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Effect of Phosphorus Solubilizing Bacteria on Nutrients uptake by Broad bean (*Vicia faba* L.) Plant and phosphorus availability at different Phosphorus Levels

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Abstract

This research was conducted at the plastic house at the College of Agricultural Sciences, University of Sulaimani, Bakrajo, during 2016 to study the effect of phosphorus solubilizing bacteria (*Bacillus megaterium*) at different phosphorus levels (0, 40, 80, 160 and 320) kg P ha^{-1} in calcareous soil on uptake of some nutrients in broad bean plants and phosphorus availability. The experiment was performed in a factorial experiment with completed randomized design (CRD) in a silty clay loam soil, with three replications for each treatments, thus the total number of experimental units was (2×5×3=30) units. After ten weeks of growth, the plants were harvested to determine plant shoot nutrients content of (N, P, k, Fe and Zn and available phosphorus in soil. The following results were obtained inoculating soil with phosphorus solubilizing bacteria was significant for increasing shoot N, P, K, and Fe content, the highest values were 11.61 g kg^{-1}, 3.82 g kg^{-1}, 16.18 g kg^{-1} and 90.66 µg g^{-1} respectively, recorded at highest P level (320 kg P ha^{-1}), while the highest value for shoot Zn content was 52.83 µg g^{-1} recorded at medium P level (80 kg P ha^{-1}) and the highest value for available P in soil was 10.36 µg l^{-1} recorded at highest P level (320 kg P ha^{-1}).

Introduction

Phosphorus (P) is one of the most important essential macronutrients limiting plant growth and yield, improving crop quality, preventing plant diseases and plays a crucial role in plant biology, phosphorus plays a significant role in several physiological and biological plant activities, it is involved in several key plant functions such as photosynthesis, respiration, energy metabolism, the synthesis of nucleic acid, transformation of sugars, nutrient movement within the plant, nitrogen fixation in legume plants, enzyme regulation and transportation of the genetic traits *[1]*. Phosphorus solubilizing bacteria refer to a group of soil microorganisms that as a component of P cycle can release it from insoluble sources through producing phosphate enzymes, and from dissolving organic phosphorus compounds by secreting different organic acids such as oxalic, citric, butyric, malonic, lactic, succinic, malic and acetic acid or which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate thereby converting it into soluble forms. The phosphorus solubilizing bacteria is effective in releasing phosphorus from inorganic and organic pools, of total soil phosphorus through solubilization and mineralization. Broad bean is an important legume crop either for protein, or yield for human and animal consumption of the world, it is a good source of minerals like Ca, Mg, K, Zn, Cu various vitamins, protein, fat, and carbohydrate ***[2]***. It improves soil fertility due to fixing atmospheric nitrogen. Phosphorus solubilizing bacteria are necessary for improving the growth, quality, and yield of broad bean. Minimized ecological pollution caused by chemical fertilizers is more important for reducing input energy costs and seeking more sustainable agricultural production ***[3]***. Since there are no detailed studies carried out on using phosphorus solubilizing bacteria as bio fertilizers for broad bean plant especially in Kurdistan region therefore, pay attention to such studies. There are problems in Iraqi-Kurdistan region such that most of the soil contains a high amount of calcium carbonate ($CaCO_3$), which fix the available phosphorus and precipitate it as tri-calcium phosphate and this leads to deficiency of available phosphorus ***[4]***.

Materials and Methods

This research was carried out at the Department of Soil and Water Sciences and plastic house of College of Agricultural Sciences, University of Sulaimani in Bakrajo, during 2016, to study the effect of phosphorus solubilizing bacteria *Bacillus megaterium* which purchased from (Dr. Adel Kamal Khider in Salahaddin University, College of Education). On uptake some nutrients by broad bean plant under different phosphorus levels (0, 40, 80, 160 and 320 kg P ha^{-1}). Rock phosphate was used in this study as a source of phosphorus which contained 8.6% P. The soil used in this study belongs to silty clay loam (Vertisol order), the soil was collected from the research field experiment of College of Agricultural Sciences at the depth of 20-40 cm, and the soils were low in phosphorus content. The soil samples were air dried and sieved to pass through a 4 mm aperture size and stored in the trashcan. (Table: 1) shows some physical and chemical properties of the studied soil. The sterilized broad bean seeds were soaked in a suspension of bacteria (*Bacillus megaterium)* for 30 min. Arabic Gum 16% was added as an adhesive agent prior to inoculation. Then broad bean seeds were planted in each pots treatment. Soil pots were also either inoculated or non-inoculated with 5 ml of a suspension of bacteria *Bacillus megaterium* which was uniformly added directly in to the planting holes in each pot 5 cm diameter by 5 cm depth, and then seeds were covered with 5 cm thick soil layer.

The pots were arranged at a plastic house in a factorial experiment conducted with completed randomized designed (CRD) by three replications per treatment,

The pots were watered and maintained at 70% of field capacity, after germination, the seedling were thinned to two plants per pot. The plants were grown under natural light in a plastic house. All pots were irrigated with tap water every three days to keep the soil at 70% of its field capacity by regular weighting of pots, during the study period. After 10 weeks of growth the plants were harvested and the shoots removed from the roots and, the shoots were dried at 70 $^{\circ}$C for 72 hours, and the oven dry shoots were ground to pass through a 0.5 mm sieve and some macro N,P,k and micro Fe,Zn nutrients content were determined and after the available p in pot soil were determined.

Table-1: Some important physical and chemical properties of the soil used in the experiment:

Soil properties	The Value
Sand (g kg^{-1})	67.70
Silt (g kg^{-1})	432.70
Clay (g kg^{-1})	499.60
Texture	Silty Clay Loam
pH	7.07
ECe $ds.m^{-1}$ at 25°C	0.15
Organic Matter (g kg^{-1})	10.00
Calcium Carbonate (g kg^{-1})	240
Total Nitrogen (g kg^{-1})	0.10
Available Phosphorus (mg kg^{-1})	2.67
Soluble K^+ ($mmol.l^{-1}$)	0.38

Results and Discussion

Shoot Nitrogen Content (g kg^{-1})

The result in figure 1: illustrates that the values of shoot N content in inoculated plants with *B. megaterium* significantly raised compared with non-inoculated plants. The highest shoot N content 11.61 g kg^{-1} was recorded from the highest P levels (320 kg P ha^{-1}), while the lowest value for shoot N content was 10.36 g kg^{-1} recorded from control treatment (Zero kg P ha^{-1}). This result is in agreement with the result found by ***[5]***. The increasing of shoot N content in *B. megaterium* broad bean plants, might be due to increasing N uptake by plant roots and excreting different organic acids which dissolve rock phosphate resulting in raised supply of available P in the soil solution that helped N_2-fixation in legume plants and improved mineral nutrients uptake, specially N by crops leading to increased N in the plants ***[5].***

Figure 2: shows the relation between phosphorus levels and shoot nitrogen content which fit the quadratic polynomial correlation with (R^2=0.9674) for inoculated with phosphorus solubilizing bacteria

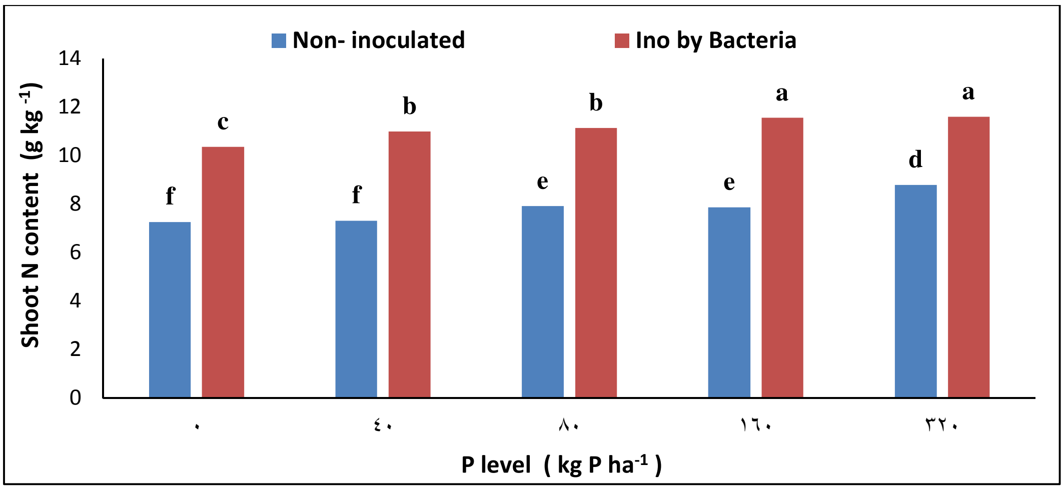
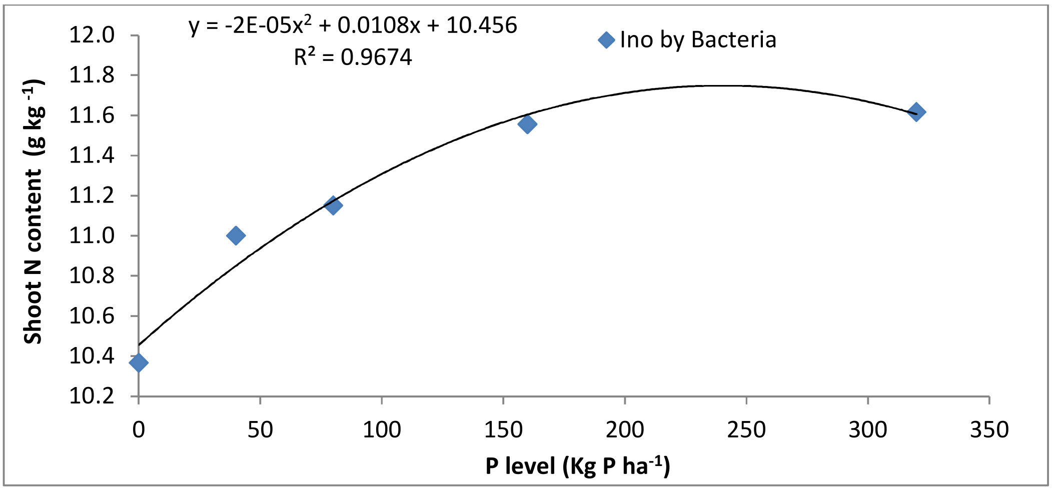


Figure-1: Effects of phosphorus solubilizing bacteria inoculated and non-inoculated on broad bean shoot N content (g kg $^{-1}$) at different phosphorus levels.



Finger-2: The relation between shoot N content and phosphorus levels for plants inoculated with phosphorus solubilizing bacteria on broad bean plant.

Shoot Phosphorus Content

It is clear from the data presented in figure3: that inoculated soil with *B. megaterium,* significantly elevated shoot P content in broad bean plants compared with non-inoculated plants. The highest value for shoot P content was 3.82 g kg $^{-1}$ recorded at the highest P levels (320 kg P ha $^{-1}$), and the lowest value was 2.20 g kg $^{-1}$ recorded at the lowest P level (Zero kg P ha $^{-1}$). In non-inoculated plants, the highest value 2.92 g kg $^{-1}$ was recorded at the highest P level (320 kg P ha $^{-1}$), and the lowest value 1.44 g kg $^{-1}$ was recorded at the lowest P level (Zero kg P ha $^{-1}$). As well as in both inoculated and non-inoculated plants with *B.megaterium,* the shoot P content increased with increasing P levels. This result is in agreement with the results of ***[7]***. Increasing broad bean shoot P content in inoculated plants with bacteria *B. megatarium* may be due to the activity of bacteria to secrete different organic acids and enzymes that act on insoluble phosphates and convert them into soluble forms that provide phosphorus to the plants ***[8]***, or may be due to reduction in soil pH in soil rhizosphere that rise the availability of P by organic acids excreted by *B. megatarium* ***[9]***. and the ability of *B. megaterium* to supply different organic acids, that dissolve insoluble P and convert them to available for the plant, and this led to rise in the concentration of P in the shoot ***[12]***.It appear from figure-2 that the shoot p content from inoculated treatments was sufficient for plant growth or above critical level (2g.kg) this explain the positive role of this bacteria.

Figure 4: Shows the relation between phosphorus levels and shoot phosphorus content which fit the quadratic polynomial correlation with (R^2=0.9487) for inoculated with phosphorus solubilizing bacteria

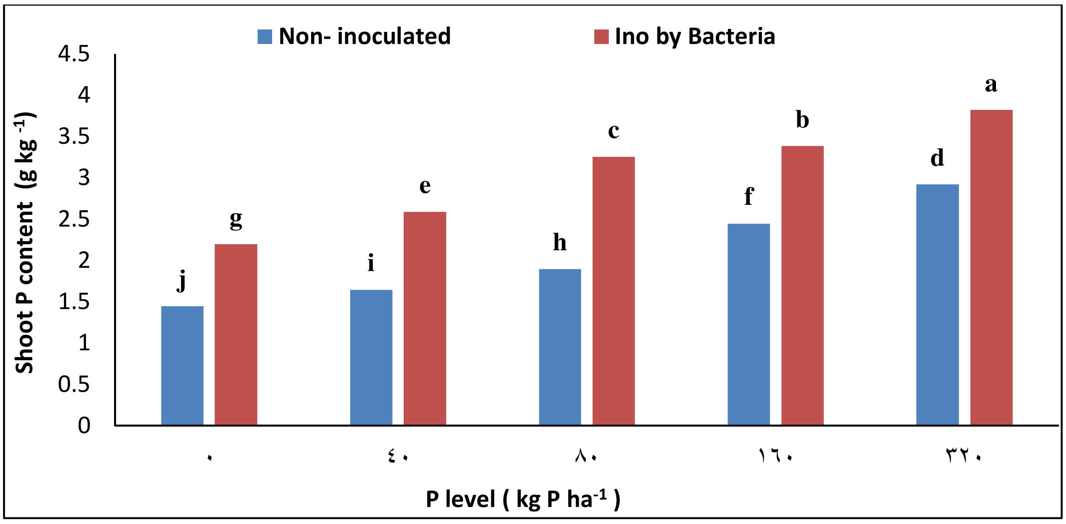


Figure-3: Effect of phosphorus solubilizing bacteria inoculated and non-inoculated on broad bean shoot P content (g kg^{-1}) at different phosphorus levels.

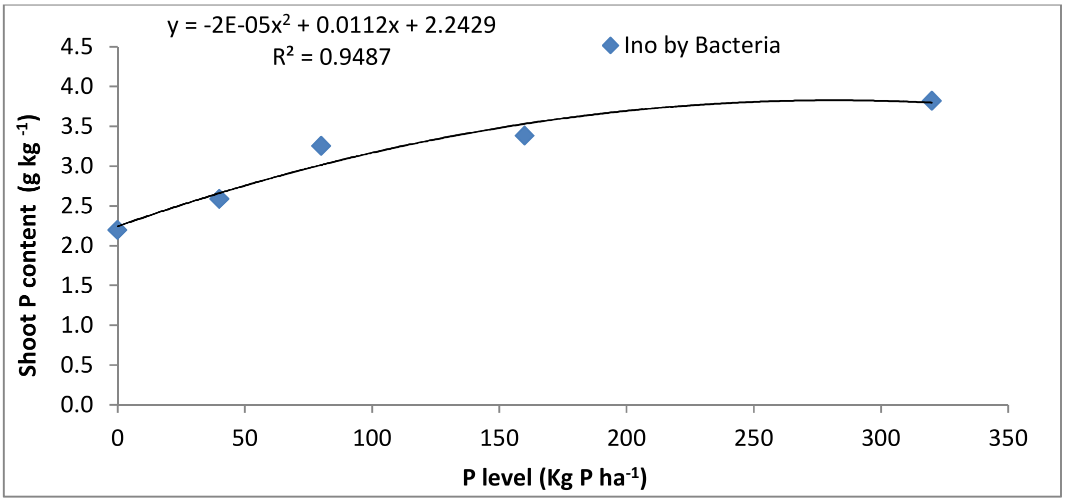


Figure-4: The relation between shoot p content and phosphorus levels for plants inoculated with phosphorus solubilizing bacteria.

Shoot Potassium Content (g kg^{-1})

The results in figure 5: indicate that when the soil inoculated with *B. megaterium,* the broad bean shoot K content significantly increased at different P levels compared with non-inoculated plants, and shoot K content elevated by increasing P levels *[10]*. The maximum value for shoot K content was 16.18 g kg^{-1} recorded at (320 kg P ha^{-1}), while the minimum value 12.63 g kg^{-1} was recorded at (Zero kg P ha^{-1}), while in non-inoculated plants, the highest value 12.16 g kg^{-1} was recorded at the maximum P levels (320 kg P ha^{-1}), while the lowest value 8.96 g kg^{-1} was recorded at the minimum P levels (Zero kg P ha^{-1}).

The increasing of shoot K content in broad bean plants inoculated with phosphorus solubilizing bacteria may be due to the elevated K uptake by plants roots in inoculated plants due to excretion of different organic acids by *B. megaterium* that dissolved rock phosphate resulting in a high supply of available P in the soil solution which helped legume plants growth and improved nutrients uptake such as K by the plants and led to increase the accumulation of K in the plants ***[11]***, or may be due to the ability of phosphorus solubilizing bacteria to convert nutritionally important nutrients from unavailable to available form such as K which raised the uptake by plant roots ***[12]***. Figure 6: shows the relation between phosphorus levels and shoot potassium content which fit the quadratic polynomial correlation with (R^2=0.8871) for inoculated with phosphorus solubilizing bacteria.

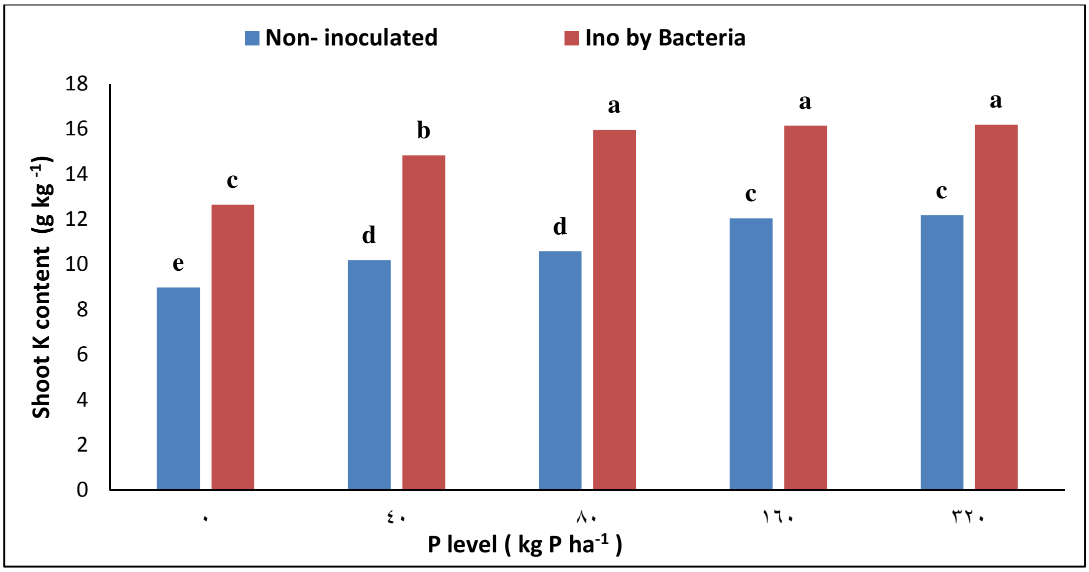


Figure-5: Effects of phosphorus solubilizing bacteria inoculated and non-inoculated on broad bean shoot K content (g kg^{-1}) at different phosphorus levels

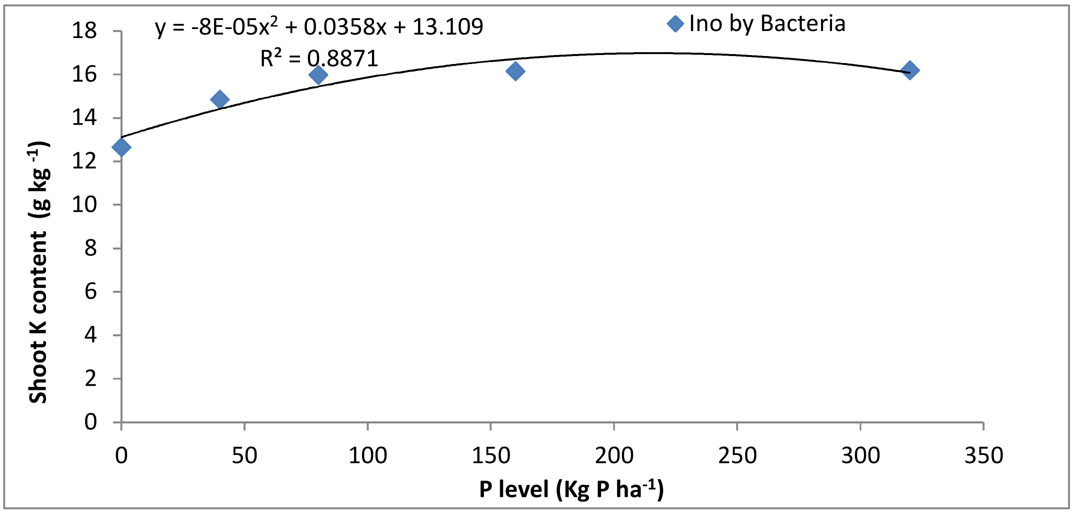


Figure-6: The relation between shoot K content and phosphorus levels for plants inoculated with phosphorus solubilizing bacteria on broad bean plant.

Shoot Iron Content (µg g^{-1})

The effect of inoculation with *B. megaterium* on broad bean plant shoot Fe content at different P levels were show in figure 7: It"s clear from the result that inoculated soil with bacteria *B. megaterium* significantly raised shoot Fe content in broad bean plants, the maximum value was 90.66 µg g^{-1} recorded at the highest P levels (320 kg P ha^{-1}), and lowest value 71.66 µg g^{-1} was recorded at (Zero kg P ha^{-1}), in non-inoculated plants. The maximum value was 68 µg g^{-1} recorded at the highest P levels (320 kg P ha^{-1}), while the lowest value 42 µg g^{-1} was recorded at the lowest P levels (Zero kg P ha^{-1}), and the results show that shoot Fe content increase by increasing P levels in both inoculated and non-inoculated plants.

The increasing of shoot Fe content in broad bean plants raised by bacteria *B. megaterium* may be due to the symbiotic relationship between bacteria and legume plants, that bacteria provide soluble phosphate to the soil solution and enhance plant production which increased more Fe in the plant ***[13]***, or might be due to the role of *B. megaterium* bacteria in phosphorus nutrients by enhancing the availability to plants through the release of inorganic and organic Fe pool by solubilization and mineralization, bacteria produce different organic acids, which increase available P, that promote plant growth and more uptake of nutrients such Fe.It means the inoculation caused increase in quality of broad bean science increase in iron to a certain level causes increase in quality. Figure8: shows the relation between phosphorus levels and shoot iron content which fit the quadratic polynomial correlation with (R^2=0.9805) for inoculated with phosphorus solubilizing bacteria

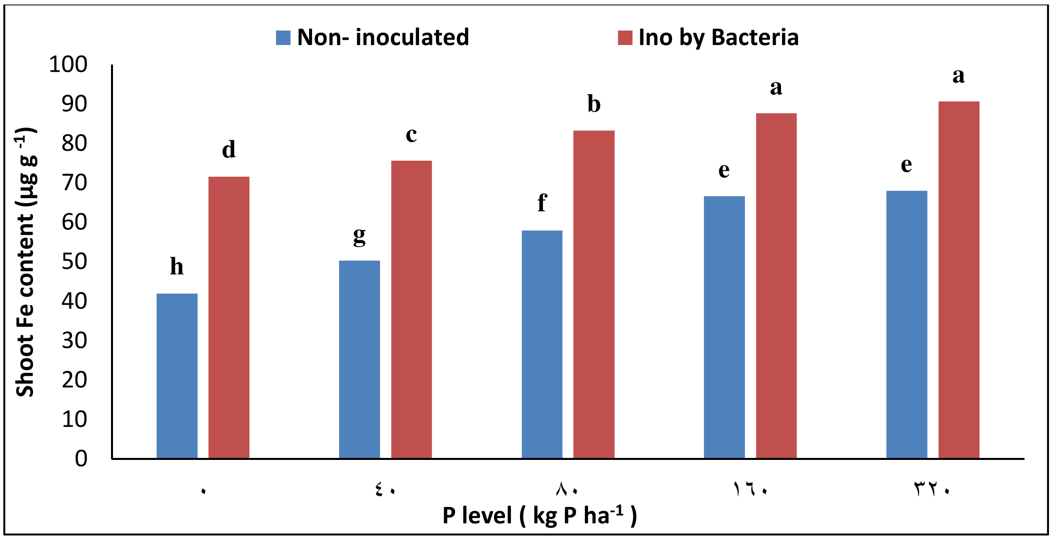


Figure-7: Effects of phosphorus solubilizing bacteria inoculated and non-inoculated them on broad bean shoot Fe content (µg g^{-1}) at different phosphorus levels.

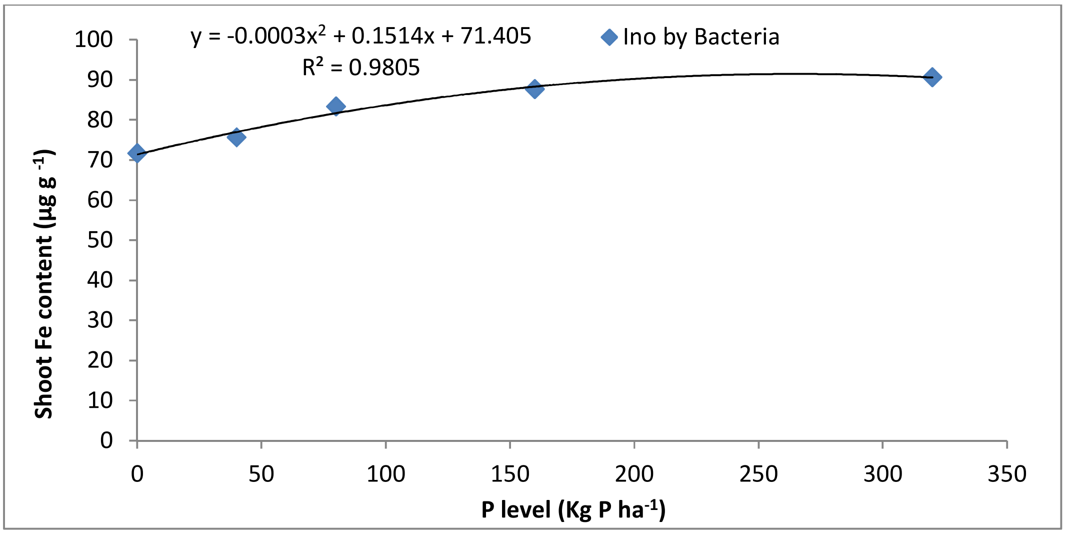


Figure-8: The relation between shoot Fe content and phosphorus levels for plants inoculated with phosphorus solubilizing bacteria.

Shoot Zinc Content (µg g^{-1})

Figure 9: shows the effect of *Bacillus megaterium* on shoot Zn concentration in the broad bean plants, the results shows when the soil inoculated by *B. megaterium,* the shoot Zn content of broad bean plants significantly rose at different P levels compared with non-inoculated plants. This result was in agreement with the result of ***[12]***.

The highest value for shoot Zn content was 52.66 µg g^{-1} recorded at the highest P levels (320 kg P ha^{-1}), while the lowest value was 30 µg g^{-1} recorded at lowest P levels (Zero kg P ha^{-1}), the maximum value for shoot Zn content in non-inoculated soil was 35.33 µg g^{-1} recorded at (320 kg P ha^{-1}), and the minimum value was 13 µg g^{-1} recorded at (Zero kg P ha^{-1}).The increasing shoot Zn content in inoculated plants by *B. megaterium* may be due to secretion of different organic acids such as gluconic and ketogluconic acids by the soil phosphorus solubilizing bacteria which dissolved the soil phosphorus along with decreasing the pH of the soil ***[14]***. Decrease in the pH and the production of organic acids had a combined effect in the solubilization of phosphate in the soil which raised plant growth, and improved Zn uptake and raised the amount of Zn in the plant ***[12]*** and ***[15]***, or might be due to the role of bacteria *Bacillus megaterium* in secreting enzymes and different organic acids which makes fixed micronutrients such as Zn convert in to mobile form available for plants, resulting in elevated a concentration of Zn in the plants ***[16].*** In addition, it may be due to excreting different organic acids by bacteria which decreased soil pH which raised the availability of micronutrients such as Zn in the soil and more uptakes by plants ***[8]***. Figure10: shows the relation between phosphorus levels and shoot zinc content which fit the quadratic polynomial correlation with (R^2=0.5249) for inoculated with phosphorus solubilizing bacteria

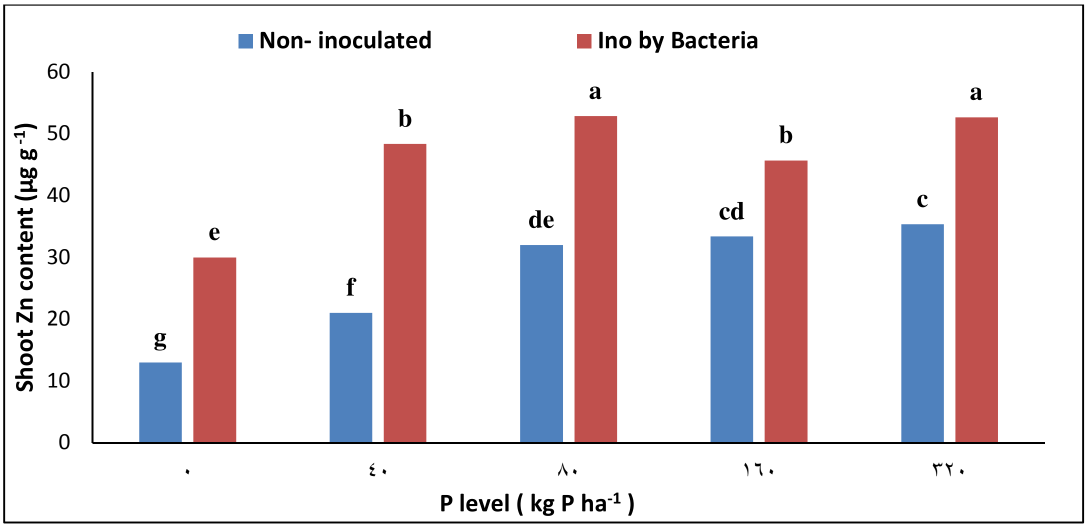


Figure-9: Effects of phosphorus solubilizing bacteria inoculated and non-inoculated on broad shoot Zn content (µg g $^{-1}$) at different phosphorus levels.

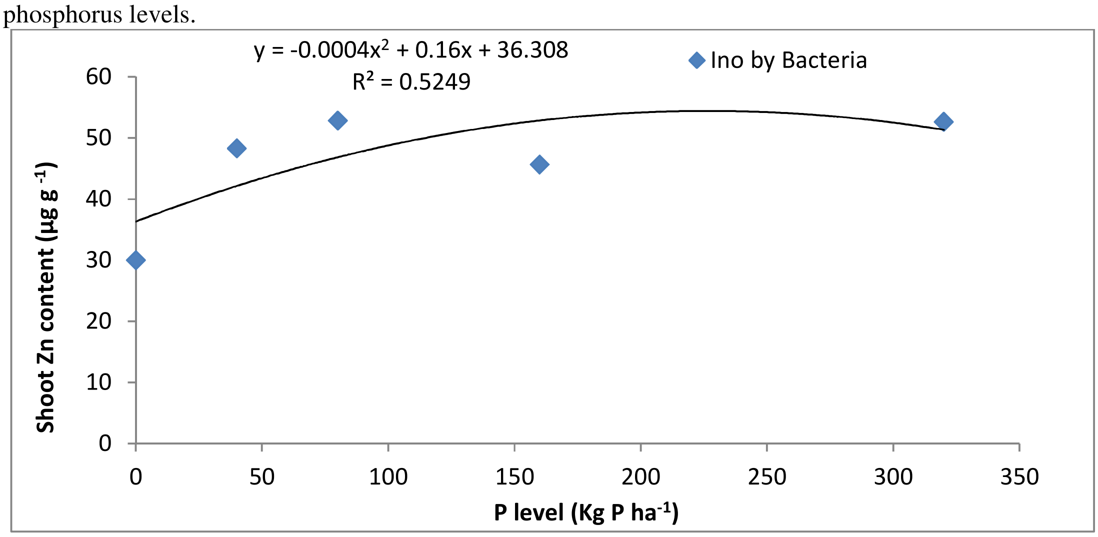


Figure-10: The relation between shoot Zn content and phosphorus levels for plants inoculated with phosphorus solubilizing bacteria on broad bean plant.

Available Phosphorus in Soil (mg l $^{-1}$)

Data presented in figure 11: shows when the soil inoculated with *Bacillus megaterium* , the available P in soil elevated significantly compared with non-inoculated plants, at different P levels and available P raised by increasing P levels. The same result was found by ***[17]***. The maximum value for available P in soil solution for inoculated plants by phosphorus solubilizing bacteria was 10.36 mg l^{-1} recorded at the highest P levels (320 kg P ha^{-1}), while the lowest value was 3.05 mg l^{-1} recorded at the lowest P level (Zero kg P ha$^{-1)}$ .It means inoculation with *Bacillus megaterium* caused raised in p in the soil from deficient level to adequate level (*more than 7mg.l*). However, for non-inoculated plant with phosphorus solubilizing bacteria , the highest value for available P in soil was 5.38 mg l^{-1} recorded at the highest P levels (320 kg P ha^{-1}), while the lowest value was 2.62 mg l^{-1} recorded at the lowest P levels (Zero kg P ha^{-1}). The mechanism of mineral phosphate solubilization by phosphorus solubilizing bacteria strains is associated with the release of low molecular weight of organic acids ***[18]***, which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms ***[19]***, or may be due to the role of bacteria *B. megaterium* excels in P availability in the soil by secreting phosphatase enzyme which promoted to change unavailable organic P to its available forms and accumulated in high concentration in the soil solution ***[12]***. Figure 12: shows the relation between phosphorus levels and available Phosphorus in Soil which fit the quadratic polynomial correlation with (R^2=0.9902) for inoculated with phosphorus solubilizing bacteria

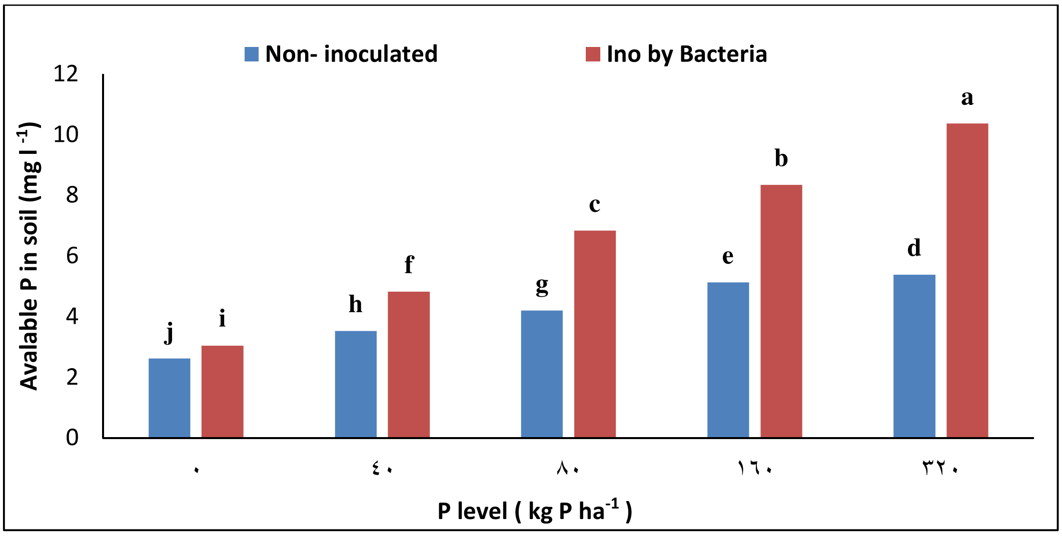


Figure-11: Effects of phosphorus solubilizing bacteria inoculated and non-inoculated on available phosphorus in soil (mg l^{-1}) at different phosphorus levels.

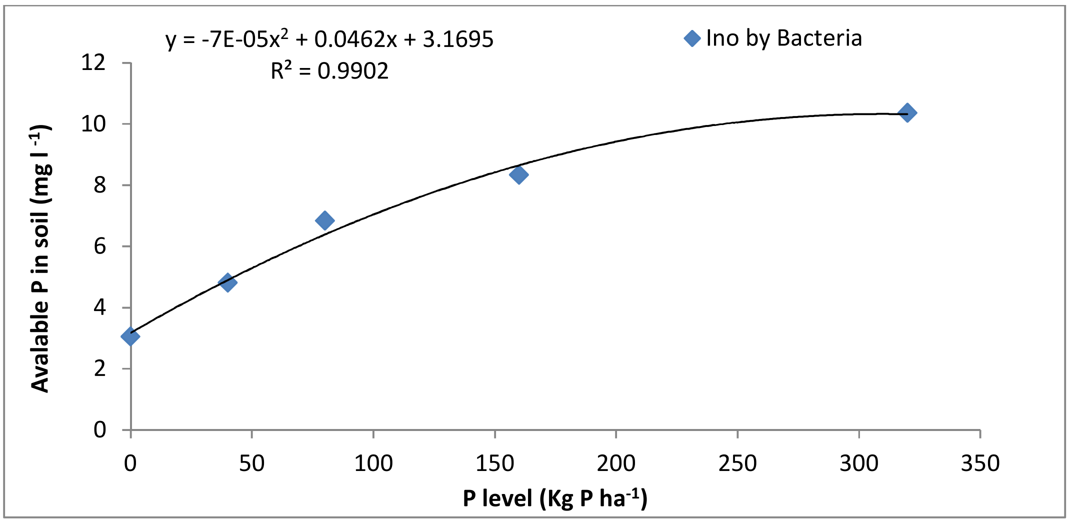


Figure-12: The relation between available phosphorus in soil and phosphorus levels for plants inoculated with phosphorus solubilizing bacteria on broad bean.

Conclusion

Inoculation of broad bean plants with phosphorus solubilizing bacteria had more effect on plant nutrients uptake than non-inoculated plants and Phosphorus solubilizing bacteria was very effective in increasing available phosphorus in the soil. On the other hand solubilizing bacteria had grate effect in directly on increasing the availability of N, k, Fe and Zn.

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