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The Associative Effect of VA Mycorrhizae with *Bradyrhizobium* as Biofertilizers on Growth and Nutrient Uptake of *Arachis hypogaea.*

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Abstract: Pot experiments were carried out in virgin sandy soil amended with either rock phosphate or super phosphate. The experiment aimed to investigate the influence of single or dual inoculation of N_2 fixer *Bradyrhizobium* and the vesicular-arbuscular mycorrhizal fungi VAM, as biofertilizers, on the growth and nutrient uptake of peanut plants (*Arachis hypogaea* L.). The pots were received half of the recommended dose of P fertilizer in the rate of 20 mg P/kg soil, whereas, ammonium nitrate was added in the rate of 10 mg N/kg soil. The growth parameters of the plant were studied, the results revealed that the biomass and grain yield were significantly improved by using the dual biopreparations of AM fungi with *Bradyrhizobium*. Data also revealed that bacterial–mycorrhizal–legume symbiosis increase nodule number, nitrogenase activity, total pigments, carbohydrate, protein and lipid content. Also, the nitrogen, phosphorus and potassium (NPK) uptake were significantly increased due to the single or dual inoculation . Generally, inoculation with VA mycorrhizae and/or *Bradyrhizobium* can, synergistically, remove the deficient effect of N and P on N_2-fixation and plant growth in the soil of low nutrient content. In all experimental interactions, rock-P amended soil gave higher values than that obtained from super-P applicable soil.

Key words: Peanut plants, *Bradyrhizobium,* VA mycorrhizae, nitrogen, phosphorus potassium

INTRODUCTION

About one-third of the land area of the world comprises arid and semiarid climates. Arid desert soils were previously considered economically unimportant; however, during the past three decades, the economic and agricultural utilization of arid lands has emerged as a critical element in maintaining and improving the world's food supply[73]. The peanut, or groundnut (*Arachis hypogaea*) is a species in the legume family. It is an important oil and protein source and is grown widely in the semi-arid tropics.

Legume crops are generally cultivated in poor environments, even recently bred cultivars are selected to grow in such a poor environment and associated with its rhizobium an associated microflora. Legume crops have a high phosphorus requirement for nodule formation, nitrogen fixation and optimum growth[71]. Mycorrhizal condition of legume crops found to increase its vegetative growth and seed yield in addition to improve nodulation on it's root system[41,47]. Legume nitrogen-fixing bacteria and arbuscular mycorrhizal symbiosis were studied by some authors[13,15,53] who reported that the analysis of arbuscular mycorrhiza and nitrogen-fixing nodules suggested that the plant have universal system for monitoring their microbial affinities that may be either mutualistic or antagonistic depending on the form of symbiosis, environmental conditions and individual genetic characters of interacting organisms. These regulatory systems were not only apparently important for evolution of the beneficial microbial interactions that contribute generally to the adaptive potential of terrestrial plants, but also, create a more favourable environment for the development of ecosystems processes.

Because of the importance of peanut as food legume in Egypt, the new reclaimed soils were brought about cultivation for enhancing the product. One of the principle production constraints is poor fertility. This problem is greatly solved by the addition of mineral fertilizers, which are scarcity and high cost. The great deep gap between supply and demand and their adverse effect on environment have led agricultural scientists to look for alternative strategies[70]. Organic wastes and biofertilizers are considered to be the alternative source to meet the nutrient requirement of crops and to bridge the future gaps[61]. The lack of indigenous soil populations of VA mycorrhizae and rhizobia has restricted potential yields of peanut cultivated in this area. Applying of the soil microorganisms which can fix atmospheric nitrogen, solubilize phosphorus or stimulate plant growth will be environmentally benign approach for nutrient management of ecosystem.

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The aim of the present work was conducted to examine the role of vesicular-arbuscular mycorrhizal (VAM) fungi with *Bradyrhizobium* spp. inoculation on the growth, yield and nitrogen, phosphorus and potassium (NPK) uptake of peanut plants grown in soil amended with either super-P or rock-P as biofertilizers, and their response to N_2 fixation by the plant.

MATERIALS AND METHODS

Soil Preparation: An agricultural virgin sandy loam soil, from New Al-Salheyia Region, Sharkia Governorate, Egypt, contains 78% sand, 10% silt, and 12% clay, pH 7.3, 4.7 g kg^{-1} total organic matter, 3.4 g kg^{-1} total nitrogen, 25 mg kg^{-1} phosphorus, 34 mg kg^{-1} potassium and 1.9 mmoh m^{-1} E.C.The soil was air dried, sieved to pass through < 2mm mesh and packed in plastic pots.

Seed: Seeds of peanut (*Arachis hypogaea* L.) cv. Giza 5 were obtained from Field Crops Res. Institute, Agric. Res. Centre, Giza, Egypt.

Experiments: Three seeds of peanut were sown in each plastic pot, separately. Pots were filled with 10 kg/pot of a sandy soil. The seedlings were thinned to one plant after 15 days of emergence. The experiment was arranged as a randomized complete block design with three replicates for each treatment. Phosphorus was applied either as super phosphate (7.2% P) at the rate 20 mg P/kg soil or fine rock-P (11.7% P), at same rate[22]. Rock phosphate (26.4 % P_2O_5) was obtained from Abu Zaabal phosphate fertilizer Co. All pots were received 10 mg N/kg soil added as ammonium nitrate, at the rate of 20 kg fed^{-1} (NH_4NO_3, 33.5%). Potassium was applied as 50 kg K_2O fed^{-1} (k_2SO_4, 48%).

Watering, weeding and spraying against pests and diseases were done when necessary. The replicates were carried out with the following treatments: uninoculated control, inoculation with *Bradyrhizobium,* inoculation with VA mycorrhizae, and inoculation with both *Bradyrhizobium* and VA mycorrhizae, all treatments were fertilized with either rock-P or super-P.

Inoculants: *Bradyrhizobium* strain USDA 3456 was obtained from the Agriculture Research Centre, Giza, Egypt, and used for an appropriate peat-based inocula (3.5×10^8 cells g^{-1} peat) for seed inoculation. Gum Arabic was used as sticker to ensure viable rhizobia per seed, before sowing. Mixed VA mycorrhizal inoculum's consisted of spores, mycelium and root segments of *Glomus clarum*; *G. mosseae* and *G. fasciculatum* isolates propagated with onion (mycorrhizal infection = 70%) roots for four months, using the wet sieving technique[24]. Approximately 450 spores pot^{-1} were injected at 6-8 cm below the seed bed before sowing to ensure a good infection.

Sampling: Peanut plants were harvest after one hundred twenty days, separated into shoots and roots. Pods were separated and their number/plant have been recorded. Fresh weights of shoots and roots were determined. The roots were dipped in water to remove adhering soil particles, and washed with distilled water. Plant height and root nodule number were estimated. Total dry weight of shoots and roots were determined after drying at 70°C till a constant weight. The oven dried plants were grounded and mineralized by sulfuric-perchloric acids[58]. Nitrogen (N) was extracted from plants with sulfuric acid using the semi-micro Kjeldahl method[30]. Phosphorus (P) was extracted by nitric-perchloric acid digestion and measured using the vanadono-molybdophosphoric colorimetric method[29]. Potassium (K) was assayed using a flame spectrophotometer[6]. The sugar and protein content of plant tissues were estimated according to Naguib[51] and Bradford[14]. Lipids were extracted with petroleum ether using Soxhlet apparatus according to AOAC[10]. Nitrogenase activity of plant was measured by using acetylene reduction assay[25]. Values of nitrogenase activity were recorded as nmoles C_2H_4 gm $plant^{-1}$ h^{-1}. The method used for the quantitative determination of photosynthetic pigments chlorophyll *a* and *b* was that of Metzner *et al.,*[49]. A subsample of washed roots was cleared in 10% KOH (w/v) and fungus stained with 0.03% (v/v) trypan blue in lactoglycerol according to the method of Philips and Hayman[57], percentage of mycorrhizal infection was determined according to Gerdemann and Nicolson[24].

Statistical Analysis: The data obtained in the present study were expressed as mean of treatment variables and the statistical analysis done by using SPSS program (Statistical Package for the Sciences System). P-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Fresh Biomass Yield: Effect of inoculation with VA mycorrhizal fungi and /or *Bradyrhizobium* on shoot and root fresh weights were presented in Table 1. Data indicated that the dependence of peanut growth on inoculation with *Bradyrhizobium* increase the fresh biomass yield of aerial and ground parts of peanut plants than the uninoculated plants in both P-amended soils. Abdel-Ghaffar[2] reported that, inoculating faba-bean with *Rhizobium* increased the yield and the quality of grain compared to uninoculated treatments. Also data revealed that single inoculation with

Table 1: Shoot and root fresh weight of peanut plants, inoculated with VA mycorrhizal fungi and /or *Bradyrhizobium*, grown in soil amended with rock-P or super-P.

Inoculation	Rock – P			Super - P		
	Fresh weight of shoot (g)	Fresh weight of root(g)	Total Fresh weight	Fresh weight of shoot (g)	Fresh weight of root (g)	Total Fresh weight
Uninoculated	476.30	16.06	492.36	458.71	15.76	474.47
Plant+Rh	519.71	19.11	538.82	498.20	19.07**	517.27
Plant+VAM	659.10**	26.62***	685.72	590.19	22.03	612.22
Plant+VAM+Rh	884.78***	38.44***	923.22	861.25**	38.40***	899.65

Comparing uninoculated with other treatment in the same column:
* = significant at P ≤ 0.05, ** = high significant at P≤ 0.01, *** = highly significant at P ≤ 0.001
Comparing the same treatment in rock-P with super-P:
a = significant at P ≤ 0.05, b = high significant at P ≤ 0.01, c = highly significant at P ≤ 0.001

mycorrhizae in presence of either rock-P or super-P significantly enhanced the total fresh shoot and root weights of peanut plants over the non-mycorrhizal plants by about 39% and 29%, respectively. With this respect, Khanizadeh *et al.,*[37] reported that vesicular-arbuscular mycorrhiza inoculation in combination with phosphorus increased dry and fresh shoot weight, leaf area and leaf number of strawberry compared to application of phosphorus alone. Lambert and Weidensaul[41] and Mathur and Vyas[47] reported that mycorrhizal condition of legume crops found to increase its vegetative growth and seed yield in addition to improve nodulation on it's root system. Highly significant improvement were reported with the inoculation of VA mycorrhizal fungi in combination with *Bradyrhizobium*, either in rock-P or super-P, where the total fresh weight content reached up to 88% and 90% for both soils, respectively, as compared with the uninoculated plants. This may attribute to the potential benefit of this mutualistic association on growth biomass. For all experimental interaction, shoot and root fresh weights were higher in case of soil amended with rock-P than super-P, but the increase was not significant. These results were in agreement with those obtained by El-Ghandour *et al.,*[23].

Dry Weight Yield: Shoot and root dry weights, as affected by inoculation of VA mycorrhizal and/or *Bradyrhizobium*, grown in soil amended with either rock-P or super-P, were shown in Table 2. Data indicated that inoculation with *Bradyrhizobium* increased the dry matter yield of shoot and root peanut plants, over the uninoculated plants cultivated in soil amended with rock-P or super-P. El-Ghandour *et al.,*[22] reported that fungal infection and rhizobial inoculation either alone or in combination increased dry matter yield as compared to uninoculated plants. Also, data showed that VA mycorrhizal colonization with peanut plant roots significantly increase dry weight of shoot and root, that the total weight exceeded that of uninoculated plants by about more than 2-fold in both rock and super phosphate applied soil. This result was in correspond to Krishna and Bagyaraj[40] who reported that inoculation with the mycorrhizal fungus *Glomus fasciculatum* enhanced peanut growth and increased its dry matter more than 2-fold compared with the non-inoculated control, in both sterilized and non-sterilized soil. Also Al-Karaki *et al.*,[4] indicated that shoot dry matter, shoot phosphorus and root dry matter were higher for mycorrhizal infected wheat (*Triticum aestivum* L.) plants than for non infected plants. Chulan and Martin[17] reported a significant shoot dry weight increment when *Theobroma cacao* was inoculated with VA-mycorrhiza. Aggangan and Dela Cruz[3] reported a dry matter yield increment of up to 631% when *L. leucocephala* was inoculated with vesicular-arbuscular mycorrhiza. Chien *et al.,*[16] marked that dry matter yield of different soyabean plant parts showed an increase in dry matter yield with different P sources as compared with check. The highest significant yields were recorded with the inoculation of both VA mycorrhizal fungi and *Bradyrhizobium* either in soil amended with rock-P or super-P. The total dry weights of shoot and root, for both soils, reached approximately more than 4-folds over the uninoculated plants. The application of bioinoculants like Am fungi and one of the plant growth-promoting rhizobacteria is an environment-friendly. The beneficial effects of these bacteria in combination with Am fungi have been reported by a number of workers[42,55]. It has been reported that these bacteria may affect Am fungi and their plant host through a variety of mechanisms that include (1) effects on the receptivity of the root; (2) effects on the root-fungus recognition; (3) effects on the fungal growth; (4) modification of the chemistry of the rhizospheric soil; and (5) effects on the germination of the fungal propagules. Moreover, Am fungi and nitrifying bacteria often act synergistically on infection rate, mineral nutrition and plant growth. Data showed that soil amended with rock-P significantly yielded

Table 2: Shoot and root dry weight of peanut plants, inoculated with VA mycorrhizal fungi and /or *Bradyrhizobium*, grown in soil amended with rock-P or super-P.

Inoculation	Rock – P			Super - P		
	Dry weight of shoot (g)	Dry weight of root(g)	Total Dry weight	Dry weight of shoot (g)	Dry weight of root (g)	Total Dry weight
Uninoculated	73.46	4.16	77.62	68.71	4.00	72.71
Plant+Rh	110.77***	7.50*** [a]	118.27	112.08***	6.02**	118.10
Plant+VAM	201.71***[a]	10.19**	211.90	191.08***	9.61***	200.69
Plant+VAM+Rh	294.92***[a]	16.82***	311.74	287.08***	16.07***	303.15

Comparing uninoculated with other treatment in the same column:
* = significant at P ≤ 0.05, ** = high significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001
Comparing the same treatment in rock-P with super-P:
a = significant at P ≤ 0.05, b = high significant at P ≤ 0.01, c = highly significant at P ≤ 0.001

shoot and root dry weights higher than that of super-P, this was in agreement with El-Ghandour *et al.,*[23] who reported that shoots and roots dry weight was higher in case of rock than super-P whereas the mean of shoots were 33.5 when rock-P was applied while it was 31.4 in case of super-P.

Height and Root Length: Table 3 showed that inoculating peanut plants with *Bradyrhizobium* significantly increased the shoot height and root length in either rock-P or super-P amended soil. Data also revealed an enhanced height increment in peanut plants with mycorrhizal symbiosis up to 51% in rock-P, and 45% in super-P amended soil, respectively, as compared to the non inoculated plants. Marschner and Dell[48] reported that mycorrhiza infection is known to enhance plant growth by increasing nutrients uptake. The higher height increment registered with inoculated plants could be as a result of enhanced inorganic nutrient absorption[19] and greater rates of photosynthesis[5], which obviously could have given rise to an increase in plant growth.

Inoculation with VA mycorrhiza also, significantly, increased the root length up to 68% and 74% in rock-P and super-P amended soil, respectively, as compared to non mycorrhizal infected soil. Huang *et al*.,[27] reported a root length increment of up to 80% when *Leucaena leucocephala* was inoculated with vesicular-arbuscular mycorrhizae. Levy and Syvertsen[43] while working on the effect of drought stress on citrus, reported that, although plant to plant variations obscured significant differences, VA mycorrhiza plants did tend to have greater total feeder root length per plant than control plants. In addition to the mycorrhiza inoculation enhancing the plants absorption of more nutrients, especially phosphorus, via an increase in the absorbing surface area[48], mycorrhiza colonization could have protected roots from soil pathogen[56], and therefore could have lead to an increase the root growth and nutrients acquisition of peanut plants.

The highest significantly increase in height and root length was recorded with inoculation of *Bradyrhizobium* and VA mycorrhiza for both P-amended soils. Rabie *et al.,*[61] indicates that dual inoculation with *Glomus clarum and Azospirillum brasilense* can increase the plant height, dry weight and root shoot ratio of cowpea plants more than single inoculation with Am fungi or NFB as well as control at all salinity levels. The higher root to shoot ratio of the inoculated plants could be attributed to the effect of mycorrhiza and/or *Bradyrhizobium* infection, which could have increased nutrients absorption, giving rise to a higher root length and height increment with a uniform growth. Clapperton and Reid[18] reported that as the colonization by vesicular-arbuscular mycorrhizal fungi increased, so did root to shoot ratios. In all experimental interactions, rock-P amended soil gave more values than super-P one. This result was in agreement with El-Ghandour *et al.,*[23].

Grain Yield: The results obtained from Table 4 indicated that the grain yield and fresh weight of one pod of peanut plants increase with the inoculation of *Bradyrhizobium*, comparing with uninoculated plants, in both P-amended soils. In other words, N_2-fixation process maximize the yield of inoculated plants as compared with uninoculated treatments[69,68]. Infection with VA mycorrhizal fungi, significantly, enhance grain yield of peanut plants, either in rock or super-P amended soil, of up to 265% and 255%, respectively. Jackson and Mason[31] found positive relationships among (P) availability, VA mycorrhizal infection and pod yield in groundnut (*Arachis hypogaea* L.). Alloush *et al.,*[7] found that chickpea plants inoculated with mycorrhizal fungus *Glomus versiforme* had higher number of nodules, shoot phosphorus content, shoot dry weight and grain yield than uninoculated chickpea plants. Further grain yield were harvested with the inoculation of both *Bradyrhizobium* and VA mycorrhizal fungi in both P-amended soils. Also solely

Table 3: Height and root length of peanut plants, inoculated with VA mycorrhizal fungi and /or *Bradyrhizobium*, grown in soil amended with rock-P or super-P.

Inoculation	Rock – P			Super - P		
	Height (cm)	Root length (cm)	R /S ratio	Height (cm)	Root length (cm)	R /S ratio
Uninoculated	36.25	17.18	0.47	34.87	15.50	0.44
Plant+Rh	47.67*** [a]	23.92**	0.50	43.65**	22.60**	0.52
Plant+VAM	54.63***	28.93***	0.53	50.70***	27.03***	0.53
Plant+VAM+Rh	68.06***	40.09***	0.59	66.25***	37.33***	0.56

Comparing uninoculated with other treatment in the same column:
* = significant at P ≤ 0.05, ** = high significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001
Comparing the same treatment in rock-P with super-P:
a = significant at P ≤ 0.05, b = high significant at P ≤ 0.01, c = highly significant at P ≤ 0.001

Table 4: Grain yield of peanut plants, inoculated with VA mycorrhizal Fungi and /or *Bradyrhizobium*, grown in soil amended with either rock-P or super-P.

Inoculation	Rock – P		Super - P	
	Number of pods /plant	Fresh weight of one pod (g)	Number of pods /plant	Fresh weight of one pod (g)
Uninoculated	72.02 [a]	2.58	69.53	2.42
Plant+Rh	107.51*** [a]	3.61	102.10***	3.01**
Plant+VAM	262.82***	3.80	246.46***	3.79**
Plant+VAM+Rh	417.24***	5.17**	393.00***	4.98***

Comparing uninoculated with other treatment in the same column:
* = significant at P ≤ 0.05, ** = high significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001
Comparing the same treatment in rock-P with super-P:
a = significant at P ≤ 0.05, b = high significant at P ≤ 0.01, c = highly significant at P ≤ 0.001

VA mycorrhizal infection increased the fresh weight of one pod up to 1.5 and 1.3 fold, compared with the non-inoculated control, in rock-P and super-P amended soil, respectively. While the dual combination of VA mycorrhiza with *Bradyrhizobium*, significantly, increased the fresh weight of pod, in both soils, of up to 2 fold. The beneficial effect of VA mycorrhizal symbiosis, as well as interaction with rhizobia, in general, greatly increase growth biomass, dry matter, and grain yield of inoculated plants as compared with the uninoculated ones. This indicated that N and P supply was influenced by VA mycorrhizal and by *Bradyrhizobium* inoculation. Rock-P soil produced more grain yield and fresh weight of pod than the super-P amended soil.

Nodule Number and Nitrogenase Activity: Data from Table 5 indicated that nodulation of the roots of peanut plants was significantly too much improved with the application of *Bradyrhizobium* biopreparation either singly or combined with Am fungi, in both P-amended soils, comparing with the uninoculated plants. Great nodule numbers (347.4 & 338.0 nodules/plant) were observed in inoculated with *Bradyrhizobium* alone, whereas, considerable numbers of nodules (299.8 & 289.4 nodules/plant) were noticed with the infected mycorrhizae plants as compared with non-infected plants in both soils. The results (Table 5) also revealed that nitrogenase activity increased within peanut plants inoculated with single *Bradyrhizobium* (82.4 & 79.2 nmol C_2H_5/g plant/h) or with Am fungi inoculation (75.0 & 67.5 nmol C_2H_5/g plant/h) in rock-P and super-P amended soils, respectively. Generally, rock- P amended soil resulted, significantly, higher values of nodule number and nitrogenase activity comparing with super-P amended soil. This result was in agree with El-Ghandour *et al.,*[23] who reported that inoculation with *Bradyrhizobium* (strain 3456 & 3339) increased nodulation and nitrogen content either rock-P or super-P were added, in the same time these parameters were higher in case of rock-P than super-P application. Pacovsky *et al.,*[54] explained that in alkaline soil and low P-deficient, rock-P enhanced nodulation and nitrogen fixation of mycorrhizal plants. Also, nodulated roots can enhance the production of root exudates which may affect the growth of soil microflora. Rhizobia may also increase the permeability of root cell to the fungus. Reports also stated that the presence of Am fungi is known to enhance nodulation and nitrogen fixation by legumes[9,36,62]. The increased phosphorus uptake conferred by the Am symbiosis is beneficial for the functioning of the nitrogenase enzyme of the bacterial symbionts, leading to increased nitrogen fixation and consequently promotion of root and

Table 5: Nodule numbers and nitrogenase activity (nmol C_2H_5/g plant/h) of peanut plants inoculated with VA mycorrhizal fungi and /or *Bradyrhizobium*, grown in soil amended with either rock-P or super-P.

Inoculation	Rock – P		Super - P	
	Nodule number / plant	N_2 activity (nmol C_2H_5/g plant/h)	Nodule number / plant	N_2 activity (nmol C_2H_5/g plant/h)
Uninoculated	101.3	35.1	92.9	32.9
Plant+Rh	347.4*** [a]	82.4***	338.0***	79.2***
Plant+VAM	299.8*** [a]	75.0*** [a]	289.4***	67.5***
Plant+VAM+Rh	421.2*** [a]	96.9*** [b]	411.3***	84.9***

Comparing uninoculated with other treatment in the same column:
* = significant at P ≤ 0.05, ** = high significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001
Comparing the same treatment in rock-P with super-P:
a = significant at P ≤ 0.05, b = high significant at P ≤ 0.01, c = highly significant at P ≤ 0.001

mycorrhizal development. Ishac *et al.,*[28] reported that the positive effect of AM on groundnut growth, nodulation, N_2 fixation and N &P uptake may be attributed to the mycorrhizal hyphae which increased the root mass spread in the soil and consequently increased the nutrient uptake. It is of interest to show that during the course of this experiment, nitrogenase activity responses followed nodule responses to different inoculants in both P-amended soils, this result was in agree with that recorded by Rabie and Humiany[61]. Highest significant nodule number and nitrogenase activity within peanut plants were recorded in the presence of dual inoculants of *Bradyrhizobium* and Am fungi. The synergistic effects of dual inoculants of Am fungi and nitrifying bacteria on nodule formation and nitrogenase activity were previously proved for different legumes plants[42,60,66]. In fact, it is easy to understand that the nodules can fix atmospheric nitrogen, but its efficiency is mostly determined by the phosphorus nutrient condition of the host plant since appropriate phosphorus nutrient support is indispensable for the process of nitrogen fixation. Mycorrhizal infection could contribute to proper phosphorus uptake in *Bradyrhizobium* and ensure the activity of nitrogen fixation enzyme. In this connection, Jha *et al.,*[34]; Valdes and Sannchez-Francia[72] and Johansson *et al.,*[36], showed that dual inoculation with mycorrhizae and nitrifying bacteria can support both the needs for N and P and increase the growth of host plant. Data also revealed significant and high significant values in case of soil fertilized with rock-P than that fertilized with super-P.

Total Chlorophyll Content and Total Carbohydrate: The results obtained from Table 6 revealed the total pigments content and total carbohydrate of leaves of peanut plants. Data showed that inoculation with *Bradyrhizobium,* significantly, increases the total pigment content of peanut plants as comparing to non-infected plants. Infection with single VA mycorrhizal fungi also, significantly, increases the total chlorophyll *a* and *b*, higher than those of both bacterial and non-infected plants, in both soils. With this respect, Hayman[26] stated that mycorrhizal associations in terrestrial plants have been shown to increase chlorophyll production compared with plants without fungal associations. The total carbohydrate also, significantly, increases with inoculated peanut plants with *Bradyrhizobium* singly, then, significant, higher increase was tabulated with the infected VA-mycorrhizal plants. However, the increase in total pigment content was positively correlated with the increase in total carbohydrate content. Several researchers have been proposed that AMF symbiosis increased leaf gas exchange and photosynthetic rate[64], enhanced water uptake through improved hydraulic conductivity and increasing leaf conductance and photosynthetic activity[39,20]. The values recorded in soil amended with rock-P were, significantly, still higher than that in super-P amended soil. The most effective co-inoculation was observed in the combined treatment with *Bradyrhizobium* and VA mycorrhizal fungi, which synergistically increased total chlorophyll *a*, *b* and carbohydrate compared with singly inoculated, as well as the control plants, in each P-amended soil. The enhancement in chlorophyll and carbohydrate content can be attributed to the increase of absorption and translocation of essential metal ions, due to VA mycorrhizal infection, which in turn accelerate the metabolic rates related to the synthesis of such constituents. The higher height increment registered with inoculated plants could be as a result of enhanced inorganic nutrient absorption[19] and greater rates of photosynthesis[5], which obviously could have given rise to an increase in plant growth.

Vam Infection Percentage, Protein Content and Total Lipid: The results presented in Table 7 revealed VA mycorrhizal infection percent, protein and lipid content of peanut plants grown in soil amended with either rock or super-P inoculated with VA mycorrhizal fungi and /or *Bradyrhizobium*. Slightly, non-significant, increase in percentage of VA mycorrhizal infection in peanut plants infected with Am fungi and *Bradyrhizobium* than those with VA solely in either P fertilized soils. With this respect, we can conclude that VA mycorrhizal fungi influenced the competitiveness of *Bradyrhizobium* strains, and that *Bradyrhizobium*

Table 6: Total Chlorophyll content (mg /g fresh weight), and total carbohydrate (mg/g dry weight) of leaves of peanut plants inoculated with VA mycorrhizal fungi, and /or *Bradyrhizobium*, grown in soil amended with either rock-P or super-P.

Inoculation	Rock – P				Super - P			
	Chloro-phyll " *a* "	Chloro-phyll " *b* "	Total pigm-ents (mg/g)	Total carboh-ydrate (mg/g)	Chloro-phyll " *a* "	Chloro-phyll " *b* "	Total pigm-ents (mg/g)	Total carboh-ydrate (mg/g)
Uninoculated	1.32 [b]	0.45 [b]	1.77	3.111	1.48	0.51	1.99	3.459
Plant+Rh	1.79*** [b]	0.59**	2.38	7.038*	1.54*	0.53	2.07	6.852*
Plant+VAM	2.24*** [b]	0.74*** [a]	2.98	11.123***	1.96***	0.64**	2.60	7.550**
Plant+VAM+Rh	2.54*** [b]	0.84*** [b]	3.38	13.495***	2.25***	0.74***	2.99	11.826***

Comparing uninoculated with other treatment in the same column:
* = significant at P ≤ 0.05, ** = high significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001
Comparing the same treatment in rock-P with super-P:
a = significant at P ≤ 0.05, b = high significant at P ≤ 0.01, c = highly significant at P ≤ 0.001

Table 7: Percentage of mycorrhizal infection, protein content (mg/g), and total lipid (mg/g) of dry seeds of peanut plants inoculated with VA mycorrhizal fungi and /or *Bradyrhizobium*, grown in soil amended with either rock-P or super-P.

Inoculation	Rock – P			Super - P		
	% 0f VAM infection	protein content (mg/g)	lipid content (mg/g)	% 0f VAM infection	protein content (mg/g)	lipid content (mg/g)
Uninoculated	30.0	17.7 [a]	46.3	27.0	15.9	45.2
Plant+Rh	34.0	23.4**	51.7** [a]	28.0	22.8**	47.8
Plant+VAM	64.0	21.7*	51.3**	58.0	20.6**	49.0*
Plant+VAM+Rh	78.0	25.7**	53.7**	69.0	25.1***	52.6**

Comparinguninoculated with other treatment in the same column:
* = significant at P ≤ 0.05, ** = high significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001
Comparing the same treatment in rock-P with super-P:
a = significant at P ≤ 0.05, b = high significant at P ≤ 0.01, c = highly significant at P ≤ 0.001

also affected the root colonization by VA mycorrhizal fungi[52]. In addition, the parameters of the plant grown in soil amended with rock phosphate were found to be more beneficial than that grown in super-P amended soil. El-Ghandour *et al.,*[23] noticed that nodulation and micorrhizal infection were higher in soil amended with rock than those amended with superphosphate. Inoculating plants with *Bradyrhizobium* solely, high significantly, increased their protein content to a great extent comparing to non-inoculating plants in both P applicable soils. In addition, inoculating with Am fungi had a significant slightly increment comparing to non-infected plants, but still lower than that of bacterial inoculation. The highest significant content was obtained in presence of *Bradyrhizobium* and Am fungi, in rock-P amended soil, which exhibited high significant values than super-P amended soil.

From the previous data, bacterial-Am fungal-legume tripartite symbiosis showed better nitrogen fixation (nodule number, nitrogen and protein contents as well as nitrogenase activities) than that of bacterial-legume symbiosis. These results were consistent with Minerdi *et al.*,[50] who demonstrated the presence of genes for fixation in endosymbiotic *Burkholderia* bacteria in AM mycorrhizal hyphae and suggested that there may be a potential for improving N supply to mycorrhizal plants through fixation of atmospheric N[62].

Lipid content was notably influenced by inoculation and fertilization of soil. Peanut plants showed high content with the inoculation of *Bradyrhizobium*, followed by mycorrhiza symbiosis. The highest was produced, significantly, with the dual inoculation of both inoculants. Table 7 also revealed that rock-P amended to the soil showed an effect on the lipid content of peanut plants. This result was in agree with those obtained by Rabie[62] who reported that rock phosphate amended to the soil showed a significant effect on the lipid content of red kidney and wheat plants, where the lipid content of the plants grown in soil with rock-P was still higher than that without rock-P especially in the presence of Am fungi.

Nutrient Uptake: Table 8 shows that nitrogen plant tissue, phosphorus and potassium concentration was much higher in the inoculated plants than non inoculated ones, either in rock-P or super-P amended soil. *Bradyrhizobium* inoculation had increased N, P and K uptake by peanut plants, in both P amended soils, comparable to the uninoculated plants. It is of interest to mention that the *Bradyrhizobium* strain has the ability to solubilize rock-P[1], this was proved from

Table 8: Nitrogen, phosphorus, and potassium (NPK) content (mg g^{-1}) of dry leaves of peanut plants inoculated with VA mycorrhizal fungi and /or *Bradyrhizobium*, grown in soil amended with either rock-P or super-P.

Inoculation	Rock – P			Super - P		
	N	P	K	N	P	K
Uninoculated	2.80	0.23	1.13 [a]	2.70	0.17	1.09
Plant+Rh	3.55	0.41***	2.04***	3.46**	0.39***	2.00***
Plant+VAM	3.34	0.46***	2.07	3.15*	0.43***	1.98***
Plant+VAM+Rh	4.01**	0.53***[b]	2.13***	3.93**	0.58***	2.11***

Comparing uninoculated with other treatment in the same column:
* = significant at P ≤ 0.05, ** = high significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001
Comparing the same treatment in rock-P with super-P:
a = significant at P ≤ 0.05, b = high significant at P ≤ 0.01, c = highly significant at P ≤ 0.001

the highly significant data of P uptake from *Bradyrhizobium* inoculated plants in rock-P and super-P amended soil, as compared to uninoculated plants. Data also reveal higher N, P and K concentration in the mycorrhizal inoculated plants, in both phosphate fertilized soils. The increased efficiency of mycorrhizal plants versus non-mycorrhizal is caused by the active uptake and transport of nutrients by mycorrhizae. The highest significant values of N, P and K were recorded with the pair inoculants combinations, where the values were higher under rock-P than super-P amended soil. With this respect, Azcón-Aguilar *et al.,*[11] recorded that inoculation with *Glomus mosseae* not only affected plant growth and nutrition in *Medicago sativa,* but also enhanced the activity of *Rhizobium meliloti* when it was applied as an inoculant.

In legume plants the importance of AMF symbiosis has been attributed to high P requirements on the nodulation and N_2 fixation process which requires enhanced P uptake[12]. Thus, AMF have been shown to improve productivity in soils of low fertility[33] and are particularly important for increasing the uptake of slowly diffusing ions such as PO_4^{3-}[32].

Plant roots alone may be incapable of taking up phosphate ions that are immobilized, for example, in soils with basic pH. The mycelium of the mycorrhizal fungus can however access these phosphorus sources, and make them available to the plants they colonize[44]. The mechanisms of increased absorption are both physical and chemical. Mycorrhizal mycelia are much smaller in diameter than the smallest root hair. For this reason they are able to explore a greater volume of soil and have a much larger surface area for absorption. Also, the cell membrane chemistry of fungi is different from that of plants. Mycorrhizae are especially beneficial for the plant partner in nutrient-poor soils. Mycorrhizal plants are often more resistant to diseases, such as those caused by microbial soil-borne pathogens[21,59], and are also more resistant to the effects of drought[67,65]. These effects are perhaps due to the improved water and mineral uptake in mycorrhizal plants. Quilambo[60] reported that inoculation with an indigenous inoculant resulted in increased leaf and root growth and prevented the expected increase in root to shoot ratio and root-weight ratio that are normally observed under phosphorus deficient and drought stress conditions in peanut. Peanut (*Arachis hypogaea* L.) plants grown in sterilized soil without VA-fungi inoculation developed visible symptoms of phosphorus and zinc deficiency[47]. Krishna and Bagyaraj[40] stated that inoculation of peanut plants with mycorrhizal fungus *Glomus fasciculatum* increased uptake of phosphorus and micronutrients such as zinc, copper, manganese and iron.

The higher plant tissue nitrogen content in inoculated plants could be attributed to hyphae uptake. It has been reported that the existence of extra-radical hyphal bridges between individual plants permits transfer of nutrients such as nitrogen[48]. They reported that about 24% of the total nitrogen uptake in mycorrhizal plants could be attributed to uptake and delivary by the external hyphae. There is also evidence that nitrogen is taken up by vesicular-arbuscular mycorrhiza hyphae from inorganic sources of ammonium and therefore, the higher nitrogen concentration in mycorrhizal plants could be attributed to the hyphae uptake. The same could be said of the higher potassium concentration in inoculated plants. In other words, mycorrhizal fungi can improve absorption of N from NH_4^+ – nitrogen mineral fertilizers, transporting it to the host plant[8,35]. Its transport and absorption can also increase biomass production in soils with low potassium[46]. In a compartment pots experiment, Li *et al.,*[45] demonstrated that about 10% of the total potassium uptake in mycorrhizal coach grass was due to hyphal uptake and transport. Kohler *et al.,*[38] reported that the foliar P and K contents increased significantly with the *Bacillus subtilis* or *Glomus intraradices* inoculation, alone or in combination.

Conclusion: We conclude that natural microbial inoculants enrich the nutritional value of the soil and it is advisable to use these microorganisms as biofertilizers in the presence of low dose of N and P fertilizers in low soil fertility. Also, rock-P could be considered as available and cheap source of P fertilization.

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