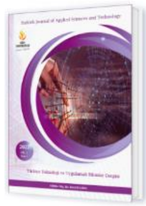
Türkiye Teknoloji ve Uygulamalı Bilimler Dergisi



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Optimizing Fenugreek (*Trigonella foenum-graecum* L.) Cultivation: Role of *Rhizobium* Inoculation and Phosphorus Fertilization on Growth, Nodulation, and Yield Attributes

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Abstract

Rhizobium inoculation and phosphorus fertilization play a crucial role in enhancing the growth, nodulation, and yield attributes of fenugreek by improving nitrogen fixation. Aim of the study is to explain co-application of *Rhizobium* and phosphorus treatment on the agronomic performance of fenugreek under rainfed conditions. This study was conducted in a split-plot randomized complete block design with four replications, where *Rhizobium* inoculation served as the main factor, and phosphorus doses as subplots. The results showed that *Rhizobium* inoculation significantly increased biological yield and seed yield by 22.3% and 52.4%, respectively, demonstrating the effectiveness of biological nitrogen fixation. The 60 kg P_2O_5 ha^{-1} fertilization caused the highest plant height (22.7 cm), number of pods per plant (15.0), and seed yield (135 kg da^{-1} seed yield). Furthermore, nodule formation showed a linear increase with phosphorus application from 3.33 to 9.00. The best agronomic performance was determined in *Rhizobium* application with 60 kg P_2O_5 ha^{-1} phosphorus dosage treated plants, therefore, this fertilization strategy optimally supports plant growth and productivity. According to results, it is recommended that fenugreek cultivation incorporate *Rhizobium* inoculation along with 60 kg P_2O_5 ha^{-1} to maximize yield potential. Future research should focus on the long-term effects of these treatments on soil fertility and sustainability to further refine fertilization strategies for fenugreek and other leguminous crops.

Keywords: Feritilization management, Legumes, Nitrogen fixation, Rhizobia, Symbiosis.

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual leguminous plant belonging to the Fabaceae and is widely cultivated for its medicinal, culinary, and agronomic benefits. It is native to the Mediterranean region and parts of Asia but is now cultivated globally, particularly in India, China, Egypt, and Türkiye (1). Fenugreek is primarily grown in the Central Anatolian and Aegean regions, with annual production increasing due to its expanding culinary and pharmaceutical applications in Türkiye.

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The global production of fenugreek is estimated at approximately 110,000 metric tons per year, with India being the largest producer, contributing over 80% of the global supply (2). Fenugreek production in Türkiye is primarily concentrated in the Central Anatolia and Aegean regions, with an annual production volume ranging between 1,500 and 2,000 tons. According to data published by the Ministry of Agriculture and Forestry, fenugreek production has increased in recent years, and its use has become widespread, particularly in the spice and pharmaceutical industries (3). This plant is renowned for its diverse applications, including use as a spice, fodder, and medicinal herb with hypoglycemic, antimicrobial, and anti-inflammatory properties (4). Additionally, fenugreek seeds contain high levels of dietary fiber, proteins, and bioactive compounds such as saponins and flavonoids, making them valuable in pharmaceutical and nutraceutical industries (5). Seed compound, quality and yield depends on environmental conditions such as climate, soil and fertilization.

Fertilization is a crucial agronomic practice that directly influences plant growth, yield, and quality. Phosphorus (P) has a pivotal role in root development, energy transfer, and overall plant metabolism (6). Phosphorus application enhances nodulation in legumes, thereby improving nitrogen fixation and plant growth (7). However, phosphorus availability is often limited due to its low mobility in the soil and required external application to optimize crop productivity. Phosphorus fertilization increases seed yield and biomass production (8). In addition to phosphorus, nitrogen (N) is another essential nutrient for plant development and root proliferation. Nair et al. (9) indicated that 45 kg P_2O_5 ha^{-1} increased seed yield by 46.7% in fenugreek. Nahar et al. (10) determined that phosphorus fertilization (45 kg ha^{-1}) promoted seed yield in fenugreek from 0.85 ton ha^{-1} to 1.92 ton ha^{-1}, i.e., by 126%. Similarly, Talaviya and Patel (11) indicated yield increase in fenugreen with phosphorus fertilization. On the other hand, fenugreek can fix atmospheric nitrogen through symbiosis with rhizobia, thereby reducing the need for synthetic nitrogen fertilizers (12). Understanding the interactive effects of phosphorus and nitrogen fertilization is therefore critical for optimizing fenugreek production while promoting sustainable agriculture.

Biological nitrogen fixation (BNF) is a key ecological process in legumes, involving symbiotic interactions with *Rhizobium* bacteria in root nodules (13, 14). The process begins when *Rhizobium* recognizes plant root exudates and invades the root hairs, forming nodules where atmospheric nitrogen is converted into ammonia through the nitrogenase enzyme system (15). However, effective nitrogen fixation requires optimal environmental conditions, including sufficient phosphorus levels, appropriate soil pH, and adequate moisture (16). *Rhizobium* inoculation is often recommended in soils with poor microbial populations or when introducing legumes into new cultivation areas. Studies indicate that inoculation can enhance nitrogen fixation efficiency, leading to increased nitrogen accumulation in plant tissues and higher crop yields (14). Nair et al. (9) reported that Rhizobium, inoculation and phosphorus feritilization exhibited a synergistic effect on seed yield and yield attributed.

Aim of the study is to explain co-application of *Rhizobium* and phosphorus treatment on the agronomic performance of fenugreek. This research seeks to explain by evaluating how phosphorus application influences *Rhizobium* effectiveness and overall plant performance. The findings will contribute to optimizing fertilization strategies for fenugreek, promoting sustainable agricultural practices, and reducing dependence on synthetic fertilizers.

2. Materials and Methods

2.1. Experimental Material

Gürarslan fenugreek (*Trigonella foenum* graecum L.) variety, which was registered by Ankara University, Faculty of Agriculture at 2004, was used in the experiment (3). Effective *Rhizobium* strain, *Sinorhizobium meliloti*, was obtained from Republic of Türkiye Ministry of Agriculture and Forestry Soil, Fertilizer and Water Resources Central Research Institute (Ankara). Triple superphosphate was used for phosphorus doses.

2.2. Experimental Area

The experiment was conducted in Van Yüzüncü Yıl University, Van, Türkiye. The experiment was laid out during 2005-2006 growing season under rainfed conditions. The experimental area was located at 38°33′ N and 43°17′ E. The altitude was 1651 m.

2.3. Climatological and Soil Characterization of Experimental Area

Total precipitation for 2005-06 was 391.9 mm, while the long-years average (LYA) was 385.7 mm. The highest precipitation was recorded in January (90.4 mm), whereas the lowest one was in June (0.1 mm). The total precipitation in 2005-06 was close to the long-term average, indicating no significant drought or excessive rainfall. The average temperature for 2005-06 was 10.5 °C, slightly higher than the LYA of 9 °C. The coldest month was January (-3.1 °C), while the hottest one was June (21.5 °C). Overall, temperatures in 2005-06 were slightly above the long-term averages. The average relative humidity for 2005-06 was 60.5%, compared to 61.3% in LYA. The highest humidity was recorded in March (77.5%), while the lowest one was in June (41.9%). The relative humidity generally followed the long-term pattern but showed a noticeable decrease during summer months (Table 1).

The soil reaction (pH) and electrical conductivity (EC) were determined using pH-EC meter that depends on 1:2.5 soil-water mixture method. Texture was analyzed using the Bouyoucus hydrometer technique. Organic matter was determined by method of De Vos et al. (18). Lime content was determined using calcimeter method. Phosphorus was quantified by spectrophotometric sodium bicarbonate method (19), while available potassium was measured using the ammonium acetate method using flame photometer (20). Soil samples were taken from 0-30 cm depth. Soil texture is sandy loam and pH is notr. It has no salt and very low organic matter. Soil is moderately calcareous. Phosphorus and potassium are high (Table 2).

Table 1. Climatological characterization for experimental area during 2005-06 and long years

Months	Precipitation (mm)		Average temperature (°C)		Relative humudity (%)	
	2005-06	LYA	2005-06	LYA	2005-06	LYA
September	9.2	13.0	17.2	17.2	55.4	44.0
October	35.4	45.2	11.2	10.6	56.9	58.0
November	29.3	47.9	4.6	4.4	69.1	66.0
December	34.3	37.3	1.9	-0.8	69.0	69.0
January	90.4	35.4	-3.1	-3.6	73.7	68.0
February	47.7	32.5	-1.3	-3.2	74.2	69.0
March	45.7	45.7	3.0	0.9	77.5	68.0
April	39.6	56.6	9.8	7.4	66.5	62.0
May	35.4	45.0	14.6	13	54.0	56.0
June	0.1	18.5	21.5	18	41.9	50.0
July	22.4	5.2	2.3	22.2	47.5	44.0
Total	391.9	385.7				
Mean			10.5	9	60.5	61.3

Table 2. Physiochemical characterization of experimental soil

Depth (cm)	Texture -	pH -	Lime (%)	OM (%)	Salt (%)	P (mg kg^{-1})	K (mg kg^{-1})
0-30	Sandy loam	7.4	14.6	0.91	0.18	4.8	154

(OM: Organic matter)

2.4. Experimental Design

The experiment was conducted in split-plot randomized complete block design with four replications. Main plots were constituted with *Rhizobium* inoculation (*Rhizobium* and control), while subplots received phosphorus doses which were set as P0 (control), P1 (20 kg kg P_2O_5 ha^{-1}), P2 (40 kg P_2O_5 ha^{-1}), and P3 (60 kg P_2O_5 ha^{-1}). A 25 kg ha^{-1} pure nitrogen in ammonium sulphate (NH_4SO_4) form was treated as starter dose in all plots during sowing time.

The inter-plot and block distances were set at 1 m and 1.5 m, respectively. The plot width and length were arranged as 1.2 m and 4 m, respectively, establishing a total area of 4.8 m^2. Row spacing was 30 cm, with each plot consisting of 5 rows. Planting rate was adjusted to 30 kg ha^{-1} (21). Seeds were manually sown on September.

Mechanical weed control was implemented from seedling emergence, as needed, throughout the growing season. The experiment was conducted without irrigation. A 0.5 m from both ends of the plant rows and the side border rows were trimmed to avoid edge effect and 1.8 m^2 of area was harvested.

2.5. Experimental Observations

Ten plants were collected from plots before harvest to determine number of nodule, plant height, number of pods per plant, number of seeds per plant and seed yield per plant. Plants were manually harvested and air-dried. Total weight was determined for biological yield. Straw and seeds were seperated and seed yield was calculated for hectare. 1000-seed weight was determined and harvest index was calculated.

2.6. Statistical Assesment of Data

Analysis of variance (ANOVA) was subjected to data and significant degree ($p<0.05$ or 0.01) were calculated. Grouping of means were determined using Tukey HSD via JMP software.

3. Results

Rhizobium inoculation showed significant differences ($p<0.05$ and0.01) on number of seeds per plant, seed yield per plant, biological yield and seed yield. On the other hand, phosphorus treatment caused significant differences in all characteristics except harvest index. RxP interaction had no significant impact on investigated characteristics.

Phosphorus treatments caused statistically significant differences ($p<0.01$) in plant height and number of pods per plant, while *Rhizobium* inoculation and RxP interaction were not statistically significant. Plant height changed between 17.1-22.7 cm according to experimental treatments. Phosphorus treatments significantly affected plant height and the lowest plant height (17.2 cm) was determined in without phosphorus treated plants, however, there was no significant difference with 2 kg P_2O_5 da^{-1} and 4 kg P_2O_5 da^{-1} treated ones. The highest plant height (22.7 cm) was obtained with 6 kg P_2O_5 da^{-1} treatment. Number of pods per plant changed between 8.8-15.7 with treatments. According to phosphorus doses, the lowest number of pods per plant (9.9) was determined with control, while the highest one (15.0) was obtained from 6 kg P_2O_5 da^{-1} treated plants (Table 3).

Table 3. Efficacy of *Rhizobium* and phosphorus implementation on plant height and number of pods per plant in fenugreek

	Plant Height (cm)				Number of pods per plant		
	RZ (-)	**RZ (+)**	**Mean P**		**RZ (-)**	**RZ (+)**	**Mean P**
Control	17.1	17.3	17.2 B	Control	8.8	11	9.9 C
P1	18.5	18.7	18.6 B	P1	10.8	12	11.4 B
P2	19.4	19.7	19.6 B	P2	11.3	12.8	12.1 B
P3	22.6	22.7	22.7 A	P3	14.3	15.7	15.0 A
Mean RZ	19.4	19.6		Mean RZ	11.3	12.9	
Sum of Square: R: 0.22, P: 97.3, RxP: 0.031				Sum of Square: R: 14.3, P: 81.9 RxP: 0.865			
F ratio: R: 0.011, P: 7.27**, RxP: 0.0023				F ratio: R: 2.695, P: 37.1**, RxP: 0.392			

(RZ (-): No *Rhizobium* inoculation, RZ(+): *Rhizobium* inoculation)

While *Rhizobium* inoculation significantly affected number of seeds per plant ($p<0.05$) and seed yield per plant ($p<0.01$), phosphorus treatment caused significant differences ($p<0.01$) in both of them. *Rhizobium* inoculation increased number of seeds per plant and seed yield per plant by 36.8% and 52.4% over control, respectively. Number of seeds per plant increased from 68.6 (control) to 128.9 (6 kg P_2O_5 da^{-1}). Similarly, the lowest seed yield per plant (0.925 g) was observed in control plants while the highest one (1.808 g) was determined with 6 kg P_2O_5 da^{-1} treated plants. Increasing in number of seeds and seed yield per plant depending on phosphorus treatmens achieved up to 87.9% and 95.5%, respectively. In general, number of seeds per plant changed between 57.7-144.3 while seed yield per plant varied from 0.733 g to 2.017 g (Table 4).

Table 4. Efficacy of *Rhizobium* and phosphorus implementation on number of seeds per plant and seed yield per plant in fenugreek

	Number of seeds per plant				**Seed yield per plant (g)**		
	RZ (-)	**RZ (+)**	**Mean P**		**RZ (-)**	**RZ (+)**	**Mean P**
Control	57.7	79.5	68.6 B	Control	0.733	1.117	0.925 B
P1	66.5	91.3	78.9 B	P1	0.817	1.373	1.095 B
P2	66	100.2	83.1 B	P2	0.917	1.693	1.305 B
P3	113.5	144.3	128.9 A	P3	1.6	2.017	1.808 A
Mean RZ	75.9 B	103.8 A		Mean RZ	1.017 B	1.550 A	
Sum of Square: R: 4677, P: 12864, RxP: 141				Sum of Square: R: 1.707, P: 2.640, RxP: 0.144			
F ratio: R: 49.7*, P: 22.7**, RxP: 0.250				F ratio: R: 104.7**, P: 14.6**, RxP: 0.797			

(RZ (-): No *Rhizobium* inoculation, RZ(+): *Rhizobium* inoculation)

Rhizobium inoculation caused statistically significant differences at a ratio of 5% in both biological and seed yield, while phosphorus treatment significantly ($p<0.01$) affected them. *Rhizobium* inoculation increased biological yield from 587.8 kg da^{-1} to 719.1 kg da^{-1}, i.e., 22.3%. Phosphorus doses lineerly increased biological yield over control in which it varied between from 530 kg da^{-1} to 809 kg da^{-1}. Biological yield changed between 57.7-144.3 kg da^{-1} according to RxP treatment, but it was not statistically significant. *Rhizobium* inoculation promoted seed yield by 20.1%. Seed yield increased from 88.6 kg da^{-1} (control) to 135 kg da^{-1} (6 kg P_2O_5 da^{-1}) and the differences ratio was about 52.4%. In general, seed yield varied between 76.9-144.9 kg da^{-1} (Table 5).

Table 5. Efficacy of *Rhizobium* and phosphorus implementation on biological yield and seed yield in fenugreek

	Biological yield (kg da-1)				**Seed yield (kg da-1)**		
	RZ (-)	**RZ (+)**	**Mean P**		**RZ (-)**	**RZ (+)**	**Mean P**
Control	461.1	606.9	530.0 C	Control	76.9	101.2	88.6 C
P1	565.5	682.4	616.3 B	P1	94.3	113.7	105.3 B
P2	591.6	714.5	658.3 B	P2	98.6	119.1	110.0 B
P3	748.2	869.5	809.0 A	P3	124.7	144.9	135.0 A
Mean RZ	587.8 B	719.1 A		Mean RZ	99.7 B	119.7 A	
Sum of Square: R: 103491, P: 245023, RxP: 1084				Sum of Square: R: 2399, P: 6616, RxP: 2383			
F ratio: R: 15.31*, P: 77.80**, RxP: 0.344				F ratio: R: 7.79*, P: 84.82**, RxP: 0.306			

(RZ (-): No *Rhizobium* inoculation, RZ(+): *Rhizobium* inoculation)

1000-seed weight was just significantly ($p<0.05$) affected with phosphorus treatments. According to phosphorus treatments, the lowest 1000-seed weight (13.1 g) was determined in control whereas the highest one (17.5 g) was observed in 6 kg P_2O_5 da^{-1} treated plants, but it was in the same statistical group with 4 kg P_2O_5 da^{-1} treated ones (17.3 g). 1000-seed weight changed between 12.2-18.3

g. Finally, harvest index was not affected by neither experimental treatments nor their interaction. Harvest index varied between 16.60-17.61% (Table 6).

Table 6. Efficacy of *Rhizobium* and phosphorus implementation on 1000-seed weight and harvest index in fenugreek

	1000-seed weight (g)				Harvest index (%)		
	RZ (-)	**RZ (+)**	**Mean P**		**RZ (-)**	**RZ (+)**	**Mean P**
Control	12.2	14	13.1 B	Control	16.7	16.74	16.72
P1	14.7	14.4	14.5 AB	P1	17.61	16.65	17.13
P2	17	17.7	17.3 A	P2	16.85	16.6	16.73
P3	16.7	18.3	17.5 A	P3	16.74	16.64	16.69
Mean RZ	15.1	16.1		Mean RZ	16.98	16.66	
Sum of Square: R: 5.61, P: 83.9, RxP: 4.41				Sum of Square: R: 0.617, P: 0.804, RxP: 0.889			
F ratio: R: 0.556, P: 5.271*, RxP: 0.277				F ratio: R: 1.411, P: 0.618, RxP: 0.684			

(RZ (-): No *Rhizobium* inoculation, RZ(+): *Rhizobium* inoculation)

Nodule formation was not observed in non-inoculated plants, therefore, data were compared just depending on phosphorus levels.and not subjected to statistical analysis. There was a lineearly increase with phosphorus doses. The lowest number of nodule (3.33) was determined in control plants whereas the hghest one (9.00) was observed in 60 kg P_2O_5 ha^{-1} treated plants. Thus, 60 kg P_2O_5 ha^{-1} caused a 2.7-fold increase in number of nodules.

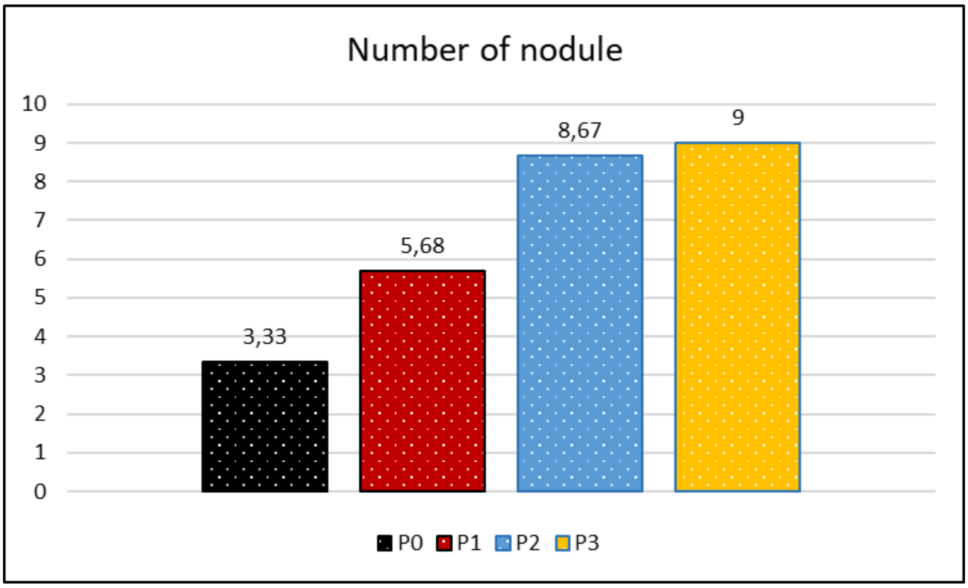

Figure 1. Number of nodules with changing phosphorus doses

4. Discussion

The results demonstrated that implementation of *Rhizobium* and phosphorus influenced the growth, nodulation, and yield attributes of fenugreek. The highest seed and biological yield were observed in co-application of *Rhizobium* and phosphorus, confirming that these factors play complementary roles in legume productivity. Similar findings have been reported in previous studies, where the combined application of phosphorus and *Rhizobium* inoculation enhanced yield and nitrogen fixation in legumes such as chickpea, soybean and lentil (22-24).

The effect of *Rhizobium* inoculation was particularly evident in increased seed yield and biological yield, as well as a higher number of seeds per plant compared to non-inoculated controls. This aligns with the role of *Rhizobium* in biological nitrogen fixation, where atmospheric nitrogen is converted into plant-available ammonia, supporting plant growth and productivity (15). Notably, nodule formation was only observed in *Rhizobium*-inoculated plants, confirming that natural soil populations were insufficient for effective nitrogen fixation. This finding is consistent with earlier reports suggesting that inoculation is necessary when native rhizobia populations are low or ineffective (16). Phosphorus deficiency has been shown to reduce nodule number and function in legumes, as nodulation is an energy-intensive process requiring ATP derived from phosphorus metabolism (7). The observed linear increase in nodule formation with phosphorus application suggests that adequate phosphorus supply enhances *Rhizobium* activity, leading to improved nitrogen fixation efficiency.

Phosphorus fertilization significantly influenced plant height, number of pods per plant, and overall yield, demonstrating its vital role in legume growth and development. The highest seed yield was obtained with the highest phosphorus dose (60 kg P_2O_5 ha^{-1}), suggesting that phosphorus was a limiting factor for fenugreek productivity. This finding aligns with previous research showing that phosphorus is essential for root development, nutrient uptake, and reproductive growth in legumes (25-27). The increased number of pods per plant in phosphorus-treated plots indicates that phosphorus contributes to enhanced reproductive success, likely by improving plant energy status and facilitating flower and pod formation (28). Ceritoglu et al. (29) determined that phosphorus fertilization boosts nutrient uptake and biofortification of lentil plants. Özyazıcı and Açıkbaş (30) persuaded 8-8.5 kg P_2O_5 ha^{-1} and 9.7 kg P_2O_5 ha^{-1} addition for the highest herbage and seed yield in narbon vetch (*Vicia narbonensis* L.), respectively. Erman et al. (31) pointed out that phosphorus addition promotes nitrogen, manganese, copper and iron content in bean (*Phaseolus vılgaris*) seeds while it increases nitrogen status of straw.

5. Conclusion

The highest seed yield (135 kg da^{-1}) and biological yield (809 kg da^{-1}) were obtained with the combined application of *Rhizobium* inoculation and 60 kg P_2O_5 ha^{-1}, highlighting the synergistic effects of nitrogen fixation and phosphorus availability. Notably, *Rhizobium* inoculation increased seed yield per plant by 52.4%, while phosphorus application enhanced plant height (22.7 cm) and pod formation (15.0 pods per plant). Nodule formation showed a linear increase with phosphorus application, confirming its essential role in enhancing *Rhizobium* activity. Based on these findings, it is recommended that fenugreek cultivation under rainfed conditions should incorporate both *Rhizobium* inoculation and phosphorus fertilization to maximize productivity. Future studies should explore the long-term effects of these treatments on soil health and optimize phosphorus application rates to prevent nutrient imbalances while maintaining sustainable crop production.

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