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Evaluation Effect of Inoculum Prepared from Local Bacterial Isolates of Rhizobium and Nitrogen Fertilization on some Growth Traits of Mung Bean Plant in Gypsiferous Soil

Marwan Abdul-Aziz Shihab[1] **Abdul Kareem Eraibi Alkurtany[2]**
Marwan.a.shehab@st.tu.edu.iq alkurtany@tu.edu.iq

[1,2]Department of Soil and Water Resources Sciences, College of Agriculture, University of Tikrit, Tikrit, Iraq.
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Abstract

The root nodule bacteria are utilized in the production of natural biological fertilizers to achieve clean agriculture by reducing chemical fertilizers. Therefore, the bacterial inoculum was prepared from local isolates of *Rhizobium leguminosarum* and *Bradyrhizobium japonicum*, which were morphologically and molecularly characterized. Subsequently, the efficacy of the prepared inoculum was tested in the formation of root nodules and some growth traits of mung bean plants in gypsiferous soil at three levels of nitrogen fertilization (0, 40, 80) kg N ha^{-1} The results showed a significant increase in all plant growth traits during the flowering stage for the treatments inoculated with *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* bacteria in comparison to the treatment without inoculation. The inoculated treatments outperformed the non-inoculated treatment significantly. Additionally, the treatment inoculated with *Bradyrhizobium japonicum* showed a significant superiority over the treatment inoculated with *Rhizobium leguminosarum* in all studied growth traits, the recorded values for the count of root nodules were (0.00, 8.33, 35.11) nodule. plant^{-1}, The wet mass of nodules was (0.00, 0.091, 1.802) g. plant^{-1}, and dry mass of nodules was (0.00, 0.010, 0.337) g. plant^{-1}. The plant height was (52.11, 56.22, 58.05) cm. plant^{-1}. The dry mass of the vegetative part, was (14.03, 20.10, 23.05) g. plant^{-1}, and ،the dry mass of the root part was (1.37, 2.57, 2.67) g. plant^{-1}. The nitrogen content was (1.547, 2.765, 3.028) % phosphorus (0.121, 0.236, 0.377) %, and potassium (1.168, 2.342, 3.562) % in the vegetative part. The absorbed nitrogen in the vegetative part was (217.41, 541.10,718.02) mg. plant^{-1}. These values were recorded for the non-inoculated treatment, *Rhizobium leguminosarum* inoculated treatment, and *Bradyrhizobium japonicum* inoculated treatment, respectively. The results showed a significant superiority for the 40 kg N ha^{-1} fertilized treatment in comparison to both the unfertilized treatment and the 80 kg N/ha^{-1} fertilized treatment in all studied traits. The interaction effect was significant, as the treatment that was inoculated with *Bradyrhizobium japonicum* and 40 kg N ha^{-1} fertilized treatment exhibited significant superiority over the other treatments in all studied traits.

Keywords: Bradyrhizobium japonicum, Rhizobium leguminosarum, Inculation, Nitrogen Fertilization, Mungbean, Gypsiferous.

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Correspondence Author: Marwan Abdul-Aziz Shihab-Marwan.a.shehab@st.tu.edu.iq

Introduction

Because of the excessive utilization of chemical fertilizers in agricultural practices especially nitrogen fertilizers, which have had negative effects on the environment and hazardous effects on human health, in addition to the direct impact on microorganisms. Therefore, the world has turned to the use of modern agricultural technologies, including bio-fertilizers, to reduce pollution problems, which are a natural and food source for plants and inexpensive when compared to chemical fertilizers in poor nutrient soils. Useful microorganisms are used in bio-fertilizers to improve agricultural productivity.[1] Bio-fertilizers are of great importance in plant fertility and its surroundings, as they provide one or more of the necessary nutrients for plant growth, which can reduce mineral fertilizers, in addition to encouraging the plant to secrete some useful hormones and growth stimulants [2]. Rhizobia are microorganisms capable of nitrogen fixation through a symbiotic relationship with the plant family. They fixing about 65% of the nitrogen used in sustainable agricultural production of legume crops [3]. Therefore, there has been an increased demand for bio-fertilizers, and their production and use have expanded compared to manufactured nitrogen fertilizers. Nitrogen-fixing rhizobia bacteria convert about 20 million tons of atmospheric nitrogen (N2) annually into ammonia, which is 50-70% of the biologically fixed nitrogen in the world. The high nitrogen fixation determines the success of the symbiotic interaction existing between rhizobia bacteria and leguminous plants [4]. Mung bean (Vigna radiata L.) ranks among the most significant leguminous crops globally, being a member of the Leguminosae family. It holds a prominent position among the prevalent crops in numerous tropical and subtropical areas[5] . it is an herbaceous or semi-erect plant, with a height between 25-125 cm, covered with fuzz and its leaves are triple compound. It is characterized by a short life cycle of 70-90 days and is relatively tolerant to drought [6]. It is a high-nutritional-value crop and a cheap source of protein [7]. Apart from serving as a high-protein resource, it also plays a pivotal role in preserving soil fertility by means of biological nitrogen fixation[8] . It is introduced annually within agricultural cycles to preserve soil fertility and sustainability [9] [10]. Due to the importance of implementing the symbiosis between *Rhizobium* bacteria and nitrogen fertilization in improving the growth of legumes, this research was undertaken to assess the impact of inoculation with locally isolated, effective, and partially characterized strains of *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* on the formation of root nodules and some growth traits of growing mung bean plants in gypsiferous soil at different levels of nitrogen fertilizer.

Materials and Methods

Selection of Bacterial Strains

Two efficient partially characterized *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* isolates were chosen for their efficiency for phosphate solubilisation, indole acetic acid production, and siderophore synthesis، as shown in Table

Table 1. Efficiency of bacterial isolates in solubilisation of phosphate, production of indole acetic acid, and siderophore generation.

Isolate	Phosphate solubilization (mg/L^{-}1)	Indole production (µg/mL^{-1})	Iron chelating
Rhizobium leguminosarum	23.7	17.2	+++
Bradyrhizobium japonicum	39.5	21.5	+++

Preparation Steps for Bacterial Inoculum

1. Cultivation of Rhizobia Bacteria

Pure isolates of *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* were grown by transferring equal-sized colonies from 24-hour-old cultures to Erlenmeyer flasks with a volume of 250 mL, containing 200 mL of sterile liquid nutrient broth medium (adjusted to pH 7) using a sterilized loop. Subsequently, the flasks were positioned within a shaking incubator set at 28°C and 100 rpm, maintained for a duration of 3 to 5 days. The bacterial cell numbers were estimated by the dilution and plating method. One millilitre from each flask was diluted and serially diluted, and then plated on plates containing selective medium for each bacterial isolate. Three replicates were prepared for each selected strain, which were then Kept in incubation at a temperature of 28°C for a period spanning 48 to 72 hours. Bacterial colonies were quantified by multiplying the colony count with the reciprocal of the dilution factor.

2. The Preparation of the Carrier

Peat moss, manufactured by the FAO (Food and Agriculture Organization) worldwide, was used as a carrier. It was sieved through a mesh with a diameter of 0.5 mm and distributed equally into plastic bags made of high-temperature-resistant polyethylene, with a weight of 250 grams. The pH value was confirmed to be 7. Then, the carrier material was moistened with water to achieve a moisture content ranging from 55% to 60%. The carrier material Undergoing sterilization via autoclaving at a temperature of 121°C and pressure of 1.5 bar for 30 minutes, with three repetitions of the sterilization process. The bags were left to cool down and securely sealed before being removed from the autoclave. Dilution was carried out to ensure the effectiveness of the sterilization process and the absence of microorganisms in the peat moss [11].

3. Mixing the Inoculant with the Carrier

100 g of inoculant were prepared by taking 45 g of sterilized peat moss and adding 5 g of calcium carbonate to adjust the pH, followed by the addition of 20 ml of tap water. The mixture was then placed in sterilized polyethylene bags and 30 ml of bacterial culture was added. The carrier was then incubated at 30°C for 7 days with daily shaking in different directions. The bacterial cell count in the inoculated carrier was determined by dilution and plating on agar plates before inoculating the seeds. 20 g of the inoculant mixed with 180 ml of sterilized water were suspended under sterile conditions and stirred for 30 minutes. A series of dilutions were then made and 0.1 ml of the 10^8-10^9dilution was spread on YEMA plates for Rhizobia bacteria and subjected to incubation at 28°C for a duration of 3 to 5 days.

4. Preparing the Adhesive Solution

An Arabic gum solution was prepared at a concentration of 40% by dissolving 40g of the material in 100mL of sterilized distilled water in 250mL glass beakers. The solution was continuously stirred while making sure that the temperature does not exceed 100°C to prevent changes in its properties. A sucrose solution was also prepared at a concentration of 15% by dissolving 15g of the material in 100mL of sterilized distilled water in 250mL glass beakers to increase the vitality and efficiency of the bacteria. Then, the adhesive solution was mixed with the vaccine loaded onto the peat moss at a ratio of 3:1 (1mL of adhesive solution for every 3g of the inoculum) [12].

5. Seed Sterilization and Inoculation

The mung bean seeds were surface-sterilized to eliminate any microorganisms present on their surface. This was achieved by washing the seeds thoroughly with water several times and then soaking them for 2 minutes in a 2% sodium hypochlorite (bleach) solution. The seeds were then washed with sterilized water 5-6 times to remove any traces of the sterilizing agent. The prepared seeds were inoculated by mixing 100g of seeds with 4g of biofertilizer. The inoculated seeds were spread out on a dry paper to air-dry in the shade, away from direct sunlight, to preserve the vitality of the inoculant. The inoculation was done one hour before planting.

6. Implementation of the Field experiment

A factorial field study was undertaken at the Soil and Water Resources Research Station, Agriculture College, Tikrit University, in gypsiferous soil with a Sandy Clay loam texture and a content of gypsum, lime, and organic matter (152,178,5) g.kg^{-1} respectively as in the following table

Table 2. Physical, Chemical, and Biological Properties of Soil Before the cultivation.

Property	Measurement Unit	Value
The pH of the soil	-	7.2
Electrical conductivity (EC)	ds.m-1	2.8
Cation-exchange capacity(CEC)	Cmol.Kg-1 soil	13.26
Organic matter (OM)	g.kg-1	5
Calcium carbonate (CaCO3)	g.kg-1	178
Gypsum (CaSO4)	g.kg-1	152
Available Nitrogen (N)	mg.kg-1	17.37
Available Phosphorus (P)	mg.kg-1	5.2
Available Potassium (K)	mg.kg-1	114
Bulk density	g.cm-3	1.24
Sand	g.kg-1	546
Silt	g.kg-1	219
Clay	g.kg-1	235
Texture		Sandy Clay loam
Total bacterial count	C.F.U	10^6×4.8
Total fungal count	C.F.U	10^3×4.5

The objective of the experiment was to investigate the impact of inoculation with two distinct strains, namely Rhizobium leguminosarum and Bradyrhizobium japonicum on the formation of root nodules and some growth traits of mung bean plants at different levels of nitrogen fertilizer.The experiment was carried out employing a completely randomized block design (RCBD) encompassing two factors:

Inoculation with three levels: no inoculation (B0), inoculation with *Rhizobium leguminosarum (B1)*, and inoculation with *Bradyrhizobium japonicum(B2)* and fertilization with three levels: (0, 40, 80)kg N ha^{-1}.

Therefore, the total number of treatment combinations was (9). Each treatment was replicated threefold, yielding a cumulative count of (27) experimental units. After the plowing and soil levelling process, the field was partitioned into three segments, each containing nine experimental units. The area of each experimental unit was (2x2) m^2. A distance of (1) m was left between each section and between the experimental units. The inoculated and non-inoculated seeds were sown on August 11, 2022, according to the treatment combinations. The planting was done in rows, spacing between rows was set at (40) cm, and the distance between plants within the same row was maintained at (10) cm. Nitrogen was applied at three levels (0, 40, 80) kg N ha^{-1} using urea fertilizer. Phosphorus was administered at a dosage of (160) kg P ha^{-1}. using triple superphosphate fertilizer (21% P). Potassium was administered at a dosage of (160) kg P ha^{-1} using potassium sulfate fertilizer (43% K) in a single application before planting، and watered The field by strip irrigation method.

The Studied experiment

The following traits were recorded during the flowering stage:

- Count of root nodules and dry, wet mass of root nodules.
- Plant height and dry weight of vegetative and root part.
- The nitrogen concentration and the absorbed nitrogen in the vegetative part.
- The phosphorus, and potassium concentration in plant.

Results and Discussion

1.Count of root nodules and dry, wet mass of root nodules

Tables 3, 4, and 5 demonstrate that the inoculation exerted a significantly impact on count of root nodules, as well as the wet and dry mass of mung bean plants during the flowering stage. The non-inoculated treatment failed to form any root nodules, while the treatment inoculated with *Bradyrhizobium japonicum* significantly outperformed the treatment inoculated with *Rhizobium leguminosarum* in terms of the count of nodules and their wet and dry mass. The average values for the non-inoculated, *Rhizobium leguminosarum*-inoculated, and *Bradyrhizobium japonicum*-inoculated treatments were (0.00, 8.33, 35.11) nodule. plant^{-1}, and (0.00, 0.091, 1.802) g. plant^{-1}, and (0.00, 0.010, 0.337) g. plant^{-1} for the count of nodules and their wet and dry mass, respectively.

Moreover, nitrogen fertilization Induced a significantly influence on the count of root nodules and their wet and dry mass in mung bean plants. 40 kg N ha^{-1} fertilized treatment surpassed the non-fertilized treatment and the 80 kg N ha^{-1} fertilized treatment in terms of the count of nodules and their wet and dry mass. The average values for the non-fertilized, the 40 kg N ha^{-1} fertilized treatment, and 80 kg N ha^{-1} treatments were (13.00, 16.11, 14.34) nodule. plant^{-1}, and (0.4590, 0.803, 0.630) g. plant^{-1}, and (0.107, 0.123, 0.116) g. plant^{-1} for the count of nodules and their wet and dry mass, respectively.

the interaction between Rhizobium bacteria inoculation and nitrogen fertilization at different levels, it had a significant effect. The *Bradyrhizobium japonicum* inoculation and treated with 40 kg N ha^{-1} fertilization outperformed all other treatments significantly in terms of the count of nodules and their dry and wet mass. It was followed by the treatment inoculated with *Bradyrhizobium japonicum* and fertilized at a rate of 80 kg N ha^{-1}. However, no substantial distinction was observed between the control treatment and the treatments that were non-inoculated, fertilized with 40 kg N ha^{-1}, or fertilized with 80 kg N ha^{-1} in terms of the measured traits.

Table 3. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interaction on the count of root nodules (nodule. plant^{-1}) of mung bean during the flowering stage.

Nitrogen fertilizer (kg.ha^{-1})	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	0.00	6.66	32.34	13.00
40	0.00	10.33	38.00	16.11
80	0.00	8.00	35.00	14.34
Inoculation rate	0.00	8.33	35.11	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.98	0.98	1.70	

Table 4. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interaction on the wet mass of root nodule(g.plant^{-1}) of mung bean during the flowering stage.

Nitrogen fertilizer (kg.ha^{-1})	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	0.00	0.009	1.370	0.459
40	0.00	0.149	2.260	0.803
80	0.00	0.115	1.776	0.630
Inoculation rate	0.00	0.091	1.802	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.16	0.16	0.29	

Table 5. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interaction on the dry mass of root nodule(g. plant^{-1})of mung bean during the flowering stage.

Nitrogen fertilizer (kg.ha^{-1})	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	0.00	0.005	0.318	0.107
40	0.00	0.016	0.354	0.123
80	0.00	0.009	0.341	0.116
Inoculation rate	0.00	0.010	0.337	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.0062	0.0062	0.010	

The failure of the non-inoculated treatment with *Rhizobium* bacteria to form root nodules may be attributed to the low colonization efficiency and population of the bacteria The significant superiority in the count of root nodules and their wet and dry mass in the treatment inoculated with *Bradyrhizobium japonicum* may be due to the efficiency of this bacteria in colonizing the root zone and forming nodules, as well as its ability to coexist with mung bean plants, resulting in higher numbers of active root nodules in the soil and increased likelihood of nodule infection and formation in the roots. This finding is consistent with [13], who found that inoculation with *Bradyrhizobium japonicum* for mung bean plants increased the count and mass of nodules, both wet and dry.

The significant increase in the count of root nodules and their wet and dry mass with fertilization at the 40 kg N ha^{-1} level may be attributed to the fact that in the early stages of growth and before nodule formation, plants require nitrogen for their biological processes. This nitrogen is known as the starter, which stimulates plant growth and subsequently enhances nodule formation, as well as increasing their size and weight, both wet and dry. This finding is consistent with [14].

The significant superiority of the treatment with The observed interaction between *Bradyrhizobium japonicum* inoculation and fertilization at 40 kg N ha^{-1} level highlights the significance of this particular synergy was beneficial and contributed to increasing the number of nodules and their wet and dry weight. This finding is consistent with [15] in their study on *Bradyrhizobium japonicum* and nitrogen fertilization for mung bean plants.

2.Plant Height and Dry Weight of Vegetative and Root Part

Table 6 shows that the inoculation had a significant effect on the height of mung bean plants during the flowering stage. The inoculated treatments outperformed The treatment without inoculation significantly. Additionally, the treatment inoculated with *Bradyrhizobium japonicum* showed a significant superiority over both The treatment without inoculation and the treatment inoculated with *Rhizobium leguminosarum*. The average plant height values were (52.11, 56.22, 58.05) cm. plant^{-1} for the non-inoculated, *Rhizobium leguminosarum*-inoculated, and *Bradyrhizobium japonicum*-inoculated treatments, respectively.

Furthermore, Table 6 demonstrates that nitrogen fertilization exhibited a noticeable impact on the height of mung bean plants. 40 kg N ha^{-1} fertilized treatment surpassed the non-fertilized treatment and 80 kg N ha^{-1} fertilized treatment. The average height values were (54.00, 56.84, 55.77) cm. plant^{-1} for the non-fertilized, 40 kg N ha^{-1}, and 80 kg N ha^{-1} treatments, respectively.

The interaction between inoculation with *Rhizobium* bacteria and nitrogen fertilization at different levels also had a significant effect on the height of mung bean plants. The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 40 kg N ha^{-1} showed significant superiority over all other treatments. It was followed by the treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 80 kg N ha^{-1}. The control treatment had the lowest average value.

Table 6. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interactionon the height of the mung bean plant (cm. plant^{-1}) during the flowering stage.

Nitrogen fertilizer (kg.ha^{-1})	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	50.50	54.50	57.00	54.00
40	53.50	57.50	59.50	56.84
80	53.00	56.66	57.66	55.7
Inoculation rate	52.11	56.22	58.05	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.64	0.64	1.21	

It is evident from Table 7 that the inoculated treatments outperformed the non-inoculated treatment significantly on the dry weight of vegetative part. Additionally, the treatment inoculated with *Bradyrhizobium japonicum* showed a significant superiority over both the treatment without inoculation and the treatment inoculated with *Rhizobium leguminosarum*, with an increase of (64.29) % compared to the treatment without inoculation and (14.67) % compared to the *Rhizobium leguminosarum*-inoculated treatment.

Moreover, nitrogen fertilization had a significant effect, as the 40 kg N ha^{-1} fertilized treatment outperformed the non-fertilized treatment and the 80 kg N ha^{-1} fertilized treatment. The average values were (17.39, 20.94, 18.79) g. plant^{-1} for the non-fertilized, 40 kg N/ha^{-1}, and 80 kg N ha^{-1} treatments, respectively.

Table 7 also demonstrates that the interaction exerted a substantial influence on the dry weight of the vegetative part of mung bean plants. The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 40 kg N ha^{-1} showed significant superiority over all other treatments. It was followed by The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 80 kg N ha^{-1}. The control treatment had the lowest average value, followed by the non-inoculated and fertilized with 40 kg N ha^{-1} treatment, and the non-inoculated and fertilized with 80 kg N ha^{-1} treatment, respectively.

Table 7. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interaction on the dry weight of vegetative part (g.plant^{-1}) of mung bean during the flowering stage.

Nitrogen fertilizer (kg.ha^{-1})	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	13.34	18.56	20.29	17.39
40	14.62	22.53	25.67	20.94
80	14.13	19.21	23.20	18.79
Inoculation rate	14.03	20.10	23.05	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.72	0.72	1.27	

Table 8 shows that the inoculated treatments outperformed the non-inoculated treatment significantly on the dry weight of root part during the flowering stage. The treatment inoculated with *Bradyrhizobium japonicum* showed a significant superiority over both The treatment without inoculation and the treatment inoculated with *Rhizobium leguminosarum.* The average values recorded were (1.37, 2.57, 2.67) g. plant^{-1} for the non-inoculated, *Rhizobium leguminosarum*-inoculated, and *Bradyrhizobium japonicum*-inoculated treatments, respectively.

Moreover, Nitrogen fertilization demonstrated a significantly impact on the dry weight of the root segment. The 40 kg N ha^{-1} fertilized treatment outperformed the non-fertilized treatment and the 80 kg N ha^{-1} fertilized treatment. The average values for nitrogen fertilization were (2.06, 2.32, 2.22) g. plant^{-1} for the non-fertilized, 40 kg N ha^{-1}, and 80 kg N/ha^{-1} treatments, respectively.

The interaction impact between inoculation with *Rhizobia* and nitrogen fertilization was significant on the root dry weight of mung bean plants. The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 40 kg N ha^{-1} showed significant superiority over all other treatments. It was followed by The treatment inoculation with *Rhizobium leguminosarum* and received fertilization at a rate of 40 kg N ha^{-1}. No considerable distinction was observed between the control treatment and the non-inoculated, 40 kg N ha^{-1} fertilized treatment in terms of the dry weight of the root part.

Table 8. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interaction on the dry weight of root part (g. plant^{-1}) of mung bean during the flowering stage.

Nitrogen fertilizer (kg.ha^{-1})	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	1.32	2.34	2.54	2.06
40	1.39	2.75	2.82	2.32
80	1.41	2.62	2.65	2.22
Inoculation rate	1.37	2.57	2.67	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.035	0.035	0.061	

The significant superiority in Plant height and dry weight of vegetative and root part resulting from the inoculation with *Bradyrhizobium japonicum* may be attributed to the isolate's ability to produce indole compounds (Table 1) and other growth regulators such as cytokinins and gibberellins. This leads to the elongation and growth of root cells, affecting the cell division rate and elongation, as well as the overall growth rate, resulting in heightened plant height and enhanced dry weight of vegetative and root part, which represents the accumulation of carbon assimilation processes due to nitrogen uptake and its conversion into amino acids and then into protein compounds. This improves plant growth and increases dry weight of vegetative part [16]. This result is consistent with [17-19].

The significant superiority resulting from fertilization with 40 kg N ha^{-1} may be attributed to the role of nitrogen, which is an essential element in the formation of proteins, enzymes, and nucleic acids that help in plant growth. Nitrogen also contributes to the biological processes of the plant, as it is involved in the synthesis of protoplasm, which affects cell division rate to provide dry weight of vegetative part growth, thereby increasing plant growth rate [20]. This is supported by [21]. Additionally, the importance of nitrogen in increasing root activity and its ability to absorb water and other nutrients from the soil may contribute to increased plant growth and the dry weight of the root part. This finding is consistent with [22-24].

The significant superiority resulting from the interaction between *Bradyrhizobium japonicum* and fertilization with 40 kg N ha^{-1} may be attributed to the beneficial interaction that led to a significant in heightened plant height and enhanced dry weight of the vegetative and root parts compared to other treatments. This finding is consistent with [25,26].

3.The Nitrogen concentration and the Absorbed Nitrogen in the Vegetative Part

The Table 9 shows that the inoculation had a significant effect on nitrogen concentration in the vegetative part during the flowering stage. The inoculated treatments outperformed the treatment without inoculation significantly, and the inoculated treatment with

Bradyrhizobium japonicum surpassed the inoculated treatment with *Rhizobium leguminosarum* significantly. The average values for the treatments were (1.547, 2.765, 3.028) % for The treatment without inoculation, the treatment inoculated with *Rhizobium leguminosarum*, and the treatment inoculated with *Bradyrhizobium japonicum*, respectively.

The nitrogen fertilization also had a significant effect on nitrogen concentration in the vegetative part. The 40 kg N ha^{-1} fertilized treatment outperformed the non-fertilized treatment and the 80 kg N ha^{-1} fertilized treatment significantly. The average values for the treatments were (2.359, 2.551, 2.431) % for the non-fertilized treatment, the treatment fertilized with 40 kg N ha^{-1}, and the treatment fertilized with 80 kg N ha^{-1}, respectively.

The Table 9 demonstrates that the interaction effect was significant on nitrogen concentration in the vegetative part. The treatment inoculated with *Bradyrhizobium japonicum* and 40 kg N ha^{-1} fertilized treatment outperformed all the other treatments significantly. It was followed by the treatment inoculated with *Bradyrhizobium japonicum* and fertilized with 80 kg N ha^{-1}. No considerable distinction was observed between the control treatment and the non-inoculated treatment fertilized with 80 kg N ha^{-1}.

Table 9. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interactionon the The nitrogen concentration in the vegetative part (%) of mung bean during the flowering stage.

Nitrogen fertilizer (kg.ha^{-1})	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	1.479	2.670	2.929	2.359
40	1.642	2.858	3.153	2.551
80	1.522	2.769	3.004	2.431
Inoculation rate	1.547	2.765	3.028	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.072	0.072	0.13	

The Table 10. shows that the inoculation had a significant effect on the absorbed nitrogen quantity in the plant in the vegetative part during the flowering stage. The inoculated treatments outperformed The treatment without inoculation significantly, and the inoculated treatment with *Bradyrhizobium japonicum* surpassed the inoculated treatment with *Rhizobium leguminosarum* significantly. The increase in absorbed nitrogen quantity was (230.26) % higher than the non-inoculated treatment and (35.27) % higher than the inoculated treatment with *Rhizobium leguminosarum*, respectively.

The nitrogen fertilization demonstrated a significantly impact on the absorbed nitrogen quantity in the plant in the vegetative part. The 40 kg N ha^{-1} fertilized treatment outperformed the non-fertilized treatment and 80 kg N ha^{-1} fertilized treatment significantly. The average values for the fertilization treatments were (451.02, 542.09, 483.42) mg. plant^{-1} for the non-fertilized treatment, the treatment fertilized with 40 kg N ha^{-1}, and the treatment fertilized with 80 kg N ha^{-1}, respectively.

The interaction effect between the inoculation with *Rhizobium* and the nitrogen fertilization was significant on the absorbed nitrogen quantity in plant in the vegetative part. The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 40 kg N ha^{-1} outperformed all the other treatments significantly. It was followed by The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 80 kg N ha^{-1}. No considerable distinction was observed between the control treatment and the non-inoculated treatments fertilized with 40 and 80 kg N ha^{-1}, respectively.

Table 10. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interaction on the absorbed nitrogen in the vegetative part (mg. plant^{-1}) of mungbean plant during the flowering stage.

Nitrogen fertilizer (kg.ha^{-1})	Bacterial inoculation B0	B1	B2	Fertilization Rate
0	209.71	497.32	646.05	451.02
40	220.67	595.23	810.37	542.09
80	221.87	530,77	697.64	483.42
Inoculation rate	217.41	541.10	718.02	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	23.09	23.09	40.004	

The significant superiority in nitrogen concentration and absorbed nitrogen quantity in the vegetative part as a result of inoculation with *Bradyrhizobium japonicum* can be attributed to the efficiency of this bacteria in nodule formation and increasing its wet and dry weight Tables 3, 4, and 5. This, in turn, leads to increased nitrogen fixation from the atmosphere and its accumulation in plant tissues. Additionally, it is possible that this bacterium has the ability to absorb 1-aminocyclopropane-1-carboxylate deaminase)ACC) and convert it into Ketobutyrate and NH3, which serve as a carbon and nitrogen source [27]. Studies have shown that rhizobia produce ACC deaminase, which plays a role in regulating plant processes and acts as an effective nitrogen fixer controlled by the nif gene responsible for nitrogen fixation [28]. These findings are consistent with previous studies [29-31].

The significant increase in nitrogen concentration and absorbed nitrogen quantity in the vegetative part at the 40 kg N ha^{-1} level can be attributed to the fact that adding this nitrogen level increases the plant's ability to absorb more nitrogen, especially in the early stages before nodule formation, where it can be converted into proteins, nucleic acids, and other compounds required by the plants. Consequently, this leads to an increase in the absorbed nitrogen quantity. This finding is also supported by previous studies [32,33].

Moreover, the significant increase observed in the treatment inoculated with *Bradyrhizobium japonicum* and fertilized at the 40 kg N ha^{-1} level suggests that the beneficial interaction between inoculation with *Bradyrhizobium japonicum* and fertilization at this level improves the plant's utilization of available nitrogen in the soil, resulting in an increased absorbed nitrogen quantity in the vegetative part. This is consistent with the findings of [34], who demonstrated that the addition of rhizobia with low fertilization leads to an increase in soil nitrogen quantity, resulting in an increased nitrogen content in plants. This finding has been confirmed by [35]. These outcomes align with the discoveries of [36,37]

The Phosphorus and Potassium concentration in the Vegetative Part

Table 11 demonstrates the significant superiority of inoculated treatments in phosphorus concentration in the vegetative part of mung bean plants compared to the treatment without inoculation during the flowering stage. The treatment inoculated with *Bradyrhizobium japonicum* showed a significant advantage over The treatment without inoculation and the treatment inoculated *with Rhizobium leguminosarum*, with an increase of (211.57) % and (59.74%) respectively, compared to the treatment without inoculation and *Rhizobium leguminosarum*-inoculated treatments.

Moreover, the table reveals that nitrogen fertilization demonstrated a significantly impact on the phosphorus concentration in the vegetative part of mung bean plants. The 40 kg N ha^{-1} fertilized treatment exhibited significant superiority over the non-fertilized treatment and 80 kg N ha^{-1} fertilized treatment with average values of (0.243, 0.248, 0.245) % for the non-fertilized treatment, the treatment fertilized with 40 kg N ha^{-1}, and the treatment fertilized with 80 kg N ha^{-1}, respectively.

Furthermore, the interaction between inoculation with *Rhizobia* and fertilization with different nitrogen levels had a significant impact on the phosphorus concentration in the vegetative part of mung bean plants. The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 40 kg N ha^{-1} showed significant superiority over all other treatments. It was followed by The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 80 kg N ha^{-1}. No considerable distinction was observed between the control, non-inoculated, and fertilized with 80 kg N/ha^{-1} treatments.

Table 11. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interaction on the phosphorus concentration in the vegetative part (%) of mung bean plant during the flowering stage.

Nitrogen fertilizer ($kg.ha^{-1}$)	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	0.119	0.234	0.374	0.243
40	0.124	0.240	0.380	0.248
80	0.121	0.237	0.377	0.245
Inoculation rate	0.121	0.236	0.377	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.0019	0.0019	0.0033	

Table 12. demonstrates the significant superiority of inoculated treatments in potassium concentration in the vegetative part of mung bean plants compared to The treatment without inoculation during the flowering stage. The treatment inoculated with *Bradyrhizobium japonicum* showed a significant advantage over The treatment without inoculation and the treatment inoculated with *Rhizobium leguminosarum*, with average values of (1.168, 2.342, 3.562) % for The treatment without inoculation, *Rhizobium leguminosarum*-inoculated treatments and *Bradyrhizobium japonicum* -inoculated treatments, respectively.

Furthermore, the nitrogen fertilization demonstrated a significantly impact on the potassium concentration in the vegetative part of mung bean plants. The 40 kg N ha^{-1} fertilized treatment exhibited significant superiority over the non-fertilized treatment and 80 kg N ha^{-1} fertilized treatment, with average values of (2.338, 2.378, 2.356) % for the non-fertilized treatment, the treatment fertilized with 40 kg N ha^{-1}, and the treatment fertilized with 80 kg N ha^{-1}, respectively.

Additionally, the interaction between inoculation with *Bradyrhizobium japonicum* and fertilization with different nitrogen levels had a significant impact on the potassium concentration in the vegetative part of mung bean plants. The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 40 kg N ha^{-1} showed significant superiority over all other treatments. It was followed by The treatment inoculation with *Bradyrhizobium japonicum* and received fertilization at a rate of 80 kg N ha^{-1}. The control treatment had the lowest value, followed by the non-inoculated treatment and the treatment fertilized with 80 kg N ha^{-1}, followed by the non-inoculated treatment and the treatment fertilized with 40 kg N ha^{-1}, respectively.

Table 12. The impact of inoculation with *Rhizobium leguminosarum and Bradyrhizobium japonicum* bacteria, fertilization with three levels of nitrogen, and their interactionon Potassium concentration in the vegetative part (%) of mungbean plant during the flowering stage.

Nitrogen fertilizer ($kg.ha^{-1}$)	Bacterial inoculation			Fertilization Rate
	B0	B1	B2	
0	1.159	2.330	3.525	2.338
40	1.179	2.362	3.593	2.378
80	1.166	2.335	3.569	2.356
Inoculation rate	1.168	2.342	3.562	
L.S.D	Inoculation	Fertilization	Interaction	
0.005	0.012	0.012	0.021	

The significant increase in phosphorus and potassium concentration in the vegetative part as a result of inoculation with *Bradyrhizobium japonicum* could be attributed to the bacteria's ability to solubilize phosphates (Table 1) and enhance their availability, thus improving overall plant traits and specifically increasing phosphorus concentration [38]. It could also can be ascribed to the function or influence of bacteria in increasing plant nutrient uptake from the soil, leading to an increase in potassium content. This finding is consistent with [39-41].

The significant increase in phosphorus and potassium concentration when fertilized with 40 kg N ha^{-1} can potentially be attributed to the significant role of nitrogen, especially in leguminous plants, in the early stages before nodule formation. This improves plant growth, activates roots, and enhances nutrient uptake efficiency, including phosphorus and potassium. This result is in agreement with [42] and [43].

The significant superiority of The treatment inoculation with *Bradyrhizobium japonicum* and fertilized with 40 kg N ha^{-1} could be attributed to the beneficial interaction that contributed to an increase in phosphorus and potassium concentration in the vegetative part. This finding is consistent with [44] and [45].

Conclusion

Based on the present study, it can be inferred that the non-inoculated treatment failed to form root nodules. Additionally, the treatment inoculated with *Bradyrhizobium japonicum* showed significant superiority over the treatment inoculated with *Rhizobium leguminosarum* in terms of all studied traits during the flowering stage. The 40 kg N ha^{-1} fertilized treatment showed significant superiority over the 80 kg N/ha^{-1} fertilized treatment in all studied traits. The interaction treatment between inoculation with *Bradyrhizobium japonicum* and fertilization with 40 kg N ha^{-1} exhibited the highest values and showed significant superiority over all other treatments for all studied traits .

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مجلة جامعة كركوك للعلوم الزراعية

تقييم تأثير اللقاح المحضر من عزلات محلية لبكتريا الرايزوبيوم والتسميد النتروجيني في بعض صفات النمو لنبات الماش في تربة جبسية

مروان عبد العزيز شهاب		عبد الكريم عريبي سبع

Marwan.a.shehab@st.tu.edu.iq		alkurtany@tu.edu.iq

[1,2,3] قسم علوم التربة والموارد المائية، كلية الزراعة، جامعة تكريت، تكريت، العراق.

- تاريخ استلام البحث2023/08/13 وتاريخ قبوله 2023/08/23.
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الملخص

يتم استغلال بكتريا العقد الجذرية في إنتاج الأسمدة الحيوية الطبيعية لتحقيق الزراعة النظيفة من خلال تقليل استخدام الأسمدة الكيميائية، لذلك تم تحضير اللقاح البكتيري من عزلات محلية لبكتريا *Rhizobium leguminosarum* و*Bradyrhizobium japonicum* التي تم تشخيصها مظهرياً وجزيئياً، ومن ثم تم اختبار كفاءة اللقاح المحضر في تكوين العقد الجذرية وبعض صفات نمو نبات الماش في تربة جبسية عند ثلاث مستويات من السماد النتروجيني (80,40,0) كغم N هكتار$^{-1}$، وأظهرت النتائج وجود زيادة معنوية في جميع صفات نمو النبات في مرحلة التزهير للمعاملات الملقحة ببكتريا *Rhizobium leguminosarum* و*Bradyrhizobium japonicum* مقارنة بالمعاملة غير الملقحة اذ تفوقت المعاملات الملقحة معنوياً على المعاملة غير الملقحة ، وتفوقت المعاملة الملقحة ببكتريا *Bradyrhizobium japonicum* معنوياً على المعاملة الملقحة ببكتريا *Rhizobium leguminosarum* في جميع صفات النمو المدروسة، اذ سجلت القيم التالية في عدد العقد الجذرية (0.00، 35.11,8.33) عقدة.نبات$^{-1}$ ووزن العقد الرطب(1.802,0.091,0.00) غم.نبات$^{-1}$ ووزن العقد الجاف (0.337,0.010,0.00)، غم.نبات$^{-1}$ وارتفاع النبات (58.05,56.22،52.11) سم.نبات$^{-1}$ وفي الوزن الخضري والجذري الجاف (20.10,14.03 ، 23.05)غم.نبات$^{-1}$ و(1.37، 2.67,2.57) ومحتوى النتروجين (1.547، 3.028,2.765)% والفسفور (0.377,0.236,0.121)% والبوتاسيوم (3.562,2.342,1.168)% في الجزء الخضري والنتروجين الممتص في النبات(718.02,541.10,217.41) ملغم.نبات$^{-1}$ وللمعاملة غير الملقحة والمعاملة الملقحة ببكتريا *Rhizobium leguminosarum* والمعاملة الملقحة ببكتريا *Bradyrhizobium japonicum* على التتابع . واظهرت النتائج تفوق المعاملة المسمدة بالمستوى 40 كغم N هكتار$^{-1}$ معنوياً على المعاملة غير المسمدة وعلى المعاملة المسمدة بالمستوى 80 كغم N هكتار$^{-1}$ في جميع الصفات المدروسة. اما تأثير التداخل فكان معنوياً اذا تفوقت المعاملة الملقحة ببكتريا *Bradyrhizobium japonicum* والمسمدة بالمستوى 40 كغم N هكتار$^{-1}$ معنوياً على باقي المعاملات في جميع الصفات المدروسة.

الكلمات المفتاحية: Bradyrhizobium japonicum، Rhizobium leguminosarum، التلقيح، التسميد النتروجيني، الماش