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### ARTICLES

- Amelioration of a degraded ultisol with hardwood biochar: Effects on soil physico-chemical properties and yield of cucumber (*Cucumis sativus* L)** 1781  
C. N. Mbah, C. Njoku, C. C. Okolo, E. E. Attoe and U.C. Osakwe
- Understanding the need for transfer of biologically-based crop protection technology for soil pest control in vegetable production in Rwanda** 1793  
Musebe R., Dusenge L., Agwanda C., Kajuga J., Waweru B., Karanja D., Hongmei L. and Day R.
- Climate conditions in the eastern amazon: Rainfall variability in Belem and indicative of soil water deficit** 1801  
Lucieta Guerreiro Martorano, Maria Isabel Vitorino, Bruna Paula Pantoja Caxias da Silva, José Reinaldo da Silva Cabral de Moraes, Leila Sheila Lisboa, Eleneide Doff Sotta and Klaus Reichardt
- Sunflower seed yield under trickle irrigation using treated wastewater** 1811  
Hamzeh Mohmmad Rawashdeh
- Insect pests infesting black pepper (*Piper nigrum* L.) in southwestern part of Ethiopia** 1817  
Teshale Daba, Habetewold Kifelew, Girma Hailemichael and Wakjira Getachew
- Genetic variability, heritability and genetic advance for quantitative traits in coffee (*Coffea arabica* L.) accessions in Ethiopia** 1824  
Getachew Weldemichael, Sentayehu Alamerew and Taye Kufa
- Knowledge and perceptions of plant viral diseases by different stakeholders in Zimbabwe's agricultural sector: Implications for disease management** 1832  
Charles Karavina, Vincent Tinashe Munyati and Augustine Gubba
- Forage productivity system evaluation through station screening and intercropping of lablab forage legume with maize under Irrigated lands of smallholder farmers** 1841  
Weldeyesus Gebreyowhens

<b>Nutritional composition of vegetables grown in organic and conventional cultivation systems in Uberlândia, MG</b>	<b>1848</b>
Luciana Nunes Gontijo, Cassiano Oliveira da Silva, Lorena Gomes Stort, Renata Maria Teixeira Duarte, Cristiane Betanho and Erika Maria Marcondes Tassi	
<b>The growth and nutrition of pineapple (<i>Ananas comosus</i> L.) plantlets under different water retention regimes and manure</b>	<b>1852</b>
Vander Mendonça, Luciana Freitas de Medeiros Mendonça, Eduardo Castro Pereira, Grazianny Andrade Leite, José Maria da Costa and Francisco Mickael Câmara de Medeiros	

*Full Length Research Paper*

# Understanding the need for transfer of biologically-based crop protection technology for soil pest control in vegetable production in Rwanda

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Despite growth in Rwanda's agricultural production ability, with food crops representing 33% of the National GDP and 80% of the population reliant on agriculture, food supply remains fragile. A factor which has significant impact on productivity is soil pests, whose effects filter through the whole value chain. Poor yields result in demand that exceeds supply, leading to higher food prices and reduced affordability by the poor. Poor quality products result in lower consumer acceptability and short shelf life. Rwandan farmers have limited access to plant protection products. A survey of farmers conducted in 2008 revealed that only 16% of the households use pesticides and few use other products for soil pest control. This paper examined the existing vegetable production situation, major soil pests for vegetables and the needs of the farmers in respect to soil pests' management in Rwanda in 2014. From interviews of 110 vegetable farmers and 18 key informants, it was established that the main vegetable crops were cassava, beans, Irish potatoes and cabbages. The main method for vegetable production was intercropping. Production of vegetables was not intensive as evidenced by the limited use of high value inputs such as fertilizers and crop protection chemicals. The vegetable production constraints starting from the most serious were insect pests, diseases, lack of high quality seeds, high cost of pesticides and fertilizers. The key insect pests were white grubs, cutworms, termites and bean fly. Most of the farmers (76%) did not control the insect pests due to lack of knowledge, lack of alternative methods for pest management and high cost of pest control products especially the chemical pesticides. Chemical pesticides were reported as the main control method by 55% of those who controlled insect pests. This translates to only 13% of the farmers reporting use of chemical pesticides to control insect pests. This represents a 3% decline in the already low (16%) use of chemical pesticides to control insect pests. Conversely, pest infestation levels have been increasing over time. Diversification of the pest control methods is therefore warranted and has indeed been lauded as a key approach to improving pest control. Biologically-based crop protection technology using entomopathogenic nematodes is critical for improving insect pest control. This is due to the possibility of the technology being maintained over a large area without major efforts on the part of the already financially resource poor farmers. Facilitating access to information about the sources of the biologically-based insect pest control technology can enhance diversification of the insect control methods.

**Key words:** Soil pests, biological control, crop protection, entomopathogenic nematodes.

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## INTRODUCTION

Despite growth in Rwanda's agricultural sector, with food crops representing 33% of the National GDP and 70% of the population reliant on agriculture, food supply remains fragile. A factor which has significant impact on productivity is soil pests, whose effects filter through the whole value chain. Poor yields result in demand that exceeds supply, leading to higher food prices and reduced affordability by the poor. Poor quality products, particularly root crops damaged by soil insect pests, result in lower consumer acceptability and short shelf life, as a result of secondary infections. The situation is more critical in vegetable production where yield losses could be up to 100% attributed mainly to soil insect pests. The key vegetables in Rwanda were cassava (*Manihot esculenta*), beans (*Phaseolus vulgaris*) and cabbages (*Brassica oleracea* var. *capitata*) in Bugesera (East) and Nyamagabe (South), and Irish potato (*Solanum tuberosum*), beans and cabbages in Musanze (North). Other vegetables were tomato (*Solanum lycopersicum*), carrots (*Daucus carota*) and sweet potatoes (*Ipomoea batatas*). The main soil insect pests of economic importance reported in Rwanda include white grubs (*Scarabeid beetle larvae: Anomala* species, *Melonthini* species, *Hoplochelus* species); cutworms (*Agrotis* species) and bean fly (*Ophiomyia phaseoli*) (Anon, 2008). The main method that is used for insect pest control is chemical pesticides. Rwandan farmers have limited access to plant protection products. A survey of farmers conducted in 2008 revealed that only 16% of households use pesticides and few use other products for soil pest control (Anon, 2008). This is worsened by the fact that there are limited alternatives for the control of pests.

Under these circumstances a question that arises is how to make alternative insect pest control methods accessible to the vegetable farmers. In the same vein, the alternative pest control methods need to be known in order for the vegetable farmers to make informed choices. Low-input, environmentally friendly and economically sustainable plant protection technologies, such as entomopathogenic nematodes (EPNs), originating from China, have demonstrated capacity to improve protection of key vegetable crops against soil insect pests and increase productivity and would prove useful if made accessible to the vegetable farmers in Rwanda.

This paper examined why biologically based crop protection technology would be useful for Rwanda. In so doing, the paper addressed the following specific objectives: to examine the existing vegetable production situation in Rwanda; identify major soil insect pests for

vegetables and the control measures used and identify the needs of farmers in respect to soil pests' control.

## MATERIALS AND METHODS

Purposive sampling was used to select Eastern, Southern and Northern Provinces of Rwanda for data collection for the study that contributed to this paper. From each of the provinces one district was selected purposively based on the prevalence of the target crops and pests. The districts selected were Musanze in Northern Province, Bugesera in Eastern Province and Nyamagabe in Southern Province. These areas were purposively selected as the sites with suitable agro-ecological conditions for production of key vegetable crops and where key pests for the study were domicile. Data for the study was obtained from vegetable farmers and key informants.

Vegetable farmers were selected from each of the study areas using systematic sampling where the first respondent was selected randomly and thereafter every fifth prospective vegetable farmer was selected until the required sample size of 110 vegetable farmers was obtained. The vegetable farmers were distributed as Musanze (38), Nyamagabe (40) and Bugesera (32). The selection process used lists that were prepared by the agricultural extension officers in each of the districts. The lists were from the various sectors that had been identified as being involved in the production of the target vegetables.

Six key informants were selected using purposive sampling from each of the districts. Thus a total of 18 key informants participated in the study. They included staff at the Ministry of Agriculture in the various districts, Rwanda Agriculture Board (RAB) experts and other officers with specialist information in the target districts. Other key informants included the cooperative president, one member of the cooperative committee, one progressive farmer, and agriculture officer at the sector level, non-governmental organization (NGO) representative and project Agronomists. After selection of the respondents, household surveys and key informant interviews were conducted.

The reference period used for the study was the last season during the crop year which was 1st April 2013 to 31st March, 2014 and the data was collected in the months of April and May 2014. Data collected included land owned, proportion of land devoted to vegetable production, types of vegetables grown, input usage, production systems, pests and diseases of the vegetables, and methods of pest control. Data was collected using structured and pre-tested questionnaires/checklists.

Descriptive statistics were estimated including means, standard deviation and percentages. Comparisons were done at the district levels to establish differences and similarities. Inferential statistics were also generated to provide the requisite cause and effect relationships for specific variables in the study.

## RESULTS AND DISCUSSION

### Farm characteristics

The farmers own small land parcels of an average of 1.48 ha and devote relatively smaller portions of their land to

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**Table 1.** Average land owned and area under different vegetables.

District	Land owned (ha.)	Area of cassava (ha)	Area of beans (ha)	Area of Irish potato (ha)	Area of cabbage (ha)
Bugesera	1.31 (0.24)	0.62 (0.15)	0.49 (0.09)	-	0.12 (0.03)
Musanze	1.77 (0.69)	-	0.33 (0.09)	0.71 (0.11)	0.01 (0.06)
Nyamagabe	1.34 (0.51)	0.10 (0.00)	0.07 (0.02)	0.12 (0.03)	0.06 (0.03)
All Districts	1.48 (0.31)	0.59 (0.15)	0.27 (0.04)	0.40 (0.07)	0.07 (0.02)

Values in parentheses are standard errors.

**Table 2.** Systems of vegetable production and reasons for the selected systems.

Vegetable	Source(s) of seeds	System of production	Reasons for the selected production system
Irish potatoes	Own, neighbours, RAB, Cooperative	mono cropping	Half of farmers use mono cropping system for Irish potatoes because it is a high yielding cropping system,
Cassava	RAB, Own, neighbours, Cooperative	Intercropping	To optimise on the use of space for effective yields
Beans	Own, RAB, Cooperative, neighbours	intercropping	To optimise on the use of space for effective yields
Cabbage	Own, RAB, Cooperative, neighbours	mono cropping	Avoid crop competition, prevent diseases spread and recommended by Government of Rwanda

vegetable production (Table 1). Land is mainly owned by individuals thereby making it possible to seek credit facilities to improve production of the vegetables as need arises. The main vegetable crops grown in order of importance that is based on area devoted to each crop were cassava, Irish potatoes, beans and cabbages.

### Vegetable production systems and input usage

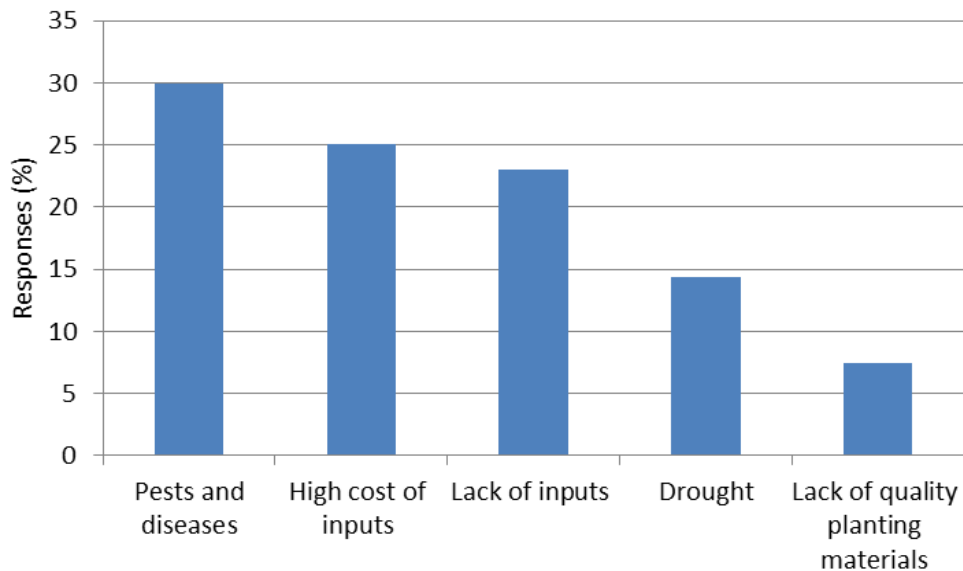
The main methods for vegetable production were intercropping and mono cropping, while the preferred method was mono cropping for varieties that are more commercial than subsistence (Table 2). Additionally, mono cropping is the system that is recommended by the government. The main sources of the seeds used for vegetable production were the cooperatives followed by Rwanda Agriculture Board (RAB) and own seed sources (farmer saved seed).

Production of vegetables was not intensive as less high value inputs were used. In particular, the use of fertilizer and crop protection chemicals was limited. The main sources of inputs were the agro-dealers who included retailers (68.4%), cooperative (10.5%) and companies (21.1%). The main products sold by the agro-dealers were pesticides and other inputs such as seeds and fertilizers. The agro-dealers had most of the highly used inputs but indicated that given financial support they would be able to supply other inputs on demand.

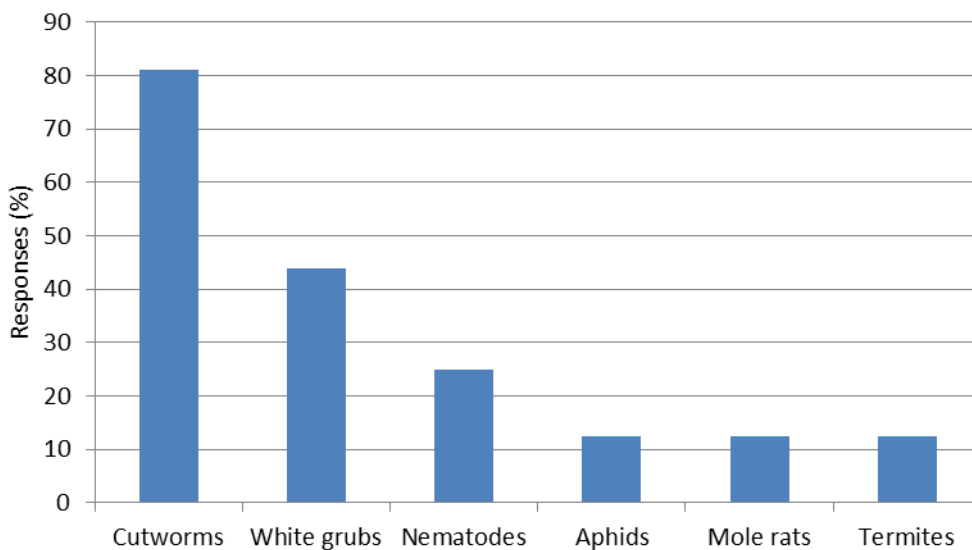
The agro-dealers provided some information on the use of the products that they sell. Twenty five percent of the agro-dealers give advice on proper use of the pesticides, while 23% reported that they advise on the dosage required for specific pest and disease scenarios. Use of protective gear was reported to have been provided by 7% of the agro-dealers. Informal discussions between agro-dealers and the buyers also involved advice on pooling resources for purchase of pesticides or even land for group production endeavours. All the interviewed agro-dealers reported that they would be willing to disseminate a technology aimed at reducing pest infestation levels. If required they would be able to provide facilities for storage of the products based on the capacity of the trader.

### Vegetable production constraints

The vegetable production constraints as reported by the vegetable farmers in order of importance were pests and diseases, high cost of inputs especially pesticides and fertilizers and lack of inputs particularly pesticides (Figure 1). In the entire grouping of constraints at the production level, pests were rated as the most serious (IITA, 2010). The farmers' views about vegetable production constraints were consistent with those of the key informants. Lack of quality planting materials referred to shortage of good quality seeds and cuttings in the case of



**Figure 1.** Vegetable production constraints.



**Figure 2.** Key vegetable pests in the survey area.

vegetative propagated vegetables. Lack of pesticides, occasioned by high costs or inability to get the required types of pesticides, suggests the need for alternative methods for pest control.

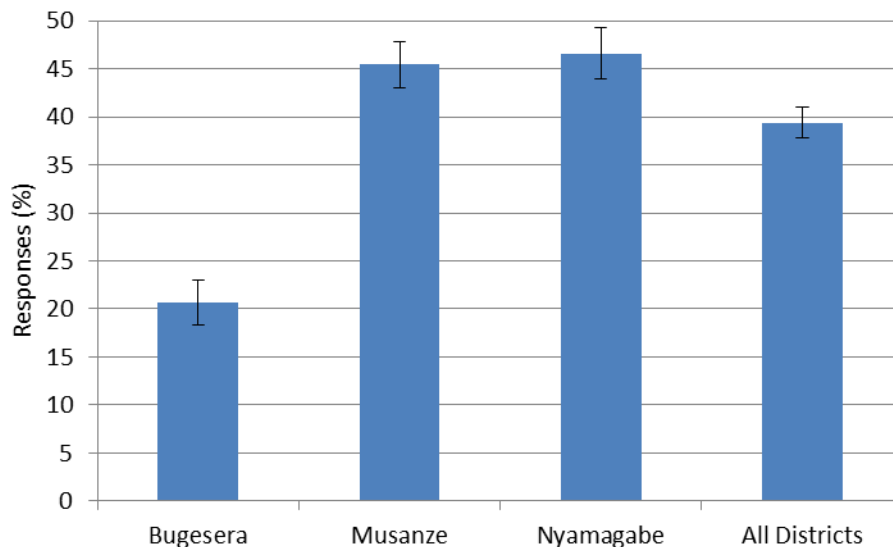
#### **Vegetable pest control and opportunities for improvement**

The key pests of vegetables in the area were cutworms, white grubs and nematodes. This is corroborated by the

percentage of farmers reporting prevalence of the pests (Figure 2). Other pests included aphids, mole rats and termites.

Vegetable crop losses due to pest damage were an average of 39%. Some farmers reported 100% loss due to damage by the pests. Key informants also agreed that there were instances where 100% crop loss occurred due to pest damage. Nyamagabe district had the highest average loss while Bugesera had the lowest loss (Figure 3).

Only 24% of the farmers reported having attempted to



**Figure 3.** Average vegetable loss due to pest infestation in different districts.

**Table 3.** Percentage of farmers reporting different levels of effective control.

Ability to control	Bugesera	Musanze	Nyamagabe	All districts
Very good	0.00	10.53	0.00	3.67
Good	16.13	42.11	0.00	19.27
Fair	35.48	5.26	2.50	12.84
Poor	48.39	42.11	97.50	64.22

control soil pests. Despite the efforts of the farmers to control pests, effective control was not achieved. The perception of effective control was the capacity to clear and or kill all pests. Most of the farmers (64.22%) were not able to effectively control the soil pests (Table 3). There were significant differences ( $p < 0.01$ ) in farmer capacity to control pests effectively in the different districts. Nyamagabe and Bugesera district had the lowest capacity to effectively control pests.

The main methods for controlling pests were reported as use of pesticides (55%) and hand picking (15%) (Figure 4). Other methods included use of quality seeds and uprooting diseased plants.

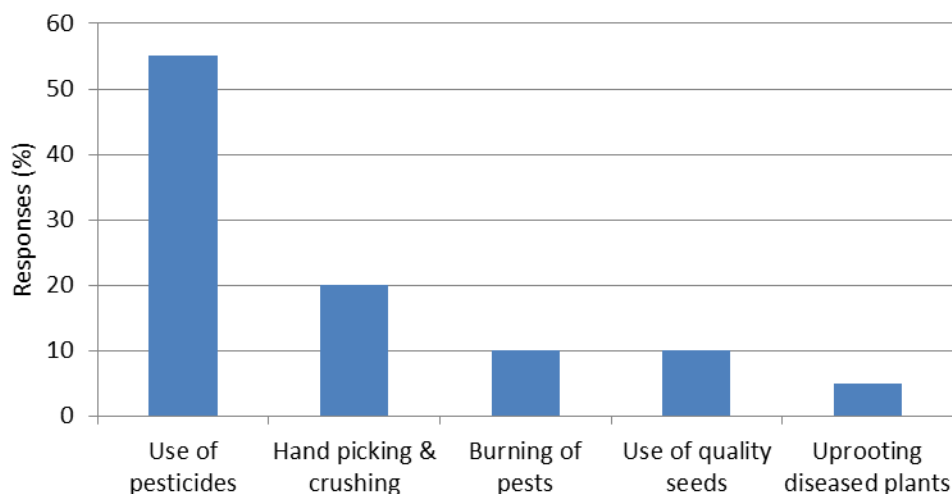
Hand picking had an advantage of not being costly and required less technical know-how. Pesticides were reported as expensive (Bruno and Henry, 2011) and also contributing to environmental contamination. Cost of controlling using pesticides was 51.70% higher than the cost of hand picking as reported by 58.33% of the farmers (Table 4). Hence, the need for alternative pest control approaches. Key informants noted that there were other control options such as cultural practices and biological control using some beneficial insects. Farmers lacked a good understanding of the beneficial insects that would be used for control of vegetable pests.

However, farmers expressed preference for the use of beneficial insects because of lower costs involved. Given successful use of entomopathogenic nematodes (EPNs) in other parts of the world (Carol et al., 2012; Clara et al., 2002; Smart, 1995), tests conducted by the research station in Rwanda and discussions it emerged that EPNs would be a good alternative control option. This is justified by problems associated with pest control and farmer efforts to undertake control.

About 76% of the vegetable farmers reported that they do not control pests. The farmers who did not control cited a number of reasons (Table 4). The main reasons for failure to control pests were lack of knowledge, high costs of the pesticides and pest resistance to pesticides.

Since use of pesticides was the main pest control option, additional assessment was conducted to establish factors that were limiting use of pesticides. It was established that costs of pesticides was the main factor (Table 5).

The factors that limit farmer capacity to control can be addressed by increasing farmer access to many pest control options. Among the approaches are options that help farmers to address the pests in soil with ease as is the case with biological control. In the case of biological control, use of EPNs is considered critical. This is



**Figure 4.** Methods used for pest control.

**Table 4.** Reasons for failure to control soil pests

Reason for no control	Responses (%)
Lack of knowledge of the control method and the pests	48.50
Difficulty to apply the pest control products in the soil	7.92
High costs of the pesticides	15.85
Lack of pesticides	10.34
Not effective/efficient pesticides	17.41

**Table 5.** Problems of accessing pesticides reported by farmers (%).

Problems of accessing pesticides	Responses (%)
Agro-dealer shops are far from the farmers' homes	5.95
Farmers do not know appropriate pesticides and the methods of use	13.10
Expensive (high cost)	58.33
Lack of capital	11.90
Not readily available	10.71

because of the many opportunities and appropriateness of biological control to small farmers who are unable to afford expensive chemicals (Greathead, 1986) as is the case in Rwanda. In addition, use of EPNs can be maintained over a large area without a lot of efforts on the part of the farmer (Divya and Sankar, 2009; Nyasani et al., 2007; Parwinder, 2001). The key soil pests in Rwanda are known to be susceptible to EPNs. For example, there is promising field efficacy of EPNs against cutworms (Georgis et al. 2006). EPNs are most efficacious in habitats that provide protection from environmental extremes, especially in the soil, which is the natural habitat (Hazir et al., 2003). The agro-ecosystems in Rwanda provide a very conducive

environment for the EPNs. EPNs are safe to humans and other non-target organisms and have no known negative effects on the environment (Hazir et al., 2003). EPNs can be used effectively in integrated pest management, which makes them very suitable for use in Rwanda. Biologically based pest management strategies present opportunities through predation or parasitism of pests and plant direct or indirect defense mechanisms that can all be important components of sustainable pest management programs (Chidawanyika et al., 2012). The use of EPNs is therefore expected to generate long-term benefits to the farmers in Rwanda.

The farmers also reported that providing training on soil pests and existing control options is a key approach. The

need for training was alluded to by 56.2% of the respondents. Other farmers noted that there was need for manuals and other materials to help in pest identification. Similarly, all plausible methods of control need to be made available to the farming community. It was noted that proven technologies would be preferred by the farmers based on financial resource base. Financial support to help purchase the crop protection products were also cited as key in the fight against pests.

Some vegetable farmers did not have clear information regarding EPNs but noted that an environmentally safe technology that is easy to apply to control soil insect pests would be preferred. In this case EPNs would be the preferred choice. The farmers who were aware of the EPNs noted that the EPNs would address the soil insect pests in a form that does not require the farmers to physically access the soil pests, especially where it is difficult to locate the pests. There is interest in the use of other methods for the control of pests that attack vegetables but no explicit information exists on alternative methods for control of pests. This in essence suggests that there is potential for the use of EPNs contingent upon the requisite promotion efforts. This assertion is consistent with the understanding among the farming community that pesticides, which is the common control method is expensive. Providing information about pest control is also necessary to facilitate endeavours aimed at pest control.

There are indications among some farmers albeit very few that there exists alternative pest control approaches including the EPNs meaning that if such farmers could be identified through the village headmen it could help in dissemination of the EPNs technology. There were no reported social norms that could hinder the dissemination of any biologically based technology aimed at improving the pest control scenario in the study area. The farmers' needs for effective pest control are a range of methods for pest control, training on the use of the various techniques for pest control and facilitation to use the requisite methods.

## Conclusions

Many vegetable types are produced in Rwanda and there is preference for the vegetables by both the farmers and consumers as reported by those involved in the vegetable trade. The main production system is intercropping although the drive is towards mono cropping. Production processes are less intensive as indicated by the limited use of fertilizers and pesticides. The overall production process is fraught with limited technical know-how and hence calls for effective extension from basic production practices to pest control and post-harvest handling.

The major soil insect pests were cutworms, white grubs and nematodes. There were few control options at the disposal of the farmers. This was aggravated by the fact

that very few farmers attempted control of pests. Only 24% of the farmers reported to have attempted control of soil pests. The main control method used was application of chemical pesticides by only about half of those that attempted control. Even for those that attempted control the success rate was low. Failure to control the soil pests and limited success rate was attributed to low financial resources and limited technical know-how. In particular, farmer access to chemical pesticides and other crop protection practices was restricted by limited financial resources. There was also limited knowledge regarding the pest control approaches and the methods for controlling pests were not diversified.

The vegetable farmers were willing to take on any technology that would help in the control of pests. There was however, preference for a technology that would have long term benefits and be compatible with integrated pest management. Hence, the use of biologically based crop protection technology would be the first choice. To this end entomopathogenic nematodes (EPNs) appear to be the best choice given environmental suitability, low farmer financial resource base and the expected long term benefits. Success in this line would be guaranteed with appropriate information dissemination about the technology. Appropriate methods for dissemination of information would be important especially if they take into account the stakeholder capacity that includes education and financial resources. Pooling of resources and group activities may be necessary in the short run.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Amelioration of a degraded ultisol with hardwood biochar: Effects on soil physico-chemical properties and yield of cucumber (*Cucumis sativus* L)

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A study was conducted in two consecutive cropping seasons to assess the effect of biochar on soil properties and yield of cucumber (*Cucumis sativus* L) in an intensive cucumber–maize rotation based system of Abakaliki, Southeastern Nigeria. Five rates of hardwood biochar (0, 2.5, 3.75, 5 and 6.25 t ha<sup>-1</sup>) were used for the study. The study was laid out as a randomized complete block design (RCBD) with five treatments and four replications. Data were collected from both soil and plant parameters. Soil samples (0 to 20 cm) were collected before and at harvest from different plots for soil chemical analyses. Results obtained from the study showed significant ( $P < 0.05$ ) improvement in soil properties. Bulk density (BD) was significantly ( $p < 0.05$ ) decreased in biochar amended plots. Total nitrogen (N), available phosphorus (P), organic carbon (C), pH and exchangeable bases ( $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$  and  $Na^+$ ) were significantly ( $p < 0.05$ ) higher in biochar amended plots relative to the control. Biochar application significantly ( $p < 0.05$ ) increased vine length, number of fruits, fruit length and yield of cucumber compared to the control. On average, 6.25 t ha<sup>-1</sup> rate of biochar application gave the highest improvement in soil properties while highest increase in yield and other agronomic parameters were observed in 5 t ha<sup>-1</sup> rate of application. The study recommended 5 t ha<sup>-1</sup> as the maximum rate of biochar application in the study area. Our results indicated that biochar application could be a possible way of improving soil properties and native soil carbon in the degraded ultisols and intensive cropping systems.

**Key words:** Mineral fertilizer, organic inputs, small holder farms, soil productivity, sustainable agriculture.

## INTRODUCTION

Unprecedented global population growth, the expansion of agricultural frontier and other human activities encroaching on fragile ecosystems in many parts of the

world, especially in Sub-Saharan Africa, has necessitated the urgent need for increased and sustainable agricultural production. Agriculture is the main source of livelihood

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and income for two-third of Africa's population (Ditto, 2013). Imhoff et al. (2004) showed that agricultural production must increase significantly to meet the needs of a growing global population with increased per capita consumption of food, fibre, building materials and fuel. Most small holder farms have soils depleted of nutrients and soil organic carbon (SOC), following years of nutrient removal in crop harvest with minimal return of crop nutrients through mineral fertilizer or organic inputs (Smalling et al., 1993). Using burnt and unburnt rice husk dust as soil amendment, Njoku and Mbah (2012) reported improved soil properties and increased maize grain yield.

Biochar is a charcoal (carbon-rich solid material) produced under high temperatures (300 to 500°C) through the process of pyrolysis using crop residues, animal manure, or any type of organic material (Bracmort, 2010). The two main methods of pyrolysis are "fast" pyrolysis (heating of biomass in the absence of oxygen, Chan et al. (2007) and "slow" pyrolysis (by natural burning or by the combustion of biomass under oxygen-limited conditions, Sohi et al. (2009). Fast pyrolysis yields 60% bio-oil, 20% biochar, and 20% syngas and can be done in seconds, whereas slow pyrolysis can be optimized to produce substantially more char (~50%), but takes on the order of hours to complete (Odesola and Owoseni, 2010). Lehmann and Joseph (2012) have distinguished the term biochar from charcoal in that it is charred organic matter that is applied to soil not only to improve soil properties but also to promote soil remediation or other environmental services while the charcoal is used as fuel or source of heat, as a filter, as a reductant in iron-making or as a colouring agent in industry or art. Researches on biochar are expanding rapidly not only because of its potential for carbon sequestration (Sohi and Shackley, 2009) but also for its several co-benefits as soil amendment, such as increase in crop yield (Akca and Namli, 2015), potential as a technology for immobilizing pollutants (Herath et al., 2015) and increasing soil fertility and nutrient retention in soils. Though previous researchers have really explored the potentials of biochar as soil amendments for agricultural production and improvement of soil quality (Ndor et al., 2015), research on accurate rate of biochar application on a degraded Ultisol and other soil types for specific arable crops is scanty and rather proceeding slowly. Furthermore, biochar's effect is soil type dependent (Nelissen et al., 2015) and also, biochar effects on soil aggregation is dependent on soil and biochar types (Herath et al., 2013). Moreover, biochar properties depend both on feedstock and production conditions, through which biochar's impact on soil properties is expected to vary (Ronsse et al., 2013).

Studies done on biochar effects on Nigerian soils are very few and scanty. Current review of available literature of biochar in Nigeria indicates that nearly all the biochar research were potted/greenhouse experiments (Fagbenro

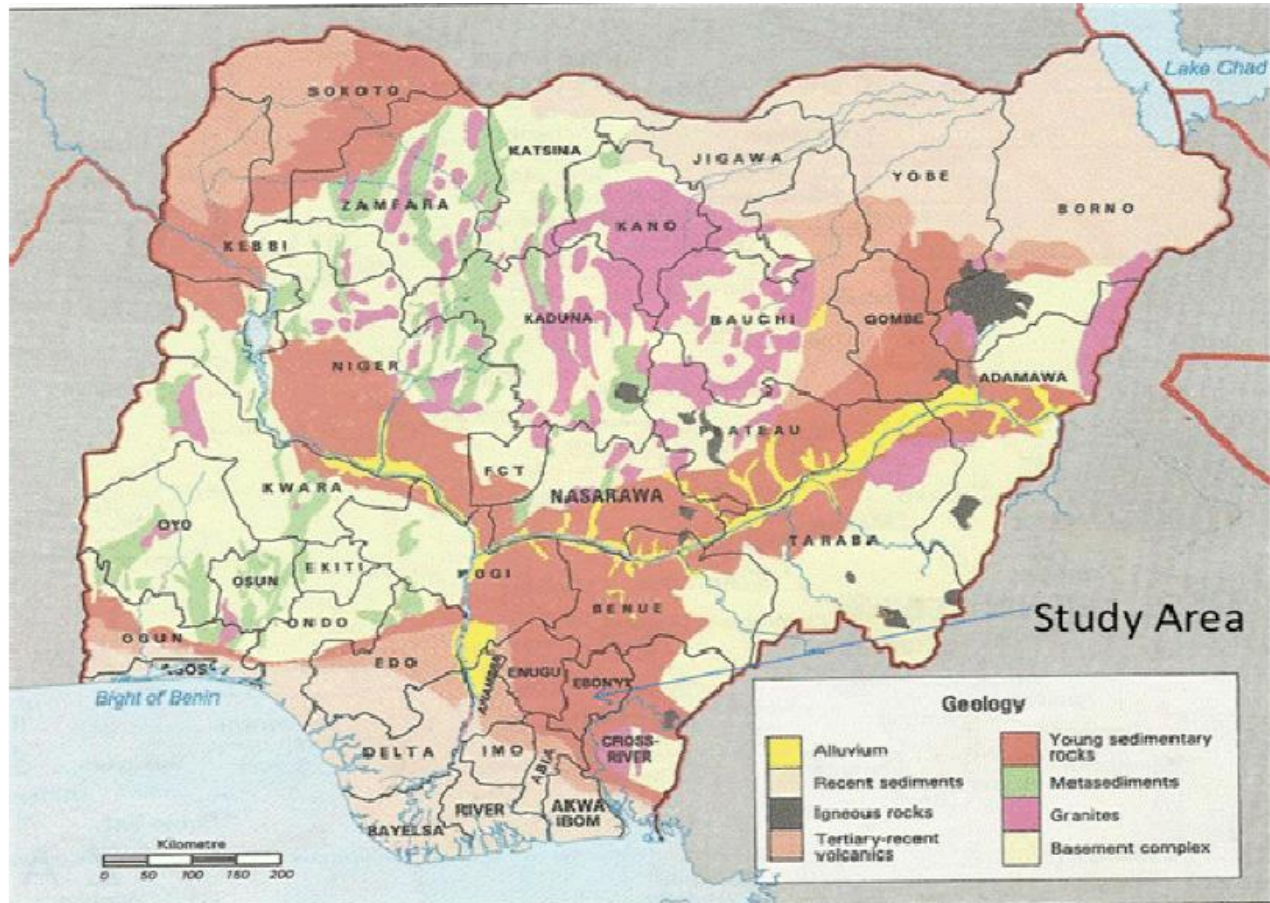
et al., 2015; Onwuka et al., 2015). Ndor et al. (2015) focused on the effect of biochar on soil properties and organic carbon sink in degraded soil of southern guinea savanna zone, Nigeria while Yilangai et al. (2014) investigated the effect of biochar and crop yield on growth and yield of Tomato (*Lycopersicum esculentus* Mill) in Jos, North central Nigeria. There is urgent need for long-term studies on biochar in field trials to better understand biochar effects and to investigate its behavior in different soil types under varying climatic settings thereby providing a framework information about their potential in improving soil quality and increasing crop productivity, as well as its resultant associated risks (if any). Many of the short-term effects of biochar on plant growth and soil behavior reported from laboratory studies were not observed in the field emphasizing the need for long term field trials to help inform agronomic management decisions involving biochar (Jones et al., 2012). More so, adequate care should be taken on the amount and type of biochar added to the soil for restoring degraded soils (Mekuria and Noble, 2012).

Soils of Southeastern Nigeria are poor in their native availability of nutrients (Mbagwu, 1989), low in organic matter content (usually <1%) and, hence are structurally degraded (Obalum et al., 2012). Soil fertility depletion in small holder farm is the fundamental cause of declining per capita food production (Sanchez et al., 1996). Agbede and Kalu (1995) opined that Nigerian farmers' access to fertilizer in vegetable growing season is limited by fund, thus the Abakaliki small holder farmers are seriously faced with the problems of scarcity and late distribution which in turn militates against optimum productivity. In the face of these challenges, there is a need for cheaper alternative which is environmentally friendly that can make fertilizer more available to small holder farmers for sustainable agricultural productivity.

Cucumber (*Cucumis sativus* L.) is a tropical vegetable that grows in warm temperate and cool tropical area. According to De luca et al. (2006), cucumber does well with temperature range of 18 and 30°C with growth reduction occurring at temperature below 16°C and above 30°C. Recently, interest in the production of cucumber by small holder farmers in Abakaliki, South east Nigeria has increased. The increased interest in cucumber production was due to increased demand and consumption of the vegetable in the study area as a result of increase in population arising from the presence of a new Federal University and production factories in the area. However, the use of biochar as an amendment has not been really explored in the study area. Thus, published articles/information on how various biochar types affect plant growth and crop yield specifically in the production of cucumber in different soil types is not available in the study area and still proceeding rather slowly.

Based on these assumptions we hypothesized that soil biochar amendment in a cucumber (*Cucumis sativus* L) crop could:





**Figure 1.** Geologic map of Nigeria showing the location of study area (Balogun, 2000).

- i) Improve soil physical quality through decreasing soil bulk density and increasing porosity,
- ii) Enhance soil properties and carbon (C) sequestration potential also in a short-term crop;
- iii) Improve soil nutrient balance in a degraded soil;
- iv) Improve quality and crop yield.

The study will also recommend appropriate rate of biochar for use in cucumber (*Cucumissativus L*) production in the study area for sustainable agricultural productivity.

## MATERIALS AND METHODS

### Study area

This research was carried out during the 2012 and 2013 cropping seasons in the Teaching and Research Farm of Faculty of Agriculture and Natural Resources Management, Ebonyi State University, Abakaliki, Nigeria (Figure 1). Abakaliki (longitude 08° 65 E, latitude 06° 04 N, temperature 27 - 31°C, rainfall 1700 to 2000 mm, relative humidity 60 to 80%) experiences bimodal pattern of rainfall (April to July and September to November) with short spell in August called "August break". The relative humidity is high during rainy season reaching 80% (Overseas Development of Natural Resources Institute, ODNRI, 1989) and declines to 65% in dry

season. The underlying geological material is Shale formation with sand intrusions locally classified as the 'ASU River' group. The soil is hydromorphic and belongs to the order Ultisol and classified as TypicHaplustult (Federal Department of Agricultural Land Resources (FDALR), 1985). Farming is the major activity of people of the area. Land uses include low land traditional rice farming; multiple (annual) cropping (cassava, plantain, cocoyam, maize, vegetables, pepper, melon seed and beans); citrus and oil palm plantations, herbaceous plants, grasses as bush fallow, and natural forest through the crest to lowlands of the upland-inland continuum (Okolo et al., 2013). The soil is sandy loam with moderate soil organic carbon (OC) content, low in pH and cation exchange capacity (CEC), with dominance of the exchange complex site by calcium and magnesium (Table 1).

### Preparation of the biochar

The biochar of four different species of hard wood (Iroko: *Chlorophora excelsa*, Obeche: *Triplochiton sleroxylon*, Oil palm: *Elaeis guineensis* and Gmelina: *Gmelina arborea*) bought from a local distributor (pyrolysed at 350°C for 3 h) was manually crushed to particle sizes smaller than 2 mm and thoroughly mixed together. Afterwards characterization was carried out according to Biochar material test categories and characteristic of the IBI Biochar Standards Version 2.0 (2014) and incorporated at different rates into the soil.

**Table 1.** Some properties of the topsoil (0 to 20 cm) and biochar before amendments (pre-planting).

Parameter	Unit	Soil	Biochar
Sand	gkg <sup>-1</sup>	680	Nd
Silt	"	178	Nd
Clay	"	142	Nd
Bulk density	gcm <sup>-3</sup>	1.60	Nd
pH (0.01M CaCl <sub>2</sub> )		5.9	7.5
Exchangeable bases	cmolk <sup>-1</sup>		
Na <sup>+</sup>	0.09	1.81	
K <sup>+</sup>	0.10	3.78	
Mg <sup>2+</sup>	1.70	1.89	
Ca <sup>2+</sup>	2.10	1.55	
Total nitrogen	gkg <sup>-1</sup>	0.9	0.88
Available phosphorus	gkg <sup>-1</sup>	3.78	22.01
Organic carbon	gkg <sup>-1</sup>	10.1	64.24
Ash content	%	Nd	21.0
SSA (Specific surface area)		Nd	0.8

Nd = Not determined.

### Field methods/preparations

The site was slashed and cleared of grasses in July, 2012. A total land area measuring 11 by 14 m (0.154 ha) was used for the study. The experiment was laid out as a randomized complete block design (RCBD) with five treatments and replicated four times to form twenty plots. The experimental plots measured 2 m by 2 m with 1 m plot alley. The soil amendment was a thorough mixture of different hardwood biochar (Iroko: *Chlorophora excelsa*, Obeche: *Triplochiton sleroxylon*, Oil palm: *Elaeis guineensis* and Gmelina: *Gmelina arborea*) applied at different rates and these included:

T<sub>1</sub> = Control; T<sub>2</sub> = 1.0 kg/plot; T<sub>3</sub> = 1.5 kg/plot; T<sub>4</sub> = 2.0 kg/plot and T<sub>5</sub> = 2.5 kg/plot (equivalent to 0, 2.5 t/ha, 3.75 t/ha, 5 t/ha and 6.25 t/ha, respectively). The experimental site was cleared, ploughed, harrowed and made into seed beds with traditional hoe. The treatment (hardwood biochar) were crushed and incorporated into the beds at the depth of 0.20 m during tillage. Cucumber (*Cucumis sativus* L. variety "market more") was sown at three (3) seeds per hill. The seeds were planted at a distance 30 cm and 50 cm and at a depth of 1.5 cm. The cucumber plants were thinned to two plants per hill ten days after germination. The same procedure was equally carried out in 2013 cropping season at the same experimental site.

### Soil sampling and data collection

A composite topsoil sample from ten observational points at a depth of 0 - 20 cm was collected from the experimental site with the aid of soil auger after site clearing for initial soil characteristics. At harvest (end of the study), three soil samples were collected from all the plots for chemical analyses to determine the changes that occurred due to treatments application. Similarly, three core samples were collected from each plot at the end of the study for determination of physical properties. The auger soil samples were composited, air dried and used for determination of pre and post nutrient content of the soil. The agronomic data collected at maturity included vine length, number of fruits, fruit length and yield. At maturity nine plants were selected per plot-based on visual evaluation and tagged. Agronomic data (vine length, fruit length, number of fruits

and yield) were collected from the tagged plants. The harvested fruits were weighed with the aid of a simple weighing balance with two decimal places.

### Laboratory analysis

The pre and post-harvest soil samples were air-dried and sieved with 2 mm sieve, and analysis done using the soil fractions less than 2 mm. Soil pH was measured in a 1:2.5 (soil:0.1 M KCl) suspensions. The soil organic carbon (SOC) was determined by the Walkley and Black method as described by Nelson and Sommers (1982). The total nitrogen was determined by the method described by Bremner and Mulvaney, (1982). Exchangeable bases (K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup>) were determined by the method of Thomas (1982) while effective cation exchange capacity (ECEC) was obtained by summation ECEC = TEB + TEA (where ECEC = effective cation exchange capacity, TEB = total exchangeable bases and TEA = total exchangeable acidity). Available phosphorus (P) was measured by the Bray II method (Bray and Kurtz, 1945). Particle size distribution was carried out by hydrometer method (Clayton and Tillers, 1979). Bulk density was determined using the core method as described Blake and Hartge (1986). Total porosity was calculated from soil bulk density value with an assumed particle density of 2.65 g cm<sup>-3</sup> as follows:

$$TP = 1 - (BD/PD) \times 100$$

Where TP = Total porosity, BD = Bulk density and PD = Particle density.

### Data analysis

Statistical analysis of all the data was performed using GENSTAT 3 7.2 Edition. Significant treatment means was separated and compared using Fisher's least significant difference (F-LSD) according to Steel and Torrie (1980), and all inferences were made at 5% Levels of probability.

**Table 2.** Effect of biochar on soil bulk density ( $\text{gcm}^{-3}$ ) and total porosity (%).

Treatment ( $\text{t ha}^{-1}$ )	BD	TP	BD	TP
	2012		2013	
0 (C)	1.50	43	1.53	42
2.5	1.47	45	1.46	45
3.75	1.47	45	1.45	45
5.0	1.46	45	1.44	46
6.25	1.45	45.3	1.44	46
FLSD = 0.05	0.21	NS	0.28	0.12

C=control, BD=Bulk density, TP=Total porosity.

## RESULTS

Table 1 showed that the soil has low total nitrogen ( $\text{g kg}^{-1}$ ), medium available phosphorus ( $\text{mg kg}^{-1}$ ) and low organic carbon ( $\text{g kg}^{-1}$ ) according to the ratings of Landon (1991). The soil is moderately acidic (pH 5.9) (USDA-SCS, 1974). Application of biochar significantly ( $p < 0.05$ ) decreased soil bulk density and increased the total porosity for the two cropping seasons (Table 2). The biochar material contained high quantity of organic carbon (64.24) prior to application. Bulk density values ranged between 1.50 to 1.45  $\text{g cm}^{-3}$  and 1.53 to 1.44  $\text{g cm}^{-3}$  in the first and second cropping seasons, respectively. In the first cropping season highest bulk density value of 1.50  $\text{g cm}^{-3}$  was observed in the control (C). This value was 2, 2, 3 and 3% higher than the bulk density values in 2.5, 3.75, 5.0 and 6.25 t/ha rate of applications, respectively. The table showed non-significant ( $p > 0.05$ ) increase in soil total porosity (TP) among the amended plots in the first cropping season. However a 5% increase over the control was observed across the treatments. The order of increase in soil total porosity in the second cropping season was 6.25 t/ha = 5 t/ha > 3.75 t/ha > 2.5 t/ha > C.

C = Control, BD = Bulk density, TP = Total porosity, NS = Not significant.

Results of the study in Figures 2 to 5 showed significant ( $p < 0.05$ ) increase in all the soil chemical properties (pH, total nitrogen, organic carbon and available phosphorus) in biochar amended plots compared to the control as shown in their strong  $R^2$  values. Specifically in the first cropping season, organic carbon (OC) ( $\text{mgkg}^{-1}$ ) in control was 6, 41, 42 and 44% lower than OC in 2.5, 3.75, 5.0 and 6.25 t/ha rate of application, respectively. Soil pH with second-order polynomial regression was strongly correlated with biochar ( $R^2 = 91.2$  and 99.7% for 2012 and 2013, respectively; Figure 2) treatments. The order of increase in soil pH was 5.0 t/ha > 6.25 t/ha > 3.75 t/ha > 2.5 t/ha > C in the second cropping season.

The trend of increase in total N ( $\text{g kg}^{-1}$ ) in the first cropping season was 6.25 > 5.0 > 3.75 > 2.5 t/ha > C. In the second cropping season, total N in the control was

33, 33, 83 and 83% lower than in 2.5, 3.75, 5.0 and 6.25 t/ha rate of application, respectively. The highest second-order polynomial regression ( $R^2 = 97\%$ ) was obtained between total nitrogen and biochar in the first year while the second year value was  $R^2 = 90.3\%$  (Figure 3). Figure 4 show higher OC in the first cropping season compared to the second cropping season. Soil organic carbon was strongly correlated with biochar ( $R^2 = 82.4\%$  and  $R^2 = 79.6\%$  for first and second year, respectively) treatments (Figure 4).

There was a remarkable increase in available phosphorus (P) in amended plots relative to the control in both cropping seasons (Figure 5). The order of increase in available P was 6.25 t/ha = 5.0 t/ha > 3.75 t/ha > 2.5 t/ha > C and 6.25 t/ha > 5.0 t/ha > 3.75 t/ha > 2.5 t/ha > C in the first and second cropping seasons, respectively. Second-order polynomial regression analysis showed that available phosphorus in the first year was most associated with biochar ( $R^2 = 99.8\%$ ) treatment more than the second year ( $R^2 = 99.3\%$ ; Figure 5).

Higher exchangeable bases ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$  and  $\text{Na}^+$ ) were observed in the amended plots relative to the control in both cropping seasons (Table 3). Potassium ( $\text{K}^+$ ) and  $\text{Ca}^{2+}$  ranged between 0.08 to 0.15 and 3.7-5.8 ( $\text{cmolkg}^{-1}$ ), respectively in the first cropping season. In the second cropping season, highest values of  $\text{K}^+$  and  $\text{Ca}^{2+}$  were observed in 6.25 t/h rate of application. The order of increase in  $\text{Na}^+$  in the second cropping season was 6.25 > 3.75 > 5.0 > 2.5 t/ha > C. Similarly, ECEC in the second cropping season was 32, 8, 18 and 11% higher than in C, 2.5, 3.75, 5.0 and 6.25 t/ha rate of application, respectively (Table 3).

Results of the study (Table 4) showed significantly ( $p < 0.05$ ) higher fruit and vine length in amended plots relative to the control. Fruit length was lower in the first than in the second cropping season. In both seasons, highest fruit length (17.1 and 17.2 cm) were observed in 5.0 t/ha rate of application. Similarly, higher number of fruits was observed in the amended plots relative to the control. The highest number of fruits (22 and 25) was observed in 5.0 t/ha rate of application in the first and second cropping seasons. The order of increase in the number of harvested fruits in the second cropping season

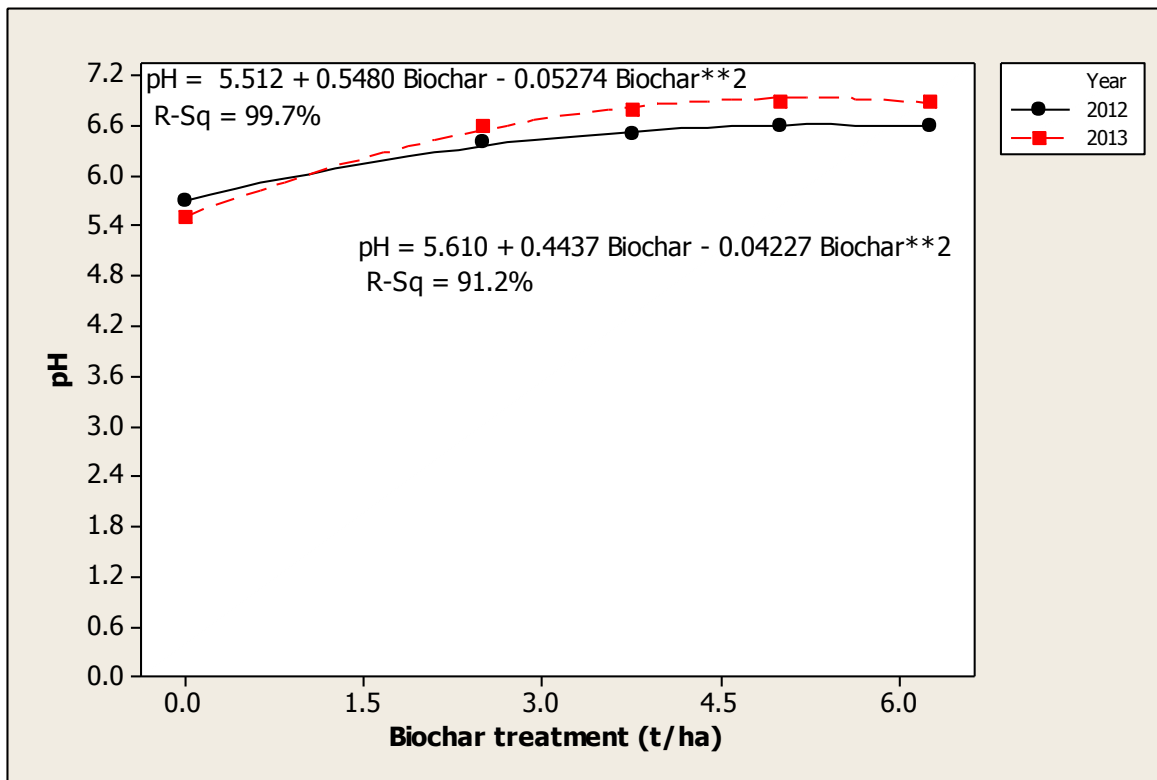


Figure 2. Second-order polynomial regression between biochar treatment and soil pH for the two seasons.

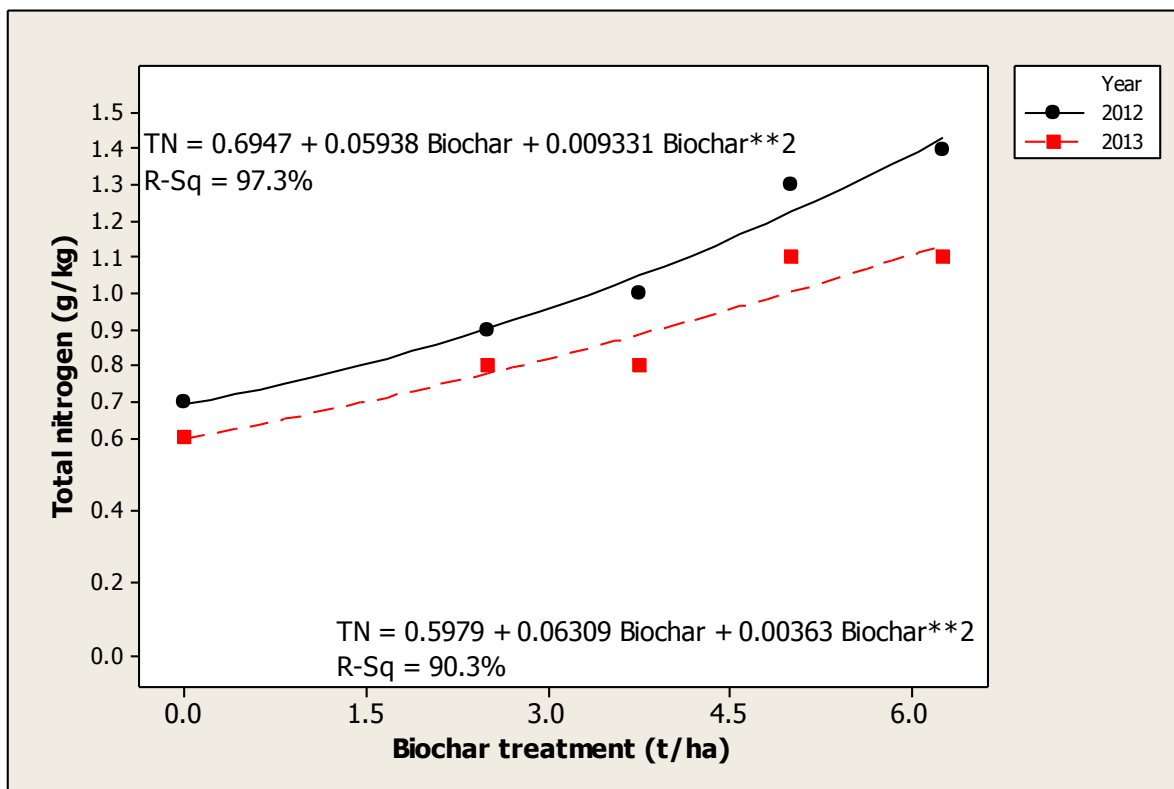


Figure 3. Second-order polynomial regression between biochar treatment and total nitrogen for the two seasons.

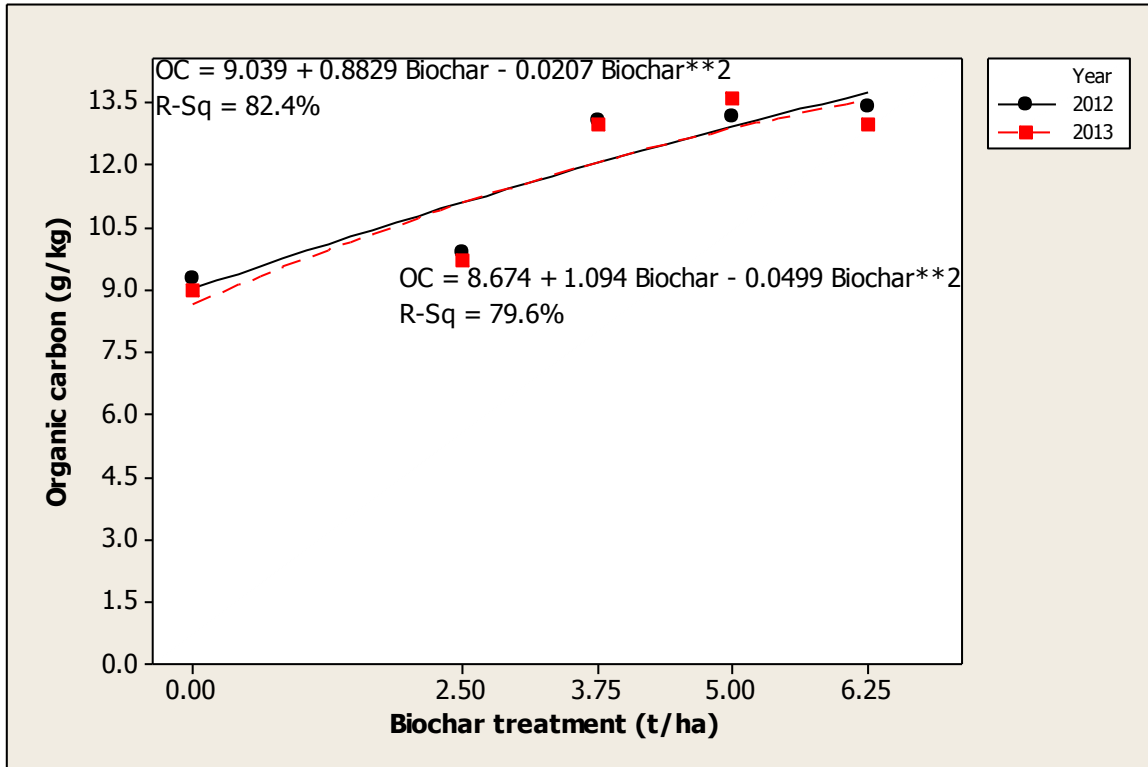


Figure 4. Second-order polynomial regression between biochar treatment and organic carbon for the two seasons.

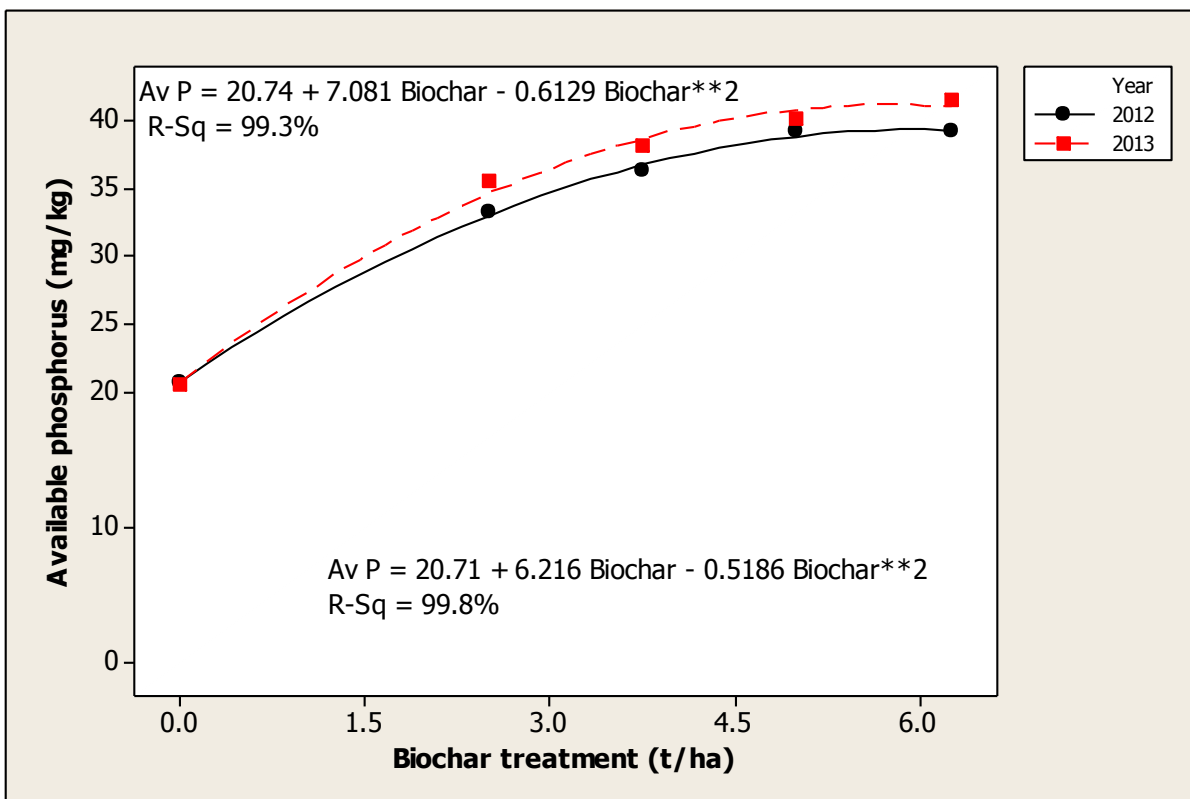


Figure 5. Second-order polynomial regression between biochar treatment and available phosphorus for the two seasons

**Table 3.** Effect of biochar on soil exchangeable bases and Effective cation exchange capacity (ECEC) ( $\text{cmolkg}^{-1}$ ).

Treatment ( $\text{t ha}^{-1}$ )	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	ECEC	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	ECEC
	2012					2013				
0 (C)	0.06	0.08	1.80	3.7	6.6	0.05	0.06	1.60	3.6	6.4
2.5	0.08	0.10	2.00	5.5	8.0	0.07	0.11	2.00	5.2	8.4
3.75	0.09	0.13	2.09	5.5	7.5	0.10	0.15	1.90	5.6	7.9
5.0	0.15	0.13	2.31	5.0	7.9	0.08	0.14	2.40	4.8	8.1
6.25	0.17	0.15	2.34	5.8	8.0	0.10	0.16	2.00	6.4	9.1
FLSD = 0.05	0.10	0.08	1.09	0.13	1.30	0.04	0.10	1.03	1.06	1.20

C = control.

**Table 4.** Effect of biochar on vine length (cm), fruit length (cm), number of fruits and yield ( $\text{t ha}^{-1}$ ) of cucumber.

Treatment	Vine length	Fruit length	Number of fruits	Yield
<b>2102</b>				
0 (c) $\text{t ha}^{-1}$	43.4	13.6	16	4.81
2.0 "	51.7	15.3	19	5.10
3.75 "	53.4	16.2	20	6.16
5.0 "	57.3	17.1	22	6.20
6.25 "	56.8	16.9	20	6.00
FLSD = 0.05	1.69	1.01	1.28	2.13
<b>2013</b>				
0 (c) $\text{t ha}^{-1}$	42.2	13.4	18	4.70
2.0 "	54.6	15.6	21	6.63
3.75 "	58.6	16.3	23	7.42
5.0 "	61.6	17.2	25	7.50
6.25 "	57.2	14.8	20	4.23
FLSD = 0.05	1.20	0.98	1.33	1.22

C = Control.

was  $5.0 > 3.75 > 2.5 > 6.25 \text{ t/ha} > \text{C}$ . The table also showed higher cucumber yield in biochar amended plots relative to the control. Application of biochar at 5.0 t/ha gave the highest yield in both first and second cropping seasons. The highest yield of 6.20 in 5.0 t/ha in the first cropping season was 37, 12, 5 and 47% higher than in C, 2.5, 3.75 and 6.25 t/h rates of application, respectively.

## DISCUSSION

Decrease in soil BD following addition of biochar in the present study is in line with the earlier report of Nelissen et al. (2015). The authors observed that addition of biochar into the soil can alter microbial population, shift functional group and reduce BD with a corresponding increase in soil total porosity. Similarly, Brady and Weil (2004) observed that biochar has a lower BD ( $0.3 \text{ Mgm}^{-3}$ ) than mineral soil ( $1.3 \text{ Mgm}^{-3}$ ) and thus can reduce soil BD

to a desirable level for plant growth. Indeed, the addition of biochar reduced bulk density from  $1.53 \text{ g cm}^{-3}$  in non-treated soil down to  $1.44 \text{ g cm}^{-3}$  in biochar treated soil (Table 2). Notably, the lowest biochar application rate (2.5 t/ha) soil treatment in the present study had similar bulk density to untreated soils.

A reduction in soil bulk density using similar rates of biochar application has been reported in other studies utilizing hardwood biochar (Ndor et al., 2015), wheat straw (Alburquerque et al., 2014); fronds of date palm (Khalifa and Yousef, 2015), and mixed feedstock obtained from prunings of fruit trees (Castellini et al., 2015). The result of the current study tends to reaffirm the postulation of Atkinson et al. (2010) that biochar could possibly be part of a long-term adaptation strategy, as it could affect soil physical properties like soil structure, soil bulk density, porosity, particle density and water storage capacity for sustainable agricultural productivity.

Remarkably, the different rates of biochar application in

the present study has the potential of enhancing the physical structure of amended soils making them favorable for the growth of cucumbers and increased aeration and water storage, thus improving the soil quality.

In the present study, pH, total N, OC, available P, exchangeable bases ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{K}^+$ ) and ECEC were used as chemical or fertility indicators of soil quality for better understanding of the changes that might have occurred as a result of biochar application. The study revealed that the soil was moderately acidic (5.9) before the incorporation of biochar (Table 1). The acidity is typical of the soils of the Southeastern part of Nigeria and is attributed to the parent materials, excessive precipitation which leads to leaching losses of most of the cations in the soil and degradation in soil physicochemical properties (Ano and Ubochi, 2007). The pH values recorded in this study are similar to pH values reported by Okolo (2014) for some other Nigerian soils.

In the present study, addition of different rates of biochar increased soil pH slightly from 5.7 to 6.4 and 5.5 to 6.9 for the two cropping seasons (Figure 2), with all biochar application rates being equally effective and remarkable. This implies that any slight addition of biochar to an acidic soil will give a resultant positive effect in regulating the soil pH. Excessive acidity in arable soils is undesirable because such acidity encourages among other things toxic conditions and also nutrient cation deficiency. It is therefore very necessary and imperative to neutralize excessive soil acidity in order to create optimum and favorable soil environment for plant growth. The increase in soil pH in this study with the application of different rates of biochar corroborates the study of Chan et al. (2008a). Vaccari et al. (2011) in a research with wood biochar pyrolysed at 550°C equally reported an increase in the pH of an acidic (pH = 5.2) silty loam soil at both elevated levels of 30 and 60 t/ha.

The application of different rates of biochar in this study had a significantly ( $p < 0.05$ ) positive effect on SOC in the biochar treated plots compared to control for the two cropping seasons, thus supporting our second hypothesis that applications of different rates of biochar will enhance soil properties and carbon (C) sequestration potential also in a short-term crop. Notably, as a pyrolysed product, biochar is protected from rapid microbial degradation and is able to securely sequester carbon, contributing to mitigation of greenhouse gas emissions (Lehmann et al., 2006). The result of the present study collaborate the recent findings of Angst et al. (2014) who reported that SOC was significantly increased due to the applications of different biochars. Haefele et al. (2011) reported a 66.5% increase in organic carbon contents at elevated level of 41.3 t ha<sup>-1</sup> (about 4%) using rice husk biochar in a near neutral soil. Similarly, Zhang et al. (2012) recorded 44% increase in soil organic carbon at 20 t ha<sup>-1</sup> (about 2%) application rate with a wheat straw biochar. Also, in another research, Khan et al. (2013)

achieved a 550% increase in total carbon contents in a 5% amendment using sewage sludge biochar in an acidic paddy soil. Markedly, biochar treatment was strongly correlated with total nitrogen ( $R^2 = 95\%$  and  $R^2 = 89.7\%$  for 1<sup>st</sup> and 2<sup>nd</sup> year, respectively; Figure 3) and SOC ( $R^2 = 82\%$  and  $R^2 = 78.4\%$  for 1<sup>st</sup> and 2<sup>nd</sup> year, respectively; Figure 4).

The improvement in soil content of total N, available P and basic cations following addition of biochar could be attributed to higher levels of these nutrients in biochar as reported by Preston and Schmidt (2006) and also indicated in the biochar characterization (Table 1). Study by Lehmann et al. (2011) showed that biochar contains high levels of essential nutrients, including P, N, C, CEC and a more neutral pH. The nature and source of biochar, method of pyrolysis and soil type could play an important role in soil properties. For example, mineralization of N could be enhanced by application of biochar produced from slow pyrolysis rather than fast pyrolysis (Bruun et al., 2012), while some studies elsewhere has shown that N in plant-based biochars may be less available than that in biochar from animal manures (Tagoe et al., 2008).

Agegnehu et al. (2015) reported that biochar and composted biochar addition increased soil N by 14 and 29%, respectively. This may be due to the amount of N added and low C: N ratio of the soil, which limits N immobilization. Previous research by Xu et al. (2013) observed that available P increased in biochar amended plots, with the source of P coming from the biochar types used. In the present study, biochar application added very significant amount of available P in amended plots compared to the control ( $R^2 = 89.7\%$  and  $R^2 = 87.7\%$  for 1<sup>st</sup> and 2<sup>nd</sup> year, respectively; Figure 5), thus inferring that biochar application contributes to the increase in soil available P.

Effective cation capacity is the sum of the cations a soil can adsorb at its natural pH, and is obtained by the summation of total exchangeable bases (TEB) and total exchangeable acidity (TEA). It was observed that the amendment of the soil with different rates of biochar significantly improved the ECEC of the soil, thus indicating that the retention of non-acidic cations by the soils increased (Agegnehu et al., 2015). It can be stated that biochar serving as a soil conditioner tends to increase the availability and retention of plant nutrients in soil, thereby potentially increasing nutrient use efficiency for increased agricultural production in degraded soils.

Glaser et al. (2002) opined that biochar inherently containing ash, adds nutrients such as K, Ca and Mg to the soil solution thereby increasing the pH of the soil and providing readily available nutrients for optimum plant growth. The finding of this study demonstrated positive effects of biochar on SOC content and nutrients levels and is in conformity with the findings of Liu et al. (2012) and Agegnehu et al. (2015). Both studies reported positive effects of biochar on SOC content and nutrients levels under field studies in Dystric Cambisol in Northeast

Germany and Ferrasol in North Australia, respectively.

Improvement in soil properties following application of biochar led to increase in vine length, number of fruits, fruit length and yield of cucumber relative to the control. Notably, the temperature of the study area is within the range that enhances cucumber growth and yield. Earlier studies attributed the effect of biochar on crop yield to associated nutrient retention, increased pH and base saturation, available P and increased plant available water (Agegnehu et al., 2015). The observed significant response of cucumber to different rates of hardwood biochar in the present study collaborates with the previous studies of Carter et al. (2013), and Fagbenro et al. (2015) on the stimulating effect of biochars on tree growth. In this study, 5 t/ha rate of application gave the highest yield, vine length, fruit length and number of leaves for the both cropping seasons.

Yilangai et al. (2014) reported significantly higher yield of tomato in beds treated with charcoal than without charcoal. Similarly, biochar application increased vegetable yields by 4.7 to 25.5% as compared to farmers' practices (Vinh et al., 2014). Most recently in an experiment using green waste biochar at 0, 10, 30, 50 and 100 t/ha rates of application. Upadhyau (2015) observed increased number of leaves, root length, plant height and final biomass using lettuce and potato as test crops.

Despite numerous reports of positive effects of biochar application to soil and improved crop production, as evidenced in the current study, some researchers elsewhere have reported negative effect of biochar on soil and crop production. In a recent investigation, Bargmann et al. (2014) observed that in some cases, biochar application can decrease soil available N and plant tissue N concentration. Also, Jones et al. (2012) did not detect differences in soil bulk density three years after biochar addition in a UK field trial, while more recently; Tammorg et al. (2014) did not observe an effect of biochar on soil bulk density and porosity in the field.

It is worthy to mention that the increased agronomic parameters recorded with addition of biochar in the present study are totally in variance with the findings of Schultz et al. (2014). In their investigation, they found a negative effect on growth and yield of oat plant with application of biochar on soil, though it was greenhouse experiment and needed field research to negate or affirm their findings.

## Conclusion

The results of our study showed that improvements in soil properties due to biochar application led to increased vine length, number of fruits, fruit length and yield of Cucumber. On the average, application of biochar at 6.25 t ha<sup>-1</sup> resulted to the highest improvement in soil properties in both seasons while the highest increase in

cucumber yield and other agronomic properties were observed in 5 t ha<sup>-1</sup> rate of application. The results indicated that different rates of biochar application added as soil amendment has the potential of improving soil quality and boosting productivity of cucumber in a degraded Ultisol. More long term and periodic field studies are urgently needed in different soil types and climatic regions to fully understand the benefits of different biochar sources/rates and equally to confirm/negate some of the observations made in view of fostering robust interdisciplinary scientific research.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## Climate conditions in the eastern amazon: Rainfall variability in Belem and indicative of soil water deficit

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The objective of this study was to evaluate the regimes of temperature and rainfall in Belém, PA, Brazil, with emphasis on the start of the dry season in order to provide planning support for agricultural activities during years of climatic anomalies in the region. An initial analysis was done of the metropolitan region of Belém comparing it to the typology of Amazonian climates using rainfall data from 1971 to 2014 and creating an annual index of precipitation anomalies (AIPA). The temperature regime was described using a homogeneous rainfall dataset from 1990 to 2014. The hydrological balance was estimated for the period 1990 to 2014 using an index of capacity of soil water availability equal to 100 mm to identify the months with deficit or excess of soil water. Box plots were analyzed by decade and maximums of daily rainfall for the month of August were used. The Pareto principle was applied to 9 indices to assess the effects of rainfall quantity in relation to anomalous years. Although the metropolitan region of Belém is for the most part categorized by the Af2 climate type it is possible to have prolonged soil water deficit from August through November, an effect that is intensified by the El Niño phenomenon. Furthermore, in the month of August there were years with extreme rainfall events, such as that of August 7th, 2010 where 72.4 mm of rainfall occurred representing 53% of total monthly rainfall. This event can be explained by the intense waves of humidity coming from the East that amplified local rainfall. During the last two decades extreme daily rainfall events have become more frequent, and rainfall reductions in the region have tended to intensify in areas that historically receive less rainfall such as the transition between the Amazonian and Savannah biomes. Therefore, in La Niña or El Niño years, the month of August can be considered to be the signal for meso- and large-scale atmospheric mechanisms that influence precipitation regimes and that can have a negative effect of the region's agricultural productivity.

**Key words:** Precipitation rate, El Niño, La Niña.

### INTRODUCTION

Over the last decades, extreme air temperature and rainfall events have been studied at a global, national,

regional, and local scale. Globally, detailed analyses of those extremes were discussed in the fourth report of the Intergovernmental Panel on Climate Change (IPCC, 2007) and reinforced in the most recent report (IPCC, 2014) due to its changes in magnitude and frequency. Extreme events reflect conditions anomalous to the climatology of a region and may last from a few days to years (De Sousa, 2015). In climate assessments and future climate projections, short-term events have been considered more important than the long-term events owing to their higher frequencies and intensities (intense rainfall, heat and cold waves, droughts) in a climate change scenario (Lewis et al., 2011; Marengo et al., 2016; Espinoza Villar et al., 2011).

The general circulation of the atmosphere (Van der Ent et al., 2010), the digital terrain models, land use, and cover patterns are determinant in the distribution, volume and intensity of rainfall globally and mainly in the Amazon Basin. The Amazon Basin has an estimated area of 6.3 million km<sup>2</sup>, of which approximately 5 million km<sup>2</sup> are in Brazilian territory. The remainder of this area belongs to countries such as Bolivia, Colombia, Ecuador, and Peru. This region is bound in the west by the Andes Mountain Range, which plays a major role in the region's climate dynamics.

In the north, the Guiana Shields act as a barrier for trade winds from the North Atlantic Ocean, while the Atlantic Ocean lies to the east, to where all water caught in the basin flows. To the south of the region, the effects on meteorological variables are impacted by the Brazilian Highlands (Amanajás, 2012). The Amazon plays an important role in energy, water and mass transport in the soil-plant-atmosphere system providing ecosystem goods and services on a continental scale. The services provided by atmospheric flows are responsible for the maintenance of the regional and global climate, such as: The storage and absorption of excess carbon from the atmosphere, the transport of trace gases, aerosols and water vapor to remote regions and, mainly, recycling of precipitation (Rocha et al., 2015).

The Amazon is considered the world's largest tropical rainforest (Verburga et al., 2014) and is very important as a source of heat to the atmospheric circulation both locally (Fu and Li, 2004; Li and Fu, 2004) and globally (Nobre et al., 2009). The region is considered to be a moisture sink for the atmosphere, indicating that it rains more than the rate of evapotranspiration. The main source of moisture is supplied by the Equatorial Atlantic Ocean, transported by trade winds (Arraut et al., 2012), which influences the region's rainfall regime. The sources of moisture come from the North and South Tropical Atlantic Ocean, with a flow from East to West during all

seasons of the year (Satyamurty et al., 2013). During the rainy season, the South Tropical Atlantic (Drumond et al., 2014) is the largest supplier of moisture in the region, and in the rainy season, 32% of precipitated water comes from evapotranspiration (Rocha et al., 2015).

According to Reibota et al. (2010), the Intertropical Convergence Zone (ITCZ) may act in two ways in the north region of Brazil. In one, convective clusters are formed along this front and reach the Amazon basin when they move west and, in the other, trade winds interact with sea breeze to form instability lines that move inland and recover when moving west, often reaching the Andes (Cohen et al., 2014). In addition, according to Marcuzzo et al. (2013), some cold fronts also contribute to rainfall in the region and may go as far as the Brazilian west-most state of Acre, dropping the temperature down to 4°C.

Those mechanisms promote oscillations in the equatorial Pacific Ocean that impact the local meteorological systems by enhancing or inhibiting the development of convection clouds, thus compromising the climatology of rainfall events (Sansigolo, 2000). In years featuring El Niño - Southern Oscillation (ENSO), which occurs when the equatorial Pacific Ocean has anomalous sea surface temperature (SST), the rainy season finishes earlier, with long dry spells. In years under the influence of La Niña, which is characterized by abnormal cooling of SST in the tropical Pacific Ocean, the Amazon experiences excessive rainfall. El Niño and La Niña are large-scale mechanisms that significantly impact meteorological conditions such as pressure, temperature, relative air humidity, cloud cover, wind, and precipitation (Boers et al., 2015).

The rainy season, as well as the extreme rainfall events in the Amazon region, deserves attention due to their negative impacts on economic and social activities. They are fundamental to the maintenance of agricultural crops in the region, because they may reduce grain productivity (Santos et al., 2013), particularly due to extended periods of soil water deficit. The objective of this study was to evaluate the thermal-water regime in Belém, highlighting the month of August as a strategy to subsidize agricultural planning in years with climatic anomalies in the region.

## MATERIALS AND METHODS

### Study region and climate conditions

The city of Belém is located in the western Amazon region, in the northeast portion of the state of Pará, and, given its proximity with the equator and the Atlantic Ocean; it features high air

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temperatures that influence the relative humidity in the region. Medium-scale meteorological systems such as sea breeze, large-scale systems such as the Easterly Waves Disturbance (EWD), the Intertropical Convergence Zone (ITCZ), Bolivia high, and instability lines, among others, are the mechanisms that modulate weather and climate in the Amazon and affect the rainfall regime in Belém (Santos, 2014).

The climatic typology map of the Brazilian Amazon generated at the Agrometeorology Laboratory of Embrapa Amazônia Oriental was utilized for the study to locate Belém and identify the predominant subtype in the capital of Pará. The Köppen climate classification is one of the most widely used climate classification systems and the Amazon typology is classified as Tropical Moist Climates, with occurrence of average temperatures above 18°C in all months that is included in the "A" group. According to Tourne et al. (2016) and Martorano et al. (2011) it is possible to identify three climatic types by the Köppen method, but according to the adaptation of Martorano et al. (1993) there are 10 typological zones in the region. In Af typology, there are variations with three patterns ( $Af_1$ ,  $Af_2$  and  $Af_3$ ), occurring predominantly in the state of Amazonas. In the typology Am, 4 subtypes ( $Am_1$ ,  $Am_2$ ,  $Am_3$ ,  $Am_4$ ) are found, which prevail in the northeast-southwest direction, and the typical areas of Aw have three subtypes ( $Aw_3$ ,  $Aw_4$  and  $Aw_5$ ) Leste-South of the Legal Amazon.

#### Data base and descriptive statistics analysis

Considering the limitation of long historical series with meteorological data in the Amazon, an analysis was made using descriptive statistics, considering a database of the meteorological station of the National Institute of Meteorology (*Instituto Nacional de Meteorologia* - INMET) in the city of Belém (01° 27' S; 48° 30' W; 10 m). Monthly rainfall records over 43 years were analyzed, comprising the period from 1971 to 2014.

Data on the synoptic charts provided by the National Institute for Space Research, more specifically the Center of Weather Forecast and Climate Studies (*Instituto Nacional de Pesquisas Espaciais/Centro de Previsão de Tempo e Estudos Climáticos* – INPE/CPTEC), were consulted. EUMETSAT's Meteosat satellite imagery (copyright 2010-2012 EUMETSAT) of 9:00 am UTC on August 7th, 2010 (Color IR 9) was also analyzed. That day is considered to have featured the largest rainfall event in the historical series evaluated.

A detailed analysis was conducted for the month of August based on meteorological data of Belém in order to identify possible fluctuations, caused by anomalous years. This water deficit is acknowledged as the main factor in the reduction of pasture availability for livestock farms in the region and of water supply to the horticultural belt surrounding Belém. Water balances were surveyed adopting the soil's available water capacity (AWC) of 100 mm based on surpluses and deficits in grass and annual crops. The mean air temperature and solar radiation data used was between 1990 and 2014, the longest homogeneous period found, to strengthen the evidence that August features a soil water scarcity process.

The calculation to determine the annual rainfall anomaly index (RAI) in Belém followed the methodology by Rooy (1965) adapted by Freitas (2005), according to Equations 1 and 2, with the classes adapted as a function of the rainfall in August:

$$RAI = 3 \left( \frac{RNF - RNFm}{x - RNFm} \right) \quad (1)$$

$$RAI = -3 \left( \frac{RNF - RNFm}{y - RNFm} \right) \quad (2)$$

Where RAI is the rainfall anomaly index; RNF (mm) is the rainfall observed; RNFm (mm) is the mean rainfall; x (mm) is the average of the ten highest values observed; and y (mm) is the average of the ten lowest values observed.

After the calculations, the RAI was classified according to nine categories, described in Table 1. Once the anomalies were determined, negative rainfall anomalies (values below -1,0), associated with large-scale mechanisms such as El Niño, and positive anomalies (values above 1,0), characterizing the influence of La Niña, were analyzed.

The data were split into four decades and a box-plot analysis was carried out. Another analysis was performed adopting Pareto principle to verify whether the quantitative effects of rainfall are prioritized regarding anomalous years, considering the indices Extremely Rainy (ER), Highly Rainy (HR), Moderately Rainy (MR), Low Rainfall (LR), Normal (NORM), Slight Reduction in Rainfall (SRR), Moderate Reduction in Rainfall (MRR), Large Reduction in Rainfall (LRR), and Extreme Reduction in Rainfall (ERR). Pareto analysis is a statistical technique in decision-making used for the selection of a limited number of tasks that produce significant overall effect, known as the 80/20 rule.

## RESULTS AND DISCUSSION

### *Climate typology in the Brazilian Amazon, with emphasis on Belém, Pará*

According to the analyzed data, in the study area the mean annual rainfall varies between 2,600 and 2,900 mm. This conditions place Belém in climatic subtype  $Af_2$  (Figure 1). Monthly rainfall can exceed 60 mm with rainfall anomalies fluctuating between the above averages.

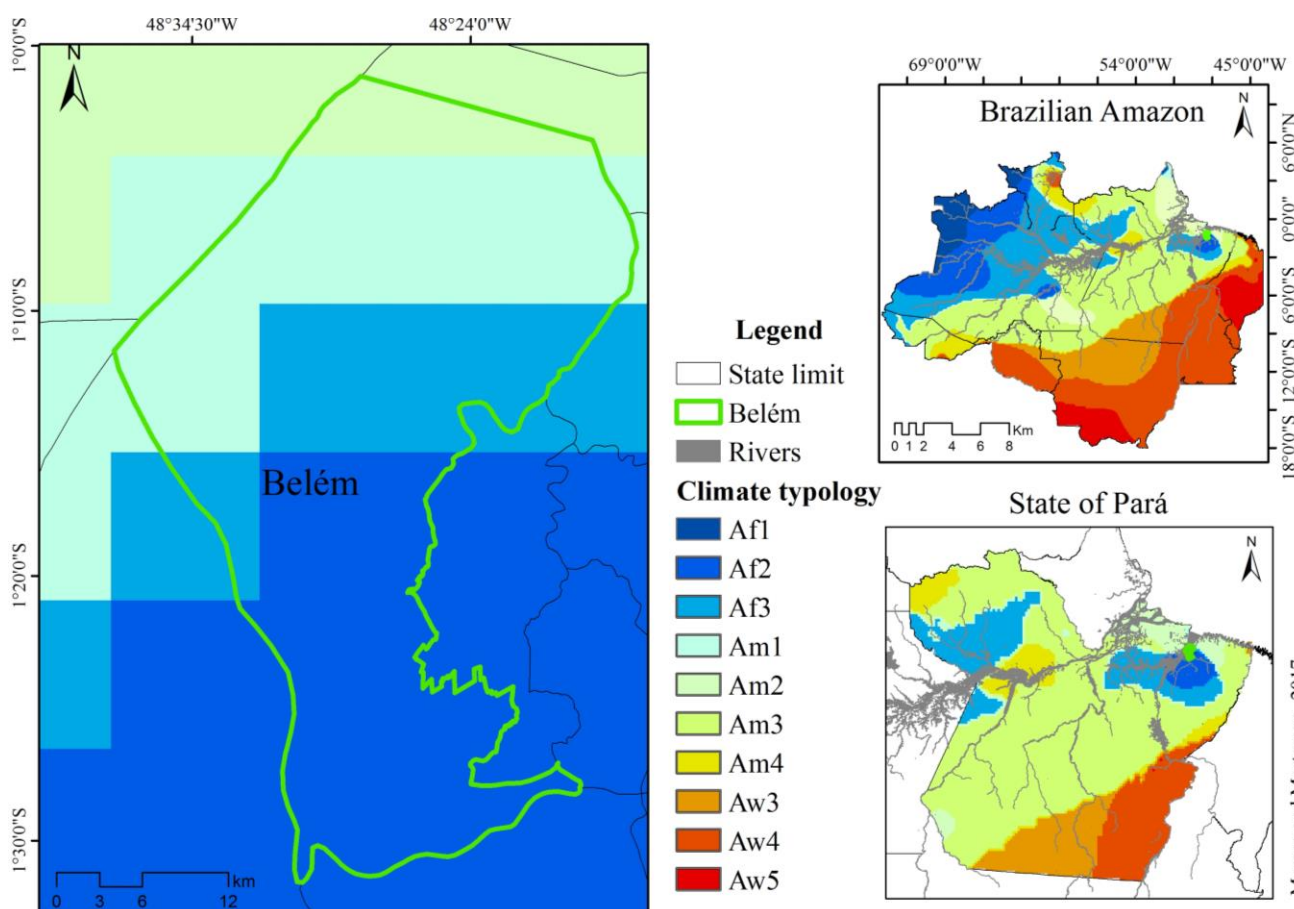
### *Climatic anomalies and soil water balance in Belém, Pará*

The rainy season goes from December to May, with values close to 470 mm in March, and the dry season goes from June to November, with mean values of 119 mm. The reduction in rainfall begins in June and reaches the lowest point in September, with the driest month features rainfall equal to or above 60 mm. Water deficits can be observed starting in August and reach values close to 30 mm between October and November. Soil water deficiency (AWC = 100 mm) in August was detected in 11 years between 1990 and 2014 in the city of Belém. Surpluses between 100 and 140 mm were observed in four years (Figure 2). Additionally, mean monthly air temperature was lowest during the rainy season, ranging from 27.0 to 27.5°C between January and May. In the dry season, confirmed by the soil water deficits between August and November, thermal conditions reach almost 28°C. However, on a yearly basis, the highest mean temperatures are close to 28°C in November and the lowest are close to 27°C in February, which indicates that the mean thermal oscillations do not exceed 1°C (Figure 2).

An analysis of the month of August per decade showed

**Table 1.** Rainfall anomaly index.

RAI	Classification	Acronym
≥ 4.00	Extremely rainy	ER
3.00 to 3.99	Highly rainy	HR
2.00 to 2.99	Moderately rainy	MR
0.5 to 1.99	Low rainfall	LR
-0.49 to 0.49	Normal	NORM
-1.99 to -0.5	Slight reduction in rainfall	SRR
-2.00 to -2.99	Moderate reduction in rainfall	MRR
-3.00 to -3.99	Large reduction in rainfall	LRR
≤ -4.00	Extreme reduction in rainfall	ERR

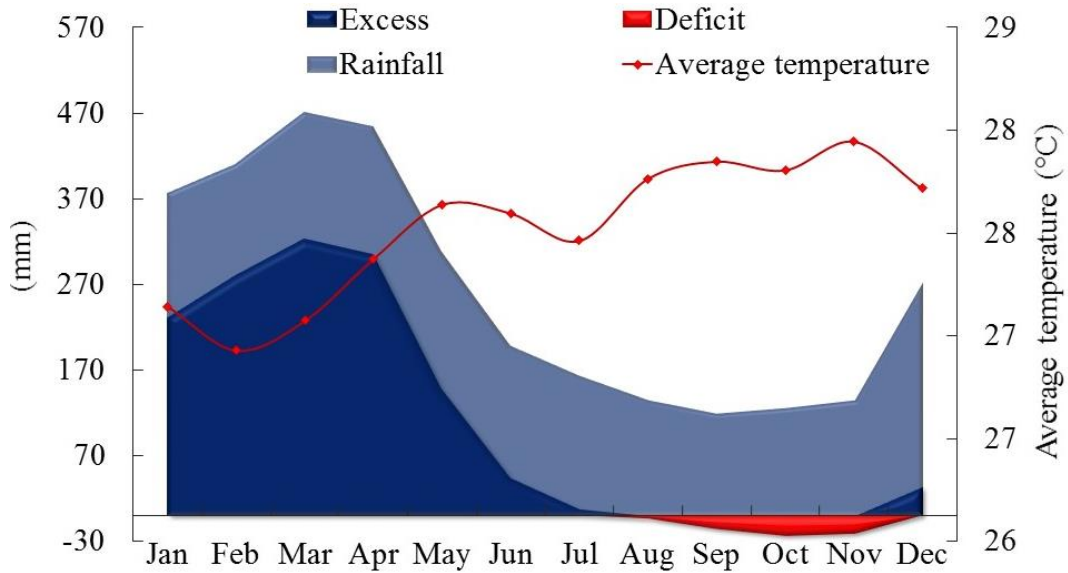


**Figure 1.** Climate typologies in the Amazon, Belém, Pará.

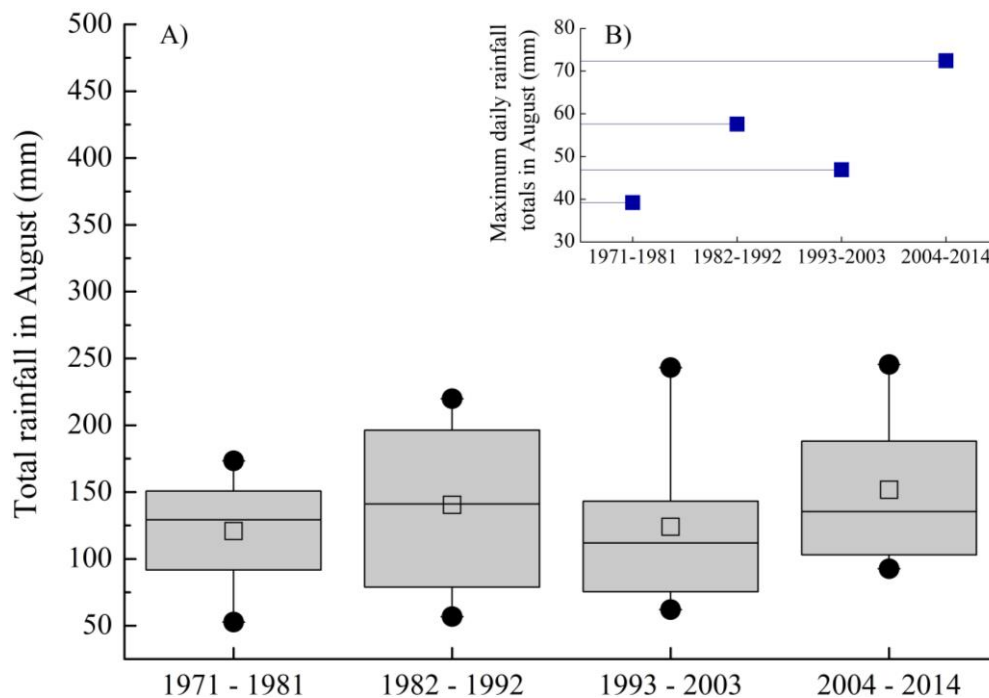
that the mean monthly rainfall is between 100 and 150 mm, reaching up to 250 mm as shown from 1993 to 2003 and from 2004 to 2014 or going as low as around 50 mm from 1971 to 1981 and from 1982 to 1992. It was also found that maximum rainfall variability was greater between 1982 and 1992. Maximum rainfall events between 1992 and 2003 were well distributed in August. The last ten years analyzed, that is, 2004 to 2014 were

the most asymmetric in the whole historical series (Figure 3A).

Isolated extreme events were concentrated in the decade 1993 to 2003 (Figure 3A), explained by the predominance of negative anomalies with rainfall reduction, which were concentrated with values in the first quartile, expressing negative asymmetry in that decade. The maximum rainfall events in August, in that



**Figure 2.** Normal water balance survey (AWC = 100 mm), rainfall (mm), and air temperature (°C) for the city of Belém - Pará, Brazil, between 1990 and 2014.



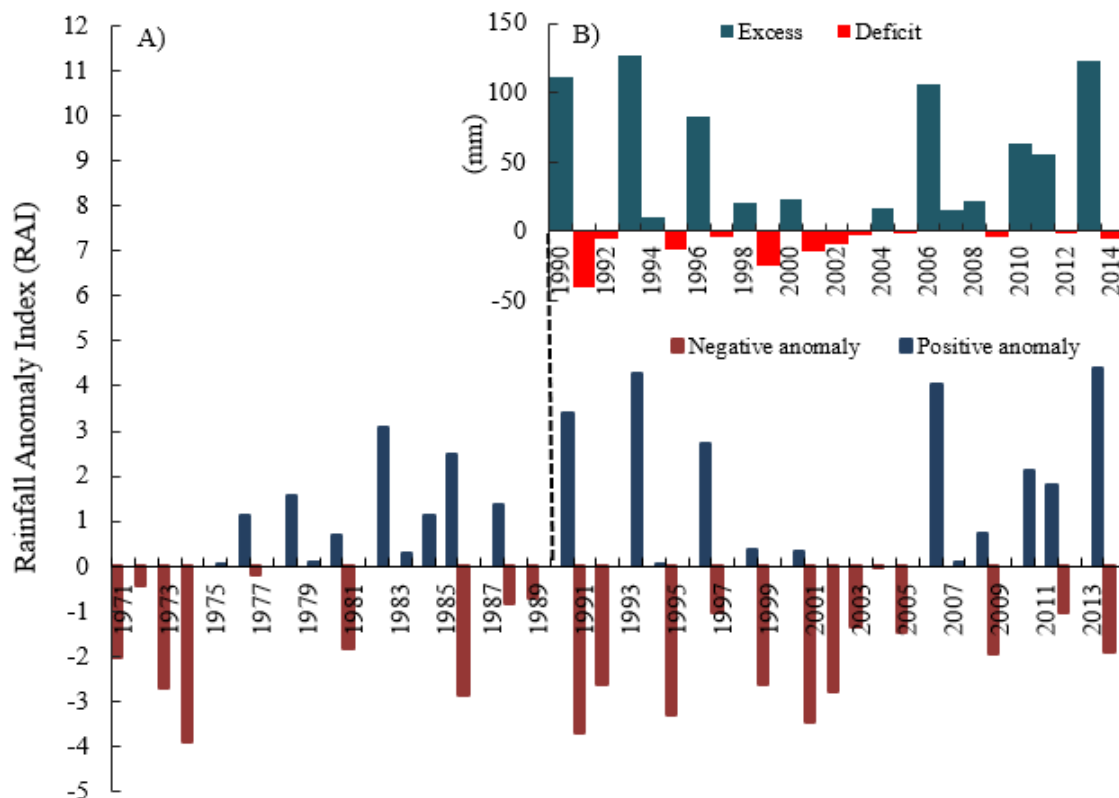
**Figure 3.** Monthly rainfall (A) and maximum rains (B) for August in Belém, Pará, Brazil.

decade, were below 50 mm (Figure 3B), reinforcing that in years of climatic extremes with low rainfall.

Specifically, August behaves as an indicator month, both registering heavy rains at short intervals, which trigger flooding in the city, as well as the occurrence of prolonged periods without rain, which limit the water

stocks in the soil and compromise crop yield and pastures in peri-urban areas.

To further support the responses in the rainfall oscillation pattern in Belém, the results presented show that, between 1971 and 1990, more positive anomalies occurred, with 11 years showing extreme events with



**Figure 4.** Rainfall anomaly index (RAI) in August in Belém (A) and sequential water balance for August between 1990 and 2014 (B), in Belém, Pará, Brazil.

higher rainfall and nine years with below-average rainfall. Between 1990 and 2014, the process was inverted, with 12 years featuring negative anomalies and nine years with positive anomalies, showing that rainfall in August has decreased over the last 23 years in Belém (Figure 4B). According to De Souza et al. (2004), negative rainfall anomalies in the Belém region are associated with positive anomalies in SST in most of the North Atlantic. Those authors highlight that the variability in regional rainfall in the Eastern Amazon and northeast Brazil is caused by a combined effect between equatorial Pacific (ENSO) and gradients in the South Atlantic.

In 1982 and 2001, two anomalous climate contrasts were identified, characterized by the simultaneous occurrences of El Niño and the SST gradients for the North and Intertropical Atlantic, with simultaneous La Niña and SST gradient in the Atlantic Intertropical. Thus, in 1982 the anomalies were positive (La Niña), but positive in 2001 negative (El Niño), being that in all analyzed series occurred more years with negative anomalies (Figure 4A). Therefore, these results reinforce that it is possible in Belém to trigger agrometeorological alerts as early as August when it is confirmed whether the years will be, for example El Niño. This information reinforces that the results presented in Figure 2 show that the water deficit initiated in August can extend until

December, requiring the replacement of water in the soil to the agricultural crops.

The analyses of the frequency of totals rainfall in August showed that 80% of the amount of rainfall in that month came from events between 60 and 180 mm. Frequencies from 60 to 80 mm and from 120 to 140 mm occur in 16% of the events, being the largest occurrences observed. The histogram shows that Belém is characterized by not having rainfall below 40 mm in August, following a normal distribution in the Gaussian curve in almost all rainfall ranges according to the historical series analyzed (Figure 5).

About 80% of the anomalies in rainfall events in August in the city of Belém can be explained by the SRR, NORM, LR, and MRR indices (Figure 6). Extreme events, such as LRR and ER, are observed in fewer than 20% of the years. However, when those events do occur, they cause large economic losses for agriculture and may be linked to large-scale factors that significantly impact meteorological conditions, such as El Niño and La Niña.

Medium-scale effects of the ENSO mechanism lead to changes in mean total rainfall in August, matching studies by Maier et al. (2016), who found significant changes in meteorological conditions such as rainfall in the region. The mean total rainfall in August during the years analyzed is 137.37 mm. It was observed that the rainfall



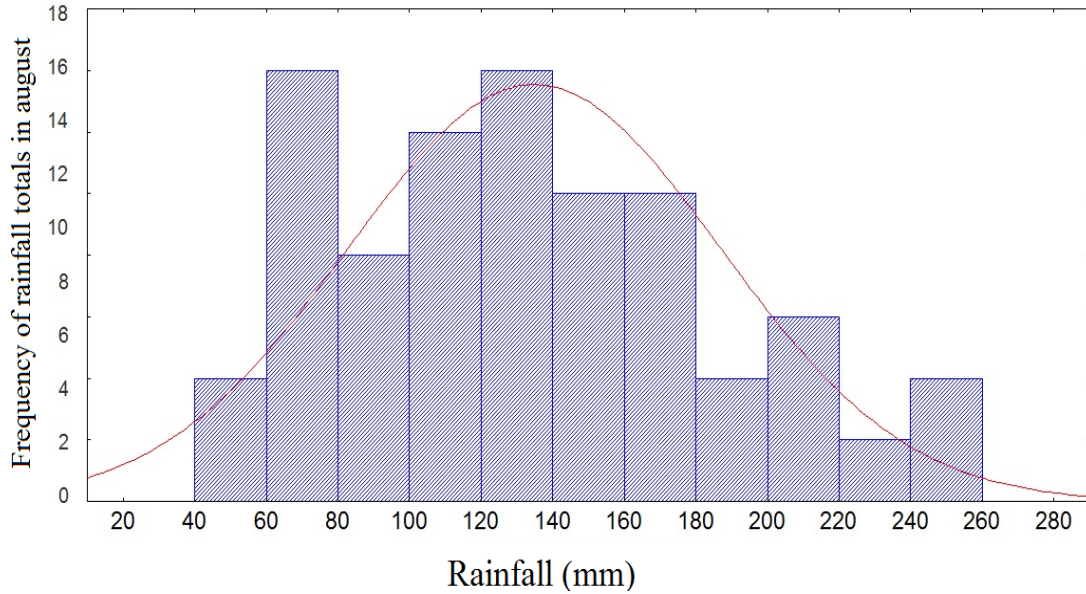


Figure 5. Frequency of total rainfalls in August between 1971 and 2014 in Belém, Pará, Brazil.

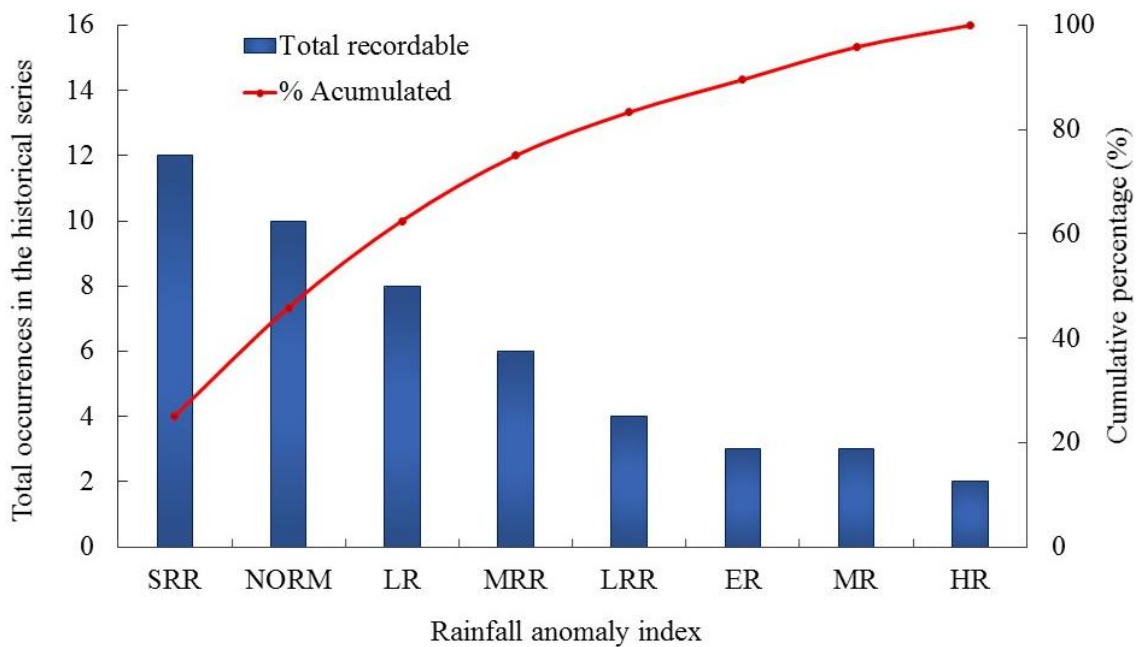
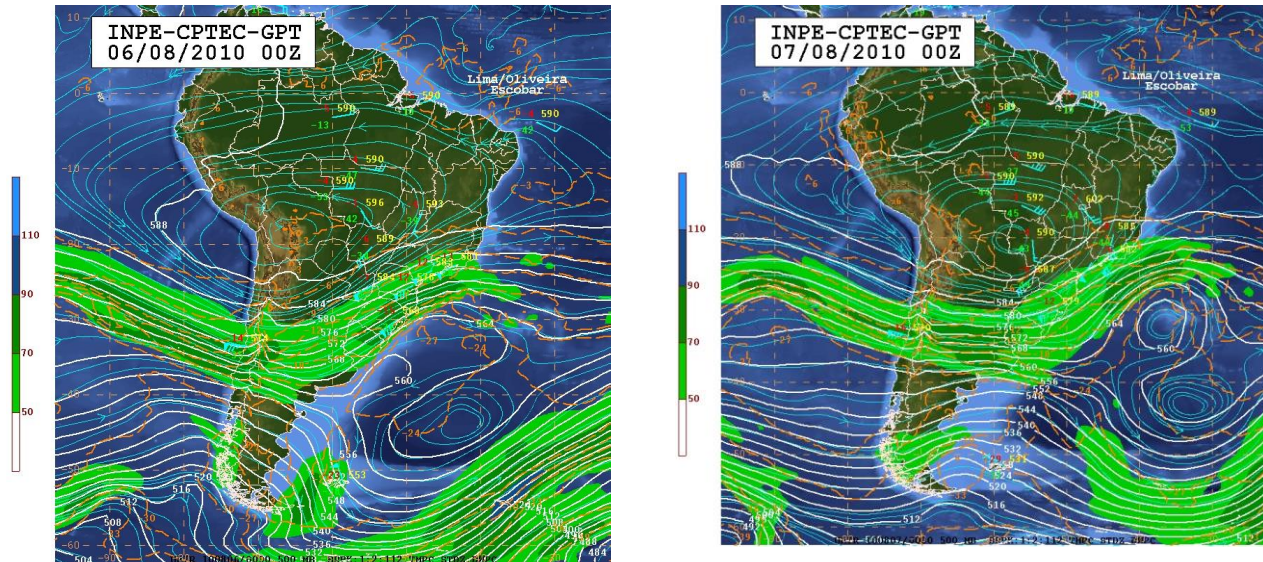


Figure 6. Pareto analysis of the rainfall anomaly index (RAI) in August in Belém, Pará, Brazil.

in 56% of the years was above average or on average, while the remaining years were below average. When the years with anomalies are analyzed, it is seen that the last 20 years had a predominance of negative anomalies, but that the intensity of positive anomalies overcome the negative ones, which justifies the rainier years in August, that is, with mean rainfall above the local conditions.

In terms of maximum rainfall over 24 h, the historical

series shows that August 7th, 2010 featured 72.40 mm of rain, which shows that, although it is a month with low rainfall, extreme events can occur during it. This episode could be explained by consulting synoptic charts provided by INPE. It was observed that, in 2010, the warnings were of a weak El Niño, which highlights the influence of this mechanism on rainfall anomalies in the Amazon. It was also observed that the wind at 500 hPa on August



**Figure 7.** 500 hPa image on August 6<sup>th</sup> and 7<sup>th</sup>, 2010 at midnight. Source: INPE/CPTEC.

6<sup>th</sup> and 7<sup>th</sup>, 2010 indicated an expansion of Bolivia high, which intensifies the effects on weather conditions in the Amazon (Figure 7).

The analyses of those synoptic charts pointed out a large anticyclone whose center was between Paraguay and the Brazilian border state of Mato Grosso near the coordinates 20° S and 55° W, whence a crest extended across Brazil's South region. This anticyclone flow also caused air subsidence and, consequently, adiabatic compression, factors that maintained weather conditions virtually cloud free and with low relative air humidity in central Brazil. The meteorological prognostics supported the existence of an intense geopotential gradient with strong south winds at 20° S coming from the Pacific, crossing Argentina, and extending into the Atlantic close to coastal areas of the South and Southeast regions of Brazil, which was associated with a cyclone vortex that fed the frontal system on the surface.

Images from NOAA (National Oceanic and Atmospheric Administration) satellites on that day (Figure 8) showed the presence of highly vertical clouds due to the moisture advection in eastern South America. The regions in shades of gray indicated little-vertical clouds and the regions in shades of white in the north portion of the Amazon indicated the presence of an ITCZ, with the presence of highly vertical clouds colder at the top, that is, mostly cumulonimbus, which are associated with more intense rains and featuring ice at the top, thus reflecting the intense white in the images.

Therefore, it is observed that those convection clouds were above Belém on August 7<sup>th</sup>, 2010, which indicates that the largest rainfall over the 43 years analyzed can be explained by the presence of this localized phenomenon. It must be pointed out that this rainfall event represented 53% of the rain of the month of August in 2010. That

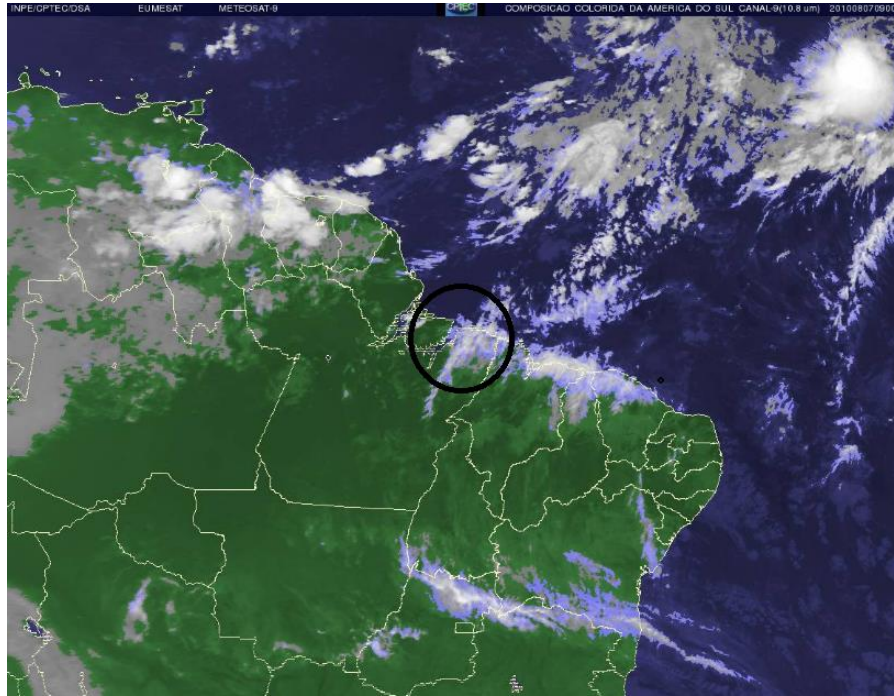
event supports the statements by Dos Santos (2015) regarding the occurrence of short-duration extreme events in the region.

The meteorological systems that modulate extreme events, intensifying or reducing rainfall in the region, may be associated with a strong El Niño and La Niña or be caused by convection clusters that intensify intense short-duration rains. Over the 43 year analyzed (1971 to 2014), specifically in August, it was observed that, since it is a transition month between the rainy to the less rainy seasons, rainfall rates are reduced, but extreme events may occur in years featuring ENSO, as observed in August 7<sup>th</sup>, 2010 (Figure 8). That event was influenced by relative air humidity coming from easterly waves, showing medium- and large-scale effects associated with the El Niño observed in that year. That suggests teleconnections between the meteorological systems that modulate the dynamics in South America with the Amazon (Reibota, 2014).

## Conclusions

Extreme single-day rainfall events compared to the monthly climatologic average were associated with large-scale mechanisms that intensify local weather conditions. The combination between atmospheric modulators on the time and climate scales account differently for the variation of extreme rainfall in August, thus contributing to the occurrence of surplus rainfall in years featuring La Niña and deficits in years of El Niño, with strong intensification of water deficit values over the last two decades.

The period between August and November can bring economic consequences for producers due to the



**Figure 8.** Meteosat image of August 7th, 2010 at 09:00 am UTC (color IR 9). Source: EUMETSAT (copyright 2010-2012 EUMETSAT), available at INPE/CPT/EC.

reduction in productivity in non-irrigated crops as well as the environmental impacts in areas that need replenishment of water in the soil to supply the water demands of the crops and cover losses due to the evapotranspiratory process. It is observed that in years with extreme events, such as a strong El Niño, the problems due to lack of water to agricultural crops are intensified. On the other hand, in climate change scenarios, the models indicate that the consequences tend to increase by the intensity and severity of these effects at a global and regional scale.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Sunflower seed yield under trickle irrigation using treated wastewater

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Filed experiment was carried out in land near Ramtha Wastewater Treatment Plant during growing seasons of 2010 and 2011 to determine the effects of using treated wastewater on plant growth, seed yield and yield components of sunflower (*Helianthus annuus* L.) under trickle irrigation. Randomized complete block design (RCBD) for three irrigation treatments namely; I<sub>1</sub>= Full irrigation (Actual crop coefficient), I<sub>2</sub>= 80% of full irrigation and I<sub>3</sub>= 60% of full irrigation randomized over three blocks. The application efficiency of trickle irrigation system was in the range of 88.2 to 90.8%. The applied irrigation water was 4990, 3992 and 2994 m<sup>3</sup> ha<sup>-1</sup> in 2010 and 5505, 4404 and 3303 m<sup>3</sup> ha<sup>-1</sup> in 2011 for I<sub>1</sub>, I<sub>2</sub> and I<sub>3</sub> irrigation treatments, respectively. Full irrigation treatment (I<sub>1</sub>) gave the highest plant height, head diameter, number of seeds plant<sup>-1</sup>, total weight of seeds plant<sup>-1</sup>, 1000 seeds weight, and seed and oil yield.

**Key words:** Sunflower production, water shortage, reclaimed wastewater reuse, oilseed crops.

## INTRODUCTION

The water availability in arid and semi-arid areas is becoming a main factor to meet the challenges of increasing population. Wastewater reclamation and its reuse have become important components of water resource management plans throughout the world including Jordan (Abdulla et al., 2016).

The scarcity of water resources is one of the major challenges for Jordan, and a limiting factor for economic development particularly for agriculture sector, although additional stress on the water resources comes from population growth, which reaches about 9.5 millions in the end of 2015 as per the population, and housing census report released in late February 2016 (Department of statistics, 2016).

Twenty seven wastewater treatment plants (WWTPs) distributed in Jordan to treat domestic wastewater. Effluent water from these plants is consider an important part of water budget due to water scarcity, and mainly used for unrestricted agricultural production such as forage.

In 2015, about 160 MCM (Million Cubic Meters) is produced from these plants (MWI, 2015). Abdulla et al. (2016) reported that Jordan is considering as a country using the highest ratio of treated wastewater for agricultural productivity in the world. About 160 MCM of treated wastewater was used for irrigation out of 560 MCM of total irrigation purposes, and this form 28.6%.

Effluent from Ramtha wastewater plant (RWWTP) is

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secondary, and nowadays is upgrading to tertiary. Effluent is successfully exploited for irrigated forage production in the surrounded land near the treatment plant (160 ha) (Rusan et al., 2007; Ayoub et al., 2016; Abdulla et al., 2016; Moradi et al., 2016). Effluent of treated wastewater used to save good water quality for domestic use; in the same time using effluent treated wastewater rich in essential nutrient for agriculture will increase agriculture productivity, and achieve economic benefits in by saving the cost of macronutrients (NPK).

Sunflower (*Helianthus annuus* L.) is the most important oilseed crop in the world containing high quality edible oil, and it is easy to grow and developed under different climatic conditions and soils. Sunflower oil is quite tasty, and contains soluble vitamins A, D, E and K. It is used in the manufacturing of margarine; and sunflower cake is used as cattle feed (Hussain et al., 2000). Furthermore, sunflower oil has a high nutritional values, and it has a higher oil percentage in seeds up to 48% (Farokhi et al., 2015). In addition, sunflower seeds are eaten as salted whole seeds as roasted nut meats (Al- Qubaie, 2011; Arshad et al., 2013).

Irrigation treatments for sunflower under semi-arid conditions significantly affected yield and yield attributes (García-López et al., 2016; Abdou et al., 2011). Also, sunflower possesses some genetic potential to grow in low to moderately salt affected areas with a threshold level of  $EC_e$  2.5  $dS\ m^{-1}$  (Heikal et al., 1980; Khatoun et al., 2000; Flagella et al., 2004; El-Kader et al., 2006).

Seed yield has been found to start decreasing beyond  $EC_e$  2.5  $dS\ m^{-1}$ , and achieve 30% less yield at 11.3  $EC_e$ , and also reported 49.21% less seed yield at 10  $dS\ m^{-1}$  (Hussain and Rehman, 1992). Sakellariou-Makrantonaki et al. (2011) found that reuse of wastewater increased seed yield of sunflower plants. The application of treated wastewater as supplementary irrigation showed significant increase in shoot growth, fruit set, fruit weight and yield as compared with rain-fed agriculture (Ayoub et al., 2016).

Irrigation with wastewater significantly increased the concentrations of Ca, Mg and Na in leaves of sunflower plants (Khan et al., 2009). Crop irrigation with treated wastewater represents ecologically sound method for its removal to the environment. Khan et al. (2009) found that the disc diameter, number of seeds per plant, plant weight and 1000 seed weight were significantly increased by the application of treated wastewater compared to either freshwater and/or freshwater with essential nutrients (NPK).

The present study was undertaken to study the effects of using treated wastewaters through surface trickle irrigation on plant growth, seed yield and yield components of sunflower plants.

## MATERIALS AND METHODS

Two field experiments were conducted in 2010 and 2011 during summer growing seasons in land near Ramtha Wastewater

Treatment Plant in northern Jordan. The site is located at 32°35'N and longitude 35°59'E with an elevation of 484 m above sea level. The long average yearly rainfall in the winter season extended from November till end of March is 275 mm. Soil samples were collected before planting from soil surface layer (0-30 cm), air dried, passed through 2 mm sieve, analysed for chemical and physical properties. Soil texture is clay based on the following particle distribution of 50.72% clay, 32.90% silt and 16.38% sand, bulk density is 1.38  $g\ cm^{-3}$ , infiltration rate is 4.8  $mm\ hr^{-1}$ , pH 7.8, EC 0.61  $dS\ m^{-1}$ , N 0.13%, P 17.13 ppm, K 210.38 ppm and organic matter is 0.53%. Randomized complete block (RCBD) design for 3 irrigation treatments namely;  $I_1$ = Full irrigation (Actual crop coefficient),  $I_2$ = 80% of full irrigation and  $I_3$ = 60% of full irrigation randomized over 3 blocks. Actual crop water requirement (ET<sub>c</sub>) was calculated based on potential evapotranspiration (ET<sub>p</sub>) determined from Penman Monteith equation multiply by crop coefficient (k<sub>c</sub>) of sunflower crop of FAO 56 (Allen et al., 1998), 3 days irrigation interval was adopted. Each experimental plot dimension 4.0 x 4.8 m, planted with 8 rows and 0.5 meter row space each row space has one single GR drip lateral line with emitter discharge of 4.0 liter per hour and space 0.40 meters (96 plants per plot). Two meters spacing separate plot to completely eliminate any interaction. At each emitter along the drip line, sunflower seeds were sown on last week of March for 2 growing seasons 2010 and 2011. After seedling growth, they were thinned, and only single seedling was kept at each emitter before starting the applied irrigation treatments.

Chemical properties of treated wastewater used for irrigation was shown in Table 1. Irrigation treatment extended from beginning of April, 10 days after seed sowing (complete seedling germination), and stopped 2 weeks before harvesting in July for both growing seasons. The life span from planting till harvesting is 128 days.

The yield components were studied on the 4 mid planting rows out of total 8 planting rows from each plot. Plants height, head diameters, number of seeds head<sup>-1</sup>, seeds weight head<sup>-1</sup>, weight of 1000 seeds and seed yield were measured. The harvest plot area (4.4 x 0.5 m) was 2.2  $m^2$  (44 plants). Seed oil content was determined according to AOAC (1995) using the Soxhlet extraction apparatus by petroleum ether (40 to 60°C boiling point).

The recorded data for yield, yield components and oil content were subjected to statistical analysis by using the analysis of variance (ANOVA) through MSTAT-C program, version 2.1 software (Russell, 1989). The mean values were compared by least significance difference (LSD) at 5% level of probability.

## RESULTS AND DISCUSSION

### Treated wastewater

Treated wastewater has  $EC=2.62\ dS\ m^{-1}$ , SAR= 3.10, pH=7.66. Whereas, nitrate content, heavy metals, BOD<sub>5</sub>, COD and fecal coliform are within Jordanian standard for reuse of wastewater for irrigated agriculture (Table 1).

### Soil characteristics

Soil after harvesting were analysed and compared with initial soil sample before planting (Table 2); the results indicated that the highest values of pH, EC, N, P, K, SAR, B, Cd, and Pb were obtained from  $I_1$  follow by  $I_2$  and  $I_3$ . There was no significant different ( $p = 0.05$ ) between  $I_1$  and  $I_2$  as well as  $I_2$  and  $I_3$  for chemical soil properties. Also, no significant differences were found between  $I_1$ ,  $I_2$  and  $I_3$  in soil B, Cd and Pb values. No fecal coliform was found in soil samples after harvesting, so the irrigation

**Table 1.** Chemical and biological analysis of treated wastewater from Al-Ramtha Treatment Plant (RWWTP) as compared to Jordanian standard for reclaimed wastewater for agriculture irrigation (Average 2 seasons).

Parameter	Treated wastewater	Max. limit Jordanian standard*
pH	7.66	6.0-9.0
EC (dS m <sup>-1</sup> )	2.62	<2.5
Cl (ppm)	639.53	360
SAR	3.10	9
NO <sub>3</sub> (ppm)	19.63	45
Cd (ppm)	< 0.002	0.01
Pb (ppm)	< 0.01	5
BOD <sub>5</sub> (ppm)	18.56	200
COD (ppm)	42.00	500
Fecal coliform(100 ml <sup>-1</sup> )	53.82	1000

\*Jordanian standard for reclaimed waste water, Ministry of Water and Irrigation, 893/2006.

**Table 2.** Effect of treated wastewater on soil chemical properties (Average 2 seasons).

Parameter	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>
pH	7.94 <sup>a</sup>	7.87 <sup>a</sup>	7.81 <sup>a</sup>
EC (dS m <sup>-1</sup> )	2.86 <sup>a</sup>	2.79 <sup>ab</sup>	2.70 <sup>b</sup>
N (%)	0.19 <sup>a</sup>	0.15 <sup>ab</sup>	0.12 <sup>b</sup>
P (ppm)	8.89 <sup>a</sup>	8.62 <sup>ab</sup>	7.54 <sup>b</sup>
K (ppm)	354 <sup>a</sup>	322 <sup>ab</sup>	291 <sup>b</sup>
SAR	2.90 <sup>a</sup>	2.36 <sup>b</sup>	2.20 <sup>b</sup>
B (ppm)	0.14 <sup>a</sup>	0.12 <sup>a</sup>	0.12 <sup>a</sup>
Cd (ppm)	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>
Pb (ppm)	<0.01	<0.01	<0.01
Fecal coliform(100mL <sup>-1</sup> )	0.00	0.00	0.00

Column means followed by the same letter are not significantly different at 0.05 probability level.

**Table 3.** Effect of treated wastewater on plant height, yield and yield components of sunflower (Average 2 seasons).

Treatments	Plant height (cm)	Head diameter (cm)	No. of Seeds plant <sup>-1</sup> (no.)	Total weight of seeds plant <sup>-1</sup> (gm)	Weight of 1000 seeds (gm)
I <sub>1</sub> (100% of full irrigation)	156.1 <sup>a</sup>	22.69 <sup>a</sup>	1034 <sup>a</sup>	102.30 <sup>a</sup>	92.77 <sup>a</sup>
I <sub>2</sub> (80% of full irrigation)	147.6 <sup>b</sup>	19.23 <sup>b</sup>	942 <sup>a</sup>	95.00 <sup>a</sup>	87.09 <sup>a</sup>
I <sub>3</sub> (60% of full irrigation)	124.7 <sup>c</sup>	11.85 <sup>c</sup>	405 <sup>b</sup>	44.39 <sup>b</sup>	60.86 <sup>c</sup>
LSD (P=0.05)	5.618	1.453	129.3	13.32	7.686

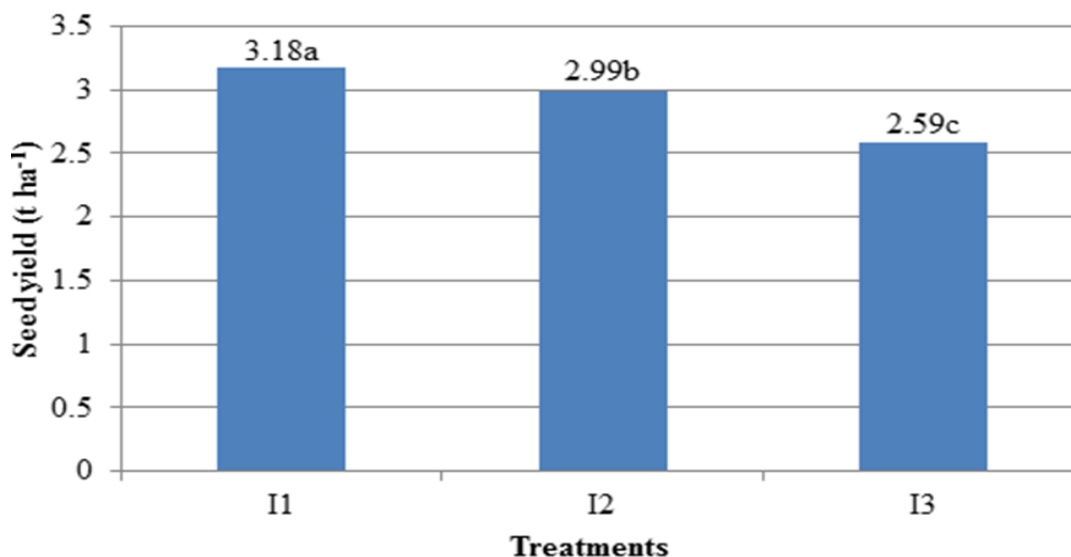
Column means followed by the same letter are not significantly different at 0.05 probability level.

with treated wastewater can be used without any expected microbial contamination for labour. The increasing in the used amounts of treated wastewater in irrigation led to increase in soil chemical properties particularly soil EC and SAR values for both growing seasons; the same trend was found by Salam et al. (2016) who reported that irrigation with treated wastewater may influence negatively on some soil properties particularly EC, SAR and Na% values, which

needs continues observing of these characteristics for long-term.

### Growth parameters (plant height)

Plant height of sunflower was significantly ( $p= 0.05$ ) affected by irrigation treated wastewater under trickle irrigation method (Table 3). The highest plant height (156



**Figure 1.** Effect of different level of treated wastewater on seed yield (Average two seasons) (Column means followed by the same letter are not significantly different at 0.05 probability level).

cm) was obtained from I<sub>1</sub>. Lowest plant height (125 cm) was produced by I<sub>3</sub>, the same trend was found by Soomro et al. (2015) were drip irrigation methods significantly increased plant height of sunflower plant.

#### Yield parameters

Head diameter, number of seeds per plant, total weight of seeds per plant and weight of 1000 seeds were significantly ( $p = 0.05$ ) affected by irrigated treated wastewater (Table 3). The highest head diameter (22.69 cm), number of seeds per head (935), seed weight per head (93 g) and weight of 1000 seeds (100.8 g) were observed in I<sub>1</sub> (100% of full irrigation). The smallest head diameter, number of seeds per head, seed weight per head and weight of 1000 seeds were obtained at I<sub>3</sub> by applying 60% of full irrigation of the sunflower water requirement. It was also observed that there were no significant differences between I<sub>1</sub> and I<sub>2</sub> in weight of 1000 seeds, total weight of seeds per head and number of seeds per head. These results are in agreement with Moradi et al. (2016) who found that oat plants irrigated by 100% wastewater had a 1000-seed weight increase of 16.5% compared to oat plants irrigated by 100% well water.

#### Seed yield

Seed yield was significantly ( $p = 0.05$ ) affected by irrigated treated wastewater (Figure 1). The highest seed yield (3.18 t ha<sup>-1</sup>) was observed in I<sub>1</sub>. The smallest seed yield (2.59 t ha<sup>-1</sup>) was obtained at I<sub>3</sub> by applying 60% of full irrigation of the sunflower water requirement. Zaki and Shaaban (2015) reported that irrigated with treated

sewage water significantly increased seed sunflower yield, and yield components over control.

#### Oil yield

Oil yield was significantly ( $p = 0.05$ ) affected by irrigated treated wastewater (Figure 2). The highest oil yield (1.04 t ha<sup>-1</sup>) was observed in I<sub>1</sub> (100% of full irrigation). The smallest oil yield (0.68 t ha<sup>-1</sup>) was obtained at I<sub>3</sub> by applying 60% of full irrigation of the sunflower water requirement.

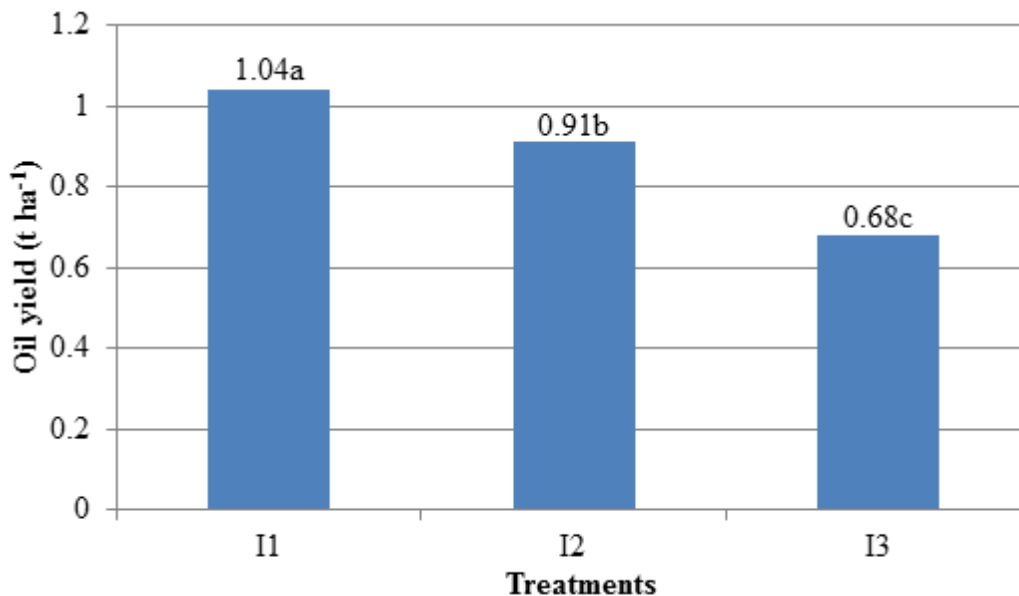
#### Total volume of treated wastewater used

Total volume of treated wastewater consumed by the sunflower crop under trickle irrigation method in 2010 was 4990, 3992 and 2994 m<sup>3</sup> ha<sup>-1</sup> whereas in 2011, 5505, 4404 and 3303 m<sup>3</sup> ha<sup>-1</sup> for I<sub>1</sub>, I<sub>2</sub> and I<sub>3</sub>, respectively. Differences in water application between the 2 seasons reflect difference in evaporative demand (Table 4). The obtained results indicate that total volume of water consumed under trickle irrigation system was effective on growth and yield components of sunflower. This result is in agreement with Qureshi et al. (2015) who reported that drip irrigation increased sunflower production as compared to the furrow irrigation method.

#### Conclusions

This study shows that highest yield components values of the sunflower were obtained at full irrigation treatment by treated wastewater water (I<sub>1</sub>). Treated wastewater can be





**Figure 2.** Effect of different level of treated wastewater on oil yield (Average two seasons) (Column means followed by the same letter are not significantly different at 0.05 probability level).

**Table 4.** Monthly evaporation measurements from a class A pan from seeding to maturity of sunflower, 2010 and 2011

Year	Month					Total
	March	April	May	June	July	
	mm					
2010	23.7 <sup>z</sup>	167.7	231.7	263.1	108.5 <sup>**</sup>	794.7
2011	13.5 <sup>+</sup>	160.0	232.5	309.7	145.4 <sup>**</sup>	861.1

\* 25 through 31 March, \*\* 1 through 12 July, + 27 through 31 March, ++ 1 through 14 July.

used for irrigation sunflower plants to maximize seed yield and oil production as well as other comparable crops to find solution for water scarcity, and availability for irrigation.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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## Full Length Research Paper

# Insect pests infesting black pepper (*Piper nigrum* L.) in southwestern part of Ethiopia

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Survey was carried out to study the distribution, infestation and damaging level of insect pests of black pepper during 2013/2014 cropping season in Southwestern Ethiopia. Insect pests were observed and identified at their sites in the surveyed areas. In addition, samples of insect pests and infected plant parts were collected and insect images were taken. The samples were diagnosed in Tepi National Spice Research Center laboratory. A total of twenty two species of insect pests were recorded as black pepper insect pest with different rate of infestation and damage level. Biting black ants (*Tetramorium* species), black pepper flea beetle (*Longitarsus* species), leaf gal trips (*Liothrips* species), and stink bugs (Pentatomidae) were recorded with relatively high infestation and damage level from all surveyed area, while others were considered as intermediate and minor pest due to low infestation and damage level. Therefore, it is important to design control options for these major insect pests to ensure plant health and pest action under economic threshold level.

**Key words:** Survey, black pepper, insect pests, infestation, damage level, spices.

## INTRODUCTION

Ethiopia is a homeland for many spices, such as korarima (*Aframomum corrarima*), long pepper (*Piper capense*), black cumin (*Nigella sativa*), bishops weed (*Trachy spermumammii*) ('Nechazmud') and coriander (*Coriandrum sativum*) (Jansen, 1981; Edossa, 1998; Girma et al., 2008a). Black pepper known as king of spices is one of the oldest spice crops that originated from India and distributed to other countries (Purseglove et al., 1981; Girma et al., 2008a). Black pepper ('Kundo-

berbere' in Amharic) was introduced to Ethiopia between 1979 and 1980 from potential producing countries (Girma et al., 2008a, b; TNSRC, unpublished data).

It is considered a high value spice crop, since it earns significant foreign exchange for a country (CSA, 2016). For instance, in 2012 around 45,000 kg of dry black pepper was exported from Ethiopia (Bebeka Coffee Plantation, currently Horizon Plantation PLC., 2012). The demand for black pepper and its product is increasing

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year by year in the world market, but production is limited to few countries. Recently, black pepper has performed well in the south west of Ethiopia, particularly, at Tepi, Gemadro and Bebeke large scale farms (Girma et al., 2008a, b). Due to its promising performance in these areas, various business plans on black pepper production, value addition and black pepper oil production are being planned. After long evaluation for adaptation, yield and quality performance, two cultivars of black pepper were selected and released for large-scale production in Ethiopia. The varieties are "Tato" and "Gacheb" and they give dry yield of 2170 and 3050 kg/ha, and 10 and 9.1% (w/w) oleoresin and 2.29 and 3.2% (v/w) essential oil contents, respectively. This performance is below their genetic potential due to several factors (Girma et al., 2008a). Black pepper cultivation has been under threat due to various diseases and insects attacking it, starting from nursery to field plantation at any growing stage (Habetewold, unpublished data). Also reports from India suggest that insects and diseases can cause considerable yield loss on black pepper. For instance, Pollu beetle is the most destructive pest causing 30 to 40% yield loss in humid, tropical evergreen forests of India (Devasahayam et al., 1988) which is similar to south western Ethiopia where black pepper is adapted (Table 1).

Hence, black pepper cultivation is at its infant stage in Ethiopia and little has been done on crop protection. Therefore, the current study aims to survey insect pest damaging black pepper in south western Ethiopia to initiate control options.

## METHODOLOGY

A survey was conducted in three zones: Bench-Maji, Mezhenjer (known as Majang) and Sheka during the 2013/2014 cropping season. From Bench-Maji zone, two districts (Guraferda, and Sheko), Majang one district (Godere), from Sheka zone two districts (Yeki and Andracha (Gemadro)) were surveyed at flowering and maturity growth stages of black pepper (Figure 1). Eighteen sites (three state farms, one on stations (Black pepper maintenance field) and fourteen farmers' field) were surveyed. Field size covered by black pepper was obtained from zonal agricultural office to determine representative samples. From each site, 10 to 15 plants were taken at random to assess pest prevalence from each of the upper (8 leaves), middle (8 leaves) and lower (8 leaves) layers of the black pepper sampled plant. Moreover, insect pests were collected from selected sites for further identification. A pocket lens (10X), insect collecting nets, camel brush, glass vials and polythene bags were used for collection of insect pests for their proper identification. Some pests were identified in the field using identification keys and some of them were brought to Tepi National Spice Research Center for detail study, using pertinent literature and internet search. Some specimens have been maintained to be identified at a later date.

The insect damage scale was assigned according to Seif and Hillocks (1999) that states very low ( $\leq 5\%$ ), low (6 to 10%), medium (11 to 20%), high (21 to 50%) and very high ( $> 50\%$ ) levels. Infestation and damage level from attacked plant leaf/parts were calculated by using the following formula. Infestation per cent = Number of affected sampled leaves/Total number of sampled

leaves  $\times$  Hundred (100); Damage level = Area of plant tissue affected/Total area of plant (tissue)  $\times$  Hundred (100).

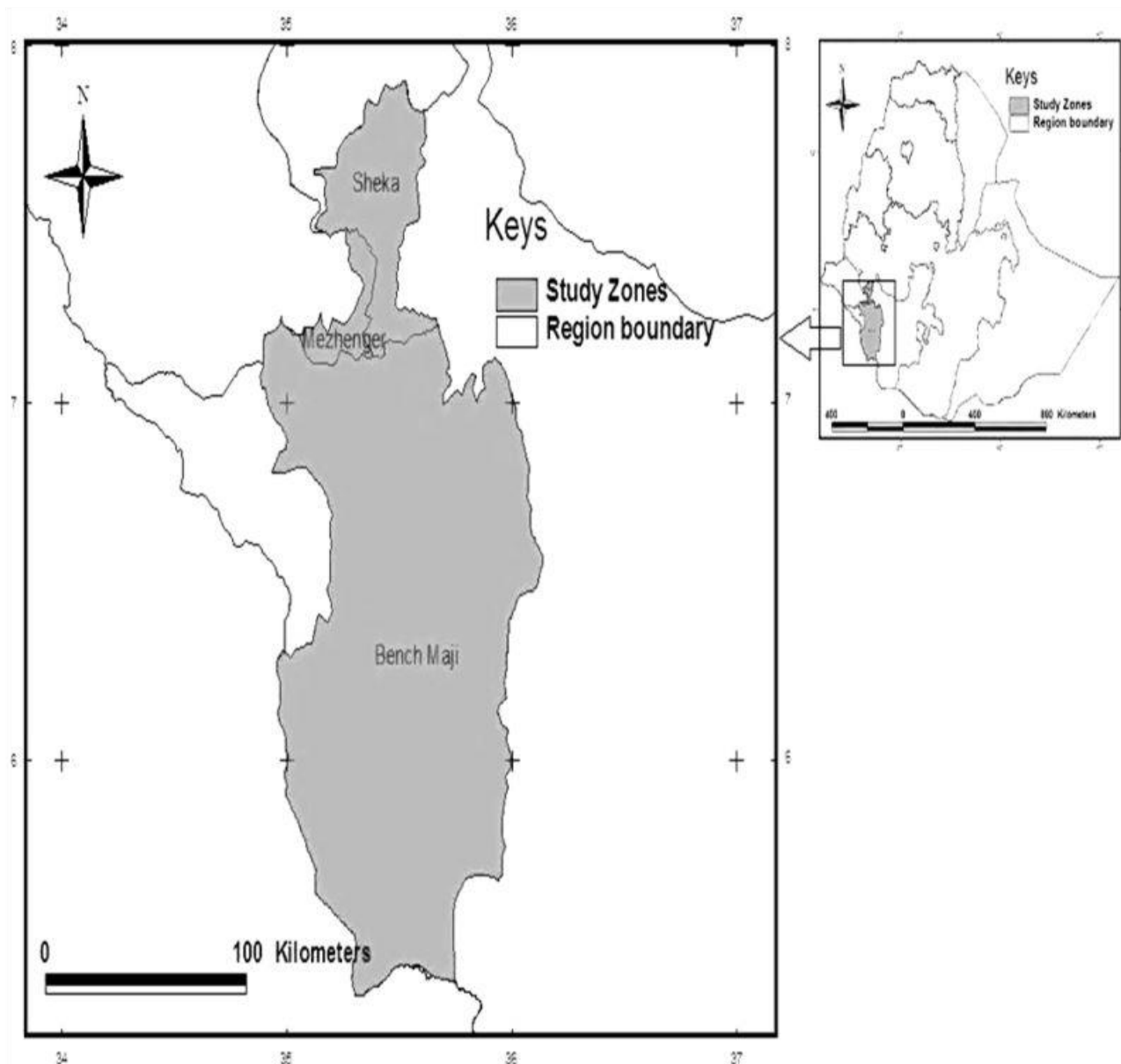
## RESULTS AND DISCUSSION

The present survey revealed distribution, infestation, and damage level of insect pests in southwestern part of Ethiopia (Table 2). Twenty two species of insect pest from six orders and one mite pest were recorded causing different infestation and damage levels. Among insect pests recorded were biting black ants (*Tetramorium* species), black pepper flea beetle (*Longitarsus* species), leaf gall thrips (*Liothrips* species), and stink bugs (Pentatomidae) were recorded relatively with high infestation and damage level from all surveyed areas (Figure 2). Insect pests of pepper have been reviewed recently by Devasahayam (2000) as pepper is infested by 56 general species of insects damaging various parts of vines such as root, stem, shoot, leaves, spikes and berries. However, depending on the severity and extent of damage, pollu beetle, top shoot borer, leaf gall thrips and scale insects could be considered as major pests.

Infestation rate of biting black ants was (Guraferda (42.8%), Sheko (35.5%), Goderre (39.2%), and Andracha (Gemadro) (26.2%) and the ant's damage level were high in Guraferda (28.3%), Goderre (23.7%) and Andracha (21.8%). Biting black ants (*Tetramorium* spp.) found with constructed nest mostly between two leaves. Leaves with ants showed wounded and deformed symptoms. Fisseha (2014) also reported that biting black ants cause indirect attack by interfering with normal agronomic practices particularly during harvesting time. Similar to this result, Hill (1983) reported that the ants spread rapidly when disturbed, bite severely and inject formic acid to the wound which cause irritation. Roger and Alain (1993) stated that urticating ant *Tetramorium aculeatum* (Mayr), the biting ant, is an African ant feared by plantation laborers. Similarly, black pepper flea beetle (*Longitarsus* spp.), were recorded with high infestation rate 36.6 and 23.8% damage level in Guraferda, while it caused 20.1% infestation rate in Sheko district. The adults were found damaging fruit and chewing leaves which cause considerable leaf and tender leaf defoliation. Similar to this finding, pollu beetles belonging to Chrysomelidae families are the most destructive pest of black pepper in India (Devasahayam et al., 1988). Verma (1988) also reported that leaf beetles or Chrysomelidae are one of the largest families with diverse species that adapt in a range of ecology. Among insect pests recorded, leaf gall thrips (*Liothrips* spp.) were also found seriously infesting black pepper plants (Guraferda (23.2%) and Godere (28.4%)) (Table 2). Apart from leaf gall, the pest infestation resulted in reduction in size and malformation of infested leaf. Banerjee et al. (1981) also recorded that the leaf thrips were the most pest of black pepper in south Wynad area in Kerala. In addition to the above pests, brown sting bugs were recorded with a high

**Table 1.** Geographical description of the study areas (CSA, 2007).

Regions	Zones	Geographic location		Altitude (masl)	Temperature (°C)	Rainfall (mm)
		Longitude (N)	Latitude (E)			
SNNPR	Bench-Maji	34.88 to 36.14	5.33-7.21	500 to 2500	15.1-27	400-2000
Gambella	Majang	7.20	35.10	2400-522	15 and 25	1400 and 2200
SNNPR	Sheka	35.24 to 37.90	7.12-7.89	1001-3000	15.1-27.5	1201-1800

**Figure 1.** Map showing the location of zones surveyed for black pepper insect pests.

infestation rate in Yeki (27.1%). However, its damaging and infestation rate are low to medium in other districts. This bug damaged plant parts by sucking plant contents. The infestation and damage level of this insect varied

from district to district (Table 2). McPherson and McPherson (2000) reported that stink bugs (Pentatomidae) and leaf-footed bugs (Coreidae) are important direct pests of many crops which agree with

**Table 2.** Distribution and infestation level of insect pests of black pepper in Southwestern parts of Ethiopia between 2013 and 2014 cropping season.

Zone	District	Insect pests				Infestation (%)	Damage level (%)
		Order	Family	Species	Common name		
Bench-Mmaji	Guraferda	Coleoptera	Chrysomelidae	<i>Longitarsus</i> spp.	Black pepper flea beetle ****	34.6	23.8
		Homoptera	Cicadellidae	<i>Poecilocardia</i> spp.	Leaf hopper*	3.1	0.4
		Thysanoptera	Phlaeotripidae	<i>Liothrips</i> spp.	Leaf gal trips****	23.2	9.7
		Coleoptera	Chrysomelidae	-	Blue leaf flea beetle*	1.2	0.06
		Hymenoptera	Formicidae	<i>Tetramorium</i> spp.	Biting black ants****	42.8	28.3
		Coleoptera	-	<i>Lophobaris</i> spp.	Black pepper stem borer	-	-
		Orthoptera	Acrididae	<i>Cyrtacanthacris</i> spp.	Brownspotted grasshopper***	18.0	7.8
		Hemiptera	Pentatomidae	<i>Euschistus</i> spp.	Brown sting bug***	15.5	11.6
	Sheko	Orthoptera	Acrididae	<i>Acanthacris</i> spp.	Grasshopper*	2.7	3.6
		Homoptera	Cicadellidae	<i>Poecilocardia</i> spp.	Leaf hopper*	0.6	0.01
		Homoptera	Pseudococcidae	<i>Ferrisia</i> spp.	Mealy bug*	2.1	0.4
		Hemiptera	Pentatomidae	<i>Euschistus</i> spp.	Brown sting bug***	19.3	16.7
		Coleoptera	Chrysomelidae	<i>Longitarsus</i> spp.	pepper flea beetle ***	20.1	13.4
		Hymenoptera	Formicidae	<i>Tetramorium</i> spp.	Biting black ants****	34.5	19.1
Hemiptera		Pyrrhocoroidea	<i>Dysdercus</i> spp.	Brown long bug*	1.2	0.11	
Thysanoptera		Phlaeotripidae	<i>Liothrips</i> spp.	Leaf gal trips**	9.1	4.3	
Hemiptera		Miridae	<i>Neurocolpus</i> spp.	Plant bug*	0.4	0.07	
Hemiptera		Pentatomidae	<i>Euschistus</i> spp.	Brown sting bug***	11.1	4.1	
Majang	Godere	Lepidoptera	Geometridae	<i>Ascotis</i> spp.	Caterpillar) *	0.3	0.67
		Coleoptera	Chrysomelidae	<i>Longitarsus</i> spp.	Black pepper flea beetle **	8.8	5.3
		Homoptera	Cicadellidae	<i>Poecilocardia</i> spp.	Leaf hopper*	0.1	0.01
		Heteroptera	Coreidae	<i>Leptoglossus</i> spp.	Leaf-footed plant bug*	4.1	2.3
		Thysanoptera	Phlaeotripidae	<i>Liothrips</i> spp.	Leaf gal trips****	28.45	16.2
		Orthoptera	Acrididae	<i>Cyrtacanthacris</i> spp.	Brownspotted grasshopper***	17.4	12.4
		Hymenoptera	Formicidae	<i>Tetramorium</i> spp.	Biting black ants****	39.2	23.7
		Coleoptera	Chrysomelidae	-	Small spotted leaf beetle***	11.3	11.54
		Hymenoptera	Formicidae	<i>Tetramorium</i> spp.	Biting black ants**	18.5	8.7
		Orthoptera	Acrididae	<i>Cyrtacanthacris</i> spp.	Brownspotted grasshopper****	17.8	12.2
Sheka	Yeki	Homoptera	Cicadellidae	<i>Poecilocardia</i> spp.	Leaf hopper*	0.3	1.1
		Coleoptera	Chrysomelidae	<i>Longitarsus</i> spp.	Black pepper flea beetle ****	24.1	6.5
		Homoptera	-	<i>Toxoptera</i> spp.	Black pepper aphids	6.7	3.2
		Acarina	Eriophyidae	<i>Oligionychus</i> spp.	Red mite*	0.1	0.01

Table 2. Contd.

	Hemiptera	Pentatomidae	<i>Euschistus</i> spp.	Brown sting bug****	27.1	13.5
	Isoptera	Termitidae	<i>Macrotermes</i> spp.	Termite*	-	-
	Heteroptera	Coreidae	<i>Leptoglossus</i> spp.	Leaf-footed plant bug*	0.4	3.2
	Homoptera	Coccidae	<i>Lepidosaphes</i> spp.	Soft brown scale*	0.9	0.02
	Thysanoptera	Phlaeotripidae	<i>Liothrips</i> spp.	Leaf gal trips***	19.7	13.6
	Homoptera	Coccidae	<i>Lepidosaphes</i> spp.	Soft brown scale*	1.8	0.73
	Hymenoptera	Formicidae	<i>Tetramorium</i> spp.	Biting black ants****	26.2	21.8
	Homoptera	Cicadellidae	<i>Poecilocarda</i> spp.	Leaf hopper*	0.05	0.02
Andracha	Orthoptera	Acrididae	<i>Catantops</i> Spp.	Green grasshopper**	9.2	2.4
	Coleoptera	Chrysomelidae	<i>Longitarsus</i> spp.	Black pepper flea beetle ***	14.8	9.3
	Orthoptera	Acrididae	<i>Cyrtacanthacris</i> spp.	Brown grasshopper***	19.1	15.75
	Hemiptera	Pentatomidae	<i>Euschistus</i> spp.	Brown sting bug***	16.9	12.2
	Isoptera	Termitidae	<i>Macrotermes</i> spp.	Termite*	-	-
	Thysanoptera	Phlaeotripidae	<i>Liothrips</i> Spp.	Leaf gal trips***	18.6	6.2
	Coleoptera	Chrysomelidae	-	Small spotted leaf beetle*	0.3	0.04

The infestation and damage of biting ants (black ants) were recorded in terms of nest constructed and leaf wounded due to ants. Key: - Very low ( $\leq 5\%$ ) =\*, low (6-10%) = \*\*, medium (11-20%) =\*\*\*, high (21-50%) =\*\*\*\*level.

current finding. Adults and nymphs suck the plant sap particularly younger plant resulting in discoloration and causing the vine to die. Aldrich et al. (1991) recorded both sexes of stink bugs and the nymphs cause damage by their feeding. Black pepper stem bores (*Lophobaris* species) were also found seriously injuring black pepper plant at Gurafereda (Bebeka). However, this pest was not observed in other areas during survey time. Girma (2015), field observations also confirm that this pest is becoming a serious issue at Bebeke State farm.

Similarly, brown spotted grasshopper (*Cyrtacanthacris* species), leaf hopper (*Poecilocarda* species), termite (*Macrotermes* species) and small spotted leaf beetle were widely distributed in all surveyed areas infestation and damage level were low to medium (Table 2).

These pests occurred with low infestation and damage level included: brown grasshopper (*Acrida* species), mealy bug (*Ferrisia* species), scale insects, leaf-footed plant bug, plant bug (*Neurocolpus* species), and black pepper aphids. These pests, which are found to be very low to medium infestation and damage level, may become serious pests in future due to environmental fluctuation and pest dynamism behaviors.

In addition to insect pests, different natural enemies were observed on black pepper which included different flies (Diptera: Syrphidae species), bugs (Hemiptera: Reduviidae species), lady beetles (Coccinellidae species), green lacewings (Chrysopidae species), spiders (Salticidae and Thomisidae species), wasps (Vespidae and Braconidae species) and praying

mantids during survey period.

## Conclusions

The survey provided some clues to understand distribution, infestation and importance of insect pests on black pepper in southwestern Ethiopia. From this survey, it can be concluded that the insects which were the most distributed and recorded with high infestation rate and damaging level could be categorized as major insect pests. These pests are biting black ants (*Tetramorium* spp.), black pepper flea beetles (*Longitarsus* spp.), leaf gall thrips (*Liothrips* spp.), and stink bugs (Pentatomidae spp.). The brown spotted grasshoppers (*Cyrtacanthacris* spp.) and leaf hoppers (*Poecilocarda* spp.) could be categorized



**Figures 2.** Throughout the survey, the major insect pests observed on black pepper.  
 Note: A = Black pepper flea beetles (*Longitarsus* spp), B = Leaf gal trips (*Liothrips* spp), C = Brown sting bug (*Euschistus* spp), D = Biting black ants (*Tetramorium* spp), E = Black pepper stem borer (*Lophobaris* spp)

as intermediate pests because they were the second most important insect pests in the surveyed areas. Termites (*Macrotermes* spp.) and small spotted leaf beetles, brown grasshoppers (*Acrida* spp.), mealy bugs (*Ferrisia* spp.), scale insects, leaf-footed plant bugs, plant bugs (*Neurocolpus* spp.), and black pepper aphids are considered as minor pests. Even though some of them were widely distributed over all surveyed areas, the very low infestation rate caused less damage. Black pepper stem borers (*Lophobaris* spp.) were recorded causing high damage level at Bebeke State farm only during survey time; therefore, other locations need to be surveyed in detail. Currently, insect pests recorded as major insect pests needed emphasis for development of suitable monitoring and management technique, while regular survey is important to access potential insect pest, because there is the probability for the current minor pest become major insect pest in the future. Farmers need awareness about these pests and follow appropriate cultural practice.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

## Genetic variability, heritability and genetic advance for quantitative traits in coffee (*Coffea arabica* L.) accessions in Ethiopia

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Estimation of genetic parameters is pertinent as variability within population determines the extent of improvement achieved through crop improvement methods. In view of this, forty-nine coffee (*Coffea arabica* L.) germplasm accessions, which were collected from Gomma Woreda, were evaluated at Agaro with the objectives of assessing genetic variability, heritability and genetic advance for morphological traits. The experiment was conducted in simple lattice design with two replications. Data on 26 quantitative characters were recorded. The result revealed significant differences ( $p < 0.05$ ) among the accessions for most of the traits studied. The phenotypic coefficients of variation (PCV) was greater than genotypic coefficients of variation (GCV) for all the characters studied, this shows the influence of environmental factors on the characters. Estimates of variability indicated that high phenotypic (PCV) and genotypic coefficients of variation (GCV) were recorded for coffee berry disease (CBD) severity and yield per tree. High heritability was recorded for hundred bean weight (80.21%), number of nodes of primary branches (67.89%), stem diameter (67.16%), height up to first primary branch (66.6%), bean length (62.79%), bean width (61.43%), average inter node length of primary branches (58.33%), angle of primary branches (53.32%), leaf width (52.94%) and canopy diameter (51.95%). The high GAM were recorded for coffee berry disease reaction (88.86%), clean coffee yield per tree (24.03%), number of secondary branches per tree (22.34%), height up to first primary branch (20%) and hundred bean weight (20%). High heritability was coupled with high genetic advance as percent of mean for characters such as hundred bean weight and height up to first primary branch. The high heritability with high genetic advance as percent of mean observed for these characters is due to the lesser influence of environment in expression of the characters and additive gene effects. The present study indicated the presence of variability for some important morphological traits among the accessions. Therefore, the variability observed for yield, disease resistance and other important traits should be utilized to improve Gomma woreda coffee. However, since high morphological variation between accessions is not a guarantee for a high genetic variation, molecular and biochemical studies need to be considered as complementary to morphological variability. On the other hand, as most of the traits exhibited low GCV and/or low GAM, there is no opportunity to improve these traits using simple selection. Therefore, heterosis breeding should be applied to improve these traits.

**Key words:** Coffee germplasm, genetic variability, disease resistance, simple lattice, hundred bean weight.

## INTRODUCTION

Coffee (*Coffea arabica* L.) belongs to the family *Rubiaceae* and the genus *Coffea* (Coste, 1992). The basic chromosome number for the genus *Coffea* is  $n = 11$ . Arabica coffee is the only polyploid and self-fertile species of the genus *Coffea*, with chromosome number  $2n = 4x = 44$ , while others are diploid ( $2n = 2x = 22$ ) and self-infertile (Silvarolla et al., 2004). The *Coffea* genus includes more than one hundred different species between which a large variation in terms of chemical composition is observed (Clifford, 1985). Coffee (*Coffea arabica* L.) is indigenous to Ethiopia and comprises about 73% of world coffee production due to its superior quality (Orozco-Castillo et al., 1994). *C. arabica* utilization has a longer history than *C. canephora*, probably more than 1,500 years ago and is now the most widespread species cultivated throughout the world (Coste, 1992).

Coffee is at the center of Ethiopian culture and economy, and contributes to about 35% of country's foreign currency earnings. It accounts for 10% of the gross domestic product, and support the livelihoods of about 25% of the population of Ethiopia (representing around 20 million people) in one way or another (Gole and Senebeta, 2008). However, despite the vast area of cultivation, wealth of tremendous genetic diversity and importance to the national economy, the productivity of coffee is very low (about 0.71 t/ha) (Alemayehu et al., 2008). Although many factors hampered production and yield per unit area, the major factors contributing to such low coffee yield include predominant use of unimproved local coffee landraces, as well as conventional husbandry and processing practices, which in turn seriously hampers the overall national coffee production and productivity of the smallholder coffee farmer in the country (Taye, 2010). Hence, it is imperative to improve the productivity of coffee using selection and cross breeding.

Assessment of variability for yield and its component characters becomes absolutely essential before planning for an appropriate breeding strategy for genetic improvement. Characterization of this variability in a population is pertinent since genetic diversity within population and within species determines the rates of adaptive evolution and the extent of response in crop improvement (Solomon, 2009). Genetic parameters such as genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are useful in detecting the amount of variability present in the germplasm. Heritability indicates the effectiveness with which selection of accessions can be based on phenotypic performance. However, heritability alone

provides no indication of the amount of genetic improvement that would result from selection of individual genotypes. Hence, heritability coupled with high genetic advance would be more useful tool in predicting the resultant effect in selection of the best genotypes for yield and its attributing traits.

The heritability of various morphological traits of coffee has been estimated in *C. arabica* L. Mesfin (1980) evaluated 68 coffee germplasm accession of national coffee collection during the year 1975-1978 at Jimma and reported broad sense heritability values of 55% for coffee yield. Cilas et al. (1998) also reported that yield, stem girth and tree height had high heritability. Bayetta (2001) has also reported high heritability estimates between 71.43 and 97.32% for all morphological characters measured in *C. arabica* L., suggesting that the effect of environment on phenotypic expression of the characters was small. Moreover, Yigzaw (2005) conducted a research at Finoteselam, Ethiopia, to see the heritability of 18 morphological traits of 16 coffee accessions and has reported high heritability values for hundred bean weight, number of secondary branches and canopy diameter. However, he reported low heritability for bean thickness; percent of bearing primary branches, average inter node length of primary branches and petiole length.

Although, valuable achievements have been registered by coffee research in Ethiopia, characterization and evaluation of coffee germplasm have not been systematic and exhaustive, for example, among 200 Limu accessions collected and conserved at Agaro station, 49 accessions characterized by Olika et al. (2011) have been reported for the existence of variability for morphological traits among these accessions. Besides, Lemi and Ashenafi (2016) characterized 64 Limu coffee accessions and reported high broad sense heritability estimates coupled with high genetic advance for number of main stem nodes, stem diameter and internodes length. However, the remaining accessions are not characterized in detail.

Thus, with the above back ground information, the present investigation aimed to evaluate variability, heritability and genetic advance of coffee morphological characters in 49 Limu coffee accessions collected from Gomma woreda of southwestern Ethiopia.

## MATERIALS AND METHODS

### Description of the study site

The experiment was conducted at Agaro Station of the Jimma

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Agricultural Research Center. The center was established in 1973 on land area of 27 ha near Agaro town, 45 km far from Jimma and 397 km from Addis Ababa. Agaro is located at 7°50'35"- 7°51'00" N latitude and 36°35'30"E longitude and at an altitude of 1650 m above sea level. The mean annual rainfall of the area is 1616 mm with an average maximum and minimum air temperatures of 28.4 and 12.4°C, respectively. The major soil type is Mollic Nitisols with pH of 6.2, organic matter 7.07%, nitrogen 0.42%, phosphorus 11.9 ppm, CEC 39.40 cmol<sup>(+)</sup>/kg (Zebene and Wondwosen, 2008)

### Experimental materials

Forty seven *C. arabica* L. germplasm accessions which have been collected from the Gomma woreda of Jimma Zone and two standard checks that are maintained in the *ex situ* field gene bank of Agaro station were used for this study. The experiment was superimposed on six year old coffee trees (Table 1) of the 49 accessions and grown under uniform coffee shade tree (*Sesbania sesban*) conditions.

### Experimental design, management and season

The experiment was laid out in a 7x7 simple lattice design with two replications and with seven genotypes per each incomplete block. Each plot was comprised of four coffee trees. Spacing between trees and plots was two meter and spacing between replications was 3 m. All the improved agronomic practices were applied uniformly according to the recommendations (Endale et al., 2008).

### Data collected

Data on 26 quantitative characteristics, namely: Height up to first primary branch (cm), total plant height (cm), number of main stem nodes (no), average inter node length of main stem (cm), main stem diameter (cm), angle of primary branches (deg), number of primary branches (no), average length of primary branches (cm), number of nodes of primary branches (no), average Inter node length of primary branches (cm), percentage of bearing primary branches (%), number of secondary branches (no), canopy diameter (cm), leaf length (cm), leaf width (cm), leaf area (cm<sup>2</sup>), fruit length (mm), fruit width (mm), fruit thickness (mm), bean length (mm), bean width (mm), bean thickness (mm), hundred bean weight (g), yield per tree (kg), coffee berry disease (%) and rust incidence (%), were collected from each accession using the standard procedures (IPGRI, 1996).

### Statistical analysis

Data for quantitative characters were subjected to analysis of variance (ANOVA) using SAS version 9.2 (SAS, 2008) to examine the presence of statistically significant differences among accessions for the characters studied. Least significant difference (LSD at P = 0.05) was employed to identify accessions that are significantly different from each other.

### Estimation of genotypic and phenotypic coefficients of variation

The variability of each quantitative trait was estimated by simple measures such as mean, range, standard deviation, phenotypic and genotypic variances, and coefficients of variation. The

phenotypic and genotypic coefficients of variation were computed based on the formula suggested by Burton and de Vane (1953) as follows:

$$\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + \sigma^2_e$$

Where:  $\sigma^2_g$  = genotypic variance;  $\sigma^2_e$  = environmental variance.

$$\text{Genotypic variance } (\sigma^2_g) = (\text{MSt} - \text{MSe})/r$$

Where: MSt = mean square due to genotypes; MSe = environmental variance (error mean squares); r = the number of replication; Environmental variance ( $\sigma^2_e$ ) = error mean squares

$$\text{Phenotypic Coefficient of Variation (PCV)} = \frac{\sqrt{\sigma^2_p}}{\bar{x}} \times 100$$

$$\text{Genotypic Coefficient of Variation (GCV)} = \frac{\sqrt{\sigma^2_g}}{\bar{x}} \times 100$$

Where:  $\bar{x}$  = mean of the character.

### Estimation of heritability and genetic advance

Broad sense heritability and expected genetic advance were estimated according to the following formula.

#### Heritability in the broad sense

Broad sense heritability values were estimated using the formula given by of Falconer (1989) as follows:

$$(H^2_b) = (\sigma^2_g / \sigma^2_p) \times 100$$

Where:  $H^2_b$  = heritability in the broad sense;  $\sigma^2_p$  = phenotypic variance;  $\sigma^2_g$  = genotypic variance.

#### Expected genetic advance

Expected genetic advance for each character at 5% selection intensity was computed using the methodology described by Johnson et al. (1955).

$$GA = k * \sigma_p * H^2_b$$

Where: GA = the expected genetic advance under selection;  $\sigma_p$  = the phenotypic standard deviation;  $H^2_b$  = heritability in broad sense and k is selection intensity.

#### Genetic advance per population mean

Genetic advance as percent of mean was calculated to compare the extent of predicted advance of different traits under selection using following formula of Johnson et al. (1955).

$$GAM = \frac{GA}{\bar{x}} \times 100$$

Where: GAM = genetic advance as percent of population mean; GA = the expected genetic advance under selection;  $\bar{x}$  = mean of the character.

**Table 1.** Geographical origin of the studied coffee (*C. arabica* L.) germplasm accessions at Agaro research station.

Farmers association where coffee was collected	Woreda/district	Number of accessions collected
Chedero Suse	Gomma	4
Gabena Abo	Gomma	8
Omo Boko	Gomma	7
Goja Kemissie	Gomma	5
Bako Juju	Gomma	6
Debi Kechamo	Gomma	5
Limu Sapa	Gomma	5
Omo Gobo	Gomma	7
Standard check varieties		2
Total		49

## RESULTS AND DISCUSSION

### Analysis of variance

Mean squares for the 26 quantitative traits from analysis of variance (ANOVA) are presented in Table 2. Significant ( $P < 0.05$ ) differences among the coffee germplasm accessions were observed for all traits except for percent of bearing primary branches, leaf area, bean thickness and rust incidence and these traits are dropped from subsequent analysis.

### Range and mean values

The range and mean values for the 22 quantitative traits are given in Table 3. Relatively wide ranges were recorded for average yield per tree (0.23 - 1.45 kg clean coffee), CBD severity (0 to 75.09%), NSB (57.26 to 210.93), NPB (39 to 79), HPB (25.53 to 45.50 cm), HBW (13.31 to 22.88 g), NNPB (17 to 27), SD (4.26 to 6.46 cm) and BL (7.68 to 11.37 mm). Some of these important agronomic traits, such as average coffee yield per tree, CBD resistance level and number of secondary branches, had the highest range between the tested materials. The ranges for the above important characters were more than two fold of their respective grand means. 18 accessions (37%) had mean yield greater than the grand mean. Similarly, 25 accessions (51%) had CBD severity level less than the grand mean.

The range and mean in this study indicated the existence of variability among the tested accessions for majority of the characters studied and there is considerable potential for coffee improvement program in the future. Yigzaw (2005) reported that average green bean yield per tree varied from 144.6 - 566.7 g and 100 green bean weight ranged from 9.3 - 16.0 g. Tree height varied from 107.5 - 182.8 cm, canopy diameter from 137.1 - 246.5 cm, trunk diameter from 24.6 - 39.6 mm, number of primary branches per tree from 35.7 - 62.0 and

number of secondary branches per tree from 21.3 - 117.7. The present finding is also partly in agreement with the finding of Olika et al. (2011) who reported wide range of variation for stem diameter, number of secondary branches and yield per tree among Limu coffee accessions.

### Genotypic and phenotypic coefficients of variation

The estimates of phenotypic and genotypic variances and phenotypic (PCV) and genotypic coefficients of variation (GCV) are presented in Table 4. According to Deshmuk et al. (1986), PCV and GCV values greater than 20% are regarded as high, whereas values less than 10% are considered to be low and values between 10 and 20% to be medium. Accordingly, high PCV and GCV values were recorded for CBD reaction and yield per tree with PCV values of 91.48 and 41.67% and with GCV values of 62.82 and 22.05%. High PCV (30%) and moderate GCV (18.03) were recorded for number of secondary branches, suggesting the existence of high genetic variability among the accessions for effective selection. In the present study, the PCV values were higher than the corresponding GCV, suggesting the existence of environmental variation.

Moderate PCV and GCV were recorded for height up to first primary branch and hundred bean weights with PCV values of 14.57 and 12.07% and with GCV values of 11.9 and 10.81%, respectively. Moderate phenotypic and low genotypic coefficients of variation were recorded for number of primary branches, stem diameter, number of main stem nodes and number of nodes of primary branches with PCV values of 14.79, 10.68, 10.46 and 10.12% and GCV values of 9.28, 8.69, 6.22 and 8.34%, respectively.

Total plant height, average inter node length of main stem, angle of primary branches, average length of primary branches, average inter node length of primary branches, canopy diameter, leaf length, leaf width, fruit

**Table 2.** Analysis of variance (mean squares) for 26 quantitative characters of 49 coffee germplasm accessions grown at Agaro.

Trait	Mean square		LSD
	Treatment	Error	0.05
HUP	38.86**	7.79	6.00
TPH	757.11**	272.62	35.14
NMSN	19.51*	9.32	6.66
AILMS	0.41*	0.17	0.89
SD	0.56**	0.11	0.72
APB	42.89**	13.06	7.76
NPB	121.37**	52.76	14.60
ALPB	49.48*	22.80	10.45
NNPB	7.79**	1.49	2.63
AILPB	0.19**	0.05	0.47
PBPB	28.24 <sup>ns</sup>	27.55	10.55
NSB	1933.00*	905.65	64.86
CD	375.06**	118.6	23.91
LL	1.54*	0.64	1.75
LW	0.26**	0.08	0.64
LA	73.28 <sup>ns</sup>	32.53	12.53
FL	0.77**	0.27	1.12
FW	0.48*	0.23	1.05
FT	0.56*	0.27	1.12
BL	0.7**	0.16	0.8
BW	0.18**	0.043	0.42
BT	0.054 <sup>ns</sup>	0.033	0.36
HBW	7.83**	0.86	1.98
CBD	452.11**	162.37	28.14
Rust	134.65 <sup>ns</sup>	90.43	20.51
Yd/tr	0.08*	0.045	0.43

\*, \*\* and ns = significant, highly significant and non-significant, respectively. HUP= height up to first primary branches, TPH= total plant height, NMSN= number of main stem nodes, AILMS= average inter node length of main stem, SD= stem diameter, APB= angle of primary branches, NPB= number of primary branches, ALPB= average length of primary branches, NNPB= number of nodes on primary branches, AILPB= average inter node length of primary branches, NSB=number of secondary branches, CD= canopy diameter, LL= leaf length, LW= leaf width, FL= fruit length, FW= fruit width, FT= fruit thickness, BL= bean length, BW= bean width, HBW= hundred bean weight, CBD =coffee berry disease, Yd/tr= yield per tree

length, fruit width, fruit thickness, bean length and bean width showed low phenotypic and genotypic coefficients of variation.

Except CBD reaction, average yield per tree, number of secondary branch, height up to first primary branches and 100 bean weights, the GCV values for most of the traits in the present study is low. This low level of variation might have resulted from natural selection in rather similar agro ecological condition of the collection

**Table 3.** Estimates of range and mean for 22 morphological characters at Agaro.

Trait	Range		Mean
	Min	Max	
HUP	25.53	45.50	33.14
TPH	215.5	285.11	250.78
NMSN	30.81	42.63	36.29
AILMS	5.03	7.12	6.21
SD	4.26	6.46	5.46
APB	57.88	75.22	67.35
NPB	39.0	79.00	63.13
ALPB	77.52	97.81	87.57
NNPB	17.00	27.00	21.29
AILPB	3.45	4.84	4.14
NSB	57.26	210.93	125.70
CD	176.4	232.31	203.85
LL	12.67	16.92	14.50
LW	5.29	6.85	5.96
FL	14.34	17.47	15.62
FW	11.65	14.34	12.69
FT	8.37	11.38	10.11
BL	7.68	11.37	9.66
BW	6.21	7.69	6.79
HBW	13.31	22.88	17.27
CBD	0.00	75.09	19.16
Yd/tr	0.23	1.45	0.61

HUP= height up to first primary branches. TPH= total plant height, NMSN= number of main stem nodes, AILMS= average inter node length of main stem, SD= stem diameter, APB= angle of primary branches, NPB= number of primary branches, ALPB= average length of primary branches, NNPB= number of nodes on primary branches, AILPB= average inter node length of primary branches, NSB=number of secondary branches, CD= canopy diameter, LL= leaf length, LW= leaf width, FL= fruit length, FW= fruit width, FT= fruit thickness, BL= bean length, BW= bean width, HBW= hundred bean weight, CBD =coffee berry disease, Yd/tr= yield per tree

areas/site, considering environmental conditions (Porceddu and Damania, 1992).

The narrow gap between PCV and GCV for hundred bean weight, number of nodes of primary branches, stem diameter, bean length and bean width, suggesting that the influence of environment in phenotypic performance is minimal. However, the wider gap between PCV and GCV for yield per tree, coffee berry disease severity, number of primary branches, number of secondary branches and number of main stem node, indicates the importance of environment in influencing the traits. The present finding is in agreement with findings of Seyoum and Bayetta (2007) and Olika et al. (2011) who reported high PCV and GCV values for yield and number of secondary branches; moderate PCV and GCV values for

**Table 4.** Estimates of phenotypic and genotypic variance, phenotypic (PCV) and genotypic coefficient of variation (GCV), broad sense heritability ( $H^2$ ), genetic advance (GA) and expected genetic advance as percent of mean (GAM%) for 22 morphological characters.

Trait	$\sigma^2_p$	$\sigma^2_g$	PCV	GCV	$H^2$	GA	GAM
			%	%	%		%
HUP	23.33	15.54	14.57	11.90	66.60	6.63	20.00
TPH	514.87	242.25	9.05	6.21	47.05	21.99	8.77
NMSN	14.42	5.10	10.46	6.22	35.35	2.76	7.62
AILMS	0.29	0.12	8.67	5.58	41.38	0.46	7.39
SD	0.34	0.23	10.68	8.69	67.16	0.80	14.67
APB	27.98	14.92	7.85	5.74	53.32	5.81	8.63
NPB	87.07	34.31	14.79	9.28	39.40	7.57	11.99
ALPB	36.14	13.34	6.87	4.17	36.91	4.57	5.22
NNPB	4.64	3.15	10.12	8.34	67.89	3.01	14.15
AILPB	0.12	0.07	8.37	6.39	58.33	0.42	10.05
NSB	1419.3	513.68	30.0	18.03	36.19	28.09	22.34
CD	246.8	128.23	7.71	5.56	51.95	16.81	8.25
LL	1.09	0.45	7.20	4.63	41.28	0.89	6.12
LW	0.17	0.09	6.92	5.03	52.94	0.45	7.54
FL	0.52	0.25	4.62	3.20	48.08	0.71	4.57
FW	0.36	0.13	4.70	2.79	35.21	0.43	3.41
FT	0.42	0.15	6.37	3.77	34.94	0.46	4.59
BL	0.43	0.27	6.79	5.38	62.79	0.85	8.78
BW	0.11	0.07	4.92	3.90	61.43	0.42	6.22
HBW	4.35	3.49	12.07	10.81	80.21	3.44	20.00
CBD	307.24	144.87	91.48	62.82	47.15	17.03	88.86
Yd/tr	0.063	0.018	41.67	22.05	28.00	0.14	24.03

$\sigma^2_p$  = Phenotypic variance,  $\sigma^2_g$  = genotypic variance, GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation,  $H^2$  heritability in broad sense, GA = genetic advance, GAM = genetic advance as percent of mean. HUP = height up to first primary branches. TPH = total plant height, NMSN = number of main stem nodes, AILMS = average inter node length of main stem, SD = stem diameter, APB = angle of primary branches, NPB = number of primary branches, ALPB = average length of primary branches, NNPB = number of nodes on primary branches, AILPB = average inter node length of primary branches, NSB = number of secondary branches, CD = canopy diameter, LL = leaf length, LW = leaf width, FL = fruit length, FW = fruit width, FT = fruit thickness, BL = bean length, BW = bean width, HBW = hundred bean weight, CBD = coffee berry disease, Yd/tr = yield per tree.

height up to first primary branch and hundred bean weight. Lemi and Ashenafi (2016) also reported high values of phenotypic coefficient of variation but medium genotypic coefficient of variation for yield and with higher GCV values for number of main stem nodes.

### Heritability and genetic advance

The estimates of broad sense heritability ( $H^2$ ) for quantitative traits are presented in Table 4. According to Verma and Agarwal (1982), heritability values >50% are considered as high, whereas values less than 20% are regarded as low and values between 20 and 50% are moderate. According to this cut point, high heritability (> 50%) was recorded for hundred bean weight (80.21%), number of nodes of primary branches (67.89%), stem diameter (67.16%), height up to first primary branch

(66.6%), bean length (62.79%), bean width (61.43%) and average inter node length of primary branches (58.33%), angle of primary branches (53.32%), leaf width (52.94%) and canopy diameter (51.95%). The estimated high heritability for these traits, suggest the greater effectiveness of selection and improvement to be expected for these characters in future breeding program. Characters such as fruit length (48.08%), Coffee berry disease severity (47.15%), plant height (47.05%), average inter node length of main stem (41.38%), leaf length (41.28%), number of primary branches (39.4%), average length of primary branches (36.91%), number of secondary branches (36.19%), number of main stem nodes (35.35%), fruit width (35.21%), fruit thickness (34.94%) and clean coffee yield per tree (28.0%) exhibited moderate heritability.

The present finding partly agree with findings of Cilas et al. (1998) who reported high heritability for stem girth,

tree height and yield. Bayetta (2001) also reported high heritability estimates between 71.43 and 97.32 for all characters measured, suggesting that the effect of environment on phenotypic expression of the characters was small. Moreover, Yigzaw (2005) has reported high heritability values for hundred bean weight, number of secondary branches and canopy diameter. However, he reported low heritability for bean thickness, percent of bearing primary branches, average inter node length of primary branches and petiole length.

The high heritability for bean characteristics, such as hundred bean weight, bean length, and bean width in the present study is in agreement with the findings of Olika et al. (2011). The observed low heritability value for yield is in agreement with the findings of Ermias (2005) and Olika et al. (2011). However, in contrast to this finding, Mesfin (1980) and Bayetta (2001) reported high heritability for yield. This is probably due to the differences in test materials and the environment.

Genetic gain (GAM) that could be expected from selecting the top 5% of the genotypes as percent of the mean, varied from 3.41% for fruit width to 88.86% for CBD reaction (Table 4). According to Johnson et al. (1955), genetic advance as percent of the mean was categorized as high ( $\geq 20\%$ ), moderate (10-20%) and low (0-10%). Accordingly, the high GAM were recorded for coffee berry disease reaction (88.86%), clean coffee yield per tree (24.03%), number of secondary branches per tree (22.34%), height up to first primary branch (20%) and hundred bean weight (20%).

The characters that exhibited moderate (10 to 20%) level of genetic advance as percent of mean were stem diameter (14.67%), number of nodes of primary branches (14.15%), number of primary branches (11.99%) and average inter node length of primary branches (10.05%). The other traits, such as total plant height, number of main stem nodes, average internodes length of main stem, angle of primary branches, average length of primary branches, canopy diameter, leaf length, leaf width, fruit length, fruit width, fruit thickness, bean length and bean width, had low level of GA as percent of mean ( $< 10\%$ ). This low estimate of genetic advance as percent of mean arises from low estimates of phenotypic variability and heritability. Selection based on those traits with a high level GAM will result in improvement of the performance of the germplasm accessions for those traits.

In the current study, high heritability coupled with high genetic advance as percent of mean was observed for characters such as hundred bean weight and height up to first primary branch. This indicates the lesser influence of environment in expression of the characters and prevalence of additive gene action in their inheritance. In addition, both traits had medium genetic coefficient of variation, hence are amenable for simple selection.

CBD severity had high genotypic coefficient of variation (62.82), moderate heritability (47.17%) and high genetic

advance as percent of mean (88.86), indicating that selection will be effective to improve this important trait.

Contrarily, stem diameter, number of nodes of primary branches, and average inter node length of primary branches showed high heritability coupled with moderate genetic advance and the scope for their improvement was restricted by the low range of genetic variability present in the population. Besides, high heritability with low genetic advance was recorded for angle of primary branches, canopy diameter, leaf width, bean length and width, indicating less influence of environment but prevalence of non-additive gene action for which simple selection will be less effective. Hence, heterosis breeding would be recommended for the improvement of such traits. Mesfin (1980) reported genetic advance for yield to be 1.4 kg fresh cherry/tree from indigenous coffee collections grown at Jimma. In Ethiopia, some reports indicated that genetic advance through selection for yield at 20% selection intensity was up to 2.2 kg of fresh cherry per tree, confirming the presence of high genetic variability within Arabica coffee population. Therefore, the opportunity to bring a reasonable improvement through selection is high (Bayetta, 1997). Lemi and Ashenafi (2016) reported high broad sense heritability estimates coupled with high genetic advance in percentage of mean for number of main stem nodes, stem diameter and internodes length.

## Conclusion

The present study exhibited the existence of variability for some morphological traits among coffee germplasm accessions. Some of the important traits showed high and moderate PCV and GCV, suggesting the incidence of variability and thus offers scope for genetic improvement through selection. Besides, traits coupled with high/moderate heritability and high/moderate estimates of genetic advance, and careful selection may lead to improvement of these traits. Hence, provides better opportunities for selecting plant material for these traits in coffee. Therefore, the observed variability should be exploited in order to improve the coffee productivity. However, most of the traits exhibited low GCV and/or low genetic advance as percent mean, indicating these traits could not be improved through simple section but through heterosis breeding.

## Conflicts of Interests

The authors have not declared any conflict of interests

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Sub Center Coffee Breeding staff.

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## Full Length Research Paper

# Knowledge and perceptions of plant viral diseases by different stakeholders in Zimbabwe's agricultural sector: Implications for disease management

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Plant viruses are major constraints to crop production worldwide, causing US\$60 billion losses annually. This study assessed various agricultural sector stakeholders' knowledge and perceptions of plant viruses in Zimbabwe. Data was collected from six provinces using surveys and participatory rural appraisal methodologies between December 2013 and October 2014. *Maize streak virus*, *Tobacco mosaic virus*, *Cucumber mosaic virus*, *Tomato mosaic virus* and *Groundnut rosette virus* were ranked as the country's five most important plant viruses by agricultural technical staff. Most (72%) technical staff rated *Maize streak virus* as the most important plant virus in Zimbabwe. Over 30% of farmers were self-taught to identify diseases, while only 15.3% were trained by agricultural extension staff. Most (95.8%) technical staff trained people in disease identification through running short courses, use of demonstration plots and field days. The majority (41.9%) of farmers recommended the use of radio/TV/newspaper broadcasts to improve virus awareness. Only 23.7% of farmers and 41.6% of technical staff had heard about TSWV/tospoviruses. While most (97.2%) technical staff rated TSWV/tospoviruses as "fairly important" to "very important" plant pathogens, only 15.7% were able to correctly identify tospoviral vectors. The study showed that there is poor knowledge of plant viruses the stakeholders in the agricultural sector. There is need to train the technical staff in plant virology so that they can disseminate their knowledge to farmers for improved virus disease management.

**Key words:** Awareness, disease identification, tospoviruses, training.

## INTRODUCTION

Zimbabwe has an agro-based economy, with over 70% of the population either directly or indirectly dependent on

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agriculture for a living (Marongwe et al., 2012). The major stakeholders in Zimbabwean agriculture are the farmers, input (seed, pesticide and fertilizer) suppliers, researchers, extension staff, and agricultural teachers and lecturers. All of them play significant roles in ensuring successful agricultural productivity which is seriously constrained by many abiotic and biotic factors. Amongst the biotic factors are plant pathogenic viruses that cause about US\$60 billion losses annually worldwide (Wei et al., 2010).

Plant pathogenic viruses cause huge agricultural losses especially in the developing world where most farmers have poor knowledge of these pathogens. This can be attributed to the fact that unlike insects, fungal mycelia and rodents that can be seen with the naked eye, viruses are microscopic entities. In addition, viruses may incite symptoms similar to those by other pathogens, nutritional and/or environmental disorders (Astier et al., 2007). So, farmers tend to apply the wrong control measures in virus-infected plants. Furthermore, plant virus studies require highly specialized equipment and study techniques which are not readily available in most developing countries (Kaitisha, 2003).

Zimbabwe is a developing country reported to have impressive agricultural training, research and extension systems for improved agricultural productivity (Mutambara et al., 2013). A common perception is that stakeholders in Zimbabwe's agricultural sector are highly knowledgeable about all farming aspects, including disease and pest identification and management. However, this may not be the case with plant viral diseases due to the reasons mentioned earlier. Furthermore, changes in Zimbabwe's economy and education system since the year 2000 may have had an impact on knowledge and perceptions of viral diseases by agricultural sector stakeholders. Globally, climate change, trade and genetic mutations have contributed to the emergence of new viruses like begomoviruses, criniviruses, carlaviruses, torradoviruses and tospoviruses in the last 30 years (Navas-Castillo et al., 2012; Pappu et al., 2009). The tospoviruses, in particular, have become very important in tropical and subtropical regions. One tospovirus species, *Tomato spotted wilt virus*, is estimated to cause US\$1 billion losses annually for several important food and ornamental crops worldwide (Parrella et al., 2003). This virus has previously been reported infecting weeds, ornamentals, and food and industrial crops in Zimbabwe (Dobson et al., 2002).

In light of these pointers, a survey was conducted to capture the understanding and perceptions of plant viral diseases by key stakeholders within Zimbabwe's agricultural sector. The survey provided a useful way to canvas ideas and opinions of the respondents about plant virus diseases. This would form the basis for identifying potential intervention points in developing viral disease management strategies. Results of the study will also assist policy makers in the Agriculture and Higher

Education ministries during policy formulation on curricula development, research and extension services on plant viral diseases.

The objectives of this study were to: (i) Identify ten plant viruses that agricultural technical staff rank as the most important in Zimbabwe; (ii) Determine farmers' trainers and methods of training for disease identification; (iii) Assess respondents' perceptions of plant viruses; (iv) Gather respondents' opinions on how to improve awareness of plant virus diseases, and (v) Evaluate respondents' knowledge of *Tomato spotted wilt virus* (TSWV)/tospoviruses (TSWV/tospoviruses).

## MATERIALS AND METHODS

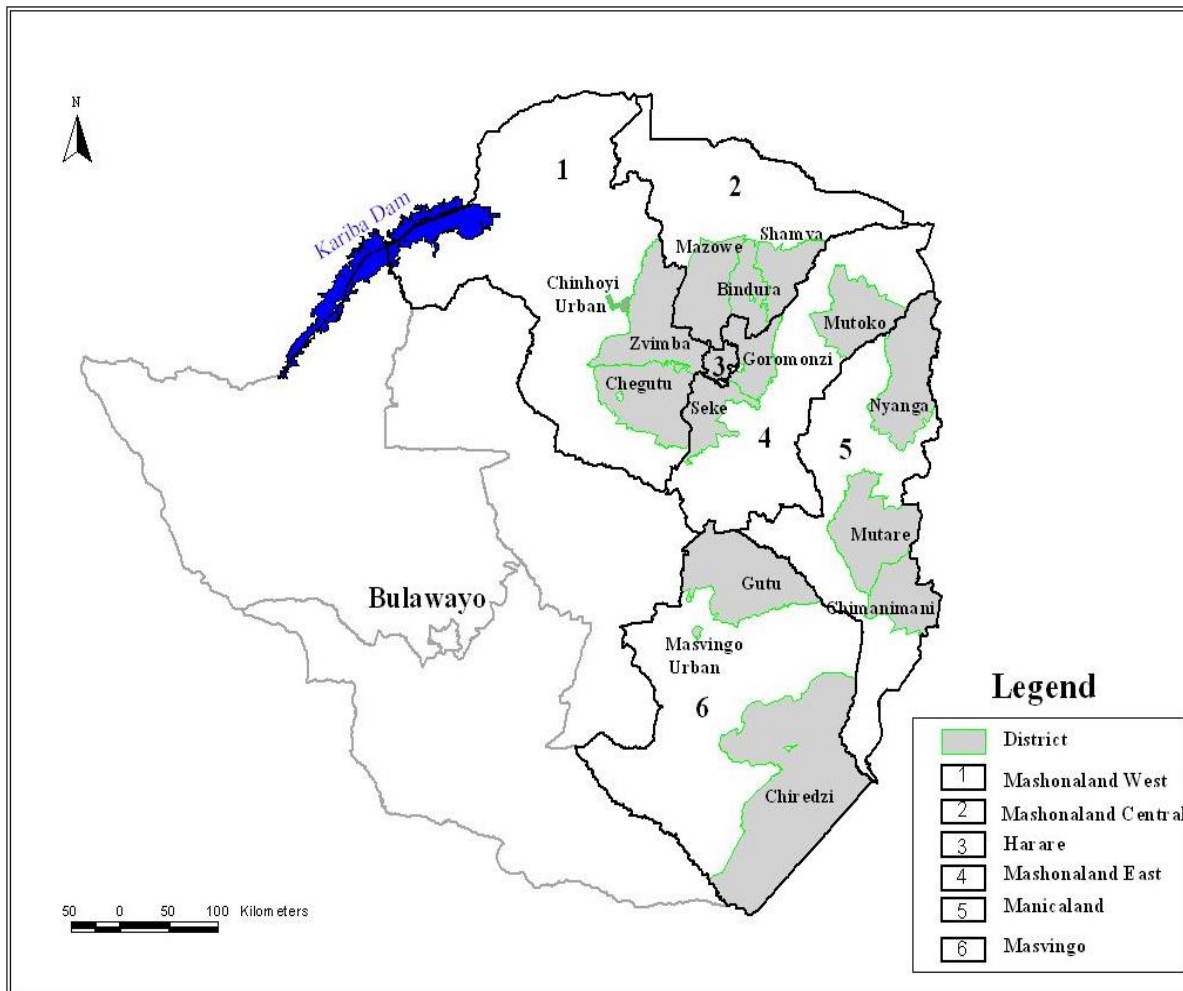
### Study area

The study was carried out in Zimbabwe (latitudes 15°13'S and 22°30'S; longitudes 25°E and 33°E), a country bordered by South Africa to the south, Mozambique to the east, Zambia to the north and Botswana to the west. Zimbabwe has five natural farming regions (NFRs) delineated primarily on the basis of rainfall, soil quality and vegetation (Chiremba and Masters, 2003). The best rainfall and land resources occur in NFR 1, while NFR 5 is very hot and unsuitable for most crops, except traditional small grains and sugarcane. Up to 80% of Zimbabwe's crops are grown in the Mashonaland Provinces, which are mainly in NFR 2. Mid-season dry spells and high temperatures occur in NFR 3 which receives 500 to 750 mm rainfall annually. NFRs 4 and 5 are low-lying, receiving not more than 650 mm rainfall per annum.

### Sampling procedure and selection of participants

The study was conducted between December 2013 and October 2014. A multistage sampling process was conducted to select provinces, districts and respondents. Six provinces, namely: Harare, Manicaland, Mashonaland Central, Mashonaland East, Mashonaland West and Masvingo, were selected. From each province, three districts were chosen for surveys. The chosen districts represented the country's NFRs as follows: NFR 1: Nyanga and Chimanimani; NFR 2: Bindura, Chegutu, Chinhoyi Urban, Goromonzi, Harare districts, Mazowe, Mutare, Seke, Shamva and Zvimba; NFR 3: Gutu and Mutoko; NFR 4: Masvingo Urban; and NFR 5: Chiredzi (Figure 1). At each district, the Principal Investigator (PI) engaged the District Agricultural Extension Officer (DAEO) who recommended wards (cluster of villages) for assessments, and agricultural extension staff (Agricultural Extension Officers and Agricultural Extension Workers) who assisted in identifying interviewed farmers. Three wards were selected per district, and fifteen farmers per ward were interviewed with questionnaires.

In addition, ten farmers per district were interviewed in Farmer Group Discussions (FGDs). Respondents from agricultural colleges, high schools, input suppliers, research stations, non-governmental and private organizations involved in agriculture in the study areas were also interviewed. In total, 810 farmers and 214 technical staff (composed of agricultural extension staff, research and training officers, agricultural teachers and lecturers, and input suppliers) were interviewed using questionnaires, and another 180 farmers were interviewed in FGDs. All protocols were followed in regards of research ethics, which included securing government permission to conduct surveys and allowing free choice of participation in the interviews.



**Figure 1.** Provinces and districts chosen for the studies on plant virus knowledge and perceptions by stakeholders in Zimbabwean agriculture. The provinces are numbered 1 to 6 while districts are shaded grey.

### Data collection

Two questionnaires were designed, one for farmers and another for the technical staff. Both questionnaires were designed in English and had closed and open-ended questions. For illiterate farmers, questionnaires were administered in Shona (a local language) and completed by the PI and his assistants. The questionnaires were pretested with fifteen farmers and eight technical staff, and modified to ensure that meanings were unambiguous. Some interviews and FGDs were recorded on audio tapes and later processed to extract information. To ensure maximum data collection, some probing and interactive sessions outside the formal data collection sessions were carried out. Printed color photographs of virus-infected plants were shown to respondents to assist with disease identification.

The questionnaires captured respondents' general knowledge of plant viruses, including major plant viruses in Zimbabwe, rating of viruses as plant pathogens and methods of improving virus awareness. Perceptions on viruses were captured as categorized variables using a scale of 1 to 5 where, 1 = Not important; 2 = Fairly important; 3 = Important; 4 = Very important and 5 = Don't know. For TSWV/tospoviruses, respondents provided the following information: Virus knowledge source, rating alongside other viruses, vectors and control measures. Respondents' socio-economic

characteristics captured on the questionnaires included province, district, gender, age, educational level, marital status, land tenure system and agricultural experience.

### Data analysis

Statistical analysis for quantitative survey data was done using the Statistical Package for Social Sciences (SPSS) Version 16.0. Survey data was coded and entered into the SPSS spreadsheet and checked before analysis. Both descriptive statistics and econometric models were used in data analysis. The logistic (logit) regression model was used to assess the respondents' awareness about TSWV/tospoviruses. The logit model is found in random utility theory and built around a latent regression.

$$Y^* = \beta x + \varepsilon_i$$

$Y^*$  is an underlying latent variable that indexes respondents' knowledge on TSWV/tospoviruses.  $\beta$  is a column vector of unknown parameters to be estimated.  $X$  is a row vector of respondent characteristics and  $\varepsilon$  is the stochastic error term. The dependent variable that was used for the model is the respondents'

**Table 1.** Explanatory variables used in assessing farmers' awareness to TSWV/tospoviruses.

Variable description	Variable type	Units
Awareness of farmers on tospoviruses	Dummy	1=aware, 0= not aware
Age of farmer	Years	Continuous
Education level	Category	1=None; 2=Primary; 3=Secondary; 3=Post-secondary
Farming experience	Years	Continuous
Tenure system	Category	1=Communal; 2=A1; 3=A2, 4=Large scale commercial; 5=Plot/Nursery; 6=Other
Land area	Continuous	Continuous

awareness of the viruses. This was chosen because the logistic model can be used in binary data; 1 = those who were aware of the viruses and 0 = those who were not aware of the viruses. The explanatory variables for the farmers' questionnaire were age, educational level, farming experience, land tenure system and land area (Table 1). For technical staff, the explanatory variables were age, gender, employer, education level and agricultural experience.

To calculate the odds ratios (which represents the constant effect of the explanatory variables on the likelihood that the respondents were aware of TSWV/tospoviruses), the formula  $ODDS = e^{a+bx}$  was used; while the probabilities from the odds ratio were calculated using the formula:  $Y = \frac{ODDS}{1+ODDS}$

The analysis used both the odds ratio and probabilities because the odds ratio is a single summary score of the effect and the probabilities are more intuitive.

## RESULTS

### Socio-economic characteristics of respondents

The proportion of male to female farmers was 60:40. Most (65.1%) farmers were from NRF 2, with only 3.7% from NR 1 and 5.6% from NR 5. The literacy rate amongst the farmers was 97.8%, and 29.6% of them had post-secondary education. All illiterate farmers were females; the literacy rate of female farmers was higher than that of their male counterparts only at primary school level. Most (76.8%) farmers were married, with 10.2% widowed and 12% single. The youngest farmer was 18 years old, while the oldest was 79 years. The largest proportion (37.9%) of farmers was communal, while 18 and 5.4% were A1 and large scale commercial farmers, respectively. Farmers with no more than 10 years' farming experience accounted for 45.9% of the respondents.

For the technical staff, the male to female ratio was 55:45. The government employed 77.1% of the technical staff, while only 20.1% were employed in the private sector. Agricultural extension staff constituted 50.5% of the technical staff. The majority (67.1%) of the agricultural extension workers (AEWs) had diplomas, while 91.4% of the agricultural extension officers (AEOs) had agriculture bachelor's degrees as their highest relevant qualifications. Only 15% of the technical staff had postgraduate degrees, with 1.9% having doctoral

degrees. Of the lecturers and teachers, 22.4% had agricultural diplomas as their highest qualification. Most (87.9%) technical staff were married. Those with 2 to 10 years' work experience accounted for 77.1% of the technical staff, while only 3.3% had more than 20 years' experience.

### Major plant viruses in Zimbabwe

According to the technical staff, the major plant viruses that occur in Zimbabwe are as shown in Table 2.

MSV was rated as Zimbabwe's most important plant virus by 72% of the technical staff. All agricultural extension staff highlighted the importance of MSV in maize production. TMV and CMV were ranked as the second and third most important plant pathogenic viruses, respectively (Table 2). GRV was reported mainly by extension staff working with smallholder groundnut farmers from NFRs 3 and 4.

### Training for disease identification

The majority of farmers were self-taught to identify diseases (Figure 2). Another 20.2% were trained by agrochemical and seed company agents, while only 16% were trained by agricultural extension and research staff (AREX/research officers). Farmers who grew greenhouse flowers hired foreign experts to assist with disease identification and management.

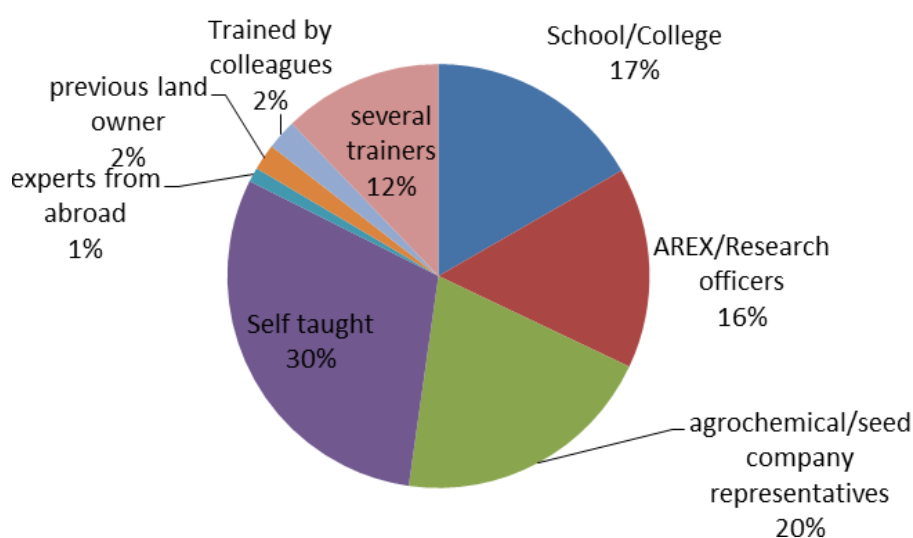
The main methods of farmer training for disease identification used by 51.9% of the technical staff included conducting of short courses, setting of demonstration plots and field days. Lectures/lessons and practicals were mainly used by teachers and lecturers to train students in disease identification.

### Respondents' perceptions of plant viruses and methods used in virus disease identification

Close to 29% of farmers did not know about plant pathogenic viruses. Only 3.1% of farmers and 2.8% of

**Table 2.** Top ten most economically important viruses affecting crops in Zimbabwe.

Rank	Acronym	Virus name	Genus
1	MSV	Maize streak virus	Mastrevirus
2	TMV	Tobacco mosaic virus	Tobamovirus
3	CMV	Cucumber mosaic virus	Cucucmovirus
4	ToMV	Tomato mosaic virus	Tobamovirus
5	GRV	Groundnut rosette virus	Umbravirus
6	PVY	Potato virus Y	Potyvirus
7	TBTV	Tobacco bushy top virus	Umbravirus
8	CTV	Citrus tristeza virus	Closterovirus
9	PVX	Potato virus X	Potexvirus
10	BCMV	Bean common mosaic virus	Potyvirus

**Figure 2.** Farmers' trainers for disease identification.

the technical staff rated viruses as “not important”, while 22.7% of farmers and 41.1% of the technical staff rated them as “very important.”

The majority (85%) of farmers who were able to identify viruses relied on field symptom assessments only. Only 5.2% of farmers sent samples to plant clinics for disease diagnosis (Figure 3).

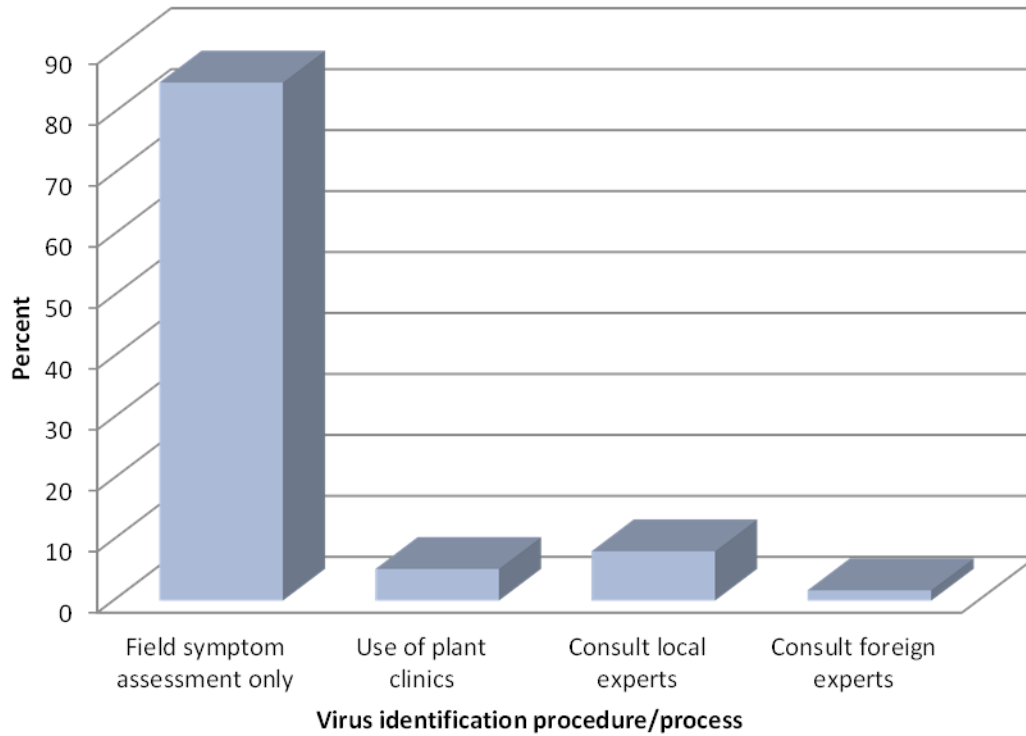
### Opinions on improving virus diseases awareness

To improve virus disease awareness, 41.9% of farmers proposed the use of radio/TV/newspaper broadcasts, while 48.1% of the technical staff recommended farmer training. Another 22.3% of farmers were of the opinion that agricultural extension staff should train farmers, while 11.4% of farmers proposed distribution of color pamphlets of virus-infected plants as a method of improving virus awareness. About 6.5% of the technical

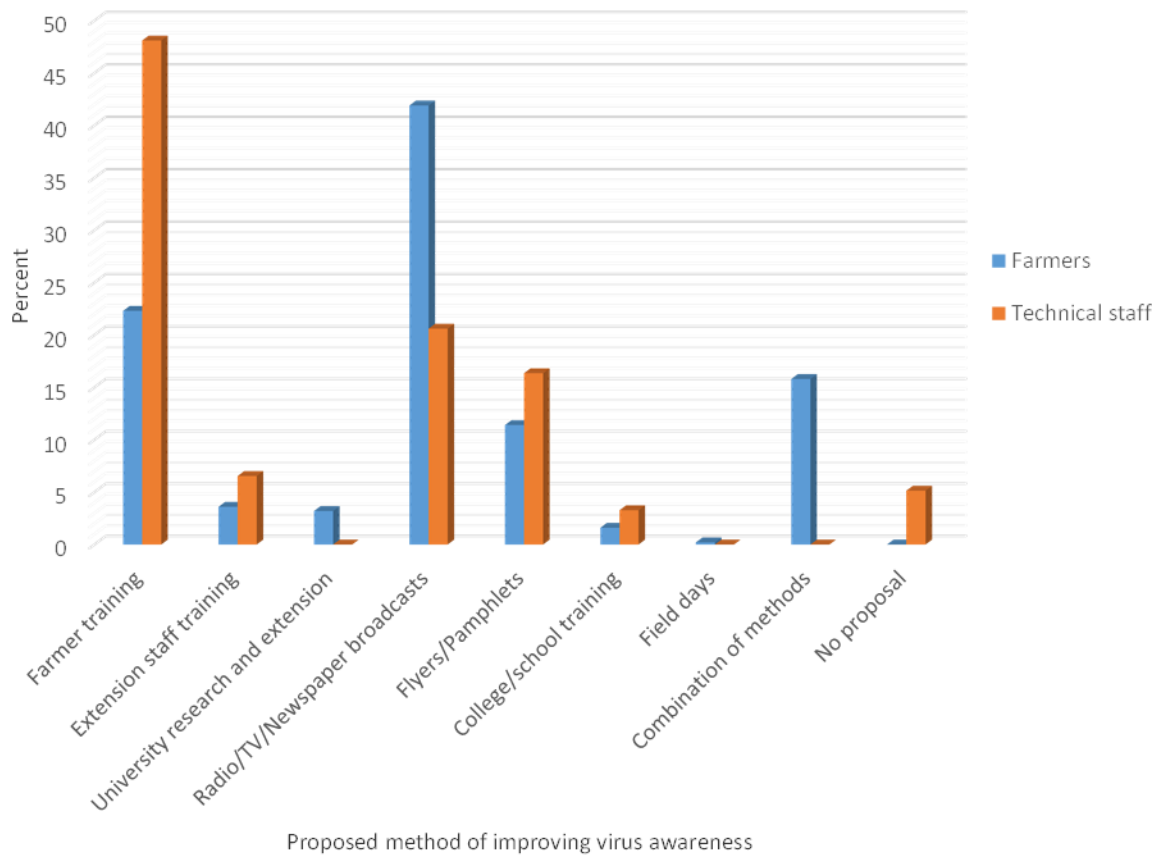
staff proposed extension staff training through workshops and short courses as methods of improving virus diseases awareness (Figure 4).

### Knowledge of TSWV/tospoviruses by respondents

Education level ( $p=0.000$ ), farmer age ( $p=0.011$ ) and agricultural experience ( $p=0.020$ ) had significant effects on respondents' knowledge of TSWV/tospoviruses (Tables 3 and 4). There were 1.042 chances that older farmers were aware of TSWV/tospoviruses, and only 0.124 chances that educated farmers were aware of the viruses (Table 3). There were 0.522 chances that technical staff respondents were aware of the viruses, and 0.541 more chances that experienced staff were aware of the viruses (Table 4). Only 23.7% of farmers and 41.6% of technical staff had heard about TSWV/tospoviruses, mainly from school/college. The



**Figure 3.** Methods of identifying virus diseases by farmers.



**Figure 4.** Respondents' opinions on how to improve virus diseases awareness.

**Table 3.** Logit regression results on factors influencing farmers' knowledge of TSWV/tospoviruses.

Variable	B	S.E.	Wald	DF	Sig.	Exp(B)
Educational level	-2.085	0.179	136.235	1	0.000	0.124
Marital status	-0.116	0.214	0.297	1	0.586	0.890
Farm experience	-0.031	0.020	2.294	1	0.130	0.970
Farm type	-0.101	0.062	2.673	1	0.102	0.904
Age	0.041	0.016	6.487	1	0.011	1.042
Constant	5.286	0.633	69.662	1	0.000	197.611

**Table 4.** Logit regression results on factors influencing technical staff's knowledge of TSWV/tospoviruses.

Variable	B	S.E.	Wald	DF	Sig.	Exp(B)
Educational level	-0.649	180	12.964	1	0.000	0.552
Agricultural Experience	-0.615	0.265	5.374	1	0.020	0.541
Age	0.064	0.035	1.692	1	0.093	1.047
Gender	0.529	0.308	2.951	1	0.086	1.696
Employer	0.342	0.321	1.135	1	0.287	1.407
Constant	1.025	1.124	0.831	1	0.362	2.787

majority (70.8%) of farmers that had heard about TSWV/tospoviruses had post-secondary education. Only 2.2% of the technical staff mentioned the electronic media as an information source for these viruses. Of those who had heard about TSWV/tospoviruses, 43.8% of farmers and 70.8% of technical staff were able to correctly identify three plant hosts to the viruses, while 39.5% farmers and 18% technical staff could only identify the tomato as a host. Close to 7% of farmers and 11% of technical staff rated TSWV/tospoviruses as "not important," while 33.2% of farmers and 31.5% of technical staff rated them as "very important." Only 14.2% of farmers and 15.7% of technical staff were able to correctly name the TSWV/tospoviruses vectors. None of the respondents were able to name tospoviral species other than TSWV.

For TSWV/tospoviruses control, insecticide use was recommended by 68.9% of farmers and 40.7% of the technical staff. The use of certified seeds and fumigation were proposed by 5.8% of farmers and 14.8% of the technical staff. To improve TSWV/tospoviruses awareness, 27.1% of the technical staff recommended "college/university/school training," while 19.2% recommended "workshops/short courses for research/technical staff" and 20.6% proposed "print and electronic media campaigns."

## DISCUSSION

There were more male than female farmer respondents because males, as household heads, were generally more willing to come forward and give information to the

researchers. This is despite the fact that women constitute the majority of workers on most farms. Similar findings were reported by Khan et al. (2014). Also, the higher literacy rate among males meant that they could confidently participate during the surveys.

There were fewer female than male agricultural technical staff respondents because fewer females graduate with agricultural professional qualifications in Zimbabwe. Historically, fewer female students study science-oriented subjects in high school and this translates to a smaller number of females who enroll for professional agricultural courses. In addition, agricultural extension is generally considered a masculine profession (Mutambara et al., 2013).

The study confirmed the changes in land demographics brought about by the country's land reform program that started in the year AD2000. The large scale commercial farming sector, previously the backbone of Zimbabwe's agriculture, has been decimated and replaced mainly by A1 and A2 farms. The fact that most farmers had no more than 10 years farming experience shows that they ventured into farming after the land reform program. Most such farmers either did not receive formal agricultural training or were poorly trained, and so are likely to be poorly knowledgeable about plant viruses, their effects and management.

Most respondents rated MSV as the most important plant virus in Zimbabwe. MSV is endemic to Zimbabwe and the sub-Saharan Africa region (Shepherd et al., 2010; Karavina, 2014). Therefore, most maize breeding and extension programs incorporate MSV researches and knowledge dissemination, respectively.

Some farmers either did not know about plant viruses



or the different groups of plant pathogens that attack crops. During the FGDs, farmers talked more about insect pests and fungal diseases than plant viruses. This observation was similar to results reported by Sibiyi et al. (2013) who found that plant diseases were lowly ranked by farmers in KwaZulu-Natal, South Africa. The major contributory factor, it appears, is poor education and training about plant viruses since even the technical staff had poor knowledge of plant viruses. While most technical staff were sufficiently trained to assist farmers to improve agricultural productivity, most could not distinguish viruses from other pathogens. A major reason for this was that most of them had diplomas as their highest relevant qualifications and so were not adequately trained in plant pathology. Even amongst the technical staff with degrees, viral diseases appreciation was poor probably because as students, most of them were poorly trained in plant pathology. The lack of qualified lecturers and training facilities in the last decade, and the “Open Distance Learning” system now in operation in the country compromised agricultural training.

The majority of respondents relied on visual symptoms assessment for disease diagnosis. This is not totally reliable, as symptom expression is influenced by the environment, host species, plant nutritional status, season, and pathogen strain (Sevik and Arli-Sokmen, 2012). It was noted that wherever maize is grown, most respondents attributed almost all mosaics, streaking and chlorosis to MSV, yet pathogens that cause similar symptoms like *Maize dwarf mosaic virus*, *Sugarcane mosaic virus*, *Maize stripe tenuivirus* and *Maize chlorotic mottle virus*, occur in Zimbabwe (Bonga and Cole, 1997). This highlights the need to employ several diagnostic tests to confirm pathogen identity. Where farmers sent diseased samples to plant clinics, the absence of qualified virologists and well-resourced laboratories also compromised viral disease diagnosis and ultimately, virus disease control.

Amongst the four major plant pathogen groups, viruses were the least appreciated by AEWs. This means the AEWs are less likely to talk about plant viruses to farmers than the other pathogens. Therefore, viruses will remain largely unknown to farmers. To remedy this situation, AEWs ought to be trained in plant virology so that they can disseminate correct information about pathogen biology, epidemiology and control. To achieve that, the agricultural training curricula must incorporate a significantly bigger section on plant virology in which virus diseases are taught.

Of the respondents who said they knew TSWV/tospoviruses, the large proportions of those who only knew tomato as a host crop and those who could not name any other tospoviruses besides TSWV, raise suspicions as to whether they really knew the pathogens. It also questions the seriousness accorded to the plant virology discipline in the country given that tospoviruses

are an emerging problem worldwide (Scholthof et al., 2011). Currently, there are at least 28 tospovirus species causing serious yield losses worldwide (Margaria et al., 2014). The fact that wrong vectors were named and wrong control methods recommended showed that respondents had poor knowledge of pathogen biology and epidemiology. This means wrong control measures are likely to be implemented against the pathogens. According to Mehle and Trdan (2012), correct vector diagnosis is the first key step in tospovirus management. The observation that most respondents recommended insecticide use to control TSWV/tospoviruses reinforces an observation by Nagaraju et al. (2002) that there is a “pesticide culture” that has been created by agrochemical companies through their extension programs and aggressive product promotion.

Only a small proportion of the technical staff mentioned the electronic media as a source of information for TSWV/tospoviruses, showing that traditional media platforms still dominate information dissemination in agricultural extension, research and training in Zimbabwe. While the country has an Information and Communication Technology (ICT) Policy that promotes the use of modern ICT tools, the agriculture sector has not adequately embraced it.

Most AEWs lacked ICT resources to enable them to do their work effectively. The majority of research and tertiary education institutions have internet connectivity challenges that further limit internet use by students, academics and researchers.

In conclusion, the study showed that plant viral diseases are poorly appreciated by stakeholders in Zimbabwe’s agricultural sector. Besides MSV, other viral disease remains largely unknown by most people. This is worrying given the fact that there are currently many emerging and re-emerging plant viruses worldwide that are causing significant crop yield losses. The survey revealed the need for concerted and multifaceted approaches to increase knowledge of plant viruses in Zimbabwe through training of all stakeholders and conveyance of information by the media. This will then enable better plant viral disease management.

### Conflict of Interests

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENTS

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*Full Length Research Paper*

# Forage productivity system evaluation through station screening and intercropping of lablab forage legume with maize under Irrigated lands of smallholder farmers

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On station, farm studies were conducted in the eastern zone of Tigray to improve feed resource through integrating forage and cereal crops, to identify suitable and compatible lablab accessions for maize lablab intercropping under smallholder farmers, to demonstrate maize/lablab intercropping on farm, and to see farmer's perception towards this technology. In the first study, eight lablab accessions were screened as monocrops adaptively, biomass and seed production. 1034, 912 and Dolichos lablab accessions were selected. Mean biomass production was estimated as 5.91, 7.12 and 8.31 DM (t/h) for 1034, 912 and Dolichos lablab accessions, respectively. These promising lablab accessions have wide adaptability and best compatible for intercropping. As follow up, 1034 and 912 lablab accessions were in farm trial, selected for intercropping with maize under irrigated lands to evaluate their contribution biomass production and adoptability. The selected legumes were row intercropped into maize and the average fresh biomass yield of maize was 18 kg under irrigated lands. The total average fresh biomass harvested from a single 10 X 10 m plot size was 18, 32 and 33 kg, for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. The mean change in total fresh biomass yield for lablab accession 912 and 1034 was 19.75 and 15.75 kg, respectively. Based on the field observation lablab accession #912 has performed best during the trial period. Hence, the total fresh biomass harvested from intercropped lablab accessions has increased up to 49% and higher in total fresh biomass harvested in sole maize plots. In general, the tendency for adoption of the forage legumes was higher as compared to other forage species.

**Key words:** Forage development, lablab intercropping, forage yield improvement.

## INTRODUCTION

Ethiopia has a diversified agro ecological and topographical feature, which serve as a home for different plant and animal species. There are promising indigenous and introduced forage plants adapted to different agro ecologies. Herbaceous legumes are

multipurpose forage plants which provide quality feed for livestock, serves as source of N for plant growth by improving soil fertility through the process of fixation of atmospheric Nitrogen. However, the utilization of improved forage species under smallholder farmers is still

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poor due to land scarcity which is the major constraint for intensive forage production system. As a result, livestock, feed scarcity also hinders the overall productivity of livestock in almost all part of the high land and lowland areas.

According to Tesema and Demekash (2001), one of the bottlenecks of livestock production in Ethiopia is shortage feed. Report on livestock feed resource study indicated that traditional feeding system is based on the dried pasture and crop residues, which are poor quality roughages, characterized by high NDF, low nitrogen contents, and slow fermentation rates (Yayneshet, 2010).

Feeding poor dietary combination leads to decreased intake, weight loss, increased susceptibility to health problem and reduced reproductive performance. Herbaceous legumes in these feeding regimes helps to solve some of the problems associated with low protein and high fiber diets. Lablab makes a better recovery after grazing, which demonstrates less susceptibility to disease and integrations of forage development strategies with cereal crop production, both at rain fed while irrigated lands is the best practices which improved livestock production during the dry season.

One of the most common goals of intercropping is to produce a greater yield on a given piece of land by making use of resources. Companion crop provides quick ground cover, helping to reduce wind and water erosion and resist invasion of weeds during forage establishment (Bula et al., 1995). As maize is the main cash crop in the area, intercropping of these crops with lablab improves feed availability for livestock. The objectives of the study were to improve feed resource through integrating forage and cereal crops, to demonstrate maize/lablab intercropping under irrigated land and to see farmers' perception towards this technology. Moreover, Introducing these improved forage legumes through intercropping is the best way for forage adoption.

## MATERIALS AND METHODS

### Description of the study area

On station, screening of eight lablab accessions was conducted for general adaptability and compatible with maize in Illala forage experimental site of Mekelle Agricultural Research Center.

The site is located in Mekelle Zone of Tigray regional state, 5 km North of Mekelle city. Its geographical location is 13°5'N altitudes and 39°6' E longitudes. It is found at an elevation of 1970 m above sea level. The center is laid on 40 hectares of land with a gentle slope and plan topography. The weather of the center is moderately hot and windy with mean annual maximum and minimum temperature of 27 and 10.1°C, respectively with relative humidity of 55.60%. It receives 528.8 mm mean annual rainfall.

### Treatments and experimental design

A Randomized Complete Block Design was used with 3 replications. Each accession was planted in 6 rows plot at 3 m \* 4 m. The space between plants and rows was 20 and 50 cm,

respectively. Data on establishment, biomass, seed production, and pest infestation were collected (Figure 1).

Intercropping of legumes with cereal crops under irrigated lands was conducted in K/Awlaelo and H/Wagerat districts selected by IFAD project, with similar agro ecologies of the on station sites. Maize was first planted under the irrigation condition and lablab accessions were sown after four weeks (knee stage of the maize). Maize was planted as a mono-crop and intercrop with lablab accessions in a row intercropping types.

The study was conducted in a single plot observation in one farmer's field and replicated in to 4 farmers. Each farmer allocated 10 \* 10 m plot size of land for each treatment (300 m<sup>2</sup> total areas). Spacing between rows and plants for both sole and intercropping maize in row-planting pattern was 75 and 15 cm, respectively. The spacing for lablab plants was 20 cm between plants (Figure 2).

The treatments are as follow:

Sole maize -----T<sub>1</sub>  
Maize + lablab acc# 1034-----T<sub>2</sub>  
Maize + lablab acc# 912- -----T<sub>3</sub>

### Data analysis

The collected data were analyzed using SAS version 9.2, for simple calculation of the arithmetic mean of forage yield during screening.

## RESULTS AND DISCUSSION

### Screening of lablab forage legume

The agronomic data for Lablab accession are presented in (Table 1). There was no significant difference ( $P < 0.05$ ) among the eight lablab accessions in terms of date of emergency and tiller number at harvest. The mean value of total forage biomass yield (ton/ha) for Dolichos lablab, accession number 912 and 1034 was 8.3, 7.2 and 5.6, respectively. There was no significant ( $P > 0.05$ ) difference between the biomass yield of Dolichos lablab and 912 lablab accessions.

The on station screening of lablab accession observed a significant ( $P < 0.05$ ) difference in forage biomass yield, among the eight lablab accessions from which Dolichos lablab, lablab accession number 1034 and 912 were best performing forage legume. The main factor for the observed difference in forage biomass yield was the variation towards tendency of trailing growth on the ground producing adequate forage biomass.

The on station study observed a significant difference between accession number 1034, and with Dolichos lablab and accession number 912. Those three Lablab accessions are best adaptive and promising forage legumes for improving forage production under smallholder farmer.

### Lablab plant growth characteristic

#### Establishment

Lablab grows well where annual rainfall is 650 to 3000 mm.



**Figure 1.** Screening of lablab forage legume for compatibility and biomass yield.



**Figure 2.** Inter row-intercropping system of lablab legume forage with maize.

It is drought tolerant when established but loses leaves during prolonged floods (Mullen et al., 2003).

Dry periods tolerates short periods of flooding, but is intolerant to poor drainage and prolonged floods. As a single crop, seeding rates for Lablab are between 12 and 20 kg/ha. Rows should be 60 to 120 cm apart with 30 to 60 cm between plants. Lablab germinates and stabilizes easily when sown into sub-surface soil to a depth of at

least 5 to 10 cm.

Management For optimum feeding value, the first cutting should be done at the beginning of flowering. The following cuttings provide forage with more stem than leaf, which has lower feed value. The recommended cutting height is about 30 cm above ground level and above the branches, to allow regrowth. If properly cut, it is possible to harvest lablab foliage (leaves and young stems) three times a year.

**Table 1.** Agronomic parameters of lablab accessions tested at Illala site.

Lablab accession	Emergency days	Tiller number at harvest	Plant height at harvest (cm)	DM yield (Kg/ha)	DM yield (t/ha)	Vigour score (1-5)	Disease and pest score (0-5)
147	5	3	57 <sup>a</sup>	4401.5 <sup>c</sup>	4.4 <sup>c</sup>	5	0
507	5	3	53 <sup>ab</sup>	6074.9 <sup>ab</sup>	6.1 <sup>ab</sup>	5	0
912	5	3	54 <sup>ab</sup>	7158.1 <sup>a</sup>	7.2 <sup>a</sup>	2	0
1034	5	3	51 <sup>ab</sup>	5914.1 <sup>b</sup>	5.9 <sup>b</sup>	1	0
6529	6	4	50 <sup>b</sup>	6374.3 <sup>ab</sup>	6.4 <sup>ab</sup>	5	0
10979	7	3	50 <sup>b</sup>	5093.3 <sup>bc</sup>	5.1 <sup>bc</sup>	4	0
11609	5	3	48 <sup>c</sup>	5786.4 <sup>b</sup>	5.8 <sup>b</sup>	5	0
Dolichos lablab	6	3	56 <sup>a</sup>	8307.8 <sup>a</sup>	8.3 <sup>a</sup>	5	0
SE	0.54	0.21	2.03	-	-	0.18	0
P < 0.05	-	-	-	-	-	-	-

SE= Standard error. Means within a column not connected by same letter, are significantly different at P <5 %.

**Table 2.** Effect of intercropping on total forage biomass yield/10 m\*10 m plot size

Cropping system	Replication	Mean ±SE	Min	Max
Sole maize (control)	4	18.0 ± 0.9 <sup>c</sup>	16	20
Lablab acc #1034 intercropped with maize	4	33.7 ± 0.5 <sup>b</sup>	33	35
Lablab acc #912 intercropped with maize	4	37.7 ± 0.9 <sup>a</sup>	36	40

### On farm evaluation of intercropping lablab accessions with maize

Lablab accession number 1034 and 912 were evaluated as best compatible for intercropping with maize. These lablab accessions had trailing behavior on the ground yielding huge foliage biomass. The mean forage biomass yield for 912 and 1034 lablab accession was 15 and 14 kg, respectively from 10 \* 10 m plot size. The total mean forage biomass yield harvested from 10 \* 10 m plot size was 18.0 ± 0.9, 33.7 ± 0.5 and 37.7 ± 0.9 kg, for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. There was a significant (P<0.05) difference between the mean forage biomass, harvested from the plot of lablab accession 1034 and 912 intercropped with maize and sole crop maize (Table 2).

The mean improvement over the sole maize cropping in the total fresh biomass yield for lablab accession 912 and 1034 was 19.7 kg (52.4%) and 15.7 kg (46.7%), respectively. According to the farm evaluation of intercropping forage legume with maize, the total mean fresh biomass for lablab accession 912 was significantly (P<0.05) higher (10%) than the total mean forage biomass of lablab accession 1034. Lablab accession 912 was best compatible for intercropping with maize of Lablab accession 1034 (Figure 3).

During the first 1 month, lablab grows slowly between the maize rows. When the maize begins to ripen, lablab start to grow more vigorously and obtain their greatest development (Figure 3). The result of the current study indicated that, intercropping of lablab forage legume has a possibility to reduce pest infestation and to retain

moisture for the component crop. The finding of the current study is in line to the previous findings reported by Abreham (2013), who observed that intercropping of Lablab with maize is best compatible.

About 38 smallholder farmers participated in evaluation of forage legume intercropping with maize. The intra raw maize intercropping of lablab forage legume was demonstrated under smallholder farmer's irrigated lands. During demonstration, the participant suggested that intercropping of lablab forage legume has a potential to enhance the availability of improved forage for improving livestock production. It resulted in higher forage yield than maize crop alone. Providing animals with green foliage of lablab is needed as a supplement to crop residue of Maize Stover, in order to produce a feed composition capable of meeting the basal nutritional requirements of ruminants (Figure 4).

### Nutritional importance of Lablab forage legume

The broad leave of lablab forage legume has a potential DM yield for improving livestock feed. The study done elsewhere reported that lablab varieties produce forage biomass of "70% DM with 18% CP and 60% digestibility of the DM" (Mullen et al., 2003). It yields about DM 10.9 tons per hectare at flowering stage with protein content of 14 to 19% (Tesfaye et al., 2010). The Fresh lablab forage has off-flavor feeding lablab as hay, which might help to avoid this problem during supplementary feeding. After the maize is harvested, cattle may be turned out to graze



**Figure 3.** Intercropped lablab accession 1034 (left) and 912 (right) images, with maize demonstration of lablab intercropping.



**Figure 4.** Potentiality of intercropping to improve livestock feeds under smallholder farmer.

the maize Stover / lablab field or used to cut and carry feeding system (Figure 5). Lablab has a potential in grazing land productivity improvement strategies. It is compatible for grass-legume mixture to over sow in degraded grazing land.

#### **Relavance of lablab to sustainable agricultural production**

Lablab forage legume has a potential for improving soil organic matter as well as Nitrogen and minerals in the

soil (Figure 6). Lablab is a companion crop for maize, important in enhancing soil conservation through greater ground cover than sole cropping (Nnadi and Haque, 2008).

Intercropping, offers farmers the opportunity to engage nature's principle of diversity on their farms (Humphreys, 1994). The study observed that, forage legume intercropping produced more forage biomass than the sole cropping, as a mixture of legume and crop residue for smallholder livestock. In addition, intercropping has a potential to improve soil fertility through Nitrogen fixation and organic matter in environmentally friendly manner



**Figure 5.** Demonstration of improved utilization of crop residue.



**Figure 6.** Potentiality of forage legume intercropping for soil fertility improvement.

(Bula et al., 1995; West and Griffith, 1992) (Figure 6).

## Conclusions

The study shows that lablab accessions 1034 and 912 are compatible for row intercropping with maize, in the high and mid land agro ecology for both irrigated and rain fed conditions, fitting into the existing maize based farming system.

The promising lablab accession are well adapted to the agro ecology which provide ample amount of fresh biomass under irrigated lands at 5 smallholder level. But their adoption rate is very low as per the plan. So continuous extensional fellow up together with enough

planting materials are the key concepts for wide adoption.

## CONFLICT OF INTERESTS

The authors has not declared any conflict of interests.

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*Full Length Research Paper*

## Nutritional composition of vegetables grown in organic and conventional cultivation systems in Uberlândia, MG

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**There is a growing concern about the presence of pesticides in food and the health problem they may cause. Awareness of this issue, consumers opt for organic food consumption. Organic agriculture is a farming system ecologically sustainable, economically viable and socially just, capable of integrating man and environment. This study evaluated the nutritional composition of some crops grown in an organic (Org) and conventional (Con) systems, produced in Uberlândia-MG. Samples of lettuce, collard greens and carrots from both certified farming systems were gathered from local supermarkets and moisture, lipids, ash, dietary fiber, carbohydrates, protein and energy were obtained from them. Moisture was greater and the amounts of lipids, proteins and ashes were lower in conventional vegetables. Dietary fiber content in organic collard greens was significantly greater ( $4.37\% \pm 0.28$  Org) than in conventional ones ( $3.15\% \pm 0.12$  Con). Differences in nutrient composition of crops grown organically and conventionally were observed and more studies are needed for better understanding of nutritional value differences.**

**Key words:** Nutrients, organic farming system, conventional farming system.

### INTRODUCTION

In recent decades, the consumption of organic food is growing and expanding at a high rate (Willer and Kilcher, 2012). Organic food consumption is increasing because the consumers are more concerned with environmental

issues and possible health problem that pesticide residues can cause (Borguini and Torres, 2006). In this context, organic agriculture is an ecologically sustainable food production system, economically viable and socially

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**Table 1.** Means of moisture, lipids, ash and proteins in vegetables grown under organic and conventional farming conditions (mean  $\pm$  standard deviation SD).

Vegetable	Moisture (%)	Lipids (%)	Ashes (%)	Proteins (%)
Carrot (Org)	89.20 $\pm$ 1.23 <sup>B</sup>	0.04 $\pm$ 0.05 <sup>A</sup>	0.86 $\pm$ 0.13 <sup>A</sup>	0.79 $\pm$ 0.05 <sup>A</sup>
Carrot (Con)	90.98 $\pm$ 0.56 <sup>A</sup>	0.03 $\pm$ 0.01 <sup>A</sup>	0.58 $\pm$ 0.03 <sup>B</sup>	0.63 $\pm$ 0.02 <sup>B</sup>
Lettuce (Org)	95.37 $\pm$ 0.20 <sup>A</sup>	0.16 $\pm$ 0.01 <sup>A</sup>	0.99 $\pm$ 0.01 <sup>A</sup>	1.39 $\pm$ 0.09 <sup>A</sup>
Lettuce (Con)	95.59 $\pm$ 0.81 <sup>A</sup>	0.17 $\pm$ 0.06 <sup>A</sup>	0.80 $\pm$ 0.16 <sup>B</sup>	1.19 $\pm$ 0.26 <sup>A</sup>
Collard greens (Org)	86.95 $\pm$ 0.75 <sup>B</sup>	0.53 $\pm$ 0.04 <sup>A</sup>	2.02 $\pm$ 0.08 <sup>A</sup>	3.35 $\pm$ 0.25 <sup>A</sup>
Collard greens (Con)	90.64 $\pm$ 0.27 <sup>A</sup>	0.27 $\pm$ 0.03 <sup>B</sup>	1.80 $\pm$ 0.08 <sup>B</sup>	3.03 $\pm$ 0.21 <sup>A</sup>

\*Averages followed by different letters for the same vegetable, differs by F-test at 5% probability.

just, able to integrate man and environment. Some studies demonstrate differences between organic and conventional foods, when attributes such as flavor and nutritional value are considered (Favaro-Trindade et al., 2007), but so far the evidence is not sufficient to affirm the superiority of the organic and the benefits of its consumption for consumer health (Arbos, 2009).

The consumption of carrots, collard greens and lettuce is recommended in the diet, since these foods have low calorie and high content of dietary fiber, vitamins and minerals. Carrots, collard greens and lettuce are consumed vegetables, being of great importance in food and human health (Boeing, 2012). The consumption of organically grown vegetables has been growing in recent times, and there is little information on the nutritional composition of these organically produced vegetables.

A stimulus to boost the choice of these foods is the most accurate knowledge on the nutritional composition of organic food. Thus, this paper investigates the nutritional composition of crops grown in organic (Org) and conventional (Con) cultivation systems, produced in the city of Uberlândia - MG.

## MATERIALS AND METHODS

Conventional and organic samples of lettuce (*Lactuca sativa* L.), collard greens (*Brassica oleracea* L. *acephala*) and carrot (*Daucus carota* L.), in commercial maturation stage and available for consumption, were obtained in March 2014 from local establishments (Uberlândia-MG). The organic samples were certified by competent bodies. The samples ( $\approx$  5 kg) were packed in polyethylene bags, transported in polystyrene boxes containing ice to the Laboratory of Bromatology of Federal University of Uberlândia, within a maximum of 2 h after collection. The samples were lightly washed with running water and distilled water and dried with paper napkin. Five replicates were collected for each sample and the quartering method was used to obtain representative samples.

Moisture of samples *in natura* were determined by drying them in an oven at 65°C until constant weight was obtained, the fixed mineral residue (ash) was determined by incineration in a muffle furnace at 550°C and total lipids by extraction method using Soxhlet extractor and employing ethyl ether as solvent for 8 h under reflux,

according to the methodology described in Analytical Standards Manual from Adolf Lutz Institute (Zenebon et al., 2008).

The protein determinations were performed according to the semimicro-Kjeldahl method for the total nitrogen measurement, described by Association of Official Analytical Chemists (AOAC, 1980). The nitrogen to protein conversion factor was 6.25. To determine the dietary fiber, the official nonenzymatic-gravimetric method for the determination of total dietary fiber (AOAC 993.21) modified by Li and Cardozo (1994) was used. The carbohydrate content of the food was calculated by subtracting the sum of the contents in the components: moisture, protein, fat, ash and total dietary fiber, from the entire set. This procedure is predicted in the DRC No. 360, of December 23, 2003, ANVISA (BRASIL, 2003).

## Statistical analysis

Five replicates were made of each vegetable and statistical analyzes were performed using the F-test at 5% probability, using the statistical program SAEG-UFV 9.1.

## RESULTS AND DISCUSSION

The results of the moisture, lipids, ash and protein values (expressed in g 100 g<sup>-1</sup>) for the chosen vegetables grown in organic and conventional systems, obtained in this study are shown in Table 1.

In this work, organic foods presented lower moisture content, therefore, they contain higher dry matter content and a higher concentration of nutrients. It is believed that the lack of nitrogen sources available in organic systems leads to an increase in the amount of dry matter. Moisture can be influenced by storage conditions, crop type, time of the year, and crop age (Silva and Queiroz, 2006). Darolt (2003) surveyed the main comparative studies between organic and conventional foods, and observed higher content of dry matter, lower level of nitrates and higher content of vitamin C, in organic products, particularly in green leaves. According to the author, this occurs due to the lack of nitrogen sources available in organic systems. Vidigal et al. (1995) observed an increase in water content of lettuce cultivated on the average organic compounds. While

**Table 2.** Means of fiber content, carbohydrates and energy in vegetables grown under organic and conventional farming conditions (mean  $\pm$  standard deviation SD).

Vegetable	Fiber (%)	Carbohydrates (%)	Energy (kcal 100 g <sup>-1</sup> )
Carrot (Org)	3.665 $\pm$ 0.27 <sup>A</sup>	5.4342 $\pm$ 1.3040 <sup>A</sup>	25.3048 $\pm$ 5.1569 <sup>A</sup>
Carrot (Con)	2.730 $\pm$ 0.19 <sup>A</sup>	5.0366 $\pm$ 0.6238 <sup>A</sup>	23.0046 $\pm$ 2.3173 <sup>A</sup>
Lettuce (Org)	1.760 $\pm$ 0.08 <sup>A</sup>	0.3164 $\pm$ 0.2004 <sup>A</sup>	8.3020 $\pm$ 0.8461 <sup>A</sup>
Lettuce (Con)	1.570 $\pm$ 0.01 <sup>A</sup>	0.6452 $\pm$ 0.3701 <sup>A</sup>	8.9964 $\pm$ 2.9388 <sup>A</sup>
Collard greens (Org)	4.370 $\pm$ 0.28 <sup>A</sup>	2.7616 $\pm$ 0.8099 <sup>A</sup>	29.3040 $\pm$ 3.1923 <sup>A</sup>
Collard greens (Con)	3.150 $\pm$ 0.12 <sup>B</sup>	1.0882 $\pm$ 0.3312 <sup>B</sup>	18.9548 $\pm$ 0.9740 <sup>B</sup>

\*Averages followed by different letters for the same vegetable, differs by F-test at 5% probability.

Souza et al. (2005) claimed that adding organic and/or mineral fertilizers does not affect the water content of lettuce.

Higher lipid content ( $p \leq 0.05$ ) was observed in organic collard greens leaves compared to conventional samples, corroborating whit Rocha et al. (2008) who studied the composition of organic and conventional collard greens. The difference in the amount of lipids from organic and conventional foods may be due to the type of food studied. The lettuce and carrot samples showed no difference in the amount of lipids. A study carried out in the metropolitan region of Curitiba, comparing organic and conventional lettuce and carrots, also did not find difference in the amount of this nutrient (Arbos, 2009). Lima et al. (2009) did not find difference in the lipid content of organic and conventional vegetables.

The amount of ashes, measured in this work were higher in organic food, which was also evidenced by Borguini (2002), that point out higher levels of minerals (phosphorus, potassium, magnesium, sulfur, sodium, iron and zinc) in organic tomato samples. The ashes represent the inorganic residue left over the combustion of organic matter, and its composition refers to the amount of mineral substance present (ARBOS, 2009).

The results on the protein content show that organic vegetables have higher values than conventional ones. For carrots, this value was significantly higher (Table 1). In the work of Arbos (2009), it was observed that the organic lettuce has a higher protein content, although not significant. Results of some studies also show a trend in protein accumulation in organic foods (Stertz et al., 2005a; Worthington, 1998; Lima et al., 2009). The greater protein content in organic foods can be assigned to the type of fertilizer used. It is true that farmers producing organic food use fertilizers with high nitrogen content, originated from animal waste and organic matter of other cultures. Weston and Barth (1997) reported a positive correlation between the protein content and the use of fertilizer rich in nitrogen.

In this study, it was observed that only the collard greens had significantly higher organic fiber than

conventional (Table 2). In the other vegetables, it is observed that there was a trend of higher fiber content on crops grown organically even with no significant difference occurred. As lignin is part of the total fiber and its structure contains phenols, it is possible that the type of cultivation may interfere with the increase of phenolic compounds since these compounds are produced by the plant as a defense against pathogens (Daniel et al., 1999).

The increase in fiber content can be explained due to the increased production of phenolic compounds by plants as a defense to insect attack (Young et al., 2005). In the work of Rocha et al. (2008), there was no difference in fiber content between organic and conventional foods, however, according to Daniel et al. (1999), the application of some herbicides can increase or decrease the secondary metabolites content, including total phenols, which leads to an uncertainty in the fiber content of conventional foods in Rocha's work.

The fibers form a group of substances resistant to the action of human digestive enzymes can be classified into water-soluble and water-insoluble fibers. Most pectins, gums and certain hemicelluloses are soluble and cellulose, other pectin, most of the hemicelluloses and lignin are insoluble. Surveys show the beneficial effects of dietary fiber in the prevention and treatment of intestinal diseases, cancer reduction and control of diabetes mellitus (Kelsay, 1978; Marques et al., 2011).

As shown in Table 2, there was no significant difference in carbohydrate concentration observed in the analyzed vegetables. Only the organic collard greens had higher levels of carbohydrates than conventional. Unlike what was found in this study, Arbos (2009) studied the composition of organic and conventional lettuce, noted that organic lettuce contains higher carbohydrate values (conventional lettuce showed 1.7 g of carbohydrates 100 g<sup>-1</sup>, while the organic was 4.87 to 5.68 g 100 g<sup>-1</sup>).

In analyzing the results, it is possible to conclude that organic vegetables studied in this work tend to have a higher content of dietary fiber than those from conventional system. The moisture content was lower in

organic, which may justify a larger amount of energy obtained. Lettuce showed no difference in moisture content, fiber or carbohydrates, resulting in no difference in the energy quantity, which is in line with the work of Stertz et al. (2005a), who studied nutritional quality and lettuce contaminants (*L. sativa*) conventional, organic and hydroponic, found no difference between the three.

On the average, the data found in this work shows that vegetables grown under organic management tend to have a higher concentration of nutrients when compared with the conventional ones.

## Conclusions

There was difference in the nutritional composition of some vegetables grown organically and conventionally, analyzed in this study. Organic ones have mostly lower moisture content and higher amount of nutrients: proteins, ash and fiber. A higher amount of lipids was observed only for collard greens.

More studies, including those related to micronutrients and controlled production must be carried out for further investigation on nutritional values. More accurate knowledge of the nutritional composition of organic vegetables may be the key for boosting the consumption of this type of food.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

## The growth and nutrition of pineapple (*Ananas comosus* L.) plantlets under different water retention regimes and manure

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Despite the many advantages of pineapple plants offered by micropropagation, there is difficulty in rooting and slowness in the growth of seedlings, requiring a long period of acclimatization in the greenhouses. The aim of this study was to evaluate organic sources and water retention polymers used for pineapple cultivar seedlings during the acclimatization phase. The experimental design was randomized blocks in a 2 × 2 × 3 factorial design. Bovine and goat manures were the organic sources that provided the greatest increases in growth characteristics for both cultivars. Goat manure was the organic sources that provided the greatest increases in growth characteristics for both cultivars. Provide seedlings with 20 leaves, height of 19.4 cm, diameter of the rosette of 26.03 mm and 23.1 cm of length of the root system at 270 days to "Vitória". For "Imperial" the goat manure promoted seedlings with 20 leaves and height of 27.8 cm in 220 days. Bovine manure provided greater nutritional gains to seedlings. The use of the hydrogel did not favor the growth of shoots. However, it resulted to the increase in root dry mass when incorporated into the manure for both cultivars. The "Imperial" cultivar had a higher macronutrient intake on seedlings' leaves than the "Vitória" cultivar at 270 days of acclimatization.

**Key words:** Fruit production, *Ananas comosus*, propagation, hydrogel, organic fertilizers.

### INTRODUCTION

In 2014, one year after a drought affected the pineapple culture in north of the country, a survey by the Brazilian

Institute of Geography and Statistics (IBGE) indicated that the area cultivated with pineapple was 66,544 ha,

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with a production of 1,762,938,000 fruits (IBGE, 2016). The states of Paraíba, Bahia and Rio Grande do Norte were major producers in the region.

The state of Rio Grande do Norte ranks sixth among states with the largest production of pineapples. It is concentrated mainly in the municipalities of Ielmo Marinho, Touros and Pureza, and consists of an activity with a great economic and social importance for Rio Grande do Norte.

With a high demand by the market, producers seek to increase the production of several pineapple cultivar seedlings. In this sense, new cultivars have been released both for a better fruit acceptance and an increase of plants resistant to the fusarium wilt disease caused by the fungus *Fusarium subglutinans* f. sp. *Ananas*, which has been one of the major diseases of this culture leading to major losses in agriculture (Matos et al., 2009; Ventura et al., 2009).

Furthermore, to obtain uniform, high quality, free of diseases and genetically superior seedlings, and especially to produce large-scale plants, micropropagation is an alternative propagation method used for this species (Cid, 2001). Despite many advantages offered by micropropagation for pineapples, there is difficulty in rooting and slowness of seedling growth (Moraes et al., 2010), requiring a long period of acclimatization in greenhouses (Teixeira et al., 2001). The decrease in such period may be an option to lower the cost of the technique and increase the production of seedlings.

In addition, the development of technologies that help plants to tolerate prolonged periods of drought and the use of more tolerant cultivars are essential to minimize the negative impacts of drought. Among the technologies available for water supply to plants, soil conditioners also known as water absorbing polymers or hydrogels have been widely used in agriculture (Ferreira et al., 2014). However, studies with fruits, particularly concerning the formation of seedlings, are still scarce. The use of organic substrates with suitable characteristics for a planted species is also an important technique available for plant propagation as it enables reducing consumption of inputs, such as chemical fertilizers, pesticides and labor (FERMINO and KAMPF, 2003). It also contributes to the reduction of the acclimatization period. Some studies have been conducted incorporating organic matter into substrates for the production of fruit seedlings (Sousa, 1994; Muller et al., 1979; Peixoto, 1986).

In this sense, the aim of this study was to evaluate organic sources and water retention polymers used for pineapple cultivar seedlings during the acclimatization phase.

## MATERIALS AND METHODS

The experiment was conducted in a greenhouse located at the

Federal Rural University of the Semi-Árido (UFERSA), Campus Leste, in Mossoró, Rio Grande do Norte (RN) state. According to Sobrinho et al. (2011), the climate of Mossoró is BSw<sup>h</sup>, is a very hot semi-arid region with a rainy season in the summer extending to the autumn. The average temperature is 27.4°C, with a very irregular annual rainfall and an average relative humidity of 68.9%. Pineapple seedlings (*Ananas comosus* L. Merrill), cultivars "Vitória" (INCAPER, 2006) and "Imperial" (EMBRAPA, 2003), propagated *in vitro* in plastic pots with a 200 ml capacity were provided by the BioClone Biotechnology Laboratory and maintained in a MS medium described by Murashige and Skoog (1962) without growth regulators and vitamins. Upon arrival at the UFERSA (June 15, 2013), the seedlings were transferred to a pre-acclimatization greenhouse of the Seedling Production Sector of UFERSA, where they remained until the installation of the experiment (July 17, 2013) receiving water every day by an automated micro-sprinkler system.

The experiment was installed when seedlings were at 30 days of pre-acclimatization in the greenhouse. The substrate used for filling the pots (2 L) was composed (v/v) by 70% of soil and 30% of the organic sources tested in the experiment. The organic sources tested were bovine manure (BM), goat manure (GM) and commercial organic compost (OC) Eco Fertil<sup>®</sup>. They were sieved and mixed until complete homogenization as recommended by Moreira (2001). A sample was taken from each material (soil + organic source) and sent to the soil analysis laboratory of UFERSA for analysis of chemical attributes (Table 1).

The hydrogel was purchased in a commercial form (Biogel Hydro Plus, Biossentes) and the dose was adapted from the dose recommended by the manufacturer for pineapple crops (4 g per pot). The dose was mixed homogeneously to the dry substrate. During the experiment, there were daily irrigations at late morning and late afternoon by a micro-sprinkler system with an average flow of 40 L h<sup>-1</sup>.

The experimental design was randomized blocks in a 2 × 2 × 3 factorial design. The factors were two pineapple cultivars ("Imperial" and "Vitória"), two hydrogel applications (with and without addition of hydrogel to the substrate), and three tested organic sources (bovine manure, goat manure and organic compost), totaling 12 treatments with 4 replications and 5 plants per plot. Evaluations of growth were performed on the day of transplantation and at 120, 150, 180, 210, 240 and 270 days after seedlings were transplanted to pots. The number of leaves, plant height (cm) and leaf rosette diameter (cm) were quantified.

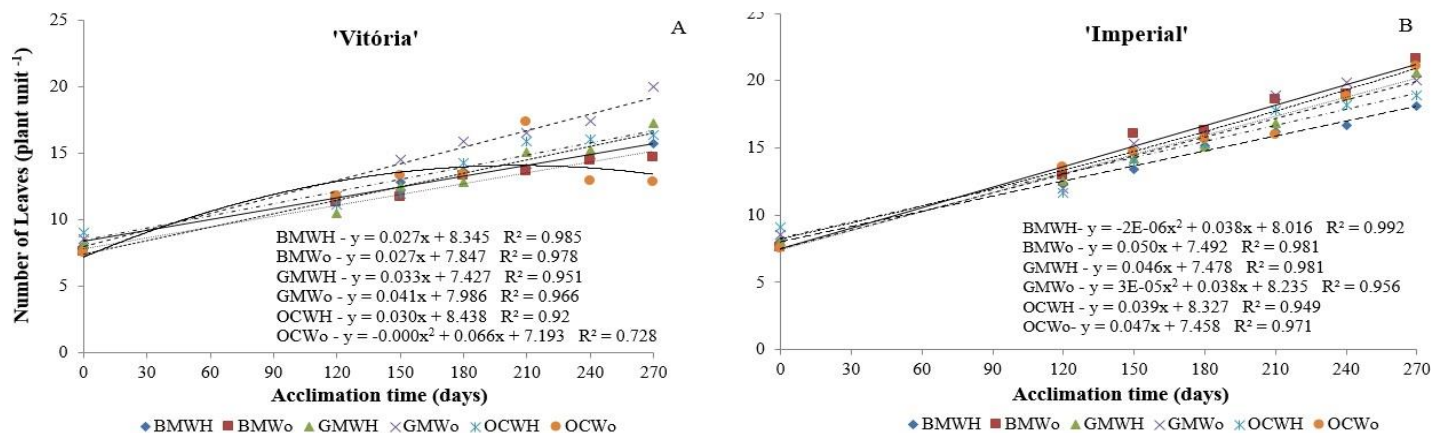
At the end of the experiment (270 days), the plants were evaluated regarding the number of leaves, plant height (cm), leaf rosette diameter (cm), root length (cm) and weight of root and shoot dry matter (g). The biometric evaluations of plants were performed using rulers. Height measurements were performed from the base of the plants to the highest point of the leaves without changing the structure of the plants. The rosette diameter was measured between the biggest opposing leaves. The length of the "D" leaf was measured after its removal. To analyze fresh and dry matter, precision scales were used. After measuring the fresh weight of shoots, the plant material was packed in paper bags and placed to dry in a forced-air circulation oven at 65°C until constant weight to obtain dry mass.

At the end of the experiment, a nutritional analysis of shoots was conducted. Shoots were separated from roots and washed in running and deionized water, dried with cotton and then sent to the plant nutrition laboratory of UFERSA to perform the foliar chemical analysis of macronutrients and micronutrients. The samples were analyzed as for levels of N, P, K, Ca and Mg according to Battaglia et al. (1983).

Data were submitted to analysis of variance using the software SISVAR (Ferreira, 2011) according to the factorial design adopted and significant means were compared by Scott Knott test at 5% probability.

**Table 1.** Chemical analysis of the substrates (30% organic sources + 70% soil) used for the acclimatization of pineapple cultivar seedlings. Mossoró-RN, UFERSA, 2014.

Treatment	N	pH	EC	O.M	P	K <sup>+</sup>	Na <sup>+</sup>	V	m	PST
	g kg <sup>-1</sup>	water	dS/m	g kg <sup>-1</sup>		mg dm <sup>-3</sup>			%	
Soil + BM	0.42	6.03	0.75	21.05	173.64	1120.2	452.86	100	0	19.25
Soil + GM	0.63	5.33	0.9	14.8	183.29	620.25	119.8	100	0	6.74
Soil + OC	0.32	5.3	1.59	36.03	204.51	289.24	1062.3	100	0	47.22

**Figure 1.** Number of leaves (plant<sup>-1</sup>) of "Vitória" (A) and "Imperial" (B) pineapple seedlings grown in different organic sources. Bovine manure (BM), goat manure (GM) and organic compost (OC) with (W) and without (Wo) hydrogel (H) at 0, 120, 150, 180, 210, 240 and 270 days of acclimatization.

## RESULTS AND DISCUSSION

For the "Vitória" cultivar, only goat manure obtained 17.3 leaves at 210 days potentiated by not using the hydrogel (Figure 1A). The "Imperial" cultivar obtained 17 leaves with all organic carbon sources tested. This happened earlier when the manure was used without the hydrogel (17.1 leaves at 190 days) (Figure 1B).

Berilli et al. (2011) claim 17 visible leaves of acclimated seedlings to be the minimum appropriate amount to transfer them to the field. The goat manure provided in general greater increases in the number of leaves for "Vitória", which may be attributed to the higher N content in its composition (Table 1). This is because this nutrient is responsible for the production of new cells and tissues as it is present in chloroplasts as a constituent of the chlorophyll molecule (photosynthesis). It also participates in the synthesis of vitamins, hormones, coenzyme, alkalis and other compounds.

For the "Imperial" cultivar, as Oliveira and Natale (2013) states, the increase in N rates linearly decreases P, K and S contents and decreases the Mn content quadratically. The increase in K doses linearly decreases leaf contents of N, P, Ca and Mg. This may have happened in this study because the organic sources

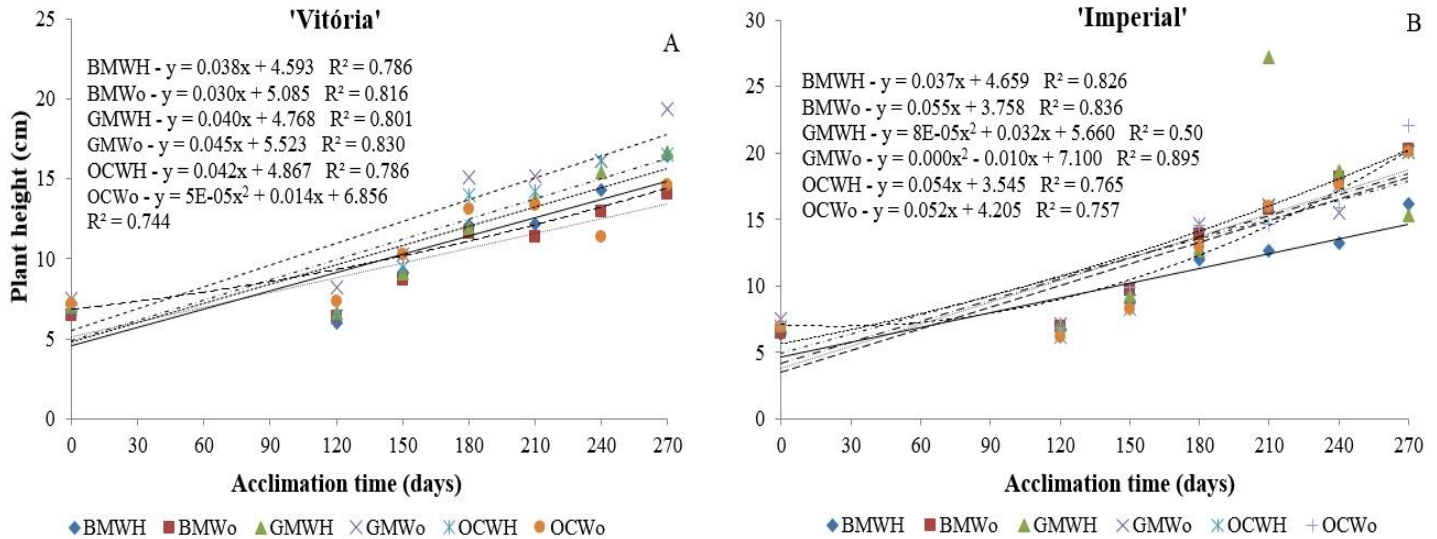
that provided a greater increase were the sources with less content of N.

Coelho et al. (2007) opine that the absence of effects on height during the first evaluations and the greater growth in the seventh month after transplantation indicate a slow growth of pineapple seedlings during the early stage of development. Also, according to Teixeira et al. (2009), a variable period (between six and eight months) in a greenhouse is necessary for plants to reach 20 to 30 cm, a size suitable for transfer to the field.

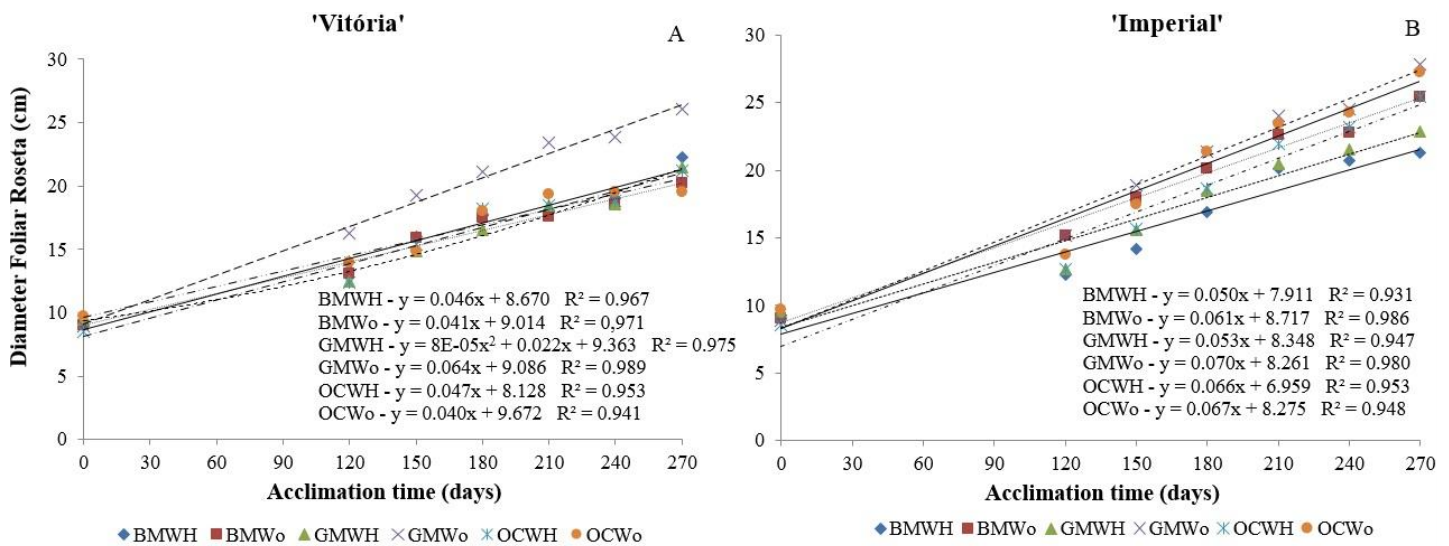
On the subject of "Vitória", that number (20 cm) was obtained by using goat manure without hydrogel. It reached 19.6 cm after 270 days (Figure 2A). The "Imperial" cultivar reached 20 cm at day 220 using goat manure and incorporating hydrogel into the substrate (Figure 2B).

Sousa Júnior et al. (2001) state that plant height is a variable that allows visual assessments. It is very important and even decisive to define the time of transplanting seedlings to the field. Coelho et al. (2007) suggest that the absence of effects on height during the first evaluations and the greater growth in the seventh month after transplantation indicate a slow growth of pineapple seedlings during the early stage of development.





**Figure 2.** Plant height (cm) of "Vitória" (A) and "Imperial" (B) pineapple seedlings grown in different organic sources: Bovine manure (BM), goat manure (GM) and organic compost (OC) with (W) and without (Wo) hydrogel (H) at 0, 120, 150, 180, 210, 240 and 270 days of acclimatization.



**Figure 3.** Leaf rosette diameter (cm) of "Vitória" (A) and "Imperial" (B) pineapple seedlings grown in different organic sources: Bovine manure (BM), goat manure (GM) and organic compost (OC) with (W) and without (Wo) hydrogel (H) at 0, 120, 150, 180, 210, 240 and 270 days of acclimatization.

Mews et al. (2015), studying the effects of hydrogel and urea on the production of *Handroanthus ochraceus* seedlings, found that for plant height and stem diameter the doses that had the highest increase values were between 2 and 4 g both for urea and hydrogel. To Lopes et al. (1999), adequate nitrogen nutrition automatically increases the foliar nitrogen and phosphorus, consequently increasing the growth and the production of high-quality seedlings.

During the study period, pineapple seedlings did not

reach the minimum value of 30 cm recommended by Berilli et al. (2011) as the ideal value for leaf rosette diameter of seedlings suitable to field conditions. The best approximation was provided by the goat manure without hydrogel, when "Vitória" obtained 28 cm at day 270 of acclimation and "Imperial" obtained 27.4 cm at day 270 (Figure 3).

However, such values were similar to those found by Baldotto et al. (2009), who studied the performance of "Vitória" pineapple cultivar in response to the application

**Table 2.** Mean values of seedlings growth parameters of "Vitória" and "Imperial" for number of leaves (NL), plant height (PHe), leaf rosette diameter (LRF), length of the root system (LRS), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), ratio between shoot and root dry matter (SDM/RDM) and chlorophyll content (CC).

Cultivars	NL	PHe	LRF	LRS	SDM	RDM	TDM	SDM/RDM	CC
'Vitória'	15.96 <sup>b</sup>	16.3 <sup>b</sup>	21.7 <sup>b</sup>	21.05 <sup>b</sup>	4.8 <sup>b</sup>	0.7 <sup>b</sup>	4.8 <sup>b</sup>	6.7 <sup>a</sup>	5.6 <sup>b</sup>
'Imperial'	20.04 <sup>a</sup>	19.54 <sup>a</sup>	25.01 <sup>a</sup>	26.4 <sup>a</sup>	8.4 <sup>a</sup>	2.5 <sup>a</sup>	11.7 <sup>a</sup>	6.6 <sup>a</sup>	9.7 <sup>a</sup>

Means followed by the same letters do not differ by Scott Knott test ( $p \leq 0.05$ ).

**Table 3.** Means for number of leaves (NL), plant height (PHe), leaf rosette diameter (LRD) and length of the root system (LRS) of seedlings of "Vitória" and "Imperial" pineapple cultivars grown on substrates containing bovine manure (BM), goat manure (GM) and organic compost (OC) with and without hydrogel at 270 days of acclimatization. Mossoró, RN, 2014.

OS ('Vit.')	NL		PHe		LRD		LRS	
	With	Without	With	Without	With	Without	With	Without
BM	15.7 <sup>Aa</sup>	14.6 <sup>Ba</sup>	16.4 <sup>Aa</sup>	14.05 <sup>Aa</sup>	22.3 <sup>Aa</sup>	20.2 <sup>Ba</sup>	21.6 <sup>Aa</sup>	22.6 <sup>Aa</sup>
GM	17.3 <sup>Aa</sup>	20 <sup>Aa</sup>	16.7 <sup>Aa</sup>	19.4 <sup>Aa</sup>	21.5 <sup>Aa</sup>	26.03 <sup>Aa</sup>	21.3 <sup>Aa</sup>	23.1 <sup>Aa</sup>
OC	16.4 <sup>Aa</sup>	12.8 <sup>Bb</sup>	16.5 <sup>Aa</sup>	14.6 <sup>Aa</sup>	21.2 <sup>Aa</sup>	19.1 <sup>Ba</sup>	19.9 <sup>Aa</sup>	17.9 <sup>Ba</sup>
OS ('Imp.')								
BM	18.1 <sup>Ab</sup>	21.6 <sup>Aa</sup>	21 <sup>Aa</sup>	24.5 <sup>Aa</sup>	21.3 <sup>Aa</sup>	25.4 <sup>Aa</sup>	24.2 <sup>Ba</sup>	25 <sup>Aa</sup>
GM	20.6 <sup>Aa</sup>	20.03 <sup>Aa</sup>	24.2 <sup>Aa</sup>	14.6 <sup>Aa</sup>	22.9 <sup>Aa</sup>	27.8 <sup>Aa</sup>	24.3 <sup>Ba</sup>	26.9 <sup>Aa</sup>
OC	18.9 <sup>Aa</sup>	21.1 <sup>Aa</sup>	22.2 <sup>Aa</sup>	20.2 <sup>Aa</sup>	25.4 <sup>Aa</sup>	27.2 <sup>Aa</sup>	29.8 <sup>Aa</sup>	28.6 <sup>Aa</sup>

Means followed by the same capital letters do not differ regarding organic sources. Means followed by the same lowercase letters do not differ regarding the use of hydrogel by Scott Knott test ( $p \leq 0.05$ ).

of humic acids during acclimatization. The authors found maximum values of 25.33 and 21.83 cm when 10 and 20 mmol/L of C derived from humic acids of a vermicompost when used respectively.

Considering these values as reference, the goat manure without hydrogel provided 19.3 cm for "Vitória" and 20 cm for "Imperial" at 165 days.

At 270 days, when a destructive analysis of pineapple seedlings was performed, there was a superiority of the "Imperial" cultivar over the "Vitória" cultivar. This superiority was recorded during the evaluation process independent of the applied treatment (Table 2). Probably, this superiority is genetic because the "Imperial" cultivar, according to Matos et al. (2016), has a size similar to the "Pérola" cultivar, with a good development and growth, and a good seedling production.

The comparative performance of the cultivars "Vitória" and "Imperial" with respect to growth, especially during the acclimatization phase of seedlings, are not described in the literature. For "Vitória", the organic sources did not promote an increase in the number of leaves, height of pineapple seedlings, leaf rosette diameter and root growth when hydrogel was incorporated into the substrate used. However, when there was the incorporation of the hydrogel, the goat manure provided higher increases in the number of leaves and leaf rosette diameter. For the length of the root system, goat and

bovine manures provided higher gains irrespective of using hydrogel. However, statistical differences for these organic sources were only observed without the incorporation of hydrogel into the substrate (Table 3).

For the "Imperial" cultivar, the hydrogel negatively affected the number of leaves when bovine manure was used as source. However, the organic sources and the hydrogel did not influence plant height and leaf rosette diameter. The length of the root system of the "Imperial" cultivar had higher average values when the hydrogel was not incorporated into the substrate, except for the organic compost, which was in turn potentialized by the incorporation of the hydrogel, statistically differing from the other organic sources (Table 3).

The superiority of bovine and goat manures for shoots of pineapple seedlings, notably "Vitória", can be explained by the higher content of nitrogen (N) and potassium (K) in these sources. These are the elements that pineapple seedlings require the most. Such elements are directly involved in photosynthesis and respiration, which possibly resulted in an increased performance of seedlings when goat and bovine manures were incorporated into the substrate.

Andrade et al. (2015), studying an organic fertilizer for pinecone plants in function of organic substrates, concluded that the substrate containing bovine manure is a great choice for the formation of pinecone seedlings.

**Table 4.** Means for shoot dry matter (SDM), root dry matter (RDM), chlorophyll index (CI) and ratio between shoot and root dry matter (SDM/RDM) of seedlings of "Vitória" and "Imperial" pineapple cultivars grown on substrates containing bovine manure (BM), goat manure (GM) and organic compost (OC) with and without hydrogel at 270 days of acclimatization. Mossoró, RN, 2014.

OS ('Vit.')	SDM		RDM		CI		SDM/RDM	
	With	Without	With	Without	With	Without	With	Without
BM	4.3 <sup>Aa</sup>	4.8 <sup>Aa</sup>	1.1 <sup>Aa</sup>	0.8 <sup>Aa</sup>	7.5 <sup>Aa</sup>	4 <sup>b</sup>	6.3 <sup>Aa</sup>	5 <sup>Aa</sup>
GM	3.5 <sup>Aa</sup>	7.7 <sup>Aa</sup>	0.53 <sup>Aa</sup>	0.9 <sup>Aa</sup>	5.1 <sup>Aa</sup>	6 <sup>Aa</sup>	7.3 <sup>Aa</sup>	4.8 <sup>Aa</sup>
OC	5.7 <sup>Aa</sup>	3.1 <sup>Aa</sup>	0.5 <sup>Aa</sup>	0.3 <sup>Aa</sup>	5.2 <sup>Aa</sup>	5.7 <sup>Aa</sup>	9 <sup>Aa</sup>	7.5 <sup>Aa</sup>
OS ('Imp.')								
BM	10.4 <sup>Aa</sup>	7.5 <sup>Aa</sup>	3.02 <sup>Aa</sup>	2.3 <sup>Aa</sup>	9.9 <sup>Aa</sup>	11.3 <sup>Aa</sup>	4.6 <sup>Aa</sup>	64 <sup>Aa</sup>
GM	6.5 <sup>Aa</sup>	8.7 <sup>Aa</sup>	1.6 <sup>Ab</sup>	3.2 <sup>Aa</sup>	8 <sup>Aa</sup>	9.9 <sup>Aa</sup>	7.4 <sup>Aa</sup>	7.4 <sup>Aa</sup>
OC	7 <sup>Aa</sup>	10.1 <sup>Aa</sup>	17 <sup>Aa</sup>	3.2 <sup>Aa</sup>	8.5 <sup>Aa</sup>	10.4 <sup>Aa</sup>	5.5 <sup>Aa</sup>	8.5 <sup>Aa</sup>

Means followed by the same capital letters do not differ regarding organic sources. Means followed by the same lowercase letters do not differ regarding use of hydrogel by Scott Knott test ( $p \leq 0.05$ ).

Alves and Pinheiro (2008) stated that goat manure is a valuable product and its use provides an important alternative source of income for producers. Some studies have examined the potential use of goat manure and all of them stress its value. Comparing it with bovine manure, however, few data exist in the literature regarding its use, mainly during the acclimatization of pineapple seedlings.

For "Imperial", the increase in N rates linearly decreases P, K and S leaf contents and quadratically decreases the Mn content. The increase in K doses linearly decreases leaf contents of N, P, Ca and Mg (Oliveira and Natale, 2013). This may have happened in this study since the organic sources (organic compost) that provided a greater increase in the length of the root system were the sources with less content of N and K, but with a higher content of organic matter and phosphorus.

According to Lopes (1989), phosphorus promotes early root formation, early root growth, improves the efficiency of water usage and, when at a high level in the soil, helps to keep its absorption by seedlings, even under high soil moisture conditions, which happened in this work due to the action of the hydrogel.

Regarding shoot dry matter, organic sources provided no significant differences for both cultivars regardless of the use of hydrogel (Table 4). However, although there were no significant differences, the goat manure without hydrogel produced greater increases in the shoot dry matter of the "Vitória" cultivar, reflecting the gain that goat manure without hydrogel provided for a number of leaves and length of shoots.

Araújo et al. (2010), studying goat manure in the composition of substrate aiming the formation of papaya seedlings, also found that shoot dry matter responded better to a treatment containing goat manure, which differed significantly from other treatments.

There were no significant differences regarding organic sources and the use of hydrogel for the two cultivars

regarding root dry matter, except for the "Imperial" cultivar because the hydrogel negatively affected the goat manure (Table 4). Nevertheless, the highest averages for both "Vitória" and "Imperial" were observed with the use of bovine manure with hydrogel, which indicates that the use of the polymer for this cultivar provided a higher number of roots/rootlets at the expense of their size.

Wofford Jr. (1992) pointed out that the roots of the plants grow inside the beads of the hydrated polymer, with a great development of root hairs providing a larger contact surface of the roots with water sources and nutrients, facilitating absorption.

For the "Imperial" cultivar, there was a considerable performance of the organic compost without hydrogel regarding root dry mass, reflecting the increase in the length of the root system with that treatment.

Flannery and Busscher (1982) point out that despite the contribution to water retention capacity offered by the polymer, it was detrimental to azalea plants not because it was toxic, but because of the lack of aeration in the root system due to presence of the hydrated polymer on the substrate. This was more evident as the polymer dose in the substrate increased. In addition, the organic compost incorporated into the substrate may have contributed to the negative performance of the hydrogel.

Almeida et al. (2011), studying alternative substrates for the production of yellow passion fruit seedlings, found that the highest shoot, root and total dry matter was observed when substrates containing soil + bovine manure, soil + goat manure and Solaris<sup>®</sup> + bovine manure were used, especially the substrate soil + goat manure, which showed the highest average for these variables.

For the ratio between shoot and root dry matter, no statistical significant effects were observed for both cultivars regarding organic sources and incorporation of hydrogel into the substrate. Regarding chlorophyll content, the incorporation of the hydrogel into the

**Table 5.** Means for contents of leaf nitrogen (LNC), phosphorus (LPC), potassium (LKC), magnesium (LMgC) and calcium (LCaC) of seedlings of "Vitória" and "Imperial" pineapple cultivars grown on substrates containing bovine manure (BM), goat manure (GM) and organic compost (OC) with and without hydrogel at 270 days of acclimatization. Mossoró, RN, 2014.

OS ('Vit.')	LNC		LPC		LKC		LCaC		LMgC	
	With	Without	With	Without	With	Without	With	Without	With	Without
BM	13.7 <sup>Aa</sup>	11.7 <sup>Aa</sup>	3.3 <sup>Aa</sup>	2.2 <sup>Ab</sup>	33.6 <sup>Aa</sup>	31.8 <sup>Aa</sup>	1.1 <sup>Aa</sup>	0.9 <sup>Ba</sup>	0.18 <sup>Aa</sup>	0.14 <sup>Aa</sup>
GM	9.6 <sup>Ba</sup>	9.3 <sup>Aa</sup>	2.7 <sup>Aa</sup>	2.6 <sup>Aa</sup>	29.7 <sup>Aa</sup>	20.6 <sup>Bb</sup>	0.8 <sup>Aa</sup>	0.9 <sup>Ba</sup>	0.14 <sup>Aa</sup>	0.13 <sup>Aa</sup>
OC	8.2 <sup>Ba</sup>	9.6 <sup>Aa</sup>	1.7 <sup>Ba</sup>	2.2 <sup>Aa</sup>	21.6 <sup>Ba</sup>	23.3 <sup>Ba</sup>	0.7 <sup>Ab</sup>	1.5 <sup>Aa</sup>	0.16 <sup>Aa</sup>	0.22 <sup>Aa</sup>
OS ('Imp.')										
BM	9.6 <sup>Aa</sup>	11.1 <sup>Aa</sup>	2.1 <sup>Aa</sup>	1.7 <sup>Aa</sup>	28 <sup>Aa</sup>	18.2 <sup>Ab</sup>	1.9 <sup>Aa</sup>	1.9 <sup>Aa</sup>	0.26 <sup>Aa</sup>	0.28 <sup>Aa</sup>
GM	9.6 <sup>Aa</sup>	11.7 <sup>Aa</sup>	2.1 <sup>Aa</sup>	2.1 <sup>Aa</sup>	19.3 <sup>Ba</sup>	19.9 <sup>Aa</sup>	1.9 <sup>Aa</sup>	2.1 <sup>Aa</sup>	0.3 <sup>Aa</sup>	0.26 <sup>Aa</sup>
OC	7.9 <sup>Aa</sup>	10.2 <sup>Aa</sup>	2 <sup>Aa</sup>	2.2 <sup>Aa</sup>	19.3 <sup>Ba</sup>	18.03 <sup>Aa</sup>	2 <sup>Aa</sup>	2.2 <sup>Aa</sup>	0.3 <sup>Aa</sup>	0.28 <sup>AA</sup>

Means followed by the same capital letters do not differ regarding organic sources. Means followed by the same lowercase letters do not differ regarding the use of hydrogel by Scott Knott test ( $p \leq 0.05$ ).

substrate containing bovine manure provided a higher increase in the chlorophyll content for the "Vitória" cultivar when compared to the non-use of hydrogel with this organic source. It was statistically different. For the "Imperial" cultivar, there was no influence by organic sources and hydrogel (Table 4).

The difference in nitrogen content between organic sources was not sufficient to trigger differences in chlorophyll content. Baldotto et al. (2009), studying the performance of the pineapple "Vitória" in response to the application of humic acids during acclimatization, found a higher intensity of the green color when 40 mmol/L of humic acid filter cake were used. This treatment showed nitrogen and magnesium contents of 23.33 and 35.08%, respectively, inferior to the treatment with higher C and Mg contents.

Furthermore, the bovine manure used for the "Vitória" cultivar may have contributed synergistically to the hydrogel for the retention of N, and was reflected in a greater increase in the chlorophyll content in this treatment. Fagundes et al. (2015), studying a water absorbing polymer for the reduction of nutrients leached during the production seedlings of yellow passion fruits, found that N losses by leaching decreased as the dose of the polymer increased: there was a decrease of 47.8% on the leached material of a Bioplant<sup>®</sup> substrate and a decrease of 33.4% on a Provaso<sup>®</sup> substrate during the evaluation of nutrients.

The bovine manure was the organic source that contributed the most to the increase in leaf nitrogen content in "Vitória" pineapple seedlings regardless of the hydrogel. However, the bovine manure was statistically higher only with the addition of the hydrogel to the substrate. As for the "Imperial", nitrogen was not influenced by organic sources and hydrogel (Table 5).

Fagundes et al. (2015), studying water absorbing polymers for the reduction of nutrients leached during the seedling production of yellow passion fruits, found that N

losses by leaching decreased as the dose of the polymer increased in relation to the leached material without the incorporation of a water absorbing polymer. Furthermore, bovine manure may have influenced the substrate and was structurally reflected in the decrease of the leachate of this nutrient.

Regarding phosphorus, bovine and goat manures performed better with the "Vitória" cultivar when the hydrogel was used, being statistically different. However, only bovine manure was positively influenced by the hydrogel. For the "Imperial", there was no influence of organic sources and hydrogel (Table 5).

Potassium also behaved similarly. Bovine and goat manures increased to a higher degree the leaf content of potassium in "Vitória" when the hydrogel was not incorporated into the substrate. When the hydrogel was used, only bovine manure provided an increase of potassium in leaves of the "Vitória" cultivar. For the "Imperial" cultivar, the bovine manure also provided a higher leaf potassium accumulation when the hydrogel was incorporated into the substrate, differing significantly from the non-use of the hydrogel for this organic source (Table 5).

It is important to note that bovine manure is the source with the highest amount of potassium in its chemical composition among the organic sources evaluated in this study (Table 1). Oliveira and Natale (2013), studying "Imperial" leaf levels of macronutrients and micronutrients in function of nitrogen and potassium doses, stated that the K contents in leaves increased linearly and positively due to the increase in potassium doses that were applied. In addition, the bovine manure may have provided greater bio stabilization to the substrate, with lower losses even without the addition of the polymer. This is possible because its characteristics contributed to increase the water storage capacity and the availability of nutrients to the plants. Combined with it, the availability of water with the addition of the polymer into the substrate

promotes the uptake of nutrients by plants (Oliveira et al., 2004), preventing it from being lost by leaching.

According to Table 5, there was a tendency of bovine manure to provide a greater increase in calcium in the "Vitória" cultivar when the hydrogel was used. However, when the hydrogel was not incorporated into the substrate, the organic compost provided higher leaf calcium content, differing statistically by Scott Knott test at 5% probability. For the "Imperial" cultivar, there was no influence of organic sources and the use of the hydrogel. For magnesium, there were no statistical differences regarding organic sources and use of the hydrogel for the two cultivars. In addition, the average values presented in this work for leaf calcium and magnesium contents were lower than those considered suitable by nutritional studies with pineapple seedlings during the acclimatization stage (Teixeira et al., 2009; Baldotto et al., 2009; Leonardo et al., 2013; Ramos et al., 2011; Cruz et al., 2015).

Oliveira and Natale (2013) studied leaf contents of macronutrients and micronutrients in "Imperial" pineapple seedlings and found that, just as Ca, Mg leaf contents decreased with a negative linear effect as the doses of K<sub>2</sub>O increased, a behavior similar to that was found by Spironello et al. (2004).

## Conclusions

Goat manure was the organic sources that provided the greatest increases in growth characteristics for both cultivars, providing seedlings with 20 leaves, height of 19.4 cm, diameter of the rosette of 26.03 mm and 23.1 cm of length of the root system at 270 days to "Vitória". For "Imperial" the goat manure promoted seedlings with 20 leaves and height of 27.8 cm in 220 days. The bovine manure provided greater nutritional gains to the seedlings. The use of the hydrogel did not favor shoot growth. However, it contributed to the increase in root dry mass when incorporated into the bovine manure for both cultivars. The "Imperial" cultivar had a higher macronutrient transfer to the seedlings' leaves than the "Vitória" cultivar at 270 days of acclimatization.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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