EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AND BIOCHAR ON SOIL PROPERTIES AND PERFORMANCE OF COWPEA (*Vigna unguiculata* (L.)Walp)

[1]Taiwo, L. B., [2]Adesokun, K. T., [2]Olatoberu, F. T., [1]Oyedele, A. O., [1]Ojo. A. O and [*2]Olayinka, A. A.

[1]Institute of Agriculture Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan

[2]Department of Soil Science and Land Resources Management, Faculty of Agriculture, Obafemi Awolowo University, Ile Ife, Nigeria.

[*]lbtaiwo@yahoo.com; lateefbtaiwo@gmail.com

ABSTRACT

A screenhouse and field experiment was carried out at Obafemi Awolowo University, Ile-Ife, in 2016 to investigate the effect of Plant Growth Promoting Rhizobacteria (PGPR) on cowpea (Vigna unguiculata) using biochar as a carrier. This was to evaluate the suitability of PGPR as fertilizers, pesticides as well as soil fertility improvement. The experimental design was a randomized complete block design with six treatments, replicated six times. Treatments used were Control, 40 ml biofertilizer (Bacillus thuringiensis, Pseudomonas putida and Klebsiella variicola), biofertilizer (40 ml) + biochar (40 g), biochar (40 g), 60 KgP_2O_5/ha Single Super Phosphate SSP and 2.5% of Lambda Cyhalothrin (Laraforce insecticide). Agronomic data were recorded and post-harvest soil analyses were carried out. The nodulation and plant heights of cowpea plants increased with the application of biofertilizer + biochar and showed about 13% and 53% increase in plant height and number of leaves respectively, over the control for the field experiment. Biofertilizer + biochar showed just about 2% decrease in the number of pods when compared with SSP which recorded the highest number. Biofertilizer + biochar treated soils recorded high microbial respiration with about 41% increase over control soils. Biochar application significantly increased the soil exchangeable K and Mg while the application of biofertilizer alone had a significant effect on Ca and the soil organic matter. The results suggest that PGPR as a potential alternative for chemical fertilizers and pesticides in cowpea production and its combination with biochar is a good technology to be adopted for soil fertility improvement.

Keywords: Biochar, Biofertilizer; Cowpea; Insecticides; Nodulation

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.)Walp, is one of the most important grain legumes, which is widely cultivated in the semi-arid areas of the tropics and subtropics for human as well as animal consumption. It is also an essential component of sustainable cropping systems in the sub-humid tropics and, generally, dry regions across the globe (Singh *et al*.,2002; Langyintuo *et al*., 2003). Cowpea constitutes an important pulse in semi-arid regions of sub-Saharan Africa and is a major and cheap source of quality protein for both rural and urban dwellers in Africa the cheapest source of dietary protein for low income sector of the population in West and Central Africa,

(Ajeigbe *et al*., 2012; Dube and Fanadzo, 2013). Cowpea leaves, green pods, green peas and dry grains are consumed as food and the haulms, which contain about 20% protein are fed to livestock. More than 11 million hectares are cultivated worldwide, 97 % of which is in Africa. Nigeria cultivates 4.5 million hectares annually representing over 60 % of total production (FAO, 2011).

Although, FAO database estimated average cowpea grain production in Nigeria as 700 kg ha^{-1} (FAOSTAT, 2009), cowpea yields remain one of the lowest among all food legume crops, averaging at 450 kg ha^{-1} in 2006-2008, which is half of the estimated yields in all other developing regions (Haruna and Usman, 2013).The causes of the low yields have been attributed to numerous factors such as insect pests, diseases, parasitic weeds, drought and low fertility, of which insects constitute the major constraint with yield losses in cowpea due to insect pests estimated to be above 80% in Nigeria farms (Oparaeke *et al*., 2000; Maina *et al*., 2014). The presence of many insects from seedling to harvest is a feature of cowpea, although flower and pod-attacking pests are the most economically important (Karungi *et al.*, 2000, Asante et al., 2001).

Despite the use of available means of plant protection, about one third of the crops produced are destroyed by pests and diseases. The discovery of synthetic chemicals has contributed greatly to the increase in food production by controlling pests and diseases. However, the use of these synthetic chemicals during the last three decades has raised a number of ecological problems such as environmental pollution, toxicity to mammals, hazards to users and consumers (Alabi *et al*., 2003). In recent years, scientists have diverted their attention towards exploring other measure one of which is the exploitation of the potentials of beneficial microbes as bio control agents for plant protection measures. Bio-control agents are easy to deliver, improve plant growth, activate resistance mechanism in the host plant and increase biomass production and ultimately boosting yield (Nakkeeran *et al*., 2005).

These beneficial microbes are vast and play several roles in promoting plant growth, either directly or indirectly through various mechanisms. Since they play several roles, a preferred scientific term given to such beneficial bacteria is "plant-growth promoting rhizobacteria" (PGPR) (Vessey, 2003). Generally, PGPR facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of bio-control agents (Ahmad *et al*., 2008; Ahemad and Kibret, 2014). The PGPRs when used as bio-control agents can also act as biofertilizers and these are expected to reduce the overdependence on chemical fertilizers and pesticides. In addition, these microorganisms restore the soil's natural nutrient cycle and build soil organic matter. Some genera of bacteria such as *Agrobacterium*, *Arthrobacter*, *Azoarcus*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Micrococcous*, *Rhizobium*, *Pantoea*, *Pseudomonas* and *Serratia* have been reported as PGPR: (Bruto et al. 2014; Ahemad and Kibret 2014) and have shown potential as biocontrol agents against different fungal pathogens (Ahemad and Kibret, 2014).

An important aspect in the formulation of PGPRs for use as biofertilizers or as bio control agents is the choice or use of a suitable carrier, organic materials are the preferred choice due to their characteristics of soil improvement through organic recycling and organic material addition to soil. One of such organic

materials is biochar, which is a carbon(C) rich product obtained by thermal decomposition of biomass at relatively low temperatures (<700 ^{o}C) and low oxygen concentration, in a process known as pyrolysis. The process resembles traditional charcoal production, but biochar is used as a soil amendment and not for energy generation (Lehmann and Joseph, 2009). It promotes carbon sequestration, improves soil organic matter, as well as soil fertility as well (Bruun *et al*., 2011; Zhang *et al*. 2013; Nelissen *et al*., 2015).

The challenge faced by Nigeria in the production of cowpea is multi-faceted, therefore an integration of an appropriate recommendation of fertilizer, which acts as a soil amendment and at the same time useful as a bio-control agent will have a multiplier effect on soil improvement and also on cowpea growth and production. This however led to the objective of the study which was to evaluate the effectiveness of biofertilizer and biochar as soil amendments as well as to compare to compare its biocontrol ability with synthetic insecticides in a soil cropped with cowpea.

MATERIALS AND METHODS

Study Location

The pot experiment was carried out at the sc reenhouse of the Faculty of Agriculture, Oba femi Awolowo University, Ile-Ife controlled at 25 - 28^{o}C and the field experiment was conducted at the Institute of Agricultural Research and Training (IAR&T) Research Farm, Obafemi Awolowo University Teaching and Research Farm, Ile-Ife during the 2016 cropping season (September 2016). The site is situated within the forest zone and located between latitude 7^{o}32'N to 7^{o}33'N and longitude 4^{o}33'E to 4^{o}40'E. The area is about 200 m above sea level.

Soil Analysis

The top soil samples (0 – 15 cm) were collected, air-dried, crushed gently and passed through 2 mm sieve to separate gravel content from other soil components. Physical and chemical properties of soils were determined by standard methods as listed: Soil particle size distribution by the hydrometer method (Bouyoucos, 1962), pH in 1:2.5 soil/water suspension by pH-meter (Rowell, 1994), organic carbon content (OC) by the Walkley-Black method (Allison and Moodie, 1965), conversions between values of organic carbon and organic matter was made using Van Bemmelen factor of 1.724 on the assumption that, on average, Soil organic matter contains 58% of organic C, total nitrogen by the Kjeldahl method (Bremner and Mulvaney, 1982) and available P by the method of Olsen *et al.* (1954). Exchangeable bases were extracted with 1 M NH4OAC (pH 7.0) to determine K and Na using flame photometer and exchangeable Mg and Ca by atomic absorption spectrophotometer (Sparks, 1996).

Screenhouse Experiment

The screenhouse experiment was a potted experiment, arranged in a completely randomized design with six treatments and six replicates. Each plastic pot was filled with 5 kg of soil and cowpea was used as the test plant. The treatments were: Control (no treatment), 40 ml per pot of Biofertilizer (a consortium of *Bacillus thuringiensis, Pseudomonas putida* and *Klebsiella variicola*) (Biofertilizer (40 ml per pot) + Biochar (40 g per pot), Biochar (40 g per pot), Single Super Phosphate (SSP) at the rate of 60 kg P_2O_5 N/ha and 2.5% of Lambda Cyhalothrin (Laraforce insecticide). Basal application of biochar was done to the required plots before sowing while folial inoculation with biofertilizer at 5 ml per stand was done at two, four and six weeks after planting where required.

Data on plant height on plot basis was taken at two, four and six weeks after planting. Plant samples were collected at two growth stages to determine the nodulation and dry matter yield. The first sampling was done 56 days after planting (DAP) to determine the nodulation by counting the number of physical nodules on the root of each plant, while the plant samples were oven-dried at 65^{o}C until constant weight was attained to determine both the shoot and root dry matter yield. The second harvest was done at 84 days after planting (DAP) that is at maturity to determine the seed yield production. Soil samples were collected at 6 WAP to determine the effect of the different treatments on some soil microbiological parameters.

Determination of bacterial and fungal abundance

Microbiological analysis to determine the effect of the different treatments on the microbial population of bacteria and fungi was carried out with 1.0 g of soil collected from each pot and diluted ten-folds using sterile normal saline. The population of viable bacterial and fungal cells in each sample was determined by inoculating 0.1 ml aliquots from the 10^{-8} dilution onto nutrient agar and potato dextrose agar respectively by the pour plating technique. Potato Dextrose agar was further made selective for fungi by the incorporation of 50 µg of chloramphenicol/ml (v/v). Incubation was at 30°C for 24 h and 30°C for 5 days for bacteria and fungi respectively. The experiment was carried out in duplicates and viable counts of the bacteria was taken after the incubation period.

Determination of soil microbial respiration

Microbial activity was measured as the heterotrophic respiration in the absence of plant roots by an incubation-alkaline absorption method (Coleman *et al.*, 1978). Sub-samples of soils from each pot equivalent to 50.0 g dry weight were adjusted to moisture of about 60% water holding capacity, which was measured according to the method described by Forster (1995), and placed in 1–l Mason jars with a suspended beaker containing 10 mL of 0.05 M NaOH. The jars were incubated at 25 ^{o}C for 3 days in the dark immediately after sealing. At the end of the incubation period, the CO_2 trapped in NaOH was titrated with 0.05 M HCl. The rate of the respiration was calculated using the method described by Eze *et al*. (2013). The final value was expressed as the amount of CO_2evolved from microbes present per gm of soil per hour (µg CO_2 g^{-1} soil h^{-1}).

Field Experiment

The six treatments described under screenhouse were laid out in a randomized complete block design (RCBD) with three replications. The treatments were Control (no treatment), a biofertilizer (consortium of *Bacillus thuringiensis, Pseudomonas putida and Klebsiella variicola*) at 40ml per plot, Biofertilizer (40 ml per plot) + Biochar (40 g per plot), Biochar (40 g per plot), Single Super Phosphate (SSP) at the rate of 60 kg P_2O_5 N/ha and Laraforce insecticide (as a reference). Basal application of biochar was done to the required plots before sowing. Folia application with biofertilizer at 5 ml per stand was done at two, four and six weeks after planting where required. Experimental plots measured 3m x 3m each with inter-plot space of 1 m. Plots inter-row and intra-row spacing was 1m making a total plot size of 24m x 11m. Planting was done at a space of 30cm x 60cm inter and intra-row respectively on 22nd September, 2016. Four seeds were planted per hole and the seedlings thinned to two plants per stand after sprouting. The variety of cowpea used for the research work is Ife Brown and was obtained from the

Institute of Agricultural Research and Training (IAR&T), Ibadan, Oyo State. Data were collected on plant height, number of leaves per plant, number of nodules per plant, and number of pods per plant, while soil samples were taken at harvest for chemical analyses using the methods stated earlier. All cultural practices were applied.

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) and where the F-values were found to be significant, the treatment means were separated using Least Significant Difference (LSD) at 5% probability level (Genstat, 2011).

RESULTS AND DISCUSSION

Soil Pre-Cropping Analysis

The result of the pre-cropping soil analysis are presented in Table 1. The soil used was observed to be sandy loam using the soil texture triangle. The pH of the soil was slightly acidic (6.1). Organic carbon and total nitrogen was moderately available while available phosphorus was low (FMANR, 2002). However, the micronutrients determined mainly manganese, iron, copper and zinc moderately available in the soil (Agboola and Ayodele, 1985) while the exchangeable bases was also moderately available in the soil.

Table1: Physical and Chemical Properties of the soil of experimental site

Properties	Values
pH (0.01 M $CaCl_2$)	6.1
Particle Size	
Sand (g/kg)	884.0
Clay (g/kg)	74.0
Silt (g/kg)	42.0
Textural class	sandy loam
Organic carbon (%)	2.2
Total Nitrogen (%)	0.2
Available P (mg/kg)	10.8
Micronutrients	
Manganese Mn (mg/kg)	53.8
Iron Fe (mg/kg)	12.0
Copper Cu (mg/kg)	0.7
Zinc Zn (mg/kg)	5.4
Exchangeable Bases	
Calcium Ca (cmol/kg)	5.0
Magnesium Mg (cmol/kg)	2.2
Sodium Na (cmol/kg)	1.1
Potassium K (cmol/kg)	0.4

Effect of the treatments on the plant height during the ScreenHouse Experiment

At 2 weeks after planting, it was observed that there was no significant difference ($p \geq 0.05$) in the plant height for all the treatments applied (Fig 1). This indicates that at 2 WAP, effect of the different treatments applied was yet to be observed, when biochar was added. It had not gone into mineralization and so impact would not have been felt.

At 4 WAP, no significant difference ($p \geq 0.05$) was also observed in plant heights for all treatments used. Biochar + biofertilizer (*Bacillus thuringiensis, Pseudomonas putida, Klebsiella variicola)* treatment led to a non-significant increase of over 3% over biofertilizer (*Bacillus thuringiensis, Pseudomonas putida, Klebsiella* variicola) and about 6% increase over control. This is probably due to the plant growth promoting abilities exhibited by the microorganisms especially when used alone (Somers *et. al.*, 2004; Lehmann and Joseph 2006) and when it was added to biochar.

At six weeks after planting, no significant difference ($p \geq 0.05$) was observed in the heights of cowpea treated with biofertilizer (*Bacillus thuringiensis, Pseudomonas putida, Klebsiella*variicola) and the ones treated with biofertilizer (*B. thuringiensis, P. putida, K.* variicola) + biochar. However, there was a significant difference in the height of cowpea treated with biofertilizer (*B. thuringiensis, P. putida, K. variicola*) and the ones treated with biochar. Biofertilizer (*B. thuringiensis, P. putida, K.* variicola) had the highest plant height. It also had a non-significant increase of about 6% over biofertilizer (*B. thuringiensis, P. putida, K.* variicola) + biochar and 13% increase over control. This is in accordance with the reports earlier stated by Lehmann (2007) that plant growth promoting bacteria have the ability to stimulate plant growth when used as biofertilizer.

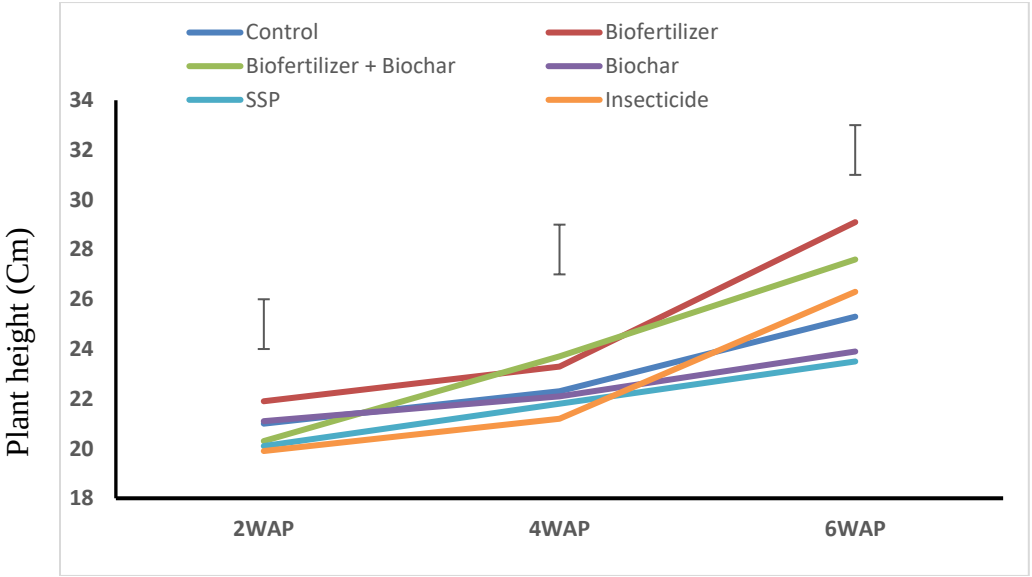

Fig 1: Effect of treatments on plant heights (cm) observed in the screenhouse from 2 weeks after planting till 6 weeks after planting.

Effect of the treaments on the number of Nodules during the ScreenHouse experiment

There was no significant difference ($p \geq 0.05$) in the number of nodules of the control plants, the biofertilizer (*B. thuringiensis, P. putida, K.* variicola) and biofertilizer (*B. thuringiensis, P. putida, K.* variicola) + biochar-treated cowpea plants (Table 2). However, a significant difference ($p \geq 0.05$) was observed in their nodule numbers when compared with biochar, SSP and insecticide treatments. Cowpea plants treated with biofertilizer (*B. thuringiensis, P. putida, K.* variicola) + biochar recorded the highest number of nodules while insecticide-treated cowpea plants gave the least number of nodules.

Effect of the treatments on the shoot weight during the screenhouse experiment

The results for shoot weight showed that there was significant difference between biofertilizer (*B. thuringiensis, P. putida, K. variicola*) + biochar and insecticide-treated cowpea plants while there was no significant difference between them and the other treatments (Table 2). Treatments of Biofertilizer (*B. thuringiensis, P. putida, K. variicola*) + biochar gave the highest shoot weight of about 10% increase over biochar alone. The effectiveness of the biofertilizer (*B. thuringiensis, P. putida, K. variicola*) + biochar treatments in having the highest shoot weight could be as a result of the addition of biochar which possibly increased the plant growth promoting activities of the microorganisms used as biofertilizers (Woolf *et al.*, 2010). The lowest shoot weight was observed in cowpea plants treated with insecticide.

Table 2: Effect of treatments on the number of nodules and shoot weight of cowpea in the screenhouse

Treatments	Number of nodules	Shoot Weight (kg)
Control	10.7a	10.7ab
Biofertilizer	16.3a	9.0ab
Biofertilizer + Biochar	17.3a	16.0a
Biochar	6.7bc	14.3ab
SSP	3.3c	10.0ab
Insecticide	2.3c	7.3b

Values followed by different letters are significantly different from each other ($p \leq 0.05$)
Single Super Phosphate applied at a rate of 60kg P_2O_5/ha
SSP = Single Super Phosphate
WAP = Weeks after planting SSP = Single Super Phosphate

Soil Microbial Population

For the microbial count, there was no significant difference ($p \geq 0.05$) among the treated soils Results as shown in Table 3 indicates that there was no significant difference ($p \geq 0.05$) among the soils with the different treatments. The soil treated with biofertilizer (*B. thuringiensis, P. putida, K.* variicola) + biochar was seen to have the highest number of soil fungi even though; it has a slightly significant increase of about 21% over the control. Biofertilizer-treated (*B. thuringiensis, P. putida, K.* variicola) soil had the lowest fungi population which may be due to the fact that the plant growth promoting rhizobacteria did not enhance fungal growth. This could be as a result of the antifungal metabolites being produced by some plant growth promoting rhizobacteria (Antoun and Prevost, 2005).

Table 3: Effect of the treatments applied on the microbial population of the screenhouse Soil

Treatment	Bacteria Count (x 10^6 CFU/ml)	Fungal Count (x 10^6 CFU/ml)
Control	81.3^b	5.3^b
Biofertilizer	63.7^d	4.0^d
Biofertilizer + Biochar	69.7^c	6.7^a
Biochar	98.3^a	5.0^c
SSP	63.01^d	3.9^e
Insecticide	62.26^e	3.9^e

Soil CO_2 Efflux

The results for soil microbial respiration (Fig 2) show that there was no significant difference in the soil respiration of the microorganisms for all treatments used. However, biofertilizer + biochar-treated soils recorded high microbial respiration with about 29% increase over control soils. This may probably be due to the introduction of plant growth promoting rhizobacteria into the soil coupled with the presence of biochar, which soil microbes actively mineralize (to derive their food) i.e. aiding microbial activities (Woolf *et. al*., 2010). Biofertilizer-treated (*B. thuringiensis, P. putida, K.* variicola) soils recorded the lowest microbial respiration.

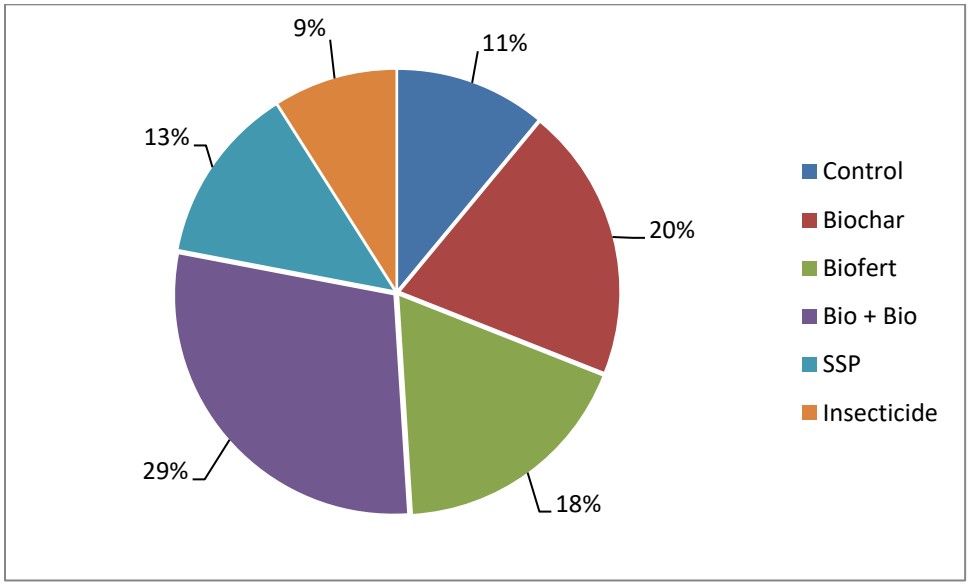

Fig 2: CO_2 efflux evaluation (million/g) in the screenhouse soil (Post-Harvest Analysis)

Chemical properties of screenhouse soil after plant harvest

Effect of the treatments on the soil chemical properties was significant (Table 4). However, there was an increase in the pH of the soil. The pH ranged from 6.0 to 7.6 among all the treatments including the control as a result of the sole and combined application of biofertilizer and biochar. Exchangeable K and Mg were significantly increased with the application of biochar (0.8 cmol/kg and 1.9 cmol/kg respectively) while application of biofertilizer had the most significant effect on exchangeable Ca. Organic C was high in the soil with the significant increase being observed with the application of biofertilizer. PGPR enhances phosphorus solubilization in plants i.e. biofertilizers and are phosphorus solubilizers. Therefore, low available P analysed in the soil after harvest could be as a result of its solubilization by the biofertilizer for uptake by the cowpea for its growth development (Nkaa *et. al.*, 2014), although the application of biofertilizer increased it as compared with the control. There was no increase in the level of available Fe as a result of the application of the treatments. Although Cu and B were low, they increased slightly as compared to the control with the application of biochar and the combined application of biofertilizer and biochar respectively. Total nitrogen did not increase in the soil. This may be probably due to the ability of cowpea to fix its own nitrogen from the air using the nodules in its roots.

Table 4: Effect of treatments applied on the Chemical properties of Screenhouse soil after plant harvest

Treatments	pH	K	Ca	Mg	Total N(%)	Org C (%)	Avail P(mg/kg)	Fe	Cu	B
			cmol/kg						mg/kg	
Control	7.3c	0.64^{b}	2.65^{c}	1.23^{b}	0.29^{b}	2.13^{d}	4.76^{d}	151.28^{a}	2.13^{b}	0.24^{d}
Biofertilizer	7.7a	0.50^{c}	3.34^{a}	1.07^{d}	0.28^{b}	2.55^{a}	6.21^{a}	110.64^{c}	2.14^{b}	0.27^{c}
Biofertilizer + Biochar	6.8d	0.49^{c}	3.04^{c}	1.16^{c}	0.25^{c}	2.23^{c}	5.68^{b}	125.81^{b}	1.79^{c}	0.37^{a}
Biochar	7.6^{b}	0.88^{a}	2.47^{d}	1.89^{a}	0.33^{a}	2.34^{b}	5.22^{c}	104.63^{d}	2.27^{a}	0.31^{b}
SSP	6.2^{e}	0.48^{d}	2.31^{e}	1.04^{e}	0.20^{d}	2.11^{e}	6.20^{a}	109.11^{c}	1.68^{d}	0.23^{d}
Insecticide	6.0^{f}	0.40^{e}	2.29^{f}	0.99^{f}	0.19^{d}	2.08^{f}	4.75^{d}	108.01^{cd}	1.60^{e}	0.20^{e}

Field Experiment

Effect of the treatments on the plant height during the field experiment

Figure 3 shows the effect of the various treatments on the plant heights of the cowpea on the field. Up till four weeks after planting, no significant difference ($p \geq 0.05$) was observed in the plant *heights of all the treatments. However, biochar + biofertilizer (B. thuringiensis, P. putida, K. variicola*) had the highest plant height with a non-significant increase of 7% over the control as observed in the screen house experiment. This also was probably due to the plant growth promoting abilities exhibited by the microorganisms (Somers *et. al.,* 2004; Lehmann et al., 2006). SSP and insecticide-treated plants recorded the lowest plant heights.

At 6 weeks after planting, there was a significant difference ($p \geq 0.05$) between biofertilizer + biochar and control. Biofertilizer + biochar had about 10% increases in plant heights when compared to the control (Fig 3). However, biofertilizer (*B. thuringiensis, P. putida, K.* variicola) + biochar recorded the highest plant heights with just about 2% increase over the insecticide-treated plants. This was probably due to the fact that biofertilizers also act as biocontrol agents as earlier reported by Antoun and Prevost, (2005). The authors stated that biofertilizers can also act as biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites).

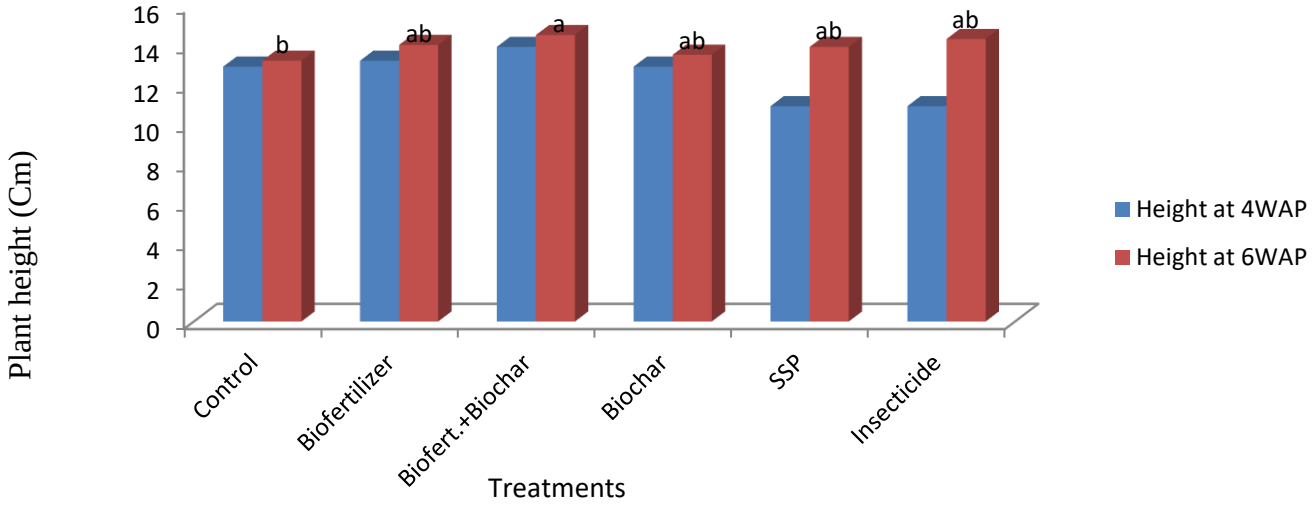

Fig 3: Plant height (cm) of cowpea plant with various treatments on the field at 4 and 6 Weeks After Planting

Effect of the treatments on the number of Leaves per plant during the field experiment

The results of the effect of the different treatments on the number of leaves per plant is shown in fig 4. At four weeks after planting, there was no significant difference ($p \geq 0.05$) in the number of leaves of the cowpea plants. The highest number of leaves was recorded by both the control and the insecticide-treated plants while the lowest number of leaves was recorded by the biofertilizer (*B. thuringiensis, P. putida, K.* variicola), biofertilizer (*B. thuringiensis, P. putida, K.* variicola) + biochar and SSP-treated plants. This is due to the fact that the mineralization of biochar had probably not begun at four weeks after planting.

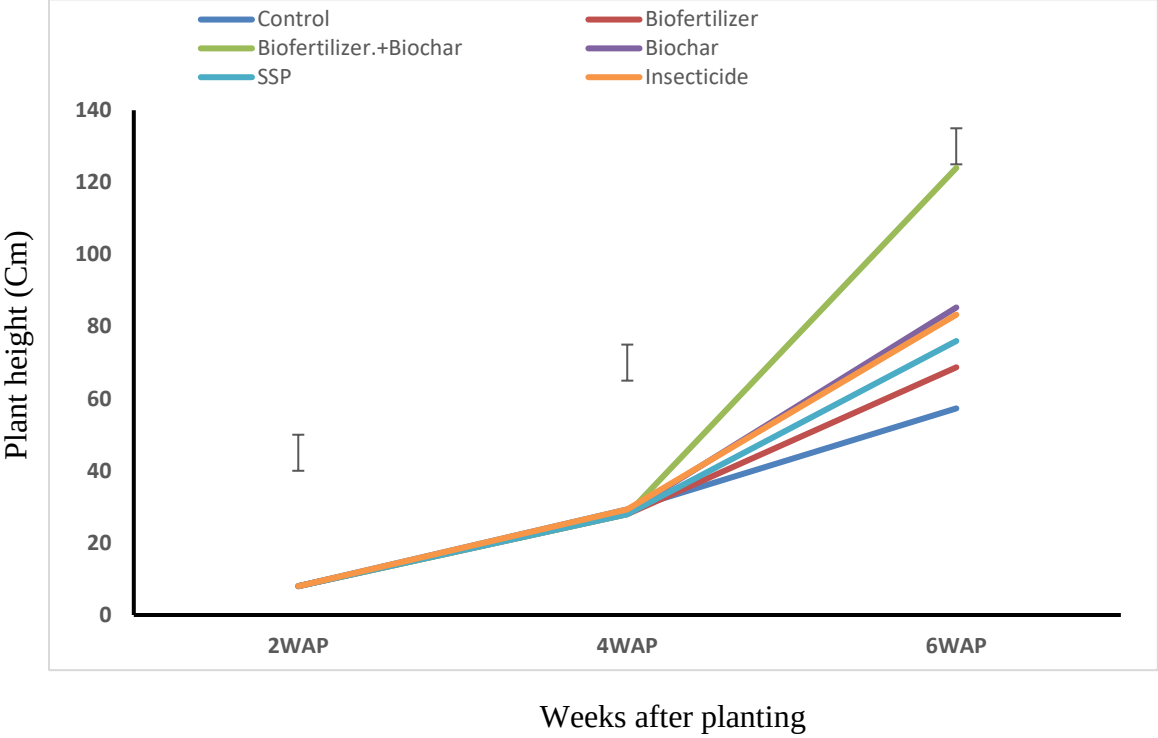

Fig 4: Number of Leaves per plant during the field experiment

Effect of the treatments on the number of Nodules during the field experiment

Results for the number of nodules shown in table 5 indicates that there were significant differences ($p \geq 0.05$) among all the treatments. Biochar + biofertilizer (*B. thuringiensis, P. putida, K.* variicola) treatment gave the greatest number of nodules with a slight and significant difference of 3% increase above insecticide treatment and 40% increase over the control which had the lowest number of nodules. The high number of nodules recorded by biofertilizer (*B. thuringiensis, P. putida, K. variicola*) + biochar is probably due to the production of phytohormones especially bacteria Indole Acetic Acid IAA which promotes greater number of nodules (Remans *et al*., 2008).

Effect of the treatments on the number of pods per plant during the field experiment

The number of pods per plant of cowpea plants is as shown in table 5. There was no significant difference in the number of pods per plant among the treatments. SSP treatment gave the highest number of pods and this was followed by biofertilizer + biochar treatments. However, SSP had a non-significant increase of about 30% over the performance of the biofertilizer + biochar treatments. The lowest number of pods was recorded by the biofertilizer treatments. The high number of pods recorded in biofertilizer + biochar shows their ability to effectively compete favorably with SSP in nutrient supply and thereby increasing number of pods in cowpea (Rodriguez et al., 2009; Singh *et. al.*, 2011).

Table 5: Effect of treatments on the number of nodules and number of pods of cowpea on the field

Treatments	Number of nodules	Number of pods
Control	6.0[e]	4.0[d]
Biofertilizer	8.0[d]	2.5[e]
Biofertilizer + Biochar	10.0[a]	9.5[b]
Biochar	9.3[b]	4.2[d]
SSP	8.7[c]	13.5[a]
Insecticide	9.0[b]	7.0[c]

CONCLUSION

Plant growth promoting rhizobacteria (PGPR) improved the growth and development of cowpea effectively as it was seen to improve plant heights, number of leaves per plant and shoot weight.. Biofertilizer (*Bacillus thuringiensis, Pseudomonas putida, Klebsiella variicola)* in combination with biochar treatments gave about 13% increase in plant height over the control and also recorded an increase of about 53% increase in number of leaves over the control. The influence of the biofertilizer + biochar was also seen on the soil pH while the organic matter content too was improved. This implies that the productive efficiency of PGPR (biofertilizers) can be optimized when used in combination with biochar. In future, they are expected to replace the chemical fertilizers, pesticides and artificial growth regulators which have numerous side-effects to sustainable agriculture. Further research and understanding of mechanisms of PGPR potentials would pave more way to find out more competent rhizobacteria strains which may work under diverse agro-ecological conditions.

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