from the pictures of insects provided?; 7 Mention at least two damages done to yellow pepper by a named insect?; 8. Do you suffer from yellow pepper yield losses from insect activities ,if yes how much?; 9. What farming practices do you employ in controlling the insect pests on your farm? The questionnaire was validated by conducting a pilot study to ensure that the questions were clear and appropriate to the level of the respondents' education. Results are grouped into sexes and presented under eight age ranges: 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-60 and <60 years. Metrics assessed through questionnaires are expressed in percentages. The questionnaires were administered in December, 2015 after the major harvesting periods. Yield reduction of above 25% was regarded as high, while cultivation period of above 5 years was regarded as long duration.

Statistical analysis

Data obtained were analyzed using statistical package for social sciences (SPSS) (version 17; SPSS Inc., Chicago, IL, USA, 2009) and descriptive statistics (average, percentage abundance, chi-square, species diversity) at P < 0.05).

RESULTS

In all the communities where the survey was conducted, females than males were involved in the production of yellow pepper as follows,55:45; 65:35; 765:36; 70:30 and 75%:25% for Edem, Lejja, Eha-Alumonah and Ibagwa Agu, respectively) (Table 1). Females between the age range 31-50 years were most involved in yellow pepper cultivation than other ranges (Table 1).Yellow pepper cultivation was the major source of income for all the farmers interviewed (100%). Manual farming was the most common and major method of farming (100%). The farmers had cultivated yellow pepper for periods ranging from 6 to 30 years (data not presented).

Insects belonging to six orders, ten families and twelve species were collected during the survey (Table 2). Pests include the green peach aphids (*Myzus persicae* Sulzer), cooton aphid (*Aphis gossypii* Glov), white flies (*Bemisia tabaci* Genn.) bugs (*Helopeltis schoutedni* Reuter), flea beetles (*Nisotra sjostedti* Jacoby), variegated grasshopper (*Zonocerus variegatus* Gestro), striped blister beetle (*Epicauta albovittata* Gestro), thrips (*Scirtothrips dorsali* Glov), leafhopper (*Zonosemata* spp.) and pepper larva (*Biston betularia* Linnaeus).Predators sampled from the crops were ladybirf beetle (*Harmoni axyridis* Pallas) and praying mantis (*Stagmonantis carolina* Johannson) (Table 2). Green peach aphid was the most encountered (27.6%), followed by white flies (*B. tabaci)* with 21.1%, cotton aphid (*A. gossypii)* (14.1%) and the least encountered was the Praying mantis (*S. carolina)* (1.4%). The shoot of the plant was significantly (P < 0.05) most attacked as seven species were collected from this plant part, followed by the leaf (2 species), while only one was collected from the fruit. Among the pests, the order Hemiptera was most abundant (4 species), while Thysanoptera and Lepidoptera were least abundant (1 species each) (Table 2). Of the total 2,279 insects collected, the

Table 2. Insect pests and predators collected from yellow pepper plants in the sampled agro-based communities.

Order	Family	Scientific Name	Common Name	%Total Number	Plant Part Attacked
Hemiptera	Aphididea	*Myzus persicae*	Green peach aphid	27.6	Shoot
Hemiptera	Aleyrodidae	*Bemesia tabaci*	White fly	21.1	Leaf
Hemiptera	Aphididea	*Aphis gossypii*	Cotton aphid	14.1	Shoot
Orthoptera	Pyrgomorphidae	*Zonocerus variegatus*	Grasshopper	7.99	Shoot
Diptera	Tephritidae	*Zonosemata spp.*	fruitfly	6.3	Shoot
Hemiptera	Miridae	*Helopeltisschoutedeni*	Bug	4.3	Shoot
Lepidoptera	Geomtridae	*Biston betularia*	pepper larva	4.2	Fruit
Coleoptera	Meloidae	*Epicauta albovittata*	Striped blister beetle	3.9	Shoot
Thysanoptera	Thripidae	*Scirtothripsdorsali*	Thrip	3.5	Shoot
Coleoptera	Chrysomelidae	*Nistorasjostedti*	Flea beetle	3.3	Leaf
Coleoptera	Coccinellidae	*Harmoniaxyridis*	Lady bird beetle larva	2.3	Predatory
Dictyoptera	Mantidae	*Stagmonantiscarolina*	praying mantis	1.4	Predatory

Table 3. Insect pests and number collected from *C. annuum*(Yellow pepper) from the agro-based communities.

Insect	Ibagwa Agu (%)	Edem(%)	Lejja (%)	Eha-Alumona(%)	Alor-Uno(%_)	Total (%)
Myzus persicae	150(23.9)	80(12.7)	100(15.9)	118(18.8)	180(28.7)	628(27.6)
Bemisia tabaci	109(22.7)	55(11.4)	77(16.0)	107(22.2)	133(27.7)	481(21.1)
Aphis gossypii	75(23.3)	43(13.4)	61(18.9)	90(28.0)	53(16.5)	322(14.1)
Zonocerus varigatus	50(27.5)	43(23.6)	10(5.5)	38(20.9)	41(22.5)	182(7.99)
Zonosemata spp.	25(17.4)	36(25.0)	20(13.9)	22(15.3)	41(28.5)	144(6.3)
Helopeltis schoutedeni	21(21.2)	10(10.1)	13(13.1)	19(19.2)	36(36.4)	99(4.3)
Biston betularia	19(20.0)	21(22.1)	16(16.8)	25(26.3)	14(14.7)	95(4.2)
Epicauta albovittata	20(22.7)	12(13.6)	16(18.2)	15(17.0)	25(28.4)	88(3.9)
Scirtothrips dorsali	16(20.0)	13(16.3)	15(18.8)	17(21.3)	19(23.8)	80(3.5)
Nistora sjostedti	16(21.1)	9(11.8)	12(15.8)	17(22.8)	22(28.9)	76(3.3)
Harmoni axyridis	7(13.2)	10(18.9)	13(24.5)	15(28.3)	8(15.1)	53(2.3)
Stagmonanti scarolina	5(16.1)	7(22.6)	4(12.9)	9(29.0)	6(19.4)	31(1.4)
Total	515(22.5)	339(14.9)	357(15.7)	492(21.6)	578(25.4)	2279(100.0)
Simpson's Index of Diversity	0.8309	0.8677	0.8345	0.8468	0.8239	

highest number was collected from Alor-Uno (578:25.4%), followed by Ibagwa Agu (515:22.5%) and the lowest from Edem (339:14.9) (Table 3).

All 12 species of insects (10 pests and 2 predators) were collected from all the sampled locations. The species diversity of insect pest found in the location was almost the same. Edem has the highest level of diversity of insects (0.8677) while Alor-Uno has the least level of diversity (0.8239) (Table 3).

In the locations that were surveyed, all the farmers were aware that some insects destroy pepper, but more female farmers than male could identify at least one of the insect pests (40:15; 50: 25;62.5:30; 20:15;and 60:10% for Edem, Lejja, Eha-Alumona, Alor Uno and Ibagwa agu respectively) (Table 4), and also were able to mention at least two damages done to yellow pepper plant by insects (35:15; 20:15; 50:20; 50:10; 50:5 for Edem, Lejja, Eha-Alumona, Alor Uno and Ibagwa Agu respectively). For all the locations, positive responses for age ranges between 36-<60 were higher than the others (Table 4).

Income generated annually ranged between ₦100,000 and 900,000, with 41% of the women earning between 800.000-900,000, while 20% of the men were found in this income range (data not presented).

In all locations, all the farmers experienced yield loses due to insect infestation (100%).80% farmers in Ibagwa-Agu experienced about 26 to 50% yield reduction, while 20% experienced 51-75% yield reduction. Most farmers in Alor- Uno (91.9%) also experienced 26 to 50% farm yield reduction, while 8.1% experienced 51-75% yield reduction. Those 100% farmers in Eha- Alumonah experienced lower yield reduction (about 1 to 25%) farm yield reduction. Most farmers in Lejja, 44, 50 and 5.6% experienced between 1 to 25%, 26 to 50% and 51 to 75% farm yield reduction respectively. Those in Edem

Table 4. Level of knowledge of the farmers on insect pests of yellow pepper in the agro-based communities

Age range (years)	Edem							Lejja							Eha-Alumona						
	No of farmers	1		2		3		No of farmers	1		2		3		No of farmers	1		2		3	
		M	F	M	F	M	F		M	F	M	F	M	F		M	F	M	F	M	F
	N(%)																				
21-25	4	4(22.2)	-	-	-	-	-	-	-	-	-	-	-		**2**	2(14.2)	-	1(8.3)	-	-	-
26-30	6	4(22.2)	2(9.0)	-	-	-	-	2	2(14.2)	-	-	-	-		2	-	2(7.6)	-	2(8)	-	2(10)
31-35	8	2(11.11)	6(27.2)	-	2(12.5)	-	-	4	2(14.2)	2(7.6)	1(10)	1(5)	-		6	2(14.2)	4(15.3)	2(16.6)	3(12)	1(12.5)	2(10)
36-40	12	4(22.2)	8(36.3)	1(16.67)	8(50)	1(16.67)	8(57.18)	4	2(14.2)	2(7.6)	1(10)	1(5)	2(33.3)		10	4(28.5)	6(23.0)	3(23)	6(24)	3(37.5)	4(20)
41-45	8	2(11.11)	6(27.2)	3(50)	6(37.5)	3(50)	6(42.2)	6	4(28.5)	2(7.6)	4(40)	-	2(33.3)		10	2(14.2)	8(30.7)	2(16.6)	8(32)	1(12.5)	8(40)
46-50	2	2(11.11)	-	2(33.3)	-	2(33.3)	-	12	-	12(46.15)	-	12(60)	-	12(60)	10	4(28.5)	6(23.0)	4(33.3)	6(24)	4(50)	4(20)
51-60	-	-	-	-	-	-	-	10	4(28.5)	6(23.03)	4(40)	4(20)	2(33.3)	6(30)	-	-	-	-	-	-	-
< 60	-	-	-	-	-	-	-	2	-	2(7.6)	-	2(10)	-	2(10)	-	-	-	-	-	-	-
Total	40	18(455)	22(55)	6(15)	16(40)	6(40)	14(35)	40	14(35)	26(65)	10(25)	20	6(15)	20(50)	40	14(35)	26(65)	12(30)	26(62.5)	8(20)	26(50)
	Alor Uno							Ibagwa Agu													
21-25	2	2(17.67)	-	-	-	-	-	4	-	4(13.33)	-	3(12)	-	2(10)							
26-30	6	2(17.67)	4(14.29)	-	3(15)	-	3(15)	6	2(20)	2(6.67)	-	1(4)	-	-							
31-35	4	2(17.67)	2(7.14)	1(16.67)	1(5)	1(25)	1(5)	6	2(20)	4(13.33)	-	4(16)	-	4(20)							
36-40	12	-	12(42.86)	-	10(50)	-	10(50)	8	2(20)	6(20)	1(25)	4(16)	-	4(20)							
41-45	10	2(17.67)	8(28.57)	1(16.67)	4(20)	1(25)	4(20)	4	2(20)	2(6.67)	1(25)	2(16)	-	2(10)							
46-50	2	-	2(7.14)	-	2(10)	-	2(10)	8	2(20)	6(20)	2(50)	5(20)	2(100)	4(20)							
51-60	2	2(17.67)	-	2(33.33)	-	1(25)	-	4	-	4(13.33)	-	4(16)	-	2(10)							
< 60	2	2(17.67)	-	2(33.33)	-	1(25)	-	2	-	2(6.67)	-	2(8)	-	2(10)							
Total	40	12(30)	28(70)	6(15)	20(5)	4(10)	20(50)	40	10(20)	30(75)	4(10)	25(60)	2(5)	20(50)							

1– No. aware that some insects destroy yellow pepper ; 2- No. that can identify at least one pest of yellow pepper; 3- No. that can mention at least two damages done to *C. annum* by a named insect pest.

experienced 1 to 25%, 26 to 50% and 51 to 75% yield reduction in the proportions of 43, 43.29 and 13.8% respectively (Figure 1). No farmer experienced farm yield reduction between 76 to 100%. There was a significant different in the level of farm yield reduction experienced during the time of infestation ($P < 0.05$) in Lejja, Eha-Alumonah and Alor Uno. No significant different existed between the level of farm yield reduction in Edem ($P > 0.05$).

Chemical control strategies were used extensively by the farmers and more by the male farmers than the female farmers (88.8:81.8; 100:73; 100:42.31; 100:79.29; 100:86.6% for male and female in Edem, Lejja, Eha-Alumonah, Alor – Uno and Ibagwa Agu respectively). The farmers used both organic based insecticide(applying palm head husk ashes to the stem and leaves of the plant and application of aqueous extract of leaves and roots of *Azadiracta indica* which were majorly their traditional control strategies) and inorganic based insecticides(spraying a combination of detergent and kerosene mixed with water, DDT, Cypermethin etc). A higher number of female farmers used the organic insecticide (68.18:0; 30.77:0; 30.77:28.57; 22.14:0 and 66.67:0% for female and male in Edem, Lejja, Eha-Alumonah, Alor–Uno and Ibagwa Agu respectively).The cultural control strategies were also practiced more by the females than the males (18.18:11.11; 15.39:0; 57.69:0; 13.57:0 and 13.33:0 for female and male in Edem, Lejja, Eha-Alumona, Alor Uno and Ibagwa Agu respectively. some traditional cultural methods most utilized were Early harvesting of crops where peppers were harvested while they were still green to minimize the effects of rot and fruit cracking, manuring using goats and cow dung, farm sanitation and crop rotation (Table 5).

In the locations, more farmers (71.16-93%) significantly ($P < 0.05$) used chemical treatments, while only 6.66-28.84% applied cultural methods. Chemical control methods were most utilized in Alor Uno, followed by Ibagwa Agu, and Lejja, while cultural control was most utilized in Eha-Alumona, followed by Edem, and Lejja.

More plants were significantly ($P < 0.05$) attacked in communities with a high chemical

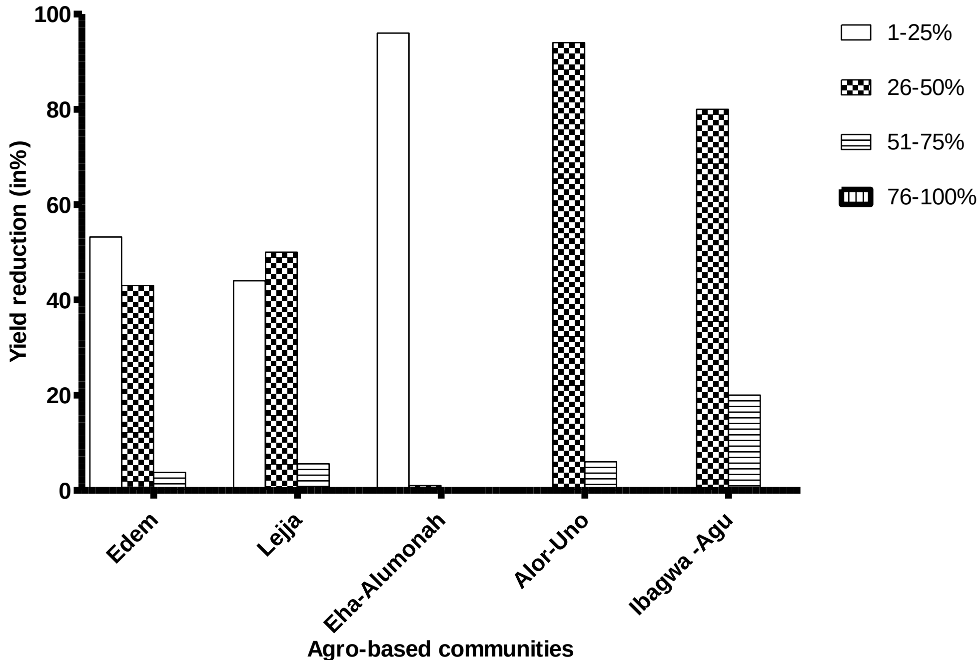

Figure 1. Farmers' perception of percentage reduction in yield from insect infestation in 2015 from the previous year (2014).

control (CH) and low cultural control methods (CC). Thus the highest number of plants attacked (48%) by insects was encountered in Ibagwa Agu, followed by Alor Uno (41%), Edem (32%), Lejja (29%) and Eha-Alumona (27%) with CH to CC ratio of 93:6.66; 98:6.78; 85.3:14.7; 89:15.37 and 71.16:28.84%. The number of pests was higher in communities with high CH and low CC. In communities with high number of predators, lower number of insect pests was encountered, while communities with lower number of predators, higher number of pests was collected (Table 6).

DISCUSSION

The survey of farmers' practices in the cropping of yellow pepper revealed that yellow pepper cultivation was the main source of income of the farmers. Indeed in Nigeria, yellow pepper is the second market gardening crop after tomato (Madu et al., 2005; Assogba-Komlan et al., 2009). In this study, income generated from yellow pepper cultivation was ₦100,000 to 900.000 annually. The cash potentials from this study, combined with previous studies indicating that it is easy to grow, harvest and process, make yellow pepper suitable for use in poverty reduction (Dagnoko et al., 2013). In all the communities sampled a higher percentage of the farmers were females, making women a crucial resource in agriculture and rural economy (FAO, 2011).

The insect pests of yellow pepper probe from Enugu State yielded a greater number of Hemiptera collections than the other orders of insects. Four species belonging to the order Hemiptera were found viz; *M. persicae, A. gossypi, B. tabaci* and *H. schoutedeni,* while two were Coleopterans (*N. sjostdi* and *E. albovittata*), one species for Orthoptera and Diptera each (*Z. variegatus* and *Zonosemata* spp. respectively). One Lepidoptera, *B. betularia* and Thysanoptera*, S. dorsalis* were collected. Praying mantis and Ladybird beetle *H. axyridis* were the only predators collected.

Insect pests have been reported as the main biotic constraints of yellow pepper cultivation (Ingerson-Mahar et al., 2015; Seal and Martin, 2016). Of the 10 insect pests identified, four: *M. persicae, B.tabaci*, *A. gossypii,* and *Z. variegatus* were the prevalent and therefore the major pests of yellow pepper in Nsukka agro-based communities sampled, while *H. schoutedeni, N. sjostedi, S. dorsalis,* E. *albovittata*, *B. betularia* and *Zonosemata* spp were minor pests. This is at variance with an earlier report where *H. schoutedeni* and *N. sjostedi* were the prevalent insest pests of pepper, while *Z. variegatus* was a minor pest (Echezona and Nganwuchu, 2006).

M. persicae which was observed attacking roots, in earlier studies, has been reported as being ubiquitous and attacking nearly130 plant families including pepper (Uzo and Williams, 1989; Helmet, 2013). It causes decreased growth, shriveling of the leaves and the death of various tissues, in addition to transmitting leaf coil and growth distortion denoviruses (Van Munster et al., 2013), while white flies *B. tabaci*, observed attacking leaves and

Table 5. Insect control methods in the agro-based communities

	Sex		Cultural control strategies				Total	Chemical control						No Control
								Organic			Total	Inorganic	Total	
			1	2	3	4		5	6	7		8		
Edem	M	18	-	-	2(100)	-	2(11.11)	-	-	-	-	16(88.88)	16(88.88)	-
	F	22			4(100)		4(18.18)	3(20)	2(13.33)	10(66.67)	15(68.18)	5(22.27)	18(81.8)	-
Lejja	M	14	-	-	-	-	-	-	-	-	-	14(100)	14(100)	-
	F	26	-	1(25)	1(25)	2(50)	4(15.39)	2(22.22)	2(22.22)	4(44.44)	8(30.77)	10(38.46)	19(73.08)	3(11.54)
Eha-alumona	M	14	-	-	-	-	-		1(25)	3(75)	4(28.57)	10(71.43)	14(100)	-
	F	26	2(24.1)	2(24.1)	3(36.14)	1.3(15.66)	15(57.69)	2(25)	1(12.5)	5(62.5)	8(30.77)	3(11.54)	1142.31)	0
Alor uno	M	12	-	-	-	-	-	-	-	-	-	12(100)	12(100)	-
	F	28	1(26.32)	1(26.32)	1.8	-	3.8(13.57)	2.2(35.48)	2(32.26)	2(32.62)	6.2(22.14)	16(57.14)	22.2(79.29)	2(7.14)
Ibagwa agu	M	10	-	-	-	-	-	-	-	-	-	10(100)	10(100)	-
	F	30	1(25)	1(25)	2(50)	-	4(13.33)	3(15)	2(10)	15(75)	20(66.67)	6(20)	26(86.67)	-

1- Early harvesting while fruits were still green; 2- farm sanitation; 3- Manuring using goats, cow dung and chicken droppings; 4- Crop rotation; 5- Farm sanitation; 6- *Elaise guinensis* husk ashes; 7- Aqueous extracts of leaves and seeds of neem (*Azadiracta indica*); 8- Synthetic chemical insecticides.

flowers in this study, have also been reported as vectors of viruses, all of which have led to loss in plant yield (Ingerson-Mahar et al., 2015). *B. tabaci* (silver leaf white fly) is one of the most destructive pests of mainly vegetables and ornamental crop world-wide and has vast host range, polyvoltinism, and short generation time, ability to transmit important plant viruses have contributed to its enormous damage potential (Shadmany et al., 2013).

In addition, *B. tabaci*, is a cryptic species complex with at least 32 biotypes based on the 3.5% divergent limit of the partial mitochondrial cytochrome oxiase subunit 1 (mt CO1) sequence (Dinsdale, 2010; Chowa-Reddy et al., 2012). Among all biotypes, B and Q are the most invasive and the huge losses caused by this pest are almost always associated with these biotypes (Chowa-Reddy et al., 2012). In many cases upon introduction into a region, they establish and partially or completely displace indigenous biotypes (Rao et al., 2011). Both invasive biotypes have already invaded and continue to invade many other countries around the world. Timely identification of the biotypes in Nsukka area can help prevent or reduce huge economic loses. Further studies are recommended to ascertain the biotype(s) in Enugu State and if it is the invasive Q type, quarantine measures can be used to hinder or delay its further spread into other States in Nigeria.

A. gossypii which was found on the shoot of the yellow pepper plants has been earlier reported as a major pest of many crops including pepper and transmits over 50 plant viruses (Blackman and Eastop, 2006).

Z. variegatus was collected from the shoot of the pepper plants in this study and previous studies report *Z. variegatus* as an important polyphagous pest of vegetable and food crops in West and central Africa and in Nigeria, yield loses have been recorded for various crops, including pepper and outside physical damage to leaves and shoots, it also transmits viruses and bacteria (Modder, 1986).

Helopeltis schoutedenti, E. albovittata, S. dorsalis, N. sjostedti collected from shoots (first three pests) and leaf (last pest)have been reported to feed on shoots, branches or whole plants and are known to cause crop failure all over the world, larvae of *B. betularia* and *Zonosemata* spp found in pepper fruits in this study, breed in pepper fruits causing enormous fruit lose (Helmet, 2013).

M. persicae and B. tabaci were more abundant in Ibagwa Agu and Akor Uno than any of the other three sampled communities and may account for the higher number of plants attacked and subsequent loss in yield in this sturdy. In Ibagwa Agu, all the farmers suffered from very high yield loss, 80 and 20% suffered from 26-50% and 51-75% respectively, while in Alor Uno, crop yield loss was also high, 91.9% and 8.1%.

Table 6. Effects of control methods on the numbers of predatory insects, predominant insect pests and plants attacked in the agro-based communities.

Agro-based communities sampled	*Predominant insect pests (% species collection)	*Plants attacked (mean%)	Predatory Insects (%)	Control methods
Ibagwa-Agu	*Myzus persicae (*23.9) *Bemisia tabaci* (22.7) *Aphis gossypii* (23.3) *Zonocerus variegatus* (7.5)	48	*Harmoniaxyridis* (13.2%) *Stagmonantiscarolina* (16.1)	CH(93.34%) CC(6.66%) NC(0%)
Edem	*Myzus persicae* (12.7) *Bemisia tabaci* (11.4) *Aphis gossypii* (13.4) *Zonocerus variegatus* (23.6)	32	*Harmoniaxyridis* (18.9) *Stagmonantiscarolina* (22.6)	CH(85.3%) CC(14.7%) NC(0%)
Lejja	*Myzus persicae* (15.9) *Bemisia tabaci* (16.0) *Aphis gossypii* (18.9)	29	*Harmoniaxyridis* (24.5) *Stagmonantiscarolina* (12.9)	CH(89%) CC(15.39%) NC(6%)
Eha- Alumona	*Myzus persicae* (18.8) *Bemisia tabaci* (22.2) *Aphis gossypii* (28.0) *Zonocerus variegatusi* (22.5)	27	*Harmoniaxyridis* (28.3) *Stagmonantiscarolina* (29.0)	CH(71.16%) CC(28.84%) NC(0%)
Alor-Uno	*Myzus persicae* (27.6) *Bemisia tabaci* (21.1) *Aphis gossypii* (14.1) *Zonocerus variegatus* (7.99)	41	*Harmoniaxyridis* (15.1) *Stagmonantiscarolina* (19.4)	CH(89.65%) CC(6.78%) NC(3.57%)

*Field observations. CH = Chemical Control method; CC= Cultural Control method; NC = No Control.

Yeild loses were lower in Edem, Lejja and lowest in Eha-Alumona, and this correlated with the lower percentage of plants attacked. Lower numbers of the major pests may also have accounted for this reduction in yield lose. This explanation may be plausible for Edem and Lejja, but not for Eha-Alumonah. In Eha-Alumonah, higher numbers of the major insect pest were encountered than Edem and Lejja, but fewer plants and yield loses were recorded in Eha-Alumonah than Lejja and Edem. This may be as a result of the presence of a higher number of the predatory insects in Eha-Alumonah, *H. axyrids* (28.3%) and *Stagmonantis carolina* (29.0%), when compared to the lower numbers in Edem (18.9:22.6% and 18.9:22.6% for *H. axyrids* and S *carolina* for Edem and Lejja respectively). Furthermore, the two communities (Edem and Lejja) with the highest number of plants attacked had the lowest number of predators (Table 3). Communities with high number of predators had low number of the insect pets. The predators may have eaten some of the insect pests reducing their numbers. Praying mantis have been reported in previous studies as large insects that feed on beetles, grasshoppers, wasps, bees, and any insect they can catch Bodson (2014), while *H. axyridis* preys on aphids and scale insects (Emden, 2011).

Chemical and cultural practices were the major control methods utilized by farmers in these communities in this study. The survey also showed that most of the farmers use insecticides to mitigate pests. Chemical insecticides therefore, seems to be the popular pest control method among famers. In assessing the pest management techniques among farmers in Cameroon, West Africa, 92% of the farmers used synthetic pesticides (Abang et al., 2014).

The chemicals utilized for chemical control included organic and inorganic based chemicals. The inorganic based chemicals were DDT, Cypermethrin, detergent and kerosene solution, etc., while the inorganic based chemicals control strategies which were mostly their traditional control methods were *Elaise guinensis* husk ashes sprinkled on the stem and leaves of the plant, aqueous extracts of leaves and seeds of neem sprayed on the plants. Some of these strategies are already being utilized such as soap solution which was reported to be effectively used in the control of *A. gossypii* (Blackman and Eastop, 2006). The use of wood ashes sprinkled around yellow pepper plants and other vegetables kept away caterpillars and was reportedly used to control insectsin ealier studies (Golob and Webley, 1980). The water extracts of the seeds and the leaves of neem tree were also previously reported by Suhmutter et al. (1984).In addition, in all the localities sampled, more farmers utilized inorganic based insecticides than organic. This study also revealed that communities with high percentage of farmers utilizing chemical control, experienced higher insect pest attack on plants and higher yield loss, and lower numbers of predatory insects. The insects may have become resistant to the insecticides as reported for *B. tabaci* which has developed resistance against almost all groups of insecticides (Rao et al., 2011). Synthetic chemical insecticides have also been reported as affecting non-target organisms. In this study, non- target organisms such as the predators may have been killed by the insecticide, reducing their numbers and off course the number of insect pests eaten. Furthermore, exposure to pesticides is one of the most important occupational risks among farmers in developing countries (Wesseling et al., 2001; Konradsen et al., 2003). Hepatic dysfunctions (El-Demerdash et al., 2001), nephrotoxic effects such as cholinesterase inhibitor poisoning have been reported (Zahm and Blair, 1992). Cancer and even death are more frequent among farmers than the general population (Gertrudis et al., 2001; Mansour, 2004). Furthermore, cytogenetic studies showed an increase in DNA damage and higher chromosomal aberrations (CAs) in exposed farmers compared to the control subjects (Naravaneni, 2007. These adverse effects from synthetic chemical insecticides mandates farmers to explore other eco-friendly, safe and efficacious insect pest control strategies such as organic based traditional chemical control methods and cultural methods. Further studies are necessary to determine the efficaciousness of the traditional organic base chemical control strategies.

A diverse number of cultural methods were used by some of the farmers in this study to control pests, these were early harvesting of crops, (a traditional method of pest control where crops were harvested while they were still green to minimize the effects of rot and fruit cracking). This method also prevented damage by fruit worms which attack pepper fruits when they begin to ripen. In the study also, some farmers claimed that chicken droppings, goat and cow dung have proved useful in plant protection, when sprayed on all green plants of vegetable and fruits. Earlier studies revealed that while the use of animal excrement may increase soil nutrient, and subsequently, increase plant growth, increased rates of poultry droppings, increased aphids (except *B. tabaci*), mired and grasshopper infestations as well as incidences and severities of pepper venial mottle virus (PVMV) symptoms, compared to where no manure was applied; but not (Echezona and Nganwuchu, 2006). Chicken droppings may have selective effects on the insect pests. In this study, the lowest percentage reduction in yield was from the community utilizing the highest percentage of cultural control (Eha Alumnoah). Further studies are needed to ascertain the efficacy of these control measures, indicating the pests that can be effectively controlled by the measure as was indicated for chicken droppings. And also the effects of the cultural control methods on non- target insects especially the predators.

The survey also showed some perceptional gender differences in the derived savannah zone of Eastern Nigeria. Males perceived the pest incidence with an equal importance, while females recognized *Z. variegatus* as one of the major pests. Males used vague attributes like harmful or harmless, while females were more specific e.g gave vivid description of *Z. variegatus* biting off the shoot of the young transplanted pepper plants. A higher percentage of the females used the cultural control methods more than the males such as sprinkling of ash on shoots and leaves, spraying with goats and cow dung, early harvesting, farm sanitation and crop rotation, while the males used chemical insecticides to control the insect pests more frequently. This choice of cultural control amongst the females was not in recognition of the reduced impact on non- target organism, or its sustainable and environment safety measures, but because it was cheap and also easier to use. The females who could afford chemical insecticides used them. A higher number of females than males also observed that yellow pepper farms close to herbaceous fallows were attacked by greater number of *Z. variegatus* than those far away from them and close to the forests. Herbaceous fallows provide favorable breeding sites for *Z. variegatus* (Modder, 1986). This observation made the fallow cultural farming system unpopular among farmers, while crop rotation was encouraged. Crop rotation is the practice of growing a series of dissimilar or different types of crops in the same area in sequential seasons. Among other advantages, it mitigates the build -up of pathogens and pests that often occur when one species is continuously cropped (Francis, 2003). This is especially important as the study revealed that yellow pepper was not intercropped with other crops, therefore monoculture was the major practice among the farmers and pests population and pathogens are commonly built up in mono culture unlike in polyculture (Francis, 2003). Crop rotation is one component of polyculture and should be encouraged amongst the farmers.

Conclusion

It is evident from the investigations, that the farmers in Edem, Eha-Alumona, Lejja, Alor-Uno and Ibagwa-Agu in Nsukka Local Government of Enugu State experience enormous economic loss in yellow pepper cultivation from the infestation of insect pests. Most of them use chemical methods to mitigate insect pests, while only a few, which were mostly females utilize cultural methods. Some of the communities with a high percentage of farmers practicing cultural control methods had fewer pest and plants attacked, while the predators' numbers were also higher than communities with fewer farmers utilizing cultural control methods. Further research is needed to ascertain the efficacy of the cultural methods. Given the wide array of human health effects from pesticide exposure, cultural control measures should be encouraged, especially crop rotation to avoid a build-up of pests and pathogens.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Seed health tests of traditional leafy vegetables and pathogenicity in plants

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Traditional leafy vegetables play a significant role in the daily diets of many people in Zimbabwe. They are produced by smallholder farmers with limited knowledge on the agronomic value of high-quality seed, on seed storage, and on plant- and seed-borne pathogens. The quality of the seed is rarely tested for seed-borne pathogens posing risks of pathogen build-up. This study was conducted in order to determine the seed-borne pathogens associated with traditional leafy vegetable seeds. Seeds were collected from five different farmers in three provinces of Zimbabwe and tested for the presence of pathogens in the plant pathology laboratory of the University of Zimbabwe. A total of 154 fungal and 233 bacterial infections were recorded on 450 seeds for each type of pathogen. Significantly high percentages of the isolates (*Xanthomonas campestris, Curvularia spp., Alternaria alternata, Fusarium oxysporum and Pseudomonas syringae*) were obtained from the three different species of traditional leafy vegetables (*Amaranthus hybridus*, *Bidens pilosa* and *Cleome gynandra*). *F. oxysporum, A. alternata, X. campestris* and *Bacillus* spp. isolates were pathogenic to traditional leafy vegetable plant species as confirmed by pathogenicity tests, hence may have significant effects on traditional leafy vegetables production. The results indicate that seed sourced from farmers 1, 2, 3 and 4 resulted in seed-borne pathogens on both the seed and plants, clearly showing that seed quality was poor. Although most pathogens observed have a broad host spectrum, pathogenicity and virulence tests confirmed that some pathogens were likely to be closely associated with these traditional leafy vegetable seed species.

Key words: Seed-borne pathogens, disease occurrence, pathogenicity, bacteria, fungi, seed quality.

INTRODUCTION

Traditional leafy vegetables (TLVs) of Africa refer to plant species, which originated on the continent and have a long history of cultivation, domestication and use in African conditions (Ambrose-Oji, 2009). *Amaranthus spp.* combine both social and socio-economic importance in Kenya and Tanzania as a multi-purpose vegetable with environmental adaptability, nutritional, medicinal and income generation properties (Weinberger and Msuya, 2004; Abukutsa-Onyango, 2007), and have gained popularity also in Zimbabwe. Mukwereza (2002) reported

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spider flower as the most preferred TLV in Zimbabwe. Together with *Bidens pilosa* and *Cleome gynandra* (spider flower) *Amaranthus spp.* also combine resilience to climate change, nutritional, medicinal and income generation properties.

Across Africa, TLVs are produced and marketed by smallholder farmers (Ngugi et al., 2007) with limited access to advanced agro-technology including limited knowledge of the agronomic value of high-quality seed (Biemond et al., 2012), lack of information on seed storage (Cernansky, 2015), on appropriate use of chemicals like pesticides and fertilizers (Schippers, 2000), plants and seed-borne pathogens. These smallholder farmers obtain most of their seeds from their neighbours or informal seed markets (Ngwerume and Mvere, 2003; Adebooye et al., 2005; Guei et al., 2011).

This seed is most likely to be of poor quality. Seed is rarely tested for germination and seed-borne pathogens, increasing risks of seed degeneration across generations. Seed-borne pathogens can prevent germination and cause serious threats to seedling establishment (Valkonen and Koponen, 1990; Walcott, 2003) and plant vigour (Anjorin and Mohammed, 2014). Farmers using poor-quality seed risk poor germination and the ultimate crop stand in their fields will be poor. This may also affect crop yields and the quality of their vegetables on the market (Biemond et al., 2012; Mancini and Romanazzi, 2013).

Most TLVs are propagated by seed (Grubben and Denton, 2004). However, seed is the most important vehicle for transmitting plant pathogens globally (Kuan, 1988). Plant pathogens are reported to have close associations with seed, as they can survive in, on or with seed, hence can be preserved in seed lots (Copeland and McDonald, 2001), at times in latent stage, are temperature dependent (Ngadze and Icishahayo, 2014), and manifest themselves and become problematic only when environmental conditions become conducive for their growth and reproduction (Copeland and McDonald, 2001). Seed has the potential to carry a wide range of pathogens including fungi, bacteria, viruses, many of which cause diseases in various plant developmental stages (from seedling to the product on the markets) (Copeland and McDonald, 2001).

A seed health test is an important component for determining the quality of seed worldwide, a prerequisite to minimise losses by assessing the quality of seed before it is sown (Copeland and McDonald, 2001). Seed health tests have been done on various seeds including tree seed, vegetable seed, and cereal seed (International Seed Testing Association - Seed Health (ISTA), 2015). Relatively very limited research on TLV seed has been reported (Sharma et al., 1980) including research on seed health tests, posing risks with seed germination (Cernansky, 2015). Plants are frequently left in the field for too long, risking seed-borne pathogens accumulation on seed (Adebooye et al., 2005).

TLV seed is affected by several seed-borne pathogens such as *Rhizoctonia spp., Sclerotinia spp., Pythium spp., Choanephora cucurbitarum, Alternaria spp.,* Mycoplasma*, Cercospora spp., Sphaerotheca fuliginea*, and *Oidiopsis taurica* (Sharma et al., 1980; Grubben and Denton, 2004), and some TLVs are important hosts of *Xanthomonas campestris pv*. *campestris* (Bradbury, 1986), which is also an important seed-borne pathogen of many plant species.

Economically, important examples of these seed-borne pathogens include *Pythium spp., Pythium aphanidermatum* and *Rhizoctonia spp.* causing a serious damping off problem in the *Amaranthus hybridus* seedbed, *Cercospora bidentis* and *Cercospora uramensis* affecting *Bidens pilosa* and *C. gynandra*, respectively (Grubben and Denton, 2004). *C. gynandra* seed is also affected by a range of seed-borne fungi including *Sphaerotheca fuliginea* and *Oidiopsis taurica*. These pathogens were reported to cause powdery mildew on *C. gynandra* plants (Grubben and Denton, 2004).

In Zimbabwe, over 75% growers of TLVs collect and retain seeds for sowing in the next season (Schippers, 2000) and the quality of the seed is rarely known. Moreover, farmers rely on traditional knowledge that is passed on through generations and often use traditional cropping technologies (Schippers, 2000). Considering that most of the seed-borne pathogens affecting TLV seed including *X. campestris*, *Cercospora spp, S. solani* and *Pythium spp.* cause serious problems in vegetable production in Zimbabwe (Handiseni et al., 2008), there is, therefore, a high risk of seed-borne infections. Moreover, Rothwell (1982) listed *Entyloma bidentis*, *Sclerotium rolfsii*, *Sphaceloma spp.*, *Uromyce bidenticola*, *Verticillium dahliae*, *Pseudomonas solanacearum* and *Oidium spp.* as pathogens that affect *Bidens pilosa*, *Amaranthus spp.* and *Cleome spp.* in Zimbabwe. Primary inoculum can also arise from other sources like contaminated tools and equipment (Jones et al., 1986), some TLVs are important host of *Xanthomonas campestris pv*. *campestris* (Bradbury, 1986), plant debris (Barak and Liang, 2008). Nevertheless, seed-borne inoculum remains paramount importance for most pathogen infections (Naseri and Mousavi, 2015).

Generally, seed pathogens transferred to plants during growth cause considerable loss of crop yields globally (Copeland and McDonald, 2001), but limited literature on the economic losses caused by these pathogens on TLVs have been reported. For example, Ngadze (2014) reported that some plant diseases may affect the plants during the growing phase and later stages in storage as well. The pathogens which are seed-borne affect the whole TLV food chain.

Initially starting with pathogen infected seed, the pathogens may result in poor crop stand due to poor germination or poor-quality seedlings which cannot withstand relative environmental changes- dying away of plants. In storage and on the markets, the quality of the product is also affected by the pathogens including those passed through the use of infected seed-borne. This may result in food and nutrition insecurity and loss of incomes to the farmers, if little attention is paid to controlling seed-borne pathogens.

The research aims at detection and identification of seed-borne pathogens on TLV seed and establishes the relationship between seed infection and pathogen which develop on the plants in the field. In addition, the study seeks to ignite researches on the diseases and their economic impact on TLV seed. The specific objectives are:

(1) To identify seed-borne pathogens in TLV seed from different sources;
(2) To investigate whether pathogens found on seed are pathogenic to the plants.

The main research question is:

(1) Do seed-borne infections contribute to the disease symptoms observed in the field?

Therefore the hypotheses tested include:

(1) Seed-borne pathogens' presence varies among seed sources;
(2) Seed-borne pathogens cause the diseases observed on the leaves of infected plants.

MATERIALS AND METHODS

The study uses three traditional leafy vegetable seed namely *C. gynandra*, *B. pilosa* and *A. hybridus* as examples. *C. gynandra* and *A. hybridus* are C_4 plants (Imbamba et al., 1977; Schippers, 2000), while *B. pilosa* is a C_3 plant. Traditional leafy vegetables have a significant role in the traditional diets of many people in Zimbabwe.

Origin of the seed sources

Seed of the three species was sourced from five different farmers. These farmers were chosen using a snow ball sampling technique, with the entry point as the traditional leafy vegetable markets. Only farmers who had seed stocks from the previous season showing that they had been producing TLV seed were chosen. About 500 g of seed for each species was sourced from the farmers and used for seed health tests. One farmer was a government research institute, that is, Horticultural Research Centre (HRC). Lot sampling was done for seed sourced from HRC because they had bigger quantities in their stock than required and hand sampling was used to come up with approximately 500 grams of seed per each species. For the remaining other seed sources, all the seed available was sourced and most of the seed lots weighed less than 1 kg per species per source. The seed collection consisted of 15 samples (three samples of three species per seed source). Subsampling to approximately 400 seeds per sample was done using the hand halving method (International Seed Testing Association (ISTA), 2016). The seed was sourced from three different agro ecological regions of Zimbabwe: Mashonaland Central, Mashonaland West and Mashonaland East. These provinces (agro ecological regions) are some of the major vegetable growing ones of Zimbabwe, because of their proximity to major cities and because they are located in areas of high agricultural potential. Our research used only five seed sources and intended to ignite more research on these vegetables across Zimbabwe. The climatic conditions during crop growth and geographical coordinates of the seed origin sites are presented in Table 1.

Detection of seed-borne pathogens

Seeds of the three TLV types were randomly selected and 150 seeds of each type of pathogen were plated on potato dextrose agar (PDA) and nutrient agar (NA) for isolation of fungi and bacteria respectively. The seeds were surface sterilized in 1% sodium hypochorite and rinsed three times with sterile distilled water. The sterile seeds were blotted with sterile filter paper (Whatman no.1) to remove excess water prior to plating. A total of 10 seeds were plated out on a 90 mm diameter petri dish. Seeds for fungal identification were plated on PDA while those for bacteria were placed on NA plates. Each Petri dish was replicated 6 times (3 plates bacteria and 3 for fungi). The Petri dishes with seeds on PDA were incubated at 25±2°C under 12/12 alternating cycles of NUV and darkness in Plant Pathology incubation room at the University of Zimbabwe for seven days. The seeds on NA were placed in an incubator (Gallenkemp- Sussex, England) and maintained at 30±2°C for 4 days. After incubation the seeds were viewed under a stereo microscope using the 16× objective.

Identification of fungi

After the incubation period the fungi, which grew on the seeds, were examined and identified under stereomicroscope (WILD M3B, Heerbrugg, Switzerland) based on the growth characters of the fungi. Slides were prepared using the associated fungi on seeds for identification under a compound microscope (JENA LABOVAL, Göttingen, Germany) for better identification following the keys described by Mathur and Olga (2001) and Commonwealth Mycological Institute descriptions.

Identification of bacteria

After the incubation period, further plating was done on the culture media, colonies of bacteria purified on the solidified potato dextrose agar or nutrient agar, respectively, were then incubated at $25^{o}C$ for 48 h. The morphological identification of the spores for fungi and bacteria was performed according to Mathur and Olga (2001) and Schaad et al. (2001). Single colonies of each isolate were used for physiological and biochemical tests. The isolates were identified by standard bacterial methods based on Cother and Sivasithamparam (1983). The tests performed were Gram reaction, oxidase activity, glucose metabolism, pectate degradation in Sutton's medium, production of phosphatase, indole production from tryptophan, gelatine hydrolysis, production of reducing substances from sucrose, production of acid from α-methylglucoside and trehalose, malonate utilisation, sensitivity to erythromycin (15 µg), growth at 37ºC which was determined after 24 h in nutrient broth (NB, Difco) and salt tolerance which was checked after 48 h growth in NB with 5 g l^{-1} NaCl.

Detection and identification of pathogens on infected plants

The aim was to investigate whether seed-borne pathogens on seed corresponded to disease symptoms observed on

Table 1. Geographical coordinates and climatic data during crop growth of the origin sites of the seed.

Farmer	Origin	Agro ecological region [a]	Mean annual temperature	Temperature range during crop growth	Geographical coordinates	Altitude (m)	Annual rainfall (mm)	Amount of rainfall during crop growth (mm)
1	Mashonaland West	2B	22-29°C	31-22°C	17°22'00" S: 30°11'59" E	1153	700-800	73-0.31
2	Mashonaland East	3	18-29°C	31-21°C	17° 38' 35.59" S: 31° 47' 2.40" E	1400	800-850	100-0.8
3	Mashonaland East	3	18-29°C	31-21°C	17° 38' 35.59" S: 31° 47' 2.40" E	1400	800-850	100-0.8
4	Mashonaland Central	3	21-31°C	31-21°C	17° 18' 56" S: 31° 34' 14" E	953	800-850	110-1.5
5	Mashonaland East	2B	18-29°C	29-27°C	18° 11' 6.97" S: 31° 33' 6.95" E)	1688	850-900	105.7-24.8

Note: Agro ecological classification also known as natural region (NR) classification of Zimbabwe, divided the country into five regions based on mean annual rainfall and was done in the 1960s (Vincent and Thomas, 1960).
Source: authors, https://www.worldweatheronline.com Historical average weather 20/04/2017 (for full details Annex 1 and 2 description).

plants. Plants were grown in the greenhouse at the University of Zimbabwe in February of 2017. A randomized complete block design with three blocks was used. The pots were labelled and randomly arranged and filled to approximately 6 cm under the rim. The soil was sterilised at 70°C for 24 h and allowed to cool. A mixture of soil and pine bark at ratio 1:3 was done to reduce soil compaction and placed in pots. Proper levelling of the growing media was done before approximately 1000 seeds were evenly distributed per pot. The seeds were then covered with 1.5 cm thick layer of the growing media. Water as needed was added to the pots. The presence of the diseases on plants was also recorded in the greenhouse (for example, damping off).

The infected plant parts showing typical symptoms of diseases were collected for further analysis. Plant pathogens were isolated from different parts of the crops that had advanced lesions and rots. The lesions and surrounding healthy tissues were cut into small pieces and washed in running tap water before sterilizing with 1% sodium hypochlorite for 2 to 3 min, followed by rinsing three times in sterile distilled water, and drying on sterile blotter papers. Disinfected pieces were placed on solidified Nutrient Agar (NA) medium (Schaad et al., 2001). The Petri dishes were incubated at 28±2°C and observed for bacterial growth 4 to 6 days later. After the incubation period, the further plating was done on the culture media, colonies of fungi and bacteria were purified on the solidified potato dextrose agar or nutrient agar, respectively, and were then incubated at 25°C for 48 h. Thereafter, the morphological identification of the spores for fungi and bacteria was performed according to Mathur and Olga (2001) and Schaad et al. (2001).

Hypersensitivity

Isolated colonies of different bacteria were tested for ability to induce a hypersensitive response on foliage of the non-host plant tobacco (*Nicotiana tabacum* cv. Burley). Five isolates were selected for each of the three bacteria species isolated from seed and infected plants. Test isolates were freshly grown on NA at 30±2°C for 24 h and suspended in sterile water, maintain an inoculums concentration of 10^8 cfu/ml (using Genie spectrophotometer at 550 nm). Tobacco seedlings were inoculated with suspensions of the selected isolates at the 5 to 6 leaf stage. 1 ml of bacterial suspension was injected using a disposable syringe into the lower surface of the tobacco leaves and the plants were sealed in plastic bags for 24 h to prevent desiccation. Plants were uncovered and kept in the laboratory at 28±2°C for 36 h. Control plants were injected with sterile distilled water and kept under same conditions.

Virulence test

For the assessment of pathogenicity of the different bacteria on TLV seedlings, inoculum suspensions were prepared in sterile water as previously described for the hypersensitive test. Young TLV seedlings were inoculated with 1 ml of inoculum using a hypodermic syringe. Control leaves were inoculated with sterile distilled water.

Data analysis

Data were analysed using GenStat 18th edition. The data were transformed using LOG10 plus 1 and subjected to analysis of variance (ANOVA) and the means of the parameters were separated using the Duncan's multiple range least significant difference test ($P \leq 0.05$). The transformation was done to normalise the data sets because some data sets had many zeros.

RESULTS

A total of 154 fungal and 233 bacterial infections were recorded on 450 seeds (for each type of pathogen) selected from seeds of three types

Table 2. Hypersensitive responses of the different isolates tested on tobacco.

Isolate	Source	Hypersensitive response (+/-)	Duration (hrs)	Virulence
Xanthomonas campestris 1	Seed	+	24	High
Xanthomonas campestris 2	Plant	+	36	Weak
Xanthomonas campestris 3	Seed	+	36	High
Xanthomonas campestris 4	Seed	+	24	Moderate
Xanthomonas campestris 5	Plant	+	72	High
Pseudomonas syringae 1	Seed	+	36	Weak
Pseudomonas syringae 2	Seed	+	24	Weak
Pseudomonas syringae 3	Plant	+	48	High
Pseudomonas syringae 4	Plant	+	72	High
Pseudomonas syringae 5	Plant	+	72	High
Bacillus spp. 1	Seed	+	24	Moderate
Bacillus spp. 2	Plant	+	72	Weak
Bacillus spp. 3	Seed	+	72	High
Bacillus spp. 4	Seed	+	24	High
Bacillus spp. 5	Plant	+	36	Weak
Control	Distilled water	-	24-72	-

of TLV. The fungi were identified based on colony morphologies as *Curvularia spp.*, *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus spp.*, *Phoma betae, Colletotrichum spp.* and *Rhizoctonia solani*. Three bacteria species were isolated from the seed and they were identified as *Xanthomonas campestris*, *Pseudomonas syringae* and *Bacillus spp*. The seed-borne infections varied according to seed species and source. The virulence test conducted on young TLV seedlings varied by pathogen species and seed species.

Hypersensitivity test

Table 2 shows hypersensitivity responses of the different isolates tested on tobacco. Ten of the fifteen isolates tested on tobacco plants induced a hypersensitivity test within 24 to 48 h after inoculation, and based on these reactions they were categorized as strongly, moderately, or mildly virulent. All isolates were considered as pathogenic since they produced a reaction within 72 h after inoculation. *X. campestris* isolates 1, 3 and 5, *P. syringae* isolates 3, 4 and 5 as well as *Bacillus* spp. Isolates 3 and 4 were regarded as highly virulent. Two isolates *X. campestris* 4 and *Bacillus* spp. 1 were moderately virulent while isolates *X. campestri* 2, *Pseudomonas syringae* 1 and 2 as well as *Bacillus spp.* 1 and 5 were considered to be weakly virulent.

Virulence tests

All isolates gave a positive response on all three TLV plant species (Table 3). The isolates were ranked as highly, moderately and weakly virulent pathogens based on host responses. Among these highly virulent isolates, symptoms in plants inoculated with *X. campestris* isolates 1, 3 and 4 were highly virulent on *B. pilosa* and *C. gynandra,* and moderately virulent on *Amaranthus hybridus* and the symptoms developed earlier than with other isolates. Plants inoculated with these isolates showed disease symptoms within 6 days. *Pseudomonas syringae* isolates 3 and 4 were highly virulent on *B. pilosa* and *A. hybridus*. No symptoms developed in controls treated with sterile water.

Isolation of pathogens from seed by plant species and source

The percentage occurrence of seed-borne pathogens for plant species and seed farmers were evaluated and are presented in Tables 4 and 5, respectively. For both cases, the percentage occurrence refers to the frequency of any single isolate observed on each seed which is measured as the proportion of the seeds per species that are infected by seed-borne pathogens. The percentage occurrence was calculated by adjusting Jager et al. (2007) calculations as number of seeds having the pathogen during the analysis divided by the total number of seeds used per species and the ratio is multiplied by 100 (following formula used) and subsequently used to determine the differences by performing the analysis of variance and separation of means using Duncan's multiple range method in GenStat.

$$Percentage\ occurrence = \frac{No.\,of\ seeds\ affected\ by\ a\ pathogen}{Total\ no.\,of\ seeds} x\ 100$$

Table 3. Virulence of the different isolates on seedlings of traditional leafy vegetables.

Isolate	*Amaranthus hybridus*	*Cleome gynandra*	*Bidens pilosa*
Xanthomonas campestris 1	++	+++	+++
Xanthomonas campestris 2	++	+	+
Xanthomonas campestris 3	++	+	+++
Xanthomonas campestris 4	++	+++	+++
Xanthomonas campestris 5	++	+++	++
Pseudomonas syringae 1	++	++	+
Pseudomonas syringae 2	++	+	++
Pseudomonas syringae 3	+++	++	+++
Pseudomonas syringae 4	+++	++	+++
Pseudomonas syringae 5	++	++	++
Bacillus spp. 1	+++	+++	++
Bacillus spp. 2	+	++	+++
Bacillus spp. 3	+	++	+
Bacillus spp. 4	+++	+	++
Bacillus spp. 5	+++	++	+++
Control	-	-	-

Note: +++, ++ and + denote highly, moderately and weakly virulent pathogens, respectively.

Table 4. The percentage occurrence of seed-borne pathogens on seed for three plant species (between brackets the transformed values and the mean separation).

Name of pathogen	Plant species			$LSD_{5\%}$
	Amaranthus hybridus	*Cleome gynandra*	*Bidens pilosa*	
Bacteria				
Xanthomonas campestris	1.3 (0.09[a])	79.3 (1.60[b])	58.7 (1.20[b])	0.57
Pseudomonas syringae	6.7 (0.36[ab])	0.0 (0.00[a])	8.7 (0.53[b])	0.40
Bacillus spp.	0.7 (0.07[a])	0.0 (0.00[a])	0.0 (0.00[a])	0.11
Fungi				
Alternaria alternata	1.3 (0.14[a])	18.7 (1.12[c])	10.7 (0.74[b])	0.38
Aspergillus spp.	0.0 (0.00[a])	0.0 (0.00[a])	4.7 (0.19[a])	0.22
Fusarium oxysporum	12.0 (0.57[a])	3.3 (0.18[a])	1.3 (0.14[a])	0.41
Curvularia spp.	2.7 (0.23[a])	25.3 (0.78[a])	18.7 (0.69[a])	0.55
Phoma betae	0.0 (0.00[a])	0.0 (0.00[a])	1.3 (0.09[a])	0.15
Colletotrichum spp.	0.0 (0.00[a])	1.3 (0.14[a])	0.0 (0.00[a])	0.16
Rhizoctonia solani	1.3 (0.09[a])	0.0 (0.00[a])	0.0 (0.00[a])	0.15

The mean separation indicates per pathogen the differences among the three species ($P \leq 0.05$).

Table 4 shows the percentage occurrence of seed-borne pathogens isolated from seeds of three species. In comparison among plant species, highest percentage occurrence of seed-borne pathogens was observed on *C. gynandra*, followed by *B. pilosa* and least on *A. hybridus* seed. Only *X. campestris*, *P. syringae* and *A. alternata* isolates were significantly different for the percentage occurrence of seed-borne pathogens on seed among the three plant species.

For *X. campestris,* the percentage occurrence of seed-borne pathogens on seed for *C. gynandra* and *B. pilosa* did not differ from each other, which was significantly higher than for *A. hybridus*. On the other hand, *C. gynandra* showed the lowest percentage occurrence on *P. syringae*. For both plant species, the percentage occurrence of *A. alternata* on seed was highest on *C. gynandra*, intermediate on *B. pilosa* and lowest on *A. hybridus*.

Table 5 shows the percentage occurrence of seed-borne pathogens per farmer. The percentage occurrence of a single bacterial or fungal isolate differed among the farmers but only significantly for *Curvularia spp.* and

Table 5. The percentages occurrence of seed-borne pathogens per seed farmer (between brackets the transformed values and the mean separation).

Name of pathogen	Seed source					*LSD 5%*
	Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	
Bacteria						
Xanthomonas campestris	35.7 (0.82[a])	64.4 (1.33[a])	66.7 (1.34[a])	33.3 (0.67[a])	32.2 (0.66[a])	0.95
Pseudomonas syringae	3.3 (0.17[a])	2.2 (0.15[a])	7.8 (0.44[a])	6.7 (0.33[a])	5.6 (0.41[a])	0.56
Bacillus spp.	0.0 (0.00[a])	0.0 (0.00[a])	0.0 (0.00[a])	0.0 (0.00[a])	1.11 (0.12[a])	0.15
Fungi						
Alternaria alternata	7.8 (0.64[a])	16.7 (0.93[a])	10.0 (0.67[a])	10.0 (0.58[a])	6.7 (0.53[a])	0.64
Aspergillus spp.	0.0 (0.00[a])	0.0 (0.00[a])	0.0 (0.00[a])	6.7 (0.20[a])	1.1 (0.12[a])	0.29
Fusarium oxysporum	11.1 (0.38[a])	2.2 (0.23[a])	0.0 (0.00[a])	6.7 (0.41[a])	7.8 (0.45[a])	0.55
Curvularia spp.	41.1 (1.32c)	3.3 (0.35[ab])	30.0 (0.81[bc])	3.3 (0.35[ab])	0.0 (0.00[a])	0.61
Phoma betae	0.0 (0.00[a])	0.0 (0.00[a])	0.0 (0.00[a])	2.2 (0.15[a])	0.0 (0.00[a])	0.19
Colletotrichum spp.	0.0 (0.00[a])	2.2 (0.23b)	0.0 (0.00[a])	0.0 (0.00[a])	0.0 (0.00[a])	0.20
Rhizoctonia solani	2.2 (0.15[a])	0.0 (0.00[a])	0.0 (0.00[a])	0.0 (0.00[a])	0.0 (0.00[a])	0.33

The mean separation indicates per pathogen the differences among the five farmers ($P \leq 0.05$).
Source: The two-way interactions between farmer and TLV species on percentage occurrence of seed-borne pathogens are seen in Annex 3 to Annex 12.

Colletotrichum spp. The lowest percentage occurrence of *Curvularia spp.* was detected for farmer 5, followed in order by farmers 3, 2, and 4 and highest for farmer 1. For *Colletotrichum spp.* the highest percentage occurrence of a single isolate was observed for farmer 2, and the remaining farmers were not significantly different from each other.

The difference between TLV species in the percentage occurrence of the *X. campestris* isolate (Annex 3) depends on the farmer, with farmer 1 showing occurrences very different from the other farmers. Similarly, a two-way ANOVA carried out on the *P. syringae* isolate (Annex 4) showed that the difference in occurrence between TLV species depended on the farmer, with *A. hybridus* and *B. pilosa* showing much more variation among farmers than *C. gynandra*. For the *Bacillus spp.* isolate (Annex 5), the results showed that the ranking of the species for each farmer was only different for Farmer 5. The results of a two-way ANOVA on the *A. alternata* isolate (Annex 6) depicted that the order of occurrence of the disease among the TLV species strongly depended on species and varied considerably.

Similarly, for the *Aspergillus spp.* isolate (Annex 7), the results showed that the difference between farmers depended on TLV species with farmer 4 showing the largest differences. For the two-way ANOVA carried out for the *F. oxysporum* isolate (Annex 8), the findings showed that the differences among, but also the ranking of the TLV species were different for each farmer. The results for the *Curvularia spp.* isolate (Annex 9) also showed that both the differences among and the ranking of the TLV species differed among farmers. For the *Phoma betae* isolate (Annex 10), the ranking of the TLV species was consistent among the farmers, but the extent of the differences among the species differed among farmers.

A two-way ANOVA was also carried out to assess the interaction between farmer and TLV species on the *Colletotrichum spp.* isolate (Annex 11), demonstrating that the variation among TLV species was larger for farmer 2 than for the other farmers. For the *Rhizoctonia solani* isolate (Annex 12), Farmer 1 showed the largest difference among TLV species, especially because *A. hybridus* had a high value.

Pathogenicity tests

Table 6 depicts the occurrence of seed- and soil-borne pathogens on plants. The occurrence here refers to the presence of seed-borne pathogens on plant parts (showing typical symptoms of diseases) and were not scored or scaled. For pathogenicity tests of seed-borne diseases on plants, two bacterial and six fungal species were identified. The two bacterial species included *X. campestris* and *Bacillus spp.* while the fungal species were *A. alternata*, *F. oxysporum*, *Trichoderma spp.*, *Penicillium spp*., *Erysiphe spp.* and *Pythium spp*. Several soil pathogens including *Bacillus spp.*, *Trichoderma spp.* and *Penicillium spp*. were also isolated from the plant parts. The farmers with an asterisk indicate that the observed pathogen were also present on seed.

Comparison of seed-borne pathogens on seed corresponds to pathogenicity on plants

The pathogenicity tests on the plants indicated that *F.*

Table 6. The occurrence of seed and soil-borne pathogens on plants.

Name of pathogen	Plant species		
	Amaranthus hybridus	***Cleome gynandra***	***Bidens pilosa***
Bacteria			
Xanthomonas campestris	Absent	Present (Farmer 2)*	Absent
Bacillus spp.	Absent	Present (Farmer 4)	Present (Farmer 2)
Fungi			
Alternaria alternata	Present (Farmer 1)*	Absent	Present (Farmer 3)*
Fusarium oxysporum	Present (Farmer 1)*	Present (Farmer 4)*	Present (Farmer 5)
Penicillium spp.	Present (Farmer 1)	Present (Farmer 2)	Present (Farmer 2)
Trichoderma spp.	Absent	Present (Farmer 4)	Absent
Erysiphe spp.	Absent	Absent	Present (Farmer 3)
Pythium spp.	Present (Farmer 4)	Absent	Absent

The words in parentheses represent the farmers; * observed pathogens were also present on seed.

oxysporum and *Penicillium spp.* had a wide range of infections as they were observed on all the three species, followed by *A. alternata* and *Bacillus spp.* as these pathogens were present on two species while *X. campestris*, *Trichoderma* spp., *Erysiphe* spp. and *Pythium* spp. had the least pathogenicity range, present only on one species. Occurrence of *X. campestris, A. alternata* and *F. oxysporum* on both seed and plants was observed. Occurrence of seed-borne pathogens on both the seed and plants was observed for farmers 1, 2, 3 and 4.

DISCUSSION

Seed heath testing is important for four reasons: reduce seed-borne inoculum incidences and give an economic value to the seed lot, its requirements to satisfy the seed movement, elucidate seedling evaluation and causes of poor germination and give recommendation and perform seed lot treatment in order to eradicate seed-borne pathogens and reduce the risk of disease transmission (ISTA, 2015).

This study aims at identifying seed-borne pathogens in TLV seed from different farmers. This is primarily useful since the literature on seed-borne pathogens for TLV seed is scanty and farmers normally rely on literature for other crops like exotic cabbages which are relatively different in terms of production requirements or they rely on traditional production knowledge that is passed on through generations (Schippers, 2000). Unlike cabbage seed from relatively improved hybrid varieties, TLV seed predominantly is from traditional unimproved varieties. Therefore, in Zimbabwe, their production requirements are totally different from cabbage and TLV farmers should not rely on information on cabbage. Furthermore, this study investigated whether pathogens found on seed showed pathogenicity to the plants. There is limited literature on pathogenicity tests for TLVs, hence this study aims at filling that gap.

Seed samples of three TLV plant species from five different farmers were compared for the percentage occurrence of seed-borne pathogen isolate on each seed. Furthermore, the study investigated whether pathogens found on seed show pathogenicity to the plants. Pathogenicity tests were conducted and showed pathogenicity effects on seeds and plants. Major pathogens included *X. campestris, A. alternata* and *F. oxysporum*. The seed-borne pathogens *X. campestris* and *Alternaria spp.* were also reported to be pathogenic to traditional leaf vegetables in several other studies (Sharma et al., 1980; Bradbury,1986; Grubben and Denton, 2004), but no such reports was made on *F. oxysporum*. The results also indicated that seed sourced from farmers 1, 2, 3 and 4 had occurrence of seed-borne pathogens on both the seed and plants (Table 6). These results clearly show that farmers use bad seed.

Further, comparison on accumulative percentage occurrence for the 10 seed-borne pathogens observed (Table 5) showed wide ranges: farmer 3 (114.4), followed by farmer 1 (101.2), then, third, farmer 2 (91.0), the fourth, farmer 4 (68.9) and least, farmer 5 (54.51). The superiority of farmer 5 over other farmers was expected, because it is a research organization, which has much better knowledge on seed production compared to its counterparts. On the other hand, differences in the percentage occurrence of seed-borne pathogens among the sources may partially be explained by different crop management practices during seed development as previously reported by Bradbury (1986) and Naseri and Emami (2013). Bradbury (1986) reported that some TLVs are important hosts of *X. campestris pv*. *campestris*, and, therefore, likely sources of inoculum if diseased plants are left to grow and subsequently seed is harvested.

Farmers need seed of good quality to start with, for them to be able to satisfy the market requirements. The quality of the seed is comprised of many different attributes that add to the performance of the seed. Being free from seed-borne diseases, is figured out the most important one (Dube and Mujaju, 2013). Observations indicating that higher levels for percentage occurrence of seed-borne pathogen isolate on each seed by farmer (statistically significant at 5%) is also confirmed by the pathogenicity tests. Although, there are not yet established the quality standards of these plant species in literature, farmers require clean seed, that is free from seed-borne diseases for them to increase their yields and get better incomes from their crops.

Many seed-borne pathogens become active when seeds are sown, which may result in seed decay or pre- or post- emergence damping off (Agarwal and Sinclair, 1996), thereby reducing desired plant population in the farmers' fields. Moreover, many seed-borne pathogens, particularly, fungi induce qualitative changes in the physio-chemical properties of seeds such as protein content (Agarwal and Sinclair, 1996) that are fundamental in the germination process. For instance, Mathur and Sehgal (1964) reported that a collective of fungal pathogens contaminants, e.g. *Fusarium spp.*, reduced seed vigour and inhibited seed germination. Notably, some isolates of *Bacillus spp*. decreased seed germination or seedling growth (Broadbent et al., 1977).

The observation of the pathogens on both seed and plants and moderate to high virulence on tested plant species may explain their strong association with seed and ability to give rise to progressive disease development in the field. Such developments influence the ultimate crop yield and reduce the commercial value of the crop as well (Naseri and Mousavi, 2015). This may, therefore, give a positive indication of possible need of control measures. For example, in a study carried by Rude et al. (1999), infection of seed by *Alternaria spp.* greatly reduced seed germination of turnip. Although in this study we did not test the germination of seed, literature regarding these TLV seed has reported difficulties with seed germination (Cernansky, 2015) which cannot be overlooked the incidences of seed-borne as an important factor reducing the seed germination.

Virulence is the degree of pathogenicity exhibited by most of the pathogens and it is a measure that effectively differentiates pathogenic and non-pathogenic strains (Russell and Herward, 2005). All bacteria isolates gave a positive response on all three TLV plant species (Table 3) and with varied magnitudes, ranking as highly, moderately and weakly virulent pathogens based on host responses. Interestingly, among the tested plant species for virulence, none were found to possess a high level of resistance to the present bacteria pathogens observed in the study. Differential virulence on these TLV plant species are further confirmed by pathogenicity test. These results suggest, although the observed pathogens have wide host range, these pathogens were sufficient to cause host-generated infections and if ignored may cause serious problems in TLV seed and vegetables production.

Erysiphe spp. was observed on *B. pilosa* during the late stage of growth, that is, seed development and seed maturation. *Erysiphe cruciferarum* in wild radish has been shown to affect the crop late and is destructive at the seed development stage (Djébali et al., 2009). Schippers (2000), Grubben and Denton (2004) also confirmed the presence of *Alternaria* spp. in East Africa causing damping off. The study results showed that the bacterium *X. campestris*, although present in high quantities on seed of all three plant species, exhibited pathogenicity on *C. gynandra* only and did not show up on *A. hybridus* and *B. pilosa* plants. *X. campestris* has already been reported as a serious pathogen, causing economic problems in vegetables production in Zimbabwe (Handiseni et al., 2008).

Considering its close association with *Cleome gynandra* seed, it poses a serious threat in *C. gynandra* production as well. On the same note, *Trichoderma* spp. was isolated from one sample of *C. gynandra* and it was detected in all the replicates. The plants had leaf spot. *Trichoderma* spp. can behave as an opportunistic pathogen. Harman et al. (2004) confirmed that particular strains of *Trichoderma* spp. in rare cases, are pathogenic to plants and have been reported to cause diseases of the crops such as apples, maize and alfalfa. Some *Trichoderma spp.* strains also grow on leaf surfaces. That probably we detected *Trichoderma* spp. in one of the study samples, may also pose a serious threat in *C. gynandra* production. Also, *Bacillus* spp. was isolated on all three plant species, *A. hybridus* seed and *C. gynandra* and *B. pilosa*. Some *Bacillus* spp. isolates can be beneficial, whereas others can be detrimental in crop production. Broadbent et al. (1977) reported that some *Bacillus* spp. increased plant growth by producing gibberellin or auxin or solubilizing phosphate, while others reduced germination.

CONCLUSION AND RECOMMENDATIONS

Although most of the pathogens observed in the study results have a broad host spectrum, pathogenicity and virulence tests substantiate that some pathogens were more likely to be closely associated with these traditional leafy vegetable seed species. All bacteria isolates gave a positive response on all three TLV plant species and with varied magnitudes, ranking as highly, moderately and weakly virulent pathogens based on host responses. However, none of the plant species were found to possess a high level of resistance to the present bacteria pathogens observed in the study. Furthermore, *X. campestris, F. oxysporum*, *A. alternata* and *Bacillus* spp. isolates were pathogenic to *A. hybridus*, *C. gynandra* and

B. pilosa plants as confirmed by pathogenicity tests. The occurrence of seed-borne pathogens on both the seed and plants for farmers 1, 2, 3 and 4 clearly showed bad seed. One further important area for future research is virulence testing for fungal pathogens using more samples collected from various places around Zimbabwe. Such tests will lead to better understanding of pathogens affecting TLV seed and production, and to more knowledge on certain pathogens prevalent in certain areas. There is limited literature reported on the economic losses caused by these pathogens on TLVs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ANNEX

Annex 1. Climatic data during crop growth of the origin sites of the seed.

Weather	Dec	Jan	Feb	March	April	May	June
Farmer 1							
Temperature (°C)	31	29	30	28	26	23	22
Rain (mm)	73	56	49	98	68.5	11.2	0.3
% cloud cover	24	36	27	33	25	17	5
% humidity	57	69	72	81	68	61	56
Bee activity	-	-	Mod	Mod	High	Low	-
Planting				Sole crop			
Farmer 2							
Temperature (°C)	31	29	30	28	26	22	21
Rain (mm)	100	49	46	85	29.5	17	0.8
% cloud cover	-	39	33	38	22	20	13
% humidity	-	67	76	79	69	67	63
Bee activity	-	-	-	Mod	High	Low	-
Planting				Intercropped with maize			
Farmer 3							
Temperature (°C)	31	29	30	28	26	22	21
Rain (mm)	100	49	46	85	29.5	17	0.8
% cloud cover	-	39	33	38	22	20	13
% humidity	-	67	76	79	69	67	63
Bee activity	-	-	Mod	Mod	High	Low	-
Planting				Intercropped with maize			
Farmer 4							
Temperature (°C)	31	29	30	28	26	22	21
Rain (mm)	110	57	63	29	15	10	1.5
% cloud cover	-	39	33	38	22	20	13
% humidity	-	67	76	79	69	67	63
Bee activity	-	-	Mod	Mod	high	-	-
Planting				Sole crop			
			Farmer 5				
Temperature (°C)	29	27	28	27	-	-	-
Rain (mm)	105.7	14.5	16.7	24.8	-	-	-
% cloud cover	24	33	30	36	29	21	17
% humidity	57	68	67	77	70	69	64
Bee activity	-	-	-	-	-	-	-
Planting				Sole crop			

https://www.worldweatheronline.com Historical average weather 20/04/2017.

Annex 2. Geographical coordinates and climatic data during crop growth of the origin sites of the seed.

The seed was sourced from three different agro ecological regions of Zimbabwe: Mashonaland Central, Mashonaland West and Mashonaland East. The five seed sources were Horticultural Research Centre (HRC) (Farmer 5), Shamva (Farmer 4), Murehwa – two farmers (Macheya- Farmer 2 and Makondora- Farmer 3) and Chinhoyi (Farmer 1). HRC is a government institute located in Mashonaland East province and is under agro ecological region 2B (18° 11' 6.97" S: 31° 33' 6.95" E) at an elevation of 1688 m above sea level (Vincent and Thomas, 1960). The mean annual rainfall for the area ranges from 850 to 900 mm. The mean annual temperature ranges from 18 to 29°C. During seed crop growth temperatures and rainfall ranged (December 2015 to March 2016) from 28.9 to 26.7°C and from 105.7 to 24.8 mm, respectively. Shamva is in Mashonaland Central province and located under agro ecological region 3 (17° 18' 56" S: 31° 34' 14" E) at an elevation of 953 m above sea level, receiving annual rainfall of 800 to 850 mm (Vincent and Thomas, 1960). The mean temperature for Shamva ranges between 21 to 31°C. During seed crop growth temperatures and rainfall ranged (December 2015 to June 2016) from 31 to 21°C and from 110 to 1.5 mm, respectively. Murehwa is located in Mashonaland East province under agro ecological region 3 (17° 38' 35.59" S**:** 31° 47' 2.40" E) at an elevation of 1400 m above sea level (Vincent and Thomas, 1960). The mean annual rainfall ranges from 800 to 850 mm. The mean annual temperature ranges from 18 to 29°C. During seed crop growth temperatures and rainfall were (December 2015 to June 2016) 31 to 21°C and 100 to 0.8 mm, respectively. Chinhoyi is located in Mashonaland West province under agro ecological region 2B (17°22′00″ S: 30°11′59″ E) at an elevation of 1153 m above sea level (Vincent and Thomas, 1960). The mean annual rainfall and temperature ranges from 700 to 800 mm and 22 to 29°C, respectively. During seed crop growth temperatures and rainfall ranged from (December 2015 to June 2016) 31 to 22°C and 73 to 0.31 mm, respectively, (authors, https://www.worldweatheronline.com Historical average weather 20/04/2017), data presented as Annex 1. In Annex 3 to 12, the mean separation indicates per pathogen the differences among the interactions between farmers and three species (Duncan's multiple range least significant difference test, $P \leq 0.05$). For all cases, all values in parentheses are transformed values and mean separation.

Annex 3. The two-way interactions between the farmers and plant species percentage occurrence of *X. campestris.*

Farmer* species	Mean
Farmer 2 *A. hybridus*	0.0(0.0^a)
Farmer 3 *A. hybridus*	0.0(0.0^a)
Farmer 4 *A. hybridus*	0.0(0.0^a)
Farmer 5 *A. hybridus*	0.0(0.0^a)
Farmer 4 *B. pilosa*	0.0(0.0^a)
Farmer 5 *B. pilosa*	0.0(0.0^a)
Farmer 1 *C. gynandra*	0.0(0.0^a)
Farmer 1 *A. hybridus*	0.67(0.44^b)
Farmer 2 *B. pilosa*	9.33(1.97^c)
Farmer 5 *C. gynandra*	9.67(1.99^c)
Farmer 1 *B. pilosa*	10.0(2.00^c)
Farmer 2 *C. gynandra*	10.0(2.00^c)
Farmer 3 *C. gynandra*	10.0(2.00^c)
Farmer 3 *B. pilosa*	10.0(2.00^c)
Farmer 4 *C. gynandra*	10.0 (2.00^c)
LSD 5%	0.33

Annex 4. The two-way interactions between the farmers and plant species percentage occurrence of *P. syringae.*

Farmer*species	Mean
Farmer 2 *A. hybridus*	0.0(0.0[a])
Farmer 1 *B. pilosa*	0.0(0.0[a])
Farmer 1 *C. gynandra*	0.0(0.0[a])
Farmer 2 *C. gynandra*	0.0(0.0[a])
Farmer 3 *B. pilosa*	0.0(0.0[a])
Farmer 3 *C. gynandra*	0.0(0.0[a])
Farmer 4 *A. hybridus*	0.0(0.0[a])
Farmer 4 *C. gynandra*	0.0(0.0[a])
Farmer 5 *A. hybridus*	0.0(0.0[a])
Farmer 5 *C. gynandra*	0.0 (0.0a)
Farmer 2 *B. pilosa*	0.67(0.44[ab])
Farmer 1 *A. hybridus*	1.0 (0.50[ab])
Farmer 4 *B. pilosa*	2.0 (0.99[bc])
Farmer 5 *B. pilosa*	1.67 (1.23[c])
Farmer 3 *A. hybridus*	2.33 (1.33[c])
LSD 5%	0.63

Annex 5. The two-way interactions between the farmers and plant species percentage occurrence of *Bacillus spp.*

Farmer*species	Mean
Farmer 1 *B. pilosa*	0.0 (0.0[a])
Farmer 1 *C. gynandra*	0.0 (0.0[a])
Farmer 2 *B. pilosa*	0.0 (0.0[a])
Farmer 2 *C. gynandra*	0.0 (0.0[a])
Farmer 3 *B. pilosa*	0.0 (0.0[a])
Farmer 3 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *B. pilosa*	0.0 (0.0[a])
Farmer 4 *C. gynandra*	0.0 (0.0[a])
Farmer 5 *B. pilosa*	0.0 (0.0[a])
Farmer 5 *C. gynandra*	0.0 (0.0[a])
Farmer 1 *A. hybridus*	0.0 (0.0[a])
Farmer 2 *A. hybridus*	0.0 (0.0[a])
Farmer 3 *A. hybridus*	0.0 (0.0[a])
Farmer 4 *A. hybridus*	0.0 (0.0[a])
Farmer 5 *A. hybridus*	0.33 (0.35[b])
LSD 5%	0.26

Annex 6. The two-way interactions between the farmers and plant species percentage occurrence of *A. alternate.*

Farmer*species	Mean
Farmer 1 *B. pilosa*	0.0 (0.0[a])
Farmer 2 *A. hybridus*	0.0(0.0[a])
Farmer 3 *A. hybridus*	0.0(0.0[a])
Farmer 4 *A. hybridus*	0.0(0.0[a])
Farmer 5 *A. hybridus*	0.0(0.0[a])
Farmer 5 *C. gynandra*	0.33(0.35[ab])
Farmer 4 *B. pilosa*	0.33(0.35[ab])
Farmer 1 *A. hybridus*	0.67 (0.69[bc])
Farmer 3 *B. pilosa*	0.67 (0.69[bc])
Farmer 1 *C. gynandra*	1.67 (1.23[cd])
Farmer 5 *B. pilosa*	1.67 (1.23[cd])
Farmer 3 *C. gynandra*	2.33 (1.33[d])
Farmer 2 *C. gynandra*	2.33 (1.33[d])
Farmer 4 *C. gynandra*	2.67 (1.38[d])
Farmer 2 *B. pilosa*	2.67 (1.44[d])
LSD 5%	0.57

Annex 7. The two-way interactions between the farmers and plant species percentage occurrence of *Aspergillus spp.*

Farmer*species	Mean
Farmer 5 *A. hybridus*	0.0 (0.0[a])
Farmer 1 *A. hybridus*	0.0 (0.0[a])
Farmer 1 *B. pilosa*	0.0 (0.0[a])
Farmer 1 *C. gynandra*	0.0 (0.0[a])
Farmer 2 *A. hybridus*	0.0 (0.0[a])
Farmer 2 *B. pilosa*	0.0 (0.0[a])
Farmer 2 *C. gynandra*	0.0 (0.0[a])
Farmer 3 *A. hybridus*	0.0 (0.0[a])
Farmer 3 *B. pilosa*	0.0 (0.0[a])
Farmer 3 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *A. hybridus*	0.0 (0.0[a])
Farmer 4 *C. gynandra*	0.0 (0.0[a])
Farmer 5 *C. gynandra*	0.0 (0.0[a])
Farmer 5 *B. pilosa*	0.33 (0.35[ab])
Farmer 4 *B. pilosa*	2.0 (0.60[b])
LSD 5%	0.51

Annex 8. The two-way interactions between the farmers and plant species percentage occurrence of *F. oxysporum.*

Mean	Farmer*species
Farmer 1 *B. pilosa*	0.0 (0.0[a])
Farmer 1 *C. gynandra*	0.0 (0.0[a])
Farmer 2 *A. hybridus*	0.0 (0.0[a])
Farmer 2 *C. gynandra*	0.0 (0.0[a])
Farmer 3 *A. hybridus*	0.0 (0.0[a])
Farmer 3 *B. pilosa*	0.0 (0.0[a])
Farmer 3 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *B. pilosa*	0.0 (0.0[a])
Farmer 5 *B. pilosa*	0.0 (0.0[a])
Farmer 5 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *A. hybridus*	0.33 (0.35[ab])
Farmer 2 *B. pilosa*	0.67 (0.69[bc])
Farmer 4 *C. gynandra*	1.67 (0.88[bc])
Farmer 1 *A. hybridus*	3.33 (1.14[c])
Farmer 5 *A. hybridus*	2.33 (1.34[c])
LSD 5%	0.67

Annex 9. The two-way interactions between the farmers and plant species percentage occurrence of *Curvularia spp.*

Farmer*species	Mean
Farmer 3 *A. hybridus*	0.0 (0.0[a])
Farmer 5 *A. hybridus*	0.0 (0.0[a])
Farmer 2 *A. hybridus*	0.0 (0.0[a])
Farmer 2 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *B. pilosa*	0.0 (0.0[a])
Farmer 5 *B. pilosa*	0.0 (0.0[a])
Farmer 5 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *C. gynandra*	0.33 (0.35[ab])
Farmer 1 *A. hybridus*	0.67 (0.44[ab])
Farmer 3 *B. pilosa*	1.67 (0.57[ab])
Farmer 4 *A. hybridus*	0.67 (0.69[ab])
Farmer 2 *B. pilosa*	1.0 (1.04[b])
Farmer 1 *C. gynandra*	5.0 (1.70[c])
Farmer 1 *B. pilosa*	6.67 (1.83[c])
Farmer 3 *C. gynandra*	7.33 (1.87[c])
LSD 5%	0.65

Annex 10. The two-way interactions between the farmers and plant species percentage occurrence of *P. betae.*

Farmer*species	Mean
Farmer 1 *B. pilosa*	0.0 (0.0[a])
Farmer 2 *B. pilosa*	0.0 (0.0[a])
Farmer 3 *B. pilosa*	0.0 (0.0[a])
Farmer 4 *A. hybridus*	0.0 (0.0[a])
Farmer 4 *C. gynandra*	0.0 (0.0[a])
Farmer 5 *B. pilosa*	0.0 (0.0[a])
Farmer 1 *A. hybridus*	0.0 (0.0[a])
Farmer 1 *C. gynandra*	0.0 (0.0[a])
Farmer 2 *A. hybridus*	0.0 (0.0[a])
Farmer 2 *C. gynandra*	0.0 (0.0[a])
Farmer 3 *A. hybridus*	0.0 (0.0[a])
Farmer 3 *C. gynandra*	0.0 (0.0[a])
Farmer 5 *A. hybridus*	0.0 (0.0[a])
Farmer 5 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *B. pilosa*	0.67 (0.44[b])
LSD 5%	0.33

Annex 11. The two-way interactions between the farmers and plant species percentage occurrence of *Colletotrichum spp.*

Farmer*species	Mean
Farmer 1 *A. hybridus*	0.0 (0.0[a])
Farmer 1 *B. pilosa*	0.0 (0.0[a])
Farmer 1 *C. gynandra*	0.0 (0.0[a])
Farmer 2 *A. hybridus*	0.0 (0.0[a])
Farmer 2 *B. pilosa*	0.0 (0.0[a])
Farmer 3 *A. hybridus*	0.0 (0.0[a])
Farmer 3 *B. pilosa*	0.0 (0.0[a])
Farmer 3 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *A. hybridus*	0.0 (0.0[a])
Farmer 4 *B. pilosa*	0.0 (0.0[a])
Farmer 4 *C. gynandra*	0.0 (0.0[a])
Farmer 5 *A. hybridus*	0.0 (0.0[a])
Farmer 5 *B. pilosa*	0.0 (0.0[a])
Farmer 5 *C. gynandra*	0.0 (0.0[a])
Farmer 2 *C. gynandra*	0.67 (0.69b)
LSD 5%	0.26

Annex 12. The two-way interactions between the farmers and plant species percentage occurrence of *R. solani.*

Farmer*species	Mean
Farmer 1 *B. pilosa*	0.0 (0.0[a])
Farmer 1 *C. gynandra*	0.0 (0.0[a])
Farmer 2 *A. hybridus*	0.0 (0.0[a])
Farmer 2 *B. pilosa*	0.0 (0.0[a])
Farmer 2 *C. gynandra*	0.0 (0.0[a])
Farmer 3 *A. hybridus*	0.0 (0.0[a])
Farmer 3 *B. pilosa*	0.0 (0.0[a])
Farmer 3 *C. gynandra*	0.0 (0.0[a])
Farmer 4 *A. hybridus*	0.0 (0.0[a])
Farmer 4 *B. pilosa*	0.0 (0.0[a])
Farmer 4 *C. gynandra*	0.0 (0.0[a])
Farmer 5 *A. hybridus*	0.0 (0.0[a])
Farmer 5 *B. pilosa*	0.0 (0.0[a])
Farmer 5 *C. gynandra*	0.0 (0.0[a])
Farmer 1 *A. hybridus*	0.67 (0.44[b])
LSD 5%	0.33

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Soybean development under soil water deficit

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One of the main factors affecting soybean development is water deficiency, especially when it occurs during the flowering and pods filling stages. Thus, this study aimed to evaluate the soybean development under soil water deficit in different phenological phases, under soil and climatic conditions of Bom Jesus, Piaui State, Brazil. The test was conducted at São Luiz Farm, located 3 km from Bom Jesus to PI, during the period from August to December, 2014. The test was conducted with a drip irrigation system using drip tape with 0.3 m between emitters and nominal flow of 1.6 L h $^{to\ 1}$. The water deficit was applied in the vegetative development stages (treatment I), flowering and grains filling (treatment II) and grain maturation (treatment III), compared to the control treatment (full irrigation throughout the cycle). Plant height, number of green leaves, leaf area index and plant dry matter were evaluated. The water deficit in the flowering and pod filling stages inhibited the BRS Sambaíba RR cultivar growth, contributing to a reduction of up to 33.2, 34.7, 41.3 and 13.7% of plant height, number of leaves, leaf area index and total dry mass, respectively compared to the control.

Key words: *Glycine max*, water balance, water availability.

INTRODUCTION

Soybean is the Brazilian crop with the highest increase in planted area during the last three decades and corresponds to 49% of the area planted with grains in the country. From the 1970s, the crop became the most important in the Brazilian agribusiness, expanding its production to occupy the 'Cerrado' lands.

Currently, the State of Piauí presents a great potential for grain production, mainly soy. During the 2015/2016 harvest season, the area planted with soybean in Piauí reached a level of 564,118 ha, with an average yield of 2099 kg ha^{-1} (CONAB, 2016). The municipalities of Baixa Grande do Ribeiro, Uruçuí, Ribeiro Gonçalves, Santa Filomena, Bom Jesus, Currais, Gilbués, Monte Alegre do Piauí, Sebastião Leal, Palmeira do Piauí and Corrente, stand out as the most soy producing municipalities in the southwest of the Piaui State (IBGE, 2015).

One of the main factors that affect the development of the crop is the soil water deficiency. The phenological phase of the crop in which the water deficit occurs leads to different morphophysiological changes. The existence of water deficit throughout the beginning of the grains filling and the beginning of the stage of green grains can

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Table 1. Chemical soil characteristics of the experimental area, Bom Jesus-PI, 2014.

Depth (m)	OM (g/kg)	pH (H_2O)	P (mg dm^{-3})	K	Ca	Mg	Na	Al	H+Al	S	CEC	V	M
				-------------------- (mmolc dm^{-3}) ----------------								%	
0.0-0.2	4.1	5.4	7.4	55.0	0.5	0.5	8.0	0.2	9.90	3.4	3.27	10.3	14.9
0.2-0.4	4.1	5.1	4.2	45.0	0.2	0.2	5.0	0.2	1.65	6.4	1.80	23.3	27.3

Table 2. Physical-water characteristics of the experimental area, Bom Jesus-PI, 2014.

Depth (m)	Granulometry (g kg^{-1})			Texture classification	FC	PWP
	Sand	Silt	Clay		(% volume)	
0.0-0.2	900.0	40.0	60.0	Loamy sand	23.11	4.3
0.2-0.4	880.0	50.0	70.0	Sand	18.8	4.3

FC, field capacity defined at – 6 kPa. PWP, permanent wilting point defined at -1500 kPa. Source: Soil Laboratory, Embrapa Mid-North, Teresina- PI, Brazil, 2014.

drastically reduce the soybean yield, because almost half of the nutrients required for the pods filling come from the soil and the biological fixation of nitrogen (Nunes, 2016).

Tavares et al. (2013) observed that the water deficit during the soybean vegetative period does not affect the first pod height, number of nodes of the main stem, number of pods with one and two seeds and the seeds yield per plant. However, it reduced plant height, stem diameter and number of pods with three seeds. The water deficit caused a reduction in the plant leaf area of 30% in stage R4 (vegetative development) and 35% in stage R6 (pod filling).

Knowledge of the water restriction interactions with soybean development stages is important for the establishment of adequate management strategies, since at various stages of crop development morphological and physiological events are responsible for the definition of the production potential. The present paper aimed to evaluate the development of soybean under soil water deficit during under the soil and climatic conditions of Bom Jesus, Piaui State, Brazil.

MATERIALS AND METHODS

The experiment was conducted at the site Luiz, located 3 km from the City of Bom Jesus to PI, in the 'Cerrado' of the southwestern region of the Piaui State, during the period from August to December, 2014 at the following geographical coordinates, obtained using GPS: 9º05'20,4 '' S, 44º20'55,1 '' W and 283 m of altitude. The annual average rainfall of the region is defined by the continental equatorial regime, with annual rainfall from 700 to 1,300 mm and the rainy season extending from November to April, with the months of January, February and March being the most humid period (Silva et al., 2013). The soil of the area is classified as Fluvic Neosol (EMBRAPA, 2013), whose chemical and physical to hydro characteristics are presented in Tables 1 and 2, respectively.

The preparation of the experimental area consisted of plowing and narrowing. The soil liming was applied 30 days before planting, with 1 t ha^{-1} of filer limestone, followed by incorporation to the soil using a harrow. Soil fertility and cover fertilization were performed based on previous soil fertility analysis of the experimental area (Table 1) and following the nutritional requirements for soybean (EMBRAPA, 2011). The base fertilization consisted of the application of 20 kg ha^{-1} of N, 100 kg ha^{-1} of P_2O_5 and 30 kg ha^{-1} of K_2O, being 30 kg ha^{-1} of K_2O applied as cover at 30 days after germination.

The soil water retention curve was determined according to the method described by Embrapa (1997) and adjusted according to the model proposed by van Genuchten (1980), with the aid of the Soil Water Retention Curve (SWPC) version 3.0 (Dourado Neto et al., 2001).

The cultivar BRS Sambaíba RR was evaluated due to its excellent adaptation to different environments. Seeding was performed on August, 15th 2014, manually, at a spacing of 0.5 m between rows, obtaining 13 plants per linear meter. The experiment was kept free of weeds by 3 manual controls and one glyphosate application. The phytosanitary treatment was carried out through two applications of the Engeo Pleno (Thiametoxan + Lambda to cyhalothrin + Inert Ingredients) insecticide at 20 and 75 DAS. The test was conducted with a drip irrigation system, using drip tape, with emitter spacing of 0.3 m, with nominal flow of 1.6 L h^{-1}, whose control of the passage of water through the water pipe administered to the plots was performed by a ½ to inch log and a pressure controller installed at the beginning of each plot. Each plot presented a total area of 33 m^2, consisting of 11 rows of 6 m in length, spaced 0.5 m between rows and 78 plants per row. The first and last rows were considered as borders.

The experiment was structured in randomized blocks, in plots subdivided in time with 6 repetitions. The plots consisted of soil water deficit in different phenological stages of the crop [T1: imposition of water deficit in the vegetative development stage (23 - 48 days after sowing - DAS), T2: imposition of water deficit in the flowering stage and pods filling (49 to 89 DAS), T3: imposition of water deficit during the pods maturation stage (90 to 120 DAS) and T4: without soil water deficiency, which means full irrigation in all development stages]. The subplots were represented by 11 plant samples collected during the growing cycle. The application of soil water deficit at each stage of soybean development was based on the 50% replacement of ETc which occurred between 2 consecutive irrigations, so that the soil moisture remained always below the critical depletion (p = 0.50). At the end of the water deficit application in each phonological stage, the full irrigation was resumed, completing 100% of the ETc replacement in other development stages. The control of the applied water was realized using hydrometers installed at the beginning of each experimental block.

Irrigation management was carried out using crop evapotranspiration (ETc) estimated based on the reference evapotranspiration (ETo) and on soybean cultivation coefficients (Kc) recommended by FAO (Allen et al., 1998). The daily values of ETo were estimated using the Penman to Monteith method, using climatic data obtained from the INMET automatic meteorological station, installed at the Federal University of Piauí to Campus Professor Cinobelina Elvas, Bom Jesus, PI, Brazil. Monitoring of soil water content was carried out by the gravimetric method, where soil samples were collected at depths of 0 - 0.2 m and from 0.2 - 0.4 m. The samples were collected 30 min before the beginning and 30 min after the irrigation was finished, as a way of assessing the values of soil water content imposed by the application of full and deficit irrigation. With this monitoring, it was possible to know the conditions of the soil water availability, both in terms of volumetric moisture and in terms of soil water potential, in which the crop was submitted, with the application of water deficit treatments.

The measured variables were: a) mean height of first pod insertion (MHFP), measured from the soil surface to the insertion of the first pod; b) number of green leaves per plant (NGP), obtained by counting all green leaves in 3 plants of the useful plot; c) leaf area index (LAI): the leaves of the plants were manually removed from the stem and then taken to an electronic meter of area LI to 3100, to determine the leaf area (cm²) and then multiplied by the number of plants in a linear meter and (d) total dry matter (TDM): after measuring the leaf area, the leaves and stems of the plant were packed in paper bags and put to drying in an air forced oven at 65°C until reaching constant weight and then weighed, in an analytical scale, with an accuracy of 0.001 g. These measurements were carried out weekly, totaling 11 samplings during the growing cycle. The variance analysis was performed, applying the "F" test using the software Assistat version 7.6 (Silva and Azevedo, 2009).

RESULTS AND DISCUSSION

During the experiment, the accumulated rainfall was 124.6 mm, in which 80.6 mm were registered during pod maturation, only compromising the water deficit management during stage IV (Figure 2).

The soil water content presented a clear variation according to water deficiency treatments, before and after irrigation, in the 2 layers of the soil, reflecting the availability of different water during water deficit imposition, as well as the extraction of soil water by the plant roots (Figures 3 and 4). The water content of the soil under full irrigation (PI) before irrigation ranged from 13.44% (in the 0 - 0.2 m depth) to 14.07% (in the 0.2 - 0.4 m depth); it always fluctuated near the critical water depletion for soybean (12.7%). After irrigation, the water content approached the field capacity, with soil moisture oscillating around 20.94% (at 0.0 - 0.2 m depth) to 21.07% (at the depth of 0.2 - 0.4 m), corresponding to 62.81 and 63.19% of the available water capacity (AWC) in the soil before irrigation, and 90.61 and 100.47% of the AWC after irrigation (Figure 3A and B).

Regarding the water content, it achieved 7.45% (0.0 - 0.2 m) and 9.28% (0.2 - 0.4 m) before irrigation for the treatment DII; always below the critical water depletion for soybean (12.7%). After irrigation the water content continued below the critical water depletion, 11.28% (0.0 - 0.2 m) and 12.23% (0.2 - 0.4 m), corresponding to 35.53 and 44.25% of the available water capacity in the soil before irrigation and 53.79 and 58.32% of the AWC after irrigation (Figure 3C and 3D).

The water content in the treatment DIII was very similar to the DII treatment, ranging from 8.51% (0.0 - 0.2 m) and 8.82% (0.2 - 0.4 m) before irrigation and 11.88% (0.0 - 0.2 m) and 12.13% (0.2 - 0.4 m) after irrigation corresponding to 40.59 and 42.07% of the available water capacity in the soil before irrigation and 56.66 and 57.82% of AWC after irrigation.

Based on the soil water retention curve (Figure 1), soil water stress values under full irrigation conditions ranged from 8 to 18 kPa (0.0 - 0.2 m) and from 6 to 8 kPa (0.2 - 0.4 m), close to the field capacity limit (FC). Normally, in 'Cerrado' sandy soils, it is defined that the water in the soil is in the FC when it is retained at a tension of 10 kPa (Andrade and Stone, 2011). Soil water tension ranged from 54 to 20 kPa (0 - 0.2 and 0.2 - 0.4m) before irrigation and from 26 to 14 kPa (0 - 0.2 and 0.2 - 0.4 m) after irrigation in DII, while it ranged from 54 to 48 kPa (0 - 0.2 and 0.2 - 0.4 m) before irrigation and from 24 - 14 kPa (0 - 0.2 and 0.2 - 0.4m) after irrigation in DIII, indicating that the plants in the layers of (0.0 - 0.2) experienced period of water deficiency in the soil, since the tension values were higher than those recommended for beginning of irrigations in the soybean, which is 37 kPa (0 - 0.2 m) (Saad and Libardi, 1992).

During the application of the DIV treatment, it was not possible to apply the desired water deficit, due to precipitation, in a few days during this phase, which raised the soil moisture to levels above the critical storage for the crop. In fact, before irrigation, in the 0 - 0.2 and 0.2 - 0.4 m layers, soil moisture ranged from 11.3 to 12.08%, while moisture after irrigation ranged from 12.97-13.7% (Figure 4C and D). In fact, the soil moisture variation in this treatment closely resembled that of full irrigation treatment (Figure 3A and 3B) and, therefore, the soil water deficit planned for this phase was compromised. In Table 3, it is possible to observe the variance analysis for the variables plant height (AP), number of leaves (NL) leaf area index (LAI) and total dry matter (TDM) during different sampling periods. There was a significant interaction for all variables among the evaluated factors. Thus, the results and discussion are presented based on the growing curves of these variables during the soybean development stages.

Figure 5 shows the variation of the height of soybean plants in response to the treatments applied throughout their phenological phases. Table 4 shows the regression equations of the curves shown in Figure 5. At the end of the cycle, it was verified that the height of the plants under full irrigation was 69.3 cm; 51.2 cm when submitted to phase II water deficit (DII); 46.3 cm when submitted to

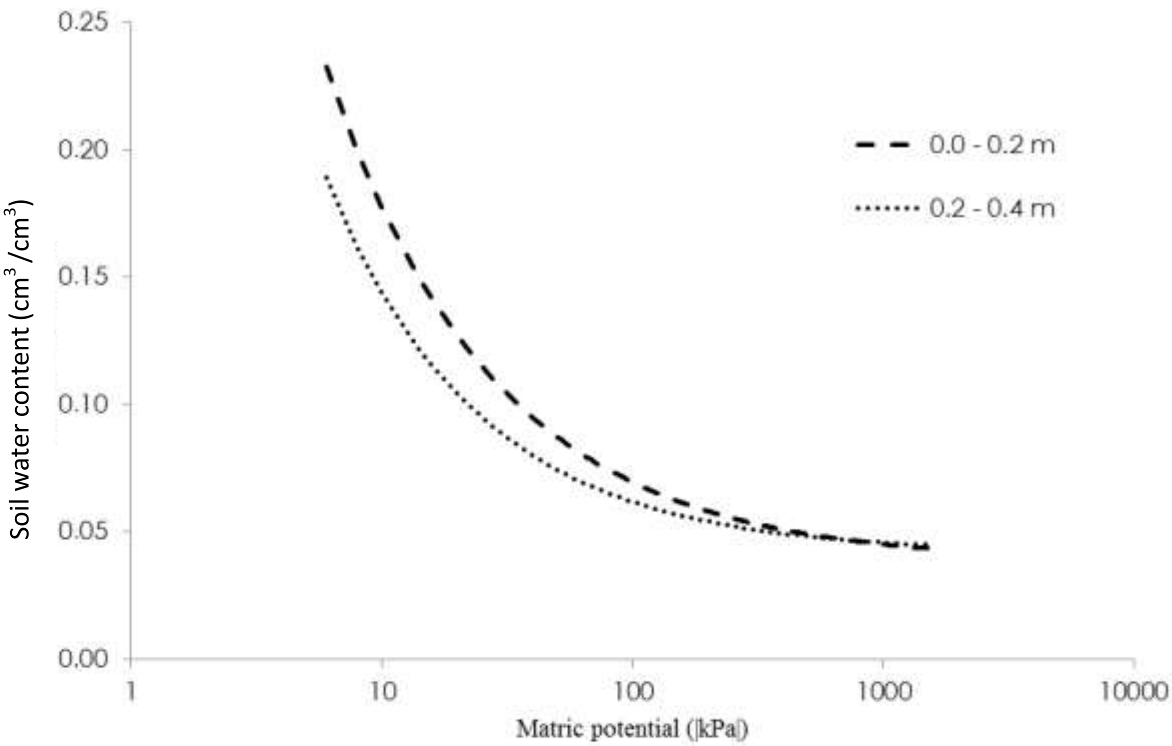


Figure 1. Curve of soil water retention for two soil depths of the experimental area.

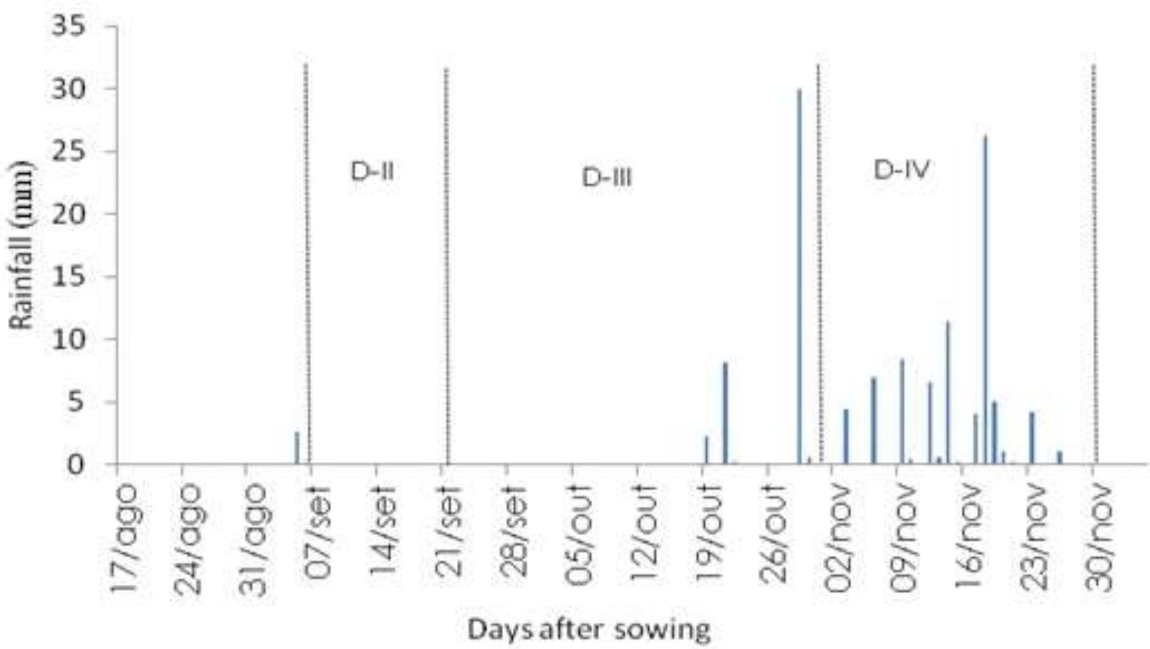


Figure 2. Daily rainfall registered during the experimental period. Bom Jesus, PI, Brazil, aug-nov, 2014.

water deficit in phase III (DIII) and 66.7 cm when submitted to phase IV water deficit (IVD), indicating that, independently of the phenological phase in which the water deficit occurred, there was a decrease in height of

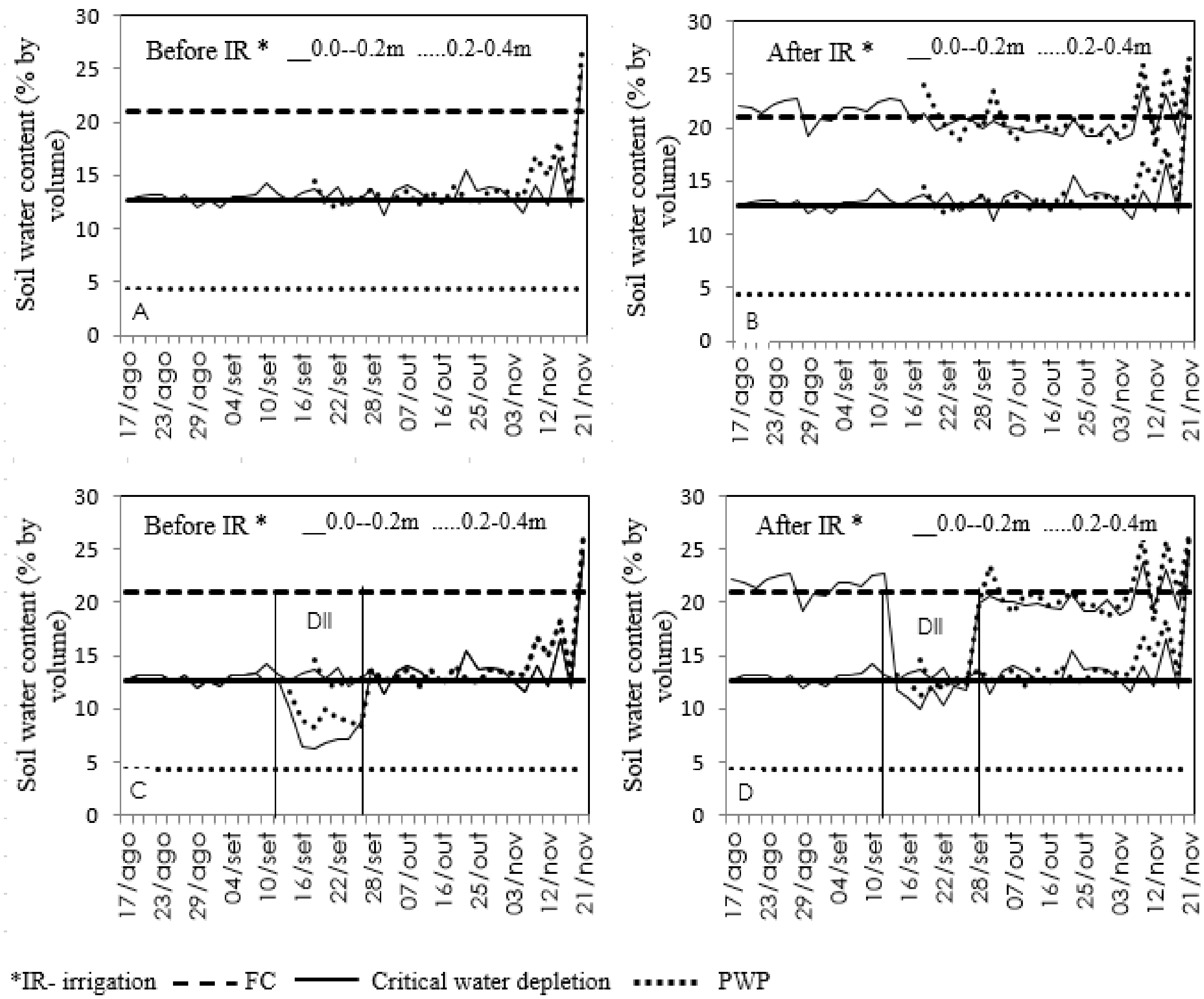


Figure 3. Soil water content in the depths 0-0.2 and 0.2-0.4m before irrigation (A) and after irrigation (B) for the control treatment (IP) and before (C) and after (B) the irrigation for the water deficit treatment during the stage II (DII). (----) Field capacity, (**—**) soy critical water depletion, (····) permanent wilting point.

plants in relation to full irrigation (PI) treatment. Although there was no significant difference in plant height among treatments in which the soil water deficit was applied in phases II and III, the reduction in plant height was more drastic when the lack of water occurred in phase III, considered critical, in which culture requires more water to satisfy its metabolic activities.

In phase IV, the water deficit effect on plant height was lower, similar to the IP treatment, because it is the final phase of the crop cycle, and the beginning of senescence and also due to the rainfall during the phase DIV (Figure 3G and 3H), which should suffer water deficit. According to Ferrari et al. (2015), the first plants response to water deficiency in the soil is the reduction of turgescence, inhibiting photosynthesis and, consequently, the growth process, during its development. The soybean height is of fundamental importance because it is a characteristic that is normally correlated with the production characteristics (Rocha et al., 2012). Fornasieri Filho (2007) affirmed that the decrease in plant height plant can be explained in part by the fact that the association between the root system and the aerial part (A/R) varies according to environmental factors in the different plant development stages. Under water deficiency in the soil, it induces a reduction in the A/R value due to inhibition of shoot development and greater root growth.

Rocha et al. (2012), studied varieties and strains of soybean in low latitude conditions in Teresina to PI, Brazil; they observed for the cultivar BRS Sambaíba, average plant height of 73.4 cm, higher than the values observed in the present study, probably because the experiment have been conducted under ideal conditions regarding the nutritional aspect or plant water supply.

Figure 6 shows the variation of soybean leaves number

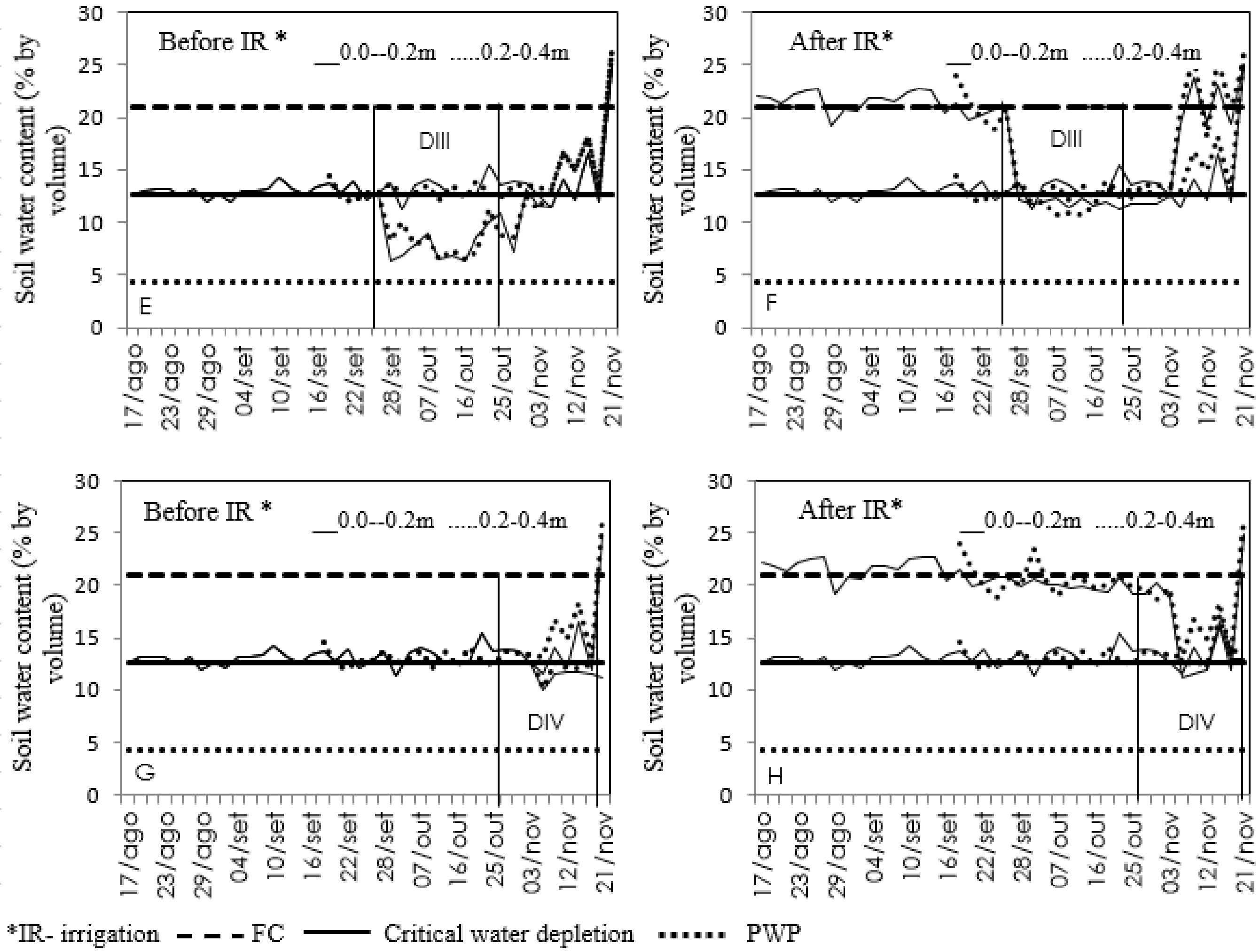
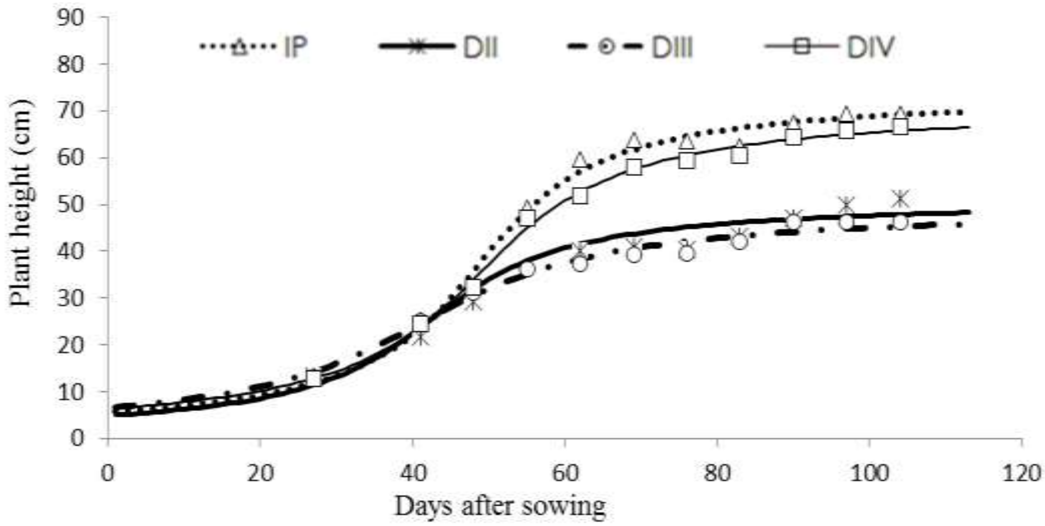


Figure 4. Soil water content in the depths of 0-0.2 and 0.2-0.4m before irrigation (A) and after irrigation (B) for the water deficit treatment during the stage III (DIII) and before (C) and after (B) the irrigation for the water deficit treatment during the stage IV (DIV). (----) Field capacity, (**—**) soy critical water depletion, (····) permanent wilting point.

Table 3. Variance analysis for plant height (PH), number of leaves (NL), leaf area index (LAI) and total dry matter (TDM) for soybean plants submitted to water deficit during their phenological stages. Bom Jesus, PI, Brazil, 2014.

Source of variation	Mean Square				
	DF	PH	NL	LAI	TDM
Blocks	5	8.69^{ns}	10.71^{ns}	0.03^{ns}	3.23**
Treatments (WD)	3	4029.33**	4552.66**	11.05**	77.16***
Residue (a)	15	14.07	23.06	0.05	1.40
Sampling period (SP)	10	5336.78**	27168.23**	16.29**	388.0**
Residue (b)	200	4.96	20.65	0.04	1.03
Interaction (WD) x (SP)	30	173.86**	565.38**	1.14**	10.83**
CV (a) %		8.48	7.52	11.18	16.00
CV (b) %		5.04	7.11	10.97	13.74
Mean		44.23	63.90	1.93	7.39

DF, Degrees of freedom; WD, soil water deficit during the stages II, III, IV and full irrigation (a); sampling period (SP); * and ** significant at 5 and 1%, respectively (F test); ns, not significant.



Treat.	27	41	48	55	62	69	76	83	90	97	104
IP	13.2 a	25.3 a	33.6 a	49.4 a	59.5 a	62.4 a	63.6 a	62.5 a	60.3 a	69.3 a	69.3 a
DII	13.3 a	21.7 a	29.3 a	46.4 a	40.1 c	41.0 b	40.5 b	43.2 b	60.3 a	49.8 b	51.2 b
DIII	13.0 a	25.2 a	31.2 a	38.3 b	39.5c	41.0 b	41.2 b	43.0 b	46.2 b	46.3 b	46.3 c
DIV	12.7 a	24.5 a	32.2 a	47.2 a	51.9 b	58.0 a	59.3 a	60.3 a	46.2 b	65.8 a	46.3 c

Figure 5. Soy plant height variation according to treatments during the crop phonological stages and mean analysis. Bom Jesus, PI, Brazil, 2014.

Table 4. Regression equation, R^2, maximum point and days after sowing (DAS) for plant height (PH) of the cultivar BRS Sambaíba RR, Bom Jesus, PI, Brazil, 2014.

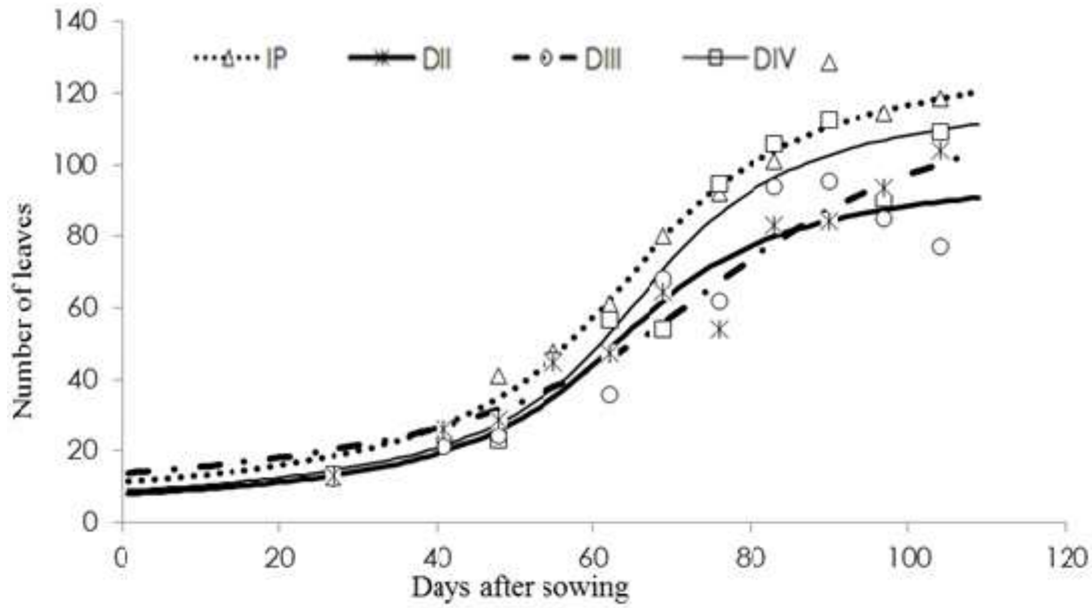
Treatment	Equations	R^2	Max	DAS
IP	PH=74.2 (atan((DAS to 48.7/11.8)+3.14/2)/3.14	0.89*	69.3	104
DII	PH=51.3 (atan((DAS to 42.7)/13.2)+3.14/2)/3.14	0.91*	51.2	104
DIII	PH=49.8 (atan((DAS to 41.4)/18.1)+3.14/2)/3.14	0.82*	46.3	104
DIV	PH=71.4 (atan((DAS to 49.0)/13.9)+3.14/2)/3.14	0.85*	66.7	104

IP to control without water deficit; DII, water deficit during the vegetative growing; DIII, water deficit during flowering and pod filling; DIV, water deficit during pod maturation.

in response to the applied treatments throughout their phenological phases. The regression equations of the curves of Figure 6 were presented in Table 5. The soybean crop presented a lower number of leaves when the water deficit was applied in phase III (DIII), with a final value of 77 leaves plant. On the other hand, the treatment under full irrigation presented a higher number of leaves per plant (118), as a result of the higher soil water availability during the crop cycle.

Nascimento et al. (2004), studied the effects of the variation of available water levels on the cowpea development and concluded that there was a reduction of approximately 11, 23 and 35%, for the number of leaves per plant, when 80, 60 and 40% of available water was applied, when compared to control. The reduction of the number of leaves in plants under water deficit can be considered as a survival strategy under adverse conditions to avoid water loss by transpiration (Ferrari et al., 2015). The existence of water deficit in extreme conditions promotes the progressive death of leaves, starting with the older ones and going to the younger ones, especially when the plant cannot maintain its water status above the RCWc (Lawn and Likoswe, 2008; Fioreze et al., 2011). Soy genotypes, when cultivated under conditions of soil water deficit, presented differences in leaf and plant survival as a function of RCWc (James et al., 2008).

According to Machado et al. (2009), the leaf senescence is a response of the water deficit and occurs after a decrease in leaf emergence. The reduction of



Treat.	27	41	48	55	62	69	76	83	90	97	104
IP	3.6 a	26.8 a	38.1 a	50.1 ab	70.0 a	71.3 a	76.6 a	88.6 a	167.0 a	113.2 a	133.8 a
DII	3.5 a	24.3 a	25.8 a	46.5 ab	70.0 a	67.5 a	69.3 a	83.3 a	82.8 b	109.0 a	99.5 a
DIII	3.5 a	24.5 a	26.6 a	41.0 b	44.3 b	64.0 a	65.8 a	105.0 a	81.0 b	93.0 a	94.2 a
DIV	3.8 a	25.0 a	29.3 a	56.1 a	66.0 ab	56.6 a	88.6 a	100.5 a	119.6 ab	108.2 a	119.8 a

Figure 6. Number of soybean leaves of according to the applied treatments during the phenological stages and mean analysis, Bom Jesus, PI, Brazil, 2014.

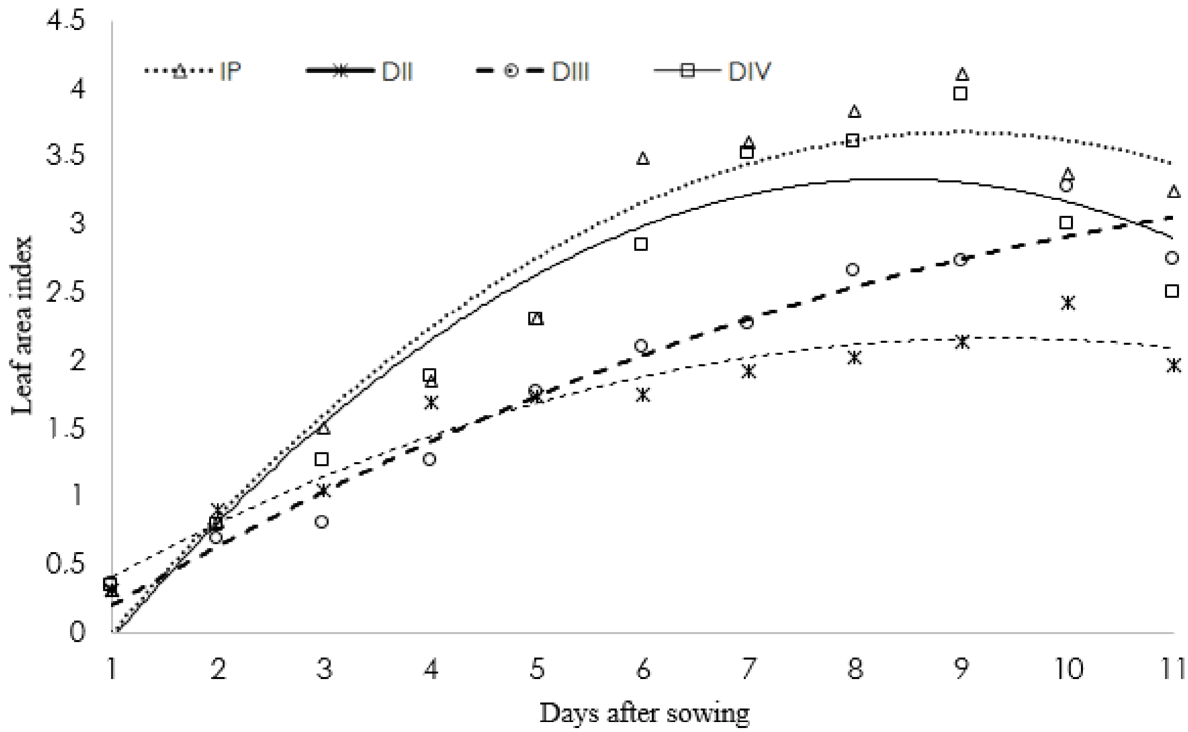
Table 5. Regression equations, R^2, maximum point and days after sowing (DAS) for the number of leaves per plant (NL) of the cultivar BRS Sambaíba RR, Bom Jesus, PI, Brazil, 2014.

Treatment	Equation	R^2	Max	DAS
IP	NL=135.86 (atan((DAS to 64.28/17.01)+3.14/2)/3.14	0.99*	128	90
DII	NL=129.92 (atan((DAS to 74.63)/25.50)+3.14/2)/3.14	0.95*	103	104
DIII	NL=101.43 (atan((DAS to 63.21)/15.59)+3.14/2)/3.14	0.90*	95	90
DIV	NL=124.45 (atan((DAS to 65.67)/14.67)+3.14/2)/3.14	0.93*	112	90

IP to control without water deficit; DII, water deficit during the vegetative growing; DIII, water deficit during flowering and pod filling; DIV, water deficit during pod maturation.

green leaves has been reported in plants with water deficiency and attributed to the strategy of decreasing the transpiring surface and the metabolic expenditure for the tissues maintenance (Inman-Bamber et al., 2008). The leaf area index of soybean in response to the applied treatments during the phenological phases is presented in Figure 7. The highest values of LAI occurred between flowering and grain filling. During the application of water deficit in phase DIII, the development was very slow when compared to other treatments, due to the water deficit in the soil, affecting the performance of the LAI, which decreased when compared to the control; about 47.4% less than the full irrigation plants (IP).

Ferrari et al. (2015) concluded that reduction of water availability in the soil decrease the leaf area index expansion. The leaf area is represented by the photosynthetically active surface of the plant, whose growth is highly related to plant production (Teixeira et al., 2015). Leaf area index (LAI) acts as an indicator of the available surface for interception and light absorption (Pavani et al., 2009). The reduction of leaf area in plants under water deficit can be translated into a survival strategy, aiming the reduction of the transpiration area (Ferrari et al., 2015), with a consequent reduction in the



Treat.	27	41	48	55	62	69	76	83	90	97	103
IP	0.3 a	0.8 ab	1.5 a	1.8 b	2.1 a	3.3 a	3.3 a	3.3 a	3.8 a	3.1 a	3.2 a
T2-DII	0.3 a	0.6 c	1.0 b	1.7 b	1.5 b	1.6 b	1.8 c	2.1 b	1.8 b	3.0 a	2.3 b
T3-DIII	0.3 a	0.9 a	1.0 b	1.7 b	1.5 b	1.9 b	1.8 c	2.0 b	1.7 b	2.1 b	1.7 c
T4-IV	0.3 a	0.7 bc	1.0 b	2.3 a	2.1 a	1.8 b	2.7 b	2.2 b	3.5 a	2.7 a	2.9 a

Figure 7. Leaf area index in soy plants according to the applied treatments during the plant phenological phases and mean analysis, Bom Jesus, PI, Brazil, 2014.

photo to assimilates production (Casaroli et al., 2007). When there is a lack of water in the soybean plant, morphophysiological changes appear, with foliar winding and wilting being an indicator of severe water scarcity (EMBRAPA, 2011; Ferrari et al., 2015).

Confalone and Dujmovich (1999), studied the influence of water deficit on solar radiation efficiency in soybean, and found LAI values of 6 and 4.1 for full irrigation and flowering and pod filling deficits, respectively. These LAI values were higher than those observed in the present study, possibly due to the use of Asgrow 4656 soybean cultivar, which has an indeterminate growth and higher seeding density than that used in the present study (29 plants per linear meter). Martorano et al. (2009), studied soil condition indicators with soybeans in no to tillage system (PD) and conventional soil preparation (CP), and observed LAI values of 6.0 in irrigated treatments, while in non to irrigated treatments the value observed was 5.7 for PD and 5.6 for CP, demonstrating similarity in terms of maximum LAI in both soil management systems, with lower values for non to irrigated treatments (Table 6).

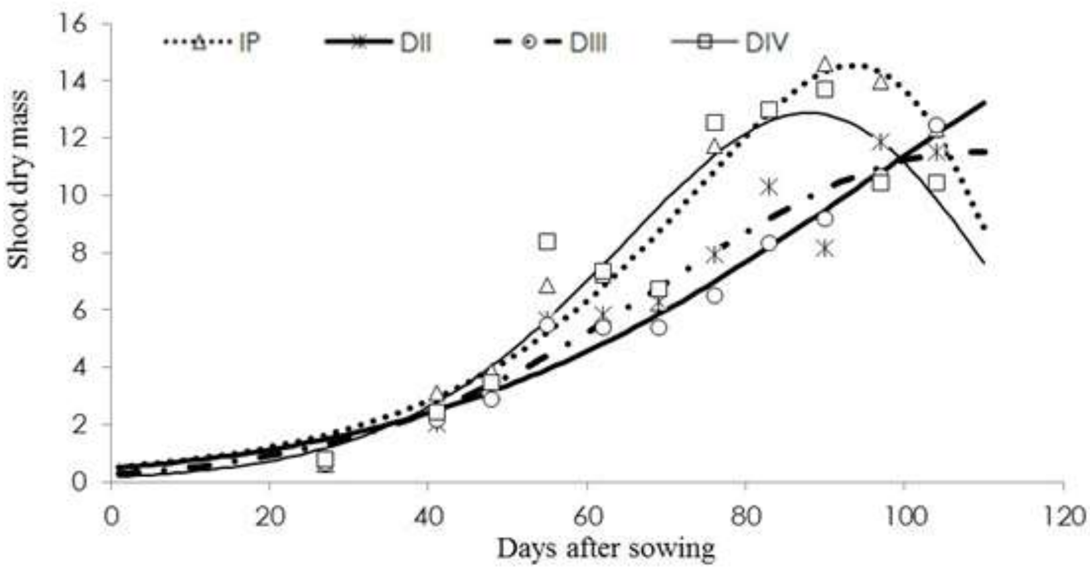
The dry matter of the aerial part of the soybean plants in response to the treatments during their phenological phases and the respective regression equations are shown in Figure 8 and Table 7. There was an increase in the total dry matter (TDM) of the aerial parts of the plants in all evaluated treatments. However, this growth in TDM was higher in plants cultivated under full irrigation and when submitted to water deficit on phase DIV, reaching maximum values of 14.4 g/plant, at 94 DAS, under IP, and 12.9 g/plant at 87 DAS with treatment DIV (Table 7). This behavior is a reflection of the higher water availability in the soil during the soybean cropping in these two conditions (Figures 3A, 3B, 4C and 4D). From these maximum values, there was decrease in the plant TDM due to the natural process of foliar senescence.

On the other hand, with the application of DII and DIII, less accumulation of TDM throughout the growing season

Table 6. Regression equations, R^2, maximum point and days after sowing (DAS) for the leaf area index (LAI) of the cultivar BRS Sambaiba RR, Bom Jesus, PI, Brazil, 2014.

Treatment	Equations	R^2	Max	DAS
IP	LAI = - 2.26 + 0.08DAS + (- 0.01) DAS^2 + (- 6.82) DAS^3 + 3.18DAS^4	0.97*	4.10	84
DII	LAI = - 4.60 + 0.25DAS + (- 0.05) DAS^2 + 5.15 DAS^3 + (- 1.95)DAS^4	0.98*	3.95	84
DIII	LAI = - 6.88 + 0.46DAS + (- 0.01) DAS^2 + 0.01 DAS^3 + (- 4.12)DAS^4	0.97*	3.27	92
DIV	LAI = - 3.59 + 0.19DAS + (- 0.03) DAS^2 + 3.24 DAS^3 + (1.38)DAS^4	0.98*	2.43	92

IP to control without water deficit; DII, water deficit during the vegetative growing; DIII, water deficit during flowering and pod filling; DIV, water deficit during pod maturation.



Treat.	27	41	48	55	62	69	76	83	90	97	103
IP	0.6 a	7.6 a	4.7 a	6.4 a	7.8 a	7.5 a	10.5ab	11.6 a	18.2 a	16.5 a	17.6 a
DII	0.8 a	6.2 a	3.0 a	6.1 a	5.6 ab	6.3 a	7.6 b	9.7 a	10.2 a	11.5 a	12.6 a
DIII	0.7 a	6.5 a	3.0 a	5.9 a	5.1 b	6.7 a	7.4 b	8.4 a	11.6 a	9.9 a	12.9 a
DIV	0.8 a	5.8 a	3.6 a	6.8 a	7.2 ab	6.7 a	13.4 a	11.3 a	17.7 a	11.1 a	15.4 a

Figure 8. Shoot dry mass in soy plants according to the applied treatments during the plant phenological phases and mean analysis, Bom Jesus, PI, Brazil, 2014.

Table 7. Regression equations, R^2, maximum point and days after sowing (DAS) for total dry matter of aerial part of soy plants, cultivar BRS Sambaiba RR, Bom Jesus, PI, Brazil, 2014.

Treatment	Equations	R^2	Max	DAS
IP	TDM=14.5exp((DAS to 93.6+16.7 to 16.7*1.4exp((DAS to clnd to 93.6)/16.7))/(16.7*1.4))	0.95*	14.61	90
DII	TDM=11.5exp((DAS to 108.5+377.8 to 377.8*0.01exp((DAS to clnd to 108.5)/377.8))/(377.8*0.01))	0.93*	11.89	97
DIII	TDM=17.2exp((DAS to 148.9+531.2 to 531.2*0.01exp((DAS to clnd to 148.9)/531.2))/(531.2*0.01))	0.96*	12.44	104
DIV	TDM=12.9exp((DAS to 87.9+50.5 to 50.5*0.2exp((DAS to clnd to 87.9)/50.8))/(50.8*0.2))	0.89*	13.42	90

IP to control without water deficit; DII, water deficit during the vegetative growing; DIII, water deficit during flowering and pod filling; DIV, water deficit during pod maturation.

was observed, due to the reduced availability of water in the soil for the plants in these two conditions. It is important to highlight that the points of maximum accumulation of TDM of these two situations were obtained only at the end of the cycle, at harvest time (11.5 g for DII treatment and 12.43 g for DIII treatment). This behavior is a reflection of the combined effect of reducing the number of leaves per plant (Figure 6) and leaf area index (Figure 7) when submitted to water deficit in these two phases, affecting TDM. Tavares et al. (2013) found that the dry mass of soybean plants up to 40 days after emergence was affected by soil water deficiency. In most cases, the deficit caused by water deficit affects the response between plants, which can be measured by the yield, growth or the primary process of CO_2 assimilation, which allow maintenance of water status during the reduction of soil moisture, where it is characterized as the drought resistance (Morando et al., 2014).

Conclusion

Soybean is sensitive to soil water deficit during the vegetative development, flowering and pod filling. The water deficit in the flowering and pod filling stages reduced the BRS Sambaiba RR cultivar growth by up to 33.2% (plant height), 34.7% (number of leaves), 41.3% (leaf area index) and 13.7% (total shoot dry matter), when compared to the control submitted to full irrigation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Rice farmers' perception of climate change and adaptation strategies in the Ketu North District, Volta Region of Ghana

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This study assessed rice farmers' perception of climate change and adaptation strategies in the Ketu North District, Volta Region of Ghana. Climate variables particularly precipitation and temperature were taken in the climate change perception study. Multistage sampling technique was used to select 340 rice farmers from six farming sections, and a structured questionnaire was used to elicit data from the respondents. Data collected from the rice farmers were analyzed using descriptive statistics and binary logistic regression. Results of the study showed that majority of the rice farmers' perceived decreasing precipitation and increasing temperature as a major climate variable affecting their agricultural practices. Farmers' level of adaptation was found with majority of them using irrigation, changing crops, changing planting dates and planting short season varieties. Findings of the study also indicate that the major barriers to climate change adaptation by rice farmers are lack of information about climate change, lack of credits and poor soil fertility. Binary logistic regression analysis found household size, education level, farming experience and financial support as significant predictors. The study concludes that rice farmers in the district perceived changes in climate and employ adaptation strategies to mitigate its effects.

Key words: Binary logistic regression, climate change, socio economics, agriculture.

INTRODUCTION

The agriculture sector is the backbone of the economies of most of the developing world. According to the World Bank report (2011), the sector employs about 60% of the workforce and contributing an average of 30%of Gross Domestic Product (GDP) in Sub-Saharan Africa. However, in recent years the sector is increasingly affected by climate change and change of weather patterns impacting food production, food security, and natural resources.

According to Trenberth et al. (2007), many developing countries have already experienced weather events manifested by floods, droughts, heat waves and tropical

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cyclones that are more frequent or intense than seen in the recent past. Generally, losses in the agriculture sector due to climate change have economy wide consequences, like loss in gross domestic output, a decline in the income/consumption of the most vulnerable population and thereby a general deterioration in households' welfare (FAO, 2007). Without appropriate responses, climate change is likely to constrain economic development and poverty reduction efforts and exacerbate already pressing difficulties especially in countries whose economies are deep rooted in climate sensitive sectors such as agriculture. Hence knowledge on the perception and adaptation of farmers to climate change and their strategies to deal with the phenomenon is quite crucial.

Adaptation is widely recognized as a vital component of any policy response to climate change. Adaptation to the adverse consequences of climate change could be viewed from two distinct perspectives. First is the awareness of the risks of climate change and the capacity to adapt to climate change, and second is how this adaptation can be carefully planned and implemented to avoid the possibility of mal-adaptation (FAO, 2007). Similarly, Taderera (2010) described adaptation as a way of reducing vulnerability, increasing resilience, moderating the risk of climate impacts on lives and livelihoods and taking advantage of opportunities posed by actual or expected climate change.

Perceptions are influenced not only by actual conditions and changes, but are also influenced by other factors. A study by Gbetibouo (2009) found that having fertile soil and access to water for irrigation decrease the likelihood that farmers will perceive climate change. However, in the same study, education, experience, and access to extension services are found to increase the likelihood that farmers perceived climate change. Against this background, this study is aimed to specifically:

(1) Determine rice farmers' perception of climate variables particularly focusing on precipitation and temperature.
(2) Identify rice farmers' choice of adaptation measures in response to these climate change variables.
(3) Identify barriers to rice farmers' adaptation measures in response to changes on these variables, and
(4) Find out the determinants of rice farmers' adaptation to changes in these climatic variables.

LITERATURE REVIEW

Climate change

The most vulnerable areas or sectors to climate change in Africa are water resources, agriculture, health, ecosystems and biodiversity, forestry and coastal zones. In general, climate change presents a substantial challenge to regional agricultural development. From food security and nutrition to sustainable management of natural resources, climate change is a significant threat to the welfare of millions of the continents rural poor. If adequate measures are not taken to adapt to the adverse consequences of climate change in sub-Sahara Africa, the region will remain vulnerable to the widespread effects of climate change (Food and Agricultural Organization (FAO), 2009). Studies have shown that while agriculture is a primary climate change impact sector, other sectors in the economy are also impacted because of the induced effect from the agriculture sector. For example, Juana et al. (2012) show that 20% reduction in water availability in South Africa due to climate change will lead to a 12% decline in agricultural output. Because of the backward and forward linkages between agriculture and the other sectors of the economy, this 12% decline in agricultural output will lead to about 8% decline in gross sectoral output. Also, this showed that 10% loss in agricultural output in Botswana due to drought will lead to about 8% decline in total sectorial output.

MATERIALS AND METHODS

Description of the study area

Ketu North District is one of the 25 districts in the Volta Region of Ghana. It is located between latitudes 6°03' N and 6°20' N and longitudes 0°49'E and 1°05'E. It shares boundaries with the Akatsi North District to the North and the Republic of Togo to the East. To the South, it is bounded by Ketu South district and Keta Municipality, and to the west by the Akatsi South District in Figure 1. The district has an estimated population of 100,000, and agriculture is the mainstay of the economy. The target population for the study includes all rice farmers farming at the Weta irrigation scheme within the Ketu North District of the Volta Region of Ghana. The estimated population of rice farmers within the Ketu North District is 1024.

Socio-economic characteristics of the target population

Age of the rice farmers

According to Hofferth (2003), the higher the age of the household head, the more stable the economy of the farm household, because older people have relatively richer experiences of the social and physical environments as well as greater experience of farming activities. Moreover, older household heads are expected to take adaptation strategies than younger heads.

Household size

This is one of the factors expected to have influence adaptation to decreasing precipitation and increasing temperature. The majority of the farm households in the study area are small-scale rice farmers. Because land and finance to purchase agricultural inputs are very limited, increasing family size tends to exert more pressure on consumption than the labor it contributes to production. Thus a positive correlation between household size and adaptation to

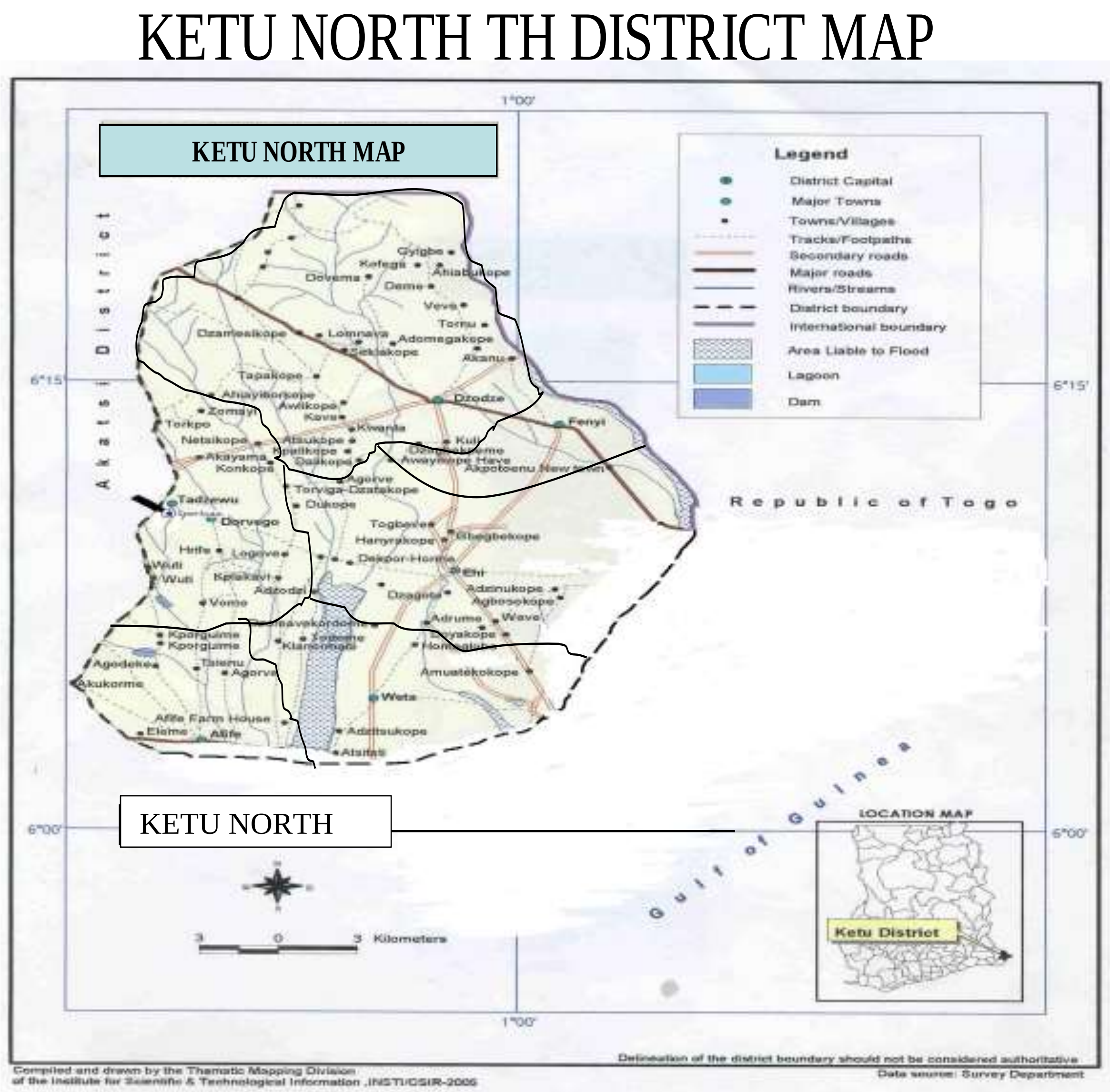

Figure 1. Ketu North District, Volta Region of Ghana.

decreasing precipitation and increasing temperature is expected.

Educational level

Education is an additional factor which is thought to influence the farmer's adaptation. Educational attainment by the household head could lead to awareness of the possible advantages of modernizing agriculture by means of technological inputs; enable them to read instructions on fertilizer parks and diversification of household incomes which in turn would enhance their adaptation to increasing temperature and decreasing precipitation.

Farm size

Farm size is a continuous variable. This study expected farm size to affect adaptation to increasing temperature and decreasing precipitation positively. According to Najafi (2003), food production can be increased extensively through expansion of areas under cultivation. Therefore, under subsistence agriculture, farm size is expected to play a significant role in influencing rice farmers' adaptation to increasing temperature and decreasing precipitation. Similarly, a study by Advancing Capacity to Support Climate Change, (ACCCA, 2010), reported that large farm size positively influenced adoption of soil and water conservation, tree planting, and use of improved varieties.

Farming experience

Farming experience is expected to positively influence rice farmers' adaptation to decreasing precipitation and increasing temperature. Farming experience increases the farmers' likelihood of adapting

Table 1. Sample size used for the study.

Rice farming section	Total number of rice farmers	Selected sample size
Section 2	74	47
Section 3	94	59
Section 5	107	67
Section 6	82	51
Section 7	95	59
Section 9	91	57
Total	543	340

climate change strategies. This is due to the fact that the knowledge of the recommendations and application of these strategies is gained over time, with practice. According to a study by Nhemachena and Hassan (2007), Temesegen et al. (2008) and Di Falco et al. (2011), farmers with more years of farming experience are more likely to notice changes in climatic conditions due to their prior experiences.

Financial support

Financial services are recognized as playing multiple roles in development so that improved access can have a far greater and more ample impact on poor households. Access to credit, which also represents the ability to purchase inputs, is expected to positively influence the decision to adopt a climate change. Access to credit increases financial resources of farmers, reduces cash constraints and allows farmers to purchase inputs (Benhin, 2006; Deressa et al., 2009; Gbetibouo, 2009).

Sampling design and sample size

The study used cross-sectional survey design to assess rice farmers' perception of climate change and adaptation strategies in the study area. Cross-sectional survey design is the appropriate design for the study because data were collected to make inferences about the population of interest at one point in time. A multistage sampling technique was used to select the respondents for the study. The sampling technique was chosen because it allows larger clusters to be subdivided into smaller, more target groupings for the purposes of surveying (Agresti and Finley, 2008). At the first stage, a simple random sampling technique was used to select 6 of the 11 rice farming sections at the Weta rice irrigation scheme in the district. At the second stage, a list of registered farmers of the 6 farming was obtained from the District Agricultural Assembly. Based on the population of these 6 farming sections, a random sampling technique was used to select 340 rice farmers using the sample size table constructed by Krejcie and Morgan (1970). Table 1 provides the summary of farmers selected from the Weta irrigation scheme, Ketu North District, Volta Region, Ghana.

Data collection

The primary data were collected through the use of self-administered questionnaires and interview. The variables were broadly categorized into socio-demographic characteristics, production activities, climate change information, adaptation measures in response to climate change and barriers to adaptation measures. Primary data were collected using open and close ended interview schedules. The questionnaire for the farmers was grouped into 38 items with five sections, I to V. Section I was made up of 12 items that is used to generate information on the socio-demographic characteristics of sampled population. Section II was on the production activities, Section III on climate change information, section IV was on adaptation measures in response to climate change and finally section V was on barriers to adaptation measures. The questionnaires for the primary data collection were pre-tested in South Tongu District. The purpose of the pre-test was to identify errors associated with the questionnaire and interview content, and omit double barreled questions and ambiguous statements. Furthermore, pre-testing was conducted to detect issues that were not anticipated and to assess:

(1) Clarity of questions regarding rice farming
(2) Whether the questions are understandable and
(3) Whether the order and wording of the questions elicited the desired responses for each question.

The total number of questions administered was 15. Based on the responses provided, modifications were made in the research instruments before administration.

Data analysis

To understand the perception and adaptation strategies of the targeted population, we developed a conceptual framework. Social, economic, and institutional factors were taken as variables that can influence farmers' perception to climate and thereby decide the adaption strategies they implement to overcome the changes in Figure 2. The study also utilized descriptive statistics and logistic regression analysis. SPSS and STATA software were used for all the analysis. Descriptive statistics such as frequencies, mean, standard deviation, and percentages were used to summarize and present rice farmers' perception on climate change, rice farmers' decision to adapt to climate change, and the barriers to adaptation. Frequencies and percentages were used to describe farmers' socio-demographic characteristics; identify farmers' choice of adaptation measures in response to climate change; and barriers to farmers' adaptation measures in response to climate change. Descriptive statistics was also used to illustrate the barriers affecting farmers. Logistic regression analysis was employed to analyze the determinants of rice farmers' adaptation to decreasing precipitation and increasing temperature. Logistic regression is a probability estimation model applied when the dependent variable is binary and the independent variables are in any form of measurement scale (Cramer, 2003). The dependent variable can take the value 1 or 0 depending on the probability of adaptation success or failure in the two variables respectively. The independent variables are the socio-economic characteristics of the rice farmers that include sex, age, household size, educational level, farming experience, farm size, and financial support. The

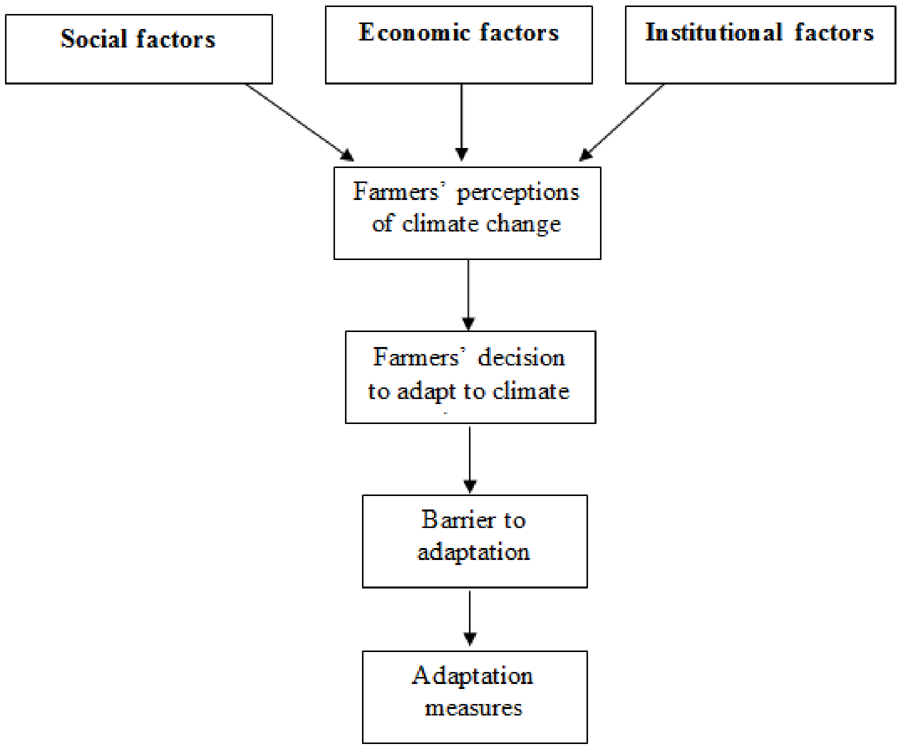

Figure 2. Conceptual framework on rice farmers' perception of climate change.

logistic regression model for estimating the probability of adaptation success or failure (P_i) is specified as follows:

Regression model specification

Probability of adaptation success is given as:

$$\Pr(Z_i=1)=P_i=\frac{1}{1+e^{-Z_i}}=\frac{e^{z_i}}{1+e^{Z_i}} \quad (1)$$

and probability of adaptation failure is given as:

$$\Pr(Zi=0)=1-P_i=\frac{1}{1+e^{Z_i}} \quad (2)$$

When dividing (1) by (2), it gives odds ratio:

$$\frac{P_i}{1-P_i}=e^{Z_i} \quad (3)$$

The logit model is a logarithmic transformation of the odds ratio.

$L_i=\ln(P_i/1-P_i)=Z_i=\alpha+\beta_1X_1+\beta_2X_2+\ldots+\beta_kX_k+\varepsilon$

Where L_i is the log of the odds ratio; e is the base of natural logarithms; α is a constant; $X_1, X_2, \ldots, X_k$ are explanatory variables; β_1 $\beta_{2,\ldots,}\beta_\kappa$ are estimated parameters corresponding to each explanatory variable; k is number of explanatory variables; and ε is the random error.

RESULTS

Rice farmers' perception of changes in precipitation and temperature

Findings of the study indicate that majority of the rice farmers interviewed perceived changes in climate specifically decreasing precipitation and increasing temperature as shown in Figure 3. This study is consistent with Sofoluwe et al. (2011), who surveyed 100 farmers to gather information on their perceptions about changes in temperature and precipitation in Osun State, Nigeria. Their results showed that majority of the respondents were aware of increase in temperature and decrease in precipitation in the region. Also, Acquah and Onumah (2011) who assessed farmers' perception and adaptation to climate change in the Western part of Ghana, found that majority of the farmers' perceived increase in temperature and decrease in precipitation. In a study to analyse farmers' perception and adaptation strategies to climate change in India, Dhaka et al. (2010), also found that significant numbers of farmers believed temperatures have already increased and the precipitation has declined along with late onset and early withdrawal of monsoon with long dry spells.

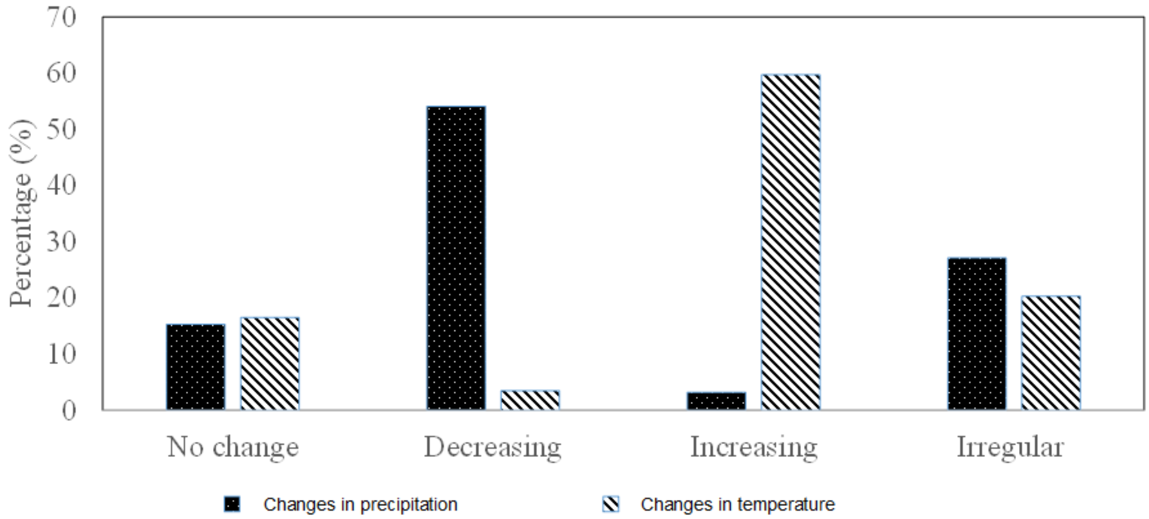

Figure 3. Rice farmers' perception of changes in precipitation and temperature.

Table 2. Rice farmers' choice of adaptation measures in response to changes in precipitation and temperature (%).

Adaptation strategies	Decreasing precipitation (%)	Increasing temperature (%)
Changing planting dates	77.9	60.0
Crop diversification	2.1	1.4
Reduce farm size	1.0	0.4
Change in crops	85.0	82.9
Find off farm jobs	1.0	3.2
Plant short season variety	12.1	11.0
No adaptation	0.3	2.5
Irrigation	99.7	82.9

Rice farmers' choice of adaptation measures in response to changes in precipitation and temperature

In Table 2, finding indicates that rice farmers' adapted some level of adaptation measures to improve their production such as changing their planting dates, change in crops types, plant short season varieties and irrigation. These findings are consistent with Deressa et al. (2008). These researchers analyzed the determinants of farmers' choice of adaptation methods in the Nile Basin, Ethiopia. Using cross-sectional data from a survey of farmers to obtain information on adaptation methods, their study found the adaptation methods currently in place in the study area. Accordingly, changing planting dates, using different crop varieties, planting tree crops, irrigation, and soil conservation were the major adaptation methods in the area. From these farmers' use of different crop varieties was the most common adaptation method, while irrigation was the least common. Also, Fosu-Mensah et al. (2010), investigated how farmers perceive long-term changes in temperature, rainfall and vegetation (91) cover over the past twenty years. The main adaptation strategies reported by the farmers were crop diversification and changing planting dates.

Barriers to rice farmers' adaptation to climate change

In Table 3, it can be seen that most rice farmers adapted to the changes in climate and faced some barriers in adapting to decreasing precipitation and increasing temperature such as, lack of information about climate change, lack of knowledge about adaptation options, lack of access to credit, no access to irrigation water and poor soil fertility. Findings of the study are also consistent with the study of Acquah and Onumah (2011) who identified lack of information on climate change impacts and adaptation options, lack of knowledge about adaptation measures, lack of access to credit and no access to

Table 3. Barriers to adaptation.

Barriers to adaptation strategies	Yes (%)	No (%)
Lack of information about climate change	74.1	25.9
Lack of knowledge about adaptation options	75.0	25.0
Lack of credit/poverty	90.9	9.1
No access to irrigation water	98.8	1.2
Changes are expensive	40.9	59.1
No barriers to adaptation	3.2	96.8
Insecure property rights	17.9	82.1
Insufficient access to inputs	36.8	63.2
Shortage of land	16.8	83.2
Poor soil facility	73.5	26.5
Others/Flooding	0.3	99.7

Table 4. Binary logistic regression of the adaptation to decreasing precipitation.

Dependent variable: Decreasing precipitation		
Independent variables	**Coefficient**	**Marginal effects**
Sex	0.479 (1.08)	0.036 -
Age	0.023 (0.82)	0.001 -
Household size	0.218 (2.44)*	0.016 -
Education level	0.867 (3.84)**	0.065 -
Farming experience	0.071 (2.13)*	0.005 -
Farm size	-0.963 (4.87)**	-0.072 -
Financial support	2.036 (4.56)**	0.153 -
Constants	-3.640 (2.24)*	- -
N	340	-
P-value of link test (_hat sq.)	0.140	-
Wald chi2(7)(P-value)	65.16 (0.000)	-
P-value of Hosmer-Lemeshow test for goodness of-fit	0.286	-

t statistics in parenthesis.
*p<0.05; ** p<0.01.

water as some of the barriers inhibiting the ability of the farmers in Western part of Ghana as the main constraints to adapt to climate change impacts. Also Nhemachena and Hassan (2007), investigated barriers to adaptation, their study indicated that farmers lack of credit facilities, information on adaptation options and insufficient inputs are the main barriers to adopting any climate change adaptation options.

Logistic regression of the determinants of adaptation to decreasing precipitation and increasing temperature

From the results, the Wald chi square value of 65.16 for decreasing precipitation and 98.31 for increasing temperature a p-value 0.000 indicates that the model provides a good fit to the data. Besides, the Hosmer–Lemeshow model fitness test shows also that we cannot reject our model which also means our model fits reasonably well. Based on the results, household size, educational level, farming experience, and financial support were found to be significant predictors to the adaption to decreasing precipitation and increasing temperature in (Tables 4 and 5).

An increase in the household size of respondents by one person increases the probability of the respondents adapting to decreasing precipitation by 1.6% and adapting to increasing temperature by 1.5% at 5% significant level holding other variables constant. This finding is consistent with Kandlinkar and Risbey (2000) and Temesegen et al. (2008). They explained that households with more labor are believed to able to take better adaptation measures in response to changes in climatic conditions compared to those with limited labor. In this sense, family size is one important variable that can determine the availability of labor.

With respect to education level of respondents, an additional year of education increases the probability of the respondents' adaptation to decreasing precipitation by 6.5% at 1% significant level, and adapting to increasing temperature by 4.9% at 5% significant level. This finding is consistent with Temesegen et al. (2008). Education is an important source of information for farm level management activities. Farmers with more formal education are believed to be better able to take adaptation measures in response to climate change compared to those without adequate education.

An additional year of farming of respondents increases the probability of the respondents adapting to decreasing precipitation by 0.5% at 5% significant level, and adapting to increasing temperature by 0.9% at 1% significant level. This finding is consistent with Nhemachena and Hassan (2007), Temesegen et al. (2008) and Di Falco et al. (2011). They recommended that elder household heads are expected to have more experience in farm practices and management.

An additional acre of farm land of respondents reduces the probability of the respondents adapting to decreasing

Table 5. Binary logistic regression of the adaptation to increasing temperature.

Dependent variable: Increasing temperature		
Independent variables	**Coefficient**	**Marginal effects**
Sex	0.450 (1.21)	0.047 -
Age	-0.003 (0.15)	-0.000 -
Household size	0.144 (2.18)*	0.015 -
Education level	0.466 (2.57)*	0.049 -
Farming experience	0.094 (3.40)**	0.009 -
Farm size	-0.809 (4.69)**	-0.085 -
Financial support	1.896 (4.81)**	0.199 -
Constants	-2.267 (1.70)	- -
N	340	-
P-value of link test (_hat sq.)	0.700	-
Wald chi2(7)(P-value)	98.31 (0.000)	-
P-value of Hosmer-Lemeshow test for goodness of-fit	0.190	-

t statistics in parenthesis.
*p<0.05; ** p<0.01.

precipitation by 7.2% at 1% significant level, and adapting to increasing temperature by 8.5% at 1% significant level. That is, farmers with smaller farm sizes are likely to adapt to climate change compared to those farmers with larger farm sizes. The study is consistent with Deressa et al. (2009). Independent variables that have demonstrated negative relationship to adaptation such as farm size could be attributed to the fact that adaptation is plot-specific. In other words it is not the size of the farm, but the specific characteristics of the farm that dictate the need for a specific adaptation method to climate change. In addition, factors identified as affecting the perception of an adaptation to climate change in the study areas are directly related to the development of institutions and infrastructure. Receiving financial support increases the probability of respondents adapting to decreasing precipitation by 15.3% at 1% significant level, and adapting to increasing temperature by 19.9% at 1% significant level as compared to respondents who do not receive any financial support.

The findings indicate that farmers who have financial assets to use more fertilizer and labor are more likely to consciously adapt to decreasing precipitation and increasing temperature. Alternatively, farmers with financial support acquire necessary inputs required to adapt to climate change and enhance their production. Such inputs include different seed varieties, fertilizers, and irrigation technologies. The result is consistent with Kandlinkar and Risbey (2000). They suggested that farmers that lack capital and other resources will fail to cover costs necessary to take up adaptation measures, and thus may not make beneficial use of the information they might have.

DISCUSSION

Climate change and weather patterns have been experiencing negative impacts on food production, food security and natural resources all over the globe. Farmers' adaption to climate change is crucial to combating food insecurity and related problems. This study sought to empirically understand Rice farmers' perception of climate change and adaptation strategies in the Ketu North District in the Volta Region of Ghana. Specifically, the study sought to achieve the following objectives:

(1) Analyse farmers' perception of precipitation and temperature patterns in the study area.
(2) Identify farmers' choice of adaptation measures in response to climate change;
(3) Investigate the determinants of farmers' adaptation to change in precipitation and temperature;
(4) Identify barriers to farmers' adaptation measures in response to climate change;
The summary of the major findings are presented with respect to the objectives of the study; which were as follows:

Rice farmers' perception of changes in precipitation and temperature patterns

Rice farmers in the study area were aware of climate change situation on their production, with majority 84.4% perceived changes in climate as a severe trend whilst 15.6% did not perceived any changes. With respect to precipitation, the study revealed that majority that is 54.1% of the rice farmers perceived a decrease in precipitation; 3.2% perceived an increase in precipitation; 27.4% of the rice farmers perceived an irregular precipitation and 15.3% of the farmers did not see any change in precipitation. Similarly, majority that is 59.7% of the rice farmer's perceived increases in temperature, 3.5% of the rice farmer's perceived decrease in temperature, 20.3% of the farmers perceived irregular pattern in temperature and 16.5% of the farmers perceived no change in temperature.

Rice farmers' choice of adaptation measures in response to climate change

Rice farmers' in the study area employ some adaptation methods due to decreasing precipitation and increasing temperature. These adaptation measures includes: Irrigation, change in crops, changing planting dates and plant short season variety were identified as the major adaptation strategies used to overcome decreasing precipitation and increasing temperature respectively.

Determinants of rice farmers' adaptation to decreasing precipitation and increasing temperature

Empirical results from the logistic regression analysis reveals household size, educational level, farming experience and financial supports positively influence rice farmers adaptation to decreasing precipitation while farm size negatively influence adaptation to decreasing precipitation. With respect to increasing temperature, household size, education level, farming experience and financial support positively influence the probability of adaptation to increasing temperature whilst farm size has negatively influence the probability of adaptation to increasing temperature.

Barriers to rice farmers' adaptations measures

The rice farmers were faced with barriers to their adaptation to climate change. These barriers include: lack of information about climate change, lack of knowledge about adaptation options, lack of credit, no access to irrigation water and poor soil fertility are the major barriers rice farmers' face in adapting to climate change.

Conclusions

From the findings of the study, the following conclusions are drawn: majority of the rice farmers perceived changes in climate specifically decrease in precipitation and increasing temperature. Rice farmers' used variety of measures to adapt to decreasing precipitation and increasing temperature. These measures include: irrigation, change in crops, changing planting dates and plant short season variety as the major adaptation measures to climate change impacts. Lack of information about climate change, lack of knowledge on adaptation, lack of credits and poor soil fertility were identified as the major barriers to adaptation. Findings from the logistic regression analysis indicate household size, education level, farming experience, and financial support as significant predictors of the probability to adaptation to decreasing precipitation and increasing temperature respectively.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Cropping systems and soil quality and fertility in south-central Uganda

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Little is known about how cropping systems influence soil quality and fertility in Uganda. Some cropping systems are more valued and as a result are given more nutrients and planted in certain soils, all of which leads to varying soil quality and fertility. This study compared soil quality (soil pH, cation exchange capacity (CEC), electric conductivity (EC), total N, and depth to restrictive layer (DRL)) and fertility (extractable P, K, Ca, Mg, and Na, and base saturation (BS) from five cropping systems (banana (*Musa × paradisiaca* L.)-dominant (B), coffee [*Coffea robusta* (L.) Linden]-dominant (C), banana-coffee (BC), annual with no crop rotation (ANR), and annual with crop rotation (AR); fertilized and unfertilized soils; and three soil types (black (Phaeozem), red (Ferralsol), and black-stony) in south-central Uganda. The analysis included farm assessments to establish management history of studied fields and soil sampling from 52 fields in Masaka District, Uganda. Main-effects ANOVA was employed to determine differences in means in soil under different cropping systems, soil types, and fertilizer use. Soil quality (pH at depths of 0 to 10 and 20 to 30 cm, CEC, and EC) and fertility (extractable Ca and Mg) varied by cropping system. The AR and B systems had higher soil quality and fertility compared to other cropping systems. Soil quality (pH at depths of 0 to 10 and 0 to 15 cm and DRL) and soil fertility (extractable P and K) varied by soil type. Black and black-stony soils had higher soil quality and fertility than red soils. Soil quality and fertility did not vary by fertilizer use. The results of this study indicate that both cropping system and soil type are associated with soil quality and fertility in south-central Uganda.

Key words: Annual cropping, perennial cropping, soil management, soil types.

INTRODUCTION

East African soils exhibit poor quality characteristics that are attributable to their geological age, climate, and land use. In Uganda, the most common soil type is Ferralsol (FAO, 2009), which is depleted in nutrients, highly weathered and comparatively infertile soil. The fertility of East African soils is further degraded by anthropogenic

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activities, primarily through agriculture. As the majority of Ugandan population depends on agriculture, looking at agricultural activities and their impact on soil is important.

Agricultural use changes soil properties through cropping and soil management. In Uganda, cropping systems are characterized by intercropping annual and perennial crops. The two most common crops in south-central Uganda are Robusta coffee [*Coffea robusta* (L.) Linden] and banana (*Musa* × *paradisiaca* L.), both of which are perennial and often intercropped (Okonya et al., 2013). Soil management practices are insufficient to maintain or improve soil quality and can include application of organic and inorganic fertilizers; practicing rotations, or fallowing; and installing trenches for soil and water conservation (Nkonya, 2002). The dynamics between cropping systems, soil management, and soil types need to be studied to better understand their influence on soil quality and fertility.

Several studies have found that cropping systems have an effect on soil quality. Intensive monocropping of banana disturbed soil's biotic structure, negatively impacted microbial respiration and water content at field capacity of Andosols and Nitisols in French West Indies (Clermont-Dauphin et al., 2004).) Annual crops were predicted to have the greatest erosion rates (93 tons of soil ha^{-1} $year^{-1}$) followed by rangelands (52 tons of soil ha^{-1} $year^{-1}$), banana-coffee (47 tons of soil ha^{-1} $year^{-1}$), and banana alone (32 tons of soil ha^{-1} $year^{-1}$) in central Uganda (Lufafa et al., 2003). Cropping system influenced culturable rhizosphere bacterial community structure irrespective of plant species in West African soils (Alvey et al., 2003).

Agricultural inputs can positively impact soil properties, moreover, coffee agroforestry systems had greater soil organic carbon than coffee monocrops in Ferrallitic soils in Uganda (Tumwebaze and Byakagaba, 2016). Low-input subsistence farming caused serious N depletion in Kenya (De Jager et al., 2001). Agricultural inputs can positively impact soil properties, especially organic and synthetic fertilizers. Frequent fertilizer use increased concentrations of exchangeable K and P in fruit and rubber tree plantation compared to the plantations with no fertilizer use in China (Zhang and Zhang, 2005). Straw application in Niger led to an increase in base saturation and pH and a decrease in extractable Al (Kretzschmar et al., 1991). Green manuring improved organic matter and soil microbial activity in the tropics (Chander et al., 1997). Application of banana stalks, field crop residues, and cattle manure increased banana yields in central Uganda (Bekunda and Woomer, 1996).

More research is needed on the effects of cropping systems on soil quality and fertility in south-central Uganda. Additionally, farmer practices need to be included in the analysis. This study looked at soil quality and fertility parameters and their variation by cropping system, fertilizer use, and soil type in south-central Uganda.

MATERIALS AND METHODS

Site description

The study site was located in Masaka District, near Lake Victoria in south-central Uganda. The district covers an area of 1,603 km^2, half of which is wetlands, with an average altitude of 1,150 m above sea level. The area is under a banana-coffee agroecological zone. Banana production has been on-going for 1000 to 1500 years (Lejju et al., 2006) while native Robusta coffee was developed as a plantation crop around 1900s (Thomas, 1947).

A favorable equatorial climate with two rainy seasons per year has allowed intensified banana production without crop rotation for millennia (Lejju et al., 2006). However, due to population increase (152 to 248 people per km^2 from 1999 to 2012) and consequent pressure on land resources, soil fertility has been deteriorating (Sebukyu and Mosango, 2012). The banana-coffee cropping history of Masaka District and its declining soil fertility make it a good area to study why and how soils are declining.

Field sampling

Farmer interviews, farm assessments, and soil sampling were conducted from June to September 2016 in Masaka District covering six sub-counties (Bukakata, Mukungwe, Buwunga, Kabonera, Kyanamukaka, Kyesiga) and one division (Katwe-Butego). In total, 52 smallholder farms were assessed representing 42 villages. Figure 1 shows the location of the sampled villages in Masaka District.

The study was designed to examine field-level soil quality and fertility under annual and perennial cropping systems in Masaka District, Uganda. Multi-stage, purposive sampling method was used to identify farms. First, one to two villages was randomly chosen from each of 26 parishes, making 42 villages in total. Then, one to two farms in each village were identified from either farmer training records kept by local extension services or by village leaders. One field was chosen per farm for assessment based on a cropping system.

Farm assessments included taking soil samples; interviewing farmers on soil management practices and history of the assessed field; and researcher observations of the soil, location, and crops grown. All farm assessments were performed by the same two people to ensure comparability of the results across fields. According to farmer recalls, field age ranged from one to 100 years of cropping with a mean of 28 years. The majority of fields (n=30) has been in agricultural production between one and twenty years following removal of bush or native forest. The number of crops per field ranged from one to five with a mean of 2.6 crops per field. Major crops included coffee, banana, common bean (*Phaseolus vulgaris* L.), and maize (*Zea mays* L.). The majority of fields were intercropped while 13 fields were monocropped. All fields fell into one of the three major local soil types with black (Liddugavu, Phaeozems), red (Limyufumyufu, Ferralsols), and black-stony types (Luyinjayinja), representing 19, 25, and 8 fields, respectively.

Cropping and soil management

The study investigated five cropping systems: banana-dominant (B), coffee-dominant (C), banana-coffee (BC), annual with no crop rotation (ANR), and annual with crop rotation (AR). The B system had banana as the main crop, which was either mono-cropped or

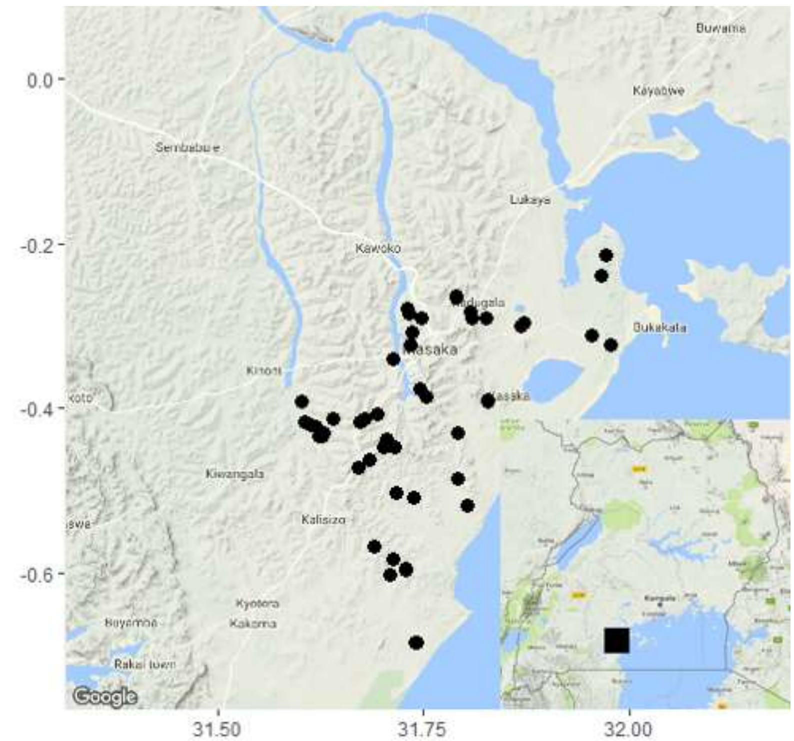

Figure 1. Map of Masaka District and sampled villages.

intercropped with one or more annual crops such as beans, maize, and cassava (*Manihot esculenta* Crantz). The C system had coffee as the main crop, which was either monocropped or intercropped with one or more annual crops such as beans, maize, and cassava. BC system had banana and coffee as two main crops, which could be intercropped with one or more annual crops such as beans, maize, and cassava. The ANR system had only annual crops such as maize, beans, and cassava, which could be mono-cropped or intercropped. The AR system also consisted of annual crops such as maize, beans, and cassava, which could be monocropped or intercropped. Farmers in this system, however, rotated crops from season to season.

All farmers were interviewed on crops they grew during the time of the interview and in previous season, and crop rotation. Based on the responses to these questions and field observations, the researcher determined categorization of the cropping system. The analysis included a binary fertilizer use variable (no vs. yes). All farmers were interviewed on any nutrient application to the fields including organic (animal manure, mulch, agricultural residues, green manure, compost) and inorganic fertilizers (diammonium phosphate (DAP), calcium ammonium nitrate (CAN), urea). The fertilizer use variable, therefore, did not differentiate between organic and inorganic fertilizers. Inorganic fertilizer application rates are too small (gross average rate of 1 kg ha^{-1}) in Uganda to cause any significant changes in soil properties (Nkonya, 2002; Ronner and Giller, 2013). As a result, the fertilizer use variable combined organic (n=26) and inorganic (n=9) nutrient applications.

The soil type variable included three levels: black, red, and black-stony. Farmers were asked to classify their soil and based on their responses; which were supplemented with field observations; each field was characterized as either under black, red, or black-stony soil. According to FAO-UNESCO soil legend, black soil corresponds to Phaeozems and is generally more fertile than other soil types (Goettsch et al., 2016). Red soil corresponds to Ferralsols (Goettsch et al., 2017) and is strongly weathered. Red soil forms more than 70% of the soil on which most of the farming is practiced in Uganda (Wortmann and Kaizzi, 1998). Black-stony soil is shallow, characterized by plinthitic and quartzitic stones, and is located on hilltops or outcrops (Mulumba, 2004).

Soil sampling and analysis

Fifteen soil properties were examined, including pH at different depths (0 to 10, 0 to 15, 10 to 20, 20 to 30, 30 to 50 cm), cation exchange capacity (CEC), electrical conductivity (EC), total N, extractable P, K, Na, Ca, Mg, base saturation (BS), and depth to restrictive layer (DRL).The CEC, EC, total N and extractable P, K, Na, Ca, and Mg were determined at depth of 0 to15 cm. Soil pH and EC were measured using the potentiometric method with soil to water ratio of 1:2. Soil CEC was estimated based on the quantities of Ca^{2+}, Mg^{2+}, and K^{+} extracted by the Mehlich-3 test (Ross and Kettering, 2011). Total N was measured by Kjeldahl digestion with sulphuric acid and selenium as a catalyst. Extractable P, K, Na, Ca, and Mg were measured by Mehlich-3 test (Mehlich, 1984). The BS was calculated based on the concentrations of Mg, K, Ca and Na. DRL was measured in the center of each field by digging vertically with a shovel until it was physically impossible to continue. Most often, the restrictive layer was characterized by parent material.

All soil parameters were separated into two categories: soil quality and soil fertility. Soil quality included soil pH, CEC, EC, total N, and DRL. These parameters represent intrinsic soil properties that are generally slow to change. Soil fertility included extractable

P, K, Ca, Mg, Na, and BS; Na is not a nutrient but it can indicate soil quality problems if high levels are found. These soil properties represent a dynamic state or health of a soil that reflects its condition under a specific management systems (Karlen et al., 1997).

Statistical analysis

Analysis of variance (ANOVA) was performed in R to examine the main effects of cropping system, fertilizer use, soil type on soil quality and fertility (RStudio Team, 2015). Following significant F-test, means were compared using Tukey's Studentized Range Test at $P \leq 0.1$. Analyses was performed on natural log-transformed EC, P, K, Ca, and Mg concentrations, which were back transformed for presentation to readers. Pearson correlations and simple linear regressions (RStudio Team, 2015) were included for better understanding of the relationships among soil parameters.

RESULTS

Cropping systems and soil quality and fertility

Soil quality and fertility varied by cropping system. Soil pH at depths 0 to 10 cm cm, CEC and 20 to 30 EC are varied by cropping system (Table 1). Soil pH at depths of 0 to 10 cm and 20 to 30 cm, CEC, and EC varied by cropping system. The AR had significantly greater soil pH at depth 0 to 10 cm compared to ANR and C systems. Soil pH at depth 20 to 30 cm was the greatest in B systems followed by AR, BC, ANR, and C systems. The AR, B, and BC had significantly greater soil pH at 20 to 30 cm depth compared to the C.

The CEC level was the greatest in AR systems followed by B, ANR, BC, and C systems. The AR had significantly higher CEC concentration compared to the rest of the systems. The B had significantly higher CEC concentration compared to C systems. EC level was the greatest in AR systems followed by B, BC, ANR, and C systems. The AR, B, and BC systems had significantly higher EC concentration compared to the C.

The C system had the lowest pH at depths 0 to 15, 10 to 20, and 30 to 50 cm. All systems had similar total N concentrations, ranging from 0.14 and 0.15 mg kg^{-1}. The C system had the greatest depth to restrictive layer followed by BC, B, ANR, and AR systems. Soil fertility varied by cropping systems. Such soil fertility parameters as extractable Ca and Mg were significant (Table 2). The extractable Ca concentration was the greatest in AR systems followed by B, BC, ANR, and C systems. The Mehlich-3 Mg concentration was the greatest in AR systems followed by B, BC, ANR, and C systems.

The AR systems had the greatest extractable P concentrations followed by B, BC, C and ANR systems. BC systems had the greatest extractable K followed by B, ANR, AR, and C while ANR and AR systems had higher Na concentration compared to B, C, and BC systems. The AR system had highest BS followed by B, BC, ANR, and C systems.

Soil types, quality and fertility

Soil quality and fertility varied by soil type. Such soil quality parameters as pH at depths 0 to 10 and 0 to 15 cm, and DRL were significant (Table 1). Black-stony soil had significantly greater pH at depth of 0 to 10 cm compared to red. Black soil also had significantly higher pH at depth 0 to 15 cm compared to red soil. Black-story and black soils had similar and higher pH at all depths compared to the red soil type. Black-stony soils had the shortest depth to restrictive layer (57 cm) followed by red (65 cm) and black soil types (70 cm). Black-stony and black soils also had higher and similar CEC concentrations compared to the red soil type. Black-stony soil had higher total N concentration compared to red and black soil types.

Soil fertility varied by soil type. Such soil fertility parameters such as extractable P and K were significant. Black soil had the greatest concentration of P followed by black-stony and red. Black-stony soil had the greatest concentration of K followed by black and red soils with red soil having significantly lower K concentration compared to black-stony. Black and black-stony soil types had the greatest and similar concentration of Ca, Mg, and BS compared to red soil type. Black and red soils had similar and lower Na concentration compared to black-stony soil.

The red soil type was the most frequent and it was primarily under BC and C cropping systems, the black soil type was the second most frequent and it was primarily under BC, B, and AR systems while the black-stony soil type was the least frequent and was under BC and ANR systems.

Fertilizer use and soil quality and fertility

Soil quality and fertility did not vary by fertilizer use. Out of a total of 52 fields, 17 received no fertilizer of any type. Fertilized soil, however, had higher soil pH, CEC, EC, and total N compared to the unfertilized soils (Table 1). Fertilized soils also had highest BS and nutrient concentrations while Na was not different between fertilized and unfertilized soils (Table 2).

Correlations among soil chemical properties

Almost all soil parameters were either highly (r > 0.8) or moderately (r of 0.5 to 0.8) positively correlated with each other at the significance level of $P \leq 0.01$ (Table 3).

Soil pH was correlated with almost all of the measured soil properties except for P, Na, and soil DRL. This indicates that soil pH is dependent on Ca, Mg, and K. Soil DRL was not correlated with any soil parameter, which could mean that it is influenced by either soil forming processes, landscape position, or erosional-depositional

Table 1. Soil quality properties from three soil types (black, black-stony, and red), fertilized and unfertilized soils, and five cropping systems (AR is annual with crop rotation, ANR is annual with no crop rotation, B is banana-dominant, BC is banana-coffee , and C is coffee-dominant). Soil collected from Masaka District, Uganda with collection period from June to September 2016.

Variables	Property	pH 0-10 cm	pH 0-15 cm	pH 10-20 cm	pH 20-30 cm	pH 30-50 cm	CEC 0-15 cm (meq $100g^{-1}$)	EC 0-15 cm (µS cm^{-1})	Total N 0-15 cm (mg kg^{-1})	DR[c] (cm)
Soil type										
Black	n=19	5.9^{ab}	5.9^{a}	6.1	6.2	6.1	10.1	60	0.14	70^{a}
Black-stony	n=8	6.0^{a}	5.9^{ab}	6.1	6.1	6.0	10.9	56	0.16	57^{b}
Red	n=25	5.7^{b}	5.5^{b}	5.9	6.0	5.9	8.7	49	0.14	65^{ab}
Fertilizer use										
Yes	n=35	5.8	5.7	6.04	6.07	6.03	8.86	67	0.14	5.8
No	n=17	5.7	5.6	5.98	6.09	6.01	7.74	63	0.13	5.7
Cropping system										
AR	n=5	6.1^{a}	6.1	6.3	6.2^{ab}	6.0	14.9^{a}	67^{a}	0.14	58
ANR	n=6	5.6^{b}	5.5	5.9	5.9^{bc}	5.9	9.3^{bc}	43^{bc}	0.15	62
B	n=9	5.9^{a}	6.0	6.2	6.3^{a}	6.3	10.7^{b}	65^{a}	0.14	65
BC	n=26	5.8^{ab}	5.6	6.1	6.1^{ab}	6.0	8.9^{bc}	56^{ab}	0.15	66
C	n=6	5.6^{b}	5.3	5.6	5.7^{c}	5.7	6.4^{c}	34^{c}	0.14	74
Significance		***P*-value**								
Soil type		*	*	NS	NS	NS	NS	NS	NS	**
Fertilizer use		NS	NS	NS	NS	NS	NS	NS	NS	NS
Cropping system		*	NS	NS	*	NS	**	**	NS	NS

**, *, and NS indicate statistical significance at $P \leq 0.05$, 0.10, and not significant, respectively. [ab] Means followed by a different letter within a column set are significantly different at $P \leq 0.10$ by LSD test. [c]DRL = depth to restrictive layer.

processes (Figure 2).

DISCUSSION

Cropping systems and soil quality and fertility

Coffee and ANR systems had the lowest soil quality and fertility while B and AR systems had the highest. The BC system exhibited moderate soil quality and fertility compared to the other systems. Low soil quality and fertility in coffee systems can be attributed to several factors. First, Mulumba (2004) found that 99% of the farmers in the Lake Victoria Basin of Uganda grow coffee because it is the main source of income. The study also reported that only 58 and 26% of the farmers mulched and controlled erosion under coffee compared to 70 and 72% of the farmers who mulched and controlled erosion under banana.

This was attributed to greater returns per hectare from the coffee compared to banana and the importance of banana as a major staple crop.

Table 2. Mehlich-3 extractable P, K, Ca, Mg, and Na concentrations from three soil types (black, black-stony, and red), fertilized and unfertilized soils, and five cropping systems (AR is annual with crop rotation, ANR is annual with no crop rotation, B is banana-dominant, BC is banana-coffee, and C is coffee-dominant). Soil collected from Masaka District, Uganda with collection period from June to September 2016.

Variables	Property	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	BS[b] (%)
Soil type							
Black	n=19	43[a]	134[ab]	856	159	32	71
Black-stony	n=8	21[b]	187[a]	841	160	36	72
Red	n=25	20[b]	94[b]	625	123	32	62
Fertilizer use							
Yes	n=35	28	121	772	149	31	67
No	n=17	25	116	660	125	31	66
Cropping system							
AR	n=5	45	114	1562[a]	262[a]	37	78
ANR	n=6	18	117	613[bc]	118[bc]	37	63
B	n=9	29	126	1080[ab]	196[ab]	32	75
BC	n=26	28	134	656[bc]	130[bc]	31	65
C	n=6	19	70	424[c]	84[c]	30	57
Significance		*P* value					
Soil type		*	**	ns	ns	ns	ns
Fertilizer use		ns	ns	ns	ns	ns	ns
Cropping system		ns	ns	*	*	ns	ns

**, *, and NS indicate statistical significance at $P \leq 0.05$, 0.1, and not significant, respectively. [b]BS: base saturation [ab]Means followed by a different letter within a column set are significantly different at $P \leq 0.1$ by LSD test.

Table 3. Pearson correlation coefficients (r) among selected soil properties.

Variables	pH	CEC	EC	N	P	K	Na	Ca	Mg
pH									
CEC	0.77[b]								
EC	0.52[b]	0.49[b]							
N	0.44[b]	0.64[b]	0.23						
P	0.18	0.34[a]	0.52[b]	0.16					
K	0.62[b]	0.59[b]	0.82[b]	0.47[b]	0.36[b]				
Na	0.16	0.23	0.17	0.29[a]	0.05	0.21			
Ca	0.87[b]	0.97[b]	0.53[b]	0.59[b]	0.34[a]	0.62[b]	0.21		
Mg	0.86[b]	0.96[b]	0.49[b]	0.61[b]	0.26	0.60[b]	0.20	0.97[b]	
DRL	-0.21	-0.23	-0.18	0.11	0.24	-0.18	-0.09	-0.24	
BS	0.98[b]	0.77[b]	0.43[b]	0.42[b]	0.14	0.52[b]	0.15	0.87[b]	-0.22[b]

[a]Correlation is significant at $P \leq 0.05$. [b]Correlation is significant at $P \leq 0.01$.

Therefore, soil quality management is motivated by food security rather than income. Second, coffee production diminishes soil quality through the removal of nutrients by harvest (Shively and Hao, 2012). In Uganda, coffee is harvested twice per year and is sold as unprocessed beans meaning that the husks from the coffee are unlikely to be returned to distant coffee fields. This slow removal of nutrients over time can lead to significant nutrient deficiencies in soil. Indeed, low P and K concentrations in the coffee system are explained by the

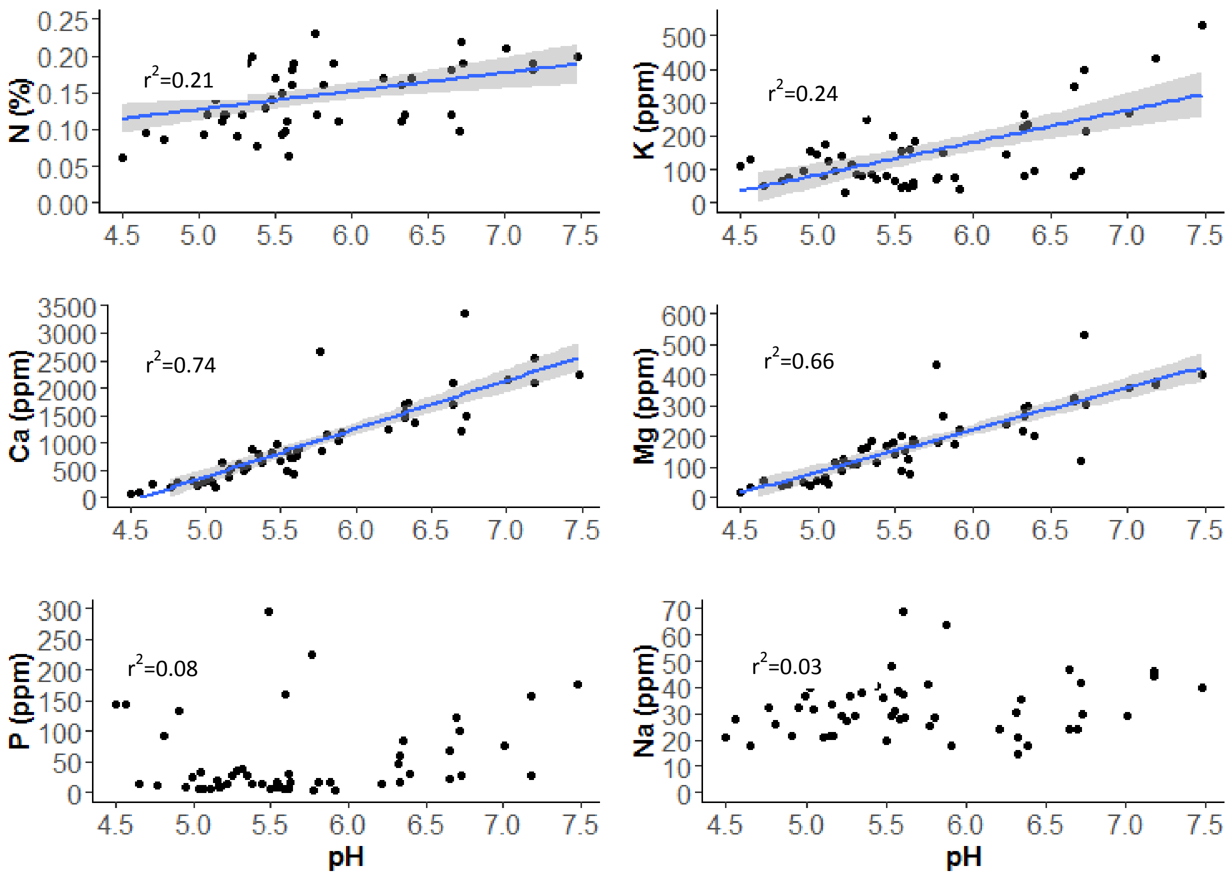

Figure 2. Linear relationships for soil pH predicting total N, extractable K, Ca, Mg, P, and Na from 53 farms in Masaka District, Uganda 2016.

removal of residues by harvest (Yamoah et al., 1990). Low Ca and Mg concentrations were also found by Nzeyimana et al. (2013) in Tanzanian coffee soil which caused Al toxicity. The sampled soils had low BS which leads to high extractable Al and limitations to crop production.

Thirdly, coffee plantations do not produce enough residues that could be used for mulch or soil cover as, for example, banana or maize do, thus further depleting the soil through erosion and leaching. Finally, coffee, as a non-staple crop, is typically grown away from home and soil fertility decreases within the farm at an increasing distances from the homestead due to limited labor (Nzeyimana et al.,, 2013). These characteristics of coffee production help explain low soil quality and fertility in coffee systems. Additionally, coffee is evenly spread across all three soil types indicating that there is either no soil preference to grow coffee or no choice in what soil to grow coffee since not every farmer has multiple soil types.

Low soil quality and fertility under ANR systems was confirmed by Mulumba (2004). He found that annual cropping systems had lower soil pH, soil organic N, and exchangeable Mg and P compared to banana and banana-coffee systems. This could be attributed to minimal soil management and the fact that annuals can be both cash and food crops. Indeed, Mulumba (2004) found that only 5% of the farmers in the Lake Victoria Basin of Uganda practiced mulching on annual cropping systems.

Banana and AR systems showed higher soil quality and fertility compared to other systems. Bananas are most often intercropped with such annual crops as beans and maize, both of which provide residues for mulching (Bekunda and Woomer, 1996). Higher nutrient content in banana soils can also be attributed to banana residue use in banana plantations and typically more organic residue use in banana systems as they tend to be closer to the homestead. Bekunda and Woomer (1996) and Wortmann et al. (1998) found that most farmers transferred annual crop residues to banana fields. Farmers also tend to allocate their best land to banana cropping because banana is essential to food security. All of these can explain greater levels and concentrations of soil pH, CEC, EC, and macronutrients in banana soils compared to coffee soils.

Additionally, the results indicate that farmers prefer to grow banana on black and red soils only. Black-stony soils are too shallow, drought-prone, and have poor water infiltration rate to be dedicated to a major food security crop like banana (Wortmann et al., 1998). Both land choice and soil management explain why fields cropped with banana contain more nutrients, especially K, than fields under coffee and annual crops. The AR systems had the highest soil quality and fertility compared to the rest of the systems. Specifically, these cropping systems had the highest levels and concentrations of CEC, EC, P, Ca, and Mg. This can be attributed to several factors. First, the rotation of crops has many benefits, some of which include pest and disease suppression and prevention. Second, crop rotations can contribute to improved nutrient and water uptake. Finally, because soil is affected by the previous crop, the type, and quantity of crop residues produced, practicing crop rotations can provide organic matter and nutrients to soil.

The rotations studied in this paper consisted of maize, beans, and cassava with different farmers rotating different combinations of these crops. Cereal/legume rotation was found to improve soil P availability and increase P uptake (Alvey et al., 2001). Additionally, it was demonstrated that soils under cereal/legume rotation had higher Mg than the soils under continuous maize (Okpara and Igwe, 2014). These findings help explain why AR systems in this study had the highest concentrations of P and Mg compared to other systems. The AR systems were found primarily in black and black-stony soils and since black soils are the most fertile, it can help explain why these systems are associated with high soil quality and fertility compared to other systems.

Soil types and soil quality and fertility

Black and black-stony soils had similar and higher soil quality and fertility compared to red soils. This indicates that there are similarities between black and black-stony soils. The fact that several cropping systems were found in black-stony soils indicates that this type of soil is common.

Fertilizer use and soil quality and fertility

Soil quality and fertility did not vary by fertilizer use which can be explained by minimal application of organic and inorganic nutrients by Ugandan farmers. Murage et al. (2000) found that 100% of the studied farmers in Kenya attributed low soil fertility to inadequate use of organic and inorganic fertilizers. Synthetic fertilizer is estimated to be used by only 10% of smallholder farmers in Uganda with the average application rate of 1 kg ha^{-1} (Benin et al., 2002).

High cost of fertilizer limits its use and it is not profitable on nutrient depleted soils (Ronner and Giller, 2013). Organic inputs such as manure, compost, and mulch are practiced by a small number of farmers due to labor requirements and lack of availability (Nkonya et al., 2004).

Conclusion

Soil quality and fertility varied by cropping system and soil type. The AR and B systems were associated with the highest soil fertility and quality while ANR and C systems were associated with the lowest soil fertility and soil quality. Black and black-stony soils were found to have similar and higher soil quality and fertility than red soils. Fertilizer use was not found to be associated with soil quality or fertility. Soil in C and ANR systems should be studied more to establish whether its quality is impacted by farmer decision to grow specific crops in certain soil types or by crop management practices.

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CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Field Assessment

1. Date
2. Farmer ID
3. Sub-county
4. Parish
5. Village
6. Geographic coordinates:
7. Slope

Crops

8. Crops grown on the plot this season
9. Crops grown on the plot last season
10. Does the plot have a rotation? If yes, what is it?
11. Provide a brief history of the plot as far back as you remember

Field Management

12. Do you use purchased fertilizers on this plot? Specify.
13. Do you return or add crop residue to this plot? If so, what are they?
14. Do you practice fallowing on this plot?
15. Do you do any other soil management practices?

Soil Description

16. How would you describe soil quality on this plot?
a) Poor b) Fair c) Good d) Excellent
17. What is soil type on this plot?
a) Red (Limyufumyufu) b) Black (Liddugavu) c) Stony (Luyijayinga) d) Sandy (Lusenyusenyu)
18. Past or present soil erosion
19. Restrictions on rooting
a) Stone layer b) hard setting E horizon c) Dense massive clay rich Bt horizon d) other (specify)

20. Description of soil horizons

Soil horizon	**Depth**	**Soil texture**	**Soil Structure**	**Soil color (Munsell)**	**Soil consistence**	**Root abundance**	**Root architecture**	**Microbial life**	**pH**

Soil texture: a) sandy loam b) loam c) silt loam d) sandy clay loam e) clay loam f) silty clay loam g) sandy clay f) clay g) silty clay
Soil structure: a) platy b) single grain c) blocky d)columnar/ prismatic e) granular f) massive
Soil consistence: a) loose b) very friable c) friable d) firm e) very firm f) extremely firm
Microbial life: a) none b) some c) vivid
Root architecture: a) vertical b) horizontal
Root abundance: a) none b) few c) common d) abundant

21. Visible nutrient deficiencies:

Crop	Growth stage	Discoloration	Other crop damage	Observations

22. Visible pest damage:

Crop	Type of damage	% of damaged crop	Any visible pests?	Observations

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Short Communication

Bacaba-de-leque (*Oenocarpus distichus*): A new wet tropics' nutritional source

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The present study aims to determine the nutritional composition of soluble and insoluble fibers, vitamins and minerals in the fruit of bacaba-de-leque (*Oenocarpus distichus* Mart.) a native Central Amazonian tropical fruit. Results show the fiber contents to be 3.95 g 100 g-^{1}, 100% of which shows to be insoluble fibers. Potassium stood out among the minerals, with 173.85 mg 100 g-^{1}. High vitamin E concentration (6.65 mg 100 g-^{1}) was observed as well. Considering the fruit's nutritional potential, we suggest its incorporation into the Amazonian population diet as an essential nutrient and health improving compounds source, to be more frequent.

Key words: Fiber, vitamins, minerals, nutrition.

INTRODUCTION

The palm tree family (Arecaceae) occurs throughout the world's tropical and sub-tropical regions. Palms trees hold high ecological and economic importance, and display complex, spatial species distributions and diversity patterns (Wolf et al., 2011). It holds 187 genera and nearly 2,466 species. Palms are well-known in warm climates. Most of them are single-stemmed with large fan leaves on top. The Amazonian region harbors many fruit species, including bacaba-de-leque (*Oenocarpus distichus* Mart.), also known as oil bacaba, which presents unique size of palm stem and distichous leaves and, stands out on account of yielding fruits bearing nutritional and economic potential to be taken advantage of by the Amazonian populations. Despite its many uses, just as for making jelly, mousse, candy, ice cream and juice or wine, very little is known regarding its nutritional composition, as of yet. Therefore, further research on its chemical composition, mainly vitamins, minerals and dietary fiber, are needed in order to contribute for improving its marketing and social inclusion programs. Even though there no published data addressing bacaba-de-leque juice chemical composition, one may still find a lot of it being published regarding another bacaba species (*Oenocarpus bacaba*) (Yuyama et al., 2013). Hence, the purpose of the present work is to provide information on health promoting phytochemicals, mainly vitamins E, C, β-carotene and complexes of vitamins B, thiamine (B1), riboflavin (B2), niacinamide (B3), pyridoxine (B6) and lipids, proteins, minerals and fiber chemical composition in the fruit bacaba-de-leque.

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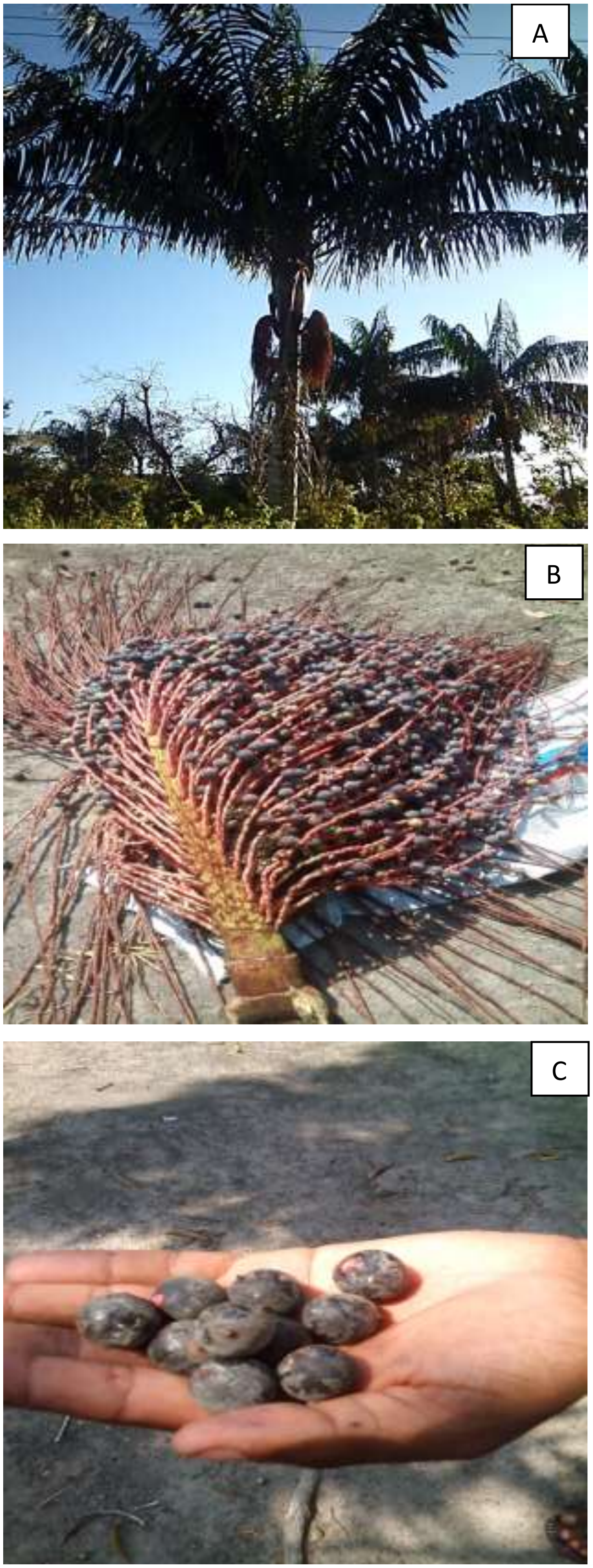

Figure 1. Bacaba of leque (*Oenocarpus distichus* Mart.) (A) tree (B) bunch and (C) fruits.

MATERIALS AND METHODS

The fruit of Bacaba-de-Leque (*O. distichus* Mart.) was harvested by hand during its ripening stage in the region of the city of Santarém, Pará, Brazil (S 02º20´57.5´´ W 054º48´0.38´´) in February 2016 (Figure 1). The sample consisted of three, 10 kg lots. They were stored into a sealed Styrofoam box and transported to the Food Physicochemical Laboratory (LFQA) of the Environment and Health Society Coordination (COSAS) at the National Research Institute of Amazonia (INPA) so as to be analyzed. Pulps were extracted with an automatic pulp remover (Itametal, 1.5 mm mesh) and homogenized in a blender prior to being analyzed in order to quantify their mineral, lipids, vitamins and fiber contents. Moisture, ash and protein contents were determined according to the method proposed by Association Analytical Chemists (AOAC) (2010) using 6.25 factor to convert the nitrogen percentage into protein content. Total lipids were extracted and determined according to Bligh and Dyer (1995). Total carbohydrates were calculated through their difference, according to equation:

$$c = 100 - (\text{Moisture} + \text{Lipid} + \text{Protein} + \text{Ash})$$

Caloric value was determined by using the indirect method, which is based on the application of the main product nutrients (carbohydrates, protein and lipids) conversion factors. Results are expressed in food kcal g^{-1}. Fiber contents were determined by the method proposed by Asp et al. (1983). Vitamin C content was measured three times by high-performance liquid chromatography (HPLC) following the method proposed by Maeda et al. (1989). β-carotene and Vitamin E content was measured three times by high-performance liquid chromatography (HPLC) following the method proposed by AOAC (2016). The methodology of analysis of vitamins from complex B was based on the procedures described by Albalá–Hurtado et al. (1997).

Determinations of the mineral content were obtained in triplicate by the atomic absorption spectrophotometry method recommended by the Adolfo Lutz Institute (IAL) (2008), and according to the Varian (2000) manual. Sample digestion was carried out in a MD-2591 MARS-Xpress brand CEM Corporation, microwave-oven. Nitric acid concentration was used to mineralize organic matter, which was then cooled and diluted with deionized water. The sample reading was performed directly on atomic absorption spectrophotometer (Spectra AA, model 220 FS, Varian, 2000), with specific lamps according to the manufacturer's manual. Ca, K, Na, Mg, Fe, Zn, Mn, Cu were the quantified mineral elements. Standard tomato leaf certified material (SRM 1573a) was employed to control the analyses according to Trahey (1992). Results are expressed in the sample's mg 100 g^{-1} fresh weight (FW).

RESULTS AND DISCUSSION

Bacaba-de-leque wine nutritional constituents showed low protein concentration and high energy level, mainly due to the presence of lipids. Compared to the other bacaba type (*Oenocarpus mapora*), the oil content of which was 6.53 g 100 g^{-1} (Yuyama, et al., 2013), that of bacaba-de-leque was shown to be much higher (7.28 g 100 g^{-1}) (Table 1). Therefore, energy supply comes to be its greatest contribution. Another attribute detected on bacaba-de-leque has shown to be the high and low content of potassium, calcium and magnesium, as well as of minor elements, such as copper and iron (Table 1), respectively. Its dietary fiber content is also very high, making it good natural source of these nutrients (3.95 g 100 g^{-1}), since in other bacaba species the fiber content

Table 1. Nutritional composition of Bacaba-de-Leque (*Oenocarpus distichus)* nutrients unit per 100 g.

Moisture (g 100 g-^{1})	**85.86**
Ash (g 100 g^{-1})	0.25
Protein (g 100 g^{-1})	1.15
Lipids (g 100 g^{-1})	7.28
Carbohydrates (g 100 g^{-1})	5.46
Soluble fiber (g 100 g^{-1})	0.0
Insoluble fiber (g 100 g^{-1})	3.95
Energy (kcal 100 g^{-1})	91.96
Vitamin C (mg 100 g^{-1})	8.10
β-Carotene (mg 100 g^{-1})	0.29
Vitamin E (mg 100 g^{-1})	6.65
Tiamin (mg 100 g^{-1})	0.01
Riboflavin (mg 100 g^{-1})	0.03
Niacin (mg 100 g^{-1})	0.01
Piridoxina (mg 100 g^{-1})	0.0
Minerals (mg 100 g^{-1})	
Calcium (Ca)	3.80
Copper (Cu)	0.20
Iron (Fe)	0.28
Manganese (Mn)	0.67
Zinc (Zn)	0.35
Potassium (K)	173.85
Sodium (Na)	1.9
Magnesium (Mg)	7.80

is very low (2.10 g 100 g-^{1}) (Yuyama et al., 2013). Studies have shown that high-fiber diets have great therapeutic potential against dyslipidemia, cardiovascular diseases and some types of cancer (World Health Organization (WHO), 2015), with the consequent reduction of glucose and blood cholesterol. Bacaba-de-leque shows a high concentration of Vitamin E 6.65 mg 100 g-^{1} as compared to the other bacaba type (*O. bacaba*) with 2.15 mg 100 g-^{1}. As to iron, bacaba-de-leque showed not to be iron-rich (0.28 mg 100g^{-1}). It should be noted that not all the iron present in food, especially vegetables, comes to be absorbed and used by the body. Therefore, further studies on the iron bioavailability are required. Bacaba-de-leque has shown to be a powerful source of antioxidants, which help to reduce the effects of premature aging caused by free radicals and prevent other diseases such as cancer and diabetes. Further studies are needed to elucidate the full potential of this fruit, which is yet to be studied.

Conclusions

Bacaba-de-leque has shown to be an excellent source of vitamin E, riboflavin, insoluble fiber, energy, minerals, especially potassium and calcium, essential for human nutrition as well as the development of different applications. Further research is paramount to exploit other palm characteristics, which may be useful for human nutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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