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Full Length Research Paper

Growth and physiological responses of coffee (*Coffea arabica* L.) seedlings irrigated with diluted deep sea water

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Concentrations of 5, 10, 20, and 40% deep sea water (DSW) were tested, with irrigation water serving as the 0% control (tap water) on coffee (*Coffea arabica* L.) seedlings. The results showed that the growth parameters were affected significantly (α < 0.05) by the irrigation of 20 and 40% deep sea water. There were significant differences (α < 0.05) among treatments in stomata density/mm^2, stomata width, and length. The highest value of stomatal measurements was obtained in the control treatment, whereas the lowest values were obtained in the 40% DSW treatments. Electrolyte leakage was enhanced in 20 and 40% DSW irrigated seedling leaves. The highest relative leaf water content (84.5%) was obtained in the control treatment and the lowest in 40% DSW (74.6%). The application of diluted deep sea water also increased the soil electrical conductivity (EC, ds/m). The overall measured parameters indicated that the control, 5, and 10% DSW treatments showed approximate results. This indicates that 5% DSW can be used as irrigation water for coffee seedlings. Also, for some period of time, the 10% DSW can be used to irrigate coffee seedlings without causing significant negative effects.

Key words: *Coffea arabica* L., electrolyte leakage, relative water content, stomata.

INTRODUCTION

Coffee is one of the most important agricultural commodities in the world trade and is considered to be the main income source in developing countries (FAOSTAT, 2008). The world coffee market is dominated by the *Coffea arabica* L. and *Coffea canephora* species, which account for about 99% of the world coffee bean production (Da Matta and Ramalho, 2006). Arabica coffee accounts for about 62% of world coffee consumption and the rest is accounted for by robusta coffee (Morais et al., 2012). In 2016/2017, the global coffee production was estimated at 153.9 million bags, a 1.5% increase on 2015/2016 (ICO, 2017). Arabica production was up by 10.2% to 97.3 million bags, while Robusta was estimated down 10.6% to 56.6 million bags. Currently, climate change is the major threat to coffee production. The availability of quality irrigation water is vital for healthy plant growth and maximize the yield. However, on reclaimed land, saline water can be used for irrigation due to an absence or limited supply of fresh water. In addition, the groundwater used for irrigating

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crops near coastal areas is frequently saline (Lee et al., 2008).

The use of saline irrigation water has adverse effects on soil-water-plant relations, occasionally severely restricting the normal physiological activity and productive capacity of the crops (Plaut et al., 2013). Abiotic stress is one of the serious constraints that limit agricultural production and cause severe yield reductions, such as salinity and drought (Bray et al., 2000). Salinity can affect plant growth in various ways, mainly as the result of toxic ion accumulation in the root zone of plants and through osmotic stress. However, several plants have developed mechanisms to tolerate these effects (Munns, 2002). The evaporation of sea water has created salt and potentially caused soil salinity in adjacent areas since ancient times. Naturally or anthropogenically, a high concentration of soluble salt occurs in terrestrial environments or aquatic environments (Larcher, 1995).

Deep sea water (DSW), generally refers to sea water from a depth of more than 200 m and is estimated at 95% of all the sea water. The use of seawater for agricultural irrigation has been studied for decades due to its high mineral content (Mount and Schuppan, 1978; Feigin, 1985; Glenn et al., 1998; Sgherri et al., 2008). Deep sea water has various trace elements that might be useful to soil lacking them, and it therefore has the potential to stimulate healthy plant growth. The abundant nutrients of deep sea water are also favorable for agriculture. Studying the use of sea water irrigation for the production of agricultural crops can provide a resource to further studies about the use of saline water for irrigation in the areas where there is a limited availability of freshwater resources.

However, the uses and impacts of deep sea water irrigation on coffee plants have not been studied. Therefore, this research was conducted to study the growth and physiological response of coffee seedlings irrigated with different concentration of diluted deep sea water, and thereby to examine the salinity effects.

MATERIALS AND METHODS

Plant and treatments applied

The experiment was carried out under greenhouse conditions at Kangwon National University, Gangwon Province, Korea, during 2016. Six-months-old healthy coffee seedlings were transplanted into small pots (each 12 cm in diameter) that were filled with soil and compost (2:1). The seedlings were well watered and kept in a shaded area so as to create a conducive environment for the transplanted seedlings to become established. The deep sea water was collected from the east sea of Korea at 600 m depth (April, 2016). After that, the water was delivered by 20 L white transparent container and kept in the coffee greenhouse. The applied treatments were different concentrations of diluted deep sea water: control (0.2 DS/m), 5% (2.3 dS/m), 10% (3.6 dS/m), 20% (6.7 dS/m) and 40% (8.1 dS/m). The dilutions were prepared by mixing the deep-sea water with normal irrigation water (tap water) at different concentrations. Finally, the electrical conductivity (EC) of each dilution was measured using an EC meter. The design of the experiment was completely randomized with 3 replications. For this experiment, a total of 15 (n=5, n×3=15) seedlings per treatment were used. Irrigation was started one week after the seedlings had become well established and continued at four-day intervals at a volume of 330 ml/seedling for 3 consecutive months. Uniform agronomic practices were applied to all of the seedlings.

Growth measurements

Measurements of growth were taken for all of the treatments once every 2 weeks. Initial measurements of seedlings' heights (cm), stem diameters (mm), leaf lengths (cm), and leaf widths (cm) were recorded (26/04/2016) and continued until the end of the experiment (26/07/2016). The leaf length and width were recorded from newly developed (top positioned) leaves and continued up to the end of the trial from the same leaves. A caliper (Mitutoyo 530-124 Vernier Caliper) and ruler were used to measure the growth parameters.

Stomata measurements

The coffee leaves were collected from all treatments and prepared for stomata assays. The epidermis from the lower parts of the leaves was peeled using forceps and placed on microscope slides. The staining solution was added to get a clear picture. Image analysis was performed using ImageJ software (https://imagej.nih.gov/ij/) to measure the stomatal length (µm) and width (µm). Thirty stomata per treatment were measured. The number of stomata per unit area (μm^2) was counted and then converted into mm^2 using the formula:

Stomatal density = number of stomata in entire FOV / area (mm^2),

where FOV is the field of view.

The stomata picture was captured by a microscope (Leica, DM 1000; 40x for counting and 100x for size measurement) from all treatments.

Relative leaf water content (%)

The leaf discs were prepared from 3 to 4 leaves to obtain about a 5 to 10 cm^2/sample and immediately weighed to obtain the fresh weight (FW). The samples were immediately soaked in deionized water in a closed Petri dishes to full turgidity for 4 h under normal light and room temperature. After 4 h, the samples were re-weighed to obtain the turgid weight (TW). After that the samples were oven dried at 80°C for 24 h and weighed to estimate their dry weight (DW) of samples. All weighing have been made to the nearest milligram (mg). Finally, the relative water content (RWC) was calculated using the formula:

RWC (%) = ((FW-DW) / (TW-DW)) × 100,

where FW is the sample fresh weight, TW is the sample turgid weight, and DW is the sample dry weight (Barrs et al., 1962).

Relative EC of leaf tissue of coffee seedlings (%)

Fifteen freshly cut leaf discs (0.5 cm^2 each) were prepared from each treatment, rinsed three times (3 min) with demineralized water and soaked in 10 mL of demineralized water. The electrolyte leakage was determined by measuring the EC of the solution (named Initial EC) after 22 h keeping at room temperature, using a

Table 1. The average growth parameters increment of coffee seedlings irrigated with deep sea water (DSW) during the experimental period.

Treatment	Plant height (cm)	Stem diameter (mm)	Leaf length (cm)	Leaf width (cm)
Control	14.3 ± 0.68^{a}	1.5 ± 0.39^{a}	14.1 ± 2.28^{a}	6.1 ± 1.31^{a}
DSW 5%	13.0 ± 0.55^{ab}	1.3 ± 0.12^{b}	13.9 ± 0.40^{a}	5.5 ± 0.43^{ab}
DSW 10%	11.4 ± 2.44^{b}	1.2 ± 0.12^{b}	13.0 ± 0.36^{ab}	5.3 ± 0.25^{b}
DSW 20%	6.7 ± 2.15^{c}	0.8 ± 0.15^{c}	8.7 ± 1.06^{b}	3.2 ± 0.25^{c}
DSW 40%	6.1 ± 0.42^{c}	0.7 ± 0.52^{d}	6.0 ± 1.65^{c}	2.0 ± 0.06^{d}
Mean	10.41	1.1	11.2	4.4
LSD	1.6	0.1	1.6	0.7
CV (%)	8.7	5.3	7.8	8.7

Results are presented as mean ± standard deviation (n = 3). Values with the same letters within the same columns are not significantly different. CV: coefficient of variation, LSD: list significant differences

conductivity meter (Mettler-Toledo AG-8603). Total EC was obtained after keeping the flasks in an oven (90°C) for 2 h. The results were expressed as % of total conductivity:

REC (%) = (Initial EC/Total EC) × 100

Soil EC (dS/m)

The soil samples were well mixed and 10 g air-dry soil (<2 mm) was weighed from each treatment to prepare a 1:5 soil:water suspension (50 ml of deionized water used). The solutions were mechanically shaken for 1 h at 15 rpm to dissolve soluble salts. The conductivity meter was calibrated according to the manufacturer's instructions using the potassium chloride (KCl) reference solution to obtain the cell constant. Then, the electrical conductivity was measured using a conductivity meter from the soil suspension by inserting the conductivity cell and the value was recorded for each treatment. The conductivity cell was carefully rinsed with deionized water between samples (Rayment and Higginson, 1992). The soil electrical conductivity was measured twice, before the treatments began and after the end of experiments.

Soil pH

The soil samples were taken from all pots (air-dried and passed through a 2-mm sieve) and well mixed. From each sample (25 g), soil was measured and mixed with 40 mL of water (distilled or de-ionized water) to each cup using a suitable volumetric container. The solution was stirred with a glass rod and the sample was allowed to sit for 30 min. The pH meter (Mettler-Toledo GmbH, CH-8603) was calibrated according to the instructions with 2 buffer solutions (pH 4.0 and 7.0). The samples were stirred again immediately before measuring the pH. The electrode was positioned in the solution just above the sand layer. The measurements were repeated 3 times to ensure accurate results. The electrode(s) was rinsed 3 times with de-ionized water after each use and before testing another sample (Hanlon and Bartos, 1993). The soil pH was measured twice, before the treatments began and right after the experiment completed.

Data analysis

ANOVA was used to determine the significance of variance among treatments based on the recorded data. In particular, the growth parameter differences (final data - initial data) during the experimental period were used for statistical analysis. The collected data were subjected to the SAS 9.0 software. The Microsoft Excel (2013) program was used to summarize the data and make a graph.

RESULTS AND DISCUSSION

The response of growth parameters

The results showed that all of the tested deep sea water (DSW) concentrations (5, 10, 20, and 40%) affected the growth and physiological parameters of coffee seedlings in comparison with the control treatment. However, the coffee seedlings that were irrigated with 5 and 10% DSW showed results that more or less approximated those of the control treatment. There were statistically significant differences ($\alpha < 0.05$) among treatments in plant height, stem diameter, leaf length, and leaf width (Table 1).

The highest growth increment in plant height was recorded in the control treatment (14.3 cm) and the lowest in coffee seedlings irrigated with 40% DSW (6.1 cm) (Table 1). This could be because of the high salt concentration present in 40% DSW. These results agree with several researchers who reported that increasing the salt concentration lead to a decrease in leaf area and plant height on bean plant (Mathur et al., 2006; Qados, 2011), sugar cane (Jamil et al., 2007) and oat (Zhao et al., 2007). Yadav et al. (2011) also mentioned that salt has two major effects on plants: osmotic stress and ionic toxicity, both of which affect all plant's primary processes. Moreover, in the present experiment, the results indicated that seedlings irrigated with 20 and 40% DSW showed significantly poor growth due to the effects of salt stress. El-Abagy et al. (2012) reported that in lettuce, salt stress negatively affects plant growth and the production of dry matter. Also, additional reports published about increasing salt concentrations in irrigation water have revealed that the practice may lead to a significant decrease in lettuce growth, yield, marketable yields,

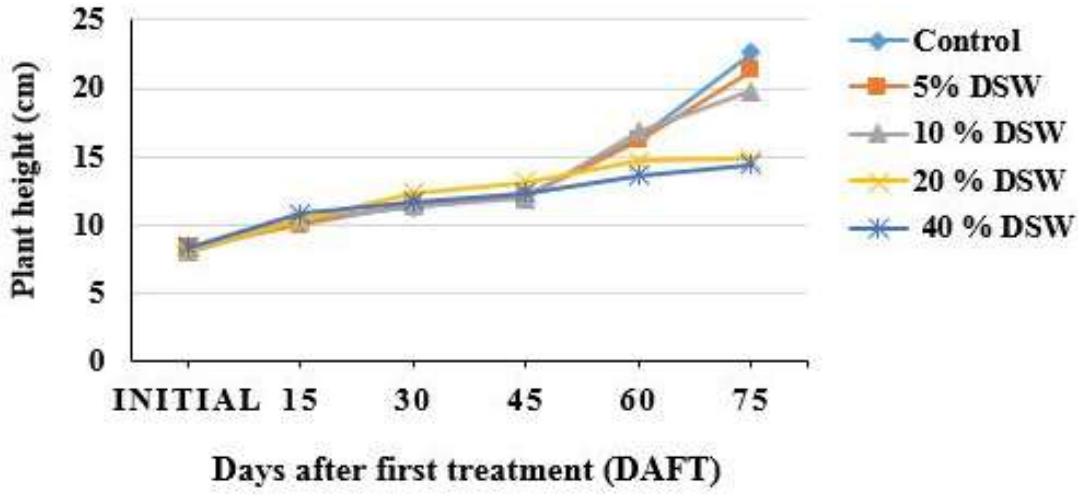

Figure 1. The effect of deep sea water treatments on plant height of coffee seedlings at every two weeks interval.

weight, and the amount of dry matter (Miceli et al., 2003; Mekki, 2007; Al-Maskri et al., 2010).

The increments in stem diameter, leaf length, and leaf width recorded in the control treatment during the experimental period were 1.5 mm, 14.1 cm, and 6.1 cm, respectively (Table 1). According to the results, there was no significant difference ($\alpha > 0.05$) in growth parameters between the control seedlings and those treated with 5% DSW except in the stem diameter. This indicated that the 5% DSW treatment can be used as irrigation water in the area where there is a shortage of fresh water for irrigation. Subsequently, the nutrients that exist in deep sea water will contribute to the growth and development of the plant. Similarly, the differences between 5 and 10% DSW treated coffee seedlings in all growth parameters were not significant. There were significant differences ($\alpha < 0.05$) among treatments in leaf length and width (Table 1). The 20 and 40% DSW treatments greatly decreased the coffee seedling leaf length and width, in comparison to other treatments. This could be because the salt concentration in 20 and 40% DSW presented in a higher amount and affected the leaf area. This leads to a reduction in the photosynthetic area, and therefore affects overall plant growth. This result is supported by Hasanuzzaman et al. (2013). They noticed that salt accumulation in leaves leads to salt toxicity in plants and later on may result in complete leaf death. It also reduces the total photosynthetic leaf area, which reduces the supply of photosynthate (food) in plants and ultimately affects the growth of the plants. Leaf length and width between the control and the 5% DSW treatment did not differ significantly. Generally, the growth performance of the control and 5% DSW treated coffee seedlings were similar. This can be an implication that 5% DSW will be used to irrigate coffee seedlings without causing adverse problems and 10% DSW can also be used to some extent considering application frequency. Frequent application of deep sea water results in an increase of salt concentration in the root zone of the plants.

Data were collected at 2 weeks intervals to study the effects of deep sea water treatment on the growth parameters of coffee seedlings. Similar plant height growth trends were observed in all treatments from the initial treatment application until 45 days after first treatment (DAFT). The similarity continued in control, 5 and 10% treated seedlings until 60 DAFT (Figure 1), whereas the 20 and 40% treated coffee seedlings showed a reduction in plant height growth starting from 45 DAFT in comparison to other treatments (Figure 1). The stem diameter growth in control, 5, and 10% DSW treated coffee seedlings had similar patterns from the initial application time to 75 DAFT. However, the 20 and 40% DSW treated seedlings stem diameter growth was inhibited and the variation became significant towards 45 DAFT, compared to other treatments (Figure 2). Salt stress greatly reduces the size of leaf area. In the present study, the 20 and 40% DSW treated seedlings leaf length and width were reduced after 45 DAFT (Figures 3 and 4). Hasanuzzaman et al. (2013) stated that the time needed to observe the response of plants to salt stress varies according to the species and salinity level. With annual species, the timescale is a day or a week, whereas, with perennial species, the timescale is months or years. However, in this experiment the salt stress effect clearly observed and the growth parameters progress declined in 20 and 40% DSW treated coffee seedlings starting from 45 DAFT.

Stomata size and density

There were significant differences ($\alpha < 0.05$) among

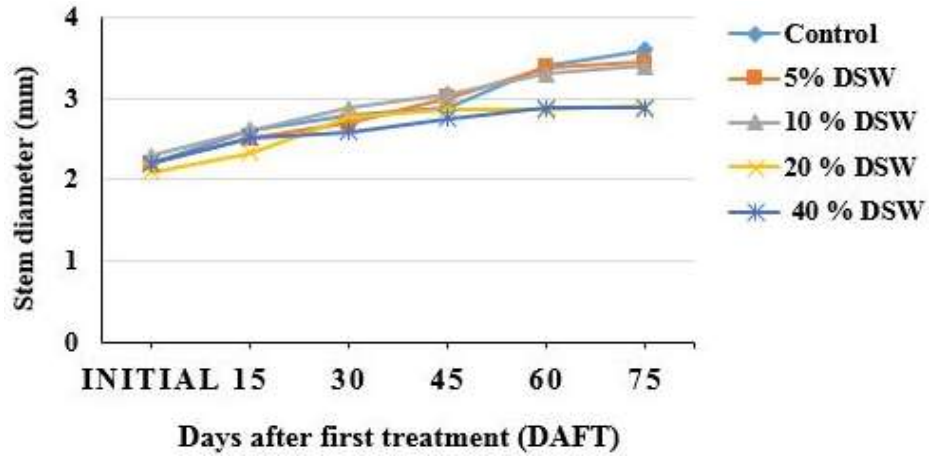

Figure 2. The effect of deep sea water treatments on stem diameter of coffee seedlings at every two weeks interval.

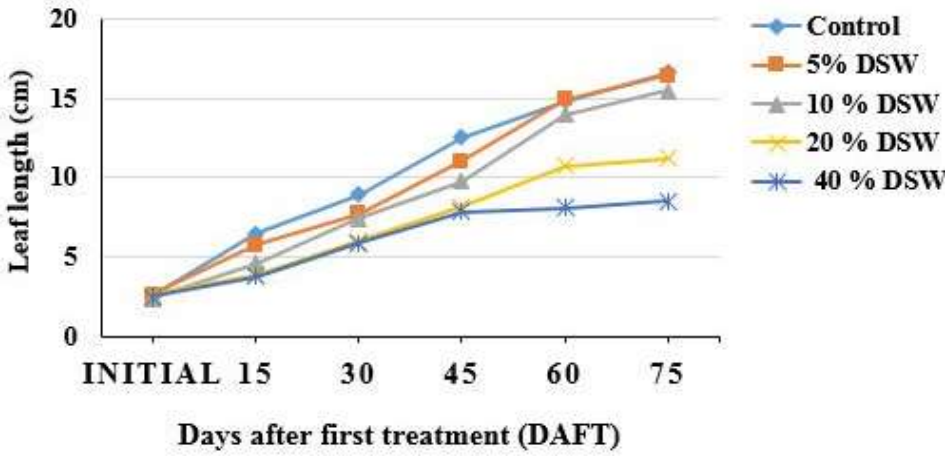

Figure 3. The effect of deep sea water treatments on leaf length of coffee seedlings at every two weeks interval.

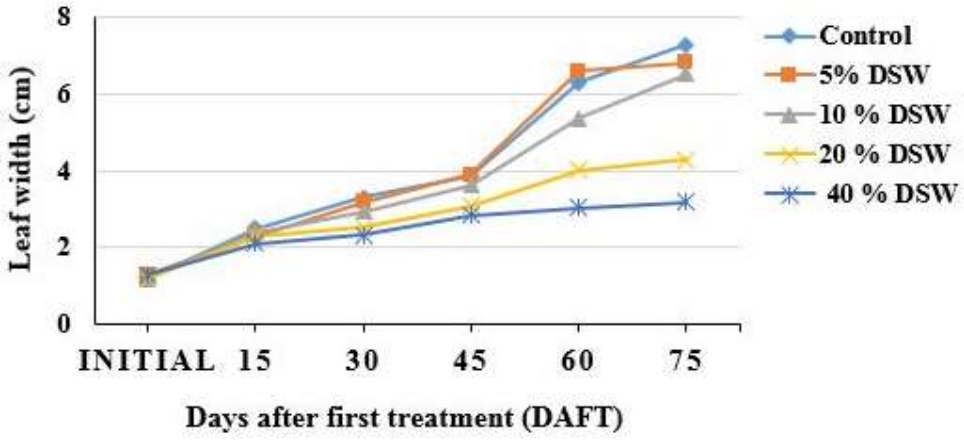

Figure 4. The effect of deep sea water treatments on leaf width of coffee seedlings at every two weeks interval.

Table 2. The stomata length, width and stomata density of coffee seedlings leaves that were irrigated with deep sea water (DSW).

Treatment	Stomata length (µm)	Stomata width (µm)	Number of stomata/mm^2
Control	20.9 ± 0.25^{a}	17.1 ± 0.36^{a}	179 ± 14.21^{a}
DSW 5%	20.3 ± 0.45^{ab}	16.6 ± 0.36^{ab}	173 ± 7.87^{ab}
DSW 10%	20.2 ± 0.39^{ab}	16.1 ± 0.29^{bc}	168 ± 8.80^{ab}
DSW 20%	19.2 ± 0.64^{bc}	15.9 ± 0.61^{c}	162 ± 10.15^{b}
DSW 40%	18.8 ±1.62^{c}	15.4 ± 0.41^{c}	160 ± 8.49^{b}
Mean	19.8	16.3	168.4
LSD	1.10	0.61	13.41
CV (%)	4.2	2.6	6.0

Results are presented as mean ± standard deviation (n = 5). Values with the same letters within the same columns are not significantly different. CV: coefficient of variation, LSD: list significant differences.

treatments in stomata length and width. A significant difference ($\alpha < 0.05$) was found between the control and 20% or the control and 40% diluted deep sea water irrigated coffee seedlings, regarded as stomatal density/mm^2. There was no significant difference between the control and 5% DSW treatment in stomata length, width and number of stomata/mm^2 (Table 2). Stomata are used as environmentally controlled gateways into the plants, regulating CO_2 uptake and transpiration. They are also involved in controlling of photosynthesis, nutrient uptake and cooling plants (Farooq et al., 2009). In plant evolution, development of stomata can be considered as a relevant feature of the plant (Brodribb and McAdam, 2011).

The highest stomata length and width have been obtained in control treatment (20.3 and 16.6 µm, respectively) treatment (Table 2). The lowest stomata length and width were recorded in 40% diluted deep sea water (18.8 and 15.4 µm, respectively) treated coffee seedling leaf. The number of stomata decreased as the salt concentration in the treatment (DSW) increased. This result of our experiment is similar to that of Pratima and Cholke (2010), who reported that the number of stomata on the leaves of *Crotalaria* species (namely, *Crotalaria rutusa* and *Crotalaria verrucosa*) decreased as the soil salinity increased. However, the number of stomata in another *Crotalaria* spp. (*Crotalaria juncea*) increased under salt stress conditions. This shows that the stomata distribution of different plant species varies under salt stress. According to Solmaz et al. (2011), the leaf area, leaf size, stomata length, and stomata width of watermelons reduced while the density of the stomata increased under salt stress conditions. The changes in stomata density and size were mainly attributed to changes in leaf area under salt stress (Curtis and Läuchli, 1987) and drought stress (Yang et al., 1995; Chaves et al., 2003; Yin et al., 2006; Gazanchian et al., 2007) conditions. The maximum number of stomata was 173 mm^{-2} in the control treatment, and the lowest was 160 mm^{-2} for the leaf of a coffee seedling irrigated with 40% DSW (Table 2). The openings of the stomata were wider in the control treatment compared with the treatment involving 20% DSW (Figure 5). Abscisic acid (ABA) level rises in the shoot as the plant is exposed to salt stress, which helps the stomata to close, decreases water loss, and transports transpirational sodium chloride (NaCl) into the shoot (Jaschke et al., 1997; Albacete et al., 2008). However, stomata closure under salt stress conditions also significantly affects the intake of CO_2 for photosynthesis.

Relative water content of leaves (%)

There were significant differences ($\alpha < 0.05$) among treatments in the relative water content (RWC) of leaves. The highest RWC was determined in the control (84.5%) treatment, whereas the lowest in 40% DSW (74.6%) irrigated coffee seedling leaves (Table 3). The result showed that as the rate of the DSW concentration increased the RWC of the leaves was decreased. This result is in line with the findings of Shaheen et al. (2013), who reported that salt stress significantly affected the relative water content of the plant. Salt treated plants often show a considerable reduction in the water uptake, which results in a decline in the water content of the various parts including the leaves (Colmer et al., 1995; Curtis and Läuchli, 1987; Machado et al., 2017). However, the RWC of leaves in control (84.5%), 5% (82.6%) and 10% (80.7%). DSW irrigated coffee seedlings, did not differ significantly ($\alpha > 0.05$) (Table 3).

Relative EC of leaf tissue of coffee seedlings (%)

Electrolyte leakage was significantly enhanced as the deep sea water concentration increased compared to the control treatment. The highest EC% obtained in 40% DSW treated coffee leaves (95%) and the lowest found in the control treatment (~0%). The electrolyte leakage of 5

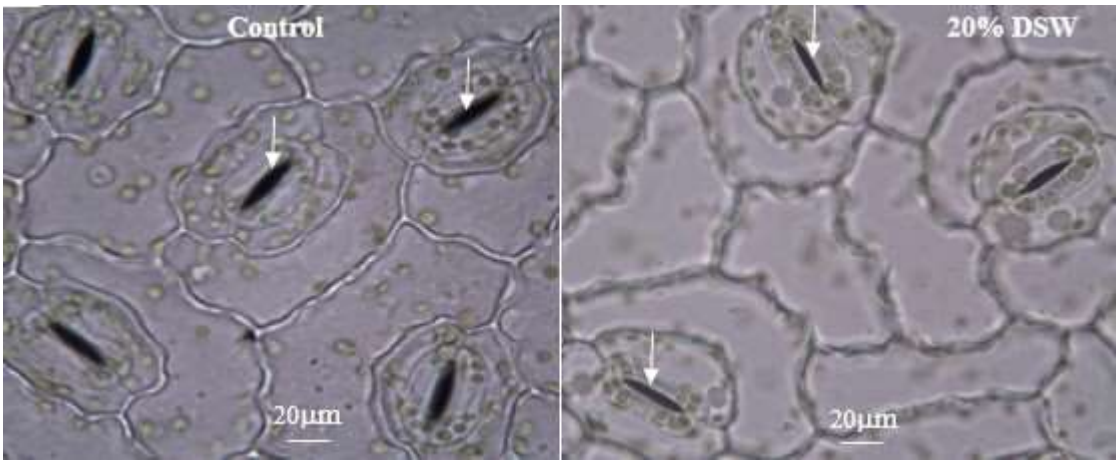

Figure 5. Leaf stomata from control and deep sea water (20% DSW) treated coffee seedlings (Control: stomata were opened widely; 20% DSW: stomata were opened narrowly than control treatment and at the same time the number of stomata in 20 % DSW treated seedling leaf were fewer than that of in the control treatment).

Table 3. The relative water content (RWC %) of coffee seedling leaves that were irrigated with deep sea water (DSW).

Treatment	RWC (%)
Control	84.5 ± 1.33a
5% DSW	82.6 ± 3.10ab
10% DSW	80.7 ± 2.37ab
20% DSW	80.1 ± 2.75b
40% DSW	74.6 ± 1.80c
Mean	80.5
LSD	4.3
CV%	3.32

Results are presented as mean ± standard deviation (n = 3). Values with the same letters within the same column are not significantly different. CV: coefficient of variation, LSD: list significant differences, RWC: relative water content.

and 10% DSW treated coffee seedling leaves were similar (14%) and the 20% DSW treated resulted in 35% (Figure 6). Several researchers reported that an increase in electrolyte leakage as plants were exposed to salinity (Dkhil and Denden, 2012; Kaya et al., 2001a, b). In this experiment also the higher electrolyte leakage was obtained due to the salt stress effect.

Soil EC (dS/m) and pH

For both soil parameters (EC and pH), we used the final data that were recorded right after the end of the experiment for statistical analysis, since the initial data were similar from all experimental pots soil. Application of deep sea water significantly increased the soil EC (Figure 7). The soil EC (dS/m) increased as the DSW concentration raised. The result agrees with the findings of Huang et al. (2011) who mentioned that the soil EC values increased as the concentration of saline irrigation water increased. The highest soil EC obtained in 40% DSW irrigated soil, and the lowest was in the control (8.97 and 2.0 dS/m, respectively) (Figure 7). The result is in line with the findings of Chadirin et al. (2008), who reported that the soil EC increased after the DSW treatment applied in tomato experiment. The 5, 10 and 20% DSW irrigated soil EC were, 5.1, 7.07 and 7.77 dS/m, respectively (Figure 7).

According to the soil salinity classification, non-saline soil EC ranges between 0 and 2 dS/m which is similar to the result of control treatment (2.0 dS/m) in this study. The other 3 treatments (5, 10 and 20% DSW) categorized under the moderately saline soil and 40% DSW irrigated soil classified under severely saline soil.

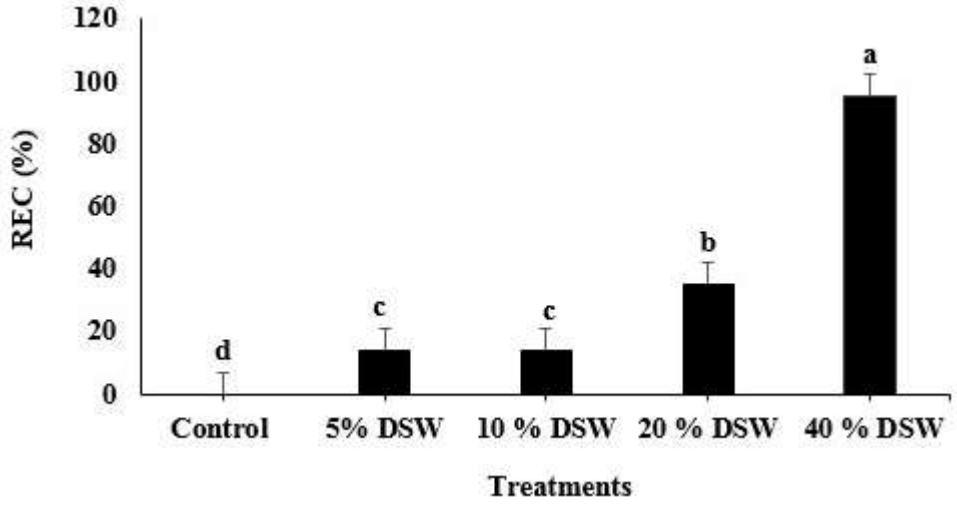

Figure 6. The relative electrolyte leakage of coffee seedling leaves irrigated with deep sea water. The vertical bars represent the means (n = 3). The bars with different letters indicate a significant difference ($p < 0.5$) among treatments. DSW: Deep sea water. REC: relative electrical conductivity.

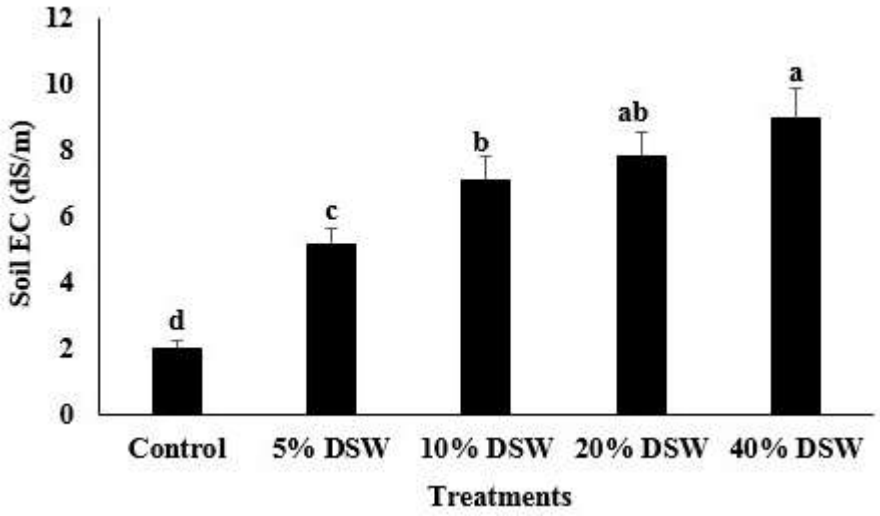

Figure 7. The effect of deep sea water irrigation on soil electrical conductivity. The vertical bars represent the means (n = 3). The bars with different letters indicate a significant difference ($p < 0.5$) among treatments. DSW: deep sea water.

The application of deep sea water during the experiment period did not significantly affect the soil pH. The soil pH was in the moderate range (5.6-6.0) (Figure 8).

Conclusion

The results indicate that all the tested diluted deep sea water concentration with a continuous four-day irrigation interval affects the growth and physiological parameters of coffee seedlings and other relevant parameters in comparison with the control treatment. However, an approximate result was obtained from the control, 5 and 10% DSW irrigated coffee seedlings. This indicates that 5% DSW can be used as irrigation water for coffee seedlings. For some period of time, 10% DSW also can be used to irrigate coffee seedlings without causing significant negative effects on their growth and physiological activities. Further investigation is crucial to understanding the optimum concentration of diluted deep

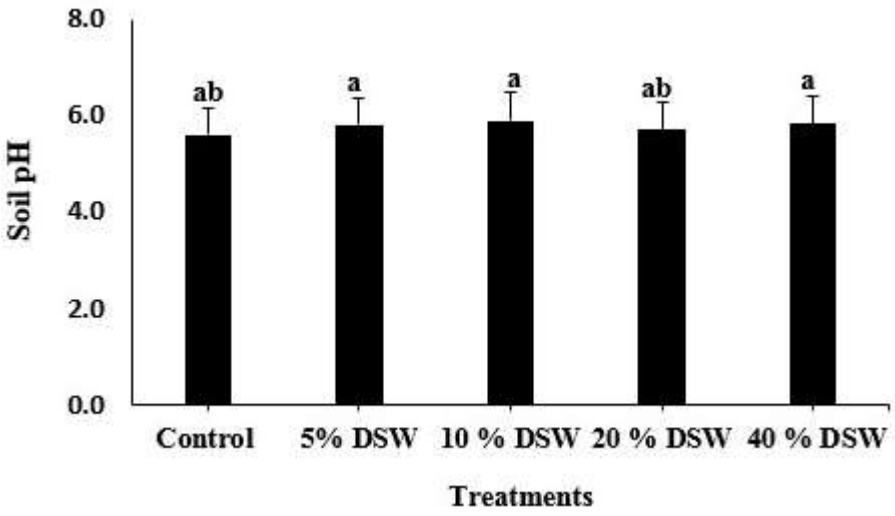

Figure 8. The effect of deep sea water irrigation on soil pH. The vertical bars represent the means (n = 3). The bars with different letters indicate a significant difference (p < 0.5) among treatments. DSW: Deep sea water.

sea water and application interval. The frequent use of diluted sea water increases the salt concentration in the root zone of the plants. Instead of the continuous use of diluted deep sea water, reducing the rate and the frequency of application will have better results in improving the growth and development of coffee seedlings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Sole and combined effect of three botanicals against cowpea seed bruchid, *Callosobruchus maculatus* Fabricius

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The bioactivity of three botanical powders in sole and combination against *Callosobruchus maculatus* (Fab.) was investigated at 29±3°C and relative humidity (R.H.) OF 65±5% in the laboratory. The appropriate mixing ratio of *Cymbopogon citratus* (C), *Alstonia boonei* (A) and *Hyptis suaveolens* involved seven combinations viz., C:A, C:H, A:H, C:A:H, H_2:C:A, A:C_2:H, H:C:A_2 in simple ratios 1:1, 1:1, 1:1, 1:1, 2:1:1, 1:2:1 and 1:1:2. The sole and combinations of botanicals were separately prepared and applied at the concentrations of 1.25% per 20 g seeds of two susceptible cowpea lines viz., Oloyin and IT845-2246 in the Kilner jars. Newly emerged ten females and five males *C. maculatus* were introduced separately into each of the Kilner jars, and replicated four times in a completely randomized design. Data were collected on adult mortality, number of eggs laid, offspring emergence, percentage seed damage, weight loss and seed viability. Results indicated that powder of *H. suaveolens* evoked significant mortality (100%) after 7 days of treatment. However, lower means were recorded in oviposition and adult bruchid emergence in cowpea seed treated with powders of *H. suaveolens* and *A. boonei*. Likewise, powder of *C. citratus* recorded the least seed damage and this implied that the three tested botanicals were observed to be effective bio-insecticide. The combination H:C:A_2 produced most desirable results causing higher adult mortality (96.33%), low offspring emergence, lower seed damage (0%), higher seed viability (88.00%), and least seed weight loss (0%) and therefore the most bio-active mixing ratio against *C. maculatus*. There was however interaction and synergism effect among the different combinations.

Key words: Bioactivity, mixing ratio, bio-insecticide, weight loss, viability.

INTRODUCTION

Cowpea, *Vignia unguiculata* is important particularly in West Africa with over 9.3 million hectares area and 2.9 million tonnes annual production (Fatokun et al., 2002). Cowpea is grown both as a leaf and pod vegetable in the humid tropics (Steele and Mehorva, 1980). Cowpea seed is important to the income of poor farmers as well as to

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the nutritional status and diets of people in the tropics (Langyintuo et al., 2003), since animal protein sources are rarely affordable in adequate quantities by majority of the populace in developing countries. Cowpea is a highly nutritive leguminous crop which contains 22% protein, 1.5% fat and 60% carbohydrate (Dolvo et al., 1976), and a valuable source of calcium, iron, thiamine and riboflavin (Ofuya, 2001). Cowpea is a veritable source of dietary protein for the teeming human population and livestock (Murdock et al., 1997), and can serve as a useful complement in diets comprising mainly of roots, tubers or cereals. Similarly, it can be boiled and consumed directly, made into flour, puddings or weaning foods for young children and thus ameliorate malnourishment and wastage (Phillips and Dedeh, 2003). Also, it can be ploughed into soil as green manure or grown as cover crop to improve soil fertility.

Cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) is responsible for over 90% of the damage done to cowpea seed (Caswell, 1982); and if left uncontrolled for over six months in storage, 100% loss may be recorded (Singh, 1977; Seck, 1993). Thus, the damage caused during storage, shipping and transportation, is a very serious problem all over the globe (Upadhyay and Ahmad, 2011). The insect pests not only damage the grain but also depreciate the weight and quality of stored grains (Rayhan, 2014). Beetle damage also causes significant reduction in seed viability because damaged seeds are riddled with holes by adult insects. The fatty acid content of seeds infested by *C. maculatus* increases, thus caused a slight denaturation of proteins and loss of the important vitamin; thiamine (Southgate, 1978). Heat, moisture and waste products produced by the bruchid result in further deterioration and the growth of molds, which renders cowpea grains unfit for consumption (Shazia et al., 2006). The quality of grains and seeds during storage depends on various factors such as crop or variety, initial seed quality, storage conditions, seed moisture content, insect pests, bacteria and fungi (Amruta et al., 2015).

Nowadays, pest control technology is mostly dependent on synthetic insecticides (Azad et al., 2013). However, the quick and effective control of pests with insecticides convinces the farmers easily compared to the non-chemical methods of pest management. Having a knockdown effect on targets, more often insecticides form the only solution of sudden outbreak of pests. Raupp et al. (2014) reported the residual effect of insecticides on insect pests and natural enemies, while inherent high mammalian toxicity and ecological safety are of great concern to both environmentalists and researchers worldwide (Zacharia, 2011). However, the development of resistance and resurgence has limited the application of single insecticides resorting to tank mixtures. Plant products, such as aqueous or organic solvent extracts are being used in many countries as protectants of stored products (Fernando and Karunaratne, 2012; Rajashekare et al., 2010 and 2012). Several workers have researched the use of single application of botanicals. It would however be germane to examine and determine the combinations of three botanicals in different mixing ratio for the farmer's use. This however engendered interaction and synergism effect among the different combinations which boosted more protectant ability of the botanicals. The combinations of more than one botanical would sustain optimal agricultural production through the management and control of insect pests of crops and food products.

MATERIALS AND METHODS

Plant materials

The three plants species viz., *C. citratus* (Dc ex Nees) Stapf, *Alstonia boonei* DeWild and *Hyptis suaveolens* Poit were sourced from Abeokuta, South West, Nigeria, and were identified at the Department of Forestry and Wildlife, College of Environmental Resources Management, Federal University of Agriculture, Abeokuta, Nigeria. The plant leaves were washed in clean water and were later air-dried in room temperature (25°C) and ground into fine powder using an electric grinder. The powder was further sieved in 100 µm aperture sieve. Ife Brown and IT845-2246 cowpea varieties were obtained from the Institute of Agricultural Research and Training, Ibadan and International Institute for Tropical Agriculture, Ibadan, Nigeria, respectively. The cowpea seeds were disinfested using cold shock treatment at 0 to 4°C for seven days.

Rearing of experimental insects

The initial 200 unsexed adult *C. maculatus* were obtained from the culture maintained on Ife Brown cowpea variety in the Department of Crop Protection, Federal University of Agriculture, Abeokuta, Nigeria. Fifty adults were introduced into a 500-ml Kilner jar containing 200 g of clean disinfested Ife Brown cowpea seeds, and 4 jars were prepared in this manner. The Kilner jars were covered with muslin cloth held in place by a screw cap in order to allow aeration and to prevent the insects from escaping. The set-up was kept under ambient temperature (27±3°C) and relative humidity (70-85%). The insects were allowed to mate for seven days and lay eggs in each of the jars after which they were removed to avoid multiple oviposition. The devoured seeds were replaced continuously with the same quantity of freshly disinfested seeds. Only the new adult bruchids emerging from the culture were used for the experiment.

Toxicity bioassay

The powders of each of the botanicals, *C. citratus* (C), *A. boonei* (A) and *H. suaveolens* (H) were admixed with 20 g of disinfested cowpea seeds of each variety in a Kilner jar. Similarly, seven combinations viz., C:A, C:H, A:H, C:A:H, H_2:C:A, A:C_2:H, H:C:A_2 in ratios 1:1, 1:1, 1:1, 1:1, 2:1:1, 1:2:1 and 1:1:2 were applied. The plant powders and their combinations were separately prepared and applied at lowest concentrations of 1.25%. Newly emerged ten females and five males of *C. maculatus* were introduced into each of the Kilner jars. Each treatment was replicated four times, and the control jar contained cowpea seeds admixed with plant powder prepared from *Azadiracta indica*. All Kilner jars containing the seeds and combined plant powders were arranged on work tables in the

Table 1. Effect of botanicals on the development and control of *Callosobruchus maculatus.*

Cowpea lines	Botanicals	Mortality at 7days post treatment	Number of eggs laid	Filial generations		
				F1	F3	F2
Oloyin	*C. citratus*	77.48^{abc}	13.50^{cdef}	26.25^{abcd}	17.75^{bc}	0.00^{b}
	A.boonei	75.83^{abc}	33.25^{bcde}	22.00^{abcd}	15.25^{bc}	0.00^{b}
	H. suaveolens	100.00^{a}	29.00^{bcde}	7.75^{d}	17.25^{bc}	0.00^{b}
	Control	0.00^{e}	68.08^{ab}	58.00^{abcd}	97.67^{a}	17.83^{a}
IT845-2246	*C. citratus*	95.00^{ab}	25.00^{bcde}	31.75^{abcd}	30.00^{bc}	0.00^{b}
	A.boonei	75.83^{abc}	18.00^{cdef}	25.75^{abcd}	27.75^{bc}	0.00^{b}
	H. suaveolens	78.30^{abc}	16.75^{cdef}	25.50^{abcd}	22.50^{bc}	0.00^{b}
	Control	0.00^{e}	51.00^{abcd}	75.33^{abc}	88.67^{b}	9.25^{b}

Means separated using Student Neumankeuls test (P<0.05). Means followed by the same letter are not significantly different from one another across the columns.

laboratory in a completely randomized design. Also, the Kilner jars containing the treated cowpea seeds were covered with a muslin cloth and tied with a rubber band. This aerated the contents and prevented other insects from entering the containers. Records of mortality were taken at 1, 3, 5 and 7 days after treatment. Thus, bruchids that showed no visible movement after 20 s of observation were turned with forceps before considering as dead. After 7th day assessment, all adult bruchids were removed from the Kilner jars and cumulative data on percentage adult bruchid mortality were corrected using Abbott (1925) formula as:

$$P_t = P_o - \frac{Pc \times 100}{100 - P_c}$$

Where, P_t, corrected % mortality; P_o, observed % mortality; P_c, control % mortality. That is, when all the bruchids had died after 14 to 21 days, the number of egg laid was counted and recorded. The F_1 progeny population was assessed on a daily basis and removed after the Kilner jars were left until 4 weeks post treatment. At the end of the twelve weeks period, the contents of each container were sieved to remove the dust, frass and any insect present in the cowpea seeds. The number of undamaged seeds was counted to assess damage to the cowpea seeds by the bruchids. The cowpea seeds were re-weighed and the percentage loss in weight was computed, thus:

$$\%W_t \text{ loss} = \frac{(W_i \times W_f)}{W_i}$$

Where, W_i is the initial weight and W_f is the final weight. The quality of the cowpea seeds was also tested through viability test. Thus, the viability of the treated seeds was tested in Petri dishes (9 cm diameter) lined with moist filter paper. Twenty cowpea seeds were randomly selected from every treatment, watered for 48 h in the Petri-dishes until the end of experiment that is 96 h. The percentage of the germinated seeds per treatment gave an indication of the relative viability of the seeds.

Statistical analysis

All data collected were subjected to analysis of variance using SAS (2002). Significant means were separated using Student's Newman-Keuls tests α = 0.05.

RESULTS

Irrespective of lines and botanicals, significantly higher bruchid mortality was recorded on treated cowpea seeds compared to the control. Hundred percent mortality, was recorded with *H. suaveolens* compared with *C. citratus* and *A. boonei* (Table 1). *Hyptis suaveolens* caused significant reduction in adult bruchid emergence (in the first and second filial generations) while all three botanicals tested caused outright inhibition and reduction in adult bruchid emergence in the third filial generation (Table 1). However, highest adult bruchid emergence was recorded on the untreated cowpea seeds (control).

Table 2 shows that the lowest seed damage was recorded on cowpea seeds treated with *C. citratus* compared to other botanicals. However, the highest seed damage was recorded on the control. Also, regardless of lines cowpea seeds treated with *C. citratus* powders gave significantly lower seed weight loss compared to other botanicals (Table 2). Nonetheless, the weight loss was lower on seeds treated with *C. ctratus, A. boonei* and *H. suaveolens* compared to the untreated. Also, mortality of bruchids after three months of storage was lower on cowpea seeds treated with the botanicals compared to control. Likewise, significantly higher seed viability was recorded on cowpea seeds treated with the three botanicals compared to untreated cowpea seeds (control) (Table 2).

Irrespective of lines, bruchid mortality varied among the different combinations. The different combinations of the botanicals gave significantly higher adult mortality compared to the control. The combinations of three botanicals, *A. boonei* (A), *C. citratus* (C) and *H. suaveolens* (H), A:C_2:H (1:2:1) recorded 100% mortality followed by H:C:A_2 (1:1:2), C:A (1:1), C:H (1:1) and H_2:C:A (2:1:1); these were significantly different from A:H (1:1) and C:A:H (1:1:1) (Tables 3 and 4).

Combinations H_2:C:A (2:1:1) and C:H (1:1) recorded significantly higher number of eggs laid relative to other

Table 2. Effect of botanicals on the development and control of *Callosobruchus maculatus.*

Cowpea lines	Botanical	Mortality after 3 months storage	Seed damage	Seed weight loss	Seed viability
Oloyin	*C.citratus*	31.25^{b}	13.13^{cd}	3.60^{cd}	69.00^{a}
	A.boonei	29.50^{b}	41.25^{abcd}	4.60^{cd}	69.75^{a}
	H. suaveolens	31.25^{b}	16.66^{abcd}	4.60^{cd}	68.25^{a}
	Control	76.25^{ab}	78.27^{abcd}	42.70^{ab}	16.67^{hi}
IT845-2246	*C. citratus*	25.00^{b}	26.67^{abcd}	4.00^{d}	50.00^{def}
	A.boonei	19.75^{b}	40.63^{abcd}	4.10^{cd}	55.00^{cde}
	H. suaveolens	16.50^{b}	51.04^{abcd}	4.10^{cd}	60.25^{abc}
	Control	87.66^{ab}	89.09^{abc}	47.07^{ab}	16.58^{hi}

Means separated using Student Neumankeuls test (P<0.05). Means followed by the same letter are not significantly different from one another across the columns.

Table 3. Assessment of combination ratios of two botanicals using teneral adult bruchid (*Callosobruchus maculatus*).

Parameter	Lines	C:A	C:H	A:H	Control
Mortality (7D)	Oloyin	93.32^{a}	92.00^{a}	73.30^{ab}	68.00^{c}
	IT845-2246	68.00^{ab}	69.98^{ab}	68.66^{ab}	60.00^{c}
Eggs laid	Oloyin	20.40^{ab}	4.60^{d}	30.00^{a}	24.71^{e}
	IT845-2246	14.80^{ab}	5.00^{d}	18.20^{ab}	19.57^{e}
F1 generation	Oloyin	0.00^{d}	0.00^{d}	1.20^{d}	42.85^{e}
	IT845-2246	0.00^{d}	0.00^{d}	2.20^{d}	31.00^{f}
F2 generation	Oloyin	0.00^{d}	0.00^{d}	4.00^{d}	48.85^{c}
	IT845-2246	0.00^{d}	4.00^{d}	2.00^{d}	58.29^{e}
F3 generation	Oloyin	0.40^{c}	0.00^{c}	2.00^{c}	30.14^{d}
	IT845-2246	0.20^{c}	0.40^{c}	1.40^{c}	28.71^{d}
Mortality (3MS)	Oloyin	58.00^{a}	18.00^{bc}	77.80^{a}	146.43^{d}
	IT845-2246	16.00^{bc}	20.00^{cd}	15.00^{cd}	142.86^{d}
Seed damage	Oloyin	4.00^{cd}	8.90^{cd}	8.10^{cd}	97.60^{a}
	IT845-2246	4.00^{cd}	8.90^{cd}	8.10^{cd}	95.00^{a}
Seed weight loss	Oloyin	12.00^{cde}	2.00^{e}	17.80^{cd}	60.86^{f}
	IT845-2246	1.80^{e}	11.80^{cde}	19.80^{cd}	62.29^{f}
Seed viability	Oloyin	72.00^{abcd}	88.00^{a}	72.00^{abcd}	30.00^{e}
	IT845-2246	84.00^{ab}	68.00^{abcd}	72.00^{abcd}	30.00^{e}

Means separated using Student Neumankeuls test (P<0.05). Means followed by the same letter are not significantly different from one another across the columns. C = *C. citratus*; A = *A. boonei*; H= *H. suaveolens*; Mortality (3MS), mortality after 3 months of storage; mortality (7 D), mortality at 7 days post treatment.

combinations. The control however recorded the highest. Cowpea seeds treated with combinations C:H (1:1) and C:A:H (1:1:1) recorded the lowest oviposition which was significantly different from other combinations except

Table 4. Assessment of combination ratios of three botanicals using teneral adult bruchid (*Callosobruchus maculatus*).

Parameter	Lines	C:A:H	A:C_2:H	H_2:C:A	H:C:A_2	Control
Mortality (7D)	Oloyin	72.66^{ab}	100.00^{a}	90.00^{a}	96.00^{a}	68.00^{c}
	IT845-2246	95.00^{a}	95.00^{a}	96.00^{a}	96.66^{a}	60.00^{c}
Eggs laid	Oloyin	6.60^{cd}	12.00^{bcd}	4.00^{d}	13.00^{bcd}	24.71^{e}
	IT845-2246	5.00^{d}	16.80^{ab}	14.40^{ab}	9.60^{cd}	19.57^{e}
F1 generation	Oloyin	0.20^{d}	14.40^{b}	0.00^{d}	0.00^{d}	42.85^{e}
	IT845-2246	0.20^{d}	29.20^{a}	9.80^{c}	0.00^{d}	31.00^{f}
F2 generation	Oloyin	0.00^{d}	24.57^{bcd}	5.80^{cd}	0.00^{d}	48.85^{c}
	IT845-2246	0.00^{d}	38.80^{a}	34.40^{ab}	0.00^{d}	58.29^{e}
F3 generation	Oloyin	7.60^{a}	1.40^{c}	3.80^{ab}	0.00^{c}	30.14^{d}
	IT845-2246	1.00^{c}	0.60^{c}	1.80^{c}	0.00^{c}	28.71^{d}
Mortality (3MS)	Oloyin	58.00^{a}	52.40^{a}	62.40^{a}	0.00^{c}	146.43^{d}
	IT845-2246	30.00^{a}	62.00^{a}	35.00^{a}	0.00^{c}	142.86^{d}
Seed damage	Oloyin	15.00^{cd}	29.00^{c}	15.60^{cd}	0.00^{d}	97.60^{a}
	IT845-2246	9.50^{cd}	56.00^{b}	21.00^{cd}	0.00^{d}	95.00^{a}
Seed weight loss	Oloyin	7.60^{de}	29.80^{bcd}	11.20^{cde}	0.00^{e}	60.86^{f}
	IT845-2246	6.20^{de}	38.60^{a}	38.80^{a}	0.00^{e}	62.29^{f}
Seed viability	Oloyin	84.00^{ab}	54.00^{bcde}	76.00^{abc}	90.00^{a}	30.00^{e}
	IT845-2246	84.00^{ab}	40.00^{def}	44.00^{de}	88.00^{a}	30.00^{e}

Means separated using Student Neumankeuls test (P<0.05). Means followed by the same letter are not significantly different from one another across the columns. C = *C. citratus*; A = *A. boonei*; H= *H. suaveolens*; Mortality (3MS) , mortality after 3 months of storage; mortality (7 D), mortality at 7 days post treatment.

H:C:A_2 (1:1:2). The control recorded the highest number of eggs laid and was significantly different from all other treated cowpea lines.

No seed damage was recorded with combination H:C:A_2 (1:1:2). Combinations C:A (1:1), C:H (1:1) and C:A:H (1:1:1) also recorded significant reduction in seed damage compared to other lines (Tables 3 and 4). There was no seed weight loss with combinations H:C:A_2 (1:1:2). Weight loss recorded with combinations C:H (1:1) and C:A:H (1:1:1) was significantly lower than the control (Tables 3 and 4). For F_1 generation, no adult emergence of bruchids was recorded with combinations H:C:A2 (1:1:2), C:A (1:1) and C:A (1:1) and C:H (1:1). With combinations H_2:C:A (2:1:1) and C:A:H (1:1:1), lowest values of F_1 generation emergence was recorded, which however was significantly lower than A:C_2:H (1:2:1) and control. Similarly, with combinations H:C:A_2 (1:1:2), C:A:H (1:1:1) and C:A (1:1), no bruchid emergence was recorded from both cowpea lines. Combinations C:H (1:1) and A:H (1:1) also recorded significant reduction in adult emergence compared to other lines.

No adult emergence was recorded with H:C:A_2 (1:1:2). Combinations C:A (1:1), C:H (1:1), A:H (1:1) and A:C_2:H (1:2:1) recorded significantly lower values of F3 generation emergence compared to C:A:H (1:1:1) and H_2:C:A (2:1:1) (Tables 3 and 4).

Combinations H:C:A_2 (1:1:2), C:H (1:1), C:A:H (1:1:1) and C:A (1:1) recorded significantly higher seed viability relative to other combinations. There were however interaction effect among the different combinations. Combination H:C:A_2 (1:1:2) recorded no bruchid mortality after three months of storage relative to other combination, while the highest percentage was recorded by the control. Combination C:H (1:1) also recorded significantly lower bruchid mortality compared to other combinations. Other combinations recorded significantly higher bruchid mortality relative to control (Tables 3 and 4).

DISCUSSION

Farmers are encouraged to resort to botanicals that have the phyto-tonic effect that would increase seed quality parameters. According to Sandeep et al. (2013), higher germination, vigour index and less infestation were

recorded during storage when *Zea may* seeds were treated with *Acorus calamus* rhizome. The results obtained from this trial showed that *H. suaveolens, C. citratus* and *A. boonei* caused bruchid mortality. Botanicals such as *Azadirachta indica*, *Acorus calamus*, *Lantana camara*, *Melia azadarach*, *Piper nigrum*, and *Adhatoda zeylanica* are biodegradable, non-residual, equally effective and easily available. Generally, all the botanicals tested caused significantly higher bruchid mortality compared with the untreated (control). Plant materials with medicinal and pharmacological properties have been found effective in botanical control of *C. maculatus* (Sofowora, 1982). In a similar experiment, Olaniran et al. (2013) reported the use of plant extracts of *Tephrosia vogelli* and *Azadirachta indica* in the control of foliage pests of *Hibiscus sabdariffa* L. The *C. citratus, H. suaveolens* and *A. boonei* caused increased in mortality, reduced progeny emergence, seed damage and weight loss. In a similar vein (Manohar et al., 2017; Azeez and Pitan 2014) reported that botanicals prove to be a better option to control field and storage pests without affecting the quality of grains or seeds and without destroying the ecosystem or environment. This is also similar to the findings of Shazia et al. (2006) who reported that black pepper powder gave significantly better results than the control in suppressing bruchid survival, higher numbers of undamaged seeds and fewer holes per cowpea seed. Rajashekare et al. (2012) however confirmed the use of botanicals as grain protectants. Previous works have demonstrated the potency of some botanicals to preserve seed quality (Khatum et al., 2011; Rana et al., 2014); reduced seed damage (Rana et al., 2014) and weight loss (Rayhan et al., 2014). Extracts of *A. boonei* possess anti-microbial activity (Omoregbe and Osaghae, 1997). Plant products, such as aqueous or organic solvent extracts are being used in many countries as protectants of stored products (Fernando and Karunarathe, 2012). Thus, some of the metabolites of plants are toxic such as pyrethrum, nicotine, rotenone etc and some are repellents, and antifeedants like azadirachtin, rape seed extract and others, like *Acorus calamus* act as sterilants (Ignatowicz and Wesolowska, 2015). *C. citratus* is effective against the yam beetle (Tobih, 2011), while the stem of *C. citratus* had been found to also cause mortality in bruchids (Dike and Mbah, 1992). Powder of *H. suaveolens* was effective in protecting cowpea seeds against insects (Adedire and Lajide, 1999). Similarly, Barbara et al. (2010) reported that topical applications of *H. suaveolens* and *H. spicigera* on insects showed that both essential oils had an effective insecticidal activity. There was neither seed damage nor weight loss in seeds treated with *A. boonei, H. suaveolens* and *C. citratus*. Botanicals affect only target pests, are effective in very small quantities, degrade rapidly and provide pesticide free food and a safe environment for living beings (Joseph et al., 2012; Rajashekare et al., 2010). Tobih (2011) had previously rated *C. citratus* as superior repellent or antifeedant botanicals to the yam beetle. Oviposition deterrence was observed on seeds treated with *C. citratus, A. boonei* and *H. suaveolens* where significantly fewer eggs were laid on the treated cowpea seeds. Rajapakse and van Emden (1997) reported that all four oils tested (corn, ground nut, sunflower and sesame) significantly reduced the oviposition of all the three bruchid species studied (*Callosobruchus maculatus, C. chinensis* and *C. rhodesianus*). Boeke et al. (2004) reported that the adult beetles died soon after they came into contact with the powder of *Tephrosia vogelli* and lay few eggs, only very few developed into adults. Musa et al. (2009) reported that seed-extract of *H. suaveolens* was significantly more effective in enhancing adult mortality, reducing egg laying and suppressing larval and adult emergence. All the three botanicals recorded significantly higher seed viability compared to control because the botanicals prevented seed damage and subsequently retained the viability of the cowpea seeds. On the other hand, damage occurred on untreated seeds resulting in destruction of the embryos and subsequent reduction in the viability of the seeds. This implied that the three botanicals are potent against *C. maculatus*. This is however underscored by the findings of Misra (2014) who reported the role of botanicals, biopesticides and bioagents in integrated pest management.

The results of the study revealed that the combinations of the botanicals gave significantly higher adult mortality compared to the control. This observation is sustainable because more complex preparations such as combination of substances present in insecticide are likely to become effective to overcome development of resistance by insect pests (Regnault-Roger and Hamraini, 1993). The combinations of three botanicals A:C_2:H (1:2:1) recorded 100% mortality at 7 days. Amruta et al. (2015) recorded effective storage insect control and higher seed quality when treated with botanicals and emamectin benzoate. This is also in agreement with the findings of Emeasor et al. (2007), who reported similar work that mixture of seed powder of *Piper guineense* and *Thevetia peruviana* at different percentage caused the highest mortality of *C. maculatus* at 7 days after infestation. The percentage mortality recorded at combination A:C_2:H (1:2:1) was not significantly different from the following combinations H:C:A_2 (1:1:2), C:A (1:1), C:H (1:1) and H_2:C:A (2:1:1). Combination H_2:C:A (2:1:1) and C:H (1:1) recorded significantly lower number of eggs laid relative to other combinations. Combinations C:H (1:1) and C:A:H (1:1:1) and H:C:A_2 (1:1:2) reduced oviposition when compared with the control. Also, H:C:A_2 (1:1:2) recorded no bruchid emergence that is F_1, F_2, and F_3 generations throughout the duration of trial. This is in agreement with the work of Dawodu and Ofuya (2000), who reported that oviposition and adult emergence of *C. maculatus* were lower in seeds treated with mixed formulation of *P. guineense* and *Dennelta tripelata*

powders compared to either applied singly. Emeasor et al. (2007) reported in another study that the mixture of *P. guineense* and *Thevetia peruviana* at different percentages caused the highest mortality, least egg counts and significantly suppressed adult emergence. Also, Rayhan et al. (2014) reported that the bio-efficacy of neem, mahogoni and their mixture were able to prevent seed damage and seed weight loss by rice weevil in storage. Although there may not be differences in the bruchid mortality recorded in the combination compared with single application, the combination is desirable due to reduction in chances of resistance development.

Neither seed damage nor weight loss, was recorded with combination H:C:A_2 (1:1:2). With combination C:A (1:1), C:H (1:1) and C:A:H (1:1:1) there was significant reduction in seed damage and weight loss compared to other lines and viability was therefore preserved. These findings would be readily accepted by the local farmers because peasant farmers in sub-saharan Africa use indigenous plants either singly or in mixtures to protect cowpeas against pest damage during storage (Ibrahim, 2012; Ignatowicz and Wesolowska, 2015; Issa et al., 2011; Khatum et al., 2011). Shazia et al. (2006) found that a combination of leaf of *A. indica* and *T. vogelli* are effective in the control of cowpea seed bruchid, *C. maculatus*. Also, Ogunwolu and Idowu (1994) reported that insecticidal activity of *Zanthoxylum zanthoxyloides* root bark powder and *A. indica* seed powder was not mitigated by mixing the two against *C. maculatus*. The mixture may give best control of a complex of pests with varying levels of susceptibility to the different components of the mixtures. Insects that are resistant to one or more insecticides may be susceptible to a combination of toxicants or synergism may be exhibited by the components (Wolfenbarger and Cantu, 1975). Mixtures of insecticides could also be used because of cost efficiency (AlII et al., 1977).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Land use effects on soil erodibility and hydraulic conductivity in Akure, Nigeria

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This research was carried out to investigate the effects of three land use categories (grazed, cropped and forest land) on soil erodibility and hydraulic conductivity. Hydraulic conductivity was determined by a steady-state flow using a mini-disk infiltrometer while soil erodibility was determined following the Wischmeier and Smith equation. A suction rate of 2 cm s^{-1} was chosen for field infiltration measurement and subsequent estimation of soil hydraulic conductivity. The USDA textural classes for the land use types in forest, cropped and grazed lands are clay, sandy clay and sandy clay loam, respectively. The mean values of the hydraulic conductivity for the land uses/land cover are: forest land (0.00162±0.002019 cms^{-1}), cropped land (0.002086±0.001299 cms^{-1}), and grazed land (0.002244±0.002176 cms^{-1}). Highest mean bulk density (1.45 ± 0.23 g cm^{-3}) and the lowest mean bulk densities (0.84 ± 0.14 g cm^{-3}) were observed in soils of forest and grazed land, respectively. Similarly, mean total porosity values ranged between 0.43 and 0.67 cm^3 cm^{-3}. Highest organic matter was found out in the grazed soil (4.90%) as a result of the urine and excreta of the cattle. High organic matter was also observed in the forest soil (3.50%) but lower relative to grazed land. The soil erodibility was high in the sampled soils of grazed land with the value of 8.73 × 10^{-2} ±0.03, while the least erodibility (6.35 × 10^{-2} ± 0.02) was recorded in the forest land. These values indicate the eroding vulnerability of the three land uses.

Key words: Infiltration rate, organic matter, bulk density, total porosity, land cover.

INTRODUCTION

Land use change is a complex process shaped by human activity and affected by ecological, economic and social drivers capable of influencing a wide range of environmental and economic conditions (Agarwal et al., 2000; MacDonald et al., 2000). Soil is one of the most essential abundant natural resources that sustain biological life. It plays a crucial role in agricultural production. A variety of farming practices often lead to

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some forms of soil degradation such as soil erosion (Ritter and Eng, 2012). Soil erosion impacts negatively on crop productivity and environmental quality and depresses the socio-economic status of farmers; it is therefore a threat to the landowners' livelihoods as well as the overall health of an ecosystem (Egbai et al., 2012). Erodibility is the susceptibility of a soil to erosion (Wischmeier and Smith, 1978). Levy et al. (2001) described erodibility as the inherent tendency of soils to erode at different rates due solely to differences in soil properties. Soil erodibility factor is an estimate of the ability of soils to resist erosion based on the physical characteristics of each soil. It is a quantitative description of the susceptibility of soil particles to detachment and transport by rainfall and runoff. Erodibility factor is the rate of erosion per unit erosion index from a standard plot. The factors that influence soil erodibility factor are soil characteristics such as permeability, infiltration, water holding capacity, distribution of particles, aggregate stability, tendency towards dispersion and abrasion, transportability, structure and humus content. Hydraulic conductivity is a property of vascular plants, soils and rocks, which describes the ease with which a fluid (usually water) can move through pore spaces or fractures. It depends on the intrinsic permeability of the material, the degree of saturation, and on the density and viscosity of the fluid. Saturated hydraulic conductivity describes water movement through saturated media. According to Kirkham (2005), hydraulic conductivity is defined as the metres per day of water seeping into the soil under the pull of gravity or under a unit hydraulic gradient. Hydraulic conductivity also shows a temporal variability that depends on different interrelated factors: including soil physical and chemical characteristics affecting aggregate stability, climate, land use, dynamics of plant canopy and roots, tillage operations, activity of soil organisms (Fuentes et al., 2004). Several studies have been conducted over the years on soil erodibility and hydraulic conductivity; for example, Fasinmirin and Olorunfemi (2011) worked on the evaluation and variability of hydraulic conductivity and soil sorptivity to water in the forest vegetative zones of Nigeria and concluded that sorptivity is largely dependent on the total porosity of soil, while increase in soil organic matter content reduces the sorptivity of soil.

In order to reduce soil erodibility effects on soil resources, adequate conservation practices such as maintaining permanent soil cover, avoiding the use of slash and burn methods and promoting minimal mechanical disturbance of soil through zero tillage systems to enhance soil and water conservation and control soil erosion and other practices that minimize soil disturbance must be employed (Fasinmirin and Olorunfemi, 2013).

High sand content and the high dispersion ratios in soils make it highly detachable. However, with remarkably good properties exhibited by majority of soils in Nigeria, particularly high infiltration rate, organic matter and adequate vegetative cover, erosion faces high resistance Ezeabasili et al. (2011).

Therefore, this research aimed to determine the effects of different land uses on hydraulic conductivity and erodibility of soils in Akure, southwestern part of Nigeria.

METHODOLOGY

Study area

This research was carried out on different land uses in Akure, the capital city of Ondo State, Nigeria. The land use types include grazed, forest and cropped land. The grazed land is located on latitude and longitude 7° 17' 00" N and 5° 13' 07" E, respectively. The forest land is located on latitude and longitude 7° 17' 01" N and 5° 08' 04" E, respectively. The cropped land is an area under continuous cultivation of arable crops such as maize, yam, cassava and vegetables. It is located on latitude and longitude 7° 17' 02" N and 5° 13' 09" E, respectively.

Sampling collection and analysis

This research adopted a random sampling method for the field measurement. Six sampling locations were chosen for the collection of different soil samples for bulk density, porosity, moisture content, organic matter content, particle size analysis and infiltration rate of each land use/land cover.

Infiltration rate

The process involved using mini disk infiltrometer to determine the hydraulic conductivity of each land use. The bubble chamber was filled up to three-quarter of its volume by running water down the suction control tube or removing the upper stopper. Immediately after the upper chamber was full, the suction control tube was slided and the infiltrometer was inverted to remove the bottom elastomer and the porous disk, and the water reservoir was then filled. The position of the end of the tube with respect to the porous disk was carefully set to ensure a zero suction offset while the tube bubbles. After filling the water reservoir, the bottom elastomer was replaced making sure the porous disk is firmly in place. No water leaked out when the infiltrometer was held vertically. Suction rate of 2 cms^{-1} was chosen on the field for the soil infiltration measurement for the different land uses soil. After the adjustment of the suction rate, the starting water volume was record at time zero, the infiltrometer was then placed on a smooth spot (scraped to remove any vegetation and ensure a level surface) on the soil surface. Instantaneously, water began to leave the lower chamber and infiltrate into the soil at a rate determined by the hydraulic properties of the soil. The infiltration measurements were recorded every 30 s for the duration of the experiment in all the land use. The infiltrometer was run for not less than 5 min on each of the land use/land cover for the accurate calculation of hydraulic conductivity. The water reservoir was refilled after the experiment. The data collected in each of the points were used to determine the water infiltration rates of the soil, then to calculate hydraulic conductivity. The hydraulic conductivity of soil in the entire plot was then calculated using the method of Zhang (1997). The method requires measuring cumulative infiltration vs. time and fitting the results with the function

A number of methods are available for calculating soil hydraulic conductivity from these data. The method proposed by Zhang

Table 1. Soil structure codes.

Soil structure	Very fine granular	Fine granular	Medium, coarse granular	Blocky, platy, massive
Code	1	2	3	4

Source: http://www.soils.wisc.edu.

(1997) is quite simple and works well for measurements of infiltration into dry soil. The method requires measuring cumulative infiltration versus time and fitting the results with the function:

$$I = \mathbf{C_1}t + \mathbf{C_2}\sqrt{t} \quad (1)$$

Where $\mathbf{C_1}$ (m s^{-1}) and $\mathbf{C_2}$ (m $s^{-1/2}$) are parameters. $\mathbf{C_1}$ is related to hydraulic conductivity, and $\mathbf{C_2}$ is related to soilsorptivity. The hydraulic conductivity of the soil (k) is then computed from

$$k = \frac{\mathbf{C_1}}{\mathbf{A}} \quad (2)$$

Where, $\mathbf{C_1}$is the slope of the curve of the cumulative infiltration vs. the square root of time, and A is a value relating the van Genuchten parameters for a given soil type to the suction rate and radius of the infiltrometer disk. A is computed from:

$$A = \frac{11.65(n^{0.1}-1)\exp(2.92(n-1.9)\alpha h_0)}{(\alpha r_0)^{0.91}} \quad n \geq 1.9 \quad (3)$$

$$A = \frac{11.65(n^{0.1}-1)\exp(7.5(n-1.9)\alpha h_0)}{(\alpha r_0)^{0.91}} \quad n \leq 1.9 \quad (4)$$

Where, 'n' and 'α' are the van Genuchten parameters for the soil, r_0 is the disk radius, and h_0 is the suction at the disk surface.

The van Genuchten parameters for the 12 texture classes of soil were obtained from Carsel and Parrish (1998) as quoted by Decagon (2008). The mini disk has a radius of 1.25 cm and a suction of 2.0.

Bulk density and porosity

The bulk density (BD) was obtained by the gravimetric soil core method described by Blake and Hartage (1986) and the particle density (PD) was assumed to be 2.65 g cm^{-3} (Osunbitan et al., 2005). The total porosity (PT) was obtained from BD and PD using the equation and relationship developed by Danielson and Sutherland (1986):

$$PT = 1 - \frac{BD}{PD} \quad (5)$$

Where, BD = Bulk density and PD = particle density (= 2.65 Mg/m^3). The default value of 2.65 g/cm^3 is used as a 'rule of thumb' based on the average bulk density of rock with no pore space (Fasinmirin and Olorunfemi, 2013).

Soil moisture

The moisture content was calculated using gravimetric method from the values recorded during the measurement of soil bulk density as:

$$\text{Moisture content wet basis} = \frac{((W_2-W_1)-(W_3-W_1))}{W_2-W_1} \times 100\% \quad (6)$$

$$\text{Moisture content dry basis} = \frac{((W_2-W_1)-(W_3-W_1))}{W_3-W_1} \times 100\% \quad (7)$$

Erodibility

The regression equation by Wischmeier and Smith (1971) (Equation 11) was used to calculate the erodibility factor.

$$100K = 2.1 \times 10^{-4} \times (silt\ \% \times (100 - \%clay)) \times ((12 - OM) + (3.25 \times (St - 2)) + (2.5 \times (Pt - 3))) \quad (8)$$

Where, OM is organic matter content %, St is soil structure code and Pt is permeability class

The soil structure is determined by physically looking at a column of undisturbed soil. The columns of soil, which were gotten using core samplers, were carefully examined physically using the eyes. Cracks were checked, the relative sizes of the particles, aggregation, ped form, and the entire structure in terms of grade, form and the entire structure and size were observed. The observations were graded according to the following codes in Table 1.

The permeability class test was done to determine the permeability of soils of the three land use. Soil samples from the three land use were put in separate measuring cylinders and 100 ml of water was added to each of the cylinders containing soil. Observation was then made on the time taken for the measured quantity of water to reach a particular level in the cylinder as it infiltrates down through the soil sample. The time was recorded and this was used for soil permeability classification according to the following codes: fast– 1, moderate to fast– 2, moderate– 3, slow to moderate– 4, slow– 5, and very slow– 6 as described by Wischmeier and Smith (1971).

Soil texture

The soil texture was determined using samples of soil collected from the site. The soil was air dried to reduce the moisture content after which it was taken to the laboratory where the soil texture was measured using the method described by Schlichting et al. (1995). Soil texture classes were defined according to FAO/USDA soil classification system.

Statistical analysis

Field data obtained were subjected to statistical analysis such as mean, standard deviation and correlation coefficients.

RESULTS AND DISCUSSION

Particle size composition of collected samples

Table 2 shows the result of variations in the particle size composition of the collected soil samples for different land use. There were variations in the percentage of sand, silt and clay among different land use soil samples. According to the USDA classification system, each land

Table 2. Textural classifications of soil of the experimental land use at 15 cm depth.

Land use/cover	Sand	Clay	Silt	USDA textural class
Forest	41.47	42.87	15.67	Clay
Cropping	46.80	35.20	18.00	Sandy clay
Grazing	66.80	22.50	10.73	Sandy clay loam

Table 3. Bulk density and porosity of different land use soil samples.

Land use	Bulk density (gcm^{-3})	Porosity ($cm\ cm^{-3}$)
Forest	1.45±0.23	0.43±0.088
Cropped	1.35±0.34	0.47±0.133
Grazed	0.84±0.14	0.67±0.053

Table 4. Moisture content (dry basis) of different land use soil samples.

Land Use	Moisture content dry (%)
Forest	27.4843±10.7501
Cropped	26.3252±13.2979
Grazed	38.5786±22.4413

Table 5. Organic matter content and organic carbon of the soils sampled.

Land use/cover	Organic matter content (%)	Organic carbon (%)
Forest	3.548±1.092	2.058±0.633
Cropped	2.833±1.316	1.643±0.764
Grazed	4.888±1.241	2.835±0.721

use soil sample has different types of soil, that is, the soil samples collected at the forest zone is predominantly clay while those at the cropped and grazed zone are sandy clay and sandy clay loam, respectively. Grazed zone has a slightly higher sand content (66.80%) than the others, as well as the lowest silt (10.73%) content and also with the lowest clay (22.50%) content. The high sand content could be attributed to the selective removal of clay particles by erosion leaving the sand particles in the freely grazed land. Forest zone has the highest clay (42.87%) content and the lowest sand (41.47%) content, respectively. Cropped zone has the highest silt (18.00%) content.

Bulk density and porosity

Table 3 presents the experimental result for both porosity and bulk density for forest, cropping and grazing zones. Of the three land uses, forest land had the highest bulk density (1.45 gcm^{-3}) but lowest porosity (0.43 cm cm^{-3}) while grazed land at 0.8 gcm^{-3} had the lowest bulk density but highest porosity 0.67 cm cm^{-3}. This observation agrees with the works of Vogelmann et al. (2010), Kay and Angers (2002), Gantzer and Anderson (2002) and Ringrose-Voase (1996).

Volumetric moisture content

Table 4 presents the moisture content of soil samples for each land use. High moisture content (38.58±22.44%) was found in the grazed zone. At 26.33±13.30% moisture content, the cropped land had the lowest moisture content. This was a result of the soil type (sandy clay loam) and the presence of crops which continuously tap moisture from the soil.

Organic matter and organic carbon

Table 5 shows the organic matter content (OMC) and organic carbon of different land use. From the results, it was observed that high organic matter was found in the

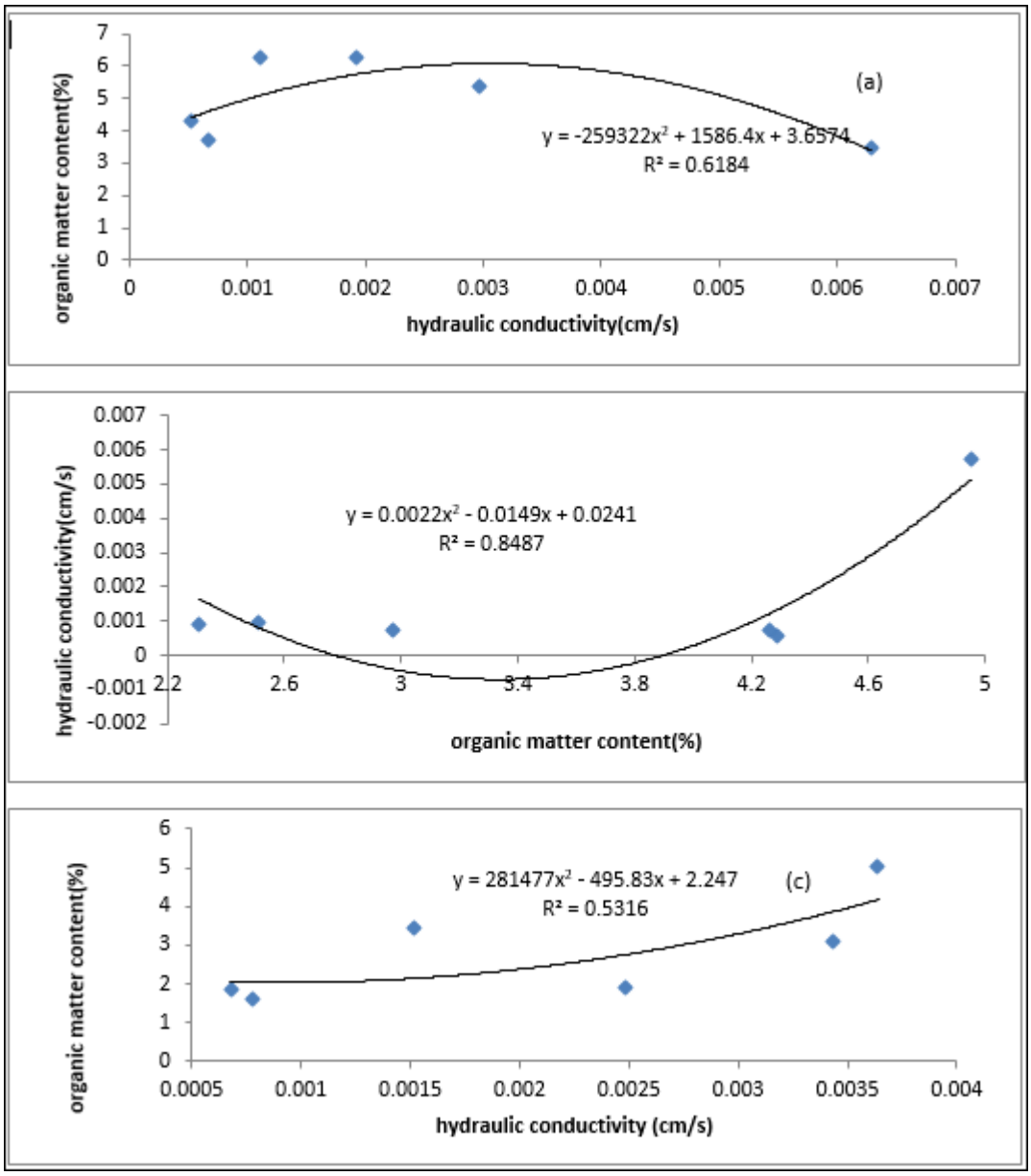

Figure 1. Relationship between hydraulic conductivity and organic matter of soils under land use/cover type, (a) grazed, (b) forest and (c) cropped.

grazed soil due to the urine and excreta of the cattle. High organic matter content was also observed in the forest soil, however at a lower quantity to grazed soil. High organic matter is attributed partly to the continuous accumulation of undecayed and partially decomposed plant and animal residues in the surface soil. The presence of high nutrient in the forest land can help to support farming. The cropped soil had the least organic matter content as a result of continuous depletion from crop use and also as a result of burning of plant residues before cropping and after harvesting. The reduction was also caused by continuous tilling of the soil for cultivation.

Hydraulic conductivity

Figures 1 and 2 present the relationship between hydraulic conductivity (HC), organic matter content (OMC) and bulk density (BD) of the different land use (grazing, forest and cropping). Observed trends between HC and OMC, BD and TP of different land use are presented in Table 7. The forest zone indicated positive correlation between HC and OMC, TP and BD. There was a perfectly negative correlation between HC and TP in the grazing and cropping zone.

Table 6 presents the average hydraulic conductivity of

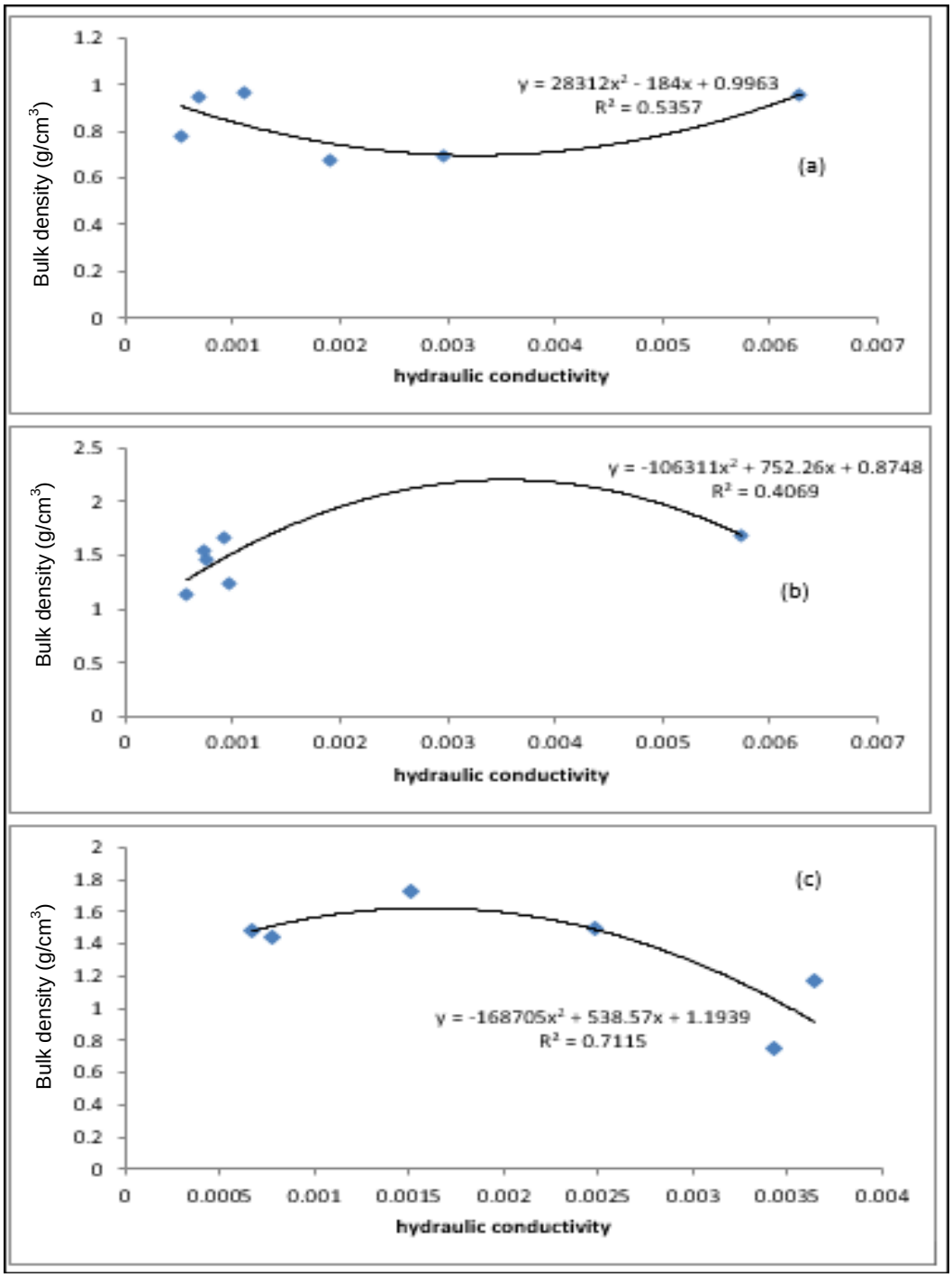

Figure 2. Relationship between hydraulic conductivity and bulk density of soils under land use/cover type, (a) grazed, (b) forest and (c) cropped.

Table 6. Soil hydraulic conductivity of different land use soil samples.

Land use	USDA textural class	Calculated hydraulic conductivity (cms^{-1})
Forest	Clay	0.00162±0.002019
Cropped	Sandy clay	0.002086±0.001299
Grazed	Sandy clay loam	0.002244±0.002176

Table 7. Spearman's rho correlation coefficient (r) among different land uses.

Land use		BD	HC	E	MC	TP	OMC
	BD	-	0.600	-0.314	-0.257	-0.314	0.086
	HC	-	-	-0.029	0.143	0.029	0.086
FOREST	E	-	-	-	-0.200	-1.00**	-0.771
	MC	-	-	-	-	0.200	0.429
	TP	-	-	-	-	-	0.771
	BD	-	-0.029	-0.314	-0.314	0.029	-0.029
	HC	-	-	0.029	0.029	-1.00**	-0.116
GRAZED	E	-	-	-	1.000**	-0.029	0.203
	MC	-	-	-	-	-0.029	0.203
	TP	-	-	-	-	-	0.116
CROPPED	BD	-	-0.486	-0.486	-0.486	0.486	-0.086
	HC	-	-	0.257	0.257	-1.000**	-0.771
	E	-	-	-	1.000**	-0.257	0.486
	MC	-	-	-	-	-0.257	0.486
	TP	-	-	-	-	-	-0.771

**Correlation is significant at the 0.01 level.

different land use soil samples. HC of sampled soils ranged from 0.00162 cms^{-1} in the forest zone to 0.00224 cms^{-1} in the grazing zone. Low hydraulic conductivity in the forest zone was as a result of low exposure of soil to sunlight and low rate of infiltration of water in the soil which was due to the effects of weight of the overlying soil. High hydraulic conductivity was caused by high soil total porosity, an indication of the infiltration rate of water into soil.

Table 8. Erodibility of different land use soil samples.

Land use	Erodibility
Forest	0.063451±0.020874
Cropped	0.076486±0.021189
Grazed	0.087351±0.032167

Erodibility

The result of soil erodibility of the land uses is presented in Table 8. Soil erodibility of sampled soils ranged from 6.35×10^{-2} in the forest zone to 8.73×10^{-2} in the grazing zone. These high values indicate vulnerability of soils on each land use to erosion. This is due to high percent of silts in each land use. The least erodibility was observed in the forest zone as it had the least clay content among the three land uses. This is in relation to the work of O'Geen et al. (2006) who concluded that erodibility is low for clay-rich soils with a low shrink-swell capacity, because clay particles come together to form large aggregates that resist detachment and transport processes. It was found that average soil loss is negatively correlated with clay content but positively correlated with very fine sand and silt plus very fine sand contents. High erodibility value of the grazed zone was due to grazing intensity of cattle which increases soil compaction thereby increasing soil density and the reduction of soil aggregate stability. Figure 3 presents the relationship between erodibility and organic matter content of the different land use (grazed, forest and cropped). Organic matter content contributes about 55% to the factors causing erodibility in the grazed zone and 77% to the forest and cropped zones.

Conclusion

This study reveals the significant differences in the soil physical properties of three land uses in Akure, southwestern Nigeria. The hydraulic conductivity is strongly correlated to bulk density and total porosity. The soil in forest zone had significantly high bulk density as compared to the low bulk density in grazed zone. However, organic matter content, moisture content and hydraulic conductivity were significantly high in the grazed zone. Erodibility values are derived solely from soil properties and factors such as slope, rainfall, surface cover, or management practices were not considered. Soil properties used for this interpretation include surface soil texture, permeability and organic matter.

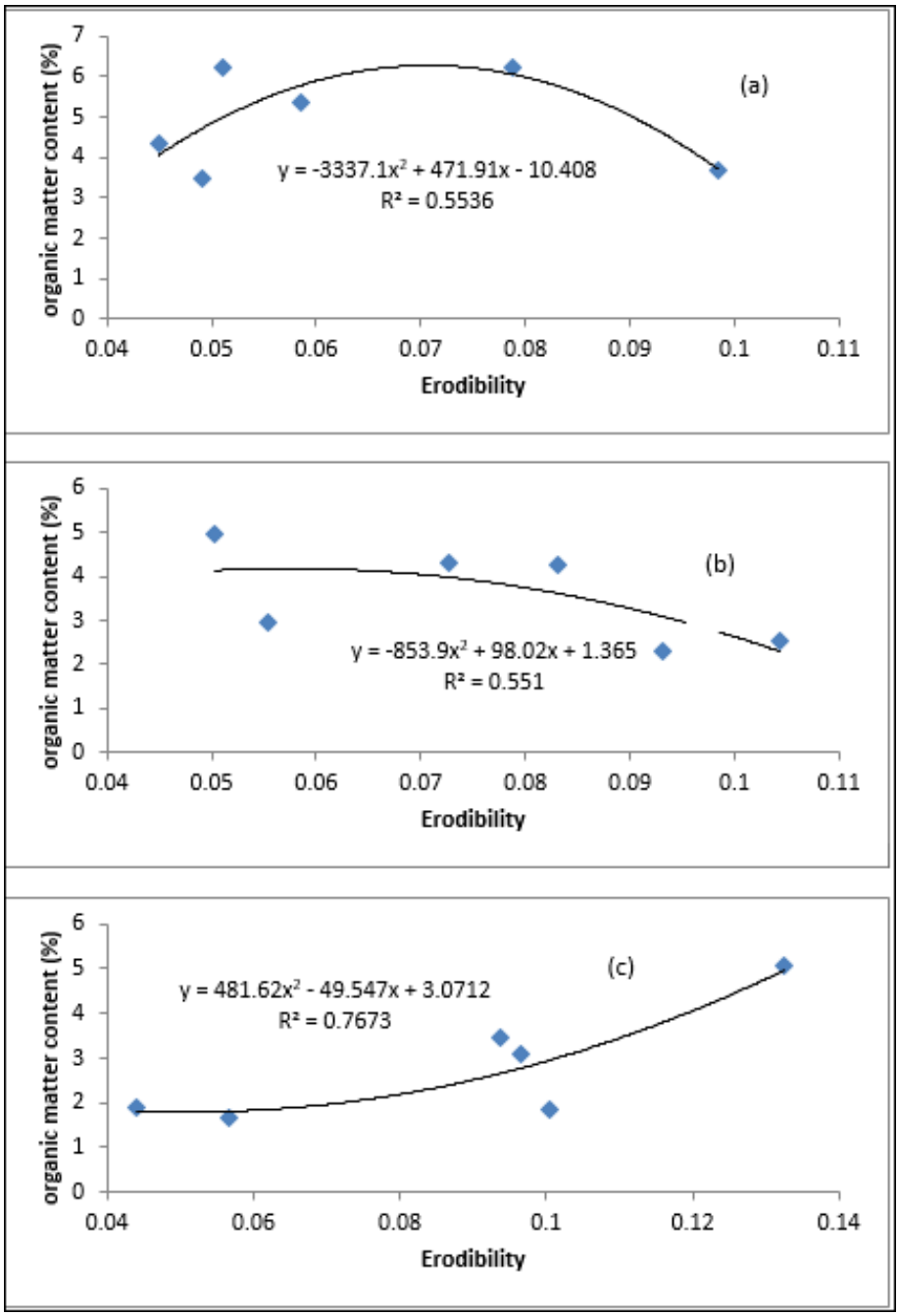

Figure 3. Relationship between erodibility and organic matter content of soils under land use/cover type, (a) grazed (b) forest and (c) cropped.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of the effect of genotype, environment and genotype X environment interaction on white common bean varieties using additive main effect and multiplicative interaction (AMMI) analysis in the mid-altitude of Bale zone, Southeastern Ethiopia

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Twelve white common bean genotypes were evaluated along two checks at three diverse locations in the mid-altitude of Bale zone, southeastern Ethiopia for two consecutive years 2014 and 2015 in order to determine their stability. The genotype by environment interaction (GEI) has an influence on the selection and recommendation of cultivars. The objective of this work was to see the effect of GEI and evaluate the adaptability and stability of productivity of twelve white common bean genotypes using additive main effect and multiplicative interaction (AMMI) model. The combined analysis of variance over locations revealed highly significant differences among the genotypes, locations and genotypes by location interaction. Among the 14 genotypes, the maximum grain yield over locations was obtained by genotype (G5) ICN Bunsi X S X B 405/5C-1C-1C-51 (2.05t/ha) followed by (G11) ICN Bunsi X S X B 405/7C-1C-1C-30 (1.96t/ha), and the site that gave the maximum grain yield was Ginir (2.16t/ha). The results of AMMI analysis indicated that the first four AMMI (AMMI-AMMI4) were highly significant (P<0.01). The GEI - was two times higher than that of the genotype effect, suggesting the possible existence of different environment groups. Based on the stability parameters like AMMI stability value (ASV), G12, G5, G7, G11, G3 and G13 were found to be as stable cultivars, respectively. As stability per se is not a desirable selection criterion and the most stable genotypes would not necessarily give the best yield performance, simultaneous consideration of grain yield and ASV in a single non-parametric index were also considered in identification of best varieties. Based on the Genotype Selection Index (GSI), which considers both the ASV and mean grain yield, genotype G5 and G11 were identified as stable genotypes for the study areas.

Key words: AMMI Stability Value (ASV), Common bean, Genotype Selection Index (GSI), GE interactions

INTRODUCTION

Common bean (P. *vulgaris* L.) germplasm was introduced into Africa from each of the two gene pools in Latin America during the past four centuries (Allen, 1995). Africa is now the second most important common bean

producing region in the tropics, following Latin America (Allen, 1995). Beans are now recognized as the second most important source of human dietary protein, and the third most important source of calories of all agricultural commodities produced in Eastern and Southern Africa (Pachico, 1993).

Bean is a major crop in many parts of Africa, especially in eastern Africa. An important food to people of all income categories, it is especially important to the poor as a source of dietary protein. Its production is agronomically diverse, being grown in many different crop associations. Bean is grown primarily by small-scale farmers in eastern Africa. Unfortunately, the rate of increase in bean production has been exceeded by the rate of population growth. The Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) recognizes research on beans as being of high importance. Bean is an important source of cash for small scale farmers in Africa, whether as part of the total farm income or for providing a marketable product at critical times when farmers have nothing else to sell such as before the maize crop is harvested (Pachico, 1993).

Common bean is a well-established component of Ethiopian agriculture, and is regarded as the main cash crop and protein source of the farmers in many lowland and mid-altitude regions of Ethiopia with an estimated production area of 239,000 ha (Wortmann and Allen, 1994). The national average yield is 500 to 700 kg/ha and yield from research station plots is in the range of 2000 to 3000 kg/ha (Mekbib, 1997). The most suitable bean production areas in Ethiopia are characterized by an altitude range of 1200 to 2200 m asl, and mean maximum temperature of less than 32°C, and well distributed rainfall of 350 to 500 mm throughout the growing season. Genotype-environment interactions are of major importance to the plant breeder in developing improved cultivars (Kang, 1993).

When cultivars are compared over a series of environments, the rankings usually differ and this may cause difficulty in demonstrating the superiority of any cultivar across environments. Since production is highly affected by the effect of environment, identifying stable cultivar for maximum yield is essential. A major challenge for plant breeders is determining the appropriate common bean genotypes due to genotype x environment (GE) interactions, which determine the differential response of genotypes among environments. To reduce the effects of GE interactions, it is convenient to know their magnitude, and to identify more stable genotypes adapted to specific environments (Cruz and Regazzi, 2007).

In this context, several methods to study adaptability and stability have been used to measure GE interactions in common bean (Coimbra et al., 1999; Carbonell et al., 2004; Ribeiro et al., 2009; Pereira et al., 2009, 2011; Torga et al., 2013), predominantly based on linear regression models (Eberhart and Russell, 1966) and multivariate analyses, such as additive main effects and multiplicative interaction analysis (AMMI) (Gauch, 2006). Traditional methods that predict genotype performances in multiple environments are based on a classic approach to statistics, which estimates one or more parameters from a set of observations.

Although there are many stability parameters, Eberhart and Russel (1996) model's parameters S^2di appeared to be very important. Since the variance of S^2di is a function of a number of environments, hence several environments with minimum replications per environmental factor being advocated to be necessary to obtain reliable estimates of S^2di. To identify the stable genotypes having adaptability over a wide range of agro-climatic conditions is of major significance in crop improvement.

Therefore, this study aimed to observe the effect of GEI and to evaluate the adaptability and stability of twelve white common bean genotypes using Additive main effect and multiplicative interaction (AMMI) model.

MATERIALS AND METHODS

In this study, 14 white common bean genotypes (Table 1) were evaluated during the main/meher seasons for two consecutive years (2014 to 2015) at three midaltitudes (Ginir, Goro and Dellomena) south eastern of bale zone, Ethiopia. The layout used at all locations was randomized complete block design with four replications. Plot size used was 6.4m^2 (4 rows at 40cm spacing and 4m long). The two central rows were used for data collection. Combined analysis of variance least significant difference (LSD) multiple range test were done using Cropstat9 software. The AMMI analysis was performed using the model suggested by Crossa et al. (1991). The stability parameters like regression coefficient (bi), deviation from regression were also calculated using Cropsta9 program. AMMI stability value (ASV), the distance from the coordinate point to the origin in a two dimensional ˆof interaction principal component axes (IPCA) 1 scores against IPCA2 scores was computed by the model suggested by Purchase et al. (2000):

$$ASV=\sqrt{\left[\frac{SSIPCA1}{SSIPCA2}(IPCA1score)\right]^2+[IPCA2]^2} \quad (1)$$

Where, $\frac{SSIPCA1}{SSIPCA2}$ is the weight given to the IPCA1 value by dividing the IPCA1 sum squares by the IPCA2 sum of squares.

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Table 1. List of genotypes used in the trial.

S/N	Genotype code	Genotype name	Source/genotypic status
1	G1	ICN Bunsi X S X B 405/4C-1C-1C-50	Breeding lines introduced from Melkasa Agriculture research center
2	G2	ICN Bunsi X S X B 405/3C-1C-1C-87	Breeding lines introduced from Melkasa Agriculture research center
3	G3	ICN Bunsi X S X B 405/9C-1C-1C-70	Breeding lines introduced from Melkasa Agriculture research center
4	G4	ICN Bunsi X S X B 405/5C-1C-1C-98	Breeding lines introduced from Melkasa Agriculture research center
5	G5	ICN Bunsi X S X B 405/5C-1C-1C-51	Breeding lines introduced from Melkasa Agriculture research center
6	G6	ICN Bunsi X S X B 405/1C-1C-1C-31	Breeding lines introduced from Melkasa Agriculture research center
7	G7	ICN Bunsi X S X B 405/7C-1C-1C-69	Breeding lines introduced from Melkasa Agriculture research center
8	G8	ECAB-0632	Breeding lines introduced from Melkasa Agriculture research center
9	G9	ICN Bunsi X S X B 405/7C-1C-1C-58	Breeding lines introduced from Melkasa Agriculture research center
10	G10	ICN Bunsi X S X B 405/3C-1C-1C-49	Breeding lines introduced from Melkasa Agriculture research center
11	G11	ICN Bunsi X S X B 405/7C-1C-1C-30	Breeding lines introduced from Melkasa Agriculture research center
12	G12	ICN Bunsi X S X B 405/4C-1C-1C-80	Breeding lines introduced from Melkasa Agriculture research center
13	G13	Roba-1	Released by Melkasa Agriculture Research Center
14	G14	Awash Melka	Released by Melkasa Agriculture Research Center

Genotype selection index (GSI) was also calculated by the formula suggested by Farshadfar et al. (2003). Here it is calculated by taking the rank of mean grain yield of genotypes (RY_i) across environments and rank of AMMI stability value ($RASV_i$) (Table 1).

$$GSI_i = RASV_i + RY_i \quad (2)$$

RESULT AND DISCUSSION

The combined analysis of variance revealed significant differences in the yield performance of the varieties were observed among the genotypes, environments and genotype by environment interactions (Table 2). Corte et al. (2002) also reported significant differences for mean grain yield of common bean for environments.

Similarly, Raffis et al. (2004), Dar et al. (2009) and Mwale et al. (2009) also reported significant differences in genotypes by environment interaction for mean grain yield of common bean. The variance due to genotypes by environment interaction was found significant for various traits by Singh et al. (2007). Mean comparison for the tested genotypes indicated that maximum grain yield was obtained from G5 (2.05t/ha) followed by G11 (1.96t/ha) and G6 (1.76t/ha) whereas the least mean grain yield was obtained from G8 (1.52t/ha) (Table 2).

The regression analysis (Table 3) revealed that the main effects of genotypes, and GE interaction were accounted only for 6.52 and 15.29% of the total sum of square (TSS), respectively (Table 3). Liner GE interaction was not significant and accounted for 5.55% of the variability in the GE interaction. As a general rule, the effectiveness of regression analysis is when 50% of the total sum squares is accounted for by liner GE interaction (Hayward et al.,1993), hence regression analysis is not useful for stability analysis of genotypes (Wade et al., 1995) (Table 3).

The result of AMMI analysis indicated that 6.52% of the total variability was justified by genotypes, 78.17% by environment and 15.29% by genotype. The partitioning of total sum of squares indicated that the environment effect was a predominant source of variation followed by GE and genotype effect. A large sum of square (SS) for environments indicated that the environments were diverse, with large differences among environmental means causing most of the variation in grain yield.

The GE interaction effect was two times higher

Table 2. Combined analysis of variance for mean seed yield of white common bean tested at three locations (Ginir, Goro and Dello mena) for two years (2014-2015).

Source of variation	DF	Mean squares
Year (Y)	1	7066210**
Location (L)	2	27962200**
Replications	3	341310**
Genotypes (G)	13	565441**
Y*L	2	12546500**
Y*G	13	361761**
L*G	26	255351**
Y*L*G	26	226430**
Residual	249	150569**
TOTAL	335	451281**
CV%	-	22.7%

**Significant at 1 % of probability level.

Table 3. Regression analysis of phenotypic stability for white common bean genotypes.

Source of variation	D.F.	S.S.	M.S.	TSS%
Genotype (G)	13	1.83768	0.14136**	6.52
Location (L)	5	22.0209	4.40418**	78.17
G X L	65	4.3073	0.066266**	15.29
G X Site Reg	13	0.239104	0.018393	5.55
Deviations	52	4.0682	0.078235**	94.45
Total	83	28.17	-	-

**Significant at 1% level of probability.

Table 4. Analysis of Variance for grain yield of white common bean for the AMMI model.

Source of variation	D.F	S.S.	TSS%	M.S. F
Genotypes (G)	13	1.83768	6.52	0.14136**
Locations (L)	5	22.0209	78.17	4.40418**
G x L	65	4.3073	15.29	0.066266**
AMMI component 1	17	1.53941	35.74	0.090554**
AMMI component 2	15	1.5262	35.43	0.101747**
AMMI component 3	13	0.888585	20.63	0.068353**
AMMI component 4	11	0.202411	4.70	0.018401**
GXE residual	9	0.150694	3.50	-
Total	83	28.1659	-	-

** Significant at 1% level of probability.

than that of the genotype effect, suggesting that there were sustainable differences in genotypic response across environments. Furthermore, the AMMI analysis revealed that there were high significant differences for IPCA1, IPCA2, IPCA3 and IPCA4. This made it possible to construct the biplot and calculate genotypes and environment effects (Guach and Zobel, 1996; Yan and Hunt, 2001; Kaya et al., 2002). The first IPCA1 accounted for 35.74% of the variability of GE, followed by IPCA2 (35.43%), IPCA3 (20.63%) and IPCA4 (4.7%).

The first two interaction principal component axes (IPCA) scores were cumulatively accounted for 71.2% of the total GE interaction. This indicates that the use of AMMI model fit the data well and justifies the use of AMMI2 (Table 4). The IPCA scores of a genotype in the AMMI analysis indicate the stability of a genotype across environment. The closer the IPCA scores to zero, the more the stable the genotypes across their testing environments.

Table 5 shows effects of genotypes and site values from the additive genotype x environment model. The large differences of effects both on genotypes and on environments were observed. Environments A (0.69t/ha) and C (0.21) have the main high significant positive grain yield effects. Environments E (-0.22t/ha) have the main significant negative grain yield effects. Genotypes G5 (0.34t/ha) and G11 (0.26 t/ha) had a positive grain yield significant effect across all environments. The majority of white common bean varieties had a small none significant main positive effect.

Table 6 indicates the different stability parameters that can determine the stability of a given genotype across the tested environment. Accordingly, the regression coefficient (bi), mean grain yield and deviation from regression should be simultaneously seen before deciding on the stability of a genotype.

Furthermore, the ASV which is the distance from the coordinate point to the origin in a two-dimensional scatter gram of IPCA1 scores against IPCA2 score should also be seen to decide the stability of a genotype (Purchase et al., 2000). In ASV method, the genotype with least ASV score is the most stable. From this study (Table 6), AMMI Stability Value (ASV) distinguished genotypes G12, G5, G7, G13 and G3 as the stable genotypes.

However, since the stability in itself should not be the only parameter for selection, as the most stable genotype wouldn't necessarily give the best yield performance (Mohammadi et al., 2007). Hence, simultaneous consideration of grain yield and ASV in single non-parametric index is needed. Therefore, based on the GSI, G5 and G11 were considered as the most stable genotypes with high grain yield compared to the others (Table 6).

The last stage of the AMMI analysis is the graphical representation of genotypes and environment in the biplot (Gabriel, 1971), and identification of mega-environment. The biplot graphics were used to analyze the description of genotypes, environments and the interaction between them. The first singular axis of the AMMI analysis

Table 5. Effects of white common bean varieties for the change in grain yield (t/ha) from the AMMI additive GE model.

Variety code	Environments						
	Gin2014 (A)	Goro201 (B)4	Gin2015 (C)	Goro2015 (D)	DM2014 (E)	DM2015 (F)	Genotypes effects
G1	0.08	-0.20	0.02	-0.07	0.34	-0.34	0.05
G2	0.02	0.89	-0.22	0.01	0.18	-0.08	-0.09
G3	0.18	-0.33	-0.07	-0.14	0.15	-0.09	0.00
G4	0.27	-0.74	-0.11	0.10	0.12	-0.30	-0.14
G5	0.41	0.13	0.10	-0.20	-0.12	-0.32	0.34**
G6	0.01	-0.15	0.30	0.06	-0.38	0.17	0.06
G7	-0.06	0.12	0.09	-0.10	-0.20	0.16	-0.14
G8	0.031	0.11	0.09	0.27	-0.41	-0.09	-0.18
G9	-0.47*	-0.17	-0.06	0.17	0.08	0.30	-0.16
G10	-0.07	-0.26	-0.59*	-0.01	0.62**	0.31	0.04
G11	0.45	0.15	-0.27	-0.05	-0.38	0.10	0.26*
G12	-0.10	0.96	-0.13	0.08	-0.16	0.22	0.02
G13	-0.21	0.54	0.33	-0.11	0.14	-0.20	-0.09
G14	-0.52*	-0.19	0.53*	0.00	0.03	0.15	0.03
Locations effects	0.69**	-0.91	0.21**	-0.17	- 0.22**	0.39	1.71**

*, ** Significant probability level at 0.05 and 0.1%, respectively.

Table 6. Regression coefficient, deviation from regression, IPCA scores, ASV and GSI of genotypes.

Genotypes	Mean	Slope (bi)	MS-DEV	IPCA1	IPCA2	IPCA3	IPCA4	ASV	GSI
G1	1.75	0.925	0.06	-0.17	-0.15	-0.43	-0.09	0.23	11
G2	1.61	0.882	0.02	-0.15	-0.19	-0.02	-0.16	0.25	18
G3	1.7	1.062	0.02	-0.20	-0.09	-0.12	0.18	0.22	13
G4	1.57	1.045	0.05	-0.31	-0.04	-0.18	-0.19	0.32	20
G5	2.05	1.072	0.09	-0.38	0.30	-0.24	0.14	0.20	3
G6	1.76	1.219	0.05	0.22	0.35	0.15	0.20	0.41	14
G7	1.57	0.995	0.02	0.11	0.16	0.14	0.16	0.20	14
G8	1.52	0.966	0.07	0.02	0.37	0.16	-0.39	0.37	24
G9	1.55	0.84	0.08	0.41	-0.24	0.23	-0.20	0.48	25
G10	1.75	1.038	0.22	-0.11	-0.82	0.20	0.15	0.83	18
G11	1.96	1.054	0.10	-0.41	0.25	0.41	0.14	0.21	6
G12	1.72	0.951	0.03	0.08	0.01	0.33	-0.07	0.09	8
G13	1.62	0.863	0.05	0.21	0.05	-0.41	-0.01	0.22	15
G14	1.74	0.987	0.15	0.67	0.04	-0.22	0.15	0.68	19

N.B. MS-DEV= deviation from regression, IPCA= Interaction Principle Component Analysis axis, ASV= AMMI Stability Value, GSI= Genotype Selection Index.

captures the highest percentage of the “pattern” of the data (Gauch and Zobel, 1988). A high percentage of the Sum Square of the GE interaction (SSGEI) is explained by the first two axes (71.2%) and the highest part of the “pattern” of the GEI will be captured.

According to the values of the two first principal components (IPCA1 and IPCA2, Figure 1), G5, G11, G6, G1 and G10 are the genotypes with more productivity in the environmental conditions prevailing during crop development. But G10, interact negatively to most of the environments, though it gives high grain yield above the grand mean. Regarding stability, G5, G11, G12, G13 and G7 are considered as the stable genotypes. However, when we see their GSI (Genotype Selection Index) which associate both the ASV and the grain yield, G5 and G11 are the more stable genotypes with high grain yield across the testing sites. G10 is more specifically adapted to environment E and G9 to environment F (Figure 1).

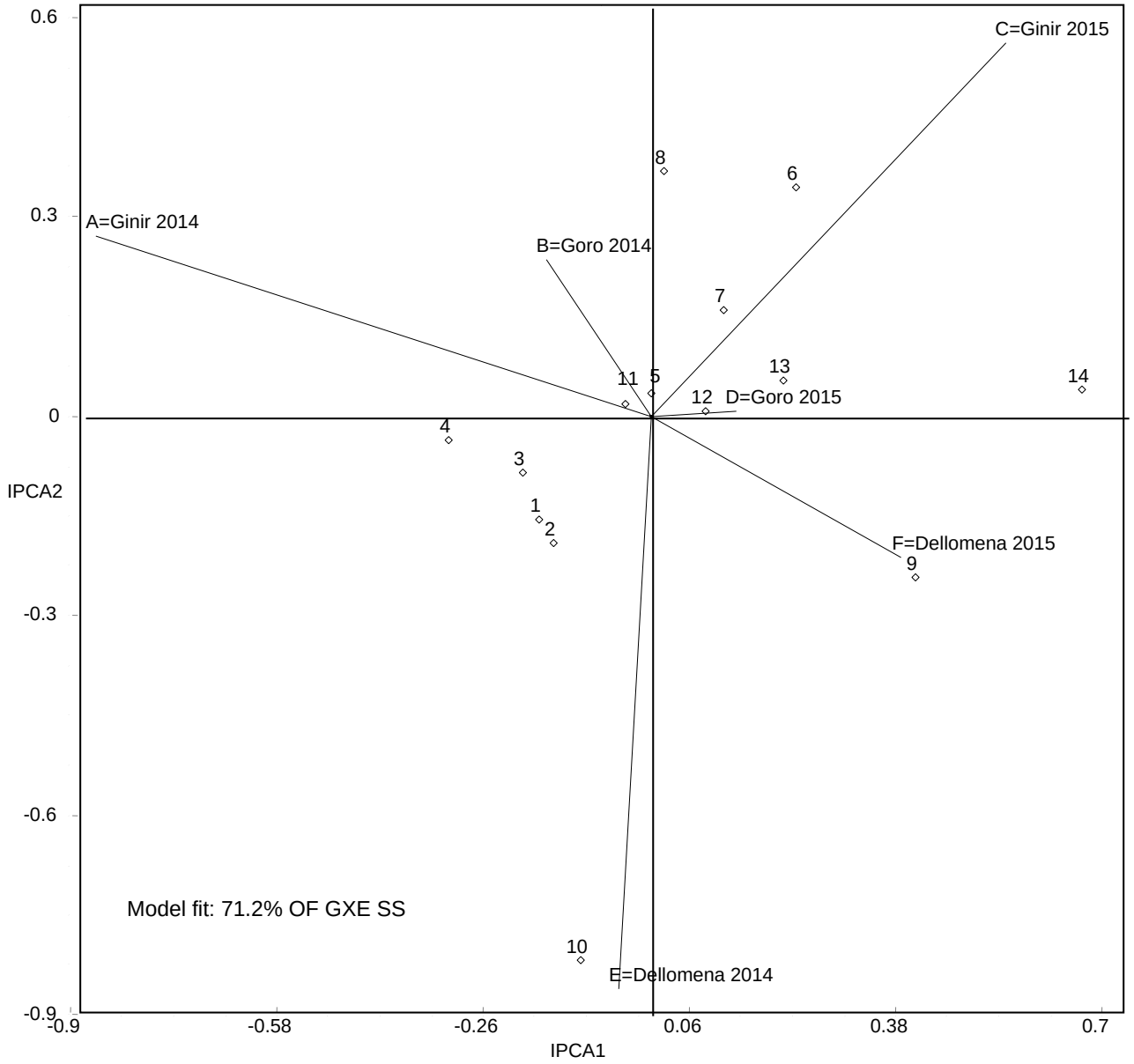

Figure 1. Biplot analysis of GE interaction based on AMM2 model for the first two interactions principal component scores.

Conclusion

AMMI analysis of multi-environment yield trials serves two main purposes:

(1) Understanding complex GEI, including delineating mega-environments and selecting genotypes to exploit narrow adaptations, and
(2) Gaining accuracy to improve recommendations, repeatability, selections and genetic gain.

Therefore, according to the present study, genotypes G5, G11 and G12 display higher adaptability and stability. Therefore, they are recommended to be used in all environments included in the study. The genotypes G13 and G7 present high mean productivity. However, they were unstable and specific adaptation to the environments of high quality that is, environment D. Environment A gives the highest mean grain yield (2.395t/ha) and environment B (0.80t/ha) gave the lowest mean grain yield. These can be considered as an example of favorable and unfavorable environments respectively. Therefore, from this study G5 and G11 were considered as the most stable genotypes and therefore,

identified as candidate genotypes for possible release.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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