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EFFECT OF BIOINOCULATION OF BACTERIA ,FUNGIA ,THE ADDITION OF PHOSPHATE ROCK IN AVAILABILITY NPK ONCORN RHIZOSPHERE

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ABSTRACT

This study was aimed to investigate effect of a field experiment was carried out at the Agricultural Research and Experiments Station College of Agricultural Engineering Sciences - University of Baghdad in the spring season of 2023 with the aim of studying the effect of two factors. The first factor is the bioinoculation at three levels (without inoculation , *Pseudomonas fluorescens*, and *Glomas mossae*). The second factor is three levels of phosphate rock (without addition, 50 and 100%) of half the fertilizer recommended equivalent used in the study. The treatment were distributed in a factorial experiment according to a completely randomized block design. the single inoculation of bacteria and fungi and the addition of phosphate rock have affected all the studied properties, while the double interaction of the biological and fungal inoculum treatments and the 50% level of phosphate rock equivalent to half the recommended fertilizer showed the highest averages for NPK properties in the corn rhizosphere, which reached (64.23, 20.24, 246.92 mg. kg^{-1} soil), respectively in comparison to the control treatment that gave the lowest averages in all the above traits which reached (48.71, 9.65, 176.07 mg kg^{-1} soil).

Keywords:, ***Glomas mossae*, Nutrients, *Pseudomonas fluorescens***
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INTRODUCTION

The rhizosphere is the area affected by the secretion of plant roots, and the presence of microorganisms in the soil is concentrated, It is the basis of the relationship between living organisms and plants, where the effects of microorganisms interaction , and it is a unique environment with its own characteristics (Bagyarj and Maiti, 2015;Singh *etal*.,2023). Phosphate dissolving bacteria live in the Rhizosphere environment and produce many organic acids, such as monocarboxylic acids (formic and acetic), dicarboxylic acids (succinic and oxalic), tricarboxylic acids (citric), and others, These organic acids dissolve phosphate by lowering the pH, as well as producing the enzyme phosphatase, which has an important role in dissolving compounds containing phosphate, which leads to increased root effectiveness and growth and its impact on the plant's content of other nutrients, including phosphorus (Adel and Ameen,2017:Rane *etal*.,2015). The *Glomus mossea* fungus is also characterized by increasing the absorption of water and nutrients such as phosphorus, nitrogen, potassium, and calcium and transferring them to the plant, thus leading to an increase in the plant's growth characteristics(AL-samaarrai and AL-Tamimi (2018);Zhang *etal*.,2021). In a laboratory study to determine the ability of (4) days bacterial strains to dissolve phosphate rock during an incubation period of (5) days at a temperature of 30°C, it was observed that

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after inoculation with phosphate-dissolving bacteria, it was observed that the pH decreased from the pH of the medium, from 7.2 at 20 days to 4.0 with the phosphate rock The reason for this is due to the production of organic acids that dissolve phosphate compounds, which in turn leads to a high concentration of availability phosphorus in the medium, as the highest concentration reached 540 ($mg.L^{-1}$) with the phosphate rock in the phosphate dissolving bacteria, The researchers will conclude that it is possible to We use certain types of bacteria as biofertilizers to dissolve insoluble phosphate compounds and other compounds, thus increasing the availability of nutrients such as phosphorus and potassium so that plants can benefit from them. Thus, phosphate rock can be used as a new alternative fertilizer (Solangi *etal*.,2024;Migual *etal*.,2020).A study was conducted to determine the effect of phosphate rock at four levels (0, 25, 50, and 75) $g.kg^{-1}$ soil and the bioinoculum of the bacteria that dissolve phosphate compounds, *Pseudomonas mallei* and *Pseudomonas cepaceae* (With two levels of no addition and addition) of 1 gm kg^{-1}. The results of the study showed that the interaction of phosphate rock at the level of 50 gm kg^{-1} with soil and *Pseudomonas mallei* bacteria reduces the pH values of the soil throughout the incubation periods in the first month,The first is due to the organic acids produced by these bacteria, but after an incubation period of 4 months, the level of 75 (g kg^{-1} soil) with phosphate-dissolving bacteria led to a decrease in organic carbon in the soil to 3.35% for the same bacteria above(Arifin *etal*.,2018;Spaepen *etal*.,2009;Singh *etal*.,2023; Singh *etal*.,2023). A field study was also conducted to determine the effect of the interaction between three factors, phosphate rock at two levels (0, 3) mg. L^{-1}, and the second factor is the fertilizer recommended for four levels of NPK (40-60-120) kg ha^{-1} (25,50, 75,100%), and the third factor is inoculation with phosphate dissolving bacteria at two levels (inoculation and non-inoculation), which gave the intervention treatment, The triple (3 $mg.L^{-1}$ + 75% of the fertilizer recommendation + inoculation with phosphate-dissolving bacteria) , The correlation coefficient of the dissolution coefficient with the availability phosphorus was 0.83%, the correlation coefficient of the phosphorus stabilized with aluminum was 0.82%, and the correlation coefficient of the phosphorus stabilized with iron was 0.84%,This may be due to the ability of bacteria that dissolve phosphate compounds to increase avalabile phosphorus, as they produce different types of organic acids, including citric, oxalic, malic, and others, which work to dissolve insoluble phosphate compounds, in addition to their role in competing with phosphate for relevant adsorption sites, High energy that increases phosphate readiness (Jediaa *et al*.,2009 ;Miguel-Rojas etal.,2022).

MATERIALS AND METHODS

The bacterial and fungal inoculum was obtained from the laboratories of the Department of Soil Sciences and Water Resources at the College of Agricultural Engineering Sciences at the University of Baghdad. Analysis were conducted for some chemical, physical and biological characteristics of the soil before planting, as shown in (Table 1).

Table 1. Some chemical, physical and fertility properties of the study soil before planting

Soil properties	Value	Unite
EC_e	3.6	ds m^{-1}
$pH_{1:1}$	7.6	
$CaCO_3$	267	g kg^{-1}
Organic matter	13	g kg^{-1}
Available N	19	mg kg^{-1}
Available P	3.1	mg kg^{-1}
AvailableK	178.95	mg kg^{-1}
Total bacteria	3 * $^{6}10$	C.F.U g^{-1} Soil
Total fangi	0.82* $^{6}10$	C.F.U g^{-1} Soil
SAND	500	g kg^{-1}
SILT	340	g kg^{-1}
CLAY	160	g kg^{-1}
Texture	Loamy	

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Phosphate rock particles were prepared by grinding and sifting the rocks with a 2 mm sieve and adding it at a distance of 4 cm around the seeds in the fallow with half the fertilizer recommended (250-100-150) kg ha^{-1} (N-P_2O_5-K_2O) K_2SO_4 50% (4). a layer of the *Glomas mossae* mycorrhizal inoculum mixture (sand - spores - infected roots) was added under the corn seeds weighing 50 grams a Seed bead a pillow, and 25 grams above the seeds to ensure the occurrence of mycorrhizal infection, so it was 75 grams. Seed $bead^{-1}$ The corn seeds were inoculated with *Pseudomonas fluorescens* and left for 12 minutes to ensure successful adhesion. Seeds were planted not free of bacteria first to avoid contamination according to the experimental design.The soil of the field was divided into experimental units with dimensions (2*3) m^2, distributed randomly and in three replicates. Within one plate there were 3 lines with a distance of 75 cm between one line and another, and the number of repair per in one line was 6 holes, between one hole and the other was 40. cm, the irrigation process was carried out using the tourist method after draining 40% of the field capacity. After 70 days of Silk Stage germination, the avalabile nitrogen in the soil was estimated using the (2N KCL) method by the microKaldahil device. The available phosphorus in the soil was extracted using sodium bicarbonate according to the Olsen method, and it was estimated using a Spectrophoto meter device, and the available potassium was extracted in the soil using the method (1M NH_4OAC) It was estimated using a flame photo meter, as stated in (Blaek,1965).

Statiisticat analysis : Use the SAS program under the Windows operating system, 2012. The means were compared with the least significant difference (L.S.D.) using the Duncan test to determine the type of significance between the means of the different coefficients.

RESULTS AND DISCUSSION

1- The effect of biofertilization with the bacteria *Pseudomonas fluorescens* and the fungus *Glomus mossea* by interaction with phosphate rock in the avalabilty of nitrogen (mg.kg^{-1} soil) in the rhizosphere soil of corn are shownin (Table 2). The results show that the concentration of availably nitrogen (mg.kg^{-1} soil) in the rhizosphere of corn plant at the silk stage (70 days) achieved a significant increase in biological Incolution and when adding phosphate rock separately, the mono interaction also achieved an increase. significantly, biological inoculation (B) with *Pseudomonas fluorescens* gave a significant increase in the concentration of avalabile nitrogen in the soil, as it reached 53.56 (mg.kg^{-1} soil). The reason is attributed to the *Pseudomonas fluorescens* due to nitrogen fixation. As for adding the mycorrhizal *Glomus mossea*, it gave a significant increase in the concentration of avalabile nitrogen in the soil, as it reached 58.19 (mg.kg^{-1} soil) compared to the control treatment 48.56 (mg.kg^{-1} soil). The reason is attributed to the role of mycorrhizae in increasing the releases of phosphorus. It is due to extract and transfering phosphorus to the plant (Al-Taie, 2005; Oudah and Al-kellabi, 2023; Aberathna *etal*.,2024). As for the levels of regular phosphate rock (Rg), the third level (kg. ha^{-1}) gave the highest significant increase in the concentration of available nitrogen, as it reached 56.30 compared to the treatment 50.57 (mg. kg^{-1} soil), and the reason is attributed to nutritional needs. Which is provided by the phosphorus liberated from phosphate rock in the formation of a large root system(Al-samarrai and AL-tamimi,*2015).*

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Table 2. The effect of biological fertilization by interaction with phosphate rock on availability nitrogen (mg.kg^{-1} soil) in the rhizosphere soil of corn plant.

The Biological factor (B)	Phosphaterok (Rg) kg Ph-1	Interaction B*Rg	Average Rg	Average B
	0	48.71		
C	50	51.39	50.57	48.56
	100	52.25		
	0	55.80		
A	50	58.79	53.44	53.56
	100	56.12		
	0	60.12		
M	50	64.23	56.30	58.19
	100	58.32		
L.S.D		B*Rg 3.0901	Rg 1.1202	B 1.1202

*** C (No inoculation) , A(*Pseudomonas fluorescens* bacteria), (M) *Glomus mossea* .**
(Rg) Phosphaterok(0,50.100)% From half the recommended chemical fertilizer

2-The effect of biofertilization by the bacteria *Pseudomonas fluorescens* and the fungus *Glomus mossea* by interacting with phosphate rock on avalabilte phosphorus (mg.kg^{-1} soil) in the rhizosphere of corn plant. (Table3). shows that the concentration of available phosphorus (mg.kg^{-1} soil) in the rhizosphere of the corn plant at the silk stage (70 days) achieved a significant increase in biological inoculation and when adding phosphate rock separately, the doubal interaction also achieved an increase significantly, biological inoculation (B) with *Pseudomonas fluorescens* bacteria gave a significant increase in the concentration of available nitrogen in the soil, reaching 15.75 compared to the control treatment. The reason is attributed to the *Pseudomonas fluorescens* due to efficiently dissolve , The results wer in accordance with(Sarikhani *etal*.,2016;Mahantaa *etal*.,2018), they observerd the importance of *Pseudomonas striota* and *Bacillus megatherium* bacteria in Efficient dissolution of phosphate rock (Biar *etal*.,2008). As for adding the mycorrhizal *Glomus mossea*, it also gave a significant increase in the concentration of available nitrogen in the soil, as it reached 18.54 (mg.kg^{-1} soil) compared to the control treatment as it 10.81 (mg.kg^{-1} soil). The reason is attributed to the ability of the mycorrhizae to extract and transfer phosphorus to the phanlant (Zhang *etal*.,2021). As for the levels of phosphate rock (Rg), the third level (100%kg. ha^{-1}) gave the highest significant increase in the concentration of phosphorus, as it reached 16.33 compared to the control (13.64 mg. kg^{-1} soil). The reason is attributed to the relative availabil of the resulting phosphorus, From the addendum (Rane *etal*.,2015; AL-falh.,*etal*.,2023). The double interaction of the biological factor (B) with the levels of phosphate rock (Rg) also gave a significant increase in the of phosphorus in the rhizosphere of the corn plant when interacting (M) with the level of (100) kg ha^{-1} of (Rg), as it reached 19.42 compared to the control treatment reached (11.65 mg.kg^{-1} soil). This increase in the plant's nitrogen content may be attributed to the ability of the Mycorrhizal fungus to dissolve metals and mineral compounds through four mechanisms: acid organic, nutrients, oxidation and reduction, in addition to primary and secondary secretions that have chelating properties such as carboxylic and phenolic compounds. Through which the available of plant nutrients, including nitrogen, is increased (Mohammed and Kareem ,2022; Shen *etal*.,2022).

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Table 3. The effect of biological fertilization by interaction with phosphate rock in avalabile phosphors (mg.kg^{-1} soil) in the rhizosphere soil of corn

The Biological factor (B)	Phosphatero (R_g) kg $_Ph^{-1}$	Interaction B*Rg	Average Rg	Average B
C	0	9.65	13.64	10.81
	50	11.07		
	100	11.73		
B	0	14.72	15.13	15.75
	50	16.56		
	100	16.95		
M	0	16.95	16.33	18.54
	50	20.24		
	100	19.42		
L.S.D		B*Rg 1.908	Rg 0.3928	R_B 0.3928

3- The effect of biofertilization of *Pseudomonas fluorescens* and the *Glomus mossea* by interacting with phosphate rock on available potassium (mg.kg^{-1} soil) in the rhizosphere soil of corn plant. (Table4). shows that the concentration of available potassium (mg. kg^{-1} soil) in the rhizosphere of corn plant at the silk stage (70 days), achieved a significant increase in bio-inoculation (B) when adding phosphate rock separately. It also achieved The double interaction showed a significant increase. Bioinoculation (B) with *Pseudomonas fluorescens* gave a significant increase in the concentration of available potassium in the soil, as it reached (216.10 mg.kg^{-1} soil). compared to the control treatment (179.38 mg.kg^{-1} soil). The reason is that *Pseudomonas fluorescens* to releases potassium from mineral rocks. The mycorrhizal *Glomus mossea* also gave a significant increase in the concentration of available nitrogen in the soil, (239.54 mg.kg^{-1} soil) compared to the control treatment as it reached 179.38 (mg.kg^{-1} soil). The reason is attributed to the ability of the fungus *Glomus mosseae* to secrete it. For some organic acids, such as carboxylic, amino, and phenolic compounds, which improve and regulate nutrient absorption, as well as growth regulators secreted by fungi, which stimulate root hairs to release nutrients from the soil and through its secretions, including potassium (Xiao *etal*.,2017). As for the levels of phosphate rock (Rg), the third level(100%) gave the highest significant increase in the concentration of available potassium (mg.kg^{-1} soil), as it reached 215.63 compared to the control (203.97 mg.kg^{-1} soil), and the reason is attributed to the content of Phosphorus in the third level of phosphate rock at the addition level compared to other levels. The dual interaction of the biological factor (B) with the levels of phosphate rock (Rg) also gave a significant increase in the available of potassium (mg. kg^{-1} of soil) in the rhizosphere of corn plant when interacting (M) with the level of (100) kg ha^{-1}of (Rg),(246.92mg.kg^{-1})soil compared to the control treatment as it reached (174.07 mg.kg^{-1} soil). The reason is due to the ability of mycorrhizae to interact with high concentrations of phosphate rock of low concentration (Solangi *etal*.,2024).

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Table 4. Effect of biological fertilization) by interaction with phosphate rock on available potassium (mg.kg^{-1} soil) in the rhizosphere soil of corn plant

The Biological factor (B)	Phosphaterok (R_g) kg $_ph^{-1}$	Interaction B*Rg	Average Rg	Average B
	0	176.07		
C	50	187.09	203.97	179.38
	100	192.99		
	0	207.45		
B	50	217.72	211.69	216.10
	100	222.14		
	0	232.71		
M	50	242.80	215.63	239.81
	100	246.92		
L.S.D		B*Rg 9.0449	Rg 3.7525	B 3.7525

CONCLUSION

1-Based on the results obtained in the laboratory, it became clear that the double interaction between bacterial and fungal inoculation with phosphate rock increases the concentration of N, P, and K in corn rhizosphere at the sillk stage (70 days)

2-The highest significant increase in the concentration of N, P, and K was obtained at the sillk stage when the two interactions between mycorrhizal fungi and phosphate rock (24, 52, and 28%), respectively.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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تأثير التلقيح الحيوي للبكتيريا, الفطريات وإضافة الصخر الفوسفاتي في جاهزية NPK في رايزوسفير الذرة الصفراء

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المستخلص

نفذت تجربة حقلية في محطة الأبحاث والتجارب الزراعية / كلية علوم الهندسة الزراعية – جامعة بغداد في الموسم الربيعي 2023 بهدف دراسة تأثير عاملين العامل الاول هو اللقاح الحيوي بثلاث مستويات (بدون لقاح و *Pseudomonas fluorescens*و *Glomas mossae*) العامل الثاني ثلاث مستويات من الصخر الفوسفاتي (بدون اضافة و50 و 100%) من نصف مكافئ التوصية السمادية المستعملة في الدراسة. وزُرعت المعاملات في تجربة عاملية وفق تصميم القطاعات العشوائية الكاملة . أثر التلقيح المفرد للبكتريا والفطر وأضافة الصخر الفوسفاتي في جميع الصفات المدروسة , في حين أظهر التداخل الثنائي لمعاملات اللقاح الحيوي البكتيري والفطري و مستوى 50% من الصخر الفوسفاتي المكافىء لنصف التوصية السمادية أعلى المتوسطات لصفات NPK في رايزوسفير الذرة الصفراء والتي بلغت (64.23 ,20.24, 246.92 ملغم . كغم $^{-1}$ تربة) على التتابع بالمقارنة مع معاملة المقارنة التي أعطت أقل المتوسطات في جميع الصفات أعلاه والتي بلغت (48.71 176.07,9.65ملغم .كغم$^{-1}$ تربة), هذا يشير الى أهمية دور اللقاح الحيوي بالتداخل مع تقنات أضافة الصخر الفوسفاتي في زيادة جاهزية NPK في رايزوسفير النبات في بعض ترب العراق الكلسية.

الكلمات المفتاحية: المغذيات , *Pseudomonas fluorescens* و *Glomas mossae*.

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