

The Effect of Different Phosphorus Doses on Fixed Oil Component and Plant Nutritional Elements of Black Cumin (*Nigella sp.*)

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Abstract

This study was conducted to determine the fixed oil components and macro-micro nutrients of black cumin grown at different phosphorus doses. Seed samples were obtained from two different black cumin genotypes grown for two years (in 2017-2018 and 2019-2020 winter growing seasons) by applying five different phosphorus doses (0, 3, 6, 9, 12 kg da⁻¹ P). According to the research results, increasing phosphorus amounts did not show a significant difference in fixed oil components. The unsaturated fatty acid ratio has a much higher ratio than saturated fatty acid ratio. Linoleic acid (58.26%), an unsaturated fatty acid, and palmitic acid (11.84%), a saturated fatty acid, were found to be the highest in black cumin oil. When macro-micro nutrients are examined, it has been observed that the difference in dose and genotype x dose interaction is important. While the macro-micro elements Fe, Ca, P, Zn, and Mn increased up to a certain dose, the elements Mg, K, Na, and N increased depending on the dose increase, and the highest value was obtained from the 12 kg da⁻¹ P application.

Key words

Çameli variety, *Nigella sativa*, Phosphorus dose, Fixed oil, Plant nutrients.

Introduction

Medicinal plants have been used for seasoning, food, and healing purposes for centuries (Bayram et al. 2010). Black cumin is an annual herbaceous plant in the Ranunculaceae family. While the genus *Nigella* is represented by 20-24 species in the world, it is known that 12-15 of the species naturally grow in our country's flora (Baser, 2010; Ayhan, 2012). Black cumin, which is among the medicinal and aromatic plants, is used as a raw material in the food industry, and is used as a flavor, odor, and flavoring agent in kitchens, can be easily grown in the climatic conditions of Turkey. (Ertas, 2016; Akgul, 1993; Kucukemre, 2009; Ceylan, 1997). It has a hairy and upright structure, which can usually be 35-70 cm in length. Black cumin seeds are black in color and 2.5-4 mm long (Baydar, 2013; Urusan, 2016). Traditionally, black cumin is used as a therapeutic for stomach ailments, gas expectorant, dysentery, diuretic, obesity, back pain, asthma, bronchitis, cough relief, appetite stimulant, menstrual regulator, jaundice reliever, and many other diseases (Ozguven, 2005; Baser, 2010). The main fatty acids in black cumin seeds are linoleic, oleic, and palmitic acid (Kizil et al. 2008). Black cumin seeds carbohydrates (25%), crude fiber (8.4%), contain protein (26%) and ash (4.8%). The seeds contain good amounts of carotene and minerals such as Cu, P, Zn, and Fe (Ahmad et al. 2013). Although yield values vary depending on annual rainfall (Kaya et al. 2023), one of the most important cultural processes that increase the quality and yield in plant production is the appropriate fertilization. (Anonymous, 2016). For this reason, the application time, shape, and amount of the fertilizer should be determined according to the region where the plant will be grown. Phosphorus needed in Turkish soil is in the first place after nitrogen. (Turan 2014). According to the dry matter principle, the P content of the plants generally varies between 0.05% and 1.0%. In plants; phosphorus is the least abundant macronutrient (N, K, Ca, Mg, P). Inorganic phosphorus compounds (Pi) entering the root are stored in the root or transported to the top organs of the plant. Phosphorus taken from the soil after various chemical reactions; It turns into various inorganic compounds, including enzymes, proteins, and nucleic acids. High-energy phosphate

compounds play an important role in all metabolic events in plants. It is known that the amount of phosphorus in the plant is very important for seed and fruit quality. Phosphorus has a positive and significant effect on root development in plants. Phosphorus increases the resistance of plants against diseases and pests because it makes plant tissues stronger. Phosphorus accelerates generative development in plants and causes plants to come to harvest earlier (Kacar, 2015). According to Kizil et al. (2008) reported that the maximum fixed oil rate (37.4%) was obtained in 160 kg ha⁻¹ P application and it was statistically similar to 120 kg ha⁻¹ P dose in a study conducted in Diyarbakir ecological conditions. In this study, the effects of different phosphorus doses on fixed oil components and plant nutrients in black cumin genotypes were determined.

Materials and Methods

In this study; Seed samples used for the determination of fixed oil components and nutrients were obtained from a two-year study established in 2018 and 2020. Two different genotypes were used, with the first genotype 1 (*N. sativa*) and the second genotype 2 (Çameli variety).

Experimental Material

Seed samples obtained from two different black cumin genotypes grown by applying five different phosphorus doses in Kahramanmaraş conditions for two years were used. Phosphorus doses applied in the experiment were 0, 3, 6, 9, and 12 kg da⁻¹, while the genotypes used were *N. sativa* and Çameli registered variety.

Design and Cultural Practices

The trial was repeated for two years in 2018 and 2020. The experiment was set up in a split-parcel trial design with 3 replications. Five different doses of phosphorus (0, 3, 6, 9, 12 kg da⁻¹) and two different black cumin genotypes were used. *Nigella sativa* genotypes were distributed to the main plots, and phosphorus doses to the sub-plots. In both years, the trial was established in November, the necessary agricultural applications were made and the harvest was made in June.

Table 1. Climate data of Kahramanmaraş Province trial years and long-term growing seasons (Anonymous 2020a)

Climatic Factory	Year	Months								Total Average
		November	December	January	February	March	April	May	June	
Precipitation (mm)	2017-2018	89.60	33.70	149.90	63.10	47.40	71.60	28.10	39.40	522.80
	2019-2020	39.10	198.50	88.00	72.70	173.40	61.80	18.50	0.30	652.30
	Long years	87.50	116.60	125.40	108.30	93.40	69.80	41.20	8.40	650.80
Average Temperature (°C)	2017-2018	12.20	8.90	7.40	9.70	14.20	18.40	21.70	25.40	14.74
	2019-2020	13.50	8.40	6.30	6.10	12.50	15.90	15.90	24.50	13.25
	Long years	11.50	6.80	4.90	6.40	10.60	15.50	20.30	25.30	12.60
Relative humidity (%)	2017-2018	64.20	69.00	69.50	69.40	60.80	45.30	52.60	49.10	59.98
	2019-2020	56.20	81.90	69.30	68.30	67.30	58.20	47.20	46.90	61.91
	Long years	66.68	79.85	69.99	65.62	60.00	57.59	54.95	49.67	63.04

It is seen that the average temperature values in both years in which the experiment was carried out were above the average temperature of Kahramanmaraş for many years. When the relative humidity values are examined, it is seen that the average relative humidity values of both years are lower than the average relative humidity values of the long years. Considering the precipitation values, the average precipitation amount in the first year of

the experiment was below the average precipitation value for many years, while the second year had a value above the average precipitation amount for long years (Table 1).

Considering the soil characteristics of the experimental area, it is clay loam, slightly alkaline, low in salinity, moderately calcareous, low in organic matter, and high in potassium (Table 2).

Table 2. Some chemical and physical properties of the study area soils (Anonymous 2020b)

Year	Texture class	Organic matter (%)	CaCO ₃ (%)	EC (dS m ⁻¹)	pH	P ₂ O ₅ (kg da ⁻¹)	K ₂ O (kg da ⁻¹)
2018	Clay (72)	1.66	3.91	0.86	7.66	6.29	53
2020	Clay-loam (69.96)	1.58	6.09	0.05	7.71	2.84	55.51

Data Collection

Fixed oil extraction

After the seed samples were ground, 15 gr samples were prepared from each sample and, the fixed oil was obtained by extraction with petroleum ether for 6 hours in the Soxhlet apparatus.

Fixed oil components (%): 0.1 g of oil was taken from the extracted oil samples, 1 ml of 2 N methanolic KOH solution was added, vortexed for 2 minutes, and waited for 15 minutes. Then, 10 ml of hexane was added to it, mixed thoroughly, and centrifuged at 7000 rpm for 10 minutes for phase separation, and 1 microliter of the upper phase was injected into the Shimadzu brand GC-FID device.

Nutrient elements (mg kg⁻¹): Necessary preparations were made using ground seed samples and measurements were made in the ICP-OES (Optima 2100 DV: Perkin Elmer Inc.) device in the ÜSKİM laboratory.

Statistical Analyses

The results obtained from the properties of plant nutrients were analyzed using the SAS 9.1 package program according to the split-parcel trial design. Differences found to be significant were subjected to the LSD multiple comparison test.

Results and Discussion

In this study, the effects of phosphorus dose-increasing applications on fixed oil components and plant nutrients in two different black cumin genotypes were investigated.

Plant nutritional elements (mg kg⁻¹)

When we look at the data regarding the nutritional elements in Tables 3 and

4, it is seen that genotype, dose, and genotype x dose interaction is significant at the p<0.01 level in all macro-micro elements. It was found in terms of genotypes that genotype 2 had a higher value than genotype 1 in all elements except zinc (Table 4). When we look at the nutritional elements in terms of doses, the lowest values for Fe, Ca, Na, and Mn were obtained from the 9 kg da⁻¹ P dose, and the

lowest value was obtained from the 0 kg da⁻¹ dose for the other elements studied. It was found that Fe, Ca, and Mn reached the highest value at the 3 kg da⁻¹ application; P and Zn at the 9 kg da⁻¹ application; and Mg, K, Na, N at the 12 kg da⁻¹ application. In terms of genotype x dose interaction, the highest values of Mg, P, and Na elements were obtained from the 12 kg da⁻¹ application of genotype 1; the highest value of Fe, Ca, and Mn from the 3 kg da⁻¹ application of genotype 2 and the highest value of N from 6 kg da⁻¹ of genotype 2 (Fig. 1F-1N). Vatansav et al. (2013) determined Fe as 117.32 mg g⁻¹, Zn as 41.42 mg g⁻¹, and Mn as 28.56 mg g⁻¹, which were lower than the values in this study. Mamun and Absar (2018) reported Fe as 41.80 mg%, Ca as 579.33 mg%, Mg as 218.33 mg%, P as 91.5 mg%, Na as 100 mg%, and K as 510.30 mg%. Studying the nigella varieties obtained from five different sources, Takruri and Dameh (1998) obtained the highest Fe ratio (91-130 mg kg⁻¹) from the Turkey source, the highest Na ratio (419-550 mg kg⁻¹) from the Indian source, the highest K ratio (4423-5606 mg kg⁻¹) from Syria 1 source, the highest Ca ratio (1544-2005 mg kg⁻¹) from Syria 1 source, the highest Zn ratio (56-66 mg kg⁻¹) from Syria 2 source, the highest P ratio (5023-5769 mg kg⁻¹) from Syria 1 source.

Table 3. Two-year averages and LSD groupings of applied phosphorus doses in terms of properties examined in black cumin

		Fe(mg/kg)	Ca(mg/kg)	Mg(mg/kg)	P(mg/kg)
Genotype	G1	317.84	b	5788.70	b
	G2	367.88	a	6533.20	a
Doses (kg/da)	0	322.71	d	6109.92	d
	3	380.03	a	6538.00	a
	6	377.27	b	6167.75	c
	9	296.66	e	5544.58	e
	12	337.62	c	6444.50	b
Year	2018	390.93	a	6933.90	a
	2020	294.79	b	5388.00	b
Genotype x Doses (GxD)	G ₁ x P ₀	391.55	b	5839.17	f
	G ₁ x P ₃	222.43	i	5809.33	f
	G ₁ x P ₆	395.05	b	5552.50	g
	G ₁ x P ₉	273.45	g	5522.67	g
	G ₁ x P ₁₂	306.73	f	6219.83	e
	G ₂ x P ₀	253.88	h	6380.67	d
	G ₂ x P ₃	537.65	a	7266.67	a
	G ₂ x P ₆	359.51	d	6783.00	b
	G ₂ x P ₉	319.88	e	5566.50	g
	G ₂ x P ₁₂	368.53	c	6669.17	c
Mean		324.86		6160.95	
CV		0.71		0.74	
LSD for genotype		1.29**		24.03**	
LSD for doses		2.04**		37.99**	
LSD for G x D		9.65**		179.41**	
				2598.53	4648.15
				0.80	0.45
				10.97**	11.09**
				17.35**	17.54**
				81.94**	82.82**

Table 4. Two-year averages and LSD groupings of applied phosphorus doses in terms of plant nutrients in black cumin

		K(mg/kg)	Zn(mg/kg)	Mn(mg/kg)	Na(mg/kg)	N(%)
Genotype	G1	8291.43	b	65.09	a	49.11
	G2	8868.07	a	63.54	b	51.89
Doses (kg/da)	0	8127.25	e	61.47	e	49.51
	3	8455.50	d	64.33	b	57.90
	6	8579.83	c	61.91	d	51.39
	9	8643.92	b	69.96	a	43.26
	12	9092.25	a	63.91	c	50.43
Year	2018	9052.70	a	58.36	b	50.12
	2020	8106.80	b	70.27	a	50.88
Genotype x Doses (GxD)	G ₁ x P ₀	7347.33	g	57.43	h	54.61
	G ₁ x P ₃	8379.00	e	67.37	c	49.46
	G ₁ x P ₆	8215.50	f	60.34	f	50.90
	G ₁ x P ₉	8539.17	d	71.62	a	42.64
	G ₁ x P ₁₂	8976.17	b	68.73	b	47.96
	G ₂ x P ₀	8907.17	bc	65.52	d	44.43
	G ₂ x P ₃	8532.00	de	61.30	f	66.35
	G ₂ x P ₆	8944.17	b	63.50	e	51.89
	G ₂ x P ₉	8748.67	c	68.31	bc	43.90
G ₂ x P ₁₂	9208.33	a	59.10	g	52.92	
Mean		8579.75		64.32		50.50
CV		0.73		0.70		0.63
LSD for genotype		33.11**		0.23**		0.16**
LSD for doses		52.35**		0.37**		0.26**
LSD for G x D		247.22**		1.77**		1.26**

**P<0.01

In a study conducted by Al-Naqeep et al (2009) on three different samples in Yemen, it was reported that calcium values of 544.00 mg 100g⁻¹, 755.00 mg 100g⁻¹, 811.00 mg 100g⁻¹ were obtained from Marib, Sadah, Taiz samples respectively, potassium values of 447.30 mg 100g⁻¹, 476.70 mg 100g⁻¹, 563.00 mg 100g⁻¹ were obtained in the same respective order, and *Nigella* seeds were rich in potassium. They reported that the phosphorus element had values of 65.00 mg 100g⁻¹, 54.20 mg 100g⁻¹, and 77.40 mg 100g⁻¹ and it was different from the data in the literature. The values of zinc 1.84 mg 100g⁻¹, 1.90 mg 100g⁻¹, 2.50 mg 100g⁻¹, sodium 44.00 mg 100g⁻¹, 73.00 mg 100g⁻¹, 80.70 mg 100g⁻¹, magnesium 219.00 mg 100g⁻¹, 260.00 mg 100g⁻¹, 234.00 mg 100g⁻¹, iron 8.60 mg 100g⁻¹, 10.70 mg 100g⁻¹, 56.60 mg 100g⁻¹ were obtained from the samples. Kabir et al. (2019) found the values as potassium 1498.3 mg

100g⁻¹, phosphorus 481.5 mg 100g⁻¹, sodium 44.8 mg 100g⁻¹, calcium 366.7 mg 100g⁻¹, magnesium 355.2 mg 100g⁻¹, zinc 6.7 mg 100g⁻¹, iron 42.6 mg 100g⁻¹, manganese 3.1 mg 100g⁻¹. Uras et al. (2010) determined the P-value as 5284 mg g⁻¹, the K value as 42.18 mg g⁻¹, the Ca value as 4214 mg g⁻¹, the Mg value as 1387 mg g⁻¹, the Na value as 367.40 mg g⁻¹, the Mn value as 25.83 mg g⁻¹, the Fe value as 77.37 mg g⁻¹, and the Zn value as 78.79 mg g⁻¹. They stated that three macro-minerals, namely phosphorus, potassium, and calcium were relatively high in seeds, *N.sativa* seeds contained relatively high P, K, Ca, and Fe, these elements played an important role in the protection of human health, and the seeds would be a good alternative source of these mineral elements as nutritional supplements (Uras et al., 2010). Similarly, the three macro minerals mentioned were also found to be high in this study.

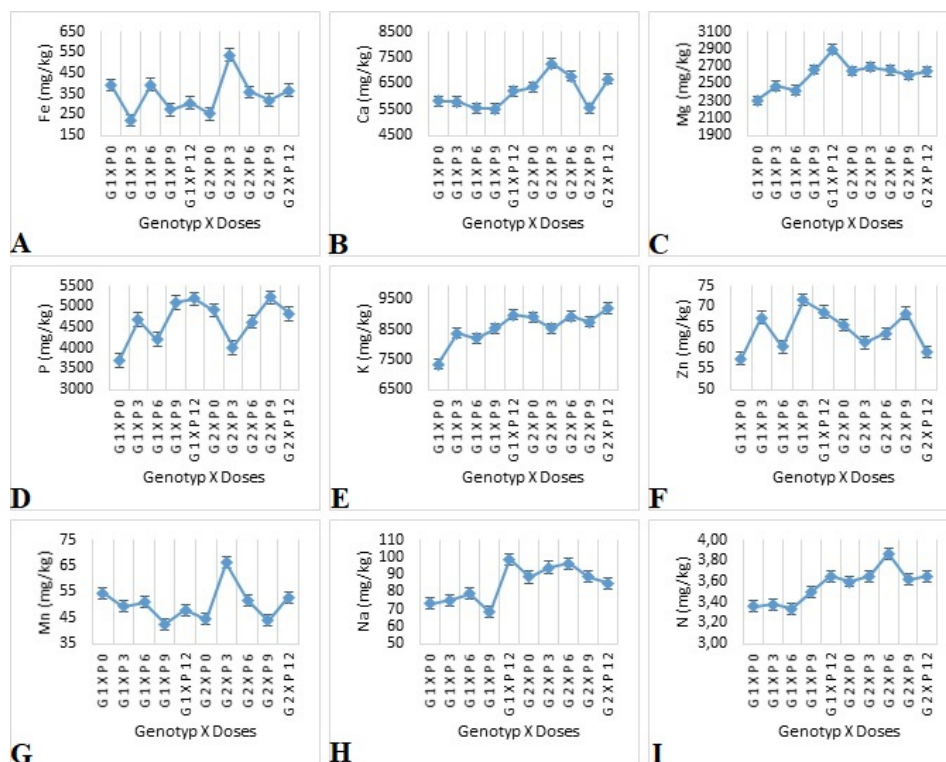


Figure 1. Two-year average values of genotype x dose interaction, which is statistically significant in the investigated characteristics

Fixed oil component (%)

When looking at the fixed fatty acids of black cumin genotypes grown in increasing phosphorus applications; In both genotypes, the rate of unsaturated fat is approximately 4.5 times higher than the rate of saturated fat. When the ratios of saturated and unsaturated fatty acids are examined in terms of doses,

it is seen that the values in all doses are close to each other. A total of 19 fixed fatty acid components were determined in both genotypes. When the percentage ratios of the components are examined, it is seen that the main fatty acid is linoleic acid and while it is 57.77% in genotype 1, it is 57.95% in genotype 2. Oleic acid is in the second place, while it is 23.46% in genotype

1 and 23.50% in genotype 2. While palmitic acid is 11.74% in genotype 1, it is 11.55% in genotype 2. The highest value in saturated fatty acids was obtained in 9 kg da⁻¹ P application in genotype 1, and 6 kg da⁻¹ P application in genotype 2. The highest value in unsaturated fatty acids was obtained from

6 kg da⁻¹ P application in genotype 1, and 9 kg da⁻¹ P application in genotype 2 (Table 5,6). Since the genotypes are composed of the same species (*N. sativa*), the fatty acid components and ratios are very close to each other.

Table 5. Two-year average values of the main fixed oil components of genotype 1 (*N. sativa*) applied different phosphorus doses

No	RT	FAMES*	Phosphorus doses (kg da ⁻¹)					Mean
			0	3	6	9	12	
1	5.11	Caproic Acid	0.36	0.13	0.46	0.51	0.10	0.31
2	17.54	Myristic Acid	0.13	0.14	0.14	0.13	0.14	0.14
3	19.60	Myristoleic Acid	0.02	0.01	0.02	0.02	0.01	0.01
4	21.75	Palmitic Acid	11.66	11.84	11.76	11.73	11.70	11.74
5	22.98	Palmiteloic Acid	0.23	0.22	0.23	0.26	0.19	0.22
6	25.07	Heptadecanoic Acid	0.05	0.04	0.04	0.04	0.05	0.04
7	26.18	Stearic Acid	2.52	2.62	2.61	2.53	2.42	2.54
8	27.20	Oleic Acid	23.31	23.86	23.57	23.23	23.33	23.46
9	28.54	Linolelaidic Acid	0.09	0.07	0.09	0.09	0.07	0.08
10	28.99	Linoleic Acid	57.89	57.56	57.41	57.72	58.26	57.77
11	30.48	Gamma-Linolenic Acid	0.39	0.10	0.08	0.07	0.08	0.14
12	31.00	Alfa-Linolenic Acid	0.21	0.19	0.21	0.21	0.23	0.21
13	31.33	Arachidic Acid	0.29	0.29	0.29	0.29	0.29	0.29
14	32.96	Heneicosanoic Acid	2.98	2.83	2.87	3.00	3.02	2.94
15	35.44	Cis-11,14,17-Eicosatrienoic Acid	0.20	0.00	0.02	0.03	0.00	0.05
16	36.14	Arachidonic Acid	0.02	0.00	0.02	0.02	0.00	0.01
17	37.08	Tricosanoic Acid	0.03	0.03	0.02	0.02	0.03	0.03
18	41.09	Nervonic Acid	0.02	0.01	0.02	0.01	0.03	0.02
19	43.37	Cis-4,7,10,13,16,19-Docosahexaenoic Acid	0.14	0.08	0.17	0.13	0.06	0.11
Saturated fatty acids %			18.02	17.92	18.19	18.25	17.75	18.03
Unsaturated fatty acids %			82.5	82.1	81.84	81.79	82.26	82.08
Total %			100	99.98	100	100	99.98	

*FAMES: Fatty acid methyl esters

In addition, it is seen that there is no significant difference in the amount of fixed oil components with increasing phosphorus doses. Similarly, Kizil et al. (2008) reported that there was no significant change in fatty acid components in terms of P application and linoleic, palmitic, and oleic acid were the main fatty acids, and the highest linoleic acid was obtained from 0 kg da⁻¹ P and 160 kg da⁻¹ P application in winter planting. Uras et al. (2010) reported that from

saturated fatty acids palmitic acid is 14.1% and stearic acid is 2.6%, from unsaturated fatty acids oleic acid is 20% and linoleic acid is 51.8%. According to Telci et al. (2014), palmitic acid 12.5%, linoleic acid 57.0%, oleic acid 22.8%, heneicosanoic acid 3.3%. In this study, heneicosanoic acid was obtained as 3.02% from 12 kg da⁻¹ P application and the data are compatible with the studies in the literature.

Table 6. Two-year average values of the main fixed oil components of genotype 2 (Çameli variety) applied different phosphorus doses

No	RT	FAMES*	Phosphorus doses (kg da ⁻¹)					Mean
			0	3	6	9	12	
1	5.11	Caproic Acid	0.13	0.76	0.55	0.19	0.54	0.43
2	17.54	Myristic Acid	0.19	0.16	0.16	0.19	0.17	0.17
3	19.60	Myristoleic Acid	0.01	0.02	0.01	0.01	0.02	0.01
4	21.75	Palmitic Acid	11.60	11.54	11.56	11.52	11.54	11.55
5	22.98	Palmiteloic Acid	0.23	0.28	0.27	0.25	0.28	0.26
6	25.07	Heptadecanoic Acid	0.05	0.04	0.05	0.04	0.05	0.05
7	26.18	Stearic Acid	2.33	2.38	2.52	2.33	2.44	2.40
8	27.20	Oleic Acid	23.85	22.62	23.84	23.73	23.48	23.50
9	28.54	Linolelaidic Acid	0.10	0.11	0.09	0.07	0.10	0.09
10	28.99	Linoleic Acid	58.01	58.27	57.40	58.35	57.72	57.95
11	30.48	Gamma-Linolenic Acid	0.05	0.07	0.07	0.10	0.08	0.07
12	31.00	Alfa-Linolenic Acid	0.20	0.20	0.19	0.19	0.20	0.19
13	31.33	Arachidic Acid	0.28	0.33	0.29	0.28	0.28	0.29
14	32.96	Heneicosanoic Acid	2.87	3.01	2.85	2.77	2.91	2.88
15	35.44	Cis-11,14,17-Eicosatrienoic Acid	0.00	0.03	0.02	0.00	0.02	0.01
16	36.14	Arachidonic Acid	0.00	0.03	0.03	0.00	0.03	0.02
17	37.08	Tricosanoic Acid	0.03	0.03	0.02	0.04	0.03	0.03
18	41.09	Nervonic Acid	0.02	0.01	0.02	0.02	0.01	0.02
19	43.37	Cis-4,7,10,13,16,19-Docosahexaenoic Acid	0.07	0.14	0.13	0.08	0.15	0.11
Saturated fatty acids %			17.48	18.25	18.00	17.36	17.96	17.80
Unsaturated fatty acids %			82.54	81.78	82.07	82.80	82.09	82.23
Total %			99.99	99.99	100	100	99.99	

*FAMES: Fatty acid methyl esters

Conclusion

This study was carried out to determine the effect of increasing phosphorus doses in black cumin on fixed oil components and plant nutrients. The nutritional elements, Fe, Ca, and Mn had the highest value at the 3 kg da⁻¹ P dose, P and Zn at the 9 kg da⁻¹ P dose, Mg, K, Na, and N at the 12 kg da⁻¹ P application. Accordingly, nutritional elements were affected at different levels by the increasing doses of phosphorus. It was observed in the applied phosphorus doses that the main fatty acids were the unsaturated fatty acids of linoleic and oleic acids, and that the main unsaturated fatty acid was palmitic acid. There was no significant difference between increasing phosphorus doses in terms of fixed fatty acid components. It was observed that the genotypes had similar ratios and characteristics in terms of fixed oil components.

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Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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