Rev. Ivoir. Sci. Technol., 23 (2014) 199 - 211
ISSN 1813-3290, http://www.revist.ci

CAN COMMERCIAL MICROBIAL INOCULANTS INCREASE GROUNDNUT PHOSPHORUS UPTAKE AND YIELD UNDER LOW NUTRIENT CONTENT SOIL IN SUDAN SAVANNA ?

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ABSTRACT

Groundnut production in Sudan Savanna is threatened by the gradual depletion of soil macro/micro-nutrient due to high population pressure on arable soil and lack of efficient farming system management. In addition, small-scale farmers encounter difficulties in using inorganic fertilizers although their application has a destructive effect on soil resources. Therefore, biofertilizers are beneficial in agricultural practices for environmental safety and sustainability of soil productivity. The aim of this work was to evaluate the effect of commercial arbuscular mycorrhizal fungi (AMF) and rhizobacteria inoculants on groundnut phosphorus uptake and grain yield. Field experiments with three commercial inoculants were carried out during 2010 cropping season in Shanono and surrounding villages in Kano State, Nigeria. The application of commercial AMF inoculant (Rhizatech) showed positive effect in shoot dry weight production (16%), shoot P concentration (2.5 g kg^{-1}), and pod dry weight (120%). The positive effect of microbial inoculants in increasing groundnut production is promising. Further investigation and mass trial implementation, taking into account soil fertility level in the agroecological zone, are needed to validate the biofertilization technology with small farmers.

Keywords : *peanut, biofertilizers, p-uptake, Sudan Savanna.*

RÉSUMÉ

La nutrition en phosphore et le rendement de l'arachide peuvent-ils être augmentés par les inoculants commerciaux microbiens dans les sols à faible teneur en nutriments de la savane Soudanienne ?

La production de l'arachide dans la savane Soudanienne est menacée par la réduction progressive des macronutriments et micronutriments des sols en raison de la forte pression démographique sur les terres arables et l'absence de gestion efficace des systèmes agricoles. Les petits agriculteurs rencontrent des difficultés dans l'utilisation des engrais minéraux, même si leur application a un effet destructeur sur les ressources du sol. Par conséquent, les biofertilisants sont bénéfiques dans les pratiques agricoles pour la sécurité environnementale et la durabilité de la productivité des sols. Cette étude avait pour objectif d'évaluer l'effet des inoculants commerciaux à base de champignon mycorhiziens arbusculaires et de bactéries rhizosphériques sur la concentration en phosphore (P) et le rendement en grain de l'arachide. Les expérimentations au champ ont été réalisées au cours de la campagne agricole de 2010 à Shanono dans l'Etat de Kano, au Nigeria. L'inoculation de Rhizatech a eu des effets positifs sur la production de la biomasse aérienne sèche (16%), la concentration en P (2.5 g kg^{-1}), et le poids sec des gousses (120%). L'effet positif des inoculants microbiens dans la production de l'arachide est prometteur, mais il est nécessaire d'établir des essais complémentaires en grand nombre afin de valider ces nouveaux produits.

Mots-clés : *arachides, biofertilisants, absorption,-phosphore, savane soudanienne.*

I - INTRODUCTION

In response to increasing population pressure, continuous or permanent cultivation has gradually replaced shifting cultivation of arable lands in West African savannas. As the savanna soils are inherently low in nutrients content [1], continuous cultivation quickly led to soil degradation and nutrient depletion through diminution of organic matter content, degradation of soil structure and reduction of available macro and micro-nutrients. These processes are accompanied by very low crop yield [2] and food insecurity. In Nigerian savanna, nitrogen (N) and phosphorus (P) are recognized to be the two most limiting nutrients [3, 4]. The replenishment of these nutrients is mostly done through application of inorganic fertilizer to the soil. For instance the fertilizer recommendation for groundnut is 20:40:20 N, P_2O_5 and K_2O kg ha^{-1} irrespective of the agroecological zone (AEZ).

However, fertilizers applied to the soil are not completely used by the plants. Recent report indicated that only 10 - 20% of the P applied is generally taken up by the plant roots in the first year [5]. Sudan Savanna has high solar radiation and is well suited to annual crops such as groundnut (*Arachis hypogea* L.), cowpea (*Vigna unguiculata* (L) Walp) and soybean [*Glycine max* (L.)] [6, 7]. Cowpea and groundnut are traditional crops of the savanna agroecological zones while soybean was introduced a few decades ago. As legumes, they are able to fix atmospheric N_2 in symbiosis with bacteria from *Rhizobium* genus. This natural nitrogen factory is a promise way to increase smallholder farmer nitrogen input in the N-deficient soils and reduces over dependence on inorganic N fertilizers.

As well, soil microorganisms involved in phosphorus solubilization or mobilization have been explored and give promising way to also overcome dependency on inorganic P fertilizer and reduce crop production cost. P biofertilizers used in agricultural systems include fungi (arbuscular-mycorrhizal fungi (AMF), *Trichoderma*…) and plant growth-promoting rhizobacteria (PGPR) based inoculants. In addition, microorganisms such as AMF are also associated to Zn and Cu uptake [8, 9], abiotic and biotic stress alleviation [10, 11] that can limit crop yield. Equally, beneficial effects of PGPR (*Pseudomonas*, *Bacillus* …) either in single or in mixed inoculants were extensively investigated by research works as other nutrients uptake enhancers, biological control agents, and also as phytohormones stimulators [12-15]. With regards to these positives impacts on crop production, microbial based inoculants are commercially produced and brought to the market. These microbial inoculants are widely available and are claimed to be effective by their manufacturers.

However, apart from the microorganism-host plant specificity, biofertilizers efficiency is influenced by soil biological and chemical properties, and other agroecological specific factors. For instance, deficiency of phosphorus (P) and molybdenum (Mo) and some environmental factors reduce rhizobial efficiency [16-20] while soil available P limit AMF root colonization and nutrient provision to the plant [21]. The survival of introduced strain could be severely affected by the chemical deficiency or toxicity. Thus, the microbial strains introduced in the soil must survive to successfully enter in symbiosis with the host plant and increase its nutrient uptake under certain circumstances. This trial was therefore carried out to evaluate the potential of commercial AMF and rhizobacteria inoculants on groundnut growth, P uptake and grain yield in Nigeria's Sudan savanna.

II - MATERIALS AND METHODS

II-1. Sites description

The experiment was established in Shanono within the Sudan Savanna (SS) agro-ecological zone located between latitudes 9°30' and 12° 31' N and longitudes 4° to 14° 30'E. The Sudan savanna covers over one quarter of Nigeria's total area [22]. It has a unimodal rainfall comprised between 600 and 1000 mm per annum and a length of growing period of about 100-150 days. Groundnut with cowpea is the most cultivated grain legumes in Shanono.

II-2. Experimental setup

Soil samples were collected from six farmers' fields. The fields selected did not have legume as previous crop. Before planting, 15 soil cores were taken randomly within each farm at a depth of 0 to 15 cm and bulked together per farm. Soil samples were processed and sent to Analytical Soil Laboratory of the International Institute of Tropical Agriculture (IITA) for chemical and physical analysis. ICGV-IS-96894 or SAMNUT 23 was used as the test crop. SAMNUT 23 is an early maturing (90-100 days), rosette resistant groundnut genotype that has been released in the AEZ by the International Crops Research Institute for the Semi-Arid Tropic (ICRISAT) and the Institute of Agricultural Research more than two decades ago. The experiment was carried out during 2010 cropping season using commercial free-living rhizobacteria and AMF inoculants (*Table 1*). Nutri-Life Myco-Tea™ is multiple bacteria-fungi inoculant while PHC® BioPak is mixed bacteria inoculant. Rhizatech is a mycorrhizal inoculant composed of spores and mycelia fragments of AMF mixed with sandy soil.

The inoculants were applied according to the respective manufactures' protocol. Three specific treatments were added: 20 kg and 40 kg P ha^{-1} and one control (no inoculant, no applied P). The P was applied as TSP at planting and no basal application of other nutrients has been done. The experiment was carried out in randomized completely block design with 6 replicates. Each replicate was represented by one farmer's field in which the treatments were randomized. The plot size was 5 m x 6 m. Three seeds were planted per hole with 20 cm between stations. Plant sampling was done at 8 WAP within the two middle rows used as net plot; plants from half meter within each row were harvested at half meter from each diagonal corner of the two rows. The plants were cut aboveground and their roots carefully excavated to preserve the nodules. Shoot and root were oven-dried for 72 h at 60 ºC to constant weight and then the shoot dry weight was recorded.

The nodules were counted, dried and weighed to determine their dry weight. After the measurement of the shoot dry weight, the shoot was separated into leaves and stems and the organs were grinded separately. One volume of milled leaves and two volumes of stems were mixed for the measurement of shoot P concentration. During sampling at 8 WAP, the pods were collected and oven-dried at 72 h at 60 ºC to determine their dry weight. At harvest, the grain yields were collected from the net plot size (3.75 m^2) to estimate the grain yield per plant. One hundred seeds were randomly selected and weighed. The seeds were oven-dried as described above. The moisture content of the 100-seed weight was then used to estimate the dried weight of grain yield. The data collected were subjected to analysis of variance (ANOVA) using the PROC GLM procedure of Statistical Analysis System (SAS) software version 9. Multiple mean comparison of factors showing a significant effect were separated with the Duncan's multiple range test at P = 0.05

Table 1 : *Sources and content of the commercial microbial inoculants*

Products/Application rates	**Companies/Countries**	**Active agents**
PHC® Biopak (4 g per m^2 at planting)	Plant Health Care plc, UK	*Bacillus licheniformis, B. megaterium, B. polymyxa, B. subtilis, B. pumilus* and *Paenibacillus azotofixans*
Nutri-Life Myco-Tea™ (1 kg ha^{-1} at planting)	Nutri-Tech Solution P/L, Australia	*Trichoderma lignorum, Chaetomium globosum, Verticillium lecanii, Paecilomyces lilacinus, Penicillium chrysogenum, Azotobacter chroococcum, Bacillus polymyxa, Saccharomyces cerevisiae*
Rhizatech (50 mL per 30 m^2 at planting)	Dudutech, Kenya	Spores and mycelia fragments of AMF

III - RESULTS AND DISCUSSION

III-1. Soils effect on groundnut growth and yield

The properties of soils at the beginning of the experiments are displayed in ***Table 2***. The pH ranged between 5.2 and 6.5, the available P ranged between 6.37 and 14.81 mg kg^{-1}. NO_3-N varied between 4.14 and 7.48 mg kg^{-1}. As indicated in ***Table 3***, the effect of the soils on shoot dry weight was significant. The highest shoot dry weight was recorded in farm 1 while the lowest was produced by the plants in farm 6. As well, the soil had significant effect on shoot P concentration. The shoot P concentration of plants from farm 3 reached 3.16 g kg^{-1}. The pod yield per plant at 8 WAP ranged from 1.45 to 5.74 g $plant^{-1}$. It was not significantly influenced by the different soils (*Table 3*). In contrary, the grain yield per plant was significantly influenced by the different soils. The grain yield range from 6.86 to 17.35 g $plant^{-1}$. The highest percentage increase was recorded with the application of 40 kg P ha^{-1} ***(Figure 1)***.

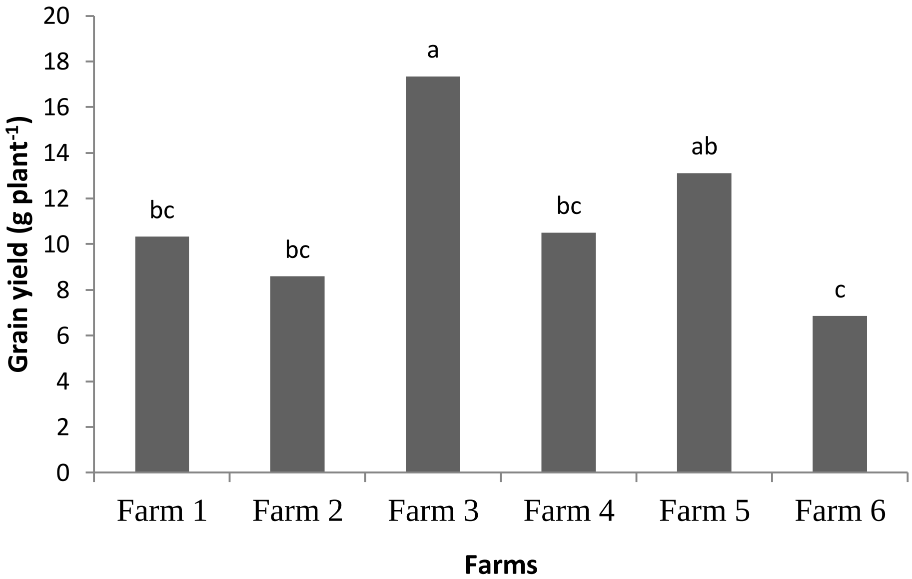

Figure 1 : *Effect of the soils on groundnut grain yield (Bar with same letters are not statistically different according to Duncan's Test at p = 0.05)*

Table 2 : *Physical and Chemical properties of Shanono soils at planting*

Properties	Farm1	Farm2	Farm3	Farm4	Farm5	Farm6	Means
pH 1:1	6.1	6.3	5.8	6.5	5.9	5.2	6.0
OC (g kg^{-1})	2.8	3.2	2.8	2.0	1.7	2.8	2.6
N (g kg^{-1})	0.33	0.37	0.52	0.25	0.24	0.29	0.33
Mehlich P (mg kg^{-1})	10.98	6.37	14.17	11.36	10.27	14.81	11.3
Ca ($Cmol_c$ kg^{-1})	5.45	2.27	2.57	4.35	2.75	2.61	3.3
Mg ($Cmol_c$ kg^{-1})	1.34	0.60	0.77	1.68	0.94	0.77	1.0
K ($Cmol_c$ kg^{-1})	0.63	0.11	0.26	0.15	0.19	0.24	0.3
Na ($Cmol_c$ kg^{-1})	0.16	0.15	0.16	0.16	0.15	0.15	0.2
Exch. Acidity	0.00	0.00	0.00	0.00	0.00	0.00	0.0
ECEC ($Cmol_c$ kg^{-1})	7.59	3.13	3.75	6.34	4.03	3.77	4.8
Zn (mg kg^{-1})	38.91	18.41	19.79	12.56	12.24	15.34	19.5
Cu (mg kg^{-1})	0.24	0.27	0.08	0.07	0.04	0.09	0.1
Mn (mg kg^{-1})	63.71	143.15	60.20	59.73	39.54	46.49	68.8
Fe (mg kg^{-1})	159.80	126.70	180.43	183.46	145.36	159.07	159.1
NO_3-N (mg kg^{-1})	7.48	4.14	6.49	4.80	5.83	8.32	6.2
NH_4-N (mg kg^{-1})	0.00	0.00	0.00	0.00	0.00	0.00	0.0
Sand (g kg^{-1})	772	792	792	772	792	792	785
Silt (g kg^{-1})	112	92	92	92	92	92	95
Clay (g kg^{-1})	116	116	116	136	116	116	119

Table 3 : *Effect of the soils on groundnut shoot dry weight and P concentration, and pods dry weight at 8 WAP in Shanono*

Farms	Shoot dry weight (g plant^{-1})	Shoot P concentration (g kg^{-1})	Pod dry weight (g plant^{-1})
Farm 1	54.11 a	2.09 cd	5.737
Farm 2	34.79 bcd	2.02 cd	3.383
Farm 3	31.09 cd	3.16 a	2.75
Farm 4	38.91 bc	2.34 cb	1.453
Farm 5	43.87 ba	2.52 b	3.493
Farm 6	23.80 d	1.94 d	3.88

Figures followed by the same letters in the same column are not statistically different according to Duncan's test ($p \leq 0.05$).

III-2. Treatments effect on groundnut growth and yield

The treatment of groundnut with microbial inoculants and soil applied P showed that the shoot dry weight per plant ranged from 34.7 g to 42.5 g. The inoculation of Rhizatech yielded 40.2 g plant^{-1} with 16% as percentage increase. The statistical analysis did not show significant difference between the treatments. The measurement of shoot P concentration showed a variation from 2.1 g kg^{-1} to 2.6 g kg^{-1}. The plants treated with Rhizatech were the greatest (2.5 g kg^{-1}) in shoot P concentration among the microbial inoculants. The shoot P concentration recorded with the application of 20 kg P ha^{-1} reached 2.6 g kg^{-1} but was not statistically different from other treatments. PHC Biopak was relatively lower than the control plot after the 40 kg P ha^{-1} application. In general, the pod yield was enhanced by the microbial inoculants ***(Table 4)***. At 8 weeks, Rhizatech increased the pod dry weight per plant up to 120% (5.5 g plant^{-1}). It was followed by the plant treated with Myco-Tea (4 g plant^{-1}). The result of the statistical analysis did not show significant difference between the treatments means.

The means of the grain yield are shown in ***Figure 2.*** The application of 40 kg P ha^{-1} recorded 13.6 g $plant^{-1}$ and was followed by the application of 20 kg P ha^{-1} which gave 12.1 g $plant^{-1}$. All the microbial inoculants increased the grain yield and PHC Biopak was the best among them with about 48% as percentage increase over the control (8.6 g $plant^{-1}$) and then followed by Rhizatech (42%). The increase of the grain yield obtained from the applications of P was not high enough to show significant difference from the microbial inoculations.

Table 4 : *Response of groundnut to biofertilizers and phosphorus application in terms of growth, shoot P concentration and pod yield in Shanono at 8 WAP*

Treatments	Shoot dry weight (g $plant^{-1}$)		Shoot P concentration (g kg^{-1})		Pod dry weight (g $plant^{-1}$)	
	means	Relative response (%)	means	Relative response (%)	means	Relative response (%)
control	34.7	-	2.3	-	2.5	-
Biopack	36.9	7	2.1	-9	2.9	16
Myco-Tea	35.6	3	2.3	0	4	60
Rhizatech	40.2	16	2.5	9	5.5	120
20 kg P ha^{-1}	42.5	23	2.6	13	3.2	28
40 kg P ha^{-1}	36.7	6	2.3	0	2.5	0

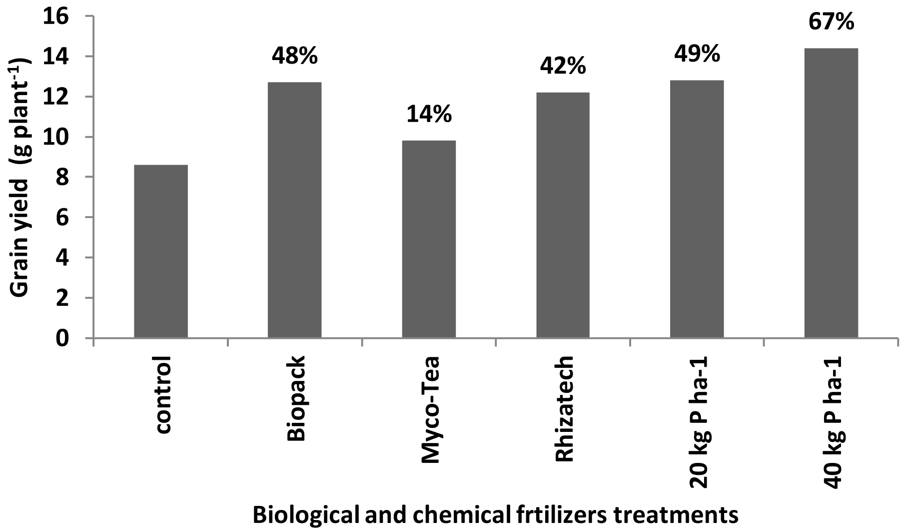

Figure 2 : *Effect of the biological and chemical treatments on groundnut grain yield*

III-3. Discussion

Improvement of crops growth and yield from bacteria and fungi inoculation is a complex process involving soil biological, chemical and physical properties. The variation of soil properties as observed on the pH and organic carbon must have influenced differently the efficacy of the microbial inoculant for P and other nutrient solubilization and/or mobilization. Variation in microbial inoculants efficiency due to soil physical and chemical properties has been also observed by Egamberdiyeva [23]. The potential of the microbial inoculants, especially the mycorrhizal inoculant (Rhizatech) in shoot dry weight, pod and grain yields was relatively higher than the control. This could be explained by an increase of nutrient uptake from microbial activity as observed by Farzaneh *et al*. [24]. However, the microbial inoculants did not increase the grain yield compared to the application of inorganic P fertilizer. This might be due to the amount of P mobilized by the microbial inoculants that may not be enough to sustain the initiated pods around 8 WAP. The release of P from biofertilizers could be affected by P mobilization which depends on the P available in the volume of soil explored by the root. Balota *et al*. observed, in greenhouse experiment, a range of soil available P (between 7.8 and 25 mg kg^{-1} of P) where mycorrhizal inoculation has highest efficiency.

This influence of range of available P on AMF efficiency could vary with crops species and soil properties under field condition. On the other hand, the difficulty of microbial inoculant to improve the grain yield compared to the use of inorganic fertilizer might be due to their limited P extraction rate resulting from high energy requirement that could have been reduced during mid-podding and grain filling [26-28].

IV - CONCLUSION

The present trials showed in general the potential of microbial inoculants to increase groundnut grain yield in Sudan savanna. The soil fertility level (chemical content) may be a constraint to biofertilization. Therefore, generalization of the biofertilizer efficacy under one agroecological zone could involve the knowledge of soil fertility threshold that suit application of the named biofertilizers. Further studies should be designed to take into account the within-AEZ soil fertility variation or farmer-to-farmer soil heterogeneity.

Acknowledgements

The authors are grateful to BMGF to have granted this work through COMPRO I Project of Tropical Soil Biology and Fertility Institute of Centro Internacional de Agricultura Tropical (CIAT-TSBF) and International Institute of Tropical Agriculture (IITA).

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