



## ***Role of Local Isolates of Paenibacillus polymyxa in Improving Soil Phosphorus Availability, Growth, and Yield of Wheat Cultivars (Triticum aestivum L.)***

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### **Abstract**

The field experiment was conducted with an objective to investigate the effect of bio-inoculation using three local isolates of *P. polymyxa* and three varieties of wheat on growth and yield of wheat plant (*Triticum aestivum* L). The field experiment was undertaken in 2024 at Al-Muthanna Governorate, under the conditions of the second Agricultural Research Station affiliated to the College of Agriculture, University of Al-Muthanna in soil texture (Silty Clay Loam). The experimental design was Randomized Block Design (RCBD), having three replications and was comprised of two factors, the first factor included four Local Isolates (level), which were designated P0 – P1 – P2 – P3 and the second factor included three different wheat varieties i.e. Rasheed Class (C1), Tamooz Class II (C2) and Ibaa 99(C3). Based on results obtained from the experimentation of bio-inoculation of the various levels of Local Isolates and the growth and yield for the three varieties of wheat, the following was noted: In the P3 bio-inoculation experiment, there were significantly higher levels of phosphorus and nitrogen in both soil and vegetative parts of treated plants during the flowering stage for both treatments, respectively (P3: 11.39 mg/kg & 1.4300%; P2: 18.22 cm & 41.22 g/1,000 grains). The Tammuz 2 variety performed best overall for phosphorus concentration in soil, spike length, and weight of 1,000 seeds (11.06 mg/kg) among the varieties tested and Abaa 99 had higher levels of nitrogen than 1,4208% as indicated by the average values for each treatment.

Third: When isolates were cross-pollinated with varieties, variations were observed. The Al-Rumaytha isolate with the Tammuz 2 variety recorded the highest average spike length and thousand-grain weight, reaching (20.33 cm - 45.70 g plant<sup>-1</sup>) respectively, while the Al-Khadar isolate with the Abaa 99 variety had the highest plant nitrogen concentration, reaching

(1.4567)%. The Al-Khadar isolate with the Tammuz 2 and Rashid varieties also had the highest soil phosphorus concentration, recording the highest average of (12.30) mg kg-soil.

**Keywords:** Wheat, *Paenibacillus polymyxa*, Local isolates, Phosphorus availability, Biofertilizer, Growth and yield.

## Introduction

Bio-fertilizers are defined as all bacteria inoculant; used in numerous ways applied to soil or seed, with the intention of using them to generate helping to create some of the nutrient necessities for crops, so that crops may grow and produce better, as well as with additional help from the visual environmental elements of water and air. Consequently, the global trend of clean agriculture technology is the reduction of waste by way of using natural resources with the aim of increasing the overall food production per acre of land, for example, using bio-fertilizers which can be combined with chemical fertilizers (17). Both organic and biological fertilisers influence nutrient availability, an important factor in decreasing the degree of environmental degradation resulting from continuous use of mineral fertilisers. They contribute to improving crop quality at the same time(26)(28). Also, as noted, the use of *Bacillus* species as biological inoculums added to an organic fertiliser leads to increases in available phosphorus in the soil, by improving both the chemical and physical properties of the soil, as well as the increased biological activity of the soil where slight decreases in the pH of the soil and bulk density of the soil occur, along with increases in the amount of available water and macronutrients. All of these effects increase both the quantity and quality of the crop yield as compared to non-amended soil. The renewal of the soil microbiome in the rhizosphere is a major factor in supporting plant growth. Interest in the study of these organisms has grown tremendously over

the past several years. One of the most significant microorganisms in this area is *Paenibacillus polymyxa* (formerly known as *Bacillus polymyxa*), as it excretes microorganisms that promote plant growth and is found in abundance in the soil of the rhizosphere(25).

Wheat (*Triticum aestivum* L.), belonging to the family Poaceae, is one of the most significant food crops, considered to be the most important cereal crop in both Iraq and worldwide, and in order of importance, amount produced, and area cultivated. Global production of wheat reached 761.6 million tons (in 2019) (16), and 4.343 million tons were produced in Iraq in 6,331,000 dunams (2019) (18). Because of its importance for food security, wheat is considered one of the most significant small-grain crops grown around the globe.

Wheat's importance to humans for food is related, in part, to wheat's gluten, which produces the highest quality breads (10). A primary factor that hampers wheat production and prevents self-sufficient wheat production is the low yield per unit area of wheat. Thus, one way to achieve higher yields from wheat production is through increasing the crop's productive capacity and/or efficient use of natural and synthetic nutrients, which can often be accomplished by applying biological fertilizers to crops. The goal of this research involving the *paenibacillus* species of bacteria isolated from wheat was to investigate the following three goals:

1. To investigate how local isolates of *P. polymyxa* are able to both stimulate plant growth and solubilize phosphorus by converting it from unavailable forms to soluble forms.

2. To investigate the influence of the various wheat varieties on how well each grew and yielded.

3. To investigate how local isolates interact with different wheat varieties and their effects on yield and growth.

**Materials and method**

*2.1 location of the experiment*

A factorial experiment was conducted in the winter of 2024-2025 at the second research station of the College of Agriculture, located in the Muthanna Governorate in Iraq. The experimental field soil was classified as composed of Clay Silty Loam, and will be used to evaluate the effects of three plant growth-promoting isolates of the bacterium *P. polymyxa* and three cultivars of wheat (*Triticum aestivum*) in a randomized complete block design (RCBD). Prior to doing any of the above-listed tasks, field

soil samples were collected randomly from the site in three different areas of the Muthanna Governorate to isolate *P. polymyxa* bacteria, before being combined and pooled together on the basis of their collected location so that they could create a more representative composite soil sample for this experiment. Once pooled, the soil samples were then sieved through a sieve with a 2 mm hole diameter before proceeding with the process of isolating *P. polymyxa*, which requires biological analyses. In addition, a portion of the sieved soil sample will be stored in the refrigerator until it is needed to complete the assessment described above.

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

Value	Unit of measure	Measured characteristic
4	ds <sup>m</sup> <sup>-1</sup>	Ece
7.5		pH
5.1	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 <sup>3</sup> *15	Cfu g <sup>-1</sup> soil	Total Bacterial Density
10 <sup>7</sup> *0.14		P. Polymyxa

## 2.2 Transactions and experimental design

The research was implemented as an on-field 2-factor experiment in a randomized complete block design (RCBD). Each factor for this experiment contained 3 treatments with 3 replicate blocks, giving 36 total experimental units. The following factors are presented here:

Factor 1: Application of 4 Local Isolates of P. Polymyxa

1 - P0 - No Application (Control)

Factor 3: Application of NPK Fertilizers

The application of NPK Fertilizers was made according to the comprehensive fertilizer recommendations for N-P-K, except for a total application rate of P (20% density): 36 total experimental units (4 x 3 x 3).

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

Due to their high efficiency in phosphorus solubilization, a local isolate of Paenibacillus (local number 3), identified as Paenibacillus polymyxa, was selected for field experiments. In order to generate the required quantity of inoculum for field experiments, three 1000 mL conical flasks were prepared with Pikovsky medium and 50 mL of liquid culture for

## 2.4 Agriculture and crop service operations

Land preparation consisted of having the soil ploughed (plowed), smoothed, and levelled, and was divided into three large blocks of land averaging 500 square meters each; with eight rows of plots within each block at a distance of 20 cm apart, each plot being 2 metres x 2 metres with an allowance of 1.5 metres between blocks

2 - P1 - Application of Samawa Isolate

3 - P2 - Application of Al-Rumaitha Isolate

4 - P3 - Application of Al-Khudhir Isolate

Factor 2: Application of 3 Wheat Varieties

1 - C1 - Rasheed Wheat

2 - C2 - Tamooz Wheat

3 - C3 - Ibaa99 Wheat

inoculation using sterile pipettes. The three inoculants were incubated using a shaking incubator at 30°C for 2 to 3 days. A McFarland Standard solution was prepared according to Yousuf et al. (2008) and the density of the inoculation was 108 x 1.5 CFU/mL. For the sterilization of the three different varieties of wheat seed, the seeds were submerged in 95% ethyl alcohol for 2 minutes followed by rinsing with sterile distilled water several times to remove any residual alcohol. The seeds were placed in sterile glass bowls, then inoculated by covering with a mixture of Arabic gum and peat moss for an appropriate period allowing the inoculum and peat moss to adhere to the seeds for planting.

to avoid interference by other treatments on the same plot.

All necessary cultural operations were carried out for each crop, and all crops were manually controlled for weeding; the crops were harvested close to the ground in and placed in paper bags for required analysis after harvesting had been completed.

## 2. 5 Soil measurements

### 2.5.1 Determination of phosphorous:

As mentioned by Olsen, the Phosphorous was extracted using 0.5 N NaHCO<sub>3</sub> (reagent grade), after which the color was developed with ammonium molybdate mixed with ascorbic acid and then measured for both accuracy and concentration using a spectrophotometer at 882 nm (28).

### 2.5.2 Nitrogen content in vegetative part (%).

The Nitrogen content was determined using the microkjeldahl method as outlined in section 3.2.5 of this protocol using a Microkjeldahl apparatus.

### 2.5.3 Spike length (cm):

Spike length was determined as the distance between the base of the spike to the terminal spike (excluding the awns) and is reported as the average of 10 randomly selected spikes from the midline of each experimental unit.

### 2.5.4 1000 grain weight (g):

In this trial, random samples of grains were taken from each experimental unit to determine the weight on a sensitive scale.

## 2.6 Statistical Analyses

A factorial experiment was conducted and analyzed statistically according to RCBD (completely randomized block design) on Genstat discovery edition program as per analysis of variance (ANOVA) and means were compared using LSD 0.05 significance level.

## Results and Discussion

### Results of Biochemical Tests:

Bacteria were classified into their groups by using the biochemical tests in Table (2). All of the groups returned positive results when tested for all types of tests except for the urease enzyme test and the indole test; both of these tests produced negative results when tested. All of these bacteria were incapable of producing the oxidase test, except for strain P1 which produced a positive result. The citrate test also produced negative results for all isolates, except for strain P3, which returned a positive result. All strains were capable of growing at 50% of the total temperature for growth.

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

Result			Tests	T
P3	P2	P1		
+	+	+	<b>Gram Stain Test</b>	1
+	+	+	<b>Growth at pH (7.5) and (6)</b>	2
+	+	+	<b>NaCl (%5) Growth at</b>	3
+	+	+	<b>Growth at 50C<sup>0</sup> Temperature</b>	4

+	+	+	<b>Methyl Red Test</b>	5
-	-	-	<b>Indole Production</b>	6
+	+	+	<b>Catalase test</b>	7
-	-	+	<b>Oxidase test</b>	8
+	+	+	<b>Gelatin Test</b>	9
+	+	+	<b>Voges- Proskauer Test</b>	10
+	-	-	<b>Simmon's Citrate Test</b>	11
+	+	+	<b>Motility Test</b>	12
+	+	+	<b>Test of phosphatase</b>	13
-	-	-	<b>Urease Test</b>	14
+	+	+	<b>Nitrate Reduction</b>	15
+	+	+	<b>Starch hydrolysis Test</b>	16

## Field experiment

### 1- Available phosphorous in soil $mg\ kg^{-1}$ .

According to Table (3), a significant difference in the level of available phosphorus within the soil was observed using bio-inoculation with *P. Polymyxa*. The treatment P3 resulted in the highest amount of available phosphorus at an average of  $11.39\ mg\ kg^{-1}$  soil, which represents an increase of 25.16% compared to the control treatment that had an average of  $9.1\ mg\ kg^{-1}$  soil; this increase may be due to *P. Polymyxa* being a bacteria that produces substances that enhance plant growth through the uptake of phosphorus by the root system and other associated nutrients. Such findings are consistent with the previously documented reports from (9), who also indicated that the increase in

phosphorus in the soil is due to the presence of bacteria that support phosphorus availability; (23) supported this by suggesting that the bacteria *P. Polymyxa* are capable of solubilizing precipitated (i.e. non-available) phosphates in soil into their solubilized form to facilitate absorption by the plant for its metabolism.

There was no significant difference between inoculants and cultivars in producing different levels of phosphorus in the soil, and also no significant interaction between the local isolates and cultivars in producing different levels of phosphorus in the soil.

The effect of using a prescribed inoculant to inoculate specific cultivar with local isolates of *P. polymyxa* and how their interactions will affect the total concentration of phosphorous in

the soil were found not to be statistically significant (Table 3).

**Table(3)** : The results indicate that no statistically significant interactions occurred between P. polysacrynhi and particular wheat cultivars regarding phosphorous content (mg/kg), as evidenced in .

Mean	C3	C2	C1	Varieties
				Isolates
9.10	9.17	9.83	8.30	P0
10.62	10.63	10.63	10.60	P1
11.30	10.10	11.50	12.30	P2
11.39	10.47	12.30	11.40	P3
10.60	10.09	11.06	10.65	Mean
PC=N.S	C=N.S		P=1.417	LSD

## 2 Nitrogen content in the vegetative part (%).

The nitrogen concentration of the available parts of wheat was analyzed and the results of table (4) indicated the presence of significant differences (at  $\alpha 0.05$ ) when the plants were inoculated with three isolates of P. Polymyxa at each test concentration for nitrogen. All concentrations of P. Polymyxa were represented in all isolates; therefore, both the highest and lowest results of all treatments were included to determine how P. Polymyxa affected the availability of nitrogen in the plant.

This is supported by the fact that the average nitrogen level of P2 was 1.416%, with an increase of 7.59% from the control average to the control treatment. The average nitrogen level for the lowest nitrogen treatment was recorded at 1.316%.

The increase in nitrogen concentration in the available parts of the wheat may be due to the effect of the organisms used as biofertilizers in

secreted hormones such as: gibberellin, auxin, indole acetic acid that stimulate root cell division and/or absorption of maximum nutrients, including nitrogen. Proven increases in root cells have been shown to increase nitrogen fixation, along with other vital nutrient fixation; (21).

As recorded in the table (4), approximately 1.420% (mean) and 4.41% increase (mean) in percentage of nitrogen concentration has been achieved by treatment C3, compared to treatment C1 (lower means). This increases can be attributed to using the nitrogen-fixing bacteria, Paenibacillus, for inoculating crops such as barley and wheat. The results show significant increases in the growth rate and yield of these crops, which supports the findings of several earlier reports. Specifically, [28] showed that there was an increase in P uptake from wheat plants due to inoculation with bacillus spp., leading to higher wheat yields. Similarly, [22] demonstrated that inoculation with phosphate-solubilizing bacteria (PSB)

contributed to biological N fixing and also enhanced root node weight, population density, and availability of certain nutrients, all leading to increased yield of crops.

components of soybean.

The outcomes from the interaction between various local isolates and cultivars account for the presence of noteworthy variations with respect to variables; the range of these yields was 1.4567% for treatment combination P3C3 - maximum value; whereas, 1.2700% was observed for treatment POC1, or minimum. The

positive growth response associated with the development of activated indole-3-acetic acid (IAA) with respect to wheat based upon the interaction between both organisms (11). This positive effect can also include the fact that there will be other types of organisms (associative bacteria) involved such as *Bacillus* spp., *A. chroococcum*, *Azospirillum brasilense* through their utilization of growth regulators such as auxins, gibberellins, and cytokinins in addition to the fact that those organisms also produce the required nutrients via mineralization and nitrogen fixation.

**Table (4)** The effect of inoculation with local isolates of *P. polymyxa* and wheat cultivars and the interaction between them on nitrogen concentration in the vegetable part%

Mean	C3	C2	C1	Varieties <i>Isolates</i>
1.3166	1.3567	1.3233	1.2700	P0
1.3989	1.4267	1.4033	1.3667	P1
1.4166	1.4433	1.4167	1.3900	P2
1.4300	1.4567	1.4200	1.4133	P3
1.3906	1.4208	1.3908	1.3600	Mean
PC=0.0201	C=0.0100		P=0.0116	LSD

### 3-

#### **Spike length (cm):**

The statistical results of the analysis (Table 5) indicated large differences in the characteristics between the isolates.

The wheat spikes had their longest average spike length of 18.22 cm in P2 treatment, a 49.10% increase over the shortest average spike length (12.22 cm) from a P0 treatment. Therefore, the

increase in spike length in the P2 treatment was probably due to the addition of the *polymyxa* bacteria biofertiliser and the additional nitrogen capacity. Nitrogen has been found to increase plant activity, especially during cell division and cell expansion, therefore improving photosynthetic efficiency. These findings correspond with (14), (7), and other researchers (13). The addition of *polymyxa* (*polymyxa*) bacteria biofertiliser and the evident increase in

nitrogen availability would also lead to an increase in the availability (increased root activity) of nutrients N, P and K. Those findings (12) and (1) relate the timing of nutrient availability to the timing of the emergence and growth of the ear of corn,

their relative responsiveness to differing environmental influences related to the growth and development of both the structure and growth patterns of the final internode that contains the spike (which distinguishes the different varieties).

According to the information provided in Table 5, a statistical analysis of the characteristics measured revealed significant variability between the different varieties of corn based on the measured spike length characteristic. In particular, variety C2 had an average spike length of 16.41 cm, which was an increase of 17.97% from the average spike length of variety C3 (13.91 cm). This significant difference is due to genetic variations among the varieties, since spike length is a morphological characteristic that has a relatively high heritability coefficient (5 and 2). Furthermore, the amount of variation between varieties for the spike length characteristic may be related to

The interaction between different local isolates and varieties of wheat had a significant impact on the length of the wheat spikes. The longest wheat spike was observed in treatment P2C1, with an average of 19 cm, while the shortest was observed in treatment POC3, which measured 8.67 cm on average. Larger root systems allow for a larger amount of water to be taken up through the roots, which is needed for photosynthesis and other physiological processes that promote growth and dry matter production in a plant, which in turn leads to a greater size of plant cells and height (8).

**Table (5):** The effect of the interaction between local isolates of *P. polymyxa* bacteria and wheat varieties on their spike lengths (cm).

Mean	C3	C2	C1	Varieties
				<i>Isolates</i>
12.22	8.67	12.33	15.67	P0
13.77	14.67	14.33	12.33	P1
18.22	15.33	20.33	19.00	P2
16.89	17.00	18.67	15.00	P3
14.22	13.91	16.41	15.50	Mean
P=2.075	C=1.797	PC=3.594		LSD0.05

4-

**Weight of 1000 grains (g)**

Table (6) shows that weight of wheat grains with exactly 1000 pieces has a highly significant effect on weight after inoculation with the biopollinating bacteria, *P. Polymyxa*. The average weight of the highest weight 41.22 g represent an increase in weight of 18.41% or 6.41 g from the average weight of the lowest weight of 34.81 g. This may have resulted from increased absorption and concentration of nutrients and waterproofers directly in the plant causing increased production and transport of nutrients to the grains thereby making their weight heavier.

The biofertilizer was added to the plant that contained the biopollinating bacteria, *P. Polymyxa* and it had an effect on overall plant growth indicating how effective biofertilization is to supply nutrients to the plant and thus increased the weight (volume) of 1000 grains of wheat. This information supports (6). Results of this experiment support the results of research which demonstrated a very important role of hormones secreted during biopollination in promoting growth characteristics and yield components of plants through the promotion of cell division and elongation, expanding root hairs; increasing the root's surface area and thus contributing to their ability to absorb materials; therefore improving all growth factors in the vegetative and dry matter produced; thus improving plant yield (19).

Weight of 1000 grains was strongly impacted by variety, with the Tamooz variety coming in with the heaviest average at 40.67 g, or 14.37% greater than the Abaa 99 variety, which came in at 35.56 g. The differences in weight between varieties and the dominance of one type of sample over another may be due to compensatory phenomena of yield components in cereal crops, such as those seen with wheat and barley. Compensation phenotype(s) is/are mainly modulated by genetic or varietal distinctions. These findings support related literature scientific evidence, including (15), (2), and (3), showing some differences have been found amongst wheat variety weights of 1,000 grains (a component of wheat yield). This study offers additional support regarding the importance of plant hormones facilitating metabolic or biological processes affected by bio-fertilization, (4).

There is a significant difference in the 1000-grain weight of the wheat plants due to the interaction of the isolate/cultivar, when measuring the bioinoculation of *P. polymyxa*. It's possible that isolate P2/cultivar C2 had a greater level of interaction, as indicated by the value of 45.70 g compared to 32.04 g for the POC2 treatment group. The rationale surrounding these differences may be the beneficial effects of the organisms utilized during the bioinoculation process, and their role in providing growth regulators, particularly auxins.

**Table (6 ):** Effect of inoculation with local isolates of *P. polymyxa* bacteria and wheat varieties, and the interaction between them, on the yield of 1000 grains of wheat (g).

Mean	C3	C2	C1	Varieties
				<i>Isolates</i>
34.81	34.10	32.04	38.29	P0

39.99	36.59	42.86	40.53	P1
41.22	38.97	45.70	39.00	P2
36.57	32.60	42.09	35.04	P3
38.15	35.56	40.67	38.21	Mean
PC= 2.388                      C=1.194 P=1.379				LSD0.05

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