International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(RESEARCH ARTICLE)

EFFECT OF BIOFERTILIZATION WITH PLANT GROWTH-PROMOTING RHIZOBACILLUS (PGPR) ON GROWTH AND PRODUCTIVITY OF MAIZE (*ZEA MAYS* L.) COMPARED TO CHEMICAL FERTILIZATION

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International Journal of Biological and Pharmaceutical Sciences Archive, 2026, 12(01), 111–117

Publication history: Received on 03 July 2026; revised on 15 August 2026; accepted on 17 August 2026

Article DOI: https://doi.org/10.53771/ijbpsa.2026.12.1.0077

ABSTRACT

Background: Maize (*Zea mays* L.) is one of the world's most important cereal crops, and improving its productivity while reducing dependence on chemical fertilizers has become a major goal of sustainable agriculture. The potential role of plant growth promoting rhizobacteria (PGPR) as a biofertilizer evolved as appropriate substitute to neutralize adverse environmental impacts wielded by manmade agrochemical.

Objective: This study aimed to evaluate the effects of Pseudomonas fluorescens and Bacillus subtilis, individually and in combination, on the growth and yield of maize compared with conventional NPK fertilization.

Methods: A field experiment was conducted during the 2025 growing season at the Field Crops Research Station, College of Agriculture, University of Samarra, using a Randomized Complete Block Design (RCBD) with three replicates. Six treatments were evaluated: Untreated control (T1), Pseudomonas fluorescens (T2), Bacillus subtilis (T3), combined inoculation (P. fluorescens + B. subtilis) (T4), combined inoculation with NPK fertilizer (T5), and NPK fertilizer (20:20:20) only (T6). Vegetative growth and yield-related traits were recorded and statistically analyzed.

Results: Inoculation of plants with PGPR bacteria resulted in a significant improvement in both vegetative growth and yield compared to the untreated control group. Pseudomonas fluorescens (T2) exhibited the highest vegetative growth rate, recording the highest plant height (148.00 cm), leaf area (365.00 cm^2), leaf area index (2.63), and number of grains per spike (688 grains) compared control group recorded (92.33 cm), (10.67 $plant^{-1}$), (151.73 cm^2), (0.70) respectively. Bacillus subtilis (T3), achieved the highest productivity, producing the largest number of spikes per plant (2.67 spikes) and the highest spike weight (283.50 g) compared control group recorded (2.00) and (161.60 g).

Conclusion: The use of PGPR, and especially Pseudomonas fluorescens and Bacillus subtilis as potential biofertilisation agents is a promising sustainable alternative to chemical fertilisation that can enhance maize growth and production, while decreasing dependence on mineral fertilisers.

Keywords: *Zea Mays*, PGPR, *Pseudomonas fluorescens, Bacillus subtilis*

1 INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops globally, ranking third in economic importance after wheat and rice[1]. The area cultivated with it worldwide reached approximately 197.2 million hectares, with a production of nearly 1,148.5 million tons, according to FAO statistics (2021). In Iraq, the cultivated area reached 40,543 hectares, with a production of 419,300 tons. This crop is of particular importance due to its multiple uses in human nutrition, animal feed production, and other applications[2]. With the increasing global demand for food due to rapid population growth, it has become essential to adopt sustainable agricultural strategies that contribute to increased productivity without harming the environment. The intensive use of chemical fertilizers has led to numerous environmental problems, such as soil degradation, reduced biodiversity, and increased pollution, necessitating the search for environmentally friendly alternatives[3, 4].

In this context, the importance of beneficial microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), has emerged as a sustainable biological solution for improving plant growth and increasing productivity[5]. These bacteria promote plant growth directly by fixing atmospheric nitrogen and increasing the availability of nutrients such as phosphorus, or indirectly by inhibiting pathogens and producing growth-regulating biochemicals such as auxins, cytokinins, and gibberellins [6].

Among the important species of these bacteria is *Bacillus subtilis*, which is characterized by its ability to solubilize unavailable phosphates, produce the enzyme urease, induce systemic resistance, and produce defensive compounds such as phytoalexins [7]. It is characterized by its ability to form spores, which enables it to survive in harsh environmental conditions such as drought and high salinity[8]. Also, *Bacillus subtilis* plays an important role in improving soil fertility by increasing the solubility and availability of mineral phosphorus to the plant, as well as contributing to atmospheric nitrogen fixation, which leads to increased dry matter accumulation and improved yield components in maize[9].

Furthermore, some strains of *Pseudomonas fluorescens* produce antibiotic compounds such as 2,4-Diacetylphloroglucinol (DAPG), which contribute to controlling root pathogens[10].

Pseudomonas fluorescens is one of the most efficient bacteria for improving root growth in maize, as it contributes to increased water and nutrient uptake through the secretion of natural plant growth regulators, which positively impacts plant vitality and productivity[10, 11]. Accordingly, our current investigation was conducted to assess the efficiency of biofertilization as compared with balanced chemical fertilization (N.P.K) (20:20:20) in terms of growth and yield parameters for maize (*Zea mays* L.) using two strains of plant growth-promoting rhizobacteria (PGPR), *Pseudomonas fluorescens* and *Bacillus subtilis*. This study also focused on accommodating the possibility of decreasing reliance on mineral fertilizer during crop development and yield through these specific biofertilizers.

2 MATERIAL AND METHODS

2.1 Experimental site and Design

The field experiment was conducted at the Field Crops Research Station, College of Agriculture, University of Samarra, during the 2025 growing season. The trial was established on August 5, 2025, after land preparation and the completion of the recommended agronomic practices, including plowing, leveling, and dividing the field into experimental units for maize (*Zea mays* L.) cultivation. The physical and chemical properties of the experimental soil before planting are presented in Table 1. The experiment was arranged in a Randomized Complete Block Design (RCBD) with six treatments and three replicates, giving a total of 18 experimental units. The treatments were designed to evaluate the effect of biofertilization using plant growth-promoting rhizobacteria (PGPR) in comparison with conventional chemical fertilization, as follows:

- **T1 (Control):** No application (comparison treatment).
- **T2:** Inoculation with *Pseudomonas fluorescens*.
- **T3:** Inoculation with *Bacillus subtilis.*
- **T4:** Combined inoculation with *Pseudomonas fluorescens* and *Bacillus subtilis*.
- **T5:** Co-inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis*, with the addition of a neutral fertilizer (NPK 20:20:20).
- **T6:** The addition of a neutral chemical fertilizer (NPK 20:20:20) only.

Table 1: Selected chemical composition of the study soil before planting

No.	Property	Value	Unit	Acceptable Limits
1	Sand	44.5	%	—
2	Silt	45.42	%	—
3	Clay	10.07	%	—
4	Soil Texture (Loam)	---	---	---
5	Field Capacity	28.4	%	—
6	Organic Matter	0.75	%	> 1.2
7	Electrical Conductivity (EC)	2.19	dS/m	> 5
8	Soil pH	7.4	—	—
9	Bulk Density	1.23	g/cm^3	—
10	Permanent Wilting Point	9.5	%	—
11	Gypsum	17.5	%	—
12	Nitrogen (N)	12.2	mg/kg	> 20
13	Phosphorus (P)	4.91	mg/kg	8–15
14	Potassium (K)	102	mg/kg	> 50
15	Calcium Carbonate ($CaCO_3$)	16	—	—
16	Sodium (Na)	—	—	—

2.2 Studied characteristics

2.2.1 Plant height (cm)

The plant height was measured from the point of contact with the ground to the end of the spike, excluding the awns, using a graduated ruler. It was measured at the end of the season for ten randomly selected plants, and then their average was taken[12].

2.2.2 Stem diameter

Stem diameter was measured in three replicates using a digital micrometer. The measurement was taken at a height of 50 cm from the soil surface, using two perpendicular planes to minimize the error of non-circular shape. The average stem diameter for each replicate was then calculated by averaging the values of the selected plants[13].

2.2.3 Number of leaves(leaf)

The number of leaves was measured by counting the complete leaves of a number of randomly selected plants from each treatment, and then calculating the average number of leaves per plant[14].

2.2.4 Leaf Area (cm^2)

The leaf area at 100% flowering was calculated for five plants randomly selected from the rows, and its average was calculated using the following equation [14].

Leaf Area = (Length of leaf below main ear leaf)^2 x 0.75

2.2.5 Leaf Area Index

The leaf area index was calculated using the following equation [15].

Leaf Area Index = Leaf Area of Plant (cm^2) / Area of Land Occupied by Plant (cm^2)

2.2.6 Grain Yield Characteristics

Five plants were randomly selected from the middle rows of each experimental unit after full maturity, and the following were calculated:

2.2.7 Number of Ears

The number of ears was manually counted, and its average was calculated.

2.2.8 Number of Grains per Ear (grain/ear – 1)

The number of grains per ear was manually counted, and its average was calculated.

2.3 Statistical analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference factors (Factorial experiment-ANOVA one way) in study parameters. Least significant difference-LSD test was used to significant compare between means in this study.

3 RESULTS

3.1 Vegetative Growth Parameters

Table (2) shows the vegetative growth indicators of under the influence of different treatments (T1-T6). The results showed significant differences with the different fertilization treatments ($P \geq 0.05$). Inoculation with plant growth-promoting rhizobia (PGPR) enhanced plant growth compared to control. Among all treatments, T2 produced the best vegetative performance, recording the highest values for plant height (148.00 cm), number of leaves (12.67 leaves per plant^{-1}), leaf area (365.00 cm^2 per plant^{-1}), and leaf area index (2.63). Treatment T3 came in second and showed similar values for most vegetative traits. In contrast, the control treatment consistently recorded the lowest values for all growth parameters. Treatments T4, T5, and T6 resulted in moderate responses.

Table 2: Effect of PGPR biofertilization and chemical fertilization on vegetative growth parameters of *Zea mays* L.

Treatment	Plant height (cm)	Number of leaves (plant^{-1})	Leaf area (cm^2 plant^{-1})	Leaf area index (LAI)
T1	92.33 C	10.67 B	151.73 D	0.6967
T2	148.00 A	12.67 A	365.00 A	2.6300 A
T3	128.67 B	11.33 AB	345.73 AB	2.2367 AB
T4	101.67 C	10.00 B	200.50 CD	1.1533 CD
T5	116.67 BC	10.33 B	267.73 BC	1.6000 BC
T6	97.00 C	10.33 B	230.73 CD	1.3893 CD

3.2 Yield characteristics

Table (3) shows the effect of different fertilization treatments (T1-T6) on the yield characteristics of *Zea mays* L. The results showed statistically significant differences ($P \leq 0.05$) between the treatments. The highest yield performance was recorded in plants treated with PGPR bacteria, especially in treatment T3, which recorded the highest spike weight (283.50 g). Treatment T2 produced the highest number of grains per spike (688 grains/spikes) compared to the control sample. Treatments T4 and T5 showed relatively low performance despite bacterial inoculation. The number of spikes per plant also increased significantly in treatment T3, indicating a positive response to bacterial inoculation. Grain weight showed only a slight difference between the treatments, except for treatment T4, which recorded a significantly lower value. These results indicate that inoculation with PGPR bacteria was more effective than chemical fertilization alone in improving maize productivity.

Table 3: Effect of PGPR biofertilization and chemical fertilization on yield and yield components of *Zea mays* L.

Treatment	Number of ears ($plant^{-1}$)	Ear weight (g)	Grain weight (g)	Number of grains ear^{-1}
T1	2.00 AB	161.60 D	0.4800 A	451.33 D
T2	2.33AB	272.00 B	0.4000 A	688.00 A
T3	2.67A	283.50 A	0.4400 A	532.33 B
T4	1.33C	164.90 C	0.2000 B	528.00 C
T5	1.67 BC	128.30 F	0.4900 A	252.67 F
T6	2.00 AB	153.00 E	0.4400 A	312.67 E

4 DISCUSSION

Vegetative growth attributes are regarded as one of the most important traits of crop performance, as they directly affect photosynthesis, nutrient acquisition efficiency and consequently productivity in maize. Plant height, number of leaves, leaf area and leaf area index combine to represent the plant's ability to make efficient use of the resources that are available during veg. In light of this, improvement in these parameters usually leads to more dry matter accumulation and grain yield at maturity[16].

The present study showed that PGPR inoculation significantly improved all vegetative growth parameters compared to the non-inoculated control. Among the tested treatments, *Pseudomonas fluorescens* (T2) produced the greatest improvement in plant height, number of leaves, leaf area, and leaf area index, followed by *Bacillus subtilis* (T3), in that order. These improvement in plant growth and health can be mainly ascribed to the diverse modes of actions of PGPR. The two types of bacteria can colonize the rhizosphere and facilitate plant nutrient uptake through phosphate solubilization, biological nitrogen fixation, siderophore production, and phytohormone synthesis (especially indole-3-acetic acid [IAA] that leads to root elongation and branching). The greater the vegetative elaboration therefore is accompanied by a more developed root system which increases water and mineral uptake[17]. In addition, *P. fluorescens* bacteria suppress many soil-borne pathogens by producing antimicrobial compounds and stimulating systemic resistance, allowing plants to allocate more resources to vegetative growth rather than defensive responses.

Bacillus subtilis significantly improved vegetative growth compared to the control group. This bacterium is a plant growth promoter, capable of secreting plant hormones, extracellular enzymes, and phosphate-soluble compounds while forming highly resistant endospores that enable it to survive for extended periods in the soil. These properties improve the nutrient availability environment around the root zone and enhance root structure in response to growth, as evidenced by the marked improvement in plant height and leaf count[18]. Previous studies, Nascimento et al., [19] and Khan *et al*., [20] have reported that *Bacillus* species are particularly efficient in increasing phosphorus uptake under nutrient-deficient soil conditions

The current findings are consistent with those of Choudhry *et al***.** [18], who demonstrated that inoculation of maize with Bacillus subtilis significantly increases plant height, aerial mass, and overall vegetative growth under field conditions. Similarly, Martins *et al*. [21] reported that inoculation with Pseudomonas fluorescein improves phosphorus utilization efficiency and promotes maize growth by enhancing nutrient uptake and root zone activity.

Yield components are considered of the vegetative growth stage physiological process is yield components. Thus, enhancing ear weight, grain number and grain yield indicates efficient nutrient uptake, photosynthesis and assimilate flow from source to developing grains. PGPR inoculation significantly improved maize productivity compared with the untreated control,. These benefits are largely related to improved nutrient availability, high phosphorus agronomic efficiency and the production of phytohormone along with rooting activity that affect assimilate accumulation in this period.

The superior performance of *Bacillus subtilis* (T3) in ear weight and number of ears per plant may be attributed to its high ability to increase phosphorus availability during the reproductive stage. Phosphorus is essential for ATP production, carbohydrate transport, and grain filling; consequently, improved phosphorus nutrition enhances assimilate partitioning toward developing ears rather than vegetative tissues[22]. In addition, Bacillus spp. produce several metabolites that stimulate root activity and improve nutrient-use efficiency during reproductive growth, resulting in better kernel development and increased yield. These findings agree with Backer et al. [23], who reported that *Bacillus* species improve nutrient acquisition and crop productivity through enhanced phosphorus solubilization and improved plant nutritional status. Although *Pseudomonas fluorescens* (T2) produced the highest vegetative growth, it did not consistently record the highest values for all yield components. This indicates that vigorous vegetative growth does not always lead to maximum grain yield due to the necessity for efficient remobilization of stored nutrients and the availability of phosphorus during the grain-filling period, rather than only biomass accumulation during vegetative growth. Similar observations were reported for Lopes et al. [24] observed that *P. fluorescens* resulted in relatively less improvement of the maize yield as compared with *Bacillus subtilis.*

The results of the current study indicate that the application of composite bacteria T4 (Combined *Pseudomonas fluorescens* and *Bacillus subtilis*), T5 (Co-inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + (NPK 20:20:20)) did not outperform the individual inoculations. This observation may indicate that simultaneous inoculation with two bacterial strains does not necessarily result in synergistic effects. This can be explained by the fact that competing bacterial populations for root secretions/colonization sites/or other available nutrients reduce the stability efficiency of one or both strains, thus decreasing their overall ability to promote plant growth [25]. The results of the current study are consistent with what Lopes *et al*. [24] reported, that excessive mineral fertilization may inhibit many of the beneficial functions of root bacteria by reducing the plant's reliance on microbial absorption of nutrients. The results of the current study disagreement with studies [26] that indicated that co-fertilization of biofertilizers with chemical fertilizers increased soil fertility around the roots, resulting in more efficient absorption of nutrients from the soil by plants and thus increased vegetative characteristics and productivity in maize plants.

5 CONCLUSION

The results of this study showed that biofertilization using plant growth-stimulating root bacteria (PGPR) was effective in improving maize yield and grain yield compared to the untreated control and chemical fertilization alone. *Pseudomonas fluorescens* proved more effective in promoting vegetative growth, while *Bacillus subtilis* outperformed several yield parameters, especially ear count and weight. PGPR inoculation is an economically viable and behaviorally friendly option that would partially eliminate the need for chemical fertilizers while maintaining or even increasing corn productivity.

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