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Effects of Different Nitrogen Dose Applications on Black Cumin (*Nigella sativa* L.): Some Vegetative Parameters and Oil Ratio

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Abstract: Nigella is a medicinal and spice plant belonging to Ranunculaceae family which is cultivated in many regions of the world and in Turkey. A field experiment was conducted to study the effect of nitrogen level (0, 40, 60, 80 and 100 kg N ha^{-1}) on seed yield, the content and composition of oil in black cumin (*Nigella sativa* L.) seeds in plain conditions in Mardin province. Ammonium sulfate ($(NH_4)_2SO_4$) was used as N source. The fatty oil content of the mature plant seeds was determined by NMR and isolated by cold press. As a result of the study, it was determined that increasing nitrogen doses had a parallel effect on the plant characteristics examined. Seed yield is ranged between 103.4 - 133.1 kg da^{-1} and the highest oil content was 39.5%.

Keywords: Black cumin, fatty oil, *Nigella sativa* L., nitrogen, seed yield

Farklı Azot Dozu Uygulamalarının Çörekotu (*Nigella sativa* L.) Bitkisinde Etkileri: Bazı Bitkisel Değerler ve Yağ Oranı

Öz: Çörekotu; dünyada ve Türkiye'nin birçok yöresinde yetiştiriciliği yapılan, Ranunculaceae familyasına ait bir tıbbi ve baharat bitkisidir. Bu çalışmada; Mardin İli ova şartlarında, çörekotu bitkisinde bitkisel özellikler ve yağ oranı üzerine, farklı azot dozlarının etkisi (0, 40, 60, 80, 100 kg ha^{-1}) araştırılmıştır. Araştırmada; bitki boyu, dal sayısı, kapsül sayısı, bin tohum ağırlığı, tohum verimi gibi bitkisel özelliklerin yanı sıra sabit yağ oranı da incelenmiştir. Azot kaynağı olarak amonyum sülfat ($(NH_4)_2SO_4$) kullanılmıştır. Olgun bitki tohumlarındaki sabit yağ oranı NMR ile belirlenmiş ve soğuk presle izolasyonu yapılmıştır. Çalışma sonucunda, artan azot dozları, incelenen bitkisel özellikleri üzerine genelde paralel yönde etkilediği ve tohum veriminin 103.4 -133.1 kg da^{-1} arasında değiştiği, en yüksek yağ oranının %39.5 olduğu tespit edilmiştir.

Anahtar Kelimeler: Azot, çörekotu, *Nigella sativa* L., sabit yağ, tohum verimi

1. Introduction

Nigella sativa (black cumin) is an annual plant in the family Ranunculaceae (Arslan et al., 2000). It is an annual plant with a height varying among 20-50 cm (İlisulu, 1992). It has a total of 20 Nigella species, 14 of which are reported to exist in the natural flora of our country (Seçmen et al., 2000). In Turkey, it is grown in Thracian, Northern Anatolian and Mediterranean regions and produced in Burdur, Afyon, Isparta, Amasya, Mersin, Istanbul, Gaziantep and Kahramanmaraş (Tonçer and Kızıl, 2004; Akgören, 2011). The seeds of *Nigella sativa* are used in the folk medicine for the treatment of stomach diseases, as antiflatulent agents in intestines and diuretically (Ceylan, 1987). Beside their use as a spice, *Nigella sativa* seeds are also known to be used for decorating baked products and to make the products tasty in food industry (Özel et al., 2009).

There are many practices to increase plant production. (İlisulu, 1992). Some of them are the practices such as the development and determination of highly productive varieties, seed time, fertilizing, crop alternation and crop frequency (Chapman and Carter, 1976).

Nitrogen is one of the macro elements which is effective in the plant in terms of not only development and yield but also seed quality (Marschener, 1995). This macro element occupies a conspicuous place in plant metabolism system. All vital processes in plants are associated with protein, of which nitrogen is an essential constituent. Consequently, to get more crop production, nitrogen application is indispensable and unavoidable. Nitrogen plays a key role in agriculture by increasing of crop yield (Leghari, 2016). Nitrogen applications play a significant role in the synthesis of the plant components with the effect of different enzymes (Jones et al., 1991). Nitrogen fertilization increases the plant growth, essential oil, fatty oil, total carbohydrate and soluble sugar amount (Khalid, 1996, 2001).

Nitrogen, one of the primary nutritional elements, also has effect on the yield in addition to the product quality. The nitrogen element plays a role in respiration, photosynthesis and protein synthesis. It gives the leaves their dark green color, promotes vegetative and shoot growth and procures more productive use of the present inputs and thus high productivity (Pawar et al., 2007).

Nigella sativa production is increasing day by day in together with some medicinal, aromatic and spice plants in Mardin and surrounding cities. In region, there are no studies conducted to increase the yield per decare and the quality of *Nigella sativa*. The implementation of studies on plant yield and quality of Nigella sativa as well as all the cultural plants could be an added value for the economy of both the region and the country.

In this study, a research under the plain conditions of Mardin Province in Turkey was conducted to determine the effect of different nitrogen dose applications on plant characteristics and oil rate of *Nigella sativa*.

2. Materials and Methods

Field experiments were conducted on *Nigella sativa L.*, during the winter seasons of 2014-2015 and 2015-2016 in a field at the plain conditions of Mardin Province (latitude 37◦13_N, longitude 40◦ 93_E, and altitude 400 m). Seeds were obtained from Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Field Crops. Ammonium sulfate ((NH4)2SO4) were used as the source of nitrogen. The N treatments used were 0, 4, 6, 8 and 10 kg da^{-1}. Nitrogen was applied with half of the planting and the remaining half when the plant height reached 10-15 cm. The experiment was laid out in a Split Plot design with three replications. Seeds of black cumin were sown in mid-October, in 4.5 m^2 (3*1.5 m) plots, and the distance of 30 cm between rows. The seed rate was 10 kg ha^{-1}. The experiment was irrigated with sprinkler irrigation system as 6 times. Weed control was performed manually when necessary. Dry and hot summers and rainy and warm winters constitute the plain conditions of Mardin Province, where the research was conducted. The application field was used under irrigated conditions and it was irrigated when needed after the soil moisture was checked. The climate data of the experimental field are given in Table 1. While the total rainfall data of January in the first year and April in the second year, during which the study was carried out, were low compared to the long years average, the total rainfall in other months was in parallel with the long years average. Monthly average temperature and moisture values were similar to those of the long years' average temperature in the first and second years.

The experimental soil has loamy texture and it is poor in organic matter (1.15%). It is light alkaline (pH = 8.08), its lime content is very high (33.39%) and it does not have any salinity problems (0.010%). The amount of phosphorus (2.92 kg da^{-1}) and potassium (111.44 ppm) in soil is sufficient. The amount of magnesium (250.6 ppm), copper (33.000 ppm), zinc (11.314 ppm) and iron (11.121 ppm) in soil is sufficient; however, the level of manganese (5.150 ppm) is insufficient (Table 2).

The data obtained in the study were analyzed in JMP 5.1 package program. Analysis results were evaluated according to LSD test.

Table 1. Some meteorological data for long years (2004-2016) and 2014 – 2015-2016 growing periods in Mardin province *
Çizelge 1. *Mardin ilinde uzun yıllar (2004-2016) ve 2014-2015-2016 vejetasyon dönemleri için bazı meteorolojik veriler*

	Rainfall (mm)			Temperature (°C)			Relative Humidity (%)		
Months	Long Years	2014-2015	2015-2016	Long Years	2014-2015	2015-2016	Long Years	2014-2015	2015-2016
November	33.3	76.8	46.0	14.0	12.7	12.4	51.6	53.1	50.3
December	54.8	100.4	34.8	9.1	5.8	7.3	54.3	72.2	51.7
January	42.8	8.3	73.2	7.1	6.8	5.2	60.3	66.6	75.2
February	47.6	76.0	35.8	8.8	8.2	11.0	60.0	68.7	65.8
March	34.2	89.9	59.9	13.1	10.8	12.0	52.0	60.3	59.0
April	37.7	25.4	9.3	17.5	14.0	17.4	49.3	53.0	41.3
May	17.3	11.1	12.3	23.7	21.2	21.0	37.0	37.3	42.0
June	2.4	0.2	0.5	30.5	26.9	29.1	22.8	29.0	28.2
July	0.4	0.0	0.0	34.1	33.1	32.5	22.0	19.6	22.4

* Sources: Turkish State Meteorological Service.

Table 2. Soil properties of the experimental area *
Çizelge 2. *Deneme alanının toprak özellikleri*

Analyzes (0-30 cm)	Limit Values	Analysis Result	Analysis Method
Phosphor (P)	< 3 Trace	2.92 kg da^{-1}	TS ISO 11263
Potassium (K)	>30 Sufficient	111.44 ppm	TS 8341
Lime (%)	>25 Excessive calcic	33.39%	TS EN ISO 10693
pH	7.5-8.5 Light Alkaline	8.08	TS ISO 10390
Organic substance (%)	1-2 Little	1.15%	TS8336
Electrical conductivity (salt) (%)	<2 Salt free	0.010%	TS ISO 11265
Mangan (Mn)	4-14 Insufficient	5.150 ppm	TÜZÜNER 1990
Iron (Fe)	>4.5 Sufficient	11.121 ppm	TÜZÜNER 1990
Copper (Cu)	>0.2 Sufficient	33.000 ppm	TÜZÜNER 1990
Zinc (Zn)	> 8 Excessive	11.314 ppm	TÜZÜNER 1990
Calcium (Ca)	1150-3500 Sufficient	1216.6 ppm	TÜZÜNER 1990
Magnesium (Mg)	160-480 Sufficient	250.6 ppm	TÜZÜNER 1990
Sodium (Na)	--	64.68 ppm	TÜZÜNER 1990
Organic Carbon	--	0.67%	TS8336
Carbon/Nitrogen (C/N)	--	0.55%	Calculation method
Texture	Sand %39.2 Silt %28.0 Clay %32.7	CL (Clayey/loamy)	TÜZÜNER A.1990

* Soil analysis were made in MARTEST Analysis Laboratory

3. Results and Discussion

Plant height (cm)

In the plant height, the difference between the years (51.4-52.2 cm) was statistically insignificant, and the difference between the nitrogen-dose average values was found to be significant ($p<0.05$). In the years average, the highest value was obtained as 56.6 cm at 8 kg da^{-1} nitrogen application and the lowest value was obtained as 43.8 cm in the control group (Table 3).

In their different nitrogen dose studies on the plant height in *Nigella sativa*, Yimam et al. (2015) stated that the plant height of *Nigella sativa* was maximum 57.3 cm at 6 kg da^{-1} nitrogen fertilization. Leghari et al (2016) said that plant height is a trait which related to plant genotype and easily affected by ecological variations in growing conditions and cultural applications. So, differences in plant height among the different ecological and soil conditions with different seed populations would be expected. Tunçtürk et al. (2012) expressed that increasing nitrogen doses enhance plant height of black cumin in their experimental years. According to two-year average values, the highest plant height (32.9 cm) was measured in 80 kg N ha^{-1}, and the lowest plant height (29.5 cm) was obtained from control plots. Tulukçu (2015) stated that nitrogen doses did not affect the plant height but, specified the range as 23.95 – 40.95 cm.

Table 3. The effect of different nitrogen doses on agronomical characteristics of black cumin (*Nigella sativa* L.).
Çizelge 3. *Farklı azot dozlarının çörekotu bitkisinin (Nigella sativa L.) agronomik özellikleri üzerindeki etkisi.*

Nitrogen doses (kg da^{-1})	Plant height (cm)			Number of branches (piece plant^{-1})			Number of capsules (piece plant^{-1})			Thousand seed weight (g)			Seed yield (kg da^{-1})		
	2015	2016	**Mean**	2015	2016	**Mean**	2015	2016	**Mean**	2015	2016	**Mean**	2015	2016	**Mean**
0	45.1	42.5	**43.8d***	4.8	4.9	**4.9d***	6.9f*	7.1f	**7.0c**	2.6	2.2	**2.4**	118.8	113.6	**116.2bc**
4	48.7	50.4	**49.6c**	6.3	6.0	**6.2c**	8.6e	10.2cd	**9.4bc**	2.6	2.6	**2.6**	121.8	119.8	**120.8ab**
6	54.6	51.9	**56.3c**	7.2	6.2	**6.7bc**	9.6de	11.8ab	**10.7a**	2.5	2.6	**2.6**	111.3	108.7	**110.0bc**
8	56.8	56.4	**56.6a**	7.3	6.4	**6.9b**	9.5de	11.1bc	**10.3a**	2.6	2.6	**2.6**	135.2	130.9	**133.1a**
10	55.6	55.5	**55.6ab**	8.2	6.9	**7.5a**	9.5de	12.4a	**10.9a**	2.6	2.5	**2.6**	108.1	98.7	**103.4c**
Mean	**52.2**	**51.4**		**6.8**	**6.1**		**8.8b**	**10.5a**		**2.6**	**2.5**		**119.0**	**114.4**	

*LSD: ($p<0.05$)

Number of branches (branch plant^{-1})

In the number of branches per plant, the difference between the years (6.8-6.1 branch plant^{-1}) was statistically insignificant and the difference between the nitrogen-dose in terms of average values was found to be significant ($p<0.05$). In the years with the regard of the average, the highest value was obtained as 7.5 pieces in 10 kg da^{-1} nitrogen application and the lowest value was obtained as 4.9 pieces in the control group (Table 3).

Nitrogen is an important nutrient that improves vegetative parts. Our results showed that the number of branches augmented with increasing nitrogen dose. In studies conducted on the nitrogen doses in *Nigella sativa,* Tulukçu (2015) stated the highest the number of branches (5.41 branch plant^{-1}) was obtained from 120 kg N ha^{-1} doses according to two-year averages. Tunçtürk et al. (2012) expressed that the number of branches increased by increasing nitrogen doses up to 60 kg ha^{-1}, there was a slight decrease in further nitrogen doses. According to two-year averages, the number of branches varied from 3.18 to 4.51 branches plant^{-1} and the highest value (4.51 branch plant^{-1}) was obtained from 60 kg ha^{-1}.

Number of capsules (capsule plant^{-1})

In the number of capsules per plant, the difference between the years (8.8-10.5 capsule plant^{-1}) and the difference between the nitrogen dose average values were found statistically significant ($p<0.05$). In the years average, the highest value was obtained as 10.9 pieces in 10 kg da^{-1} nitrogen application and the lowest value was obtained as 7.0 pieces in the control group (Table 3).

In their previous studies, Tunçtürk et al. (2012) discovered the number of capsules within the range of 5.3 – 7.5 pieces plant^{-1}. Tulukçu (2015) stated that the number of capsules augmented by increasing nitrogen doses and they produced more capsules, the highest number of capsules (8.58 capsule plant^{-1}) was determined in 80 kg ha^{-1} nitrogen doses and the lowest number of capsules (7.72 capsule plant^{-1}) were obtained from control plots.

Shah (2008) reported number of capsules as 15.25 - 35.74, in the study of nitrogen application and gibberellic acid (GA_3) application on plant leaves.

Thousand Seed Weight (g)

In the thousand seed weight, the difference between the years (2.5-2.6 g) and the difference between the nitrogen-dose in terms of average values were found statistically insignificant. In the years regarding the average, the highest value was obtained as 2.6 g in 2, 4, 6 and 8 kg da^{-1} nitrogen applications and the lowest value was obtained as 2.4 g in the control group

(Table 3). Thousand seed weight has high hereditary value. It is usually the least affected feature from applications. However, seed weight is also affected in some dry years (Bicer and Sakar, 2005)

In their previous studies on thousand seed weight, Yimam et al. (2015) found the highest value as 2.3 g, Tulukçu (2015) obtained it between 2.55 - 4.18 g, and Tunçtürk et al. (2012) reported it within the range of 2.20 - 2.33 g.

Seed Yield (kg da^{-1})

In the seed yield per decare, the difference between the years (114.4-119.0 kg da^{-1}) was statistically insignificant, and the difference among the average values was found to be significant ($p<0.05$). In the years for average, the highest value was obtained as (133.1 kg da^{-1}) in 8 kg da^{-1} nitrogen application and the lowest value was obtained as (103.4 kg da^{-1}) in 10 kg da^{-1} nitrogen application (Table 3). Seed yield increased by increasing nitrogen doses, but some slight decrease in the seed yield. Similarly, Tunçtürk et al. (2012), Yimam et al. (2015) and Tulukçu (2015) reported the seed yield increased with increasing nitrogen doses, falls after a certain dose.

Oil Rate (%)

The effect of different nitrogen-dose applications on the oil rate, the difference between the years (38.4 – 38.6%) was statistically insignificant, and the difference between the nitrogen-dose average values was found to be significant. In the years average, the highest value (39.4%) was obtained in 10 kg da^{-1} nitrogen application and the lowest value (36.7%) was obtained in 8 kg da^{-1} nitrogen application (Table 4).

For different nitrogen-dose studies on the oil rate, Ashraf et al. (2006) and Tulukçu (2015) stated the ranges were changed between 32.41 – 37.82% and 15.21 – 25.10%, respectively. Shah (2008) determined this rate as 36.41 – 37.54% in the study where gibberellic acid (GA_3) was applied on the plant leaves together with different nitrogen fertilization.

Table 4. The effect of different nitrogen doses on the fatty oil rate of black cumin (*Nigella sativa* L.)

Çizelge 4. *Farklı azot dozlarının çörekotunun (Nigella sativa L.) yağ oranı üzerindeki etkisi*

Nitrogen doses (kg da^{-1})	First year (%)	Second year (%)	Mean (%)
0	38.2	38.5	**38.4a**
4	39.4	39.6	**39.5a**
6	38.5	38.7	**38.6a**
8	38.6	36.8	**36.7b**
10	39.2	39.5	**39.4a**
Mean (%)	**38.4**	**38.6**	

4. Conclusion

It was concluded from the results noted that nitrogen plays an important role in growth of black cumin plant. As a result of the study, it was revealed that nitrogen fertilization contributed to the increase in the quantitative values in *Nigella sativa* L. such as plant height, number of branches, number of capsules, thousand seed weight, seed yield and oil rate in seed. It was determined that the most optimum nitrogen dose was 8 kg da^{-1} for seed yield. The oil yield was not significantly affected by the fertilizer doses.

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