



Response of three wheat varieties (*Triticum aestivum* L.) to inoculation with local isolates of *Polymyxa paeinbacillus* and its role in improving soil phosphorus availability, plant growth, yield, and components

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Abstract

A field trial was conducted at the Agricultural Research Station to study the effect of local strains of *P. polymyxa* used as bioinoculants on the growth and yield of wheat (*Triticum aestivum*). The field trial was conducted as a two-factorial experiment that included biological inoculation (using different strains of *P. polymyxa*) and 3 different types (cultivars) of wheat. The trial was a randomized complete block design (RCBD), located in Al-Muthanna Governorate in 2024, on loamy silty soil with three replications, with two main factors of B0 -B1-B2-B3 (4 levels of *P. polymyxa* bioinoculant) and wheat variety A1-Bohuth 22, A2-Rashid, and A3-Wafia (3 cultivars). The results found that:

In summary: The B2 treatment of the biopollination of *P. polymyxa* improved the leaf area, dry weight, and total biomass of the wheat more than the other treatment types; with average leaf area, dry weight, and total biomass, respectively, of 55.94 cm²/plant, 10.61 g, and 18.35 Mg/ha at full bloom, was greater than any of the other treatments (BA-B1, B3). Conversely, the plant height was greater for the BO treatment with an average of 92.6 cm.

In the second finding: The wheat varieties showed variations in many of the measured characteristics. The Rashid variety demonstrated superiority in flag leaf area and biological yield with an average of (49.03 cm² plant⁻¹ - 18.28 Mg), while the Wafia variety showed the greatest height with an average of 88.9 cm, and the Buhouth 22 variety exhibited the highest dry weight average of (10.44 g).

Third:- When the isolates coincided with the different varieties, a change was noted. The Samawah isolate paired with the Rashid variety achieved the highest mean in flag leaf area and biological yield, attaining (62.73) cm² plant⁻¹ - 22.73 Mg e⁻ (respectively, while that isolate combined with the Buhouth 22 variety stood out in terms of plant height, reaching 99.8) cm. Moreover, the isolate that did not include the inoculant with the Wafia variety performed best in dry weight, hitting the top average of (11.40) g.

Keywords: wheat varieties, soil phosphorus, *Polymyxa paeinbacillus*.

Introduction

As one of the most important food crops globally and domestically (in Iraq), the wheat plant (*Triticum aestivum* L.) is also classified as a grassy species. In 2019 alone, there were 761,600,000 tons of global wheat produced (14). Countries that produced wheat include Iraq at approximately 4,343,000 tons grown on 6,331,000 dunams (20). Given its status as one of the major small grain crops for people throughout the world, it has a strategic importance to guarantee food security. The role of wheat in human nutrition stems from the fact that it creates the world's highest-quality bread. (2)

All of these inoculants are biofertilizers containing microorganisms that are added to the soil. They contain essential nutrients for plants which allow them to grow longer. Some of the essential nutrients for the growth and production of wheat are nitrogen, phosphorus and potassium. When fertilizing with these three nutrients, there are several problems that occur, especially because of the fact that most soils in Iraq contain high amounts of calcium carbonate, have high pH, and low organic matter. The nitrogen in the soil is subject to great loss due to the fact that it is lost as gas when it goes through the process known as ammonia volatilization and reverse nitrification. Almost all ionic phosphates added to the soil as phosphates, along with those found in the soil, will have been converted to sedimentary phosphates and are thus not available for plant use; there is some evidence supporting the above (10). Although repeated applications of chemicals at a high rate can increase the amount of vegetative growth of plants, contemporary chemical applications can negatively impact yield. The chemicals also have adverse effects on the environmental and the people

in the area in which they are applied, in addition to direct effect of the chemicals. The global issue of environmental harm and agricultural erosion caused by the disruption of fragile ecosystems and beneficial soil microorganisms has led to an urgent need for the agricultural industry to address a shortfall of fertilizers through bio-fertilization as there are many advantages associated with this practice, including the ability to reduce reliance on costly synthetic fertilizers and improve soil fertility in the Rize and Sive region. Identification and research into beneficial microbial populations has increased over the past few years due to the availability of funding from government programs and the increasing availability of technology.

Some of the other beneficial properties of soil microbes that positively affect plant growth include their ability to fix or convert nitrogen from the atmosphere into forms that can be taken up by plants. The beneficial properties of soil microorganisms also include their ability to increase the solubility and availability of phosphorus by converting insoluble phosphorus into soluble phosphorus for use by plants, and their ability to improve the physical characteristics of soil (e.g. increase porosity).

The low productivity of wheat per unit area, presents a significant barrier to the further increase of wheat production and to achieving self-sufficiency from this crop. Thus, there is a need to take measures to enhance its productive efficiency by providing required plant nutrients from the use of biofertilizers that would enhance production.

The study of the plants, paenibacillus and their ability to stimulate growth, dissolve phosphorus and convert phosphorus from non-available to available forms, will attempt to produce three types of results:

1. Examining the effects of local isolates of *P. polymyxa* and the effects of stimulating plant growth and their ability to dissolve

phosphorus and convert it from one form to another.

2. Examining the impact of different wheat varieties on the growth and yield of the plants.

3. Examining the effect of interacting local isolates of *P. polymyxa* with different wheat varieties on plant growth and yield.

Materials and method

2.1 location of the experiment

A factorial study took place in the Muthanna Governorate at the College of Agriculture's research facility during the winter period of the year 2024-2025 to investigate the impacts of three distinct isolates of *P. polymyxa* along with three varieties of *Triticum aestivum* within a Randomized

Complete Block Design (RCBD). Soil was randomly collected in three separate samples from the Muthanna Governorate and mixed together at different sites to create representative composite samples for the three regions. These combined samples were subsequently passed through a sieve with a 2mm mesh size to facilitate the isolation process using biological techniques. One of the composite samples will be kept at a temperature of 4 degrees Celsius until it is analyzed

Table (1) some chemical, physical and biological characteristics of the study soil before planting.

Value	Unit of measure	Measured characteristic
4	ds ^m ⁻¹	Ece
7.5		pH
51	mg.kg-1 Soil	Available Nitrogen
6.9		Available phosphorous
124		Available Potassium
1.16	g kg-1 soil	Organic matter
19.5	g kg-1 soil	Sand
44.3		silt
36.2		Clay
silty clay loam	—	Tissue type

10 ³ *15	Cfu g ⁻¹ soil	Total Bacterial Density
10 ⁷ *0.14		P. Polymyxa

2.2 Transactions and experimental design

A randomized complete block design (RCBD) was used to conduct a two-factor, field experiment in which there were two treatment factors that were randomly allocated into three replicate blocks. The experiments performed were as follows:

Factor One: Four local *P. polymyxa* bacterial (P) isolates have been assigned a code or label (B) and have been added to the experiment.

1. B0: No Inoculation
2. B1: Rumaytha isolate added (B)
3. B2: Samawah isolate added (B)
4. B3: Khader isolate added (B)

Factor 2: Three wheat varieties, designated A

- 1- A1: Bohuth wheat variety
- 2-A2: Rashid wheat variety
- 3-A3 : Wafia wheat variety

Third: NPK mineral fertilizers

NPK mineral fertilizers were applied according to the complete fertilizer recommendation for nitrogen, potassium and 20% phosphorous and the number of treatment units was 36 experimental units (4 * 3 * 3).

2.3 Preparation of the *P. polymyxa* inoculant and its use as a biofertilizer

A specific local isolate of the bacteria genus *Paenibacillus* bearing a local identifying number (3) and classified as type (*P. polymyxa*) was selected as a candidate for use in field trials

based on its ability to dissolve phosphorous efficiently. In order to produce enough inoculant to use in field trials, (3) conical flasks containing (1000) ml of a Pikovsky medium and (50) ml of the inoculum taken from the prepared liquid culture were prepared. The inoculum was placed in a vibrational incubator (30° C) for approximately (2-3) days. Colony-forming unit counts, was established using the McFarland standard solution, and the density of the inoculum used was formatted (810 * 1.5) colony-forming unit.ml-1.. Several (3) varieties of wheat seeds were sterilized with ethyl alcohol for (2) minutes, and rinsed with distilled water several times to remove traces of the sterilization solution, transferred to sterile glass container for the inoculant, (made with a gum arabic and peatmoss solution) was pm-ordered and incubated with the inoculant and peatmoss for (x) period of time to allow for attachment of the inoculant and peatmoss, before the seeds were taken to a field site for planting.

2. 4 Agriculture and crop service operations

After the process of land preparation (Interconnected Plowing, Flattening, Leveling), the 500-square-meter land has been divided into three large blocks. The units are separated in 8 rows with a 20 cm distance between each row, the plot width was 2*2m, and a 1.5m distance between each block would reduce the overlap of the sampling/experimental factors.

Prior to the harvest, all necessary crop care operations were performed, manually weeding to remove any damaging bush or creeping plants, and after the harvest, all plants were cut to their lowest point and packed into cardboard boxes for necessary analyses of the sampled/experimental factors post-harvest.

2. 5 Crop measurements after planting

2.5.1 Plant height (cm)

The height of ten randomly selected plants from each experimental unit was measured from the soil surface to the top (17).

2.5.2 Flag Leaf Area (plant cm):

Leaf length was measured at a rate of ten plants per experimental unit and randomly selected leaves during the flowering stage and measured using the following equation:

Leaf Area - Maximum Length * Maximum Width * 0.95 for plant leaves

2.5.3 Dry weight of the vegetative system (g):

Ten plants were randomly selected from each experimental unit and weighed using a sensitive balance to determine the plant's dry weight of the vegetative system at harvest.

2.5.4 Biomass (megagrams ha⁻¹):

Calculated from the same area as the grain yield in each experimental unit. The entire plant (grain + straw) was weighed and the weight converted to megagrams ha⁻¹.

2.6 Statistical analyses

The data from this factorial experiment will be analysed with an analysis of variance using the GENSTAT Discovery Edition Software programme and then using least significant difference (LSD) to compare the means at a significance level of $p < 0.05$.

Results and Discussion

Results of Biochemical Tests

The bacterial isolates identified in the previous section were characterised based on the biochemical testing results provided in Table 2. All bacterial isolates tested produced positive

results in all of the tests performed except for indole production and urease activity, which had negative results. The oxidase activity tests were negative except isolate B1 that produced an oxidase positive result. All bacterial isolates tested negative for citrate utilisation except isolate B3 that produced positive results. All bacterial isolates could be grown at 50 °C.

Table (2) represents the biochemical tests of *Paenibacillus Polymyxa* isolates

Result			Tests	T
P3	P2	P1		
+	+	+	Gram Stain Test	1
+	+	+	Growth at pH (7.5) and (6)	2
+	+	+	NaCl (%5) Growth at	3
+	+	+	Growth at 50C⁰ Temperature	4
+	+	+	Methyl Red Test	5

-	-	-	Indole Production	6
+	+	+	Catalase test	7
-	-	+	Oxidase test	8
+	+	+	Gelatin Test	9
+	+	+	Voges- Proskauer Test	10
+	-	-	Simmon's Citrate Test	11
+	+	+	Motility Test	12
+	+	+	Test of phosphatase	13
-	-	-	Urease Test	14
+	+	+	Nitrate Reduction	15
+	+	+	Starch hydrolysis Test	16

Field experiment

1-PlantHeight(cm):

Table 3 reveals that Bacterial Inoculation with *P. Polymyxa* resulted in considerable variances in abilities of Germination, the Use of BO (Bio-Organic) vs. Control (Untreated) Treatments, which proved to have the greatest creating average of 92.6 cm of height, which reflected an increase of 81.41% over Control (Untreated), with the Control being at 78.2 cm for average Plant Height (loss of 81.41%). Increased biological activity which occurs as a result of Incorporating this type of Inoculant/Bacteria leads to reduced pH levels of soil within the Rhizosphere of Plants; therefore, bio addition of Nutrients and Absorption occurs and creates favourable conditions for Plants to thrive (Increased Ability of Plants to Absorb

Micronutrients enables them to have improved Characteristics). Increasing Plant Height is also attributed to the bacteria being able produce Phosphorous, by means of Organic Acids and Phosphatase Enzymes, and will lead to the plants receiving Growth Factors through Producing Indoles, Phenols, Gibberellins and Cytokinins, thus stimulating Plant Growth, and Yields Continued Height (11).

There were however no Additional Significant Differences amongst Varieties with regards to Plant Height.

The previous results (Table 4) also indicate a significant difference in plant height between the different varieties based on their binary interaction with each of the local isolates. The highest recorded value of 99.5 cm was achieved using BOA3, which represents a 48.06 % increase compared to B1A1, which produced the lowest value of

67.2 cm. This is due to the biofertilizer's role in providing phosphorus through antibiotic secretion and the secretion of IAA, which promotes cell elongation and division; therefore, these biofertilizers promote the

production of proteins through these two actions, thus positively affecting plant height. This finding corresponds with the citations of (1) and (6).

Table (3) Effect of inoculation with local isolates of *P. Polymyxa* bacteria and wheat varieties, and the interaction between them, on the wheat plant height trait (cm).

Mean	A3	A2	A1	Varieties <i>Isolates</i>
92.6	99.5	83.8	94.5	B0
78.2	82.4	85.2	67.2	B1
86.8	83.0	77.6	99.8	B2
88.6	90.7	89.9	85.3	B3
86.6	88.9	84.1	86.7	Mean
B =16.55 A=N.S BA=28.67				LSD0.05

2-LeafArea(cm²Plant)

As Table (4) shows, using the bioinoculant had a significant impact on the average leaf area of wheat plants, with treatment B2 having the highest average at 55.94 cm²/plant, a 89.24% increase from the control group B0, whose average was 29.56 cm²/plant. The increase in the size of the leaf area can be attributed to the ability of the bioinoculant *P. Polymyxa* to increase surface area of roots in contact with the soil, by making available more nutrients in the soil (especially NPK). This provides the plants with more opportunities to absorb nutrients and water creating greater growth and development for both the roots and the shoot

of the plant. Results are in line with (11) and (7).

As indicated by the results in Table (4), the variety of the crops had a very large impact on the flag leaf area. For instance, sample A2 produced an average of 49.03 cm or 12.55% more than the average of 43.56 cm produced by sample A3. The increase in the average flag leaf area could be due to the different genetic structures of the two samples resulting in differences in growth traits, such as flag leaf area. These findings are in agreement with other researchers who have reported large differences between the flag leaf area of various types of bread wheat (9).

Analyzing the interacting local isolates and cultivars in the leaf area trait in the table provides a very interesting result, where the treatment B2A2 has the greatest number of 62.73 cm² per plant for average leaf area, and the isolate BO producing the least number of plant per average leaf area 25.97 cm² per plant, if combined with the cultivar A1 Buhouth 22 without addition. The reason for this very large average leaf area of treatment B2A2 is because when

phosphorus-solubilizing bacterium inoculum is applied, it helps to build a more effective root system which helps to maximize at the same time plant uptake of water and nutrients, allowing for greater surface area of leaf area to be produced through synthesis of carbohydrate and protein materials that accumulate throughout the various plant tissues that allow the dry weight of the plant to be increased. According to Makkar & Anjanappa (21)

Table (4) Effect of inoculation with local isolates of *P. Polymyxa* bacteria and wheat varieties and the interaction between them on the characteristic of leaf area (cm² plant⁻¹).

Mean	A3	A2	A1	Varieties <i>Isolates</i>
29.56	29.10	33.63	25.97	B0
52.46	53.30	53.97	50.13	B1
55.94	53.83	62.73	51.27	B2
45.05	38.03	45.80	51.33	B3
45.76	43.56	49.03	44.67	Mean
B=3.404	A=2.948	BA=5.896		LSD0.05

3-The dry weight of the shoot plant-1 gm:

Table 5 displays substantial variations between the dry weights of the wheat plant's vegetative tissue in all groups of seedlings treated with *P. polymyxa* bacteria via bio-inoculation; with treatment B2 averaging 10.61 g/seedling, which is significantly more than the isolate BO that is averaging 10.40 g/seedling. This represents a 10.98% increase over the control treatment's average

of only 9.56 g/seedling. The dry weight of the vegetative biomass with *P. polymyxa* inoculation may be due to the secretion of plant growth hormones including gibberellins, indoles, and organic acids. The presence of these plant growth hormones solubilize nutrients from their insoluble sources and make them available to plants. An example of soluble compounds is phosphorous, which is a growth nutrient

energy source, and promotes cell division. Therefore, the increase in dry weight of the vegetative biomass may be due in part to the presence of growth-promoting hormones from the *P. polymyxa* inoculation. (15)

Regarding the different types, there is no notable impact on the dry mass of wheat plant shoots.

The findings presented in the same Table (5) also indicate remarkable variations concerning the interaction between the different varieties and the isolates. The highest mass recorded was 11.40 g per plant with the BOA3 treatment, showing a significant difference when compared to isolates B1A1 and B1, which had masses of 11.00 g and 11.03 g, respectively.

The weight of the plants exhibited a sequential increase of 38.51% when compared to the lowest value noted at 8.23 g. This enhancement may result from the microorganisms introduced through various

processes. Nutrients can be solubilised from the soil by means of these mechanisms. As well as secreting organic acids, hormones and growth-promoting chemical substances that promote cell division and aid in plant growth, these secretions will help to support plant growth, which can lead to an increase in dry mass. In addition to the solubilisation of nutrients, I believe that bio-fertiliser inoculation improves the availability of nutrients and the release of growth-promoting substances. Through the potential for the bio-fertiliser to encapsulate the seed and enhance its concentration around the rhizosphere and surrounding area of root growth, I have noted that the dry mass increase due to the bio-fertiliser is related to the ability of the microorganism to infect the roots and use root exudates. If the bio-fertiliser is merely placed on the soil, it will be present at lower concentrations in the area of the roots and will not be as effective.

Table (5) Impact of inoculation with *P. polymyxa* local isolates + wheat cultivars x interaction on dry weight of wheat plant shoots (g/plant)

Mean	A3	A2	A1	Varieties
				<i>Isolates</i>
10.4	11.40	10.77	9.03	B0
9.90	8.23	10.47	11.00	B1
10.61	10.37	10.43	11.03	B2
9.56	9.60	8.37	10.70	B3
10.12	9.90	10.01	10.44	Mean
BA=1.515	A=N.S	B=0.875		LSD

4- Bio-yield (megagrams ha⁻¹)

Table 6 illustrates that bio-inoculation using bacteria of the genus *Penicillium Polymyxa* significantly influenced the bio-yield of wheat. The inoculated treatment B2 showed the highest yield average of 18.35 megagrams per hectare, which was 21.36% more than the B0 treatment, showing the least yield and yielding 15.12 megagrams per hectare. This could then mean bio-fertilization is one factor that aided growth and improved nutrient availability. The bio-fertilizer basically has phosphate-solubilizing and nitrogen-fixing organisms, in addition to organic fertilizer enhanced with several nutrients. This had a positive effect on the yield of the vegetative part in dry matter and total yield itself. This agrees with studies (3), (19), and (16).

Significant differences in biological yield were also observed among the evaluated wheat varieties in Table 6. The Rashid variety outperformed the Buhouth 22 and Wafia varieties, with averages of 15.95 and 15.86 μg per hectare, respectively. The difference due to genetic characters in their response to environmental conditions shows in part the variation in yield.

Furthermore, the interaction between local isolates and varieties revealed significant differences. The highest value came from treatment B2A2, which reached 22.73 μg per hectare. This represented a 74.10% increase compared to treatment B0A1, which had the lowest yield of 14.76 μg per hectare. Study (18) found that inoculating with the bacterium *P. Polymyxa* improves plant phosphorus nutrition and stimulates wheat growth under conditions of phosphate

deficiency. Study (5) noted increased growth and yield of wheat planted in various desert soils when inoculated with a biofertilizer containing phosphate-solubilizing bacteria. It was also observed that using organic or mineral fertilizer alone, without phosphate-solubilizing bacteria, led to lower growth and yield compared to when the bacteria were present.

Table (6) Effect of inoculation with local isolates of *P. polymyxa* and wheat varieties and the interaction between them on the biological yield of wheat plants (megagrams ha).

Mean	A3	A2	A1	Varieties <i>Isolates</i>
15.12	14.79	15.82	14.76	B0
17.08	16.08	17.78	17.39	B1
18.35	16.63	22.73	15.70	B2
16.23	15.94	16.81	15.96	B3
16.70	15.86	18.28	15.95	Mean
BA=2.273		B=1.312	A=1.136	0.05LSD

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