

**IMPROVING THE EFFICIENCY OF INORGANIC PHOSPHORUS AS
AFFECTED BY PHOSPHATE DISSOLVING BACTERIA UNDER
CALCAREOUS SOILS**

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ABSTRACT

A field experiment in split-split plot design was conducted on a calcareous soil at Nubaria Agriculture Research Station, El-Behera Governorate, Egypt, during the growing seasons of 2012/2013 and 2013/2014 to study the effectiveness of seed inoculation by *Bacillus megaterium* (as a phosphate dissolving bacteria (PDB)) on enhancement the P availability and improvement use efficiency of many inorganic phosphorus forms and rates in soil to improve the production of faba bean (*Vicia faba L.*) cultivar sakha 4 . Three fertilizer P forms namely **P₁**; single super phosphate 15.5% P₂O₅ (SSP), **P₂**; triple super phosphate 46% P₂O₅ (TSP) and **P₃**; phosphoric acid (H₃PO₄) 72.4% P₂O₅ (PA), at 75% and 100% of recommended doses (45 kg P₂O₅ fed⁻¹ RD).

The results revealed that PDB inoculation increased their population in soil and significantly increased the availability of P in calcareous soil whether applied alone or combined with added P forms compared without it, where the treatments of TSP at 75 and 100% RD combined with PDB gave the highest percentage (125.8, 136.4%) at the 50 days age and the lowest percentage (50.9, 53.3%) at harvest, respectively. The results indicated to increase macronutrients uptake by faba bean plants, which positively reflected on quantities and qualities of yields. It can be concluded that adding TSP at 75% RD combined with PDB inoculation is the recommended for improving the productivity of faba bean crop, while the means values of seed yield relatively increased about with 41.4% over the same treatment without PDB and about 34.2, 36.9% for PDB inoculation and TSP forms as individual effect respectively. It was noticed that the PDB-inoculated seeds increased phosphorus use efficiency (PUE), phosphorus agronomic efficiency (PAE) and phosphorus recovery efficiency (PRE) by about 33.04, 101.1 and 138.1%, respectively over the uninoculated. Triple superphosphate (TSP) has the greater efficiency whether it was alone or combined with PDB inoculation at 75% RD addition compared to other forms.

Key word: calcareous soil, mineral P forms, phosphate dissolving bacteria (PDB), faba bean.

INTRODUCTION

Calcareous soils are characterized by the presence of calcium carbonate in the parent material and in some cases by a calcic horizon. Thus phosphorus may be immobilized under pH values above 8.0 forming tri-calcium phosphate or hydroxyapatite (Balba, 1995). So, the low availability of phosphorus is due to the insoluble complexes with calcium and magnesium under alkaline soil conditions as di- or tri-calcium phosphates (Tindall, 2007). The poor P fertilizer recovery is mainly due to P adsorbing by the soil in unavailable form to plants (70-90% of the added phosphorus) lacking specific adaptations (Cordell *et al.*, 2011). It means that the soil contains high amount of total phosphorus, but its availability to plant is very low and it is often a limiting factor for the plant growth (Mikanova and Novakova, 2002).

Phosphorus (P) as one of the most essential plant nutrients which profoundly affect the overall growth of plants, is a major growth-limiting nutrient, (Ezawa, *et al.*, 2002). Plants can only absorb P in two soluble forms, the monobasic (H_2PO_4^-) and the dibasic (HPO_4^{2-}) ions (Glass, 1989), It can convert into insoluble forms (Bolland and Gilkes, 1998), with not more than 25 % efficiency (Isherword, 1998). The concentration of available bio-P in soil is very low (1.0 mg kg^{-1} soil), (Goldstein, 1994). However, enhancing microbial activity through solubilizing P inoculants may contribute considerably in plant P uptake. (Khan, *et al.*, 2009). Thus, phosphate solubilizing bacteria (PSB) play an important role in reducing P deficiency in soil (Aipova, *et al.*, 2010).and have attracted the attention of agriculturalists as soil inoculums to improve the plant growth and yield (Fasim, *et al.*, 2002). It has provided their worth as biological alternative to compensate agrochemicals and to sustain environment-friendly crop production (Dobbelaere, *et al.*, 2003).

Phosphate dissolving bacteria like *Bacillus megaterium* are able to affect the solubility of low dissolvable inorganic acids. The other bacteria of the same group are able to release phosphorus from phosphorus organic compounds, by producing phosphate enzymes. Phosphate solubilizing bacteria not only release phosphorus but also produce other biological compounds like hormones such as auxin and gibberellic acid as well as vitamins (Mehrvarz and Chaichi, 2008). So, exploitation of phosphate solubilizing bacteria through bio-fertilization has enormous potential for making use of ever increasing fixed P in the soil (Khan, *et al.*, 2009). Faba bean (*Vicia faba* L.) is one of the principal winter food legume crops in Egypt as a low-cost food rich in proteins and carbohydrate (Sepetoglu, 2002). Its root system can enhance the solubility of inorganic P and make it available to plant (Li *et al.*, 2007).

MATERIALS AND METHODS

The field experiment was carried out in the experimental farm of Nubaria Agriculture Research Station, El-Behera Governorate, Egypt, during

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two winter seasons of 2012/2013 and 2013/2014 to study the effect of *Bacillus megaterium* as phosphate dissolving bacteria (PDB) on increasing the availability and the use efficiency of different P fertilizer forms and rates which added to calcareous soil and its effects on the production of faba bean cultivar sakha 4. The experimental design was split- split plots with three replicates. The main plots were uninoculation or PDB inoculation, which half amount of seeds were soaked overnight in large basin in contact with the bacterial suspension of *Bacillus megaterium*, produced by Dept. of Microbiology, Soils, Water & Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt, and the other portion from seeds were soaked in water. The soaked seeds were planted on November 18 and 25 in 2012 and 2013, respectively. The sub main plots were three phosphorus fertilizers forms, i.e. **P₁**: calcium superphosphate (is available in powder contents 15.5% P₂O₅), it is known as single superphosphate (SSP), **P₂**: triple superphosphate (TSP) (is available in granular contents 46% P₂O₅), and **P₃**: phosphoric acid (PA) (72.4% P₂O₅), respectively. These forms were applied at two rates (75% and 100% from the recommended dose; 45 kg P₂O₅ fed⁻¹) as sub- sub plots. The two solid phosphorus fertilizers were added broadcasting before planting while the diluted (H₃PO₄) was added through sprinkler over rows. Other usual agronomy practices were conducted as recommended by Ministry of Agriculture. The inoculants was (200 g./50 kg. seeds) mixed with soaked seeds for N fixation, Nitrogen mineral fertilizer at a rate 30 kg Nfed⁻¹ in the form of ammonium nitrate (33.5% N) was added in two equal doses after 20 and 40 days from planting. K fertilizer at a rate 24 kg K₂O fed⁻¹ in the form of potassium sulphate (48% K₂O) was added just after 40 days from planting.

Preparation of bacteria inocula:

Bacillus megaterium was grown on nutrient broth (Difco, 1984) incubated for 24 hr at 30°C to obtain population of 5×10⁹cfu/ml culture. Seeds were soaked overnight in large basin in contact with the bacterial suspension; each inoculated seed of faba bean received about 10⁶ bacterial cells using Arabic gum as adhesive agent. (according to the Dept. of Microbiology, Soils, Water & Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt.).

Soil samples: Initial soil samples were collected from experimental area, air-dried ground, mixed thoroughly and sieved through a 2mm screen for analyzed, texture was determined by the hydrometer method, soil organic C was analyzed using Walkley and Black method. Soil pH was determined in 1: 2.5 soil/water suspensions after 0.5 h. EC_e was determined in a soil paste extract, total CaCO₃ was determined using the calcimeter method and some physical properties were determined according to (Page, *et al.*, 1982 and Klute, 1982). Available P was extracted according to Olsen, *et al.*, 1954 and determined colorimetrically after Murphy and Riley, 1962. The chemical characteristics of the experimental soil are presented in Table (1). Other

samples (0-25 cm surface layer) were collected at 50 days after sowing and at harvest.

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

Depth (cm)	Organic matter (%)	Total CaCO ₃ (%)	Particle size Distribution (%)			Texture class	Available Macro-nutrients (mg.kg ⁻¹ Soil)		
			Sand	Silt	Clay		N	P	K
0-25	0.45	28.4	56.7	24.1	19.2	Sandy Loam	41.5	5.4	85.7
pH (1:2.5)	EC (dS m ⁻¹ , soil paste extract)	Bulk density (g cm ⁻³)	Field capacity (%)	Available water (%)	Wilting point (%)				
8.22	3.50	1.34	38.30	25.80	12.50				

Densities of *Bacillus megaterium*:

From each sample, 10 g of soil was aseptically weighed after 80 days from sowing and transferred to an Erlenmeyer flask containing 100 ml of sterilized water, and shaken for 30 min at about 150 rpm. Immediately after shaking, each suspension was diluted 10-fold by pipetting 1-ml aliquots into 9 ml of sterilized water. A 10⁸-fold final dilution was obtained and 0.1 ml of each dilution of the series was placed on a petri dish with Bunt and Rovira (1955), modified by Abdel-Hafez, (1966). For each dilution 3 replicate dishes were made. The agar plates were incubated at 30°C for 7 days (aerobically). After the incubation period, the colonies forming unit (cfu) of the bacteria that developed on the respective agar plates was counted with the standard method. Data from each replicate were averaged for a soil sample and expressed as cfu per gram of oven-dried soil.

Plant sample: Faba bean samples were taken from each treatment at two studied growth periods which separated to shoots and roots at tillering stage (50 days after planting) and separated at maturity to seed and straw, and prepared to analysis. At harvest, ten plants were randomly taken from the three central rows for each plot to determine the number of branches/plant, number of pods/plant and 100-seed weight. Seed and straw yields were determined and calculated in (kg fed⁻¹). Plant portions (shoot, root, seed and straw) were dried at 70 C⁰ and digested for chemical determinations according to Wolf, (1982). Nitrogen, P and K content in the digests were determined according to the methods described by Cottenie, *et al.*, (1982) and Page *et al.*, (1982). Crude protein content in seeds was determined by multiplying the nitrogen percentage by 5.75 according the method described by A.O.A.C., (1984).

Statistical analysis: The obtained data were tabulated and subjected to statistical analysis of variance (ANOVA) by using Minitab computer program and least significant difference (L.S.D) at 5% level of error was calculated, according to Barbara and Brain, (1994). Combined analysis was made for the two growing seasons hence the results of two seasons followed similar trend.

Item Efficiencies: The efficiency of phosphorus was calculated as follows as suggested by Dobermann, (2005):

$$\text{Phosphorus Use Efficiency (PUE)} = \frac{\text{GrainYield (kg /fed)}}{\text{Fertilizer applied (kg P}_2\text{O}_5 \text{/fed)}}$$

$$\text{Phosphorus Agronomic Efficiency (PAE)} = \frac{(\text{Yield (fertilized)} - \text{Yield (control)}) \text{ kg /fed}}{\text{Fertilizer applied (kg P}_2\text{O}_5 \text{/fed)}}$$

$$\text{Phosphorus Recovery Efficiency (PRE)} = \frac{(\text{P total uptake (fertilized)} - \text{P total uptake (control)}) \text{ kg /fed}}{\text{Fertilizer applied (kg P}_2\text{O}_5 \text{/fed)}}$$

RESULTS AND DISCUSSION

Phosphate Dissolving Bacteria (PDB) Population:

Fig. (1) indicated that seeds inoculated by *B. megaterium* as PDB had highly positive effects on the population of these bacteria in soil rhizosphere. All the treatments involved P fertilizer + PDB improved the PDB Population and the available P level in the soil significantly over the P-fertilized treatments without PDB. Also, Fig. (1) shows that PDB population numbers increased with increasing of P fertilizer rates compared with control (without P fertilizer). While the population numbers of (PDB) in soil rhizosphere were differently increased with addition of different P fertilizer forms. It increased with increasing the rate of both single and triple calcium superphosphate fertilizers up to 100% recommended dose. In contrast, the population of (PDB) were increased with increasing phosphoric acid application up to 75% RD then decreased with the highest rate (100% RD), which the role of inoculation was vanished approximately at this treatment which were equivalent with those without inoculation. Sundara, *et al.*, 2002 reported that PDB populations were insignificantly influenced by the changes in the P fertilizer rates whether applied with PDB or otherwise. The results presented in Fig. (1) showed that triple super phosphate fertilizer was the best form positively on the population of (PDB) in soil rhizosphere at both used rates compared to the others.

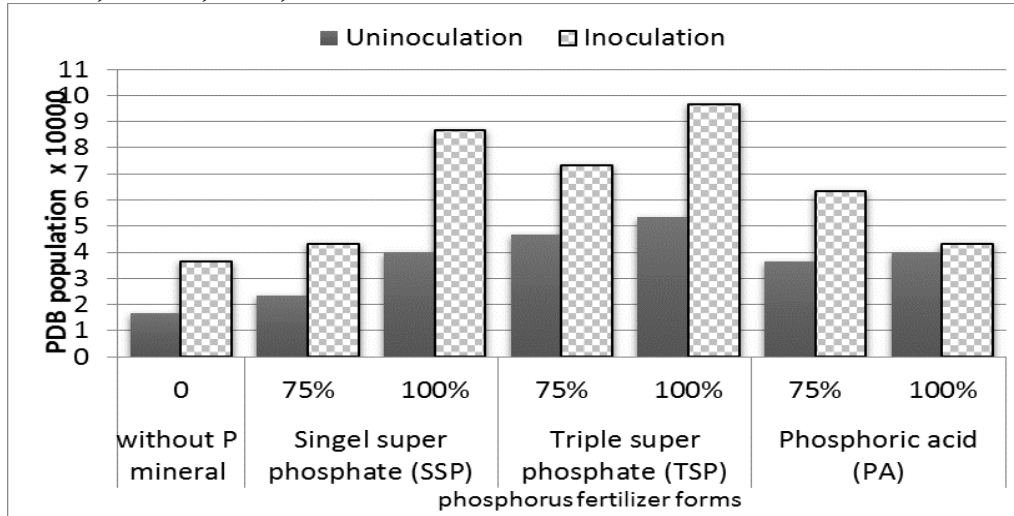


Fig. (1): Effect of phosphorus forms and rates and seed inoculation on PDB population numbers in faba bean rhizosphere.

Soil phosphorus availability:

Regarding to available P in soil presented in Table (2), it was noticed significant differences among treatments of each individual factor recording superiority of seed inoculated by (PDB) to uninoculated, also the application rate 100% corresponding to 75% RD and triple super phosphate compared with other used forms at two different growth intervals (after 50 days and maturity, respectively). The availability of soil phosphorus was greatly affected by PDB inoculation particularly after 50 days from plant sowing. PDB enhanced the P availability by 96% (from 8.2 to 16.1 mg kg⁻¹) and 84% (from 4.5 to 8.3 mg kg⁻¹) compared with those uninoculated at 50 days and harvesting stage, respectively. It's meaning that available P in soil were significantly increased by using PDB due to increase the population and its role in solubility process of insoluble phosphate in addition to other biological materials such as auxin, gibberellic acid, vitamins and hormones that increase the dissolution of phosphate, (He, et al., 2002).

Table (2): Effect of treatments on soil available P (mg kg⁻¹) (mean values for two seasons at two different growth stages).

Soil available P	Treatments	Uninoculation (b)				Inoculation (b)				mean 0%	mean 75%	mean 100%	mean of P forms
		Rate of phosphorous forms (r)											
		0%	75%	100%	mean	0%	75%	100%	mean				
at 50 days	SSP	5.1	8.9	9.3	7.8	9.1	17.2	21.3	15.9	7.1	13.1	15.3	11.8
	TSP	5.1	9.1	9.9	8.0	9.1	20.6	23.4	17.7	7.1	14.8	16.7	12.9
	PA	5.1	10.5	11.2	8.9	9.1	17.9	16.8	14.6	7.1	14.2	14.0	11.7
	mean	5.1	9.5	10.1	8.2	9.1	18.6	20.5	16.1	7.1	14.0	15.3	12.1
LSD at 0.05 level		(b:0.51)	(f:0.63)	(r:0.63)	(b*f:0.89)	(b*r:0.89)	(f*r:1.09)	(b*f*r:1.54)					
at harvesting	SSP	3.4	3.6	4.6	3.8	6.7	8.7	10.7	8.7	5.0	6.1	7.6	6.3
	TSP	3.4	5.6	5.9	5.0	6.7	8.5	9.0	8.1	5.0	7.1	7.4	6.5
	PA	3.4	5.3	5.4	4.7	6.7	7.8	9.6	8.0	5.0	6.6	7.5	6.4
	mean	3.4	4.8	5.3	4.5	6.7	8.3	9.7	8.3	5.0	6.6	7.5	6.4
LSD at 0.05 level		(b:0.24)	(f:ns)	(r:0.30)	(b*f:0.42)	(b*r:0.42)	(f*r:0.52)	(b*f*r:0.732)					

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These results were in agreement with Park, *et al.*, (2010) who concluded that PDB increase the solubility of insoluble P compounds such as rock Phosphate through the production of organic acids and phosphatase enzymes produced by microorganisms. Available P decreased with time in calcareous soil which reached at harvest down to 8.3 mg kg⁻¹ and in uninoculated treatments down to 4.5 mg kg⁻¹. It may be due to increasing the consumption with increasing plant growth and braiding of precipitation process. These results are in agreement with those obtained by El Azab, *et al.*, (2015). Data presented in Table (2) showed significant difference among P forms addition on P availability in soil by 152.9%, 131.4% and 129.4% after 50 days from sowing compared with the control for individual triple super phosphate (TSP), single super phosphate (SSP) and phosphoric acid (PA) respectively, due to the augmentation of PDB population in soil rhizosphere fertilized by TSP compared to other P forms. In contrast, the differences at harvest were insignificant among different P forms recording maximum value 6.5 mg kg⁻¹ (91.2% over) at TSP addition compared the control.

With different P rates addition as individual factor, data in Table (2) showed that soil available P values were increased significantly up to 14.0 and 15.3 mg kg⁻¹ after 50 days compared with control (7.1 mg kg⁻¹) and increased significantly up to 6.6 and 7.5 mg kg⁻¹ respectively compared with control (5.0 mg kg⁻¹) by addition 75 and 100% RD respectively. These results mean that percentage was decline with increasing the addition rates and with the time to the end of season.

The double interactions were generally significant among them, while the interaction of inoculation by PDB and P rates (b*r) and that between PDB and P forms (b*f) following the trend of their individual elements. This trend differed occasionally according to kind of mechanism in soil reaction, while the mean values of P available in soil at harvest were highest with the treatment (inoculation without addition fertilizer P) compared with treatments (75% or 100% from the recommended dose of addition fertilizer P without inoculation) but, the same rates of single super phosphate (SSP) combined with PDB inoculation gave the top values of P available at harvest compared with other forms.

The interaction between the all studied factors (b*f*r) gave at the 1st age (after 50 days) superiority at treatment of PDB inoculation with 100% RD of TSP significantly to others (23.4 mg kg⁻¹) due to the positive relation between TSP and PDB consequently increasing the population and its production. On the other hand, at the 2nd stage (at harvest) treatment of PDB inoculation with 100% RD of SSP was significantly superior to others (10.7 mg kg⁻¹). The difference may be due to decreasing the removed to plant and increasing the residual through plant growth.

Macronutrients contents (%) in plants:

At tillering stage:

The data presented in Table (3) showed that there were significant increases in shoot and root macronutrient contents with PDB inoculation due to increasing macronutrients availability in soil rhizosphere consequently the activity

of PDB in rhizosphere, these increases were 11.1, 25.6 and 16.9 % in shoot and 6.9, 14.2 and 12.3 % in root for N, P and K, respectively, compared with the treatments non-treated by (PDB). These increments might be due to the production of growth promoting substances such as indole acetic acid, gibberellic acid and auxin in rhizosphere and could increase root growth and nutrient absorption (Ibrahim, et al. 2010). These results are in agreement with those obtained by (El-Tantawy and Mohamed, 2009, and El-Edfawy, 2012). Also, could be due to its promotion of free living nitrogen fixing bacteria and enhancing nitrogen fixation, and then supplying of different nutrients like nitrogen, phosphorous, sulfur, iron and copper according to El-koumey, et al., 1993.

Table (3): Effect of treatments on macronutrients contents (%) in shoot and roots of faba bean plant at tillering stage, (mean values for two seasons).

Item Studied	Treatments	Uninoculation (b)				Inoculation (b)				mean 0%	mean 75%	mean 100%	mean of P forms	
		Rate of phosphorous forms (r)												
		0%	75%	100%	mean	0%	75%	100%	mean					
Macronutrients content in shoot (%)	N	SSP	2.1	2.5	2.5	2.4	2.6	2.7	2.7	2.7	2.4	2.6	2.6	2.5
		TSP	2.1	2.4	2.4	2.3	2.6	2.7	2.8	2.7	2.4	2.6	2.6	2.5
		PA	2.1	2.7	2.9	2.6	2.6	2.8	2.8	2.7	2.4	2.8	2.8	2.7
		mean	2.1	2.5	2.6	2.4	2.6	2.7	2.8	2.7	2.4	2.6	2.7	2.6
	LSD at 0.05 level		(b:0.167) (f: ns) (r:0.205)				(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)	
	P	SSP	0.24	0.32	0.35	0.30	0.33	0.41	0.40	0.38	0.28	0.36	0.38	0.34
		TSP	0.24	0.32	0.34	0.30	0.33	0.41	0.43	0.39	0.28	0.36	0.39	0.34
		PA	0.24	0.34	0.34	0.31	0.33	0.38	0.40	0.37	0.28	0.36	0.37	0.34
		mean	0.24	0.33	0.34	0.30	0.33	0.40	0.41	0.38	0.28	0.36	0.38	0.34
	LSD at 0.05 level		(b:0.04) (f: ns) (r: 0.05)				(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)	
	K	SSP	2.7	3.1	3.0	2.9	3.2	3.3	3.4	3.3	2.9	3.2	3.2	3.1
		TSP	2.7	3.1	2.8	2.9	3.2	3.3	3.5	3.3	2.9	3.2	3.2	3.1
		PA	2.7	3.1	2.8	2.9	3.2	3.5	3.8	3.5	2.9	3.3	3.3	3.2
		mean	2.7	3.1	2.9	2.9	3.2	3.4	3.5	3.4	2.9	3.2	3.2	3.1
	LSD at 0.05 level		(b:0.20) (f: ns) (r: 0.24)				(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)	
	Macronutrients content in root (%)	N	SSP	1.4	1.5	1.6	1.5	1.6	1.7	1.7	1.6	1.5	1.6	1.6
TSP			1.4	1.6	1.6	1.5	1.6	1.7	1.7	1.6	1.5	1.6	1.6	1.6
PA			1.4	1.5	1.5	1.5	1.6	1.6	1.6	1.6	1.5	1.6	1.6	1.5
mean			1.4	1.5	1.6	1.5	1.6	1.7	1.6	1.6	1.5	1.6	1.6	1.6
LSD at 0.05 level		(b: ns) (f: ns) (r: ns)				(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)		
P		SSP	0.19	0.22	0.22	0.21	0.21	0.24	0.26	0.23	0.20	0.23	0.24	0.22
		TSP	0.19	0.22	0.21	0.21	0.21	0.25	0.26	0.24	0.20	0.23	0.23	0.22
		PA	0.19	0.18	0.24	0.20	0.21	0.25	0.25	0.24	0.20	0.22	0.24	0.22
		mean	0.19	0.21	0.22	0.21	0.21	0.24	0.25	0.24	0.20	0.23	0.24	0.22
LSD at 0.05 level		(b:0.02) (f: ns) (r: 0.02)				(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)		
K		SSP	0.29	0.34	0.33	0.32	0.32	0.33	0.38	0.34	0.30	0.33	0.36	0.33
		TSP	0.29	0.35	0.36	0.33	0.32	0.42	0.39	0.37	0.30	0.38	0.37	0.35
		PA	0.29	0.31	0.34	0.31	0.32	0.39	0.39	0.36	0.30	0.35	0.36	0.34
		mean	0.29	0.33	0.34	0.32	0.32	0.38	0.39	0.36	0.30	0.36	0.36	0.34
LSD at 0.05 level		(b: 0.04) (f: ns) (r: 0.05)				(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)		

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On the other hand, there are significant increases in macronutrients content with P application and increasing its addition rate, these increases were insignificant among them. In contrast, the effect of P forms and their double and triple interactions with rates or PDB inoculation were insignificant on N, P and K content either in shoot or root at tillering stage.

At harvest stage:

Table (4): Effect of treatments on macronutrients contents (%) in seed and straw of faba bean at harvesting stage, (mean values for two seasons).

Item Studied at harvest	Treatments	Uninoculation (b)				Inoculation (b)				mean 0%	mean 75%	mean 100%	mean of P forms			
		Rate of phosphorous forms (r)														
		P forms (f)		0%	75%	100%	mean	0%	75%					100%	mean	
Macronutrients contents in seed (%)	N	SSP	3.72	3.81	3.80	3.78	4.11	4.33	4.14	4.19	3.92	4.07	3.97	3.99		
		TSP	3.72	3.90	3.97	3.86	4.11	4.46	4.18	4.25	3.92	4.18	4.08	4.06		
		PA	3.72	3.56	3.76	3.68	4.11	4.54	4.46	4.37	3.92	4.05	4.11	4.03		
		mean	3.72	3.76	3.84	3.77	4.11	4.44	4.26	4.27	3.92	4.10	4.05	4.02		
	LSD at 0.05 level		(b: 0.21)		(f: ns)		(r: ns)		(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)	
	P	SSP	0.12	0.17	0.17	0.15	0.18	0.25	0.27	0.23	0.15	0.21	0.22	0.19		
		TSP	0.12	0.17	0.19	0.16	0.18	0.28	0.36	0.27	0.15	0.22	0.28	0.22		
		PA	0.12	0.14	0.17	0.14	0.18	0.18	0.19	0.18	0.15	0.16	0.18	0.16		
		mean	0.12	0.16	0.18	0.15	0.18	0.24	0.27	0.23	0.15	0.20	0.22	0.19		
	LSD at 0.05 level		(b: 0.03)		(f: 0.03)		(r: 0.03)		(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)	
	K	SSP	0.96	1.13	1.08	1.06	1.12	1.01	1.00	1.04	1.04	1.07	1.04	1.05		
		TSP	0.96	1.03	1.13	1.04	1.12	1.14	1.04	1.10	1.04	1.09	1.09	1.07		
		PA	0.96	1.02	1.13	1.04	1.12	1.11	1.04	1.09	1.04	1.07	1.09	1.06		
mean		0.96	1.06	1.11	1.05	1.12	1.09	1.03	1.08	1.04	1.07	1.07	1.06			
LSD at 0.05 level		(b: ns)		(f: ns)		(r: ns)		(b*f: ns)		(b*r: 0.10)		(f*r: ns)		(b*f*r: ns)		
Macronutrients contents in straw (%)	N	SSP	2.25	2.31	2.31	2.29	2.30	2.35	2.32	2.32	2.27	2.33	2.32	2.31		
		TSP	2.25	2.30	2.34	2.30	2.30	2.30	2.36	2.32	2.27	2.30	2.35	2.31		
		PA	2.25	2.32	2.53	2.37	2.30	2.38	2.35	2.34	2.27	2.35	2.44	2.35		
		mean	2.25	2.31	2.39	2.32	2.30	2.34	2.34	2.33	2.27	2.33	2.37	2.32		
	LSD at 0.05 level		(b: ns)		(f: ns)		(r: ns)		(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)	
	P	SSP	0.36	0.40	0.42	0.39	0.44	0.47	0.54	0.48	0.40	0.43	0.48	0.44		
		TSP	0.36	0.44	0.45	0.42	0.44	0.52	0.56	0.51	0.40	0.48	0.50	0.46		
		PA	0.36	0.41	0.41	0.39	0.44	0.55	0.43	0.47	0.40	0.48	0.42	0.43		
		mean	0.36	0.42	0.43	0.40	0.44	0.51	0.51	0.49	0.40	0.46	0.47	0.44		
	LSD at 0.05 level		(b: 0.04)		(f: ns)		(r: 0.05)		(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)	
	K	SSP	1.44	1.49	1.62	1.52	1.57	1.62	1.71	1.63	1.51	1.56	1.66	1.57		
		TSP	1.44	1.48	1.75	1.56	1.57	1.65	1.73	1.65	1.51	1.57	1.74	1.60		
		PA	1.44	1.53	1.26	1.41	1.57	1.55	1.46	1.53	1.51	1.54	1.36	1.47		
mean		1.44	1.50	1.54	1.50	1.57	1.61	1.63	1.60	1.51	1.55	1.59	1.55			
LSD at 0.05 level		(b: ns)		(f: ns)		(r: ns)		(b*f: ns)		(b*r: ns)		(f*r: ns)		(b*f*r: ns)		

Data in Table (4) revealed that PDB inoculation significantly increased N and P content in seed about (13.2, 52.4 %) respectively compared with uninoculation, whereas K content was insignificantly affected by inoculation. The PDB inoculation recorded a significant increase in straw P content by 21.2% over the control. These results are in agreement with those obtained by Abd El-Hameed, *et al.*, (2014) who reported that inoculation with PDB significantly affected on growth parameters, yield and yield component of peanut and faba bean crops. Also, P content was increased significantly with increasing application P rate about 33.4, 51.5% in seed and 14.6, 15.6 % in

straw by using 75, 100% RD, respectively, compared with control. Treatment interactions had insignificant effect on macronutrients contents.

Macronutrients uptake by faba bean plants:

Data presented in Table (5) showed the superiority effect of PDB inoculation in comparison with control treatment, consequently causing significant increases in macronutrients uptake (mg kg⁻¹) with about 34.1, 51.3 and 27.7% for N, P and K, respectively compared to those without inoculation. These results were in agreement to those obtained by El-Tantawy and Mohamed, 2009 who detected accelerate nutrient absorption than uninoculated plants resulting in accumulation of more N, P, and K in the leaves. However, phosphate solubilize is not the only way for promoting plant growth by PDB but also help plant growth by stimulating the efficiency of plant hormone production such as auxins, cytokines, gibberellins and also some volatile compounds (Podile and Kishore, 2006). These results also are in line with those obtained by Ekin, (2011).

It is worthy to mention that the amounts of NPK removed to plant were significantly increased also with 75% RD addition P rate by 39.26, 52.47 and 27.67% over the control for NPK respectively, but insignificantly increased amounts in macronutrient uptake by using 100% RD compared with 75% RD. The increases in N and K contents can be explained by increasing available P in soil rhizosphere whether consequently PDB inoculation or increased fertilizer P source, where contributed for building new tissues and increase of the nodular number, size, and mass, which in turn increases N₂-fixation by bacteria, these results were in agreement by El-koumey, et al., (1993). Other treatment interactions were insignificantly effective on macronutrient uptake.

Table (5): Effect of treatments on NPK uptake (Kg fed⁻¹) by faba bean plants, (mean values for two seasons).

Item Studied	Treatments	Uninoculation (b)				Inoculation (b)				mean 0%	mean 75%	mean 100%	mean of P forms	
		Rate of phosphorous forms (r)												
		0%	75%	100%	mean	0%	75%	100%	mean					
Total uptake (kg fed ⁻¹)	N	SSP	76.9	95.5	97.0	89.8	104.7	128.7	131.3	121.6	90.8	112.1	114.1	105.7
		TSP	76.9	101.6	108.4	95.6	104.7	135.7	133.9	124.8	90.8	118.6	121.2	110.2
		PA	76.9	89.6	102.2	89.6	104.7	134.6	128.3	122.5	90.8	112.1	115.3	106.1
		mean	76.9	95.5	102.5	91.7	104.7	133.0	131.2	123.0	90.8	114.3	116.9	107.3
		LSD at 0.05 level	(b:5.99) (f: ns) (r:7.33)				(b*f: ns) (b*r: ns)		(f*r: ns)		(b*f*r: ns)			
	P	SSP	6.7	10.0	10.2	8.9	10.3	14.2	17.1	13.8	8.5	12.1	13.6	11.4
		TSP	6.7	11.3	12.0	10.0	10.3	16.6	18.9	15.3	8.5	13.9	15.5	12.6
		PA	6.7	9.1	10.3	8.7	10.3	15.4	12.6	12.7	8.5	12.2	11.4	10.7
		mean	6.7	10.1	10.8	9.2	10.3	15.4	16.2	13.9	8.5	12.8	13.5	11.6
		LSD at 0.05 level	(b:0.92) (f:1.32) (r:1.32)				(b*f: ns) (b*r: ns)		(f*r: ns)		(b*f*r: ns)			
K	SSP	32.3	43.5	44.8	40.2	44.3	52.4	59.8	52.1	38.3	47.9	52.3	46.2	
	TSP	32.3	44.8	52.3	43.1	44.3	57.7	57.0	53.0	38.3	51.2	54.6	48.0	
	PA	32.3	40.6	40.5	37.8	44.3	54.5	49.9	49.6	38.3	47.6	45.2	43.7	
	mean	32.3	43.0	45.9	40.4	44.3	54.9	55.6	51.6	38.3	48.9	50.7	46.0	
	LSD at 0.05 level	(b:4.24) (f: ns) (r:5.19)				(b*f: ns) (b*r: ns)		(f*r: ns)		(b*f*r: ns)				

Some growth parameters and production of faba bean:

Regarded to the effect of the studied treatments on growth parameters, biological yield and quality yields of faba bean, the statistical analyses for data in Table (6) showed that inoculation of faba bean seeds with phosphate dissolving bacteria insignificantly differed in number of branches/plant and number of pods/plant, these results were corresponded with results obtained by Abd El-Hameed, *et al.*, (2014) on peanut crop. While biological yield and seed quality parameter were highly significant increased with PDB inoculation, the corresponding relative increments were 12.2, 34.27, 11.3 and 13.36% over the uninoculated ones for straw, seed yields, 100- seed weight and protein percentage, respectively. These results were in accordance with those obtained by Abdallah, (2014).

Table (6): Effect of treatments on some growth parameters of faba bean, biological yield (kg. fed⁻¹) and seed quality, (mean values for two seasons).

Item Studied	Treatments	Uninoculation (b)				Inoculation (b)				mean 0%	mean 75%	mean 100%	Mean of P forms	
		Rate of phosphorous forms (r)												
		0%	75%	100%	mean	0%	75%	100%	mean					
Yield components (growth parameter)	Number of branches / plant	SSP	2.0	3.3	3.0	2.8	1.7	2.3	2.3	2.1	1.8	2.8	2.7	2.4
		TSP	2.0	2.3	2.3	2.2	1.7	3.0	2.3	2.3	1.8	2.7	2.3	2.3
		PA	2.0	2.3	2.0	2.1	1.7	2.3	2.3	2.1	1.8	2.3	2.2	2.1
		mean	2.0	2.7	2.4	2.4	1.7	2.6	2.3	2.2	1.8	2.6	2.4	2.3
	LSD at 0.05 level		(b: ns)	(f: ns)	(r: 0.46)	(b*f: ns)	(b*r: ns)	(f*r: ns)	(b*f*r: ns)					
	Number of pods /plant	SSP	11.9	14.4	15.4	13.9	12.5	15.7	14.9	14.4	12.2	15.0	15.2	14.1
		TSP	11.9	14.5	15.3	13.9	12.5	14.9	15.6	14.3	12.2	14.7	15.5	14.1
		PA	11.9	12.3	14.2	12.8	12.5	14.1	13.7	13.4	12.2	13.2	13.9	13.1
		mean	11.9	13.7	15.0	13.5	12.5	14.9	14.7	14.1	12.2	14.3	14.9	13.8
	LSD at 0.05 level		(b: ns)	(f: ns)	(r: 1.17)	(b*f: ns)	(b*r: ns)	(f*r: ns)	(b*f*r: ns)					
	Wt. of 100 seed (g.)	SSP	80.7	82.1	85.8	82.9	92.2	92.7	91.2	92.1	86.5	87.4	88.5	87.5
		TSP	80.7	86.0	86.1	84.3	92.2	95.0	89.5	92.3	86.5	90.5	87.8	88.3
		PA	80.7	85.7	86.3	84.2	92.2	98.7	96.8	95.9	86.5	92.2	91.5	90.1
		mean	80.7	84.6	86.1	83.8	92.2	95.5	92.5	93.4	86.5	90.0	89.3	88.6
	LSD at 0.05 level		(b: 5.45)	(f: ns)	(r: ns)	(b*f: ns)	(b*r: ns)	(f*r: ns)	(b*f*r: ns)					
Biological yield	straw yield (kg fed ⁻¹)	SSP	1471	1913	1830	1738	1650	2038	2333	2007	1561	1976	2082	1873
		TSP	1471	2064	1991	1842	1650	2134	2071	1952	1561	2099	2031	1897
		PA	1471	1783	1938	1731	1650	2213	2131	1998	1561	1998	2035	1864
		mean	1471	1920	1920	1770	1650	2129	2178	1986	1561	2024	2049	1878
	LSD at 0.05 level		(b: 140.4)	(f: ns)	(r: 172)	(b*f: ns)	(b*r: ns)	(f*r: ns)	(b*f*r: ns)					
	Seed yield (kg fed ⁻¹)	SSP	1189	1343	1451	1328	1627	1892	1854	1791	1408	1617	1653	1559
		TSP	1189	1394	1557	1380	1627	1971	2032	1877	1408	1683	1795	1629
		PA	1189	1347	1421	1319	1627	1820	1764	1737	1408	1583	1593	1528
		mean	1189	1361	1476	1342	1627	1894	1883	1802	1408	1628	1680	1572
	LSD at 0.05 level		(b: 42.2)	(f: 51.7)	(r: 51.7)	(b*f: ns)	(b*r: 73.1)	(f*r: 89.5)	(b*f*r: ns)					
Seed quality	Crude protein %	SSP	21.4	21.9	21.8	21.7	23.6	24.9	23.8	24.1	22.5	23.4	22.8	22.9
		TSP	21.4	22.4	22.8	22.2	23.6	25.7	24.1	24.5	22.5	24.0	23.4	23.3
		PA	21.4	20.5	21.6	21.2	23.6	26.1	25.6	25.1	22.5	23.3	23.6	23.1
		mean	21.4	21.6	22.1	21.7	23.6	25.6	24.5	24.6	22.5	23.6	23.3	23.1
	LSD at 0.05 level		(b: 1.20)	(f: ns)	(r: ns)	(b*f: ns)	(b*r: ns)	(f*r: ns)	(b*f*r: ns)					

On the other hand, the majority of growth parameter and biological yield were significantly increased with addition of different P fertilizer forms under 75% or 100% RD compared with control without significant difference among them, this means that the 75% RD is suitable to be a recommended rate

which gave the highest quantities and quality yields. The relative increases were 44.4, 17.21, 29.7 and 15.6% over the control for number of branches /plant, number of pods/plant, shoot yield and seed yields respectively.

Phosphorus efficiency:

The results in Table (7) revealed that seed inoculated with PDB resulted in improving phosphorus use efficiency (PUE), phosphorus agronomic efficiency (PAE) and phosphorus recovery efficiency (PRE) over uninoculated seed. The relatively increases were 33.0, 101.1 and 138.1% respectively. The increasing rates of different phosphorous forms were found to be decreased all efficiencies, this is may be due to the utilization of nutrient decreased with increasing the rate of nutrient application as fixed by law of limiting factor (Blackman,1905) and Liebig's law of the minimum (Liebig, 1840). Concerning P form efficiencies, it showed that triple super phosphate (TSP) has the greater values for item Phosphorus efficiency at 75% RD addition compared other forms.

Table (7): Effect of experimental treatments on phosphorus efficiencies

Treatments		PUE (kg kg ⁻¹) P ₂ O ₅	PAE (kg kg ⁻¹) P ₂ O ₅	PRE (kg kg ⁻¹) P ₂ O ₅
(PDB) (-) or (+)	P- forms P- rats (RD)			
Uninoculation	SSP	0%	-----	-----
		75%	39.78	17.62
		100%	32.24	13.79
	TSP	0%	-----	-----
		75%	41.32	23.63
		100%	34.60	19.71
	PA	0%	-----	-----
		75%	39.92	13.91
		100%	33.09	18.00
	sum		220.95	106.67
Inoculation	SSP	0%	-----	-----
		75%	56.06	37.62
		100%	41.20	33.92
	TSP	0%	-----	-----
		75%	58.41	42.81
		100%	45.15	32.05
	PA	0%	-----	-----
		75%	53.91	40.65
		100%	39.21	27.43
	sum		293.94	214.48
Relatively Increases (%)		33.04	101.07	138.119
PUE = phosphorus use efficiency			PAE = phosphorus agronomic efficiency	
PRE = phosphorus recovery efficiency				

CONCLUSIONS

From the aforementioned results, it could be concluded that phosphate dissolving bacteria (PDB) inoculation improved PDB population and P availability in faba bean rhizosphere particularly at 75% RD of TSP addition, hence 25% reduction in the amount of P mineral fertilizer applied is possible. Also, it has greater impact on macronutrients and their uptake by plants due to its positive effect on soil fertility, and in turn on plant productivity. So, it is recommended by inoculating faba bean with BDP (*Bacillus megaterium var.*) in calcareous soil as an environment-friendly, economically feasible and viable substitute to obtain the highest efficiency of mineral P fertilizers and the highest productivity under the experiment conduction.

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**تحسين كفاءة الفوسفور الغير عضوى باستخدام البكتريا المذيبة للفوسفات فى الاراضى الجيرية
أحمد خليل عامر – قدرية مصطفى العزب – عبدالله مختار عبد الفتاح – مدحت يمانى ابوزيد
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أجريت تجربة حقلية بنظام القطع المنشفه لمرتين بالأراضى الجيرية بمحطه بحوث النوبارية – محافظة البحيره مصر . خلال الموسمين الزراعيين (٢٠١٢-٢٠١٣ و ٢٠١٣-٢٠١٤) لدراسه فعالية تلقيح بذور الفول البلدى (صنف سخا ٤) بالبكتريا المذيبة للفوسفات بجانب صور ومعدلات مختلفه من الأسمدة الفوسفاتية على زيادة تيسير الفوسفور فى التربة وتحسين كفاءة إستخدام تلك الأسمده المضافة اليها لتحسين إنتاجية الفول البلدى . أستعملت ثلاث صور مختلفه من الأسمدة الفوسفاتية وهى سوبر فوسفات الكالسيوم ١٥.٥% - ثلاثى سوبر فوسفات ٤٦% و حامض الفوسفوريك ٧٢.٤% بمعدل : ٧٥% أو ١٠٠% من المعدل الموصى به.

أوضحت النتائج أن التلقيح بالبكتريا المذيبة للفوسفات زادت من كثافة ومستعمرات تلك البكتريا فى منطقه انتشار الجزور كذلك ايضا زادت من تيسير الفوسفور بالتربة بدرجة معنوية سواء استعملت بمفردها او كانت بجانب الصور المضافة من الأسمده الغير عضوية مقارنة بعدم استعمالها. إضافة ٧٥% و ١٠٠% من الموصى به من ثلاثى سوبر فوسفات مصحوبا بالتلقيح بالبكتريا أعطت أعلى نسبة مئوية لتيسير الفوسفور حيث بلغت ١٢٥.٨% و ١٣٦.٤% عند العمر الأول (٥٠ يوم من الزراعة) وأقل نسبة مئوية (٥٠.٩% و ٥٣.٣%) عند الحصاد على التوالي . تشير النتائج الى زياده الممتص من المغذيات الكبرى والتي إنعكست إيجابيا على زيادة كمية المحصول وجودته. ويمكن التوصية بإضافة ٧٥% من المعدل الموصى به من سماد ثلاثى سوبر فوسفات (التريل) بجانب التلقيح بالبكتريا المذيبة للفوسفات لتحسين إنتاجية الفول البلدى وزيادة محصول الحبوب بالتلقيح حوالى ٤١,٤% من عدمة فى وجود الأسمدة الفوسفاتية وحوالى ٣٤,٢ و ٣٦,٩% لكل من التلقيح وسماد ثلاثى الفوسفات على التوالي كعوامل فردية . كما لوحظ أن تلقح البذور بالبكتريا المذيبة للفوسفور بجانب الأسمده الفوسفاتيه المستخدمة زاد من كفاءة إستخدام الفوسفور والكفاءة الزراعية للفوسفور وكفاءة إسترداد الفوسفور بحوالى ٣٣ و ١٠١% و ١٣٨% على التوالي مقارنة بالمعاملات غير الملقحه. أظهر سماد ثلاثى سوبر فوسفات أفضل القيم لهذه الكفاءات خاصة عند استخدام ٧٥% من المعدل الموصى به مقارنة بصور الأسمده الأخرى المستخدمة.