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Effect of Carrier-based *Rhizobium leguminosarum* Inoculants on the Soil Physicochemical Characteristics, Nodulation and Growth of Soybean

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Authors' contributions

This work was carried out in collaboration between all authors. Author ONC designed the study and wrote the protocol. Author OSC supervised the research and managed the literature research. Author ODK performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate the effect of carrier-based inoculants on the soil physicochemical characteristics, nodulation and growth of soybean.
Study Design: Examination of different carrier.
Place and Duration of Study: Department of Applied microbiology and Brewing, Nnamdi Azikiwe University, Awka, from February 2014 and March, 2015.
Methodology: Field experiment of soybean mixed with sawdust and cassava peels inoculated with *Rhizobium leguminosarum*. Growth parameters of soybean and physicochemical characteristics of the soil were checked after 40 days of planting.

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Results: There was significant increase in the calcium, magnesium and potassium contents, total nitrogen and conductivity of the soil after the field experiment. The shoot height was significantly different from the control at $p \leq 0.05$, though the growth parameters were not statistically significant from the control. Nonetheless, there was marked increase over the control in nodulation and other growth parameters with the highest found in soybean plant inoculated with cassava-based *Rhizobium leguminosarum*.
Conclusion: This study thus showed since the carrier-based *Rhizobium leguminosarum* inoculants increased the physicochemical characteristics of the soil, nodulation and soybean growth; therefore such inoculant is an effective alternative to chemical fertilizers in enhancing the plant growth.

Keywords: Carrier-based inoculants; soybean; growth parameters; field experiment.

1. INTRODUCTION

Biofertilizers are products containing active or latent strains of soil microorganisms, either bacteria alone or in combination with algae or fungi that increase the availability and uptake of mineral nutrients [1]. In general, they contain free-living organisms associated with root surfaces but they may also include endophytes, microorganisms that are able to colonize the intercellular or intracellular spaces of plant tissues without causing apparent damage to the host plant. The use of bio-fertilizers can prevent the depletion of the soil organic matter [2].

The use of microbial inoculants as a bio-fertilizer do not just increase crop yield, but it's environment-friendliness and can be utilized as an alternative or to complement to inorganic nitrogen fertilizer [3]. The application of selected carrier materials for the bacterial inoculants proves to be beneficial to protect the bacteria and have long been practiced. The success of microbial inoculation to promote growth of plant is vastly influenced by the number introduced into the soil [4].

Simultaneously the selected carrier materials must also have the properties such as being cost effective, ability to dissolve well in water so that bacteria can be released and be able to tolerate harsh environmental conditions [5].

Leguminosae has major impacts on agriculture, environment, animal/human nutrition, and health, of which soybean [*Glycine max*] is one of the world's most important pulse crops. It accounts for 29.7% of the world's processed vegetable oil and is rich in dietary protein both for human food and animal feed [6]. Soybean production worldwide is estimated to be in the range of 12–20 x 10^6 ha [7].

This study was therefore conducted to evaluate the effect of carrier-based *Rhizobium leguminosarum* inoculants on the soil physicochemical characteristics, nodulation and growth of soybean.

2. MATERIALS AND METHODS

2.1 Collection of the Carriers

Cassava peel was collected from a local farmer in Awka while the sawdust was obtained from the timber processing complex, Umuokpu, both in Anambra State, Nigeria. They were all oven-dried at 70ºC for 24 hours, then ground; sundried and sieved to obtain the particle sizes ranging from 0.5 to 3.0 mm.

2.2 Isolation of *Rhizobium* Strain from Soil

Soil sample was collected from Nnamdi Azikiwe University, Awka, Anambra State. The soil sample was stored in sterile polythene bags and transported to the laboratory. A ten-fold serial dilution of one g of the soil sample was done, with one ml aliquots of 10^5, 10^6, 10^7 and 10^8 dilutions plated on yeast extract mannitol agar and incubated for 48 hours at room temperature. Subculturing was done on Congo red yeast extract mannitol agar and incubation was carried out at room temperature for 24 hours to obtain pure culture. Rhizobium strain was characterised biochemically and genetically for further confirmation of the isolate.

2.3 Determination of the Physicochemical Characteristics of the Soil in the Experimental Field Site

Total nitrogen of the soil was determined by the macro Kjeldahl digestion method [8]. Available phosphorus was determined by the Bray No. one method [9] and determined by blue molybdocolometric method [10]. Metal analysis for calcium, magnesium, potassium, zinc, iron,

cadmium, chromium was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to the method of APHA 1995 (American Public Health Association).

Determination of soil conductivity

a) Calibration of instrument using 0.01 N KCl solution: 74.5 mg KCl was dissolved in 100 mL conductivity water free from CO_2. This is the standard reference solution whose conductance is 1412 µS/cm at 25°C. The KCl solution was transferred into a 100 mL beaker. The electrode was dipped in it. (If the conductivity meter does not read 1412 µS/cm, then adjust the instrument to read 1412).
b) Conductivity measurement: Five g of the soil was weighed into a beaker and added 25 mL distilled water, stirred well for about 5-10 minutes and allowed to settle.
c) The distillate was taken into another beaker and used for the conductivity measurements. The sample conductivity was read directly from the conductivity meter. Taken into another beaker and used for the conductivity measurements. The sample conductivity was read directly from the conductivity meter.

Determination of organic matter- One gram of the air-dried ground soil sample was weighed and transferred to a 500 mL Erlenmeyer flask. The sample was added 10 ml of 0.167 M potassium dichromate ($K_2Cr_2O_7$) by means of a pipette. Concentrated sulphuric acid (H_2SO_4) (20 ml) was added by means of a dispenser and swirled gently to mix. This was allowed to stand for 30 minutes. The suspension of the resulted mixture was diluted with 200 ml of deionized water to provide a cleaner suspension for viewing the endpoint. Ten ml of 85% phosphoric acid (H_3PO_4) and 0.2 g of sodium fluoride (NaF) were added. 10 drops of ferroin indicator were added prior to titration to avoid deactivation by adsorption onto clay surfaces. The resulting mixture was titrated against 0.5 M ferrous ammonium sulphate solution to a burgundy endpoint. The colour of the solution at the beginning was dark green but changes to wine red at the end point.

$$\text{Percentage of Oxidizable Organic Carbon (w/w)} = \frac{(V_{blank} - V_{sample}) \times 0.3 \times M}{Wt}$$

Percentage Total Organic Carbon (w/w) = 1.334 x % Oxidizable Organic Carbon

Percentage Organic Matter (w/w) = 1.724 x % Total Organic Carbon

where,

M = Molarity of ferrous ammonium sulphate solution (~ 0.5M).
V_{blank} = Volume of ferrous ammonium sulphate required to titrate the blank (ml); *blank* contains all reagents but no soil.
V_{sample} = Volume of ferrous ammonium sulphate solution required to titrate the test samples (mL).
Wt = Weight of air-dried soil (g).

$0.3 = 3x10^{-3} \times 100$, where 3 is the equivalent weight of carbon [10].

2.4 Cultivation of *Rhizobium leguminosarum*

*T*he cultures were grown in 50 mL nutrient broth and incubated for 4 days on a rotary shaker at room temperature.

2.5 Preparation of Carrier-based Inoculums

Fifty grams (50 g) each of the sterile carriers sterilized by autoclaving at 121°C for 15 minutes were weighed and collected separately in 100 ml beaker. They were each mixed with 50 ml nutrient broth containing the *Rhizobium leguminosarum* inoculum (1.4×10^7 cfu/ml) in a conical flask and then mixed together with sterile spatula. The conical flasks were stoppered with cotton wool and kept for 24 hours for curing. During this time, acclimatization of the *Rhizobium leguminosarum* with the carrier was allowed.

2.6 Preparation of Soybean Seeds

Soy bean seeds were bought from Eke Awka market, Awka, Anambra State, Nigeria and taken to the laboratory for surface sterilization. They were first rinsed in 95% alcohol for 10 seconds to remove waxy material and trapped air. The seeds were immersed completely for 3-5 minutes in 2.5% sodium hypochlorite solution. Seeds were rinsed at least five times with sterile distilled water. In order to ensure that all the applied inoculum stuck to the seed, the required quantity of inoculant was suspended in 1:1 ratio in 10% sugar solution which served as an adhesive. The thick slurry of the inoculant was gently mixed with

the seed so that all the seeds received a thin coating of the inoculants.

2.7 Cultivation Experiment

The field experiment was carried out at the premises of the Faculty of Biosciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria. The completely randomized experimental design with three replicates per treatment was adopted. Fifty surface-sterilized soybean seeds were added per 50 mL of sawdust-based *Rhizobium leguminosarum* and cassava peel-based *Rhizobium leguminosarum*, and thoroughly mixed. Control treatments consisted of surface-sterilized seeds without any carrier-based inocula. All inoculations were done just before planting to maintain the viability of the bacterial cells. Seeds were allowed to air dry for a few minutes and were then sown at the required rate and spacing with 9 seeds per plot. Plots with uninoculated seeds which served as control were planted first to avoid contamination. The inoculated and uninoculated seeds of the varieties were then planted at a depth of 4cm and at a spacing of 10cm between plants. Seeds were immediately covered with soil after sowing.

2.8 Measurement of Growth Parameters of the Soy Bean Plant

Three plants from each plot were randomly selected for height measurement which was taken after 40 days of planting. The height measurements for shoot and root were measured using a calibrated centimeter ruler. The nodules were counted. The number of leaves per plant uprooted were also counted. The nodule fresh weight was measured with a weighing balance while the dry weight was measured after they were oven-dried at 65°C for 24 hours.

2.9 Determination of the Physicochemical Characteristics of the Soil after 40 days of Inoculation with *Rhizobium leguminosarum*

These characteristics (pH, total nitrogen, conductivity, calcium, magnesium, potassium, organic matter) were re-assayed after a 40-day planting period with sawdust- and cassava peel-based *Rhizobium leguminosarum.*

2.10 Statistical Analysis

The results of the growth parameters collected were subjected to a one-way Analysis of Variance to ascertain the level of significance of the carrier-based inocula against the control using SPSS v.16.

3. RESULTS AND DISCUSSION

All carriers had high water holding capacity and free from toxic materials, easily available and also had optimal moisture content for *Rhizobium* strain. There was also increase in the pH, calcium, magnesium, potassium and organic matter contents of the soil containing carrier based *Rhizobium leguminosarum* inoculant after 40 days of planting (Table 2). The results of the field experiment carried out on soybean seeds inoculated with carrier based *Rhizobium leguminosarum* are illustrated in Figs. 1 – 5. The longest shoot and root lengths were observed in cassava based *Rhizobium leguminosarum* with the control having the shortest shoot and root lengths. The shoot length was significantly different from the control at $p \leq 0.05$, even though other growth parameters were not statistically different from that of the control. Nonetheless, there was an increase in other growth parameters such as root length and nodule number of carrier based inocula treatment over control. The highest root weight was observed in sawdust based *Rhizobium leguminosarum* with the control having the lowest weight. Significant increase in nitrogen content may be due to contribution of total pool of nitrogen in the soil by the legume [11]. Higher P content may be due to inoculation and availability of P nutrients in soil by microbes and also nutritional elements of the soil. There was also increase in the pH and calcium, magnesium, potassium and organic matter contents of the soil containing carrier based *Rhizobium leguminosarum* inoculant after 40 days of planting, while a significant decrease was noticed in the physicochemical characteristic of the soil containing the uninoculated seed. High number of nodules and leaves were observed in soybean seed pelleted with carrier based *Rhizobium leguminosarum* in comparison to the control (Figs. 2 and 3). A significant increase in nodule number as a result of rhizobia inoculation due to more competitive ability of microbes in carrier than in pure culture against native rhizobial population [12]. This proves that carrier-based *Rhizobium* inoculants have positive impact on nodulation of soybean plant. Cassava peel-based *Rhizobium leguminosarum* had the highest effect on the shoot and root height, shoot and root dry weight, shoot and root fresh weight which make up the growth parameters of

soybean (Figs. 1, 4 and 5), this could be due to the higher concentrations of essential elements in cassava peel [13].

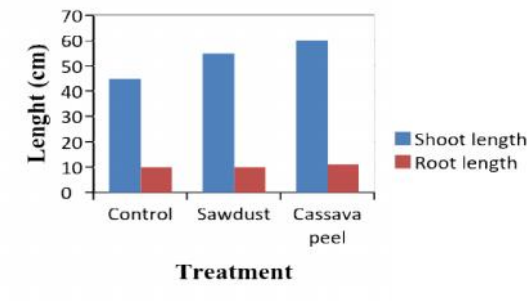

Fig. 1. Shoot and root height after 40 days of planting

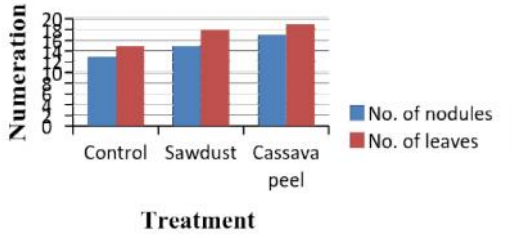

Fig. 2. Nodule and leaf number after 40 days of planting

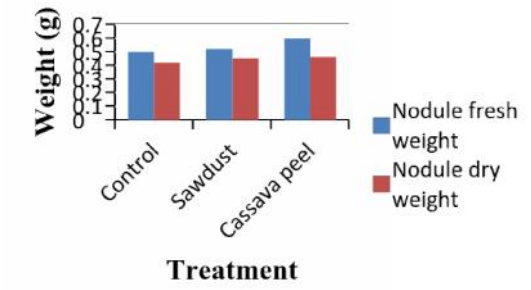

Fig. 3. Nodule weight (fresh and dry) after 40 days of planting

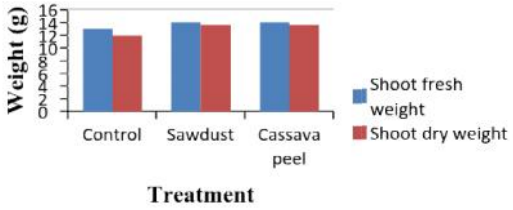

Fig. 4. Shoot weight (fresh and dry) after 40 days of planting

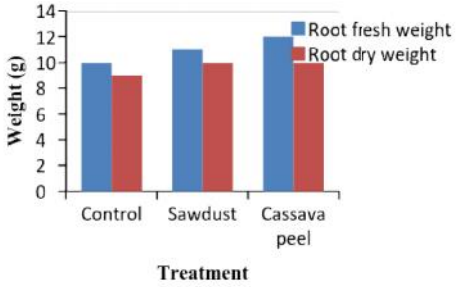

Fig. 5. Root weight (fresh and dry) after 40 days of planting

Table 1. Physicochemical characteristics of the soil sample in the experimental field site before the field experiment

Characteristics	Values
pH	6.65
Total nitrogen	1.57
Conductivity (MS/cm)	5.24
Calcium (ppm)	9.25
Magnesium (ppm)	19.08
Potassium (ppm)	8.59
Organic matter	18.26
Texture	Sandy clayey

Table 2. Physicochemical characteristics of the soil at the end of the field experiment

Characteristics	Cassava-based treatment values	Sawdust-based treatment values	Control treatment values
pH	6.68	6.66	6.52
Total nitrogen (%)	2.37	2.06	1.51
Conductivity (MS/cm)	5.45	5.33	5.15
Calcium (ppm)	9.56	9.37	9.50
Magnesium (ppm)	20.24	20.04	19.21
Potassium (ppm)	9.49	9.01	8.23
Organic matter (%)S	25.00	21.00	15.00

4. CONCLUSION

The objective of this study which was to find the effect of carrier based *Rhizobium leguminosarum* inoculants on the soil physicochemical characteristics, nodulation and growth of soy bean was accomplished successfully. There was significant increase in the growth parameters examined and also increase in physicochemical characteristics of soil after inoculation with carrier-based *Rhizobium* inoculant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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