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SYNERGISTIC INTERACTION OF *BRADYRHIZOBIUM* AND *ARBUSCULAR MYCORRHIZAL* FUNGI WITH LEVELS OF MINERAL NITROGEN, PHOSPHORUS AND MOLYBDENUM ON PEANUT GROWN IN SANDY SOILS

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ABSTRACT: ***A field experiment was conducted in the newly reclaimed sandy soils at El-Bostan area, to investigate the influence of bio and mineral fertilizers on yield and yield components of peanut plants (Arachis hypogaea L.). Treatments include single or dual inoculation of Bradyrhizobium (BR) and the vesicular-arbuscular mycorrhizal fungi (VAM), as biofertilizers. In addition, eight mineral fertilizer treatments, nitrogen (75, 100 and 120% of recommended dose), phosphorus (50, 75 and 100% of recommended dose) and two molybdenum treatments (with 200 g ha^{-1} Mo as ammonium molybdate and without) under randomize complete block statistical design (RCBD). The results indicate that inoculation with VAM+BR significantly increase hundred kernel weight, kernel, pods, hay and biological yield by 34.00, 26.12, 45.81, 26.80 and 34.32 %, respectively compared to uninoculated treatments followed by single treatments of BR then VAM. Data also revealed that bacterial–mycorrhizal–legume symbiosis increase significantly VAM infection and number of spores, nodule number and BR count, nitrogenase activity and alkali phosphatase compared with uninoculated. Also, the plant nitrogen, phosphorus and potassium (NPK) concentrations, protein content and shelling percentage were significantly increased due to dual biofertilizers followed by the single inoculation. Application of molybdenum increased nodules number per plant and BR count by 4.35 and 10.97% when compared with without molybdenum application. Generally, inoculation with VAM and BR can, synergistically, remove the deficient effect of N and P in the soil of low nutrient content. At the same time, increase the NUEs and PUEs. Bio-Dependency of pods, hay and biological yields increased significantly by 29.70, 36.89 and 45.87; 15.08, 21.02 and 26.93; and 20.76, 27.18 and 34.28%, respectively with inoculation with VAM, BR and VAM+BR.***

Key words: ***Mycorrhiza, Bradyrhizobium, Biofertilizers, peanut, sandy soils.***

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is considered one of the most important edible oil crops in Egypt, which is due to its seeds' high nutritive value for humans and the green leafy hay for feeding livestock, moreover to the seed oil's importance for industrial purposes. The main growing zones are sited in the north of the country; they include reclaimed desert to the east and west of the Nile Delta (Fageria *et al*., 1997). Peanut yields are known to be influenced by N uptake and P mobilization. It is a crop ideally suited for studies on the tripartite association between legume, nitrogen fixing bacteria and mycorrhizal fungi (El-Azouni *et al*., 2008).

Arbuscular mycorrhizal (AM) symbiosis that is formed by AM fungi and the roots of vascular plants is the most widespread mutualistic associations in environment (Willis *et al.,* 2013; Chen *et al.,* 2018). AM fungi play a very important role in ecosystems through nutrient

cycling (Shokri and Maadi, 2009; Wu *et al.,* 2011 and Tabassum *et al.,* 2012). Mycorrhizae can also increase the availability and supply of slowly diffusing ions, such as phosphate to the plant (Sharda and Koide, 2010). In addition to their significant role in P acquisition, AM fungi can also provide other macro- and micro-nutrients such as N, K, Mg, Cu and Zn, particularly in soils where they are present in less soluble forms (Meding and Zasoski, 2008 and Smith and Read, 2008). In general, mycorrhizae stimulate plant growth not only by providing nutrients necessary for plant growth, but also help the plant to tolerate environmental stress (Kaya *et al*., 2009; Sheng *et al*., 2009 and Abiala *et al*., 2013). The formation of VAM symbiosis is considered to be one of the most promising strategies evolved by plants for handling nutrient-deficiency stresses. Although the role of P nutrition in VAM associations has been a major attention of this field, an increasing number of studies have exposed that plants can also take up substantial amounts of N through the so-called mycorrhizal pathway (Bücking and Kafle, 2015).

One of the well-known N_2-fixing plant-microorganism interactions is the legume–rhizobia symbiosis, which is considered the most efficient and important process in crop production, so as to improve soil fertility and farming system flexibility (Mylona *et al*., 1995). Rhizobia (including Rhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium) are generally regarded as microbial symbiotic partners of legumes and are mainly known for their role in the formation of nitrogen-fixing nodules (Antoun and Prévost, 2005). In particular, Rhizobium species are a vast group of soil borne rhizobia with representatives that have proven plant growth promoting activities through nitrogen fixation. These bacteria can equally produce plant growth regulators and solubilize organic and inorganic phosphates that would have a role in their plant growth promoting activities (Antoun *et al*., 1998).

The legume-nodulating rhizobia have the ability to reduce dinitrogen to ammonia and supply nitrogenous compounds to the plants. Thus, the plants gain independence of the existence of nitrogenous compounds in the soil environment (Mwenda *et al*., 2010).

Improved legume nutrition has been observed with AM fungi and Rhizobium (Guo *et al*., 2010; Tavasolee *et al*., 2011). The use of AMF gives promising results in legumes which can form tripartite symbiotic associations with nodule-inducing rhizobia and AMF simultaneously, benefitting the plant by increasing both P and N use efficiency (Ossler *et al*., 2015; Chang *et al*., 2017).

A number of research articles shows the role of Bradyrhizobium and mycorrhizae alone and/or in combination in enhancing plant growth under stress conditions. However, in contrast, a few review papers are available which discuss the synergistic interactions between Bradyrhizobium and mycorrhizae for enhancing plant growth under normal or stressful environments. The present study comprehensively studies the effectiveness of Bradyrhizobium and mycorrhizal fungi for enhancing peanut yield and seed quality of peanut by biofertilizers include VA mycorrhizal fungi and Bradyrhizobium under different levels of nitrogen, phosphorus and molybdenum fertilizers.

MATERIALS AND METHODS

Summer season field experiment was conducted in the newly reclaimed sandy soils at El-Bostan area, Mohamed Reffat village (30o 12′ N and 30o 30′ E, 7.4 m above sea level). The soil of the experimental site is coarse sand.

Experimental Design:

This experiment was carried under fixed system sprinkler irrigation with spacing 18 X 18 m. The average precipitation rate was 9.78 mm hr^{-1} and discharge of 3.17 m3 hr^{-1} for each sprinkler. The total experimental area was 30 x 60 m (1800 m^2). The area was chiseled and harrowed. Then it was divided into 72 plots, the area of each experimental unit was 5 x 5 m (25 m^2) with 0.5 m border between plots. The area was cultivated with peanut (Giza 5). Planting was carried out at 16 May 2016 by hand sowing; 0.5 m apart between rows, 0.2 m between plants in the row and two seeds in each site. The randomize complete block statistical design (RCBD) was carried out with four biofertilizers applications (Bradyrhizobium (BR), Vascular Arbuscular Mycorrhiza Fungi (VAM), BR+VAM and without). In addition, eight mineral fertilizer treatments, three N levels [80.5 (75%), 107.0 (100%) and 128.5 (120%) kg N ha^{-1} as ammonium nitrate (33.5 % N)], three P levels [35.7 (50%), 53.6 (75%) and 71.4 (100%) kg P_2O_5 ha^{-1} as mono calcium superphosphate (15.5 % P_2O_5)]. Nitrogen and phosphorus fertilizers were split into three equal doses. Two molybdenum treatments (200 g ha^{-1} Mo as ammonium molybdate and without). All treatments received 57.1 kg K_2O ha^{-1}. At harvesting, peanut plants of each experimental unit were taken separately to determine peanut yield and yield components. Then soil samples were taken separately from each experimental unit at soil depth of 0-30 and 30-60 cm.

Soil Analyses:

Soil paste extracts were obtained according to (Page *et al*., 1982). Main soil chemical and nutritional characteristics are presented in Table (1). Soluble cations and anions, pH and EC were also measured. Total calcium carbonate content ($CaCO_3$ %) was determined using the pressure Calcimeter method (Page *et al*., 1982). Organic carbon content (OC%) was determined using Walkely and Black method (Nelson and Sommers, 1982). Available potassium was extracted by ammonium acetate extraction and analyzed using Flame Photometer apparatus (Knudsen *et al*., 1982). Total-N was measured using Kjeldahl. Available phosphorus was extracted by ammonium bicarbonate and measured colorimetric using spectrophotometer (Olsen and Sommers, 1982). Particle size distribution of the soil samples was carried out using hydrometer method (Gee and Bauder, 1986).

Table (1): Mean soil physical, chemical and nutritional characteristics at the experimental site.

Depth (cm)	Particle size distribution (%)			Texture	pH*	E.C ** dSm^{-1}	$CaCO_3$ %	Chemical analysis							
	Sand	Silt	Clay					Soluble cations (meq/l)				Soluble anions (meq/l)			
								Na^+	Ca^{++}	Mg^{++}	K^+	CO_3^-	HCO_3^-	Cl^-	SO_4^{--}
0-30	96.1	1.5	2.4	Sand	8.05	1.46	7.0	5.1	5.5	3.5	0.7	--	5.6	6.0	3.2
30-60	94.5	1.2	4.3	Sand	8.13	1.36	5.7	6.2	4.5	3.0	0.7	--	5.1	6.5	2.8

Available nutrients mg/kg								
Depth (cm)	N	P	K	Zn	Mn	Fe	Cu	Mo
0-30	106.5	7.2	61.6	0.84	1.78	4.35	0.04	0.02
30-60	107.5	5.8	102.5	0.72	1.49	4.12	0.03	0.02

*(1:2.5) soil : water suspension

** Soil paste extract

Bio-Analyses:

Rhizosphere soil sample were collected at harvest and analyzed for number of AM spores and *Bradyrhizobium* count.

Assessment of VA-mycorrhizal infection was measured on composite samples of three plants per plot: The staining method of Phillips and Hayman (1970) was used for preparing root samples for microscopic observation. The gridlines intersect method of Giovannetti and Mosse (1980) was used to estimate the VA-mycorrhizal infection percentage.

$$Mycorrhizal\ Colonization\ (\%) = \frac{Total\ number\ of\ root\ segments\ colonized}{Total\ Number\ of\ root\ segments\ studied} x100$$

Nitrogenase activity was determined on a detached root system, using gas chromatograph (Thermo Scientific TRACE GC Ultra equipped with FID detector and Capillary column CP-PoraBOND Ufused silica plot 25 m × 0.32 mm, df = 7_m) as described by (Abd-Alla, 2011). For determination of phosphatase activity disodium phenylphosphate served as enzyme substrate (Öhlinger, 1996).

Bio dependency (BD) is the increase of yield or yield components which refer to the bio-inoculation and calculate as:

$$Yield\ Bio-Dependency\ (\%) = \frac{Yield\ (Inoculated) - Yield\ (UnInoculated)}{Yield\ (UnInoculated)}\ x\ 100$$

Plant analysis:

Plant samples were taken at harvesting from each treatment, dried at 70°, and ground for the determination of N, P, K. The samples were digested using H_2SO_4 and H_2O_2 for determination of phosphorus and potassium content. Total phosphorus content in the extract was determined using Vanado-Molybdate yellow color method as described by (Jackson, 1970). Total potassium content was determined using Flame photometer according to (Cottenie *et al*., 1982). Total nitrogen was determined using the micro kjeldahl method (Bremner and Mulvaney, 1982). Protein content of seeds and hay were determined by multiplying total N by the standard factor 6.25 (Hunter, 1984).

Yield and yield components:

At harvest, one square meter from each plot was taken to determine kernel, pods, hay and biological yields (kg ha^{-1}). Shelling percentage was calculated as:

$$Selling\ Percentage\ (\%) = \frac{(Weight\ of\ kernels/plant)}{(Weight\ of\ Pods/plant)}\ x\ 100$$

Statistical analyses:

The obtained data were carried out using COSTAT Software Ver. 6.4. Analysis of Variance (ANOVA) was used to test pods, kernel, hay and biomass production of Peanut. Differences in mean values of various treatments were evaluated by Duncan's multiple range test as described in (Gomez *et al*., 1984).

Nitrogen Efficiency Analysis:

Analysis of the nitrogen fertilizer efficiencies was carried out using the efficiency parameters defined by Huggins and Pan (2003) as follows:

$$Nitrogen\ \ use\ \ efficiency(NUE) = G_w / N_s$$

$$Nitrogen\ \ utilization\ \ efficiency(NUTE) = G_w / N_t$$

$$Grain\ N\ accumulation\ \ efficiency(GNAE) = N_g / N_s$$

Where

(G_w) Grain yield

(N_g) Grain nitrogen

(N_s) Nitrogen supply

(N_t) Aboveground plant nitrogen

Phosphorus Efficiency Analysis:

Analysis of the Phosphorus fertilizer efficiencies was carried out using the efficiency parameters defined by Huggins and Pan (2003) as follows:

$$Phosphorus\ \ use\ \ efficiency(PUE) = G_w / P_s$$

$$Phosphorus\ \ utilization\ \ efficiency\ (PUTE) = G_w / P_t$$

$$Grain\ P\ accumulation\ \ efficiency\ (GPAE) = P_g / P_s$$

Where:

(G_w) Grain yield

(P_g) Grain Phosphorus

(P_s) Phosphorus supply

(P_t) Aboveground plant Phosphorus

RESULTS AND DISCUSSION

Root colonization and number of spores in soil rhizosphere:

Data in Table 2 illustrated the root colonization and number of spores. It is very interesting to observe that the biofertilizers significantly increased mycorrhizal infection from 16.55% in uninoculated to 53.13 and 55.07% and from 9.01 in uninoculated to 13.87 and 13.95 spores/g soil for VAM and VAM+BR treatments, respectively. Root colonization and number of colonizations were enhanced significantly by the influence of VAM in presence of Bradyrhizobium compared to VAM or uninoculated treatments separately. At the same time, the highest mycorrhizal infection and number of spores/g under mineral fertilizers treatments were observed in P1 treatment (39.87% and 11.67 spores/g soil) followed significantly by P2 treatment (37.52% and 11.60 spores/g soil), while the lowest percentage was observed in N1 (34.19%) for root colonization and N3 (11.3 spores/g soil) for number of spores. The influence of VAM in presence of Bradyrhizobium was more effective by applying 50 and 75% of P fertilizer recommended dose. (Kobae *et al*., 2016) established that high phosphorus supply exerts strong inhibitory effect on AM colonization. N starvation may be able to trigger a signal that counteracts the inhibition of high P availabilities on AM colonization (Nouri *et al*., 2014; Breuillin-Sessoms *et al*., 2015). This result is in agreement with ARM (2014), root colonization (percent) was enhanced significantly by the influence of VAM in presence of Rhizobium compared to in absence of any one. Solaiman *et al*. (2005) also recorded enhanced root colonization in chickpea due to the influence of VAM and Rhizobium. Similar results showed by (Khanam *et al*., 2005).

Number of nodules and *Bradyrhizobium* count:

The influence of different combinations of VAM, BR and mineral fertilizers on number of nodules per plant and BR count per g soil is exhibited in Table 2. Nodulation of the roots of peanut plants was significantly increased and improved with the application of Bradyrhizobium either singly or combined with VAM under the mineral fertilizer treatments comparing with the uninoculated treatments. The maximum number of nodules (363.37/plant) and BR count (58.22 x 104 cfu/g soil) were observed with interaction of VAM and BR. The individual of BR treatment significantly followed the mixed treatment and has the second highest number of nodules (332.52 / plant). The maximum number of nodules and BR count were observed under mineral treatment N1 (75% of N recommended dose). At the same time, Application of Mo increased nodules number per plant and BR count by 4.35 and 10.97% when compared with without Mo application. (Bulgarelli *et al*., 2017) stated that VAM promoted plant biomass and nodule formation compared to non-VAM plants. At the same time, Roots of inoculated soybean plants were highly colonized, with high abundance of arbuscules. Also, Mirdhe and Lakshman (2009) found that Mycorrhizal fungi and Rhizobium were found to be synergistic with respect to nitrogen fixation and per cent of root colonization.

Table (2): Effect of biological and mineral fertilizers on VAM infection and count, nodules number, Bradyrhizobium count, Nitrogenase and Alkali phosphatase

Fertilizers: Mineral	Fertilizers: Bio	VAM infection (%)	VAM count /g soil	nodule number/ plant	Bradyrhizobium count 10^4 cfu g^{-1} soil	Nitrogenase activity (nmol C_2H_5 g^{-1} plant h^{-1})	Alkali phosphatase (mg phenol g^{-1} hr^{-1})
N1	Un-Inoc.	16.1	8.9	91	22.4	31.4	36.1
	VAM	52.1	13.7	278	23.5	67.6	43.8
	BR	18.3	9.0	354	85.2	79.4	37.2
	VAM+BR	50.1	13.8	420	88.4	84.8	44.9
	Mean	34.2	11.4	285.8	54.9	65.8	40.5
N2	Un-Inoc.	17.3	9.1	95	20.8	30.4	37.2
	VAM	51.1	13.6	287	21.6	65.7	43.5
	BR	20.4	8.9	341	73.5	75.4	37.5
	VAM+BR	52.1	13.7	418	74.3	82.7	44.1
	Mean	35.2	11.3	285.3	47.6	63.6	40.6
N3	Un-Inoc.	17.9	9.0	97	18.4	31.1	36.2
	VAM	53.1	13.5	294	19.2	62.1	43.4
	BR	19.2	9.1	335	51.4	70.4	37.9
	VAM+BR	54.8	13.6	410	52.6	78.8	43.8
	Mean	36.3	11.3	284.0	35.4	60.6	40.3
Mean		35.2	11.3	285.0	45.9	63.3	40.5
P1	Un-Inoc.	15.2	9.1	98	18.7	30.7	40.0
	VAM	62.1	14.2	251	19.1	57.6	50.5
	BR	17.9	9.2	297	49.7	63.4	39.5
	VAM+BR	64.3	14.2	310	50.8	64.1	51.2
	Mean	39.9	11.7	239.0	34.6	54.0	45.3
P2	Un-Inoc.	16.4	9.0	104	17.4	29.5	38.2
	VAM	55.8	14.1	285	18.0	56.1	48.5
	BR	19.1	9.1	311	47.4	62.4	37.5
	VAM+BR	58.8	14.2	320	48.7	63.7	48.8
	Mean	37.5	11.6	255.0	32.9	52.9	43.3
P3	Un-Inoc.	17.9	8.9	110	17.8	29.9	35.2
	VAM	50.1	14.0	295	18.7	55.7	44.4
	BR	17.3	9.1	315	49.0	61.8	33.1
	VAM+BR	54.8	14.1	332	49.7	61.9	43.8
	Mean	35.0	11.5	263.0	33.8	52.3	39.1
Mean		37.5	11.6	252.3	33.8	53.1	42.6
Without Mo	Un-Inoc.	15.5	9.0	93	19.1	29.3	35.4
	VAM	48.7	13.8	281	20.1	45.6	38.9
	BR	25.2	9.2	345	47.2	58.4	36.9
	VAM+BR	52.1	13.9	338	48.4	61.5	39.1
	Mean	35.4	11.5	264.3	33.7	48.7	37.6
With Mo	Un-Inoc.	16.1	9.1	101	24.1	33.5	36.7
	VAM	52.1	14.1	284	20.0	48.2	39.8
	BR	24.3	9.4	359	52.4	61.2	38.7
	VAM+BR	53.6	14.1	359	52.9	62.4	40.2
	Mean	36.5	11.7	275.8	37.4	51.3	38.9
Mean		36.0	11.6	270.0	35.5	50.0	38.2
$LSD_{0.05}$	Mineral	0.107	0.037	1.152	0.247	0.251	0.141
	Bio	0.076	0.026	0.814	0.174	0.178	0.1

Nodule Activity:

Specific nitrogenase (acetylene-reduction) activity per gram fresh weight nodule was significantly increased by mixed inoculation with VAM+BR (65.97 nmol C2H5 g-1 plant h-1) followed by BR (66.63 nmol C2H5 g-1 plant h-1) (Table 2). Mixed inoculation treatment with N1 mineral fertilizer recorded the highest nitrogenase activity (84.8 nmol C2H5 g-1 plant h-1). While under mixed inoculation treatment and without-Mo record the lowest nitrogenase activity (61.5 nmol C2H5 g-1 plant h-1). Table (2) also showed that phosphatase activity recorded significant increase due to mixed inoculation treatments. Mixed inoculation treatment with P1 treatment recorded the highest phosphatase activity (51.2 mg phenol g-1 hr-1) followed by VAM under P2 treatments (50.5 mg phenol g-1 hr-1). Bulgarelli *et al*. (2017) found that VAM symbiosis increased nitrogenase activity, P content in nodules and leaf N content and reduced the metabolic limitation of photosynthesis under P starvation, which indicated the VAM stimulus to symbiotic N2 fixation and photosynthesis. Then mycorrhizal symbiosis could not completely meet soybean P demand compared with well-nourished soybean plants, which produced higher nodules biomass. Badawi *et al*. (2011) stated that bacterization of peanut seeds with bradyrhizobia utilized considerable improvement in number and mass of root nodules, increased the rate of acetylene reduction and all growth characters in compared to comparison to the uninoculated.

Yield and yield components (pods, seeds, hay, 100 seeds):

Data in Table 3 indicated that the yield parameters (100 kernel weight, kernel, pods, hay and biological yield) increased significantly with inoculation with VAM+BR, BR and VAM under all mineral fertilizer treatments when compared with uninoculated treatments. In other words, N2 -fixation process and P solubility maximizes the yield of inoculated plants as compared with uninoculated treatments. Inoculation with VAM+BR significantly increase hundred kernel weight, kernel, pods, hay and biological yield by 34.00, 26.12, 45.81, 26.80 and 34.32 %, respectively compared to uninoculated treatments.

The beneficial effect of interaction between VAM and Bradyrhizobium, in general, greatly increase yield parameters of inoculated plants as compared with the uninoculated ones. This indicated that N and P supply was influenced by VAM and by bradyrhizobium inoculation. The data also cleared that the yield parameters increased in Mo treated plants when compared to untreated ones.

This increase refers to the role of Mo to enhances the N2 fixation efficiency in bradyrhizobium nodules. Jackson and Mason (1984) found positive relationships among P availability, VA mycorrhizal infection and pod yield in groundnut (*Arachis hypogaea* L.). Alloush *et al*. (2000) found that chickpea plants inoculated with mycorrhizal fungus had higher number of nodules, shoot phosphorus content, shoot dry weight and grain yield than uninoculated chickpea plants.

Further grain yield was harvested with the inoculation of both Bradyrhizobium and VA mycorrhizal fungi in both P-amended soils. El-Azouni *et al*. (2008) reported that the biomass and grain yield were significantly improved by using the dual bio-preparations of AM fungi with Bradyrhizobium. Significantly maximum shelling percentage (64.46%) was obtained in VAM+BR combination treatment followed by BR treatment (60.66%) when compared to uninoculated treatment. The present results in

agreement with the findings of (Malligawad, 2010) which found that application of Rhizobium and trichoderma increases shelling percent in groundnut. Also, Zalate and Padmani, 2009 and Mahrous *et al*., 2015) finding the same.

Table (3): Grain, Pod, Hay yields of peanut plants, inoculated with VAmycorrhizal Fungi and /or *Bradyrhizobium,* grown in soil amended with either nitrogen, phosphorus or molybdenum fertilizers.

Fertilizers		100 Kernel weight (g)	Kernel yield (kg ha^{-1})	Pods yield (kg ha^{-1})	Hay yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Shelling percentage (%)	Kernel yield B.D.* (%)	Pods yield B.D.* (%)	Hay yield B.D.* (%)	Biological yield B.D.* (%)
Mineral	Bio										
N1	Un-Inoc.	68.1	1411	2235	3179	5414	58.84	-----	-----	-----	-----
	VAM	80.8	1694	2692	3808	6500	60.07	20.06	20.45	19.79	20.06
	BR	86.7	1688	2810	3999	6809	62.93	19.63	25.73	25.79	25.77
	VAM+BR	91.0	1717	2918	4186	7104	63.13	21.69	30.56	31.68	31.22
	Mean	81.6	1628	2664	3793	6457	61.2	20.5	25.6	25.8	25.7
N2	Un-Inoc.	68.9	1426	2246	3411	5657	53.68	-----	-----	-----	-----
	VAM	82.5	1775	3077	3906	6983	56.04	24.47	37.00	14.51	23.44
	BR	88.4	1796	3205	4155	7360	57.69	25.95	42.70	21.81	30.10
	VAM+BR	92.7	1810	3372	4309	7681	63.49	26.93	50.13	26.33	35.78
	Mean	83.1	1702	2975	3945	6920	57.7	25.8	43.3	20.9	29.8
N3	Un-Inoc.	71.4	1471	2367	3572	5939	54.34	-----	-----	-----	-----
	VAM	83.3	1771	3060	4002	7062	57.26	20.39	29.28	12.04	18.91
	BR	91.0	1825	3187	4232	7419	57.88	24.07	34.64	18.48	24.92
	VAM+BR	93.5	1870	3441	4407	7848	62.15	27.12	45.37	23.38	32.14
	Mean	84.8	1734	3014	4053	7067	57.9	23.9	36.4	18.0	25.3
Mean		83.2	1688	2884	3931	6815	59.0	59.0	35.1	21.5	26.9
P1	Un-Inoc.	64.6	1409	2157	3218	5375	60.50	-----	-----	-----	-----
	VAM	89.3	1762	2635	3727	6362	64.59	25.05	22.16	15.82	18.36
	BR	85.9	1751	2711	3892	6603	66.87	24.27	25.68	20.94	22.85
	VAM+BR	91.0	1777	2937	4094	7031	65.32	26.12	36.16	27.22	30.81
	Mean	82.7	1675	2610	3733	6343	64.3	25.1	28.0	21.3	24.0
P2	Un-Inoc.	69.7	1433	2100	3405	5505	55.34	-----	-----	-----	-----
	VAM	91.0	1784	3036	3858	6894	56.48	24.49	44.57	13.30	25.23
	BR	86.7	1761	3118	4053	7171	58.76	22.89	48.48	19.03	30.26
	VAM+BR	93.5	1856	3354	4290	7644	68.24	29.52	59.71	25.99	38.86
	Mean	85.2	1709	2902	3902	6804	59.7	25.6	50.9	19.4	31.5
P3	Un-Inoc.	72.3	1430	2231	3533	5764	54.10	-----	-----	-----	-----
	VAM	92.7	1814	3068	4007	7075	55.10	26.85	37.52	13.42	22.74
	BR	87.6	1761	3196	4156	7352	59.13	23.15	43.25	17.63	27.55
	VAM+BR	94.4	1873	3462	4446	7908	64.10	30.98	55.18	25.84	37.20
	Mean	86.7	1720	2989	4036	7025	58.1	27.0	45.3	19.0	29.2
Mean		84.9	1701	2834	3890	6724	60.7	60.7	41.4	19.9	28.2
without Mo	Un-Inoc.	66.4	1400	2179	3179	5358	58.51	-----	-----	-----	-----
	VAM	79.6	1584	2510	3808	6318	57.78	13.14	15.19	19.79	17.92
	BR	85.7	1608	2783	3999	6782	63.11	14.86	27.72	25.79	26.58
	VAM+BR	91.0	1692	2892	4186	7078	64.25	20.86	32.72	31.68	32.10
	Mean	80.7	1571	2591	3793	6384	60.9	16.3	25.2	25.8	25.5
with Mo	Un-Inoc.	70.2	1425	2190	3572	5762	52.19	-----	-----	-----	-----
	VAM	82.4	1698	2879	4002	6881	55.66	19.16	31.46	12.04	19.42
	BR	90.4	1789	3214	4232	7446	58.98	25.54	46.76	18.48	29.23
	VAM+BR	92.3	1796	3441	4407	7848	65.07	26.04	57.12	23.38	36.20
	Mean	83.8	1677	2931	4053	6984	58.00	23.6	45.1	18.0	28.3
Mean		83.1	1650	2785	3912	6697	59.90	59.90	37.2	21.2	27.3
$LSD_{0.05}$	Mineral	0.30	6.0	10.2	14.3	24.5	0.21	0.07	0.10	0.08	0.08
	Bio	0.21	4.3	7.2	10.1	17.3	0.15	0.05	0.07	0.05	0.06

***Bio Dependency**

Bio-Dependency of pods, hay and biological yields, in general, increased significantly by 29.70, 36.89 and 45.87; 15.08, 21.02 and 26.93; and 20.76, 27.18 and 34.28%, respectively with inoculation with VAM, BR and VAM+BR as illustrate in Table 4. Shibata and Yano (2003) found that, inoculation with VAM greatly increased P uptake in pigeonpeas and peanuts more than in soybeans, indicating greater mycorrhizal dependency in both these plants. Also, Elhindi *et al*. (2017) found that the sweet basil plants appeared to have high dependency on AMF which improved plant growth, photosynthetic efficiency, gas exchange and water use efficiency under salinity stress.

Results in Table (4) observed that kernel and hay nitrogen, phosphorus and potassium concentrations were much higher in the inoculated plants than uninoculated. The VAM+BR and BR treatments recorded significantly highest kernel nitrogen content (3.54 and 3.45%) which increases by 34.09% and 30.68% compared with uninoculated treatment. In the same time, VAM+BR and BR treatments recorded highly hay nitrogen content (3.05 and 2.95%) with increase of 43.86 and 39.15% compared to uninoculated treatment. Bagayoko *et al*. (2000) reported that P application could led to large increase in early root growth severely P deficient soils, following prerequisite for early mycorrhizal colonization and a subsequent significant contribution of AM to increase plant growth and nitrogen uptake. On the other hand, Govindarajulu *et al*. (2005) found that the VAM fungi are able to provide enough N for optimal plant growth and development knowing that inorganic N taken up by the fungi can be incorporated into amino acids that are further transferred to the plant.

Also, it is clear in Table 4 that the highest nitrogen content in hay (3.34%) was observed in with-Mo treatment under bradyrhizobium inoculation. That's indicate the role of molybdenum to enhance nitrogen fixation process. Data also reveal higher N, P and K concentration in the single inoculation of VAM and/or BR under the mineral fertilizers when compared with uninoculated treatments. It is of interest to mention that two inoculants enhance other to fix nitrogen and solubilize P, this was proved from the highly significant data of N and P content in kernel and hay. The increased efficiency of mycorrhizal plants versus non-mycorrhizal is caused by the active uptake and transport of nutrients by mycorrhizae. Antunes *et al*. (2006) and Javaid (2010) stated that, in Mediterranean soils with high phosphate fixing capacity, N fixation by rhizobia is weak due to limited supply of phosphorus and other minor nutrients. The AM fungi are commonly associated with legumes in these soils and, therefore, can increase plant nutrient uptake. The nitrogen percentage in peanut plants was lower in bradyrhizobium only inoculated plants than that in co-inoculated plants. Nodules on co-inoculated peanut roots fixed more nitrogen compared with the nodules on bradyrhizobium only inoculated roots (Zhang *et al*., 2016). Protein content in peanut kernel and hay was significantly higher over control when the plants were inoculated with VAM and Bradyrhizobium or combination of them (Table 4). The maximum protein percentage (21.77 and 27.83%) for kernel and hay was obtained from the VAM+BR treatment followed by BR and VAM where protein percentage (20.67 and 19.30) for kernel and (18.35 and 17.00) for hay, respectively. The maximum protein percentage was noticed in N3 (120% of recommended nitrogen dose) followed by N2 (100% of recommended

Table (4): Nitrogen, Phosphorus and Potassium content (%) of dry leaves of peanut plants, inoculated with VA mycorrhizal Fungi and /or *Bradyrhizobium*, grown in soil amended with either nitrogen, phosphorus or molybdenum fertilizers

Fertilizers		Kernel			Hay			kernel protein (%)	hay protein (%)
Mineral	Bio	N	P	K	N	P	K		
N1	Un-Inoc.	2.44	0.30	1.81	2.10	0.34	1.62	15.25	13.13
	VAM	3.06	0.36	1.95	2.61	0.42	1.75	19.13	16.31
	BR	3.54	0.32	1.90	3.04	0.38	1.70	22.13	19.00
	VAM+BR	3.70	0.37	2.21	3.17	0.49	1.90	23.13	19.81
	Mean	3.19	0.34	1.97	2.73	0.41	1.74	19.91	17.06
N2	Un-Inoc.	2.86	0.28	1.84	2.43	0.30	1.64	17.88	15.19
	VAM	3.24	0.37	1.97	2.77	0.41	1.81	20.25	17.31
	BR	3.60	0.33	1.92	3.08	0.37	1.77	22.50	19.25
	VAM+BR	3.78	0.38	2.15	3.25	0.50	1.92	23.63	20.31
	Mean	3.37	0.34	1.97	2.88	0.40	1.79	21.06	18.02
N3	Un-Inoc.	3.04	0.32	1.86	2.64	0.30	1.62	19.00	16.50
	VAM	3.26	0.38	2.00	2.79	0.43	1.79	20.38	17.44
	BR	3.64	0.34	1.94	3.12	0.41	1.76	22.75	19.50
	VAM+BR	3.66	0.39	2.19	3.14	0.50	1.92	22.88	19.63
	Mean	3.40	0.36	2.00	2.92	0.41	1.77	21.25	18.27
Mean		3.32	0.35	1.98	2.85	0.40	1.77	20.74	17.78
P1	Un-Inoc.	2.84	0.22	1.76	1.89	0.18	1.62	17.75	11.81
	VAM	3.11	0.38	1.89	2.33	0.53	1.78	19.44	14.56
	BR	3.50	0.31	1.85	2.71	0.40	1.68	21.88	16.94
	VAM+BR	3.64	0.41	2.17	2.83	0.55	1.88	22.75	17.69
	Mean	3.27	0.33	1.92	2.44	0.42	1.74	20.45	15.25
P2	Un-Inoc.	2.74	0.24	1.79	1.93	0.22	1.67	17.13	12.06
	VAM	3.05	0.39	1.92	2.36	0.54	1.84	19.06	14.75
	BR	3.44	0.33	1.94	2.71	0.41	1.79	21.50	16.94
	VAM+BR	3.51	0.42	2.11	2.82	0.56	1.92	21.94	17.63
	Mean	3.19	0.35	1.94	2.46	0.43	1.81	19.91	15.34
P3	Un-Inoc.	2.70	0.36	1.82	1.95	0.26	1.64	16.88	12.19
	VAM	2.96	0.41	2.01	2.38	0.54	1.85	18.50	14.88
	BR	3.32	0.37	1.95	2.77	0.41	1.83	20.75	17.31
	VAM+BR	3.40	0.43	2.19	2.86	0.58	1.91	21.25	17.88
	Mean	3.10	0.39	1.99	2.49	0.45	1.81	19.34	15.56
Mean		3.18	0.36	1.95	2.46	0.43	1.78	19.90	15.39
without Mo	Un-Inoc.	2.21	0.29	1.59	2.04	0.33	1.58	13.81	12.75
	VAM	2.78	0.35	1.71	2.52	0.40	1.66	17.38	15.75
	BR	3.11	0.33	1.68	2.94	0.37	1.65	19.44	18.38
	VAM+BR	3.20	0.36	1.94	3.05	0.47	1.68	20.00	19.06
	Mean	2.83	0.33	1.73	2.64	0.39	1.64	17.66	16.48
with Mo	Un-Inoc.	2.32	0.28	1.66	2.00	0.31	1.60	14.50	12.50
	VAM	2.87	0.33	1.73	2.55	0.49	1.67	17.94	15.94
	BR	3.46	0.33	1.72	3.21	0.35	1.66	21.63	20.06
	VAM+BR	3.47	0.34	1.93	3.34	0.52	1.69	21.69	20.88
	Mean	3.03	0.32	1.76	2.78	0.42	1.66	18.94	17.34
Mean		3.01	0.34	1.81	2.62	0.41	1.69	18.83	16.40
$LSD_{0.05}$	Mineral	0.012	0.001	0.007	0.01	0.001	0.006	0.076	0.066
	Bio	0.009	0.001	0.005	0.007	0.001	0.004	0.054	0.047

dose) fertilizer mineral treatment. Fries *et al*. (1998) documented similar findings. Moreover, Elsheikh and Mohamedzein (1998) reported that Bradyrhizobium with nitrogen fertilizers significantly increased protein content in groundnut seed. The relationship between NPK uptake and protein content (%) was linearly and positively correlated to colonization. Minerdi *et al*. (2001) and Badawi *et al*. (2011) stated that bacterial-AM fungal-legume tripartite symbiosis showed better nitrogen fixation (nodule number, nitrogen and protein contents as well as nitrogenase activities) in peanut plants than that of bacterial-legume symbiosis.

Nitrogen and phosphorus use efficiencies:

Nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUTE) and grain nitrogen accumulation efficiency (GNAE) has the highest values under inoculation with VAM+BR followed by BR then VAM treatments when compared to uninoculated treatments (Table 5). Totally NUE increased by 25.80, 23.22 and 21.67% for VAM+BR, BR and VAM, respectively when compared with uninoculated treatment. While totally NUTE increased by 42.31, 24.13 and 4.87% for VAM+BR, BR and VAM, respectively when compared with uninoculated treatment. Whereas, totally GNAE increased by 72.5, 62.5 and 42.5% compared to uninoculated treatment. For different treatments, NUE and GNAE was significantly high in N1 and N2 mineral fertilizer treatments. Increasing plant N uptake efficiency can reduce the consumption of N fertilizers, and is of great significance for development of sustainable agriculture. Although the contribution of VAM fungi to plant N nutrition varies widely in diverse symbiotic systems, VAM fungi can transfer substantial amounts of N to their hosts (Chen *et al*., 2018).

CONCLUSION

The data of this study concluded that the dual application of Bradyrhizobium and AMF was more effective than single inoculation. Inoculation with VAM and/or BR can, synergistically, remove the deficient effect of N and P in the soil of low nutrient content. This could be important to enhance the production of peanut plants cultivated in sandy soils and therefore improve the fitness of plants. Development of such a sustainable biofertilizers technology for maximum and environmentally friendly crop production and preserve soil sustainability is highly desirable.

Table (5): Nitrogen and Phosphorus Use Efficiencies of peanut, inoculated with VA mycorrhizal Fungi and /or *Bradyrhizobium*, grown in soil amended with either nitrogen, phosphorus or molybdenum fertilizers

Fertilizers		Total N-Applied	NUE	NUTE	GNAE	Total P-Applied	PUE	PUTE	GPAE
Mineral	Bio	Kg ha^{-1}	kg kg^{-1}			Kg ha^{-1}	kg kg^{-1}		
N1	Un-Inoc.	53.55	26.35	13.94	0.64	71.4	19.76	93.81	0.06
	VAM	53.55	31.63	11.20	0.97	71.4	23.73	76.68	0.09
	BR	53.55	31.52	9.31	1.12	71.4	23.64	81.95	0.08
	VAM+BR	53.55	32.06	8.75	1.19	71.4	24.05	63.91	0.09
	Mean	53.55	30.39	10.80	0.98	71.40	22.79	79.09	0.08
N2	Un-Inoc.	80.44	17.73	11.53	0.51	71.4	19.97	100.24	0.06
	VAM	80.44	22.07	10.71	0.71	71.4	24.86	78.60	0.09
	BR	80.44	22.33	9.32	0.80	71.4	25.15	84.32	0.08
	VAM+BR	80.44	22.50	8.68	0.85	71.4	25.35	63.68	0.10
	Mean	80.44	21.16	10.06	0.72	71.40	23.83	81.71	0.08
N3	Un-Inoc.	107.1	13.73	10.58	0.42	71.4	20.60	95.38	0.07
	VAM	107.1	16.54	10.46	0.54	71.4	24.80	73.98	0.09
	BR	107.1	17.04	9.20	0.62	71.4	25.56	77.47	0.09
	VAM+BR	107.1	17.46	9.04	0.64	71.4	26.19	63.76	0.10
	Mean	107.10	16.19	9.82	0.55	71.40	24.29	77.65	0.09
Mean		80.36	22.58	10.23	0.75	71.40	23.64	79.48	0.08
P1	Un-Inoc.	107.1	13.16	13.97	0.37	35.7	39.47	158.45	0.09
	VAM	107.1	16.45	12.44	0.51	35.7	49.36	66.62	0.19
	BR	107.1	16.35	10.50	0.57	35.7	49.05	83.40	0.15
	VAM+BR	107.1	16.59	9.84	0.60	35.7	49.78	59.63	0.20
	Mean	107.10	15.64	11.69	0.52	35.70	46.91	92.02	0.16
P2	Un-Inoc.	107.1	13.38	13.65	0.37	53.55	26.76	131.10	0.06
	VAM	107.1	16.66	12.26	0.51	53.55	33.31	64.19	0.13
	BR	107.1	16.44	10.33	0.57	53.55	32.89	78.52	0.11
	VAM+BR	107.1	17.33	9.97	0.61	53.55	34.66	58.33	0.15
	Mean	107.10	15.95	11.56	0.51	53.55	31.90	83.04	0.11
P3	Un-Inoc.	107.1	13.35	13.30	0.36	71.4	20.03	99.76	0.07
	VAM	107.1	16.94	12.17	0.50	71.4	25.41	62.39	0.10
	BR	107.1	16.44	10.14	0.55	71.4	24.66	74.76	0.09
	VAM+BR	107.1	17.49	9.81	0.59	71.4	26.23	55.35	0.11
	Mean	107.10	16.06	11.36	0.50	71.40	24.08	73.07	0.10
Mean		107.10	15.88	11.53	0.51	53.55	34.30	82.71	0.12
without Mo	Un-Inoc.	107.1	13.07	14.62	0.29	71.4	19.61	96.22	0.06
	VAM	107.1	14.79	11.31	0.41	71.4	22.18	76.24	0.08
	BR	107.1	15.01	9.60	0.47	71.4	22.52	79.99	0.07
	VAM+BR	107.1	15.80	9.31	0.51	71.4	23.70	65.67	0.09
	Mean	107.10	14.67	11.21	0.42	71.40	22.00	79.53	0.07
with Mo	Un-Inoc.	107.1	13.31	13.64	0.31	71.4	19.96	94.60	0.06
	VAM	107.1	15.85	11.26	0.46	71.4	23.78	67.35	0.08
	BR	107.1	16.70	9.05	0.58	71.4	25.06	86.36	0.08
	VAM+BR	107.1	16.77	8.57	0.58	71.4	25.15	61.88	0.09
	Mean	107.10	15.66	10.63	0.48	71.40	23.49	77.55	0.08
Mean		107.10	15.40	11.12	0.47	65.45	26.60	79.93	0.09
$LSD_{0.05}$	Mineral	----	0.095	0.037	0.003	----	0.095	0.299	0.003
	Bio	----	0.067	0.026	0.002	----	0.067	0.211	0.002

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التأثير التفاعلى للبرادى رايزوبيم وفطريات الميكوريزا مع مستويات من النيتروجين والفسفور والموليبدنيم المعدنى على الفول السودانى النامى في الاراضى الرملية

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الملخص العربى:

أجريت تجربة حقلية في الأراضي المستصلحة حديثاً بمنطقة البستان لدراسة تأثير الأسمدة الحيوية والكيميائية على إنتاجية ومكونات محصول الفول السوداني (*Arachis hypogaea* L.) تشمل المعاملات التلقيح الفردي أو المزدوج لبكتيريا البرادي رايزوبيوم (BR) والفطريات الميكوريزا الداخلية (VAM) كأسمدة بيولوجية. بالإضافة إلى ثمانية معاملات للأسمدة الكيميائية وتشمل النيتروجين (٧٥ ، ١٠٠ و ١٢٠٪ من الجرعة الموصى بها) ، الفوسفور (٥٠ ، ٧٥ و ١٠٠٪ من الجرعة الموصى بها) واثنين من معاملات الموليبدينوم (أضافة ٢٠٠ جرام هكتار$^{-1}$ Mo كأمونيوم موليبدات وبدون اضافة) تحت تصميم إحصائي القطاعات العشوائية الكاملة (RCBD). تشير النتائج إلى أن التلقيح باستخدام VAM + BR يزيد معنوياً وزن المائة حبة ، ومحصول كل من الحبوب والقرون والتبن والمحصول البيولوجى بنسبة ٣٤,٠٠ ، ٢٦,١٢ ، ٤٥,٨١ ، ٢٦,٨٠ و ٣٤,٣٢٪ ، على التوالي مقارنة بالمعاملات غير الملقحة متبوعة بالمعاملات المفردة لـ BR ثم VAM. كشفت البيانات أيضا عن أن التكافل بين البكتيريا – الميكوريزا – البقوليات يزيد معنوياً من نسبة الإصابة بالميكوريزا (VAM infection) وعدد جراثيم الميكوريزا بالتربة وعدد العقد الجذرية وعدد بكتريا BR بالتربة وكذلك نشاط أنزيمى النيتروجينيز والفوسفاتاز القاعدى مقارنة مع الغير ملقحة. كما ازدادت معنوياً تركيزات النتروجين والفسفور والبوتاسيوم بالنبات وكذلك محتوى البروتين ونسبة الحبوب بالقرون (Shelling %) مع إضافة الأسمدة البيولوجية معاً ويليها التلقيح الفردى من كل من VAM و BR. إضافة الموليبدنيم عمل على زيادة العقد الجذرية بالنبات وكذلك عدد بكتيريا BR بنسبة ٤,٣٥ و ١٠,٩٧ ٪ بالمقارنة بمعاملة عدم الإضافة للموليبدنيم. عموما التلقيح بالميكوريزا الداخلية VAM و بكتريا BR بالتعاون بينهما يؤدى الى إزالة تأثير النقص في عنصرى النيتروجين والفسفور في التربة ذات المحتوى المنخفض منهما وفي نفس الوقت يؤدى لزيادة كفاءات استخدام النيتروجين والفوسفور NUEs و PUEs. الاعتماد الحيوي (Bio-Dependecy) لمحصول القرون والتبن والمحصول البيولوجى زاد معنوياً بنسب ٢٩,٧٠ ، ٣٦,٨٩ ، ٤٥,٨٧ و ١٥,٠٨ ، ٢١,٠٢ و ٢٦,٩٣ و ٢٠,٧٦ ، ٢٧,١٨ ، ٣٤,٢٨٪ على التوالي مع التلقيح الفردى بفطرات الميكوريزا (VAM) وبكتريا (BR) والتلقيح المزدوج من BR + VAM معاً.

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