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Original article

Effect of cultural condition on seed growth and content of essential oil of two populations and one cultivar of genus *Nigella*

Mahmut Camlica[•] and Gulsum Yaldiz

Department of Field Crops, Faculty of Agriculture and Natural Sciences, Bolu Abant Izzet Baysal University, Bolu-14280, Turkey

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Abstract

Nigella is an important genus of family Ranunculaceae, used in traditional medicine for centuries to cure diseases like cancer, cardiovascular complications, diabetes, asthma, kidney disease, *etc.* The aim of this study is to evaluate the growth and essential oil content of one cultivar of *Nigella sativa* L. (Cameli) and two cultivars of *Nigella damascena* L. (Ankara and Rize) populations under controlled conditions of Bolu. On average days to 50% seedling and flowering determined as 15.67-18.0 days in 2017, 14.33-16.0 days in 2018, 69.0-75.33 days in 2017, 65.67-75.33 days in 2018, respectively. Seed weights per plant were 17.11-27.08 g in 2017 and 11.51-12.43 g in 2018; the 1000 seeds weights were between 2.18-2.84 g in 2017 and 2.37-2.77 g in 2018. Seed yield in the first year (38.02-60.18 kg da⁻¹) was higher than the yield in the second year (31.88-37.20 kg da⁻¹). The essential oil content of the populations were between 5.68-51.06%. The fatty oil rate in populations and cultivar varied between 20% and 29%, and fatty acid compositions were between 66.83-91.58%. The major inorganic matters were found as K⁺ (177.50-310.5 mg g⁻¹) and Ca⁺² (58.90-128.0 mg g⁻¹) and followed by Mg⁺² and Na⁺. The plant height, the number of branches, the number of capsule and 1000-seed weight of populations varied according to years. Generally, as compared to populations and cultivar, the best results were obtained in *N. sativa* cultivar.

Keywords: Essential oil, growth, Nigella sp., quality, yield

1. Introduction

The seeds of *Nigella sativa* L. (black cumin or black seed) have been used for thousands of years as a spice and food preservative. It is an annual herbaceous plant which is cultivated for its seeds and known as an edible plant, belongs to the Ranunculaceae family (Yaldiz and Camlica, 2018).

It is indigenous to Southern Europe, North Africa and Southeast Asia and extensively cultivated in many countries as Middle Eastern, Mediterranean Region, South Europe, India, Pakistan, Syria, Turkey and Saudi Arabia in the world (Karna, 2013).

Traditionally, black cumin is used as a medicament in a variety of disorders in the respiratory system, digestive tract, cardiovascular system, kidney, liver, and immune system as a medicament. It is also used as antiseptic and local anesthetic (Karna, 2013). Black cumin has been studied for biological activities and therapeutic potential and found to have wide spectrum of activities as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory,

E-mail: mcamlica25@outlook.com **Tel.:** +90-5315265942

Copyright © 2019 Ukaaz Publications. All rights reserved. Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com antimicrobial and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties (Khaled, 2009; Abel-Salam, 2012).

The seeds of black cumin contain important components as protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fiber (8.4%), total ash (4.8%), essential oil (0.5-1.6%), fatty oil (35.6-41.5%) (Karna, 2013), cellulose (6.8-7.4%) and moisture (8.1-11.6%) (Heshmati and Namazi, 2015). They are also rich in various vitamins as A, B1, B2, B3 and C and minerals such as Ca, K, Se, Cu, P, Zn, Fe (Islam *et al.*, 2017).

The aim of this study is to determin the important agricultural and quality characters of one cultivar of *N. sativa* (Cameli) and two *N. damascena* (Ankara and Rize) populations in controlled ecological conditions of Bolu.

2. Material and Methods

The study was carried out during growing seasons in 2017 and 2018 at Bolu Abant Izzet Baysal University, Bolu. The field experimental site was located at research and application area of Agriculture and Natural Sciences Faculty, is between 40°44'45" N latitude, 31°37'46" E longitudes with altitude of 881 m. Seeds of black cumin were obtained from Ankara, Rize (populations) and Transitional Zone Agricultural Research Institute-Eskibehir (TZARI) (Cameli cultivar) (Table 1).

Author for correspondence: Mr. Mahmut Camlica

Research Assistant, Department of Field Crops, Faculty of Agriculture and Natural Sciences, Bolu Abant Izzet Baysal University, Bolu-14280, Turkey

 Table 1: Information about black cumin populations and cultivar used in the study

Black cumin cultivar/populations	Obtained from					
Ankara population	Ankara					
Rize population	Rize					
Cameli cultivar	TZARI					

These seeds were sown (29th April 2017 and 2018) in a randomized complete block design (RCBD), having 3 replications with a distance of 0.3 m between rows and 0.2 m between plants. Average climatic data were recorded as 16.08°C, 17.10°C; temperature; 41.37 kg.m⁻²; 71.18 kg.m⁻² rainfall; 69.2 kg.m⁻², 53.68 kg.m⁻² humidity during the vegetation periods for 2017 and 2018, respectively (Anonymous, 2019). Experimental area soils are clay-loam with a pH value of 7.5, organic matter content of 1.6%, phosphorus ratio of 23.74 kg da⁻¹ and potassium ratio of 38 kg da⁻¹ (Anonymous, 2019).

In the experiment, 4 kg da⁻¹ diammonium phosphate (DAP) as base fertilizer and 8 kg da⁻¹ ammonium sulfate (AN) as top fertilizer were applied. These populations and cultivar were harvested between 29 August to 2 September, 2017, and 25-28 August, 2018.

Ten competitive plants from each parcel were randomly selected and morphological and some yield properties were taken for recording of data pertaining to black seed from these plants.

Essential oil analyses were carried out in accordance with method TS 8882. Approximately, 20 g sample was taken from the seeds of cultivar and populations and placed into glass clevenger flasks. 200 ml distilled water was added and samples were subjected to hydrodistillation for 3 h. The amount obtained was recorded (in ml) from the graduated section of the flask and then used to calculate percentage essential oil yields.

The essential oil composition of samples was analyzed using a gas chromatography (model 7890A, USA), coupled with a flame ionization detector and mass spectrometry (model 5975C) with a capillary column (HP-innowax capillary; $60.0 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ µm}$). Essential oils were diluted (1:50) with hexane. GC MS/FID analysis was carried out, using a split mode of 50:1. Injection volume and temperature were adjusted to 1 µl and 250°C, respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 1 ml min⁻¹. The oven temperature programme comprised: 60° C for 10 min, increasing at 20°C/min to 250°C, and then holding at 250°C for 8 min. MS spectra were monitored between 35 and 450 amu and the ionization mode used was electronic impact at 70eV. The relative percentage of the components was calculated from GC-FID peak areas and components were identified using the WILEY, NIST and FLAVOR libraries (SITARC, 2019).

5 g samples from each black cumin populations and cultivar were extracted with n-hexan for 6 h, using Soxhlet apparatus for determining the seed oil content (%).

The data were analyzed using XLSTAT statistical software program and obtained results were subjected to analysis of variance and least significant difference test (LSD) in order to find differences among the black cumin populations and cultivar at p=0.05.

3. Results

3.1 50% seedling period (days)

There was not a significant differences among the black cumin populations and cultivar as statistically both in 2017 and 2018 years (Table 2). The 50% seedling periods of black cumin changed between 14.33-18.00 days between two years. The earliest seedling period was determined in Cameli cultivar (14.33-15.67 days). The latest seedling period was found in Ankara population with 18.0 and 16.0 days in both the years.

3.2 Days to flowering time (days)

Days to flowering time (50%) ranged from 65.67 (Cameli) to 75.33 days (Cameli and Rize population) (Table 2). While the earliest days to flowering time was seen in Rize population (69.0 days) and Cameli (65.67 days), the latest days-to-flowering time was seen in Cameli (75.33 days) and Rize population (75.33 days) in 2017 and 2018, respectively. Flowering time of Ankara population was found similar in both the years (71.0-72.67 days).

3.3 Plant height (cm)

The variation with respect to plant height at harvest time ranged from 33.84-44.17 cm (Table 2). The highest plant height was recorded in Cameli cultivar, and follwed by Rize population (35.57 cm) while Rize population had the lowest plant height (39.50 cm) in first year, Ankara population (33.84 cm) had in second year. Cameli cultivar was found higher than Ankara population in both the years.

3.4 Number of branches per plant

The number of branches were seen between 1.97-6.93 number plant⁻¹ (Table 2). While the highest number of branches were obtained from Ankara (6.93 number) and Rize (3.03 number) in 2017 and 2018, respectively. The least number of branches were obtained from Cameli cultivar (5.90 and 1.97 number) in both the years. Ankara population was found lower than Rize population in terms of number of branches in 2018.

3.5 Number of capsule in per plant (capsule plant⁻¹)

The number of capsule was seen between 2.93-14.67 number plant⁻¹ (Table 2). While the highest number of capsule was obtained from Cameli (14.67 number) and Rize population (3.67 number) in 2017 and 2018, respectively. The least number of capsule was obtained from Rize population (9.67 number) in first year and Cameli cultivar (2.93 number) in second year. Ankara population was found between the Cameli cultivar and Rize population in first and second year.

3.6 Seed weights per plant (g plant⁻¹)

The variation in seed weights per plant ranged from 11.51-27.08 g among the populations and cultivar (Table 2). While the highest value was found from Cameli cultivar (27.08 g) in 2017, and it was found from Ankara population (12.43 g) in 2018. The lowest values were found in Rize population in both the years as 17.11 and 11.51 g. Seed weights per plant of 2017 year were found higher than 2018 including populations and cultivar.

3.7 Seed weight (g)

The variation in 1000-seed weight ranged from 2.18 to 2.84 g in 2017 and 2018 (Table 2). There was a significant differences among the populations and cultivar in terms of 1000-seed weight in 2017, but there was no difference in 2018. Cameli cultivar had the highest 1000-seed weight per plant (2.84 and 2.77 g), and the lowest 1000-seed weight was found in Ankara population (2.18 and 2.37 g) in first and second year, respectively. Rize population was found between Cameli and Ankara population in both the years with 2.32 and 2.44 g.

3.8 Seed yield (kg da⁻¹)

Seed yield is the most important part of the plant because of including essential oil, fatty oil or using as spice or medicine.

The range for seed yield was $31.88-60.18 \text{ kg da}^{-1}$ in cultivation years (Table 2). Cameli cultivar (60.18 kg da^{-1}) gave the highest seed yield in first year, however, Ankara population had the highest seed yield in second year. The lowest seed yield was registered for Rize population (38.02 kg da^{-1}) and Cameli cultivar (31.88 kg da^{-1}) in 2017 and 2018, respectively.

Table 2: Morphological and yield properties of black cumin populations and cultivar	
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Populations	SP(d	lays)	FT	(days)	PH	(cm)	Ν	В	N	С	SV	V (g)	1000	SW	SY ((kg/da)
/cultivar							(No/plant)		(No/plant)							
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Ankara	18.0	16.0	71.0	72.67AB	44.17	35.17	5.90	1.97	14.67	2.93	27.08	11.93B	2.84A	2.77	60.18	31.88
Rize	16.67	15.33	69.0	75.33A	41.77	33.84	6.93	2.63	13.00	3.53	24.00	12.43A	2.32B	2.44	53.34	37.20
Çameli	15.67	14.33	75.33	65.67B	39.50	35.57	6.80	3.03	9.67	3.67	17.11	11.51C	2.18B	2.37	38.02	34.17
LSD (5%)	3.54	4.78	6.59	7.56	12.29	5.51	4.14	0.68	15.09	3.85	26.58	0.035	0.36	0.34	59.06	21.96

SP: Seedling period; FT: Flowering time; PH: Plant Height; NC: Number of Capsule; SW: Seed Weight; 1000SW: 1000 Seed Weight; SY: Seed Yield. Different letters indicate significant differences at (P < 0.05) using LSD test

3.9 Essential oil (%)

3.10 Essential oil components (%)

There was a significant differences among the populations and cultivar (Table 3). The essential oil ranged from 0.10 to 0.31% in populations and cultivar in 2017. Cameli cultivar gave the lowest value (0.1%) and Ankara population had the highest value (0.31%).

Generally, 20 essential oil components were determined among the populations and cultivar (Figure 1). The major essential oil components were found p-cymen-2-ol (12.81%) in Cameli cultivar, β -terpinolen (35.13%) in Ankara population and farnesol (3.49%) in Rize population (Figure 1).

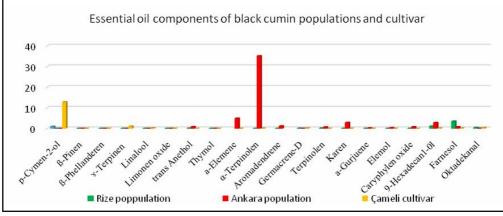


Figure 1: Essential oil components of black seed populations and cultivar.

3.11 Seed oil (%)

There was no significant difference among the populations and cultivar (Table 3). The seed oil ranged from 20.0 to 29.33% in populations and cultivar in 2017. Cameli cultivar (20.0%) gave the lowest value, Ankara population (25%) had the highest value in terms of seed oil.

3.12 Fatty acid compositions (%)

The fatty acid compositions (ratio of unsaturated to saturated fatty acids) in edible oils and fats are very significant for human nutrition.

Seed oil of black cumin is also used for edible purpose, because it is thought as one among newer sources of edible oils. It shows an important role in human nutrition and health (Tulukcu, 2011). 18 fatty acid compositions (10 saturated fatty acid, 8 unsaturated fatty acid) were found in black cumin populations and cultivar. The main fatty acid compositions were found as linoleic acid (38.48-41.69%) and oleic acid (13.26-17.08%). Fatty oil components of black cumin populations and cultivar also contained palmitic acid (8.6-9.77%), stearic (2.07-2.85%), arachidic acid (2.67-8.26%) and trace amount of other acids (Figure 2).

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Populations /cultivar	Essential oil (%) 2017	Seed oil (%) 2017
Ankara	0.31A	25.00
Rize	0.25B	29.33
Cameli	0.10C	20.00
LSD (5%)	0.06	28.32

 Table 3: Essential oil and seed oil of black cumin populations and cultivar

Different letters indicate significant differences at (p < 0.05) using LSD test

3.13 Inorganic matter (mg g⁻¹)

The inorganic matter of populations and cultivar was found between 177-310 mg/g for K^+ , 58.90-128.0 mg/g for Ca^{+2} , 14.85-33.55 mg/g for Mg^{+2} and 16.14-26.10 mg/g for Na^+ (Figure 3).

K⁺ was found the highest in Cameli cultivar (310 mg/g) and Ca⁺², Mg⁺² and Na⁺ were (determined) the highest values in Rize population with 128, 33.55, and 26.10 mg/g, respectively. It was noted that the inorganic matters of black cumin were found for K⁺ (61.2-67.5 mg/g), P (37.0-43.1 mg/g), Na⁺² (35.0-46.2 mg/g), Fe (1.0-1.5 mg/g), Ca⁺² (27.8-30.7 mg/g) and Mg⁺ (13.4-14.7

3.14 Dendrogram and biplot analysis

The dendrogram analysis separated as *N. sativa* and *N. damascena*. It divided two main groups (Group A and B). Group A consisted only the black cumin cultivar and Group B included black cumin populations (Ankara and Rize populations). *N. sativa* was different from *N. damascena*, based on examined properties.

10 measured quantitative and qualitative morphological traits were observed and analyzed are presented in Figure 5. The first principal component (PC1) accounted for 84.7% of the total variance in terms of SY, SW and essential oil. The second principal component (PC2) had high contributing factor, loadings from the analyzed traits seed per pod and pod length, and contributed 15.3% to the total variation. SY and SW were determined in the same region and seed oil was took place in another region and others were seen together in the same region. The black cumin cultivar and populations were seen in different regions in biplot analysis.

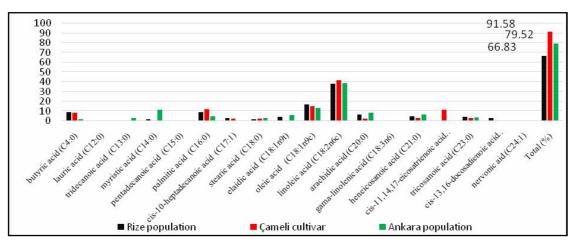


Figure 2: Fatty acid components of black seed populations and cultivar.

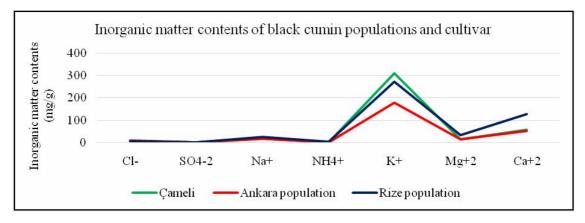


Figure 3: Inorganic matter of black seed populations and cultivar.

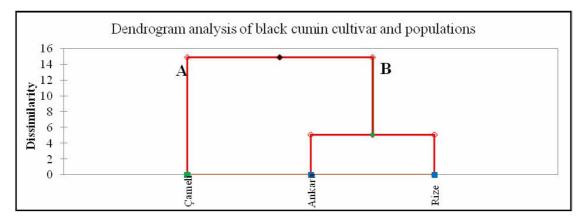


Figure 4: Dendrogram analysis of the black cumin cultivar and populations based on examined properties.

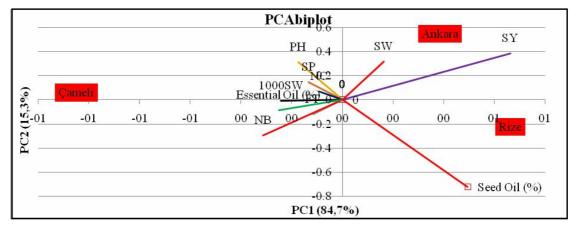


Figure 5: Distribution of the 3 analyzed black cumin in the first two principal components based on the main sources of variance.

4. Discussion

It was noted that plant height was changed between 16.1-47.1 cm (Kara *et al.*, 2018), 29.67-38.53 cm (Ghamarnia *et al.*, 2010). Similarly, Tektas (2015) reported that plant height was determined as 63.87-70.37 cm, Kilic and Arabaci (2016) as 39.33-78.90 cm and Sahin (2013) 17.2-53.1 cm, Senyigit and Arslan (2018) as 23.3-68.1 cm in black cumin. Our results were found partly similar with other reserachers except Tektas (2015). These differences could be due to probably variations in ecological conditions, agronomic applications, genotypes and soil properties.

Kara *et al.* (2018) reported 2.0-5.3 number range for number of branches in 5 black cumin populations in Isparta and Eskisehir ecological conditions. Branch number of black cumin changed between 3.7-7.6 number in Afyonkarahisar condition (Senyigit and Arslan, 2018). The results were found similar as reported by other researchers.

It was noted that number of capsule per plant of black cumin varied between 2.1-7.7 number by Kara *et al.* (2015). The number of capsule varied between 3.17-5.60 (Özel *et al.*,2002), 4.5-4.9 (Taqi, 2013), 4.03-7.63 (Kosar and Ozel, 2015) and 5.9-17.9 (Senyigit and Arslan, 2018). Our results were found partly similar with these researchers.

Kosar and Ozel (2018) reported that 1000-seed weight ranged from 1.67 to 3.40 g in 33 different black cumin variety and populations. It was also noted that 1000-seed weight changed between 1.21-

2.62 g (Akgören, 2011), 2.22-2.69 g (Kulan *et al.*, 2012), 2.57-2.78 (Taqi, 2013), 2.45-2.67 g (Kara *et al.*,2015), 2.40-2.90 g (Tektas, 2015). The obtained values were found partly similar with other researchers. The differences can be explained due to different black cumin genotypes, environmental and growing conditions. In addition, the difference between the years can be because of the environmental conditions as temperature, amount of precipitation in the seed development periods of plants (Table 2).

Kara *et al.* (2018) reported seed weight range between 19.77-94 kg da⁻¹. Similarly, Ghamarnia and Jalili (2013) reported 2.13-113.1 kg da⁻¹range for seed yield. The seed yield obtained in the present study was lower than top findings of other researchers in the literature. The observed differences may be due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants. In addition to this, Kara *et al.* (2018) reported that essential oil ranged from 0.10% to 0.78% in Isparta and Eskischir ecological conditions. The essential oil was found between 0.48-0.55% (Tunçtürk *et al.*, 2005), 0.08-0.20 (Tektas, 2015). The obtained values were found partly similar with other researchers.

Generally, the highest essential oil component was found in Ankara population with 51.06% and the lowest was found in Rize population with 5.68%. The essential oil component was found as 98.7% and it was also noted that the main essential oil components were determined as p-cymene (60.2%) by Wajs *et al.* (2008). P-cymene was found between 0.33-38.0% as main component in different

techniques as reported by Benkaci-Ali *et al.* (2013). The results obtained in the present study are different to those reported in previous studies. It was thought that environmental conditions, genetic differences and growth conditions may be the cause of differences.

Kara *et al.* (2018) reported that seed oil varied between 20.6-33.6%. Similarly, seed oil changed between 29.10 -30.40% (Ghamarnia *et al.* 2010), 27.93-41.20% (Tektas, 2015), 31.73-38.40% (Kilic and Arabaci, 2016), 36.42-40.17% (Kosar and Özel, 2018). The results were found partly similar with other researchers, except Kilic and Arabaci (2016) and Kosar and Özel (2018). It can be considered that the differences in results are based on genotypes, ecological and grown conditions.

The highest fatty acid component was found in Cameli cultivar with 91.58% and the lowest was found in Rize population with 66.83%. The linoleic and oleic acids were found as 53.14-56.82%, 23.02-24.42% by Parhizkar *et al.* (2011), respectively. The linoleic acid was noted as 61.25% and oleic acid was reported as 17.63% by Thilakarathna *et al.* (2018). Linoleic and oleic acids were found as major components in different nitrogen applications with 53.72-54.66% and 23.87-25.69%, respectively (Ashraf *et al.*, 2006). The main fatty acid of our study was found lower than reported by other researchers in terms of linoleic and oleic acid. This result can be explained because of different genotypes, ecological and growning conditions.

The obtained results were found partly similar with Ashraf *et al.* (2006) in terms of Ca^{+2} and found similar with Yaldiz and Camlica (2018) in terms of Ca^{+2} , Mg⁺². Other inorganic matters were found lower than other researchers. This situation can be explained due to genotypes, other growing and ecological conditions.

4. Conclusion

The plant height, number of branches and number of capsules, and 1000-seed weight of selected populations varied according to years. Generally, as compared to populations and cultivar, the best results were obtained in *N. sativa* cultivar in terms of morphological properties. For quality characterizations, Ankara population showed the highest values as compared the Rize population and Cameli cultivar except inorganic matter.

Conflict of interest

The authors declare that no conflict of interest exists in the course of conducting this research. Both the authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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