

The effect of biological inoculation (*Bacillus megatrium*, *Pseudomonas fluorescense*) to increase phosphorus availability and growth standards plant (barbin) *Portulaca oleracea* L.

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Received 6th October 2024; Accepted 22nd February 2025; Available online 24th August 2025

Abstract: A field experiment was carried out in the fields of the College of Medicinal and Industrial Plants, University of Kirkuk, to study the effect of biological inoculation in increasing available phosphorus in the soil and some growth characteristics of the barbin plant, and the interaction between them. A randomized block design with three replicates of the first factor, calcareous and gypsum soil, and the second factor, two types of bacteria and P fluorescence B megatrium., the results showed that the treatments inoculated with bacteria increased the amount of phosphorus available in the soil, plants, and all the studied plant characteristics compared to the non-inoculation treatment. The best treatment was the inoculation treatment with B megatrium bacteria in the calcareous soil the amount of available phosphorus in the soil reached 10.1 mg p kg⁻¹, and the amount of phosphorus in the shoot system was 0.22. %, and the amount of phosphorus in the root system was 0.12%. The plant height reached 26.1 cm, the root length reached 11 cm, the dry plant weight was 5.1 gm. plant⁻¹, the weight of the root system was 1.5 gm. plant⁻¹, and the vegetative fresh weight was 162.8 gm. plant⁻¹ the root fresh weight was 29.8 gm. plant⁻¹ compared to the non-inoculation treatment, as the amount of available phosphorus in the calcareous soil was 6.5 mg p kg⁻¹, the amount of phosphorus in the shoot system was 0.15%. the interaction treatments significantly affected most growth characteristics.

Keywords: *Bacillus megatrium*. *Pseudomonas fluorescense*. Phosphorus, calcareous soil, gypsum soil.

Introduction

Soil is one of the most important natural resources. Its importance increases with time as the need for agricultural products increases due to the increase in population numbers and to sustainably secure its resources and provide plants with the necessary nutrients for this man searched for alternatives in providing these

elements by making optimal use of the effectiveness of microscopic organisms in the soil as an environmentally safe and cheap alternative source compared to chemical fertilizers due to their effective role in dissolving metals phosphate and converting it from the insoluble form to the form available to the plant (Kalayu , 2019), reducing environmental degradation and adopting clean

agriculture. Therefore, researchers turned to developing beneficial microorganisms and employing them to improve the natural, chemical and biological characteristics of the soil, including several genera of bacteria, the most important of which are *Bacillus* and *Pseudomonas* (Paul & Sinha, 2013; Ríos-Ruiz *et al.*, 2024) for their ability to dissolve phosphate minerals and fill part of the plant's requirements for dissolved phosphorus. Phosphorus is an essential nutrient for plant growth, but its availability in soil is often limited by its fixation in insoluble forms. Biofertilization, particularly using phosphate-solubilizing bacteria (PSB) such as *Bacillus megaterium*, plays an important role in increasing available phosphorus in soil (Chen *et al.*, 2006, Huda *et al.*, 2019).

This research aimed to evaluate the ability of some bacterial species to liberate and increase the available phosphorus in limestone and gypsum soils and to increase the growth of the hops plant (*barbin*) and the importance of the phosphorus element for the plant, meaning that it encourages root growth and increases its spreads and increases its ability to absorb nutrients

Materials & Methods

Gypsum soil samples were taken from Tikrit and calcareous from Kirkuk. They were air-dried and ground. They were sieved with a sieve with a diameter of 2 mm and mixed well to homogenize them. A sample was taken from each soil, physical and chemical analyses were conducted, and the amount of phosphorus was measured before planting, as shown in (Table 1). Two bacterial isolates were selected: *B. megaterium* and *P. fluorescence*; we obtained a previous diagnosis from the soil science laboratories / College of Agriculture - Tikrit. These isolates were preserved at a temperature

of 4°C, and the soil was sterilized to eliminate microorganisms. Then, 2 kg of sterilized soil was weighed in each pot; the pots were inoculated with bacteria and planted with foolish plant seeds. The treatments were randomly distributed according to a completely randomized block design (C.R.B) with three replicates diluted. The seedlings were divided into three plants per pot. The weed control process was carried out manually. Plant samples were taken 50 days after planting. The root was separated from the shoot in the crown area and placed in marked bags. The following characteristics were studied: plant height, root length, dry weight of the shoot, and dry weight of the root system. The phosphorus concentration in the vegetative part and the fresh weight of vegetables and roots. The statistical analysis used the least significant difference (L.S.D) (Al-Zubaidi, 2016) test under the 5% probability level

Results & Discussion

Firstly, the concentration of available phosphorus

The soil is shown in Table 2. The significant effect of inoculation with phosphorus-dissolving bacteria, *P. fluorescence* bacteria (B1) and *B. megaterium* bacteria (B2) in gypsum and calcareous soils on the phosphorus concentration, as treatments B1 and B2 gave an average phosphorus concentration of (8.45, 9.65) mg Per kg⁻¹, respectively, compared to the control treatment B0, which gave the average phosphorus concentration reached (5.4) mg p kg⁻¹. As for the soil type, treatments S2 and S1 gave an average phosphorus concentration of (7.23 and 8.43) mg p kg⁻¹, respectively. The presence of *B. megaterium* bacteria and phosphate-dissolving *P. fluorescence* bacteria in the soil environment

contributes to the increase in available phosphorus in the soil by dissolving precipitated phosphate compounds and converting them into phosphorus dissolved (Dahich *et al.*, 2011). The table results show that the soil type affected the phosphorus concentration, meaning that in gypsum soils, the amount of released phosphorus increased

for all types of bacteria. The reason is attributed to the presence of sulphates and calcium ions and their combination with phosphorus ions in the gypsum soils, which leads to precipitation. Phosphorus and its transformation into unprocessed compounds (Ingle & Padole, ,2017).

Table (1): Some chemical and physical characteristics of the study soils

property	Unit	Calcareous soil	Gypsum soil
pH		7.75	7.50
EC	dS m ⁻¹	2.35	2.32
CaSO ₄ .2H ₂ O		31.16	58
CaCO ₃	gm kg ⁻¹	225	202
O.M		8.3	10
CEC	Cmol(+) Kg ⁻¹	12.32	11.66
Sand		460	632
Silt		298	107
Clay	gm kg ⁻¹	242	261
Texture		L	S C L
Available elements			
N		14.1	17.6
P	mg Kg ⁻¹	4.98	5.30
K		92.54	116.31
Soluble ions			
Na		1.27	1.25
Ca		7.84	5.12
Mg		4.65	5.91
Cl		2.51	2.82
CO ₃	mmol L ⁻¹	Nil	Nil
HCO ₃		2.35	1.97
SO ₄		9.85	8.92

Table (2): The effect of inoculation with *B. megatrium* bacteria, *P fluorescence* bacteria, and two types of soil and the interaction between them on the availability of phosphorus in the soil (mg P kg⁻¹)

Inoculation type	S1	S2	Inoculation average
B0	6.3	4.5	5.4 c
B1	8.9	8.0	8.45 b
B2	10.1	9.2	9.65 a
Soil average	8.43	7.23	

S1calcareous soil ,S2Gypsum soil B1 B0 without inoculation *P fluorescence* B2 :*B. megatrium*

Second-Phosphorus concentration in the shoot and root system (%).

Table 3 shows the effect of inoculation with bacteria (B1) and inoculation with bacteria (B2), as treatments B1 and B2 gave an average phosphorus concentration for the shoot system amounting to (0.175 and 0.205) % and for the root system. (0.09) and (0.11%), respectively, compared to the non-inoculation treatment B0, which gave an average phosphorus concentration of (0.14%,0.07%) for the shoot and root system, which respectively, It is clear from the table that the phosphorus concentration was affected by the type of soil, and treatment S1 and S2 gave the average concentration of phosphorus reached (0.186 and 0.16%) for the shoot and(0.1and 0.08 %.) root system the table shows that treatment B2S1 had the highest concentration of phosphorus, amounting to 0.22, 0.12% for the shoot and root for system respectively, compared to the non- inoculation treatment B0S2, which gave the lowest concentration of 0.06%. 0.13 for shoot and root system in gypsum soil.

Table (3): The effect of inoculation with *B. megatrium* bacteria, *P fluorescence* bacteria, and two types of soil and the interaction between them on the phosphorus concentration (%) of the root and shoot system plant

Inoculation type	Shoot system			Root system		
	S1	S2	Inoculation rate	S1	S2	Inoculation rate
B0	0.15	0.13	0.14	0.0	0.0	0.0
				6	6	7
B1	0.19	0.16	0.175	0.0	0.0	0.0
				8	8	9
B2	0.22	0.19	0.205	0.1	0.1	0.1
				0	0	1
Soil average	0.18	0.16		0.0	0.0	
	6			8	8	

S1 calcareous soil S2Gypsum soil B0 without inoculation, B1 ,*P. fluorescence* B2 : *B. megatrium*

The resulting increase may be due to its efficiency in dissolving phosphate in limestone soils, which is higher than in gypsum soils. In turn, it encourages plant growth by increasing the absorption of nutrients, especially phosphorus, which increases its concentration in the plant (Saharan & Nehra, 2011. Alsaman & Al-Ansari ,2023). The reason for the difference may be attributed to Between the treatments; the ability of the selected isolates to dissolve phosphorus differs genetically.

Third - Plant growth characteristics: plant height, root length (cm), and vegetative and root dry weight.

Table 4 shows the effect of inoculation with isolates of bacteria (B1) and bacteria (B2) and two types of soil and the interaction between them on the height of the plant. It is clear that the height of the plant was affected by the

interference between inoculation and soil type, as the treatments inoculated with B2 were superior. It gave plant height and root length of treatment B2S1 gave 26.1 cm and treatment B2S1 gave 11 cm, and gave the highest vegetative and root dry weight of treatment B2S1 gave 5.1 gm plant⁻¹ and treatment B2S1 gave 1.5 gm plant⁻¹ respectively, compared to the non-inoculation treatment B0, which gave plant height and reached treatment B0S1 15.2

cm and length root reached treatment B0S1 5cm. It also gave a vegetative and root dry weight of B0S1 0.8 and B0S1 2.6 gm Plant⁻¹ respectively. It is also clear from Table 4 that the calcareous soil with inoculation with *B. megatrium* bacteria caused an increase in the height of the plant, the length of the roots, and the dry weight of the vegetative and root system

Table (4): The effect of inoculation with *B. megatrium* bacteria, *P. fluorescence* bacteria, and two types of soil, and the interaction between them, on the growth characteristics of the barbin plant

Inoculation type	Plant length cm			Root length cm			Vegetable dry weight gm. plant ⁻¹			Root dry weight gm. plant ⁻¹		
	S1	S2		S1	S2	Inoculation average	S1	S2	Inoculation average	S1	S2	Inoculation average
B0	15.2	14.7	14.95	5	5	5	2.6	2.1	2.35	0.8	0.6	0.7
B1	22.7	20.2	21.45	8	7	7.5	4.2	3.6	3.9	1.2	1.0	1.1
B2	26.1	24.7	25.4	11	8	9.5	5.1	4.1	4.6	1.5	1.3	1.4
Soil average	21.3	19.8		8	6.6		3.96	3.2		1.16	.960	

S1 calcareous soil S2 Gypsum soil B0 without inoculation S2 limestone soil B1 *P. fluorescence* B2 *B. megatrium* s

The reason for the superiority of the inoculated treatments over the non-inoculated treatments may be attributed to the bacteria's high ability to dissolve insoluble phosphate compounds and make them available for the plant, as well as through their ability to produce growth regulators such as indole acetic acid (IAA), (Kumar *et al.*, 2012). The reason for the superiority of the treatment of calcareous soil over the treatment of gypsum soil may be attributed to the increased availability of phosphorus in the calcareous soil due to the decreased presence of calcium and sulfate ions, which combine with phosphorus ions and lead to the precipitation of phosphorus. It becomes more stable compounds (Ingle & Parole, 2017). Transaction B2S1 on the remainder of the coefficients can be attributed to the positive effect of the interaction between the two factors, the calcareous soil factor and the pollination factor in the second isolate B2,

which was reflected in the growth characteristics, including plant height and dry weight, and this confirms the importance of inoculation in the soil (Hatem, 2023).

Fourthly. Fresh weight of shoots (gm plant⁻¹)

Table (5) shows the effect of inoculation with two isolates of *B. megatrium* bacteria (B1) and *P. fluorescence* bacteria (B2) and two types of soil and the interaction between them on the fresh weight of the vegetative total of the hops plant after 50 days of planting. It appears from the table that the fresh weight of the shoot was significantly affected by inoculation with bacteria, as the inoculated treatments B1 and B2 were significantly superior to the non-inoculated treatment, and the inoculated treatment B2S1 gave 162.8 gm.plant⁻¹ treatment B1S1 gave 143.5 gm.plant⁻¹ for the calcareous soils and treatment B2S2 gave 154.3 gm.plant⁻¹ and treatment B1S2 gave 140.1 gm.plant⁻¹ for

the gypsum soils on Sequence compared to the non-vaccinated treatment B0, which B0S1 gave 130 gm. Plant⁻¹ and treatment B0S2 gave 121.2 gm. Plant⁻¹ for the calcareous and gypsum soils, respectively. It is clear from the table that inoculation of the calcareous soils led to a significant increase in the fresh weight of the vegetative system. However, the effect of the interaction was significant, as treatment B2S1 gave the highest fresh weight of the vegetative system, reaching B2S1 162.8 gm. Plant⁻¹. compared to the comparison treatment B0S2, which amounted to 121.6 g plant⁻¹.

Table 5: The effect of inoculation with *B megatrium* s bacteria, *P fluorescence* bacteria, and two types of soil and the interaction between them on the vegetative fresh weight of hops plants (gm. plant⁻¹)

Inoculation type	Soil type		Inoculation average
	S1	S2	
B0	130	121.2	125.6 c
B1	143.5	140.1	141.8b
B2	162.8	154.3	158.5 a
Soil average	145.4	138.5	

These results are consistent with the findings of Jarak *et al.*, (2012). The superiority of the calcareous soil treatments over the gypsum soil treatments may be due to the low presence of calcium and sulphate ions, which combine with phosphorus ions and lead to phosphorus precipitation. It becomes a more stable compound (Ingle & Padole ,2017). The reason for the superiority of interaction B2S1 over the rest of the interaction is possible this is due to the positive effect of the interaction between the two factors, which are both the calcareous soil factor and the inoculation factor in the S1

S1calcareous soil S2Gypsum soil B0 without inoculation, B1 ,*P fluorescence* B2 :*B. megatrium* s, second isolate, which was reflected in the growth characteristics, including the fresh weight of the shoots.

As for the superiority of the inoculation treatments, it appears that there is a clear response to inoculation under the conditions of this calcareous soil with a low phosphorus availability. The reason may be the great role that phosphorus plays in the plant, as it plays an important role in increasing the root and vegetative growth of the plant (Storey, 2007).

#Fifth, fresh weight of the root system (gm. plant⁻¹)

Table 6 shows the effect of inoculation with two isolates of *B. megetrium* bacteria (B1) and *P. fluorescence* bacteria (B2) and two types of soil and the interaction between them on the fresh weight of the root system of the barbin plant after 50 days of planting. It had a significant effect on the fresh weight of the root system, as treatment B1S1 and B2S1 gave the fresh weight of the root system amounting to 27.4 and 29.8 gm. plant⁻¹, respectively. The treatment B1S2 and B2S2 gave 24.5 and 26.3 gm plant⁻¹, compared to the non-inoculated treatment B0, in which B0S2 gave 25.6, and B0S gave 215.8. gm. plant⁻¹ for calcareous and gypsum soils on mixed soils. The results also showed that the type of soil had a significant effect on the fresh weight of the root system, as the calcareous soil treatments were significantly superior to the gypsum soil treatment, and treatments S1 and S2 gave an average fresh weight of 27.6 and 22.2 gm. plant⁻¹ for the calcareous and gypsum soils. Consecutively, as for the effect of the interaction between bacterial isolates and soil type, it was also significant, as treatment B2S1

excelled and gave the highest average fresh weight, which amounted to 29.8 gm. plant⁻¹, while treatment B0S2 gave the lowest fresh weight value, which amounted to 15.8 gm. plant⁻¹.

Table 6: The effect of inoculation with *B megatrium* bacteria, *P fluorescence* bacteria, and two types of soil and the interaction between them on the root fresh weight of sedge plants (gm. plant⁻¹)

Inoculation type	Soil type		Inoculation average
	S1	S2	
B0	25.6	15.8	20.7 c
B1	27.4	24.5	25.29 b
B2	29.8	26.3	28.05 a
Soil average	27.6	22.2	

S1 calcareous soil S2Gypsum soil B0 without inoculation, B1 ,*P fluorescence* B2 :*B. megatrium* s

The reason for the superiority of isolate B2 over the rest of the isolates may be due to it giving the highest average fresh weight of the root system because it is the most efficient among the isolates in dissolving phosphate, producing indole acetic acid, and producing chelating compounds. It may also be due to the superiority of the treatments inoculated with *P. fluorescence* bacteria and *B. megatrium* bacteria over the inoculated treatment. The ability of bacteria to increase soil phosphorus availability through their production of organic acids such as ketochloric acid, oxalic, and citric, which has been confirmed by Hwangbo *et al.*, (2002), as well as its ability to secrete some hormones and growth regulators that work to stimulate and increase the growth of the plant and thus increase its dry weight, and this was confirmed by Tahir & Sarwar (2013). As for the reason for the superiority of the calcareous soil treatments and inoculation, there is a clear response to inoculation under

the conditions of this soil, in addition to increasing the efficiency of the roots in absorbing nutrients and transporting them to the upper parts, and then their positive effect is reflected in the fresh weight of the roots (Al-Rawi *et al.*, 2001). The reason for the superiority of the treatment of the binary interaction B2S1 on all interaction factors showed a beneficial effect for both factors, which are the calcareous soil factor and the inoculation factor in the second Recommendation.

Conclusion

Treatments inoculated with bacteria (especially *Bacillus megaterium*) showed a significant increase in the rate of available phosphorus in the soil compared to the uninoculated treatment, and an improvement in all studied growth traits of barberry plants, including plant height, root length, and plant dry weight. Calcareous soil was also more effective than gypsum soil in enhancing growth traits and increasing available phosphorus.

Acknowledgements

The authors would like to thank staff of Department of soil science and water Resources, College of Agriculture for space and support provided for the current study.

Contributions of authors

S.H.S.: Collection of specimens, Laboratory techniques, wrote and revised the manuscript.

I.T.D.: Suggestion the proposal of the article, wrote and revised the manuscript, identification of the parasite.

A.A.Q: Suggestion the proposal of the article, revised the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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Ethical approval

All ethical guidelines related to Soil Biology and care issued by national and international organizations were implemented in this report.

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تأثير التلقيح البيولوجي (*Bacillus megatrium*, *Pseudomonas Fluorescent*) في زيادة توفر

الفسفور ومعايير النمو لنبات البربين (الحمقاء) *portulaca Oleracea* L.

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المستخلص: نفذت تجربة حقلية في حقول كلية النباتات الطبية والصناعية جامعة كركوك لدراسة تأثير التلقيح البيولوجي في زيادة الفسفور المتوفر في التربة وبعض صفات النمو لنبات البربين (الحمقاء)، والتفاعل بينهما. واستخدم تصميم القطاعات العشوائية وبثلاث مكررات العامل الأول نوعين من التربة كلسية وجبسية والعامل الثاني نوعين من البكتيريا و *P. fluorescent megatrium*. بالإضافة إلى معاملة المقارنة أظهرت النتائج أن المعاملات الملقحة بالبكتيريا زادت من معدل الفوسفور المتوفر في التربة وفي النباتات وجميع صفات النبات المدروسة مقارنة بالمعاملة غير الملقحة وأفضل معاملة كانت معاملة التلقيح ببكتيريا *B megatrium* في التربة الكلسية إذ بلغت كمية الفوسفور المتوفر في التربة 10.1 ملغم كغم⁻¹ وكمية الفسفور في المجموع الخضري بلغت 0.22%. ونسبة الفوسفور في المجموع الجذري بلغت 0.12%. وارتفاع النبات بلغ 26.1 سم، وطول الجذر 11 سم، ووزن النبات الجاف 5.1 غم. نبات⁻¹، ووزن المجموع الجذري الجاف 1.5 غم نبات⁻¹، والوزن الخضري الطري 162.8 غم نبات⁻¹ والوزن الطري للجذر بلغ 29.8 غم نبات⁻¹ مقارنة بالمعاملة غير الملقحة إذ بلغت كمية الفوسفور المتوفر في التربة الكلسية 6.5 ملغم كغم⁻¹، وبلغت كمية الفسفور في المجموع الخضري 0.15%. وكمية الفسفور في المجموع الجذري بلغت 0.08%، وارتفاع النبات بلغ 15.2 سم، وطول الجذر 5 سم، ووزن النبات الجاف 2.6 غم نبات⁻¹، ووزن الجذر بلغ 0.08 غم نبات⁻¹ والوزن الخضري الطري 130.0 غم نبات⁻¹. وبلغ وزن الجذر الطري 25.6 غم نبات⁻¹، وأثرت معاملات التفاعل معنوياً في معظم صفات النمو. كما بينت النتائج أن معاملة بكتيريا *B megatrium* كانت أفضل معاملة إذ زادت من صفات النمو وكمية الفوسفور المتوفر في التربة وفي نبات البربين (الحمقاء)، وكانت التربة الكلسية أفضل من التربة الجبسية في جميع الصفات.

الكلمات المفتاحية: *Bacillus megatrium*، *P. fluorescent*، الفوسفور، التربة الكلسية، التربة الجبسية.