International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 3 Issue 5, August 2019 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Effect of Rhizobium Innoculation on Growth, Nodulation Count and Yield of Soybeans (Glycine Max) Grown in Biochar Amended Soil of Sounthern Guinea Savana of Nigeria

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How to cite this paper: Ndor, E | Jayeoba O. J | Dauda, S. N "Effect of Rhizobium Innoculation on Growth, Nodulation Count and Yield of Soybeans (Glycine Max) Grown in Biochar Amended Soil of Sounthern Guinea Savana of Nigeria" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-3 | Issue-5, August 2019, pp.1486-1489, **https://doi.org/10.31142/ijtsrd26648**

IJTSRD26648

ABSTRACT

Replenishment of depleted soil nitrogen depends largely on addition of inorganic fertilizers; but due to problems of scarcity and high the cost of these fertilizer. Rhizobia inoculation is a cheaper, easier and safer option to improve soilnitrogen fixation and increase productivity of grain legumes.The experiments were conducted during 2016 and2017 rainy season; with the aimed of evaluating the potentials of rhizobium inoculation on growth, nodulation count and yield of soybean planted in biochar amended soil. The results showed that Rhizobium inoculation of soybeans and biochar soil amendment didnot showed any significant increase on allgrowth parameter at 8 weeks after planting in 2016 and 2017 cropping seasons. However, inoculated soybeans produced significantly higher number of 25.35 and 23.42 roots nodules per plant compare to the control. Rhizobium inoculation and biochar soil amendment had a significant increase on all the yield parameter assessed in both years of cropping. All the soybeans that were inoculated with rhizobium produced the highest number of flowers (58.45 and 56.84); highest number of pods per plant (48.15 and 35.34); and highest seed weight of 2.67 and 2.85 t ha$^{-.1}$ Application of 10 t ha^{-1} of biocharproduced the highest number of flowers (63.26 and 68.21); highest number of pods per plant (53.45 and 54.25); and highest seed weight of 2.53 and 2.69 t ha^{-1} in 2016 and 2017 cropping seasons respectively.

KEYWORDS: *Soybean, Biochar, Rhizobium, Growth, Yield, Nodulation*

INTRODUCTION

Soybean (Glycine max L. Merrill) has its origin from China where it has been cultivated since the 11th century.

Soybean was first introduced into Nigeria in1908 (Fennel, 1966) while attempt to grow it at Moor Plantation, Ibadan at that time failed. It is now widely grown in Nigeria especially in the middle belt states: Benue, Kogi, Niger, Kwara and Kaduna (Olufajo and Pal, 1991). Over the years, several species of legumes were screened and adopted for inclusion into the farming systems of farmers in guinea savanna agro-ecological zone for soil fertility improvement and restoration; but none has received the expected acceptance and inclusion like soybean by the smallholder farmers as it meets the food, fodder and fertilizer needs of the farmers (Misiko, 2007).Soybean production in Nigeria was for many years centered on the savanna ecological zone where soils are characteristically low in nitrogen and phosphorous (Chiezey and Yayock, 1991).The unique ability of legumes to fix atmospheric nitrogen through symbiotic association with root nodule bacteria could be used to improve the yields of legumes in sub-Saharan Africa, since current yields are only a small fraction of their potential (Abaidoo et al., 2007). Moreover, most of the soils used for legumes production in Nigeria are poor in terms of their nutrient status, especially total-N, therefore, relatively unproductive (Machido et al., 2011; Laditi et al., 2012). The soils are also low in organic carbon and available phosphorus; consequently, these soils are inherently low in fertility. The situation is further worsened by nutrient depletion by crops and other related processes, such as leaching, denitrification, volatilization and removal of crop residues for alternative uses (Yakubu et al., 2010; Machido et al., 2011). Hence, replenishment of depleted nutrients, especially nitrogen depends largely on addition of inorganic fertilizers, which in turn contributes substantially to environmental pollution. On the other hand, most farmers cannot afford the inorganic fertilizers due to their high cost and non-availability on time in the region (Sanginga, 2003; Yakubu et al., 2010).This makes rhizobia inoculants a cheaper, easier and safer option to improve soil nitrogen fixation and productivity of grain legumes. The use of rhizobium inoculants for improvement in nitrogen fixation and productivity of grain legumes has been well established in developed countries; but in Sub – Saharan African countries like Nigeria, it is still in the developing stage. Studies on the use of these inoculants were conducted on Soybean (*Glycinemax* (L.) Merrill), mostly the "US type" which requires specific inoculation with *Bradyrhizobiumjaponicum* for optimum productivity, becausethis rhizobium species was scarce in Nigerian soilsat that time; it was recommended to apply Rhizobium inoculant to soybean seed each time you plant soybeans.

This is especially true on sandy soils, where it is more difficult for the Rhizobia to survive from one season to the next. Also, it is important to inoculate on any soil if soybeans are to be grown for the first time. Biochar has been described as a possible means to improve soil fertility as well as other ecosystem services and sequester carbon (Sohi et al., 2010). This is because biochar can improve soil nutrients retention and plant absorption capacity; increased in pH of acidic soil; increased in cation exchange capacity; increased in soil water retention capacity and reduced soil nutrients leaching and increased the abundances and activities of biological communities in the soil. These physicochemical properties of biochar can sustain rhizobium life: highly porous structure and surface area can be inhabited by rhizobiumbacteria and protect microorganisms from predation; high water holding capacity can prevent bacterial desiccation; reduced carbon is a potential source of energy, finally biochar can provide some mineral nutrients for survival of the bacteria. Therefore, it is believed that biochar can sustain the growth and survival of rhizobium over time. This study aimed at evaluating the potentials of rhizobium inoculation on growth, nodulation count and yield of soybean planted on biochar amended soil.

MATERIALS AND METHODS

Climate Conditions of the Experimental Site

The experiments were conducted during 2016 and2017 rainy season at the research and teaching farm of the college of agriculture, Lafia, Nasarawa state, Nigeria. The study area falls within southern guinea savanna agroecologicalzone of Nigeria, and is located between Latitude08.33 N and Longitude 08.32 E. Rainfall usually starts from May – October and the average monthly rainfall figures ranges from 40 mm-350mm. The months of July and August usually records heavy rainfall. The daily maximum temperature ranges from 20.0ºC – 38.5ºC and daily minimum ranges from 18.7ºC – 28.2ºC. The relative humidity rises as from April to a maximum of about 75- 90 percent in July (NIMET, 2017).

Soil and Vegetation of the Study Area

The soil type of the study area composed of highly leached alfisols with low base saturation. The soil is moderately acidic and has high content of iron and Aluminium oxides hence reddish brown in colour with very low organic matter content, low cation exchange capacity, low total nitrogen andavailable phosphorus, but high base saturation. The dominant clay type is Kaolinite. The vegetation of the study area is that of the southern Guinea Savanna with interspersion of thicket, grassland, trees, fringing woodlands or gallery forest along the streams

Treatments and Experimental design

The treatment consisted of bacteria *Bradyrhizobiumjaponicum* (Inoculated and Not inoculated) three levels of biochar (0, 5, and 10tha^{-1}). It was a factorial experiment laid into a Randomized Complete Block Design (RCBD) and replicated three times to form eighteen plots. The land was cleared ploughed and harrowed; then biochar was incorporated at different rates into the soil. The soybeans seeds that were inoculated and not inoculated were planted on the plots at spacing of 75cm X 20cm and agronomic practices like weeding, fertilizer application etc were carried out. .

Data Collection

Soil samples were taken at a depth of 0-15cm before planting of soybeans and were analyzed as presented in (Table 1). Four plants were selected from the net plot and tagged and the following data were taken from them: Growth parameter (Plant height, number of branches, number of leaves, Nodulation count) Yield parameters: pods/plants, no. seed/plant, seed weight/plant (g) and seed weight per (kg/ha)

Data Analysis

The data collected will be subjected to analysis of variance using GENSTAT, and where there isa significant difference; the means will be separated using F-LSD at 5% probability level.

Inoculation of soybeans with Bradyrhizobiumjaponicum strain

Soybeans Seeds were surface sterilized with 70% ethanol and washed afterwards several times in distilled water. Gum Arabic was dissolved in warm water which serve as the bidding agent and allowed to cool, then *bradyrhizobiumjaponicum* was introduced into the liquid and finally the seeds of soybeans were mixed and stirred to form a slurry that is used to coat the seed.

2.2 Local Production of Biochar

Rice husk feed stock were collected from rice mill in Lafia for the production of biochar. An improvised kiln was constructed, which was an empty drum that was perforated but had a cover. The rice husk materials were poured inside the drum half full, then was ignited inside the drum and the drum lid was covered to encourage slow burning and the content in the drum was consistently stirred to enhance uniformity of burning. After 3-4 hours the content of drum is poured out and the fire was quenched by sprinkling water on the hot char. Rice husk biochar was finally dried in the sun and ready to be use.

RESULTS

Soil and Biochar Analysis

The soil of the experimental site was sandy loam(Table 1); low in nitrogen, phosphorus, potassium, organic carbon and alsocation exchange capacity. However, the soil was slightly acidic in nature (6.08, 6.10); high in percent sand fraction (85.00, 84.00) and also very high in percentage base saturation (87.00%, 90.39%) in both 2016 and 2017 cropping seasons. While, the chemical properties of biochars used for soil amendment are: the pH was almost neutral (7.13); total nitrogen of 0.59gkg^{-1}; organic carbon 3.78 gkg^{-1}; ashes (13.36 gkg^{-1}). Then organic matter was 6.50gkg^{-1}; available phosphorus 3.01gkg^{-1}; and low C.E.C of 5.18gkg^{-1}but high %base saturation of 96.52%

Table1: Laboratory analysis of soils at 0-30cm and biochar before cropping in both years

Properties	2016	2017	Biochar
Mech. Composition			
Clay (%)	11.6	12.6	ND
Silt (%)	3.4	3.4	ND
Sand (%)	85.0	84.0	ND
TCL (USD)	SL	SL	ND
Chemical composition			
pH(H2O)	6.08	6.10	7.34
pH(0.01MKCl2)	6.00	5.44	7.13
Ashes(gkg^{-1})	ND	ND	13.56
T N%	0.04	0.07	0.59
% OC	0.64	0.86	3.78
% O M	1.10	1.48	6.50
Avail. P(gkg^{-1})	4.57	12.29	3.01
K($mgkg^{-1}$)	0.31	0.38	0.31
Mg($cmolkg^{-1}$)	1.78	1.28	1.34
Ca($cmolkg^{-1}$)	3.41	4.83	3.01
Na($cmolkg^{-1}$)	0.67	0.42	0.52
Al + H(acidity)	0.83	0.76	0.20
CEC($cmolkg^{-1}$)	6.17	7.91	5.18
0%Base Saturation	87.00	90.39	96.52

ND = Not Determine

Effect of Rhizobium Inoculation on Soybean Grown in Biochar amended Soil

Rhizobium inoculation of soybeans had shown significant increase on allgrowth parameter at seven weeks after planting (Table 2) in 2016 and 2017 cropping seasons. The inoculated plants were better in terms of all the growth parameters (number of leaves, plant height and number of branches) assessed. Inoculated soybeans produced significantly produced higher number of leaves (90.59 and 93.19); plant height (27.95cm and 28.63cm) and number of branches (5.08 and 5.11). Also inoculated plants roots nodules (25.35 and 23.42) where higher compare to the uninoculated soybeans plants that were only 9.12 and 8.83 roots nodules in both years of cropping. However, biocharrates did not produce any significant increase on the growth parameters of soybeans. Although, additional increase in biochar application resulted to proportional increase in all growth parameters of soybeans in both years of cropping.

Table2: Effect of rhizobium inoculation on growth and nodulation count of soybean grown on biochar amended soil at seven weeks after planting

Treatments	No. of branches	No. of branches	Plant height (cm)	Plant height (cm)	No. of Leaves	No. of Leaves	Nodulation count	Nodulation count
	2016	2017	2016	2017	2016	2017	2016	2017
Rhizobium (bacteria)								
Control	4.34	4.44	24.89	26.72	84.74	87.31	9.12	8.83
Inoculated	5.08	5.11	27.95	28.63	90.59	93.19	25.35	23.42
LSD(0.05)	0.09	0.10	1.26	1.01	2.56	2.55	5.54	7.24
Biochar (t/ha)								
0	4.82	4.75	23.54	26.65	78.53	82.45	15.25	14.73
5	5.04	5.08	28.27	27.24	86.45	90.34	15.95	15.89
10	5.56	5.50	29.12	30.78	90.24	94.25	16.76	17.87
LSD (0.05)	0.18	0.15	1.72	1.58	2.12	3.14	4.24	5.18

Effect of Rhizobium inoculation on Growth Parameters of Soybean grown on Biochar amended soil

Rhizobium inoculation and biochar soil amendment had a significant increase on all the yield parameter assessed (Table 3) in both years of cropping. All the soybeans that were inoculated with rhizobium produced the highest number of flowers (58.45 and 56.84); highest number of pods per plant (48.15 and 35.34); and highest seed weight of 2.67 and 2.85 t ha.[1] Application of 10 t ha-1 of biochar produced the highest number of flowers (63.26 and 68.21); highest number of pods per plant (53.45 and 54.25); and highest seed weight of 2.53 and 2.69 t ha^{-1} in 2016 and 2017 cropping seasons respectively.

Table3: Effect of rhizobium inoculation on yield parameters of soyabean grown on biochar amended soil at harvest

Treatments	No. of flowers	No. of flowers	No. of pods/plant	No. of pods/plant	Seed weight (t/ha)	Seed weight (t/ha)
	2016	2017	2016	2017	2016	2017
Rhizobium(bacteria)						
Control	41.56	45.32	37.23	41.12	2.23	2.34
Inoculated	58.45	56.84	48.15	45.34	2.67	2.85
LSD(0.05)	6.42	4.21	3.26	2.45	0.12	0.23
Biochar (t/ha)						
0	26.45	39.45	28.43	34.63	1.95	2.05
5	40.79	49.65	36.15	43.25	2.21	2.38
10	63.26	68.21	53.45	54.25	2.53	2.69
LSD (0.05)	7.34	6.06	4.32	3.35	0.22	0.25

DISCUSSION

The soil of the experimental site is already exhausted and acidic (Table 1); due to intensive and continuous cultivation and without adequate application of replenishment measures to sustain it productivity. This result agrees with finding of (Jayeoba, et. al., 2013). The significant increase in growth parameters (number of leaves, plant height, number of branches and root nodules) of inoculated soybeans could be attributed to the ability of the *bradyrhizobiumjaponicum* to increase the number of root nodules (Table 2), which means increase in number of nitrogen fixing bacterial and subsequent increase in Biological Nitrogen Fixation (BNF). This BNF led to soil nitrogen improvement; then biochar application aided nitrogen use efficiency of soybean plant. Since nitrogenis known to improve cell activity, enhance cell multiplication and development of luxuriant vegetative plant (Akanbi et al., 2005). This results is in consonance with the findings of Khain*et al* (2003) who work on the Organic and inorganic fertilization on the growth, yield and seed quality of soybean. The significant increase in yield parameters (number of flower per plant, number of pod per plant and seed weight t ha^{-1}) of soybeans as a result of rhizobium inoculation and biochar soil amendment may be attributed to decreased exchangeable acidity levels and increase in the number of leaves. Therefore, macronutrient like phosphorus was readily available for plant uptake thereby, resulting in the synthesis of more photo-assimilates, which is used in producing seeds. This result is supported by the work of Adeyeye et al (2017) who recorded 35% grain yield increase in inoculated soybeans against the uninoculated.

CONCLUSION

Rhizobium inoculation and biochar soil amendment at a rate of 10 t/ha significantly increased grain yield of soybean. Also, substantial increase in soybean nodulation which directly affected both growth and yield of soybeans. The present study therefore, recommends treating soybean seeds with rhizobium inoculants before planting and amending the soil with 10 t/ha of biochar for increased grain yield

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