

Response of Peanut (*Arachis hypogaea* L.) to Co-inoculation With *Bradyrhizobium* spp. and Phosphate Dissolving Bacteria Under Different Levels of Phosphorus Fertilization in Sandy Soils

G. A. A. Mekhemar*, F. M. Ismail**, F. Sh. F. Badawi*, and B. A. A. Kandil*

* Agric. Microbiol. Dept., Soils, Water and Environ. Res. Inst., Agric. Res. Center, Giza, Egypt.

** Oil Crops. Field Crops. Res. Inst. Agric. Res. Center, Giza, Egypt.

Received: 5/3/2007

Abstract: Two field trials were conducted at Ismailia Agricultural Research Station during the two successive summer growing seasons of 2005 and 2006 to study the effect of phosphorus levels (0,8,16 and 32 kg P₂O₅/fed) and inoculation with *Bradyrhizobium* spp. and phosphate dissolving bacteria (*Bacillus megaterium* var. *phosphaticum*) either alone or in combinations on nodulation status (number and dry weight of nodules/plant), growth, nitrogen and phosphorus uptake, yield and its attributes of groundnuts in a sandy soil. Results showed that inoculation with *Bradyrhizobium* and phosphate dissolving bacteria either solely or in combinations improved nodulation status, growth, N and P uptake, yield and its components. However, dual inoculation with (*Bradyrhizobium*+ phosphate dissolving bacteria) were found to surpass the single inoculation for the aforementioned characters, in both growing seasons. Increasing phosphorus levels up to 16 and 32 kg P₂O₅/fed significantly increased each of number and dry weight of nodules, dry matter, N and P uptake, number of pods/plant, pod weight/plant, seed weight/plant, 100-pod weight, 100-seed weight, shelling %, pod yield/fed and crude protein compared with the plants received 8 kg P₂O₅ /fed and unfertilized treatments. Moreover, results showed that most of the aforementioned characters increased significantly when the two phosphorus levels (16 and 32 kg P₂O₅/fed) were added in combinations with mixed inoculation with (*Bradyrhizobium*+ phosphate dissolving bacteria) compared to other treatments. It can be concluded that improvement of peanut yield could be achieved by application of 16 kg P₂O₅/fed as rockphosphate in addition to inoculation with (*Bradyrhizobium*+ phosphate dissolving bacteria) under sandy soils conditions.

Keywords: Nitrogen fixation, *Bradyrhizobium* spp., peanut, phosphate dissolving bacteria, *Bacillus megaterium* var. *phosphaticum*, phosphorus fertilization.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is considered to be one of the most important edible oil crops in Egypt, which due to its seeds high nutritive value for human and the produced cake as well as the green leafy hay for livestock, in addition to the seed oil importance for industrial purposes. It is one of main crops grown in sandy soils. These soils are usually poor in plant nutrients and devoid of root-nodule bacteria which fix atmospheric nitrogen symbiotically in legumes.

The greatest source of nitrogen for peanut is symbiotic nitrogen fixation. This account for 50-80% of the total nitrogen in the plant (Boddey *et al.*, 1990). The inoculation of peanut seeds with effective and efficient strains of *Bradyrhizobium* spp. prior to sowing, is recommended, especially when the soil is void of the specific *Rhizobium* (Abdel-Wahab *et al.*, 2006 and El-Sawy *et al.*, 2006).

Phosphorus is one of the essential elements, which plays a highly recognized role in the growth and metabolism of leguminous plants.

Phosphorus is a constituent of nucleic acids (DNA and RNA) and is considered as high strong energy compounds (Miller and Donahue, 1995), stimulate cell division and metabolic processes such as photosynthesis and synthesis of protein, carbohydrate and lipids (Marschner,1986) and enhances root growth (Russel, 1973), nodulation and N₂-fixation (Knany *et al.*, 2004). The phosphorus content in the poor sandy soils is low, moreover, the available phosphorus in Egyptian soils is too low to face the plant requirements from this element

(Koreish *et al.*, 1998). Furthermore, Egyptian soil pH is high, which resulted in decreasing phosphorus availability. Under such conditions, phosphorus fertilization has always been important (Wahba *et al.*, 2000).

Biological fertilization becomes an important factor to increase availability of P and micronutrients as well as to improve their uptake. EL-Habbasha *et al.* (2005) reported that phosphate dissolving bacteria has an important role in solubilizing of P and its absorption which in turn, improves seed germination and yield of plant, which could be attributed mainly to N₂-fixation.

The current work aimed to evaluate the effect of inoculation with *Bradyrhizobium* spp. and phosphate dissolving bacteria (PDB) used solely or in combination with four rates of phosphorus fertilizer (0,8,16 and 32 kg P₂O₅/fed as rockphosphate) on nodulation, growth, shoot contents of N and P, yield and yield attributes of groundnut.

MATERIALS AND METHODS

Microorganisms:

Bradyrhizobium spp. (strain USDA 3339) and phosphate dissolving bacteria (*Bacillus megaterium* var. *phosphaticum*) were kindly obtained from Biofertilizers Production Unit, Agric. Microbiology. Dept, Soils Water and Environ. Res. Inst. (SWERI) Agric. Res. Center (ARC), Giza, Egypt.

Preparation of bacterial inocula:

Bradyrhizobium spp. strain USDA 3339 was grown on yeast extract mannitol medium broth (Vincent, 1970)

for 3 days at 28°C on a rotary shaker until early log phase (5×10^9 cfu/ml).

Bacillus megaterium var. *phosphaticum* was grown in kings medium B (Atals, 1995) and incubated at 28°C for three days on rotary shaker (giving 3×10^9 cfu/ml). Neutral-powdered vermiculite supplemented with 10% fresh peat was used as a carrier. The carrier packed in polyethylene bags (200 g carrier per bag) then sealed and sterilized by gamma irradiation (5.0×10^6 rads). Inoculants of each strain were prepared by injected 140 ml of log phase growing culture to 200 g sterilized carrier mixed thoroughly and left for a week for curing.

Field experiments:

Two field experiments were carried out at Ismailia Agric. Res. Farm Station, (ARC) during two successive summer seasons of 2005 and 2006 to study the effect of dual inoculation with *Bradyrhizobium* spp. (strain USDA 3339) and phosphate dissolving bacteria (*Bacillus megaterium* var. *phosphaticum*) under different levels of phosphate fertilization on nodulation, growth, N, P content, yield and yield attributes. The uniform agricultural practices for peanut production were applied at the proper time. The main physical and chemical properties of representative soil samples are shown in Table (1).

Table (1): Physical and chemical properties of the experimental soil.

Characters	Value		
	Season 2005	Season 2005	
Physical properties	Sand %	90.1	89.8
	Silt %	2.4	89.9
	Clay %	7.5	7.5
	Texture grade	Sandy	Sandy
	S.P (%)	22.20	22.0
Chemical properties	pH	7.36	7.35
	EC (dSm ⁻¹ at 25°C)	0.30	0.32
	Organic matter (%)	0.21	0.20
	Total soluble N (ppm)	17.62	16.93
	Available P ppm)	8.00	7.79
Soluble cations (meq L ⁻¹)	Ca ⁺⁺	0.49	0.50
	Mg ⁺⁺	0.29	0.34
	Na ⁺	1.69	1.76
	K ⁺	0.52	0.57
Soluble anions (meq L ⁻¹)	CO ₃ ⁼	0.00	0.00
	HCO ₃ ⁻	0.80	0.84
	CL ⁻	0.57	0.61
	SO ₄ ⁻	1.62	1.72
DTPA-extractable (ppm)	Fe	1.10	1.20
	Mn	0.34	0.36
	Zn	0.4	0.39
	Cu	0.22	0.23

The following treatments were conducted:

- 1-Uninoculated and received recommended doses of N, P and K(40 kg N, 30 kg P₂O₅ and 24 kg K₂O
- 2-*Bradyrhizobium* (Br)+ zero P₂O₅/fed
- 3- Br. + 8 kg P₂O₅/fed.
- 4- Br.+ 16 kgP₂O₅/fed.
- 5- Br.+ 32 kg P₂O₅/fed.

- 6-Phosphate dissolving bacteria (PDB)+ zero P₂O₅/fed
- 7- PDB+ 8 kg P₂O₅/fed.
- 8- PDB+ 16 kg P₂O₅/fed.
- 9- PDB+ 32 kg P₂O₅/fed.
- 10- Br.+ PDB+ zero P₂O₅/fed.
- 11- Br.+ PDB+ 8 kg P₂O₅/fed.
- 12- Br.+ PDB+ 16 kg P₂O₅/fed.
- 13- Br.+ PDB+ 32 kg P₂O₅/fed.

A completely randomized block design with four replicates was used. Seeds of peanut were sown on 17th and 22nd May in 2005 and 2006 growing seasons, respectively.

Each plot was 3 m in width and 3.5 m length (1/400 fed). Peanut seeds (variety Giza 6) were inoculated with various inoculants at a rate of 400g inoculum/40 kg seeds, just before sowing by wetting them with an Arabic gum solution (16%), then inoculant was added and thoroughly mixed.

Rockphosphate (25% P₂O₅) was broadcasted and incorporated into the soil before planning at the rates of zero, 8, 16 and 32 kg P₂O₅/fed equal zero,32, 64 and 128 kg rockphosphate/fed, respectively.

All inoculated plots received potassium sulphate (48% K₂O) at the rate of 50 kg/fed. Nitrogen fertilizer was also applied at rate of 20 kg N/fed in form of ammonium sulphate (20.5% N) presowing as activator dose for *Bradyrhizobium* spp and *Bacillus megaterium*.

Five guarded plants were taken at random from the second row of each plot after 75 days from sowing to determine the number and dry weight of nodules, dry weight of shoots and total nitrogen and phosphorus contents.

At harvest time, ten guarded plants were randomly taken from the second inner two rows of each experimental unit to determine number of pods/plant, weight of pods/plant, weight of seeds/plant, 100-seed weight. The plants on the middle three row in each plot with 3m² area were harvested and their pods were air dried to calculate pod yield (kg/feddian).

Also,

$$\text{shelling \%} = \frac{\text{Seed Weight/plant}}{\text{Pod Weight/ Plant}} \times 100$$

and crude protein percentage of seeds, were determined.

Methods of analyses:

-Soil characters were determined according to Page *et al.* (1982).

-Total nitrogen and phosphorus in shoots as well as total- N of seeds were assumed according to Page *et al.* (1982).

-Crude protein of seeds was calculated by multiply N% of seeds by a factor of 6.25 (Tripathi *et al.*, 1971).

All data were statistically analyzed according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Nodulation status:

The influence of inoculation with *Bradyrhizobium* spp. and *Bacillus megaterium* under different levels of phosphorus fertilization (0, 8, 16 and 32 kg P₂O₅/fed) at 2005 and 2006 growing seasons are shown in Table (2).

Irrespective of phosphorus rate, data revealed that uninoculated peanut plants (control) formed 12.67 and 11.67 nodules/plant in 2005 and 2006 seasons, respectively. The corresponding nodules dry weight were 82.33 and 80.00 mg/plant. Results suggest that the presence of native rhizobia of peanut in the experimental soil, which was reported by (Abdel-Wahab *et al.*, 2006 and El-Sawy *et al.*, 2006). In general, inoculation with *Bradyrhizobium* spp. and *Bacillus megaterium* either solely or both in combination enhanced the nodulation status of peanut plants in both seasons. The highest nodulation status was obtained by dual inoculation with (*Bradyrhizobium* spp.+ *Bacillus megaterium*) followed by single inoculation with *Bradyrhizobium* followed by *Bacillus megaterium*. These results are in harmony with those obtained by Srinivasan *et al.* (1997) who found that co-inoculation with *Rhizobium* and *Bacillus* isolates resulted in increasing number of nodules, nodules fresh weight, nitrogenase activity and leghaemoglobin content in the root nodules of *Phaseolus vulgaris*. Also, Hassanein *et al.* (2006) found that co-inoculation with *Rhizobium* and *Bacillus subtilis* caused significant increases in number and dry weight of nodules compared with the uninoculated plants.

Also, data in Table (2) demonstrated that phosphorus fertilization increased number and dry weight of nodules in both seasons. The highest number and dry weight of nodules were obtained by adding 16

or 32 kg P₂O₅/fed as rockphosphate, while the corresponding lowest ones were scored when no phosphorus fertilization was added. Similar observation has been reported by Saleh *et al.*, (2000) & Zaki and Radwan (2006) who reported that nodule number was enhanced by increasing phosphorus level, and there was, almost, a two fold increase in nodules number with the addition of phosphorus.

Application of rockphosphate at the rates of 16 or 32 kg P₂O₅/fed to treatments inoculated with *Bradyrhizobium* and *Bacillus megaterium* resulted significant increases in number and dry weight of nodules in both growing seasons. The highest values of nodules number/plant (87.33 and 78.67) and nodules dry weight /plant (876.0 and 879.33mg) were obtained by using dual inoculation (*Bradyrhizobium*+ *Bacillus megaterium*) and 16 kg P₂O₅/fed followed by inoculation with *Bradyrhizobium*+ *Bacillus megaterium* and 32 kg P₂O₅/fed, which recorded (77.67 and 74.00) for nodules number and (697.33 and 835.00 mg/plant) for nodules dry weight in both seasons, respectively.

These results could be explained as increasing phosphorus level up to 16 or 32 kg P₂O₅/fed may enhance the *Bradyrhizobium* bacteria to form more nodules. These results agree with those obtained by El-Habbasha *et al.*(2005) who reported that nodules number and dry weight were enhanced by increasing application rate of phosphorus up to 30 kg P₂O₅/fed.

Table (2): Effect of co-inoculation with *Bradyrhizobium* spp. and phosphate dissolving bacteria on number and dry weight of nodules

Treatments	Season 2005		Season 2006	
	Number of nodules/ plant.	Dry weight of nodules mg/ plant.	Number of nodules/ plant.	Dry weight of nodules mg/ plant.
Uninoculated (Recommended NPK)	12.67	82.33	11.67	80.00
Br.+ zero Kg P ₂ O ₅ / fed.	37.33	264.67	39.00	240.00
Br.+ 8 Kg P ₂ O ₅ / fed.	53.33	323.00	55.00	350.30
Br.+ 16 Kg P ₂ O ₅ / fed.	54.00	350.00	58.00	355.67
Br.+ 32 Kg P ₂ O ₅ / fed.	75.00	354.67	59.00	434.30
PDB+ zero Kg P ₂ O ₅ / fed.	16.00	138.00	18.00	129.33
PDB+ 8 Kg P ₂ O ₅ / fed.	20.33	144.33	24.67	168.00
PDB+ 16 Kg P ₂ O ₅ / fed.	25.67	160.00	26.67	202.67
PDB+ 32 Kg P ₂ O ₅ / fed.	25.33	149.33	25.67	198.00
Br.+ PDB+ zero Kg P ₂ O ₅ / fed.	71.67	361.00	64.33	452.00
Br.+ PDB+ 8 Kg P ₂ O ₅ / fed.	71.33	579.33	66.67	746.00
Br.+ PDB+ 16 Kg P ₂ O ₅ / fed.	87.33	876.00	78.67	879.33
Br.+ PDB+ 32 Kg P ₂ O ₅ / fed.	77.67	697.33	74.00	835.00
L. S. D 0.05	10.88	71.75	14.91	81.18

Br = *Bradyrhizobium* spp., PDB = Phosphate dissolving bacteria.

Peanut growth, nitrogen and phosphorus contents:

Shoots dry weight and its nitrogen and phosphorus contents as affected by inoculation with *Bradyrhizobium* spp. and phosphate dissolving bacteria (PDB) used either solely or in combination with four rates of phosphorus as (rockphosphate) in both growing seasons are given in Table (3). Results revealed that uninoculated plants fertilized with full dose of NPK produced high shoots dry weight (20.23 and

21.07g/plant), nitrogen content (799.96 and 789.81 mg N/plant) and phosphorus content (284.67 and 266.11 mg P/plant) in both growing seasons, respectively. All inoculation treatments improved the observed plant growth parameters mainly, dry weight of shoot, N and P contents. However, dual inoculation with *Bradyrhizobium* spp and *Bacillus megaterium* were found to surpass the single inoculation for shoot dry weight, nitrogen and phosphorus contents. These results

are in an agreement with those obtained by El-Dsouky and Attia (1999) and Abo El-Soud *et al.* (2003) who found significant increases in plant dry weight, N and P-contents, wherever, the tripartite association of rhizobia, phosphate dissolving bacteria and legumes were found.

With respect to the influence of phosphorus fertilization on dry matter, N and p-contents, obtained results showed that increasing application rate of phosphorus fertilization up to 16 kg P₂O₅ caused significant increases of the all studied characters mentioned above compared with the unfertilized plants in both growing seasons. However, application of 32 kg P₂O₅ /fed resulted less values for these characteristics compared with 16 kg P₂O₅ /fed without significant differences between the two rates. Such effects may be due to that phosphorus encourages the growth of root system, nodulation and the fixation and utilization of N as well as the role of P in enhancing photosynthesis, carbohydrates metabolism and protein synthesis and this in turn increased the amount of metabolites synthesized by the peanut plants resulting increasing dry weight of its organs i.e, roots, leaves and stem. These results are confirmed by those reported by Ali and Mowafy (2003) & El-Sayed and Youssef (2003).

Data in Table (3) show also that *Bradyrhizobium* inoculation coupled with 32 kg P₂O₅ recorded increases in shoot dry weight by (22.71 and 14.20%), N-content by (29.23 and 23.00%) and P-content by (22.19 and 13.23%) over those recorded by the plants inoculated with the same strain but had not received any phosphorus fertilization. *Bacillus megaterium* inoculation combined with 16 kg P₂O₅ /fed caused

significant increases over the plants inoculated with the same strains and had not received any phosphorus fertilization. These increases were 40.14 and 29.62% for dry matter, 52.68 and 44.76% for N-content and 61.56 and 45.89% for P-content in both growing seasons, respectively. Also, data in Table (3) showed that dual inoculation with *Bradyrhizobium* and *Bacillus megaterium* combined with phosphorus application at the rate of 16 kg P₂O₅/fed gave increases in shoot dry weight by 33.87 and 20.73%, nitrogen content by 30.55 and 38.97% and P-content by 85.89 and 93.72% over the plants inoculated with *Bradyrhizobium* only and received phosphorus fertilization at the rate of 32 kg P₂O₅/fed. In this respect, Zayed (1998) noted that the ability of bacteria to dissolve the precipitated phosphorus depends on its efficiency in producing inorganic, organic acids and/or CO₂. Therefore, the unavailable forms of phosphorus can be partially dissolved by the action of phosphate dissolving bacteria naturally occurring or inoculated in the soil. These results are in harmony with those obtained by Abo El-Soud *et al.* (2003) and Mekhemar *et al.* (2005) who reported that inoculation with *Rhizobium* either alone or in combined with helper bacteria increased dry weight of shoot, and its N-content and that could be attributed to nitrogen fixation, consequently increasing plant growth. Also Abdel-Wahab *et al.* (2007) found that co-inoculation with *Bradyrhizobium* and *Bacillus megaterium* recorded higher values of nodular tissues, shoot dry weight as well as shoot nitrogen and phosphorus contents of chickpea plants.

Table (3): Effect of co-inoculation with *Bradyrhizobium* spp. and phosphate dissolving bacteria on dry weight, N and P-contents of shoot of peanut plant.

Treatments	Season 2005			Season 2006		
	Dry weight of shoot (g)/ plant	Shoot N-content mg/ plant	Shoot P-content mg/plant	Dry weight of shoot (g)/ plant.	Shoot N-content mg/plant	Shoot P-content mg/ plant
Uninoculated (Recommended NPK)	20.23	799.96	284.67	21.07	789.81	266.11
<i>Br.</i> + zero Kg P ₂ O ₅ / fed.	16.60	485.83	141.10	16.90	479.92	145.84
<i>Br.</i> + 8 Kg P ₂ O ₅ / fed.	18.80	567.32	155.36	18.23	544.50	150.00
<i>Br.</i> + 16 Kg P ₂ O ₅ / fed.	20.17	622.78	172.73	19.07	589.82	160.40
<i>Br.</i> + 32 Kg P ₂ O ₅ / fed.	20.37	627.84	172.41	19.30	590.30	165.13
PDB+ zero Kg P ₂ O ₅ / fed.	12.63	298.16	118.75	12.73	292.95	118.77
PDB+ 8 Kg P ₂ O ₅ / fed.	15.17	372.58	144.09	15.40	370.33	146.35
PDB+ 16 Kg P ₂ O ₅ / fed.	17.70	455.25	191.85	16.50	424.07	173.28
PDB+ 32 Kg P ₂ O ₅ / fed.	16.66	422.49	168.30	14.90	381.01	149.50
<i>Br.</i> + PDB+ zero Kg P ₂ O ₅ / fed.	18.10	603.35	204.51	18.23	618.01	209.43
<i>Br.</i> + PDB+ 8 Kg P ₂ O ₅ / fed.	22.97	795.66	310.49	20.93	711.65	272.84
<i>Br.</i> + PDB+ 16 Kg P ₂ O ₅ / fed.	27.27	819.65	320.49	23.30	820.34	319.89
<i>Br.</i> + PDB+ 32 Kg P ₂ O ₅ / fed.	23.03	801.63	312.16	22.47	789.99	314.44
L. S. D 0.05	1.96	57.66	20.45	2.234	67.93	26.29

Peanut yield and some yield components:

The effect of inoculation with *Bradyrhizobium* and *Bacillus megaterium* under different levels of phosphorus on number of pods/plant, pod weight g/plant, seed weight g/plant and 100-pod weight g/plant are presented in Table (4). Results show that there are significant increases in the abovementioned parameters

by inoculation with *Bradyrhizobium* and *Bacillus megaterium* either individually or in combined application.

In general, the inoculation with *Bradyrhizobium* was more effective in this respect effective than *Bacillus megaterium*. The highest increase in values of all studied characters were observed for inoculated

treatments with mixture of *Bradyrhizobium* and *Bacillus megaterium*. The positive effect of inoculation could be attributed to producing some biologically active substances, i.e., indole acetic acid, gibberilline and cytokinine like substances. These substances greatly

help in increasing the root biomass and thus indirectly enhance absorption of nutrients from surrounding environment (Saubidet *et al.*, 2000 and Tilak *et al.*, 2005).

Table (4): Effect of co-inoculation with *Bradyrhizobium* spp. and phosphate dissolving bacteria on number of pods, pods weight, seed weight and 100 pods weight of peanut plants.

Treatments.	Season 2005				Season 2006			
	No. of pods/plant	Pods weight (g/plant)	Seed weight (g/plant)	100-pods weight (g)	No. of pods/plant	Pods weight (g/plant)	Seed weight (g/plant)	100-pods weight (g)
Uninoculated (Recommended NPK)	57.37	67.40	43.13	155.67	58.13	70.20	45.07	159.33
Br.+ zero Kg P ₂ O ₅ / fed.	51.77	64.73	40.80	156.67	50.97	61.60	37.83	155.00
Br.+ 8 Kg P ₂ O ₅ / fed.	57.43	67.07	44.33	159.00	56.60	67.70	43.00	156.33
Br.+ 16 Kg P ₂ O ₅ / fed.	59.03	70.03	44.23	156.00	56.67	71.43	46.53	161.67
Br.+ 32 Kg P ₂ O ₅ / fed.	59.90	71.40	48.23	164.00	64.53	70.10	48.17	165.67
PDB+ zero Kg P ₂ O ₅ / fed.	47.07	62.10	37.00	145.00	48.51	62.67	36.83	148.67
PDB+ 8 Kg P ₂ O ₅ / fed.	50.03	63.77	38.63	154.00	49.00	64.10	37.60	154.00
PDB+ 16 Kg P ₂ O ₅ / fed.	58.07	70.50	47.93	165.67	64.33	70.53	48.00	164.67
PDB+ 32 Kg P ₂ O ₅ / fed.	55.17	65.90	40.43	154.67	55.10	65.67	40.30	157.33
Br.+ PDB+ zero Kg P ₂ O ₅ / fed.	50.67	66.83	41.03	161.33	57.57	67.03	42.27	159.33
Br.+ PDB+ 8 Kg P ₂ O ₅ / fed.	60.00	71.73	47.23	164.33	60.20	68.47	44.23	160.33
Br.+ PDB+ 16 Kg P ₂ O ₅ / fed.	69.30	74.03	51.23	171.33	70.07	75.33	53.13	169.33
Br.+ PDB+ 32 Kg P ₂ O ₅ / fed.	61.33	72.13	48.40	166.33	60.50	72.33	50.47	164.00
L. S. D 0.05	8.54	4.922	7.128	10.68	9.159	5.810	6.603	7.456

Data in Table (4) also, revealed that number of pods, pod weight, seed weight/plant and 100-pods weight increased significantly with increasing application rate of phosphorus from zero to 32 P₂O₅ kg/fed in both growing seasons. The highest number of pods/plant (69.30 and 70.07), pod weight (74.03 and 75.33 g/plant), seed weight (51.23 and 53.13 g/plant) and 100-pods weight (171.33 and 169.33) were due to dual inoculation with (*Bradyrhizobium* and *Bacillus megaterium*) + 16 kg P₂O₅/fed followed by inoculation with *Bradyrhizobium* + 32 kg P₂O₅ then *Bacillus megaterium* + 16 kg P₂O₅ in the first and second seasons, respectively. These increases may due to the effect of co-inoculation with *Bradyrhizobium* and *Bacillus* spp, which exert their influence via the production of specific activator compounds through different mechanisms that have the ability to enhance the nodulation, N₂- fixation, nutrient availability as well as improving the nutrient and water uptake in sandy soil. These results are in accordance with those reported by Kloepper (2003), Tilak *et al.* (2005) and Abdel- Wahab *et al.* (2006). Data in Table (5) revealed that inoculation of peanut with either *Bradyrhizobium* and/or phosphate dissolving bacteria in combination with phosphorus fertilization gave different responses. The highest pod yield, shelling %, 100-seed weight and crude protein % were due to the treatment inoculated with *Bradyrhizobium* and *Bacillus megaterium*. Also, dual inoculation gave a similar effect of the full dose of NPK. These effects may be attributed to auxin, gibberellin and cytokinine produced by rhizobacteria. These results are in harmony with those obtained by Dileep Kumar *et al.* (2001) and Bai *et al.* (2003).

Data in Table (5) indicated that pod yield/fed, shelling %, 100-seed weight and crude protein increased as phosphorus fertilizer increased from 0 to 32 kg P₂O₅/fed. The positive effect of phosphorus fertilization on peanut yield and its attributes might be due to the favorable effects of phosphorus in stimulating nodulation and the growth of root system, consequently increasing the efficiency of the roots in absorbing various nutrients enhance the vegetative growth of peanut plants as expressed by plant height, number of branches/plant, which in turn increased number of flowers and pods/plant. Moreover, phosphorus increased flowers number/plant and pods setting. These results are in agreement with those obtained by Hafiz and El-Kholy (2000) & El-Sayed and Youssef (2003). Also, P stimulate nodulation and N₂ fixation as well as the important role of P in protein synthesis. These results are in parallel with those reported by Abdel Wahab *et al.* (1999) and El-Habbasha *et al.* (2005).

Concerning the effect of inoculation combined with phosphorus fertilizer, data in Table (5) declared that dual inoculation with *Bradyrhizobium* and *Bacillus megaterium* combined with 16 kg P₂O₅/fed induced the highest pod yield/fed (2198.00 and 2193.30), shelling% (69.07 and 70.57), 100-seed weight g (84.33 and 83.33) and crude protein% (29.07 and 29.04) in both growing seasons, respectively.

These results may be attributed to the nature of root exudates, which act as suitable substrates for the associative bacteria, that release plant promoting substances mainly indole acetic acid, gibberellins and cytokinins. The plant growth promoters could stimulate plant growth, absorption of nutrients and their efficiency as well as the metabolism of photosynthates. These

results stand in accordance with those obtained by Kloepper (2003) and Tilak *et al.* (2005).

Generally, on the basis of the obtained results it is concluded that yield and its attributes of peanut plants inoculated with dual inocula (*Bradyrhizobium* and phosphate dissolving bacteria) and fertilized with 32 kg P₂O₅/fed rockphosphate almost corresponded with *Bradyrhizobium*+ PDB and fertilized with 16 kg

P₂O₅/fed of rockphosphate. Therefore, the use of phosphorus at a rate of 16 kg P₂O₅/fed combined with dual inoculation with *Bradyrhizobium* plus PDB may be recommended as an alternative for phosphorus fertilization at a rate of 32 kg P₂O₅ as well as the recommended NPK doses to reduce the production costs of peanut grown in sandy soils and to avoid soil pollution.

Table (5): Effect of co-inoculation with *Bradyrhizobium* spp. and phosphate dissolving bacteria on 100- seed weight, shelling %, pod yield/fed and crude protein% of peanut plants.

Treatments.	Season 2005				Season 2006			
	100-Seed weight (g)	Shelling (%)	Pod yield/ fed (Kg)	Crude protein of seed (%)	100-Seed weight (g)	Shelling (%)	Pod yield/ fed (Kg)	Crude protein of seed (%)
Uninoculated (Recommended NPK)	82.33	64.03	1784.2	28.78	79.33	64.20	1725.1	29.12
Br.+ zero Kg P ₂ O ₅ / fed.	76.33	62.87	1498.0	24.44	72.33	61.77	1575.8	24.97
Br.+ 8 Kg P ₂ O ₅ / fed.	78.33	63.27	1757.8	25.35	75.67	63.53	1680.8	25.00
Br.+ 16 Kg P ₂ O ₅ / fed.	79.33	65.80	1754.7	25.44	77.67	65.03	1773.3	25.07
Br.+ 32 Kg P ₂ O ₅ / fed.	80.67	67.57	1774.9	25.41	80.67	68.63	1841.8	25.17
PDB+ zero Kg P ₂ O ₅ / fed.	73.67	49.40	1403.1	20.95	70.30	58.73	1260.0	20.30
PDB+ 8 Kg P ₂ O ₅ / fed.	73.67	60.47	1569.6	21.09	74.00	56.60	1487.1	21.04
PDB+ 16 Kg P ₂ O ₅ / fed.	77.33	68.00	1594.5	22.07	76.67	68.05	1669.1	22.21
PDB+ 32 Kg P ₂ O ₅ / fed.	77.00	61.40	1596.0	21.65	75.00	61.43	1498.0	21.69
Br.+ PDB+ zero Kg P ₂ O ₅ / fed.	76.67	61.40	1756.2	26.00	76.33	62.90	1731.3	25.93
Br.+ PDB+ 8 Kg P ₂ O ₅ / fed.	76.67	65.83	1793.6	28.97	77.33	65.70	1795.1	28.89
Br.+ PDB+ 16 Kg P ₂ O ₅ / fed.	84.33	69.07	2198.0	29.07	83.33	70.57	2193.3	29.04
Br.+ PDB+ 32 Kg P ₂ O ₅ / fed.	77.67	67.07	2048.7	29.03	79.00	69.70	2026.9	28.90
L. S. D 0.05	4.086	4.551	401.2	0.354	4.863	4.835	422.3	0.353

Br. = *Bradyrhizobium* sp

PDB = phosphate dissolving bacteria RP= Rockphosphate.

REFERENCES

- Abdel-Wahab, A. F., F. Sh. F. Badawi, G. A. A. Mekhemar and W. M. El-Farghal (2007). Effect of enriched compost tea and rhizobacteria on nodulation, growth and yield of chickpea in sandy soil. *Minufiya J. Agric. Res.* 32: 297-321.
- Abdel-Wahab, A. F., G. A. A. Mekhemar, H. Sh. Shehata and A. A. Hanafi (2006). Effect of plant growth bioprotecting and promoting rhizobacteria an compost on the healthy and productivity of peanut crop in sandy soil. *Minufiya. J. Agric. Res.* 31: 1323-1348.
- Abdel-Wahab, A. M., G. M. Yakout, E. A. Ali, and M. H. Greish (1999). Response of soybean crop to phosphate fertilization, organic manuring and biofertilization under new reclaimed sandy soil conditions. *Egypt. J. Appl. Sci.*, 14: 125-138.
- Abo El-Soud, A. A., A. A. Ragab, G. A. A. Mekhemar and F. T. Mekhaeel (2003). Response of faba bean to inoculation with N₂ fixers and phosphate dissolving bacteria as influenced by different sources of phosphorus *Egypt. J. Appl. Sci.*, 18: 73-9.
- Ali, A. A. G. and S. A. E. Mowafy (2003). Effect of different levels of potassium and phosphorus fertilizers with the foliar application of zinc and boron on peanut in sandy soils. *Zagazig. J. Agric. Res.*, 30: 335-358.
- Atlas, R. M (1995). *Handbook of Media for Environmental Microbiology*. CRC. Press, Boca Raton, FL.
- Bai, Y, X. Zhou and D. L. Smith (2003). Enhanced soybean plant growth resulting from co-inoculation of *Bacillus* strains with *Bradyrhizobium japonicum* *Crop Ecology, Management and quality. Crop. Sci.* 43: 1774-1781.
- Boddey, R. M, S. Urguiage, M. C. P. Neves, A. R. Sohet and J. R. Peres (1990). Quantifications of the contribution of N₂-fixation to field grown grain legume. A strategy for the practical of N¹⁵ isotope dilution technique. *Soil. Biol. Biochem.*, 22: 649-655.
- Dileep Kumar, B. S, I. Berggren and A. M. Martensson (2001). Potential for improving pea production by co-inoculation with fluorescent pseudomonas and Rhizobium. *Plant and Soil.*, 229: 25-34.
- El-Dsouky, M. M. and K. K. Attia (1999). Effect of inoculation with phosphate solubilizing bacteria, organic manuring and phosphate fertilization on peanuts grown on sandy calcareous soil. *Assiut. J. of Agric. Sci.*, 30: 177-187.
- El-Habbasha, S. F, A. A. Kandil, N. S. Abu-Hagaza, A. K. Abd El-Haleem, M. A. Kalafallah and T. Gh. Behairy (2005). Effect of phosphorus levels and some biofertilizers on dry matter, yield and yield

- attributes of groundnut. Bull. Fac. Agric. Cairo Univ., 56: 237-252.
- El-Sawy, W. A., G. A. A., Mekhemar and B. A. A. Kandil (2006). Comparative assessment of growth and yield responses to two peanut genotypes to inoculation with *Bradyrhizobium* conjugated with cyanobacteria or rhizobacteria. Minufiya. J. Agric. Res., 31: 1031-1049.
- El-Sayed, M. A. A. and H. Y. M. Youssef (2003). Effect of phosphorus fertilizer rates on peanut productivity under some irrigation intervals in sandy soil. Egypt. J. Appl. Sci., 18: 184-199.
- Hafiz, S. I. and M. A. El-Kholy (2000). Response of two lupin varieties to foliar nutrition with potassium and magnesium under different levels of phosphatic fertilization in sandy soils. J. Agric. Sci. Mansoura Univ., 25: 33-51.
- Hassanein, A. M., A. M. El-Garhy and G. A. A. Mekhemar (2006). Symbiotic nitrogen fixation process in faba bean and chickpea as affected by biological and chemical control of root-rot. J. Agric. Sci. Mansoura. 31: 963-980.
- Knany, R. E., A. M. Masoud and Y. B. El-Warakly (2004). Comparative study between biofertilization and sulphur on availability of added phosphorus to Faba bean plants under high p^H soil conditions. J. Agric. Sci. Mansoura Univ., 29: 4801-4809.
- Klopper, J. W. (2003). A Review of Mechanisms for plant growth promotion by PGPR. 6th Internat PGPR Workshop, 5-10 October, Calcutta, India.
- Koreish, E. A., H. M. Ramadan, M. E. E. Fayoumy and H. M. Gaber (1998). Response of faba bean and wheat to bio- and mineral fertilization in newly reclaimed soils. J. Adv. Agric. Res., 6: 2001-2010.
- Marschner, H. (1986). Mineral nutrition of higher plants. Academic Press INC, USA, 674 pp.
- Mekhemar, G. A. A., M. Shaaban, A. A. Ragab and A. M. M. Blomy (2005). Response of faba bean to inoculation with *Rhizobium*. Leguminosarum bv. Viceae and plant growth promoting *Rhizobacteria* under newly reclaimed soils. Egypt. J. Sci., 20: 126-144.
- Miller, R. W. and R. L. Donahue (1995). Soils in our environment. Prentic Hall, Engle Wood Cliffs. 649 pp.
- Page, A. L., R. H. Miller and D. R. Keeney (1982). Methods of soil analysis part 2: chemical and microbiological properties. Second Edition, Am. Sac. Agron. Inc, Soil. Sci. Am., PP: 595-624.
- Russel, E. W. (1973). Soil conditions and plant growth. Langauge Book Society and Longman. London. PP. 30-37.
- Saleh, S. A., M. A. El-Deeb and A. A. Ragab (2000). Response of faba bean (*Vicia Faba. L*) to *Rhizobium* inoculation as affected by nitrogen and phosphorus fertilization. Bull. Fac. Agric., Cairo. Univ., 51: 17-30.
- Saubidet, Maria. I, Nora Fatta and Atilio. J. Barneix (2000). The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. Plant and Soil. 245: 215-222.
- Snedecor, G. W. and W. G. Cochran (1980). Statistical Methods 7th ed., Iowa State Univ. Press, USA, PP: 255-269.
- Srinivasan, M, D. J. Peterson and F. B. Holl (1997). Nodulation of *phaseolus vulgaris* by *Rhizobium etli* is enhanced by the presence of *Bacillus*. Can. J. Microbiol., 43:1-8.
- Tilak, K. V. B. R, N. Ranganayaki, K. K. Pal, R. De, A. K. Saxena, C. Shekhar Nautiyal, Shilpi Mittal, A. K. Tripathi and B. M. Tohri (2005). Diversity of plant growth and soil helth supporting bacteria. Current, Sci., 89: 136-150.
- Tripathi, R. D, G. P. Srivastava, M. S. Misra and S. C. Pandey (1971). Protein to some varieties legume. The Allah Abad Farmer. 16:291-294.
- Vincent, J. M. (1970). A Manual for the practical study of the Root Nodule Bacteria. IBP Hand book, No. 15, Blackwell Publishers, Oxford.
- Wahba, M. M., S. M. El-Ashry and A. M. Zaghloul (2000). Kinetics of phosphate absorption as affected by vertisols properties. Egypt. J. Soil. Sci., 42: 571-588.
- Zaki, R. M. and T. E. E. Radwan (2006). Impact of Microorganisms activity on phosphorus availabiltiy and its uptake by faba bean plants grown on some newly reclaimed soils in Egypt. Int. J. Agric. Biol., 8: 221-225.
- Zayed, G. (1998). Can the encapsulation system protect the useful bacteria against their bacteriophages. Plant and Soil. 197: 1-7.

استجابة الفول السوداني للتلقيح المشترك بكل من البرادى ريزوبيوم والبكتريا المذيبة للفوسفات تحت مستويات مختلفة من التسميد الفوسفاتى فى الاراضى الرملية

جمال عبدالفتاح أحمد مخيمر*، فوزى محمد اسماعيل**، فريد شوقى فريد بدوى*، بلال عبد السميع أحمد قنديل*
 * قسم بحوث الميكروبيولوجيا الزراعية معهد بحوث الاراضى والمياه والبيئة- مركز البحوث الزراعية- الجيزة- مصر.
 ** قسم بحوث المحاصيل الزيتية- معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية- الجيزة- مصر.

أجريت تجربتان حقليتان بمحطة البحوث الزراعية بالإسماعيلية خلال موسمى النمو ٢٠٠٥ و٢٠٠٦ لدراسة تأثير التسميد بمعدلات مختلفة من الصخر الفوسفاتى (صفر، ٣٢، ١٦، ٨ كجم فو.أه/فدان) والتلقيح بالبرادى ريزوبيوم والبكتريا المذيبة للفوسفات (باسيلس ميجاتيريم) إما بصورة منفردة أو مشتركة على حالة التعقيد (عدد ووزن العقد الجاف/نبات)، النمو والمحتوى من النيتروجين والفوسفور ومحصول الفول السودانى وبعض مكونات المحصول فى الاراضى الرملية.

أظهرت النتائج أن التلقيح بالبرادى ريزوبيوم والبكتريا المذيبة للفوسفات اما بصورة منفردة أو مشتركة أدت إلى تحسين حالة التعقيد والنمو والمحتوى من النيتروجين والفوسفور والمحصول وبعض مكوناته. بينما أعطى التلقيح المشترك بالبرادى ريزوبيوم والبكتريا المذيبة للفوسفات قيم تفوق القيد المتحصل عليها من التلقيح المنفرد بكل منها لجميع القياسات تحت الدراسة خلال موسمى الزراعة.

أدى زيادة التسميد الفوسفاتى حتى ٣٢، ١٦ كجم فو.أه إلى زيادة معنوية فى كل من عدد ووزن العقد والوزن الجاف للمجموع الخضرى ومحتواه من النيتروجين والفوسفور وعدد القرون/نبات ووزن القرون/نبات ووزن البذور/نبات ووزن ١٠٠ قرن ونسبة التقشير (التصافى%) ومحصول القرون والبروتين الخام مقارنة بالنباتات المسددة بالمعدل المنخفض من الفوسفور (٨ كجم فو.أه/فدان)

أشارت النتائج الخاصة بالتلقيح المشترك+ المعدلات المختلفة من الصخر الفوسفاتى إلى أن معظم القياسات السابقة تحت الدراسة زادت معنويا عند استخدام معدل التسميد الفوسفاتى (٣٢، ١٦ كجم فو.أه/فدان) فى وجود التلقيح المشترك بكل من البرادى ريزوبيوم والبكتريا المذيبة للفوسفات وذلك مقارنة بالمعاملات الأخرى تحت الدراسة

وعلى ذلك فإنه يمكن تحسين محصول الفول السودانى ومكونات المحصول عن طريق استخدام معدل التسميد الفوسفاتى ١٦ كجم فو.أه/فدان فى صورة صخر فوسفاتى مشتركا مع التلقيح الثانى بالبرادى ريزوبيوم والبكتريا المذيبة للفوسفات تحت ظروف الاراضى الرملية ويؤدى ذلك أيضا إلى تقليل تكاليف الانتاج والحد من تلوث التربة.