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Effect of Sewage Sludge Treatment and Rhizobium Inoculation on Growth, Nodulation Yield Attributes in Lentil (*Lens culinaris* Medik.) under Field Conditions

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Abstract: This study investigated the effects of sewage sludge treatment and Rhizobium inoculation on the growth, yield, and nodulation of lentil (*Lens culinaris* Medic.) under field conditions. The experiment was conducted at Van Yüzüncü Yıl University over two growing seasons using a randomized complete block design with three replications. Four doses of sewage sludge (0, 10, 20, and 40 t ha^{-1}) were applied with and without Rhizobium inoculation. The results revealed significant seasonal variations, with biological yield decreasing from 8306 kg ha^{-1} in 2003-04 to 3262 kg ha^{-1} in 2004-05, and grain yield declining from 2716 kg ha^{-1} to 633 kg ha^{-1}. The application of 20 t ha^{-1} sewage sludge resulted in the highest biological yield (6724 kg ha^{-1}) and grain yield (1854 kg ha^{-1}), while higher doses (40 t ha^{-1}) negatively impacted seed yield. The number of plants per square meter significantly varied between years, and reduced from 183 in 2003-04 to 74 in 2004-05, thereby altering growth and yield attributes. Sewage sludge increased plant density, reaching 153 at 40 t ha^{-1}, while Rhizobium inoculation slightly reduced it. In contrast to grain and biological yields, the number of seeds per plant and seed yield per plant decreased with increasing sewage sludge doses. Overall, the study suggests that 20 ton ha^{-1} sewage sludge application optimizes lentil productivity in semi-arid conditions, but higher doses may lead to reduced yield.

Keywords: Inoculation, *Lens culinaris*, organic fertilizer, symbiotic nitrogen fixation, waste

1. Introduction

Lentil (*Lens culinaris* Medik.) is one of the most important leguminous crops due to its high nutritional content, adaptability to diverse climates, and improvement of soils by nitrogen fixation (Tena et al., 2016). However, improvement of lentil productivity remains a challenge in regions where soil fertility is poor and climatic fluctuations Aaffect plant development (Gan et al., 2005; Ceritoglu et al., 2025). Although chemical fertilizers and pesticides help to prevent yield and quality losses, they have threaten human and public health since the early 1900s (Açıkbaş et al., 2017). This situation increased the importance of reducing chemicals and increasing organic resources in plant nutrition. Moreover, the conversion of organic waste into valuable plant nutrition products plays a critical role in terms of sustainability in agricultural production (Ceritoglu et al., 2018; Sharma et al., 2019; Ceritoglu and Erman, 2020; Ashokkumar et al., 2022).

One widely researched approach to enhancing soil fertility is the application of sewage sludge, a byproduct of wastewater treatment rich in organic matter, and macro- and micronutrients (Özyazıcı and Özyazıcı, 2012; Börjesson and Kätterer, 2018). Several studies reported that sewage sludge improves soil physical structure, water retention, and microbial activity, leading to increased plant growth and yield (Rebah et al., 2002; Erman et al., 2024). However, excessive application may result in heavy metal accumulation in soils, which can reduce microbial diversity and plant productivity (Nunes et al., 2021; Achkir et al., 2023).

In addition to organic amendments, biological nitrogen fixation through Rhizobia plays a crucial role in improving legume growth and productivity as well as soil health (Barbieri et al., 2023). Rhizobium bacterias form symbiotic relationships with lentil roots facilitating atmospheric nitrogen fixation, which enhances soil fertility and reduces the need for synthetic fertilizers (Sekhon et al., 1978). Research suggests that Rhizobium inoculation can increase lentil grain yield by 23-32% in nutrient-deficient soils (Bhuiyan et al., 2011).

Despite these individual benefits, the combined effects of sewage sludge and Rhizobium inoculation on lentil growth and yield remain largely unexplored. Some studies suggest that while sewage sludge increases soil organic matter, it may also introduce toxic elements that negatively impact Rhizobium activity (Paliya et al., 2019). On the other hand, Rhizobium inoculation may exhibit synergistic interaction with sewage sludge treatment and mitigate some of the negative effects by improving plant nutrient uptake and microbial balance (Hasan et al., 2023). It was reported that the presence of high concentrations of zinc and cadmium in sewage sludge suppresses nodulation in legumes reducing the efficiency of nitrogen fixation (Angle et al., 1993). The aim of this study is to enhance understanding of strategies for optimizing soil fertility and promoting sustainable lentil (*L. culinaris*) production in nutrient-deficient environments.

2. Materials and Methods

2.1. Experimental area

The study was carried out at Van Yüzüncü Yıl University, Eastern of Türkiye, (35° 57' 84 N and 42° 74' 61 E) during the 2003-04 and 2004-05 growing seasons. The altitude of the experimental area was 1725 m.

2.2. Climatological and soil characterization of experimental area

Mean temperatures were 9.9 °C in 2003-04 and 9.6 °C in 2004-05. The lowest temperatures were seen in January 2003-04 (-0.9 °C) and December of 2004-05 (-3.7 °C). The highest mean temperatures were observed in August during both experimental seasons and they were 22.2 °C and 23.4 °C, respectively. Total precipitation was 349.7 mm and 421.2 mm during the 2003-04 and 2004-05 experimental seasons, respectively. Total precipitation during the 2003-04 season was lower than long years average (385.7 mm), while the second one was higher (Figure 1).

Before establishing the trials, soil samples were taken from a depth of 0-20 cm in both years. The collected soil samples were analyzed for basic fertility, macro and micro elements, as well as certain heavy metals, and the results are presented in Table 1. The texture type was determined as sandy loam (SL) in both years. There is no salinity problem in the soils. The soil reaction (pH) is slightly alkaline, and the organic matter content is classified as 'very low,' lime content as 'moderate,' and nitrogen (N) and phosphorus (P) levels as 'low'. The contents of potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) in the soils are at sufficient levels. Cd and Pb could not be determined in the first year, wherease they were 0.11 and 0.06 mg kg^{-1} in the second year, respectively. The cobalt (Co) values in the soils ranged from 0.098 to 0.132 (Table 1). Additionally, the heavy metal content of the trial soils is below the threshold limits set by the Regulation on the Control of Soil Pollution (Anonymous, 2005).

2.3. Experimental materials

One red registered lentil cultivar (cv. Sazak-91) was tested in the experiment. Sazak-91 is a high-

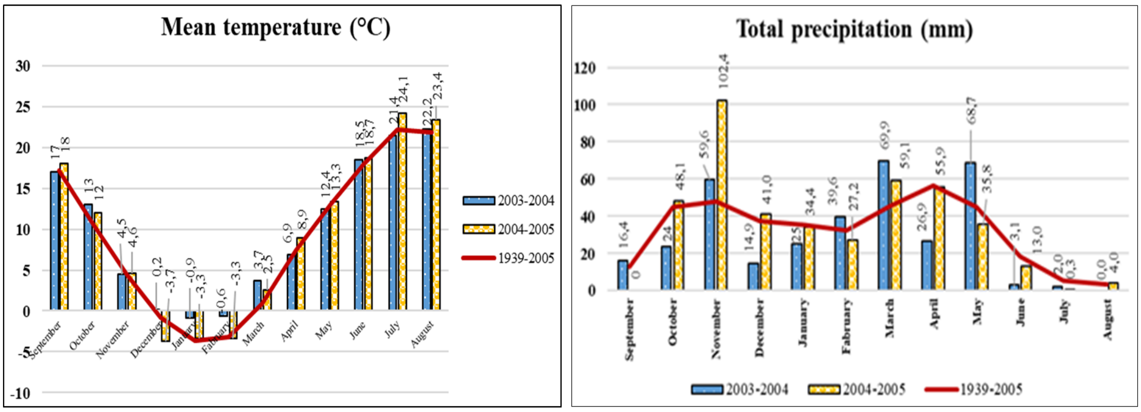

Figure 1. Climatological data of Van Province through 2003-04 and 2004-05 experimental seasons

Table 1. Physiochemical characterization of experimental soil and sewage sludge in both years*

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
SS	1.Year	0.04	29.4	-	-	1.32	7.98	1544	2430	2100	567	956	440	560	149	0.96	4.56	2.89
	2.Year	0.05	30.2	-	-	1.4	8.01	1645	2301	2081	497	889	413	529	152	1.02	4.12	3.01

SS: Sewage sludge, OM: Organic matter, SL: Sandy loam, *: Determined heavy metals are extractable elements tolerant cultivar to cold, drought, and lodging. Also, it has a high seed size with 63-63.5 g of 1000-seed weight (Anonymous, 1991).

Sewage sludge was obtained from the waste collection area of Van Metropolitan Municipality sourced from the disposal site. The chemical properties of sewage sludge were determined and given in Table 1. Sewage sludge materials have high organic matter and very high nutritional composition in both years. The maximum permissible heavy metal concentrations in sewage sludge intended for agricultural use are 4000 ppm for Zn, 1750 ppm for Cu, 40 ppm for Cd, and 1200 ppm for Pb in Türkiye (Anonymous, 2005). Accordingly, the heavy metal contents of the sewage sludge used in the soil were found to be below these threshold values. Furthermore, microbiological analysis of the sewage sludge showed no detectable levels of Fecal Coliform, which could otherwise limit its use.

Seeds were inoculated using peat inoculants containing a mixture of nodule-forming strains of *Rhizobium leguminosarum* biovar viciae specific to lentils. The peat inoculant was prepared with commercial peat cultures provided by the Soil, Fertilizer and Water Resources Central Research Institute, Ankara, Türkiye.

2.3. Sewage sludge treatment and Rhizobium inoculation process

Sewage sludge (average moisture about 70-80%) was seasonally collected and air-dried up to 20-30% moisture content. Sewage sludge (SS) was applied at a rate of 0 (SS0: Control), 10 (SS10), 20 (SS20), and 40 (SS40) tons ha^{-1} into the soil 3-4 weeks before planting twice years (Yang et al., 2021).

Four doses of sewage sludge were mixed before planting, soil cultivation was carried out with a hoe engine to both prepare the seed-bed and mix the treatment sludge homogeneously into the plot. The method described by Erman et al. (2011) was used for seed inoculation. The peat inoculants were stored at +4°C in a refrigerator and seeds were sown in 24 hours. The content and activity of the peat inoculants were assessed before the experiment. The peat inoculants were utilized after adjusting the cell count to $1x10^8$ Rhizobium per gram of peat inoculant using the most probable number method for estimating viable cells (Somesagaran and Hoben, 1994).

2.4. Experimental design

Four doses of sewage sludge were tested with/without Rhizobium inoculation under field conditions. A randomized complete block design was used in the experiment with three replications. Plot size was adjusted to 7.5 m^2 (1.5 x 5). Each plot had 6 rows with 30 cm row-spacing. Peat inoculant application on the seeds was carried out using water containing 2% sugar just before sowing. Each plot received a basal dose of 140 kg ha^{-1} diammonium phosphate fertilizer. The seeding rate was 230-250 seeds per square meter (Ceritoglu et al., 2024) and seeds were manually sown on 20 October 2003 and 25 October 2004, respectively. The experiment was laid out under rain-fed conditions. Plots were manually weeded twice per season. Plants were harvested in late June for two consecutive years (22 June 2004 and 27 June 2005).

2.5. Experimental characteristics

The number of plants per square meter was determined in randomly selected area of plots before harvest. Ten plants were collected from each plot to determine plant height, number of first and second branches, number of nodules, number of pods per plant, number of seeds per plant, and seed yield per plant. After collecting the samples, plants were cut from the stem node. Roots were shaken to remove soils and carefully washed and collected nodules. While harvesting, 0.5 meters from both ends of plots and external rows were abolished to reduce the edge effect. The area of 3.6 m^2 (0.9x4) was harvested and grain yield, biological yield and harvest index were calculated according to hectare. Finally, randomly selected seeds were weighed and 1000-seed weight was calculated.

2.6. Statistical analysis

Two years of experimental data were subjected to analysis of variance (ANOVA) to determine the significance degree ($p<0.05$ and 0.01) among treatments according to a randomized complete blocks design with factorial arrangement. Means were grouped by Tukey HSD using JMP statistical software.

3. Results

ANOVA results indicated that experimental years were statistically significant on the number of second branches at 5% and on the number of first branches, number of nodules, biological yield, seed yield, harvest index, and 1000-seed weight at 1%. Rhizobium inoculation caused no significant difference in investigated characteristics except for the number of plants per square meter. Sewage sludge treatment significantly affected ($p<0.05$) seed yield per plant and the number of seeds per plant, while it caused statistically significant differences ($p<0.01$) in biological yield, seed yield, and 1000-seed weight. YxR caused statistically significant differences ($p<0.01$) in plant height, number of second branches, number of pods, and number of seeds per plant, whereas it did not affect other characteristics. YxS was significant ($p<0.01$) for biological yield, seed yield, and harvest index. RxS caused statistically significant differences by 5% and 1% in seed yield and harvest index, respectively. YxRxS interaction significantly affected ($p<0.05$) the number of second branches and biological yield. The number of plants per square meter was statistically ($p<0.01$) affected by year, Rhizobium, sewage sludge, and their all interactions (Table 2).

Rhizobium inoculation, sewage sludge treatment or experimental years caused no statistical significant difference on plant height that varied between 25.3-29.0 cm. However, alterations in plant height depending on YxR were noteworthy, in which the lowest plant height (26.7 cm) was determined with Rhizobium inoculation at the 2004-05 season, while the highest one (28.8 cm) was obtained from non-Rhizobium treatment at the same season (Table 3).

The number of first branches in the 2003-04 season (19.4) was higher by 44.7% compared with the 2004-05 season (13.4). However, experimental treatments and their various interactions were not effective in the number of first branches and it changed between 11.0-23.0 (Table 4). On the other hand, the number of second branches increased by 20.7% in 2004-05 season compared with 2003-04. In addition to experimental years, YxR and YxRxSS were decisive factors in the number of second branches. According to YxR, the lowest number of second branches (14.4) was determined with no Rhizobium inoculation in the first experimental season, whereas the highest one (23.2) was observed with Rhizobium inoculation in the second year. YxRxSS revealed a different scenario where the lowest number of second branches (11.0) was determined with 40 ton ha^{-1} treatment without Rhizobium at the first experimental season, whereas the highest one (28.3) was obtained from with 40 ton ha^{-1} treatment without Rhizobium at the second experimental seasons (Table 5).

There are significant differences among experimental years for the number of plants per square meter. The number plants per square meter was higher in the first year (183) compared with the second year (74) by 147%. Rhizobium inoculation reduced number of plants per square meter from 135 to 121. In contrast to Rhizobium, sewage sludge enhanced the number of plants per square meter. The lowest number of plants per square meter (96)

Table 2. Analysis of variance for experimental years and characteristics

Traits	Sum of square/F probablity						
	Year	Rhizobium	Sewage sludge	YxR	YxSS	RxSS	YxRxSS
PH	2.475^{ns}	7.442^{ns}	1.971^{ns}	22.01**	2.602^{ns}	12.56^{ns}	22.98^{ns}
NFB	432**	33.33^{ns}	5.67^{ns}	16.33^{ns}	50^{ns}	16^{ns}	27.7^{ns}
NSB	168.8*	108^{ns}	219.1^{ns}	300**	92.4^{ns}	4.50^{ns}	323.2*
PSM	147076**	2423**	22856**	4661**	5209**	2180**	2275**
NN	120.8**	4.738^{ns}	20.29^{ns}	1.689^{ns}	15.37^{ns}	14.57^{ns}	11.49^{ns}
NP	1508^{ns}	105^{ns}	6059^{ns}	10296**	1476^{ns}	1636^{ns}	319^{ns}
SYP	8.33^{ns}	2.52^{ns}	48.61*	20.28^{ns}	18.07^{ns}	18.91^{ns}	27.59^{ns}
NSP	300^{ns}	290^{ns}	11184*	15194**	2054^{ns}	2368^{ns}	862^{ns}
BY	305252794**	59080^{ns}	28465039**	464527^{ns}	8370574**	1467550^{ns}	2404780*
SY	52062502**	29601^{ns}	2528504**	25854^{ns}	1345476**	669003*	205144^{ns}
HI	2208**	11.19^{ns}	90.41^{ns}	3.16^{ns}	266**	174**	50^{ns}
SW	441.1**	1.39^{ns}	100.6**	5.31^{ns}	20.1^{ns}	22.9^{ns}	23.8^{ns}

PH: Plant height, NFB: Number of first branches, NSB: Number of second branches, PSM: Plants per square meter, NN: Number of nodules, NP: Number of pods, SYP: Seed yield per plant, NSP: Number of seeds per plant, BY: Biological yield, SY: Seed yield, HI: Harvest index, SW: 1000-seed weight, ns: No significant difference, *: $p<0.05$, **: $p<0.01$

Table 3. Effect of Rhizobium and sewage sludge on plant height (cm)*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	26.7	28.5	29.0	27.3	27.6	28.2	27.8	27.9	27.9
10	26.7	28.2	28.2	27.0	27.5	27.6	27.5	27.6	27.5
20	28.4	25.2	28.8	27.2	26.8	28.0	28.6	26.2	27.4
40	26.3	28.4	29.4	25.3	27.4	27.4	27.9	26.9	27.4
	Y x R				Year		Rhizobium		
Mean	27.0 AB	27.6 AB	28.8 A	26.7 B	27.3	27.7	27.9	27.1	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

Table 4. Effect of Rhizobium and sewage sludge on the number of first branches

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	23.0	19.3	12.7	11.7	21.2	12.2	17.8	15.5	16.7
10	16.3	18.7	15.0	13.3	17.5	14.2	15.7	16.0	15.8
20	19.7	20.0	16.0	11.0	19.8	13.5	17.8	15.5	16.7
40	19.7	18.7	15.7	12.0	19.2	13.8	17.7	15.3	16.5
	Y x R				Year		Rhizobium		
Mean	20.0	19.2	14.8	12.0	19.4 A	13.4 B	17.3	15.6	

Y: Year, R: Rhizobium, SS: Sewage sludge

Table 5. Effect of Rhizobium and sewage sludge on the number of second branches*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	20.7 ab	23.0 ab	21.7 ab	24.0 ab	21.8	22.8	21.2	23.5	22.3
10	11.3 b	14.3 ab	20.0 ab	18.7 ab	19.5	23.5	19.8	23.2	21.5
20	14.7 ab	24.7 ab	22.7 ab	20.3 ab	19.7	21.5	18.7	22.5	20.6
40	11.0 b	27.7 ab	28.3 a	21.7 ab	12.7	20.8	15.5	18.0	16.8
	Y x R				Year		Rhizobium		
Mean	14.4 B	22.4 A	21.2 A	23.2 A	18.4 B	22.2 A	18.8	21.8	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

was determined in control plots, while the highest one (153) was observed in 40 ton ha^{-1} treated plots. YxR indicated that the lowest number of plants per square meter (70) was determined in the second year without Rhizobium whereas the highest one (200) was observed in the first year without Rhizobium. According to YxSS, the lowest number of plants per square meter (58) was determined in control plots in the 2004-05 season, while the highest one (209) was observed in 40 ton ha^{-1} treated plots in the 2003-04 season. According to RxSS, the lowest number of plants per square meter (78) was determined with Rhizobium inoculation without sewage sludge, while the highest one (155) was detected in 40 ton ha^{-1} treated plots without Rhizobium. YxRxSS indicated that the lowest number of plants per square meter (50) was determined with Rhizobium inoculation and sewage sludge 10 ton ha^{-1} treated plots in 2004-05, whereas the highest one (225) was detected in 20 ton ha^{-1} treated plots without Rhizobium in 2003-04 (Table 6).

The difference among experimental years on the number of nodules was significantly different in that it was higher in the first year by 131% over the second one. Rhizobium inoculation and sewage sludge treatment varied between 3.69-4.32 and 3.11-4.09, respectively. In general, number of nodules changed between 1.28-8.25 (Table 7).

The number of pods per plant was not affected by experimental years, Rhizobium inoculation, or sewage sludge treatment and changed between 101-116, 109-112, and 101-129, respectively. On the other hand, Rhizobium inoculation exhibited different characterization in different years such that the lowest number of pods per plant (92) was determined in 2003-04 without Rhizobium

Table 6. Effect of Rhizobium and sewage sludge on number of plants per square meter*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	168 d	101 e	60 h	56 hi	135 c	58 f	114 d	78 e	96 D
10	188 c	173 d	68 g	50 i	180 b	59 f	128 c	111 d	120 C
20	225 a	191 c	60 h	91 f	208 a	76 e	143 b	141 b	142 B
40	219 a	199 b	91 f	104 e	209 a	97 d	155 a	152 a	153 A
	Y x R				Year		Rhizobium		
Mean	200 A	166 B	70 D	75 C	183 A	74 B	135 A	121 B	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

Table 7. Effect of Rhizobium and sewage sludge on the number of nodules*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	5.10	6.26	1.64	2.50	5.68	2.07	3.37	4.38	3.87
10	3.15	5.15	1.87	1.28	4.45	2.08	3.01	3.22	3.11
20	8.25	6.45	2.48	2.57	7.35	2.53	5.37	4.51	4.94
40	3.85	6.51	2.18	3.82	5.18	3.00	3.01	5.17	4.09
	Y x R				Year		Rhizobium		
Mean	5.09	6.09	2.29	2.54	5.59 A	2.42 B	3.69	4.32	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

inoculations, whereas the highest one (132) was obtained in 2004-05 without inoculation. Number of pods per plant varied between 78-145 depending on treatments and growing seasons (Table 8).

Seed yield per plant varied between 7.55 and 8.38 g depending on the years, while it changed from 7.73 to 8.19 g with Rhizobium inoculation. Depending on the sewage sludge applications, Seed yield per plant varied between 6.83 and 9.32 g. While the lowest value was obtained from the 40 ton ha^{-1} sewage sludge application, the highest one was determined in the control. In general, the seed yield per plant changed between 5.37 and 11.43 g (Table 9).

The number of seeds per plant varied between 127 and 132 depending on the years and Rhizobium inoculation. Sludge treatments significantly altered the number of seeds per plant, ranging from 115 to 154. The lowest number of seeds per plant was obtained from the application of 40 tons ha^{-1} of sewage sludge, while the highest number of seeds per plant was obtained from the control group without sewage sludge. Finally, the number of seeds per plant varied between 86 and 181 (Table 10).

The biological yield was determined as 8306 kg ha^{-1} in the first year and 3262 kg ha^{-1} in the second year. In the sewage sludge application, the lowest value was obtained from the 10-ton ha^{-1} application with 6156 kg ha^{-1}, while the highest was obtained from the 20-ton ha^{-1} sewage sludge application with 6724 kilograms. According to the YxRxSS interaction, the lowest biological yield was determined as 2.668 kg in plants that were not treated with sewage sludge and were treated with Rhizobium in the 2004-05 season, while the highest one was determined in plants that were not treated with Rhizobium but were treated with 40-ton ha^{-1} sewage sludge in the 2003-04 season (Table 11).

Seed yield was 2716 kg ha^{-1} and 633 kg ha^{-1} in the 2003-04 and 2004-05 seasons, respectively. In contrast to morphological characteristics and plant individual yield indices, grain yield increased with sewage sludge treatment. The lowest grain yield (1387 kg ha^{-1}) was observed in control plants while the highest grain yield (1941 kg ha^{-1}) was determined in 40 ton ha^{-1} sewage sludge treated plants, however, it was in the same statistical group with 20 ton ha^{-1} treated ones. YxSS interaction indicated that the lowest grain yield (514 kg ha^{-1}) was determined in the second experimental season without sewage sludge treatment whereas the highest one (3174 kg ha^{-1}) was reported with 20-ton ha^{-1} sewage sludge in the 2003-04 season. According to RxSS, the lowest grain yield (1285 kg ha^{-1}) was observed in Rhizobium inoculated plants without sewage sludge, while the highest grain yield was seen Rhizobium inoculation with 40 ton ha^{-1} sewage sludge (2083 kg ha^{-1}) and non-Rhizobium inoculation with 20 kg ha^{-1} (2019 kg ha^{-1}). In general, grain yield changed between 454-3459 kg ha^{-1} depending on treatments and experimental years (Table 12).

The harvest index was a pivotal indicator of gigantic yield differences among experimental

Table 8. Effect of Rhizobium and sewage sludge on the number of pods per plant*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	105	143	145	124	134	124	125	133	129
10	90	119	123	109	116	105	106	114	110
20	90	118	122	78	100	104	106	91	102
40	82	93	139	90	115	87	111	98	101
	Y x R				Year		Rhizobium		
Mean	92 B	118 AB	132 A	100 B	104	116	112	109	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

Table 9. Effect of Rhizobium and sewage sludge on seed yield per plant (g)*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	7.90	11.40	9.63	8.33	9.65	8.98	8.77	9.87	9.32 A
10	6.90	7.80	7.97	11.43	7.35	9.70	7.43	9.62	8.53 AB
20	6.50	7.90	8.83	5.50	7.20	7.17	7.67	6.70	7.18 AB
40	5.37	6.60	8.77	6.57	5.98	7.67	7.07	6.58	6.83 B
	Y x R				Year		Rhizobium		
Mean	6.67	8.43	8.80	7.96	7.55	8.38	7.73	8.19	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

Table 10. Effect of Rhizobium and sewage sludge on the number of seeds per plant*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	136	181	166	135	158	150	151	158	154 A
10	111	143	136	125	127	131	124	134	129 AB
20	109	132	151	86	120	119	130	109	119 AB
40	91	112	156	100	102	128	123	106	115 B
	Y x R				Year		Rhizobium		
Mean	112 B	112 B	152 A	142 AB	127	132	132	127	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

Table 11. Effect of Rhizobium and sewage sludge on biological yield (kg ha^{-1})*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	6877 de	6179 e	2808 g	2668 g	6528 a	2738 e	4843	4424	5623 B
10	8138 b-d	7893 cd	3476 fg	2984 fg	8016 b	3230 de	5807	5439	6156 B
20	8741 a-c	9440 ab	3133 fg	3312 fg	9091 a	3222 de	5937	6376	6724 A
40	10001 a	9177 a-c	3378 fg	4339 f	9589 a	3859 d	6690	6758	4633 C
	Y x R				Year		Rhizobium		
Mean	8439	8172	3499	3326	8306 A	3262 B	5819	5749	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

Table 12. Effect of Rhizobium and sewage sludge on seed yield (kg ha^{-1})*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	2402	2116	454	574	2259 c	514 e	1488 bc	1285 c	1387 B
10	2427	2585	501	558	2506 bc	530 de	1492 bc	1543 bc	1518 B
20	3459	2889	489	579	3174 a	534 de	2019 a	1689 a-c	1854 A
40	2770	3083	1082	829	2926 ab	956 d	1799 ab	2083 a	1941 A
	Y x R				Year		Rhizobium		
Mean	2764	2668	635	632	2716 A	633 B	1700	1650	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

years. The harvest index decreased by about 70% in the second year. While Rhizobium inoculation and sewage sludge treatment were not individually effective on harvest index, YxSS and RxSS interactions caused significant differences. According to YxSS, the lowest harvest index (16.7%) was seen with 10 ton ha^{-1} sewage sludge-treated plants in 2004-05, while the highest harvest index (35.3%) was determined in 20 ton ha^{-1} sewage sludge-treated plants at the first growing season. RxSS indicated that the lowest harvest index (22.7%) was seen in Rhizobium inoculation and 20 ton ha^{-1} sewage sludge treatment, whereas the highest one (29.3%) was determined in 40 ton ha^{-1}

sewage sludge with Rhizobium or 20 ton ha^{-1} sewage sludge without Rhizobium. In general, the harvest index varied between 16.3-40.0% (Table 13).

1000-seed weight was 63.7 and 57.7 g in the 2003-04 and 2004-05 seasons, respectively. Sewage sludge was effective on 1000-seed weight in which the lowest 1000-seed weight (62.4 g) was determined in control seeds while the highest one (62.4 g) was obtained from 20 ton ha^{-1} sewage sludge-treated plants. In general, 1000-seed weight varied between 14.9-40.0 g (Table 14).

Table 13. Effect of Rhizobium and sewage sludge on harvest index (%)*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	35.0	33.7	20.6	17.4	34.3a	19.0 cd	27.8 ab	25.5 ab	26.7
10	29.8	32.7	16.3	17.1	31.3a	16.7 d	23.1 ab	24.9 ab	24.0
20	40.0	30.6	18.7	14.9	35.3a	16.8 d	29.3 a	22.7 b	26.0
40	27.7	33.7	24.7	25.0	30.7ab	24.9 bc	26.2 ab	29.3 a	27.8
	Y x R				Year		Rhizobium		
Mean	33.1	32.7	20.1	18.6	32.9 A	19.3 B	26.6	25.6	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same rw in the same group are not statistically significant

Table 14. Effect of Rhizobium and sewage sludge on 1000-seed weight (g)*

Sewage sludge (ton ha^{-1})	Year x Rhizobium x Sewage sludge				Y x SS		R x SS		SS mean
	2003-04		2004-05						
	R (-)	R (+)	R (-)	R (+)	2003-04	2004-05	R (-)	R (+)	
0	62.2	61.2	57.4	62.2	61.7	56.4	58.5	59.3	59.0 C
10	61.5	62.5	56.5	61.5	62.0	57.1	59.5	59.5	59.5 BC
20	64.7	65.8	59.8	64.7	65.3	59.6	62.0	62.8	62.4 A
40	68.5	63.4	57.6	68.5	66.0	57.7	63.2	60.5	61.8 AB
	Y x R				Year		Rhizobium		
Mean	64.2	57.5	63.2	57.8	63.7 A	57.7 B	60.9	60.5	

Y: Year, R: Rhizobium, SS: Sewage sludge, *: Differences between means indicated by the same letter within the same row in the same group are not statistically significant

4. Discussion and Conclusion

The study shows significant differences between the 2003-04 and 2004-05 seasons. In this context, the number of primary branches, the number of plants per square meter, the number of nodules, biological yield, grain yield, harvest index, and 1000-seed weight were superior in the 2003-04 season, whereas only the number of secondary branches per plant was observed to be higher in the second year. Both the direct and indirect effects of climatological factors that have emerged over the years contributed to these results.

A significant decrease in the number of plants per square meter was observed between the 2003-04 and 2004-05 seasons. This decline is more related to environmental conditions than genetic factors. When climate data were examined, lower average temperatures in 2004-05 (-3.7°C) and an increase in total precipitation (421.2 mm) compared to the previous season were identified as key factors affecting plant losses (Figure 1). Vadez et al. (2012) stated that low temperatures reduce germination rates and increase seedling losses, particularly in legumes.

On the other hand, it was found that the number of pods per plant, number of grains per plant, and seed yield per plant did not show significant differences between years. This suggests that yield and growth parameters are directly related to the number of plants per square meter. The higher number of secondary branches per plant in the second year indicates that lower plant density led to increased lateral branch development (Ghaffari Neamat Abad and Saba, 2024; Mahanta and Konjengbam, 2024).

The study demonstrated that the effect of Rhizobium inoculation on lentil growth was limited. It was observed that inoculation had a significant effect only on number of plants per square meter. The decrease in the number of plants in the inoculated group can be explained by the soil's naturally rich Rhizobium population. In other words, the additional bacteria introduced through inoculation may have disrupted the natural microbial balance in the soil, negatively affecting the development of some young seedlings. Similarly, high-dose inoculation could lead to competition with antagonistic microorganisms in the soil (Hibbing et al., 2010). This may have created stress in lentil seedlings, resulting in early-stage mortality. On the other hand, Rhizobium inoculation did not create a significant difference in other experimental parameters. Meanwhile, Erman (1998) showed that Rhizobium inoculation increased root nodulation and nitrogen fixation in lentils. However, the findings from this study did not indicate a significant effect of Rhizobium inoculation on increasing the number of nodules ($p>0.05$). This can be explained by the already sufficient levels of natural Rhizobium bacteria present in the soil.

The study revealed that sewage sludge treatment resulted in statistically significant differences in the number of plants per square meter, seed yield per plant, number of seeds per plant, biological yield, grain yield, and 1000-seed weight. Among these characteristics, number of seeds per plant and seed yield per plant exhibited a negative relationship with sewage sludge treatment, while the highest number of plants per square meter was achieved at 40-ton ha^{-1}, and the highest values for the other characteristics were observed at 20 ton ha^{-1}.

At higher doses (40 ton ha^{-1}), sewage sludge had a negative effect on grain yield, which aligns with the findings of Alvarenga et al. (2015), who reported that excessive application of sewage sludge could lead to soil toxicity. The decline in seed yield per plant at 40 ton ha^{-1} supports this hypothesis. Furthermore, it was reported by Çığ et al. (2019) that increasing doses of sewage sludge application increased soil N and P content but reduced Ca, Mg, Mn, Zn, and Cu concentrations, indicating a disruption in soil ion balance at high doses. However, the fact that 20 and 40 kg ha^{-1} sewage sludge increased biological yield and grain yield can be attributed to the increase in number of plants per square meter. Erman et al. (2024) indicated that 40 kg ha^{-1} sewage sludge improved barley biofortification and restricted heavy metal accumulation in plant tissues. The application of sewage sludge was found to increase the number of plants per square meter from 96 to 153, suggesting that sewage sludge is rich in organic matter and nutrients, and enhanced germination rates. Kumar et al. (2022) reported that sewage sludge improved germination rates in legumes and enhanced soil water retention capacity, thereby supporting seedling development. Different researchers reported that organic fertilizers improve soil structure, promote root development, and reduce early seedling mortality (Ceritoglu et al., 2018; Chen et al., 2021).

In conclusion, extreme climatic events that vary between years in the Van region can lead to significant differences in lentil yield. Therefore, the application of sewage sludge to the soil may help improve seedling emergence rates and increase plant survival, preventing plant density from dropping below a critical threshold under harsh winter conditions. On the other hand, a sewage sludge application of 20 kg ha^{-1} is recommended to avoid economic inefficiencies and soil ion imbalance issues. The regional soils appear to have a sufficient Rhizobium population, and inoculation was not found to provide any additional beneficial contribution. It was concluded that sewage sludge can positively affect lentil cultivation, however, both soil and characteristics of sewage sludge must be taken into account while determining the application dose.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

Conceptualization, Material, Methodology, Investigation, Data Curation, Writing-Review & Editing, *M. ERMAN*; Conceptualization, Material, Methodology, Investigation, Data Curation, Writing-Review & Editing, *F. ÇIĞ*; Formal Analysis, Visualization, Writing-Original Draft Preparation, *M. CERİTOĞLU*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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