



Rhizobacterial Inoculation to Enhance Growth and Productivity of Fenugreek

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ABSTRACT

The use of rhizobacterial inoculants represents a promising strategy to improve crop productivity and sustainability. This study investigates the impact of four different rhizobacterial strains on the growth and productivity of fenugreek (*Trigonella foenum-graecum*). Conducted under field conditions, the experiment followed a randomized complete block design with four replications. The treatments included inoculation with individual strains as well as a control with no inoculation. Key growth parameters, such as germination rate, plant height, biomass, nodulation, and seed yield, were systematically recorded. The results demonstrated significant enhancements in fenugreek growth and yield in response to rhizobacterial inoculation compared to the control. Strain F1 showed the highest increase in germination rate and plant height, while Strain F2 resulted in the maximum biomass accumulation. Strain F3 significantly improved nodulation and overall root development, contributing to better nutrient uptake. Strain F4 produced the highest seed yield, indicating its potential in enhancing fenugreek productivity. All inoculated treatments exhibited improved soil health indicators, such as increased microbial activity and nutrient availability, compared to the control. The combined analysis revealed that the use of these rhizobacterial strains not only promoted plant growth but also contributed to sustainable agricultural practices by enhancing soil fertility. This study underscores the potential of specific rhizobacterial strains in boosting fenugreek production and provides a foundation for the development of effective microbial inoculants. Further research on the synergistic effects of these strains and their application methods could optimize their benefits, offering a viable solution for enhancing crop yield and soil health in legume cultivation.

INTRODUCTION

The global demand for food production has significantly increased due to rapid population growth and urbanization, placing immense pressure on agricultural systems. In response, modern agriculture has traditionally relied heavily on synthetic fertilizers and chemical inputs to

maximize yields. However, these practices have raised serious concerns regarding their long-term environmental and economic sustainability. Continuous use of synthetic fertilizers can lead to soil degradation, loss of biodiversity, water contamination, and a decrease in soil organic

matter, ultimately reducing soil fertility and productivity over time ¹. Moreover, these inputs can be costly, particularly for small-scale farmers in developing countries, limiting their accessibility and long-term viability. Thus, there is a pressing need for sustainable agricultural practices that can maintain or enhance crop productivity while minimizing environmental impacts and reducing dependence on synthetic chemicals.

In recent years, biological solutions have gained prominence as sustainable alternatives in agriculture, focusing on leveraging natural processes to enhance soil health and crop growth. Among these solutions, beneficial rhizobacteria have emerged as promising microbial inoculants due to their multifaceted roles in promoting plant growth, enhancing nutrient availability, and improving soil structure. Rhizobacteria, commonly referred to as plant growth-promoting rhizobacteria (PGPR), are root-associated bacteria that establish symbiotic or associative relationships with plants, conferring multiple growth benefits. These bacteria play essential roles in nutrient cycling, such as nitrogen fixation, where atmospheric nitrogen is converted into a form usable by plants, and phosphate solubilization, which increases the bioavailability of phosphorus—a critical nutrient for plant growth ².

One of the most notable advantages of PGPR is their ability to produce phytohormones, such as auxins, gibberellins, and cytokinins, which directly stimulate plant growth and enhance root development. These hormones can improve the plant's ability to absorb water and nutrients, leading to healthier and more resilient crops. Additionally, rhizobacteria can produce secondary metabolites and enzymes that help plants withstand biotic stresses, such as diseases caused by pathogens, and abiotic stresses, including drought, salinity, and extreme temperatures ³. This attribute is particularly valuable in the context of climate change, where unpredictable weather patterns can negatively impact crop yields. Thus, integrating rhizobacterial inoculants into agricultural systems aligns with the goals of sustainable agriculture by not only promoting higher productivity but also enhancing crop resilience and reducing the ecological footprint of farming practices.

Fenugreek (*Trigonella foenum-graecum*), a leguminous plant, is highly valued for its seeds and leaves, which are used for culinary, medicinal, and

industrial purposes. Its seeds are rich in proteins, fibers, and bioactive compounds such as diosgenin, which is associated with various health benefits, including antidiabetic, anti-inflammatory, and antioxidant properties ⁴. The crop's ability to fix atmospheric nitrogen through symbiotic relationships with rhizobia makes it particularly suitable for sustainable farming systems, as it reduces the need for nitrogen fertilizers. Despite these advantages, fenugreek productivity is often limited by inadequate soil fertility, particularly in nutrient-poor or degraded soils. Additionally, poor nodulation—a process critical for nitrogen fixation—can further constrain fenugreek growth and yield potential. This limitation underscores the importance of exploring effective bio-inoculants to enhance fenugreek productivity.

The application of rhizobacterial inoculants holds significant promise for improving fenugreek production by promoting nutrient uptake, enhancing root development, and improving soil structure. Several studies have demonstrated the effectiveness of rhizobacteria in enhancing growth parameters such as root biomass, nodulation, and yield in various legume crops, including chickpea, soybean, and lentils. However, research specifically targeting fenugreek remains limited, creating a knowledge gap regarding the potential benefits of rhizobacterial inoculants for this crop ⁵. Given fenugreek's growing economic importance and its potential as a sustainable crop, identifying and utilizing effective rhizobacterial strains could offer valuable agronomic and environmental benefits.

This study aims to investigate the impact of four selected rhizobacterial strains on the growth and productivity of fenugreek under field conditions. This study supports the broader goal of integrating biological solutions into modern agriculture, aligning with global efforts to create resilient, efficient, and environmentally friendly farming systems.

MATERIALS AND METHODS

Study Site and Experimental Conditions

The field experiment was conducted at the research area of Agricultural Biotechnology Research Institute, Ayub Agriculture Research Institute, Faisalabad over a growing season 2023-2024. The study area is characterized by an arid climate with average rainfall of 300 mm and 4-49 °C

temperature. Soil at the experimental site was analyzed prior to planting, revealing a light textured sandy loam soil having 8.2 pH, 0.56% organic matter content, and very low 0.1% N and 7.4 ppm P contents.

Plant Material and Rhizobacterial Strains

Fenugreek (*Trigonella foenum-graecum*) seeds were sourced from a certified supplier. Four rhizobacterial strains—designated as F₁, F₂, F₃, and F₄—were selected for this study based on previous screening for their plant growth-promoting properties. These strains were isolated from the rhizosphere of fenugreek.

Experimental Design and Treatments

The experiment followed a randomized complete block design (RCBD) with four replications to ensure statistical robustness. Each replication included five treatments:

Control (No inoculation)

Strain F₁

Strain F₂

Strain F₃

Strain F₄

Each treatment plot measured 5x5 m² with a buffer zone between plots to prevent cross-contamination of inoculants. Inoculation was performed by coating fenugreek seeds with a standardized bacterial suspension (10⁸ CFU/ml) before planting.

Agronomic Practices

Standard agronomic practices, including soil preparation, irrigation, and weed control, were followed uniformly across all plots. Fertilizers (DAP and SOP) were applied at recommended rates as basal dose, nitrogen was enough as provided by DAP and need not to apply in splits as nitrogen fixation by rhizobacterial inoculants was expected to supplement nitrogen needs.

Data Collection

Data were collected at key growth stages to assess the impact of rhizobacterial inoculation on fenugreek. The parameters recorded included:

- **Germination rate:** measured as the percentage of seeds germinated within the first two weeks.
- **Plant height:** measured at regular intervals from germination to harvest.
- **Biomass:** assessed by harvesting plants at maturity and recording fresh and dry weights.

- **Nodulation:** the number and size of nodules per plant were recorded at flowering.
- **Seed yield:** total seed weight per plant was recorded at harvest.

Additionally, soil samples were taken before planting and after harvest to assess changes in microbial activity, nutrient availability, and overall soil health.

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) to determine the significance of treatment effects. The Least Significant Difference (LSD) test was applied for post-hoc comparisons between treatments at a 5% significance level ⁵. Statistical analysis was performed using Statistix 8.1® software.

RESULTS AND DISCUSSION

Germination Rate and Early Growth

Rhizobacterial inoculation significantly improved the germination rate compared to the control ($p < 0.05$) depicted in table 1. Strain F₁ resulted in the highest germination rate (93.45%) followed by Strains F₂ and F₃, suggesting that these strains may enhance seed vigor through phytohormone production or other mechanisms. This finding aligns with previous studies indicating that specific rhizobacteria can accelerate germination by modulating hormonal balances ⁶.

Table 1

Effect of rhizobacterial inoculation on germination plant height and biomass of fenugreek

Rhizobacterial Strains	Germination Rate (%)	Plant Height (cm)			Plant Biomass (g)
		14 DAS	28 DAS	42 DAS	
F ₀	79.18 c	6.42 c	15.02 d	21.32 d	42.31 d
F ₁	93.45 a	7.92 a	18.21 a	25.01 a	52.48 b
F ₂	91.02 b	7.86 a	18.18 a	24.78 ab	54.06 a
F ₃	90.91 b	7.71 b	18.00 b	24.63 b	51.62 bc
F ₄	91.13 b	7.72 b	17.78 c	24.16 c	51.22 c
LSD	0.81	0.08	0.14	0.29	1.23

Plant Height and Biomass Accumulation

Plant height measurements taken at 2-week intervals revealed that inoculation with Strains F₁ and F₂ resulted in significantly taller plants compared to the control (Table 1). By maturity, plants treated with Strain F₂ exhibited the highest

biomass, with an increase of 27.8% over the control. The increased biomass in inoculated plants is likely due to improved nutrient uptake facilitated by rhizobacterial activity in the rhizosphere, which enhances root growth and access to soil nutrients ².

Nodulation and Root Development

Table 2 depicts that the application of strain F₃ demonstrated a pronounced effect on nodulation, with treated plants exhibiting a higher number of nodules and improved root architecture compared to other treatments. Enhanced nodulation is a key indicator of effective symbiotic relationships between legumes and rhizobacteria, facilitating nitrogen fixation and benefiting overall plant health. These results support previous findings that highlight the role of specific rhizobacterial strains in promoting nodulation ⁸.

Seed Yield and Productivity

Table 2 depicts that the application of strain F₄ led to a significant increase in seed yield, achieving an average of 1.99 g/plant compared to 1.12 g/plant in the control. This suggests that strain F₄ may enhance reproductive success, possibly through improved nutrient acquisition or by modulating stress responses. Notably, all inoculated treatments showed higher seed yields than the control, indicating the general efficacy of rhizobacterial inoculants in boosting fenugreek productivity ⁹.

Table 2

Effect of rhizobacterial inoculation on nodulation, root development and seed yield of fenugreek

Rhizobacterial Strains	Nodules Count (Plant ⁻¹)	Root Length (cm)	Seed Yield (g plant ⁻¹)
F ₀	12.13 d	9.63 d	1.12 c
F ₁	13.51 b	13.52 b	1.93 a
F ₂	11.42 c	10.88 c	1.67 b

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F ₃	16.91 a	10.91 c	1.70 b
F ₄	11.12 c	14.22 a	1.99 a
LSD	0.63	0.39	0.07

Soil Health Indicators

Post-harvest soil analysis revealed in Table 3 indicates enhanced microbial activity and nutrient availability in inoculated plots. Treatments with Strains F₂ and F₃ were particularly effective, showing increased levels of soil organic matter and nutrients. This improvement in soil health highlights the potential of rhizobacterial inoculants not only for immediate crop productivity but also for long-term soil sustainability.

Table 3

Effect of rhizobacterial inoculation on soil organic matter and nutrients

Rhizobacterial Strains	Organic Matter (g kg ⁻¹)	Soil N (%)	Soil P (mg kg ⁻¹)
F ₀	42.3 d	0.08 c	9.17 d
F ₁	63.92 c	0.12 b	13.23 c
F ₂	73.10 a	0.15 a	15.77 a
F ₃	73.03 a	0.14 a	15.71 a
F ₄	71.12 b	0.14 a	15.06 b
LSD	1.27	0.01	0.27

CONCLUSION

The positive effects of rhizobacterial inoculation on growth, nodulation, and yield underscore the potential of these strains as bio-inoculants for fenugreek. Each strain exhibited unique benefits, with Strain F₁ enhancing early growth, strain F₂ boosting biomass, strain F₃ improving nodulation, and strain F₄ increasing seed yield. These findings suggest that a combination or consortium of these strains might further optimize growth and yield, warranting further investigation.

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