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Growth and yield response of cowpea (Vigna unguiculata) to bio-fertilizers produced from Aspergillus niger and animal waste materials

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Abstract–This study involved the production of bio-fertilizers from plant and animal waste materials such as saw dust, cow dung and poultry droppings inoculated with a fungus (*Aspergillus niger*). The saw dust and the cow dung and or poultry droppings were mixed in a ratio of 30:1. The substrates were mixed with water in line with standard method after which they were placed separately into a locally constructed bio-digester. The substrates (cow dung-saw dust mixture and or poultry droppings-saw dust mixture) were stirred daily, while they were allowed to be degraded by the fungus (*A. niger*). The process of degradation of the different waste materials lasted for a period of two weeks after which the nitrogen, phosphorus and potassium (NPK) contents of the bio fertilizers were analyzed. After biodegradation process, the various substrates were used to amend the soil used for growing cowpea (*Vigna unguiculata*). Plant growth parameters and yield of cowpea plant grown on soil amended with bio-fertilizers were measured and compared with those grown on soil amended with organic and inorganic fertilizers. The growth performances of the cowpea plants grown on soil amended with the two bio-fertilizers and those grown on the soil amended with inorganic fertilizer showed no significant difference at (p>0.05). The results of this study is very important in that farmers that cannot afford the expensive inorganic fertilizers can fall back on bio-fertilizers which are comparatively for the cultivation of food crops.

Keywords- ***Vigna unguiculata*****, bio-fertilizer,** ***Aspergillus niger*****, animal waste materials.**

I. Introduction

Global warming and climatic change have resulted in unexpected drought, stormy rainfalls, extremely high temperature, cold damage, hurricanes and tornadoes in many places around the world where such disastrous tragedy had never occurred in the past decades (Chun- Li *et al*., 2014). Heavy casualties and agricultural loses in these areas therefore significantly affected the lives and security of residents and consequently, the regional economy. Establishing an environmental friendly co-existing mechanism on earth is of vital importance. In recent years agrochemicals such as chemical pesticides and fertilizer are extensively applied to obtain high yield. Intensive application of agrochemicals leads to several agricultural problems and poor cropping systems. Some farmers use more chemical fertilizers than the recommended levels for many crops. This practice accelerates soil acidification but also risks of contaminating ground water and the atmosphere and also weakens the roots of plants and making them easy prey to unwanted diseases (Chun- Li *et al*., 2014).

Bio-fertilizers are important components of integrated nutrient management. These potential biological 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 (Khosro and Yousef, 2012). Bio-fertilizers are renewable sources of plant nutrient which supplement chemical fertilizers (Hari *et al*., 2010). Utilization of bio-fertilizer is one of the ways to increase crop production by naturally optimizing the nitrogen and phosphorous level of the soil and by enriching the compost waste used as a natural fertilizer (Khosro and Yousef, 2012). The main sources of bio-fertilizer are bacteria, fungi and cyanobacteria (blue-green algae). Vessey (2003) defined a bio-fertilizer as a substance which contains living microorganisms which, when applied to seeds, plants surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. According to Hari *et al*. (2010) bio-fertilizer is most commonly referred to as selected strains of beneficial soil microorganisms cultured in the laboratory and packed in suitable carriers.

Cowpea (*Vigna unguiculate*) is a legume grown in savannah region, the tropics and sub-tropics. It is largely grown in the West and Central African

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countries. Its value lies with its high protein content. Its ability to tolerate drought and poor soil makes it an important crop in the savannah region where these constraints restrict other crops. Cowpea seed is nutritious and is a cheap source of protein for both rural and urban consumers. The seed contains about 25% protein and 64% carbohydrate (Chinma *et al*., 2008). The study therefore, is aimed at producing cost effective and environmental friendly bio-fertilizers using waste materials, and phosphorus and potassium solubilizing fungus (*A. niger*) to meet increasing nutritional food requirements and to determine, based on the results, whether bio-fertilizer is a possible option for improving the productivity of cow pea (*Vigna unguiculata*) especially when compared with synthetic fertilizer.

II. Materials and methods

A. *Sample collection*

With the aid of a shovel about 50kg of fresh cow dung was collected from abattoir (slaughter house) in Jos, Nigeria. Also about 50kg each of poultry droppings and saw dust were collected from the section of a local poultry farm and timber market respectively in the above mentioned city. About 30kg of potato peels were collected from food sellers, sundried and milled into powder using a grinding machine to serve as one of the substrates for the growth of the fugus (*Aspergillus niger*). Soil samples were collected from the garden in the study area and screened for the presence of *A. niger*. Ten gram of soil sample was dissolved in 100ml of sterile distilled water and mixed thoroughly to obtain the dilution of 10-1. The soil sample was serially diluted in sterile distilled water up to 10-7 (each tube containing 9ml of sterile water). After then, 10-5, 10-6, 10-7 dilutions were taken for spread plate technique culture. Sterilized Potato Dextrose Agar (PDA) was prepared and poured into Petri dishes. After solidification of the medium, 0.1ml of each dilution was poured into agar medium plate in duplicates. By using L-rod, the sample was spread evenly over the agar surface and then incubated at room temperature ($25^{O}C$) for 3days.

B. *Identification of fungal culture*

To evaluate the colony characteristics of *A. niger*, it was necessary to subculture the suspected colony of the fungus to PDA to obtain a pure culture. The Morphology and cultural characteristics were determined and used for identification of the fungi. The fungal isolates were identified by making references to Domsch and Anderson (1980).

C. *Preparation of growth medium and inoculum*

The growth medium used for preparing the *A. niger* inoculum consisted of 30g of milled potato peels, peptone, 0.1%; malt extract, 0.1% (w/v), calcium carbonate 0.2% (w/v); ammonium phosphate, 0.2% (w/v), and ferrous sulphate.$7H_2O$, 0.001% (w/v) (Abouzeid and Randy, 1986). *A. niger* inoculum was prepared in $250cm^3$ cotton–plugged conical flasks containing $100cm^3$ of the growth media. The flasks containing the growth medium were sterilized and inoculated with *A. niger* spores. A sterile glass rod was inserted in each of the flasks and the flasks were kept at room temperature for 7 days to allow for sufficient mycelial growth and sporulation of the fungus.

D. *Experimental Protocol*

Production of bio-fertilizer- With the aid of a shovel, the saw dust and the cow dung/poultry droppings were mixed in a ratio of 30:1 respectively. The C:N of manure is a very important factor that affects the whole degradation process because microbes need 30 times more carbon than nitrogen to growth and carry out their biochemical activities. The ratio used in this experiment was obtained by weighing the materials with the aid of a weighing balance. Twenty kilograms of the cow dung-saw dust mixture and the poultry droppings- saw dust mixture were placed in locally constructed plastic container bio-digester of 20 liters having a cover and a stirrer. After this, some quantity of water was added to the manure to ensure active degradation. The bio-digesters were covered tightly and left for a period of two weeks during which the substrates were turned regularly to ensure faster and complete degradation. The untreated cow dung and poultry droppings without saw dust were dried and kept at room temperature so that their effects on plant parameters can be compared with those of biodegraded manures.

Aging of the Substrates-After the process of bio-digestion, the substrates were kept in a cool dry place to dry. Sun drying was not employed because elements such as nitrogen can escape when exposed to high temperatures (Swathi, 2010). Aging was done in order to dry the degraded substrates and end the activity of microorganisms that might have survived degradation temperatures. Aging lasted for two weeks after which the substrates were sieved to remove lumps.

Packing and Storage-The bio-fertilizer was packaged in a black polythene bag, sealed and labeled according to the type of organic manure, microorganisms and the quantity of NKP. They were stored in a cool dry place away from direct sunlight.

Analysis of Nutrient Content of Bio-fertilizers-The two bio-fertilizers produced from cow dung (BCD), poultry droppings (BPD), and the two untreated manures cow dung (CD) and poultry droppings (PD) were analyzed for the presence of nitrogen, phosphorous and potassium according to methods described by Reginald (2012). Nitrogen and phosphorous content were determined using calorimeter method, while potassium was measured with an ion-selective electrode.

Experimental Design and Measurement of Plant Parameters-The experimental design employed in this study was the complete randomized design. Six treatments plus control with 5 replicates per treatment were used. The manure types were weighed and 1.6kg of each manure type mixed with soil at ratio of 1:4 respectively (Adeoye *et al*., 2011) and put in pots. The manure types included; BCD, BPD, CD, PD, and combination of BCD and BPD, and inorganic fertilizer (IF). Pots containing mixture of sandy and loamy soils that were not amended with any of the manure served as the control. All the pots were kept in a green house to avoid wilting which may result from harsh weather of the experimental site. The applied manure was allowed to mix well with the soil by light irrigation with a portable sprinkler system, once daily for 3 days. TVx3236 cowpea variety was then planted at a rate of 3 seeds per pot. Treatment with manure was done ones every three weeks after the establishment of crops. The plant parameters measured during the six weeks of the experiment included plant height (measured with a meter rule), leaf area index (calculated as a product of total length and breadth at the broadest point of the longest leaf on the plant), and stem girth (measured with a venier caliper). After fruiting, the harvested pods were taken and assessed for parameters needed to calculate the yield such as number of pods, length of pods, and number of seeds per pod and weight of seeds (Adeoye *et al*. 2011).

Randomized complete block design (RCBD) was employed to analyze the data (Rangaswamy, 2007).

III. Results and discussion

The mean daily temperatures of the substrates recorded during the biodegradation process carried out in the bio-digester are presented in Fig. 1. The mean temperatures of the substrates ranged from 21^{O}C- 32^{O}C for cow dung–sawdust mixture and 20^{O}C to 32^{O}C for poultry droppings–sawdust mixture during the biodegradation process, indicating that both substrates had similar temperature ranges. The similarity in the temperatures of the substrates could be as a result of the balancing of the C:N ratio of the substrates before being subjected to biodegradation process. Hence, there is the possibility that by the microorganisms resident in the in the substrates would exhibit similar metabolic activities. The decrease in temperature towards the end of the biodegradation can be attributed to decrease in metabolic activities as a result of the death of most of the organisms. Microbial death was caused by interplay of factors such as changes in temperature, and pH as result of secretion of organic acids by acid producing organisms which lower the pH in their vicinity (Akhtar and Siddiqui, 2009).

The results in Fig. 2 represent the nutrient content of bio-fertilizer produced from cow dung-sawdust mixture (BCD), poultry dropping- sawdust mixture (BPD) and organic manure (cow dung (CD) and poultry droppings (PD)). The results showed that nutrient contents of organic manure samples varied widely, while the nutrient contents of bio-fertilizers were comparable. In general, PD had the highest concentrations of nitrogen (482ppm), followed by CD (465ppm), while BPD and BCD had similar concentrations of potassium, 379ppm and 376ppm respectively which were higher than those of PD and CD (271ppm) and (250ppm) respectively. Although, the phosphorous contents were very low in all the manure samples but slight increases were recorded for BCD and BPD. The rise in potassium and phosphorus levels in the bio-fertilizers is as result of the activities of *A. niger* inoculated into the substrates. *A. niger* has the ability to secrete metabolites capable of converting the insoluble forms of phosphorus and potassium to soluble forms. The mean NPK contents of the bio-fertilizers obtained in this study is suitable for plant growth, as the mean NPK requirement of plants is usually in the ratio of 1.5-0.2-1.0. It has also been reported that excess phosphorus in soil causes early maturity of plants and low crop yield in the plants, whereas appreciable amount of nitrogen and potassium are needed for good crop yield (Scalenghe, 2012).

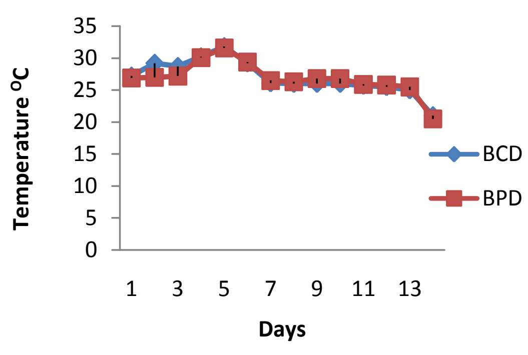

Fig. 1: Temperature of substrates in bio-digesters during biodegradation process. BCD & BPD = Bio-fertilizers from cow dung and poultry-saw dust mixtures respectively, undergoing degradation

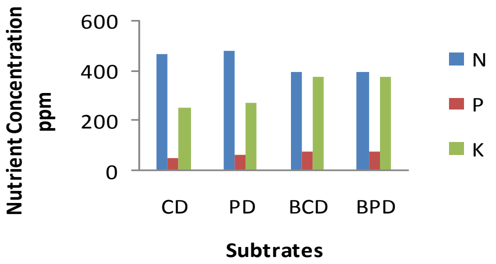

Fig. 2: Nutrient contents of bio-fertilizers and organic manures. (N=Nitrogen, P=Phosphorus, K=Potassium) CD=Cow dung (organic manure), PD= Poultry dropping (organic manure), BCD= Bio-fertilizer from cow dung, BPD=Bio-fertilizer produced from poultry droppings

The results of the plant parameters are presented in Table 1. Both bio-fertilizers and the inorganic manures effected growth parameters of the cowpea variety used at varying degrees. There was a remarkable increase in all the growth parameters for all the treatments over the control (Cowpea grown on soil not amended with fertilizer) with the exception of the cow dung. All the growth parameters were affected by manure application. The application of inorganic fertilizer and poultry droppings (PD) led to remarkable difference in stem height, number of leaves and leaf area index of the cowpea plant. However, the combination of poultry droppings- sawdust mixture and cow dung- sawdust mixture bio-fertilizer (BCD+ BPD) gave the best yield (49.5%) of cowpea. This was followed by BPD (38.7%) and BCD (38.1%). The improvement in cowpea yield is associated with increase in the phosphorous and potassium concentrations in the soil amended with bio-fertilizers is affirmed by Reyhan and Amiraslani (2006). *A. niger*, a phosphorus and potassium-solubilizating fungus, can enhance plant growth by increasing the uptake of phosphorus and potassium by the crop. In addition, *A. niger* has the ability to enhance the availability of other elements such as iron to the plants (Fe) zinc (Zn) (Ngoc *et al*., 2006). Also, it can synthesize enzymes that modulate plant hormone level and can also kill pathogen with antibiotic (Akhtar and Siddiqui, 2009). The lesser numbers of pods and longer length of pods recorded in cowpea plants grown in soil amended with poultry droppings and the inorganic manure than those grown in soil amended with bio-fertilizer could be as result of very high concentration of nitrogen in the manures (PD&IF). This agrees with the report of Davis *et al*. (1991) which stated that excess nitrogen in cowpea promotes lush vegetable growth, suppresses nitrogen fixation and may reduce pod and seed yield. The excessive application of poultry manure in some cropping systems has also resulted in nitrate contamination of ground water (Chun- Li *et al*., 2014). Thus, proper estimation of nitrogen content of poultry manure and proper manure handling are necessary for optimum crop growth and minimal nitrate leaching. One will expect that the soil treated with cow dung (CD) should give a better yield than the control but the reverse is the case. This present result is in line with the findings of Reyhan and Amiraslani (2006) which stated that cowpea yield, can be inhibited by cattle manure because of its high salinity level.

Table 1: Average Plant Parameters 6 Weeks after Seed Sowing.

Treatment (Manure types)	Height (cm)	Leaf area (cm^2)	Stem girth (cm)
CD	22.0	1.125	0.061
PD	32.6	1.192	0.074
BCD	27.5	1.176	0.080
BPD	28.1	1.179	0.086
BCD+BPD	28.5	1.185	0.098
IF	33.1	1.198	0.079
Control	24.3	1.161	0.067

Table 2: Effects of Various Manure Types on Cowpea Yield.

Treatment (Manure types)	Average number of pod per plant	Average length of one pod(cm)	Average number of seed per pod	Average number of seed per pot	Total weight of bean seed (g) harvested	Yield (%)
CD	11.00	0.10	8.00	264	61	18.3
PD	15.00	0.18	10.00	450	85	25.5
BCD	18.00	0.15	13.00	702	127	38.1
BPD	19.00	0.11	14.00	741	129	38.7
BCD+BPD	23.00	0.16	16.00	1104	165	49.5
IF	14.00	0.19	11.00	462	94	28.2
Control	12.00	0.11	9.00	324	68	20.4

CD=Cow dung (organic manure), PD=Poultry droppings (organic manure), BCD= Bio-fertilizer produced from cow dung-saw dust mixture, BPD=Bio-fertilizer produced from poultry droppings-saw dust mixture, BCD+BPD= Bio-fertilizer produced from cow dung saw dust mixture combined with Bio-fertilizer produced from poultry droppings-saw dust mixture, IF= Inorganic fertilizer

IV. Conclusion

In conclusion, the present study has shown that using bio-fertilizers offers a better option for the growth and yield of cowpea and will help in reducing the use of agrochemical, and also help to maintain soil fertility and strength. The yield components of cowpea plants were increased remarkably as a result of the soil being amended with bio-fertilizers. However, the combination of both bio-fertilizers gave the best yield. Thus, for optimum yield, it is recommended that the combination of the bio-fertilizers produced from cow dung-saw dust mixture and poultry droppings-saw dust mixture be employed in amending the soil used for growing cowpea. The results of this study is very relevant, in that farmers can utilize the less expensive and eco-friendly bio-fertilizers for the cultivation of food crops.

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