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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Quality Characteristics of Numerous Different Origin Black Cumin (*Nigella sativa* L.) Seed Genotypes Grown with Good Agricultural Practices under Ankara Conditions*

Ankara Koşullarında İyi Tarım Uygulamaları ile Yetiştirilen Çok Sayıda Farklı Kökenli Çörek Otu (*Nigella sativa* L.) Genotiplerinin Kalite Özelliklerinin Belirlenmesi

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Abstract

In this study; during the spring vegetation period of 2019-2020, fixed and essential oil ratios, their components and, oil yield were determined in 31 black cumin genotypes at advanced yield level, developed by phenotypic recurrent selection method, under in Ankara ecological, fertilizer-free and pesticide-free growing conditions. Fixed oil ratio is determined by the solvent extraction method in the soxhlet device; Its components were determined by gas chromatography. The amount of thymoquinone was determined according to the UV spectrum (PDA). In the two-year evaluation, significant differences were seen between the lines. The results indicate that the USDA-Egyptian population (No. 26) exhibited an average fixed oil content of 34.8%-33.5% in 2019 and 2020, with the highest rate reaching 38.9%. Additionally, the oil yield average from the Burdur genotype (No. 4) was 13.2 kg da^{-1} -12.3 kg da^{-1}, and the highest oil yield recorded was 17.5 kg da^{-1}. Analysis of fixed oil components over the years revealed linoleic acid as a major component at 52.4%, 56.3% and 54.3%, oleic acid at 23.2%, 24.8% and 24.0%, palmitic acid at 10.4%, 12.2% and 11.3%, and stearic acid at 4.3%, 3.2% and 3.7%, respectively. Essential oil results showed variations ranging from 0.01% to 0.08%, with thymoquinone (54.4%-83.1%), *p*-Cimen (1.0%-16.4%), cis-4-Methoxy thujane (1.8%-5.8%), and longifolene (0.5%-8.5%) identified as the primary components. These results obtained from the study and the high-quality characteristics (ratio and yield) of the naturally grown black cumin plant, reveal the positive impact of good agricultural practices. In addition, the lines that stand out as a result of the research can be used in hybridization studies and can be safely evaluated as a new, multipurpose potential product for pharmaceutical, industrial, and cosmetic uses.

Keywords: Black cumin (*Nigella sativa* L.), Essential oil, Fixed oil, Thymoquinone, Essential and fixed oil component

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*This study was summarized from the "Determination of yield and quality characteristics of different black cumin (*Nigella sativa* L.) genotypes grown by good agriculture practices under Ankara ecological conditions" PhD thesis.

Öz

Bu çalışmada; 2019-2020 yıllarının ilkbahar vejetasyon döneminde, Ankara ekolojik koşullarında, fenotipik tekrarlamalı seleksiyon metoduyla geliştirilen ileri verim kademesindeki 31 çörek otu genotipinin gübresiz ve ilaçsız yetiştirme koşullarında sabit ve uçucu yağ oranları, bunların bileşenleri ve yağ verimi belirlenmiştir. Sabit yağ oranı, soxhelet cihazında solvent ekstraksiyonu yöntemiyle; bileşenleri, gaz kromatografisiyle belirlenmiştir. Timokinon miktarı ise UV spektrumuna (PDA) göre tespit edilmiştir. İki yıllık değerlendirmede, hatlar arasında önemli farklılıklar görülmüştür. Çalışma sonucunda, sabit yağın 2019 ve 2020 deneme yılları ortalaması sırasıyla %34.8-%33.5 ve ortalama en yüksek sabit yağ oranı %38.9 ile 26 numaralı USDA-Mısır popülasyonundan; yağ verimi ise yıllar itibariyle sırasıyla ortalama 13.2 kg da^{-1}-12.3 kg da^{-1} ve ortalama en yüksek yağ verimi 17.5 kg da^{-1} ile 4 numaralı Burdur genotipinden elde edilmiştir. 2019-2020 ve birleşik yıl deneme ortalamasına göre sabit yağın majör bileşenlerinden linoleik asit yıllar itibariyle sırasıyla ortalama %52.4, %56.3 ve %54.3; oleik asit %23.2, %24.8, %24.0; palmitik asit %10.4, %12.2, %11.3; stearik asit %4.3, %3.2 ve %3.7 olarak belirlenmiştir. Tek yıllık uçucu yağ sonuçlarına göre uçucu yağ oranı %0.01-0.08 arasında değişmiş ve uçucu yağın ana bileşenleri timokinon (%54.4-83.1), p-Simen (%1.0-16.4), cis-4-Metoksi tuyan (% 1.8-5.8) ve longifolen (% 0.5-8.5) olarak tespit edilmiştir. Çalışmadan elde edilen bu sonuçlar ve doğal (organik) yetiştirilen çörek otu bitkisinin kalite özelliklerinin (oransal ve verim) yüksek olması, üretimde sürdürülebilirlik açısından iyi tarım uygulamaları/organik tarımın olumlu etkisini ortaya koymuştur. Araştırma sonucunda öne çıkan hatlar melezleme çalışmalarında kullanılabilmenin yanı sıra, farmasötik, endüstriyel ve kozmetik kullanımlar için yeni, çok amaçlı potansiyel bir ürün olarak güvenle değerlendirilebilecektir.

Anahtar Kelimeler: Çörek otu (*Nigella sativa* L.), Uçucu yağ, Sabit yağ, Timokinon, Uçucu ve sabit yağ bileşenleri

1. Introduction

Throughout human history, the search for healing in nature has persisted, with medicinal plants playing an irreplaceable role in various industries, including spices, beverages, perfumery, cosmetics and pharmaceuticals. Despite advancements in medicine, medicinal plants continue to hold vital importance. It is estimated that globally, 4-6 thousand medicinal plants are popular, with three thousand species (Acıbuca and Bostan Budak, 2018) traded and 900 cultivated for commercial purposes (Arslan et al., 2015). In Australia, 48% of the population, in Belgium 31%, in Canada 70%, in the United States 42%, and in France 49% are among the countries where at least once traditional treatment methods have been used (Kalaycı,2016). Notably, countries such as the USA, Germany, Greece, Poland, Vietnam, Italy, Holland, Belgium, Brazil, Japan, France, and Canada are significant consumers of traditional herbal treatments. Japan holds the highest per capita consumption of herbal medicines, while Türkiye exports medicinal and aromatic plants to approximately 100 countries worldwide, with major sales to North America, Latin America, the European Union, the Far East and North Africa (Karık and Tunçtürk, 2019; Mathe and Turgut, 2023).

Black cumin (*Nigella sativa* L.), originating in Eastern Mediterranean countries, Eastern and Southern Europe and West Asia, has historical significance and is widely grown in temperate and warm climates, including the Balkans, the Middle East, Southern Europe, India, and North Africa. Its seeds contain 30-45% fixed oil, 0.01-0.5% essential oil, 20-30% protein, alkaloid, and saponins. The fatty acid composition in black cumin seed includes linoleic, oleic and palmitoleic acids, accounting for about 74% of unsaturated fatty acids, with linoleic acid being the predominant component. Saturated fatty acids constitute approximately 26% of *Nigella* seeds. Thymoquinone, a key element in essential oil, exhibits a range of pharmacological effects, including antitumor, antibacterial, antioxidant, antihistamine, antidiabetic, anti-hypertensive, anti-inflammatory, and antimicrobial properties. The plant's therapeutic, preventive and protective effects have sustained its role in human health over the years. Additionally, black cumin has demonstrated success in preventing oxidation in foods and treating certain animal and plant diseases. For instance, it has been successfully used against Moniezia infection in sheep (Akhtar and Javed, 1991). Boyraz and Özcan (1997) reported that a 1% *Nigella sativa* L. extract inhibited the mycelial development of *Colletotrichum coccodes*, *Fusarium oxysporum* f. sp. *melonis*, and *Rhizoctonia solani* fungi by 30.63% to 67.78%. They also mentioned that a 2% dose of the extract inhibited the mycelial development of all fungi, including *Alternaria solani*, to varying extents. Another study discovered that adding 3.5 ml/kg of *Nigella sativa* L. oil to hens' diet greatly reduced their serum cholesterol ratio; adding 2.5 ml/kg of *Nigella sativa* L. oil to their feed significantly raised their serum globulin concentration (Bölükbaşı et al., 2009). Due to its composition of fixed and volatile oils, the *Nigella sativa* L. plant has influenced various industries, impacting production and cultivation areas. Official records indicate an increase in both data sets from 2012 to the present (in 2012, 2,299 da, 161 tons, and in 2022, 108,029 da, 10,089 tons). The export revenue has exceeded one million dollars since 2017 (with the 2022 export value being 2,117,597 dollars) (Anonymous, 2023). Like all other medicinal and aromatic plants, sustainable production and market potential for black cumin also require the product to meet the desired quantity and quality standards. The yield and quality characteristics of *Nigella sativa* L. are influenced by environmental factors (edaphic, orographic, and biotic factors), climate factors (temperature, light, water, CO_2, wind, precipitation) and cultivation/agronomic practices (sowing time/depth/frequency, fertilization, spraying).

Given its significance in the food sector and its health-related benefits, it is crucial to produce black cumin under conditions that closely mimic natural environments. Medicinal and aromatic plant species and drugs grown naturally and organically have high market value. Notably, black cumin lacks a licensed herbicide, and the use of fertilizers, even if organic, poses a risk due to potential contamination with pathogens such as *E. coli* 0157:H7, *Salmonella*, and *Campylobacter* (Arslan et al., 2015). The fight against existing chemicals results in a disruption of the natural balance, environmental pollution by mixing the chemicals in the groundwater, residue problems, adverse impact on non-target organisms, and resistance of agricultural pests to the pesticides used (Budak et al., 2022). This study aims to assess the quality characteristics of black cumin genotypes, especially local ones, under good agricultural practices and ecological conditions, avoiding the use of fertilizers and pesticides.

In recent years, the black cumin plant, which has taken a prominent place among the products exported by Türkiye, has increased in importance due to the foreign exchange revenue it brings to the country and the development of various industries with different areas of use. This has made it mandatory to conduct studies in line with the changing and evolving world standards. In order to address consumers' legitimate concerns, there is a limited number of long-term quality studies on a large number of black cumin plants grown through reliable plant production methods that do not harm people and the environment. Consequently, there are no quality results for standardized lines developed within the breeding program. With this study, advanced black cumin lines (especially local genetic resources of black cumin) were aimed to be evaluated for quality characteristics in a good and ecologically friendly agricultural practice without the use of fertilizers and any chemicals (herbicides or insecticides) for future scientific studies. With the lines that stand out in terms of results and quality; It is aimed to develop standardized varieties that are productive as well as high quality and have the qualities needed for both direct use and industry.

2. Materials and Methods

Field trials for this study, encompassing 31 black cumin genotypes, including one variety, were conducted in the experimental fields of the Central Research Institute of Field Crops (CRIFC) Research and Application Farm and under Ankara's ecological conditions during the summers of 2019-2020. As material, a total of 31 black cumin genotypes, including one variety from the black cumin breeding program, were utilized, originating from both domestic and international sources (*Table 1*). The study, designed with three repetitions in Randomized Complete Block Design (parcel size 7.5m^2), excluded fertilization and pest control processes. While irrigation was performed once in May 2019, it was unnecessary in 2020.

The soil analysis revealed that the trial fields in 2019 were clayey (C), slightly alkaline (pH 7.95), calcic (30.5%), and salt-free (0.60 dS/m). In 2020, the soil was clayey-loamy (CL), slightly alkaline (pH 7.73), calcic (30.0%), and salt-free (0.64 dS/m). Both years showed low levels of available phosphorus (P), manganese (Mn) and organic substance, zinc (Zn); high potassium (K); medium iron (Fe); sufficient copper (Cu); and good levels of calcium (Ca) and magnesium (Mg).

Table 1. Genotypes of black cumin used in the study

GN*	Origin	GN*	Origin
1	Denizli-1	17	BCSMM-1*
2	Denizli-2	18	BCSMM-2*
3	Burdur-1	19	BCSMM-3*
4	Burdur-2	20	BCSMM-4*
5	Ethiopia	21	India-2
6	Syria-1	22	Bursa/ Keles
7	India-1	23	Gazi Osmanpaşa (GOP) Univ. Eskişehir
8	Syria-2	24	Afyonkarahisar
9	Denizli-3	25	USDA- Pakistan
10	Burdur-3	26	USDA- India
11	Burdur-4	27	Burdur-5
12	Konya/ Akören-1	28	Kırıkkale/Halitli
13	Konya/ Meram	29	Ankara-1
14	Konya/ Akören-2	30	Ankara-2
15	Diyarbakır	31	Çameli (variety)
16	Syria-3		

* GN: Genotype number, BCSMM: Black Cumin Stock Market Material

The average temperatures for the research field during the vegetation period (March-August) were 14.4°C in 2019, 15.0°C in 2020, and a long-term average of 15.4°C. Total precipitation measured 103.8 mm, 111.6 mm, and 171.9 mm, respectively, with relative humidity at 55.3%, 50.9%, and 58.5% (*Table 2*). Climatic variations, including heavy rain and hail above seasonal averages in June and July, led to irregular precipitation distribution during the research years. Seed planting occurred on March 12th-13th, 2019, and March 4th-5th, 2020, with harvesting on August 2nd-10th, 2019, and July 23rd-August 5th, 2020. Fixed oil and fatty acid component analysis was conducted in both years, while essential oil and its components were analyzed only in 2019.

Table 2. Meteorological data of long years and growing season of 2019 and 2020 at Ankara

Climatic Factors	Years	March	April	May	June	July	August	Average	Total
Minimum Temperature(°C)	2019	-1.0	2.4	8.6	13.6	12.5	14.0	8.3	-
	2020	-0.1	2.3	6.4	10.8	15.1	14.8	8.2	-
	LY*	-13.6	-5.1	-0.1	4.7	2.8	5.9	-0.9	-
Maximum Temperature(°C)	2019	11.2	14.0	21.9	25.7	26.8	27.6	21.2	-
	2020	12.2	14.3	20.2	24.2	30.6	30.5	22.0	-
	LY	24.5	28.0	31.7	34.9	39.6	38.4	32.9	-
Average Temperature (°C)	2019	4.6	7.8	15.0	18.7	19.2	20.7	14.4	-
	2020	5.6	8.2	13.2	17.2	23.1	22.7	15.0	-
	LY	5.1	9.7	14.4	18.6	22.1	22.5	15.4	-
Relative Humidity (%)	2019	58.5	61.3	55.2	59.6	50.2	47.1	55.3	-
	2020	60.6	54.7	56.2	53.8	42.3	37.5	50.9	-
	LY	72.0	64.3	63.1	59.3	46.4	45.6	58.5	-
Precipitation (mm)	2019	20.6	23.4	3.8	15.0	31.8	9.2	-	103.8
	2020	14.8	26.0	42.8	27.4	0.6	0.0	-	111.6
	LY	42.1	24.3	47.8	38.9	10.1	8.7	-	171.9
Soil Temperature 5 cm (°C)	2019	10.6	14.6	23.7	27.3	28.8	28.9	22.3	-
	2020	11.0	15.7	22.0	24.0	33.8	34.4	23.5	-
	LY	5.9	10.9	16.6	21.3	25.1	25.8	17.6	-
Soil Temperature 10 cm (°C)	2019	8.7	12.1	19.3	23.9	25.1	25.4	19.1	-
	2020	9.0	13.0	18.6	20.9	27.7	28.0	19.5	-
	LY	6.0	10.8	16.2	20.6	24.5	25.1	17.2	-

* LY: Long years, Data were obtained from Turkish State Meteorological Service, Ankara

Fixed oil ratio (%): The 10 g seed samples homogenously taken from each plot were ground, and solvent (96% pure hexane from ISOLAB company) extraction was performed using a Soxhlet apparatus at the Oilseed Crops Unit Laboratory of the Field Crops Central Research Institute (Soxtherm 2000 fat determination device) according to the ISO 659:2009 method.

Fixed oil yield (kg da^{-1}): Calculated using fixed oil ratio along with seed yield per decare.

Fatty acids composition (%): The analysis of fatty acids was determined by gas chromatography at the Oilseed Crops Unit Laboratory of the Central Research Institute of Field Crops. 0.1 g of oil was mixed with 10 mL n-hexane (98% purity, company SIGMA-ALDRICH), shaken, and then 0.5 ml of 2N methanol (99.9% purity, company SIGMA-ALDRICH), KOH (86% purity, company SIGMA-ALDRICH) was added to the mixture. Esterification was achieved by stirring and allowing it to stand for 30 minutes. Samples taken from the upper phase were placed in the Shimadzu AOC-20i automatic injector. Shimadzu GC-2010 (Japan), flame ionization detector (FID), and Teknokroma Capillary column (100 m × 0.25 mm and 0.2 µm film thickness) were used to determine the fatty acid composition. Helium was applied as the carrier gas with a flow rate of 0.94 mL min^{-1}. The split ratio was set at 1:100. The operating temperatures were set at 250°C for the injection block and detector. The isothermal condition of the column oven was programmed to wait at 140°C for 5 minutes, then increase at a rate of 4°C/minute to 240°C, and finally wait for 20 minutes. Restek 35077, Food Industry FAME mix (USA) was used as a standard for the identification of fatty acids.

The volatile oil components, the ratio of volatile oil, and the ratio of thymoquinone were determined at The Plant, Drug and Scientific Research Application and Research Center (AÜBİBAM/Eskişehir) laboratories.

Essential oil ratio (%): The fixed oil samples of the cumin seeds from each plot were subjected to micro-distillation to capture the volatile components with hexane. Subsequently, the hexane phase was removed under nitrogen gas, and the weight percentage of the volatile component in the oil was provided. The micro-distillation process was carried out using the Eppendorf Microdistiller®.

Essential oil components (%): The components of the volatile oil of the cumin seeds from each plot were identified using Gas Chromatography-Mass Spectrometry (GC-MS). The method employed Gas

Chromatography/Mass Spectrometry (Hewlett Packard 5973 Mass Selective Detector System, Carrier Gas: Helium 1.0 mL/min) for the identification of the components of the samples, and Gas Chromatography (Hewlett Packard 6890 GC System, Carrier Gas: Helium 1.0 mL/min) for determining their relative percentages.

Sample preparation: The sample material was subjected to micro-distillation to capture volatile components with hexane, and subsequently injected into the system at a 2:1 split ratio as a 1 µL sample (10% h/h).

Gas Chromatography (GC) conditions

System: Hewlett Packard 6890 GC System

Column: Agilent HP5MS (30 m x 0.25 mm internal diameter x 0.25 µm film thickness)

Detector: Flame Ionization Detector (FID)

Injection temperature: 250°C

Detector temperature: 300°C

Temperature program: 60°C (10 min.), 4°C/min to 240°C (5 min.), Total 60 min.

Carrier gas: Helium (1.0 mL/min)

Gas Chromatography/Mass Spectrometry (GC/MS) conditions

System: Hewlett Packard 5973 Mass Selective Detector System

Column: Agilent HP-Innowax (30 m, 0.25 mm internal diameter, 0.25 µm film thickness)

Injection temperature: 250°C

Ion source temperature: 230°C

Ionization mode: Electron Impact

Electron energy: 70 eV

Mass range: 35-450 m/z

Temperature program: 60°C (10 min.), 4°C/min to 240°C (5 min.), Total 60 min.

Carrier gas: Helium (1.0 mL/min)

Identifications: Wiley 9-Nist 11 Mass Spectral Database

Thymoquinone ratio (mg thymoquinone/100 mg oil): The amount of thymoquinone in each parcel's black seed samples was determined using the Waters Acquity UPC^2 system based on the UV spectrum (PDA).

Chromatographic Conditions

System: Waters Acquity UPC^2

Column: Waters Acquity UPC^2 HSS C18 SB 1.8 µm (3.0x100 mm)

Column Temperature: 40ºC

Mobile Phase: 94% CO2 - 6% MeOH at 0.6 mL/min

Back Pressure: 2000 psi

Injection Temperature: 15ºC

Detector: 1) PDA

3D scan: 210-500 nm

2D scan: 238 nm

Sample preparation: 0.5% solution of the sample was prepared with 2-isopropyl alcohol and 1 µL was injected into the system.

Statistical analysis: All data were subjected to analysis of variance (ANOVA) using the MSTAT-C computer statistical software. The significant differences between the group means were separated using Duncan's test.

3. Results and Discussion

3.1. Fixed oil ratio (%)

Combined analysis revealed a significant 1% difference between genotypes, years, and their interaction (*Table 3*). Genotype 26 exhibited the highest fixed oil content (40.60% in 2019, 37.16% in 2020, and 38.88% in the combined analysis), although it was not statistically different from genotype 10 in the combined analysis. The lowest values were recorded for genotype 30 in 2019 (27.82%) and for genotype 2 in 2020 and in the combined years (22.26% and 27.75%), which, however, did not differ significantly from genotype 1 in 2020 (*Table 4*).

Table 3. Combined variance analysis results for fixed oil percentage data of black cumin genotypes in the experiment

Source of Variance	D.F. (Degree of Freedom)	S.S. (Sum of Square)	M.S. (Mean Square)	F Value
Replication	4	2.58	0.65	0.27
Year	1	76.32	76.32	31.38**
Genotypes	30	872.30	29.08	11.95**
Year×Genotypes	30	811.08	27.04	11.11**
Error	120	291.90	2.43	
Sum	185	2054.20		
CV (Coefficient of Variation) (%)	4.53			

(**) Significant at the 0.01 level, (*) Significant at the 0.05 level

Table 4. Mean values and LSD groups for the fixed oil ratio (%) determined in black cumin genotypes included in the trial

Genotypes	2019		2020		2019-2020	
1	35.4	dg	24.0	l	29.7	m
2	33.2	gj	22.3	l	27.8	n
3	37.3	be	34.8	bg	36.1	bc
4	37.0	be	31.5	ik	34.2	di
5	37.2	be	32.5	gk	34.9	bh
6	39.0	ab	31.4	jk	35.2	bf
7	33.9	gj	30.5	k	32.2	jl
8	37.6	bd	30.7	k	34.1	di
9	37.7	bc	34.8	bg	36.3	b
10	39.1	ab	37.0	ab	38.0	a
11	32.6	ij	33.7	di	33.1	hk
12	33.9	gj	35.7	ad	34.8	bh
13	34.2	fi	35.0	af	34.8	bh
14	33.3	gj	35.5	ae	34.4	ci
15	32.9	hj	33.8	dh	33.4	gk
16	34.7	fi	36.3	ac	35.5	be
17	31.9	jk	35.2	af	33.5	fk
18	35.2	eh	32.4	hk	33.8	ej
19	33.2	gj	35.1	af	34.1	di
20	33.3	gj	33.1	fj	33.2	hk
21	32.3	j	35.4	ae	33.8	ej
22	33.4	gj	36.9	ab	35.2	bf
23	36.6	cf	35.2	af	35.9	bd
24	35.2	eg	35.4	ae	35.3	bf
25	33.9	gj	32.6	gk	33.2	hk
26	40.6	a	37.2	a	38.9	a
27	37.2	be	35.2	af	36.2	b
28	36.8	bf	33.3	ej	35.1	bg
29	29.6	kl	34.0	ch	31.8	kl
30	27.8	l	34.3	ch	31.1	lm
Çameli	32.0	j	33.7	di	32.9	ik
Mean	34.8		33.5		34.1	
LSD	2.3		2.3		1.8	
CV (%)	4.0		4.2		5.5	

Our findings regarding the fixed oil content values obtained from black cumin genotypes in the research (22.3-40.6%) are within the limits reported by Türker and Bayrak (1997) (25.0-37.2%), D'Antuono et al. (2002) (19.1-22.7%), Rchid et al. (2004) (40%), Matthaus and Özcan (2011) (28-36.4%), Hosseini et al. (2019) (27.6-33.0%), Kamçı (2019) for summer planting (31.3-32.4%), Özdemirel and Kaçar (2021) (29.1-33.0%), Sağlık (2020) (12.2-20.0%), Gülhan and Taner (2020) (26.2-38.2%), Yılmaz et al. (2020) (25.6-32.9%), Anil Kumar et al. (2021) (23.96-38.74%), Bozdemir et al. (2022) (27.2-30.7%), Karer and Beyzi (2022) (34.02-39.84%), and Durmaz and Kara (2023) (32.60-34.63%), being generally higher than the maximum reported value. They are also partially similar to the values reported by Al-Naqeeb et al. (2009) (32-48%) but lower than the maximum value they reported.

The fixed oil content in black cumin cultivation is crucial, serving as one of the significant yield parameters directly influencing the plant's quality. The reasons for the research results differing from studies conducted in different ecologies are thought to be primarily due to the materials having diverse genetic diversity. Additionally, cultural practices, climate and soil conditions, varied harvesting times, and oil processing techniques applied play essential roles.

For instance, the oil obtained through cold press extraction may result in a lower oil content compared to solvent-based extraction methods due to the inability to extract all the available oil in the seeds. The sensitivity of black cumin to water stress, limiting seed yield, also negatively affects the fixed oil content. Studies suggest that the best values are obtained when the plant's water requirement is met at 100%, while conditions where 80% of the water requirement is met (water stress threshold of 80%) are recommended for quality parameters (Ghamarnia and Jalili, 2013). In black cumin, the fixed oil content is significantly influenced by cultural practices such as fertilization (Ghiyasi et al., 2017; Muhammad et al., 2017; Sağlık, 2020; Shah, 2007), different row spacings and plant distances (Gholinezhad and Abdolrahimi, 2014), sowing time (winter and summer sowing), irrigation (Ghamarnia and Jalili, 2013; Yiğitbaşı, 2019) and the method of oil extraction from seeds (Gharby, 2015; Sağlık, 2020), as determined by various studies.

3.2. Fixed oil yield (kg da^{-1})

The combined analysis revealed statistically significant differences at the 1% level between genotypes and the year x genotype interaction and at the 5% level between years in terms of fixed oil yield (*Table 5*). The highest fixed oil yield was obtained in 2019 and the combined analysis from genotype 4 with 23.1 kg da^{-1} and 17.5 kg da^{-1}, respectively. In 2020, genotype 16 yielded the highest with 18.1 kg da^{-1}. The lowest fixed oil yield was recorded for genotype 30 in 2019 (5.3 kg da^{-1}), genotype 2 in 2020 (6.3 kg da^{-1}), and genotype 21 in the combined analysis (8.9 kg da^{-1}). However, in the combined analysis, genotype 21 did not differ significantly from genotype 30 (9.0 kg da^{-1}). The average for the years was 13.2 kg da^{-1} in 2019 and 12.3 kg da^{-1} in 2020 (*Table 6*).

Table 5. Combined variance analysis result for the fixed oil yield data of black cumin genotypes in the experiment

Source of Variance	D.F. (Degree of	S.S. (Sum of	M.S.	F Value
Replication	4	77.23	19.31	1.94
Year	1	39.68	39.68	3.99*
Genotypes	30	812.09	27.07	2.72**
Year× Genotypes	30	1576.39	52.55	5.28**
Error	120	1194.40	9.95	
Sum	185	3699.79		
CV (Coefficient of Variation) (%)	24.51			

(**) Significant at the 0.01 level, (*) Significant at the 0.05 level

The findings regarding fixed oil yield values (5.3-23.1 kg da^{-1}) were similar to and higher than results reported by Kamçı (2019) for summer cultivation (6.1-23.9 kg da^{-1}), higher than the minimum and lower than the maximum values reported by Özdemirel and Kaçar (2021) (12.1-27.3 kg da^{-1}) and Ekren and Koç (2024) (20.9-26.0 kg da^{-1}) and lower than the results obtained by Yılmaz et al. (2020) (31.6-55.6 kg da^{-1}).

Oil yield is a crucial criterion for black cumin seeds, given the plant's utilization of both seeds and oil. It is influenced by genetic characteristics, climatic and environmental conditions, cultivation practices and oil production techniques. Climate-related issues, such as excessive rain, hail near harvest, contributed to low yields in the experiment, affecting oil yield consistently.

Table 6. Mean values and LSD groups for fixed oil yield (kg da^{-1}) values determined in the Nigella sativa L. genotypes included in the experiment

Genotypes	2019		2020		2019-2020	
1	20.3	ab	9.0	gı	14.6	af
2	17.2	bd	6.3	i	11.7	ch
3	16.5	bd	10.2	ei	13.3	bg
4	23.1	a	11.8	ch	17.5	a
5	10.1	fj	14.1	af	12.1	ch
6	14.3	cf	13.0	bg	13.6	bg
7	14.4	cf	15.4	ad	14.9	ad
8	17.0	bd	12.1	ch	14.5	af
9	18.5	ac	13.1	bg	15.8	ab
10	16.4	bd	14.1	af	15.2	ad
11	14.4	cf	8.0	hi	11.2	eh
12	15.6	be	10.9	di	13.2	bg
13	13.7	cg	9.8	fi	11.8	ch
14	12.4	dh	9.8	fi	11.1	fh
15	17.0	bd	12.3	ch	14.7	ae
16	7.9	hj	18.1	a	13.0	bg
17	14.6	cf	17.5	ab	16.1	ab
18	10.4	ej	12.0	ch	11.2	eh
19	8.0	hj	13.1	bg	10.6	gh
20	6.8	ij	14.6	af	10.7	gh
21	6.7	ij	11.1	ch	8.9	h
22	20.3	ab	10.3	ei	15.3	ac
23	11.7	di	14.8	ae	13.2	bg
24	12.9	dh	12.8	bh	12.8	bg
25	10.3	ej	15.8	ac	13.0	bg
26	15.7	be	10.4	ei	13.1	bg
27	9.9	fj	13.5	ag	11.7	dh
28	9.9	fj	10.4	ei	10.2	gh
29	8.4	gj	12.6	ch	10.5	gh
30	5.3	j	12.8	bh	9.0	h
Çameli	10.4	ej	12.1	ch	11.2	eh
Mean	13.2		12.3		12.8	
LSD	5.5		4.8		3.6	
CV (%)	25.3		24.0		24.5	

3.3. Fatty acids composition (%)

When examining the fatty acid composition of black cumin oil in both years of the study, the ratios were determined in the following order from highest to lowest: linoleic, oleic, palmitic and stearic acids, identified as the major fatty acid components. Other fatty acid components identified in black cumin for the year 2019 included caproic, behenic, arachidic, cis-11-eicosenoic, cis-11,14-eicosadienoic, linolenic, cis-4,7,10,13,16,19-docosahexaenoic, palmitoleic, heptadecanoic, cis-10-heptadecenoic and erucic acids (*Table 7*). For the year 2020, arachidic, cis-11-eicosenoic, cis-11,14-eicosadienoic, linolenic, cis-4,7,10,13,16,19-docosahexaenoic, nervonic, palmitoleic, tricosanoic, heptadecanoic, behenic and myristic acids were found (*Table 8*). The average values for oleic and linoleic acids over the years were determined as follows: in 2019, oleic acid was 23.2% and linoleic acid was 52.4%; in 2020, oleic acid was 24.8% and linoleic acid was 56.3%. Across both years, the average oleic and linoleic acid contents were 24.0% and 54.3%, respectively.

In the genotypes, the maximum values for the major components identified in the fatty acid composition were as follows: in 2019, oleic acid was 29.0% from genotype 11 (min. 20.5%), linoleic acid was 54.1% from genotype 21 (min. 46.0%), palmitic acid was 11.4% from genotype 23 and stearic acid was 4.6% from genotype 11; in 2020, oleic acid was 27.2% from genotype 26 (min. 24.0%), linoleic acid was 57.2% and 57.2% from Çameli variety and genotypes 14 and 28, respectively, (min. 54.2%), palmitic acid was 12.7% from genotype 27 and stearic acid was 3.4% from genotype 17. According to the average results of the two years, genotype 11 gave the highest values for oleic acid (27.2%, min. 22.5%) and genotype 14 had the highest linoleic acid ratio (55.6%, min. 51.0%). In

2019, the average composition was linoleic acid 52.37%, oleic acid 23.19%, palmitic acid 10.42%, and stearic acid 4.28%. In 2020, linoleic acid increased to 56.25% (+3.88 units), oleic acid to 24.83% (+1.64 units), and palmitic acid to 12.19% (+1.77 units), while stearic acid decreased to 3.20% (-1.08 units). These findings demonstrate that the composition varied significantly across years, with the genotypes being richer in linoleic acid in 2020 (*Table 9*). The findings indicate that both genetic factors (differences among genotypes) and environmental conditions (year-to-year variation) play critical roles in determining fatty acid composition. The values for trace components were similar for all genotypes in both years. When examining the fatty acid profile of the black cumin genotypes in the study, it was found that linoleic acid was the most abundant unsaturated fatty acid, followed by oleic, palmitic and stearic acids, respectively. The results obtained show some similarities and differences with previous studies. Overall, these differences depend on the techniques used to determine black cumin oil content, the sensitivity of the process (Ramadan and Mörsel, 2002), the genetic characteristics of the genotypes, the method of obtaining the seeds (wet-dry cultivation, irrigation time and frequency, harvest time, climate and soil characteristics (ecology in which it is grown), disease and pest status, storage conditions, etc.) and the method of oil extraction.

Table 7. Results of 2019 analysis of fatty acid composition (%) values in black cumin (Nigella sativa L.) genotypes

G	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	24.7	51.2	10.2	4.1	0.3	0.3	0.2	2.5							4.0
2	22.8	53.3	10.4	4.2	0.2	0.3	0.2	2.9							4.6
3	24.0	52.8	9.7	4.2	0.2	0.3	0.2	2.6	0.1	0.1					4.7
4	24.9	52.3	10.1	4.6	0.2	0.6		2.8							4.5
5	23.3	53.1	10.2	4.6	0.2	0.6		3.0							5.1
6	26.4	49.1	9.7	4.2	0.2	0.6		2.8			1.2				4.8
7	23.3	51.5	9.9	4.5	0.2	0.2	0.2	2.7			1.1				5.3
8	25.2	47.9	9.4	4.5	0.3	0.3	0.2	2.4		0.2	2.1	0.2	0.3		5.9
9	22.5	50.8	9.6	4.2	0.1	0.5	0.3	2.6		1.3	1.1	0.2	0.3		5.4
10	23.5	50.9	9.7	4.1	0.3	0.6	0.5	2.7		1.8	0.5	0.1	0.2	0.1	5.0
11	29.0	46.0	9.4	4.6	0.2	0.2	0.2	2.3			2.0	0.2	0.3	0.1	5.5
12	23.0	52.1	10.3	4.5	0.3	0.2	0.2	2.7			1.3	0.1	0.3		5.1
13	23.5	51.8	10.4	4.3	0.3	0.3	0.2	2.8			1.9		0.3		4.3
14	22.3	53.9	11.0	4.4	0.2	0.3	0.2	3.0			1.3				3.5
15	23.2	53.9	11.2	4.1	0.2	0.2	0.2	3.0			0.7	0.2			3.1
16	20.5	54.0	11.0	4.3	0.3	0.2	0.2	2.9			1.3	0.1	0.2		4.0
17	22.3	53.8	10.7	4.3	0.3	0.2	0.2	3.0			1.1	0.2	0.2	0.1	3.7
18	21.2	53.9	10.7	4.3	0.3	0.3	0.2	2.9			1.4	0.2			3.6
19	22.9	52.4	10.6	4.3	0.3	0.2	0.2	2.8			1.9	0.2	0.2		4.1
20	23.3	54.0	10.8	4.4		0.3	0.2	3.0			0.9				3.2
21	22.6	54.1	11.2	4.0	0.2			2.6			1.5	0.1	0.2		3.4
22	22.3	53.4	10.8	4.2	0.2	0.3	0.2	3.1			1.5	0.2	0.2		3.7
23	21.3	53.8	11.4	4.5	0.2	0.1	0.1	2.7			1.0				3.8
24	21.9	52.9	10.8	4.2	0.2	0.3	0.2	2.9			1.3	0.2	0.2		3.9
25	21.9	52.9	10.8	4.2	0.2	0.3	0.2	2.9			1.3	0.2	0.2		3.9
26	23.7	52.6	10.7	4.2	0.3	0.2	0.2	2.5			1.5	0.2	0.2		3.7
27	21.6	52.9	10.5	4.2	0.3	0.2	0.2	2.8			2.0	0.2	0.3		4.9
28	22.2	52.6	10.5	4.3	0.2	0.3	0.2	2.7			1.5		0.3		5.2
29	22.8	53.1	10.5	4.2	0.3	0.3	0.2	2.9			1.2	0.2	0.3		0.3
30	22.7	54.1	10.8	4.1	0.2	0.3	0.2	2.8			1.0	0.2	0.2		3.7
Ç	24.1	52.3	10.4	4.1	0.2	0.27	0.15	2.8			1.2	0.2	0.3		4.0

G: genotypes, **A:** oleic acid (C18:1n9c), **B:** linoleic acid (C18:2n6c), **C:** palmitic acid (C16:0), **D:** stearic acid (C18:0), **E:** arachidic acid (C20:0), **F:** cis-11-eicosenoic acid (C20:1), **G:** linolenic acid (C18:3n6), **H:** cis-11,14-eicosadienoic acid (C20:2), **I:** behenic acid (C22:0), **J:** erucic acid (C22:1n9), **K:** cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n3) DHA, **L:** palmitoleic acid (C16:1), **M:** cis-10-heptadecanoic acid (C17:1), **N:** heptadecanoic acid (C17:0), **O:** caproic acid (C6:0), **Ç:** Çameli

Tablo 8. Results of 2020 analysis of fatty acid composition (%) values in black cumin (Nigella sativa L.) genotypes

G	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	24.8	56.7	12.4	3.0	0.1	0.4		2.6			0.2				
2	24.9	55.8	12.5	3.2	0.2	0.5		2.8	0.4	0.3	0.2				
3	25.0	56.3	12.3	3.2	0.2	0.5		2.6			0.2				
4	24.7	55.9	12.5	3.2	0.2	0.5		2.9	0.3	0.2	0.2		0.1	0.2	
5	24.4	56.8	12.2	3.3	0.1	0.6		2.6							
6	24.7	56.2	12.1	3.2	0.2	0.5	0.3	3.1							
7	24.5	56.8	12.3	3.4	0.2	0.6		2.6							
8	24.2	56.5	12.5	3.3	0.2	0.6		2.7	0.3	0.2					
9	24.3	57.0	12.3	3.1	0.2	0.5	0.3	2.6		0.1	0.2	0.2			
10	25.8	55.3	12.4	3.3		0.5		2.7	0.2						
11	25.3	56.1	11.8	3.4	0.2	0.6		2.6			0.2	0.2			
12	24.6	56.4	12.3	3.3	0.2	0.5		2.5			0.2				
13	24.7	56.4	12.4	3.2	0.2	0.6		2.5			0.2				
14	24.0	57.2	12.4	3.2	0.2	0.5	0.3	2.5							
15	24.3	56.2	9.2	3.4	0.2	0.6		2.6	0.4	0.3	0.3				
16	24.5	56.3	12.4	3.3	0.3	0.5	0.3	2.6							
17	24.7	56.2	12.3	3.4	0.2	0.4	0.3	2.8			0.2				
18	25.5	54.7	12.1	3.4	0.2	0.6	0.3	2.7		2.4	0.1				
19	25.2	56.1	12.3	3.4	0.3	0.4		2.4			0.1				
20	24.4	56.7	12.3	3.4	0.2	0.3	0.2	2.6			0.2				
21	25.3	56.5	12.3	2.8	0.2	0.6		2.4	0.4	0.2	0.1				
22	26.1	55.1	12.6	3.1	0.2	0.4		2.3			0.1	0.4			
23	24.9	56.5	12.1	3.1	0.2	0.6		2.8			0.1				
24	24.8	56.8	12.1	3.1	0.2	0.5		2.5			0.1				
25	25.2	56.1	12.3	3.1		0.6		2.6			0.2	0.2			0.2
26	27.2	54.2	12.2	3.0	0.2	0.5		2.3	0.4		0.2				
27	24.0	56.4	12.7	3.2	0.2	0.6		2.7	0.3	0.2		0.2			
28	24.0	57.2	12.3	2.9	0.2	0.5		2.6	0.3	0.2	0.2				
29	24.6	56.2	12.4	3.2	0.2	0.4		2.7		0.1	0.2	0.3			
30	24.5	56.3	12.3	3.3	0.2	0.4		2.8			0.2				
Ç	25.1	57.2	12.0	3.0	0.2	0.5	0.3	2.9	0.4	0.3	0.1	0.4	0.2	0.2	

G: genotypes, **A:** oleic acid (C18:1n9c), **B:** linoleic acid (C18:2n6c), **C:** palmitic acid (C16:0), **D:** stearic acid (C18:0), **E:** arachidic acid (C20:0), **F:** cis-11-eicosenoic acid (C20:1), **G:** linolenic acid (C18:3n6), **H:** cis-11,14-eicosadienoic acid (C20:2), **I:** cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n3) DHA, **J:** nervonic acid (C24:1), **K:** palmitoleic acid (C16:1), **L:** tricosanoik acid (C23:0), **M:** heptadecanoic acid (C17:0), **N:** behenic acid (C22:0), **O:** miristic acid (C14:0), **Ç:** Çameli

Table 9. Descriptive statistics of major acids

Fatty Acid	2019				2020				Mean Difference (2019-2020)
	Mean	**Std.**	**Min.**	**Max.**	**Mean**	**Std.**	**Min.**	**Max.**	
LA	52.37	1.87	45.99	54.1	56.25	0.69	54.2	57.23	+3.88
OA	23.19	1.64	20.45	29.03	24.83	0.67	23.95	27.22	+1.64
PA	10.42	0.54	9.38	11.4	12.19	0.58	9.22	12.67	+1.77
SA	4.28	0.16	4.04	4.61	3.2	0.16	2.83	3.44	-1.08

LA: linoleic acid**, OA:** oleic acid**, PA:** palmitic acid**, SA:** stearic acid

According to research results conducted by various researchers in different ecologies, the major fatty acid components and their percentages in black seed oil are as follows; Nickavar et al. (2003) linoleic acid 55.6%, oleic acid 23.4%, palmitic acid 12.5%; Al-Naqeeb et al. (2009) linoleic acid 57.0%, oleic acid 20.6%, palmitic acid 11.2%; Amin et al. (2010) linoleic acid 50.2%, oleic acid 19.9%, margaric acid 10.3%, stearic acid 2.5%; Toma et al. (2013) linoleic acid 63.7%, oleic acid 19.4%, palmitic acid 8.9%; Palabıyık and Aytaç (2018) linoleic acid 39.2-43.7%, oleic acid 33.4-37.8%; Thilakarathna et al. (2018) linoleic acid 50.2-61.3%, oleic acid 17.6-19.1%; Kamçı (2019) linoleic acid 52.5-55.5%, oleic acid 20.6-28.3%, palmitic acid 12.2-12.6%, miristic acid 0.2-3.2%; Bozdemir et al. (2022) linoleic acid 45.5-55.6%, oleic acid 18.8-25.0%, palmitic acid 7.0-11.7%, stearic acid 3.5-4.8%; Ekren and Koç (2024) linoleic acid 22.79-23.93%, oleic acid 52.77-54.69%, palmitic acid 12.56-13.41%.

In the study conducted by Yiğitbaşı (2019), the highest amount of oleic acid was obtained from plants grown under the highest irrigated conditions (25.4%) and the lowest under dry conditions (23.4%). The highest amount of linoleic acid was obtained under dry conditions (54.5%) and the lowest under irrigated conditions (50%).

Among saturated fatty acids, the highest amount of palmitic acid was obtained from plants grown under irrigated conditions (15.1%) and the lowest was obtained under dry conditions (13.0%).

Kamçı (2019) reported that the ratios of fatty acid composition were influenced by irrigation practices at different growth stages of the plant. Sağlık (2020) stated that different fertilizer applications affected the amounts of oleic, linoleic, palmitic and stearic acids, with the least obtained from worm compost and the highest from nitrogen, phosphorus, potassium (NPK) application. Gharby et al. (2015) found that the composition of oil in Moroccan black cumin is similar to studies conducted in the Mediterranean countries. The highest ratio of linoleic acid was found to be 58.5% and 56.5% for cold press and solvent extraction, respectively. Oleic acid was determined to be 25% and 24% and palmitic acid was 12% and 13%, respectively, based on the extraction methods. The averages of the main fatty acid components obtained in the research showed similar results over the years. In 2020, the increase in linoleic and palmitic acids, along with the decrease in stearic acid, enhanced the nutritional quality of the oil, while the rise in oleic acid contributed positively to oxidative stability. For breeding purposes, genotypes with higher linoleic acid content may be prioritized for nutritional polyunsaturated fatty acids (PUFAs) enrichment, whereas those with higher oleic acid content may be targeted for improved stability and processing quality.

3.4. Essential oil ratio (%)

The results for the volatile oil content (%) values determined in the genotypes of black cumin (*Nigella sativa* L.) for the year 2019 are presented in *Table 10*. The volatile oil content varied between 0.01% and 0.08% in the black cumin genotypes included in the trial. The highest volatile oil content was observed in genotype 24, while the lowest volatile oil content was found in genotypes 8, 9, 10, 12, 17, 19, and in the Çameli variety.

In various studies on *Nigella sativa* L. conducted in different ecologies, the volatile oil content has been reported as follows: Akgül (1993) found it to be between 0.5-0.7%, Türker and Bayrak (1997) reported 0.1-0.4%, Geren et al. (1997) found it to be 0.67-0.68%, D'Antuono et al. (2002) reported 0.3-0.5%, Rchid et al. (2004) found it to be 0.3%, Kıralan (2006) reported 0.5%, Ramadan (2007) mentioned 0.5–1.6%, Yiğitbaşı (2019) reported an average of 0.18% under irrigated conditions, 0.16% under rainfed conditions, and an average of 0.17% depending on the sowing time, Gülhan and Taner (2020) reported it to be in the range of 0.2-0.5%, while Durmaz and Kara (2023) reported a range of 0.41-0.71%. The results obtained in the research for the year 2019 were lower than these values, higher than the lower limit (0.01-0.50) detected by Nickavar et al. (2003).

In various studies aiming to determine the effective applications on the volatile oil content, it has been observed that the application of phosphorus fertilizer does not significantly affect the volatile oil content (Geren et al., 1997). However, nitrogen has a positive effect on the volatile oil content of the *Nigella sativa* L. plant and the average volatile oil content increases up to a certain level with increasing nitrogen dosage (Shah, 2007). On the other hand, increasing row spacing has a positive effect on the volatile oil content (El Deen and Ahmed, 1997) and shortening the vegetation period leads to reductions in volatile oil quantities (D'Antuono et al., 2002). It has been observed that planting in different seasons and under dry or irrigated conditions does not have a statistically significant effect on volatile oil yields but the interaction is significant (Yiğitbaşı, 2019).

Ceylan (1997) reported that the effective substances in medicinal plants, such as volatile oils and alkaloids, vary according to morphogenetic, ontogenetic, and diurnal variability, which are referred to as climate, environmental, topographic conditions, genetic structure, and individual variability. The reason for the difference between the results obtained and the literature in the research can be explained by the presence of *Nigella sativa seeds* with different genetic characteristics, cultivation conditions, cultural practices, and the method used to extract the volatile oil. The variations in results compared to the literature were attributed to differences in genetic characteristics, growing conditions, cultural practices, and essential oil extraction methods.

Table 10. Analysis results for the volatile oil (%) values in black cumin (Nigella sativa L.) genotypes in the year 2019

Genotypes	Fixed Amount of Oil Taken to	Amount of Essential Oil Obtained After
1	0.5005	0.0007
2	0.2513	0.0003
3	0.5001	0.0006
4	0.5003	0.0003
5	0.5004	0.0006
6	0.5001	0.0004
7	0.5005	0.0002
8	0.5009	0.0001
9	0.5003	0.0001
10	0.5005	0.0001
11	0.2517	0.0003
12	0.5008	0.0001
13	0.5009	0.0003
14	0.5001	0.0004
15	0.5003	0.0003
16	0.5007	0.0003
17	0.5000	0.0001
18	0.5001	0.0002
19	0.5006	0.0001
20	0.5001	0.0004
21	0.5003	0.0003
22	0.5000	0.0006
23	0.5008	0.0003
24	0.5003	0.0008
25	0.5004	0.0004
26	0.5003	0.0006
27	0.5010	0.0005
28	0.5003	0.0003
29	0.5030	0.0002
30	0.2515	0.0002
Çameli	0.2514	0.0001

3.5. Essential oil components (%)

The present study reports the analysis of volatile oil components in various genotypes of *Nigella sativa* L. (black cumin) identified in the year 2019. The results pertaining to the percentage composition of these volatile oil components are presented in *Table 11*, while the quantities of thymoquinone in milligrams are detailed in *Table 12*. The identified components collectively constitute 78.7% to 98.2% of the total volatile oil. Thymoquinone emerges as the primary component, ranging from 54.4% to 83.1%, with p-Cymene (1.0-16.4%), cis-4-Methoxy thujan (1.8-5.8%) and longifolene (0.5-8.5%) following in prominence after thymoquinone. Other components include limonene (0.5-6.9%), γ-Terpinene (0.5-0.6%), trans-4-methoxy thujan (0.5-0.8%), terpinen-4-ol (0.5-0.9%), α-longipinene (0.6-1.2%), longipinene (0.5-5.7%), (E)-2-heptanal (0.5-2.7%), sandracopimaradiene (0.5-1.7%), palmitic acid (0.9-4.4%), linoleic acid (1.7-11.4%), (E,E)-2,4-decadienal (0.5-11.2%), and carvacrol (1.3-3.3%).

In addition to the common volatile oil components listed in *Table 9*, genotype 1 exhibits octane (13.1%), hexanal (1.2%), α-pinene (0.9%), sabinene (0.5%), β-pinene (0.8%), myrcene (0.9%), α-phellandrene (0.5%), 1.8-cineole (2.6%) and linalool (0.6%). Genotype 2 shows ent-pimara-8(14)-15-dien (0.9%), genotype 3 has hexanal (0.8%), genotype 21 contains α-thujene (0.7%) and α-pinene (0.5%), genotype 23 has α-thujene (0.5%) and α-pinene (0.7%), genotype 24 features α-pinene (0.5%), genotype 29 contains 1-octen-3-one (0.5%) and genotype 30 reveals amyl furan (0.9%) and (E,Z)-2,4-decadienal (3.9%).

The main component of the volatile oil has been identified differently by various researchers; Türker and Bayrak (1997) identified limonene; Geren et al. (1997), D'Antuono et al. (2002), Rchid et al. (2004), Yiğitbaşı

(2019), Kabir et al. (2020) identified p-cymene; Palabıyık and Aytaç (2018) identified thymoquinone; Nickavar et al. (2003) identified trans-anethole (38.3%); and Kıralan (2006) identified linalool.

When comparing the thymoquinone ratio obtained in our study, where the main component of the volatile oil was identified as thymoquinone, with other research results that also identified thymoquinone as the main component, it is observed that our results fall higher than the lower limit but lower than the upper limit of Palabıyık and Aytaç's (2018) results (67.7%) which ranged from 54.4 to 83.1%, but higher than results obtained by Farhan et al. (2021) (25.4%) and Ahmed et al. (2024) (25%). In contrast, our findings are considerably lower than the results obtained in all other studies. Another component of the volatile oil, p-cymene, was measured between 1.0 and 16.4% in our study. This is lower than the findings of Akgül (1993) (31.7%), Türker and Bayrak (1997) (1.8-28.5%), Geren et al. (1997) (52.2-69.0%), D'Antuono et al. (2002) (33.8%), Rchid et al. (2004) (47.4%), Yiğitbaşı (2019) (22.7-25%), and Kabir et al. (2020) (36.35-41.80%), but higher than Nickavar et al.'s (2003) result of 14.8%.

Table 11. Results of 2019 analysis of essential oil components (%) values in black cumin (Nigella sativa L.) genotypes

Genotypes	S	L	γ-T	MT	TMT	TO	T	LF	α-L	LP	*E*-H	S	PA	LA	*E*-D	K	Total
1	6.1	6.9	0.6	4.0		0.7	58.3	2.1									78.7
2	2.4			2.4		0.6	83.1	8.5	1.2								98.2
3	1.6			1.8		0.5	77.0	4.2	0.6		0.9	0.6	3.7	5.0			95.9
4	1.0			2.3		0.8	80.2	5.6	1.0				2.3	3.8			97.0
5	1.4			2.8		0.9	80.2	6.2	1.2				1.1	2.4			96.2
6	3.4			4.1		0.9	78.9	3.8	0.8				1.6	2.7			96.2
7	2.8			3.4		0.8	70.3	3.1	0.6		2.1	0.7	4.0	7.0	0.6		95.4
8	3.5		0.6	3.8	0.5	0.8	69.4	2.7	0.6		2.7	0.6	4.4	6.6	0.5		96.7
9	2.2			2.6		0.7	60.0	6.5	1.1		1.4	0.7	2.5	9.4	0.6		87.7
10	2.9			3.9	0.5	0.9	70.9	4.7	0.9		0.7	0.5	1.5	3.8			91.2
11	10.4	0.5		5.8	0.8	0.8	54.4	2.9			1.0	0.5	3.0	11.4	0.6	1.3	93.4
12	6.9			2.8		0.5	61.2	3.6			1.7	0.7	3.1	6.2	0.5	2.5	89.7
13	6.0			2.8		0.6	65.0	3.6			1.7	0.6	2.4	4.9	0.7	2.9	91.2
14	10.5	0.5	0.5	4.0	0.6	0.7	64.0	3.5		0.6	0.9	0.5	1.3	3.6		2.7	93.9
15	11.9	0.7	0.5	4.6	0.6	0.8	64.4	2.6		0.5	1.0		1.0	2.1		2.3	93.0
16	8.7	0.5	0.5	3.9	0.5	0.8	66.1	2.7		0.5	1.4		1.3	4.8		2.5	94.2
17	11.7	0.6	0.6	4.1	0.6	0.6	62.7	3.4		0.6	1.2	0.5	1.9	3.0		2.4	93.9
18	10.0	0.7	0.5	4.0	0.6	0.7	66.7	3.3		0.6	1.2		1.2	1.8		2.8	94.1
19	12.8	0.6		4.9	0.7	0.8	62.2	0.5		3.3	1.3		1.2	2.4	0.5	2.1	93.3
20	7.7			4.1	0.5	0.8	69.2	4.4		0.8	0.8		0.9	1.7		2.3	93.2
21	16.4	0.8		4.8	0.7	0.7	66.5	3.4		0.6						1.9	95.8
22	5.9			3.3	0.5	0.8	75.1			5.7						3.3	94.6
23	9.8	0.5		3.4	0.5	0.6	72.8	3.8		0.5						2.0	93.9
24	8.0			4.2	0.5	0.8	73.7	4.1		0.8						2.3	94.4
25	8.5			4.6	0.6	0.9	73.4	3.7		0.7						2.4	94.8
26	10.6	0.5		4.8	0.6	0.9	73.1	2.8								2.1	95.4
27	10.6	0.5		4.6	0.6	0.8	68.3	4.9		0.8	0.5					2.7	94.3
28	10.5	0.5		4.6	0.6	0.7	69.0	4.9		0.9	0.8		0.5			1.8	94.8
29	3.3			2.3		0.5	77.5	4.9		0.7	1.2	0.9			0.8	2.1	94.2
30	4.4			2.8		0.6	59.3	5.4		0.8	2.0	1.7			11.2	1.7	89.9
Çameli	2.8			2.0		0.6	76.5	7.7		1.0		1.1			0.6	3.1	95.4

S: *p*-cymene**, L:** Limonene**, γ-T:** γ-Terpinene**, MT:** *cis*-4-methoxy thujane**, TMT:** T*rans*-4- methoxy thujane**, TO:** Terpinen-4-ol**, T:** Thymoquinone**, LF:** Longifolene**, α-L:** α-Longipinene**, LP:** Longipinene**, *E*-H:** (*E*)-2-heptanal**, S:** Sandracopimaradiene**, PA:** Palmitic acid**, LA:** Linoleic acid**, *E*-D:** (*E,E*)-2,4-Decadienal**, K: C**arvacrol

Nigella sativa L. oil is one of the substances that contribute significantly to preventing oxidation in foods and repairing potential cell damage in the human body (Bulca, 2014). In the volatile oil composition of the seed, numerous components containing high levels of omega fatty acids, primarily responsible for antioxidant activities, are found. However, the key bioactive component responsible for many pharmacological effects has been identified as thymoquinone, with a molecular weight of 164.2 g/mol ($C_{10}H_{12}O_2$; 2-isopropyl-5-methylbenzo-1,4-quinone) (Ramadan, 2007; Salem, 2005). In vitro and in vivo studies have demonstrated that thymoquinone exhibits a broad range of effects, strengthening the immune system, and showing activities such as antihistaminic, antifungal, antidiabetic, antihypertensive, antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic properties.

The selection of the appropriate extraction method for black cumin is also a crucial issue (Rooney and Ryan, 2005). It has been observed that applying the cold-press method instead of heat treatment or solvent use in the production of black seed oil results in a more natural and high-quality oil with enhanced nutritional and antioxidant properties (Kıralan et al., 2014; Maghoub et al., 2013). Some studies have determined that the volatile and fixed oil of black seed obtained through the cold-press method exhibit different pharmacological properties and component compositions compared to oils obtained by other methods (Kıralan et al., 2014; Lutterodt et al., 2010; Ramadan, 2007). However, it has been identified that the oil richest in thymoquinone is obtained through the microwave extraction method and can be used as a natural source of thymoquinone (Kıralan et al., 2014). Furthermore, the quality of the oil and the mechanism of action of its components vary significantly depending on how the plant is cultivated. According to Gordon et al. (2001), antioxidants help maintain the quality of the product and eliminate oxidation problems in foods when used in conjunction with good quality raw materials, appropriate production techniques, and packaging and storage methods. This emphasizes the importance of cultivating the plant through good agricultural practices.

The chemical composition of black seed varies according to factors such as the planting and harvesting time, variety, climate, and region where the plant is cultivated (Al-Jassir, M.S. (1992). In a related study, Yiğitbaşı (2019) found the highest thymoquinone content in volatile oil under aqueous conditions (16.54-17.98%) and the lowest under dry conditions (14.33-16.75%).

Table 12. Results of 2019 analysis of thymoquinone (mg) values for different genotypes of Nigella sativa L.

G	% Thymoquinone (mg thymoquinone/100 mg oil)	G	% Thymoquinone (mg thymoquinone/100 mg oil)
1	0.050	17	0.030
2	0.081	18	0.062
3	0.052	19	0.058
4	0.173	20	0.150
5	0.248	21	0.052
6	0.135	22	0.261
7	0.016	23	0.080
8	0.021	24	0.156
9	0.039	25	0.237
10	0.094	26	0.192
11	0.048	27	0.119
12	0.022	28	0.102
13	0.038	29	0.015
14	0.061	30	- (not detected)
15	0.051	Ç	0.045
16	0.085		

4. Conclusions

This study was conducted on advanced black cumin lines belonging to the breeding program of the Central Research Institute of Field Crops under Ankara conditions. Today's world drug trade encourages countries to work for good agricultural practices and organic farming for quality and reliable raw materials. Products grown in this way are considerably more valuable in the market compared to conventional products. Therefore, no fertilizers or pesticides were used in any year of the breeding program. The local material used in the research has different genotypic richness, collected from various geographical locations where black cumin farming is carried out in Türkiye. The material differs from previous studies in terms of material diversity and agricultural history.

In the research findings, genotypes 26 (USDA-Egypt), 10 (Burdur-3), 9 (Denizli-3), 27 (Burdur-5), and 3 (Burdur-1) were highlighted for their fixed oil content, while genotypes 4 (Burdur-2), 17 (Stock Market-1), 9 (Denizli-3), 22 (Bursa), and 10 (Burdur-3) stood out in terms of fixed oil yield. The primary constituents of fixed oil were identified as linoleic, oleic, palmitic, and stearic acids, while the major components of volatile oil were found to be thymoquinone, p-cymene, cis-4-methoxy thujan and longifolene.

Based on the one-year volatile oil results, genotypes 2 (Denizli-2)-4 (Burdur-2)-5 (Ethiopia)-6 (Syria-1)-29

(Ankara-1) were notable in terms of thymoquinone proportion, while genotypes 4 (Burdur-2)-5 (Ethiopia)-6 (Syria-1)-20 (Stock Market-4)-22 (Bursa)-24 (Afyonkarahisar)-25 (Pakistan)-26 (Egypt)-27 (Burdur-5)-28 (Kırıkkale) were prominent in milligrams.

As a result of the evaluation of seed yield, phenological, morphological observation and measurement results together with the quality results in the study, Denizli line number 9 was recommended as a commercial variety. In 2024, it was officially registered as a variety named Yusuf1941 by the Ministry of Agriculture and Forestry, Variety Registration and Certification Center.This study revealed a substantial variation among trial materials, particularly in terms of quality, encompassing fixed and volatile oil percentages and their component ratios. This variability can be attributed to genetic differences, climatic factors, and the method employed for obtaining fixed and volatile oils from black cumin seeds. However, the noteworthy level of fixed oil and its components underscores the necessity of popularizing such production models. The ratio of fixed and volatile oils and their components in black cumin, especially the quantity of thymoquinone as the crucial bioactive compound in genotypes, suggests promising prospects for the region where the study was conducted. Investigating the impact of climate and organic/good agricultural practices on this component is essential, emphasizing the need to replicate this study in subsequent years.

The results indicate that *Nigella* spp. seed oil, obtained through good agricultural practices, has the potential to be a versatile product for industrial, cosmetic, and pharmaceutical applications. Furthermore, the strong emphasis on continuing breeding studies with prominent lines is evident from these findings. In future studies, it is important to make crosses on lines that stand out in terms of oil (thymoquinone, fixed oil ratio, components) and seed yield, and to develop separate black cumin variety candidates suitable for the purpose as spice (high seed yield) or oil (high-quality values). In addition, studies should be carried out on the diseases that occur in black cumin (especially fungal diseases) due to climatic variations, the determination of different extraction methods suitable for the purpose and the association of all results with genetic characterization. In the future, more emphasis should be placed on biotechnological studies aimed at increasing this ratio, especially in lines where there is hope for thymoquinone. For lines with superior quality features to be used as medicine, advanced research stages should be carried out to determine different activities such as antioxidant, antimicrobial, antidiabetic, antiviral and anticancer.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Bozdemir, Ç., Uranbey, S.; Design: Bozdemir, Ç.; Data Collection or Processing: Bozdemir, Ç.; Statistical Analyses: Bozdemir, Ç.; Literature Search: Bozdemir, Ç.; Writing, Review and Editing: Bozdemir, Ç., Uranbey, S.

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