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Growth and Yield Components of *Pisum Sativum* L. (Pea) in Response to *Rhizobium* Bio-fertilizer Supplemented with Carrier Materials (Earthworm Castings and Poultry Litter)

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The indiscriminate use of chemical fertilizers to increase the soil nutrients and the use of pesticides is one major problem facing crop farming. Hence, the use of bio-fertilizers can be a very good complimentary to the chemical fertilizers as they not only promote crop growth and yield but also maintain soil health for sustainable agriculture. The growth and yield components of pea (*Pisum sativum* L.) in response to *Rhizobium* bio-fertilizer supplemented with earth worm casts and poultry litter was evaluated in this study. Soya bean (*Glycine max* L.) was cultivated to obtain the root nodules needed for the isolation of *Rhizobium* species. The nodules were sterilized, crushed, serial dilutions prepared, inoculated on Yeast Extract Mannitol Agar (YEMA) medium and incubated at 28±℃. Pure culture of *Rhizobium* was isolated, mass produced and then mixed with autoclaved earthworm casts and poultry litter and separately for application unto the experimental crops. The *Rhizobium* broth and the supplements were mixed in the ratio of 2 litres to 100 kg. Analyses of experimental soil, poultry litter and earthworm casts were carried out to determine their physico-chemical properties. Four treatments (three fertilizer types and control were replicated four times and arranged in a Randomized Complete Block Design (RCBD). These treatments are: Chemical fertilizer (CHF), *Rhizobium* bio-fertilizer plus poultry litters (BP), *Rhizobium* bio-fertilizer plus earthworm cast (BEC) and a negative control (without fertilizer). Ten kilogram of the fertilizer types each was applied to the ridges grouped into 4 plots (A, B, C and D) according to the fertilizer types and the control. Plant's growth and yield parameters of pea grown on soil amended with the bio-fertilizers supplemented with earthworm casts and poultry litter, inorganic fertilizers and the control were measured and compared. The results of the analysis of physico-chemical properties of the soil, earthworm casts and poultry litters reveal that experimental soil had a mean pH value of 6.23, while those of the earthworm cast and the poultry litter were 5.78 and 8.63 respectively. Poultry litter had the highest organic matter content of 58.25% followed by earthworm cast which had 6.00%, while the experimental soil had the least (1.41%). The percentage of nitrogen was highest in poultry droppings (3.20%), followed by earthworm casts (0.19%) and the least value (0.0 %) was obtained from the soil. The present findings showed an improvement in the growth and yield parameters of pea grown on soil amended with *Rhizobium* bio-fertilizers and chemical fertilizer over the control. Although, the highest improvement recorded in the growth and yield of pea was from bio-fertilizers supplemented with poultry droppings and earthworm casts, there was no significant difference at ($p>0.05$) in the growth and yield parameters of pea in relation to various fertilizer treatments. Hence, the total yield of pea seed was found to be uniform 2.4 t/ha each for all the treatments except in the control which had the lowest yield (0.7 t/ha). The outcome of this study is important in that farmers can fall back on *Rhizobium* bio-fertilizer for the cultivation of pea, since the inorganic fertilizers are very expensive such that most poor farmers cannot afford them.

Keywords: Growth, yield, *pisum sativum*, *Rhizobium*, bio-fertilizer, earthworm casting, poultry litter.

INTRODUCTION

For optimum plant growth, nutrients must be available in sufficient and balanced quantities (Chen, 2006). The most important constraint limiting crop yield in developing nations worldwide and especially among resource-poor farmers is soil infertility. Unless the fertility is restored in these areas, farmers will gain little benefit from the use of improved varieties and more productive cultural practices (Eifediyi and Remison, 2010). Therefore, maintaining soil quality can reduce the problems of land degradation, decreasing soil fertility and rapidly declining production levels that occur in large parts of the world needing the basic principles of good farming practices (Khosro and Yousef, 2012).

Soil infertility can be restored effectively through adopting the concept of integrated soil fertility management (ISFM) encompassing a strategy for

nutrient management based on natural resource conservation, biological nitrogen fixation (BNF) and increase efficiency of the inputs (Khosro and Yousef, 2012). Bio-fertilizers can be a very good complimentary to the chemical fertilizers as they not only kill the harmful insects but also the beneficial insects such as pollinators (Giri and Joshi, 2010). Vessy, (2003) and Rockhzadi *et al.* (2008) defined a bio-fertilizer as a substance which contained living microorganism which when applied to seeds, plant surfaces or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients (nitrogen, phosphorus). The practice of using excessive chemical fertilizers and agrochemicals not only accelerates soil acidification but also results contaminating ground water, the atmosphere and weakens roots (Chun- Li, 2014).

Bio-fertilizers are important components of integrated nutrients management (Itelima *et al*., 2018). These potential bio-fertilizers would play key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers (Eifediyi and Remison, 2010). They are cost effective, eco-friendly and renewable sources of plant nutrients to supplement chemical fertilizers in sustainable agricultural system (Khosro and Yousef, 2012). Beneficial microorganisms in bio-fertilizers accelerate and improve plant growth and protect plants from pests and diseases (El-yazeid *et al.,* 2007). When bio-fertilizers are applied to the seed and the soil, they increase the availability of the nutrient to the plant and increase the yields up to 10–20% without producing any adverse effect to the environment (Ritika and Uptal, 2013). Utilization of bio-fertilizers is one of the ways to increase crop production by naturally optimizing the nitrogen and phosphorus levels of the soil and by enriching the compost waste used as a natural fertilizer (Khosro and Yousef, 2012).

Bio-fertilizers are gaining momentum recently due to the increasing emphasis on maintenance of soil health, minimize environmental pollution and cut down on the use of chemicals in agriculture (Hari and Perumal, 2010). In rain fed agriculture, these inputs gain added importance in view of their low cost; as most of the farmers are small and marginal and cannot afford to buy expensive chemical fertilizers (Tulberk *et al*., 2017). Bio-fertilizers are also ideal input for reducing the cost of cultivation and for practicing organic farming. It can be easily found that bio-fertilizers are identified as plant extract, composted urban wastes, and various microbial mixtures with unidentified constituents, and chemical fertilizer formulations supplemented with organic compounds (Itelima *et al*., 2018).

However, bio-fertilizer is most commonly referred to the use of soil microorganisms to increase the availability and uptake of mineral nutrients for plants (Hari and Perumal, 2010).

Bio-fertilizers are classified based on the type of microorganisms they contain. These include: nitrogen fixing bio-fertilizer (NFB) e.g. *Rhizobium* sp, Phosphate Solubilizing bio-fertilizer (PSB) e.g. *Aspergillus* species, Phosphate Mobilizing Bio-fertilizers (PMB) e.g. *Mycorrhiza*, Potassium Solubilizing Bio-fertilizers e.g. *Aspergillus niger* and Growth Promoting Bio-fertilizers (GPB) e.g. *Pseudomonas* sp. are most common (Hari and Perumal, 2010). The main sources of bio-fertilizers are the bacteria, fungi and Cyanobacteria (blue-green algae) (Khosro and Yousef, 2012).

Bio-fertilizers are usually prepared as carrier based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy handling, long term storage and high effectiveness of bio-fertilizers (Khosro and Yousef, 2012). Various types of materials can be used as carrier for seed or soil inoculation. The carrier material should have good pH buffering capacity, easy to process and sterilized by either autoclaving or gamma radiation (Khosro and Yousef, 2012). Examples of carrier materials include: clay, mineral, diatomaceous soil, rice bran, wheat bran, peat, lignite, peat soil, humus, wood charcoal, animal manure, discarded feed as organic matter. However, clay, mineral and rice bran and animal manure are most often used as carrier materials (Ritika and Uptal, 2013).

Earthworm castings are an organic form of fertilizer produced from earthworms also known as vermicasts. Worm castings manure is essentially earthworm wastes (Hakim *et al*., 2010), otherwise known as worm “poo”. As the worms eat through compost, their waste creates an optimal soil enricher (Nikki, 2010). According to Datko (2012), worm casts have over 60 micronutrients and trace minerals, including calcium, magnesium, nitrogen, phosphates and potassium. Also, they act as barrier to help plants grow in soil where the pH levels are too high or too low (Datko, 2012).

In agriculture poultry litter is a mixture of poultry excreta, spilled feed, feathers, and material used as bedding in poultry operations (Enujeke, 2013). Historically, application for as fertilizer for crop is an old time practice. Poultry litter is a source of nutrients to the crops. It contains high level of nutrients such as N, P and C. Rhizobia are soil habitat bacteria which are able to colonize the legume roots and fix atmospheric nitrogen symbiotically. *Rhizobium* fixes 50–100 kg/ha nitrogen with legumes (Graham and Vance, 2001). *Rhizobium* association has been extensively exposed in the root nodules of legumes where they fix atmospheric nitrogen but recent studies also suggest that *Rhizobium* can exhibit Plant Growth Promoting (PGP) activities with certain non-legumes such as cereals. In India, Rhizobia bio-fertilizer was produce on a commercial scale (Giri *et al*., 2010). Studies by Ali and Kumar, (2006) on Mungbean *(Vigna radiata* L.) and soybean (Glycine max

L.) respectively found that *Rhizobium* culture significantly affected the growth and yield components like number of pod bearing branches per plant, number of pods per plant, number of seeds per pod and 1000-seedweight were significantly affected by inoculation. Another study by Ravikumar, (2012) on the effects of *Rhizobium* inoculation in *Vigna mungo* and *Vigna radiate* found that both *V. mungo* and *V. radiata* varieties inoculated plants possessed greater height, greater fresh weight, greater number of roots, nodules, greater number of leaves, shoots, pods, greater length of pods, greater seed weight, over their respective controls. The study by Tairo and Ndakidemi, (2013) on soybean found that plant height for field experiment increased with *Rhizobium* inoculation for the entire interval of the soybean growth. Also it was found that inoculation significantly increased the stem girth in the glasshouse and field experiments respectively. In another study by Nyoki and Ndakidemi (2014 a), rhizobia inoculation in cowpea significantly improved the plant height measured at four, six and eight weeks after planting (WAP) in both screen house and field experiments relative to the control treatment (Nyoki and Ndakidemi, 2014a).

Soya bean (*Glycine max* L.) is one of the most widely adopted grain legumes in the world. Soya bean is used in the production of oil and protein. World area soyabean cultivation was approximately 79, 410, 495 acres in 2010 (Fernia and Gudiny, 2014). Just like other legumes, *Rhizobium* forms a symbiotic association with the root nodules of soya bean. Nodulation of soya bean requires specific *Bradyrhizobium* species. In soils where the soya bean crop has not been grown previously, compatible populations of *Bradyrhizobium* are seldom available. The nitrogen demand of soya bean can be supplied via biological nitrogen fixation through inoculation with selected *Bradyrhizobium japonicum*, *B. elkanii* strains (Fernia and Gudiny, 2014).

Pea (*Pisum sativum* L.) is one of the most important ancient vegetable and belongs to the family Leguminaceae. It ranks third or fourth in world-wide production, amongst the grain legumes (Farrington, 1974). The pea generally called as legumes (pod bearing plants). Because, they are characterized by the pods with a single cavity ovary which splits along two margins when dry, legumes thus have the ability to improve the soil fertility and structure. The plants of pea are 35-60 cm tall. The plant is a short leaved, herbaceous and annual which climbs by leave let tendrils. The stem is slender, circular and weak. The root system is not strongly developed except tap roots. Peas are grown particularly on all types of soil from light sandy to heavy clay. Frequent irrigation tends to increase vegetative growth at the expense of pod formation (Mfilinge *et al*., 2014) peas have specific requirement in respect of seasonal changes in temperature during their growth cycle. Tulbek *et al.* (2017) reported that pea crop can grow within several climate and soil zones; however pea plants grow best on fertile, light, well-drained, and humus rich soils. These authors also reported that soil salinity and extreme acidity can be detrimental for pea production whereby the ideal soil pH for pea production is 6.5-7.0. Pea seeds are rich in protein, carbohydrate and dietary fibre, vitamins and minerals and can be utilized as milled ingredients such as proteins, starches, flours and fibres. Ingredients derived from peas provide unique attributes in food systems such as egg replacement solution in pasta, cakes, cookies, batters and breading systems; as high-protein ingredients in snack bakery, pasta and meal product. Consumption of whole peas reduces blood glucose, improves gastrointestinal health, and enhances satiety (Tulbek *et al.,* 2017).

The excessive use of chemical fertilizers to increase the soil nutrients and the use of agrochemicals (pesticides) is one major problem facing crop farming (Chen, 2006). The use of chemical fertilizers and agrochemical degrade the soil year after year making it difficult to sustain soil fertility (Santosh *et al.,* 2012). These chemicals not only cause immense damage to the soil but also create a chain of economic and ecological problem (Khosro and Yousef, 2012). This practice also risk contaminating ground water, the atmosphere and weakens the roots of plants and made them easy prey to unwanted diseases (Chun-Li, 2014). Moreover, due to the several health hazards associated with the consumption of food produced from chemical fertilizers, consumers preferences shift towards the use of the organic food grown without the use of chemical fertilizer. These potential biological fertilizers will play key role in productivity and sustainability of soil and protect the environment as eco-friendly and cost effective inputs for the farmers as reported by Khosro and Yousef (2012). Although bio-fertilizer can be produced from cheap waste materials which are abundant in Nigeria, there is still dearth of knowledge of bio-fertilizer production in the country (Itelima *et al*., 2018). Farmers in Nigeria depend mostly on imported inorganic fertilizers. Importation of these fertilizers has many disadvantages which include drain of foreign reserve, insufficient supply due to high cost of importation and transportation which makes the price of fertilizer very high thereby making it inaccessible to resource poor farmers (Itelima *et al.,* 2018). The increasing demand for production of food crops for vast population of Nigeria and the above mentioned reasons have led to the production of fertilizers from other sources. So far, there is a little information on the performance of synergistic effect of *Rhizobium* inoculation and carrier materials such as earthworm casts and poultry litters on the growth and yield of pea plants, hence a need for research to exploit the potential of this bacterium and the carrier materials. The aim of the study was to determine the growth and yield response of *Rhizobium* sp. to bio-fertilizer supplemented with

earthworm casts and poultry litter.

MATERIALS AND METHODS

The study area

The field experiment was conducted at Mangu town, located in Mangu local government Area of Plateau State, Nigeria. Mangu is found in the central zone of Plateau State between the following coordinates: 9° 26'N, 9° 08' E and 9.433°N, 9.133°E. It is about 70 km away from Jos, the capital of Plateau State. The mean annual temperature as recorded by the Gindiri College of Education Meteorological Station varies between 21.6°C to 24°C, while the annual rainfalls are between 1192.7 mm to 1, 317.5 mm. The relative humidity hovers around 50 – 78% on the average (Gilbert, 2009).

Sample collection

The soya bean seed was purchased and planted on sandy – loamy soil in Mangu-Gindiri for the isolation of *Rhizobium* sp. from the root nodules. The seeds were planted within the first week of May, 2015. 2 – 3 seeds per hole were planted at a depth of 1.5 – 2 inch and 2 – 4 inch apart. At about 8 weeks when the root nodules were fully formed, the soya bean plants were carefully uprooted without damaging the root nodules and then transported to the laboratory for the isolation of *Rhizobium* species (Vishal and Abhishek, 2014). Seeds of proven variety of pea seeds were purchased from the market in Jos. About 100 kg of poultry litter was collected into clean bags using shovel from a deep litter pen of poultry farm in Jos. It was left to decompose for about three months as described by Adeoye *et al*. (2011). About 100kg of earthworm casts was collected within the University of Jos premises, Bauchi road campus using shovel and packed into clean bags. The physicochemical properties of both samples were analyzed using appropriate method as described in IITA manuals (1979). A composite soil sample weighed about 10grams was collected from different locations of the experimental soil into clean polyethylene bags using spatula from 0–30 cm depth prior to planting before the application of fertilizer to determine the physicochemical properties of the soil (Eifediyi and Remison, 2010). The soil sample was taken to Agricultural Screening and Training Centre (ASTC) laboratory, Kassa Barkin Ladi Local Government of Plateau State for physico-chemical analysis.

Isolation of *rhizobium* species from soya bean root nodules

The method used to isolate *Rhizobium* species is as described by Vishal and Abhishek, (2014). At about 8 weeks old, when the root nodules have developed fully, the young plants were carefully uprooted and transported to the Laboratory to collect their root nodules for the isolation of the bacteria. The roots were washed in sterile running water to remove the soil as well as reducing the surface microorganisms associated with the roots. The pink root nodules were then detached carefully and dipped in 3 – 5% hydrogen peroxide (H_2O_2) for about 3 – 4 min to surface sterilized the root nodules. The root nodules were then washed successively four to five times with sterile water to remove traces of hydrogen peroxide completely.

The sterile root nodules were then transferred to test tubes containing 1 ml of distilled water and the nodules (1 – 2) crushed using sterilized glass rod to obtain a milky suspension extract or suspension of bacteriodes. A serial dilution was prepared by transferring 1ml of the bacteriodes suspension into a test tube containing 9 ml of sterile water to obtain10^{-4}. One mililitre of each suspension was spread on Yeast Extract Mannitol Agar (YEMA). YEMA media was prepared according to standard procedures as described by Vishal and Abhishek, (2014). The media constitute K_2HPO_4 (0.5 g), K_2SO_4. $7H_2O$ (0.2 g), NaCl (0.1 g), Mannitol (10.0 g), Yeast extract (1.0 g), Agar (20.0 g) and 1% Congo red dye solution (2.5 ml). The weighed amounts of all the constituents (except K_2HPO_4 which was dissolved separately) in distilled water and mixed in the agar solution. The volume was made to 1000 ml and then autoclaved. Congo red solution was sterilized separately and added to the medium at the time of pouring in Petri dishes. The plates were inoculated with suspension of *Rhizobium* and then incubated at 28$\pm$1°C for 24-72 h.

Sub-culturing to obtain pure isolate

The streak plate method was employed in order to obtain a pure culture of *Rhizobium* species. A loopfull of the *Rhizobium* culture was taken from the preserved broth slant and inoculated on YEMA agar surface. The inoculums were streaked and incubated at 28$\pm$1°C for 24-72 h. The *Rhizobium* cultures were maintained on YEMA broth slant after periodic subculturing at interval of 15–30 days. The pure isolate was preserved as per need culture as broth slant by putting it in refrigerator (Cheesbrough, 1991).

Identification of *rhizobium* species

After obtaining the pure culture, the morphological and cultural characteristics were observed. Gram's staining and other biochemical tests were carried out to distinguish *Rhizobium* sp. from other bacteria (Vishal and Abhishek, 2014).

Gram's staining technique

Gram's staining technique was done according to the method described by Cheesbrough, (1991). Fairly thin smears were made from suspected pure colonies of the cultures on clean grease-free glass slides with sterile physiological saline and allowed to air – dry. The smears were gently heat- fixed, and flooded with 0.5% crystal violet stain for 1 min, before being flushed off with Lugol's iodine to remove the scum. The Lugol's iodine (a mordant) was further applied for 1 min, after which it was thoroughly rinsed off with distilled water. It was then finally counter-stained with safranin for 20 seconds, rinsed with distilled water and allowed to air dry. The stained smears were finally examined with x 100 objective of a binocular light microscope for typical Gram – negative rods.

Biochemical test

Catalase test

This tests the ability of an organism to produce the enzyme, catalase which catalyzes the decomposition of hydrogen peroxide to water and oxygen.

Procedures

A drop of 20 – 30% hydrogen peroxide was placed on a clean grease – free glass slide and a portion of the suspected colony of the organism obtained in pure culture was picked with the edge of another clean glass slide (to forestall false positive reaction elicited by some wire loops when in contact with hydrogen peroxide) and brought into contact with the drop of hydrogen peroxide. The release of oxygen bubbles indicates positive tests, while its absence indicates negative test.

Hydrogen sulphide production test

A strip of filter paper was impregnated with 10% lead acetate solution and dried in an incubator at 37°C. The test organism was inoculated onto prepared blood agar slope containing 0.02% cysteine hydrochloride close to a Bunsen burner flame. The dried lead acetate paper strip was then placed on the cysteine hydrochloride blood agar sloop plugged with non-absorbent cotton wool and incubated at 42°C for 48 h. A browning or blackening of the test paper strip indicates a positive test.

Acid reaction in litmus milk

The test is based on the ability of bacteria to reduce litmus milk by enzyme action; shown by decolonization of the litmus. A large sterile loop was used to inoculate 0.5 ml of sterile litmus incubated at 27 – 30°C for up to 24 h, examining at 30 min intervals for a reduction reaction. A change in colour from white to pale yellow colour is positive but when the colour remains the same or pink is negative.

Sugar fermentation

One gram of each of these sugars: glucose, lactose, maltose, sucrose, fructose, sorbitol and mannitol, was dissolved in 100 ml of peptone water containing an inverted Durhams tube and sterilized. After cooling, each of the bottles were inoculated with a colony of the test organism and incubated at 29°C for 24 h. The formation of gas displaced the water inside the Durham's tube.

Indole production

The suspected colonies of the test organisms were cultured in peptone water within 24-48 h. Indole production was detected by the use of Kovac's reagent which contains P-dimethlaminobenz–aldehyde. Reddening of the medium indicates a positive result.

Sterilization of carrier materials

Sterilization of carrier materials was necessary to keep high number of inoculation bacteria on the carrier for long storage period. As reported by Hari and Perumal, (2010), the carrier materials can be sterilized by autoclaving. The carrier materials (earthworm casts and poultry droppings) were packed in partially opened, thin-walled polythene bags and autoclaved for 60 min at 121°C.

Mass production of r*hizobium* species and formulation of bio-fertilizers

To mass produce *Rhizobium* species isolated from various regions and grown as per need culture from slant were transferred to liquid broth of YEMA medium in conical flask measuring 1000 ml each. The broth was used as inoculants. For easy handling, packaging storing and transporting, the broth was mixed with the sterilized carrier material containing sufficient amount of Rhizobial cells. In this study, poultry droppings and earthworm casts were used as carrier materials. Each carrier material was mixed separately with the inoculant in the ratio of 1000 kg to 2000 ml. after proper mixing of the carrier and inoculant, the carrier-inoculant was left for 4 – 10 days by covering with polyethene at 24 – 25°C to

allowed the microorganisms to multiply in the remaining broth and the formulated bio-fertilizer (microbial inoculant) was then packed in dark sterile polyethene bags, sealed and labeled according to the type of carrier material used. The bio-fertilizer was then stored in dark cool environment at room temperature for subsequent soil application to the crops (Hari and Perumal, 2010).

Determination of physicochemical properties of experimental soil, earthworm casts and poultry litter

Samples of the experimental soil, earthworm casts and poultry litter were taken to Agricultural Screening and Training Centre (ASTC) Laboratory, Kassa in Barkin Ladi Local Government Area of Plateau State for the analysis of physico-chemical characteristics (pH, nitrogen, phosphorus, potassium and organic matter content,). The pH was determined using Pye Unican model MK_2 pH meter in a 1:2.5 soil/water suspension ratio. Organic carbon was determined by Walkley–Black wet oxidation method. Total nitrogen was determined by micro Kjaldahl distillation Technique (Bremier and Mulvaney, 1982). Available phosphorus was determined by Bray No. 1 method (IITA, 1979). Potassium was determined by flame photometer (Enujeke, 2013).

Experimental design

Four treatments (three fertilizer types and control were replicated four times and arranged in a Randomized Complete Block Design (RCBD). These treatments are: Chemical fertilizer (CHF), *Rhizobium* bio-fertilizer plus poultry litter (BPD), *Rhizobium* bio-fertilizer plus with earthworm casts (BEC) and a control (without fertilizer).

Field study

A total land area of 320 cm^2 (20 x 16) cm was selected for the study and prepared by using hoe to plough and harrow the land (Enujeke, 2013) four Blocks separated by 1m alleys and consisting of 16 plots (4 × 4) m each which were separated by a one meter alley to minimize inter – plot interference and also for easy access to the plots was provided. Each block consist of 4 plots and in each plots are ridges which were made 100 cm apart. 10 kg of the various fertilizers each were applied to the cultivated ridges in plot A to C plot D (the control) was not treated with fertilizer (Adeoye *et al.,* 2011). Fertilizer was applied once at the time of planting the seeds.

Planting of pea seeds

In each case the seeds were sown on the ridges at the rate of 3 seeds per stand at a spacing of (1 × 1) m and at a depth of 2.5 cm, but the seedlings were later thinned to 2 seedlings per stand as reported by Enujeke, (2013). Planting was done in May, 2016.

Weeding and pesticides application

Weeding was done twice; 7 days after germination and the second 14 days after germination using hoe (Eifidiyi and Remison, 2010). There was no occurrence of insects, hence no pesticide was used.

Measurement of growth and yield parameters

During the experimentation, three plants per plot were randomly selected and tagged from the two middle ridges for bi-weekly observation and measurement of growth and yield parameters beginning two weeks after planting. Crops growth and yield parameters measured include: number of leaves per plant by visual counting height of plant (cm) using metre rule, girth (mm) of plants using venier caliper, number of pods per plant. Plant parameters such as the number of flowers per plant, weight (g) of pods per plant, number of seeds per pods per plant, length of pods were also recorded using appropriate methods. All the pea pods, the seeds and the plants harvested from the sample plants per plots were used to determine the yield as described by Adeoye *et al.* (2011). The pea pods and seed yields per plot were computed and expressed on per hectare basis using the relationships shown below:

$$Y = \frac{Pfw \times Pdw}{F_{sw}} \times 1000$$

Where

Y= plant yield
P_{fw} = plant fresh weight
P_{dw} = plant dry weight
F_{sw} = fresh sub-weight

Statistical analysis

One-way Analysis of Variance (ANOVA) was performed on agronomic data such as (number of leaves per plant, height and girth of plants, number of pods per plant, number of flowers per plant, weight (g) of pods per plant, number of seeds per pods and length of pods) using general linear SPSS software (Version 22, SPSS Inc., USA). When the F tests were significant, treatments means of the parameters were separated using Tukey's least significant difference at 5% probability level.

RESULTS

Plate 1 represents the cultivated soya bean plants ready for collection of root nodules at about 8 weeks of planting when the nodules were fully formed. At this stage, some of the leaves were light green and some pale yellow. Plate 2 shows the healthy root nodules collected from soya bean plants, while Plate 3 reveals the culture of *Rhizobium* colonies. The culture was observed to be white, translucent, elevated and mucilaginous after 48 h of incubation at 28 ±1°C. The bacterial colonies did not absorb red colour when cultured in YEMA medium containing congo red (Plate 3). The results of the biochemical tests carried out to further identify *Rhizobium* species is presented in (Table 1). The bacterium was gram negative and motile. *Rhizobium* failed to grow in glucose peptone agar medium and did not hydrolyze urea. The bacterial isolate produced catalase but did not show acid reaction in litmus milk. The organism did not hydrolyse starch but was found to utilize D–glucose, mannitol, D–fructose, L–arabinose and sucrose as fermentation sugar.

Plate 1. Soya bean plant cultivated to obtain root nodules for isolation of *Rhizobium* species.

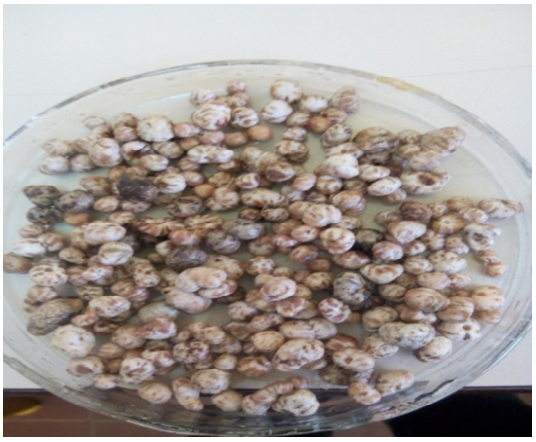

Plate 2. Root nodules obtained from the roots of soya bean plants.

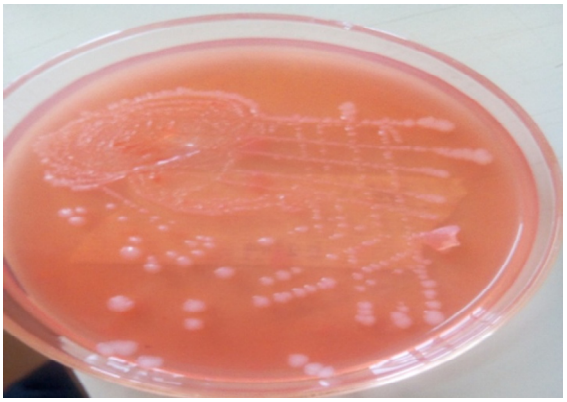

Plate 3. Culture of *Rhizobium* species on YEMA medium.

Table 1. Biochemical test of *Rhizobium* species isolated from soya bean plant.

Biochemical Test	Test Result
Gram reaction	–
Motility test	+
Absorption of Congo Red on YEMA	–
Catalase	+
Acid reaction in litmus	–
Indole	+
Hydrolysis of urea	–
Starch hydrolysis	–
D –glucose	+
D –fructose	+
L – arabinose	+
Mannitol	+
Sucrose	+

– = negative
+ = positive

Physicochemical characteristics

The results of the analysis of physicochemical properties of the soil, earthworm casts and poultry litters are presented in (Table 2). The pH of soil was observed to be weakly acidic with a mean value of 6.23, while the pH of earthworm cast was more acidic (5.78) and the mean pH of poultry litter was found to be basic (8.63). Poultry litter had the highest organic matter content of 58.25 % followed by earthworm cast had 6.00 %, while the experimental soil had the least (1.41%). The percentage of nitrogen was highest in poultry droppings (3.20 %), followed by earthworm casts (0.19 %) and soil had the least value (0.04 %).

Effect of fertilizer treatments on pea girth

The result of the effect of different fertilizer treatments on

Table 2. Physicochemical properties of soil, earthworm casts and poultry litter

Substrate	pH	N (%)	P (ppm)	K(ppm)	OM (%)
Soil	6.23	0.04	6.95	26.50	11.41
Worm casts	5.78	0.19	12.60	44.30	6.00
Poultry Droppings	8.63	3.20	2.50	1.42	58.25

pH = Hydrogen ion concentration, N = Nitrogen, P = Phosphorus
K = Potassium, OM = Organic matter, ppm = Part per milligram

Table 3. Effect of different types of fertilizers on yield parameters of pea plant.

Treatment	Parameter				
	Weight of pod (g)	No. of pods	Length of pod (cm)	No. of seeds per pod	Yield of seeds (t/ha
CHF	173.0±10.78	53±2.87	7.3±0.12	4.0±0.28	2.4±1.50
BEC	206.5±17.68	63±3.79	7.6±0.12	4.0±0.23	2.4±1.10
BDP	227.2±14.82	65±3.35	7.7±0.08	4.0±0.25	2.4±1.23
CON.	144.5±04.17	47±2.25	6.7±0.11	3.0±0.40	0.17±3.40
LSD(0.05)	37.99	9.62	0.35	NS	6.32

NS = not significant (p>0.05), CHF= Chemical fertilizer, BEC= Bio-fertilizer plus earth worm casts, BDP= Bio-fertilizer plus poultry litter, CON= Control.

pea girth is shown in (Figure 1). The application of BPD and BEC gave the highest mean girth size of (5.3±0.10 mm and 5.3±0.0.5 mm respectively) and the least mean girth size (4.3±0.4 mm) was observed in the control treatment. Pea girth size increased significantly ($p<0.05$) with the application of *Rhizobium* bio-fertilizer mixed with poultry litters (BPD) and *Rhizobium* bio-fertilizer mixed with earthworm casts (BEC). BPD and BEC treatments produced similar effect on the girth size of the plant.

Effect of fertilizer treatments on pea height

Pea height was observed to be highest in peas treated with BEC and BPD (55.5±1.39 cm and 55.5 ±1.33 cm), followed by treatments under chemical fertilizer (CHF) (50.8±2.55 cm), while the control treatment gave the least height of 35.7±2.55 cm (Figure 2). Statistical analysis showed that there was a high significant difference ($p<0.05$) between the pea treated with fertilizers and the control with respect to the height of the plant. Thus, treatments with CHF, BPD and BEC produced almost the same effect in terms of the height of peas.

Effect of fertilizer treatment on number of leaves of pea

There was a high significant difference in the number of leaves under fertilizer treatments. The highest number of leaves (51.0±1.37) per plant was observed in treatments receiving BPD. This was closely followed by BEC and CHF with 50.0±1.79 and 48.0±1.10 respectively, while the least (38.0±1.77) was observed in the control (Figure 3). Similarly treatments with BPD, BEC and CHF produced almost the same effect ($p> 0.05$) in terms of the number of the leaves of the pea plants. However, there was a significant difference between the plants treated with fertilizers and the control with respect to the number of the leaves of the plants.

Effect of fertilizer treatments on number of flowers of pea

The statistical analysis of data in (Figure 4) shows that the number of flowers produced by pea plant was not significantly ($p>0.05$) influenced by the fertilizer treatments when compared to the control. Hence, the number of flowers was observed to be 25.0±1.25, 24.0±0.85, 21.0±1.25 and 18.0±1.03 in plants treated with BEC, BDP, CHF and the control respectively.

Effect of fertilizer treatments on weight of pods

The result presented in (Table 3) shows that the weights of pods of pea vary significantly under fertilizer treatments. Peas treated with BPD having the highest weight of pods 227.2±14.82 g followed by PEC 206.5±17.6 g, while the (control) produced pods that had the least weight (144.5±4.17 g).

Effect of Fertilizer Treatments on Number of Peas Pods

There was a significant difference in the number of pod of peas in relation to the fertilizer treatments. Peas that

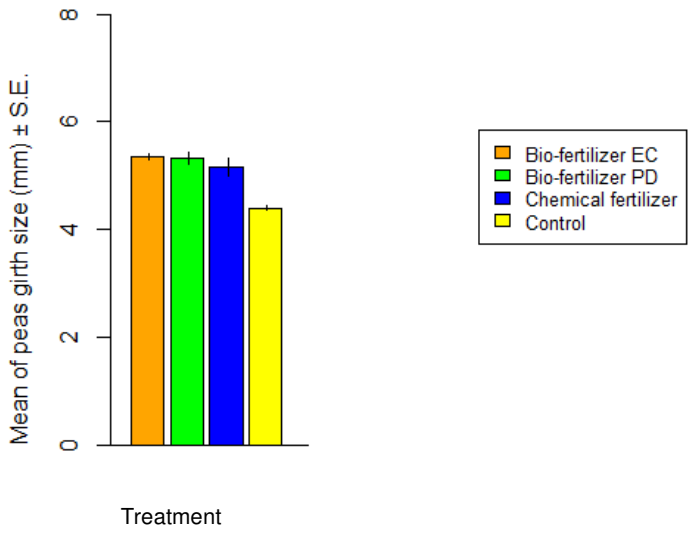

Figure 1. Mean of pea girth in relation to fertilizer treatments. EC= Earth worm cast, PD= Poultry litter

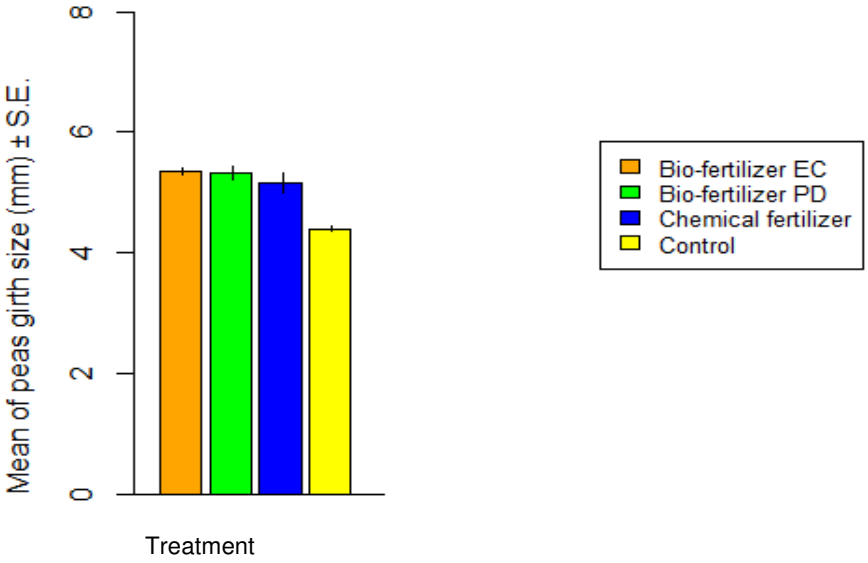

Figure 2. Mean of pea height in relation to fertilizer treatments.

received BPD produced the highest number of pods (65±3.35), while control was observed to have the lowest number (47±2.25) of pods. Plants that received CHF had same effect with those under the control (Table 3).

Effect of fertilizer treatments on number of pea seeds per pod

There was no significant difference in the number of seed of peas in relation to fertilizer treatments. However, the highest mean seed number of peas was observed in the plots that were treated with BPD, BEC and CHF (4), while peas in control plot had the least number of seeds (3) per plant (Table 3). The total yield of pea seed (t/ ha) was found to be uniform in all the treatments except in control which had the lowest yield.

DISCUSSION

The Bacterium isolated from the root nodules of soya bean plants did not absorb red colour from YEMA medium and all the biochemical tests confirmed that

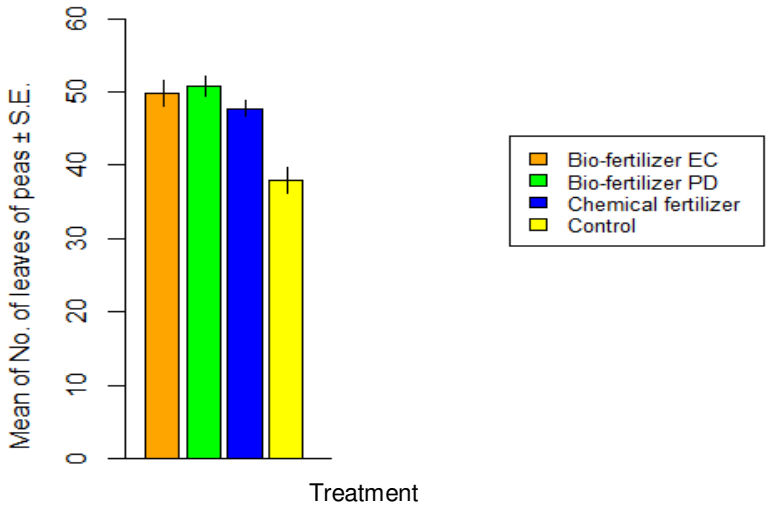

Figure 3. Mean number of leaves of pea in relation to fertilizer treatments.

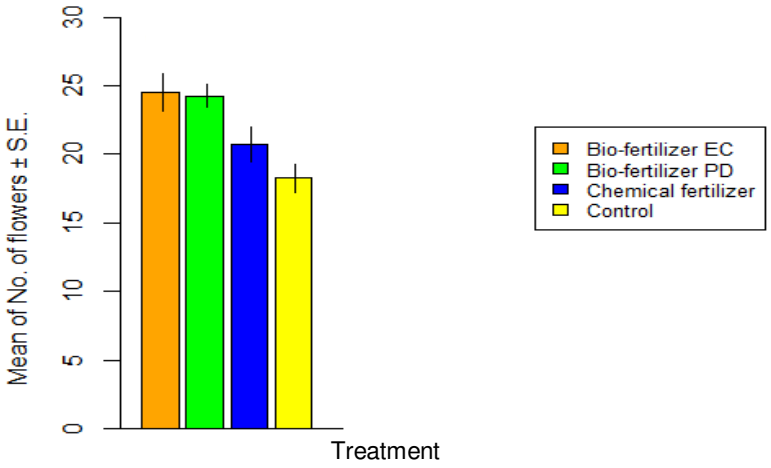

Figure 4. Mean number of flowers of pea in relation to fertilizer treatments.

isolated strain was *Rhizobium* sp. Although, the results of the physico-chemical analysis of the experimental soil showed that the pH (6.22) is favourable for the cultivation of pea as reported by Tulbek *et al.* (2017), but its low contents of nitrogen (N), phosphorus (P), potassium (K) and organic matter may lead to low productivity of pea crop. Also, Tulbek *et al.* (2017) reported that pea crop can grow within several climate and soil zones; however pea plants grow best on fertile, light, well-drained, and humus rich soils. Thus, addition of supplements such as worm casts and poultry litter to *Rhizobium* sp. used for soil amendment in order to obtain better growth and yield of the crop is paramount as these materials contain nutrients such as nitrogen, phosphorus, potassium and organic matter necessary for plant growth and productivity. Nitrogen is the critical limiting element for growth of most plants due to its unavailability in most tropical soil (Graham and Vance, 2000; Bambara and Ndakidemi, 2010). According to Wortmann, (1998) beans need nitrogen more than any other nutrient. Deficiency in N causes reduced growth, leaf yellowing, reduced branching and small trifoliate leaves in beans. Nitrogen is a building block of proteins and is highly needed for all enzymatic reactions in a plant. It is a major part of the

chlorophyll molecules and plays a necessary role in photosynthesis and also is a major component of several vitamins (Mfilinge *et al*., 2014). It has been reported by some researchers that phosphorous is among the important elements needed for growth and production of legumes in many tropical soils (Buerkert *et al*., 2001; Kisinyo *et al*., 2012). Potassium plays important roles in major plant processes such as photosynthesis, respiration, osmoregulation, growth and yield of plants (Mfilinge *et al*., 2014).

The result of the study shows that there was increase in growth parameters and yield parameters of pea plants that were treated with bio-fertilizer supplemented with poultry litter and earthworm casts over the control.. This agrees with findings of previous workers that stated that *Rhizobium* inoculation in legumes is accredited for stimulation growth and is an alternative to the expensive inorganic nitrogen fertilizers (Ndakidemi *et al*., 2006; Abbasi *et al*., 2010; Mahmoodi *et al*., 2013). The present findings also agree with those of Mfilinge *et al*. (2014). These researchers reported that the use of appropriate strains of inoculants in nitrogen deficient soil may offer an excellent opportunity for improving legume growth and development. The improvement in pea yield which is associated with increase in the phosphorus and potassium concentration in the soil amended with bio-fertilizers is affirmed by Mfilinge *et al*. (2014) evaluated the effects of *Rhizobium* inoculation and supplementation with phosphorus and potassium, on growth, leaf chlorophyll content and nitrogen fixation of bush bean varieties. Further support to the present findings was affirmed by Khosro and Yousef (2012) who reported that inoculation of legumes with *Rhizobium* bio-fertilizer resulted to an increased yield by 15 – 20%. Also, Giri *et al.* (2010) that the inoculation of *Rhizobium* as bio-fertilizer was very significant in production of number of nodules, increase in the length of roots, shoots, number of leaves and pods in *Cicera rietinum*. Hoque and Haq, (1994) reported that inoculation of seed with *Rhizobium* significantly increase plant height of lentil. Also the effect of combined inoculation with VAM – fungi and *Rhizobia* has been reported to further increase the growth and yield of some legumes including soya bean. Higher number of leaves per plant was produced by plants that received BPD and BEC possible because the poultry manure established and maintained soil physical condition for plant. This is consistent with the reports of Lombin *et al* .(1992); Mangila *et al.* (2007) and Enujeke *et al.* (2013) which indicated that poultry manure (the richest known animal manure) is essential for establishing and maintaining the optimum and physical condition for plant growth. It is also synonymous with findings of Ewulo *et al.* (2008) who reported that poultry manure is not only a cheap carrier but also an effective source of nitrogen for sustainable crop production, but also improved soil physical properties by reducing temperature, bulk density and increasing total porosity. Ramaswami and Oblisami, (1986) reported the increase in nodules and yield due to inoculation application of *Rhizobium* bio-fertilizer. The high organic matter content of the carrier materials used to supplement the bio-fertilizer is suspected to be partly responsible for the increase growth and yield in pea. This agrees with the findings of Hakim *et al*. (2010) that vermi-compost increase the growth of pea. Reddy *et al.* (1998) also recorded maximum plant height at harvest, number of seeds per pods with the application of vermin-compost. Pea being legume requires high amount of nitrogen for maximum production especially in soil (Khosro and Yousef, 2012). Thus, the increase in growth and yield parameters of pea that received bio-fertilizer application could partly be as a result of high percentage of nitrogen contained in the carrier materials. Also, on comparing the yield and growth parameters of pea plants exhibited by bio-fertilizers and that of chemical fertilizer, there was no significant difference between them in most cases. This is an indication that Rhizobia bio-fertilizer can serve as an alternative to the convention fertilizers. Generally, *Rhizobium* biofertilizers supplemented with worm casts and poultry litter used for soil amendment have been found to not only stimulate pea plants' growth but also improved their yield significantly and thus serve as an alternative to the expensive inorganic nitrogen fertilizers. The use of appropriate strains of inoculants in nitrogen deficient soils may offer an excellent opportunity for improving legume growth and development.

Conclusion

The result of this study has buttressed earlier findings that Rhizobia bio-fertilizer improves yield of legumes. The results of the study also showed that plants that received the bio-fertilizer amended with poultry litter and earthworm casts were superior in all most all the growth and yield parameters tested. The high organic matter content of poultry litter and earthworm casts might have also contributed to the increased growth and yield of the crops since pea require high organic matter for healthy growth and development. Based on the findings of the study, it is recommended that pea farmers should use Rhizobia bio-fertilizers amended with carrier material rich in organic matter such as poultry manure and worm castings in place of the commonly used inorganic fertilizer which is expensive and harms both the plant and the environment if applied in excess. Government should adopt or encourage the use of bio-fertilizer for their farming activities since bio-fertilizer offers a better option for growth and yield of crops and will help in reducing the use of agrochemicals and also to maintain soil fertility and strength. Furthermore, these findings would tend to encourage more and quality production of pea by farmers using Rhizobia bio-fertilizer. Thus,

farmers can utilize the less expensive and eco-friendly bio-fertilizers for the cultivation of food crops.

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