Evaluation of local commercial Bradyrhizobium inoculum and phosphorus in improving mung bean yield and its components in Gypsipherous soil

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Abstract

Root nodule bacteria are exploited to produce natural biofertilizers to achieve clean agriculture by reducing the use of chemical fertilizers .The efficacy of the formulated inoculum was evaluated by comparison with a commercial inoculum to study yield-related traits of mung bean grown in gypsiferous soil under four phosphorus fertilization levels: 0, 40, 80, and 120 kg P ha^{-1}.The results showed a significant increase in all yield parameters in treatments inoculated with either the local or commercial *Bradyrhizobium japonicum* inoculate compared to the uninoculated control. Inoculated treatments were significantly superior to the uninoculated ones. Moreover, the treatment inoculated with the local *Bradyrhizobium japonicum* isolate significantly outperformed the treatment with the commercial inoculum in terms of pod number, recording averages of 10.37, 12.64, and 18.47 pods $plant^{-1}$ for the uninoculated, commercial inoculated, and locally inoculated treatments, respectively .These treatments also recorded total seed yields of 1.890, 2.229, and 2.782 t ha^{-1}, respectively. The corresponding nitrogen, phosphorus, and protein contents in the seeds were 1.524%, 0.148%, and 12.701% for the uninoculated control; 2.438%, 0.186%, and 15.237% for the commercial inoculum; and 3.009%, 0.226%, and 18.806% for the local inoculum treatment, respectively. Furthermore, the treatment fertilized with 80 kg P ha^{-1} significantly outperformed both the unfertilized treatment and the treatment receiving 40 kg P ha^{-1} in all studied parameters. A significant interaction effect was also observed between inoculation and phosphorus fertilization. The treatment combining inoculation with Bradyrhizobium japonicum and fertilization at 80 kg P ha^{-1} showed a significant superiority over all other treatments in all measured traits.

Keywords: *Bradyrhizobium japonicum*, inoculum, mung bean, Carrier

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Introduction

Gypsiferous soils negatively affect the growth and productivity of mung bean (*Vigna radiata* L.) due to their poor physical, chemical, and biological properties. These soils are characterized by weak structure and the formation of hard surface crusts, which reduce soil aeration and hinder root proliferation. In addition, their low water-holding capacity further exacerbates growth limitations, resulting in reduced vegetative growth indicators such as plant height, number of branches, and biomass, as well as decreased yield-related traits such as pod number, seed number, and total seed yield. [1] Mung bean (*Vigna radiata* L.) is a leguminous crop of high nutritional value widely grown in many Asian and African countries. It is rich in high-quality plant protein (20–30%), essential amino acids, vitamins, and minerals that contribute to overall human health. Beyond its nutritional value, mung bean plays an important ecological role through its ability to fix atmospheric nitrogen in symbiosis with Bradyrhizobium spp., thereby enhancing soil fertility and reducing the need for chemical nitrogen fertilizers. Recent studies have also shown that mung bean cultivars can tolerate drought and heat stress, making the crop a promising candidate for agricultural expansion in dry and semi-arid environments affected by climate change. [2] Although the excessive use of chemical fertilizers has led to short-term improvements in agricultural productivity, overdependence on nitrogenous and phosphate fertilizers has raised serious environmental and sustainability concerns. Prolonged reliance on such inputs results in soil fertility degradation, decreased microbial activity, and accumulation of harmful salts, which ultimately impairs nutrient uptake efficiency and disturbs soil nutrient balance. Moreover, a large proportion of applied fertilizers is not absorbed by plants but instead leaches into groundwater and surface water, contributing to water pollution and eutrophication [3]. Biofertilizers, including Rhizobium spp., are considered key components of sustainable agriculture. These nitrogen-fixing bacteria effectively contribute to biological nitrogen fixation in leguminous plants, reducing the dependency on synthetic nitrogen fertilizers. In addition, Rhizobium enhances plant growth by improving nutrient uptake, producing phytohormones, and enhancing soil structure through increased organic matter content and microbial diversity [4]. Phosphorus is an essential macronutrient for plants, involved in energy transfer (e.g., ATP), nucleic acid synthesis, root development, and flowering. Although total phosphorus may be abundant in the soil, much of it exists in insoluble forms that are not available to plants. Phosphorus fertilization is therefore required to improve its availability. Recent studies have demonstrated that balanced phosphorus supply enhances drought and heat tolerance, regulates stomatal function, and improves plant physiological responses related to growth and yield under harsh environmental conditions [5].The combined application of phosphate fertilization and Rhizobium inoculation is one of the most effective agronomic strategies for improving mung bean growth and productivity. Phosphorus activates vital physiological processes, particularly those related to root and nodule development, while Rhizobium contributes to atmospheric nitrogen fixation, reducing the need for synthetic nitrogen fertilizers. Recent findings have shown that this integrated approach improves nodule efficiency, vegetative growth, flowering, and yield parameters such as pod and seed number, resulting in significantly higher total yields compared to using either input alone [6].

Due to the lack of studies, especially in gypsum soils, and the importance of applying the interaction between Rhizobium bacteria and phosphate fertilization in improving the growth of legumes, this study was conducted for the purpose of:

Material and Methods

Selection of Bacterial Strains:

The effective and partially molecularly characterized isolate Is-27 of *Bradyrhizobium japonicum* was selected for biofertilizer preparation, based on its ability to solubilize phosphate and produce siderophores and iron-chelating compounds, as shown in Table (1).

Table 1. Efficiency of bacterial isolates in phosphate solubilization, indole-3-acetic acid (IAA) production, and siderophore formation

Iron chelating	Indole production (μg/mL-1)	Phosphate solubilization (mg/L-1)	Isolate
+++	22.6	37.311	Is-27 (Bradyrhizobium japonicum)

Preparation Steps for Bacterial Inoculum:

Cultivation of Rhizobia Bacteria

The pure isolate Is-27 of *Bradyrhizobium japonicum* was cultured by transferring a loopful from a 24-hour-old culture into flasks containing an appropriate nutrient medium. The bacterial cell count was estimated using the serial dilution and plate count method. Specifically, 1 mL from each flask was serially diluted and plated on selective media specific to the bacterial isolate. Three replicates were prepared for each dilution. The plates were incubated at 28 °C for 48–72 hours. Bacterial colony numbers were calculated by multiplying the number of colonies by the reciprocal of the dilution factor.

The Preparation of the Carrier

Peat moss, prepared according to the specifications of the Food and Agriculture Organization (FAO), was used as a carrier material for the preparation of the bacterial inoculum. It was sieved using a 250-micron mesh sieve, then evenly distributed into heat-resistant polyethylene plastic bags, each containing 250 grams. The pH of the carrier material was adjusted to 7, and moisture content was brought to 55–60% by adding sterile water. The carrier was sterilized in an autoclave at 121 °C and 1.5 bar pressure for 30 minutes. The sterilization cycle was repeated three times to ensure complete decontamination.After sterilization, the bags were allowed to cool and were tightly sealed before removal from the autoclave. To verify the effectiveness of the sterilization process and confirm the absence of microbial contamination, the carrier material was subjected to microbial testing using the serial dilution plate count method [7].

Mixing the Inoculant with the Carrier

The bacterial inoculum was prepared based on a formulation in which 100 grams of the final product consisted of 45 grams of sterilized peat moss mixed with 5 grams of calcium carbonate to adjust the pH, followed by the addition of 20 mL of tap water. The mixture was transferred into sterile polyethylene bags, to which 30 mL of an active bacterial culture was added.The inoculated carrier material was incubated at 30 °C for seven days with daily shaking in multiple directions to ensure uniform bacterial distribution within the carrier. The bacterial population in the carrier was estimated prior to seed inoculation using the serial dilution and plate count technique. Specifically, 20 grams of the inoculated carrier were suspended in 180 mL of sterile water under aseptic conditions and shaken for 30 minutes. A series of serial dilutions was then performed, and 0.1 mL of the 10^8 –10^9 dilution was plated onto YEMA medium specific for Rhizobium isolation. The plates were incubated at 28 °C for 3 to 5 days [7].

Preparing the Adhesive Solution

An adhesive solution was prepared using 40% gum arabic by dissolving 40 grams of the gum in 100 mL of sterile distilled water in 250 mL glass beakers, with continuous stirring during dissolution. The temperature was carefully maintained below 100 °C to prevent any alteration in the physical or chemical properties of the solution.Additionally, a 15% sucrose solution was prepared by dissolving 15 grams of the sugar in 100 mL of sterile distilled water, with the aim of enhancing the viability and efficiency of bacterial cells in the inoculum.The adhesive solution was then mixed with the peat-based inoculum at a ratio of 1:3 (i.e., 1 mL of adhesive per 3 grams of inoculum) to ensure better bacterial adhesion to the seed surface during inoculation [8].

Seed Sterilization and Inoculation

Mung bean seeds underwent surface sterilization to eliminate any microorganisms adhering to their surface. The seeds were first thoroughly washed under running tap water several times, followed by soaking in a 2% sodium hypochlorite solution for two minutes.Subsequently, the seeds were rinsed five to six times with sterile distilled water to ensure complete removal of any residual disinfectant. Seed inoculation was performed by mixing 100 grams of seeds with 4 grams of the biofertilizer inoculum.The inoculated seeds were then spread on clean, dry paper and air-dried in the shade, away from direct sunlight, to maintain bacterial viability and activity. The inoculation process was carried out precisely one hour before sowing to ensure maximum effectiveness of the inoculum upon seed placement in the soil [9].

Implementation experiment of the Field:

A factorial field experiment was conducted at the Soil and Water Resources Research Station, College of Agriculture, University of Tikrit.

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Table 2. Physical, chemical, and biological properties of the soil before planting

Value	Unit	Property
2.4	$dS \cdot m^{-1}$	Electrical Conductivity (EC)
7.6	–	pH
12.05	$cmol(+) \cdot kg^{-1}$	Cation Exchange Capacity (CEC)
0.7	$g \cdot kg^{-1}$	Organic Matter (O.M)
169	–	Gypsum ($CaSO_4$)
180	–	Lime ($CaCO_3$)
21	$mg \cdot kg^{-1}$	Available Nitrogen (N)
4.5	$mg \cdot kg^{-1}$	Available Phosphorus (P)
116	$mg \cdot kg^{-1}$	Available Potassium (K)
1.24	$g \cdot cm^{-3}$	Bulk Density (B.D)
544	$g \cdot kg^{-1}$ (soil)	Sand
220	$g \cdot kg^{-1}$ (soil)	Silt
236	$g \cdot kg^{-1}$ (soil)	Clay
Sandy Clay Loam	–	Soil Texture
106×10^6	$CFU \cdot g^{-1}$ (soil)	Total Bacterial Count

The experiment aimed to evaluate the effects of local and commercial Bradyrhizobium spp. inoculants and phosphorus fertilization on the yield and its components of mung bean grown in gypsiferous soil.A factorial field experiment was conducted using a randomized complete block design (RCBD) with two factors: Inoculation factor at three levels: uninoculated, inoculated with a commercial inoculant containing Bradyrhizobium japonicum, and inoculated with a locally prepared inoculant containing Bradyrhizobium japonicum.Phosphorus fertilization factor at four levels: 0, 40, 80, and 120 kg P ha^{-1}.Each treatment combination was replicated four times, resulting in a total of 48 experimental units.After plowing and leveling, the field was divided into four blocks, each containing 12 plots. Each plot measured 2 × 3 m^2, with 1-meter buffer spaces between blocks and between individual plots within each block.Prior to planting, the soil was disinfected using 2% formalin and covered with polyethylene sheets for three days.Seeds (both inoculated and uninoculated) were sown on August 3, 2023, in hills spaced 10 cm apart along five rows per plot, with 40 cm spacing between rows.Phosphorus was applied at the designated levels (0, 40, 80, and 120 kg P ha^{-1}) as a single dose before

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planting, using triple superphosphate (21% P).Additionally:Nitrogen was applied at a rate of 40 kg N ha^{-1} using urea fertilizer (46% N),Potassium was applied at a rate of 120 kg K ha^{-1} using potassium sulfate (43% K), both also applied as a single dose before planting [8].Irrigation was carried out using a drip irrigation system, with five lines per block connected to a mainline that was linked to a water pump to ensure adequate pressure and water flow.

The Studied Traits:

The following yield-related traits were measured and analyzed: number of pods per plant, pod weight, 100-seed weight, and total seed yield (grain yield). Additionally, the concentrations of nitrogen and phosphorus in the seeds were determined, along with the seed protein content, to evaluate the nutritional quality of the produced grains.

Results and Discussion

Number of pods:

Table 3 illustrates the effect of inoculation with local and commercial *Bradyrhizobium japonicum* inoculants, phosphorus fertilization, and their interaction on the number of pods per plant at the harvest stage of mung bean.The results showed that bacterial inoculation had a significant effect on pod number. Inoculated treatments were significantly superior to the uninoculated control. Furthermore, the treatment inoculated with the local inoculum significantly outperformed the commercial inoculum, recording 18.47 and 12.64 pods $plant^{-1}$, respectively, compared to 10.37 pods $plant^{-1}$ in the uninoculated treatment.As shown in Table 3, phosphorus fertilization also had a significant effect on pod number. Fertilized treatments were superior to the unfertilized control, and the 80 and 120 kg P ha^{-1} levels were significantly superior to the 40 kg P ha^{-1} level. The highest pod number was recorded at the 80 kg P ha^{-1} level, with an average of 16.87 pods $plant^{-1}$, which was significantly higher than the 15.47 pods $plant^{-1}$ recorded at the 120 kg P ha^{-1} level and the 10.21 pods $plant^{-1}$ in the unfertilized treatment.Regarding the interaction between bacterial inoculation and phosphorus fertilization, the interaction effect was also significant. The treatment combining local inoculation with 80 kg P ha^{-1} recorded the highest pod number, reaching 25.13 pods $plant^{-1}$, followed by the treatment with local inoculation and 120 kg P ha^{-1}, which gave 21.14 pods $plant^{-1}$. In contrast, the control treatment recorded the lowest value of 8.30 pods $plant^{-1}$.

Table 3. Effect of local and commercial *Bradyrhizobium japonicum* inoculation, phosphorus fertilization, and their interaction on the number of pods per plant of mung bean

Fertilization Mean	Bacterial Inoculation			Phosphorus Fertilizer (kg ha⁻¹)
	Local Inoculum	Commercial Inoculum	No Inoculum	
10.21	11.94	10.39	8.30	0
12.75	15.68	12.97	9.62	40
16.87	25.13	14.13	11.37	80
15.47	21.14	13.08	12.19	120
	18.47	12.64	10.37	Inoculation Mean
	Interaction	Fertilization	Inoculation	L.S.D. (0.05)
	0.925	0.534	0.462	

Pod weight per plant:

Table 4 shows that inoculated treatments outperformed uninoculated ones in terms of pod weight. Moreover, the treatment inoculated with the local *Bradyrhizobium japonicum* inoculum was significantly superior to the treatment inoculated with the commercial inoculum, recording average pod weights of 15.39 and 12.20 g plant^{-1}, respectively, compared to 9.39 g plant^{-1} in the uninoculated treatment.The results also indicate that phosphorus fertilization had a significant effect on pod weight. Fertilized treatments outperformed the unfertilized control, and the 80 and 120 kg P ha^{-1} levels were significantly superior to the 40 kg P ha^{-1} level. The recorded pod weights for the 0, 40, 80, and 120 kg P ha^{-1} treatments were 8.59, 11.01, 14.95, and 14.77 g plant^{-1}, respectively.As for the interaction effect between bacterial inoculation and phosphorus fertilization, it was also significant. The combination of local inoculation and 80 kg P ha^{-1} fertilizer recorded the highest average pod weight of 18.44 g plant^{-1}, representing a 229% increase compared to the control treatment, which gave only 5.60 g plant^{-1}.

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Table 4. Effect of local and commercial *Bradyrhizobium japonicum* inoculation, phosphorus fertilization, and their interaction on pod weight (g plant^{-1}) of mung bean

Fertilization Mean	Bacterial Inoculation			Phosphorus Fertilizer (kg ha^{-1})
	Local Inoculum	Commercial Inoculum	No Inoculum	
8.59	11.63	8.54	5.60	0
11.01	14.23	11.08	7.72	40
14.95	18.44	14.78	11.65	80
14.77	17.28	14.43	12.62	120
	15.39	12.20	9.39	Inoculation Mean
	Interaction	Fertilization	Inoculation	L.S.D. (0.05)
	0.770	0.445	0.358	

100-seed weight:

Table 5 illustrates the effect of local and commercial *Bradyrhizobium japonicum* inoculation, phosphorus fertilization, and their interaction on the 100-seed weight of mung bean at the harvest stage.The results revealed that bacterial inoculation had a significant effect on 100-seed weight, with inoculated treatments significantly outperforming the uninoculated control. Furthermore, the treatment inoculated with the local inoculum showed a significant advantage over the treatment with the commercial inoculum, recording average 100-seed weights of 8.95 and 8.08 g, respectively, compared to 7.02 g in the uninoculated treatment.The results also showed that phosphorus fertilization had a significant impact on 100-seed weight. All fertilized treatments outperformed the unfertilized control, and the 80 and 120 kg P ha^{-1} levels were significantly superior to the 40 kg P ha^{-1} level. Statistical analysis revealed no significant differences between the 80 and 120 kg P ha^{-1} levels, which recorded 9.10 and 8.91 g, respectively, compared to 6.63 g in the unfertilized treatment.The interaction effect between bacterial inoculation and phosphorus fertilization was also significant. The treatment combining local inoculation with 80 kg P ha^{-1} recorded the highest average 100-seed weight of 10.56 g, followed by the local inoculation with 120 kg P ha^{-1}, which gave 9.13 g. In contrast, the control treatment recorded the lowest average value of 5.84 g.

Table 5. Effect of local and commercial *Bradyrhizobium japonicum* inoculation, phosphorus fertilization, and their interaction on 100-seed weight (g plant^{-1}) of mung bean

Fertilization Mean	Bacterial Inoculation			Phosphorus Fertilizer (kg ha^{-1})
	Local Inoculum	Commercial Inoculum	No Inoculum	
6.63	7.59	6.48	5.84	0
7.42	8.52	7.58	6.18	40
9.10	10.56	9.31	7.43	80
8.91	9.13	8.97	8.65	120
	8.95	8.08	7.02	Inoculation Mean
	Interaction	Fertilization	Inoculation	L.S.D. (0.05)
	0.987	0.638	0.552	

Grain yield:

Table 6 presents the effect of local and commercial *Bradyrhizobium japonicum* inoculation, phosphorus fertilization, and their interaction on the total seed yield of mung bean.The results showed that bacterial inoculation had a significant effect on grain yield. Inoculated treatments significantly outperformed uninoculated ones. Moreover, the treatment inoculated with the local inoculum was significantly superior to that with the commercial inoculum, recording the highest values. The average grain yields for the uninoculated, commercial, and locally inoculated treatments were 1.890, 2.229, and 2.782 t ha^{-1}, respectively.The table also indicates that phosphorus fertilization had a significant effect on total seed yield. Fertilized treatments were significantly superior to the unfertilized control, with the 80 and 120 kg P ha^{-1} levels significantly outperforming the 40 kg P ha^{-1} level. The 80 kg P ha^{-1} treatment showed the highest yield among all phosphorus levels, giving an average of 2.748 t ha^{-1}, compared to 1.629 t ha^{-1} in the unfertilized treatment.The interaction between bacterial inoculation and phosphorus fertilization was also significant. The treatment combining local inoculation with 80 kg P ha^{-1} recorded the highest grain yield, reaching 3.361 t ha^{-1}, followed by the local inoculation combined with 120 kg P ha^{-1}, which recorded 3.080 t ha^{-1}. These represent increases of 175.71% and 152.66%, respectively, compared to the control treatment, which recorded an average yield of 1.219 t ha^{-1}.

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Table 6. Effect of local and commercial Bradyrhizobium japonicum inoculation, phosphorus fertilization, and their interaction on total seed yield (t ha^{-1}) of mung bean

Fertilization Mean	Bacterial Inoculation			Phosphorus Fertilizer (kg ha^{-1})
	Local Inoculum	Commercial Inoculum	No Inoculum	
1.629	2.038	1.632	1.219	0
2.179	2.651	2.056	1.830	40
2.748	3.361	2.747	2.138	80
2.645	3.080	2.482	2.374	120
	2.782	2.229	1.890	Inoculation Mean
	Interaction	Fertilization	Inoculation	L.S.D. (0.05)
	0.122	0.070	0.061	

Discussion

The significant improvements observed in the number of pods and other yield components of mung bean as a result of inoculation with *Bradyrhizobium japonicum* inoculants may be attributed to the formation of effective root nodules, which facilitated biological nitrogen fixation and stimulated root system development. This, in turn, enhanced the efficiency of nutrient uptake from the soil. Inoculation with these bacteria improves the growth of leguminous plants by increasing shoot and root dry matter, as well as plant height, which positively affects the absorption of nitrogen and other essential nutrients.Improved nitrogen uptake promotes the synthesis of amino acids and proteins within plant tissues. Additionally, Bradyrhizobium bacteria produce plant growth-promoting substances such as indole-3-acetic acid (IAA), which stimulate flower formation and development, thereby increasing the number and weight of seeds per plant. These processes also contribute to the development of reproductive organs and better seed filling. These findings are in agreement with the results of Beshah et al .[10] .The observed increases in the number and weight of pods, number of seeds per pod, seed weight per plant, and other yield components in response to phosphorus fertilization may be attributed to the vital role of phosphorus in enhancing root growth and density, which improves nutrient uptake efficiency and supports both vegetative and reproductive development. Phosphorus is a key component of energy molecules (ATP, ADP), essential for cell division and sugar translocation from leaves to developing pods and seeds.It also stimulates the synthesis of nucleic acids (DNA, RNA) and contributes to the formation of phospholipids required for membrane biogenesis, thus supporting chloroplast stability and improving photosynthetic

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efficiency. These physiological functions ultimately enhance pod and seed productivity, as confirmed by several previous studies [11]. The significant interaction observed between *Bradyrhizobium japonicum* inoculation and phosphorus fertilization suggests a synergistic and physiologically beneficial effect. This interaction improved root growth and nutrient uptake efficiency, resulting in notable increases in pod number and weight, seeds per pod, seed weight per plant, and overall yield-related traits.The complementary mechanism is likely due to the provision of biologically fixed nitrogen by the bacteria, while phosphorus activates key enzymes and supports energy distribution within the plant, enhancing metabolic and reproductive processes. Consequently, plant productivity is significantly improved. These findings are consistent with those reported by [12].

Nitrogen and phosphorus content in seeds:

Table 7 illustrates the effect of local and commercial *Bradyrhizobium japonicum* inoculation, phosphorus fertilization, and their interaction on nitrogen concentration in mung bean seeds at the harvest stage.The results revealed that bacterial inoculation had a significant effect on seed nitrogen concentration, as all inoculated treatments significantly outperformed the uninoculated control. Moreover, the treatment inoculated with the local inoculum was significantly superior to the commercial inoculum, with average nitrogen concentrations of 2.88%, 3.25%, and 3.74% in the uninoculated, commercially inoculated, and locally inoculated treatments, respectively.The data also showed that phosphorus fertilization had a significant effect on seed nitrogen content. All fertilized treatments outperformed the unfertilized control, and the 80 and 120 kg P ha^{-1} levels were significantly superior to the 40 kg P ha^{-1} level. The highest nitrogen concentration was recorded at the 120 kg P ha^{-1} level (3.48%), followed closely by the 80 kg P ha^{-1} level (3.45%), with no significant difference between the two. In contrast, the unfertilized treatment recorded 3.03%.The interaction between bacterial inoculation and phosphorus fertilization was also significant in enhancing seed nitrogen concentration. The highest value (4.08%) was obtained from the treatment combining local inoculation with 120 kg P ha^{-1}, followed by local inoculation with 80 kg P ha^{-1}, with no significant difference between them. This represented a 308% increase compared to the control treatment, which recorded the lowest nitrogen concentration of 2.80%.

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Table 7. Effect of local and commercial *Bradyrhizobium japonicum* inoculation, phosphorus fertilization, and their interaction on nitrogen concentration (%) in mung bean seeds

Fertilization Mean	Bacterial Inoculation			Phosphorus Fertilizer (kg ha^{-1})
	Local Inoculum	Commercial Inoculum	No Inoculum	
3.03	3.27	3.04	2.80	0
3.20	3.58	3.17	2.85	40
3.45	4.06	3.38	2.92	80
3.48	4.08	3.44	2.93	120
	3.74	3.25	2.88	Inoculation Mean
	Interaction	Fertilization	Inoculation	L.S.D. (0.05)
	0.072	0.041	0.036	

Table 8 shows that bacterial inoculation had a significant effect on phosphorus concentration in mung bean seeds. Inoculated treatments significantly outperformed the uninoculated control. Moreover, the treatment inoculated with the local inoculum was significantly superior to the commercial inoculum, recording phosphorus concentrations of 0.226% and 0.186%, respectively, compared to 0.148% in the uninoculated treatment.The table also indicates that phosphorus concentration in seeds was significantly affected by phosphorus fertilization. Fertilized treatments outperformed the unfertilized control, and the 80 and 120 kg P ha^{-1} levels were significantly superior to the 40 kg P ha^{-1} level. The highest phosphorus concentration was recorded at the 120 kg P ha^{-1} level (0.240%), which was significantly higher than the 0.209% recorded at the 80 kg P ha^{-1} level, while the unfertilized treatment recorded 0.143%.Regarding the interaction between inoculation and phosphorus fertilization, the effect was also significant. The combination of local inoculation with 80 kg P ha^{-1} gave the highest seed phosphorus concentration at 0.301%, followed by local inoculation with 120 kg P ha^{-1} at 0.262%. These values represent increases of 161.73% and 127.82%, respectively, compared to the control treatment, which recorded the lowest phosphorus concentration of 0.115%.

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Table 8. Effect of local and commercial *Bradyrhizobium japonicum* inoculation, phosphorus fertilization, and their interaction on phosphorus concentration (%) in mung bean seeds

Fertilization Mean	Bacterial Inoculation			Phosphorus Fertilizer (kg ha^{-1})
	Local Inoculum	Commercial Inoculum	No Inoculum	
0.143	0.169	0.145	0.115	0
0.179	0.247	0.154	0.136	40
0.209	0.262	0.210	0.156	80
0.240	0.301	0.235	0.186	120
	0.226	0.186	0.148	Inoculation Mean
	Interaction	Fertilization	Inoculation	L.S.D. (0.05)
	0.041	0.023	0.020	

Discussion

The significant increases in nitrogen, phosphorus, and potassium concentrations in mung bean seeds following inoculation with *Bradyrhizobium japonicum* may be attributed to the high efficiency of this bacterium in biological nitrogen fixation, which positively affects nitrogen accumulation in plant tissues and consequently in seeds. Moreover, this bacterium plays an important role in producing enzymes and organic compounds that help solubilize organic phosphate in the soil, converting it into plant-available forms.The formation of effective root nodules enhances the plant’s ability to absorb and efficiently utilize phosphorus, explaining the increased phosphorus concentration in seeds (Table 32). Additionally, *Bradyrhizobium japonicum* secretes biologically active compounds such as plant hormones (e.g., auxins), amino acids, and phytohormones that stimulate plant growth and improve nutrient uptake efficiency—including potassium, which is essential for protein synthesis and regulating various cellular functions. These findings are in agreement with those reported by Beshah et al [13] .The significant increase in seed nitrogen, phosphorus, and potassium contents due to phosphorus fertilization may be ascribed to the critical physiological role of phosphorus in enhancing metabolic reactions related to growth and development. Phosphorus is a structural component of ATP and ADP, which are essential for cellular energy transfer and metabolic activities. It also participates in the synthesis of nucleic acids responsible for cell division and tissue growth.This positive effect enhances root development and efficiency, which in turn improves nodule formation and nitrogen fixation when Bradyrhizobium is present. Moreover, phosphorus addition increases the

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availability of soil phosphorus, enhancing its translocation to seeds, especially in the form of phytin. Since improved root growth also enhances the uptake of other nutrients such as potassium and biologically fixed nitrogen, phosphorus fertilization is considered a key factor not only in increasing seed phosphorus content but also in facilitating the uptake of nitrogen and potassium. Potassium plays a vital role in enzyme activation and osmotic regulation, ultimately increasing nutrient accumulation in seeds. These results are consistent with the findings of several studies [14].The significant increase in seed nitrogen, phosphorus, and potassium contents in the treatment combining Bradyrhizobium japonicum inoculation with phosphorus fertilization may be attributed to the synergistic interaction between the bacterial inoculant and phosphorus. This beneficial interaction improved nutrient uptake efficiency and promoted the plant’s ability to accumulate nutrients, which was reflected in enhanced nutrient concentrations in the seeds. These findings are in agreement with those of [15].

Seed protein content (%):

Table 9 illustrates the effect of inoculation with both the commercial and locally prepared Bradyrhizobium japonicum inoculants, along with phosphorus fertilization and their interaction, on the protein content of mung bean seeds at harvest stage.

The results reveal a significant effect of bacterial inoculation on seed protein content, as inoculated treatments significantly outperformed the uninoculated control. The treatment inoculated with the locally prepared inoculant showed a statistically significant superiority over the commercial inoculant, with mean protein contents of 23.46%, 20.41%, and 18.03% for the local inoculant, commercial inoculant, and control, respectively. Furthermore, phosphorus fertilization had a significant impact on seed protein content. Fertilized treatments significantly surpassed unfertilized ones, with the 80 and 120 kg P ha^{-1} levels being statistically superior to the 40 kg P ha^{-1} level. The 120 kg P ha^{-1} treatment recorded the highest protein content; however, no significant difference was found between this level and the 80 kg P ha^{-1} level. The average protein contents were 19.03%, 20.04%, 21.62%, and 21.85% for the 0, 40, 80, and 120 kg P ha^{-1} levels, respectively.Regarding the interaction between inoculation and phosphorus fertilization, the effect was also significant. The highest protein content was recorded in the treatment inoculated with the local inoculant and fertilized with 120 kg P ha^{-1}, with a mean of 25.56%, followed by the local inoculant combined with 80 kg P ha^{-1}, which recorded 25.40%, compared to the control treatment which recorded 17.53%.

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Table 9. Effect of inoculation with commercial and locally prepared *Bradyrhizobium japonicum* inoculants and phosphorus fertilization and their interaction on seed protein content (%) of mung bean

Fertilization Mean	Bacterial Inoculation			Phosphorus Fertilizer (kg ha⁻ ¹)
	Local Inoculum	Commercial Inoculum	No Inoculum	
19.03	20.50	19.06	17.53	0
20.04	22.40	19.86	17.86	40
21.62	25.40	21.16	18.30	80
21.85	25.56	21.56	18.43	120
	23.46	20.41	18.03	Inoculation Mean
	Interaction	Fertilization	Inoculation	L.S.D. (0.05)
	0.458	0.265	0.229	

Discussion

The significant increase in seed protein content as a result of inoculation with *Bradyrhizobium japonicum* may be attributed to the bacterium's efficiency in root infection and the formation of effective nodules. This, in turn, enhances the amount of biologically fixed nitrogen utilized in the synthesis of amino acids and proteins within the plant. This is evident from the increased number and weight of root nodules. Moreover, the biological activity of the bacteria may have contributed to promoting both root and shoot development, thereby improving the plant's overall productivity and ultimately enhancing seed yield and protein content. These findings are in agreement with those reported by [16] .The significant increase in protein content in seeds following phosphorus fertilization at a level of 80 kg P ha⁻ ¹ may be attributed to the essential role of phosphorus in the formation of nucleic acids (DNA and RNA) and the activation of vital processes related to protein synthesis. Phosphorus also promotes the development of the root system, which improves nitrogen uptake—an essential element in protein construction. These results are consistent with the findings of. [17] Furthermore, the observed increase in protein content under the combined treatment of *Bradyrhizobium japonicum* inoculation and phosphorus fertilization suggests a positive interaction between these two factors. This synergistic effect enhanced the uptake of essential nutrients required for protein biosynthesis. These findings are in line with those reported by [18].

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Conclusions

The locally prepared inoculum containing the indigenous isolate *Bradyrhizobium japonicum* (Is-27) demonstrated a significant superiority in enhancing the yield components of mung bean compared to the commercial inoculum and uninoculated treatments. Gypsiferous soils are generally characterized by a low native population of effective root nodule bacteria, which underscores the importance of inoculating with nitrogen-fixing bacteria to improve plant growth and productivity. Phosphorus fertilization, particularly at the level of 80 kg P·ha^{-1}, proved effective in supporting yield traits and increasing nutrient concentrations, highlighting the vital role of phosphorus in interacting with Rhizobium bacteria under gypsiferous soil conditions.The interaction between bio-inoculation and phosphorus fertilization showed a pronounced synergistic effect, as the treatments inoculated with the local biofertilizer and supplemented with phosphorus significantly outperformed other treatments in most of the evaluated traits. This research represents a crucial step toward the development of an environmentally friendly local biofertilizer that enhances legume growth in arid and semi-arid regions. It also lays a foundation for reducing dependency on chemical fertilizers in the context of sustainable agriculture.

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