

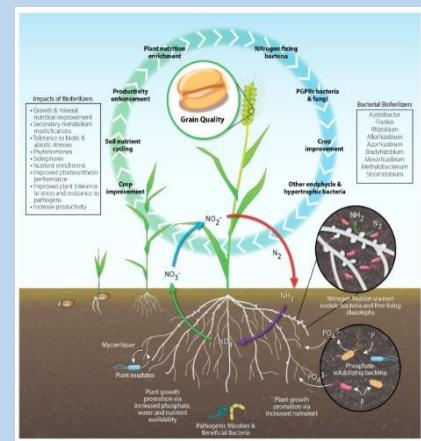


## Comparative Study of Biological and Chemical Fertilizers as a Stimulator of Physiological and Growth Parameters of *Triticum aestivum* L.

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**Abstract:** Chemical fertilizers have been extensively used in agriculture to boost crop production. Despite their proven effectiveness, they pose significant environmental and health risks. In response, researchers have explored sustainable alternatives that can enhance productivity while reducing ecological impact. Biofertilizers have emerged as a promising solution. Hence, the idea of this research came to conduct a comparison between a chemical fertilizer (NPK) and two types of biological fertilizers (*Bacillus megaterium* and *Pseudomonas putida*) in their effect on some physiological and growth characteristics of three varieties of *Triticum aestivum* (Abu Ghraib, Al-Fath and Al-Rashidiya). Ten milliliters of biofertilizers (as broth culture) were added to the rhizosphere and NPK was added with 1% concentration. The variables measured on the plants under examination included shoot dry weight, total chlorophyll and carotenes content, soot content of N, proteins, K, and P. Results showed that the biofertilizers used in the current study increased the level of all studied traits when compared to the NPK fertilizer. Biofertilizers augmented the nitrogen concentration within the plants by 51.8% and 60.4%, with identical percentages observed for the protein content. Furthermore, they enhanced the potassium concentration in the plants by 26.8% and 27.32%, as these percentages appeared under the influence of *Bacillus* and *Pseudomonas* respectively. The highest phosphorus content appeared for *Bacillus* treatments, represented by the value 1.93%. The three varieties of wheat showed different responses to the fertilizers used in the study, but Al-Rashidia variety showed the greatest response to the fertilizers for most of the studied traits.



**Keywords:** Biofertilizers, Wheat, NPK, *Bacillus*, *Pseudomonas*.

### Introduction

Wheat is recognized as a paramount cereal crop of significant economic importance. Its relevance in Iraq is underscored by the inadequacy of local production to meet the annual consumption demands; consequently, the nation has resorted to importing wheat for the past six decades, as indicated by numerous reports from the Central Statistical Organization affiliated with the Ministry of Planning over recent decades. The optimal regions for wheat cultivation, in terms of soil quality, are characterized by elevated levels of humus, adequate aeration, and an abundance of essential nutrients [1]. Scholars have projected that approximately 60% of farmland globally is deficient in nutrient availability for agricultural crops [2]. Iraq imports more than two thirds of the wheat grains needed to feed its people, with the remaining third coming from domestic production [3]. Due to the use of antiquated, traditional methods and the lack of widespread adoption of contemporary scientific technology in the production sector, the crop runs the risk of having low productivity. Consequently, it has become necessary to consider new strategies for achieving this objective and raising the yield per unit area. One such strategy is the application of the

plant growth regulator technique, which is now a standard practice in contemporary agriculture [4]. The use of synthetic fertilizers facilitated the Green Revolution, and millions of tons of chemical fertilizers are still applied worldwide [5]. Although there are many different kinds of fertilizers with various compositions, NPK, compost, and manure fertilizer are the most commonly utilized varieties [6]. However, overuse of chemical fertilizers has led to problems with ecology and negative effects on the ecosystem [7]. Several of these adverse consequences encompass: land degradation, contamination of food and agricultural products, exacerbation of the greenhouse effect and depletion of the ozone layer, pollution of aquatic ecosystems leading to eutrophication, and the proliferation of algal blooms, adverse human health effects, and overall ecosystem imbalance [8]. As a result of these negative impacts, microbial inoculants and biofertilizers designed to enhance plant growth and development have been formulated [9]. These include microorganisms that promote plant growth, such as those that fix nitrogen and microbes that solubilize phosphate [10]. Living microbes can be found in biofertilizers. Applying biofertilizers to surfaces or the rhizosphere boosts the concentrations of mineral

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elements available to the crops, which encourages plant development [11].

Biofertilizers may be characterized as a substance that comprises living microorganisms, which, upon introduction to the soil, colonize the rhizosphere and enhance plant growth by facilitating the availability of essential nutrients to the plants [12]. Biofertilizers are ecologically sustainable and economically advantageous, possessing the significant capacity to provide essential nutrients for plant growth. Additionally, they can reduce the application rates of chemical fertilizers by 25-50% [13]. Microorganisms utilized as biofertilizers encompass bacteria that are capable of nitrogen (N) fixation, potassium (K) solubilization, phosphorus (P) solubilization, and phosphorus mobilization [14]. Scientists have particularly concentrated their research efforts on the genera *Pseudomonas* and *Bacillus*. The spore-forming characteristics of *Bacillus* species are garnering increasing attention in their application as biofertilizers or biopesticides [15]. The beneficial impact of bio fertilizers can be attributed to the phenomenon whereby microorganisms enhance root functionality within the rhizosphere, stimulate hormonal processes, and consequently, augment the assimilation of essential plant nutrients [12,16,17].

In light of the aforementioned considerations, this investigation was undertaken with the objective of executing a comparative analysis between NPK fertilizers and microbial-based fertilizers, through an examination of the physiological responses and growth outcomes associated with these distinct fertilizer types.

## Materials and Methods

### Planting Process

The experiment was carried out in the balcony of the botany research laboratory of the Life Sciences Department - College of Science – University of Anbar during the winter season of 2022-2023 (so the experiment was under normal environmental conditions). Seeds of the three varieties of wheat (Abu Ghraib, Al-Fath, and Al-Rashidiya) were obtained from the Desert Studies Center - Department of Conservation Agriculture - University of Anbar. The process for seed sterilization was conducted utilizing a 10% sodium hypochlorite solution, which was applied for 2 minutes, then it was washed with distilled water. Seeds were first planted in sterile Petri dishes containing a layer of cotton and two filter papers that contained the grains inside. These dishes were watered with 10 ml of distilled water for each dish, then incubated at a temperature of 20°C for six days. The germinated and homogeneous grains were transferred from the dishes to black plastic pots filled with planting medium 5 kg, which was prepared by mixing mixed soil and peat moss at a ratio of 5:1, Some physical and chemical properties of soil are given in Table 1.

Seven seedlings were transferred into each pot, and after ten days the plants were thinned to five more uniform plants. The plants transferred to the pots were watered with equal amounts of tap water, 300-500 ml per pot. Fifteen days after transferring the germinated seedlings to the pots, biological and chemical fertilizers *Bacillus megaterium*, *Pseudomonas putida*, and NPK were added to the area near the roots. Ten milliliters of biofertilizers, in the format of liquid cultures (broth), were administered into the rhizosphere, and NPK fertilizer was added at a concentration of 0.1% (according to the recommended concentration). The fertilization process was repeated three times during the growing season, 15 days apart between each addition and the other. The treatments were labeled as a follow:

F0= without adding any fertilizer to the plants (control)

F1= *Bacillus* fertilizer

F2= *Pseudomonas* fertilizer

F3= NPK fertilizer

Bacterial isolates were isolated and purified and diagnosed by a specialized staff in the microbiology laboratory of the Center for Desert Studies at University of Anbar by using cultural and biochemical diagnostic methods.

**Table (1):** Chemical and physical properties of the soil used in this study.

P (mg/kg)	K <sup>+</sup> (mg/kg)	Ca <sup>++</sup> (mg/kg)	Mg <sup>++</sup> (mg/kg)	Cd <sup>++</sup> (mg/kg)	Ec <sup>+</sup>	TDS (ppm)	NaCl (%)
17.337	9.13	114.5	26.5	0.06642	2637	1318	5.2

### Statistical analysis

The experimentation was carried out in a completely randomized design (CRD) encompassing three replicates (pots) for every treatment condition. The obtained data were subjected to statistical analysis utilizing GenStat statistical software, twelve editions. Analysis of variance (ANOVA) was performed to test the significance of fertilizer effect. The least significant difference (LSD) was computed to compare treatment means at the probability threshold ( $P \leq 0.05$ ).

### Shoot dry weight (g. plant-1)

One plant from each pot was cut from contact with soil at the age of 92 days after planting and dried at 80 °C in a hot air oven until the weight was stable after drying. The weight was measured and the values were expressed in grams/plant.

#### Total chlorophyll and carotenoid estimation

The pigments were isolated from freshly collected leaves, utilizing three replicates for each treatment; approximately 0.2 g of fresh leaf material was swiftly homogenized in 10 ml of 80% acetone. The concentrations of total chlorophyll and carotenoids were ascertained via spectrophotometry, adhering to the methodologies delineated by [18] for total chlorophyll and by [19] for total carotenoids. Absorbance readings were taken at the wavelengths of 470, 645, and 663 nm. The quantification of photosynthetic pigment concentrations was achieved through the application of the following equations:

$$"Ca \text{ (mg/g tissue)} = 12.7A663 - 2.69A645 * (V/1000*W)"$$

$$"Cb \text{ (mg/g tissue)} = 22.9A645 - 4.68A663 * (V/1000*W)"$$

$$\text{Total chlorophyll} = Ca + Cb$$

$$"Cx+c \text{ (mg/g tissue)} = 1000 A470 - 1.82 Ca - 85.02 Cb / 198"$$

Where Ca = chlorophyll a; Cb = chlorophyll b;  
Ca + Cb = total chlorophyll;

Cx + c = carotenoid, Aλ = absorbance at λ (nm)

### Nitrogen percentage estimation

Nitrogen was estimated according to the Kjeldahl method [20].

### Total protein content

From calculated nitrogen values, protein value was estimated by the following equation:

$$\text{Protein \%} = \text{Nitrogen \%} * 6.24 [21]$$

### Minerals estimation

#### Sample preparation (digestion)

Wheat plants were harvested after completing the experiment at an age of about 160 days. The aerial parts were taken and dried to measure some of chemical contents. Approximately 0.5 grams of dried and finely ground aerial parts were taken and digested by a mixture of concentrated acids H<sub>2</sub>SO<sub>4</sub>, HClO<sub>4</sub>, and HNO<sub>3</sub> in a ratio of 10:4:1, respectively. The samples were placed in Kjeldahl flasks with a long neck and 10

ml of the above mixture was added. Then, they were left on the heaters until the color disappeared, after which it was cooled down and the volume was completed to 50 ml with distilled water

### Potassium Estimation

One ml of the digested solution was taken and diluted 50 times with deionized water to determine the content of K in the digested solution of the plant samples. Two ml from this dilute was used to estimate K by flame photometer [22]. Potassium concentrations of plant samples were calculated by interpolation of the reading on the standard curve conducted in the same assay as for the samples using the standard curve fitting equation.

### Phosphorus estimation

Approximately 10  $\mu$ l of dilute solution was used to estimate phosphorus by colorimetric method according to the US Environmental Protection Agency [23]. A standard solution of phosphorous was prepared with a concentration of 50mg/ L by dissolving 0.2195 grams of KH<sub>2</sub>PO<sub>4</sub>, which was dried at 105°C in a liter of distilled water. The phosphate reagent was prepared from: (a) Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) at a concentration of 4.9 N, which was prepared from dilution of 136.11 ml in a liter of distilled water. (b) 4% solution of ammonium molybdate, which was prepared by dissolving 4 grams in 100 ml of distilled water. (c) The ascorbic acid that was prepared by dissolving 9 gm in a liter of distilled water. (d) Antimony potassium tartrate that was prepared by dissolving 3 grams in a liter of distilled water.

The reagent was mixed by taking 100 ml of solution a, 15 ml of solution b, 30 ml of solution c, and 5 ml of solution d. The solutions were mixed well after each addition; then 5 ml of the reagent was added to 100 ml of the sample. Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form a Complex of antimony-phospho-molybdate. This complex has been reduced to an intensely blue-colored ascorbic acid complex. The color is proportional to the color of the concentration of phosphorus.

## Results

### Shoot dry weight (g. plant -1)

As recorded in Table 2, the NPK fertilizer was significantly superior to the control and the biofertilizers. Bacillus and pseudomonas increased shoot dry weight by 22.5%, and 22.8% respectively, compared to the control treatment. However, there were no significant disparities observed among them. Al-Rashidia variety was significantly superior to the other two varieties in its response to the fertilizers used (average values of 0.3746, 0.4106 and .4590 g. plant -1, for Abu Graib, Al-Fath, and Alrashidiya, respectively)

**Table (2):** Shoot dry weight of wheat plant varieties (g. plant<sup>-1</sup>) under different types of fertilizers.

Variety name	Fertilizer type				Variety Average
	F0	F1	F2	F3	
Abu Ghraib	0.2800	0.3787	0.3837	0.4560	0.3746
Al-Fath	0.3470	0.3670	0.3480	0.5803	0.4106
Al-Rashidiya	0.2733	0.3580	0.3740	0.8307	0.4590
LSD <sub>P&lt;0.05</sub>	0.08109				0.04055
Fertilizer Average	0.3001	0.3679	0.3686	0.6223	
LSD <sub>P&lt;0.05</sub>	0.04682				

F0= Control treatments F1= *Bacillus* fertilizer

F2= *Pseudomonas* fertilizer F3= NPK fertilizer

### Total chlorophyll content in leaves (mg. g-1 fresh weight)

Total chlorophyll values shown in Table 3 demonstrated that biofertilizers used in the study increased the chlorophyll values of plant leaves by 13.9% and 11.5% when adding Bacillus and Pseudomonas respectively, but this increase was in fact not significant compared to the values that appeared in the case of adding NPK to the soil, which increased chlorophyll values significantly by 45.3% over the control treatment of 1.537 mg.g-1 fresh weight, which also significantly exceeded the biological fertilizers used. The results also showed that Abu Ghraib variety was significantly superior to the other varieties in chlorophyll content of its leaves (2.065, 1.751, and 1.613 mg.g-1 fresh weight, for Abu Graib, Al-Fath, and Alrashidiya, respectively).

**Table (3):** Total leaf chlorophyll content of wheat plant varieties (mg. g<sup>-1</sup>) under different types of fertilizers.

Variety name	Fertilizer type				Variety Average
	F0	F1	F2	F3	
Abu Ghraib	1.666	1.968	2.277	2.349	2.065
Al-Fath	1.420	1.831	1.574	2.179	1.751
Al-Rashidiya	1.524	1.458	1.294	2.174	1.613
LSD <sub>P&lt;0.05</sub>	0.5632			0.2816	
Fertilizer Average	1.537	1.752	1.715	2.234	
LSD <sub>P&lt;0.05</sub>	0.3252				

F0= Control treatments F1= *Bacillus* fertilizer

F2= *Pseudomonas* fertilizer F3= NPK fertilizer

### Total carotenoid content in leaves (mg. g-1)

Carotene values of wheat varieties are shown in Table 4. The results revealed a positive effect of adding biofertilizers, which significantly increased carotene values by 32.4% and 28.6% under Bacillus and Pseudomonas effect respectively, when compared to control average value of 3.596 mg. g<sup>-1</sup>, without any significant differences among them.

The NPK fertilizer had the greatest value among the experimental treatments, being significantly higher than the control treatment by 49.8%. Abu Gharib variety has the highest content of carotene (4.953 mg. g<sup>-1</sup>), which is significantly different from the carotene of the other two varieties.

**Table (4):** Leaves total carotenoids content of wheat plant varieties (mg. g<sup>-1</sup>) under different types of fertilizers.

Variety name	Fertilizer type				Variety Average
	F0	F1	F2	F3	
Abu Ghraib	4.208	5.074	5.161	5.370	4.953
Al-Fath	2.584	4.928	4.117	5.200	4.207
Al-Rashidiya	3.995	4.286	4.607	5.594	4.621
LSD <sub>P&lt;0.05</sub>	0.5965			0.2982	
Fertilizer Average	3.596	4.763	4.628	5.388	
LSD <sub>P&lt;0.05</sub>	0.3444				

F0= Control treatments F1= *Bacillus* fertilizer

F2= *Pseudomonas* fertilizer F3= NPK fertilizer

### Shoot nitrogen content (%)

Adding biological inoculators to the soil (Bacillus and Pseudomonas) had a positive effect on the nitrogen content of the plant's dry shoots, which increased its nitrogen values significantly by 51.8% and 60.4% compared to the control treatment (Table 5). There were no significant differences between the two biofertilizer types, but these fertilizers had significantly lower average values compared to the NPK fertilizer, which increased the nitrogen content of plants to more than double compared to the control. Al Rashidia variety had the highest nitrogen content with a value of 6.48%, which was significantly different from the other two varieties.

**Table (5):** Shoot nitrogen content of wheat plant varieties (%) under different types of fertilizers.

Variety name	Fertilizer type				Variety Average
	F0	F1	F2	F3	
Abu Ghraib	2.85	3.65	3.94	5.11	3.89
Al-Fath	2.53	3.08	4.37	4.76	3.69
Al-Rashidiya	3.35	6.53	5.68	10.36	6.48
LSD $P_{0.05}$	1.874			0.937	
Fertilizer Average	2.91	4.42	4.67	6.75	
LSD $P_{0.05}$	1.082				

F0= Control treatments F1= *Bacillus* fertilizer

F2= *Pseudomonas* fertilizer F3= NPK fertilizer

### Shoot protein content (%)

Results of plant shoots protein content (Table 6) show the effect of biofertilizers in raising plant content of protein, as *Bacillus* significantly increased plants protein by 52.4%, *Pseudomonas* also raised plant protein values significantly by 60.7% over the control treatment of 18.1%, with no significant differences between the two biofertilizers. The highest percentage of protein appeared when NPK was added to the soil, which reached 42.12% with a highly significant difference from the control treatment itself. Al- Rashidia variety recorded the greatest value in its total protein content estimated by percentage, which amounted to 40.4%, which differed significantly from the protein values of the other two varieties.

**Table (6):** Shoot protein content of wheat plant varieties (%) under different types of fertilizers.

Variety name	Fertilizer type				Variety Average
	F0	F1	F2	F3	
Abu Ghraib	17.8	22.8	24.6	31.9	24.3
Al-Fath	15.8	19.2	27.3	29.7	23.0
Al-Rashidiya	20.9	40.8	35.5	64.7	40.4
LSD $P_{0.05}$	11.69			5.85	
Fertilizer Average	18.1	27.6	29.1	42.1	
LSD $P_{0.05}$	6.75				

F0= Control treatments F1= *Bacillus* fertilizer

F2= *Pseudomonas* fertilizer F3= NPK fertilizer

### Shoot potassium content (ppm)

The results of the statistical analysis, listed in Table 7, reveal that there are significant differences in the potassium content of wheat plant shoots in response to all fertilizers used in the study. The percentage increase in potassium content was largest in the presence of NPK (51.8%), when compared to the percentage increase in the presence of *Bacillus* and *Pseudomonas* (26.8% and 27.32%). The results showed that Abu Ghraib variety had the highest potassium content, which reached 178.9 ppm with a significant difference from the values that appeared in the other two varieties.

**Table (7)** Shoot potassium content of wheat plant varieties (ppm) under different types of fertilizers.

Variety name	Fertilizer type				Variety Average
	F0	F1	F2	F3	
Abu Ghraib	148.2	193.2	191.2	182.9	178.9
Al-Fath	130.7	139.4	134.6	202.7	151.8
Al-Rashidiya	97.0	143.9	152.9	185.1	144.7
LSD $P_{0.05}$	15.02			7.51	
Fertilizer Average	125.3	158.9	159.6	190.2	
LSD $P_{0.05}$	8.67				

F0= Control treatments F1= *Bacillus* fertilizer

F2= *Pseudomonas* fertilizer F3= NPK fertilizer

### Shoot phosphorus content (%)

When looking at the results listed in Table 8, it is clear that the plants treated with *Bacillus* showed the highest phosphorus

content, which reached 1.9283 %, followed by plants treated with *Pseudomonas*, whose phosphorus content reached 1.6817 %. Both significantly differed from the control treatment (1.2383 %) and the NPK (1.5933%). Al- Rashidiya plants recorded the highest phosphorus content (2.008%), which differed significantly from the plants of the other two varieties, Abu Ghraib and Al-Fath.

**Table (8):** Shoot phosphorus content of wheat plant varieties (%) under different types of fertilizers.

Variety name	Fertilizer type				Variety Average
	F0	F1	F2	F3	
Abu Ghraib	1.2850	1.3650	1.6450	1.3450	1.4100
Al-Fath	1.0750	1.6550	1.2700	1.6500	1.4125
Al-Rashidiya	1.3550	2.7650	2.1300	1.7850	2.0088
LSD $P_{0.05}$	0.02983			0.01492	
Fertilizer Average	1.2383	1.9283	1.6817	1.5933	
LSD $P_{0.05}$	0.01723				

F0= Control treatments F1= *Bacillus* fertilizer

F2= *Pseudomonas* fertilizer F3= NPK fertilizer

### Discussion

Bio fertilizers serve a crucial function in promoting the growth and increasing the yield of agricultural crops. They participate in numerous biotic interactions and contribute to sustainable agricultural production [24]. The biofertilizers used in the current study increased the dry weight of the plants used in the study, and this may reflect their effect on the growth rate. This is consistent with what founded by Noreen and Noreen, [25] who recorded that application of bio fertilizers significantly enhances the growth attributes and biological yield of wheat. This is primarily attributed to the ability of bacteria to fix atmospheric nitrogen within the soil, which consequently leads to an increase in production. Therefore, the combined application or the exclusive utilization of bio fertilizers should be regarded as advantageous for both the growth and yield of wheat. The findings presented by Khiniab, [4] indicated that bio-fertilization exerted a pronounced influence on the height (cm) of wheat plants. The enhancement in soil enzyme activity contributed to an augmentation in plant growth, the outcomes of which were distinctly observable through various metrics including plant dry weight, the quantity of tillers per plant, the nutritional status of the plant, and overall yield, as demonstrated by Namli et al., [26]. In addition, microorganisms excrete growth-promoting substances (Plant Growth Promoting Rhizobacteria (PGPR), which enhance the physical, chemical and biological characteristics of the soil, thereby augmenting soil fertility through an increase in microbial populations within the soil, resulting in a beneficial effect on plant development [27, 28]. Elhag, [29] concluded that treating wheat plant with nitrogen fertilization and bio fertilizers significantly enhanced the productivity of the plant.

Chlorophyll pigment is integral to the photosynthetic mechanism that generates a product utilized as energy for the plant's growth and various physiological processes. Furthermore, the concentration of chlorophyll exhibits variability across different plant species, influenced by a multitude of internal and external factors; thus, each plant possesses a unique photosynthetic capacity [30]. Our results showed a significant increase in photosynthetic pigments (chlorophyll and carotene) when using fertilizers. This may align with the findings of Abdulfatah and Naji, [31] refers to the fact that used of biofertilizers reduces the negative impact of salinity on oats' chlorophyll content. Kamal et al., [17] results showed that *Bacillus megaterium* strain BM18-2 increased chlorophyll production, as well as root and shoot elongation, biomass accumulation, dry weight, and the overall nitrogen content ratio.

Furthermore, BM18-2 has been identified as an economically viable and efficient product at the commercial scale, serving as a proficient alternative to synthetic fertilizers. The application of fertilizers utilized in the present investigation on wheat plants results in an enhancement of the nitrogen, potassium, and phosphorus concentrations within the dry shoot biomass. This is supported the findings of El Tarabily et al., [32] who reported that biofertilizers synthesized utilizing rock-derived phosphorus (P) and potassium (K) that are inoculated with oxidizing bacteria are capable of producing sulfuric acid. Lima et al., [33] added that acid play a significant role in the reduction of soil pH. Consequently, it becomes imperative to incorporate organic matter characterized by elevated pH levels to mitigate the acidic repercussions associated with rock-based bio fertilizers. They undertake the biological fixation of atmospheric nitrogen, thereby facilitating a decrease in the reliance on nitrogenous mineral fertilizers. Furthermore, certain organisms are capable of solubilizing phosphate in calcareous soils, rendering them conducive to plant development. Additionally, these organisms contribute to the enhancement of the root and vegetative systems of plants through the synthesis of phytohormones, including gibberellins, cytokinins, and auxins [34]. The mineral phosphate transforms into soluble phosphates, specifically primary and secondary orthophosphates, through the action of these genera, which facilitate the production of organic acids such as acetic acid, malic acid, citric acid, and oxalic acid [35,36]. *Bacillus megaterium* is recognized for its capacity to facilitate the dissolution of phosphorus and potassium within terrestrial substrates [37] and fosters the enhancement of plant development [38]. The findings presented by Naji et al. [39] indicated that the application of *Bacillus megaterium* on bean seeds augmented the resilience of the resultant seedlings against heavy metal stress through an elevation in the amino acid proline, subsequently enhancing protein synthesis. This phenomenon elucidates the observed enhancement in the protein content of the shoots of the wheat plant varieties analyzed in the present investigation.

## Conclusions

During the current study, the wheat plant showed a clear response to all the fertilizers used in the study. Although the highest values for most of the measured parameters were achieved by NPK fertilizer, the bio fertilizers had an effective role in raising the values of these traits when compared to the control treatment. As it is possible to dispense with or even reduce the concentration of chemical fertilizers in order to avoid the environmental and health damage resulting from their use in order to achieve a clean environment and optimally sustainable agriculture.

## Disclosure Statements

- **Ethics approval and consent to participate:** The authors confirm that they respect the publication's ethics and consent to their work's publication.
- **Consent for publication:** The authors consent to the publication of this work.
- **Author's Contribution:** The authors confirm their contribution to the research as follows: Main idea, novelty and experimental design: Enas F. Naji Analysis and interpretation of results based on previous studies: Enas F. Naji and Hiba F. Abdulfatah Manuscript preparation: Enas F. Naji and Hiba F. Abdulfatah. Provision of identified bacterial isolates and classified seeds: Ali A. Alrawi and Ahmad Sh. A. Lafi. Journal correspondence: Ali A. Alrawi. All authors reviewed the results and they approved the final version of the manuscript.

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