J. Agric. Sci. Mansoura Univ., 33(11): 8137 - 8153, 2008

INFLUENCE OF PHOSPHATE DISSOLVING AND NITROGEN FIXING BACTERIA ON FABA BEAN UNDER DIFFERENT LEVELS OF PHOSPHORUS FERTILIZATION

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ABSTARCT

A field experiment was conducted in a silty clay soil at Damas village, Mit Ghamr, Dakahlia using faba bean during 2007-2008 winter season to study the response of faba bean (*Vicia faba* L.) cv. Giza 3, to inoculation with *Rhizobium leguminosarum* bv. *Viciae* either solely or dually with *Bacillus megatherium var.* phosphaticum under three levels of phosphorus fertilizer (0, 15 and 30 kg P_2O_5 fed^{-1}) on leaves chlorophyll content, nodulation, seed yield and its components and seed protein. Also on N, P and K content of seed and straw, available nitrogen and phosphorus in soil and numbers of phosphate dissolving bacteria in rhizosphere plant after 30, 60 and 90 days from sowing. Results revealed that the dual inoculation with *Rhizobia* and *Bacillus* gave significant increases in chlorophyll a and a + b, number of nodules/plant, dry weight of nodules/ plant and nitrogenase activity after 75 days from sowing. Yield and its components, seed protein % and seed protein content increased significantly by inoculation or increasing of phosphorus fertilizer rate or their combination where, the increase in seed yield and seed protein content recorded about 35 and 57%, respectively, over control (uninoculated treatment).

Regarding N, P and K content in faba bean seed and straw, data showed significant increases as a result of increasing in P fertilizer or due to bacterial inoculation especially a dual inoculation. The soil available P and N increased significantly by either inoculation or P-fertilizer, in response to the use of 30 kg P_2O_5 fed^{-1}.

Finally, results confirmed the necessity of dual inoculation with *Rhizobia* and *Bacillus* in combination with applying 30 kg P_2O_5 as P-fertilizer for faba bean growth and productivity and for improvement of soil fertility.

INTRODUCTION

The use of symbionts is more economical and much better than the use of chemical fertilizers which, had oil ready raised serious objection and real concern about the pollution of the environment. The symbiotic relation between higher plants and soil microorganisms represents one of the most striking biological phenomena. The use of a symbiotic bacterium, *Rhizobium*, produces enough nitrogen to support the building up of the whole protein requirements of the legumes (Bedrous *et al.*, 1990).

Mineral nutrients deficiencies are major constraints limiting legume nitrogen fixation and yield (O'Hara *et al.*, 1988). Among the necessary nutrients, legumes need relatively large amounts of phosphorus Nodules formation and function are both adversely affected with phosphorus deficiencies (Van Schreven, 1958). However, phosphorus application gave a highly significant increase in faba bean yield (Hussein *et al.*, 1993). Also, it is very important element to plant growth and plays a key role in metabolic processes such as the conversion of sugar into starch and cellulose (Mengel and Kirkby, 1987). It 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 enhance root growth (Russel, 1973), nodulation and N_2-fixation (Knany *et al.,* 2004).

Biological fertilization becomes an important factor in increase availability of P and micronutrients as well as to improve their plant 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 N_2-fixation.

The interaction of diazotrophic bacteria in the rhizosphere of leguminous crops inoculated with the N_2-fixing bacteria has been discussed in earl publications (Burnus *et al.,* 1981) and (Iruthayathas *et al.* 1983). Field trials showed a simultaneous inoculation of non symbiotic N_2-fixing bacteria and naturally rhizobia-colonizing legumes resulted in substantial increases in nitrogenase activity, greater number of nodules and eventually yield increase (Del Gallo and Fabbri, 1991). The naturally occurring soil bacteria that are capable of stimulating plant growth named as Plant-Growth-Promoting Rhizobacteria (PGPR) (Klepper and Schorth, 1981).

Bacillus spp. play a dual role by fixation of atmospheric nitrogen and producing antimicrobial agents against deleterious rhizobacteria (Hassanein and Mekhemar, 2003 and Kloepper, 2003). Another effect includes an increase in mobilization of insoluble nutrients followed by enhancement of uptake by the plants (Lifshutz *et al.*, 1987). *Rhizobia* are widely used in agriculture for inoculating the leguminous crops because of their ability to fix atmospheric nitrogen. Logically, potentiality for improving plant yield by combining these PGPR with rhizobia have been reported by many workers (Dileep-Kumar *et al.,* 2001 and Nassef *et al.*, 2005).

The current work aims to evaluate the effect of inoculation with phosphate dissolving bacteria (PDB) viz. *Bacillus megatherium var.* phosphaticum and *Rhizobium leguminosarum* bv. *Viciae* used each solely or in combination with three rates of phosphorus fertilizer, i.e., 0, 15 and 30 kg P_2O_5 fed^{-1} on nodulation, growth, chlorophyll contents, seed and straw contents of N, P and K, soil available P, yield and yield attributes of faba bean, as well as total count of PDB in its rhizosphere.

MATERIALS AND METHODS

A faba bean (Variety Giza 3) field experiment was carried out during the winter season of 2007-2008 at Damas village, Mit Ghamer, Dakahlia Governorate, Egypt, to study the effect of different phosphorus of 0, 15 and 30 kg P_2O_5/fed^{-1}, bacterial inoculation with *Bacillus megatherium* var. Phosphaticum (phosphate dissolving bacteria) and/or *Rhizobium leguminosarum bv. Viciae* (nitrogen fixing bacteria) and a mixture of both on faba bean yield and its components, number and dry weight of nodules, N, P

& K percentages of faba bean seeds and straw, seed protein yield, plant leaf pigments (Chlorophyll a & b) evaluated after 75 days from sowing, soil available N at harvest (as NH_4^+ and NO^-_3) and the soil available P at 45, 75 days from sowing and at harvest, as well as the soil total count of phosphate dissolving bacteria (*Bacillus megatherium* var. Phosphaticum) done at 30, 60 and 90 days from sowing.

The experiment was designed in a split- split plot design and comprises 12 treatments in three replicates. The main plots were phosphorus levels and the sub plots were for biofertilizer inoculation. The treatments are as in the following:

A) Phosphorus treatments (Main plots)
   1- Without phosphorus
   2- 15 kg P_2O_5/fed^{-1}
   3- 30 kg P_2O_5/fed^{-1}

B) Biofertilizer treatments (Sub plots)
   1- Uninoculated
   2- Inoculation with *Rhizobium leguminosarum* (a).
   3- Inoculation with *Bacillus megatherium* (b)
   4- Inoculation with a mixture of a + b.

Physical and chemical analyses of the experimental soil were conducted as described by Hesse (1971) and are Present in Table (1).

Table (1): Some physical and chemical properties of the experimental soil

Properties	Value
Particle size distribution (%)	
Sand	15.70
Silt	39.30
Clay	45.00
Textural class	Silty clay
$CaCO_3$ (%)	2.80
OM (%)	1.02
H (1: 2.5 soil suspension)	7.50
CEC (Cmol /Kg)	49.91
EC dS/m (soil paste)	2.63
Cations (meq /L):	
Ca^{++}	17.20
Mg^{++}	3.71
Na^+	7.03
K^+	1.55
Anions (meq /L):	
CO_3^{--}	0.00
HCO_3^-	8.43
Cl^-	6.32
SO_4^{--}	14.74
Available macronutrients	
N (ppm)	33.50
P (ppm)	12.50
K (ppm)	379
Available micronutrients	
Zn (ppm)	2.30
Mo (ppm)	0.19

The plot area was 10.5 m^2 (3 x 3.5 m), i.e., 1/400 faddan. Faba bean seeds were cultivated in rows (50 cm in width) with hills containing two seeds each with 20 cm apart at 7th November in the winter season of 2007 -2008.

Samples and determinations:

Plant samples:

Three plants were randomly taken at 75 days from sowing from each plot to determine number of nodules $plant^{-1}$, dry weight of nodules (mg) and nitrogenase activity of nodules (n mole C_2H_4 g^{-1} dry nodule) as described by Hardy *et al.* (1976). Disc samples from the fourth upper leaves 75 days were obtained, both chlorophyll a and b were determined colorimetrically according to the method described by Wettstein (1957).

At harvest plants were cut just above the soil surface to determine both on faba bean yield and its components, N, P & K percentages (Jackson, 1976) of faba bean seeds and straw, seed protein content A.O.A.C. (1980).

Soil samples:

Soil samples were taken from rhizosphere faba bean plants at 30, 60 and 90 days from sowing to determine the count of phosphate dissolving bacteria (PDB) using modified Bunt and Rovira medium (Abdel-Hafez, 1966). Also, other soil samples were collected at 45, 75 and at harvest to determine the soil available phosphorus (ppm) (Olsen et al., 1954). Soil available was determined in as NH_4^+ and NO^-_3 (ppm) using Fechnican Auto Analyzer according to Markus *et al.* (1982).

Bacteria used:

Rhizobium leguminosarum bv. Viciae and *Bacillus megatherium* var. Phosphaticum were kindley supplied by Department of Microbiology, SWERI, ARC, Giza.

Faba bean seeds inoculation:

Faba bean Seeds were divided into four parts. The first part, was cultivated without inoculation and the second part, was inoculated with rhizobial inoculant and the third part was inoculated with *Bacillus megatherium* and the last part was inoculated with a mixture of *Rhizobium leguminosarum* bv. *Vicae* and *Bacillus megatherium*. Faba bean seeds were coated with in each part using Arabic gum an adhesive agent Just prior to sowing.

Fertilizers application:

Nitrogen fertilizer as urea (46% N) was applied at a rate of 20 kg N fed^{-1} into two equal split doses at 35 and 6o days from sowing, while potassium in the form of potassium sulphate (48% K_2O) was added at the rate of 50 kg K_2O fed^{-1} after 35 days from sowing. Agricultural processes were done as ecommended by the Ministry of Agriculture and Land Reclamation, Egypt.

Preparation of bacterial inoculum:

Rhizobium strain was grown on yeast extract manitol broth medium (Vincent, 1970) incubated at 30°C for 3 days until early log phase (5 x 10^9 cfu/ml culture). Vermiculate supplemented with 10% Iresh peat was packed in polyethylene bag (300 g carrier per bag), then sealed and sterilized by gamma irradiation (5 x 10^6 rads). *Rhizobium* culture was injected into the carrier to satisfy 60% of the maximal water holding capacity. *Bacillus* *megatherium* were grown on a broth medium (Difico Mamual, 1984), incubated for 24 hr at 30°C to obtain population of 5 x 10^8 cfu/ml culture and injected in sterilized carrier as mentioned before.

Counting of phosphate dissolving bacteria:

After 30, 60 and 90 days from sowing *Bacillus megatherium* was counted in the rhizosphere of faba bean plants using modified Bunt and Rovira medium (Abdel-Hafez, 1966).

All obtained results were statistically analyzed by the method described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Faba bean leaves chlorophyll:

Response of faba bean leaves chlorophyll to bacterial inoculation under different rates of phosphorus fertilizer is given in Table (2).

Table (2): Chlorophyll a & b concentrations of faba bean plant leaves (µg / g dry leaves) as affected by bacterial inoculation and different rates of phosphorus fertilizer

P_2O_5 (Kg/fed) (A)	Inoculation treatments (B)	Chlorophyll a	Chlorophyll b	Chlorophyll a+b
0	Uninoculated	5.37	2.25	7.62
	Bacillus (S)	5.89	2.48	8.37
	Rhizobium (R)	6.10	2.71	8.81
	(S + R)	6.29	2.88	9.17
Mean		5.92	2.58	8.49
15	Uninoculated	6.03	2.39	8.42
	Bacillus (S)	6.31	2.62	8.93
	Rhizobium (R)	6.44	2.89	9.33
	(S + R)	6.59	3.03	9.62
Mean		6.34	2.73	9.08
30	Uninoculated	6.23	2.61	8.84
	Bacillus (S)	6.51	2.92	9.43
	Rhizobium (R)	6.65	3.05	9.70
	(S + R)	6.77	3.16	9.93
Mean		6.54	2.94	9.48
Means of bacterial inoculation treatments				
Uninoculated		5.88	2.42	8.29
Bacillus (S)		6.24	2.67	8.91
Rhizobium (R)		6.40	2.88	9.28
(S + R)		6.55	3.02	9.57
L.S.D. at 0.05				
(A)		0.037	N.S.	0.045
(B)		0.024	N.S.	0.033
(A x B)		0.041	N.S.	0.057

Data revealed that bacterial seed inoculation before sowing increased significantly chlorophyll a and a + b, while chlorophyll b had not significantly increased as a result of inoculation. Dual inoculation with *Rhizobium and Bacillus* gave the highest values of chlorophyll a and a + b where it scored mean values of 6.55 and 9.57, respectively.

The increase in photosynthetic pigments as a result of bacterial inoculation may be attributed to the increase of fixed nitrogen in plant, which led to increase root nodules number (as shown in Table 3), where nitrogen is a major component of chlorophyll. These findings are in line with those obtained by Massoud *et al.* (2005). However, due to phosphorus fertilizer application, the use of 30 kg P_2O_5 fed^{-1} gave significantly the highest values of chlorophyll a & b and a + b. The corresponding values were 6.54, 2.94 and 9.48, respectively.

Table (3): Nodulation status and nitrogenase activity of faba bean plants after 75 days from sowing as affected by bacterial inoculation and different rates of phosphorus fertilizer

P_2O_5 (Kg/fed) (A)	Inoculation treatments (B)	Nodules number/ plant	Nodules dry weight (mg/plant)	N_2-ase activity (n mole C_2H_4 g^{-1} h^{-1})
0	Uninoculated	18.0	28.9	101.2
	Bacillus (S)	21.0	36.3	121.6
	Rhizobium (R)	37.3	81.3	278.2
	(S + R)	39.7	91.4	292.4
Mean		29.0	59.5	198.0
15	Uninoculated	22.3	40.1	145.1
	Bacillus (S)	28.0	52.7	181.3
	Rhizobium (R)	44.0	97.6	336.7
	(S + R)	47.0	104.1	359.1
Mean		35.3	73.6	255.3
	Uninoculated	24.9	44.1	160.4
30	Bacillus (S)	32.7	61.8	218.1
	Rhizobium (R)	52.7	121.4	421.5
	(S + R)	56.7	129.4	448.6
Mean		41.8	89.2	311.8
Means of bacterial inoculation treatments				
Uninoculated		21.7	37.7	135.3
Bacillus (S)		27.2	50.3	173.3
Rhizobium (R)		44.7	100.1	345.0
(S + R)		47.8	108.3	366.3
L. S. D. at 0.05				
(A)		3.144	1.239	6.454
(B)		3.624	3.269	10.031
(A x B)		N.S.	2.477	12.908

With regard to bacterial inoculation with *Rhizobium* and\or *Bacillus* individually or both in combination, all the inoculated treatments increased significantly all chlorophyll components over those uninoculated ones. Also, the use of 30 kg P_2O_5 fed^{-1} combined with dual inoculation with *Rhizobium and Bacillus* recorded significantly the highest values of chlorophyll a & b and a + b.

These results are in harmony with those obtained by El-Shikha and Gaafar (2006) who found that application of phosphorus at a high rate of 45 kg P_2O_5fed^{-1} was the most effective treatment for increasing chlorophyll a, chlorophyll b and chlorophyll (a + b) in leaves of faba bean. These findings may be explained on the assumption that P occurs in chlorophyll indicated that high pigments activity was produced and hence more photosynthetic activity was appeared (Mohamed, 2005).

Nodulation and N_2-ase activity of faba bean:

Table (3) noted that dual inoculation with *Rhizobium* and *Bacillus megatherium* gave the highest number and by dry weight of nodules where it recorded 47.8 nodules/ plant (as mean value) and 108.3 mg/plant, respectively. Also the dual inoculation caused the highest mean value of N_2-ase activity (366.3 n mole C_2H_4/ g dry nodules / h). All these increases of these parameters were positively significant compared to either uninoculated treatments or to those inoculated with each individually.

Considerable information are available regarding the ecological interactions of helper bacteria that are able to colonize the roots of legumes and non-legumes and subsequently fix nitrogen either in asymbiotic or as associative manner (Rai, 1983). Also, Fayez *et al.* (1988) indicated that there was either synergistic or additive effect of the introduced *Azotobacter* and/or *Azospirillum* with *R. leguminosarum* for faba bean.

These results are/in line with those obtained by Saleh *et al.* (2000) and Massoud *et al.* (2005) who indicated that *Rhizobium* inoculation increased significantly the number and dry weight of nodules. Co-inoculation of *R. leguminosarum* and *Bacillus megatherium* var. phosphaticum caused stimulative effect on number and dry weight of nodules. This may be due to the effect of phosphate solubilizing bacteria which could utilize some organic compounds as carbon and energy sources and produce organic acid which solubilize insoluble inorganic phosphate (Gained and Gaur, 1991). Additionally, phosphate dissolving bacteria may produce growth promoting substances such as auxins, gibberellins and cytokinins (Hauka *et al.*, 1990) which might improve plant growth and stimulate the microbial development (Emskine *et al.* 1993).

The presence of active nodules in nitrogenase enzyme for uninoculated plants is owing to the indigenous *Rhizobia* in soil (Moawad and Abd El-Rahim, 2002).

Regarding the effect of phosphorus fertilization on nodulation and N_2-ase activity, results indicated that the use of 30 kg P_2O_5 fed^{-1} had achieved the highest significant mean values of nodules number/plant, dry weight and N_2-ase activity compared to those received 15 kg P_2O_5 fed^{-1}. The corresponding values were 41.8 nodules/plant, 89.2 mg/plant and 311.8 n mole C_2H_4/g dry nodules /h). These results are in harmony with those obtained by Radwan *et al.* (2007).

Concerning the combination between P_2O_5 fertilization and bacterial inoculation, data showed that the application of 30 kg P_2O_5 fed^{-1} combined with dual inoculation with *Rhizobium* and *Bacillus* recorded the highest significant mean values of nodules/plant (56.7), 129.4 mg nodules/plant and 448.6 n mole C_2H_4/ g dry nodules / h).

These results are in line with those obtained by Saleh *et al.* (2000) who reported that phosphorus fertilization combined with rhizobial inoculation resulted in better nodulation, nitrogenase activity and high increases in yield of soybean and faba bean crops.

Phosphate dissolving bacteria count of faba bean rhizosphere plants:

Table (4) explains the response of inoculation with *Rhizobium* and/or *Bacillus* and different rates of phosphorus fertilizer after 30, 60 and 90 days from sowing on number of phosphate dissolving bacteria (PDB) in faba bean rhizosphere plants. Results indicated that the number of phosphate dissolving bacteria due to all the inoculation treatments had increased as compared to uninoculated ones at all tested growth periods.

Table (4): Numbers of phosphate dissolving bacteria (BDP) in rhizosphere of faba bean plants after 30, 60 and 90 days from sowing as affected by bacterial inoculation and different rates of phosphorus fertilizer

P_2O_5 (Kg/fed) (A)	Inoculation treatments (B)	PDB numbers X10^3		
		30 days	60 days	90 days
0	Uninoculated	18	33	30
	Bacillus (S)	290	410	450
	Rhizobium (R)	35	43	40
	(S + R)	270	520	560
15	Uninoculated	27	60	41
	Bacillus (S)	380	670	560
	Rhizobium (R)	31	69	44
	(S + R)	400	620	650
30	Uninoculated	31	50	48
	Bacillus (S)	420	520	530
	Rhizobium (R)	34	62	52
	(S + R)	500	610	650

Regarding the effect of phosphate fertilization on bacterial counts, generally, the numbers of PDB increased gradually with the increase of P-rate for the three growth periods.

The highest count of PDB (670 x 10^4/g dry rhizosphere soil) is recorded by the plants inoculated with *Bacillus megatherium* combined with 15 kg P_2O_5/fed. These results are agreement with those obtained by Mallarino *et al.* (1991) and Shady *et al.* (1992).

Yield and its components of faba bean:

The response of faba bean (*Vicia faba* L.) cv. Giza, 3 grown in clayey soil to inoculation with *R. Luguminosarum* bv. *Vicia* and/or *Bacillus megatherium* var. *phosphaticum* under different levels of P_2O_5 is present in Table (5). Results show that with increasing P rate combined with bacterial inoculation and control (uninoculated) increased significantly increase No. of tillers/plant, No. of pods/plant, 100-seed yield, seed yield, straw yield, seed crude protein percentage and seed protein yield. The use of 30 kg P_2O_5 fed^{-1} recorded the highest mean values of these parameters.

Regarding the effect of bacterial inoculation, data revealed that inoculation with *Rhizobium* and *Bacillus* gave positively significant increases in faba bean yield and its components (Table 5). The order increases in these parameters according to inoculation was dual inoculation with *Rhizobium* and *Bacillus* followed by that of *Rhizobium* then that of *Bacillus* and control (uninoculated).

Table (5): Faba bean Yield and its components as affected by bacterial inoculation and different rates of phosphorus fertilizer

P_2O_5 (Kg/fed) (A)	Inoculation treatments (B)	No. of tillers/ plant	No. of pods/ plant	100 seed weight (g)	Seed yield (Kg/fed)	Straw yield (Kg/fed)	Crude protein (%)	Seed protein content (Kg/Fed)
0	Uninoculated	2.79	12.7	57.3	1348.5	950	18.75	252.78
	Bacillus (S)	3.51	16.1	63.9	1503.5	1070	20.25	304.40
	Rhizobium(R)	3.63	18.2	64.5	1596.5	1150	20.69	330.35
	(S + R)	3.71	20.1	65.9	1689.5	1210	20.94	353.73
Mean		3.41	16.8	62.9	1534.5	1090	20.16	310.31
15	Uninoculated	3.05	14.9	63.8	1550.0	1100	20.75	321.69
	Bacillus (S)	4.25	18.1	66.8	1767.0	1260	22.69	400.82
	Rhizobium(R)	4.41	20.3	67.7	1937.5	1390	23.63	457.65
	(S + R)	4.51	22.1	68.7	2154.5	1550	24.38	525.14
Mean		4.06	18.9	66.8	1852.3	1330	22.86	426.33
30	Uninoculated	3.91	16.3	66.4	1751.5	1250	21.56	377.64
	Bacillus (S)	4.43	20.3	68.9	2092.5	1510	23.69	495.77
	Rhizobium(R)	4.63	22.0	69.9	2216.5	1590	24.63	454.75
	(S + R)	4.95	23.5	73.9	2449.0	1750	25.12	615.16
Mean		4.48	20.6	69.8	2127.4	1530	23.75	508.58
Means of bacterial inoculation treatments								
Uninoculated		3.25	14.7	62.5	1550.0	1100	20.35	317.37
Bacillus (S)		4.06	18.17	66.5	1787.7	1280	22.21	400.33
Rhizobium (R)		4.22	20.17	67.4	1916.8	1380	22.98	444.58
(S + R)		4.39	21.91	69.5	2.97.7	1503	23.48	498.01
L. S. D. at 0.05								
(A)		0.049	1.366	0.505	39.015	47.174	0.167	7.84
(B)		0.043	0.7426	1.023	73.629	79.179	0.100	11.71
(A x B)		0.087	N.S.	1.011	78.031	94.347	0.333	20.29

The increases as a result of dual bacterial inoculation with *Rhizobium* and *Bacillus* and over control (uninoculated) were about 35%, 49%, 11%, 35%, 15% and 57% over control for No. of tillers/plant, No. of pods/plant, 100 seed weight, seed yield, straw yield, seed crude protein % and seed protein content, respectively.

Concerning phosphorus fertilizer rate when combined with bacterial inoculation, data revealed that dual inoculation with *Rhizobium* and *Bacillus* combined with 30 kg P_2O_5 fed^{-1} recorded the highest significant values of the tested faba bean yield components parameters compared to the other tested treatments. These results are in harmony with those obtained with faba bean by Mekhemer *et al.* (2005) who reported that either single inoculation with *Rhizobium* or combined with helper bacteria increased dry weight of shoots, N-content, nodulation and yield, which could be attributed to nitrogen fixation, consequently increasing plant growth.

The recorded results due to uninoculated treatments were acceptable even they were lower than the other inoculated treatments. This behavior could be due to the positive role of native bacteria mainly *Rhizobia* and other PGPR inhabiting soil among several decades ago this view agreed with Radwan *et al.* (2007).

NPK concentration in faba bean seed and straw:

Data presented in Table (6) show the values of NPK percentages in faba bean seeds and straw as affected by bacterial inoculation and different rates of phosphorus fertilizer. Results revealed that bacterial inoculation with *Rhizobium* and\or *Bacillus* increased significantly NPK percentages in faba bean seeds and straw. This effect could be arranged for N and K in response to the tested treatments in the following order: the treatments inoculated with a mixture of *Rhizobium* and *Bacillus* > inoculated with *Rhizobium* > inoculated with *Bacillus* > control (uninoculated treatment), while for P the order was a mixture of *Rhizobium* and *Bacillus* > *Bacillus* > *Rhizobium* > control. Also, dual inoculation with *Rhizobium* and *Bacillus megatherium* increased P-uptake and dry weight of faba bean compared to the sole P-fertilization (Saber *et al.*, 1983) and resulted in the highest increases in P and N contents in faba bean (Abdallah *et al.* 1984). Chabot *et al.* (1996) explained the important role of helper inoculated bacteria where they showed that *Bacillus megatherium* and *Azotobacter* without rhizobial inoculation increased straw yield of maize as compared to soil inoculated only with *Rhizobia.* The positive effect of *Rhizobium* inoculation on N, P and K may be due to increasing nitrogen fixation, which enhance the vegetative and root growth of plant and consequently increased N, P and K and other nutrients. Similar results were obtained by Massoud *et al.* (2005).

With regard to the effect of P-fertilization with on N, P and K in faba bean seed and straw, data indicated that 30 kg P_2O_5/fed gave the greatest percentages of NPK compared to the control and the law rate of P_2O_5, in seed or straw. Also, it can be seen that there was a gradual response in NPK concentration of faba bean owing to the increase in phosphorus rate. These results may be due to that the application of high rate (30 kg P_2O_5/fed) resulted in much amount available P amounts in soil that led to a better plant root growth, which in turn absorbs more N from the soil resulting in increasing N content of faba bean seeds and straw. Also, these results may be attributed to the role of phosphorus for organization in rapid alteration of nutritional compounds with plants through its effect on the enzyme and other metabolism activity. Similar results were obtained by Hanna *et al.* (1996) who reported that addition of phosphorus increased significantly P and K content in broad bean, which due to the significantly increased in root surface area and this was important in supplying the growing plants with the required nutrients.

Table (6): NPK concentrations in faba bean seeds and straw as affected by bacterial inoculation and different rates of phosphorus fertilizer

P_2O_5 (Kg/fed) (A)	Inoculation treatments (B)	Seeds			Straw		
		N%	P%	K%	N%	P%	K%
	Uninoculated	3.00	0.451	0.605	0.421	0.135	1.14
	Bacillus (S)	3.24	0.500	0.663	0.454	0.151	1.25
	Rhizobium (R)	3.31	0.468	0.725	0.465	0.141	1.37
	(S + R)	3.35	0.511	0.751	0.473	0.153	1.42
Mean		3.23	0.483	0.679	0.453	0.145	1.03
15	Uninoculated	3.32	0.541	0.763	0.475	0.162	1.25
	Bacillus (S)	3.63	0.623	0.855	0.514	0.188	1.38
	Rhizobium (R)	3.78	0.581	0.923	0.531	0.173	1.53
	(S + R)	3.90	0.651	0.981	0.551	0.195	1.59
Mean		3.66	0.599	0.881	0.518	0.180	1.44
30	Uninoculated	3.45	0.604	0.884	0.493	0.181	1.36
	Bacillus (S)	3.79	0.676	0.981	0.533	0.202	1.51
	Rhizobium (R)	3.94	0.648	1.05	0.551	0.194	1.68
	(S + R)	4.02	0.712	1.13	0.569	0.214	1.78
Mean		3.80	0.660	1.01	0.537	0.198	1.58
Means of bacterial inoculation treatments							
Uninoculated		3.26	0.532	0.751	0.463	0.159	1.25
Bacillus (S)		3.55	0.600	0.833	0.500	0.180	1.38
Rhizobium (R)		3.68	0.566	0.899	0.516	0.169	1.53
(S + R)		3.76	0.625	0.954	0.531	0.187	1.60
L. S. D. at 0.05							
(A)		0.035	0.013	0.012	0.007	0.002	0.031
(B)		0.041	0.018	0.011	0.010	0.003	0.038
(A x B)		0.071	0.026	0.024	0.014	0.005	N.S.

The increasing percentages of K in seeds may be explained by greater ATP formed, which could increase nutrients availability to the plants.

Concerning the combination effect between P-fertilization and seed inoculation with *Rhizobium* and\or *Bacillus*, data explained that, the greatest effect of biofertilizer inoculation on N, P and K concentration as percentages in seed or straw were recorded with the inoculated treatment by mixture of *Rhizobium* and *Bacillus* at rate of 30. kg P_2O_5/fed

Whereas, the lowest values of N, P and K percentages were recorded with uninoculated treatment at Zero P_2O_5/fed. The increasing of P percentages due to phosphorus fertilization together with the bacterial inoculation may be attributed to the effect of the availability of soil phosphorus, which is consequently led to the high efficiency of the roots in absorbing various nutrients. Nadia *et al.* (1993) reported that the higher rates of phosphorus were associated with increases in N and P content of faba bean plants.

Soil available phosphorus and nitrogen:

Results of soil available P and N as affected by the bacterial inoculation and different rates of phosphorus fertilizers added at 45, 75 days and harvesting stages are given in Table (7). Data reveal that bacterial inoculation with *Rhizobium* and\or *Bacillus* and their combination gave significantly the highest available P concentration due to all the other uninoculated treatments at all examined periods. This phenomenon was true when bacterial inoculation was combined with different phosphorus fertilizer rates. However, the highest soil available P concentration was due to the inoculation with a mixture of *Rhizobium* and *Bacillus* combined with 30 kg P_2O_5/fed. The corresponding value was 31.7 ppm available P (45 days). Obviously, increasing phosphorus fertilizer rate from 15 to 30 kg P_2O_5/fed increased significantly the soil available P concentration especially when they were applied in combination with any bacterial inoculation. Due to the tested growth period, the soil available P concentration had decreased as the growth period increased. The relative values were 31.7 ppm (45 days), 33.9 ppm (75 days) and 14.9 ppm (at harvest) soil available phosphorus.

Table (7): Available phosphorus and nitrogen (ppm) of soil cultivated by faba bean plants as affected by bacterial inoculation and different rates of phosphorus fertilizer

P_2O_5 (Kg/fed) (A)	Inoculation treatments (B)	Available P at			Available N at harvesting	
		45 days	75 days	Harvesting	NH^+_4	NO^-_3
0	**Uninoculated**	10.3	9.3	8.7	2.3	16.9
	Bacillus (S)	18.8	15.8	10.7	7.7	22.4
	Rhizobium (R)	13.7	12.7	9.5	12.4	34.1
	(S + R)	19.8	16.6	11.3	14.5	40.7
Mean		15.7	13.6	10.1	9.23	28.5
15	**Uninoculated**	13.8	11.8	9.9	3.8	19.5
	Bacillus (S)	23.5	19.5	13.5	9.1	27.3
	Rhizobium (R)	17.5	15.5	11.9	16.5	40.3
	(S + R)	27.4	21.7	13.6	19.8	49.2
Mean		20.6	17.1	12.2	12.8	34.1
30	**Uninoculated**	17.5	15.5	10.1	5.1	24.5
	Bacillus (S)	27.7	21.7	15.7	10.8	31.8
	Rhizobium (R)	21.4	17.4	12.7	20.3	51.2
	(S + R)	31.7	23.9	14.9	22.8	62.3
Mean		24.6	19.6	13.4	14.7	42.5
Means of bacterial inoculation treatments						
Uninoculated		13.9	12.2	9.6	3.73	20.3
Bacillus (S)		23.3	19.0	13.3	9.20	27.17
Rhizobium (R)		17.5	15.2	11.4	16.4	41.84
(S + R)		26.3	20.7	13.3	19.0	50.73
L. S. D. at 0.05						
(A)		1.365	0.166	0.255	0.858	0.144
(B)		1.814	0.172	0.175	0.261	0.349
(A x B)		2.731	0.332	0.510	0.452	0.605

This may be due to the continuous phosphorus uptake by faba bean plants during the growth period. The same conclusion was obtained by Foaad (2002) in soil cultivated with faba bean. Also, it may be due to the alkaline conditions of the studied soils.

With respect to available N (NO^-_3 and NH^+_4) at harvesting, results revealed that bacterial inoculation treatments increased significantly NH^+_4-N and No^-_3-N concentration compared with uninoculated ones. Similar results were obtained by Massoud (2001). The highest mean values for NH^+_4 and NO^-_3 were 19.0 ppm and 50.73 ppm, respectively, when plants inoculated with dual inoculation with *Rhizobium* and *Bacillus*. Also, data showed that increasing P-fertilizer rate increased significantly the available nitrogen forms in soil. However, the highest concentrations of nitrogen forms in soil of 22.8 and 62.3 ppm for NH^{+}_{4} and NO^{-}_{3}, respectively, had recorded due to the application of 30 kg P_2O_5/fed combined with dual inoculation with *Rhizobium* and *Bacillus*.

In conclusion, the present results confirmed that dual inoculation with *Rhizobia* and *Bacillus* in combination with 30 kg P_2O_5 as P-fertilizer for faba bean enhanced its growth and productivity and improved the soil nutrients status and in turn its fertility. However, this work requires to be repeated in different sites to reach the degree of recommendation.

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تأثير البكتريا المذيبة للفوسفات والمثبتة للنيتروجين على نبات الفول البلدى تحت تأثير مستويات مختلفة من السماد الفوسفاتى

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أجرى هذا البحث في قرية دماص - ميت غمر - الدقهلية على نبات الفول البلدي خلال الموسم الشتوي 2007 - 2008 لدراسة استجابة محصول الفول البلدي صنف Giza 3 للتلقيح البكتيرى ببكتريا الريزبيوم *Rhizobium leguminosarum* bv. Viciae منفردة أو مزدوجة مع بكتريا *Bacillus megatherium* var. Phosphaticum تحت تأثير ثلاث مستويات من السماد الفوسفاتى (صفر ، 15 ، 30 كجم فو$_2$أ$_5$/فدان) وذلك على محتوى النبات من الكلوروفيل وتكوين العقد الجذرية والمحصول ومكوناته وبروتين الحبوب كذلك محتوى القش والحبوب من النيتروجين والفوسفور والبوتاسيوم بالإضافة إلى النيتروجين والفوسفور الميسر في

التربة وكذلك عدد البكتريا المذيبة للفوسفات في منطقة جذور النبات (rhizosphere) بعد 30 ، 60 ، 90 يوم من الزراعة .

أظهرت النتائج المتحصل عليها إن التلقيح المزدوج بالبكتريا الباسيلاس والريزوبيا أعطى زيادة معنوية لمحتوى الكلوروفيل a, a + b كذلك أعطى زيادة معنوية للعقد لكل نبات والوزن الجاف وانزيم النيتروجينيز بعد 75 يوم من الزراعة .

بالنسبة للمحصول ومكوناته وكذلك البروتين في الحبوب ، محتوى النيتروجين للحبوب بالتلقيح البكتيرى كلا من البكتريا المستخدمة تحت الدراسة أو الزيادة في السماد الفوسفاتى أو التفاعل بينهما وقد بلغت نسبة الزيادة في نتيجة التلقيح بكلا البكتريا المستخدمة إلى حوالى 35 % كما زاد محتوى البروتين في الحبوب إلى حوالى 57 % مقارنة با الكنترل (معاملة غير ملقحة) .

وكانت هناك زيادة معنوية في محتوى البذور والقش من النيتروجين والفوسفور والبوتاسيوم نتيجة التلقيح خاصة التلقيح المزدوج كذلك بزيادة السماد الفوسفاتى زادت هذه العناصر معنوياً في كل من القش والبذور .

وقد ازداد الفوسفور الميسر وكذلك النيتروجين الميسر نتيجة التلقيح الحيوى حيث أعطى التلقيح المزدوج أعلى زيادة معنوية وكذلك زيادة السماد الفوسفاتى المعدنى عند 30 كجم فو$_2$أ$_5$/فدان أعطى زيادة معنوية .

لذا يوصى البحث بتطبيق معاملة التلقيح المزدوج من الريزوبيا والباسيلوس مع إضافة 30 كجم فو$_2$أ$_5$/فدان عند الزراعة حيث أحدثت تأثيرا إيجابيا على إنتاجية نبات الفول البلدى كذلك أدت إلى تحسين خصوبة التربة.