J. Agric. Sci. Mansoura Univ., 28(8): 6371 - 6388, 2003

EFFECT OF PHOSPHORUS FERTILIZATION AND SEEDS INOCULATION WITH PHOSPHATE DISSOLVING BACTERIA ON MICROBIOLOGY OF RHIZOSPHERE, FABA BEAN YIELD AND YIELD COMPONENTS

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ABSTRACT

Two field experiments were carried out at Sakha Agricultural Research Station using inoculated faba bean seeds variety Improved Giza 3 during the two successive seasons of 1998/1999 and 1999/2000. The inoculation was done using phosphate dissolving bacterial strain namely *Bacillus megatherium* var.*phosphaticum*. Different levels of superphosphate (P_2O_5 kg/fed) were also used with the bacterial inoculation to examine their effect on microbiology of faba bean rhizosphere, yield and yield components and NPK uptake as well.

Obtained data showed significant differences ($P< 0.01$) between inoculated treatments and unincculated ones regarding the five bacterial groups examined under all level of superphosphate applied. These bacteria are P-dissolvers, total viable bacteria, *Rhizobium spp., Azotobacter spp.* and *Azospirillum spp.*

Concerning the growth characters of faba bean, the data of plant height and number of branches showed significant increase with bacterial inoculation and superphosphate levels used during the two successive cultivation seasons.

The highest values of pod seeds, 100-seed weight, seed yield and straw yield were achieved under bacterial inoculated treatment at all levels of superphosphate used. After harvesting, the three main elements, nitrogen, phosphorus, and potassium recorded significant increase towards the bacterial inoculation together with superphosphate either for seed, shoot, or for straw. Obtained data proved also the importance of biofertilizer represent in P-dissolving bacteria as well as using superphosphate fertilization less than the recommended level. This can also take part in reducing the pollution of the soil from the chemical showed be added every year. Furthermore, this also increased the yield of faba bean and increase the nutritive values as well.

INTRODUCTION

Bean is one of the most important food crops for many of the world population especially in the developing countries, such as Egypt. There are several factors that affect the production of faba bean and the most important one of these factors is the fertilization. The intensive use of expensive mineral fertilizers in recent years, which results in environmental pollution problems has focused the attention of research on the possibility of using biofertilizers as an alternative or complementary for mineral fertilization (Raju *et al.*, 1997). Phosphorus comes next to nitrogen as a vital nutrient for plants and soil microorganisms. The inorganic forms of the element in soil are compounds of calcium, iron, aluminum and fluorine. The organic forms are compounds of phytins, phospholipids and nucleic acids, which come mainly by way of decaying vegetation (Subba-Rao, 1988). Beside the other important functions of P in plants, it plays as an agent of energy transfer and deficiency of available P is more likely to limit crop production than any other material except water.

In Egyptian soils, most of the total P content is present in the inorganic form (Balba, 1981). The organic phosphorus form constitutes the following three main groups: (1) phytin (inositol) hexaphosphate, (2) nuclic acids (phytin) derivative, and (3) phospholipids. The inorganic forms can be classified to alaminum phosphate (Al-P) and iron phosphate (Fe-P), which occur frequently in acidic soils, and calcium phosphate (Ca-P) occurring in calcareous soils and in soils of arid and semiarid regions having pH values in the alkaline side. Calcium phosphate exist in several forms, most important forms are $Ca(H_2PO_4)_2.H_2O$ monocalcium phosphate, which is water soluble, and the dominant phosphate of superphosphates. This form is the available form for plant. It represents in little percent from the total P in soils (Russell, 1973). $CaHPO_4.3H_2O$ dicalcium phosphate (hydrated) and $CaHPO_4$ (dehydrated form). The hydrated is metastable and goes over to the dehydrated form relatively easily, and both are only slightly soluble in water. Biofertilizers denote preparations containing living micro-organisms, such as bacteria; *Rhizobia, Azotobacter, Azospirillum* and phosphate dissolvers, such as; *Bacillus megatherium*. These microorganisms can improve the soil fertility by changing unavailable sources of atmospheric nitrogen and soil phosphorus into available form for growing crops. Bio-fertilizers are considered to be cheap way to recycle the elements to conserve natural resources and to act as protection factor against increasing pollution due to the extensive use of mineral fertilizers (Neeru *et al.*, 2000). In developing countries, the exploration of the possibilities of using inexpensive and easily available biofertilizers should therefore, be one of the immediate tasks, to meet the increasing needs for plant nutrients and for a less polluted globe. In the developed countries, chemically produced water-soluble P fertilizers are routinely applied to crops. The P in these fertilizers is initially available for plant use, then rapidly reacts with soil components and becomes progressively less available for plant uptake. In the developing countries, where chemical fertilizers are not available or are too expensive, ground rock P offers a less costly option (Nahas, 1996). This form of P is much less available to plants than standard fertilizers, except in acidic soils. Many soil microorganisms are able to solubilize otherwise unavailable forms of bound P. These organisms are found in most soils but the numbers and types vary from soil to soil. The ability of microorganisms to solubilize P in soil, and make it available for plant use was demonstrated. In the 50 years since this discovery, researchers world-wide have worked to isolate P-solubilizing organisms, study their characteristics, and use them in inoculants to make P more readily available in commercial agriculture (Singh and Kapoor, 1999).

So, the aim of this investigation is to examine the effect of bacterial inoculation of faba bean seeds and using different levels of superphosphate (P_2O_5 kg/fed) on microbiology of rhizosphere, yield and yield components together with NPK uptake were also included in this work.

MATERIALS AND METHODS

This investigation was carried out at Sakha Agricultural Research Station. In this investigation, faba been seeds variety improved Giza 3 were used in field experiments during the two successive cultivation seasons of 1998/1999 and 1999/2000.

Soil samples:

Twenty surface soil samples were taken at ten different locations at 0-20 cm depth. Mixed sample was obtained and saved in plastic bags. For soil particle size determination, mechanical analysis was carried out using the method described by Piper (1950). The organic carbon content of soils sample was determined using the method of Jackson (1958). The soluble cations and anions were determined according the method described by Richards (1954). The electrical conductivity was determined according to the method described by Richards (1954). Their analysis either physical or chemical are presented in Table 1.

Table (1): Some physical and chemical properties of the experimental clayey soil during 1998/1999 and 1999/2000 seasons.

Soil properties	Season of cultivation	
	1^{st} (1998/1999)	2^{nd} (1999/2000)
Clay (%)	49.19	49.95
Silt (%)	25.74	24.95
Sand (%)	24.28	23.92
$CaCO_3$ (%)	2.10	2.15
Organic matter (%)	0.96	1.05
pH (1:2.5 soil water suspension)	8.10	8.00
EC mmhos cm^{-1} (Soil paste extract at 25°C)	2.10	2.30
Total phosphorus (ppm)	1200	1250
Avaialble macronutrients (ppm):		
N	22.00	35.00
P	8.30	8.50
K	825.5	914.50
Avaialble micronutrients (ppm):		
Zn	0.98	1.10
Fe	7.85	8.30
Mn	7.50	8.20
Soluble cations meq/liter:		
Ca^{++}	5.55	5.35
Mg^{++}	4.63	4.00
Na^{+}	10.55	11.11
K^{+}	0.40	0.36
Soluble anions meq/liter:		
$CO^{=}_3$	--	--
HCO^{-}_3	2.50	2.00
Cl^{-}	10.86	11.17
$SO^{=}_4$	7.77	7.65

Bacterial strain used for inoculation:

An active phosphate dissolving bacterial strain namely *Bacillus megatherium* var. *phosphaticum* was provided from Microbiology Department, Agric. Res. Center, Ministry of Agriculture, Egypt. This strain was maintained on nutrient agar slant at 5°C till used. Strain of *Bacillus megatherium* var. *phosphaticum* was grown on a liquid medium of modified Bunt and Rovira (Abdel-Hafez, 1966) using 250 ml Erlenmeyer flasks containing 100 ml of the medium. The flasks were incubated at 28°C for 7 days. The bacterial growth was washed and suspended in a physiological mineral solution. Seeds of faba bean plants were washed and soaked for 30 minutes in the physiological mineral solution contained about 10^6 cells/ml. An adhesive agent Arabic gum was used to obtain coated seeds. The coated seeds were then air dried in the shade for 30 minutes and sown immediately.

Planting:

The two field experiments. were designed in a split design. The arrangement of the field experiment involves two factors. The first is the microbial inoculation with phosphate dissolving bacteria as main plots, two treatments were conducted for faba bean included uninoculated and inoculated one. The second factor is P-fertilization using superphosphate (15.5 P_2O_5). The treatments in case of faba bean plant were 0, 5, 10, 15, 20 and 25 kg P_2O_5/fed. Thus, all treatments were 12 treatments which replicated 4 times, and the plot area was 3 x 3 = 9 m^2. In the 1st season sowing of faba bean was on the 8th November, 1998, while in the 2nd season was on 10th of November, 1999. 15 kg N/fed in the form of NH_4NO_3 (33% N) were added at sowing time, which followed by the irrigation. Phosphorus fertilizer was applied in one dose after three weeks from planting, which followed by the irrigation. Harvesting was done on 20th April, 1999 and 23rd April, 2000 for the 1st and 2nd season, respectively.

Before harvesting, 10 plants faba bean were randomly collected, put in paper bags, and immediately carried to the laboratory. These plants were then used to study the behaviour of plant growth as affected by addition of P_2O_5 and bacterial inoculation with phosphate dissolving bacteria as well. The subjected examination were plant height (cm) and number of branches per plant.

Yield and yield components:

The seed yield of faba bean was obtained by threshing the harvested plants and collecting the seeds of the area of 9 m^2 (plot), separated from straw, weighed and determined per plot and the obtained results were expressed as kg/fed. Straw yield was obtained by subtracting the values of seed yield (kg/fed) from the values of total yield of accumulated weight (seed + straw). The obtained results were expressed as kg/fed. Seed index was determined as the mean number of seeds in pod.

Plant analysis:

For determination of nitrogen content, samples of 60 days for faba bean seed, as well as straw at maturity from treatments were ground in a mill, portions of each seed and straw (0.2 g) were digested in 5 ml of H_2SO_4 and 1 ml of perchloric acid in conical flask and the digested samples were distilled by micro-kjeldahl procedure. The nitrogen content (%) of distillate was determined by titration according to Black *et al.* (1965). For determination of phosphorus content, the digested samples were serially diluted in measuring flasks and the phosphorus content (%) was colorimetrically measured according to the method described by Snell and Snell (1967). For determination of potassium content, the collected samples were dried and digested using a mixture solution of H_2SO_4 and $HClO_4$ and the potassium content (%) was measured by the method described in AOAC (1980) using the Perkin Elmer flame photometer.

Microbiological measurements:

The count of total viable bacteria was determined in rhizosphere soil samples according to Vincent (1970) using the soil extract agar medium. Determination of phosphate dissolving bacteria was carried out using Bunt and Rovira medium modified by Abdel-Hafez (1966), these bacteria were counted by the decimal plate count technique. Symbiotic N-fixers expressed as *Rhizobium spp.* were determined by yeast extract-mannitol agar (YMA) medium.

Non-symbiotic N-fixers both *Azotobacter spp.* and *Azospirillum spp.* were determined. The total number of *Azotobacter spp.* were counted in rhizosphere samples using the technique of most probable number (MPN) as described by Vincent (1970). The medium of Ashby modified by Abdel-Malek and Ishac (1968) was used. The positive tubes were distinguished by the presence of the pellicle and examining stained preparation. The total number of viable *Azospirillum spp.* were counted in rhizosphere soil samples using the semi-solid malate medium of D.bereiner (1978). The technique of most probable number (MPN) described by Vincent (1970) was used. The number of viable bacteria were calculated using Cochran's tables (Cochran, 1950) and related to oven dry weight sample.

Statistical analysis:

The obtained results were subjected for statistical analysis according to the procedure outlined by Gomes and Gomes (1984).

RESULTS AND DISCUSSION

The periodical changes in colony counts of the total microorganisms, phosphate dissolving bacteria, *Rhizobium spp.*, *Azotobacter spp.*, and *Azospirillum spp.* were followed in applied field experiment. The initial population densities of uninoculated case were relatively low compared to inoculated treatments. The population count is significantly increased during the first two months reaching the maximum values. Then the values gradually decreased during the next two months reaching nearly the minimum value at the 4th month after planting of faba bean plants either in case of uninoculated or inoculated treatments.

Single culture inoculations of faba bean seeds by *Bacillus megatherium* var. *phosphaticum* is significantly enhanced the colonies counts, which increases compared to the uninoculated treatments to reach the maximum values at the 2nd month after faba bean cultivation. Phosphate fertilization significantly increased the colonies count of different microorganisms tested in rhizosphere. The counts in the rhizosphere of uninoculated and inoculated plants increased as the amount of superphosphate increased.

Microbiological value of faba bean rhizosphere:

The obtained data in Table (2) clearly show that the counts of phosphate dissolving bacteria in rhizosphere of faba bean increased to reach the maximum value (105 x 10^4 cfu/g) under inoculation treatments at the 2nd month and fertilized with 25 kg P_2O_5 / fed. The lowest count (31 x 10^3 cfu/g) was recorded under uninoculated treatments at the 4th month and fertilized with 5 kg P_2O_5/fed. The mean value of P-dissolving bacterial counts increased from 39.8 x 10^3 cfu/g unfertilized soil to reach 57.5 x 10^3 cfu/g soil fertilized with 25 kg P_2O_5 / fed and from 53 x 10^4 cfu/g soil (without P) to 70.5 x 10^4 cfu/g soil in case of using 25 kg P_2O_5/fed in case of uninoculated and inoculated treatments, respectively. El-Borollosy (1999) reported that P-dissolving bacteria have the capability to bring insoluble phosphate in soil into soluble forms by producing organic acids such as formic, acetic, propionic, lactic, glycolic and succinic acids.

Table (2): Changes in counts of phosphate dissolving bacteria in rhizosphere of faba bean plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	P-dissolving bacteria x 10^3/cells/g soil					P-dissolving bacteria x 10^4/cells/g soil				
0	33	58	38	30	39.8	35	85	60	32	53.0
5	32	60	40	31	40.8	45	90	65	34	58.5
10	35	75	50	34	48.5	44	95	70	35	61.0
15	35	80	55	38	52.0	48	98	75	45	66.5
20	38	85	60	40	55.8	50	100	78	44	68.0
25	40	88	61	41	57.5	52	105	80	45	70.5
Mean	35.50	74.33	50.67	35.67	49.0	45.67	95.5	71.33	39.17	62.9

	P	T	I
F test	**	**	**
LSD 0.05	0.54	0.11	0.22
0.01	0.73	0.15	0.20

It is clearly evident from the data presented in Table (3) that the counts of total bacteria in rhizosphere of faba bean increased to reach the maximum value of 66 x 10^6 cfu/g under inoculation and 20 kg P_2O_5/fed at the 2nd month. The lowest count was found to be 30 x 10^5 cfu/g were obtained under uninoculated treatments at the 1st and 4th month in case of 0.0 and 5 kg of P_2O_5/fed. The mean value of the total colonies counts was increased from 36 x 10^5 cfu/g (without P) to reach 40 x 10^5 cfu/g with 25 kg P_2O_5/fed and from 41 x 10^6 cfu/g (without P) to 43.0 x 10^6 cfu/g with 20 kg P_2O_5/fed in case of uninoculated and inoculated treatments, respectively. Obtained increase could be explained by the formation of available phosphorus and the production of growth promoting substances such as gibberellins, cytokinins and auxins by inoculated P-dissolvers. These results are in a good agreement with those obtained by Abdel-Hamid (1995), who noticed remarkably increased in the densities in total count and some other groups in soil.

Table (3): Changes in counts of the total viable bacteria in rhizosphere of faba bean plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	Total viable bacteria x 10^5/cells/g soil					Total viable bacteria x 10^6/cells/g soil				
0	30	52	32	30	36.0	32	60	40	32	41.0
5	30	56	33	30	37.3	32	61	41	32	41.5
10	31	57	35	31	38.5	33	64	42	32	42.8
15	31	58	35	30	38.5	34	64	41	30	42.3
20	32	58	38	31	39.8	34	66	42	31	43.3
25	32	58	40	30	40.0	33	65	42	32	43.0
Mean	31.00	56.50	35.50	30.33	38.3	33.00	63.33	41.33	31.50	42.3
	P	T	I							
F test	**	**	**							
LSD 0.05	0.03	0.008	0.01							
0.01	0.04	0.010	0.02							

Obtained data of Table (4) reveal that inoculation of faba bean seeds with *Bacillus megatherium* resulted in a considerable increase in the densities of *Rhizobium* colonies in the rhizosphere region. The mean increases recorded were from 38.5 x 10^3 cfu/g of uninoculated soil to 58.6 x 10^5 cfu/g of inoculated soil. The maximum value of *Rhizobium* density was found to be 105 x 10^5 cfu/g, which was observed at the 2nd month of inoculated treatment and fertilized with 25 kg P_2O_5/fed. At the later stages, the counts sharply dropped and reached their lowest values being 14 x 10^3 cfu/g at the 4th month without P addition and uninoculated treatments. Srinivasan *et al.* (1997) observed that the presence of both *Rhizobium sp.* and *Bacillus megatherium* is necessary for enhancing nodulation of *Phaseolus vulgaris* by *Rhizobium* microsymbiont. They added that the dual inoculation by symbiotic N_2-fixers in combination with a P-dissolver resulted in increasing roct hair proliferation and lateral root formation.

Data recorded in Table (5) reveal that the counts of *Azotobacter spp.* in rhizosphere of faba bean increased to reach the maximum value of 85 x 10^3 cfu/g under inoculation treatment and fertilization with 20 kg P_2O_5 / fed at the 2nd month. The lowest counts was found to be 10 x 10^2 cfu/g, which was recorded under uninoculated treatments at the 4th month either without fertilization or with 25 kg P_2O_5 / fed or with 10 kg/fed. The mean count of *Azotobacter spp.* was increased from 27.5 x 10^2 cfu/g (without P) to reach 30.8 x 10^2 cfu/g (25 kg P_2O_5 / fed) and from 37.0 x 10^3 cfu/g (without P) to 46.3 x 10^3 cfu/g (25 kg P_2O_5/fed) under uninoculated and inoculated treatments, respectively. The results obtained by Mohammad and Ram-Prasad (1988) revealed that the addition of *Bacillus megatherium* with *Azotobacter chroococcum* led to increase the seedling growth of pea when it compared to the control.

Table (4): Changes in counts of *Rhizobium spp,* in rhizosphere of faba bean plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	Rhizobium spp. x 10^3/cell/g soil					*Rhizobium spp.* x 10^5/cell/g soil				
0	18	65	45	14	35.5	32	90	70	30	55.5
5	20	70	50	15	38.8	33	95	70	30	57.0
10	20	70	45	15	37.5	32	95	75	30	58.0
15	20	75	50	15	40.0	34	90	70	30	56.0
20	18	75	55	14	40.5	34	100	80	32	61.5
25	20	75	45	14	38.5	34	105	85	30	63.5
Mean	19.33	71.67	48.33	14.50	38.5	33.17	95.83	75.00	30.33	58.6
	P	T	I							
F test	**	**	**							
LSD 0.05	3.64	1.21	1.48							
0.01	4.98	1.63	2.04							

Table (5): Changes in counts of *Azotobacter spp.* in rhizosphere of faba bean plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	Azotobacter spp. x 10^2/cells/g soil					*Azotobacter spp.* x 10^3/cells/g soil				
0	15	55	30	10	27.5	25	70	35	18	37.0
5	15	55	32	11	28.3	35	75	40	20	42.5
10	18	60	35	10	30.8	33	75	42	20	42.5
15	20	62	33	12	31.8	35	80	40	20	43.8
20	20	60	32	12	31.0	40	85	45	25	48.8
25	20	62	31	10	30.8	40	80	40	25	46.3
Mean	18.00	59.00	32.17	10.83	30.0	34.67	77.50	40.33	21.33	43.5
	P	T	I							
F test	**	**	**							
LSD 0.05	2.54	1.00	1.04							
0.01	3.43	1.35	1.42							

The recorded data in Table (6) reveal that inoculation of faba bean seeds with *Bacillus megatherium* led to increase the mean densities of *Azospirillum spp.* in the rhizosphere region. The increases were found to change from 28.9 x 10^2 cfu/g (uninoculated) to 42.8 x 10^3 cfu/g in case of inoculated treatment. The maximum value of *Azospirillum spp.* densities being 80 x 10^3 cfu/g was observed at the 2^{nd} month of inoculated case and fertilized either with 20 kg P_2O_5 / fed or 25 kg P_2O_5/fed. At the later month, the counts sharply dropped and reached to their lowest values of 11 x 10^2 cfu/g at the 4^{th} month without P addition and uninoculated treatments.

Table (6): Changes in counts of *Azospirillum spp.* in rhizosphere of faba bean plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	Azospirillum spp. x 10^2/cell/g soil					*Azospirillum spp.* x 10^3/cell/g soil				
0	15	50	25	11	25.3	20	65	35	15	33.8
5	15	50	30	12	26.8	30	65	40	15	37.5
10	15	55	30	12	28.0	35	70	45	20	42.5
15	20	55	35	12	30.5	35	75	45	25	45.0
20	20	60	33	12	31.3	38	80	50	30	49.5
25	20	60	35	11	31.5	40	80	45	30	48.8
Mean	17.50	55.00	31.33	11.67	28.9	33.00	72.50	43.33	22.50	42.8
	P	T	I							
F test	**	**	**							
LSD 0.05	2.57	0.90	1.05							
0.01	3.53	1.21	1.44							

Growth of faba bean:

Data presented in Table (7) show the effect of phosphorus (P_2O_5) application level and inoculation of faba bean seeds with *Bacillus megatherium* var. *phosphaticum* on some growth parameters during the two seasons of 1998/99 and 1999/2000. The data reveal that increasing phosphorus application level significantly increased the plant height and number of branches / plant, in case of inoculated and uninoculated treatments in the two successive seasons. In the 1st season, increasing P levels from 0 to 5, 10, 15, 20 and 25 kg/fed for uninoculated treatment increased the plant height from 104.75 to 104.5, 108.25, 107.0, 107.25 and 105.25, respectively. In the 2nd season, these values are 103.0, 103.25, 103.25, 104.50, 105.00 and 105.00 cm for the same level of P_2O_5 under uninoculation conditions. In case of inoculation, the applying of 25 kg P_2O_5 / fed gave the highest value being 114.13 and 107.25 cm in 1st and 2nd season, respectively. The results reveal that plant height was significantly increased with P-dissolving bacterial inoculation. The mean values of plant height were 106.2 and 112.5 cm in the 1st season, while these values were 104.0 and 106.7 cm in the 2nd season for uninoculated and inoculated treatments, respectively. Regarding the number of branches / plant, it can be noticed that the same trend with plant height was recorded. The values of branches per plant were significantly increased from 2.50 and 2.75 (without P addition) to 3.75 and 4.00 at 25 P_2O_5/fed level in case of uninoculation in the 1st and the 2nd season, respectively. The values of branches number / plant under the inoculation were increased from 3.75 (without P_2O_5 addition) to 4.75 at 25 kg P_2O_5/fed for both seasons.

Table (7): Effect of P-fertilization and bacterial inoculation with *Bacillus megatherium* var *phosphaticum* on faba bean growth.

P_2O_5 (kg/fed)	1998/1999		1999/2000	
	Plant Height (cm)	No. of branches / plant	Plant Height (cm)	No. of branches / plant
	Uninoculated			
0	104.75	2.50	103.00	2.75
5	104.50	2.50	103.25	2.75
10	108.25	2.75	103.25	2.75
15	107.00	2.75	104.50	3.00
20	107.25	3.50	105.00	3.50
25	105.25	3.75	105.00	4.00
Mean	106.20	3.00	104.00	3.10
F test	*	**	*	**
LSD 0.05	1.3	0.34	0.17	0.79
0.01	--	0.72	--	1.09
	Inoculated			
0	111.00	3.75	105.75	3.75
5	111.13	3.75	106.50	3.50
10	111.75	3.75	106.25	3.75
15	113.00	4.25	107.00	4.50
20	113.75	4.50	107.50	4.50
25	114.13	4.75	107.25	4.75
Mean	112.50	4.10	106.70	4.10
F test	**	**	**	**
LSD 0.05	0.54	0.14	0.89	0.32
0.01	1.34	0.29	1.22	0.44

The positive effect of phosphorus may be due to the role of phosphorus in plant photosynthesis and respiration in addition to its role in cell division and development at meristematic tissues.Similar results were obtained by Ashour (1998). Plant roots can absorb the phosphorus from the soil solution and in either H_2PO_4 or HPO_4 form and both forms are quickly changed in the soil to insoluble form $Ca_3(PO_4)$. There is a strong competition between plants and soils for P in the soil solution.The winner usually is soils, so, it is essential to apply phosphate fertilization or using biofertilizer to mobilize soil phosphorus. This led to increase the plant growth, P-uptake and microbial population in legumes rhizosphere (Ghosh and Poi, 1998).

Yield and yield components:

Data in Table (8) show the value of No. of seeds / pod, 100-seed weight, seeds yield and straw yield as affected by phosphorus application at different levels and seed inoculation with phosphate dissolving bacteria in 1998/99 and 1999/2000 growing seasons in field experiments. In uninoculated treatments, data in Table (8) clearly show that increasing phosphorus application levels increased the No. of seeds in pod. The increase was insignificant in the 1st season, while it reached to be significant in the 2nd season. The highest value of pod (3.48 seeds/pod) seeds was recorded with 20 kg P_2O_5/fed in the first season, while it is 4.10 in the second season when using 25 kg P_2O_5/fed. Regarding the 100-seed weight as affected by the phosphorus application under the inoculated treatments, data in Table (8) show that the values of 100-seed weight were gradually increased as the phosphorus fertilization levels increased. The increases were highly significant in both seasons. The maximum value of 100-seed weight was found to be 63.63 and 63.72 g with using 25 kg P_2O_5/fed in the 1st and 2nd seasons, respectively.

Table (8): **Effect of P-fertilization and bacterial inoculation with *Bacillus megatherium* var. *Phosphaticum* on faba bean yield and yield components.**

P_2O_5 (kg/fed)	First season				Second season			
	No. of seeds / pod	100-seed weigh (g)	Seed yield (ton/ fed)	Straw yield (ton/ fed)	No. of seeds / pod	100-seed weigh (g)	Seed yield (ton/fed)	Straw Yield (ton/fed)
	Uninoculated							
0	2.85	61.43	1.05	2.93	3.00	62.15	1.17	2.93
5	3.10	61.45	1.23	3.08	3.08	62.00	1.21	3.08
10	3.35	62.20	1.32	3.16	3.23	62.28	1.32	3.17
15	3.33	62.55	1.37	3.24	3.55	62.70	1.37	3.25
20	3.48	63.00	1.39	3.95	3.03	63.20	1.41	3.31
25	3.43	63.63	1.42	3.32	4.10	63.72	1.43	3.35
Mean	3.26	62.36	1.30	3.28	3.33	62.66	1.32	3.18
F test	NS	**	**	**	*	**	*	*
LSD 0.05	--	0.42	0.04	0.05	0.26	0.46	0.03	0.09
0.01	--	0.88	0.06	0.09	--	0.64	--	--
	Inoculated							
0	3.73	63.38	1.22	3.06	3.10	63.38	1.24	3.08
5	3.65	63.70	1.28	3.14	3.15	63.53	1.30	3.10
10	4.05	64.08	1.37	3.25	3.35	63.85	1.36	3.20
15	4.03	64.30	1.41	3.35	3.78	63.95	1.41	3.44
20	4.10	64.63	1.45	3.40	4.03	64.60	1.45	3.41
25	4.10	65.20	1.47	3.42	4.35	65.35	1.47	3.43
Mean	3.94	64.22	1.37	3.27	3.63	64.12	1.37	3.28
F test	**	**	**	**	NS	**	*	*
LSD 0.05	0.15	0.17	0.01	0.02	--	0.19	0.02	0.04
0.01	0.31	0.36	0.02	0.02	--	0.26	--	--
P x I	NS	NS	NS	NS	NS	NS	S	NS

The data in Table (8) show that the increasing of phosphate application levels, in case of uninoculated treatments, significantly increased seed and straw yield of faba bean. Increasing P application led to increase the seed yield, since the highest values were 1.42 and 1.43 ton/fed in case of using 25 kg P_2O_5/fed without bacterial inoculation in the first and second season, respectively. Data in Table (8) show that inoculation with P-dissolving bacteria significantly increased No. of seeds / pod in the 1st season as a result with raising P_2O_5 levels. Also, the weight of 100-seeds high significantly increased in both seasons. The increases due to raising P_2O_5 level with inoculation of P-dissolving bacteria are higher than without inoculation. Seed and stem yields were significantly affected by the inoculation under the used phosphorus levels. The seed yield are 1.22, 1.28, 1.37, 1.41, 1.45 and 1.47 ton/fed at the level of P_2O_5 of 0, 5, 10, 15, 20 and 25 kg P_2O_5/fed under inoculation condition in the 1st season, respectively. The increase in seed yield in the 2nd season was significant. Straw yield increased from 3.06 at zero P_2O_5 / fed to 3.24 ton/fed at 25 kg P_2O_5/fed in the 1st season. These values were 3.08 and 3.43 ton/fed in the 2nd seasons. The present results are in good agreement with those found by Abdallah *et al.* (1984) and Dubey and Billore (1992). They found increases in seed yield of soybean and peanut as a result of inoculation with P-dissolving bacteria. The increase in seeds/pod, 100-seed weight, seed and straw yield due to inoculation with P-dissolver bacteria may be attributed to the increase in the available P in root media. The decreasing in pH values of soil will lead to an increase in micronutrient contents. Also, as found in Table (4) and (5), the inoculation with P-dissolver bacteria resulted increases in the count of *Rhizobium* and *Azotobacter* in rhizosphere of faba bean so increase the available nitrogen for plant. These results are in harmony with those obtained by Amara and Nasr (1995) and Singh and Kapoor (1999).

N, P and K content:

Data presented in Table (9) show the values of N % in faba bean plant as affected by the bacterial inoculation as well as the fertilization by different ratios of P_2O_5/fed. The values of N content gradually increased with the increase of P-fertilizer. The tabulated N content are for the different parts of faba bean plants, i.e. shoots after 60 days from planting, seeds and straw (at maturity), either in case uninoculated or inoculated treatments. In the 1st season, in case of uninoclation treatment, the lowest values of N % were recorded for the zero P_2O_5/fed to be 3.43, 3.78 and 0.428 for shoots, seeds and straw, respectively. On the other hand, the highest values of N% were found to be 3.98, 4.13 and 0.680 in case of using 25 kg P_2O_5/fed for shoots, seeds and straw, respectively. So increasing the P_2O_5 ratio from zero to 25 kg/fed increased the N content from 3.43 to 3.98, 3.78 to 4.13 and from 0.428 to 0.680% for shoots, seeds and straw, without inoculation treatments, respectively.

In the 2nd season, the trend was the same for the 1st season, where the lowest values of the N% content were found to be 0.51, 3.63 and 0.468 without phosphorus fertilization and uninoculated treatment for faba bean shoots, seeds and straw, respectively. Under the same conditions, the highest values of N% were achieved with the treatments of using 25 kg P_2O_5 / fed, which are 3.75, 4.11 and 0.627 for faba bean shoots, seeds and straw, respectively.

These results may be due to that the application of high levels (25 kg P_2O_5/fed) resulted in much amount of P in soil to be available for better root plant growth which consequently absorbs more N from the soil resulting in increasing N contents of different plant parts (Table 9). These results are in agreement with those recorded by Shady *et al.* (1992).

As shown from Table (9) inoculation with P-dissolvers increased the N content for all studied plant organs over that of P_2O_5 treatment only,in both

Table (9): Effect of P-fertilization and bacterial inoculation with *Bacillus megatherium* var. *Phosphaticum* on N content (%) of faba bean plants.

P_2O_5 (kg/fed)	First season			Second season		
	Shoot after 60 d.	**Seeds at maturity**	**Straw at maturity**	**Shoot after 60 d.**	**Seeds at maturity**	**Straw at maturity**
	Uninoculated					
0	3.43	3.78	0.428	3.51	3.63	0.468
5	3.58	3.93	0.495	3.56	3.71	0.505
10	3.67	3.96	0.590	3.63	3.76	0.545
15	3.73	4.00	0.613	3.70	3.85	0.585
20	3.87	4.06	0.640	3.72	3.93	0.610
25	3.98	4.13	0.680	3.75	4.11	0.627
Mean	3.71	3.98	0.558		3.83	0.557
F test	**	**	**	**	**	**
LSD 0.05	0.08	0.03	0.012	0.04	0.04	0.015
0.01	0.10	0.05	0.015	0.06	0.05	0.021
	Inoculated					
0	3.59	3.82	0.460	3.54	3.68	0.505
5	3.68	3.94	0.550	3.63	3.76	0.568
10	3.70	4.00	0.622	3.64	3.80	0.587
15	3.86	4.03	0.650	3.71	3.94	0.600
20	3.92	4.09	0.670	3.75	4.02	0.627
25	4.02	4.21	0.720	3.77	4.17	0.653
Mean	3.80	4.02	0.597	3.67	3.90	
F test	**	**	**	**	**	**
LSD 0.05	0.07	0.01	0.010	0.04	0.02	0.013
0.01	0.10	0.02	0.013	0.06	0.02	0.020
P x I	NS	NS	NS	NS	NS	NS

The increase may be attributed to the increase in the available P in soil as well as the increase in the count of symbiotic and non symbiotic nitrogen fixing bacteria as found previously.

Data presented in Table (10) clearly show that the increasing applied ratio of phosphorus (P_2O_5/fed) resulted in a highly significant increase of P content in different parts of faba bean under uninoculated or inoculated treatments. In the 1st season, the lowest values of P% were found to be 0.360, 0.390 and 0.100 for shoots, seeds and straw, respectively, for treatment without phosphorus fertilization and uninoculated treatments. The highest values of P% were achieved under the treatments of 25 kg P_2O_5/fed without seed inoculation with phosphate solubilizing bacteria. These values were found to be 0.505, 0.535, and 0.165 for faba bean shoots, seeds and straw, respectively. In the 2nd season, the lowest values of P% were found to be 0.338, 0.400 and 0.105 for shoots, seeds and straw, respectively. These values were obtained without phosphorus fertilization and uninoculated treatments as shown in Table (10). Whereas, the highest values of P% were achieved under the treatments of using 25 kg P_2O_5/fed without seed inoculation with phosphate solubilizing bacteria being 0.433, 0.515 and 0.143 for shoots, seeds and straw, respectively. The increases of P content in different faba bean plant parts due to phosphorus fertilization together with the bacterial inoculation may be attributed to the effect of the availability of soil phosphorus and consequently the high efficiency of the roots in absorbing various nutrients. Emam *et al.* (1993) reported that the higher levels of phosphorus were associated with increase in N and P content of plant. Data in Table (10) show clearly that the inoculation with P-dissolving bacteria led to high significantly increased of the P content in the studied plant organs in both seasons. At zero level of P_2O_5, the P content increased from 0.360 to 0.363, 0.390 to 0.410 and from 0.100 to 0.108% in shoot, seed and straw due to inoculation, respectively. This increases are from 0.505 to 0.525, 0.535 to 0.555 and from 0.165 to 0.175% under 25 kg P_2O_5/fed for the above mentioned plant organs, respectively.

Table (10): Effect of P-fertilization and bacterial inoculation with *Bacillus megatherium* var. *Phosphaticum* on P content (%) of faba bean plants.

P_2O_5 (kg/fed)	First season			Second season		
	Shoot after 60 d.	Seeds at maturity	Straw at maturity	Shoot after 60 d.	Seeds at maturity	Straw at maturity
	Uninoculated					
0	0.360	0.390	0.100	0.338	0.400	0.105
5	0.380	0.428	0.115	0.335	0.426	0.118
10	0.405	0.453	0.125	0.365	0.450	0.125
15	0.443	0.480	0.135	0.375	0.473	0.128
20	0.475	0.502	0.150	0.395	0.493	0.135
25	0.505	0.535	0.165	0.433	0.515	0.143
Mean	0.428	0.465	0.132	0.374	0.459	0.126
F test	**	**	**	**	**	
LSD 0.05	0.012	0.011	0.011	0.012	0.010	0.004
0.01	0.005	0.015	0.014	0.016	0.014	0.012
	Inoculated					
0	0.363	0.410	0.108	0.353	0.410	0.115
5	0.393	0.445	0.123	0.365	0.433	0.123
10	0.418	0.478	0.133	0.383	0.463	0.130
15	0.465	0.490	0.153	0.400	0.480	0.138
20	0.485	0.518	0.155	0.415	0.910	0.145
25	0.525	0.555	0.175	0.458	0.528	0.148
Mean	0.442	0.483	0.141	0.396	0.471	0.133
F test	**	**	**	**	**	**
LSD 0.05	0.01	0.004	0.004	0.005	0.004	0.004
0.01	0.02	0.006	0.005	0.006	0.006	0.005
P x I	NS	NS	NS	NS	NS	NS

Concerning the interaction effect between P fertilization and seed inoculation by phosphate solubilizing bacteria on P content in faba bean plant, data show that no significant effect was observed in the two seasons. The highest values for shoots, seeds and straw were observed in case of using 25 kg P_2O_5/fed to be 0.525, 0.555, 0.175 and 0.458, 0.528, 0.148 for the first and the second season, respectively.

Data recorded in Tables (11) show that increasing the phosphorus application ratio to faba bean plant resulted in high significant increase of K (%)in different parts of faba bean, after 60 days age, seeds and straw under uninoculated and incculated treatments. In the 1st season and in case of uninoculation treatment, the lowest mean values of K% were found in control (zero P_2O_5/fed) which are 4.051, 0.518 and 1.3 for shoots, seeds and straw, respectively. The highest values of K% were achieved under the treatments of using 25 kg P_2O_5/fed without seed inoculation with *Bacillus megatherium*. These values were determined to be 4.42, 0.650, and 1.52 for shoots, seeds and straw, respectively. In the second season the trend was the same, where the lowest values of K % were measured to be 4.00, 0.565 and 1.23 without phosphorus fertilization for shoots, seeds and straw, respectively. On the other hand, raising the P_2O_5 levels to 25 kg/fed gave the highest values of K%. These values were found to be 4.31, 0.768 and 1.40 for shoots, seeds and strew, respectively.

Table (11): Effect of of P-fertilization and bacterial inoculation with *Bacillus megatherium* var. on *Phosphaticum* K content (%) of faba bean plants.

P_2O_5 (kg/fed)	First season			Second season		
	Shoot after 60 d.	Seeds at maturity	Straw at maturity	Shoot after 60 d.	Seeds at maturity	Straw at maturity
	Uninoculated					
0	4.05	0.518	1.30	4.00	0.565	1.23
5	4.16	0.538	1.39	4.06	0.597	1.25
10	1.24	0.573	1.39	4.10	0.660	1.27
15	4.29	0.600	1.41	4.16	0.700	1.31
20	4.38	0.638	1.46	4.20	0.715	1.31
25	4.42	0.650	1.52	4.31	0.768	1.40
Mean	4.26	0.586	1.41	4.14	0.668	1.30
F test	**	**		NS	**	NS
LSD 0.05	0.06	0.032	0.02	--	0.015	--
0.01	0.09	0.044	0.03	--	0.020	--
	Inoculated					
0	4.10	0.540	1.31	4.02	0.597	1.26
5	4.22	0.560	1.36	4.08	0.615	1.28
10	4.28	0.580	1.42	4.13	0.673	1.30
15	4.34	0.613	1.44	4.20	0.712	1.34
20	4.44	0.650	1.49	4.26	0.742	1.37
25	4.46	0.673	1.54	4.33	0.800	1.40
Mean	4.31	0.603	1.43	4.17	0.690	1.33
F test	**	**	**	NS	**	NS
LSD 0.05	0.02	0.021	0.008	--	0.006	--
0.01	0.03	0.015	0.011	--	0.008	--
P x I	NS	S	NS	NS	NS	NS

The inoculation treatment increased the K% in faba bean organs in both seasons compared to the application of P_2O_5 levels only. In the 1st season, K% increased from 4.10 to 4.46%, 0.540 to 0.673% and from 1.31 to 1.54% for shoot, seeds and straw, respectively due to inoculation zero and 25 kg P_2O_5/fed. In the 2nd season, the values of K% increased from 4.02 (Zero P_2O_5/fed) to 4.33% (25 kg P_2O_5/fed) for shoot due to inoculation. Concerning the interaction effect between P fertilization and seed inoculation by *Bacillus megatherium* on K % (content), data show that no significant effect was observed, except with K% with seeds in the first season.

On the light of the obtained results, it can be, generally, concluded that the inoculating of faba bean plants (*Vicia faba* L. Improved Giza 3) cultivated in clayey soil with an active bacterial strain as phosphate dissolver namely *Bacillus megatherium* var. *phosphaticum* is of great importance. This led to significant increase in the biofertility of soil as well as the yield of the plant growth. The biofertility of soil expressed in increasing the number of different bacterial groups in rhizosphere area. These groups are the phosphate dissolving bacteria, the total viable bacteria, *Rhizobium spp., Azotobacter spp.* and *Azospirillum spp.* These groups have active effect in releasing phosphorus in addition to the N_2-fixation process and the degradation of the organic materials by the enzymatic systems they have. This can also take part in reducing the pollution of the soil from the chemical showed be added every year. Furthermore, this also increased the yield of faba bean and increase the nutritive values as well.

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تأثير التسميد الفوسفاتى وتلقيح بذور الفول البلدى بالبكتيريا المذيبة للفوسفات على ميكروبيولوجيا الريزوسفير ومحصول الفول الناتج ومكوناته

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تم دراسة تأثير تلقيح بذور الفول البلدى (جيزة ٣ محسن) بالبكتيريا المذيبة للفوسفات وذلك على ميكروبيولوجيا ريزوسفير النبات وكذلك على محصول الفول الناتج ومكوناته وذلك خلال التجارب الحقلية.

أجريت تجربتان حقليتان بمحطة بحوث سخا بكفر الشيخ فى أراضى طمية خلال موسمى الزراعة ١٩٩٨ / ١٩٩٩ ، ١٩٩٩/٢٠٠٠ حيث تم زراعة بذور الفول البلدى جيزة ٣ محسن والتى تم تلقيحها بالبكتيريا المذيبة للفوسفات متمثلة فى ميكروب *Bacillus megatherium* var. *phosphaticum*.

بينت النتائج المتحصل عليها زيادة تعداد البكتيريا المذيبة للفوسفات فى منطقة الريزوسفير مقارنة بالكنترول هذا علاوة على زيادة بعض المجاميع البكتيرية الأخرى مثل التعداد الكلى للبكتيريا الحية وتعداد ميكروبات الريزوبيم وكذلك بكتيريا الآزوتوباكتر والأزوسبيريلم.

وبالنسبة لقياسات النمو النباتى فقد إزدادت أطوال النباتات مع التلقيح البكتيرى جنباً إلى جنب مع زيادة الأفرع للنبات تحت الدراسة.

دلت القياسات الخاصة بمحصول الفول البلدى ومكوناته زيادة ملحوظة فى أعداد البذور بقرن الفول ، وزن ١٠٠ بذرة ، محصول البذور وكذلك محصول القش الناتج.

ولقد تم تحليل النبات الناتج كيماوياً والتى دلت نتائجه على زيادة فى النسبة المئوية لكل من النيتروجين والفوسفور والبوتاسيوم لكل من البذور والقش وزيادة محصول الفول وصلاحية العناصر الغذائية بالتربة. ولقد أثبتت نتائج هذا البحث أهمية تعظيم إستخدام الأسمدة الحيوية والتى أدت إلى تقليل نسبة إستخدام السماد الكيماوى مما يقلل من تلوث التربة الزراعية بالكيماويات علاوة على حل مشكلة الفوسفات المترسبة فى الأراضى المصرية نتيجة إضافة الأسمدة الفوسفاتية سنوياً نظراً لطبيعة الأراضى المصرية لكونها أراضى تميل للقلوية ومحتوية على كربونات كالسيوم.