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Influence of Rhizobacteria Inoculant Application Methods and Phosphate Fertilizer Rates on Dry Matter Accumulation, Yield of Bambara Groundnut [*Vigna subterranea* (L.) Verdc] and Soil Total Nitrogen Content in a Degraded Ultisol in Southeast Nigeria

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Abstract

Bacterial inoculants contribute to increased agronomic efficiency in the rhizosphere via its effects on biological nitrogen fixation, phosphate solubilization and other plant growth promotion (PGP) traits. This study evaluates the influence of rhizobacteria inoculant application methods and phosphate fertilizer rates on dry matter accumulation, nodulation, yield of Bambara groundnut [*Vigna subterranea* (L.) Verdc] and post-harvest soil total nitrogen content in a Typic Paleudult in Southeast Nigeria (6° 29′ N; 7° 54′ E). Field trials were conducted in 2015 and 2016 cropping seasons in a 2 × 4 factorial in a randomized complete block design with three replications. The treatments were four rates of single super phosphate fertilizer (0 kg P ha^{-1}, 25 kg P ha^{-1}, 50 kg P ha^{-1} and 75 kg P ha^{-1}) and two rhizobacteria inoculant application methods (seed applied method and soil applied method). The results from the study showed that soil applied rhizobacteria inoculant increased leaf, stem and root dry weight of Bambara groundnut significantly (P<0.05) by between 19%-25% when compared with seed applied rhizobacteria inoculants. Similarly, soil applied rhizobacteria inoculant increased number of root nodules, fresh pods of Bambara groundnut and soil total nitrogen content at 90 days after planting (DAP) significantly (P<0.05) by 29%, 22% and 19% respectively when compared with seed applied rhizobacteria inoculants. Soils fertilized with 75 kg P ha^{-1} produced the highest dry matter (above and below) yield of Bambara groundnut (97.37 g plant) and this was significantly higher than when P was applied at 0 kg P ha^{-1}, 25 kg P ha^{-1} and 50 kg P ha^{-1} by 54%, 32% and 15% respectively for the two cropping seasons. The interaction effect showed that soils fertilized with 75 kg P ha^{-1} combined with soil application method of rhizobacteria inoculation produced the highest fresh pod yield of Bambara groundnut (178.4 g plant) and postharvest soil total N content (0.29%) and this was significantly higher in plots without P in seed applied rhizobacteria plots by 52% and 88% for the two cropping seasons. The results of our study showed that the inoculation method plays an important role in determining the success of microbial inoculation. Soil inoculation rather than seed application of exogenous rhizobacteria and up to 75 Kg P ha^{-1} is recommended that for optimal dry matter yield and increment in postharvest soil total nitrogen content for cultivation of Bambara groundnut in degraded Ultisols and soils with similar edaphic conditions.

Keywords: Bambara groundnut; Rhizobacteria; Dry matter accumulation; Phosphate fertilizer

Introduction

Bambara groundnut is an African indigenous legume that has been cultivated for centuries in sub-Saharan Africa, mainly the semi-arid regions, and has in the past contributed to food security [1-4]. Bambara plant makes little demand on soil nutrients, thus is useful for climate change adaptable agriculture. Access to the nitrogen allows the plants to produce leaves fortified with nitrogen that can be recycled throughout the plant which in turn yields nitrogen-rich seeds [5]. Through symbiotic relationships with rhizobacteria, it fixes atmospheric nitrogen to the soil, thereby benefitting crop rotations and intercropping systems [6,7]. Bambara groundnut has been reported to fix up to 28.42 kg N ha^{-1} in the Sudano-Sahelian zone of Nigeria [8]. Bambara nuts contain 63% carbohydrates, 19% protein and 6.5% oil [9,10] characterized its micro nutrient content per 100 mg as calcium (95.5 mg to 99.0 mg), iron (5.1 mg to 9.0 mg), potassium (11.45 mg to 14.36 mg) and sodium (2.9 mg to 10.6 mg). Its high protein content confers advantage in alleviating nutritional disorders in both humans and animals [11].

In Enugu area southeast Nigeria, preliminary investigation showed that the soils are strongly acidic. The organic carbon, nitrogen and available phosphorus were very low [12] considering bench mark for tropical soils. The results depict soils that are poor, degraded and of low fertility status. This condition is caused by over exploitation, nutrient volatilization, erosion or leaching [13]. The agronomic practices such as plant spacing and phosphate fertilizer requirements and inoculation technologies for the improvement of productivity of Bambara groundnut have not yet been fully determined in southeast Nigeria.

It is, therefore, considered necessary to introduce the use of plant growth promoting rhizobacteria inoculant as an ameliorant in order to reclaim and improve the fertility and productivity of

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the soil. As side the biological nitrogen fixation potentials of plant growth promoting bacteria such as *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*, [14], these bacteria are also helpful in a variety of mechanisms that involve soil structure formation, decomposition of organic matter, recycling of essential elements, solubilization of mineral nutrients, producing numerous plant growth regulators, degrading organic pollutants, stimulation of root growth crucial for soil fertility, bio-control of soil and seed borne plant pathogens and in promoting changes in vegetation [15]. Plant growth promoting rhizobacteria can reduce chemical fertilizers use and it is economically and environmentally beneficial for lowering production cost. It is suitable for sustainable crop production and soil fertility restoration [16].

Application of beneficial microorganisms to seeds or directly in the soil are mechanisms for placement of microbial inocula into soil where they will be well positioned to colonize seedling roots and protect against soil-borne diseases and pests. However, despite the long history of inoculation of legume seeds with *Rhizobia* spp. and clear laboratory demonstration of the ability of a wide range of other beneficial microorganisms to improve crop performance, there are still very few commercially available microbial seed or soil inoculants [17]. Seed and soil inoculation techniques used for research purposes are often not feasible at a commercial scale and there are significant technical challenges in maintaining viable microbial inocula on seed throughout commercial seed treatment processes and storage. Further research is needed before the benefits of a wide range of environmentally sensitive potential seed or soil inoculants can be captured for use in agriculture, ecosystem restoration and bioremediation [17].

Legumes are phosphorus loving plants. They require phosphorus for growth, nodulation and seed development, and most especially in nitrogen fixation which is an energy driving process [18]. Investigations by [18], revealed that the highest application rate of single super phosphate fertilizer (70 kg P ha^{-1}) increased the number of leaves per plant, number of branches per plant, plant height, leaf area index and weight of pod after harvest of *Mucuna flagellipes* in a degraded acid tropical Ultisol. Careful application of phosphorus fertilizer to legumes must be geared towards enhancing not only their growth and yield, but also nitrogen fixation. In Nigeria, legumes rarely receive any form of mineral phosphorus fertilizer. They, therefore, rely entirely on the natural available soil phosphorus and other nutrients for nitrogen fixation and growth and this has resulted in lower yield and low nitrogen fixation by these legumes [18].

The general objective of this study is to determine the influence of rhizobacteria inoculant application methods and phosphate fertilizer rates on dry matter accumulation, nodulation and yield of Bambara groundnut [*Vigna subterranea* (L.) Verdc] and post-harvest soil total nitrogen content in a Typic Paleudult in Southeast Nigeria.

The specific objectives are:

To determine the effects of soil-and seed applied rhizobacteria inoculant application methods and phosphate fertilizer rates (0 kg P ha^{-1}, 25 kg P ha^{-1}, 50 kg P ha^{-1} and 75 kg P ha^{-1}) on dry matter accumulation, yield of Bambara groundnut [*Vigna subterranea* (L.) Verdc.] and soil total nitrogen content.

To determine the relationship between the number of nodules, fresh pod weight, soil total nitrogen content and phosphate fertilizer rates in Bambara groundnut plots.

Materials and Methods

Soil characterization

This experiment was carried out in 2015 and 2016 planting season respectively at the Teaching and Research Farm of the Faculty of Agriculture and Natural Resources Management, Enugu State University of Science and Technology, Nigeria (06° 52'N, 07° 15'E and elevation 450 m above sea level) [19]. The area has an annual rainfall which ranges from 1700 mm to 2010 mm. The rainfall pattern is bimodal and is between April and October, and the dry season is between November and March. The soil's textural class is sandy loam with an isohyperthermic raulic conductivity were 41% and 21.72 cm hr^{-1} respectively.

Field methods

soil temperature regime [19] and is classified as Typic Paleudult [20]. The pre-planting percentage organic carbon, nitrogen and available P were 0.65%, 0.038% and 7.91% cmol kg^{-1} respectively. The soil pH in KCl was 5.4, CEC 8.10, Mg 0.72, K 0.17, Na 0.90, and Al 3.24 cmol kg^{-1}. The soil dry bulk density was 1.52 Mg m^{-3}.

The experiment was a 2 × 4 factorial trial in a randomized complete block design with three replications. The treatments were four rates of single super phosphate fertilizer (0 kg P ha^{-1}, 25 kg P ha^{-1}, 50 kg P ha^{-1} and 75 kg P ha^{-1}) and two rhizobacteria inoculant application methods (seed applied method and soil applied method). Healthy Bambara groundnut seeds (Cultivar TVSU 1061 from IITA Ibadan Nigeria) were selected, washed with 95% ethanol, surface sterilized with 3.5% sodium hypochlorite (commercial bleach) and then rinsed with distilled water before inoculation.

Preparation of inoculant slurry (Figure 1a)**:** Materials: 500 ml of bottled water can, wooden stirring spoon plastic basin, 3 liter capacity Rhizobacteria inoculant (Nodumax legume inoculant manufactured by IITA business incubation platform, Ibadan Nigeria), Bambara groundnut seeds, white paper.

Seed inoculation procedure (Figure 1b)

The enclosed gum Arabic (sticker) inside the sachet of Nodumax inoculant was dissolved into 300 ml of warm water to form a slurry, 15 kg of Bambara groundnut seeds were poured into a basin, the slurry formed was added to the seeds and mixed uniformly, 100 g of nodumax inoculant was added uniformly to the seeds and covered for 10 min to avoid direct sunlight. The inoculated Bambara groundnut seeds (Figure 1c) were planted into a moist seed bed.

Soil inoculation procedure (Figure 2a)

Planting holes were made to a depth of 10 cm using a plant spacing of 20 cm × 45 cm (intra × inter row spacing) on each seed bed. Inside the planting holes, ten grams of rhizobacteria inoculants were measured out and placed into each of the planting hole then the un-inoculated Bambara groundnut seeds (Figure 2b) and covered with the soil.

Field operations

A total land area measuring 190 m^2 (22 m × 10 m) was used for the experiment. The land was divided into three blocks (columns: north-south direction), and each was sub-divided into eight plots (rows: east-west direction) making a total of twenty-four plots. Plots (beds) measuring 2 m × 2 m (4 m^2) was separated by 1 m × 1 m pathway between and in between plots. The Bambara seeds were planted at 10 cm depth using 0.20 m × 0.45 m planting distance at one seed per

(1a) Inoculant slurry (1b) Seed inoculation (1c) Soil inoculation

Figure 1: Preparation of inoculant slurry, seed inoculation and soil inoculation.

(2a) Rhizobacteria inoculated seeds (2b) Uninoculated seeds

Figure 2: Rhizobacteria inoculated and uninoculated Bambara groundnut seeds [Vigna subterranea (L.) Verdc.].

hole giving a plant population of 111,111 plants hectare^{-1}. Lost stands were replaced. Prophylactic application of 15 ml of Karate (Pyrethroid insecticide) in five liters of water was applied at one week after planting and at four, six and eight weeks after planting to avert pest incidence. Three plants at the center rows were sampled during data collection. One weeding regime was carried out manually using small hoe at 21 DAP. Subsequently, rouging was employed to reduce weed competition in each of the seasons.

Observation and data collection

Number of nodules per plant was obtained at 60 DAP whereas number of fresh pods were determined at harvest (120 DAP). In determining the number of nodules and fresh pods, a spade was used to carefully scoop out the soil containing the plant roots. The soil with the roots was then immersed in a basin of water to remove the soil, the roots were recovered and the nodules counted manually. The number of fresh pods per plant at harvest was manually counted. Fresh pods per plant were weighed using electronic weighing balance. Sampled plants were separated into leaves, stems and roots and put in a paper envelope and oven dried at 80°Celsius to a constant weight for three days for the dry matter determination of leaf, stem and root at harvest.

Laboratory methods

Soil samples were collected from the top soil at a depth of 0 to 15 cm before planting and at harvest. Three representative soil samples were randomly collected per plot and bulked to form a composite soil sample for each plot. A total of twenty-four composite soil samples were collected. Samples were air dried ground and passed through a sieve of 2 mm standard mesh size. The soil pH was determined with a pH meter using 1:2.5 soil to water ratio and 1: 2.5 soil to 0.1 N KCl (potassium chloride) suspension [21,22]. Soil organic carbon was determined using the Walkley and Black wet digestion method. Soil organic matter content was obtained by multiplying the value of organic carbon by 1.724 (Van Bemmeler factor). Total nitrogen was determined by Micro-Kjeldahl procedure before planting and at harvest. The exchangeable cations and cation exchange capacity (CEC) were determined by the method described by Thomas (1982). Aluminium and hydrogen content (exchangeable acidity) was determined by titrimetric method after extraction with 1.0 N KCl [21]. Available phosphorus was extracted with Bray II extractant as described by Bray and Kurtz [23] and determined colorimeterically using ascorbic acid method [24]. Particle size distribution analysis was done by the hydrometer method [25] and the corresponding textual class determined from the United State Department of Agriculture Soil Texture Triangle. Dry bulk density was determined by the core method [26]. Total porosity values were derived from bulk density data. Saturated hydraulic conductivity (Ksat) was determined [27].

Data analysis

Data collected was subjected to analysis of variance (ANOVA) test for randomized complete block design as outlined by [28]. Significant means was separated using Fisher's least significant difference (F-LSD) at 5% probability level. Three simple linear regression analyses was done to derive a model for the estimation of number of nodules per plant versus SSP fertilizer rates (Y1), fresh pod weight per plant at harvest versus single super phosphate (SSP) fertilizer rates (Y2) and soil total nitrogen at harvest versus SSP fertilizer rate (Y3). Statistical analysis was executed using Statistical Software [29].

Results and Discussion

Pre-planting soil analysis

The pre-planting analyses of soil properties in both years are presented in the Table 1. The result indicated that the textural class of the study site is loamy sand. Percentage organic carbon (0.65%) and nitrogen (0.038%) are low indicating values below critical levels for the study area. However, these values were higher in 2016 planting season compared 2015 planting season (0.65% and 0.041%), respectively. As expected, this indicated that the soil had higher values for soil nutrient at the start of the experiment in 2016 when compared to 2015 planting season because of the influence of the legumes planted in the previous season. The soil pH in KCl ranged from 5.4 to 6.2 in both years indicating slight acidity according to the rating by Landon (1991). The pre-planting exchangeable cations content and exchangeable acidity of the soil in 2015 were (Mg^{2+} 0.70, K^+ 0.17, Na^+ 0.09, Al^{3+} 3.27 and CEC 8.1 cmol kg^{-1}) whereas the values obtained in 2016 were (Mg^{2+} 0.90, K^+ 0.10, Na^+ 0.17, Al^{3+} 3.20 and CEC 8.10 cmol kg^{-1}).

Effect of rhizobacteria inoculant application methods on the dry matter accumulation, yield of Bambara groundnut and soil total nitrogen content

The data presented in Table 2 shows the main effect of rhizobacteria inoculant application methods on the dry matter accumulation, yield of Bambara groundnut [*Vigna subterranea* (L.) Verdc.] and soil total nitrogen at harvest in 2015 and 2016 planting seasons. The results from the study showed that soil applied rhizobacteria inoculant increased leaf, stem and root dry weight of Bambara groundnut significantly ($P<0.05$) by between 19-25% when compared with seed applied rhizobacteria inoculants. Higher plant dry matter accumulation at harvest were found in soil applied rhizobacteria inoculant plots [leaf dry weight was 40.74 g to 43.81 g plant, stem dry weight was 30.85 g to 33.11 g plant and root dry weight was 8.01 g to 8.44 g plant for both seasons] when compared with seed applied rhizobacteria inoculants plots with leaf dry weight of 35.50 g to 38.81 g plant, stem dry weight of 24.89 g to 27.08 g plant and root dry weight of 6.30 g to 6.78 g plant at harvest for both seasons. In this study, bacteria applied by soil inoculation significantly enhanced plant biomass, nodulation, and seed yield of Bambara groundnut to a higher degree than seed applied rhizobacteria. Studies by [30] showed that soil inoculation with rhizobacteria resulted in better crop yields and more efficient colonization than direct inoculation of seeds [31].

Parameters	2015	2016
Particle size distribution (%)		
Coarse sand	46	47
Fine sand	41	40
Clay	5	5
Silt	8	8
Textural class	Loamy sand	Loamy sand
Bulk density (Mg m^{-3})	1.57	1.52
Total porosity (%)	41	43
Saturated hydraulic conductivity (Cm hr^{-1})	21.72	22
pH (water)	6.1	6.3
pH (KCl)	5.4	5.7
Organic carbon (%)	0.65	0.69
Total nitrogen (%)	0.038	0.041
Available phosphorus (c mol kg^{-1})	7.91	8.82
Exchangeable bases (cmol kg^{-1})		
Calcium	1.2	1.6
Magnesium	0.72	0.79
Potassium	0.17	0.16
Sodium	0.9	0.98
Exchangeable acidity (cmol kg^{-1})		
Hydrogen	2.66	2.4
Aluminum	3.24	3.06
Cation exchangeable capacity (cmol kg^{-1})	8.1	8.9

Table 1: Initial soil characteristics before planting in 2015 and 2016 cropping season.

This outcome is attributed to the ability of the rhizobacteria to spread quickly in the rhizosphere, convert atmosphere nitrogen (N_2) into nitrate and ammonia forms of nitrogen needed by plant for growth and development. More so, the application of rhizobacteria inoculant to the soil increased the microbial population of the soil thereby creating a suitable niche for the plant microbe interaction.

Soil applied rhizobacteria inoculant increased number of root nodules, fresh pods of Bambara groundnut and soil total Nitrogen content at 90 days after planting (DAP) significantly ($P<0.05$) by 29%, 22% and 48% respectively when compared with seed applied rhizobacteria inoculants.

Higher number of nodules per plant at eight weeks after planting [52-55] number of fresh pods per plant [9], fresh pod weight per plant [150.00-154.10 g plant] and post-harvest soil Total N [0.18% to 0.20%] were found in soil applied rhizobacteria inoculant plots for both seasons when compared with seed applied rhizobacteria inoculants plots with the number of nodules per plant at eight weeks after planting, number of fresh pod at harvest [7] and fresh pod weight at harvest [112.70 g to 117.68 g plant] and post-harvest soil Total N [0.09% to 0.11%] for both seasons. The beneficial effect of inoculating the soil with effective rhizobacteria is that it promotes nodulation and yield of leguminous crops. The mechanisms by which rhizobacteria stimulates nodulation is that is promotes the availability of nutrients and phosphate solubilization and production of phytohormones. It is postulated that observed differences between soil and seed inoculation could be that seeds may have released exudates before germination in soil, which favored the growth of other seed-residing microorganisms and out-competed the inoculated bacteria on the seed coat [31,32]. Nelson (2004) also contended that cells may have been stressed or injured during seed plantation and germination. Seed exudates can consist of many different molecules including sugars, amino acids, organic acids, and phenolic compounds among others, but little is known about the specific response of microorganisms to these. Additionally, various sulphur-containing compounds are produced by seeds and some of these have been found to have anti-microbial effects (Lanzotti 2006), which might reduce the number of inoculated cells.

Methods	Leaf dry weight (g $plant^{-1}$)		Stem dry weight (g $plant^{-1}$)		Root dry weight (g $plant^{-1}$)		Number of nodules per plant at eight weeks after planting		Number of fresh pod		Fresh pod weight (g $plant^{-1}$)		Soil total nitrogen (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Seed applied	35.5	38.81	24.89	27.08	6.3	6.78	37	39	7	7	112.7	117.68	0.09	0.11
Soil applied	40.74	43.81	30.85	33.11	8.01	8.44	52	55	9	9	150	154.1	0.18	0.2
$LSD_{(0.05)}$ for any 2 methods	1.65	0.04	1.09	0.05	0.42	0.04	2.23	0.04	0.39	0.39	5.22	0.03	0.02	0.03

$LSD_{(0.05)}$ – Least significant difference at 0.05 probability level

Table 2: Main effects of Rhizobacteria inoculant application method on dry matter accumulation yield of Bambara groundnut [Vigna subterranea (L.) Verdc.] and soil total nitrogen content at harvest in 2015 and 2016 planting season.

Effect of phosphate fertilizer rates on the dry matter accumulation, yield of Bambara groundnut and soil total nitrogen content

Soils fertilized with 75 kg P ha^{-1} produced the highest dry matter (above and below) yield of Bambara groundnut (97.37 g) and this was significantly higher than when P was applied at 0 kg P ha^{-1}, 25 kg P ha^{-1} and 50 kg P ha^{-1} by 54%, 32% and 15% respectively for the two cropping seasons (Table 3). The leaf dry weight, stem dry weight and root dry weight at harvest increased (P<0.05) with an increasing rate of single super phosphate fertilizer addition to the soil in the order of influence of 51.07 kg P ha^{-1}<72.05 kg P ha^{-1}<82.33 kg P ha^{-1}<97.37 kg P ha^{-1} for plots amended with 0 kg P ha^{-1}<25 kg P ha^{-1}<50 kg P ha^{-1}<75 kg P ha^{-1} respectively. The increase in dry matter accumulation recorded in this study is due to increased supply of phosphorus (Reedy et al., 2000), which might have accelerated cell division and system development in plants as reported by [32]. [33] Also reported that the application of 60 kg P_2O_5 ha^{-1} increased dry matter production due to increased respiration and photosynthetic ability. More so, Wabekwa et al. in 2014) reported that dry matter production also increased with increasing phosphorus application.

The result also shows that application of single super phosphate fertilizer at varying rates significantly (P<0.05) affected the number of fresh pod at harvest, fresh pod weight at harvest and post-harvest soil Total N for both seasons. Bambara groundnut grown on soils fertilized with 75 kg P ha^{-1} produced the highest number of nodules per plant [60-63], number of fresh pod at harvest [10-11] and fresh pod weight at harvest [158.60 g to164.60 g plant] followed by Bambara groundnut grown on plots which received 50 kg P ha^{-1} that produced 50-54 nodules per plant, 9 fresh pod at harvest and 146.60 g to 149.75 g plant fresh pod weight at harvest whereas plots amended with 25 kg P ha^{-1} produced 40 to 43 nodules per plant, fresh pod at harvest (7) and fresh pod weight at harvest [127.80 g to 134.56 g plant]. The plots that did not receive P (0 kg P ha^{-1}) produced the least number of nodules per plant [27-28], number of fresh pod at harvest (6) and fresh pod weight at harvest [92.30 g to 94.66 g plant] respectively when compared with the other SSP fertilizer rates. Phosphorus deficiency is common in most soils in southeastern Nigeria because of problems associated with P fixation. Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars, and other such functions, which directly or indirectly influence nitrogen fixation by legume plants [34,35].

The significant response to phosphorus fertilizer application observed in terms of nodulation count and yield may be attributed to the fact that phosphorus stimulates root and plant growth, initiates nodule formation as well as influences the general efficiency of the rhizobium-legume symbiosis, thereby optimizes the biological nitrogen fixation (BNF) system of legume. This result agreed with the findings of [36,37].

Post-harvest soil total nitrogen content was significantly (P<0.05) influenced by the SSP fertilizer rates. These results show that, at the different SSP rates, post-harvest soil, total nitrogen concentration increased by a magnitude of between 79% to 81% in the two cropping seasons. SSP fertilizer rate increases (0 kg P ha^{-1}<25 kg P ha^{-1}<50 kg P ha^{-1}<75 kg P ha^{-1}) resulted in corresponding post-harvest total soil total nitrogen levels (0.04%<0.09%<0.15%<0.22%) respectively for the two seasons of the study. Considering that some rhizobacteria possess ability of phosphate solubilization, they could be useful in crop production improvement by increasing P content in the soil and enhancing nodulation and N fixation in legumes. According to [38] the inoculation of plants with rhizobacteria can increase native population through various mechanisms that convert insoluble inorganic and organic soil P into plant available forms and therefore improve plant nutrition.

Interaction effects of bacterial inoculant application method and phosphate fertilizer rates on the dry matter accumulation, yield of Bambara groundnut and soil total nitrogen content

The data presented in Table 4 show the interaction effects rhizobacteria inoculant application methods and phosphate fertilizer rates on the dry matter accumulation, yield of Bambara groundnut [*Vigna subterranea* (L.) Verdc.] and soil total nitrogen at harvest in 2015 and 2016 cropping seasons. At harvest, soil applied rhizobacteria inoculant plots which received 75 kg P ha^{-1} had significantly (P<0.05) the highest leaf dry weight [53.07-59.11 g plant), stem dry weight [40.73g to 44.17 g plant] and root dry weight [9.83-10.23 g plant] respectively in the two seasons of study. The results showed that leaf, stem and root dry weight increased by between 49% to 58% when compared with seed applied rhizobacteria plots that did not receive SSP fertilizers which gave the lowest dry matter yield of Bambara groundnuts in the two seasons.

Results also show that soil applied rhizobacteria inoculant plots which received 75 kg P ha^{-1} had significantly (P<0.05) higher number of nodules per plant at eight weeks after planting higher number of fresh pod at harvest [12], higher fresh pod weight at harvest [175.90g to180.90 g plant] and higher post-harvest soil total N content [0.27% to 0.29%] respectively in the two seasons of study. These results showed that in soil applied rhizobacteria plots, the number of nodules per plant, number of fresh pod at harvest, fresh pod weight at harvest and post-harvest soil total N content at 75 kg P ha^{-1} increased by 66%, 58%, 52% and 76% respectively when compared with their corresponding seed applied rhizobacteria inoculant plots that did not receive SSP fertilizers in the two seasons.

Relationship between number of nodules, fresh pod weight, total soil nitrogen and phosphate fertilizer rates

The results in Table 5 shows that there is a significant (P<0.05)

SSP fertilizer rates (kg P ha^{-1})	Leaf dry weight (g $plant^{-1}$)		Stem dry weight (g $plant^{-1}$)		Root dry weight (g $plant^{-1}$)		Number of nodules per plant at eight weeks after planting		Number of fresh pod per plant		Fresh pod weight (g $plant^{-1}$)		Soil total nitrogen (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
0	27.53	29.72	18.15	19.67	4.83	5.23	27	29	6	6	92.3	94.66	0.04	0.04
25	35.33	39.46	25.95	28.13	7.37	7.86	41	43	7	7	127.8	134.56	0.07	0.09
50	41.58	43.12	30.8	33.2	7.78	8.17	50	54	9	9	146.6	149.75	0.12	0.15
75	48.03	52.94	36.58	39.39	8.63	9.17	60	63	10	11	158.6	164.6	0.19	0.22
$LSD_{(0.05)}$ for any 2 rates	2.33	0.06	1.54	0.06	0.59	0.06	3.15	0.05	0.55	0.06	7.38	0.05	0.03	0.04
$LSD_{(0.05)}$ – Least significant difference at 0.05 probability level														

Table 3: Main effects of phosphate fertilizer rates on dry matter accumulation yield of Bambara groundnut [Vigna subterranea (L.) Verdc.] and soil total nitrogen content at harvest in 2015 and 2016 planting season.

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Method x rate (kg P ha^{-1})	Leaf dry weight (g plant^{-1})		Stem dry weight (g plant^{-1})		Root dry weight (g plant^{-1})		Number of nodules per plant at eight weeks after planting		Number of fresh pod per plant		Fresh pod weight (g plant^{-1})		Soil total nitrogen (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Seed applied × 0	27.17	29.44	17.1	19.22	4.53	5	25	27	5	5	84.4	86.12	0.04	0.03
Soil applied × 0	27.9	30	19.2	20.11	5.13	5.46	28	30	7	7	100.3	103.2	0.08	0.08
Seed applied × 25	32.87	38.92	22.87	24.14	6.33	6.79	37	39	7	7	100	109.11	0.09	0.12
Soil applied × 25	37.8	40	29.87	32.11	8.4	8.92	45	47	7	7	155.7	160	0.14	0.15
Seed applied × 50	38.97	40.11	27.17	30.33	6.9	7.2	40	44	8	8	125	127.2	0.17	0.18
Soil applied × 50 kg	44.2	46.12	34.43	36.06	8.67	9.14	61	64	9	10	168.2	172.3	0.2	0.23
Seed applied × 75 kg	43	46.77	32.43	34.61	7.43	8.11	44	46	9	9	141.3	148.3	0.23	0.24
Soil applied × 75 kg	53.07	59.11	40.73	44.17	9.83	10.23	76	80	12	12	175.9	180.9	0.27	0.29
$LSD_{(0.05)}$ for any 2 methods × rates	3.3	0.08	2.18	0.09	0.83	0.09	4.45	0.08	0.78	0.08	10.44	0.07	0.02	0.03

$LSD_{(0.05)}$ – Least significant difference at 0.05 probability level

Table 4: Interaction effects of Rhizobacteria inoculant application method and phosphate fertilizer rates on dry matter accumulation yield of Bambara groundnut [Vigna subterranea (L.) Verdc.] and soil total nitrogen content at harvest in 2015 and 2016 planting season.

Predictors variable (Y)	Regression model	R	R²	Standard error of estimate	n
Y1-Number of nodules per plant	Y1=28+0.44P	0.774*	0.599*	10.514	24
Y2-Fresh pod weight per plant	Y 2=98.1+0.87P	0.750*	0.563*	22.39	24
Y3-Soil total nitrogen	Y 3=22.0-355.30P	0.990*	0.981*	4.062	24

Y1, Y2, and Y3-Predictors variables, P-Explanatory variables (phosphate fertilizer rates), *-significant at P < 0.05, n-number of observations, R-correlation coefficient, R^2-correlation of determination

Table 5: Relationship between number of nodules, fresh pod weight, soil total nitrogen and phosphate fertilizer rates.

positive linear relationship between number of nodules per plant (R^2=0.60*), fresh pod weight per plant (R^2=0.56*), total soil nitrogen content (R^2=0.99*) and SSP fertilizer rates respectively. These results indicate that a better relationship was established using SSP fertilizer rate in improving the post-harvest soil total nitrogen concentration, fresh pod weight per plant and number of nodules per plant. Various workers [32,39,40] noted that phosphorous deficiency has been shown to affect symbiosis by decreasing the supply of photosynthates to the nodule, which reduces the rate of bacterial growth and the total population of legume-nodulating microorganisms [41-46]. Reported that enhancement in nodule number, nodular mass due to inoculation might lead to the expansion in root length and mass, thus more number of active sites for nodulation by the rhizobial strains.

Conclusions

Agronomic practices like methods of delivery of plant growth promoting bacteria and application of phosphate fertilizer at the appropriate rates can have profound influence on plant growth. Soil inoculation of *Rhizobium* and supplemental application of 75 kg ha^{-1} SSP enhanced the shoot, root dry weights, nodulation, yield and post-harvest soil total N content of Bambara groundnut as compared to seed inoculation of *Rhizobium* and sub-optimal rates of P fertilizer. This is due to direct and indirect enhancement of plant growth by a variety of mechanisms such as production of growth promoting substance and solubilization of minerals such as P in the soil.

A combination of soil inoculation and appropriate rates of P fertilizer increased growth and yield, compared to seed inoculation, probably due to the synergistic effect of balanced nutrition, and improved absorption of nitrogen, phosphorus, and other mineral nutrients. Phosphorous availability increased the number and size of nodules and the amount of nitrogen assimilated per unit weight of nodules, increasing the percent and total amount of nitrogen in the harvested portion of the host legume and improving the density of Rhizobia bacteria in the soil surrounding the root. The positive effect of combined soil inoculation of rhizobacteria with optimum rate of phosphorus fertilization resulted in a general improvement in dry matter yield, number of nodules, and increased post-harvest soil total N content.

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