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The Influence of The Isolated *Enterobacter spp* and *Pantoea sp*., on Barley's Phosphorus Uptake Grown in Calcareous Soil

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ABSTRACT

The purpose of this study was to determine the possibility of three phosphate-releasing bacteria (PSB) increasing the bioavailability of phosphorus (P) in a calcareous soil fertilized with single superphosphate (SSP) and its absorption by the barley (*Hordeum vulgare* L.) variety Giza 123. The pot experiment, a trial using calcareous soil, was conducted at the experimental farm located in Borg Al-Arab. The inoculated and uninoculated barley seeds were sown on December 1st, 2020 for 60 days. The bacterial isolates were *Enterobacter aerogenes* (ENPSB 1), *Pantoea sp.* (ENPSB 2), *Enterobacter sp*. (ENPSB 3*)*, and their mixture in the ratio (1:1:1) was tested in combination with four levels of single super phosphate SSP (0%, 50%, 75%, and 100% of the recommended dosage for barley (150 kg/Fed.). In a randomized full-block design, each treatment included three replicates. Treatments and their interactions significantly affected the plant's dry weight, and bioavailable P in the soil. When these PSB strains were inoculated together (in a mixed culture), they were able to operate synergistically and were responsible for an increase in plant growth, P absorption, and accessible P in the soil when compared to a single inoculation. Also, plants inoculated with the different PSB isolates had a significant impact on total amino acids as compared with un-inoculated plants. Accordingly, we can reduce SSP application to 75% of the recommended dosage + inoculation with a mixed culture of these PSB to have a major impact on barley growth more than the application of 100% SSP of the recommended dosage without PSB inoculation to maintain environmental and soil health.

INTRODUCTION

Barley (*Hordeum vulgare* L.), a member of the Poaceae family, is the fourth most significant crop in the world and the principal crop farmed on a large scale in Egypt's North Coastal Region, as well as in freshly reclaimed soils. Barley is considered one of the most adapted cereals to environmental conditions, that are not suitable for growing other cereal crops. Its grains are used for food and malting purposes, while straws provide an important source of roughage for feeding animals (El-Sheshtawy *et al.*, 2018).

Plants require phosphorus (P) as the second most important macronutrient after nitrogen. P is one of the lithosphere's less abundant elements (with the exception of nitrogen), and as a result, it is frequently viewed as a limiting nutrient in agricultural soils when compared to other key macronutrients (with the exception of nitrogen). Therefore, using mineral phosphorus fertilizers to increase plant P nutrition has become relatively widespread (Maharajan *et al.,* 2018). Phosphorus is rapidly fixed in the soil after the application of P fertilizers by producing an unavailable complex with Al or Fe in acid soils or with Ca in calcareous soils (Toro, 2007). Inorganic P is abundant in calcareous soils, but due to P-fixation, only a small amount is accessible for crop use. On the other hand, frequent use of P fertilizers is well-known to be both expensive and unfavorable in the agroecosystem. Unlike chemical fertilizers, biofertilizers are generally based on plant growth-promoting rhizo-bacteria (PGPR), which boost crop productivity and soil fertility without harming the environment. PGPR promotes crop growth and health in a variety of ways. They've been linked to nitrogen fixation pathways, mineral solubilization (Zn, Fe, P), and increased tolerance to biotic and abiotic stressors (Santos *et al.,* 2019; and Ramakrishna *et al.,* 2020).

Instead of phosphorus soil additives, several soil bacteria that have a role in soil P dynamics can be employed. Phosphate solubilizing microorganisms (PSM) are microorganisms that solubilize phosphate, possibly providing plants with the accessible forms of P, boosting P uptake and so presenting a viable alternative to chemical fertilizers (Sharma *et al.,* 2013). The most important phosphate-solubilizing bacteria have been identified as *Azotobacter, Azospirillum, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Pantoea, Flavobacterium, Microbacterium Pseudomonas, Rhizobium,* and *Serratia* (Sudhakar et al., 2000; Mehnaz and Lazarovits, 2006; Bhattacharyya and Jha, 2012). The excretion of organic acids has often been blamed for the microbial solubilization of soil phosphorus. PSM's organic acids may improve the solubilization of insoluble phosphate by decreasing pH. chelating cations compete for adsorption sites in the soil with phosphate. (Nahas, 1996). According to Chen and Liu (2018), rice seedling development inoculated with S32 (*Pantoea sp*.) demonstrated the most significantly enhanced plant height, biomass, root growth, and P absorption. The isolated S32 strain has a high P-solubilization ability for both Pi and Po, as well as a reclaimed soil alleviation effect. Several bacteria identified from the rhizosphere of hard plants, such as (*Advenella mimigardefordensis, Bacillus cereus, Bacillus megaterium,* and *Burkholderia fungorum*) were able to dramatically enhance levels of absorbed phosphate and dry weight of ears when compared to non-inoculated plants. Because these strains were able to boost barley crop growth and productivity, they might be used as biofertilizers (Ibáez *et al.,* 2021).

As a result, soil inoculation with appropriate strains is necessary to enhance and raise their levels (Igual *et al.,* 2001). In this view, being suited to the soil conditions and successfully competing with the soil microflora is a prerequisite for bringing these beneficial bacteria into the environment, alongside the potential to support plant development (Taurian *et al.,* 2010).

PSMs have been commonly applied as inoculants to enhance phosphorous uptake and crop production due to their ability to release phosphorous from insoluble inorganic sources of total soil phosphorous (Khalid *et al.,* 2004; Hameeda *et al.,* 2006; Chen *et al.,* 2008). Besides P solubilization, these microorganisms promote plant growth and development by other activities, such as nitrogen fixation and production of plant phytohormones (Zaidi *et al.,* 2009). Organisms having phosphate-solubilizing capability enhance soluble phosphate availability and may promote plant development by generating plant growth-

promoting regulators such as indole acetic acid (IAA) (Ponmurugan and Gopi 2006; Liu *et al.,* 2018). IAA affects plant cell division, extension, and differentiation, as well as the production of lateral and adventitious roots. It also increases root surface area and length, allowing the plant to absorb more nutrients from the soil. In addition, rhizobacterial IAA loosens plant cell walls and stimulates an increase in root exudation, which provides more nutrients to help rhizosphere bacteria grow (Glick, 2012). Two PGPR strains (*Enterobacter aerogenes* (LJL5) and *Pseudomonas aeruginosa* (LJL13)) isolated from the rhizosphere of (*Medicago sativa* L.) were found to promote plant growth, and in a saline-alkali setting, shoot height, fresh and dry weights, yield, and crude protein content all increased significantly (Liu *et al.,* 2018). Furthermore, the majority of PGPR candidates may be hired as eco-friendly biofertilizers and biocontrol agents in organic farming (Utama *et al.,* 2021). Shen et al., (2021) were investigated those amino acids and their derivatives were better to understand the metabolomic mechanism of microbial inoculants on plant growth-promoting effects.

The identification and characterization of local bacteria might lead to the acceptance of acceptable strains for inoculation in certain soils. The goal of this study was to investigate the role of Egyptian native phosphate solubilizing bacteria (ENPSB) and different single super phosphate (SSP) levels that might help barley plants develop on Egypt's calcareous soil on the northern-western coast. In addition to bioavailability in soil, the native PSB strains *Enterobacter aerogenes* (ENPSB 1), *Pantoea sp*. (ENPSB 2), *Enterobacter sp*. (ENPSB 3) and a mixture of them (1:1:1) were evaluated as inoculants when combined with different levels of single super phosphate fertilizer (SSP) on barley (*Hordeum vulgare* L.) growth and P uptake.

MATERIALS AND METHODS

Soil:

Soil collected from the Arid Lands Cultivation Research Institute (ALCRI) farm in the City of Scientific Research and Technological Applications in Borg Al-Arab City (30° 53' 33.17" N and 29° 22' 46.43" E) was taken from the experimental farm there. Analyses were done on the air-dried soil sample after it had been ground up and sieved at 2-mm intervals (Page et al. 1982). After shaking for 30 minutes, a pH meter was used to measure the soil's pH in a 1:2.5 soil-water solution, and the soil's pH was found to be low (Pansu and Gautheyrou, 2006a). WTW InoLab was used to measure electrical conductivity (EC, dS m-1) in the saturated extract of soil paste (WTW cond 720, Weilheim, Germany) The Calcimeter method was used to measure the total calcium carbonate content. b): (Pansu and Gautheyrou, 2006a) Walkley and Black used a method to figure out how much organic matter was in the soil (Nelson and Sommers, 1982). The Olsen method was used to figure out how much phosphorus could be found (Olsen and Sommers, 1982). The amount of available K was extracted and as determined by a flame photometer (Knudsen et al., 1982). The amount of total nitrogen (TN) was calculated by the Kjeldahel distillation technique (Bremner and Mulvaney, 1982). The amount of available nitrogen was extracted by a 2.0 M KCl solution (to extract available NH_4^+ and NO_3^-), then determined by the Vapodest 30s Gerhardt Kjeldahel distillation unit (Keeney and Nelson 1982). The particle size distribution of sand, silt, and clay, as well as the soil characteristics, were examined using the hydrometer method with sodium hexameta-phosphate as a dispersion agent (Gee and Bauder, 1986). Table (1) shows the chemical characteristics of the tested soil texture.

Table 1. The experimental soil's basic chemical characteristics and texture

Properties	value
Soil texture class	Sandy Loam
Sand %	65.3
Clay %	16.0
Silt %	18.7
Organic matter, %	0.97
E C_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
Available K, mg Kg^{-1}	440
Total N, mgKg^{-1}	300
Total P, mgKg^{-1}	492.5

Bacterial Isolates Suspension Preparation:

Three efficient PSB strains, *Enterobacter aerogenes* (ENPSB 1), *Pantoea sp.* (ENPSB 2), and *Enterobacter sp.* (ENPSB 3), were previously isolated from soils sampled from the rhizosphere of grown barley using the technique described by (Nautiyal, 1999). After being put into 500 ml flasks containing nutrient broth and cultivated aerobically on a revolving shaker (150 rpm) for 48 hours at 30 °C, the cells were collected by centrifuging at 3000 rpm for 20 minutes and then suspended in sterile 0.85 percent NaCl solution. For use in soil inoculation investigations, the bacterial solution was diluted in sterile distilled water to a final concentration of 108 CFU ml^{-1} before being added to the soil. For the mixed inoculation, an equal amount of each strain (108 CFU ml^{-1}) was blended with an equal number of the other strains (1:1:1).

Testing the Bacterial Ability for The Phosphate Degradation Process:

1. The Bacterial Ability to Degrade Phosphate Was Tested Using Pikovskaya's Agar Medium:

The three tested P-solubilizing isolates were cultivated at 28° C on Pikovskaya's agar medium (Pi culture medium), which contends with P-solubilizing ability (yeast extract 0.5; dextrose 10.0; calcium phosphate 5.0; ammonium sulphate 0.5; potassium chloride 0.2; magnesium sulphate 0.1; manganese sulphate 0.0001; ferrous sulphate 0.0001, and Agar 15.0). The cells were then incubated at 30° C. After the development of the colonies in 4–5 days and the formation of a distinct phosphate-releasing zone. The size of the phosphate-solubilizing zone was measured for each bacterial colony (Nair et al., 1995; and Chen and Liu, 2018).

Farm's Experiment:

A pot experiment was conducted at the ALCRI experimental farm. Pots with a diameter of 17 cm and a depth of 20 cm were sterilized with 1.5 percent sodium hypochlorite, then filled with 2 kg of prepared soil and sterile water to remove the surplus hypochlorite. Giza 123 barley seeds were received from Egypt's Agricultural Research Centre. The seeds were surface sterilized by soaking them in 70% ethanol for 3 minutes and then in 1% sodium hypochlorite (bleach) for 10 minutes. The seeds were washed 10 times with sterile tap water, air dried, and then soaked for 10 minutes in the culture broth of each PSB inoculant to remove any leftover chemicals (excluding un-inoculated pots). To guarantee soil inoculation, each seed was infected with 1 ml of PSB inoculant (10^8 CFU ml^{-1}) after planting.

On December 1st, 2020, barley seeds were sown at a depth of 1 cm (10 seeds/ pot^{-1}). After seven days of planting, seed germination percentages (90-100%) were

determined, and seedlings were trimmed to five seedlings per pot. The pots were watered once a week to keep the soil moisture at 25% of field capacity. In all treatments, recommended N fertilizer dosages (300 kg fed^{-1} of $NH4NO_3$ (33.5) percent N at a rate of 100 kg N fed^{-1}) were administered as a primary dose and added in three equal doses before irrigation. The first dosage was given when the seeds were planted, the second dose after 20 days, and the third dose after 40 days. Before planting seedlings, a recommended quantity of K fertilizer (50 kg fed^{-1} potassium sulphate 48 percent K_2O) was administered. 100 %, 75 %, 50 %, and 0 percent not treated with phosphorus fertilizer, a single super phosphate (SSP) were applied as 15 percent P_2O_5 as a percent of the recommended dose (150 SSP kg fed^{-1}) with or without PSB were added before seeding and well combined with the soil. With three replicates, the experiment was set up in a randomized complete block design (RCBD). There were 20 treatments in the experiment four levels of SSP (0, 50, 75, and 100%), and five inoculation treatments (ENPSB 1, 2, 3, mixed culture and control without inoculation) as follows:

Table 2. Treatments of PSB inoculation and SSP application

	Treatments
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

Determination Phosphorus Uptake:

After 60 days of culture, the plants were harvested, the root and shoot sections of the plants were separated, and growth (shoot and root dry weight) was measured. They were milled after being oven-dried to a constant weight at 70°C. Dry ashing was conducted to determine total P by burning in a muffle furnace at 500 °C for 6 hours using 1.00 g of plant material in a crucible. When the ashing phase is over, the crucible was removed from the muffle furnace, cooled, and the ash is dissolved by adding 10 ml of dilute aqua regia (concentrated HNO_3 + HCl (1: 3)) and the digested solution was filtered into a 100-ml volumetric flask and brought to volume with de-ionized water after cooling to room temperature. The ammonium paramolybdate-vanadate technique was used to determine total P in the dry-ash extraction.

The biomass dry weight was calculated by the P concentration to get the P uptake. Each treatment's rhizosphere soil samples were gathered by gently uprooting the plants without injuring the root structure. The roots of each treatment were gently shaken to remove loosely adhering soil particles, and the soils were then evaluated for accessible P content, which was extracted using the bicarbonate technique and quantified using the molybdate blue colour method (Olsen et al., 1954).

Some Free Amino Acids Arginine, Proline, And Phenylalanine Were Evaluated:

The content of free amino acids arginine, proline, and phenylalanine was measured according to Umbreit *et al*., (1972). The same extraction was carried out by grinding dry matter in Macllavaine buffer (sodium citrate buffer, pH 6.8). Homogenized for 3 minutes and centrifuged at 4000 rpm for 15 min. The supernatant was then used to determine the content of some free amino acids such as arginine, proline, and phenylalanine (Umbreit *et al.,* 1972). 0.5 mL of extract, 1 mL citrate buffer (pH 5), 0.5 mL of ninhydrin, and 3.5 mL of isopropanol solution were used for this purpose. The optical density of proline was determined spectrophotometrically at 450 nm, 492 nm for phenylalanine, and 515 nm for arginine. In addition, instead of extract, 0.5 ml of distilled water was utilized in the reference cuvette. The concentration of each amino acid was calculated using a standard curve created for the relevant amino acids.

Statistical Analysis:

The data were examined using a two-way analysis of variance (ANOVA) at $p \leq_{0.05}$ with the statistical tools of the Co-Stat programmer for statistics (2004). The Least significant difference ($LSD_{0.05}$) test was also employed to distinguish between significant and non-significant data.

RESULTS

Phosphorus Bioavailability in The Tested Soil:

The soil sample shows 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 bioavailable P content according to the critical value of (Olsen *et al.,* 1954) comparatively to the high total P in the soil. The reaction of P with calcium, which has the lowest solubility of these calcium phosphate minerals, causes lower P bioavailability in calcareous soil (alkaline soil).

Testing the Bacterial Phosphorus Breakdown Efficiency:

Pikovskaya's agar medium (PVK) is usually used to be the detection of phosphate-solubilizing bacteria from soil. The selective media contain insoluble tricalcium phosphate that was used to test the three bacterial isolates' ability for Phosphate solubilization. They were tested according to the formation of the transparent zone on Pikovskaya's agar medium (PVK), (Fig. 1) by measuring the bacterial transparent zone. From Figure (1) we observed that the isolation *Pantoea sp* had the largest clear zone, as measured by the diameter of the transparent zone (D) to colony (d) ratio, compared to *Enterobacter sp* and *Enterobacter aerogenes*. The three PSBs isolates that had a morphological colony were emulsus, yellow, opaque, glossy, and orderly. The colonies appeared clear halo zones surrounding the bacterial growth were considered as phosphate solubilizers. Estimated the bacterial (PSB) ability to convert insoluble form of phosphorous to an available form. That lead us to apply the tested PSB bacteria as inoculants to increase the phosphorus availability and uptake on barley plants.

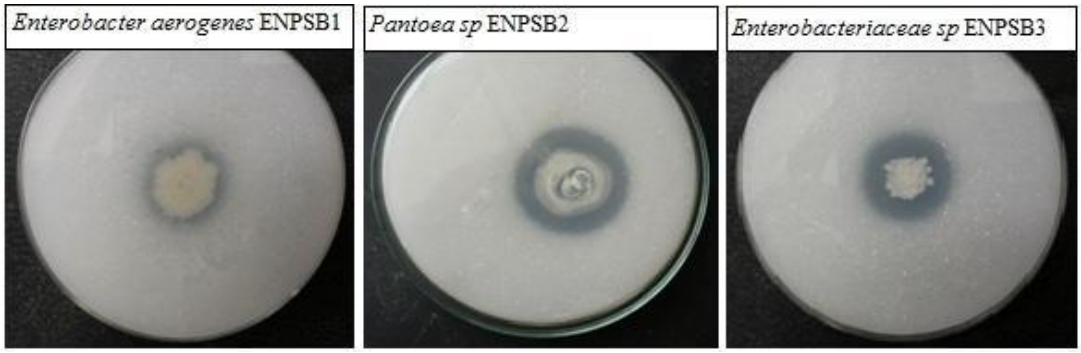

Fig.1. The three bacterial isolates formed a transparent zone on Pikovskaya's agar medium (PVK).

Determination Of Certain Plant Growth Parameters:

The interaction effect of bacterial inoculation and SSP application on plant growth parameters (Table 3) revealed that bacterial inoculation affected root and shoot dry weights depending on the isolate type. The highest values of plant biomass were (0.229 and 1.323 g/ plant) dry weights in such a bacterial strain mixed cultural with 75 percent of the SSP phosphate fertilizers.

In comparison to any single inoculation, co-inoculation of all three PSB strains resulted in a considerable increase in the plant dry weight of barley seedlings (Table 3), showing that the three PSB strains may work synergistically to improve barley development. This is especially true when reducing the mineral phosphate fertilizer percentage by 25 %. Also, the bacterial isolate *Enterobacter aerogenes* showed the most effective isolates came after the mixed culture for the three isolates together. It recorded 0.179, 1.185, and 1.364 g dry weights per plant for root, shoot, and total dry plant with 75 % single super phosphate (SSP).

Table 3. The Effect of four different doses of single super phosphate (SSP %) and phosphate-solubilizing bacteria inoculation (PSBs) 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
$LSD_{0.05}$	**0.036**	**0.183**	**0.200**

Phosphorus Bioavailability:

The data in Table (4) showed the effect of bacterial strains (PSB) and Single Super Phosphate (SSP %) levels on phosphorus bioavailability in soil. The available phosphorus content in the rhizospheric soil samples revealed that the PSB threshed soil samples exhibited significantly ($P \leq_{0.05}$) higher levels of available phosphorous compared to the rhizosphere soil samples collected from the control treatment. Also, the interaction affects the bioavailable phosphorus in the soil it was significant; the maximum bioavailable phosphorus was obtained by the co-application of 100% SSP + mixed culture (15 mg/kg) in soil 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 (4) PSB strains were able to increase available phosphorus in soil without the 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 capacity of these PSB strains to enhance the concentration of soil accessible P and boost plant development in soils without the addition of SSP suggested that phosphate solubilization by these PSB was a contributing factor in plant growth promotion.

Table 4. The Effect of four different doses of single super phosphate (SSP %) and phosphate-solubilizing bacteria inoculation (PSBs) 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
LSD $_{0.05}$	**2.54**			

Phosphorus Uptake:

The interaction effect between bacterial inoculation and SSP application on P concentrations of root and the phosphorus uptake (Table 5) 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). On the other hand, shoot and root phosphorus concentrations were increased by the increments of the different doses of single super phosphate (SSP %) while, there were no significant differences were observed with the third and the fourth doses of SSP percentage. Furthermore, the mixed culture of the three isolates recorded the greater amount of p concentration on the shoot (1.555 mg/g DW) with the 75 % of phosphate fertilizers doses. The same trend was revealed with phosphorus uptake. The obtained results from (Table, 5) reported that the inoculated barley plants with even mixed bacterial culture or *E. aerogenes* had the highest P uptake in case of decreasing the mineral fertilizers to 25 % from the recommended dose.

Table 5. The Effect of four different doses of single super phosphate (SSP %) and phosphate-solubilizing bacteria inoculation (PSBs) 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**
$LSD_{0.05}$	**0.33**	**0.33**	**0.97**

The larger responses in dry matter and P absorption following inoculation with combination specific strains in SSP-fertilized soil compared to unfertilized soil is likely due to the existence of sufficient amounts of readily accessible P in the soil. However, available P in soil that had not been treated with SSP proved to be a limiting factor for plant development (Table 6). As a result, any release of P from native P in soil solution caused by PSB would have a favorable impact on barley plant development and P absorption. Greater responses in dry matter and P absorption in SSP-treated soil were linked to PSB inoculation rather than single isolates. The significant and positive correlations between available P, P absorption, and dry matter (Table 6) suggest that both P uptake and dry matter yield are influenced by soil P availability.

Table 6. For each treatment, the coefficients of correlation between soil accessible P, P absorption, and dry matter of barley plants were calculated.

	P uptake	Dry matter
Available P	0.739^{**}	0.715^{**}
Dry matter	0.952^{**}	

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

Based on our findings, we can reduce SSP percent application to 75% of recommended dosage + inoculation with a mixed culture of this PSB with the significant promotion of barley growth compared to 100% SSP of recommended dosage without

PSB inoculation to save chemical fertilizer and maintain soil health. The good impacts of these strains on barley plant development and phosphorus absorption demonstrate the beneficial role of these PGPR, which might be related to IAA generation, phosphate releasing process, or perhaps other non-evaluated PGPR features that drive plant growth.

Content of Some Free Amino Acids (Phenylalanine, Arginine and Proline):

1. Arginine

Arginine content was significantly increased due to the inoculation of phosphate solubilizing bacteria (PSB), at the different levels of single super phosphate. The highest value of arginine content (0.910 mg/g DW) was found in case of P75% with *Pantoea sp* bacteria Tables (7). While there were no significant variations in the phosphate dosages.

2. Proline

Significant differences were recorded in proline content among different phosphate treatments. Meanwhile, bacterial inoculation had a significant impact. Data recorded that the proline content effect by the different bacterial isolates and the mixture of them also, *Enterobacter sp* with 75 % single super phosphate recorded the high value.

3. Phenylalanine

Results of Table (7) indicated that there was a significant increase in phenylalanine content with barley plants that inoculated with the three bacterial isolates (*Enterobacter aerogenes* (ENPSB 1), *Pantoea sp.* (ENPSB 2), *Enterobacter sp.* (ENPSB 3) and a mixture of them with percent (1:1:1) as compared with control plants in the presence of all different doses of phosphorus. Furthermore, the data illustrated that the inoculated plants with *Pantoea sp*. with the full dose of phosphorus recorded a significantly highest increase with the phenylalanine content. At the different levels of phosphorus fertilizer, it was (0.859 mg/g DW). While the lowest mean value of phenylalanine content was noticed in control plants in the case of P50% of the mineral P fertilizer, it was (0.581 mg/g DW).

Table 7 The effect of phosphate-solubilizing bacteria inoculation on some free amino acids of barley (*Hordeum vulgare* L.) in the presence of four different doses of single super phosphate SSP.

Treatments	Arginine (mg/g DW)	Proline (mg/g DW)	Phenylalanine (mg/g DW)
0 % + Control	0.694	0.520	0.594
0 % + *E. aerogenes*	0.678	0.522	0.655
0 % + *Pantoea sp.*	0.730	0.555	0.630
0 % + *Enterobacter sp.*	0.699	0.532	0.647
0 % + Mix culture	0.648	0.507	0.646
50 % + Control	0.713	0.534	0.581
50 % + *E. aerogenes*	0.706	0.536	0.615
50 % + *Pantoea sp.*	0.714	0.554	0.736
50 % + *Enterobacter sp.*	0.724	0.580	0.761
50 % + Mix culture	0.821	0.512	0.732
75 % + Control	0.730	0.505	0.673
75 % + *E. aerogenes*	0.835	0.541	0.762
75 % + *Pantoea sp.*	0.910	0.562	0.829
75 % + *Enterobacter sp.*	0.872	0.611	0.769
75 % + Mix culture	0.772	0.598	0.773
100 % + Control	0.794	0.521	0.709
100 % + *E. aerogenes*	0.788	0.549	0.811
100 % + *Pantoea sp.*	0.804	0.601	0.859
100 % + *Enterobacter sp.*	0.826	0.587	0.698
100 % + Mix culture	0.888	0.542	0.685
$LSD_{0.05}$	0.0733**	0.0548*	0.0621**

DISCUSSION

Our data showed that the maximum values of plant growth were obtained practically by co-application of mixed culture + 75% SSP and it was significantly higher than control treatment supplemented with 100% SSP. Inoculation by PSB with inorganic P increased the growth of barley and other crops as reported by many researchers (Tomar *et al.,* 1996; Chaykovskaya *et al.,* 2001). These results were nearly identical to those of Afzal et al. (2005), who reported that phosphate solubilizing microorganisms, alone or in combination with other combinations, had a significant impact on wheat growth and biological yield. Pre-sowing inoculation of wheat seeds with phosphate-solubilizing bacteria increased yield compared to non-inoculated groups, according to Dwivedi *et al.* (2004).

Individual inoculation with single strains may be a good alternative for mixed inoculation with diverse strains, owing to the combination of distinct mechanisms employed by each strain in the consortium. When *Pseudomonas striata* and *Bacillus polymyxa* strains with phosphate solubilizing capacity were combined with a strain of *Azospirillum brasilense,* grain and dry matter yields improved significantly, and P uptakes increased as well (Alagawadi and Gaur 1992). Seeds or soil inoculation with phosphate-solubilizing organisms and other plant growth-promoting rhizobacteria improved the production of many crops, according to Afzal and Bano (2008). This study shows that P solubilization is critical for bacteria that thrive in rhizosphere conditions, and it backs up the theory that barley, like other plants, may modify the root microbiome by producing root exudates that function as chemo-attractants for a small number of microorganisms (Sasse *et al.,* 2018). To investigate the influence of PGPR co-inoculations on cereal crop development and yield, several authors conducted studies on cereal crops in pots or the field. *B. megaterium, A. chlorophenolicus,* and *Enterobacter s*p. were utilized by Kumar *et al.* (2014) to significantly increase wheat plant height, grain production, and straw yield. Co-inoculation with *Bacillus subtilis* 05U142, *Bacillus megaterium* M3, and *Azospirillum brasilense* Sp245 generated higher plant nutritional element concentrations than mineral fertilizer treatment, according to Baris *et al.,* (2014).

The plant growth promotion bioassay was supported by the synergistic influence on phosphate solubilization of single- and co-inoculation of two phosphate solubilizing bacteria, *Burkholderia anthina* PSB-15 and *Enterobacter aerogenes* PSB-16. Co-inoculated golden gramme seedlings grew faster than control. As a result, co-inoculation of the strains *B. anthina* and *E. aerogenes* outperformed solo vaccination with each strain in terms of increasing plant development (Jin-Hee *et al.,* 2016). Ibáez *et al.,* (2021) on the other hand, discovered that various isolates of phosphate solubilizing bacteria (PSB) from the barley rhizosphere may enhance the dry weight of plant ears.

There are also several cases in cereal crops where distinct plant growth-promoting rhizobacteria (PGPR) have synergistic effects. In terms of wheat growth and nutrient absorption, co-application of mixes and bio-inoculants (*Anabaena torulosa* + *Pseudomonas striata* and/or *Anabaena torulosa* + *Azotobacter chroococcum*) was superior to single inoculation and chemical fertilizer control (Swarnalakshmi *et al.,* 2013). Manjunath *et al.,* (2011) found that co-inoculation of wheat with two proteobacteria (*Providencia sp*. and *Alcaligenes sp*.) and two cyanobacterial (*Anabaena oscillarioides* and *Anabaena torulosa*) inoculants improved nutrient absorption and root physiology. Another study found that seed bacterization with *P. fluorescens* BAM-4 and *B. cepacia* BAM-12, either alone or in combination, boosted growth and yield, but the co-inoculation treatment raised the bacterial population, ear length, shoot P content, and kernel yield more than the single treatment. For P content with free and immobilized cells,

B. cepacia BAM-12 + TCP and *B. cepacia* BAM-12 + *P. fluorescens* BAM-4 + TCP were the best bio inoculation treatments (Minaxi *et al.* 2013).

As shown in (Table 4), PSB strains were able to increase available phosphorus in soil without the 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 capacity of these PSB strains to enhance the concentration of soil accessible P and boost plant development in soils without the addition of SSP suggested that phosphate solubilization by these PSB was a contributing factor in plant growth promotion. Phosphate-solubilizing bacteria (PSB) have recently emerged as a kind of PGPR that aids and improves phosphorus uptake by plants (Sharma *et al.,* 2013). Insoluble phosphorus is abundant in dry and semi-arid soils, largely in the form of tri-calcium phosphate (TCP), of which only 2.4–3.9 percent is accessible to plants, the rest is unavailable organic (15–20 percent), and the other 77–82 percent is unavailable inorganic form (TCP) (Rao and Tarafdar, 2002). Furthermore, the strains studied were isolated from alkaline calcareous soil from an arid location (Northern-western coast of Egypt), which includes TCP as the most prevalent insoluble P source. According to Chen and Liu (2018), rice seedling development infected with S32, *a Pantoea sp*, demonstrated the largest substantial increase in accessible phosphorous. Sundara *et al.* (2002) discovered that adding PSB (*B. megaterium* var. *phosphaticum*) to the soil improved P availability. PSB lowered the needed P dosage by 75% when used in combination with P fertilizers. PSB inoculation, *Bacillus* M-13, with and without varying levels of phosphorus (P) fertilizer was found to efficiently mobilize P in sunflower and boost seed quality and oil output. PSB, on the other hand, had a significantly stronger effect when applied in conjunction with P fertilizers (Zehra 2010). When utilized in conjunction with PSB, the largest seed of sunflower achievable with 100 kg P2O5 ha^{-1} fertilizer was rendered with roughly 50 kilograms of P_2O_5 ha^{-1} fertilizer.

Phosphate solubilizers have been identified in a variety of microorganisms. The most well-known belong to the genera *Pseudomonas, Burkholderia, Bacillus, Serratia,* and *Enterobacter*, among others, and all can help plants absorb more P (Zhu *et al.,* 2018). Inoculation of PSB and parental transconjugants improved plant biomass output and P absorption in pot culture, according to Gera *et al.* (2005). Green gramme plants showed similar increases in phosphorus absorption after being inoculated with PSB strains (Ghanem and Abbas, 2009). Wheat from *Azotobacter chroococum* (Kumar *et al.,* 2001), peanut from *P. fluorescens* (Dey *et al.,* 2004), walnut from *Bacillus cereus* and *Pseudomonas* sp. (Yu *et al.,* 2011), and tomato from *Paenibacillus polymyxa* and *B. megaterium* have all shown increased phosphorus absorption and growth (EI-Yazeid and Abou-Aly, 2011). In comparison to each solo inoculation, combined inoculation of all three PSB strains resulted in an increase in dry weight and P absorption of barley seedlings, demonstrating that the three PSB strains might function synergistically in boosting barley growth. Our findings are consistent with those of (Ibáez *et al.,* 2021) who used slightly infected PSP plants. Acidic and alkaline phosphatase activities were observed in all PSB, varying across isolates when compared to control and identification of phosphatase activities implicated in P solubilization. When compared to single PSB or N2 fixer inoculations, Inoculation of PSB strain *P. striata* with N2-fixing bacteria *Rhizobium* sp. dramatically increased accessible P in soil, as well as plant dry matter and P absorption by chickpea (Wani *et al.,* 2007). Ibáñez *et al.,* (2021) recorded the PSB isolated from barley rhizosphere had significant differences in phosphate solubilization capacity, they found that strain *Enterobacter cloacae* PSB8 and *Pseudomonas koreensis* PSB6 had the highest level of solubilization capacity. Furthermore, in laboratory circumstances, the *Bacillus*

cereus PSB3 strain was only able to release a little quantity of soluble orthophosphate into the growth medium. Besides, the *B. megaterium* PSB1 strain displayed a restricted solubilization ability under test conditions. In reclaimed soil, the application of PSB highly effective PSB strains S32 greatly boosted rice seedling development in terms of plant height, biomass, root growth, and P uptake (Chen and Liu, 2018).

From data illustrated in Table (7), the total amino acids were differed by the isolate's differences, and our results closer to the data obtained from Ibáñez *et al.,* (2021) PSB had a favorable impact on barley development, resulting in an increase in metabolic activity of several active chemicals., PGPR traits tested in vitro are generally associated with the ability of beneficial bacteria to promote plant growth and health was increased according to siderophore and HCN production. Because of their active participation in various processes of plant growth and development, Shen *et al.*, (2021), demonstrated that amino acids were the focus.

Conclusion

In summary, the current research showed that the plants inoculated with the three different PSPs isolates such as *Enterobacter aerogenes*, *Pantoea sp*., *Enterobacter sp*., and their mixture in the ratio (1:1:1) had a significant impact on barley growth and phosphate absorption at 75% and 100 % of single superphosphate (SSP) while, there is no significant differences were observed between the two doses. This leads us to reduce SSP application up to 25% from the recommended dosage + inoculation with mixed culture to maintain environment and soil health.

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