

Effect of Microbial and Chemical Fertilization on Lentil Growth, Yield and Quality under Semi-Arid Conditions

Yarı Kurak Koşullarda Mikrobiyal ve Kimyasal Gübrelemenin Mercimek Gelişimi, Verimi ve Kalitesi Üzerine Etkisi

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Abstract

Sustainable agricultural practices and effective plant nutrition strategies are increasingly important for both yield improvement and the conservation of natural resources. This study aimed to investigate the synergistic effects of microbial and chemical fertilization on lentil (*Lens culinaris* Medik.) growth, yield, and nutrient composition under the ecological conditions of Van, Türkiye. The experiment evaluated the interaction between *Rhizobium leguminosarum* (RHZ), phosphate-solubilizing bacteria strain (B), and nitrogen fertilizer (NF) across two growing seasons. The findings revealed that both year-to-year climatic variability and treatment combinations significantly affected plant performance. Among treatments, RHZ+NF+B consistently resulted in superior outcomes in biological and grain yields of 2992 and 1302 kg ha⁻¹, representing 19.5% and 28.9% increases over the control, respectively. This integrated approach also boosted grain N, P, and K contents by 11.5%, 34.6%, and 35%, respectively, thereby indicating improved nutrient uptake and mobilization. In contrast, combined application of NF with RHZ and B reduced yield productivity since nitrogen has inhibitory effect on nodulation and nitrogenase enzyme activity. Number of plants per square meter, which ranged from 55.3 to 120.8, was a key determinant of total biomass and pod number per plant, therefore, the higher emergence rates in the first season were likely driven by more favorable climatic conditions. Nutrient analysis showed that integrated microbial-chemical treatments enhanced macronutrient accumulation, however, micronutrient responses exhibited differences. Notably, Fe and Cu concentrations decreased in high-yielding plots, whereas Zn and Mn levels remained relatively stable. The study highlights the importance of tailoring nutrient input strategies to support both biological nitrogen fixation and optimal nutrient composition. It also reinforces the potential of integrated biofertilization practices in promoting sustainable legume production under semi-arid conditions. These results highlight balanced N fertilizer to support biological N₂ fixation. Further research is recommended to focus on long-term soil health impacts the long-term impacts on soil health.

Keywords: Bio-priming, *Lens culinaris*, Plant nutrition, Sustainable agriculture, Synergistic action

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Öz

Sürdürülebilir tarım uygulamaları ve etkili bitki besleme stratejileri hem verim artışı hem de doğal kaynakların korunması açısından giderek daha fazla önem kazanmaktadır. Bu çalışma, mikrobiyal ve kimyasal gübrelemenin mercimek (*Lens culinaris* Medik.) gelişimi, verimi ve besin bileşimi üzerindeki sinerjik etkilerini Van ekolojik koşullarında araştırmayı amaçlamıştır. Çalışmada, *Rhizobium leguminosarum* (RHZ), fosfat çözücü bakteri straini (B) ve azotlu gübre (NF) uygulamalarının etkileşimi, iki büyüme sezonu boyunca değerlendirilmiştir. Bulgular, yıllar arası iklim değişkenliği ve uygulama kombinasyonlarının bitki performansı üzerinde önemli etkiler yarattığını göstermiştir. Uygulamalar arasında RHZ+NF+B kombinasyonu, biyolojik ve tane verimi açısından en yüksek değerleri (sırasıyla 2992 ve 1302 kg ha⁻¹) üretmiş, bu da kontrole göre sırasıyla %19.5 ve %28.9 oranında artış anlamına gelmiştir. Bu entegre yaklaşım aynı zamanda tanede N, P ve K içeriğini sırasıyla %11.5, %34.6 ve %35 oranlarında artırarak besin alımı ve mobilizasyonunun iyileştiğini ortaya koymuştur. Buna karşılık, NF'nin RHZ ve B ile birlikte uygulanması, azotun nodül oluşumu ve nitrojenaz enzim aktivitesi üzerindeki baskılayıcı rolü nedeniyle verimliliği azaltmıştır. Ayrıca, metrekaredeki bitki sayısı 55.3 ile 120.8 arasında değişmiş olup, toplam biyokütle ve bitki başına bakla sayısının belirleyici bir faktörü olmuştur. Bu nedenle, birinci sezondaki daha yüksek çıkış oranlarının daha elverişli iklim koşullarından kaynaklandığı düşünülmektedir. Besin analizleri, entegre mikrobiyal-kimyasal uygulamaların makro besin birikimini artırmış, ancak mikro besin elementlerinin tepkilerinde farklılıklar sergilemiştir. Özellikle Fe ve Cu konsantrasyonları yüksek verimli parsellerde düşerken, Zn ve Mn seviyeleri nispeten sabit kalmıştır. Çalışma hem biyolojik azot fiksasyonunu destekleyecek hem de optimum besin bileşimini sağlayacak şekilde besin girdisi stratejilerinin dikkatle uyarlanması gerektiğini vurgulamaktadır. Ayrıca, entegre biyo-gübreleme uygulamalarının yarı kurak bölgelerde sürdürülebilir baklagil üretimi açısından taşıdığı potansiyeli bir kez daha ortaya koymaktadır. Bu sonuçlar biyolojik N₂ fiksasyonunu desteklemek için dengeli bir N gübrelemesinin altını çizmektedir. Toprak sağlığı üzerindeki uzun vadeli etkilere odaklanmak için daha fazla araştırma yapılması önerilmektedir.

Anahtar Kelimeler: Biyo-priming, *Lens culinaris*, Bitki besleme, Sürdürülebilir tarım, Sinerjik etki

1. Introduction

Lentils (*Lens culinaris* Medik.), one of the oldest domesticated legume crops, originated from Southwest Asia and have been cultivated since the Neolithic period. Its high protein content, drought tolerance, and adaptability to low-input systems have made it a staple crop in arid and semi-arid regions (Doğan et al., 2014). As of 2023, Türkiye ranks among the top lentil-producing countries, with an annual production of approximately 270,000 tons. Lentil plays a vital role not only in food security but also in the economic stability of smallholder farmers. Additionally, lentil positively contributes to soil health enriching organic matter, reducing erosion, and promoting biological nitrogen fixation, thereby forming an essential component of sustainable agricultural systems.

One of the key properties is biological nitrogen fixation (BNF) in legumes, which supports sustainability (Takıl and Kayan, 2023). Lentil roots form nodules that convert atmospheric nitrogen into a form usable by plants, thereby reducing dependency on synthetic nitrogen fertilizers and improving soil fertility (Howieson et al., 2008). This symbiosis is especially critical in nitrogen-deficient soils. Walley et al. (2005) reported that Rhizobium inoculation increased grain yield in lentil by 18% and significantly enhanced nitrogen fixation capacity. Ögetürk and Karaaslan (2025) reported that Rhizobium inoculation increased protein ratio in *Arachis hypogaea* over control and chemical fertilizer. In addition to symbiotic systems, a wide range of free-living or associative microorganisms such as *Bacillus*, *Azospirillum* and *Paenibacillus* spp., have also demonstrated growth-promoting effects through mechanisms including phosphorus solubilization, phytohormone production, and stress alleviation (Bhattacharyya and Jha, 2012). Shaharoon et al. (2008) reported that *Bacillus* seed inoculation increased root development, improved phosphorus uptake by 22%, and positively affected productivity in winter legumes. Such findings highlight the potential of integrating symbiotic and free-living bacterias to enhance agricultural productivity.

However, modern agriculture's reliance on intensive use of chemical fertilizers and pesticides has had detrimental effects on soil health. These practices have been linked to reduced microbial diversity, increased soil salinity, and accumulation of heavy metals (Savci, 2012). Prolonged application of nitrogen fertilizers can lead to soil acidification and suppress microbial activity, thereby lowering yield (Fan et al., 2005). Nitrate leaching, biodiversity loss, and groundwater pollution also pose broader environmental threats. Despite these concerns, abandoning fertilizer not viable meeting global food demands (Tilman et al., 2002). Therefore, integrated nutrient management strategies that balance productivity and environmental protection are urgently needed. Egamberdieva et al. (2017a) found that the combined application of plant growth-promoting bacteria (PGPB) and nitrogen fertilizer enhanced nutrient uptake, increased biomass, and maintained biological activity in soil. Authorities suggest that biofertilizers are not merely alternatives but essential tools for sustainable agriculture.

This study investigates the effects of RZH, B, NF and the combination of these treatments on lentil growth, yield, and seed nutrient composition under Van ecological conditions. Numerous studies have addressed the separate roles of RZH and B, however, their interactive effects under field conditions and in long-term trials remain poorly understood. The originality of this research lies in its comparative evaluation of microbial and chemical treatments within a field experiment, which provides practical insights into efficient and sustainable lentil production systems.

2. Materials and Methods

2.1. Experimental materials

Sazak 91 lentil (*Lens culinaris*) variety was used in the study. RZH strain was obtained from the Agriculture and Forestry Soil, Fertilizer and Water Resources Central Research Institute (Ankara). PGPB strain, i.e., *Bacillus invictae* (BA142), was taken from Atatürk University. BA142 exhibits nitrogen fixation and phosphorus solubilizing abilities, as determined through laboratory tests conducted at Van Yüzüncü Yıl University. Ammonium sulphate was used as a chemical fertilizer.

2.2. Experimental conditions

A two-year field experiment was conducted in the production area of Van Yüzüncü Yıl University from the 2003-04 to the 2004-05 seasons. An analysis of the climatic data for the 2002-03 and 2003-04 seasons reveals

that the mean temperatures, precipitation, and relative humidity in the study area followed seasonal patterns. Mean temperatures reached their highest levels in September and June, while the lowest values were recorded in January and February at both years. The mean annual temperatures were calculated as 8.78°C for 2002-03 and 8.44°C for 2003-04. In terms of precipitation, the 2002-03 season was significantly wetter with a total of 459.2 mm compared to 285.7 mm in 2003-04. This difference is mainly attributed to higher rainfall amounts in December, January, February, and March of the first year. Regarding relative humidity, the 2003-04 period exhibited generally higher values than the previous year, especially during the autumn months (September, October, and November). When compared to the long-term average (1937-04), experimental seasons displayed temperature and humidity patterns consistent with historical trends, although considerable interannual variability was observed in precipitation (Table 1).

Table 1. Climatological characteristics of the experimental area

Mean Temperature		September	October	November	December	January	February	March	April	May	June
	2002-03	19.0	11.0	3.7	-2.6	-1.4	-1.4	-0.1	8.4	14.5	18.2
2003-04	17.0	13.0	4.5	0.2	-0.9	-0.6	3.7	6.9	12.4	18.5	
LYA	17.2	10.6	4.4	-0.8	-3.6	-3.2	0.9	7.4	13.0	18.0	
Precipitation		September	October	November	December	January	February	March	April	May	June
	2002-03	10.0	34.0	42.9	72.9	26.1	54.5	83.4	78.8	6.4	50.2
2003-04	16.4	23.6	59.6	14.9	25.0	39.6	69.9	26.9	68.7	3.1	
LYA	13.0	45.2	47.9	37.3	35.4	32.5	45.7	56.6	45.0	18.5	
Relative Humidity		September	October	November	December	January	February	March	April	May	June
	2002-03	55.2	63.2	67.0	75.6	77.6	72.5	69.8	73.0	64.2	61.5
2003-04	64.5	71.0	74.3	76.7	78.8	76.1	72.3	66.4	67.8	57.8	
LYA	44.0	58.0	66.0	69.0	68.0	69.0	68.0	62.0	56.0	50.0	

(LYA: 1937-2004)

The soil salinity and low organic matter content were low in both seasons. The soil texture was classified as sandy loam (SL), which may limit water-holding capacity. The pH was slightly alkaline. Total nitrogen was insufficient. Phosphorus was found to be inadequate, whereas potassium was present in sufficient quantities. Calcium and magnesium levels were adequate in both years. Micronutrients such as iron, manganese, zinc, and copper were generally enough, although a noticeable decrease in zinc was observed in the second year. Among heavy metals, cadmium was detected only in the second year and remained low. Cobalt was at a borderline level, and lead was measured only in the second year at a low concentration (Table 2).

Table 2. Characteristics of experimental soils during 2003-04 and 2004-05 seasons

Source	Year	Salt %	OM %	Texture	Lime %	N	pH	P	K	Ca	Mg	Fe	Mn	Zn	Cu	Cd	Co	Pb
mg kg ⁻¹																		
Soil	1.Year	0.03	0.82	SL	4.99	0.08	7.93	3.92	360	2130	201	10.37	19.0	1.57	1.61	-	0.132	-
	2.Year	0.03	0.80	SL	5.01	0.07	7.95	3.87	351	2201	211	9.70	17.6	1.03	1.52	0.11	0.098	0.06

2.3. Experimental design

The experiment was laid out according to randomized complete block design (RCBD) with four replications. Experimental treatments were control, NF (40 kg NH₄SO₄ ha⁻¹), RHZ (*Rhizobium leguminosarum*), B (*Bacillus invictae*), B+NF, RHZ+B, RHZ+NF and RHZ+B+NF. RHZ inoculated in peat material was applied to seeds before sowing with 4% sugar-water. The 500 g of RHZ containing peat (10⁹ colony forming unit (cfu)) was applied to 50 kg seed (Erman, 1998). BA142 was sown in nutrient agar and a single colony was isolated and transferred to the nutrient broth medium (Ceritoglu et al., 2024). One hundred milliliters of PGPB containing nutrient broth solution was applied to PGPB-treated plots. The plot size was arranged as 7.5 m² with 5 rows. Sazak-91 was sown at a rate of 160 kg ha⁻¹. The distance between plots and blocks were 1 and 2 meters, respectively. The experiments were started on 18.10.2002 and 20.10.2003 at the first and second seasons, respectively. Weeds were controlled manually throughout experiment time. Herbicide and insecticide were not used in both years. Plants were harvested by hand on June 24, 2003, and June 17, 2004, respectively.

2.4. Data collection

The number of plants per square meter was determined in a randomly selected area of the plots before harvest. Ten plants were collected from each plot to determine plant height, number of branches and number of pods per plants. The 0.5-meter buffer was established from both ends of the plots and external rows to reduce edge effects. The area of 3.6 m² (0.9 × 4) was harvested. Grain and biological yield were calculated on a per hectare basis. Straw and seed samples were collected from each plot to observe nutrient status. A 0.5 g of the ground sample was weighed into porcelain crucibles and they were burned in a muffle furnace at 500 ± 20 °C. The burnt samples were filtered into volumetric flasks using filter paper and made up to the line with pure water (Erman et al., 2024). The totals N concentration was determined in straw and grain using the Kjeldahl method (Horwitz and Latimer, 2006). P, K, Ca, Mg, Mn, Fe, Zn and Cu concentrations of grain were determined using ICP-OES (Inductively Coupled Plasma-optical Emission Spectrometry, Perkin Elmer Optima 2100 DV Atomic Emission Spectrometer) by the methods of Hansen et al. (2012).

2.5. Statistical analysis

Analysis of variance (ANOVA) was subjected based on the RCBD. Tukey's Honestly Significant Difference (HSD) multivariate test was used to group the data using the "agricolae" package (Mendiburu, 2010). Statistical calculations were conducted using R software (V.4.4.1).

3. Results and Discussion

3.1. Results

Years showed significant ($p < 0.01$) differences in the number of plants per square meter, biological yield, and grain yield. Additionally, P and Zn in the grain were also significantly affected at $p < 0.01$, while Ca showed significance at $p < 0.05$. Plant height, number of branches and pods, N in straw and grain, K, Mg, Fe, Cu, and Mn were not significantly influenced by year. Grain yield, N in straw and grain, and P in grain showed strong responses at the $p < 0.01$ level. Cu and Fe were significantly affected at $p < 0.05$, while number of pods per plant, K and Mg also responded at either $p < 0.05$ or $p < 0.01$. In contrast, plant height, number of branches, Ca, Mn, and Zn showed no statistically significant response to treatment. Regarding the $Y \times T$ interaction, only two traits were significantly affected. Biological yield showed a significant response at the $p < 0.05$ level, while grain yield was influenced at $p < 0.01$ (Table 3).

Table 3. Results of variance analysis for experimental data according to the randomized complete block design

Variation sources	Significance degree		
	Year	Treatment	Y × T
Number of plants per square meter	0<01	ns	ns
Plant height	ns	ns	ns
Number of branches	ns	ns	ns
Number of pods per plant	ns	0<01	ns
Biological yield	0<01	ns	0<05
Grain yield	0<01	0<01	0<01
N Straw	ns	0<01	ns
N Grain	ns	0<01	ns
P Grain	0<01	0<01	ns
K Grain	ns	0<05	ns
Ca Grain	0<01	ns	ns
Mg Grain	ns	ns	ns
Fe Grain	ns	0<01	ns
Cu Grain	ns	0<01	ns
Mn Grain	ns	ns	ns
Zn Grain	0<01	ns	ns

(ns: No significant difference)

The number of plants per square meter was higher in the first year (120.8) than the second year (55.3). Biological yield decreased from 3205 kg ha⁻¹ in 2002-03 to 2353 kg ha⁻¹ in 2003-04, while grain yield declined from 1309 kg ha⁻¹ to 853 kg ha⁻¹. Ca increased from 9.49 mg kg⁻¹ in the first year to 10.92 mg kg⁻¹ in the second. P also showed a slight increase from 3.25 mg kg⁻¹ to 3.44 mg kg⁻¹. Other parameters showed relatively small variations such as plant height ranging from 26.9 to 28.2 cm, the number of branches from 39.6 to 40.2, and the 0.2 number of pods per plant from 167 to 171. Straw N ranged from 4.63 to 4.66, and grain N from 22.9 to 23.1. K ranged from 325 to 344 mg kg⁻¹, Mg from 46628 to 49440 mg kg⁻¹, Fe from 661 to 739 mg kg⁻¹, Cu from 15.7 to 18.0 mg kg⁻¹, Mn from 12.83 to 13.09 mg kg⁻¹, and Zn from 85.2 to 90.6 mg kg⁻¹ (Table 4).

Table 4. Alterations of experimental observations during 2002-03 and 2003-04 growing seasons

Observations	2002-03	2003-04
Number of plants per square meter	120.8 a	55.3 b
Plant height (cm)	26.9	28.2
Number of branches	39.6	40.2
Number of pods per plant	171	167
Biological yield (kg ha ⁻¹)	3205 a	2353 b
Grain yield (kg ha ⁻¹)	1309 a	853 b
N Straw (%)	4.63	4.66
N Grain (%)	23.1	22.9
P Grain (mg kg ⁻¹)	325 b	344 a
K Grain (mg kg ⁻¹)	2.718	2.024
Ca Grain (mg kg ⁻¹)	9.49 b	10.92 a
Mg Grain (mg kg ⁻¹)	49440	46628
Fe Grain (mg kg ⁻¹)	661	739
Cu Grain (mg kg ⁻¹)	12.83	13.09
Mn Grain (mg kg ⁻¹)	85.2	90.6
Zn Grain (mg kg ⁻¹)	15.7 b	18.0 a

According to Figure 1, number of plants per square meter, plant height and number of branches changed between 85-100, 24.5-28.1 cm and 34.7-42.5, respectively. The lowest number of pods per plant was recorded in the B treatment (160), while the highest number was observed in the RZH+NF+B treatment plants (175), representing an 8% increase compared to the control. In terms of biological yield, the lowest value was obtained from the RL+B treatment (2273 kg ha⁻¹) and the highest from the RL+N treatment (2992 kg ha⁻¹), representing a 19.5% increase over the control (2504 kg ha⁻¹). Similarly, grain yield ranged from 781 kg ha⁻¹ in the B treatment to 1302 kg ha⁻¹ in RL+N, indicating a 28.9% increase compared to the control (1010 kg ha⁻¹). These results highlight the notable improvements in yield components achieved through the combining Rhizobium and nitrogen fertilizer (Figure 1).

According to the Figure 2, straw N ranged from 4.3% in the control to 5.2% with RZH+NF+B, while grain N varied between 22.6% (control) and 25.2% RZH+NF+B). The lowest P was observed in the B treatment (283 mg kg⁻¹) whereas the highest P was determined with RZH+NF+B (381 mg kg⁻¹), corresponding to a 34.6% increase over the control (283 mg kg⁻¹). K ranged from 2.0% in B to 2.7% in RZH+NF+B, thereby increasing 35% compared to the control (2.0%). In contrast, Ca ranged from 9.4 to 10.9 mg kg⁻¹, and Mg content varied between 44,297 and 50,004 mg kg⁻¹ across treatments. These findings indicate that the RZH+NF+B treatment notably enhanced nutrient accumulation in lentil grains, particularly for N, P, and K (Figure 2).

According to Figure 3, Fe was highest in the control (826 mg kg⁻¹) and lowest in the RZH+B (590 mg kg⁻¹), representing a 28.6% decrease compared to the control. Similarly, Cu peaked at 18.0 mg kg⁻¹ in the NF, while the lowest value was recorded in RZH+NF+B (14.1 mg kg⁻¹), corresponding to a 12.4% reduction relative to the control (16.1 mg kg⁻¹). In contrast, Zn ranged from 15.4 to 17.1 mg kg⁻¹ and Mn content varied between 76.3 and 92.7 mg kg⁻¹ across treatments, indicating a relatively narrow distribution for these elements (Figure 3).

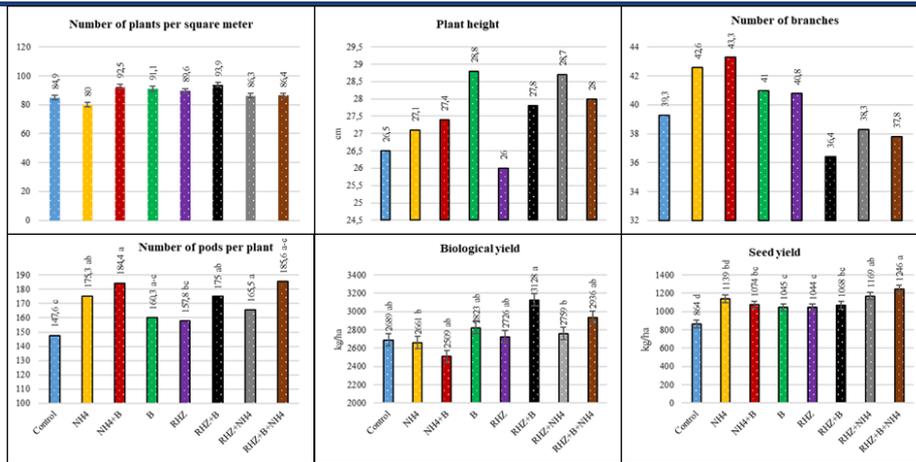


Figure 1. Changes in agronomic observations with different fertilization strategies

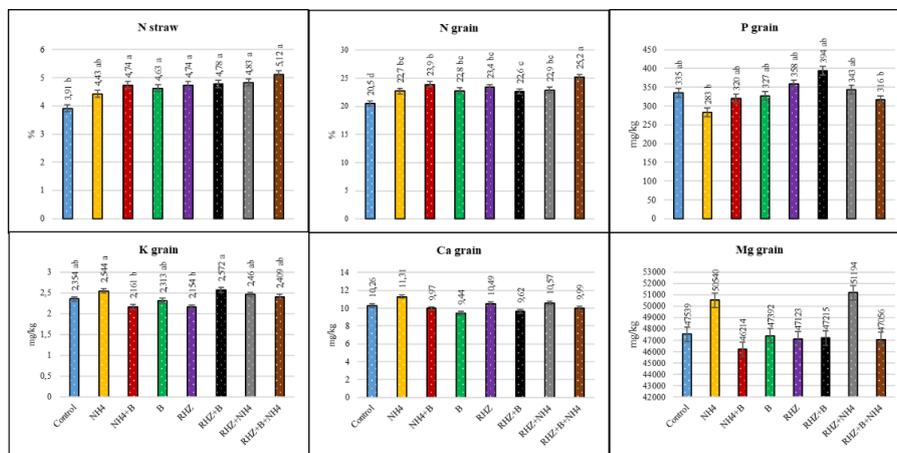


Figure 2. Changes in macronutrients with different fertilization strategies

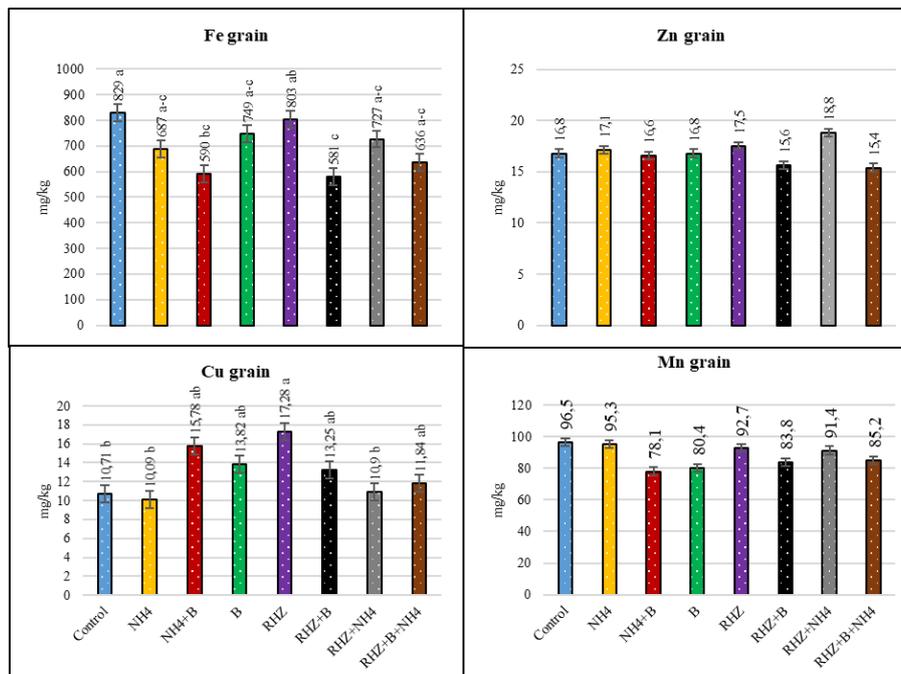


Figure 3. Changes in micronutrients with different fertilization strategies

Biological yield in 2002-03 ranged from 2588 kg ha⁻¹ (control) to 3652 kg ha⁻¹ (RHZ+B), while it changed between 1889-2791 kg ha⁻¹ in 2003-04. The highest biological yield overall was observed in 2002-03 under the RHZ+B treatment. Grain yield ranged between 1055 kg ha⁻¹ (control) and 1509 kg ha⁻¹ (RHZ+B+NH₄) in the first experimental season, whereas it ranged from 672 kg ha⁻¹ (control) to 983 kg ha⁻¹ (RHZ+B+NH₄) in 2003-04. The highest grain yield was recorded with RHZ+B+NF in 2002-03 (Table 5).

Table 5. Alteration of biological yield and grain yield depending on YxT interaction

Treatment	Biological yield (kg ha ⁻¹)		Grain yield (kg ha ⁻¹)	
	2002-03	2003-04	2002-03	2003-04
Control	2588 a-e	2791 a-e	1055 de	672 h
NH ₄	2911 a-e	2411 b-e	1378 ab	900 ef
NH ₄ +B	3129 a-d	1889 e	1268 bc	880 fg
B	3367 a-c	2278 c-e	1181 cd	910 ef
RHZ	3023 a-e	2430 b-e	1256 bc	832 f-h
RHZ+B	3652 a	2603 a-e	1411 ab	725 gh
RHZ+NH ₄	3485 ab	2032 de	1415 ab	922 ef
RHZ+B+NH ₄	3486 ab	2386 b-e	1509 a	983 ef

3.2. Discussion

Agronomical growth and yield attributes

The results clearly demonstrate that year and treatments significantly influenced both biological and grain yields. The highest values were recorded NF when the combined application of RZH, B, and NF was used. Studies conducted under nutrient-deficient conditions have shown that co-inoculation strategies in lentil, such as the combined use of Rhizobium and phosphate-solubilizing bacteria, significantly enhance yield (Altunkaynak and Ceyhan, 2018; Ceritoglu et al., 2024). These approaches promote plant development by improving both biological nitrogen fixation and phosphorus availability to the plant. Consistent with these findings, the present study also demonstrates that integrated microbial and chemical fertilization exerts a synergistic effect, contributing to sustainable yield improvement in lentil cultivation under semi-arid conditions (Ceritoglu et al., 2024). Co-application of Rhizobium with NF did not lead to yield enhancement, however, it resulted in reduced total biomass and pod number per plant in some treatments. This finding aligns with previous research indicating that excessive nitrogen can suppress biological N₂ fixation by inhibiting nodule formation and nitrogenase activity in legumes (Zahran, 1999; Salvagiotti et al., 2008). The inhibitory effect is more pronounced under conditions where available nitrogen in the soil reduces the need for symbiotic association, leading to a physiological shift in the plant's nitrogen acquisition strategy. On the other hand, partial substitution of NF with biofertilizers in our trial, particularly the combination of *Rhizobium* and *Pseudomonas*, appeared to support improved emergence rates and pod set, suggesting a potential synergistic effect under moderate nutrient regimes. These results support previous findings that integrated use of microbial and chemical fertilizers can improve resource-use efficiency and stimulate plant growth by producing phytohormones, solubilizing nutrients, and enhancing root development (Egamberdieva et al., 2017b; Soysal and Erman, 2020; Tadayon et al., 2025).

In addition to treatment effects, plant density was a critical determinant of yield performance. The difference in plant density between the two experimental years (120. in 2002-03 and 55.3 in 2003-04) can be attributed not only to germination and emergence conditions but also to climatic influences throughout the entire growing period. During the 2002-03 season, the September–November period experienced an average temperature of 11.6 °C, a total rainfall of 83.0 mm, and an average relative humidity of 61.7%, resulting in balanced soil moisture that supported healthy germination and emergence. Despite low winter temperatures (e.g., -2.6 °C in January), moderate humidity levels (77.6% in January, 72.5% in February) supported plant survival through winter. Moreover, lentil has a high adaptability to extreme cold conditions and can survive with no foliar damage by -5 °C (Ali et al., 2000). Additionally, 78.6 mm of rainfall in April promoted continued vegetative growth and reduced plant loss. Conversely, in the 2003-04 season, the same autumn period had higher temperatures (13.3 °C), greater rainfall (99.3 mm), and elevated humidity (70.8%), conditions that likely caused soil saturation and hypoxia, thereby inhibiting seedling emergence (Ashraf, 2012; Ahmed et al., 2013). Winter months were also consistently humid (78.8% in January, 76.1% in February), increasing the risk of root diseases and seedling

decay. Furthermore, rainfall in April dropped to just 26.9 mm, which may have exposed young plants to early drought stress during a critical growth phase. Altogether, these cumulative climatic stresses in 2003-04 contributed to a significantly reduced plant density by the time of harvest. These findings underscore the importance of evaluating seasonal climatic variables when interpreting plant density and crop performance outcomes (Table 1).

Higher plant density contributed to increased total pod number in plants and biomass per unit area, thereby elevating biological yield. The positive correlation between plant density and yield components has been consistently documented in grain legumes (López-Bellido et al., 2005; Cutforth et al., 2013). The number of plants per square meter remained within agronomically optimal ranges, ensuring that intra-specific competition did not outweigh the productivity benefits. Moreover, the effects of microbial and chemical treatments were more pronounced under high-density conditions, further emphasizing the importance of considering planting density when evaluating fertilization strategies. Efficient nodulation and rhizosphere function depend on balanced nutrient availability and early-stage root development, which may be suboptimal under single-inoculant treatments without starter nitrogen (Howieson et al., 2008; Egamberdieva et al., 2017a).

Notably, the RHZ+NF and B+NF treatments consistently underperformed compared to the control. This yield suppression is attributed to nitrogen feedback inhibition, where high external nitrogen levels inhibit nodulation and biological nitrogen fixation. Specifically, elevated levels of ammonium or nitrate suppress nod gene expression, limit rhizobial colonisation, and reduce symbiotic function (Walley et al., 2005). Excess nitrogen also interferes with oxygen regulation in root nodules. Nitrogenase, the key enzyme for BNF, is highly sensitive to oxygen and depends on the buffering capacity of leghemoglobin. High nitrogen availability disrupts this system via reducing nitrogenase activity (Guinel, 2009; Streeter, 1988). Cabeza et al. (2014) further reported that excess nitrate affects carbon allocation and hormonal signaling, thereby leading to nodular dysfunction. These findings indicate that the effectiveness of microbial inoculants depends not only on the microbial strains themselves but also on the form, dose, and timing of nutrient inputs. The highest yields were achieved through integrated strategies where microbial and chemical agents complemented each other.

Nutrient Uptake and Grain Mineral Composition

The nutrient accumulation trends further support the agronomic outcomes. The RHZ+NF+B treatment resulted in significant increases in grain N, P, and K contents compared to the control by 11.5%, 34.6%, and 35%, respectively. These increases are consistent with improved microbial mobilization of nutrients and expanded root surface area, facilitated by the synergistic activity of RHZ and B (Meena et al., 2017; Bhattacharyya and Jha, 2012). In particular, the elevated phosphorus content confirms the role of B in converting insoluble phosphate to plant-available forms through acidification and enzymatic activity (Ceritoglu et al., 2025).

Micronutrient responses, however, were more variable. Notably, Fe and Cu concentrations in grain declined in some inoculated treatments. Fe decreased by 28.6% in RHZ+B compared to the control, and Cu dropped by 12.4% in RHZ+NF+B. This could be attributed to a dilution effect, wherein increased biomass reduces mineral concentration per unit of grain mass (Fan et al., 2005), or to nutrient antagonism triggered by excess N. Indeed, studies have shown that high nitrogen availability can inhibit Fe uptake and disrupt its transport due to rhizospheric pH shifts or altered expression of transporters (Gao et al., 2020). By contrast, Zn and Mn concentrations remained relatively stable (15.4-17.1 mg kg⁻¹ and 76.3-92.7 mg kg⁻¹, respectively), suggesting that these micronutrients were not significantly affected by treatment conditions or remained within adequate soil supply thresholds.

The nutrient uptake and seed nutrient composition were markedly influenced by the microbial treatments, particularly when co-applied with reduced levels of chemical fertilizers. The use of Rhizobium in combination with *Pseudomonas fluorescens* resulted in higher N, P and micronutrient (Fe, Zn, Mn) in lentil grains. This can be attributed to the complementary mechanisms of the two inoculants, while *Pseudomonas* enhances nutrient solubilization and mobilization, especially of poorly available forms of P and micronutrients (Khan et al., 2019). Several studies have reported that microbial consortia promote a more balanced nutrient supply to plants by increasing rhizospheric activity and enzyme secretion such as phosphatases and siderophores (Meena et al., 2017). In contrast, treatments combining Rhizobium with chemical N led to comparatively lower nutrient use

efficiency, likely due to suppression of microbial activity and reduced colonization efficacy under high inorganic N levels (Kobua et al., 2025). These findings emphasize the need for nutrient balance and microbial compatibility in integrated fertilization strategies to optimize both yield and nutritional quality in legumes under field conditions. Overall, the RHZ+NF+B combination proved effective not only in maximizing yield but also in enhancing macro-nutrient accumulation. However, the results also emphasize the need for careful nitrogen management, as excessive ammonium-based fertilization may compromise microbial efficiency and disrupt the micronutrient balance. These findings align with those of Smith et al. (2011), who emphasized the importance of optimizing inputs for both productivity and environmental sustainability in legume-based systems.

4. Conclusions

This study demonstrated that the combined use of *Rhizobium leguminosarum*, a phosphorus-solubilizing *Bacillus* strain, and ammonium-based N fertilizer (RHZ+NF+B) resulted in the highest lentil yields and improved seed nutrient content, particularly for N, P, and K. The synergistic relationship between microbial inoculants and moderate nitrogen input enhanced both plant growth and nutrient uptake. In contrast, treatments combining microbial agents with excess ammonium (RHZ+NF, B+NF) were less effective, likely due to nitrogen-induced suppression of nodulation and nitrogenase activity. These results underscore the importance of balanced nitrogen management in supporting biological nitrogen fixation. Micronutrient responses varied, with Fe and Cu levels declining in high-yield treatments, possibly due to dilution effects, while Zn and Mn remained stable. In summary, integrated microbial and chemical fertilization offers a promising strategy for sustainable lentil production, provided nutrient inputs are carefully calibrated.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authorship Contribution Statement

Concept: Erman, M.; Design: Erman, M.; Data Collection or Processing: Çiğ, F.; Statistical Analyses: Erman, M., Ceritoglu, M.; Literature Search: Ceritoglu, M.; Writing, Review and Editing: Erman, M., Çiğ, F., Ceritoglu, M.

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