YIELD AND QUALITY OF BLACK CUMIN (*Nigella sativa* L.) ACCORDING TO LEONARDITE AND NITROGEN DOSES

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Abstract. This study aimed to reveal the effect of different levels of leonardite and nitrogen fertilizer applied to the soil on seed yield and some quality parameters of black cumin (*Nigella sativa* L.). The study was carried out in the Southeastern Anatolia Region of Turkey between the years 2016-2018. In the study, 4 different doses of leonardite and nitrogen fertilizer were administered. In the study, the highest plant height and number of branches were found in L_2N_{90}, the highest number of capsules was found in L_2N_{60}, and the highest number of seed per capsule was found in L_2N_{60} and L_3N_{60}. As a result of the study, the highest seed yield was obtained from L_2 and L_3 doses of leonardite and from N_{60} dose of nitrogen. It was determined that the fixed oil ratio, essential oil ratio contained in the black cumin seeds, and essential oil components parallel with the increase in leonardite and nitrogen doses. It was concluded that leonardite, a soil conditioner, could be used in black cumin cultivation under semi-arid climate conditions and that 2000 kg ha^{-1} leonardite and 60 kg ha^{-1} nitrogen could be applied to improve the yield and quality of black cumin.
Keywords: *soil conditioner, thymoquinone, seed yield, Nigella sativa, essential oil, fixed oil*

Introduction

The blackcumin is a annual herbaceous plant from the Ranunculaceae family. It is distributed over a wide area from southern and eastern shores of Indian continent to mediterranan basin (Egypt and Turkey) (Seyyedi et al., 2015; Kılıç and Arabacı, 2016). It has 12 species distributed in Turkey. *Nigella sativa* and *Nigella damascena* species are commonly cultivated (Baydar, 2013). One of the most famous books in the history of medicine by İbn-i Sina, the author of “El Kanun Fi‟ ttıb”, states that black cumin stimulates metabolism, prevents drowsiness and fatigue, regulates body energy and restores vitality lost by disease (Nasr, 2008). The seeds of black cumin have high economic value and contain fixed oil, essential oil, protein and carbohydrates. It also contains nigellidin, nigellisin and nigellimin in alkaloids in seeds (Baydar, 2013).

Black cumin is an important spice species and have high production especially in East and Southeast Asia. There is no specific report of production area and amount for black cumin, but for spice production India, China and Turkey leads world production (Dessie et al., 2020).

Humic acids, which are among the most important components of humic substances, are heterogeneous natural resources with high molecular weight and colors varying from yellow to black (Akıncı, 2011), and they have significant benefits in terms of both improving soil properties and agricultural production such as increasing the cation exchange capacity of the soils and neutralizing the pH of the soil (Stevenson, 1994), making plant nutrients bound to soil colloids available (Yılmaz, 2007), slowing down the evaporation of water in the soil (Akıncı, 2011; Sesveren and Taş, 2018), improving soil microflora (Larcher, 2003; Calvo et al., 2014; Li et al., 2019), improving plant growth, yield, and quality (Sharif et al., 2002; Esringü et al., 2015; Selladurai and Purakayastha, 2016; Çöl and Akınerdem, 2017; Ahmad et al., 2018), and increasing the

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uptake of mineral elements in plants (Mackowiak et al., 2001; Eyheraguibel et al., 2008; Khaled and Fawy, 2011). Leonardite constitutes the raw material of humic acids that are classified as soil conditioners (Pekcan et al., 2018). Leonardite, which is a sedimentary rock formed by the changes of plant and animal remains during a period of millions of years (Pekcan et al., 2018), is an all-natural organic material that has not reached the level of coal (Sesveren and Taş, 2018). The use of materials such as leonardite to increase the organic matter content of the soil, especially in agricultural soils containing low organic matter with an intense polyculture production pattern, takes an important place nowadays.

It was reported that humic acid applications in *Brassica napus* ssp. *oleifera* L. plant had positive effects on plant height, the number of sub-branches attached to the main stem, the number of capsules in the main stem, the number of seeds per capsule, thousand grain weight, seed yield, oil ratio, and oil yield properties (Gürsoy and Kolsarıcı, 2017), that better tuber yields were obtained with humic acid and fulvic acid applications in the potato plant especially in dry and cool periods (Wadas and Dziugieł, 2019), and that potassium fertilizer and humic acid doses applied in increasing amounts in *Helianthus annuus* L. plant had significant effects on the yield and yield components and mineral content of the plant (Yağmur and Okur, 2017). Similarly, it was determined that the tuber yield in potato (Şanlı et al., 2013), wheat grain yield (Kolay et al., 2016), rye dry matter yield (Adiloğlu et al., 2017) increased in parallel with the increase in leonardite doses.

Obtaining high yields and quality products in agricultural production depends on giving the nutrients needed by the plant to plants in the appropriate period and dose, along with the accurate and timely implementation of many other cultural practices. In this sense, nitrogen (N) is the primary element among nutrients that are absolutely essential for many cultivated plants. The unconscious and excessive use of fertilizers, which are the most important inputs of agricultural activities, is shown to be one of the most important causes of soil pollution nowadays, and high doses of fertilizer applications also lead to toxic accumulation in plants, the inhibition of the intake of some other nutrients, and of course, economic losses. Therefore, the determination of nutrient needs of cultivated plants in different ecologies and the preparation of fertilization programs according to the soil analysis results constitute the most important branches of sustainable agriculture techniques.

Moreover, it is necessary to carry out cultural application techniques that minimize agricultural inputs such as fertilizer. For this purpose, it is essential to use soil conditioners such as leonardite in plant production, especially in agricultural soils where soil organic matter is low and, consequently, the intake of nutrients is prevented, and to reveal the interaction with other nutrients, and to show its effects on product yield and quality. In this study, it was aimed to reveal the effect of leonardite and nitrogen fertilizer applied to the soil at different levels on seed yield and some quality parameters of black cumin.

Materials and methods

The study was carried out between 2016-2018 under the ecological conditions of Siirt province located in the Southeastern Anatolia Region of Turkey, which has a semi-arid climate. Siirt is located at 37° 58' 7.37" N and 41° 51' 3.87" E coordinates with 894 m altitude (*Figure 1, Figure 2*).

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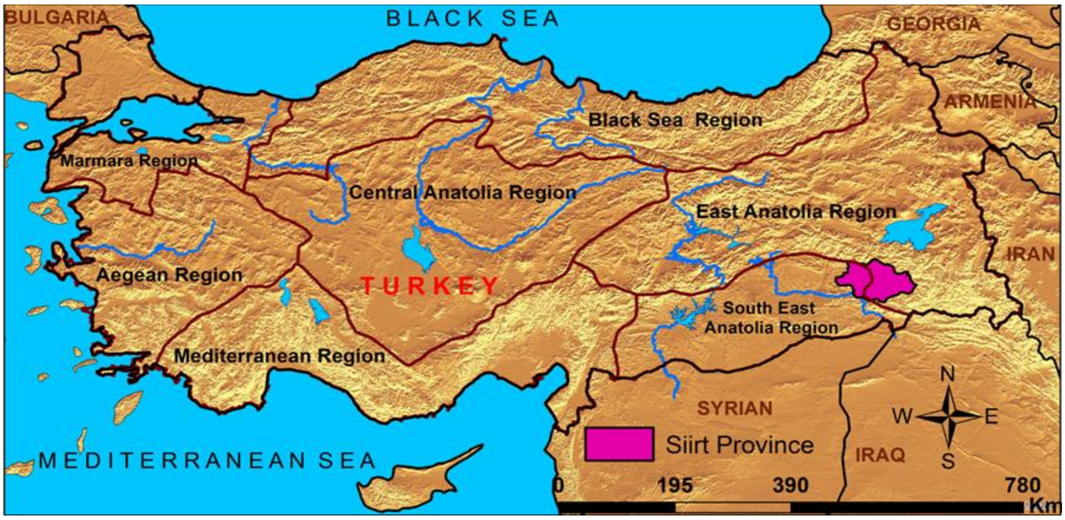

Figure 1. *Location of the study area in Turkey*

Figure 2. *A photo of the research area*

In the second year of the study, less precipitation (522.8 mm) occurred in comparison with the first year (574.2 mm), while the long-term annual precipitation was 634.1 mm. While the highest precipitation occurred in March and April during the 2016-2017 vegetation period, the highest precipitation was recorded in May during the 2017-2018 vegetation period (*Table 1*). The mean temperature during the study years and the long-term mean temperature (38 years) were 11.2 ^oC, 13.8 ^oC, and 11.4 ^oC, respectively (*Table 1*) (Anonymous, 2018).

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Table 1. *Climate characteristics of trial area*

Climate parameters	Research years	Months								
		Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**Mean/Sum**
Average temparature (ºC)	2016-2017	10.4	3.3	3.0	2.7	9.6	14.0	19.5	26.9	11.2
	2017-2018	11.2	8.0	5.7	8.2	13.7	16.8	19.8	27.4	13.8
	Long term*	10.3	4.9	3.0	4.5	8.8	14.3	19.5	26.2	11.4
Monthly precipitation (mm)	2016-2017	55.4	116.6	46.4	29.2	119.2	132.8	74.6	0.0	574.2
	2017-2018	86.0	47.4	56.4	74.2	47.6	61.6	139.6	10.0	522.8
	Long term*	85.1	91.1	82.2	96.6	108.7	96.3	64.3	9.8	634.1

*: 1980-2018

In the study, some physical and chemical analysis results of the soils taken before establishing the field trial were presented in *Table 2*. In the first year (2016-2017), the trial area soils were loamy textured, and they were clay-loam textured in the second year (2017-2018); both trial area soils were slightly alkaline, salt-free, their lime content was "medium calcareous," the organic matter content was "low," and the available potassium (K) content was "sufficient." The available P content of the soils in the first year was determined to be "very little," and the available P content of the soils in the second year was determined to be "low" (*Table 2*).

Table 2. *Some physical and chemical properties of the study area soils (0-20 cm)**

Properties	Value	
	2016-2017	**2017-2018**
Clay, %	47.56	34.16
Silt, %	12.11	26.00
Sand, %	40.33	39.84
pH	7.72	7.53
Electrical conductivity (EC), mS cm^{-1}	0.363	0.150
Lime ($CaCO_3$), %	12.0	8.2
Organic matter, %	1.31	1.78
Available phosphorus, kg P_2O_5 ha^{-1}	24	49
Available potassium, kg K_2O ha^{-1}	1430	1250

*: Analyses were carried out in Siirt University, Science and Technology Application and Research Center Laboratory

As the plant material in the study, black cumin (*N. sativa* L.) seeds, belonging to the "Isparta population" and seeded locally in the Mediterranean Region of Turkey, were used.

In this study, the field trial was established as three replications in randomized blocks according to the factorial trial design. In the study, 4 different leonardite doses (L_0= 0, L_1= 1000, L_2= 2000, and L_3= 3000 kg ha^{-1} leonardite) and 4 different nitrogen doses (N_0= 0, N_{30}= 30, N_{60}= 60, and N_{90}= 90 kg N ha^{-1}) constituted the subject of the study. “Leonagro” was used as the source of leonardite, and urea (46% N) was used as the source of nitrogen fertilizer. The content of the leonardite material is given in *Table 3*. According to the research subjects, both fertilizer forms were mixed by applying them to the soil before seeding.

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Table 3. *Some properties of leonardite using the trial*

pH	Humic + Fulvic acid (%)	Organic Matter (%)
6-8	40	40

The seeding process was performed manually on 15 November 2016 in the first year and on 17 November 2017 in the second year on the grooves opened with the help of a marker. In the study, row distance was 30 cm and parcel distance was 100 cm. Each parcel constituted from four rows, while length and width of the parcels were 3 meter by 1.2 meter each with a total area of 3.6 m^2 per parcel. Sowing norm was 30 kg ha^{-1}, and sowing was done manually in rows opened with the help of a marker.Weed control was performed mechanically by hand several times in both years. At the harvest, two border rows and 50 cm from each side were excluded to eliminate border effects.

Plant height, number of branches, number of capsules, number of seed per capsule, seed weight were determined in 10 plants randomly selected in each parcel before harvest. The harvest was carried in the entire plot area, excluding borders in the first week of July in both years. The harvested plants were dried in the shade for 3-4 days, and seed yields were calculated per decare. Thousand seed weight, fixed oil and essential oil analysis were performed. For the fixed oil percentage grinded samples were dried at 105 ^{o}C for 3 hours and 10 g sample was fed into a Soxhlet apparatus (SOXTHERM® 2000). The extraction was performed on a water bath at 60 ^{o}C for 4 h with 200 mL petroleum ether. For the essential analysis percentage; seeds dried at 35 ^{o}C in room were crushed bu using grinder. Distillation process was carried out using the Clevenger apparatus. Distilled water (250 ml) was used, and 25 g crushed fruit were watered with 250 ml distilled water (1:10). Distillation lasted for approximately 5 h at boiling point. The essential oil collected at the end of distillation was measured in mL and calculated as % (v/w). The essential oil components of black cumin seed were determined using Headspace GC-MS. Crushed black seed samples was taken 1 g and placed in a 25 mL Chromacol Headspace vial. The vial was heated in a Triplus RSH Headspace oven for 90 minutes at 120 °C. The heated Headspace was sent from the vial to the GC-MS with an injection volume of 2.5 mL. The analysis was carried out in a Trace 1310 gas chromatograph equipped with an ISQ single quadrupole mass spectrometer (Thermo Fisher Scicentific, Austin, TX). The procedure was set to an initial temperament 60 °C for 10 min, then ramp at 1 °C/min to 140 °C, 1 min in 140 °C, then ramp at 15 °C/min to 230 °C and finally 5 min in 230 °C. The ion source and detector temperature was 220 °C and 220 °C, respectively. Separation of sample was performed on a Thermo TG-WAXMS GC column (60 m x 0.25 mm ID x 0.25 µm) using helium as carrier gas at 1.2 ml/min. Mass spectral scan range was set at the rate of 55–550 (amu). Peak identification was conducted by comparison of the known components stored in the NIST Demo, Wiley7, Wiley9, redlip, mainlip, WinRI.

The data were analyzed by JMP statistical software. A homogeneity test was applied to the data obtained from the study. According to the results of the homogeneity test, they were subjected to combined variance analysis (ANOVA) according to the factorial trial design in randomized blocks. According to the F-test results, differences between the groups were determined by the LSD multiple comparison test (Yurtsever, 1984).

Results and discussion

Plant height

According to the results of a two-year study, the effects of leonardite and nitrogen fertilizer doses on the plant height of black cumin were found to be statistically significant at a level of p<0.01 (*Table 4*).

Table 4. *Means of yield components at different leonardite and nitrogen doses in black cumin*

L doses	N doses	Plant height (cm)			Number of branches (unit/plant)			Number of capsules (unit/plant)		
		2017	2018	Mean*	2017	2018	Mean*	2017	2018	Mean*
L_0	N_0	49.87	50.87	50.37 g	3.47	3.60	3.53 g	4.63	4.77	4.70 h
	N_{30}	53.27	56.20	54.73 efg	3.60	3.70	3.65 d-g	5.47	6.07	5.77 cd
	N_{60}	61.70	64.20	62.95 bc	3.80	3.83	3.82 ab	6.57	7.13	6.85 a
	N_{90}	59.23	65.97	62.60 bc	3.80	3.83	3.82 ab	5.07	5.57	5.32 d-g
L_0 Mean		56.02	59.31	57.66 C	3.67	3.74	3.70	5.43	5.88	5.66
L_1	N_0	51.50	54.09	52.80 g	3.63	3.73	3.68 b-f	4.93	5.27	5.10 fgh
	N_{30}	61.13	62.97	62.05 bc	3.57	3.63	3.60 fg	4.97	5.30	5.13 e-h
	N_{60}	62.80	61.25	62.02 bc	3.80	3.80	3.80 bc	6.27	5.67	5.97 bc
	N_{90}	58.53	58.80	58.67 cde	3.70	3.90	3.80 bc	5.33	6.07	5.70 cde
L_1 Mean		58.49	59.28	58.88 BC	3.68	3.77	3.72	5.38	5.58	5.48
L_2	N_0	52.73	55.71	54.22 fg	3.70	3.70	3.70 b-f	4.87	5.00	4.93 gh
	N_{30}	51.13	56.89	54.02 fg	3.47	3.60	3.53 g	4.70	4.80	4.75 gh
	N_{60}	64.46	62.31	63.39 b	3.53	3.70	3.62 efg	7.40	6.43	6.92 a
	N_{90}	65.30	71.47	68.38 a	3.97	3.93	3.95 a	5.40	5.90	5.65 c-f
L_2 Mean		58.41	61.60	60.00 AB	3.67	3.73	3.70	5.59	5.53	5.56
L_3	N_0	56.03	59.13	57.58 def	3.77	3.80	3.78 bcd	4.92	4.95	4.94 gh
	N_{30}	61.30	62.56	61.93 bcd	3.70	3.80	3.75 b-e	5.60	5.63	5.62 c-f
	N_{60}	58.40	63.87	61.13 bcd	3.70	3.80	3.75 b-e	6.48	6.53	6.51 ab
	N_{90}	62.50	66.97	64.74 ab	3.67	3.67	3.67 c-g	5.83	5.88	5.86 cd
L_3 Mean		59.56	63.13	61.35 A	3.71	3.77	3.74	5.71	5.75	5.73
N Mean										
N_0		52.53	54.95	53.74 c	3.64	3.71	3.68 b	4.84	4.99	4.92 d
N_{30}		56.71	59.66	58.18 b	3.58	3.68	3.63 b	5.19	5.45	5.32 c
N_{60}		61.84	62.91	62.37 a	3.71	3.78	3.75 a	6.68	6.44	6.56 a
N_{90}		61.39	65.80	63.60 a	3.78	3.83	3.81 a	5.41	5.86	5.64 b
Means		58.12 B	60.82 A		3.68 B	3.75 A		5.53	5.69	
CV (%)		6.40			3.23			8.99		
Year (Y)		**			**			ns		
Leonardite (L)		**			ns			ns		
Nitrogen (N)		**			**			**		
LxN		**			**			**		
LxNxY		ns			ns			ns		

L_0= Control, L_1= 1000 kg ha^{-1} leonardite, L_2= 2000 kg ha^{-1} leonardite, L_3= 3000 kg ha^{-1} leonardite, N_0= Control, N_{30}= 30 kg ha^{-1} nitrogen, N_{60}= 60 kg ha^{-1} nitrogen, N_{90}= 90 kg ha^{-1} nitrogen, *: The difference between the means indicated by the same letter in the same column and group is not significant, CV: Coefficient of variation, ns: Not significant, **: p<0.01

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The plant height values increased in parallel with the increase in leonardite and nitrogen doses, and the highest plant height was measured at the highest doses of both leonardite and nitrogen. In the study, when the interaction of organic material and nitrogen doses was evaluated together, according to two-year averages, the highest plant height value was obtained in L_2N_{90} (68.38 cm) subject, and the lowest values were found in L_0N_0 (50.37 cm) subject. LxN interaction was found to be statistically significant at a level of $p<0.01$ (*Table 4*).

Nitrogen is an essential plant nutrient that promotes vegetative growth in plants. Therefore, nitrogen fertilizer applications affect plant height. In their studies carried out with black cumin, Shah (2004), Özgüven and Şekeroğlu (2007), Tuncturk et al. (2012), Yimam et al. (2015), Muhammad et al. (2017), Kızılyıldırım (2019), and Sultana et al. (2019) reported that plant height values increased in parallel with the increase in nitrogen fertilizer doses and/or plant height was positively affected by nitrogen dose applications, similarly to the results of our study.

It has also been reported in the results of many studies that organic material applications such as humic acid and leonardite, which is a source of humic acid, improve the soil properties, and therefore, plant height is positively affected in plants (Laz, 2011; Demirkıran et al., 2012; Betül et al., 2016; Eleroğlu and Korkmaz, 2016; Çöl and Akınerdem, 2017; Yağmur and Okur, 2017).

In the study, the difference between the years was also found to be significant, and the highest plant height values were determined in the second year of the study (*Table 4*). These differences in plant height values between the years can be explained by the difference in precipitation regimes over the years.

Number of branches

With respect to the number of branches, the effects of N doses alone were found to be statistically significant ($p<0.01$), while the effects of leonardite doses were found to be insignificant. As an average of leonardite doses, the highest number of branches was statistically found in N_{60} (3.75 unit plant^{-1}) and N_{90} (3.81 unit plant^{-1}) nitrogen doses that constituted the first group, and the lowest values were found in other doses of nitrogen. In the study, when LxN interaction was examined, while the highest value for the number of branches was found in L_2N_{90} (3.95 unit plant^{-1}) subject, the lowest value was found in L_0N_0 (3.53 unit plant^{-1}) subject (*Table 4*).

According to the results of the two-year study carried out with black cumin by Tuncturk et al. (2012), it was reported that nitrogen fertilizer doses increased the number of branches and that the difference between 40-80 kg nitrogen applications per hectare in terms of the number of branches was insignificant. While Özgüven and Şekeroğlu (2007) reported that the highest number of branches was obtained from 90 kg N ha^{-1} application dose among nitrogen fertilizer dose applications, Kızılyıldırım (2019) and Sultana et al. (2019) reported that it was obtained from 100 kg N ha^{-1} and 60 kg N ha^{-1} application doses, respectively. These results in the literature were found to be relatively compatible with the results of our study.

Number of capsules

With respect to the number of capsules per plant, while the effects of N doses alone were found to be statistically significant ($p<0.01$), the effects of leonardite doses were insignificant. As an average of leonardite doses, the highest number of capsules was found in N_{60} (6.56 unit plant^{-1}), and the lowest number of capsules was found in the

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control subject in which nitrogen fertilization was not applied (N_0) with 4.92 unit plant^{-1}. In the study, when LxN interaction was examined, the highest values for the number of capsules were statistically found in L_0N_{60} (6.85 unit plant^{-1}) and L_2N_{60} (6.92 unit plant^{-1}) subjects that constituted the first group. The lowest number of capsules per plant was found in L_0N_0 subject with 4.70 units. In the study, LxN interaction was found to be statistically very significant ($p<0.01$) (*Table 4*).

The number of branches per plant affects the number of capsules obtained per plant (Tuncturk et al., 2012). Indeed, in our study, nitrogen doses increased the number of branches, and consequently, more capsules were produced by plants. In their study, Tuncturk et al. (2012) investigated the effects of different nitrogen doses (0, 20, 40, 60, and 80 kg N ha^{-1}) in black cumin and reported that the number of capsules per plant increased up to the nitrogen dose of 60 kg N ha^{-1}, that the highest number of capsules was achieved with 7.5 capsules at this dose according to the average of two years, and that there was a statistically significant decrease in the number of capsules after 60 kg N ha^{-1} nitrogen dose. These results were in parallel with the results of our study. It was also reported in some other studies (Özgüven and Şekeroğlu, 2007; Rana et al., 2012; Yimam et al., 2015; Kızılyıldırım, 2019) that the number of capsules in black cumin increased depending on the level of nitrogen fertilizer.

Number of seeds per capsule

According to the results of the two-year study, the effects of leonardite and nitrogen fertilizer doses on the number of seeds per capsule in black cumin were found to be statistically significant at a level of $p<0.01$. When the two-year data were considered, the number of seeds per capsule increased in parallel with the increase in leonardite doses, and as an average of nitrogen doses, the highest and lowest values were obtained at L3 dose (92.52 seeds) and L_0 dose (62.32 seeds), respectively (*Table 5*). The positive effect of leonardite on the number of seeds per capsule can be explained by its contribution by improving the soil structure. Namely, humic substances such as leonardite serve as a reserve in terms of elements such as carbon, nitrogen, Sulphur, and phosphorus, therefore, in the study soil where soil organic matter was quite low (*Table 2*), it was considered that leonardite, known as the soil conditioner, made some plant nutrients in the soil available to plants, and consequently, flower and seed formation increased. Gürsoy and Kolsarıcı (2017) reported that humic acid doses in the soil covered with leonardite increased the number of seeds per capsule in the colza plant.

When the effects of nitrogen levels were evaluated, it was determined that the number of seeds per capsule increased up to N_{60} nitrogen dose and decreased statistically significantly after this dose and that the highest value for the number of seeds per capsule, as an average of leonardite doses, was found at N_{60} nitrogen dose with 100.69 seeds. The lowest values in terms of the number of seeds per capsule were found at N_0 dose (53.81 seeds) (*Table 5*). As in the results of our study, Shah (2004) and Mollafilabi et al. (2010) reported in their study on black cumin that there were significant decreases in the number of seeds per capsule after a certain level of nitrogen. In their study carried out with the datura plant, Esendal et al. (2000) reported that the number of seeds per capsule increased in parallel with the increase in nitrogen doses. On the contrary, Tuncturk et al. (2012) and Kızılyıldırım (2019) determined that nitrogen fertilizer levels in black cumin did not affect the number of seeds per capsule and that the number of seeds per capsule varied between 52.4-55.1 and 110.70-126.73,

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respectively, depending on the levels of nitrogen fertilizer applied. Unlike these data in the literature, significant differences between nitrogen doses in terms of the number of seeds per capsule in our study can be explained by the fact that the soil-plant nutrition relationship occurred differently in terms of nitrogen and other plant nutrients depending on the soil and climatic conditions, in addition to different varieties used.

Table 5. *Means of yield components at different leonardite and N doses in black cumin*

L doses	N doses	Number of seeds per capsule (number)			Seed weight capsule (g)			Thousand grain weight (g)		
		2017	**2018**	**Mean[1]**	**2017**	**2018**	**Mean[1]**	**2017**	**2018**	**Mean[1]**
L_0	N_0	42.00	43.94	42.97 ı	0.44	0.47	0.46	2.10	2.26	2.18
	N_{30}	47.32	49.31	48.31 h	0.51	0.52	0.51	2.30	2.30	2.30
	N_{60}	80.68	85.07	82.88 d	0.54	0.55	0.54	2.35	2.28	2.32
	N_{90}	73.72	76.49	75.11 e	0.54	0.57	0.56	2.30	2.33	2.32
L_0 Mean		60.93	63.70	62.32 D	0.51	0.53	0.52 B	2.26	2.29	2.28 C
L_1	N_0	48.95	50.69	49.82 h	0.46	0.46	0.46	2.28	2.25	2.27
	N_{30}	54.91	58.00	56.45 g	0.49	0.50	0.49	2.37	2.41	2.39
	N_{60}	91.35	93.54	92.45 c	0.54	0.56	0.55	2.29	2.36	2.33
	N_{90}	72.09	77.65	74.86 e	0.56	0.56	0.56	2.27	2.26	2.26
L_1 Mean		66.82	69.97	68.40 C	0.51	0.52	0.52 B	2.30	2.32	2.31 BC
L_2	N_0	59.52	61.29	60.41 fg	0.50	0.51	0.50	2.34	2.33	2.34
	N_{30}	74.63	75.90	75.27 e	0.49	0.54	0.51	2.35	2.36	2.36
	N_{60}	111.37	114.13	112.75 a	0.54	0.57	0.56	2.34	2.36	2.35
	N_{90}	93.67	94.55	94.11 c	0.55	0.57	0.56	2.36	2.38	2.37
L_2 Mean		84.79	86.47	85.63 B	0.52	0.54	0.53 A	2.35	2.36	2.35 A
L_3	N_0	62.00	62.12	62.06 f	0.51	0.52	0.51	2.23	2.28	2.26
	N_{30}	91.10	91.34	91.27 c	0.53	0.53	0.53	2.27	2.29	2.28
	N_{60}	114.57	114.97	114.77 a	0.53	0.57	0.55	2.38	2.37	2.37
	N_{90}	102.00	102.06	102.03 b	0.55	0.60	0.58	2.35	2.37	2.36
L_3 Mean		92.42	92.62	92.52 A	0.53	0.55	0.54 A	2.31	2.32	2.32 AB
N Mean										
N_0		53.12	54.50	53.81 d	0.47	0.49	0.49 d	2.24	2.28	2.26 b
N_{30}		66.99	68.62	67.81 c	0.50	0.52	0.51 c	2.32	2.34	2.33 a
N_{60}		99.49	101.88	100.69 a	0.54	0.56	0.55 b	2.34	2.34	2.34 a
N_{90}		85.37	87.68	86.53 b	0.55	0.57	0.56 a	2.32	2.34	2.33 a
Means		76.24 B	78.17 A		0.52 B	0.54 A		2.31	2.32	
CV (%)			5.93			4.19			7.17	
Year (Y)			*			**			ns	
L			**			**			**	
N			**			**			**	
L x N			**			ns			ns	
L x N x Y			ns			ns			ns	

L_0= Control, L_1= 1000 kg ha^{-1} leonardite, L_2= 2000 kg ha^{-1} leonardite, L_3= 3000 kg ha^{-1} leonardite, N_0= Control, N_{30}= 30 kg ha^{-1} nitrogen, N_{60}= 60 kg ha^{-1} nitrogen, N_{90}= 90 kg ha^{-1} nitrogen, [1]: The difference between the means indicated by the same letter in the same column and group is not significant, CV: Coefficient of variation, ns: Not significant, *: $p<0.05$, **: $p<0.01$

When LxN interaction was examined, the highest value for the number of seeds per capsule was statistically obtained from L_3N_{60} (114.77 seeds) and L_2N_{60} (112.75 seeds) applications, which constituted the first group. The lowest value was found in L_0N_0 (42.97 seeds). In the study, the highest values for the number of seeds per capsule were found in the second year of the study as an average of leonardite and nitrogen doses.

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With respect to the number of seeds per capsule, the difference between the years ($p<0.05$) and LxN interaction ($p<0.01$) were found to be statistically significant (*Table 5*).

Seed weight capsule

The effects of leonardite and nitrogen fertilizer doses on the seed weight capsule in black cumin were found to be statistically significant at a level of $p<0.01$. When the two-year data were considered, seed weight capsule increased in parallel with the increase in leonardite doses, and as an average of nitrogen doses, the highest values were obtained at L_3 dose (0.54 g) and L_2 dose (0.53 g), respectively (*Table 5*). The highest seed weight capsule value was 0.56 with N_{90} nitrogen doses as an average of leonardite doses (*Table 5*).

Thousand-seed weight

In the study, nitrogen doses had a statistically significant effect at a $p<0.01$ level on the thousand grain weight of black cumin, and the highest thousand seed weight values were found at other nitrogen doses (N_{30}, N_{60}, and N_{90}), except for the control (N_0) subject. The effects of leonardite applications were found to be significant at a $p<0.01$ level (*Table 5*). The highest thousand seed weight value was obtaned from L_2 (2.35 g), followed by L_3 (2.32 g) leonardite doses. In the studies carried out by Başalma (1999) in the colza plant, by Esendal et al. (2000) in the datura plant, and by Ashraf et al. (2006) and Muhammad et al. (2017) in black cumin, the researchers determined that nitrogen fertilizer doses significantly increased thousand grain weight compared to the control. On the other hand, Tuncturk et al. (2012) and Kızılyıldırım (2019) reported that no significant differences were observed between nitrogen fertilizer doses in terms of thousand grain weight in black cumin.

Seed yield

In the study, when the effects of leonardite applications were examined, the highest seed yield, as an average of nitrogen doses, was statistically found at L_3 (1673.3 kg ha^{-1}) and L_2 (1645.8 kg ha^{-1}) leonardite doses included in the first group. In terms of seed yield, the lowest results were obtained at L_0 and L_1 doses. This difference between leonardite applications was found to be statistically very significant ($p<0.01$) (*Table 6*). It was considered that leonardite applied to the soil prevented the evaporation of water in the plant root zone and conserved the water in the soil, and consequently, drought and temperature stress occurred, and it increased the effectiveness of the existing water in the soil under the climate and soil conditions of Siirt province. Accordingly, the application of leonardite in these and similar soils, which are low in organic matter, plays a role in the conversion of plant nutrients in the soil into receivable form and increases the efficiency of the use of nutrients by plants. Therefore, humic substances such as leonardite have indirect effects on the yield increase in plants by increasing the uptake of minerals. In our study, it was considered to be effective on the increase in the seed yield of black cumin, depending on the increase in leonardite doses. Similar studies carried out in different plants on this subject support the results of our study. For example, it was reported that humic acid dose applications increased the seed yield in colza (Gürsoy et al., 2016) and tuber yield in the potato plant (Çöl and Akınerdem, 2017) compared to the control, that leonardite applications increased the tuber yield in

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potato (Şanlı and Karadoğan, 2011) compared to the control, and that leonardite applications increased the amount of dry matter and the levels of some macronutrients (Adiloğlu et al., 2017) in the rye (*Secale cerale* L.) plant compared to the control.

Table 6. *Means of seed yield, fixed oil and essential oil ratio at different leonardite and nitrogen doses in black cumin*

L doses	N doses	Seed yield (kg ha^{-1}) 2017	2018	Mean[1]	Fixed oil ratio (%) 2017	2018	Mean[1]	Essential oil (%) 2017	2018	Mean[1]
L_0	N_0	736.7	790.0	763.3	36.08	37.00	36.54	0.23	0.25	0.24
	N_{30}	1100.0	1120.0	1110.0	36.34	37.22	36.78	0.26	0.26	0.26
	N_{60}	1783.3	1837.7	1810.0	36.80	37.74	37.27	0.28	0.29	0.28
	N_{90}	1580.0	1710.0	1645.0	37.30	37.63	37.47	0.28	0.30	0.29
L_0 Mean		1300.0	1364.2	1332.0 B	36.63	37.40	37.02 B	0.26	0.28	0.27 B
L_1	N_0	1023.3	1053.3	1038.3	36.66	37.58	37.12	0.25	0.26	0.25
	N_{30}	1150.0	1170.0	1160.0	36.45	37.39	36.92	0.26	0.28	0.27
	N_{60}	1810.0	1863.3	1836.7	37.58	38.25	37.92	0.28	0.31	0.29
	N_{90}	1636.7	1756.7	1696.7	37.87	38.20	38.03	0.29	0.31	0.30
L_1 Mean		1405.0	1460.8	1432.9 B	37.14	37.86	37.50 A	0.27	0.29	0.28 B
L_2	N_0	1290.0	1356.7	1323.3	36.61	37.59	37.10	0.26	0.28	0.27
	N_{30}	1226.7	1493.3	1360.0	36.40	37.30	36.85	0.27	0.30	0.29
	N_{60}	1886.7	2046.7	1966.7	37.00	37.27	37.13	0.30	0.32	0.31
	N_{90}	1910.0	1956.7	1933.3	38.27	38.60	38.43	0.30	0.33	0.31
L_2 Mean		1578.3	1713.3	1645.8 A	37.07	37.69	37.38 A	0.28	0.31	0.30 A
L_3	N_0	1120.0	1206.7	1163.3	36.44	37.11	36.77	0.25	0.28	0.27
	N_{30}	1566.7	1606.7	1586.7	36.46	37.36	36.86	0.27	0.30	0.28
	N_{60}	2043.3	2110.0	2076.7	37.69	38.02	37.85	0.29	0.31	0.30
	N_{90}	1870.0	1863.3	1866.7	37.86	37.93	37.89	0.30	0.32	0.31
L_3 Mean		1650.0	1696.6	1673.3 A	37.11	37.61	37.36 A	0.27	0.30	0.29 A
N Mean										
N_0		1042.5	1101.6	1072.1 d	36.45	37.34	36.89 c	0.25	0.27	0.26 c
N_{30}		1260.8	1347.5	1304.2 c	36.41	37.35	36.88 c	0.26	0.29	0.27 b
N_{60}		1880.8	1964.2	1922.5 a	37.27	37.83	37.55 b	0.29	0.31	0.30 a
N_{90}		1749.2	1821.6	1785.4 b	37.83	38.09	37.96 a	0.29	0.32	0.31 a
Means		1483.3 B	1558.7 A		36.99 B	37.65 A		0.27 B	0.29 A	
CV (%)			11.85			1.33			4.60	
Year (Y)			*			**			**	
L			**			**			**	
N			**			**			**	
L x N			ns			ns			ns	
L x N x Y			ns			ns			ns	

L_0= Control, L_1= 1000 kg ha^{-1} leonardite, L_2= 2000 kg ha^{-1} leonardite, L_3= 3000 kg ha^{-1} leonardite, N_0= Control, N_{30}= 30 kg ha^{-1} nitrogen, N_{60}= 60 kg ha^{-1} nitrogen, N_{90}= 90 kg ha^{-1} nitrogen, [1]: The difference between the means indicated by the same letter in the same column and group is not significant, CV: Coefficient of variation, ns: Not significant, *: $p<0.05$, **: $p<0.01$

When the effects of nitrogen doses alone were examined, as an average of leonardite doses, the highest seed yield was found to be 1922.5 kg ha^{-1} at N_{60} dose, and the lowest seed yield was found to be 1072.1 kg ha^{-1} at N_0 dose, and seed yield decreased significantly at N_{90} dose. With respect to seed yield, this difference between nitrogen fertilizer doses was found to be statistically significant at a $p<0.01$ level (*Table 6*). It was also reported in the results of some studies that nitrogen fertilization in black cumin

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had significant and positive effects on seed yield. When these studies were reviewed, it was reported that the highest seed yield in black cumin was obtained from 60 kg N ha^{-1} nitrogen dose under the ecological conditions of India (Shah, 2004), Çukurova-Turkey (Özgüven and Şekeroğlu, 2007), Van-Turkey (Tuncturk et al., 2012), Ethiopia (Yimam et al., 2015), and Eskişehir-Turkey (Sağlam, 2018), from 30-60 kg N ha^{-1} nitrogen dose under the conditions of South Korea (Ashraf et al., 2006), from 30 kg N ha^{-1} nitrogen dose in the ecology of Iraq-Sulaymaniyah (Muhammad et al., 2017), and from 80 kg N ha^{-1} nitrogen dose under the climatic and soil conditions of Kahramanmaraş-Turkey (Kızılyıldırım, 2019).

It can be said that the results of our study are generally compatible with these data in the literature. The fact that nitrogen doses have different effects in different ecologies in terms of the seed yield of black cumin can be explained by different physical and chemical properties (especially organic matter) of soils where the study was carried out, along with the genotypic difference of the plant material used.

In the study, the difference between the years in terms of seed yield was also found to be statistically significant ($p<0.05$), and the highest values, as the average of leonardite and nitrogen doses, were found in the second year of the study (*Table 6*). It was considered that this difference between the years in terms of seed yield was due to differences in precipitation and temperature between the years.

In the study, although the LxN interaction was found to be statistically insignificant, it was remarkable that the seed yield was high in the treatments in which leonardite and nitrogen fertilizer were applied together at increasing ratios (e.g., such as L_2N_{60} and L_3N_{60}) (*Table 6*), which was considered as a significant result in that soil-conditioning organic materials such as leonardite increase the effectiveness of chemical fertilizers.

Fixed oil

One of the most critical factors that determine the seed quality in black cumin is the fixed oil ratio (Akgül, 1993). In the study, it was observed that leonardite applications increased the fixed oil ratio in the seeds of the black cumin plant. It was determined that this increase was statistically very significant at a ($p<0.01$) level compared to the subject without leonardite application (L_0) and that the highest values were obtained from other applications, except for L_0. The fixed oil ratio of black cumin varied between 37.02-37.50% along with leonardite applications (*Table 6*).

When nitrogen fertilizer doses were examined alone, it was determined that the fixed oil ratio of black cumin seeds increased in parallel with the increase in nitrogen doses and that the highest fixed oil ratio was obtained from N_{90} nitrogen dose with 37.96% as an average of leonardite doses. This difference between nitrogen fertilizer doses in terms of fixed oil ratio was found to be statistically significant at a $p<0.01$ level (*Table 6*).

While the fatty acid composition of seeds varies by species and genotypes (Karaca and Aytaç, 2007), different cultural applications in the planting-harvesting process (Küçükemre, 2009; Arslan et al., 2011; Kulan et al., 2012) and the ecological (Karaca and Aytaç, 2007) and topographic (Yüksek et al., 2016) differences also affect the oil ratio. In this sense, different results were obtained with respect to the effects of nitrogen fertilization on the fixed oil ratio consisting of saturated and unsaturated fatty acids. For example, it was reported that different doses of nitrogen fertilization did not generally affect the fixed oil ratio in black cumin (Shah, 2004; Özgüven and Şekeroğlu, 2007; Kızılyıldırım, 2019). On the contrary, it was reported that significant differences

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occurred between nitrogen fertilizer doses in terms of fixed oil ratio in the fennel (*Foeniculum vulgare* Mill.) plant (Tunçtürk et al., 2011) and oil ratio in the safflower (*Carthamus tinctorius* L.) plant (Katar et al., 2012).

It was considered that the emergence of significant differences between leonardite and nitrogen applications in terms of fixed oil ratio in the research soil, where soil organic matter, and accordingly, the amount of nitrogen in the soil were low, was affected by the increased availability of plant nutrients and the synergistic relationship between some nutrients. Nevertheless, it was reported that black cumin seeds contained fixed oil at ratios varying between 21.83-40.58% (Akgül, 1993; Türker and Bayrak, 1997; Kalçın, 2003; Kulan et al., 2012; Ertaş, 2016; Selicioğlu, 2018; Kızılyıldırım, 2019), and it was observed that the fixed oil ratio values obtained as a result of leonardite and nitrogen applications in our study were within these limits in the literature.

Essential oil

In the study, both leonardite and nitrogen fertilizer doses had statistically very significant ($p<0.01$) effects on the essential oil of black cumin seeds. While the highest essential oil in leonardite applications was found at L_2 and L_3 doses (0.30% and 0.29%, respectively) as an average of years and nitrogen doses, the highest essential oil in nitrogen fertilizer applications was found at N_{60} (0.30%) and N_{90} doses (0.31%) as an average of years and leonardite doses (*Table 6*).

According to the result of the study, it was observed that leonardite applications and nitrogen fertilizer applications, also known as organic fertilizer sources, increased the essential oil of black cumin seeds. Our results obtained by nitrogen fertilizer dose applications were found to be consistent with the results obtained by Türközü (2005), Özgüven and Şekeroğlu (2007), and Kızılyıldırım (2019). For example, in some other studies in which nitrogen fertilizer applications were performed, Yıldırım and Kan (2006) and Tunçtürk et al. (2011) reported that nitrogen fertilizer doses had no effect on essential oil in fennel, contrary to the results of our study.

Essential oil components

Essential oil components obtained by the Headspace GC-MS analysis of black cumin essential oil are presented in *Table 7*. A total of 6 components were found in black cumin essential oil. The main component of black cumin essential oil is thymoquinone. In our study, the effects of leonardite and nitrogen doses alone and together on thymoquinone content were found to be statistically significant ($p<0.01$). Accordingly, it was observed that the composition of thymoquinone of the essential oil of black cumin seeds increased in parallel with the increase in both leonardite and nitrogen doses and that the highest values were obtained at high doses. Indeed, this situation also manifested itself in the LxN interaction, and the highest thymoquinone content was found in L_3N_{90} subject by 47.09% (*Table 7*). Another major component of black cumin essential oil is P-cymene, and leonardite and nitrogen doses had a very significant ($p<0.01$) effect on P-cymene content. The highest P-cymene content and the lowest P-cymene content were found at L_3 dose by 41.66% and at L_0 (41.27%) leonardite dose, respectively, as an average of years and nitrogen doses. When the effects of nitrogen doses alone were reviewed, the highest P-cymene content and the lowest P-cymene content were found at N_{90} dose by 43.11% and at N_0 nitrogen dose by 38.67%, respectively, as an average of years and leonardite doses (*Table 7*).

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Table 7. *The effect of leonardite and nitrogen fertilizer doses on the components of black cumin essential oil*

LxN	Thymoquinone			Beta-pinene			Sabinene		
	2017	2018	Mean*	2017	2018	Mean*	2017	2018	Mean*
L_0N_0	44.52	44.09	44.31 d	5.28	5.30	5.29 f	2.56	2.64	2.60
L_0N_{30}	44.58	44.67	44.63 cd	5.31	5.35	5.33 ef	2.62	2.66	2.64
L_0N_{60}	45.65	45.60	45.63 b	5.34	5.38	5.36 de	2.68	2.74	2.71
L_0N_{90}	45.70	45.80	45.75 b	5.57	5.60	5.59 bc	2.84	2.88	2.86
L_1N_0	44.68	44.72	44.70 cd	5.31	5.33	5.32 ef	2.57	2.61	2.59
L_1N_{30}	44.75	44.73	44.74 cd	5.34	5.35	5.35 de	2.66	2.69	2.68
L_1N_{60}	45.73	45.73	45.73 b	5.52	5.55	5.54 c	2.72	2.76	2.74
L_1N_{90}	45.72	45.74	45.73 b	5.61	5.63	5.62 b	2.86	2.86	2.86
L_2N_0	44.74	44.71	44.72 cd	5.33	5.35	5.34 ef	2.59	2.66	2.62
L_2N_{30}	44.84	44.85	44.84 c	5.37	5.36	5.35 de	2.67	2.69	2.68
L_2N_{60}	45.81	45.89	45.85 b	5.55	5.60	5.58 bc	2.73	2.75	2.74
L_2N_{90}	47.50	47.34	47.42 a	5.61	5.61	5.61 b	2.87	2.90	2.89
L_3N_0	44.77	44.75	44.76 c	5.36	5.34	5.35 de	2.59	2.66	2.63
L_3N_{30}	44.85	44.86	44.86 c	5.37	5.42	5.40 d	2.66	2.71	2.68
L_3N_{60}	45.84	45.82	45.83 b	5.59	5.63	5.61 b	2.74	2.77	2.76
L_3N_{90}	47.03	47.15	47.09 a	5.65	5.72	5.69 a	2.87	2.89	2.88
Mean	45.42	45.40		5.43	5.47		2.70 b	2.74 a	
CV (%)	1.85			2.16			2.09		
L_0			45.08 b			5.39 c			2.70
L_1			45.23 b			5.46 b			2.72
L_2			45.71 a			5.47 b			2.73
L_3			45.63 a			5.51 a			2.74
N_0			44.62 c			5.33 d			2.61 d
N_{30}			44.77 c			5.36 c			2.67 c
N_{60}			45.76 b			5.49 b			2.74 b
N_{90}			46.50 a			5.63 a			2.87 a
	L^{**}, N^{**}, LxN^{**}			L^{**}, N^{**}, LxN^{**}			Y^{**}, N^{**}		

LxN	Limonene			P-cymene			Linalool		
	2017	2018	Mean*	2017	2018	Mean*	2017	2018	Mean*
L_0N_0	2.02	2.05	2.03	37.94	39.08	38.51	1.86	1.97	1.91
L_0N_{30}	2.24	2.17	2.21	41.55	42.35	41.95	2.22	2.25	2.24
L_0N_{60}	2.25	2.24	2.25	41.47	41.81	41.64	2.37	2.33	2.35
L_0N_{90}	2.32	2.27	2.30	42.67	43.27	42.97	2.48	2.39	2.44
L_1N_0	2.10	2.12	2.11	38.21	38.93	38.57	1.93	1.95	1.94
L_1N_{30}	2.27	2.25	2.26	41.57	42.25	41.91	2.21	2.25	2.23
L_1N_{60}	2.29	2.29	2.29	41.81	42.62	42.21	2.36	2.32	2.34
L_1N_{90}	2.32	2.34	2.33	42.76	43.35	43.05	2.48	2.47	2.48
L_2N_0	2.15	2.12	2.13	38.42	38.85	38.63	1.94	1.93	1.94
L_2N_{30}	2.29	2.28	2.29	41.57	41.96	41.76	2.27	2.28	2.27
L_2N_{60}	2.32	2.31	2.32	41.83	42.50	42.17	2.38	2.40	2.39
L_2N_{90}	2.37	2.37	2.37	42.81	43.67	43.24	2.50	2.52	2.51
L_3N_0	2.16	2.15	2.16	38.67	39.23	38.95	1.89	1.98	1.94
L_3N_{30}	2.32	2.27	2.29	41.85	42.41	42.13	2.29	2.33	2.31
L_3N_{60}	2.28	2.30	2.29	41.99	42.79	42.39	2.39	2.43	2.41
L_3N_{90}	2.35	2.39	2.37	42.89	43.46	43.17	2.51	2.50	2.50
Mean	2.25	2.24		41.12 b	41.78 a		2.25	2.27	
CV (%)	2.89			1.06			1.94		
L_0			2.20 b			41.27 c			2.23 b
L_1			2.25 a			41.44 bc			2.25 b
L_2			2.28 a			41.45 b			2.28 a
L_3			2.28 a			41.66 a			2.29 a
N_0			2.11 c			38.67 c			1.93 d
N_{30}			2.26 b			41.94 b			2.26 c
N_{60}			2.28 b			42.10 b			2.37 b
N_{90}			2.34 a			43.11 a			2.48 a
	L^{**}, N^{**}			Y^{**}, L^{**}, N^{**}			L^{**}, N^{**}		

L: Leonardite, N: Nitrogen, CV: Coefficient of variation, Y: Year, *: The difference between the means indicated by the same letter in the same column and group is not significant, **: $p<0.01$

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In the study, other components found in black cumin essential oil were beta-pinene, sabinene, limonene, and linalool. It was determined that the amounts of these components were affected by leonardite (except for sabinene) and nitrogen dose applications and that the components generally had the highest values at the highest leonardite and nitrogen doses (*Table 7*).

In other studies on black cumin, the main component was determined to be p-cymene (47.4%, 49.06%, and 43.58%, respectively) by Orchid et al. (2004), Ashraf et al. (2006), and Toma et al. (2010). Moretti et al. (2004), Orchid et al. (2004), Toma et al. (2010), and Harzallah et al. (2011) determined that thymoquinone ratios in black cumin essential oil were 3.8%, 20.8%, 1.65%, and 0.79%, respectively. On the other hand, Akgören Palabıyık and Aytaç (2018) reported that black cumin oil contained thymoquinone by 67.7% as essential oil components, followed by carvacrol by 8.4%, and junipene by 4.8%. In our study, the main component of black cumin essential oil was thymoquinone by 45.41%. The fact that the main components of black cumin essential oil were different from some studies may also be due to different climatic characteristics along with the different genotypic features of the plant material used. Namely, the fact that the temperature is high but the amount of precipitation is low especially in May and June (*Table 1*) was considered to lead to a high amount of thymoquinone. A similar situation was also indicated by Akgören Palabıyık and Aytaç (2018), and Herlina et al. (2017) reported that high temperature had an effect on high thymoquinone content.

Conclusions

According to the results of this study carried out under semi-arid climatic conditions, the applications of leonardite, known as a soil conditioner, and nitrogen fertilizer doses significantly affected the seed yield and yield components of black cumin. The highest seed yield was obtained from 2000 kg ha^{-1} leonardite and 60 kg ha^{-1} nitrogen applications. If some amount is waived in terms of essential oil and components of essential oil, the L_2N_{60} application can be recommended with respect to yield and quality.

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