

YIELD AND QUALITY OF BLACK CUMIN (*Nigella sativa* L.) POPULATIONS: THE EFFECT OF ECOLOGICAL CONDITIONS

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ABSTRACT

The experiment was carried out in two vegetation seasons of 2013 and 2014 at the Isparta and Eskisehir ecological conditions. The main purpose of the study was to determine the effects of years and locations on seed yield, some yield-related traits, essential oil content and fixed oil ratio of black cumin populations. In the study, black cumin populations were obtained from Burdur, Tokat, Usak, Eskisehir and Antalya provinces. The experiments were arranged according to a randomized complete-block design with three replications. The average seed yield varied between 201.0-407.1 kg ha⁻¹ in 2013 and 458.9-790.3 kg ha⁻¹ in 2014. In compared to locations; while yield components of black cumin populations significantly varied according to locations and years, seed yield in both years were higher in Isparta location on all populations due to higher rainfall than Eskisehir. In compared to populations; the highest seed yield was determined in Usak population and it was followed by Eskisehir population. But the essential oil wasn't detected in Usak population. The highest essential oil content was obtained from Tokat population in both locations and years, it was followed by Eskisehir population. The fixed oil ratios were higher in Tokat, Usak and Antalya populations in both locations and years.

Keywords: Black cumin, Yield, Essential oil, Fixed oil

INTRODUCTION

Black cumin (*Nigella sativa* L.) is a medicinal plant annual belonging to *Ranunculaceae* family which grown naturally in Southwest Asia and the Mediterranean Region (Toncer and Kizil, 2004). Black cumin seeds contain protein, alkaloids (nigellicines and nigelledine), saponin (α -hederin) fixed and essential oil (Ozel et al., 2009). The fixed and essential oil of black cumin contain various bioactive molecules such as thymoquinone, thymol, tocopherol, trans retinol and selenium (Sultan et al., 2009).

12 species of black cumin are naturally grown in Turkey's flora. The seeds of *Nigella sativa*, *Nigella damascena* and *Nigella arvensis* are used in folk medicine and as a spice. The black cumin is cultivated commonly in Afyon, Isparta, Burdur and Konya regions in Turkey (Baytop, 1984) and only the species of black cumin (*Nigella sativa* L.) is traded (Kar et al., 2007). Black cumin is intensively used in as uncrushed in bakery products (bread, muffins, biscuits, etc.) and in some cheese (brynza, cottage cheese, etc.) in Turkey (Akgul, 1993). However, the production is limited compared to many other crops and at the level that will correspond to a

very little part of domestic consumption (Kar et al., 2007). Recently, industry facilities based on the evaluation of medicinal and aromatic plants in which included the black cumin. Industrialists emphasize that the demand to fixed oil of black cumin is much and so, the necessity of increasing local production for the supply of raw material (Yilmaz, 2008). For this reason, black cumin is a plant having potential in Turkey in the future. However, there is very little studies about adaptation and quality in our country and the studies are generally related to row spacing and fertilizing. Local population are generally used as seed production and breeding materials by breeders and farmers due to the lack of cultivars. Toncer and Kizil (2004) mentioned that seed yield in black cumin was 828 kg ha⁻¹ and essential oil content was between 0.27% and 0.35%. Ozel et al. (2009) reported that seed yield in black cumin at 1406.3-2482.3 kg ha⁻¹, and essential oil content was at 0.24-0.43%. Sener et al. (1985) detected that there was 0.26% lauric, 1.06% myristic, 20.4% palmitic, 1.56% stearic, 4.75% oleic, 64.6% linoleic and 7.18% arachidonic acid in fixed oil of black cumin. Nergiz and Otles (1993) stated that there was about 32% oil in black cumin seeds.

Climate and soil conditions that are the main factors on growth and development of the plants, the physiological growth of the plants, being synthesized of the active substances, the amount and quality of obtained essential oil. Especially, the effect of ecological factors on yield and essential oil of medicinal plants is higher compared to the other plants. Because the quality in medicinal and aromatic plants is as important as yield, the ones below a certain quality cannot be cultivated even if they are very high yield. Therefore, they should only be cultivated in the convenient regions for the ecologies of these plants (Yasar, 2005).

This study was conducted in order to determine the seed yield, some yield components, essential oil content and fixed oil rate of black cumin populations in Isparta and Eskisehir ecological conditions having different altitudes and climate conditions (especially rainfall) where black cumin cultivation is performed.

MATERIALS AND METHODS

Experimental conditions

The experiment was conducted during the growing seasons of 2013 and 2014 years at the Isparta and Eskisehir ecological conditions in Turkey. In this study, black cumin populations were used obtained from Burdur, Tokat, Usak, Eskisehir and Antalya because there is currently no black cumin cultivar registered in Turkey.

The province of Isparta located in the transition region in the inner part of the western Mediterranean region in between the Mediterranean Sea and the Central Anatolia Region bear the climate features (annual average rainfall is 524 mm) of both of the regions with 1050 meters altitude. The province of Eskisehir located in Aegean, Marmara and Central Anatolia Region with 788 meters altitude has semi-arid (annual average rainfall is 349.8 mm) and cool continental climate. Winter months are frosty and summer months are hot and the nights are cool. There are big differences between day and night temperatures.

The experiment land was plowed, cultivated and then prepared for planting with a single pass of a disk-harrow. The experiments were arranged according to a randomized complete-block design with three replicates. Seeds were sown by hand in the first week of March in the ratio of 15 kg ha⁻¹ in both the experimental years in Isparta location and were sown in the second week of March in both the experimental years in Eskisehir location. The distance between rows was 20 cm and with in the rows were 5 cm. Each plot area was 7.2 m² (6 m x 1.2 m) and consisted of 6 rows. Seeds were sowed at 1-2 cm depths using a dibbler.

Nitrogen and phosphorus fertilizers were applied at a rate of 60 kg ha⁻¹ and 40 kg ha⁻¹ in the form of ammonium sulphate and P₂O₅, respectively (Tunçtürk et al., 2012).

The total quantity of phosphorus fertilizer was applied at the time of sowing. Total nitrogen fertilization was applied in two equal doses, at the time of sowing and plant height when 15 cm. In both years and locations, the experiments were non-irrigated at any growing stage. All the necessary cultural practices were similarly applied to the plots during vegetation periods in both locations.

Climatic data of the experimental locations

Meteorological data for the growing seasons of locations are shown in Table 1. During the vegetative periods (from March to end of August) in 2013 and in 2014 a total precipitation of 289.5 and 284.2 mm, average temperature of 20.6 and 19.7 °C and an average humidity of 61.0 and 66.1% were recorded in Isparta, a total precipitation of 122.9 and 143.6 mm, average temperature of 16.7 and 16.6 °C and an average humidity of 55.6 and 61.8% were recorded in Eskisehir.

Soil structure

Soil at a depth of 60 cm was sampled before the experiment and subjected to a physicochemical analysis. Data for the years and locations are shown in Table 2.

Yield and its components

When the seeds ripen, plants from 4 rows in the center of each plot were harvested manually. Seed yield (kg ha⁻¹) and its components including plant height (cm), the number of branches (branches plant⁻¹), the number of capsule (capsule plant⁻¹) and 1000-seed weight (g) were determined.

Essential Oil Distillation

In order to determine the seed essential oil content, 100 g powdered black cumin samples in 0.5 L water from each population were extracted by hydro-distillation for 3 hours using Clevenger apparatus according to the standard procedure described in European Pharmacopoeia (Stainier, 1975) for determining the oil content (v/w, %).

Fixed Oil

4 g dried and powdered black cumin samples from each population were extracted with *n*-hexan for 6 hours using soxhlet apparatus (Buchi Universal Extraction System B-811, Germany) for determining the fixed oil content (%).

Statistical Analysis

All the data were analyzed according to the analysis of variance (ANOVA) using SAS Statistical Package Program, the significant differences between the group means were separated using the LSD (Least Significant Difference) test.

Table 1. Meteorological data of the experiment locations*

ISPARTA								
Climatic factors	Years	Months						Total or Average
		March	April	May	June	July	August	
Precipitation (mm)	2013	25.1	59.9	66.5	34.4	88.2	15.4	289.5
	2014	78.6	44.8	107.0	42.8	0.8	10.2	284.2
	Long Years	52.8	56.6	50.8	28.4	18.4	0.8	207.8
Average Temperature (°C)	2013	7.2	11.9	17.1	20.5	23.0	23.7	20.6
	2014	7.2	11.0	14.5	19.1	23.7	23.2	19.7
	Long Years	5.9	10.8	15.6	20.1	22.3	23.9	19.7
Relative humidity (%)	2013	60.0	58.0	52.0	50.0	44.0	41.0	61.0
	2014	63.7	60.4	62.4	52.7	45.3	45.9	66.1
	Long Years	61.3	64.2	50.3	53.0	45.8	44.5	63.8
ESKISEHIR								
Climatic factors	Years	Months						Total or Average
		March	April	May	June	July	August	
Precipitation (mm)	2013	40.1	30.9	18.5	31.3	2.1	0.0	122.9
	2014	23.1	15.2	27.2	70.6	7.5	0.0	143.6
	Long Years	27.6	43.1	40.0	23.7	13.1	9.2	156.7
Average Temperature (°C)	2013	7.1	10.8	18.2	20.0	21.6	22.4	16.7
	2014	6.5	11.3	16.4	19.9	23.7	21.9	16.6
	Long Years	4.9	9.6	14.9	19.1	22.1	21.8	15.4
Relative humidity (%)	2013	59.8	63.2	51.5	53.9	51.9	53.1	55.6
	2014	69.0	63.7	63.3	64.1	57.8	52.8	61.8
	Long Years	64.2	62.7	59.5	55.2	51.9	53.6	57.9

*Data were taken from Isparta and Eskisehir Regional Meteorological Service

Table 2. Physical and chemical characteristics of soil in experiment locations

ISPARTA						
Years	Structure	Lime (%)	Available Phosphorus (P ₂ O ₅) (kg ha ⁻¹)	Available Potassium (K ₂ O) (kg ha ⁻¹)	pH	Organic Matter (%)
2013	Clay-Loam	10.79	9.61	705	8.1	1.69
2014	Clay-Loam	9.62	9.00	650	7.9	1.38
ESKISEHIR						
2013	Clay-Loam	8.57	8.95	1290	7.71	1.58
2014	Clay-Loam	7.13	6.69	1050	8.0	1.43

RESULTS AND DISCUSSION

Seed yield, plant height, the number of branches, the number of capsule and 1000-seed weight of black cumin populations in Isparta and Eskisehir locations are presented in Table 3. In this research, differences among of years (except for 1000-seed weight, essential oil content and fixed oil rate), populations, locations (except for 1000-seed weight in loc II of the first year) and population x location interaction were found to be statistically significant for both years (Table 3).

Seed yield

Seed yields of black cumin populations varied according to locations and years. Mean seed yield in the second year (576.0 kg ha⁻¹) were higher than yield in the first year (291.9 kg ha⁻¹) (Table 3). These differences resulted from higher rainfall in growing period of plant in

the second year according to first year in both locations (Table 1).

In compared to locations, the mean seed yield in Isparta location was higher than Eskisehir location in both years. These differences in the mean seed yield could be explained by the higher rainfall in Isparta location (Table 1 and 3).

In compared to populations, while the highest seed yield was determined in Usak population (626.3 and 940.3 kg ha⁻¹, respectively) in Isparta location in both years, in Eskisehir location the highest seed yield were obtained from Tokat population (244.4 kg ha⁻¹) in the first year and from Usak population (640.3 kg ha⁻¹) in the second year.

The average seed yield in population x location interaction varied between 201.0-407.1 kg ha⁻¹ during 2013 and 458.9-790.3 kg ha⁻¹ during 2014 (Table 3)

Table 3. Yield, yield components, essential oil content and fixed oil rate of black cumin populations

Populations / Locations	Plant height (cm)						Number of branches (branches plant ⁻¹)					
	2013			2014			2013			2014		
	Loc I	Loc II	Average	Loc I	Loc II	Average	Loc I	Loc II	Average	Loc I	Loc II	Average
Burdur	22.7 b	16.1 d	19.4 c	33.5 bc	26.4 b	29.9 b	2.8 b	2.4 cd	2.6 b	3.6 b	4.4 ab	4.0 c
Tokat	34.1 a	22.2 a	28.1 a	47.1 a	24.5 b	35.8 a	3.7 ab	3.5 a	3.6 a	5.2 a	4.1 b	4.6 ab
Usak	28.7 ab	16.6 cd	22.6 b	30.9 c	26.0 b	28.4 b	4.4 a	2.7 bc	3.5 a	4.0 b	5.0 a	4.5 b
Eskisehir	33.1 a	19.8 b	26.4 a	44.1 ab	29.4 a	36.7 a	3.4 ab	2.0 d	2.7 b	5.3 a	4.5 ab	4.9 a
Antalya	26.3 ab	17.8 c	22.0 b	32.5 bc	25.8 b	29.1 b	3.9 ab	3.3 ab	3.6 a	4.1 b	4.8 ab	4.5 b
Mean	29.0 A	18.5 B	23.7 B	36.8 A	26.0 B	31.4 A	3.6 A	2.8 B	3.2 B	4.4	4.6	4.5 A
CV	9.95	2.82	8.72	12.65	3.71	10.73	12.05	9.56	8.57	4.88	6.21	5.60
Lsd	7.90	1.43	2.53	13.04	2.69	4.20	1.19	0.73	0.732	0.59	0.77	0.31
F value	8.10 *	66.67**	17.28**	7.26**	10.01**	6.86*	4.66*	17.76**	4.61*	34.85**	4.46*	21.73**

Populations / Locations	Number of capsule (capsule plant ⁻¹)						1000 seed weight (g)					
	2013			2014			2013			2014		
	Loc I	Loc II	Average	Loc I	Loc II	Average	Loc I	Loc II	Average	Loc I	Loc II	Average
Burdur	3.6 b	2.1 c	2.8 c	4.4 b	3.36	3.88 c	2.95 a	2.63	2.79 a	2.84 a	2.80 a	2.82 a
Tokat	5.7 ab	4.0 a	4.8 a	6.7 a	3.63	5.2 ab	2.12 b	2.46	2.29 b	2.10 c	2.73 ab	2.41 b
Usak	7.1 a	2.6 bc	4.9 a	7.7 a	3.80	5.7 a	3.14 a	2.60	2.87 a	2.91 a	2.10 c	2.50 b
Eskisehir	5.6 ab	2.2 bc	3.9 b	7.2 a	3.81	5.5 a	2.49 ab	2.80	2.64 a	2.49 b	2.40abc	2.44 b
Antalya	6.1 ab	2.7 b	4.4 ab	6.0 ab	3.70	4.8 b	2.64 ab	2.76	2.70 a	2.50 b	2.23 bc	2.37 b
Mean	5.6 A	2.7 B	4.1 B	6.4 A	3.6 B	5.0 A	2.67	2.65	2.66	2.57	2.45	2.51
CV	7.77	7.85	11.31	10.41	8.02	10.4	10.50	7.93	9.31	4.33	8.04	6.38
Lsd	2.73	0.56	0.88	1.82	ns	0.63	0.76	ns	0.30	0.30	0.54	0.19
F value	4.85 *	38.60**	4.57*	10.99**	1.11	6.98*	5.95 *	1.23	3.74 *	25.37**	7.21**	15.67**

Populations / Locations	Seed yields (kg ha ⁻¹)						Essential oil content (%)					
	2013			2014			2013			2014		
	Loc I	Loc II	Average	Loc I	Loc II	Average	Loc I	Loc II	Average	Loc I	Loc II	Average
Burdur	204.3 c	197.7 b	201.0 d	494.0 c	493.5bc	494.0cd	0.17 bc	0.14 c	0.15 c	0.15 c	0.11 b	0.13 c
Tokat	313.0 c	244.4 a	278.7 c	499.6 c	418.1 c	458.9 d	0.50 a	0.78 a	0.64 a	0.48 a	0.66 a	0.58 a
Usak	626.3 a	187.8 b	407.1 a	940.3 a	640.3 a	790.3 a	-	-	-	-	-	-
Eskisehir	468.3 b	192.2 b	330.3 b	690.3 b	558.5ab	624.4 b	0.33 ab	0.64 b	0.48 b	0.32 b	0.57 a	0.44 b
Antalya	274.6 c	210.6ab	242.6 c	485.3 c	540.2 b	512.8 c	0.14 bc	0.12 c	0.13 c	0.10 d	0.11 b	0.11 c
Mean	377.3A	206.5B	291.9B	621.9A	530.2B	576.0A	0.23 B	0.34 A	0.28	0.19 B	0.29 A	0.23
CV	11.64	7.38	11.26	8.02	5.77	7.18	3.14	7.31	9.85	6.54	11.76	10.64
Lsd	120.43	41.81	40.27	136.72	83.89	50.67	0.208	0.068	0.069	0.035	0.095	0.032
F value	44.60**	6.71*	49.08**	49.99**	21.50**	16.41**	18.46**	583.87**	13.98**	802.24**	223.5**	30.85**

Populations / Locations	Fixed oil rate (%)					
	2013			2014		
	Loc I	Loc II	Average	Loc I	Loc II	Average
Burdur	24.0 b	28.0 b	26.0 b	22.6 c	26.3 b	24.5 c
Tokat	32.0 a	32.3 a	32.1 a	30.3 a	31.0 a	30.6 a
Usak	29.3 a	33.6 a	32.5 a	28.6 b	32.3 a	30.5 a
Eskisehir	21.6 b	31.3 a	26.5 b	20.6 d	30.6 a	25.6 b
Antalya	31.3 a	32.0 a	31.6 a	30.2 a	30.6 a	30.4 a
Mean	27.6 B	31.4 A	29.5	26.5 B	30.3 A	28.4
CV	4.09	3.23	3.64	1.19	2.58	2.10
Lsd	3.10	2.78	1.31	0.86	2.15	0.73
F value	49.35**	13.00**	18.32**	621.00**	26.22**	58.74**

Loc 1: Isparta location, Loc 2: Eskisehir location

*, **: significant at P<0.05 and P<0.01 probability levels, respectively, ns: non significant

Means in the same columns followed by the same letters are not significantly different as statistically

These differences among the populations could be explained by the genetic potential of populations, and generally having higher the plant height, the number of branches, the number of capsule and 1000-seed weight in compared to others populations. Usak population is more early than the others (Table 4). Unlike known, early genotypes may be more yield because of its better use of available moisture and they don't coincide to high temperatures in the flowering period. In a study conducted on another plant (wheat) by Ludwig and Asseng (2010) were reported that yield of early genotypes may be higher according to late varieties due to their better use of available moisture in arid and semi-arid climatic

conditions. D'Antuono et al. (2002) reported that the major yield limiting factor for *Nigella sativa* genotypes seemed low vegetative growth and reduce of assimilate supply during the pre-anthesis phase short duration of its vegetative cycle. The results obtained from the study were comparable with Tuncturk et al. (2012) findings, but were lower than those of Karaman (1999), Toncer and Kizil (2004), Tuncturk et al. (2005) and Ozel et al. (2009). These differences were due to probably variations in climatic conditions, agronomic practices, genotype and soil properties. In addition to, environmental conditions during pollination at the first stage of seed set, determine the number of capsule and branche (Sadeghi, 2009).

Table 4. Harvest times of populations in experiment locations

Populations	2013		2014	
	Loc I	Loc II	Loc I	Loc II
Burdur	23.07.13	2.08.2013	26.07.14	07.08.2014
Tokat	25.07.13	5.08.2013	29.07.14	10.08.2014
Usak	17.07.13	28.07.2013	20.07.14	07.08.2014
Eskisehir	25.07.13	5.08.2013	29.07.14	10.08.2014
Antalya	23.07.13	5.08.2013	26.07.14	10.08.2014

Loc 1: Isparta location, Loc 2: Eskisehir location

Yield components

The plant height, the number of branches, the number of capsule and 1000-seed weight of populations varied according to locations and years. Generally, the tallest plant height was determined in Eskisehir and Tokat populations, the highest numbers of branches and number of capsule were determined in Usak and Tokat populations and the highest 1000-seed weight was obtained from Usak and Burdur populations in both years and locations (Table 3). Considering the locations and years in terms of total precipitation in Table 1, plant height, the number of branches and the number of capsule were statistically higher in rainy locations and years (Table 3). In the study, the number of branches and the number of capsules increased with the elongation of plant height due to rainfall, and seed yield was positively affected increasing yield components. Ghamarnia and Jalili (2013) reported that black cumin is a medicinal plant sensitive to water stress. The same authors stated that seed yield and its components were decreased rapidly according to increased of water stress during growing periods under semi-arid climatic conditions. Sadeghi (2009) reported that there was a positive correlation between seed yield and aboveground biomass. Ozguven and Sekeroglu (2007) and Tuncturk et al. (2012) stated that yield components such as the number of branches and capsules affects directly seed yield in the black cumin (Ozguven and Sekeroglu, 2007; Tuncturk et al., 2012).

Essential oil content

The essential oil content of black cumin populations in Isparta and Eskisehir locations are presented in Table 3. In the research, population, location and population x location interaction were found significant. No significant differences between the years were found (Table 3).

The essential oil content of the years was found to be very close to each other with 0.28% in the first year and 0.23% in the second year (Table 3).

In both years (2013 and 2014), the mean essential oil content (0.34% and 0.29%, respectively) in Eskisehir location was higher than Isparta location (0.23% and 0.19%, respectively).

In compared to populations, the highest essential oil content was obtained from Tokat population in both years and locations. The essential oil wasn't obtained from Usak population (Table 3).

The mean essential oil content in population x location interaction varied between 0.13-0.64% in 2013 and 0.11-0.58% in 2014.

Most researchers stated that the essential oil composition of black cumin varied depending on the genotype of the plant, growing, climatic and environmental conditions as well as distillation method (Ozguven and Tansi, 1989; Toncer and Kizil, 2004; Tuncturk et al., 2005; Ozel et al., 2009; Matthaus and Ozcan, 2011). Lei (2004) reported that the essential oil ratio of some essential oil plants was decreased in high rainfall and humidity conditions.

Our results agree with findings of the researchers who reported that essential oil varied between 0.36% and 0.49% (Ozguven and Tansi, 1989; Geren et al., 1997), 0.27% and 0.35% (Toncer and Kizil, 2004), 0.48.0% and 0.51% (Tuncturk et al., 2005), 0.01% and 0.50% (Ozel et al., 2009) and 0.28% and 0.36% (Matthaus and Ozcan, 2011).

Fixed oil rate

In term of fixed oil rate, population, location and population x location interaction were found significant. No significant differences between the years were found (Table 3).

The fixed oil rate of years was found to be very close to each other with 29.5% in the first year and 28.4% in the second year (Table 3).

In compared to locations, average fixed oil rate of Eskisehir location (31.4% and 30.3%, respectively) was higher than Isparta location (27.6% and 26.5%, respectively) in both 2013 and 2014 (Table 3).

In compared to populations, the highest fixed oil rate was obtained from Tokat and Usak populations in the both years and locations.

The mean fixed oil rate in population x location interaction varied between 26.0% and 32.5% during 2013 and 24.5% and 30.6% during 2014.

Many researchers determined that the fixed oil of black cumin vary between 30.4% and 36.4% (Sener et al., 1985; Matthaus and Ozcan, 2011). Ustun et al. (1990) reported that the oil contents of black cumin seeds collected from the Kutahya, Denizli and Konya provinces were 24.4%, 29.5%, and 29.7%, respectively. Our results are similar with the findings of the above-mentioned researchers. The differences between seed oil fixed oil rate

can be probably due to genotype, growing, localities, climatic and environmental conditions (Sener et al., 1985; Ustun et al., 1990; Matthaus and Ozcan, 2011; Hendawy et al., 2012).

CONCLUSION

The results obtained from present study indicated that locations (Isparta and Eskisehir) had significant effects on seed yield, some yield components, essential oil and fixed oil contents of black cumin populations. While yield components of black cumin populations varied according to locations and years, seed yield was higher in Isparta location on all populations due to higher rainfall than Eskisehir. Of the average of locations, the highest seed yield was determined in Usak population, followed by Eskisehir population. But, it was not detected essential oil in Usak population. The highest essential oil content was obtained from Tokat population in both locations and years. Tokat, Usak and Antalya populations on fixed oil rate were in the same statistical group at the $P < 0.01$ level on the basis of the LSD test.

Based on the results of the research: Tokat and Eskisehir populations for high seed yield, essential oil and fixed oil contents, and Usak population for high seed yield and fixed oil content could be evaluated in the breeding programme for line and cultivar development.

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