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INFLUENCE OF *BRADYRHIZOBIUM JAPONICUM*, MYCORRHIZA AND POULTRY MANURE ON NODULATION, NITROGEN UPTAKE, GROWTH AND YIELD OF SOYBEAN (*GLYCINE MAX* L.) UNDER MANURE APPLICATION

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Abstract: This study was carried out to assess the effects of *Bradyrhizobium japonicum*, mycorrhiza fungi and poultry manure on nodulation, growth and yield of soybean under manure application. Four types of treatments were used (poultry manure, *Bradyrhizobium* inoculation, mycorrhiza and Single Super Phosphate) and were added to each experimental sub-unit. Different rates of poultry manure were added 0 t ha^{-1}, 5 t ha^{-1} and 10 t ha^{-1} and left for two weeks to decay while *Rhizobium* inoculation comprises of inoculated and un-inoculated, the plot was sub-divided into 36 units and arranged in randomized completed block design. Leaf area, number of leaves, plant height and canopy diameter were determined at 4, 6, 8, 10, 12 and 14 Week After Planting. Number and weight nodules, fresh and dry weight of shoot and root were determined at the 6th and 8th weeks after planting and grain yield were determined at harvest. Soil samples were also collected before and after planting for laboratory analysis. The poultry manure used was also analyzed for the amount of N: P: K present. *Rhizobium* inoculation with addition of mycorrhiza fungi significantly increased the nitrogen fixation of the soybean and also helps the plant to withstand or tolerate water stress. Combination of Brad+Myc+5PM had effect on leaf area of plant. Myc+5PM had significant effect on stem girth of soybean and combination of Brad+Myc+5PM significantly increased the height of plant. The application of SSP helps to provide phosphorus to the plant. The 5 kg\ha and 10 kg\ha of poultry manure and mycorrhiza fungi significantly affects and improves the growth, nitrogen uptake and yield of soybean. *Bradyrhizobium* also helps in effective nodulation of the soybean.

Keywords: Nodulation, *Rhizobium*, Nitrogen fixation, Water stress, Soybean, Yield.

INTRODUCTION

Glycine max is an herbaceous annual legume with a bushy, erect and rather leafy plant structure. It originated from China around 1100 to 1700 BC but was introduced into Europe only in the 17th century (Osho *et al.*, 2009). Ezedinma

(1964), who reviewed the history of the crop in Nigeria, reported that soybean was first introduced in 1908 by the British looking for new sources of supply from their colonies. Attempts to grow the crop at Moor Plantation, Ibadan, at that time failed. In 1928 the soybean was successfully introduced to Samaru, where it spread into the other parts of Northern Nigeria. To meet the high European demand for oilseeds during World War II, acreage expanded rapidly and in 1947 the first exports of 9 ton were recorded. Soybean soon became a cash crop in the Tiv division and Benue Valley of Benue Province, which thereafter was the leading center of production (Shurtleff and Aoyagi, 2007). About 111.519 million Nigerians live in relative poverty which when compared to the country's population (163 million) is about 69 % (Punch, 2012), this has an obvious implication on food insecurity. There is also the contribution of myriad of problems facing the agriculture and therefore food production sector. Nigerian farmers are often being constrained by several factors which have direct or indirect effects on agricultural productivity and invariably on production.

Some of these problems or factors had been addressed by successive Nigerian Governments through different intervention programmes or policies in the past but many of these policies are inconsistent with unintended consequences as identified by Idachaba (1991, 2000) and Olaitan (2007) which then bring about the poor performance of Nigerian agriculture. Over the last two decades, IITA has made substantial efforts to improve the productivity of the crop by developing high yielding, early maturing varieties capable of nodulating in association with local rhizobia and possessing other good agronomic traits (IITA, 1994). Improved soybean varieties released in Nigeria include TGx 849-313D, TGx 1019-2EN, TGx 1019-2EB, TGx g1447-2E, TGx 536-02D, TGx 306-036C, TGx 1485-1ED, and TGx 1440-1E (IITA 1994). Others are TGx 1448-2E, TGx 1835-10E, SAMSOY 1 (M-79), and SAMSOY 2 (M-216) (SG 2000, 2010).

The major staple foods in Africa are grains and tubers whose protein contents are low, animal protein is insufficient, and of low quality therefore not a good protein substitute (IITA, 1985). Soybean (*Glycine max*) is more nutritive in terms of protein content and amino acids composition (IITA, 1985). In many countries, especially developing ones like Nigeria, farmers intercrop legumes with millet, sorghum, maize and cassava or include them in the crop rational cycle because of their capability to restore back soil fertility. Nigeria is the largest producer of soybean (*Glycine max* L.) for food in the West and Central Africa (Otitoju and Arene, 2010). Soybean is among the major industrial and food crops grown in every continent, it is a globally important oilseed crop and source of high quality protein content for human consumption, used as fodder for animal and also important in improved crop rotation system (Manyong *et al.*,1998; Carsky *et al.*,

2002). Soybean can be grown in wide range of tropical soils but preferably on sandy or clay loamy soil depending on the fertility of the soil and it matures early (65-150 days) depending on the cultivar (Adigun and Babalola, 2016). It is predominantly self pollinating and completely self fertile, but some very low levels of cross pollination do take place. Due to ability of soybean to enhance the efficiency of nitrogen for protein manufacture (Samuel and Agnes, 2011) and oilseed production, soybean production has been on the increase worldwide.
Soybean like other nodulated legumes utilizes two sources of nitrogen for its growth-mineral N in the soil (in form of Nitrite and Ammonium) and atmospheric nitrate is fixed in nodules (Mengel and Kirkby, 1987; Scott and Aldrich., 1983). It has been found that inoculation of soybean with *Bradyrhizobium japonicum* significantly increased nodulation, yield and seed quality (Sadowsky *et al.*, 1987; Okereke and Onochie, 1996; Okereke *et al.*, 2001). If soybean is abundantly nodulated, it is capable of fixing substantial amounts of its required nitrogen from BNF and in other to produce a higher protein meal because larger amount of nitrogen is needed as nitrogen is a constituent of amino acids, which plays a positive role in almost all plant metabolic process (Samuel and Agnes, 2011).

MATERIALS AND METHODS

Location of the Experiment: The experiment was carried out at the Federal University of Agriculture, Abeokuta, Ogun State. Source of organic materials: Poultry manure free from bedding/litter material was obtained from COLANIM farm (battery cage system). The rate of poultry manure applied in this experiment was 0, 5, 10 t/ha respectively, which was analysed through digestion with HNO_3 and H_2SO_4 for macro and micro nutrients (% N, P, % K) by standard procedures (Kalra and Maynard, 1991).

Test crop: The test crop used was soybean-TGX 1448-2E variety which was sourced from IITA, Ibadan Oyo state.

Sources of *Bradyrhizobium*: The *Bradyrhizobium japonicum* sourced from IITA Ibadan and was inoculated into the soybean by boiling 250 m/s of distilled water to dissolve the Gum Arabic used for 500 g of soybean and 2.5 g of *Bradyrhizobium* which was weighed into the soybean and was then mixed thoroughly for even distribution.

Source of Mycorrhiza: The mycorrhiza fungi (*Glomus moseae*) used was cultured in FUNAAB and was inoculated into soil at planting by drilling along the row.

Single super phosphate: Single super phosphate (SSP) was sourced from the Department of Plant Physiology and Crop Production, COLPLANT, Federal University of Agriculture, Abeokuta.

Land Preparation: Total area was 625 m^2 plus alley, the land was cleared manually with cutlass and hoe, each plot was 3 m by 3 m making 9 m^2, planting distance was 5 cm by 75 cm, plant population/ha was 266,000/ha. Soil preparation for routine analysis: Soil sample was collected before and after planting. It was air dried at room temperature for 48 hours, ground with mortal and pestle and passed through a 2 mm sieve and the following parameters were measured: pH, organic matter, particle size, total Nitrogen, available Phosphorus. The pH was determined (1:1 soil: water) using pH meter glass electrode (Mclean, 1982). 10 g of air – dried soil was weighed into a sample bottle, 10 ml of distilled water was added into the soil by filtering the distilled water with whatman number 42 filter paper before adding it into the soil, and the mixture was shaken for thirty minutes in a mechanical shaker, and then allowed to settle for five minutes, pH value of 4.0 was used to standardized the pH meter before the pH was determined using electrode pH meter.

The organic matter content of the samples was determined using the wet – oxidation method. Walkley – Black (1934) method modified by Allison and Moodie (1965). 1 g of soil was digested with 10 cm^3 of K_2CrO_7 and 2 ml of conc. H_2SO_4 was added to the solution, 100 ml of distilled water was added and titrated with ferrous sulphate using four drops ferroin indicator.

Formula:

$$\% OC = \frac{(\text{blank} - \text{titre value}) \times 0.5N \times 0.003 \times 1.33}{\text{Weight of soil sample}}$$

$$\% OM = \%OC \times 1.724$$

Particles size using the hydrometer method (Bouyoucos, 1962) and the proportion of sand, clay and silt was used to determine the textural class of the soil using USDA textural triangle. 50 g of soil sample was weighed into conical flask and the soil was dispersed with 5 % calgon solution for twenty four hours. The soil mixture was shaken thoroughly and introduced into 1.0 ml measuring cylinder. First hydrometer readings were taken after forty seconds for silt and clay, while second reading was taken after two to three hours. Total nitrogen was determined using Kjeldahl distillation method (Bremner, 1996). 0.5 g of soil sample was weighed in digestion tube and 4 ml of H_2SO_4 and kjeldahl tablet were added, the sample was placed in the HD40 block digester and digested at 350° C for two hours. The sample was then removed from the digester block and cooled. 50 ml of distilled water was mixed vigorously and the mixture was washed into a

100 ml flask and made up to mark. The flask was shaken thoroughly and allowed to cool and settle down. Available phosphorus was determined in soil using Bray 1 method. 1 g of soil sample was weighed into 15 ml centrifuge tube and 7 ml of extracting solution was added and shaken for one minute on mechanical shakers and centrifuged at 2,000 rpm for 15 minutes. 2 ml of clear supernatant was pipette into a 20 ml test tube; 5 ml of distilled water and 2 ml ammonium molybdate solution were added and mixed thoroughly. After fifteen to twenty minutes spectrophotometer reading of the sample was taken. Exchangeable base was determined when 5.0 g of air dried soil was weighed and 500 ml of 1 N Ammonia Acetate of pH 7.0 was poured into it. It was later shaken for 30 minutes in a mechanical shaker, and then filtered through a No 1 Whatman filter. Calcium and magnesium was read on a flame photometer while potassium and sodium was read on Atomic absorption spectrophotometer.

Data Collection: Plant heights, number of leaves, stem girth and leaf area were measured weekly from 4 WAP. Fresh weight and dry weight of the soybean plant root and shoot was taken at 6 WAP. Data was also taken for number of days to flowering for the plants.

Measurement of Dry Matter Production: The soybean seedling (5 tagged plant) was uprooted from the field at peak flowering and the shoot was separated from the root (destructive sampling), nodules were counted and weighed before been placed in the oven at 70° C to constant weight. After oven – drying, the fresh and dry weight of shoot, root and nodules were taken.

Determination of Nitrogen in Plant Tissue: Nitrogen in the plant tissue was determined by grinding the plant tissue with a mechanical grinder after oven drying to constant weight; wet ashing method was used to digest the plant tissue by weighing 1 g of plant sample and 1 g of catalyst (Potassium sulphate K_2SO_4 and 0.0075 g of selenium specs) for the digestion. Then 1 g of the mixed sample was weighed into digestion tube and 25 ml of concentrated H_2SO_4 was added into the tube and the solution was heated until it became clear, the solution was allowed to cool, then 100 ml of the solution was mixed with de-ionised water to make up the solution, the N content was determined by using Buchi auto-kjeldahl.

Statistical Analysis: Data collected was subjected to analysis of variance(ANOVA) using the SAS package (Statistical Analysis System). The means were separated using Least Significant Difference (LSD at 5 % probability).

RESULTS AND DISCUSSION

The properties of initial field soil

Table 1 below shows the physical and chemical properties of soil used for the experiments. The soil used was sandy loam with near neutral pH value of 6.8, low organic carbon of 0.60 %, moderate organic matter, total nitrogen of 1.38 % which was considered moderately low as value of at least 2.5-3.0 % was recommended as optimum at which no addition of nitrogen may be needed. Available phosphorus was 17.73 mg/kg, exchangeable potassium for the soil was 1.02 cmol/kg. Exchangeable acidity which was the summation of available hydrogen ion and aluminium ion in the soil was moderately low, exchangeable bases: magnesium, calcium, potassium and sodium were considered moderately low for tropical soil. Cation exchangeable capacity was 2.86.

Table 1: Preplanting Physical and Chemical Properties of Soil

PROPERTIES	VALUES
% Sand	91.00
% Silt	4.60
% Clay	4.40
Soil textural class	Sandy –Loam
pH (Soil/ Water)	6.8
% Organic carbon	0.6
% Nitrogen	1.4
Available phosphorus (mg/kg)	17.73
% Organic matter	1.03
Ca (cmol/kg)	0.64
Mg (cmol/kg)	0.58
K (cmol/kg)	0.18
Na (cmol/kg)	0.21
Exchangeable acidity (cmol/kg)	1.25
ECEC (cmol/kg)	2.86

Response of soybean height to treatments at different WAP.

Table 2 shows the effect of treatments on height of plant at 4 - 14 weeks after planting. All the treatments were comparable, treatments with 0, 5, 10 t/ha of poultry manure have no significant difference on soybean height from 4 to 14 WAP but treatment with 5 t/ha of poultry manure was higher compared to 10 t/ha which had lower value. Plant height increased with increase in week of plant but mycorrhizal had the highest effect on plant height compared to single super phosphate. At 6, 8, 10, 12 WAP there were no significant difference between treatments with *Bradyrhizobium* and without *Bradyrhizobium* but treatment without *Bradyrhizobium* had higher value compared to treatment with *Bradyrhizobium*.

Table 2: Effect of Poultry Manure, *Bradyrhizobium*, SSP and Mycorrhiza on Height (Cm) of Soybean at 4 to 14 weeks after Planting.

Treatments	4WAP	6WAP	8WAP	10WAP	12WAP	14WAP
Poultry manure						
Source						
0 (ton)	12.22[a]	21.55[a]	29.58[a]	38.20[a]	42.78[a]	47.99[a]
5 (ton)	12.22[a]	21.55[a]	29.58[a]	35.20[a]	42.78[a]	47.99[a]
10 (ton)	12.39[a]	22.12[a]	30.54[a]	36.22[a]	42.89[a]	46.34[ab]
P value	12.94[a]	21.29[a]	29.24[a]	34.15[a]	42.85[a]	44.13[b]
P source	0.18	0.37	0.39	0.19	0.34	0.04
Mycorrhiza	13.23[a]	22.09[a]	30.69[a]	36.32[a]	43.56[a]	48.11[a]
SSP	11.80[b]	21.21[a]	28.89[b]	34.07[a]	40.79[b]	44.19[b]
P value	0.00	0.10	0.02	0.01	0.03	0.00
Bradyrhizobium						
Source						
Bradyrhizobium-	12.28[a]	22.24[a]	31.03[a]	36.42[a]	44.99[a]	47.00[a]
Bradyrhizobium+	12.75[a]	21.06[b]	28.54[b]	33.97[b]	36.35[b]	45.30[a]
P value	0.20	0.02	0.00	0.01	0.00	0.18

Means with the same letter are not significantly different at 5% LSD level of probability.

Response of plant height to interactive application of treatments at different WAP.

Table 3 shows the interactive effect of treatments where plant height was influenced by the combination of Myc + 10PM which has the highest value for plant height at 4 (13.75) and 6 (24.31) WAP. Brad + Myc + 5PM had the highest effect on plant height at 8 (34.90) WAP, Myc + 10PM significantly affect plant height at 10 (34.76) and 12 (40.93) WAP while Myc only had the highest effect on plant height at 14 (53.70) WAP. Lower plant height was observed in SSP at 4 WAP while 10PM + SSP only at 6, 10 and 14 WAP.

Table 3: Response of Soybean Height (Cm) to Interactive Application of Brady, Mycorrhiza, SSP and Poultry Manure at 4 to 14 Weeks after Planting.

	4WAP	6WAP	8WAP	10WAP	12WAP	14WAP
BM+5PM	13.50ab	22.11	34.90	30.80	37.82	45.71
BM+10PM	12.30a	19.30	21.50	25.84	30.40	41.73a
BM	13.02a	20.02	27.10	28.38	32.18	45.70
BS+5PM	12.92ab	21.90	30.72	28.65	34.11	46.95
BS+10PM	13.30a	22.50	30.20	31.67	36.98	44.50
BS	11.50ab	20.41	25.96	26.61	32.30	47.30
M+5PM	13.20a	22.84	33.70	32.20a	36.67	48.67
M+10PM	13.75	24.31	33.63	34.76	40.93	53.24
M	13.70	23.99	34.63	33.20	39.90	53.70
S+5PM	9.99	21.62	31.32	30.52	36.24	44.03
S+10PM	13.40	18.92	23.00	24.40	28.29	37.04
S	10.71	21.80	31.52	31.20	36.47	45.40
P Value	0.001	0.001	0.001	0.001	0.001	0.001

Means with the same letter are not significantly different at 5% level of probability using LSD.

Response of soybean stem girth to treatment at various WAP

Table 4 shows the main effect of treatments on soybean stem girth. At 4 to 14 weeks after planting, all parameters were comparable. The treatments did not have significant effect on the stem girth of plant but treatments without manure application had higher value compared to others. The P source has no significant effect but treatment with mycorrhiza is higher in value than treatment with single super phosphate. Treatment with *Bradyrhizobium* had higher value compared to treatment without *Bradyrhizobium*.

Table 4: Effect of Brady, Mycorrhiza, SSP and Poultry Manure on Soybean Stem Girth (Cm) at 4, 6, 8, 10, 12, 14 weeks after Planting.

Treatments	4WAP	6WAP	8WAP	10WAP	12WAP	14WAP
Poultry manure Source						
0 (ton)	0.10[a]	0.15[a]	0.21[a]	0.28[a]	0.37[a]	0.46[a]
5 (ton)	0.10[a]	0.27[a]	0.23[a]	0.27[a]	0.36[ab]	0.44[a]
10 (ton)	0.10[a]	0.74[a]	0.22[a]	0.27[a]	0.34[b]	0.45[a]
P value	0.16	0.18	0.20	0.51	0.10	0.10
P source						
Mycorrhiza	0.10[a]	0.37[a]	0.22[a]	0.27[a]	0.36[a]	0.46[a]
SSP	0.10[a]	0.40[a]	0.22[a]	0.28[a]	0.35[a]	0.44[a]
P value	0.16	0.90	0.20	0.77	0.85	0.10
***Bradyrhizobium* Source**						
Bradyrhizobium-	0.10[a]	0.15[a]	0.21[a]	0.28[a]	0.35[a]	0.46[a]
Bradyrhizobium+	0.10[a]	0.40[a]	0.21[a]	0.27[a]	0.36[a]	0.44[a]
P value	0.16	0.94	0.10	0.38	0.25	0.10

Means with the same letter are not significantly different at 5% LSD level of probability.

Interactive effect of treatment application on stem girth at various WAP.

Table 5 shows the interactive effect of treatments on soybean stem girth at 4, 6 and 8 WAP, there were no significant difference on stem girth.

Table 5: Interactive Effect of *Bradyrhizobium*, Mycorrhiza, SSP and Poultry Manure on Stem Girth (Cm) at 4 to 14 weeks after Planting.

	4WAP	6WAP	8WAP	10WAP	12WAP	14WAP
BM+5PM	0.1a	0.14a	0.22a	0.28ab	0.35ab	0.48abc
BM+10PM	0.1a	1.30a	0.21a	0.27b	0.35ab	0.42cd
BM	0.1a	0.17a	0.21a	28.14	0.36a	0.45bcd
BS+5PM	0.1a	0.49a	0.23a	0.28ab	0.37ab	0.41d
BS+10PM	0.1a	0.15a	0.01a	0.28ab	0.33ab	0.45bcd
BS	0.1a	0.14a	0.21a	0.26a	0.40a	0.46bcd

M+5PM	0.1a	0.28a	0.23a	0.27b	0.35ab	0.40d
M+10PM	0.1a	0.17a	0.23a	0.27b	0.35ab	0.51ab
M	0.1a	0.15a	0.23a	0.31a	0.37ab	0.53a
S+5PM	0.1a	0.16a	0.23a	0.28ab	0.36ab	0.49ab
S+10PM	0.1a	1.34a	0.22a	0.28ab	0.32b	0.42cd
S	0.1a	0.15a	0.21a	0.27b	0.33ab	0.41cd
P Value	0.001	0.009	0.003	0.001	0.001	0.001

Means with the same letter are not significantly different at 5% level of probability using LSD.

Response of soybean leaf area to treatments at different WAP

Table 6 shows the main effect of treatments on soybean leaf area. Leaf area increased with increase in growth of plant. At 10 and 14 weeks after planting, 5 kg/ha of poultry manure has the highest effect on leaf area of plant. At 4 and 6 WAP, control had significantly higher value of plant leaf area than plot treated with 5 and 10 kg/ha of poultry manure. Phosphorus source treatments had no significant difference on leaf. Treatments with mycorrhiza have highest effect on leaf area compared to treatments with single super phosphate. *Bradyrhizobium* had no effect on leaf area except at 8 and 10 WAP but treatment without *Bradyrhizobium* has the highest effect on leaf area compared to treatments with *Bradyrhizobium.*

Table 6: Effect of Brady, Mycorrhiza, SSP and Poultry Manure on Soybean Leaf Area (Cm^2) at 4 to 14 weeks after Planting.

Treatments	4WAP	6WAP	8WAP	10WAP	12WAP	14WAP
Poultry manure Source						
0 (ton)	5.58^a	13.81^a	23.69^{ab}	29.78^{ab}	38.32^a	41.83^{ab}
5 (ton)	5.58^a	13.81^a	25.77^a	32.64^a	36.69^a	43.43^a
10 (ton)	5.59^a	12.18^b	21.82^b	27.07^b	35.41^a	38.28^b
P source						
Mycorrhiza	5.45^a	13.34^a	25.13^a	30.89^a	38.05^a	41.91^a
SSP	5.58^a	12.93^a	23.39^b	28.78^{ab}	35.57^a	40.45^a
***Bradyrhizobium* Source**						
Bradyrhizobium-	5.73^a	13.65^a	25.50^a	31.30^a	37.65^a	41.99^a
Bradyrhizobium+	5.31^a	12.61^a	22.02^b	28.38^b	35.96^a	40.36^a
P value	0.25	0.13	0.00	0.03	0.24	0.30

Means with the same letter are not significantly different at 5% level of probability using LSD.

Response of leaf area to interactive application of treatments at various WAP.

Table 7 shows the interactive effect of treatments on soybean leaf area. At 4 (7.49), 6 (16.45) and 10 (38.83) WAP, Myc had significant effect on leaf area of plant. However, at 8 (27.94) and 14 (47.82) WAP, leaf area was significantly influenced by the combination of Brad+Myc+5PM. Lower leaf area was observed at 4 (4.09), 6 (9.57), 10 (31.73) and 14 (31.73) WAP with the combination of Brad+Myc+10PM, SSP+10PM had lower effect at 8 (17.82) WAP while SSP+5PM had effect at 12 WAP.

Table 7: Effect of Interactive Application of Brady, Mycorrhiza and Poultry Manure on Soybean Leaf Area (Cm²) at 4 To 14 weeks after Planting.

	4WAP	6WAP	8WAP	10WAP	12WAP	14WAP
BM+5PM	4.84cd	13.70abc	27.94a	30.73abc	36.97a	47.82a
BM+10PM	4.09d	9.57d	18.39d	27.80bcd	28.70b	31.73d
BM	4.36d	10.48cd	20.67cd	28.14d	37.75a	41.30abc
BS+5PM	5.73bcd	14.01abc	22.28bcd	36.00cd	40.90a	39.50bc
BS+10PM	7.31ab	13.45bcd	22.66abcd	28.95abc	35.33a	44.85ab
BS	5.49bcd	14.10abc	20.19cd	29.77d	36.10a	36.92bcd
M+5PM	5.48bcd	14.44ab	27.89b	31.94bc	42.00d	42.61ab
M+10PM	6.45abc	15.01cd	28.44a	33.96a	41.90a	42.04abc
M	7.49a	16.45a	27.45a	38.83bcd	40.95a	45.94a
S+5PM	5.80abcd	11.48bcd	24.95abc	25.00ab	26.89a	43.77ab
S+10PM	4.17d	10.67cd	17.82d	28.61d	35.70a	34.48cd
S	4.97cd	13.85abc	26.44ab	34.57d	38.48a	43.15ab
P Value	0.001	0.009	0.003	0.001	0.001	0.001

Means with the same values are not significantly different at 5% level of probability using LSD.

Response of soybean dry matter parameters to treatments at 8WAP

Table 8 shows the main effect of treatments on dry matter parameters of soybean plant. The treatments are comparable. Treatments did not have significant effect on number and weight of nodules. However, 10 kg/ha of poultry manure had the highest effect on weight of nodules over control. The treatments did not have significant effect on root, shoot and dry matter of plant but *Bradyrhizobium* had the highest effect on shoot and dry matter of plant. 5 kg/ha had the highest effect on nitrogen content of soybean.

Table 8: Main Effect of Treatments on Shoot, Root, Nodules and Nitrogen Uptake of Soybean at 8 weeks after Planting.

Treatments	Nodules No	Nodules Wgt(g)	Root (g/plant)	Shoot (g/plant)	Dry Matter	N Content	N uptake (mg/kg)
Bradyrhizobium							
Bradyrhizobium+	7.22[a]	0.28[a]	0.32[a]	2.35[a]	2.67[a]	1.73[a]	4.92[a]
Bradyrhizobium-	5.67[a]	0.21[a]	0.44[a]	2.22[a]	2.66[a]	1.65[a]	4.73[a]
P- sources							
Mycorrhiza	6.33[a]	0.25[a]	0.40[a]	2.44[a]	2.84[a]	1.90[a]	5.37[a]
SSP	6.56[a]	0.24[a]	0.37[a]	2.13[a]	2.49[a]	1.74[b]	4.28[b]
Poultry manure							
0 kg/ha	7.00[a]	0.27[a]	0.34[a]	2.52[a]	2.86[a]	1.71[a]	4.95[a]
5 kg/ha	4.92[a]	0.26[a]	0.44[a]	2.09[a]	2.53[a]	1.92[a]	4.82[a]
10 kg/ha	7.42[a]	0.20[a]	0.36[a]	2.25[a]	2.61[a]	1.83[a]	6.68[a]

Mean with the same letters are not significantly different at 5% LSD level of probability.

Response of soybean dry matter parameters to interaction of treatments.

Table 9 show the interactive effect of treatments on dry matter parameters. It shows that Brad+Myc had the highest value for dry matter accumulation and nitrogen uptake by plant. Myc +10PM had significant effect on number of nodules. Myc+5PM had the highest effect on weight of nodules produced. Brad+Myc+5PM had the highest effect on root of plant while Brad+Myc+10PM had the highest effect on shoot of soybean plant.

Table 9: Interactive Effect of *Bradyrhizobium*, Mycorrhiza, Poultry Manure and SSP on Soybean Dry Matter (g/plant) Parameters and Nodulation at 8 weeks after Planting.

Treatments	Number Of nodules	Weight of nodules	Root (g/plant)	Shoot (g/plant)	Dry Matter	N uptake (mg/kg)
Brad/Myc	6.33[a]	0.27[a]	0.38[a]	2.41[a]	3.69[ab]	6.54[a]
Myc	9.00[a]	0.28[a]	0.40[a]	2.28[ab]	3.15[ab]	5.98[ab]
SSP	6.00[a]	0.23[a]	0.37[a]	2.14[a]	2.72[ab]	4.25[a]
Brad /SSP/5PM	4.00[a]	0.22[a]	0.30[a]	2.45[b]	2.71[ab]	5.29[ab]
SSP/10PM	4.67[a]	0.25[a]	0.20[b]	2.32[a]	2.80[ab]	3.94[a]
Brad/Myc/5PM	3.00[a]	0.19[a]	0.50[ab]	2.78[ab]	2.72[ab]	5.29[ab]
Brad /SSP/10PM	10.0[a]	0.19[a]	0.29[b]	2.60[b]	2.59[ab]	4.82[ab]
Myc/10PM	11.0[a]	0.25[a]	0.24[a]	2.55[a]	2.61[ab]	5.25[a]
Brad/Myc/10PM	4.00[a]	0.21[a]	0.45[a]	2.95[ab]	2.44[ab]	4.82[ab]
Myc/5PM	4.67[a]	0.40[a]	0.25[a]	2.48[a]	2.42[ab]	4.60[a]
SSP/5PM	8.00[a]	0.24[a]	0.30[ab]	2.32[b]	2.26[ab]	4.35[ab]
Brad/SSP	6.68[a]	0.28[a]	0.22[a]	2.14[a]	1.87[a]	3.06[b]

Mean with the same letters are not significantly different at 5% LSD level of probability.

Influence of treatments on soybean yield.

Table 10 shows the main effect of treatments on soybean yield (kg/ha) and one thousand seeds weight at the end of planting. The poultry manure has no significant difference on seed yield. 10 ton of poultry manure has higher value compared to 5 and 0 ton. Phosphorus source has significant difference on seed yield. Mycorrhiza is significantly different over single super phosphate.

Bradyrhizobium has no effect on seed yield. Treatments without *Bradyrhizobium* have higher value than treatment with *Bradyrhizobium*.

Table 10: The Main Effect of Brady, Mycorrhiza, Poultry Manure and SSP on Soybean Yield.

Treatments	Yield	HSW (Kg/plot)
Poultry manure		
0 (ton)	100.1[a]	8.84[a]
5 (ton)	103.5[a]	9.04[a]
10 (ton)	106.8[a]	8.32[a]
LSD	8.20	1.12
P source		
Myc	109.8[a]	9.07[a]
SSP	97.1[b]	8.40[a]
LSD	5.12	0.89
Bradyrhizobium+	106.1[a]	8.66[a]
Bradyrhizobium-	100.1[a]	8.82[a]
LSD	6.54	0.92

The influence of interaction on yield parameters.

Table 11 shows the interaction of treatments on yield parameters. There were significant difference on seed yield (kg/ha). Treatment with Myc+10PM and Myc had the highest effect on seeds yield than other treatments while treatments with SSP has the lowest effect on seed yield. There were no significant difference on one thousand seed weight (kg/ha). Treatments with Myc/10PM and Brad/Myc have higher significant difference compared to other treatments while treatments with Brad/Myc/10PM has the lowest effect.

Table 11: The Interactive Effect of Brady, Mycorrhiza, Poultry Manure and SSP on Soybean Yield Parameters.

Treatments	TSW(kg/ha)	Yield(kg/ha)
M+10	90.97[a]	106.3[a]
B+M	85.93[a]	89.74[abc]
B+M+5	84.83[ab]	93.57[c]
M+5	83.5[abc]	102.17[ab]
B+S+5	81.37[abc]	91[cde]
S	80.6[abc]	82.6[c]
S+5	78.57[a]	84.37[a]
M	78.43[abc]	104.77[a]
B+S+5	76.23[abc]	92.5[a]
B+S	73.1[abc]	83.64[de]
S+10	64.57[bc]	90.5[cde]
B+M+10	63.5[c]	94.8[bc]

TSW: Thousand seed weight

YPP: Yield per plot

Post planting analysis

Table 12 shows that available phosphorus before planting was 17.73 mg/kg while that of after planting was 4.37 mg/kg, this reduction was due to the fact that the Phosphorus available in the soil has been utilized by the plant.

Nitrogen content of soil before planting was 1.38 while after planting was 1.49, the soybean plant helps to fix nitrogen into the soil after planting. Exchangeable potassium for the soil was 1.02 cmol/kg before planting and 0.95 cmol/kg after planting.

Table 12: The Post Planting Analysis of Soil.

PROPERTIES	BEFORE PLANTING	AFTER PLANTING
Available P (mg/kg)	17.73	4.37
Nitrogen content of soil	1.38	1.49
Exchangeable potassium	1.02	0.95

DISCUSSION

Based on the values obtained in the experiment there was a significant difference for the factor doses of poultry manure for the variables in all the agronomic stages and nodulation. Pavanelli and Araújo (2009) noticed low nodulation of soybean plants in certain soils and attributed that to low fertility levels. In a work with sewage sludge in soybeans, Lobo (2012) observed an increase in number and dry matter of nodules due to the increase of the sludge doses up to 20 t ha^{-1} and after this there was a decrease. The shoot response may have been influenced by the nutritional status of the crop, whereas the root response by physical soil properties. It is possible that addition of poultry litter resulted in soil physical characteristics that favored root development and has not been affected by nutritional imbalance in higher doses as happened with the crop shoots. There is a positive correlation between shoot dry matter at flowering and grain yield. Therefore, it is reasonable to assume that plants with more dry mass would be more productive (Lobo, 2012).

Regarding the soil properties, Blum *et al.* (2003) found that the use of poultry litter promotes the addition of organic matter, improving related soil properties such as greater water retaining capacity, better aeration, increasing pH, and reduction of exchangeable aluminum levels, creating a more suitable environment for the proper development of the microbial flora and root system. Nogueira *et al.* (2010), working with mineral N, found linear responses for both variables, but Lobo *et al.* (2012) found quadratic response to shoot dry matter with decrease after 26 t $ha^{-1}$of sewage sludge. Brandelero, Peixoto and Ralisch *et al.* (2009) observed a significant correlation among soybean yield, nodulation, and dry matter of leaves. Thus, since the poultry litter favored nodulation, it is expected under field conditions a good correlation of doses of poultry litter with soybean yield. Soybean fertilization with poultry litter in greenhouse conditions increases the chlorophyll content of leaves, plant height, shoot dry matter, root dry matter, and nodulation. The use of poultry litter on soybean favors its growth with decreasing increments, except for root dry matter, and with an optimum dose for each variable. The supply of poultry litter can replace mineral fertilizers in terms of soybean growth (Vilmar and Darly, 2013). The reduction in agronomic yield in tropical soil can be improved by the different combination of biofertilizers supplementation which helps plants to grow spontaneously by increasing the quality of nutrients available for plant uptake (Adigun and Babalola, 2016). This also helps to supply important nutrients crucial for overall productivity of the soil. However, care must be taken on the application of nutrients.

CONCLUSION

Based on the experiments carried out on the soybean, mycorrhiza with 5 kg/ha of poultry manure is recommended for maximum yield and production of soybean. Also, *Bradyrhizobium* can be used to aid nodules formation in soybean production.

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