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ISOLATION AND DIAGNOSIS OF *LYSINIBACILLUS FUSIFORMIS* OBTAINED FROM SOIL AND ITS USE AS BIOFERTILIZER IN WHEAT CROP

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SUMMARY

The study materialized at the Plant Protection Directorate, Ministry of Agriculture, Iraq to know the effects of adding plant growth-promoting microorganisms (PGPM) (A*zospirillum brasilense*, *Lysinibacillus fusiformis, Rhizobium ciceri CP-93, Pseudomonas fluorescens, Bacillus megaterium,* and *Trichoderma harzianum*) as biofertilizers with 25% mineral fertilizer in wheat crops using the wheat cultivar IPA-99. The laboratory study included isolating and identifying *Lysinibacillus,* which showed no antagonism among these microorganisms in vitro. The study results revealed that the T2 treatment was superior in most of the traits under analysis, including the number of tillers (4.00 tillers plant^{-1}), spike length (10.50 cm), number of spikelets per spike (19.50 spikelets spike^{-1}), weight of 100 grains (3.50 g), and the number of grains per spike (35.43 grains spike^{-1}). The said treatment also excelled in the attributes, such as the grain content of nitrogen (4.870%), phosphorus (1.943%), potassium (4.156%), and protein in the grain (30.43%). The T2 outperformed all treatments, except for the biological yield characteristic, where treatment T5 (62.30 g plant^{-1}) excelled, and the harvest index, with treatment T1 (23.10%) excelled. However, they did not differ significantly from treatment T2.

Keywords: Wheat, *Lysinibacillus fusiformis*, biofertilizer, PGPMs, growth and yield traits

Key findings: *Lysinibacillus fusiformis* treatment as biofertilizer combined with 25% of the recommended mineral fertilizer doses significantly enhanced the wheat's growth and yield parameters. Additionally, the biofertilizer also increased the NPK availability in wheat plants.

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INTRODUCTION

The exponential growth of the global population necessitated a corresponding acceleration in agricultural output. Globally, the primary sources of human nourishment comprise the most significant grain crops, such as wheat, maize, and rice (Majeed, 2013; Majeed *et al*., 2017). Wheat (*Triticum aestivum* L.) crop leads for cultivated area and production. Wheat is the primary staple meal for almost one-third of the global population, providing over 25% of the caloric intake and essential nutrients, such as carbohydrates, proteins, and specific amino acids (Mohamed and Al–Shamary, 2022; Farhood *et al*., 2020). This significant contribution by wheat to human nutrition has earned the title 'king of cereals' (Costa *et al*., 2013). In a wheat crop, one of the primary benefits to human beings lies in its grains, which possess a harmonious composition of proteins and carbohydrates. The elevated gluten content in wheat grains is the primary factor contributing to the dough's significant elasticity and subsequent manufacturing of bread with substantial dimensions and superior characteristics (Knezevic *et al.,* 2017).

Iraq, one of the original regions for the inception of wheat and possessing the required environmental conditions for its cultivation, continues to reveal suboptimal productivity levels compared with global standards. Specifically, in Iraq, the wheat output is at 1.930 t ha^{-1}. However, it is worth noting that the world production rate has attained a value of 3.51 t ha^{-1} (USDA, 2022). Given the low wheat production, focusing on the wheat crop's improvement in quantity and quality is imperative. Its achievement could be through effectively executing contemporary agricultural techniques and harvest management. The increased wheat production can be possible by implementing recommended cultural practices, focusing specifically on fertilization techniques. Utilizing environmentally friendly approaches, such as biofertilizers, can be vital here.

Biofertilizer refers to a material comprising viable microorganisms, such as bacteria and fungi, commonly known as plant growth-promoting microorganisms (PGPM). In agriculture, biofertilizers seek to support sustainable and secure agricultural practices (Gupta *et al*., 2015). Upon application to the seed and soil of crop plants, these microorganisms establish a rhizosphere, even inside the plant itself, to facilitate plant growth and development and suppress plant diseases (Kaur *et al*., 2016). Biofertilizers enhance plant biomass production, and the optimal utilization and harnessing of particular microorganisms and their vital activities can be evident in the seed coating (Mashkoor and Kisko, 2021).

These microorganisms are crucial in stabilizing nitrogen, dissolving phosphorus and potassium, and producing growth-stimulating hormones, amino acids, and vitamins. Consequently, these bioactivities enhance soil properties, resulting in heightened crop productivity. The efficacy of biofertilizers in enhancing nutrient accessibility is apparent through diverse mechanisms, such as the mitigation of interaction levels via the excretion of organic acids and the synthesis of phytohormones and antibiotics to safeguard both the biofertilizers themselves and the associated crop plants against the bacterial and fungal ailments (Khanna *et al.,* 2019).

The present strategy aligns with sustainable agriculture principles, as it incorporates diverse microbial species that assist plants in nutrient possession and enhance their flexibility to specific environmental stresses, thereby mitigating potential risks associated with mineral fertilizer usage and ensuring human safety (Jawad *et al*., 2015). With the biofertilizer's importance and influence on wheat crops, the presented study focused on *Lysinibacillus fusiformis* isolation and identification and determined its effects using biofertilizer in the wheat crop.

MATERIAL AND METHODS

Selection of biofertilizers

In wheat crops, the use of a biofertilizer combination relied on microorganisms, i.e., *Rhizobium ciceri CP-93* + *Azospirillum brasilense* + *Trichoderma harzianum* + *Pseudomonas fluorescens* + *Bacillus*

megaterium, and 25% mineral fertilizer (Majeed *et al*., 2020). This study sought to determine the effects of *Lysinibacillus fusiformis* addition in improving the wheat crop. In the latest research, the microorganisms used appear in Table 1. The laboratory experiment transpired in the Biofertilizer Laboratory, Directorate of Plant Protection, Ministry of Agriculture, Abu-Ghraib, Baghdad.

Isolation and identification of *Lysinibacillus fusiformis*

Three soil samples randomly taken with a spatula had a depth of 15 to 25 cm, bagged in sterile containers, and kept in refrigerator are available in Table 2 (Sapkota *et al*., 2019). Then, the 250 ml conical flask containing 10 g of soil sample mixed with 90 ml of sterile distilled water sustained heating at 80 °C for 10 min. The serial dilution technique comprised taking 0.1 ml from 10^{-6} and spreading on the Petri dish plates with nutrient agar, using the spread plate technique, and then incubating at 50 °C for 24-48 hours (Khadka *et al*., 2020).

Microscopic characteristics

For the screening process, swabbing the bacterial isolates from the growing bacterial colonies on the nutrient agar culture media, fixing it, and staining it with gram stain helped to observe the shape and arrangement of the bacterial cells and their interaction with the stain (positive or negative), as explained in Bergey's manual of determinative bacteriology (Bergey, 1994).

Molecular characteristics

The bacterial genomic DNA extraction, amplification, sequencing, and assemblage used the PCR on 16S rRNA with 16SrRNA F and 16SrRNA R primers (Table 3), producing at least 1,300 bp of sequencing data. The molecular characterization of the pure bacterial colony progressed in the Advanced Scientific Bureau (ASCO), AL-harthia, Al-Kindi ST, Baghdad, Iraq, as described by Raimi and Adeleke (2023) using 16S rRNA gene sequencing methods. Using the rRNA database at the National Center for Biotechnology Information (NCBI), sequence analysis and confirmation of homogenic data occurred after the bacterial RNA amplification. The PCR conditions are in Table 4.

Table 1. Microorganisms used as biofertilizer and their sources.

Microorganisms	Source
Rhizobium ciceri CP-93 (Rh.)	Biofertilizer laboratory /plant protection directory
Azospirillum brasilense (Az.)	-do-
Pseudomonas fluorescens (P.F.)	-do-
Bacillus megaterium (B.m)	-do-
Trichoderma harzianum (Tr.)	Plant pathology laboratory /plant protection directory
Lysinibacillus fusiformis (Ly)	Isolation from the soil

Table 2. Soil samples used in wheat cultivation and their sources**.**

Soil samples	Source
Soli 1	Directorate of Plant Protection, Ministry of Agriculture, Abu-Ghraib field previously cultured with wheat
Soil 2	Directorate of Plant Protection, Ministry of Agriculture, Abu-Ghraib greenhouse previously cultured with cucumber
Soil 3	Baghdad University, Al-Jadriya fields

Table 3: Primer used in determining the genetic diversity of the tested bacteria.

Primer Name	Seq.	Annealing Temp. (°C)	Product size (bp)
16SrRNA F	5`-AGAGTTTGATCCTGGCTCAG-3`	60	1500
16SrRNA R	5`-TACGGTTACCTTGTTACGACTT-3`		

Table 4. Amplification reaction program of PCR.

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	55	00:30	
Extension	72	01:00	
Final Extension	72	07:00	1
Hold	10	10:00	

Interaction test between *Lysinibacillus* and other bacterial genera

The execution of this experiment sought to determine the relationship between *Lysinibacillus fusiformis* and other bacterial genera in the biofertilizer, according to Hewedy *et al.* (2010) as follows:

Bacillus megaterium against *Lysinibacillus fusiformis,*
Rhizobium ciceri CP-93 against *Lysinibacillus fusiformis,*
Pseudomonas fluorescens against *Lysinibacillus fusiformis,* and
Azospirillum brasilense against *Lysinibacillus fusiformis.*

Producing each stock solution of bacteria began by taking a full load of stock slant by a loop, which proceeded inoculation at 250 ml of nutrient broth. Each bacterial concentration test used a McFarland tube number 5 with a concentration of 10^8. Adding one drop of a stock solution of bacteria in Petri dishes with 10 ml of nutrient agar ensued, placing the drop on one side of the plate, streaked as a vertical line; meanwhile, the opposite side of the plate sustained culturing with one drop of different bacteria at the same concentration and streaked as a horizontal line, without touching each other. All of the examined bacterial species underwent three replications of this stage. Each genus of the examined bacteria employed three Petri dishes of nutrient agar as the control treatment. Incubating the plates was at 28 °C ± 2 °C (Majeed, 2013).

Interaction test between *Lysinibacillus* and *Trichoderma harzianum*

The experiment determined whether any antagonism between *Lysinibacillus* and *Trichoderma harzianum* existed (Malleswari, 2014). Inoculation of Petri dishes with 10 ml of PDA medium transpired with a disk of *T. harzianum* (5 mm in diameter) from a 7-day-old culture on one side of the plate and one drop of activated stock *Lysinibacillus* broth (10^8 cfu/ml) on the opposite side of the plate, then streaked vertically. With three replicates for each treatment, three plates of inoculation ensued with *Trichoderma harzianum* only and three other plates with *Lysinibacillus* only as a control treatment. Incubation of plates was at 26 °C ± 1 °C in a cold incubator.

Pot experiment

The study comprised three different treatments, as shown in Table 1. Wheat cultivar IPA-99 is a widely cultivated variety in various regions of Iraq. Bacteria activated and grown in 1000 ml of nutrient broth, incubated for two days at 28 °C in a cold shake to obtain a uniform cell density of 10^{7-8} cfu/ml as a viable count method helped calculate the bacterial concentration. Bacteria inoculation on a particular sterilized carrier (charcoal, peat,

3:1, and 10% Arabic gum) had the temperature at 105 °C for 55 min at 1 bar and incubated at 28 **°**C for three days with daily shaking. The seeds reached well-mixing with biofertilizer and sugar solution until achieving a perfect coating with the carrier (Majeed *et al*., 2017).

The coated wheat seeds underwent air drying for 1 to 1.5 hours in the shade (Majeed *et al.,* 2017). Sowing the inoculated seeds continued in pots, each containing 10 kg of loamy soil and peat (2:1). Planting ten seeds in each pot had three replicates. Irrigation followed just after sowing. After a week of sowing, nutrient broth with all PGPMs proceeded to irrigate all pots (50 ml in each pot). The wheat plant thinning process ensued after two weeks, with six plants for each pot.

Data recorded

The data recording on the number of spikelets per spike, number of tillers per plant, 100-seed weight, and harvest index occurred at the physiological maturity of the wheat crop. The harvest index calculation used the following formula:

$$Harvest\ Index\ (HI) = \frac{Grain\ +yield}{Biological\ yield} \times 100$$

Grain analysis

The wheat crop harvesting emerged in the first half of May. Analysis of matured wheat grains for N, P, and K transpired. The crushed seeds provided 0.2 g from each treatment and received 4 ml of concentrated sulfuric acid, keeping it the next day until it turned black. After adding 1 ml of concentrated perchloric acid, its placement on a hot plate for half an hour completed the digestion process until the color of the solution became colorless, which is evidence of complete digestion, making the following estimates (Cresser and Parsons, 1979).

Estimation of macronutrients and proteins

The total nitrogen determination proceeded by distillation after adding sodium hydroxide (10 M) and using the Micro Kjeldahl device. The total phosphorus estimation continued by the modified ammonium molybdate method after adjusting the reaction degree of the used solution measurement by spectrophotometer. The total potassium estimation used a flame photometer. The protein's estimation employed the following formula:

$$protein = N \times factor\ 6.25$$

These laboratory tests followed the method of Haynes (1980) at the Directorate of Plant Protection, Ministry of Agriculture, Abu-Ghraib, Baghdad, Iraq.

Statistical analysis

The Statistical Analysis System-SAS (2018) program aided the analysis of all the data for various parameters using the analysis of variance (ANOVA) to detect the effect of different factors on the studied parameters. The Least significant difference ($LSD_{0.05}$) test helped compare and separate the varied means.

RESULT

Laboratory experiment

Microscopic characteristics

From soil sample number two, the obtained bacterial isolates grown on nutrient agar media (NA) showed the characteristics of the bacterial isolate as moist, flat, irregular, slightly convex colonies, and Gram-positive rods.

Molecular characterization by *16S rRNA* gene

By using the BLAST analysis, identical sequences of other microorganisms recovered from the GenBank attained a comparison with the sequence of this isolate (16S ribosomal RNA). The PCR bands showed that the replicated isolate had a 1500 bp (Figure 1). Using this band helped sequence the *16S rRNA* gene (Jebur and Auda, 2020). The newly

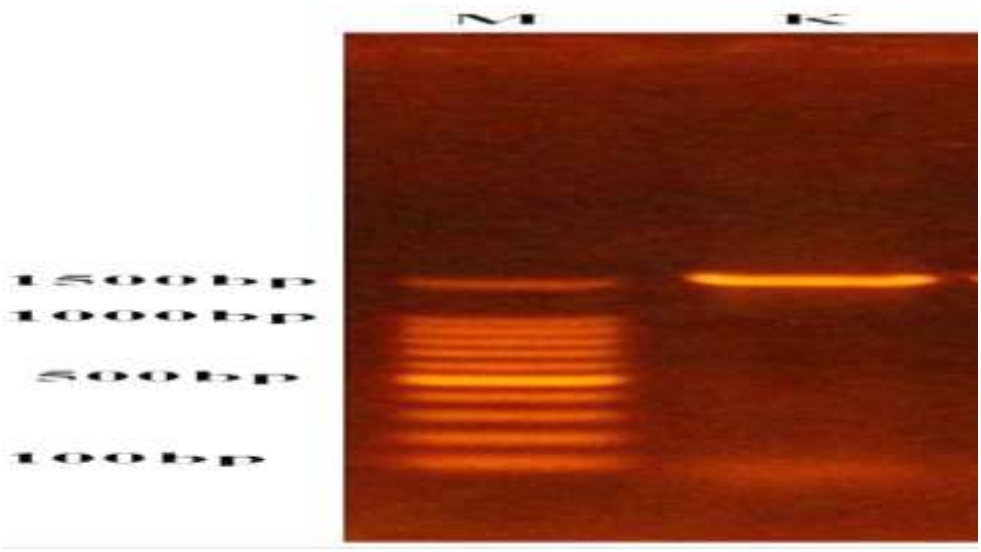

Figure 1. PCR banding profile of *16s rRNA* gene of *Lysinibacillus fusiformis.*

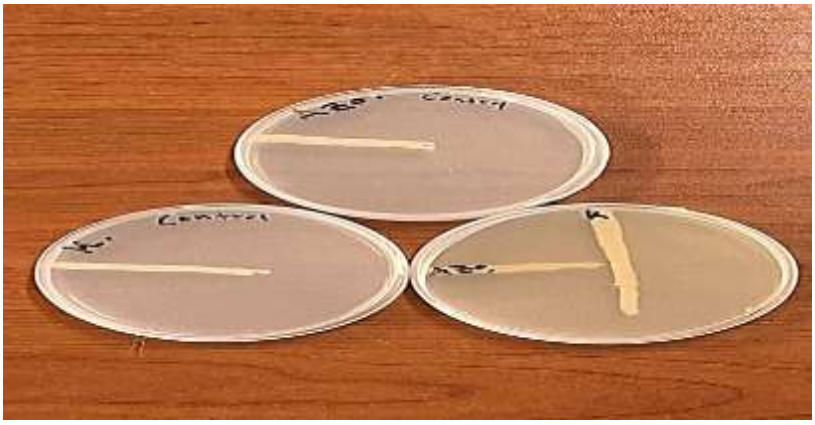

Figure 2. Interaction test between bacteria on nutrient agar (N.A) medium after inoculation at 28 °C for three days.

obtained *16S rRNA* fragment with known bacterial sequences in the GenBank database showed a sequence identical to nitrogenous bases with 100% *Lysinibacillus fusiformis* using the BLAST analysis. In the GenBank (NCBI), this sequence had a publication under the accession number OQ729826 *Lysinibacillus fusiformis* strain SHD.

Interaction test

The results indicated no antagonistic relationship between any examined bacteria on the NA medium and *Trichoderma harzianum* on the PDA medium. All the microorganism's development ensued regularly identical to how they did in the control treatment, without any inhibition zone between them (Figures 2 and 3). The results revealed that these microorganisms have an affinity and synergism with *Lysinibacillus fusiformis*, and the combination of these microorganisms was compatible. The presented results were analogous to past findings, which showed a synergistic relationship between the used microorganisms (Majeed *et al*., 2020).

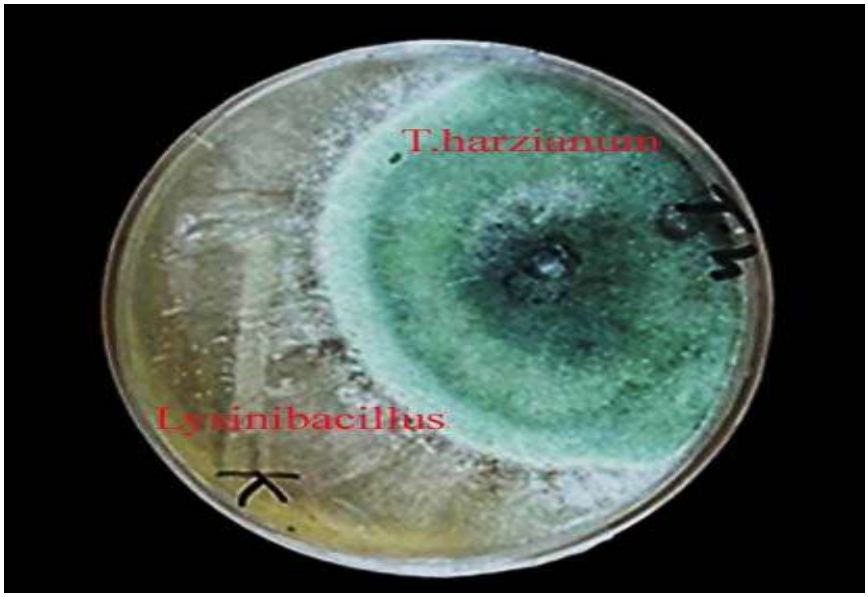

Figure 3. Interaction test between *Trichoderma harzianum* and *Lysinibacillus fusiformis* on PDA medium after inoculation at 26 °C for three days.

Biofertilizer effect on wheat

The findings showed that treatment T2, which combined several bacterial strains with the fungus *Trichoderma harzianum* and 25% mineral fertilizer, outperformed the other treatments (Tables 5 and 6). In the majority of evaluated characteristics, the treatment T2 proved to be superior. However, based on the biological yield, treatments T1 and T5 had the highest recorded values (61.70 and 62.30 g/plant, respectively). Additionally, no discernible difference between them emerged, and treatments T1 and T2 had the highest harvest index values (23.10% and 23.00%, respectively), with no discernible difference between them. Grains per spike with T5 increased significantly by 35.43 and 34.16 in T2, and spikelets per spike above T5 increased remarkably by 19.50 and 18.00 spikelets/spike), respectively.

The length of the spike and the harvest index showed a substantial rise above T6, and the number of tillers/plants (T1, T2, T4, and T5) grew significantly above T3 and T6 without substantially differing between them. Notable increases were evident with T1, T2, T3, and T4 (3.26, 3.50, 3.23, and 3.30 g, respectively) (without significant differences between both). When comparing T6 (2.56 g) with T5 (3.10 g) for weight, no discernible changes appeared for the two. On the grain number and spike, T1 and T2 (34.93 and 35.43, respectively) showed considerable dominance (with no significant differences between them) over T3, T4, T5, and T6 (31.16, 31.66, 34.16, and 27.43, correspondingly). Meanwhile, T3 and T6 recorded the lowest value compared with other treatments; T2 and T5 highly outperformed all other treatments for biological yield, with no remarkable differences between these. When using biofertilizer instead of mineral fertilizer, Table 7 shows a demonstrated increase in nutrient viability (NPK).

Compared with other treatments (T1, T3, T4, T5, and T6), T2 demonstrated a statistically significant advantage for the grain nitrogen, potassium, and protein contents. Except for T5, which did not record meaningful differences with T2, T5 (1.930%) showed a considerable rise over T1 (1.806%) in the grain phosphorus concentration. T2 also exhibited significant superiority over other treatments in this regard. For nitrogen, potassium, phosphorus, and protein concentrations in the grain, every treatment showed a notable advantage above T6.

Table 5. Treatments used in the study.

Treatments	Details of microorganisms + mineral fertilizer
T1	*Rhizobium ciceri CP-93* + *Azospirillum brasilense* + *Trichoderma harzianum.* + *Pseudomonas fluorescens* + *Bacillus megaterium* + 25% chemical fertilizer
T2	*Rhizobium ciceri CP-93* + *Azospirillum brasilense* + *Trichoderma harzianum.* +*Pseudomonas fluorescens* + *Bacillus megaterium* + *Lysinibacillus fusiformis* + 25% chemical fertilizer
T3	*Rhizobium ciceri CP-93* + *Azospirillum brasilense* + *Trichoderma harzianum.* + *Pseudomonas fluorescens* + *Bacillus megaterium*
T4	*Rhizobium ciceri CP-93* + *Azospirillum brasilense* + *Trichoderma harzianum* + *Pseudomonas fluorescens* + *Bacillus megaterium* + *Lysinibacillus fusiformis*
T5	Seeds + 100% chemical fertilizer control (standard amount of 100% chemical fertilizer 0.05 DAP + 0.075 Urea typical to 10 kg soil)
T6	Seeds + 25% chemical fertilizer control (standard amount of 25% chemical fertilizer 0.0125 Dap + 0.01875 Urea typical to 10 kg soil)

Table 6. Effects of biofertilizer on the growth and yield parameters of wheat.

Treatments	Tillers plant^{-1}	Spike length (cm)	Spikelets spike^{-1}	100-grain weight (g)	Grains spike^{-1}	Harvest index (%)	Biological yield (g plant^{-1})
T1	3.54	10.33	19.33	3.26	34.93	23.10	60.00
T2	4.00	10.50	19.50	3.50	35.43	23.00	61.70
T3	2.66	9.66	17.50	3.23	31.16	21.66	39.00
T4	3.50	10.33	19.00	3.30	31.66	21.00	53.30
T5	3.83	9.83	18.00	3.10	34.16	22.51	62.30
T6	2.17	6.67	10.00	2.56	27.43	19.80	39.00
$LSD_{0.05}$	0.532	1.18	1.48	0.607	0.944	1.775	1.32

Table 7. Effects of biofertilizer on the nutrient content of wheat grains.

Treatments	N (%)	P (%)	K (%)	Protein (%)
T1	4.120	1.806	3.197	25.74
T2	4.870	1.943	4.156	30.43
T3	3.696	1.640	2.663	23.10
T4	3.880	1.740	2.876	24.25
T5	4.070	1.930	3.566	25.44
T6	2.716	1.526	1.946	16.97
$LSD_{0.05}$	0.122	0.052	0.213	0.767

DISCUSSION

Biofertilizers have essential roles in improving the soil structure and fertility to restore the soil's natural conditions, building the soil's organic matter and nutrient cycle. Biofertilizers protect against abiotic stress, such as drought and salinity (Itelima *et al*., 2018). Applying PGPM can promote plant growth and development and enhance grain yield by fixing atmospheric nitrogen, potassium, and phosphate solubilization and producing siderophore compounds. The reimbursement from these bacteria can include increased nutrient availability, phytohormone production, shoot and root development, protection against several phytopathogens, and reduced diseases (De-Andrade *et al*., 2023). In alkaline soil, PGPMs can make available the nutrients for crop plants, such as in the soils of Iraq, where biofertilizers are essential because the soil particles contain complex mineral fertilizer residues unavailable to plants due to their complexity. Biofertilizers can reduce the pH and break down soil-held elemental particles into soluble forms, causing easy absorption by crop plants (Majeed *et al*., 2023).

The results enunciated that the biofertilizer treatment T2 containing *Lysinibacillus* above other treatments performed better for most studied traits. *Lysinibacillus* plays a synergistic role with other microorganisms, which helps the treatment perform better. *Lysinibacillus* with *Azospirillum* can fix the nitrogen. Shabanamol *et al.* (2018) showed that nitrogen fixation by *Lysinibacillus* resulted in a catalytic action of a complex enzyme system known as nitrogenase encoded by Nif genes. *Lysinibacillus* spp. has the Nif genes and produces nitrogenases. *Pseudomonas fluorescens*, *Lysinibacillus,* and *Azospirillum* can solubilize the insoluble phosphate in the soil, such as HPO_4 and H_2PO_4, into the soluble form for crop plants because these PGPM can secret organic acid, lowering the soil pH and causing the dissolution of bound form of phosphate like Ca_3 $(PO_4)_2$ in calcareous soil rendering them available to plants (Kaur *et al*., 2016). Several *Lysinibacillus* strains can solubilize other crucial insoluble minerals, such as potassium, iron, zinc, and silicate (Naureen *et al*., 2017). These PGPM microorganisms are vital in producing phytohormones, such as cytokinins, auxins, and gibberellins, inhibiting ethylene production. Auxin (IAA indol-3-acetic acid) can also stimulate cell elongation, expansion, and differentiation (Lopes *et al*., 2021).

Several *Lysinibacillus* can also synthesize indole-3-acetic acid (IAA), gibberellins, and cytokinins (Duo *et al*., 2018). Moreover, *Bacillus* sp., *Pseudomonas* sp*., Rhizobium* sp*.,* and *Azospirillum* sp. could produce gibberellins and cytokinins. The gibberellin promotes stem tissue development and root elongation, stimulating the lateral root extension, while the cytokinin controls root development and promotes cell division in crop plants (Kaur *et al*., 2016). Some PGPMs, such as *Bacillus* sp., *Pseudomonas* sp., and *Azospirillum* sp., produced ACC-deaminase (1.Aminocyclopropane – 1 –carboxylate) (Danish *et al*., 2020; Khoshru *et al*., 2020). The ACC deaminase enzyme reduces ethylene levels, alleviates stress in plants and high ethylene levels that may cause toxic effects to plants, i.e., leaf chlorosis, necrosis, senescence, reduction in fruit yield, root development, and leaf expansion (Lopes *et al*., 2021). The ACC deaminase splits ethylene into ammonia and α-ketobutyrate, which helps reduce the ethylene level (Ferreira *et al.*, 2019).

Azospirillum, *Bacillus*, *Pseudomonas*, *Trichoderma,* and *Lysinibacillus* produce siderophores to obtain iron from the environment (Passera *et al*., 2020; Poveda and Eugui 2022). The Fe^{+3}, also known as ferric ion, is the most common form of iron found in nature, and neither bacteria nor plants can readily absorb this form. In the iron-limited condition, PGPM secretes siderophores to supply iron to crop plants. Additionally, the siderophore compounds compete with PGPMs, allowing it to colonize the roots and remove additional microbes like phytopathogens from the ecological niche, by absorbing and accumulating Fe^{+3} in the surrounding roots, preventing the pathogen from obtaining iron (Kaur *et al.*, 2016).

Biofertilizers also significantly affect the grain content, including nitrogen, phosphorus, and potassium, due to the ability of microorganisms to secrete hormones and growth regulators. The increased NPK concentration in the plant's transition to grains (from the source to the downstream grain) promotes the successful development of grains (Farhood *et al*., 2022). Al-Juthery *et al.* (2020) showed that using nano-biological fertilizers revealed the highest significant content of NPK in wheat grains and straw. Mohamed *et al.* (2019) also stated that compost with biofertilizers better promotes yield components and nutrient content of the wheat grains than with mineral fertilizers.

The results were also in harmony with past findings as they observed that inoculation with a mixture of PGPMs to wheat seeds increased the 1000-seed weight, spikes m^{-2}, spikelets $spike^{-1}$, grain yield, and harvest index in wheat due to synergistic effects of PGPM (Majeed *et al*., 2017, 2020). Al-Shamma *et al*. (2020) found using microorganisms (*Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma harzianum*, *Verticillium* sp., and *Paecilomyces lilacinus*) reduced the disease incidence of seed gall disease in wheat crops. Moreover, the results showed that PGPMs

increased the flag leaf area and plant height in all the treatments with a significant difference compared with the control. Although, it had the highest values for spike weight, biological, and grain yields.

Adding biofertilizer (*Pseudomonas fluorescens* and *Pseudomonas putida*) to wheat crops significantly affected supplementing with mineral fertilizer in all the studied parameters (Abd *et al*., 2016). The presented results were analogous to the findings of Sheirdil *et al*. (2019), who concluded that using PGPM mixtures (*Pseudomonas* spp. and *Bacillus* spp.) with half-recommended chemical fertilizers could promote the wheat's root and shoot lengths and fresh and dry weights, increasing the grain yield under field and pot conditions. Akinrinlola (2018) used *Lysinibacillus fusiformis* as a plant growth-promoting agent in wheat, increasing plant biomass. Sule *et al*. (2020) concluded that *Lysinibacillus sphaericus* and *Aspergillus niger* can solubilize fixed phosphate in the soil, making the nutrient more available for the growth of maize plants and enhancing its growth. Findings from Sodiq *et al*. (2022) showed that applying biofertilizer (*Bacillus cereus* and *Lysinibacillus* sp.) with 75% of chemical fertilizer positively impacted paprika plants, increasing the phosphorus and potassium nutrient contents.

CONCLUSIONS

Using PGPM as a biofertilizer in different formulas and adding *Lysinibacillus fusiformis* combined with 25% mineral fertilizer is crucial in enhancing wheat productivity. Biofertilizers remarkably influence increasing grains per spike, spikelets per spike, 100-seed weight, biological yield, and harvest index.

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