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Growth and nodulation of cowpea (*Vigna unguiculata*) in response to compost and inoculation with *Glomus etunicatum* and *Bradyrhizobium*

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ABSTRACT

Growth and nodulation of cowpea (*Vigna unguiculata*) inoculated with *Glomus etunicatum* and *Bradyrhizobium* IRc 25^B were assessed in two cropping cycles in an Iwo series soil amended with farm waste compost in the greenhouse.

In the first cropping, separate or dual inoculation of the test crop with two types of microorganisms had no significant effect on plant height but the treatments led to varying increases in nodule number, plant tissue phosphorus (P), nitrogen (N) and percentage of N derived from the atmosphere. Vesicular-arbuscular mycorrhiza (VAM), when separately used, and rhizobium when used along with compost led to slight depressions in nodule weight. *Glomus etunicatum*, when used alone and in combination with rhizobium led to significant increases in root infection of the fungus but the root weights were not affected by the treatments.

In the second cropping cycle, compost alone or when used in combination with VAM or rhizobium led to significant increases in plant height, compared with the control. Dual or separate use of the 2 microorganisms with or without compost led to marked differences in nodule number and weight, shoot and root dry weight, plant tissue P and N and N derived from the atmosphere.

Pod number and seed weight were significantly increased by five fold over the control when the combination of VAM and compost was used. There was a progressive decrease in the nutrient levels after the first and second cropping. The endomycorrhiza used enhanced nutrient uptake while the added farm waste compost supplied additional nutrients.

Key words: Nodulation, cowpea, compost, inoculation, *Glomus*, *Bradyrhizobium*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a very important grain legume in sub-saharan Africa not only from the dietary viewpoint but because of its ability to withstand drought. The world production was estimated at 3 million tons/ha, out of which Nigeria production equals 1.7 million tons (Singh *et al.* 1997). However, yield potentials have not been fully realized due to inherent low fertility status of many tropical and sub-tropical soils and non-optimal utilization of the available soil nutrients by many cowpea varieties. In order to satisfy its nutrient requirements and increase yields, extraneous supply of some of the required nutrients such as N, P and K are made through inorganic fertilizer addition. However, prohibitive costs and problem of procurement of the fertilizers and the desire to reduce environmental pollution had made biological fertilization a rational option rather than the use of mineral fertilizers.

Positive response of cowpea to Bradyrhizobium and vesicular-arbuscular mycorrhizal inoculation is reported in literature. Chhabra *et al.* (1990) reported increases in cowpea plant height, total dry matter of root and shoot compared with the uninoculated controls. Extensive mycorrhizal development (53.78%) was also reported to have taken place in the roots of inoculated cowpea plants. Inoculation was also found to have increased the N, P and K contents significantly. In an experiment conducted to establish the role of VAM in the P nutrition and early growth of cowpea by Ikombo *et al.* (1991), an increase in dry matter yield and P content of whole cowpea top above that of non-mycorrhizal plants grown in sterilized soil was observed at 30 days after sowing (DAS). It was concluded by the authors that recovery of cowpeas from early P deficiency stress resulted from increased P absorption following the development of a mycorrhizal association with the roots. Other authors (Islam et al. 1980; Rajapakse, 1987) reported various increases in nodulation, N_2 fixation and yields of cowpea due to myicorrhizal inoculation.

In Nigeria, the use of farm waste compost to supply nutrients due to the unavailability of mineral fertilizer is now being practiced. Adebayo and Olayinka (1987) found growth response to organic fertilization in cowpea. It should however, be noted that nutrients in composts are mainly in organic forms not readily available for crop uptake. Adequate mobilization of these nutrients mediated by VAM fungus for cowpea uptake may enhance growth and increase grain yields. Reports of such response to VAM fungal-Bradyrhizobium-cowpea symbiosis in a soil enriched with farm waste composts are poorly documented.

The objective of this study here reported therefore, was to evaluate the response of cowpea to separate or dual inoculation of VAM and Bradyrhizobium in a soil amended with farm waste compost.

MATERIALS AND METHODS

The two cropping cycle greenhouse study was carried out on an Iwo series loamy sand soil (alfisol) containing the following properties in Table 1.

Vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus etunicatum* and a Bradyrhizobium strain IRc 25^B peat-based culture were used as the test microorganisms. The VAM was supplied by the second author while the Bradyrhizobium strain was obtained from the International Institute of Tropical Agriculture (IITA) Ibadan. They were selected on their proven effectiveness in enhancing nutrient uptake and N_2 fixation respectively when in symbiotic association with cowpea in preliminary studies (Taiwo umpublished). In treatments where *G. etunicatum* was used, soil culture of the fungus containing infective progagules that were made up of external hyphae, spores and VAM fungal root fragments was mixed with seeds before planting. The Bradyrhizobium strain used was grown in a Yeast Extract Mannitol (YEM) broth on a gyratory shaker and centrifuged at 5000g three times in sterile distilled water by resuspension and recentrifugation. The cells were finally suspended in sterile distilled water at a density of about 10^7 to 10^8 cells per ml as determined by plate count on YEM agar. The suspension was inoculated into sterile peat at 40mls/5g of peat (Thompson per. Comm.). The peat culture, containing about 10^6 to 10^7 cells/gram peat (MPN counts, Vincent, 1970) was inoculated on seeds at the rate of 5g inoculant/kg seeds. Dual inoculation of the two microorganisms was similarly carried out at planting. Compost application (equivalent of 2.5t/ha) was made a week before planting. The compost contained the following nutrients, namely: N, 2.43% and total P, 1.7%. The K, Ca, Mg and iron (Fe) levels were 2.51, 2.74, 0.39 and 0.69% respectively. Zinc (Zn), manganese (Mn) and copper (Cu) were 147, 300 and 34ppm respectively. In one of the treatments, compost was used without any inoculant. The uninoculated and unfertilized cowpea seeds served as the control.

Table 1. Precropping Soil Properties

Parameters	Values
% N	0.17
Available P (mg/kg)	2.03
% C	1.60
Exchangeable catirons (meq/100g soil)	
Ca	4.17
Mg	0.73
Na	0.31
K	0.51
H^+	0.08

Five kilogrammes of the soil in plastic pots were used in each case and each of the treatments (8) were replicated 6 times, to provide for sampling at both physiological and maturity stages. The treatments were laid out in a Compleletely Randomised Block Design(CBRD). At the end of first week cropping cycle, plants were harvested and the parameters measured. Soil in each pot was not sieved nor further processed again as was done before cropping in order not to remove root fragments or decaying composts. Soil samples were taken from each pot for nutrient analysis. Planting was subsequently carried out with 3 viable seeds, the seedlings of which were later thinned to 2 per pot. At the end of the second six week growing cycle, plant and soil samples were taken from 3 of each of the 6 replicates for each treatment and relevant measurements of the parameters taken.

Two weeks after planting (WAP), the seedlings were thinned to 2 per pot and ammonium sulphate with 10.09% ^{15}N atom excess (a.e) was applied in solution into each pot. Non-nodulating clay soybean used as the reference crop, was equally treated with labelled N. The heights of the above ground portion of the plants were taken 4 WAP. At 6 WAP, the dry matter produced in the shoots, roots and nodules were recorded. The dry shoots were ground and analysed for %N on a kjeldahl digest (Eatin, 1978) and the N isotope ratio analysis was performed on emission spetrometer (Fiedler and Proksch, 1975). Atmospheric N_2 fixed was estimated using isotope dilution method (McAuliffe *et al.*, 1958; Fried and Middleboe, 1977). Plant tissue P was determined after dry ashing and digestion with HCl by the vanadomolybdate method. Clearing and staining of fungal roots for the determination of rates and intensities of root infected were by the method of Kormanic *et al.* (1980). Intensities were scored as follows: Intensity 1 = small infection sites widely scattered along roots; intensity 2 = larger infection sites more uniformly distributed through the infested roots rarely coalescing, and intensity 3 = feeder roots almost solidly infected with no easily indentified, isolated patches of infection.

All data collected were subjected to Analysis of Variance (ANOVA). Treatment comparisons were carried out by the Duncan's Multiple Range Test (Steel and Torrie 1960).

RESULTS AND DISCUSSION

Declining yield of cowpea grown under rainfed conditions in the Guinea savanna zone of sub-humid and semi-arid soils of Nigeria had been attributed mainly to moisture stress and low nutrient fertility status. Even distribution of rainfall and adequate biological and/or chemical fertilization have, in many cases, led to improved yields. In this greenhouse study, biological fertilization with Bradyrhizobium and endomycorrhiza led to varying increases in the growth parameters assessed. For example, there was a general increase in nodule number in response to compost application when used alone or when combined with Bradyrhizobium or VAM (Table 2). The nodule weight was over 70% heavier when fertilized with compost and inoculated with endomycorrhiza. The roots were also more heavily infected when inoculated with VAM when compared with the uninoculated control. This indicated that the nutrients in soil and compost were more optimally utilized than the variant without compost or VAM. Islam *et al.* (1980) working on Alagba and Araromi series soil reported rapid VAM fungal infection of cowpea roots. Nodulation, N_2 fixation and enhanced utilization of P was also reported due to inoculation with VAM fungus. However, there were slight depressions in nodule dry mass in the first cropping cycle when VAM alone or when the combination of compost and Bradyrhizobium was used. This did not have much adverse effect on the other parameters used except shoot dry weight. Dual inoculation of the cowpea with *G. etunicatum* and Bradyrhizobium strain led to a 20% non-significant increase in dry matter weight (DMW) of plant top and about 15% increase in the same parameter when Bradyrhizobium was used along with compost. Dual inoculation similarly led to over 200% significant increase in DMW of root when compared with the control treatment. Various increases recorded in nodulation, DMW of shoot and root were reflected in plant tissue N, N_2 derived from the atmosphere and plant tissue P. However, plant tissue K only increased over the K content in control plant when the test crop was inoculated with VAM fungus or Bradyrhizobium or when each of the microorganisms was used along with compost. Dual inoculation or compost application plus the 2 microorganisms led to depression in the amount of K in plant tissue. Berta *et al.* (1990) attributed a similar observation to competition between fungus and the roots for photosynthates. In most cases, there was a larger shoot: root dry weight ratio in favour of the shoots in mycorrhiza plants.

Table 2. Effect of compost, mycorrhizal and Bradyrhizobial inoculation on cowpea (*Vigna unguiculata*) plant height, nodulation, root infection rate and intensity of mycorrhizal fungus in the 1st cropping cycle.

Treatments	Height (Cm)	No. No.	Nod. Wt (g)	Root infec.(%)	Infec. Intensity
Control	76.1^a	49.0^b	0.4^{ab}	9.7^c	1
VAM	88.0^a	55.3^{ab}	0.3^b	38.3^b	1
VAM+IRc	86.3^a	52.0^{ab}	0.5^{ab}	61.7^a	2
VAM+IRc+Comp.	83.7^a	64.3^{ab}	0.5^{ab}	61.7^a	2
IRc	86.5^a	55.3^{ab}	0.6^{ab}	18.3^{cd}	1
Comp	99.5^a	60.0^{ab}	0.5^{ab}	21.7^c	1
VAM+Comp	97.0^a	62.3^a	0.7^a	40.0^b	1
IRc+Comp	102.0^a	52.0^{ab}	0.3^b	11.7^{dc}	1

Value within column followed by the same letter are not significantly different at the 5% level according to Duncun Multiple Range Test.

In the second cropping cycle, a more clear-cut effect for all treatments was obtained (Table 3). Application of compost alone or when used along with *G. etunicatum* or Bradyrhizobium resulted in significant increase in height. Application of compost alone led to about 70% increase in height over the control.

As regards nodulation, increases obtained in nodule number in response to the treatments, ranged between 300 and 1500% with the combination of *G. etunicatum* inoculation and compost application giving the highest value. The same treatment also led to the highest nodule mass production. The infection rate of VAM fungus on the roots increased with inoculation. Dual inoculation led to the heaviest infection of the roots by the fungus. It is noteworthy

Table 3. Effect of compost, mycorrhizal and Bradyrhizobial inoculation on N.P.K. content of shoot and root dry weight of cowpea (*Vigna unguiculata*) in the 1st cropping cycle.

Treatments	Shoot DW (G)	Root DW (g)	% N in shoot	% NdfA	% P in shoot	% K in shoot
Control	12.2^a	0.9^b	2.3^d	40.0^b	0.6^a	2.5^{ab}
VAM	11.3^a	0.9^b	2.8^{bc}	59.0^{ab}	0.8^a	2.9^a
VAM+IRc	14.7^a	2.9^a	2.9^b	66.0^a	0.8^a	2.3^b
VAM+IRc+Comp.	12.8^a	1.7^b	2.6^c	70.0^a	0.7^a	2.0^c
IRc	10.2^a	1.2^b	2.9^b	69.0^a	0.8^a	2.5^{ab}
Comp.	12.5^a	1.4^b	3.8^a	55.0^{ab}	0.8^a	2.5^{ab}
VAM+Comp.	10.5^a	1.5^b	3.6^a	60.0^a	0.8^a	2.6^{ab}
IRc+Comp.	13.9^a	1.5^b	2.8^{bc}	61.0^a	0.9^a	2.8^a

Values within column followed by the same letter are not significantly different at the 5% level according to Duncan Multiple Range Test.

that fungal hyphae and spores were found even in the roots of the uninoculated control plants. This observation indicated the presence of endomycorrhiza fungi in soil prior to inoculation. Rate of VAM infection depends on the capacity of the fungus to use carbon substances from host roots (Pearson and Jakobsen, 1993). This infection may have possibly enhanced nutrient uptake from soil and optimal utilization of organic nutrients embeded in compost where it was applied.

Data in Table 4 showed that DMW of shoots was significantly increased in response to the treatments over the control. The combination of Bradyrhizobium inoculation and compost application resulted in over 300% increase over check. Separate inoculation with Bradyrhizobium or VAM fungus plus compost application led to significant increase in DMW of root over the control. Positive responses to VAM inoculation have been widely reported (Chhabra *et al.* 1990; Aziz and Habte 1999; Islam and Ayanaba 1981; Berta *et al.* 1990;Ikombi *et al.*1991; Islam *et al.* 1980 and Rajapkse 1987).

The plant tissue N data showed that separate inoculation with VAM fungus or dual inoculation with the two microorganisms with or without compost application led to significant enchancements in N uptake. The values of percent N derived from the atmosphere (%NdfA) indicated that dual inoculation with or without compost or separate inoculation with compost led to significant increases in the parameter. Separate use of Bradyrhizobium or compost similarly enhanced %NdfA. The tissue P was also significantly increased over the control by separate or dual inoculation of cowpea with or without compost. While Bradyrhizobium inoculation along with compost application resulted in plant tissue P increase, the same treatment had no observable effect on plant tissue K. But inoculation with VAM alone led to increase in the parameter.

Table 4. Effect of compost, mycorrhizal and Bradyrhizobial inoculation on cowpea (*Vigna unguiculata*) plant height, nodulation, root infection rate and intensity of mycorrhizal fungus in the 2nd cropping cycle.

Treatments	Height (Cm)	No. No.	Nod. Wt (g)	Root infec.(%)	Infec. Intensity
Control	37.3^{c}	3.3^{c}	0.03^{c}	11.6^{c}	1
VAM	43.8b^{c}	15.6^{d}	0.08^{bc}	50.0^{b}	1
VAM+IRc	45.6b^{c}	27.3^{c}	0.09^{bc}	65.0^{a}	2
VAM+IRc+Comp.	45.9b^{c}	30.3^{bc}	0.13^{bc}	68.3^{a}	2
IRc	44.9b^{c}	28.0 ^{c}	0.07^{bc}	28.3^{c}	1
Comp	63.3^{a}	28.6^{bc}	0.16^{ab}	35.0^{c}	1
VAM+Comp	50.6^{b}	49.6^{a}	0.24^{a}	45.0^{b}	1
IRc+Comp	54.1^{ab}	38.6^{b}	0.16^{ab}	13.3^{d}	1

Value within column followed by the same letter are not significantly different at the 5% level according to Duncun Multiple Range Test.

Separate inoculation with either of the two microorganisms with or without compost led to significant increases in pod number, with the compost application leading to over 400% increase. Even though separate use of VAM had the least effect on this parameter, an increase of over 100% over the control was recorded.

The seed weight data similarly showed varying increases in response to the treatments. The capability of VAM to mobilize compost nutrients for increase grain yield was shown with an increase of over 40% over the control from the soil environment especially at a time when the available nutrients in soil were becoming depleted. For example, the Bradyrhizobium was able to supply over 70% of cowpea nitrogen requirement in the second cropping as against less than that amount in the first cropping. Though, the DMWs of shoot were generally lower in the second cropping cycle, it was observed that about 200% increase was obtained in this parameter (when VAM inoculation was carried out) over the uninoculated control in the same cropping cycle as against the depressive effect of VAM uninoculated variant recorded in the first cropping. The seemingly lack of response in some of the treatments may be attributed to adequate nutrients supply. In this experiment, the post-harvest soil analysis showed that percent carbon (%C) and N level after the 1^{st} cropping cycle (Fig. 1 and 2) indicated that there were some significant increases in these nutrients

Table 5. Effect of compost, mycorrhizal and Bradyrhizobial inoculation on N.P.K. content of shoot and root dry weight of cowpea (*Vigna unguiculata*) in the 2nd cropping cycle.

Treatments	Shoot DW (g)	Root DW (g)	% N in shoot	% NdfA	% P in shoot	% K in shoot	Pod No.	Seed wt. Wt. (g)
Control	2.0^{c}	0.9^{c}	1.2^{b}	55.0^{b}	0.4^{b}	1.0^{b}	1.3^{d}	2.3^{c}
VAM	5.4^{b}	1.0^{bc}	1.9^{a}	68.2^{ab}	0.6^{a}	1.2^{a}	2.6^{c}	4.6^{dc}
VAM+IRc	4.9^{b}	1.2^{bc}	1.8^{a}	71.2^{a}	0.6^{a}	1.0^{b}	4.0^{b}	7..2^{bc}
VAM+IRc+Comp.	5.4^{b}	0.9^{c}	1.9^{a}	75.5^{a}	0.6^{a}	1.1^{b}	3.3^{bc}	5.8^{cd}
IRc	5.3^{b}	2.5^{a}	1.7^{ab}	72.4^{a}	0.5^{ab}	1.1^{b}	4.6^{b}	8.4^{b}
Comp.	5.7^{b}	1.3^{bc}	1.7^{ab}	69.6^{a}	0.5^{ab}	1.0^{b}	4.3^{b}	8.3^{b}
VAM+Comp.	5.9^{b}	1.6^{b}	1.6^{ab}	70.4^{a}	0.7^{a}	1.0^{b}	7.0^{a}	11.8^{a}
IRc+Comp.	8.1^{a}	1.4^{bc}	1.6^{ab}	71.5^{a}	0.6^{a}	1.1^{a}	4.6^{b}	8.7^{b}

Values within column followed by the same letter are not significantly different at the 5% level according to Duncan Multiple Range Test.

over the initial levels. These increases may possibly be attributed to root fragments and the compost applied. This was not however the case at the end of the second cropping cycle when significant decreases were generally recorded. It should, however be noted that the use of VAM fungus led to significant enhancement in nutrient uptake in both

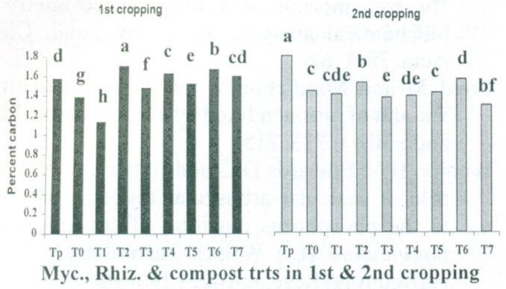

Fig. 1: Percent C in soil after 1st & 2nd crop harvest Tp, pre-cropping; T0, control; T1, Myc.; T2, Myc. + Rhiz; T3, Myc. + Rhiz+comp.; T4, Rhiz.; T5, comp.; T6, Myc.+comp.& T7, Rhiz.+comp.

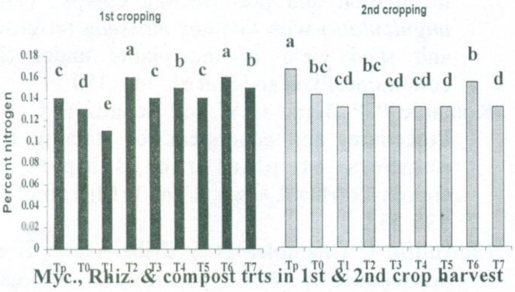

Fig. 2: Percent Nitrogen in soil after 1st & 2nd crop harvest Tp, pre-cropping; T0, control; T1, Myc.; T2, Myc. + Rhiz; T3, Myc. + Rhiz+comp.; T4, Rhiz.; T5, comp.; T6, Myc.+comp.& T7, Rhiz.+comp.

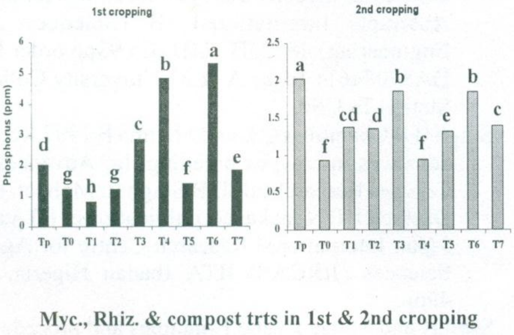

Fig. 3: P (ppm) in soil after 1st & 2nd crop harvest. Tp, pre-cropping; T0, control; T1, Myc.; T2, Myc. + Rhiz; T3, Myc. + Rhiz+comp.; T4, Rhiz.; T5, comp.; T6, Myc.+comp.& T7, Rhiz.+comp.

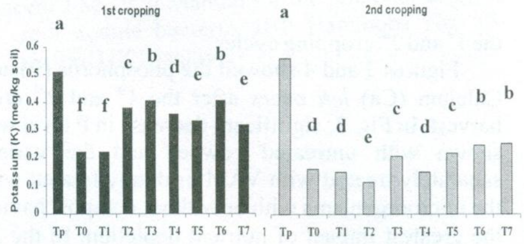

Fig. 4: K (meq/kg soil) in soil after 1st & 2nd crop harvest. harvest. Tp, pre-cropping; T0, control; T1, Myc.; T2, Myc. + Rhiz; T3, Myc. + Rhiz+comp.; T4, Rhiz.; T5, comp.; T6, Myc.+comp.& T7, Rhiz.+comp.

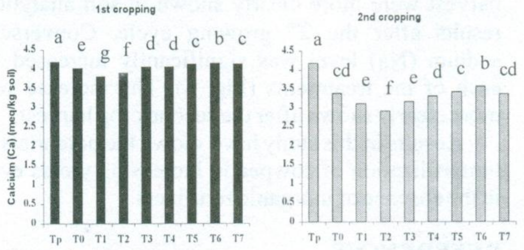

Fig. 5: Ca (meq/kg soil) in soil after 1st & 2nd crop harvest. harvest. Tp, pre-cropping; T0, control; T1, Myc.; T2, Myc. + Rhiz; T3, Myc. + Rhiz+comp.; T4, Rhiz.; T5, comp.; T6, Myc.+comp.& T7, Rhiz.+comp.

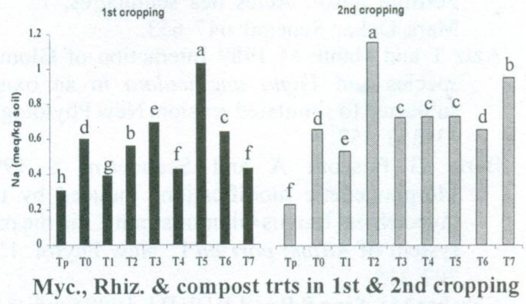

Fig. 6: Na (meq/kg soil) in soil after 1st & 2nd crop harvest. harvest. Tp, pre-cropping; T0, control; T1, Myc.; T2, Myc. + Rhiz; T3, Myc. + Rhiz+comp.; T4, Rhiz.; T5, comp.; T6, Myc.+comp.& T7, Rhiz.+comp.

the 1st and 2nd cropping cycles.

Figures 3 and 4 showed the phosphorus (P) and Calcium (Ca) *left overs* after the 1st and 2nd crop harvest. In Fig. 3, significant decrease in P level was shown with untreated cowpea and the variant separately treated with VAM or dually treated with the microorganisms with or without compost having the greatest impact of nutrient depletion. In the 2nd cropping, separate inoculation with mycorrhiza was combined with compost led to significant reduction in soil P while P levels in soil dually treated with the 2 microorganisms with or without compost, Rhizobium with or without compost alone increased. For the soil Ca, and potassium (K) (Figs. 4 and 5), significant reduction in their levels after the 1st crop harvest were more clearly shown in soil analytical results after the 2nd growing cycle. Conversely, sodium (Na) level was significantly increased by each of the treatments (Fig. 6). The increase was more clearly shown after the second crop harvest.

Results in this study have shown the potentials of biofertilization of cowpea in increasing yields even in the absence of inorganic fertilizers.

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