Performance of Egyptian Native Phosphate Solubilizing Bacteria for Improving Growth and Phosphorus Uptake of Wheat (*Triticum aestivum L.*) Grown in a Calcareous Soil

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ABSTRACT: A study was carried out to evaluate the potential of three isolated Egyptian native phosphate solubilizing bacteria (PSB) exhibiting phosphate-dissolution ability and indole acetic acid (IAA) production to test whether these bacteria are capable of increasing the available P in a calcareous soil treated with single superphosphate (SSP) and its uptake by wheat (*Triticum aestivum* L.) variety Gemiza-11. Therefore, a pot experiment trial using a calcareaous soil was conducted at the experimental farm of Arid Lands Cultivation Research Institute - City of Scientific Research and Technological Applications. The inoculated and uninoculated wheat seeds were sown on 1st, December, 2015 for 60 days. The isolates were *Enterobacter aerogenes*, *Pantoea sp.*, *Enterobacter sp.*, and their mixture in the ratio (1:1:1) were tested in combination with four levels of SSP (0%, 50%, 75% and 100% of recommended dose for wheat (150 kg/Fed.). Each treatment was set up in triplicate in a randomized complete block design. Phosphorus application or phosphorus solubilizing bacterial inoculation and their interaction significantly affected the plant dry weight, available P in soil and P uptake. Co-inoculation of these PSB strains (mixed culture) could act synergistically and were responsible for the increase in plant growth, P uptake and available P in soil in comparison with single inoculation. Accordingly, we can reduce SSP application to 75% of recommended dosage + inoculation with mixed culture of these PSB with significant promotion of wheat growth more than application of 100% SSP of recommended dosage without PSB inoculation to save chemical fertilizer application and maintain environment and soil health.

Keywords: available phosphorus, calcareous soil, single superphosphate ,phosphate solubilizing bacteria.

INTRODUCTION

Phosphorus (P) is the second most important macro-nutrient required by plants, next to nitrogen. Compared to other essential macronutrients (with exception of nitrogen), P is one of the less-abundant elements in the lithosphere (Jones and Oburger, 2011), thus often regarded as a limiting nutrient in agricultural soils. Therefore, it becomes quite common to use P fertilizers to improve P nutrition for plants. Upon application P fertilizers to soil, phosphorus rapidly is fixed by forming un available complex with Al or Fe in acid soils or with Ca in calcareous soils (Toro, 2007). Calcareous soils contain relatively enormous amounts of

inorganic P, but due to P-fixation little is available for crop use. Frequent application of P fertilizers, on the other hand, is well-known to be a costly affair and environmentally undesirable too.

Alternatively to P fertilization, many soil microorganisms that play a role in soil P dynamics, often termed as phosphate solubilizing microorganisms (PSM), could provide the available forms of P to the plants which increase P uptake and for that reason a plausible alternative to chemical phosphatic fertilizers (Khan et al., 2006). Bacterial genera like *Azotobacter*, *Azospirillum*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Pantoea*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria (Sudhakar et al., 2000; Mehnaz and Lazarovits, 2006; Bhattacharyya and Jha, 2012). The microbial solubilization of soil phosphorus has often been due to the excretion of organic acids. The role of organic acids produced by PSM in solubilizing insoluble phosphate may be due to the lowering of pH, chelation of cations and by competing with phosphate for adsorption sites in soil (Nahas, 1996).

Although these bacteria can be used as plant growth promoters, in general their amounts in soil are usually not enough to compete with different well-established bacteria in the rhizosphere, accordingly it is critical to improve and increase their levels by soil inoculation with adequate strains (Igual *et al*., 2001). In this sense, a pre-requisite for introducing these beneficial bacteria in the environment, alongside the ability to promote plant growth, is to be adapted to the soil conditions and efficiently compete with the soil microflora (Taurian *et al*., 2010). In this regard, the isolation and characterization of native bacteria could provide adequate strains for inoculation in particular soils where these microorganisms are well adapted.

Due to phosphorous solubilizing ability from insoluble inorganic pools of total soil phosphorous, PSMs have been widely used as inoculants to improve phosphorous uptake and crop yield (Khalid et al., 2004; Hameeda *et al*., 2006; Chen *et al*., 2008). Plant growth promotion and increased phosphorous availability due to inoculation of PSMs have been assessed in several studies under green house as well as field conditions (Reyes *et al*., 2002; Zaidi *et al*., 2003).

The organisms with phosphate-solubilizing potential increase the availability of soluble phosphate and might improve plant growth by production of plant growth-promoting regulators such as indole acetic acid (IAA) as stated by Ponmurugan and Gopi (2006). Generally, IAA influences plant cell division, extension and differentiation, initiates lateral and adventitious root formation; increases root surface area and length and thereby provide the plant greater access to soil nutrients. Also, rhizobacterial IAA loosens plant cell walls and

facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria (Glick, 2012).

The present study aimed to evaluate the potential of Egyptian native phosphate solubilizing bacteria (ENPSB) and different single super phosphate (SSP) levels to promote growth of wheat plants grown in a calcareous soil of Northern-western coast of Egypt. For that, the native PSB strains, *Enterobacter aerogenes* (ENPSB 1), *Pantoea sp.* (ENPSB 2), *Enterobacter sp.* (ENPSB 3) and mixture of them (1:1:1) were evaluated as inoculants in combination with different levels of single super phosphate fertilizer (SSP) on growth and P uptake of wheat (*Triticum aestivum L*.) in addition to available P in soil.

MATERIALS AND METHODS

1. Soil:

Calcareous soil sample (0-30 cm) was collected from the experimental farm of Arid Lands Cultivation Research Institute (ALCRI) - City of Scientific Research and Technological Applications located in Borg Al-Arab City (30° 53´ 33.17" N, 29° 22´ 46.43" E), West Alexandria, Egypt. The soil sample was air-dried, grinded and passed through a 2-mm sieve. This sample was analyzed according to the method described by Page et al. (1982).

Soil pH was measured in 1:2.5 soil-water suspension after shaking for 30 min, the pH was measured by pH-meter (Pansu and Gautheyrou, 2006a). Electrical conductivity (EC, dS m^{-1}) was measured in the saturated extract of soil paste using a conductivity meter WTW InoLab (WTW cond 720, Weilheim, Germany) The total content of calcium carbonate was determined by calcimeter method (Pansu and Gautheyrou, 2006b). Soil organic matter content was determined by Walkley and Black method by oxidizing the organic carbon with potassium dichromate, H_2SO_4, and H_3PO_4, and titration the excess of dichromate by ammonium ferrous sulfate (Nelson and Sommers, 1982) and organic matter was calculated by the following equation:

$$OM\ \% = OC * 1.72$$

The amount of available phosphorus was determined by Olsen method (0.5 M $NaHCO_3$, pH 8.5) and phosphorus in the clear solution (1:20, soil : 0.5 M $NaHCO_3$ suspension) was measured depending on color density by the ascorbic acid method using PG Instruments T80 UV/VIS Spectrophotometer (Olsen and Sommers, 1982). The amount of available K was extracted by neutral normal ammonium acetate solution (1 N, NH_4OAc of pH 7) in a 1:10 soil: solution ratio, shaken for 15 min and centrifuged at 2000 rpm for 5 min (this step repeated 2 more times). The supernatant was decanted into a 100 ml volumetric flask and K^+ was measured by flame photometer (Knudsen *et al*., 1982).The amount of total

nitrogen (TN) was determined by Kjeldahel distillation method (digested and determined according to Bremner and Mulvaney, 1982). This method consists of three steps: 1) Digestion of the soil sample in sulfuric acid with catalyst (100 g K_2SO_4:10 g $CuSO_4$:1g Se). The nitrogen contained in the sample is converted to ammonia and ammonium sulphate is formed. 2) Distillation of ammonia; its release from ammonium sulphate by addition of an excess of sodium hydroxide and ammonia is trapped in a trapping solution (Boric acid). 3) Back-Titration of the excess of the trapping solution. The amount of available nitrogen was extracted by 2.0 M KCl solution (to extract available NH_4^+ and NO_3^-), then determined by Vapodest 30s Gerhardt Kjeldahel distillation unit (Keeney and Nelson 1982). The particle size distribution: sand, silt and clay were determined by the hydrometer method using sodium hexametaphosphate as a dispersing agent (Gee and Bauder, 1986) and the soil texture was determined. The tested chemical properties and soil texture of soil are presented in Table 1.

Table (1). Some initial chemical properties and soil texture of the experimental soil

Properties	value
Soil texture class	Sandy Loam
Sand %	65.3
Clay %	16.0
Silt %	18.7
Organic matter, %	0.97
EC_e dS/m (saturation extract)	2.27
pH (1:2.5 w/v)	8.39
Field Capacity, %	25
$CaCO_3$, %	31.40
Available P, mg kg^{-1}	6.12
Avalable K, mg kg^{-1}	440
Total N, mg kg^{-1}	300
Total P, mg kg^{-1}	492.5

2. Preparation of inoculums:

Three efficient PSB strains *Enterobacter aerogenes* (ENPSB 1), *Pantoea sp.* (ENPSB 2) and *Enterobacter sp.* (ENPSB 3), previously isolated from soils sampled from rizosphere of grown wheat using the technique described by (Nautiyal, 1999). The isolated strains were characterized as phosphate solubilizing and IAA producing bacteria (data not shown) and grown on nutrient agar, then pure single colony of each strain was transferred into 500 ml flasks containing nutrient broth and grown aerobically on a rotating shaker (150 rpm) for 48h at 30 ^oC, after

that cells were harvested by centrifugation at 3000 rpm for 20 min and re-suspended in sterile 0.85 % NaCl solution. The bacterial suspension was then diluted in sterile distilled water to give a final concentration 10^8 CFU ml^{-1} for use in soil inoculation studies. For mixed inoculation, an equal volume containing (10^8 CFU ml^{-1} of each strain) were mixed (1:1:1).

3. Pot experiment:

A pot experiment was carried out at (ALCRI) experimental Farm. Pots of 17 cm in diameter and 20 cm in depth were sterilized with 1.5% sodium hypochlorite, then sterile water to remove the excess of hypochlorite and filled with 2 kg of prepared soil. Wheat seeds (*Triticum aestivum* L.) variety Gemiza-11 was obtained from Agricultural Research Centre, Egypt. The seeds were surface sterilized by soaking in 70% ethanol for 3 min and then in 1% sodium hypochlorite (bleach) for 10 min. To remove the residual bleach, the seeds were washed ten times with sterile tap water and air dried and soaked (except un-inoculated pots) separately with the culture broth of each PSB inoculants for 10 min before sowing. In addition, each seed was inoculated with 1 ml of PSB inoculants 10^8 CFU ml^{-1} after sowing to ensure soil inoculation.

Wheat seeds were sown at 1 cm depth (10 seeds pot^{-1}) on 1st, December, 2015. Seed germination percent was calculated after 7 days of sowing (90-100 %) and then were thinned to five seedlings in each pot. Experiment was set up in an open environment covered with wire. The pots were watered weekly to maintain soil moisture at field capacity 25%. Recommended doses of N fertilizer (300 kg fed^{-1} of NH_4NO_3 33.5% N at the rate of 100 kg N fed^{-1}) were applied as a main dose in all treatments and added in three equal doses before irrigation. The first dose was added at the time of sowing, the second was applied after 20 days from cultivation and the third one was after 40 days from planting. Recommended dose of K fertilizer (50 kg fed^{-1} of potassium sulphate 48% K_2O) was applied before sowing of seeds. Four levels of P fertilizer was applied as single super phosphate (SSP) 15% P_2O_5, as percent of recommended dose (150 SSP kg fed^{-1}) which are, 100%, 75%, 50% and 0% (not treated with phosphorus fertilizer) with or without PSB. Phosphorus dose was added before sowing and mixed well with soil. The experiment was set in a randomized complete block design (RCBD) with three replicates. There were 20 treatments in the experiment (4 levels of SSP 0, 50, 75 and 100%, and 5 inoculation treatments ENPSB 1, 2, 3, mixed culture and control without inoculation) as follows (Table 2):

Table (2). Treatments of PSB inoculation and SSP application

No.	Treatment
T1	Control + 0 % SSP
T2	Control + 50 % SSP
T3	Control + 75 % SSP
T4	Control+ 100% SSP
T5	*Enterobacter aerogenes* + 0 % SSP
T6	*Enterobacter aerogenes* + 50 % SSP
T7	*Enterobacter aerogenes* + 75 % SSP
T8	*Enterobacter aerogenes* + 100% SSP
T9	*Pantoea sp.* + 0% SSP
T10	*Pantoea sp.* + 50 % SSP
T11	*Pantoea sp.* + 75% SSP
T12	*Pantoea sp.* + 100 % SSP
T13	*Enterobacter sp.* + 0% SSP
T14	*Enterobacter sp.* + 50 % SSP
T15	*Enterobacter sp.* + 75 % SSP
T16	*Enterobacter sp.* + 100% SSP
T17	Mixed culture (1:1:1) + 0 % SSP
T18	Mixed culture (1:1:1) + 50 % SSP
T19	Mixed culture (1:1:1) + 75 % SSP
T20	Mixed culture (1:1:1) + 100% SSP

After 60 days of cultivation, plants were harvested, root and shoot portions of plants were separated and data regarding growth (shoot dry weight and root dry weight) were measured. They were oven dried at 70°C to a constant weight and grinded after drying. Dry ashing was conducted to determine total P by burnt in a muffle furnace at 500 °C for 6 hours using 1.00 g of plant material in crucible. At the end of the ashing period, the crucible was removed from the muffle furnace, cooled, and the ash is dissolved by adding 10 ml dilute aqua regia (concentrated HNO_3 + HCl (1 : 3)) and after cooling to room temperature, the digested solution was filtered into a 100-ml volumetric flask and brought to volume with de-ionized water. Total P in the extract of dry-ashing extraction was determined by ammonium paramolybdate-vanadate method (Olsen and Sommers, 1982). The P uptake was calculated by multiplying the biomass dry weight with its P concentration. Rhizosphere soil samples were collected from each treatment by uprooting the plants carefully without damaging the root system. Roots were shaken gently to remove loosely adhering soil particles from each treatment and then the soils were analyzed for measuring available P content which extracted by the bicarbonate method and determined using the molybdate blue color method (Olsen et al., 1954).The data were analyzed by two way analysis of variance (ANOVA) at $p \leq 0.05$ using statistical functions of Co-Stat software for statistics (2004). Further Least

significant difference ($L.S.D_{0.05}$) test was used to differentiate between significant and non-significant results.

RESULTS AND DISCUSSION

1. Soil:

The soil sample show different chemical properties (Table1). The soil is typic calciorthids since the soil has high $CaCO_3$ content (31.4%) and pH value of 8.39. The total P content was about 492.5 mg/kg soil. On the other hand, the available P content was 6.12 mg/kg which suggests little available P content (according to the critical value of Olsen et al., 1954) comparatively to the high total P in the soil. This effect of reduced P availability in the calcareous soil (alkaline soil) is driven by the reaction of P with calcium, with the lowest solubility of these calcium phosphate minerals.

2. Plant growth:

The data in Table 3 showed significant effect of bacterial strains inoculation and Single Super Phosphate (SSP %) level on shoot, root and whole plant dry weights. The main effects of PSB and SSP fertilizer level showed that the maximum values were obtained by mixed culture and application of SSP at level of 75% from recommended dose. The interaction effect of bacterial inoculation and SSP application on the plant growth parameters (Table 4) showed that the maximum values were obtained practically by co-application of mixed culture + 75% SSP and it was significantly higher than control treatment supplemented with 100% SSP.

Table (3). Main effects of SSP % and PSB on plant growth

Treatments	Root dry weight (g/plant)	Shoot dry weight (g/plant)	Plant dry Weight (g/plant)
SSP Level:			
0 %	0.13 b	0.722 c	0.852 c
50 %	0.14 b	0.848 b	0.988 b
75 %	**0.18 a**	**1.032 a**	**1.212 a**
100 %	**0.17 a**	**1.016 a**	**1.186 a**
L.S.D (5%)	**0.016**	**0.08**	**0.087**
PSB strain:			
Control	0.13 d	0.636 e	0.766 e
E. aerogenes	0.16 b	1.041 b	1.201 b
Pantoea sp.	0.14 cd	0.801 d	0.941 d
Enterobacter sp.	0.15 bc	0.909 c	1.059 c
Mix culture	**0.19 a**	**1.139 a**	**1.329 a**
L.S.D (5%)	**0.018**	**0.089**	**0.098**

Values marked with the same alphabetical letter(s) within comparable means in the same column do not differ significantly using the revised L.S.D. test at 0.05 levels.

Inoculation by PSB with inorganic P increased growth of wheat and other crops as reported by many researchers (Tomar et al*.,* 1996; Chaykovskaya et al*.,* 2001). These findings were almost similar to those of Afzal *et al.* (2005) who observed that phosphate solubilizing microorganisms alone or along with other combinations showed profound influence on growth and biological yield of wheat. Dwivedi *et al.* (2004) reported that pre sowing inoculation of wheat seeds with phosphate solubilizing microorganisms led to a yield increase over non-inoculated treatments.Co-inoculation of these three PSB strains showed a significant increase in plant dry weight of wheat seedlings compared to either individual inoculation (Table 3), indicating that all the entire three PSB strains might act synergistically with each other in improving wheat growth.

Inoculation with mixed different strains could be a suitable substitute to individual inoculation with single strains, likely reflecting the combination of different mechanisms used by each strain in the consortium. Co-application of *Pseudomonas striata* and *Bacillus polymyxa* strains showing phosphate solubilizing ability, with a strain of *Azospirillum brasilense*, resulted in a significant improvement of grain and dry matter yields, with a concomitant increase in P uptakes (Alagawadi and Gaur 1992). Afzal and Bano (2008) reported improvement in the yield of different crops in response to seeds or soil inoculation with phosphate-solubilizing organisms and other plant growth-promoting rhizobacteria.

Table (4). Effect of SSP % and PSB interaction on plant growth

Treatments	Root dry weight (g/plant)	Shoot dry Weight (g/plant)	Plant dry Weight (g/plant
0% + Control	0.090	0.379	0.469
0 % + *E. aerogenes*	0.146	0.859	1.005
0 % + *Pantoea sp.*	0.128	0.711	0.839
0 % + *Enterobacter sp.*	0.135	0.720	0.855
0 % + Mix culture	0.157	0.938	1.095
50 % + Control	0.128	0.629	0.757
50 % + *E. aerogenes*	0.160	0.998	1.158
50 % + *Pantoea sp.*	0.130	0.777	0.907
50 % + *Enterobacter sp.*	0.135	0.759	0.894
50 % + Mix culture	0.162	1.077	1.239
75 % + Control	0.149	0.782	0.931
75 % + *E. aerogenes*	0.179	1.185	1.364
75 % + *Pantoea sp.*	0.154	0.858	1.012
75 % + *Enterobacter sp.*	0.167	1.020	1.187
75 % + Mix culture	**0.229**	**1.323**	**1.552**
100 % + Control	0.151	0.753	0.904
100 % + *E. aerogenes*	0.164	1.121	1.285
100 % + *Pantoea sp.*	0.147	0.857	1.004
100 % + *Enterobacter sp.*	0.156	1.135	1.291
100 % + Mix culture	0.218	1.217	1.435
L.S.D (5%)	**0.036**	**0.183**	**0.200**

Several authors conducted experiments on wheat under pot or field conditions to examine the effect of co-inoculations of PGPR on the growth and yield of wheat. Kumar *et al.* (2014) found that *B. megaterium, A. chlorophenolicus* and *Enterobacter sp.* significantly improved plant height, grain yield and straw yield. Baris et al. (2014) concluded that co-inoculation with *Bacillus subtilis* 05U142, *B. megaterium M3*, and *Azospirillum brasilense* Sp245 provided greater plant nutrient element concentrations than mineral fertilizer application.

Jin-Hee *et al*. (2016) assessed the synergistic effect on phosphate solubilization of single- and co-inoculation of two phosphate solubilizing bacteria, *Burkholderia anthina* PSB-15 and *Enterobacter aerogenes* PSB-16. Based on the plant growth promotion bioassay, co-inoculated mung bean seedlings recorded higher growth than control. Therefore, co-inoculation of the strains *B. anthina* and *E. aerogenes* displayed better performance in promoting plant growth than single inoculation with each strain.

Also, there are numerous examples in wheat whereby synergistic effects of different plant growth promoting rhizobacteria (PGPR) are observed. Among those, notable is the co-application of mixtures and biofilmed bio-inoculants (*Anabaena torulosa* + *Pseudomonas striata* and/or *Anabaena torulosa* + *Azotobacter chroococcum*) were superior over single inoculation and chemical fertilizer control in term of plant growth and nutrient uptake (Swarnalakshmi et al., 2013). The benefits can be on nutrient uptake, but also in root physiology as exemplified by Manjunath *et al*. (2011) as co-inoculation of wheat with two proteobacterial (*Providencia* sp. and *Alcaligenes* sp.) and two cyanobacterial (*Anabaena oscillarioides* and *Anabaena torulosa*) inoculants. Minaxi *et al.* (2013) found that Seed bacterization with strains, *P. fluorescens* BAM-4 and *B. cepacia* BAM-12 single or combined significantly increased growth and yield, but increase in bacterial population, spike length, P content of shoots and grain yield was more in co-inoculation treatment than single. The best among the bio inoculation treatments was *B. cepacia* BAM-12 + TCP and *B. cepacia* BAM-12 + *P. fluorescens* BAM-4 + TCP for P content with free and immobilized cells.

3. Available phosphorus:

The data in Table 5 showed the effect of bacterial strains (PSB) and Single Super Phosphate (SSP %) levels on soil available phosphorus. Available phosphorous contents in rhizospheric soil samples revealed that soil samples bacterized with PSB showed significantly (P≤0.05) higher level of available phosphorous compared to the rhizospheric soil samples collected from control treatment. Also, the interaction effect on soil available phosphorus was significant; the maximum soil available phosphorus was obtained by co-application of 100% SSP + Mixed culture (15 mg/kg) followed by 75% SSP + Mixed culture (14.8 mg/kg) with no significant difference; they were significantly higher than the control treatment supplemented with 100% SSP (7.75 mg/kg).

As shown in Table 5, PSB strains were able to increase available phosphorus in soil without addition of SSP. The highest available P value was obtained by mixed culture (9.9 mg P / kg soil) followed by *Enterobacter sp.* (9.6 mg P/ kg soil), *Pantoea sp.* (7.9 mg P / kg soil) and *E. aerogenes* (7.2 mg P/ kg soil) compared to control (6.1 mg P/kg soil). The ability of these PSB strains to increase the concentration of soil available P and to promote plant growth in soils without addition of SSP indicated that phosphate solubilization by these PSB was partly responsible for the promotion of plant growth.

Arid and semi-arid soils contain a high amount of insoluble phosphorus mostly in the form of tri-calcium phosphate (TCP) of which only 2.4–3.9% is present in available form for plants, and rest in unavailable organic (15–20%) and remaining 77–82% is unavailable inorganic form (TCP) (Rao and Tarafdar, 2002). Therefore, TCP needs to be solubilized and mobilized by the inoculants to improve crop yield. Moreover, the strains used under the study were isolated from alkaline calcareous soil of arid region (Northern-western coast of Egypt) which contains TCP as most dominant insoluble P source.

Table (5). Effect of SSP % and PSB interaction on available P in soil

PSB strains	SSP levels			
	0%	50%	75%	100%
Control	6.1	6.6	6.6	7.7
E. aerogenes	7.2	12.6	12.9	13.5
Pantoea sp.	7.9	9.5	11.8	11.3
Enterobacter sp.	9.6	10.3	11.6	13.0
Mix culture	9.9	12.7	14.8	15.0
L.S.D. (5%)	**2.54**			

Sundara *et al*. (2002) found that the application of PSB, *Bacillus megaterium var. phosphaticum*, increased the P availability in the soil. When used in conjunction with P fertilizers, PSB reduced the required P dosage to 75%. Zehra (2010) evaluated the effect of PSB inoculation, *Bacillus* M-13, with and without various levels of phosphorus (P) fertilizer and found that the PSB application was able to mobilize P effectively in the sunflower and increased seed quality and oil yield. However, when PSB was used in co-application with P fertilizers, a much greater effect was observed. It was found that the highest seed yield of sunflower possible with 100 kg P_2O_5 ha^{-1} fertilizer was achieved with about 50 kg P_2O_5 ha^{-1} when used in conjunction with PSB.

4. Phosphorus uptake:

The data in Table 6 showed significant effect of bacterial strains inoculation and Single Super Phosphate (SSP %) level on P concentrations of root and shoot of wheat plants in addition to the P uptake by the whole plant. The main effects of

PSB and SSP fertilizer level showed that the maximum P concentrations for root and shoot or the P uptake by the whole plant were obtained by mixed culture and application of SSP 100% followed by 75% SSP and Mixed culture with no significant difference. The interaction effect between bacterial inoculation and SSP application on P concentrations of root and the phosphorus uptake (Table 7) showed that the maximum value was obtained practically by co-application of mixed culture + 75% SSP, but the maximum value of shoot P concentration was obtained by co-application of mixed culture + 100% SSP followed by mixed culture + 75% SSP with no significant difference; it was significantly higher than control treatment supplemented with 100% SSP or control treatment (0.93 mg/plant).

Table (6). Main effects of SSP % and PSB on P concentration and uptake

Treatments	Shoot P Concentration (mg/g)	Root P Concentrati (mg/g)	P Uptake (mg/Plant)
SSP Level			
0 %	1.77 c	0.82 c	2.29 c
50 %	2.02 b	1.04 b	3.08 b
75 %	**2.42 a**	**1.32 a**	**4.60 a**
100 %	**2.45 a**	**1.43 a**	**4.62 a**
L.S.D (5%)	**0.145**	**0.14**	**0.42**
PSB strain			
Control	1.97 b	0.9 c	2.31 e
E. aerogenes	2.31 a	1.16 b	4.24 b
Pantoea sp.	2.07 b	1.11 b	3.04 d
Enterobacter sp.	2.10 b	1.24 ab	3.59 c
Mix culture	**2.38 a**	**1.35 a**	**5.06 a**
L.S.D (5%)	**0.16**	**0.16**	**0.47**

Values marked with the same alphabetical letter(s) within comparable means in the same column do not differ significantly using the revised L.S.D. test at 0.05 levels.

Gera *et al.* (2005) reported that inoculation of PSB transconjugants as well as parent increased the plant biomass production and P uptake in pot culture. Phosphorus uptake was improved by pearl millet with rock phosphate application and seed inoculation with PSB (Kundu *et al*., 2006). Similar increases in phosphorous uptake of green gram plants due to inoculation of PSB strains was observed by Ghanem and Abbas (2009). Increased phosphorous uptake and growth have been reported in wheat from *Azotobacter chroococum* (Kumar *et al*., 2001), peanut from *Pseudomonas fluorescens* (Dey *et al*., 2004), walnut from *Bacillus cereus* and *Pseudomonas sp.* (Yu et al., 2011), and tomato from *Paenibacillus polymyxa* and *Bacillus megaterium* (El-Yazeid and Abou-Aly, 2011). Combined inoculation of these three PSB strains showed an improvement in dry weight, and P uptake of wheat seedlings compared to either individual inoculation, indicating that all the three PSB strains could act synergistically with each other in promoting wheat growth.

Table (7). Effect of SSP % and PSB interaction on P concentration and uptake

Treatments	Shoot P Concentration (mg/g)	Root P Concentration (mg/g)	P Uptake (mg/Plant)
0 % + Control	1.40	0.586	0.93
0 % + *E. aerogenes*	1.97	0.897	2.88
0 % + *Pantoea sp.*	1.61	0.691	1.92
0 % + *Enterobacter sp.*	1.91	0.810	2.32
0 % + Mix culture	1.96	1.128	3.39
50 % + Control	1.76	0.689	1.85
50 % + *E. aerogenes*	2.19	0.939	3.62
50 % + *Pantoea sp.*	2.02	1.083	2.80
50 % + *Enterobacter sp.*	1.95	1.254	2.88
50 % + Mix culture	2.19	1.223	4.24
75 % + Control	2.31	1.083	3.16
75 % + *E. aerogenes*	2.53	1.399	5.37
75 % + *Pantoea sp.*	2.28	1.173	3.52
75 % + *Enterobacter sp.*	2.29	1.407	4.37
75 % + Mix culture	**2.67**	**1.555**	**6.57**
100 % + Control	2.40	1.252	3.31
100 % + *E. aerogenes*	2.55	1.417	5.09
100 % + *Pantoea sp.*	2.38	1.483	3.91
100 % + *Enterobacter sp.*	2.24	1.467	4.78
100 % + Mix culture	**2.69**	**1.500**	**6.01**
L.S.D (5%)	**0.33**	**0.33**	**0.97**

Similarly, inoculation of PSB strain *P. striata* and N_2-fixing bacterium *Rhizobium sp.* (Vigna) appreciably improved available P of soil, as well as dry matter of plants, and P uptake by chickpea (*Cicer arietinum*) compared to single inoculation of either PSB or N_2 fixers (Wani *et al*., 2007).

The higher responses in dry matter and P uptake to inoculation with combined specific strains in the soil fertilized with SSP in comparison with soil untreated with SSP is probably related to the presence of adequate quantities of readily available P in the soil. Available P in the soil unfertilized with SSP appeared however, to be limiting factor for plant growth (Table 2). Accordingly, any release in P from the native P in soil solution induced by PSB would be reflected positively on growth and P uptake of wheat plant. Greater responses in dry matter and P uptake in the soil treated with SSP was more associated with inoculation of PSB than with single isolates. The significance and positive relationships between available P, P uptake and dry matter (Table 8), indicate that both P uptake and dry matter yield depend on the availability of P in soil.

Table (8). Correlation coefficients among soil available P, P uptake and dry matter of wheat plants for the different treatments

	P uptake	Dry matter
Available P	0.739**	0.715**
Dry matter	0.952**	

* Significant (P<0.05) ** Significant (P<0.01)

According to these results, we can reduce SSP% application to 75% of recommended dosage + inoculation with mixed culture of these PSB with significant promotion of wheat growth more than application of 100% SSP of recommended dosage without PSB inoculation to save chemical fertilizer application and maintain environment and soil health. The positive effects of these strains on plant growth, and phosphorus uptake of wheat plants show the beneficial role of these PGPR which possibly be attributed to IAA production, phosphorus solubilization, or even other non-evaluated PGPR traits that stimulate plant growth.

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الملخص العربي

فعالية استخدام سلالات بكتيرية مصرية أصيلة مذيبة للفوسفات لتحسين نمو القمح وامتصاص الفوسفور في الأرض الجيرية

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تهدف الدراسة الحالية لتقييم كفاءة ثلاث عزلات بكتيرية مصرية أصيلة مذيبة للفوسفات والتي لها القدرة العالية على اذابة الفوسفات وانتاج اندول حمض الخليك واختبار قدرتها على زيادة الفوسفور الميسر في الأرض الجيرية مع اضافة سماد السوبر فوسفات الأحادي وامتصاص الفوسفور بواسطة القمح صنف جميزة ١١. تم اجراء تجربة أصص باستخدام أرض جيرية في المزرعة التجريبية لمعهد بحوث الأراضي القاحلة – مدينة الأبحاث العلمية والتطبيقات التكنولوجية – مدينة برج العرب الجديدة – اسكندرية. تم زراعة حبوب القمح الملقحة بالبكتيريا وغير الملقحة في الأول من ديسمبر ٢٠١٥ ولمدة ٦٠ يوم. وكانت السلالات التي تم استخدامها هي *Enterobacter aerogenes*, *Pantoea sp.*, *Enterobacter sp.* وخليط من الثلاثة معا بنسبة (١:١:١) وباستخدام اربع مستويات من السوبر فوسفات الأحادي (صفر و ٥٠ و ٧٥ و ١٠٠ %) من الجرعة الموصي بها وهى ١٥٠ كجم / فدان. تم اجراء المعاملات باستخدام ثلاث مكررات باستخدام تصميم القطاعات العشوائية الكاملة. أظهرت النتائج أن استخدام الاسمدة الفوسفاتية مع التلقيح بالبكتيريا المذيبة للفوسفات له تأثير معنوي على الوزن الجاف للنبات والفوسفور الميسر في التربة وامتصاص الفوسفور بواسطة النبات. وأدى التلقيح باستخدام خليط من الثلاث سلالات معا الى زيادة اكبر في نمو النبات والفوسفور الميسر في التربة وامتصاص الفوسفور عن التلقيح باستخدام كل سلالة على حدا. وبناء عليه يمكن من خلال الدراسة تقليل جرعة سماد السوبر فوسفات الأحادي الى ٧٥ % من الجرعة الموصي بها مع التلقيح بخليط من الثلاث سلالات معا حيث أدى ذلك الى زيادة معنوية في نمو القمح أفضل من استخدام ١٠٠ % من الجرعة الموصي بها من السماد بدون التلقيح بالبكتيريا وذلك لتوفير استخدام الأسمدة الكيماوية وصيانة البيئة والتربة.