Changes in Phosphorus Fractions and its Availability Status as Affected by Appling of Bio and Mineral Fertilizers in The Calcareous Soils

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ABSTRACT

The important main reason to enhance the studied calcareous soil and support changes of phosphorus fractions to reach to availability status for plant uptake. Two kinds of phosphate solubilization bacteria (PSB), (*Lysinibacillus boronitolerans* and *Bacillus megatherium*) were used to reach this target. The greatest significant results of labile P % in soil were obtained by employing a 75% P with (*L. boronitolerans*) treatment and 75% P with (*B. megatherium* + *L. boronitolerans*) treatment. The combine *(B. megatherium* and *L. boronitolerans*) treatments with (P_{50}) or (P_{75}) were superior in increasing labile phosphorus compared to other treatment alone. The results revealed that (PSB) treatments and also P rates affect significantly soil pH values compared to control where the highest pH decrease was showed in soil treated with combine (PSB) treatment either with (P_{50}), or (P_{75}).

***L. boronitolerans* produced acidic phosphatase enzyme and produced alkaline phosphatase enzyme. *B. megatherium* doesn't produced acidic phosphatase enzyme, but produced alkaline phosphatase enzyme. *L. boronitolerans* produced many kinds of organic acids, while *B. megatherium* produced all of them except butyric acid. Treatment of 75% P with (*L. boronitolerans* + *B. megatherium*) gave high results of Spinach lengths, shoot weights, P (%), Chlorophyll A & B, Vitamins A, B, C and E., Nitrogen fixers, PSB, total microbial counts and soil Dehydrogenase enzyme.**

We recommend using treatment of 75% P with (*L. boronitolerans* + *B. megatherium*) to release P in calcareous sandy soils and increase crop productivity.

Key words: *B. megatherium, L. boronitolerans*, Soil phosphorus fractions, Sequential fractionation.

INTRODUCTION

Phosphorus (P) is the most important nutrients for plant (Mengel *et al.,* 2001; Goll *et al.,* 2012; Bilal *et al.,* 2021; Dokwal *et al.,* 2021 and Elrys *et al.,* 2021). In plants it performs an important role in several physiological activities such as division of cell, energy reaction, photosynthesis; improve carbohydrate utilization and good root system (El-Shinnawi *et al.,* 2015 and Muhammad *et al.,* 2022). Phosphorus is most frequently deficient nutrient in most agricultural soils especially calcareous soils. It is the least accessible macronutrient. Its low availability in soils, because P-fixation either it is adsorbed on the soil minerals or get precipitated by forming insoluble compounds with cations as Al^{3+} and Fe^{3+}in acid soil and with Ca^{2+} and Mg^{2+} in alkaline soil especially in calcareous soil (Sharma *et al.,* 2013; Cordell *et al.,* 2011 and Muhammad *et al.,* 2022).

P exists in soils in organic and inorganic forms; inorganic P almost is in the forms of calcium compound in alkaline and neutral soils, and also in the forms of aluminum and iron compounds in acid soil and their availability is generally determinated by soil P fractionation (Cross & Schlesinger, 1995 and Negassa & Leinweber, 2009). Phosphorus Fractionation is considered an applicable technique applied to define its forms in soils quantitatively as well as qualitatively. This information is potentially valuable for predicting P leachability, bioavailability and transformations between chemical forms in soils (Hedley *et al.,* 1982a,b and Sui *et al.,* 1999). Soil P fractionation has been investigated since 1957and was applied recently to sediments and soils to overcome the little information that provided by total P analysis only (Zhou *et al.,* 2001). Phosphorus in soil is distributed in many geochemical forms that include soluble and exchangeable, OM -bound, Ca-bound, and Fe and Al-bound forms (Hedley *et al*., 1982a and b). The degree of P association with different geochemical forms strongly depends upon physical and chemical properties of the soils due to, climate, management practices (Motavalli and Miles, 2002) and soil type (Tiessen *et al*., 1984).

Calcareous soils are the most spread soil particularly in semi-arid and arid areas (800 million hectares worldwide) (Torrent *et al.,* 1990). Ninety percent of these soils are scarcer in bio-available P (Bieleski, 1973). These soils contain high content of $CaCO_3$ especially the active $CaCO_3$ that keeps large quantities of P applied fertilizers get fixed by the active and rapid chemical reactions with cations as Ca^{2+} to form a calcium phosphate $Ca_3(PO_4)_2$ and/or by sorption on calcite surfaces (Vassilev *et al.,* 2001; Alharbi *et al*., 2018 and Silva *et al.,* 2023). Therefore, plants can utilize only a small fraction of applied P (about 20 % of applied P) and this makes them bad in performance

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(Sharma *et al*., 2013 and Blanco-Vargas *et al.,* 2020). Consequently, to fulfill P requirements of plant; these soils are supplemented with phosphate fertilizers by regularly way (Goldstein, 1995), which are expensive and rise cost of production, so improvement of P availability and plant uptake is highly needed for increase productivity (Vance *et al.*, 2003).

P availability can be increased by being able to solubilize fixed inorganic phosphorus (P) by introducing microorganisms that dissolve precipitated inorganic phosphate and mineralize organic P in soil (PSBs) (Chen *et al.,* 2006 and Zheng *et al.,* 2018). Phosphate Solubilizing Bacteria (PSB) improves the solubilization of insoluble P compounds through the release of organic acids and phosphatase enzymes (Khan *et al.,* 2014). Using of phosphorus solubilizing bacteria (PSB) as inoculants promote P uptake and enhance P availability in calcareous soils (Attia *et al.,* 2016 and El-Shazly *et al.,* 2017). The formation of several forms of organic acids as glutamic, citric, lactic, succinic, malic, fumaric, oxalic and tartaric acid has been identified as the primary process responsible for phosphorus solubilization (Khan & Joergensen, 2009 and Muhammad *et al*., 2022). The kind of raw materials and *Bacillus* species utilized in solubilization had a considerable effect on the organic acids discovered in the broth culture and their overall concentration, which had a direct influence on the quantity of phosphorus released (Saeid *et al*., 2018). In recent years, the respect for helpful bacteria is rising as a result of their non-toxic and eco-friendly characteristics and may be used as an alternate to expensive mineral P fertilizers to enhance crop yield in calcareous soil (Adnan *et al.,* 2020; Ahsan and Shimizu, 2021). The combination of PGPR and PSB could reduce addition of P fertilizer by 50 % without any negative effect on crop yield (Elkhatib *et al.,* 2008 and Jalili *et al.,* 2009).

Lysinibacillus species have lately the researchers attention as potential substitutes for agro-chemicals in the control and promotion of plant development and disease, (Ahsan and Shimizu, 2021). The PSB strains have inorganic P solubilizing abilities ranging between (25–42 µg P mL^{-1}) and organic P mineralization abilities between (8–18 µg P mL^{-1}) (Tao *et al*., 2008). The PSB with single super phosphate and rock phosphate reduce the P fertilizer used by 25 and 50 %, respectively (Sundara *et al*., 2002). Reduction of applied of phosphorus is a very importance for socioeconomic development and environmental sustainability. Therefore, options to the administration of this element are needed, and the applied of P-solubilizing microorganisms is an alternative to optimize its application by crops, allowing the search of low available fractions of the element in soils and decreasing the request for fertilizers of phosphate (Silva *et al.,* 2023).

In this direction the aim of our current study was to assess the effects of adding both *B. megatherium* and *L. boronitolerans* as bio-fertilizers in response to different application rate of mineral fertilizer to calcareous soil on changes of phosphorus fractions and its transformations to labile P to reduce P fertilizer application dose.

MATERIALS AND METHODS

1. Sequential P fractionation

Phosphorus sequential fractionation of samples of soil was processed according to the methods described by Hedley *et al*. (1982 a) and modified by Chen *et al.* (2000). 1.0 g (1 mm sieved) of soil sample was shaken with 30 mL of 0.5 M NH_4Cl for 30 min, and centrifuged at 4000 rpm for 10 min to extract soluble-P. The exchangeable-P was extracted by shaking the soil residues left from the previous extract with 30 mL of 0.5 M $NaHCO_3$, at pH 8.5 for 16 h continuously. The remaining soil was shaken with 0.1 M NaOH after washing with distilled water for 16 h to extract P bound to Al and Fe hydroxide minerals (NaOH-P), and then the Ca-bound P was extracted by shaking the remaining soil with 1 M HCl for 16 h continuously (HCl_D-P). Then the remaining soil was heated at 80°C for 10 min with 10 mL of concentrated HCl in a water bath, then add 5 mL 12 M HCl, and bring the solution to 50 mL with distilled water (HCl_C-P) to extract hardly soluble P; Fe- and Al–P. Finally, residual P was obtained as difference between total P and sum of different fractions (Frossard *et al*., 1989). Organic and inorganic fractions of P were measured using the 0.5 M $NaHCO_3$, 0.1 M NaOH, and 12 M HCl extracts individually. Where the phosphorus in these aliquots were digested by potassium persulfate to determine its total content of P (Pi+Po), organic P fractions were measured by the difference of total P and the inorganic P as described by Bowman (1989). Phosphorus in the extracts was measured spectro-photometrically, according to Jackson (1973).

2. Strains of Bacteria

The bacterial strain was used (*Bacillus megatherium*) kindly acquired from biofertilizers production unit (BPU), Agric. Microb. Dept. Soil, water and environment Res. Inst, (SWERI), Agriculture Research Center, (ARC) Giza, Egypt., while (*Lysinibacillus boronitolerans*) was isolated from olive mill wastewater sample and identified by Bergy's Manual of Determinative Bacteriology (1994). According to Berg *et al.* (2002), the isolates of bacteria were also identified using a partial 16S rRNA gene sequence analysis. PCR was used to magnify bacterial

16S rRNA gene sequences (Lane, 1991; Omar and Ibrahim, 2023).

3. Microbiological and Biochemical analysis of bacteria

Determinations of phosphate solubilization bacteria

Pikovskaya medium inoculated with the individual strains were incubated for 48 hours and observed for yellow color as positive result (Pikovskaya, 1948).

Determination of phosphatase enzyme

One phosphatase enzyme unit was defined as quantity of enzyme that hydrolyzed 1mM of p-nitrophenol hour^{1}. The sample determined in Soil, Water and Environmental Research Institute - Agriculture Research Center. Determination free-cell supernatant assayed. After incubation at 35°C for 10 days used 0.5ml inoculum from both broth cultures individually and control sample (without inoculation) on broth cultures of bacteria then phosphorus was measured for acid and alkaline phosphatase activity according to Tabatabai and Bremner (1969).

Determination of Organic acids by HPLC

HPLC analysis was performed using an Inert Sustain in Central lab. of National Research Center – Cairo – Egypt. The separation was obtained using Eclipse AQ-C_{18} HP column (4.6 mm x 150 mm i.d., 3 µm). The mobile phase contain of 0.005N H_2SO_4. The mobile phase was identified consecutively in a linear gradient for flow rate as follows: 0 - 4.5 min (0.8 ml / min); 4.5-4.7 min (1 ml / min); 4.7- 4.71 min (1 ml / min); 4.71-8.8 (1.2 ml / min); 8.8-9 (1.3 ml / min); 9-23 (1.3 ml / min) and 23-25 (0.8 ml / min). The Diode Array detector (DAD) was measured at 210 nm. The volume of injection was 5 µl for each of the sample solutions. The column temperature was at 55°C.

4. Field experiment

A field experiment was performed during winter growing season of 2021/2022 at Agricultural Experimental Station of Desert Research Center (DRC), Maryout Alexandria governorate, Egypt. The Spinach seeds (*Variety Thessaloniki*) were provided by Agriculture Research Center, Ministry of Agriculture and Land Reclamation (MALR), Giza – Egypt. The study concerned with increase the productivity of spinach by inoculation with phosphate solubilization bacteria (PSB) (*Bacillus megatherium* and *Lysinibacillus boronitolerans*) in calcareous sandy soil.

The design of experiment was arranged in a split plot design with three replicates and each plot area was (175 m^2). Organic fertilizer (farmyard manure) applied (10-15 m^3/ Feddan) before planting. Inorganic fertilizers added in two doses: the first one after three weeks from planting and the second one added after 2 weeks from the first one. Inorganic fertilizer treatments were added for all plots as full recommended dose of Ammonium nitrate (33.3% N) as 250Kg/ Feddan, full recommended dose of Potassium sulphate (48.5% K_2O) as 50Kg/ Feddan and (0%, 50% and 75% P) of recommended dose of Super phosphate (15.5% P_2O_5) as 200Kg/ Feddan. Control plot were assigned to the flood irrigation only and treated with full recommended doses of NPK and without biofertilizers. 50 and 75% doses of P without biofertilizers were used. All plots were allocated to bacterial inoculation treatments with flood irrigation. Phosphate solubilizing bacteria (PSB) were added as soil drench for all treatments except treatment of (without biofertilizers). Phosphorus was added as (0, 50 and 75%) with biofertilizers. The biological yield was collected after 55 days from cultivation.

After finishing the experiment, the surface layers (0-30 cm) of soil samples were collected from each treatment plot to determinate of physo-chemical properties according to the methods described by Page *et al*. (1982). Soil organic matter, cation exchange capacity and (EC) values in soil paste extract as dSm^{-1} were determined by (Jackson, 1973). pH was determined electrometrically in the soil paste using bench type Beckman glass electrode pH-meter. Total carbonates content were determined using Collin's calcimeter.

Determination of Spinach Plant growth parameters

The following plant growth parameters were determined in field experiments. Biological yield was expressed whole plant as lengths / cm and fresh & dry weights (Kg / Feddan) of yield were determined according to Black *et al*. (1965). The main physo-chemical properties of the experimental soil and the initial chemical analysis for water used in the field experiment are shown in Tables (1 and 2).

5. Chemical analysis of Spinach

Determination of Phosphorus percent in Spinach

At harvest, 1 m^2 at the center of each plot was chosen to be harvested for the estimation of whole plant biological parameters (Fresh weight, dry weight (Kg/ Feddan) and plant heights/ cm). P was obtained in acid digested solution, according to Cottenie *et al*. (1982). According to Jackson (1973), the plant was dried by oven and digested using a 1:1 combination of pure $HClO_4$ and H_2SO_4. Using ammonium molybdate and stannous chloride reagents, phosphorus was measured at 880 nm by Spectrophotometrically (Jackson, 1973).

Determination of chlorophyll and carotenoids content

Leaf chlorophyll content measurement by spectrophotometer, the measure at three wave lengths: (662 nm) chlorophyll A, (644 nm) chlorophyll B and (440 nm) carotenoids (Schwartz and Lorenzo, 1990).

Table 1. Some physo-chemical properties of the experimental soil

Soil property	Values
Physical Soil Properties	
Particle size distribution (%)	
Coarse sand	5.29
Fine sand	50.82
Silt	18.38
Clay	26.51
Soil texture class	Sandy Clay Loam
Field capacity%	17.38
Wilting point%	7.55
Available water%	9.83
Chemical Soil Properties	
pH	7.87
EC dS/m	2.49
Organic matter (%)	0.89
$CaCO_3$ (%)	28.61
CEC ($Cmolkg^{-1}$)	10.88
Available Macronutrients ($mgkg^{-1}$)	
N	75.85
P	9.93
K	122.65

Table 2. Chemical characteristic of the applied water of irrigation

pH	EC (dS/m)	TDS (ppm)	Soluble Cations (meq/l)				Soluble Anions (meq/l)			SAR
			Na^+	K^+	Ca^{+2}	Mg^{+2}	CO_3^{-2}	HCO_3^-	Cl^-	SO_4^{-2}	
7.62	2.25	1440	18.54	0,57	3.09	0.29	-	5.45	17.05	-	14.23

6. Determination of Spinach rhizosphere microbial counts

Spinach rhizosphere microbial counts were performed on different media as the following: Ashby's medium (Abd El-Malek and Ishac, 1968) was applied for nitrogen fixers counts by M.P.N technique and calculated by Cochren's tables, (Cochran, 1950). Total microbial counts used nutrient agar medium (Jacobs and Gerstein, 1960). Phosphate solubilizing bacteria counts (Bunt and Rovira, 1955).

Soil dehydrogenase activity (µgTPF/g dry soil/24hr.) was analyzed 2, 3, 5-triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) this evaluated by Casida (1977). To prepare inoculum which used in field experiment, nutrient broth culture (Jacobs and Gerstein, 1960) was utilized in 100 ml of nutrient broth medium or Pikovskaya broth medium (Pikovskaya, 1948) in 250 ml Erlenmeyer flask used to develop of individual bacterial growth. After inoculating the cultures and incubating them at 30°C for 10 days, both bacterial cultures densities reached to (10^8 CFU).

Statistical analysis

The statistical of the experiment design was split plot design with three replicates, whereas inorganic fertilizers treatments formed the main plots and PSB were arranged in sup main plots. The present work data was statistically analyzed and the differences between the means of the treatments were important, as they were more than the least significant differences (L.S.D) at the 5% level by using computer program of Statistix version 9 (Analytical software, 2008).

RESULTS AND DISCUSSION

1. Sequential P fractionation

Addition of PSB and superphosphate fertilizers to calcareous soil had a significant effect on soil properties, including the soil pH and P fractions. Tables (3 and 4) show the effects of treatments of *B. megatherium and L. boronitolerans* as bio- fertilizers with three rates of superphosphate fertilizer (0, 50, and 75% of recommended dose) on soil pH and P fractions, as discussed below.

Soil pH value

There were statistically significant differences in soil pH degree between soils treated with PSB treatments and those without the treatment. Superphosphate rates also decreased soil pH. The pH of rhizosphere is lowered this may be refered to the action of organic acids (mainly gluconic and keto gluconic acids) and inorganic acids (e.g. hydrochloric) secreted by PSB (Goldstein, 1995 and Deubel *et al*., 2000), in addition to biotical production of proton / bicarbonate release (anion / cation balance) and gaseous (O_2/CO_2) exchanges. Data in Table (3) showed that Phosphorus solubilization ability of PSB has direct correlation with soil pH decrease. *L. boronitolerans* treatment alone was more effective in decreasing soil pH than that of *B. megatherium* where *B. megatherium* decreased soil pH by 8.26% (P_0), 10.16% (P_{50}), and11.16% (P_{75}), while *L. boronitolerans* treatment decreased soil pH by 15.76% (P_0), 16.77% (P_{50}), and 20.58% (P_{75}). The highest pH decrease in soil was obtained with combine *B megatherium* and *L boronitolerans* treatment at P_{75} where soil pH decreased by19.70% (P_0), 20.84% (P_{50}), and 23.36% (P_{75}), as compared to the samples without PSB application. These results are in an agreement with that stated by Rodriguez & Fraga (1999) and Turan *et al.* (2007) who found a direct correlation between the decrease in pH value and bacterial solubilization of P.

Phosphorus fractions

P forms were categorized into three groups according to Niederberger *et al.* (2015), labile P includes NH_4Cl-P and $NaHCO_3$-P (Pi and Po); moderately labile P contains NaOH-P (Pi and Po) and HCl_D-Pi; non labile or P stable was HClc (Pi and Po) and residual P.

Labile fraction

Labile P forms the most biologically available form of P and direct exchangeable with soil solution with rapid turnover. Geochemically, P is non-occluded and adsorbed on crystalline compounds surface and soil colloids. The labile P includes soluble P (NH_4Cl-P) and exchangeable P ($NaHCO_3$-P). The results in Table (3) showed that the labile P was less concentration among all P fractions in the studied soil which constituting 1.58% of the total P and addition of the experimental treatments cause a significant increase at ($p < 0.05$) in the labile P compared to control. The highest concentration was observed at P_{75} with combine *B. megatherium* and *L. boronitolerans* treatment, which constituted about 23.60 % of total P. Data showed in Table (3) indicate that the concentration of NH_4Cl-P constitute 0.31% of total P without PSB at P_0, *B. megatherium* treatments raised NH_4Cl-P to 1.63 % (P_0), 3.20 % (P_{50}) and 5.23 % (P_{75}) of total P while the increments of NH_4Cl-P reached to 2.05 % (P_0), 5.24% (P_{50}) and 7.35 % (P_{75}) of total P with *L. boronitolerans* treatments Meanwhile, the combine *B. megatherium* and *L. boronitolerans* treatment led to increase the proportion of NH_4Cl-P to 2.66 % (P_0), 5.46 % (P_{50}) and 8.54 % (P_{75}) of total P. Also, results reveal that the concentration of $NaHCO_3$-P gradually increased from 6.26 to 82.75 mg kg^{-1} (from 1.28 % to 15.03% of total P) with increasing P rates. Combine *B. megatherium* and *L. boronitolerans* treatments led to increase the proportion of $NaHCO_3$-P to 5.96 % (P_0), 9.19 % (P_{50}) and 15.03% (P_{75}) of total P, combine *B. megatherium* and *L. boronitolerans* treatments were superior in increasing labile phosphorus compared to other treatment alone where labile -P significantly increased at ($p < 0.05$) from 1.58% to 8.72% (P_0), 14.33% (P_{50}), and 23.60% (P_{75}) of total P. These results are in the agreement with those findings by Turan *et al.* (2007) who found that the amount of available form of soil phosphorus fractions increase with PSB application and was also statistically significant. The rates turned by around 20% for all applied fertilizer types and also Aye *et al.* (2021) who found that addition of PSM by inoculating in soil was an efficient way to convert the insoluble P forms to plant-available P form.

Moderately labile fractions

Moderately labile P represents potentially bioavailable form of P and contains Al-Fe bound P and Ca-bound P., Al-Fe bound P (NaOH-P). Geochemically P is non-occluded and chemi-adsorbed to amorphous and crystalline Al and Fe oxides (NaOH-Pi) and that associated with humic compounds (NaOH- Po), lesser plant available with slow turnover while P adsorbed or receipted with Ca (HCl_D-P), represents a more stable form than labile and Fe-Al-bound fractions (Diez *et al*., 2006), P in these two fractions likely represents P inputs recently from fertilization (Wright, 2009).

The results in Table (3) indicated that NaOH-P had the second-lowest P content of all chemical fractions in studied soil. This was likely caused by the high concentration of Ca ions in the soil, which determine the ion speciation in soil solution (Bohn *et al*. 2001). Data present in Table (3) revealed that the application of PSB treatments along with P rates to soil led to decline in NaOH-P. Data also showed that *B. megatherium* alone was the least in decreasing NaOH-P where decreased by 19.63% (P_0), 18.53% (P_{50}), and 22.16% (P_{75}), while *L. boronitolerans* treatment decreased NaOH-P by 32.35% (P_0), 41.81% (P_{50}), and 46.37% (P_{75}). The highest NaOH-P decrease in soil was obtained with combine *B. megatherium* and *L. boronitolerans* treatment at P_{75} where NaOH-P decreased by 43.29% (P_0), 52.21% (P_{50}), and 57.76% (P_{75}), as compared to the samples without PSB application. This is due to solubilization of Fe-Al-bound P which occurs via acids release by PSB

where the solubilization of mineral phosphate takes place as a result of PO_4^{3-}anion exchange by acid anion, or by chelation of Fe and Al ions associated with phosphate releasing available phosphate for plant uptake (Omar, 1998).

The most abundant P fraction in the soils was Ca-bound P, constituting 59.93% of the total P The result in Table (3), showed that Ca-bound P significantly increased as P application rate increases where ranged from 294.26 mg kg^{-1} at P_0 to 357.95 mg kg^{-1} at P_{75} this is due to the high content of free $CaCO_3$ in the soil, P added forms Ca-associated P forms that are of low solubility and availability for plant (Alharbi *et al.* 2018). Under application of PSB at different P rates. The concentration of HCl_D-P reduced. *L. boronitolerans* treatment was more effective than *B. megatherium* in solubilization of HCl_D-P where *L. boronitolerans* treatment decreased soil HCl_D-P by 12.54% (P_0), 17.25% (P_{50}), and 22.46% (P_{75}), while *B. megatherium* decreased HCl_D-P by 10.30% (P_0), 13.02% (P_{50}), and17.52% (P_{75}). The greatest significant effect of the treatments was combine *B. megatherium* and *L. boronitolerans* treatment at P_{75} where HCl_D-P decreased by 14.07% (P_0), 25.04% (P_{50}), and 29.57% (P_{75}). The observed decreased amounts of HCl_D-P, and NaOH-P fractions were redistributed on NH_4Cl-P, $NaHCO_3$-P, many previous studies supported the findings of our study (Sundara *et al*., 2002; Shen *et al*., 2004 and Turan *et al*., 2006**).**

Stable fractions

Non Labile or stable P represents the recalcitrant P fraction that is highly fixed and non-available for plants (HCl_C-P and residual P). Hardly soluble Fe- and Al-bound P (HClc-P), non-occluded while residual P is P occluded within primary and second minerals (Aulakh *et al.,* 2003 and Zicker *et al.,* 2018). Data presented in Table (3) showed that HCl_C-P slightly increased by adding superphosphate from 67.38 to 71.17 mg kg^{-1}(13.72% to 14.50%) from P_0 to P_{75} treatments, respectively, while decreased insignificantly with *B. megatherium* applied at P_0 and P_{50} in the soil under study. While decrease significantly at P_{75} treatment (10.75%). The obtained data also indicate that *L. boronitolerans* treatment significantly decreased the HCl_C-P at $p < 0.05$ by 10.17% (P_0), 11.45% (P_{50}), and 14.50% (P_{75}), compared to the control. Meanwhile, combine of *B. megatherium* and *L. boronitolerans* led to decrease HCl_C-P by 11.91%, 12.94% and 18.20%, at levels of P_0, P_{50} and P_{75} respectively. Data in Table (3) showed that residual P had slightly increasing trend which ranged from 111.46 to 122.25 mg kg^{-1}(22.70% to 24.90% of total P) in P_0 and P_{75} treatments, respectively. These results are consistent with the findings of Dobermann *et al.* (2002) who stated that the used of P fertilizers had low impacts on residual P fractions, the results obtained from this experiment also showed a significant decrease in residual P fraction with PSB treatments as compared to control (Table 3). Where combine of *B. megatherium* and *L. boronitolerans* was the most effective treatment compared to other solely treatments where the concentration of residual P fraction tended to decrease by 5.48 % (P_0), 6.34% (P_{50}), and 14.07% (P_{75}), reduced amounts of residual P fraction due to the studied treatments may be transformed to other P fractions, especially $NaHCO_3$-Pi and NH_4Cl-P.

Content of total P

Total phosphate content of soil in all treatments is showed in Table (3). The results revealed that there were statistically significant differences in total P contents at P addition rates with/without PSB application. The total P concentrations of treatments without PSB application showed a significant effect in response to P application compared to control, where total P increased from 490.98 to 667.35 mgkg^{-1}. Data also showed total P concentrations of treatments without PSB application were higher than those with PSB application. The results in Table (3) showed that significant decrease in total P content with PSB treatments as compared to control where total P content decreased by 4.62%, 6.98% and 9.46% with *B. megatherium*, *L. boronitolerans* and combine of *B. megatherium* and *L. boronitolerans* respectively this is due to microbial solubilization of inorganic and organic phosphorus forms in soil and convert a part of insoluble phosphate form into soluble forms for plant utilization. These results are in agreement with Aye *et al.* (2021) who found that the PSB treatments cause statistically significant differences in total P content and a significant reduction in the soil total P content due to solubilization of inorganic and organic phosphorus forms in soil due to microbial release.

Organic P (Po) Fractions

Organic P may constitute a small portion that is, 3.41% of the total soil P while the remaining 96.59% was contributed by the inorganic P. The differences in Po fractions response to PSB addition and different P rates is shown in Table (4). The concentration of Po followed the order of $NaHCO_3$-Po > NaOH-Po >HCl_C-Po. compared with the P_0 treatment, all organic fractions had more Po with the increase in the P rate. In without PSB treatments Po ranged from 3.25 to 5.65 mg kg^{-1}, 5.23 to 6.22 mg kg^{-1}, and 8.25 to 13.71 mg kg^{-1} for $NaHCO_3$-Po, NaOH-Po, and HCl_C-Po, respectively. The application of PSB treatments along with P rates to soil led to reduce in Po.

Table 3. Mean concentration of P (mgkg^{-1}) in the different forms of P fractionation as affected by applying of PSB and P fertilizer in the calcareous soil

	Treatments	pH	Labile P		Moderately labile P		Non-labile P		Total P
			0.5M NH_4Cl-P	0.5 M $NaHCO_3$ -P	0.1 M NaOH-P	1.0 M HCl-P	HClc-P	Residual P	
	100%NPK Without (PSB)	7.53^{b}	13.49^{e}	23.70^{e}	25.45^{a}	397.82^{a}	82.85^{a}	129.04^{a}	667.35^{a}
P_0	Without	7.87^{a}	1.48^{g}	6.26^{f}	10.14^{d}	294.26^{c}	67.38^{c}b	111.46^{d}	490.98^{ef}
	B. megatherium	7.22^{c}	7.82^{f}	19.43^{ef}	8.15d^{e}	263.95^{d}	62.02^{d}	110.45^{c}	471.14^{f}
	L. boronitolerans	6.63^{e}	9.88^{f}	25.85^{e}	6.86^{e}	257.35^{d}	60.53^{d}	107.85^{d}	463.32^{f}
	B. megatherium + L. boronitolerans	6.32^{f}	12.80^{ef}	27.56^{d}	5.75^{e}	252.85^{d}	59.35^{de}	105.35^{d}	462.75^{f}
P_{50}	Without	7.65^{b}	9.92^{f}	17.39^{ef}	19.21^{b}	334.24^{bc}	69.55^{b}	119.85^{b}	568.61^{c}
	B. megatherium	7.02^{d}	15.37^{e}	34.59^{d}	15.65^{c}	290.71^{c}	68.85^{bc}	117.93^{bc}	543.15^{d}
	L. boronitolerans	6.55^{e}	20.45^{d}	43.85^{c}	11.28^{d}	276.60^{d}	61.59^{d}	116.53^{bc}	530.32^{d}
	B. megatherium + L. boronitolerans	6.23^{f}	26.25^{c}	46.93^{c}	9.18d^{e}	250.55^{e}	60.55^{d}	112.25^{d}c	510.71^{e}
P_{75}	Without	7.69^{b}	12.80^{ef}	21.12^{ef}	20.23^{b}	357.95^{b}	71.17^{b}	122.25^{b}	605.50^{b}
	B. megatherium	6.99^{d}	25.16^{c}	49.84^{c}	15.75^{c}	295.25^{c}	63.52^{d}	115.75^{c}	573.02^{c}
	L. boronitolerans	6.25^{f}	35.35^{b}	62.15^{b}	10.85^{d}	277.55^{d}	60.85^{d}	107.75^{d}	553.50^{cd}
	B. megatherium + L. boronitolerans	6.01^{g}	47.05^{a}	82.75^{a}	8.55d^{e}	252.10^{e}	58.22^{de}	105.05^{d}	550.72^{cd}
	LSD $_{(0.05)}$	0.033	0.25	0.75	1.052	2.033	0.55	0.15	1.32

*Mean values followed by letters indicate significant differences among P levels with and without PSB by the LSD test at P < 0.05.

The obtained data in Table (4) showed that *B. megatherium* alone was the least in decreasing Po where total Po decreased by 27.62% (P_0), 34.94% (P_{50}), and 46.21% (P_{75}), while decreased by 43.57% (P_0), 59.22% (P_{50}), and 67.27% (P_{75}) with *L. boronitolerans* treatment. The highest decreasing of Po in soil was obtained with combine *B. megatherium* and *L. boronitolerans* treatment where Po decreased by 59.00% (P_0), 73.14% (P_{50}), and 81.27% (P_{75}), as compared to the samples without PSB application. This is due to P mineralization which occurs via produce organic anions, and production of siderophores & acid phosphatase by plant roots / microbes (Yadaf and Tarafdar, 2001) or alkaline phosphatase (Tarafdar and Claasen, 1988), the soil organic P is hydrolyzed by these enzymes or split P from organic residues. The largest portion of extracellular soil phosphatases is derived from the microbial population as reported by Dodor and Tabatabai (2003).

Inorganic P (Pi) Fractions

The P fertilizer and PSB application practices had a significant effect on the proportions of Pi fractions and significantly enhanced their proportions in the soil, data in Table (4) shows that among all Pi fractions, HCl_D-P is the highest in content (252.10 - 397.82 mgkg^{-1}). The Pi fractions in response to P rate treatments showed an increasing trend compared to control take the order HCl_D-Pi > HCl_C-Pi > NH_4Cl-P > $NaHCO_3$-Pi> NaOH-Pi. Pi fractions in response to PSB application at different P rates is shown in Table (4) where the available NH_4Cl-P & $NaHCO_3$-Pi raised significantly from 1.48 to 37.05 mg kg^{-1}&3.01 to 81.70 mg kg^{-1} respectively compared to samples without PSB treatment, with the highest value observed in combine *B. megatherium* and *L. boronitolerans* treatment at P_{75}. In contrast, the application of PSB treatments along with P rates to soil led to reduce in the NaOH-Pi and HCl_D-P where decreased with *B. megatherium* treatment by 25.92% (P_0), 11.45 % (P_{50}), and 16.70% (P_{75}) & 10.30% (P_0), 13.02% (P_{50}), and17.52% (P_{75}),respectively while decreased by 37.00% (P_0), 38.47% (P_{50}), and 40.91% (P_{75})& 12.54% (P_0), 17.25% (P_{50}), and 22.46% (P_{75}), respectively under *L. boronitolerans* treatment. The highest decreasing was obtained with combine *B. megatherium* and *L. boronitolerans* treatment where NaOH-Pi and HCl_D-P decreased by 43.64% (P_0), 49.39% (P_{50}), and 55.33% (P_{75}) & 14.07% (P_0), 25.04% (P_{50}), and 29.57% (P_{75}), respectively as compared to the samples without PSB application. Data in Table (4) show that, the HClc- Pi values were decreased insignificantly with PSB applied in the soil under study.

Table 4. Mean values for organic and inorganic P fractions (mgkg^{-1}) as affected by applying of PSB and P fertilizer in the calcareous soil

	Treatments	Organic fractions (mgkg^{-1})			Total	Inorganic fractions (mg/kg)				
		$NaHCO_3$-Po	NaOH-Po	HClc-Po	Po	NH_4Cl-P	$NaHCO_3$-Pi	NaOH-Pi	HCl_D-P	HClc-Pi
	100%NPK Without (PSB)	5.75^{a}	6.21^{a}	13.27^{a}	25.23^{a}	13.49^{e}	17.95^{e}	19.24^{a}	397.82^{a}	69.58^{a}
	Without	3.25^{b}	5.23^{b}	8.25^{b}	16.73^{b}	1.48^{g}	3.01^{f}	6.21^{e}	294.26^{c}	58.13^{c}
	Bacillus megatherium	2.5 5^{c}	4.55^{c}	6.01^{d}	12.11^{c}	7.82^{f}	16.88^{e}	4.60^{f}	263.95^{d}	56.01^{d}
P_0	*Lysinibacillus boronitolerans*	2.03^{c}	2.96^{d}	4.45^{e}	9.44^{d}	9.88^{f}	23.82^{de}	3.90^{f}	257.35^{d}	56.08^{d}
	B. megatherium + L. boronitolerans	2.00^{c}	2.25^{d}	2.61^{g}	6.86^{e}	12.80^{ef}	27.56^{d}	3.50^{f}	252.85^{d}	56.74^{d}
	Without	5.13^{a}	6.11^{a}	13.26^{a}	24.51^{a}	9.92^{f}	12.26^{ef}	13.10^{b}	334.24^{bc}	56.31^{d}
	Bacillus megatherium	3.53^{b}	4.05^{c}	8.35^{b}	15.94^{b}	15.37^{e}	31.06^{d}	11.60^{c}	290.71^{c}	60.50^{b}
P_{50}	*Lysinibacillus boronitolerans*	2.55^{c}	3.22^{d}	4.22^{e}	9.99^{d}	20.45^{d}	41.85^{c}	8.06^{d}	276.60^{d}	57.37^{c}
	B. megatherium + L. boronitolerans	2.02^{c}	2.55^{e}	2.01^{g}	6.58^{e}	26.25^{c}	44.91^{c}	6.63^{e}	250.55^{e}	58.54^{c}
	Without	5.65^{a}	6.22^{a}	13.71^{a}	25.58^{a}	12.80^{ef}	15.47^{e}	14.91^{b}	357.95^{b}	57.10^{d}
	Bacillus megatherium	3.21^{b}	3.33^{d}	7.22^{c}	13.76^{c}	25.16^{c}	46.63^{c}	12.42^{c}	295.25^{c}	56.30^{d}
P_{75}	*Lysinibacillus boronitolerans*	1.22^{d}	2.04^{e}	5.02^{e}	8.28^{d}	35.35^{b}	60.93^{b}	8.81^{d}	277.55^{d}	55.83^{e}
	B. megatherium + L. boronitolerans	1.05^{d}	1.89^{e}	1.85^{g}	4.79^{f}	47.05^{a}	81.70^{a}	6.66^{e}	252.10^{e}	55.17^{e}
	LSD $_{(0.05)}$	0.045	0.33	0.055	0.21	1.033	0.055	0.55	2.033	1.011

**Mean values followed by letters indicate significant differences among P levels with and without PSB by the LSD test at P < 0.05.

2. Strains of bacteria

Bacillus megatherium and *Lysinibacillus boronitolerans* used as growth promoting bacteria and phosphate solubilizing bacteria. As soil drench on plants with concentration up to (10^8 CFU) in nutrient broth media, they added individually by dilution **1**(bacteria broth culture)**: 3** (equal volume of water). This agrees with Aliyat *et al*. (2022) who tested 9 strains of phosphate solubilizing bacteria (PSB) for their ability to solubilize phosphate and turn insoluble phosphorus to plant-available type. This (PSB) specificity allows for increased P availability, which is an immobile element in soil. All of the strains investigated a substantial significant capacity to dissolve three forms of inorganic phosphates: Tricalcium phosphate Ca_3 $(PO_4)_2$, Aluminium phosphate ($AlPO_4$), and Iron phosphate ($FePO_4$) for use as biofertilizers in calcareous or acidic soil.

3. Microbiological and Biochemical analysis of bacteria

Determinations of phosphate solubilization bacteria

On Pikovskaya medium, *Lysinibacillus boronitolerans* and *Bacillus megatherium* grown and gave clear zone around bacterial colonies and observed for yellow colour change as positive result, which mean the bacteria produce some organic acids or enzymes, as coming in results. This agree with Odeniyi and Turaki (2022**)** who studied that the phosphorus is an important nutrient for plant growth and it is abundant in the soil but inaccessible because it is Slightly soluble and complexes forms with metals. Insoluble phosphate can be mineralized and solubilized by microorganisms into bio-available shapes. On Pikovskaya, the goal of this study was for isolation and identification phosphate-solubilizing microorganisms, create alkaline phosphatase, and estimate their plant growth supporting capacities. On the third day of incubation, *Bacillus* sp. produced the highest alkaline phosphatase at pH 8, 42^{o}C, and solubilized quantities was 848.89 g/ ml of phosphates, respectively.

Determination of phosphatase enzyme

Bacillus megatherium doesn't produced acidic phosphatase enzyme, but produced alkaline phosphatase enzyme equal (9.5±0.05uM), while *Lysinibacillus boronitolerans* produced acidic phosphatase enzyme equal (7.74±0.05 uM) and produced alkaline phosphatase enzyme equal (5.6±0.02 uM), this agree with Abdelgalil *et al*. (2021) who investigated increasing the alkaline phosphatase up to 16.5- fold compared to baseline medium. The unregulated pH batch culture condition, on the other side, the greatest production of alkaline phosphatase was (7119.4 U L^{-1}) and specific growth rate ($\mu = 0.188\ h^{-1}$) at 15 hours from incubation time, which was increased > 20.75- fold above the baseline medium.

Determination of Organic acids by HPLC

Table (5), showed that a *Lysinibacillus boronitolerans* produced many kinds of organic acids as: formic, lactic, acetic, citric succinic, propionic and butyric acid. *Bacillus megatherium* also produced all of them except butyric acid. These organic acids important to keep phosphorus in soil available to plant absorption by decrease pH of soil rhizosphere and improve the rhizosphere conditions, this agree with Saeid *et al.* (2018) who discovered that *Bacillus* species generated organic acids (gluconic, acetic, lactic, propionic and succinic) and solubilized P_2O_5, with the exception of *B. megaterium*, which did not create propionic acid. Lactic and acetic acids were the most abundantly produced. An inhibition in pH was noticed in the plurality of the discussed cases.

4. Field experiment

Determination of Spinach Plant growth parameters

Table (6), showed that high significant result of spinach lengths compared to control was (17.6%) by using 75% P with (*Lysinibacillus boronitolerans* + *Bacillus megatherium*) treatment. The highest significant result of shoot weight compared to control was (23.4%) by using treatment of 75% P with (*Lysinibacillus boronitolerans* + *Bacillus megatherium*). These agree with Jabborova *et al*. (2022) who suggested the useful microbes as an actual method for improvement of plant growth, nutrient uptake and soil nutrients plant evolution. Ginger physical features and soil enzyme activities was affected. Furthermore, the co-inoculation of *Bacillus endophyticus* 33 increased height of plant (81%), quantity of leaf by (70%) and leaf length by (82%) and leaf width by 40% when compare to control. When compare to control treatment, *B. endophyticus* was increased height of plant by (51%), quantity of leaf by (56%) and leaf length by (67%) and leaf length by (27%). This on the same line with Agurre-Monory *et al*. (2019) who investigated that the *Lysinibacillus sphaericus* was used in soil review in the repair steps because it is able to fix nitrify, nitrogen and release phosphorus, supporting soil nutrients used for plant growth. Using a mixture of *L. sphaericus* OT4b.31, OT4b.49, CBAM5, III (3) 7, and 2362 strains, we evaluated nitrites, nitrates, ammonium, phosphorus and indole acetic acid levels in soil. Soils containing many *L. sphaericus* more differences in ammonium, nitrates, nitrites, phosphorus and indole acetic acid levels compared to control. *L. sphaericus* perhaps was an efficient nutrition improver and plant growth promoter for recultivation treatments, according to our results.

5. Chemical analysis of Spinach

Determination of Phosphorus percent in Spinach

Table (7), showed that highest significant result of Phosphorus (%) was increased up to (38.55 %) by using treatment of 75% P with (*B. megatherium* + *L. boronitolerans*) compared to control. It was taken from whole plant of spinach. That agree with Bhatt and Maheshwari (2020) who evaluated whole treatments, infected seeds with *Bacillus megatherium* (CDK25) exhibited a significant increase in many oncoming elements as crude fiber (3.31%), crude protein (3.84%) and ash (2.53%) compared to control. The study recommends using "*B. megatherium*" to realize the long-term potential for strengthening and supplementing plant functional, biological and nutritional functions, consequently improving *C. annuum* L. Total edible quality and soil minerals, these the same opinion with Zheng *et al*. (2018) who found a significant relationship between dissoluble P content and the 168 h time of incubation of these four strains. The quantities of soluble P in the medium was inversely exponentially linked to pH, and the succinic acid concentration was significantly linearly connected to the amount of P soluble (P 0.001), suggesting that organic acid perhaps mobilize microbial P.

Determination of total chlorophyll and carotenoids content

Table (8), showed that the high significant results of Chlorophyll A and Chlorophyll B (mg/g) compared to controls. The results increased up to (73.34 and 81.25 %, respectively) by using treatment of 75% P + (*B. megatherium* + *L. boronitolerans*), compared to control. Carotenoids values were no significant, because they went to build Vitamin A. These results in agreement with that found by Jabborova *et al*. (2022) whom discovered that *Bacillus endophyticus* IGPEB 33 and AMF increased chlorophyll a (81-58%), chlorophyll b by (68-37%), total chlorophyll by (74-53%), and carotenoids content by (67-55%), respectively. However, as compared to control, the co-inoculation of *B. endophyticus* IGPEB 33 and AMF dramatically promoted chlorophyll a (86%), chlorophyll b by (72%), total chlorophyll by (82%) and carotenoids content by (83%). Furthermore, compared to other treatments, plant-growth-promoting *B. endophyticus* IGPEB 33 and AMF inoculation improved soil nutrients and soil enzyme activity. In nutrient-shortage soil, we think that a combination of *B. endophyticus* as plant-growth-promoting and AMF inoculation might be a more sustainable and environmentally good method.

Table 5. Determination of organic acids by HPLC

Bacteria	Organic acids	Area	Conc. (µg/ml)
Lysinibacillus boronitolerans	Formic acid	5.25	172.20 ^{f}
	Lactic acid	5.88	351.64^{c}
	Acetic acid	9.20	522.74^{b}
	Citric acid	1.40	40.78^{g}
	Succinic acid	4.61	318.02^{d}
	Propionic acid	2.45	243.10^{e}
	Butyric Acid	4.99	664.66^{a}
LSD $_{(0.05)}$		-	3.2025
Bacillus megatherium	Formic acid	7.36	241.26^{d}
	Lactic acid	35.06	2097.98^{a}
	Acetic acid	16.04	911.66^{b}
	Citric acid	1.79	51.94^{e}
	Succinic acid	4.74	326.97^{c}
	Propionic acid	0.86	85.98^{e}
	Butyric Acid	ND	ND
LSD $_{(0.05)}$		-	2.5159

Table 6. Determination of Spinach Plant growth parameters

Treatments		Lengths (Cm)	Fresh weight (Kg/Feddan)	Dry weight (Kg/Feddan)
Without biofertilizers	Control (100%NPK)	64.33^{a}	53.79^{c}	3.17
	75%P	45.5^{cd}	27.43^{g}	2.73
	50%P	43.16^{df}	38.25^{e}	1.21
P_0	*Bacillus megatherium*	37.66^{f}	59.47^{b}	3.01
	Lysinibacillus boronitolerans	41.66^{e}	42.39^{d}	1.58
	B. megatherium+ L. boronitolerans	33^{g}	18.83^{i}	1.95
P_{50}	*Bacillus megatherium*	36^{fg}	29.76f^{g}	3.5
	Lysinibacillus boronitolerans	43.33^{df}	52.17^{c}	1.86
	B. megatherium+ L. boronitolerans	46.66^{c}	57.14^{b}	2.29
P_{75}	*Bacillus megatherium*	34^{g}	31.11^{f}	2.53
	Lysinibacillus boronitolerans	45^{cd}	22.98^{h}	1.17
	B. megatherium+ L. boronitolerans	53^{b}	66.38^{a}	3.32
LSD $_{(0.05)}$		3.2633	2.6645	NS

*P_0= without phosphorus fertilizer. P_{50}=50% phosphorus fertilizer. P_{75}=75% phosphorus fertilizer.

Table 7. Determination of Phosphorus percent in Spinach

Treatments		P (%)
Without biofertilizers	Control (100%NPK)	0.236^{ef}
	75%P	0.222^{fg}
	50%P	0.211^{g}
P_0	*Bacillus megatherium*	0.233^{ef}
	Lysinibacillus boronitolerans	0.260^{d}
	Bacillus + Lysinibacillus	0.293^{b}
P_{50}	*Bacillus megatherium*	0.239^{e}
	Lysinibacillus boronitolerans	0.274^{cd}
	B. megatherium+ L. boronitolerans	0.288^{bc}
P_{75}	*Bacillus megatherium*	0.283^{bc}
	Lysinibacillus boronitolerans	0.323^{a}
	B. megatherium+ L. boronitolerans	0.327^{a}
	LSD $_{(0.05)}$	**0.0152**

*P_0= without phosphorus fertilizer. P_{50}=50% phosphorus fertilizer. P_{75}=75% phosphorus fertilizer.

Table 8. Determination of total chlorophyll and carotenoids content

Treatments		Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Carotenoids (mg/g)
Without biofertilizers	Control (100%NPK)	4.69^{cd}	3.2^{e}	2.55
	75%P	7.82^{ab}	3.62^{d}	2.09
	50%P	6.12^{bc}	4.30^{c}	1.83
P_0	*Bacillus megatherium*	7.46^{ab}	5.17^{b}	1.91
	Lysinibacillus boronitolerans	6.13^{bc}	4.15^{c}	1.78
	B. megatherium+ L. boronitolerans	7.43^{ab}	5.27^{b}	1.74
P_{50}	*Bacillus megatherium*	8.52^{a}	5.07^{b}	1.98
	Lysinibacillus boronitolerans	7.09^{ab}	3.69^{d}	2.02
	B. megatherium+ L. boronitolerans	2.57^{d}	0.89^{g}	1.18
P_{75}	*Bacillus megatherium*	6.95^{ab}	5.12^{b}	1.98
	Lysinibacillus boronitolerans	4.43^{cd}	2.69^{f}	1.70
	B. megatherium+ L. boronitolerans	8.13^{ab}	5.8^{a}	1.79
	LSD $_{(0.05)}$	2.2366	0.2079	NS

*P_0= without phosphorus fertilizer. P_{50}=50% phosphorus fertilizer. P_{75}=75% phosphorus fertilizer.

6. Determination of Spinach rhizosphere microbial counts

Table (9), showed that the highest significant results in nitrogen fixers counts was (69 MPN/ml), phosphate dissolving bacteria was (73 CFU/ml), total bacterial counts was (175 CFU/ml) and Dehydrogenase enzyme was (69.4 µg TPF/g) by using treatment of 75 % P + (*B. megatherium+ L. boronitolerans*) compared to controls. These agree with Zhao *et al.* (2021) who studied the *Bacillus megaterium* can enhanced the richness of soil bacterial and fungal communities significantly. High-throughput identified of sequencing that the application of *B. megaterium* did not change the predominant bacterial compositions of *Acidobacteria*, *Actinobacteria*, and fungal in soil, but increased the abundances of the bacterial genera beneficial and fungal orders. Moreover, soil pH, total P was related to the changes of soil bacterial community structure. The findings indicated that the application of *B. megaterium* development of a more vegetables sustainable production system by enhancing the properties of soil microbial community and the bioavailability of soil P.

Table 9. Determination of Spinach rhizosphere microbial counts

Treatments		Nitrogen fixers count (10^3X MPN/ ml)	PSB counts (10^3XCFU/ gm dry soil)	Total count (10^5XCFU/ml)	Dehydrogenase (enzyme µg TPF/g dry soil/24h)
Without biofertilizers	Control (100%NPK)	39	58	144	53.7^{cde}
	75%P	36	56	140	53.2^{df}
	50%P	33	43	137	51.5^{de}
P_0	*Bacillus megatherium*	50	61	152	56.9^{bcd}
	Lysinibacillus boronitolerans	48	40	120	52de
	Bacillus + Lysinibacillus	45	38	140	51.9^{de}
P_{50}	*Bacillus megatherium*	56	59	157	60b^{c}
	Lysinibacillus boronitolerans	37	57	166	61.7^{b}
	B. megatherium + L. boronitolerans	60	62	158	62.2^{b}
P_{75}	*Bacillus megatherium*	38	35	117	50^{e}
	Lysinibacillus boronitolerans	42	65	163	62.4^{b}
	B. megatherium+ L. boronitolerans	69	73	175	69.4^{a}
	LSD $_{(0.05)}$	-	-	-	6.5917

*P_0= without phosphorus fertilizer. P_{50}=50% phosphorus fertilizer. P_{75}=75% phosphorus fertilizer.

CONCLUSION

Bacillus megatherium and *Lysinibacillus boronitolerans* were used in this work, both of which produced phosphatase enzyme and organic acids. *B. megatherium* produced alkaline phosphatase enzyme but no acidic phosphatase enzyme, whereas *L. boronitolerans* produced both acidic and alkaline phosphatase enzymes. *L. boronitolerans* produced a diverse variety of organic acids. Except for butyric acid, *B. megatherium* produced all of them. These are the primary reasons for improving the examined calcareous soil and supporting variations in phosphorus fraction available for plant uptake. The treatments with 75% P with (*B. megatherium* + *L. boronitolerans*) yielded the most significant outcomes in terms of P % in soil. When compared to controls, the treatment of 75% P with (*B. megatherium* + *L. boronitolerans*) yielded the more plurality significant results in terms of Spinach lengths, shoot weights, P (%), Chlorophyll A & B, Vitamins A, B, C and E, bacterial counts of nitrogen fixers, phosphate solubilizing bacteria, total bacterial counts, and Dehydrogenase enzyme**.**

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الملخص العربي

تغيرات فى صور الفوسفور وحالة تيسره تحت تأثير استخدام الأسمدة الحيوية والمعدنية فى الأراضى الجيرية

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من أهم الأسباب الرئيسية لإجراء هذه الدراسة هوتعزيز التربة الجيرية المدروسة ودعم تغيرات صور الفسفور للوصول إلى حالة تيسرها لامتصاص النبات لذلك تم استخدام نوعين من البكتيريا المذيبة للفوسفات PSB ، (*Lysinibacillus boronitolerans* و *Bacillus megatherium*) مع معدلات مختلفة من السماد المعدني للفسفور للوصول إلى هذا الهدف. وكانت المعاملات بـ (P 50%) أو (P 75%) متفوقة في زيادة الفوسفور الميسر مقارنة بالمعاملات الأخرى وحدها. تم الحصول على أعلي نتائج معنوية للـ P% الميسرفي التربة باستخدام معاملة (P 75% مع *L. boronitolerans*) ومعاملة P 75% مع(*B. megatherium + L. boronitolerans*). كانت معالجات الجمع بين *B. megatherium* و *L. boronitolerans* متفوقة في زيادة الفسفور الميسر مقارنة بالمعاملات الأخرى وحدها حيث زاد labile-P بشكل ملحوظ عند ($P < 0.05$). وكشفت النتائج أن معاملات PSB ومعدلات الـP المضاف تؤثر معنوياً علي قيم الرقم الهيدروجيني للتربة بالمقارنة مع الكونترول، حيث ظهر أعلى انخفاض في الرقم الهيدروجيني في التربة عند المعالجة بالدمج بين (P_{50}) أو (P_{75}) مع (*B. megatherium* + *L. boronitolerans*). كما اوضحت النتائج ايضا ان *L. boronitolerans* أنتج إنزيم الفوسفاتيز الحمضي والقلوي، بينما *B. megatherium* أنتجت إنزيم الفوسفاتيز القلوي فقط. كما أنتج *L. boronitolerans* العديد من الأحماض العضوية بينما *B. megatherium* أنتجها كلها فيما عدا حمض البيوتريك. أعطت معاملة 75% P مع (*L. boronitolerans* + *B. megatherium*) نتائج جيدة وعالية في أطوال السبانخ وأوزان المجموع الخضري و P (%) وكلوروفيل A و B وفيتامينات A، B، C و E، المثبتات النيتروجينية ، البكتيريا المذيبة للفوسفات، العدد الميكروبي الكلي وأنزيم الهيدروجيناز فى التربة.

نوصي باستخدام المعاملة 75% من الفسفور مع (*L. boronitolerans* + *B. megatherium*) لتيسيرالفسفور في التربة الرملية الجيرية وزيادة إنتاجية المحصول.