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Chemical Composition of the Fixed and Essential Oils of Nigella sativa L. from Turkey

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Abstract

Nigella sativa L. is an annual herbaceous plant which has medicinal and culinary uses. The seeds and extracts have antioxidant and antimicrobial effects. Besides, the seed oil is rich in unsaturated fatty acids. This research was conducted to determine the essential and fixed oil components of ten *Nigella sativa* populations in Eskisehir ecological conditions. Besides, yield component were also studied. Ten population were used in the research. The experimental design was randomized block design with three replications.

Statistically significant differences were found between populations in terms of seed yield and yield components. The highest seed yield was obtained from Kozluca Town, Burdur population (1885 kg/ha), the highest fixed oil yield was obtained from Kure, Sogut, Bilecik population (410.8 kg/ha).

Main essential oil components were 67.7 % thymoquinone, 8.4 % carvacrol, 4.8 % junipene, % 2.3 *p*-cymen, 1.9 % 4-Terpineol, 0.6 % longipinene, 0.5 % bornylacetate. The main unsaturated fatty acid was linoleic acid (39.20-43.74 %) followed by oleic acid (33.41-37.75 %).The total MUFAs, PUFAs, and SAFAs compositions were between 36.31-38.55%, 39.27-44.17% and 19.51- 22.23%, respectively. There were variations between populations in terms of seed yield and yield components, essential and fixed oil composition. This differences would be helpful for selecting genotypes in order to obtain new varieties with high seed and fixed oil yields. In addition, high amount of thymoquinone content in the essential oil was found for the Kure, Sogut, Bilecik population.

Key words: *Nigella sativa*, black cumin, fixed oil yield, fixed oil composition, essential oil components, thymoquinone

1. Introduction

Black cumin (*Nigella sativa* L.) is an ancient crop which originated in the Eastern-southern Europe and it has a very astonishing historical and religious background (Riaz et al., 1996; Salem, 2005). It is one of the most important medicinal plants because it has various uses. Seed of this plant have been used for centuries as a spice and food preservative. Besides, it is used in traditional medicine for the treatment of various diseases, including skin infections, for various respiratory and gastro-intestinal diseases (Riaz et al., 1996; Abu-Al-Basalc, 2009).

The seeds and the extracts have antihistaminic, anti-inflammatory, antidiabetic, antimicrobial, antitumor, antihypertensive, galactagogic and insect repellant effects (Baytop, 1984; Burits and Bucar 2000; Morsi 2000; Selamai and Hossain 2000; Ramadan 2015; Saleh 2018).

Twelve black cumin species are naturally grown in the flora of Turkey. However, cultivation aromatic plants are important to obtain sustainable products. Therefore, genotypes, cultural practices, environmental conditions like climatic and soil conditions are main factors for an efficient cultivation of black cumin. These factors affect the quantity and quality of the black cumin oil (Kara and et al, 2015; Saxena and et al, 2017). Many studies indicated that fixed oil yield was between 37- 27 % when solvent or cold press extraction methods were used, respectively. Oil yield and composition of *Nigella* seed oil is affected from location and genotype (Ashraf et al, 2005; Gharby 2015; Hosseinia et. al, 2018). Fixed oil of Nigella seed is rich in linoleic, oleic and palmitic acids. The dominating fatty acid is linoleic acid, which is an essential fatty acid and accounts for more than 50% of the total fatty acids (Atta 2003; Nickavar et. al, 2003).

The essential oil of black cumin seed which has valuable phytomedicinal features contains a variety of active compounds, of which thymoquinone, α-thujene and p-cymene are the major ones (Burits and Bucar 2000; Nickavaret. al, 2003). Especially thymoquinone is one of the most attractive component in phytomedicinal studies. Genotypes, cultural practices and environmental conditions affect the essential oil composition (Burits and Bucar 2000; Nickavaret. al, 2003).

This research was conducted to determine the essential and fixed oil components of ten *Nigella sativa* populations with their yield components in Eskisehir semi-arid ecological conditions.

2. Material and Methods

This study was carried out during the year 2009 at the research area of the Faculty of Agriculture of Eskisehir Osmangazi University, Eskisehir (39°48′ N, 30°31′ E, 789 m elevation). Soil samples were air-dried, passed through a 2 mm sieve and analyzed for pH, $CaCO_3$, organic matter and texture using standard procedures (Rowell, 1996). The soil of the research area was sandy-loam in texture, low in organic matter (0.91 %). Soluble salt, available P and available K were 0.024 %, 6.41 kg da^{-1} and 239 kg da^{-1}, respectively. Ten population were used in the research (Table 1).

Table 1. Populations of *Nigella sativa* L., their region of origin

No	Origin	No	Origin
1	Dereyalak village, İnonu, Eskisehir	6	Belevi village, Cameli, Denizli
2	Kure, Sogut, Bilecik	7	Denizli
3	Buyuksevin village, Afsin, K.maras	8	Burdur
4	Belevi village, Cameli, Denizli	9	Buyukalan, Cavdir, Burdur
5	Belevi village, Cameli, Denizli	10	Kozluca district, Burdur

The long-term (1991-2010) total rainfall, average temperature and relative humidity at the study location were 160.1 mm, 15.4 °C and 58.0%, respectively (Table 2). The precipitation and relative humidity were less in long term than in 2009. The long-term average temperature was approximately equal to the average temperature during the 2009 growing season.

First irrigation was done just after sowing while second was during flowering. Weeds controlled by hand weeding. Seed yields were taken at maturity by harvesting 0.25 m^2 area each plot by hand. At maturity, yield components such as biological yield per plant (BY) (g), plant height (PH) (cm), number of primary branches (NB) (pieces/plant), number of capsule (NC) (pieces/plant), capsule diameter (CD) (cm), number of seed per main capsule (NSC) (pieces/capsule), seed weight per main capsule (SWC) (g/capsule), seed yield per plant (SY) (g) and number of seed per plant (NS) (pieces/plant)were measured from 10 randomly selected plants.1000-seed weight (TSW) (g) was determined for each genotype. For the fixed oil content grinded samples were dried at 105° C for 3 hours and 10 g sample was fed into a Soxhlet apparatus (SOXTHERM® 2000 - C. Gerhardt GmbH & Co. KG). The extraction was performed on a water bath at 40–60° C for 4 h with 200 mL petroleum ether. The fixed oil yield was calculated by seed oil content (%) × seed yield (kg ha^{-1}).

Table 2. Monthly temperature, precipitation and relative humidity, Eskisehir (2009)

Months	Temperature (OC)		Precipitation (mm)		Relative humidity	
	2009	Long term*	2009	Long term *	2009	Long term *
March	4.6	4.9	39.8	29.6	60.5	64.2
April	10.0	9.7	26.0	44.3	55.7	62.3
May	14.8	14.9	28.9	39.4	50.7	59.3
June	20.4	19.2	7.9	24.4	41.0	55.0
July	22.2	22.0	11.4	13.4	42.9	51.9
August	21.0	22.0	2.0	9.0	42.2	53.0
Mean	**15.5**	**15.4**	**19.3**	**26.7**	**48.8**	**58.0**
Total	**93**	**92.4**	**116**	**160.1**	**293**	**345.7**

*Long-term average, 1991-2010

The fatty acid composition of the oil samples was analyzed after derivatization to fatty acid methyl esters (FAMEs) with 2 N KOH in methanol at room temperature. Analysis of FAMEs was carried out with a gas chromatograph (Agilent 6850, USA). The fatty acids were identified by the comparison of retention times to known standards. The results were expressed as percentage of total fatty acid.

Essential oil composition of the oil samples was analyzed with a gas chromatograph (Shimadzu QP2010 Plus) equipped with a Flame Ionization Detector and a capillary column (CPSil-5CB): (25 m length, 0.25 mm ID, 0.25 µm film). The injector temperature was 200 °C. The oven temperature was increased at 60 °C ramp to 200 °C at the rate of 5.0 °C /min and finally increased to 260 °C at the rate of 20 °C /min. The carrier gas was helium with a flow rate of 8 mL /min; the split rate was 1/50. The components were identified by the comparison of retention times to known standards. The results were expressed as percentage.

The results for yield and yield components, were subjected to analysis of variance (ANOVA) taking $p<0.05$ as significance according to LSD test.

3. Results and Discussion

Yield and Yield Components: Significant differences were high for all components at 5 % level ($p< 0.05$). Mean values of yield components and seed yield of different populations are shown at Table 2 and Table 3. The results indicated that“Burdur(Kozluca district)”(1881.3 kg/ha) and“Bilecik(Kure, Sogut)”(1697.2 kg/ha) outyielded for SY followed by population “Buyukalan, Cavdir, Burdur” (1605 kg/ha) and “Burdur” (1457 kg/ha) (Table 3). BY, PH, NB and NC were high for population “Kozluca district, Burdur"(3.28 g, 25.21 cm, 4.57 pieces/plant)followed by"Kure, Sogut, Bilecik"(2.54 g, 23.16 cm, 4.27 pieces/plant) (Tablo 2). NS (589.5), NSC (94.17), SY (18.81 kg/ha) were also high for population "Kozluca district, Burdur"(Table 3 and 4).Plant heightis an important characteristic that is essential under drought conditions (Alizadeh, 2005). PH was significantly different among populations between16.63-25.21 cm which was in accordance with Geren et al. (1997), Ozel et al. (2002), Toncer and Kizil (2004) and İpek et al. (2005). "Dereyalak village, İnonu, Eskisehir"was found to be the shortest (16.63 cm) population(Table 3). PH was similar with Bannayan et al. (2008) whereas capsule per plant and seeds per plant was higher than their results.

Table 3. Mean values of yield components and analysis of variance of ten *Nigella sativa* L populations.

Pop	BY (g)	PH (cm)	NB (pieces/plant)	NC (pieces/plant)	TSW (g)	CD (cm)
1	3.44 ± 1.53a	16.63 ± 0.85c	3.10 ± 0.10c	6.35 ± 0.05cd	2.06 b	1.30 ± 0.15a
2	2.54 ± 0.15b-e	23.16 ± 0.95ab	4.27 ± 0.29ab	7.43 ± 0.35bc	2.46 ab	1.16 ± 0.07b
3	1.79 ± 0.10e	17.02 ± 0.52c	3.67 ± 0.57bc	5.60 ± 0.35d	1.21 c	1.10 ± 0.08b
4	2.35 ± 0.13c-e	23.02 ± 1.70ab	3.70 ± 0.26bc	7.43 ± 1.00bc	2.56 a	1.08 ± 0.07b
5	2.38 ± 0.04c-e	21.73 ± 0.87b	4.23 ± 0.42ab	6.63 ± 0.49cd	1.29 c	1.19 ± 0.08ab
6	2.30 ± 0.05de	22.74 ± 1.49ab	3.57 ± 0.80bc	6.37 ± 0.23cd	2.53 a	1.11 ± 0.03b
7	2.72 ± 0.22a-d	22.02 ± 2.25ab	4.20 ± 0.30ab	7.17 ± 0.32c	2.62 a	1.14 ± 0.11b
8	2.67 ± 0.09a-e	21.96 ± 2.11b	4.53 ± 0.31a	7.40 ± 0.26bc	1.23 c	1.09 ± 0.04b
9	3.20 ± 0.24a-c	23.13 ± 0.80ab	3.83 ± 0.49a-c	8.67 ± 0.65ab	1.28 c	1.13 ± 0.03b
10	3.28 ± 0.01ab	25.21 ± 0.87a	4.57 ± 0.06a	9.17 ± 1.01a	1.24 c	1.10 ± 0.01b
LSD(0.01)		3.301**		1.390**	0.43**	
LSD(0.05)	0.89*		0.74*			0.14*
C.V. (%)	24.20	13.24	14.37	15.79	34.79	7.92

Mean±SE; F-test significant at P ≤ 0.05:*; F-test significant at P ≤ 0.01:**; ns: non-significant

BY (Biological yield per plant); PH (Plant height); NB (Number of branch per plant); NC (Number of capsule per plant); CD (Capsule diameter); TSW (Thousand seed weight); Pop (Populations)

NSC and SWC differed significantly between 60.53-94.17 pieces and 0.24-0.319 g, respectively. These results agreed with Toncer and Kizil (2004) and Ozel et al. (2002) in similar ecological conditions. TSW, which is associated with the seed yield, showed significant differences between 1.21 and 2.67 g, and these results agreed with Ahmed and Haque (1986), Geren et al. (1997), Ozel et al. (2002), Kalcin (2003), Ashraf (2005). The highest TSW was obtained from the population "Denizli".

SY differed significantly between 905.3 and 1881.3 kg/ha and agreed with Ahmed and Haque (1986), Ozguven and Tansi (1989), Koc (1999), D'Antuono et. al. (2001), Ashraf (2005), Shah et al. (2007).Seed yield was higher than the yields found by Tuncturk et al. (2005).Number of seed per capsule and seed oil content was similar with Toncer and Kizil (2004) and seed yield per plant was higher than their results.

The fixed seed oil yield which is multiplied by fixed seed oil content (%) with seed yield (kg ha^{-1}) is an important yield component. İn this study, fixed oil yield differed significantly between 188.5-410.8 l/ha (Figure1.) Kilic and Abaci (2016), found lower amount of seed oil yield (99-348.1 l/ha) and they reported that this difference was related to agronomic practices like sowing time and seeding rate.

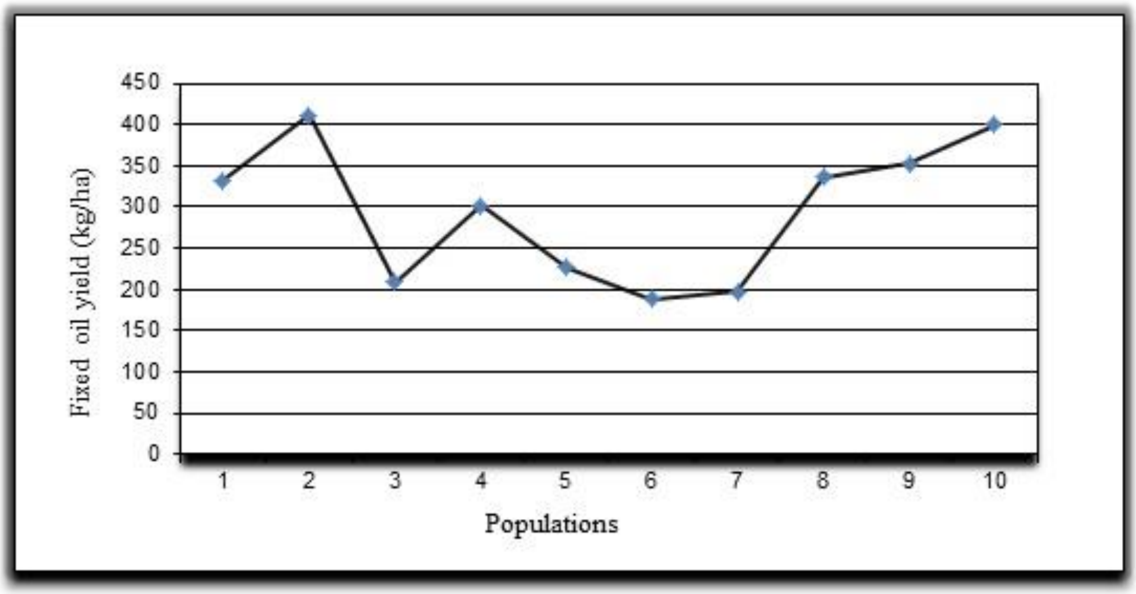
Table 4. Mean values of yield components, seed yield and analysis of variance of ten *Nigella sativa* L populations

Pop	NSC (pieces/capsule)	NS (pieces/plant)	SWC (g/capsule)	SYP (g)	SY (kg/ha)
1	60.53 ± 2.84c	317.0 ± 19.31d	0.29 ± 0.03ab	1.33 ± 0.09cd	1691.0 ± 34.20c
2	92.87 ± 1.42a	451.2 ± 44.86bc	0.29 ± 0.01a	1.29 ± 0.06bc	1697.2± 30.78b
3	73.83 ± 2.14bc	299.7 ± 10.57d	0.24 ± 0.00b	1.02 ± 0.17d	1033.3 ±27.69g
4	83.57 ± 1.12ab	455.7 ± 53.17bc	0.28 ± 0.05ab	1.24 ± 0.16b-d	1143.8 ± 39.21f
5	82.63 ± 7.77ab	393.4 ± 7.00c	0.28 ± 0.02ab	1.28 ± 0.07bc	978.7 ± 22.48i
6	87.43 ± 16.43ab	392.9 ± 35.06c	0.28 ± 0.06ab	1.16 ± 0.09cd	905.3 ± 23.04j
7	83.87 ± 4.66ab	441.7 ± 23.73bc	0.29 ± 0.02ab	1.45 ± 0.04b	1012.0 ±30.93h
8	86.60 ± 8.52ab	464.5 ± 41.27b	0.32 ± 0.03a	1.35 ± 0.06bc	1457.1 ± 49.88e
9	93.03 ± 0.50a	575.1 ± 38.58a	0.31 ± 0.02a	1.74 ± 0.04a	1605.3 ±16.38d
10	94.17 ± 4.64a	589.5 ± 18.84a	0.29 ± 0.02ab	1.70 ± 0.02a	1881.3 ± 37.66a
LSD(0.01)	15.59**	69.70**		0.233**	0.130**
LSD(0.05)			0.05*		
C.V. (%)	13.54	21.77	11.20	17.97	26.15

Mean±SE; F-test significant at P ≤ 0.05: *; F-test significant at P ≤ 0.01: **; ns: non-significant

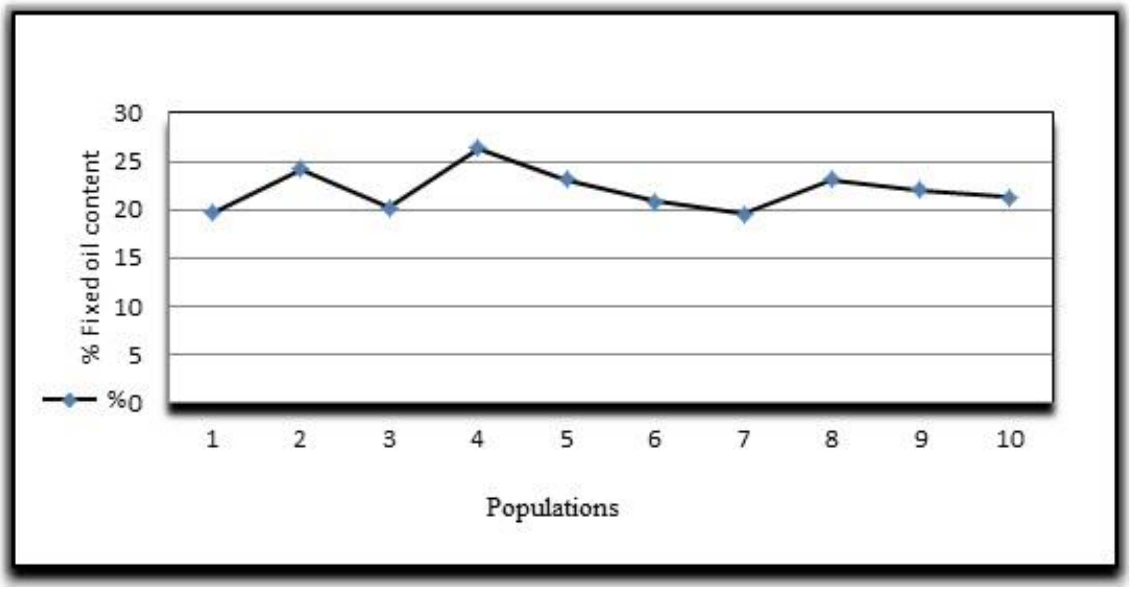
NSC (Number of seed per capsule); NS (Number of seed per plant); SWC (Seed weight per capsule); SYP (Seed yield per plant); SY (Seed yield); Pop (Populations)

Figure 1. Mean values of fixed oil yield of ten *Nigella sativa* L. populations



Phytochemical Constituents: Fixed oil content (26.34 %) was high for “Belevi village, Cameli, Denizli” (figure2) and followed by “Kure, Sogut, Bilecik” and “Burdur”. These results agreed with Toncer and Kizil (2004). Al-Neqeeb et al (2009), found higherfixed oil contents in Yemen ecological conditions which the difference can be explained by the genotypic differences of the seeds and the warmer climatic conditions.

Figure 2. Fixed oil content (%) of ten *Nigella sativa* L. populations



The results showed that UFA (palmitoleic acid, oleic acid, linoleic acid, linolenic acid, eicosenoic acid and eicosadienoic acid) account for 76.96 and 80.47%; the main unsaturated fatty acid was linoleic acid (39.20-43.74 %) followed by oleic acid (33.41-37.75 %). The total MUFAs, PUFAs, and SAFAs compositions were between 36.31-38.55%, 39.27-44.17% and 19.51- 22.23%, respectively (Table 5). Gharby and et al. (2015), found higher level of linoleic acid and lower level of oleic acid in their study (58.5 % linoleic acid, 23.8 % oleic acid). These variations could be explained by the diversity in maturity, phenotype and the origin of the investigated seeds (Kazemi, 2014; Gharby 2015). Likewise, Atta (2003) and Nickavar et al. (2014) found the major saturated fatty acids of *Nigella* seed oil as palmitic and stearic acids whereas the main unsaturated fatty acids was oleic and linoleic acids.

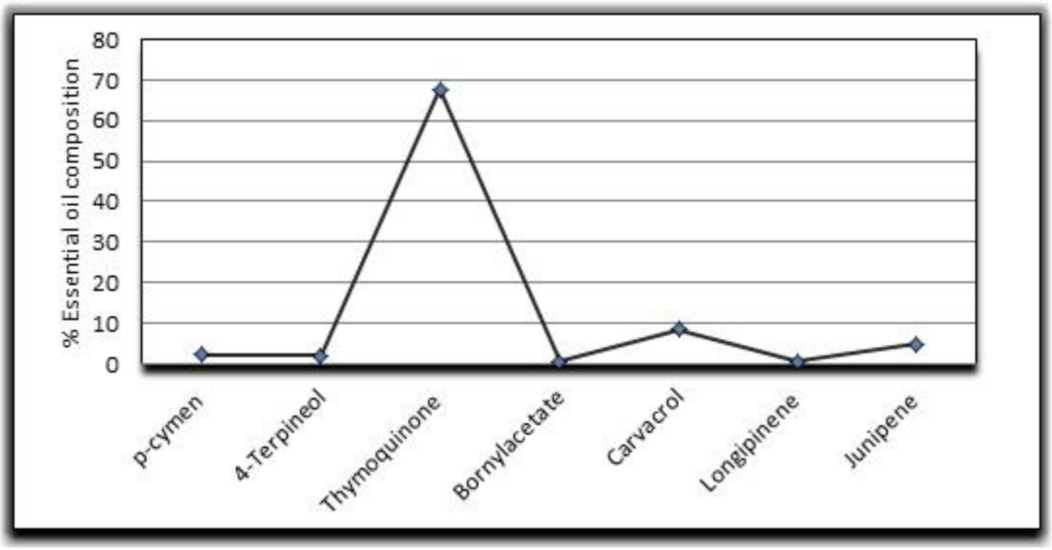
The main components of the essential oil are shown at Figure 3. Main essential oil components were 67.7 % thymoquinone, 8.4 % carvacrol, 4.8 % junipene, 2.3 %*p*-cymen, 1.9 % 4-Terpineol, 0.6 % longipinene, 0.5 % bornylacetate. Nickavar et.al (2003), determined different rates. They reported that the oil was characterized by a large amount of phenyl propanoids. The oil presented high levels of trans-anethole (38.3%) and p-cymene (14.8%). Other important constituents were limonene (4.3%), carvone (4.0%), carvacrol (1.6%), 4-Terpineol (0.7%), thymoquinone (0.6%). These differences may be related to the variations of cultivated regions. The thymoquinone content found in this study was rather high and therefore valuable which is known to have Hepatoprotective activity (Daba et.al., 1998) and is a potential cure for inflammatory disorders and cancer (Woo et al., 2011). Many other researchers reported lower thymoquinone contents in *Nigella sativa* essential oil (Burits and Bucar, 2000; Harzallah et al, 2011). The high amount of the thymoquinone content may be because of the semi-arid conditions during the seed maturation stage of the vegetation. As it seen on the climatic datas on table 2 the temperature especially of June and July were higher; the relative humidity and precipitation amounts were lower than the long term data during the seed maturation stage (June and July).

Table 5. Fatty acid composition (% of total fatty acid) of some *N. sativa* populations

Fatty acids	Populations						
	Eskisehir	Bilecik	K.Maras	Denizli	Denizli	Denizli	Burdur
Myristic (14:00)	0.38	0.36	0.32	0.38	0.37	0.35	0.36
Palmitic (16:00)	15.98	15.29	15.36	16.26	16.09	16.00	16.04
Palmitoleic (16:01)	0.33	0.33	0.30	0.36	0.46	0.34	0.40
Margaric (17:00)	0.09	0.09	0.10	0.39	0.06	0.12	0.33
Heptadecenoic (17:01)	0.06	0.13	0.08	0.21	0.31	0.12	0.41
Stearic (18:00)	4.51	3.37	3.43	3.74	4.26	3.37	3.63
Oleic (18:01)	36.09	35.43	36.61	37.02	37.71	35.41	37.75
Linoleic (18:02)	40.87	43.74	42.26	39.20	37.76	39.34	39.55
Linolenic (18:03)	0.51	0.43	0.56	1.00	1.51	0.98	0.72
Arachidic (20:00)	0.67	0.27	0.38	1.24	1.20	1.14	0.56
Ekosanoic (20:01)	0.51	0.42	0.50	0.10	-	2.51	-
Behenic (22:00)	-	0.14	0.10	-	-	-	-
Lignoceric (24:00)	-	-	-	0.09	0.27	0.32	0.25
Total	99.99	99.99	99.91	99.06	99.98	99.59	99.98
SAFA	21.63	19.51	19.62	22.09	22.23	20.89	21.16
MUFA	36.99	36.31	37.47	37.68	38.48	38.38	38.55
PUFA	41.38	44.17	42.82	39.28	39.27	40.32	40.27
total UFA	78.36	80.47	80.28	76.96	77.75	78.70	78.82

SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA (unsaturated fatty acids)

Figure 3. Essential oil components (%) of “Kure, Sogut, Bilecik” population



4. Conclusion

Statistically significant differences were found in *Nigella sativa* L. populations in terms of all yield and yield components. In terms of seed yield, “Burdur (Kozluca Town)” population had higher values compared to the recent populations, whereas fixed oil yield was higher in “Bilecik (Sogut-Kure)” population.

There were variations between populations in terms of seed yield, yield components, fixed oil yield and fixed oil composition. These differences would be helpful for selecting genotypes in order to obtain new varieties with high oil yields and fixed oil composition in further studies. “Kure, Sogut, Bilecik” Population had high amount of thymoquinone which could be a valuable source for the isolation of the component. It is recommended to cultivate it in different ecological conditions in further studies to see the variations affected from environmental conditions.

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