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Soil Microbial Activities in Soybean Rhizosphere Inoculated with Bradyrhizobium and Mycorrhizal Fungi

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Authors' contributions

This work was carried out in collaboration between both authors. Author OAB designed the study, wrote the protocol and author MOA wrote the first draft of the manuscript. Author OAB managed the literature searches, analyses of the study performed the structural equation modelling and author MOA discuss the conclusion. Both authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to investigate the activities of soil microorganisms in the soybean rhizosphere inoculated with bradyrhizobium and mycorrhizal fungi. It involves a field experiment carried out at Federal University of Agricultural, Abeokuta, Nigeria, using Randomized Complete Block Design (RCBD). Treatments applied included 4.5 kg/ha poultry manure, 40 kg/ha Single Superphosphate, Mycorrhizal and 200 kg/ha of soybean seed and 1 g/ha *Bradyrhizobium*. Treatments were replicated thrice. Result showed significant difference between treatments on yield, growth and biomass activities in soybean production. Poultry manure, Phosphorus source, and *Bradyrhizobium* gave highest plant height of 47.99 and respectively at 14 WAP. Mycorrhizal with 10 tons poultry manure gave highest yield (0.12 t/ha). Microbial activities were significantly

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influenced by treatments with *Bradyrhizobium* inoculation in giving higher total bacteria count, total fungal count, microbial biomass C. Single Superphosphate in total bacterial count and microbial biomass N; poultry manure of 5 tons in total fungal count, microbial biomass C, N and P ($p \geq 0.05$): *Bradyrhizobium*, and 5 tons of poultry manure gave highest total fungal count (0.07 CFU/g), 10 tons poultry manure gave the highest total bacterial count (1.26 CFU/g), and 5 tons poultry manure gave highest microbial biomass C (198.1 mg/kg), N (124.9 mg/kg) and P (232.2 mg/kg). Soil N (0.16%) and organic C (17.11%) were highest in plot treated with 5 tons poultry manure (0.16% for N and 17.11% for organic C), while *Bradyrhizobium* gave highest available P (14.16 mg/kg). Cellulase activities was highest in plots without *Bradyrhizobium* (0.14 mg/kg), mycorrhizal (0.14 mg/kg), 5 tons poultry manure (0.4 t/ha), 10 tons poultry manure (0.14 t/a), while protease and urease activities were highest in plots with 5 tons poultry manure (0.13 mg/kg for protease and 0.17 mg/kg for urease). Addition of 5 tons of poultry manure application increased soil microbial activities, growth and yield of soybean.

Keywords: Bradyrhizobium; poultry manure; single superphosphate; mycorrhiza; enzymes; soybean.

1. INTRODUCTION

The interest in agro ecology had been focused on studying soil and fertilizer management in agricultural systems to improve soil quality and minimize possible deleterious effects on environment (e.g., soil erosion and euthrophication of natural ecosystems) [1]. Today, a more appropriate definition of rhizosphere is ‘the field of action or influence of a root. The rhizosphere is generally considered to be a narrow zone of soil subject to the influence of living roots, where root exudates stimulate or inhibit microbial populations and their activities. Therefore, it is the part of the soil ecosystem where plant roots, soil and the soil biota interact with each other.

Materials deposited by roots into the rhizosphere can be divided roughly into two main groups. Firstly, water-soluble exudates such as sugars, amino acids, organic acids, hormones and vitamins, and secondly, water-insoluble materials such as cell walls, sloughed-off materials and other root debris and mucilage such had lysates released when cells autolyse [2]. In addition, carbon dioxide from root respiration often accounts for a large proportion of the carbon released from roots. Secretions such as polymeric carbohydrates and enzymes depending on metabolic processes for their release may also be regarded as root exudates. The soil environment itself plays a role in determining root exudation and the activity and diversity of rhizosphere microbial populations [3].

The soil microbial biomass is involved in the decomposition of organic materials and, thus, the cycling of nutrients in soils. It is also frequently used as an early indicator of changes in soil chemical and physical properties resulting from soil management and environmental stresses in agricultural ecosystems [4,5,6]. Although the soil microbial biomass C constitutes only 1-3% of total soil C and the biomass N up to 5% of total soil N, they are the most labile C and N pools in soils [7].

Therefore, nutrient availability and productivity of agro ecosystems mainly depend on size and activity of the microbial biomass [8]. The turnover time for N immobilized in the microbial biomass was found to be about ten times faster than that derived from plant material [9]. The determination of N is, therefore, important for the qualification of N dynamics in agricultural ecosystems because it controls soil inorganic N availability and loss, especially in high input systems.

Mycorrhizal fungal excrete powerful chemicals that dissolve mineral nutrients, absorb water, retard soil pathogen and glue soil particles together into porous structure. In return the mycorrhizal fungal receive sugar and other compounds from the symbiotic relationship. Research had documented improved plant nutrient, water uptake and resistance to a wide range of environmental extremes. Over 50 thousand university studies have highlighted the benefits of mycorrhizal colonization on the health and yield of plants [10].

Bradyrhizobium japonicum belongs to the genus of Gram-negative soil bacterial which are rod shaped, many of which fix nitrogen. They are a common soil dwelling microorganisms that can form symbiotic relationships with leguminous plant species, they are able to fix atmospheric nitrogen into forms readily available for plant use. Moreover, nitrogen fixation is an important part of the nitrogen cycle. Plant cannot use atmospheric nitrogen (N_2) directly, they must use nitrogen compounds such had nitrates to infect the roots and establish a nitrogen fixing symbiosis. Nodulation is host-specific, and each species can only nodulate a restricted number of legume species had both nodulation and nitrogen fixation are complex processes, involving interactions between a number of bacterial and plant gene products. Specific compounds released by roots of young legumes such as soybean are involved in attracting these symbiotic bacteria to their roots.

Among the legumes, soybean is classified as an oilseed and is prominent for its high (38-45%) protein content as well as its high (20%) oil content. Soybeans are the second-most valuable agricultural export in the United States behind corn. The bulk of the soybean crop is grown for oil production; with the high-protein defatted and "toasted" soy meal used had livestock feed. A smaller percentage of soybeans are used directly for human consumption. Soybean is the largest single source of edible oil and accounts for roughly 50% of the total seed oil production worldwide [11]. Soybean oil has also been found effective as an insect repellant in some studies [12].

Soybean cultivation in Nigeria started in the late seventies. Soybean is now being produced in Benue, Niger, Kaduna, Kwara, Bauchi, Oyo, Ogun, Delta, Edo and Delta states [13]. The newly developed varieties are yielding and store relatively better than other varieties. These improved varieties especially released for the large scale production, where small-scale farmers took the advantages of them because of their yield and agronomical potential.

2. MATERIALS AND METHODS

2.1 Description of Study Area

The field experiment was carried out in one of the experimental fields behind the female hostel in the Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB). The longitude and latitude of the location are N 7° 14.30', E 3° 26.21'. Soil USDA classification was Alfisol.

2.2 Sources of Material

The material used are arbuscular Mycorrhizal fungi (*Glomus moseae*) which was cultured in FUNAAB, *Bradyrhizobium* was prepared by dissolving *Bradyrhizobium* (Histick) in Arabic gum, a sticky liquid for gluing of soybean seeds to inoculums at a quantity of 500g of soybean seed to 2.5 g of *Bradyrhizobium* so also Single superphosphate which was sourced at OGADEP and finally poultry manure free of litter was obtained from COLANIM farm in FUNAAB. The mycorrhizal fungi and *Bradyrhizobium* inoculants as well as soybean seed (TGX-1448-2E) were obtained from IITA.

2.3 Experimental Design and Treatment

The trial was laid out in a Randomized Complete Block Design (RCBD) and the treatments consisted of three factors which include;

Bradyrhizobium (with or without)

Phosphorus source (SSP and Mycorrhizal Fungi)

Poultry manure (0, 5 and 10t/ha). The table below shows how the field was laid out with different treatments.

2.4 Land Preparation and Plot Layout

The land used for the experiment was cleared and stumped in order to remove the debris and for easy penetration of plant root. The total field area was 625 m^2 i.e. 25 m by 25 m plus alley with 36 plots and each plot size is 3 m by 3 m. Planting distance was 5 cm by 75 cm and the plant population was 266,000/ha.

2.5 Planting and Cultural Practices

The seeds were drilled along the rows and weeding was done manually when necessary with the use of hand hoe to clear the weed grown round soybean plants and cutlasses to clear the mid row weed.

2.6 Data Collection

The following data were collected:

Plant height: The plant height was taken weekly via the use of meter rule

Leaf area: The product of leaf length and breadth and multiply by a factor 0.75.

Branch numbers: Was taken weekly (Visual counting).

Stem girth: Was taken weekly (Via compass). Also at harvest, number of pods and seeds per plant were determined.

2.7 Statistical Analysis

The data collected was subjected to analysis of variance using SAS. Significant means was also separated by least significant difference (LSD).

3. RESULTS

3.1 The Physical and Chemical Properties of the Soil Used for the Experiment

Table 2 shows the pre-planting physical – chemical properties of soil pH (in H_2O) was 6.8 in which the value indicated that the soil could be neutral. The organic carbon was 0.6% and this was considered low. The exchangeable Na was 0.21 (cmol/kg), exchangeable Ca was 0.64 (cmol/kg) and exchangeable K was 0.18 (cmol/kg). The total N value of soil was 1.4% and it was considered moderately low, the critical value for P in this soil was 1.4% and it was considered moderate, the particle size analysis of the soil showed that the textural class was sandy-loam having 91% sand, 4.4% clay, and 4.6% silt.

3.2 The Effect of Poultry Manure, P Source and *Bradyrhizobium* on Plant Height from 4 – 14 WAP

Table 3 shows the main effect of treatment on plant height in which mycorrhizal had the highest value at 4, 8, 10, 12 and 14 WAP while no *Bradyrhizobium* had the highest value at 6, 8, 10 and 12 WAP and mycorrhizal had the highest value at 14 WAP.

Table 4 shows the response of plant height. Mycorrhizal + 10 tons application of poultry manure had the highest at 4, 6, 8, 10 and 12 WAP, and Mycorrhizal had highest at 14 WAP.

3.3 The Effect of Poultry manure, Phosphorus Source and *Bradyrhizobium* on Stem Girth from 4 – 14 WAP

Table 5 shows the main effect response to stem girth, in which poultry manure, phosphorus source and *Bradyrhizobium* source all had equal response to stem girth at 4 WAP; 10 tons application of poultry manure had the highest at 8 WAP; 0 ton application of poultry manure, Single superphosphate and no *Bradyrhizobium* all had the highest at 10 WAP; 0 ton application of poultry manure had the highest at 12 WAP; 0 ton application of poultry manure, mycorrhizal and no *Bradyrhizobium* all had the highest at 14 WAP.

Table 6 shows the interactive response of stem girth to application of treatments in which all the treatments had the same value at 4WAP, Single superphosphate + 10 tons application of poultry manure had the highest value at 6WAP, *Bradyrhizobium* + Single superphosphate the highest value at 12 WAP and mycorrhizal had the highest at 10 and 14 WAPs.

3.4 The Effect of Poultry Manure, P Source and *Bradyrhizobium* on Leaf Area from 4 – 14 WAP

Table 7 shows the response of main effect of poultry manure, phosphorus source and *Bradyrhizobium* source to leaf area in which no *Bradyrhizobium* had the highest at 4 WAP compared with other sources of treatment; 0 ton application of poultry manure had the highest at 6 and 12 WAP; 5 tons application of poultry manure had the highest at 8, 10 and 14 WAP; Plots treated with mycorrhiza had the highest value at 8 WAP.

Table 8 shows the interactive response of leaf area. *Bradyrhizobium* + mycorrhizal + 5 tons application of poultry manure had the highest at 10 and 14 WAP, mycorrhiza + 10 tons application of poultry manure had highest at 8 WAP, mycorrhizal + 5 tons application of poultry manure highest at 12 WAP, and mycorrhizal had highest at 4 and 6 WAP.

3.5 The Effect of Treatment on Yield Parameters

Table 9 shows the main effect of treatments on yield in which 5 tons application of poultry manure had the highest yield compared with other tons of application, mycorrhizal had the highest yield compared with other P source, and no *Bradyrhizobium* (control) had the highest yield.

Table 10 shows the interactive effect of treatment on yield in which mycorrhizal + 10 tons application of poultry manure had the highest yield compared with other interactive treatments.

Table 1. Plot layout for soybean

M/0PM	M/10PM	M/5PM	B/SSP/0PM	SSP/0PM	B/M/5PM
B/SSP/10PM	SSP/5PM	B/SSP/5PM	SSP/10PM	B/M/0PM	B/M/10PM
M/10PM	M/0PM	B/SSP/0PM	M/5PM	B/SSP/5PM	B/SSP/10PM
SSP/5PM	SSP/0PM	B/M/0PM	SSP/10PM	B/M/10PM	B/M/5PM
M/5PM	M/10PM	B/SSP/10PM	SSP/10PM	B/SSP/5PM	M/0PM
B/SSP/0PM	SSP/5PM	B/M/5PM	SSP/0PM	B/M/0PM	B/M/10PM

Key: B = Bradyrhizobium; M =Mycorrhiza; SSP =Single superphosphate; PM=Poultry manure.
In the plot, 0 kg, 5 kg & 10 kg were applied to their corresponding plots

Table 2. The physical and chemical properties of the soil used for the experiment

Properties	Values
% Sand	91.0
% Silt	4.60
% Clay	4.40
Soil textural class	**Sandy-loam**
pH (Soil/H_2O)	6.80
% Organic carbon	0.60
% Nitrogen	1.40
% Organic matter	1.03
Exchangeable acidity (cmol/kg)	1.31
Exchangeable cation (cmol/kg)	
Ca^+	0.64
Mg^+	0.58
K^+	0.18
Na^+	0.21
CEC (cmol/kg)	2.92
Available phosphorus (mg/kg)	17.73

3.6 The Response of Microbial Count, Biomass, N, P, C to Main Treatments at 8 WAP of Soybean

Table 11 shows the main effects of treatments on some soil microbial properties. *Bradyrhizobium* treatment had the highest value of total bacteria count, total fungal count, micro biomass C, N and P.

Likewise in the phosphorus source, single super phosphate had the highest value in total bacteria count, total fungal count (same value with mycorrhizal treatment), microbial biomass C, microbial biomass N and microbial biomass P.

In poultry manure, 5 tons application had the highest value in the total fungal count, microbial C (same value with 10 tons application), microbial biomass N and microbial biomass P: so also 10 tons application had the highest value in total bacterial count.

Table 12 shows the interactive response in which *Bradyrhizobium* + mycorrhizal + 5 tons application of poultry manure had the highest of total fungal count and microbial biomass N, *Bradyrhizobium* + Mycorrhizal + 10 tons application of poultry manure had the highest of total bacterial count, *Bradyrhizobium* + Mycorrhizal and *Bradyrhizobium* + Single Super Phosphate + 10 tons application of poultry manure had the highest of total fungal count, and *Bradyrhizobium* + Single Super Phosphate + 5 tons application of poultry manure had the highest of total fungal count, microbial biomass C, microbial biomass N and microbial biomass P.

3.7 Response of Protease, Urease and Cellulase Main Treatment at 8 WAP of Soybean

Table 13 shows that *Bradyrhizobium* had highest value of protease, Urease; but control had the highest value of Cellulase.

Moreover, phosphorus source shows that mycorrhizal had highest value of Protease (same value with single super phosphate), Cellulase; but single super phosphate had the highest value of Urease.

In poultry manure, 5 tons application had highest value of Protease, and 5 and 10 tons/ha had higher values Urease and Cellulase than control.

Table 14 shows the interactive response of *Bradyrhizobium* + mycorrhizal + 5 tons application of poultry manure has the highest value of Urease, *Bradyrhizobium* + Single super phosphate + 5 tons application of poultry manure had the highest value of Protease, and mycorrhizal had the highest value of Cellulase.

3.8 Response of Soil Nitrogen, Phosphorus and Organic Carbon to Treatments at 8 WAP of Soybean

Table 15 shows that 5 tons application of poultry manure had the highest value of percentage N, Moreover, *Bradyrhizobium* shows the highest value of available P, while in poultry manure, 5 tons application had highest value of percentage organic C.

Table 16 shows the effect of *Bradyrhizobium* + Mycorrhizal had the highest value of percentage N and available phosphorus, *Bradyrhizobium* + Single super phosphate + 5 tons application of poultry manure had the highest value of percentage N, and Mycorrhizal + 10 tons application of poultry manure had the highest value of percentage N and percentage organic carbon.

4. DISCUSSION

From the results of growth analyzed, it was also observed that the plant height with the main treatment Mycorrhiza had the highest value at 4 and 14 weeks after planting; No *Bradyrhizobium* (control) had the highest value at 8, 10, and 12 weeks after planting; Mycorrhizal had the highest value at 14 weeks after planting on plant height of soybean.

On stem girth, poultry manure at 0, 5 and 10 tons, Mycorrhizal, Single Super phosphate, with and without *Bradyrhizobium* all had the highest value at 4 weeks after planting; Poultry manure of 10 tons had the highest at 6 weeks after planting; Poultry manure of 5 tons had the highest at 8 weeks after planting; Poultry manure of 0 ton, Single super Phosphate and no *Bradyrhizobium* had the highest at 10 weeks after planting; Poultry manure of 0 ton had the highest 12 weeks after planting; Poultry manure of 0 ton, mycorrhizal and no *Bradyrhizobium* had the highest at 14 weeks after planting. While on interactive, the treatments all had the same value at 4 weeks after planting: *Bradyrhizobium*, Single superphosphate with 5 tons of poultry manure had the highest at 6 weeks after planting; mycorrhizal with poultry manure of 10 tons had the highest at 8 weeks after planting; mycorrhizal had the highest at 10 and 14 weeks after planting; finally, *Bradyrhizobium* with Single superphosphate had the highest at 12 weeks after planting.

Furthermore, it was also observed that the main treatments on leaf area with treatment of no *Bradyrhizobium* (control) had the highest at 4 weeks after planting; Poultry manure of 0 ton had the highest at 6 and 12 weeks after planting; poultry manure of 5 tons had the highest at 8, 10 and 14 weeks after planting; *Bradyrhizobium*, mycorrhiza with poultry manure of 5 tons had the highest at 10 and 14 weeks after planting; mycorrhizal had the highest at 4 and 6 weeks after planting.

From the yield analyzed, it was observed that mycorrhizal as one of treatments had the highest yield in tons per hectare. Whereas on interactive effects, mycorrhizal + poultry manure of 10 tons application had the highest yield.

In addition, poultry manure of 10 tons had the highest total bacteria count; *Bradyrhizobium* and poultry manure of 5 tons had the highest total fungal count; finally poultry manure of 5 tons had the highest microbial biomass of carbon, nitrogen and phosphorus. Whereas, on the interactive *Bradyrhizobium* + Mycorrhizal with poultry manure of 5 tons had the highest total fungal count and microbial biomass nitrogen; *Bradyrhizobium* + Mycorrhizal with poultry manure of 10 tons had the highest total bacterial count; *Bradyrhizobium* + Mycorrhizal had the highest total fungal count; *Bradyrhizobium* + Single Super phosphate with poultry manure of 5 tons had the highest total fungal count, microbial biomass carbon, nitrogen and phosphorus.

However, the main effect of poultry manure at 5 tons had the highest protease, urease and cellulase; no *Bradyrhizobium* (control), Mycorrhizal and poultry manure of 10 tons all had the highest cellulose had the highest cellulose. Whereas, on interaction of *Bradyrhizobium* + Mycorrhizal with poultry manure of 5 tons had the highest urease; *Bradyrhizobium* + Single super phosphate with poultry manure of 5 tons had the highest protease; and Mycorrhizal had the highest cellulase.

Finally, the main effect of poultry manure at 5 tons had the highest percentage of nitrogen and organic carbon; *Bradyrhizobium* had the highest available phosphorus. While on interaction *Bradyrhizobium* + Mycorrhizal had the highest percentage of nitrogen and available phosphorus; *Bradyrhizobium* + Single Super phosphate with poultry manure of 5 tons had the highest percentage of nitrogen; Mycorrhizal with poultry manure of 5 tons had the highest percentage of nitrogen; while Mycorrhizal with poultry manure of 10 tons had the highest percentage of nitrogen and organic carbon.

Table 3. The main effect of Poultry manure, Phosphorus source and *Bradyrhizobium* on plant height (cm) from 4 – 14 WAP

Treatments	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP	14 WAP
Poultry manure source						
0 (tons)	12.22^{a}	21.55^{a}	29.58^{a}	35.20^{a}	42.78^{a}	47.99^{a}
5 (tons)	12.39^{a}	22.12^{a}	30.54^{a}	36.22^{a}	42.89^{a}	46.34^{ab}
10 (tons)	12.94^{a}	21.29^{a}	29.24^{a}	34.15^{a}	40.85^{a}	44.13^{b}
P value	0.18	0.37	0.39	0.19	0.34	0.04
Phosphorus source						
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^{b}	40.79^{a}	44.19^{b}
P value	0.00	0.10	0.02	0.01	0.03	0.00
Bradyrhizobium source						
No 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}	28.50^{b}	33.97^{b}	36.35^{b}	45.30^{a}
P value	0.20	0.03	0.00	0.01	0.00	0.18

WAP = Week after planting

Table 4. The response of plant height (cm) to interactive effects of treatments at 4 – 14 weeks after planting

Treatments	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP	14 WAP
MB + 5PM	13.45^{a}	21.90^{bcd}	30.81^{bcd}	37.83^{abc}	38.47^{acd}	45.71^{bc}
BM + 10PM	12.47^{ab}	19.27^{e}	25.84^{ef}	30.39^{de}	35.59^{de}	41.73^{cd}
BM	13.02^{ab}	20.02^{de}	27.38^{def}	32.18^{de}	38.77^{cd}	45.65^{bc}
BS + 5PM	12.92^{ab}	21.76^{bcd}	28.65^{cde}	34.11^{abc}	45.70^{b}	46.95^{bc}
BS+ 10PM	13.39^{a}	22.65^{ab}	31.97^{abc}	36.98^{abc}	38.58^{cd}	44.49^{bc}
BS	11.45^{bc}	20.41^{cde}	26.61^{ef}	32.30^{de}	39.00^{cd}	47.29^{bc}
M +5PM	13.19^{a}	22.85^{ab}	32.18^{abc}	36.57^{bc}	47.05^{ab}	48.67^{ab}
M + 10PM	13.75^{a}	24.31^{a}	34.76^{a}	40.93^{a}	52.15^{a}	53.25^{a}
M	13.69^{a}	23.99^{ab}	33.16^{ab}	39.90^{ab}	49.32^{ab}	53.67^{a}
S +5PM	9.99^{c}	21.62^{bcd}	30.52^{bcd}	36.25^{bc}	40.34^{cd}	44.03^{bc}
S +10PM	12.27^{ab}	18.92^{e}	24.40^{f}	28.29^{e}	37.06^{d}	37.05^{d}
S	10.71^{c}	21.76^{bcd}	31.18^{abc}	36.47^{bc}	44.05^{abc}	45.49^{bc}
P value	0.001	0.001	0.001	0.001	0.001	0.001

WAP = Week after planting

Table 5. The main effect of poultry manure, Phosphorus source and *Bradyrhizobium* on stem girth (cm) from 4 – 14 WAP

Treatments	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP	14 WAP
Poultry manure source						
0 (tons)	0.10^{a}	0.15^{a}	0.21^{a}	0.28^{a}	0.37^{a}	0.46^{a}
5 (tons)	0.10^{a}	0.27^{a}	0.23^{a}	0.27^{a}	0.36^{ab}	0.44^{a}
10 (tons)	0.10^{a}	0.74^{a}	0.22^{a}	0.27^{a}	0.34^{b}	0.45^{b}
P value	0.16	0.18	0.20	0.51	0.10	0.10
Phosphorus 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^{b}	0.28^{b}	0.35^{a}	0.44^{a}
P value	0.16	0.90	0.20	0.77	0.85	0.10
Bradyrhizobium source						
No Bradyrhizobium	0.10^{a}	0.38^{a}	0.22^{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

WAP = Week after planting

Table 6. The interactive response of stem girth (cm) to application of treatments from 4 – 14 WAP

Treatments	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP	14 WAP
MB + 5PM	0.1^{a}	0.14^{a}	0.22^{a}	0.28^{ab}	0.35^{ab}	0.48^{abc}
BM + 10PM	0.1^{a}	1.30^{a}	0.21^{a}	0.27^{b}	0.35^{ab}	0.42^{cd}
BM	0.1^{a}	0.17^{a}	0.21^{a}	0.27^{ab}	0.36^{ab}	0.45^{bcd}
BS + 5PM	0.1^{a}	0.49^{a}	0.23^{a}	0.28^{ab}	0.37^{ab}	0.41^{d}
BS+ 10PM	0.1^{a}	0.15^{a}	0.01^{a}	0.28^{ab}	0.33^{ab}	0.45^{bcd}
BS	0.1^{a}	0.14^{a}	0.21^{a}	0.26^{ab}	0.40^{a}	0.46^{bcd}
M +5PM	0.1^{a}	0.28^{a}	0.23^{a}	0.27^{b}	0.34^{ab}	0.40^{d}
M + 10PM	0.1^{a}	0.17^{a}	0.23^{a}	0.27^{b}	0.34^{ab}	0.51^{ab}
M	0.1^{a}	0.15^{a}	0.23^{a}	0.31^{a}	0.37^{ab}	0.53^{a}
S +5PM	0.1^{a}	0.16^{a}	0.23^{a}	0.28^{ab}	0.36^{ab}	0.49^{ab}
S +10PM	0.1^{a}	1.34^{a}	0.22^{a}	0.28^{ab}	0.32^{b}	0.42^{cd}
S	0.1^{a}	0.15^{a}	0.21^{a}	0.27^{a}	0.33^{ab}	0.41^{cd}
P value	0.001	0.009	0.003	0.001	0.001	0.001

WAP = Week after Planting

Table 7. The main effect of poultry manure, phosphorus source and *Bradyrhizobium* on leaf girth (cm^2) from 4 – 14 WAP

Treatments	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP	14 WAP
Poultry manure source						
0 (tons)	5.58^{a}	13.81^{a}	23.69^{ab}	29.78^{ab}	38.32^{a}	41.83^{a}
5 (tons)	5.47^{a}	13.41^{ab}	25.77^{a}	32.64^{a}	36.69^{a}	43.43^{a}
10 (tons)	5.59^{a}	12.18^{b}	21.82^{b}	27.07^{b}	35.41^{a}	38.28^{b}
P value	0.97	0.12	0.03	0.00	0.26	0.02
Phosphorus 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^{a}	35.57^{a}	40.45^{b}
P value	0.73	0.55	0.02	0.12	0.09	0.36
Bradyrhizobium source						
No Bradyrhizobium	5.73^{a}	13.65a	25.50^{a}	31.30^{a}	37.65^{a}	41.99^{a}
Bradyrhizobium	5.31^{a}	12.61^{a}	22.02^{a}	28.38^{a}	35.96^{a}	40.36^{a}
P value	0.25	0.13	0.00	0.03	0.24	0.30

WAP = Week after Planting

Table 8. The main effect of poultry manure, phosphorus source and *Bradyrhizobium* on leaf girth (cm^2) from 4 – 14 WAP

Treatments	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP	14 WAP
MB + 5PM	4.84^{cd}	13.71^{abc}	27.94^{a}	34.86^{a}	39.51^{a}	47.82^{a}
BM + 10PM	4.10^{d}	9.57^{d}	18.39^{d}	21.48^{d}	28.70^{b}	31.75^{d}
BM	4.36^{d}	10.84^{cd}	20.67^{bcd}	27.07^{bcd}	37.75^{a}	41.30^{abc}
BS + 5PM	5.73^{bcd}	14.01^{abc}	22.28^{abcd}	30.74^{abc}	39.51^{a}	39.51^{abc}
BS+ 10PM	7.31^{ab}	13.45^{abc}	22.66^{abcd}	30.16^{abc}	35.33^{a}	44.85^{ab}
BS	5.50^{bcd}	14.11^{abc}	20.19^{cd}	25.96^{d}	36.10^{a}	36.92^{bcd}
M +5PM	5.49^{bcd}	14.44^{ab}	27.89^{a}	33.66^{ab}	42.00^{a}	42.61^{ab}
M + 10PM	6.45^{abc}	15.01^{ab}	28.44^{a}	33.64^{ab}	41.91^{a}	42.04^{abc}
M	7.50^{a}	16.45^{a}	27.46^{a}	34.63^{a}	40.95^{a}	45.94^{a}
S +5PM	5.81^{abcd}	11.48^{bcd}	24.95^{abc}	31.32^{abc}	26.89^{b}	43.77^{ab}
S +10PM	4.17^{d}	10.67^{cd}	17.82^{d}	21.48^{d}	34.17^{a}	34.48^{cd}
S	4.98^{cd}	13.85^{abc}	26.44^{ab}	31.52^{abc}	38.46^{a}	43.15^{ab}
P value	0.001	0.009	0.003	0.001	0.001	0.001

WAP = Week after Planting

Table 9. The effect of main treatment on yield parameters

Treatments	YPP (kg/ha)	TSW (kg/plot)
Poultry manure source		
0 (tons)	100.1[a]	8.84[a]
5 (tons)	103.5[a]	9.04[a]
10 (tons)	106.8[a]	8.32[a]
P value	0.05	1.12
Phosphorus source		
Mycorrhiza	109.8[a]	9.07[a]
SSP	97.1[b]	8.40[a]
P value	0.05	0.89
Bradyrhizobium source		
No Bradyrhizobium	106.1[a]	8.82[a]
Bradyrhizobium	100.1[a]	8.66[a]
P value	0.01	0.92

Key: TSW – Thousand seed weight; YPP – Yield per plot

Table 10. Interactive effects of treatments on yield parameters

Treatments	YPP (kg/ha)	TSW (kg/plot)
MB + 5PM	0.104[c]	93.567[c]
BM + 10PM	0.105[bc]	94.800[bc]
BM	0.100[cde]	89.733[cde]
BS + 5PM	0.103[cd]	92.500[cd]
BS+ 10PM	0.101[de]	91.000[cde]
BS	0.090[de]	83.633[de]
M +5PM	0.114[ab]	102.167[ab]
M + 10PM	0.118[a]	106.300[a]
M	0.116[a]	104.767[a]
S +5PM	0.090[de]	84.367[de]
S +10PM	0.101[de]	90.500[cde]
S	0.090[de]	82.600[e]

Key: TSW – Thousand seed weight. YPP – Yield per plot

Table 11. Response of microbial count (CFU/g), biomass, N, P, and C (mg/kg) to main treatments at 8 WAP of soybean

Treatments	Total bacterial Count	Total fungi Count	Microbial biomass C	Microbial biomass N	Microbial biomass P
Bradyrhizobium inoculants					
Bradyrhizobium	1.16[a]	0.07[a]	186.0[a]	120.2[a]	211.2[a]
Control (0)	0.89[b]	0.05[a]	143.8[b]	117.2[a]	200.7[a]
P value	0.000	0.011	0.002	0.361	0.514
Phosphorus source					
Mycorrhiza	0.99[a]	0.06[a]	155.3[a]	114.3[a]	205.3[a]
SSP	1.05[a]	0.06[a]	174.7[a]	123.2[a]	206.0[a]
P value	0.171	0.721	0.124	0.010	0.990
Poultry manure					
0 (tons)	0.75[c]	0.05[b]	138.7[a]	111.4[a]	186.7[a]
5 (tons)	1.06[b]	0.07[a]	198.1[a]	124.9[a]	232.2[a]
10 (tons)	1.26[a]	0.06[ab]	158.1[b]	119.9[a]	198.8[ab]
P value	0.000	0.019	0.002	0.007	0.070

Table 12. Response of microbial count (CFU/g), biomass, N, P, and C (mg/kg) to main treatments at 8 WAP of Soybean

Treatments	Total bacterial count	Total fungi count	Microbial biomass C	Microbial biomass N	Microbial biomass P
MB + 5PM	1.27^{b}	0.08^{a}	222.70^{b}	139.00^{a}	203.70^{bcd}
BM + 10PM	1.49^{a}	0.03^{ef}	118.30^{f}	100.70e	143.98^{d}
BM	0.70^{de}	0.08^{a}	176.42^{cd}	121.95^{b}	215.45^{bc}
BS + 5PM	1.26^{b}	0.08^{a}	276.40^{a}	139.00^{a}	286.93^{a}
BS+ 10PM	1.29^{b}	0.08^{a}	194.37^{c}	121.97^{ab}	222.33^{abc}
BS	0.97^{c}	0.05^{cd}	128.13^{ef}	115.15^{c}	194.70^{a}
M +5PM	0.80^{d}	0.05^{cd}	135.43^{ef}	120.00^{bc}	249.70^{ab}
M + 10PM	1.01^{c}	0.06^{bc}	145.83^{a}	121.28^{b}	246.30^{ab}
M	0.71^{cd}	0.04^{de}	132.87^{ef}	99.05^{e}	175.80^{cd}
S +5PM	0.93^{c}	0.07^{ab}	157.77^{cd}	118.07^{a}	188.30^{bcd}
S +10PM	1.25^{b}	0.06^{bc}	173.80^{cd}	135.60^{a}	182.87^{bcd}
S	0.63^{e}	0.02^{f}	117.42^{f}	109.23^{d}	161.00^{cd}
P value	0.0001	0.0001	0.0001	0.0001	0.0026

Table 13. Response of protease, Urease and Cellulase to main treatments at 8 WAP of soybean

Treatments	Protease (mg/kg)	Urease (mg/kg)	Cellulase (mg/kg)
Bradyrhizobium inoculants			
Bradyrhizobium	0.11^{a}	0.15^{a}	0.12^{a}
Control (0)	0.10^{a}	0.12^{a}	0.14^{a}
P value	0.481	0.051	0.074
Phosphorus source			
Mycorrhiza	0.10^{a}	0.12^{a}	0.14^{a}
SSP	0.10^{a}	0.12^{a}	0.12^{b}
P value	0.975	0.249	0.045
Poultry manure			
0(tons)	0.07^{b}	0.09^{b}	0.11^{b}
5(tons)	0.13^{a}	0.17^{a}	0.14^{a}
10(tons)	0.09^{b}	0.14^{a}	0.14^{a}
P value	0.001	0.001	0.000

Table 14. Response of protease, Urease and Cellulase activities to interactive treatments at 8 WAP of soybean

Treatments	Protease (mg/kg)	Urease (mg/kg)	Cellulase (mg/kg)
MB + 5PM	0.12^{cd}	0.21^{a}	0.14^{bcd}
BM + 10PM	0.04^{g}	0.04^{e}	0.12^{edf}
PM	0.14^{abc}	0.21^{a}	0.17^{ef}
BM	0.12^{cd}	0.14^{cd}	0.13^{bcde}
BS + 5PM	0.16^{a}	0.18^{ab}	0.15^{ab}
BS+ 10PM	0.13^{bcd}	0.20^{a}	0.15^{ab}
BS	0.07^{ef}	0.11^{d}	0.06^{g}
M +5PM	0.14^{abc}	0.15^{c}	0.16^{a}
M + 10PM	0.15^{ab}	0.16^{bc}	0.15^{a}
M	0.05^{fg}	0.04^{e}	0.18^{ef}
S +5PM	0.11^{d}	0.13^{cd}	0.13^{cdef}
S +10PM	0.09^{e}	0.16^{bc}	0.14^{abc}
S	0.05^{fg}	0.07^{e}	0.11^{f}
P value	0.0001	0.0001	0.0001

Table 15. Response of soil nitrogen, available phosphorus and organic carbon to main treatments at 8 WAP of soybean

Treatments	% nitrogen	Available phosphorus (mg/kg)	% organic carbon
Bradyrhizobium Inoculants			
Bradyrhizobium	0.14^{a}	14.16^{a}	15.56^{a}
Control (0)	0.14^{a}	12.48^{a}	15.22^{a}
P value	0.627	0.010	0.769
Phosphorus source			
Mycorrhiza	0.14^{a}	13.83^{a}	15.08^{a}
SSP	0.14^{a}	13.80^{a}	15.70^{a}
P value	0.745	0.124	0.596
Poultry manure			
0(tons)	0.11^{a}	13.10^{a}	13.38^{a}
5(tons)	0.16^{a}	13.87^{a}	17.11^{a}
10(tons)	0.15^{a}	12.98^{a}	15.67^{a}
P value	0.010	0.452	0.044

Table 16. Response of soil nitrogen, phosphorus and organic carbon to interactive effects treatments at 8 WAP of soybean

Treatments	% nitrogen	Available phosphorus (mg/kg)	% Organic carbon
MB + 5PM	0.16^{a}	15.72^{a}	15.60^{cde}
BM + 10PM	0.07^{de}	9.37^{g}	9.33^{f}
BM	0.18^{a}	15.75^{a}	17.52^{abc}
BS + 5PM	0.18^{a}	15.06^{ab}	18.07^{abc}
BS+ 10PM	0.15^{b}	14.77^{ab}	17.17^{abc}
BS	0.12^{c}	14.27^{bc}	13.60^{de}
M +5PM	0.17^{a}	11.87^{e}	19.13^{ab}
M + 10PM	0.18^{a}	13.47^{cd}	19.87^{a}
M	0.09^{a}	10.79^{f}	12.75^{e}
S +5PM	0.14^{b}	12.84^{d}	15.65^{cde}
S +10PM	0.17^{a}	14.33^{bc}	16.32^{bcd}
S	0.06^{e}	11.56^{ef}	9.65^{f}
P value	0.0001	0.0001	0.0001

5. CONCLUSION

From the results, it was observed with soybean gave significantly higher response to treatment of 5 tons poultry manure applied solely in term of plant height, stem girth, leaf area microbial count, biomass nitrogen, phosphorus, and carbon, cellulase, urease and protease. In addition, mycorrhizal solely; *Bradyrhizobium*, mycorrhizal with 5 tons application of poultry manure also had a significant effect on soybean growth and yield. Whereas, *Bradyrhizobium* and single superphosphate do not really have significant effect on soybean production.

However, the colonization level in the mycorrhizal and non- AM-rhizobial plants was higher as compared to uninoculated plants. According to a report, the AM fungi associated with legumes are an essential link for effective phosphorus nutrition, leading to enhanced nitrogen fixation that in turn promotes root and mycorrhizal growth [14]. Even though mycorrhizae can greatly enhance the acquisition of mineral nutrients [15], but in return scavenge lots of carbon from the host plants [16]. Therefore, if the host plants have the ability to take up enough nutrients, such as P, they might not harbour many mycorrhizae due to the balance of the C and P budgets [17].

In conclusion from the experiment carried out, it was evident that the use of 5 tons of poultry manure can increase yield of soybean production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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