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Yield and Quality Traits of Black Cumin (*Nigella sativa* L.) Genotypes in Response to the Different Sowing Dates

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ABSTRACT

Black cumin has been used in many countries for the treatment of diseases such as cancer and diabetes, and for thousands of years as a spice, flavoring in products such as bread, and as a food preservative in pickles. Too much delay in the sowing of black cumin has a negative effect on plant growth. In order to determine the most suitable sowing dates for different black cumin genotypes, an experiment was conducted in the open-field conditions of the Eastern Mediterranean region of Türkiye at Çukurova University, for two years, in 2020 and 2021, in three different sowing dates (October 15th, November 01st, and November 15th) with three different black cumin genotypes (Çameli cultivar (G1), Adana population (G2) and Iraq population (G3)). The findings of this research demonstrated significant differences in the agronomic characteristics and overall quality of black cumin. The main components were p-cymene (51.45%-66.33%), trans-4-Methoxythujane (8.40%-11.90%), thymoquinone (0.11%-19.26%), γ-Terpinene (1.28%-9.09%), and limonene (2.93%-4.50%). The main fatty acids were determined as linoleic acid (53.97%-57.56%), oleic acid (20.98-26.40), and palmitic acid (13.73%-15.02%). Consequently, the low number of flowers and the high temperatures observed in May, along with the early spring frosts, negatively affected the fertilization of the flowers. The seed yield was adversely affected because some of the seeds could not mature.

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Introduction

Black cumin (*Nigella sativa* L.) is an annual plant from *Ranunculaceae* family, native to the eastern Mediterranean, northern Africa, the Indian subcontinent, and Southwest Asia (Hannan et al., 2021). Black cumin is also known as black seed, small fennel, Roman coriander, black caraway, and black sesame (Hossain et al., 2021). The name Nigella derives from the Latin word niger, which means "black" (Sadiq et al., 2021). Although the Nigella genus contains 12 species in the Turkish flora, only *N. sativa* and *N. damascena* are cultivated in different regions of Türkiye (Can et al., 2021).

From ancient times to the present black cumin has been included in many beliefs, religions, and holy books due to its medicinal properties. Archaeological evidence for the earliest cultivation of black cumin is insufficient, but studies are reporting that black cumin seeds have been found at various sites in Egypt, including Tutankhamun's tomb (Hannan et al., 2021). Black cumin seeds as a whole, with their extracted forms and fatty and essential oils, have been used as a preventive and therapeutic in many diseases, as well as in cosmetics (Majeed et al., 2021). Today, black cumin seeds are used as herbal medicine in developed countries to treat diseases such as fever, skin diseases, cough, rheumatism, jaundice, headache, paralysis, eczema, and loss of appetite (Haque et al., 2022). For thousands of years, black cumin seeds have been used as spice, and have also been added to coffee, tea, wine, and vinegar (Burdock, 2022).

Black cumin seeds possess significant economic worth and comprise essential oil and fatty oil (Ozyazici, 2020). Its essential oil is yellowish-brown and has a sharp bitter taste, and can be used as a flavouring additive and functional food (Sabriu-Haxhijaha et al., 2020). Furthermore, many studies reported that black cumin has been found that the medicinal value of black cumin is because of the presence of the quinone component, phenolics, and alkaloids (Ojueromi et al., 2022). Therefore, black cumin oil is used for anticancer (Malik et al., 2021), anti-inflammatory (Noël Nyemb et al., 2022), antioxidant (Hwang et al., 2021), antibacterial (Iqbal et al., 2021), antidiabetic (Akhtar et al., 2020) and antimicrobial (Alshwyeh et al., 2022) activities.

Global climate change is a multifactorial stress that has increased in recent years and exposing plants to extreme climatic conditions such as drought, salinity, and flooding, which adversely affect plant growth and developmental processes. In the coming years, an increase in temperature and a decrease in precipitation are expected in Türkiye and especially in the Mediterranean region due to the effects of global warming (Mairech et al., 2021). It is estimated that the annual warming rate in the Mediterranean region will be 20% higher than the global annual average in the coming years (Caretta et al., 2022).

Stress factors such as high temperatures due to global climate change are the major cause of plant failure and yield reduction of more than 50% (Mahajan et al., 2020). Also, winter plants are sensitive to high temperatures during their breeding phase (Moniruzzaman et al., 2015). In Türkiye, black cumin grows with an average temperature of 16.6°C, and monthly precipitation of 122.9 mm (Cahyo et al., 2020). Researchers are working to identify appropriate management options to maintain crop productivity under climate change scenarios and to understand its impact on growth and yield (Kalra et al., 2008). One of the most important factors is the choice of the appropriate sowing dates for agriculture.

The correct sowing date is very important for the successful production of a crop. Sowing date is of great importance for plant growth and controls phenological development that affects seed production (Sharangi and Roychowdhury, 2014). Late sowing shortens the vegetative period of the plants, causing the plant to complete its life cycle in a shorter time and have a short plant height, while early sowing causes higher plant height, especially with a longer vegetative period (Mehmood et al., 2018). Therefore, the benefit of correct timing of sowing is incomparable to any other agronomic management. Achieving high yield values in black cumin, which is cultivated in our country and has economic importance, depends on meeting the temperature and water requirements during planting at the right time. Therefore, the objective of our study was to evaluate the yield and quality characteristics of some black cumin genotypes at different sowing dates under Çukurova conditions.

Material and Methods

Plant Material and Growth Environment

The field study was conducted under field conditions of the eastern Mediterranean Region in Türkiye at Çukurova University in Adana (37°00′55.30″ N, 35°21′26.30″ E) for two years, both in 2020 and 2021, in three different sowing dates (October 15th, November 01st, and November 15th) with three different black cumin (*Nigella sativa* L.) genotypes (Çameli cultivar (G1), Adana population (G2) and Iraq population (G3)) (Figure 1). Seeds of the Çameli cultivar were obtained from the Transitional Zone Agricultural Research Institute, the Adana population from a local producer, and Iraq population from a local grower in Duhok. The climate is typically Mediterranean, with hot, dry summers and temperate, rainy winters (Figure 2).

The soil at the location was clay-loam in texture, with a very low organic matter content (1.10%). The soil was plowed to a depth of 30–40 centimeters and then cultivated with a field cultivator. As plant fertilizers, N and P_2O_5 were applied to the plots at a rate of 25 kg ha^{-1} as diammonium phosphate (DAP) (18–46–0). The field experiment was designed with 3 replicates based on a split-plot design. The size of each plot was 3.6 m2 (3 × 1.2 m) and each subplot occurred in four rows. The seeds of each genotype were sown at a depth of 1-2 cm on October 15th, November 01st, and November 15th, respectively. After the seeds were sown, sprinkler irrigation was applied, and in the absence of rainfall, irrigation was done as needed.

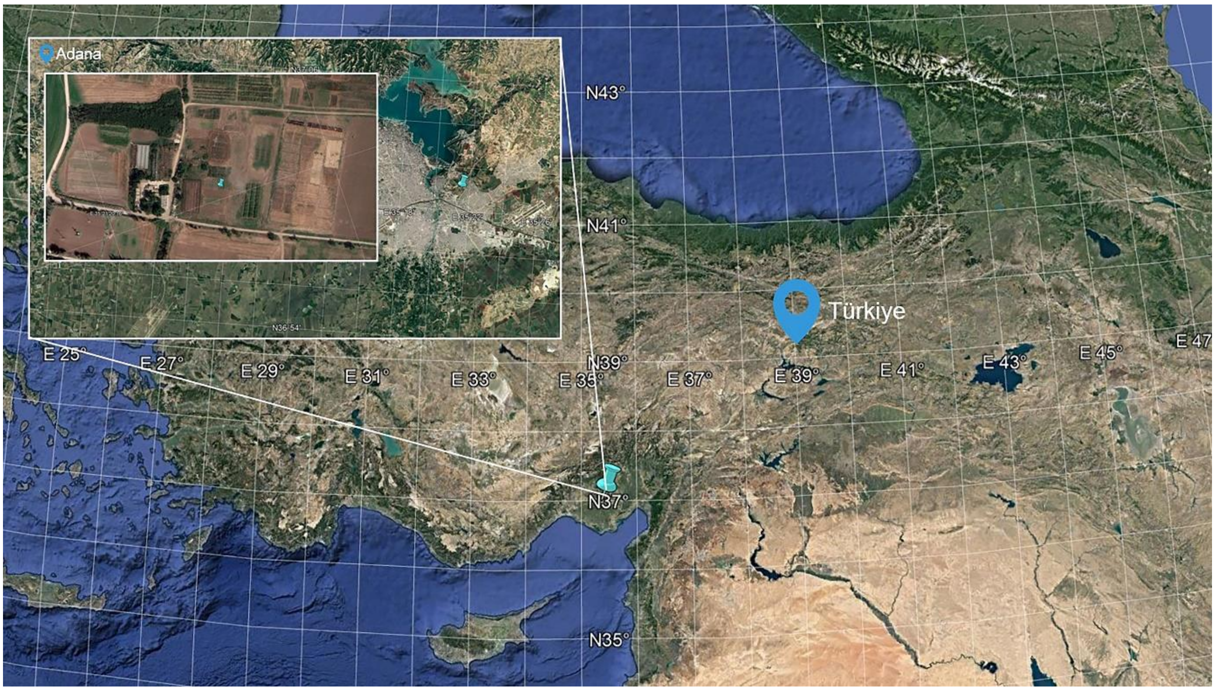

Figure 1. Çukurova University Field Crops Research and Application Area (37°00′55.30″ N, 35°21′26.30″ E)

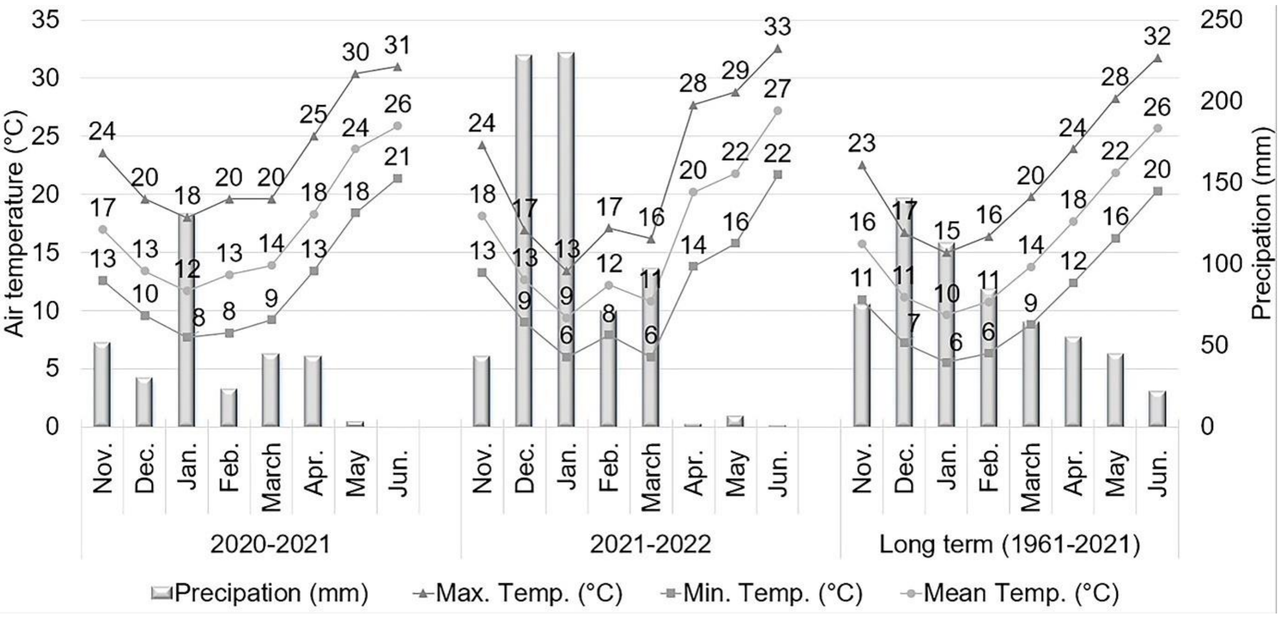

Figure 2. Meteorological data recorded during the field experiment and over the long term (1961–2021). The data were derived from the Meteorology Directorate of Adana, Türkiye

A hoe was used for weed control. During the experiment, no pesticide was applied. In each plot, ten plants were randomly chosen for analysis of agronomic and quality traits. Harvesting was done when the pods began to turn brown and the seeds began to turn black at the beginning of June. In this research, plant height-PH (cm), number of branches-NOB (pcs plant^{-1}), number of capsules-NOC (pcs plant^{-1}), number of seeds-NOS (pcs capsules-1), thousand seed weight-TSW (g), seed yield-SY (kg ha^{-1}), essential oil content-EOC (%), seed fatty oil content-SOC (%), and seed oil yield-SOY (kg ha^{-1}) were investigated as agronomic traits.

Essential oil Extraction

Black cumin seeds were ground, 30 g dried and ground seed samples from each treatment were placed in a glass balloon with 300 ml of distilled water. The Clevenger apparatus was operated for 3 hours, then the amount of extracted sample value was recorded (in mL). The essential oil content was stated as the weight of dry tissue. Chemical analyses were performed three times for each sample.

Seed oil Extraction

Black cumin seeds were ground, and 117 ml of n-hexane was added to 5 g sample, the seed oil was extracted using an ultrasonic bath at 55°C for 45 min, and then the n-hexane was evaporated from the extract at 70°C in a rotary evaporator and the remaining oil was weighed (Barut et al., 2023).

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

GC-MS analyses were carried out in the Department of Biology at Kahramanmaras Sutcu Imam University. Essential oil components, seed oils methyl-esterification, and fatty acid composition protocol were performed as specified in previous studies (Barut et al., 2021; Barut et al., 2022).

Statistical Analysis

Agronomic traits were analyzed based on the split-plot design JMP 14.0 (SAS Institute Inc., Cary, NC, 1989–2019). Sowing dates and the genotypes were organized as the main plot and subplot, respectively. PCAbiplot was performed with the same software. Flourish studio was used to create the heat map. The Corrplot Package of R Studio software was utilized to calculate correlations.

Results and Discussion

Agronomic Traits

To investigate the effects of different sowing dates on different black cumin genotypes, analysis of variance and LSD test were performed on 9 agronomic traits that were recorded (Table 1). The plant agronomic traits studied revealed many variations among the evaluated black cumin genotypes. In our study, three black cumin genotypes were exposed to different sowing dates and statistically significant changes were observed in the studied traits.

There were significant differences in years and genotypes in terms of PH values. When Table 1 is examined, it is observed that 12% higher PH value (53.8 cm) was obtained in the 1st year compared to the PH in the 2nd year (48.0 cm). The higher PH observed in the 1st year compared to the 2nd year is thought to be due to the low temperatures that occurred in January, February, and March 2022. While the average minimum temperature in January 2021 was 8°C, it was lower in 2022 at 5.9°C. Plants that developed earlier in the 2nd year and were taller in the rosette stage were damaged at low temperatures below 0°C (-1.4°C) in January but recovered later on, but this had a negative effect on PH, and 1st year produced taller plants. In terms of genotype, the highest PH was found in the G1 genotype, 85% and 50% higher than the G2 (37.4 cm) and G3 (45.9 cm) genotypes, respectively. The higher PH observed in the G1 genotype compared to the G2 and G3 genotypes is due to the fact that it is a commercially registered cultivar improved through plant breeding.

Table 1. ANOVA and mean comparisons of the yield and yield components of black cumin based on years, the sowing dates, and genotypes.

Factor		PH	NB	NC	NS	TSW	SY	EOC	SFOC	SFOY
2021		53.8a	5.6	20.0	83.6b	3.1a	550.6b	0.4	27.6a	152.0b
2022		48.0b	6.1	18.2	92.9a	2.9b	1069.8a	0.4	24.5b	262.1a
S1		53.8	6.0	19.4	82.4b	2.9	780.0	0.4	25.4	198.1
S2		48.6	5.7	18.0	83.7b	3.0	727.8	0.4	26.2	190.7
S3		50.3	6.0	19.8	98.8a	3.1	922.9	0.4	26.7	246.4
G1		69.3a	7.0a	19.7ab	109.8a	2.7b	978.1a	0.9a	26.9a	263.1a
G2		37.4c	4.7c	15.8b	71.2c	3.1a	538.1b	0.1b	26.8a	144.2b
G3		45.9b	5.9b	21.7a	83.9b	3.1a	914.5a	0.2b	24.5b	224.1a
S1	G1	75.0	7.1	18.3	98.0	2.7	699.9b-e	0.9a	26.4	184.8
	G2	37.2	4.1	13.7	68.3	3.0	451.0de	0.1cd	25.9	116.8
	G3	49.2	6.7	26.3	81.0	3.1	1189.0a	0.1d	23.8	283.0
S2	G1	67.2	7.2	20.1	106.1	2.8	1147.4ab	0.8b	27.5	315.5
	G2	36.4	4.6	15.6	67.8	3.2	367.3e	0.1cd	27.5	101.0
	G3	42.1	5.3	18.3	77.1	3.0	668.8cde	0.2c	23.5	157.2
S3	G1	65.8	6.7	20.8	125.2	2.7	1087.1abc	0.9a	27.0	293.5
	G2	38.6	5.5	18.1	77.7	3.3	796.0a-e	0.1d	27.0	214.9
	G3	46.4	5.7	20.6	93.4	3.3	885.6a-d	0.2c	26.1	231.1
Mean		50.9	5.9	19.1	88.3	3.0	810.2	0.4	26.1	211.5
LSD_Y (%5)		3.8**	ns	ns	4.8**	0.1*	251.0**	ns	2.4*	64*
LSD_S (%5)		ns	ns	ns	5.9**	ns	ns	ns	ns	ns
LSD_G (%5)		3.1**	0.8**	4.1*	6.0**	0.2**	271.0**	0.05**	1.6**	72**
$LSD_{S \times G}$ (%5)		ns	ns	ns	ns	ns	470.0*	0.1*	ns	ns

PH: Plant Height (cm); NB: Number of Branches (pcs plant^{-1}); NC: Number of Capsules (pcs plant^{-1}); NS: Number of seeds (pcs capsules^{-1}); TSW: Thousand seed weight (g); SY: Seed yield (kg ha^{-1}); EOC: Essential oil content (%); SFOC: Seed fatty oil content (%); SFOY: Seed fatty oil yield (kg ha^{-1}); S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype, ns: not significant, *: P<0.05, **: P<0.01, *Levels not connected by the same letter are significantly (P<0.05) different according to the LSD test.

Other researchers have also documented variations in PH for black cumin, ranging from 62 to 73 cm (Toncer and Kizil, 2004), 62 cm (Mehmood et al., 2018), 21 to 46 cm (Hosseini et al., 2018), 15 to 45 cm (Golkar and Nourbakhsh, 2019), 24 to 45 cm (Inan, 2020), 35 to 44 cm (Beyzi, 2020), 37 to 44 cm (Ozer et al., 2020), 26 to 60 cm (Varun et al., 2020), and 38 to 43 cm (Kara et al., 2021). When comparing the results of the PHs obtained by the researchers with the results in our study, it is observed that there are low, high, and similar values. The variation in the PH detected across these studies may reflect the genotype of the plant and the environmental influences.

Regarding the NOB, significant differences were found between genotypes. The highest NOB was obtained from the G1 genotype (7.0 pcs plant^{-1}), while it was found to be 49% and 19% higher than the G2 (4.7 pcs plant^{-1}) and G3 (5.9 pcs plant^{-1}) genotypes, respectively. The NOB observed in this study is in agreement with other results, ranging from 4 to 6 pcs plant^{-1} (Toncer and Kizil, 2004), 3 to 6 pcs plant^{-1} (Hosseini et al., 2018), 5 to 10 (Iqbal et al., 2019), 6.5 to 6.9 pcs plant^{-1} (Kara et al., 2021), 4.7 pcs plant^{-1} (Ozer et al., 2020). However, the results herein were lower than 4 to 16 pcs plant^{-1} reported by (Golkar and Nourbakhsh, 2019).

Concerning the NOC, significant differences were observed in genotypes. The highest capsules were obtained from the G3 (21.7 pcs plant^{-1}) genotype, while it was 10% and 37% higher than the G1 (19.7 pcs plant^{-1}) and G2 (15.8 pcs plant^{-1}) genotypes, respectively. Other researchers reported similar results in terms of NOC, ranging from 7 to 9 pcs plant^{-1} (Toncer and Kizil, 2004), 6 to 17 pcs plant^{-1} (Hosseini et al., 2018), 6 to 11 pcs plant^{-1} (Inan, 2020), 16 to 37 pcs plant^{-1} (Varun et al., 2020), 17 to 18 pcs plant^{-1} (Ozer et al., 2020), 16 to 20 pcs plant^{-1} (Sarkar et al., 2022), and 6 to 7 pcs plant^{-1} (Kara et al., 2021). However, the present results were less than 8 to 72 pcs plant^{-1} (Golkar and Nourbakhsh, 2019), and 10 to 65 pcs plant^{-1} (Iqbal et al., 2019).

Statistically significant differences were observed in NOS values between different years, sowing dates, and genotypes. It was found that 11% higher NOS value (92.9 pcs capsules^{-1}) was obtained in the 2nd year compared to the NOS value (83.6 pcs capsules^{-1}) in the 1st year. In terms of sowing dates, the highest NOS value was obtained at S3 sowing date (98.8 pcs capsules^{-1}), while it was 20% and 18% higher than S1 (82.4 pcs capsules^{-1}) and S2 (83.7 pcs capsules^{-1}) sowing date, respectively. The significant decrease of NOS in early sowing processes may be associated with the destructive effect of low minimum temperatures in the period when the number of leaves of the plant is high. Thus, the plants did not have enough opportunities for photosynthesis and produced fewer seeds. In terms of genotypes, while the highest NOS was obtained from the G1 genotype (109.8 pcs capsules^{-1}), which was 54% and 31% higher than the G2 (71.2 pcs capsules^{-1}) and G3 (83.9 pcs capsules^{-1}) genotypes, respectively. Other researchers reported similar results regarding NOS, ranging from (Toncer and Kizil, 2004), 39 to 94 pcs plant^{-1} (Hosseini et al., 2018), 29 to 39 pcs plant^{-1} (Rezaei-Chiyaneh et al., 2018), 56 to 88 pcs plant^{-1} (Bosh et al., 2019), and 73 to 78 pcs plant^{-1} (Ozer et al., 2020). However, the results were lower than 54 to 170 pcs plant^{-1} reported by (Golkar and Nourbakhsh, 2019).

Significant differences were found between years and genotypes with respect to TSW. It is seen that 7% higher TSW value (3.1 g) was obtained in the 1st year compared to the thousand-grain weight value (2.9 g) in the 2nd year. It is thought that the reason for the thick and full grains of black cumin sown in the first year is that the grain filling period coincides with more optimum temperatures. In terms of genotypes, the highest TSW was obtained from the G2 and G3 genotypes (3.1 g), while it was 15% higher than the G1 genotype (2.7 g). Consistent findings regarding the TSW have also been reported by other researchers, with values ranging from 1.8 g (Toncer and Kizil, 2004), 2.3 to 2.8 g (Hosseini et al., 2018), 1.5 to 1.9 g (Rezaei-Chiyaneh et al., 2018), 0.7 to 6.6 (Iqbal et al., 2019), 2.5 to 2.6 g (Ozer et al., 2020), 2.8 to 3.0 g (Sarkar et al., 2022), and 2.3 to 2.6 g (Kara et al., 2021). TSW is a significant yield-contributing trait in the majority of plant. However, the negative correlation between TSW and yield should not be ignored.

In terms of SY values, there were significant differences between years, genotypes, and the interaction of sowing date × genotype. It is observed that 94% higher SY value (1069.8 kg ha^{-1}) was found in the second year compared to the SY value in the first year (550.6 kg ha^{-1}). In terms of genotypes, the highest SY value was obtained in G1 (978.1 kg ha^{-1}) and G3 (914.5 kg ha^{-1}) genotypes, while the lowest was found in G2 genotype (538.1 kg ha^{-1}). In terms of interaction, S1G3 had the highest SY value (1189.0 kg ha^{-1}), while S2G2 had the lowest SY value (367.3 kg ha^{-1}). Other researchers reported similar results regarding the SY, ranging from 564 to 866 kg ha^{-1} (Toncer and Kizil, 2004), 824 to 1866 kg ha^{-1} (Hosseini et al., 2018), 515 to 707 kg ha^{-1} (Rezaei-Chiyaneh et al., 2018), 908 to 1277 kg ha^{-1} (Bosh et al., 2019), 176 to 662 kg ha^{-1} (Bayati et al., 2020), 1129 to 1255 kg ha^{-1} (Ozer et al., 2020), 307 to 542 kg ha^{-1} (Kara et al., 2021), and 556 to 1007 kg ha^{-1} (Moradzadeh et al., 2021). Many open flowers fell off due to late frosts without capsule formation. Deterioration of pollen formation in response to heat stress was the major contributor to yield reduction.

Significant differences in EOC were observed between different genotypes and the sowing date × genotype interaction. In terms of genotypes, the highest EOC was found in the G1 genotype (0.9%), while the lowest was found in the G2 (0.1%) and G3 genotypes (0.2%). In terms of interaction, the highest EOC was obtained from the S1G1 and S3G1 (0.9%) genotypes, while S1G3 and S3G2 had the lowest EOC (0.1%). Similar results based on the EOC were also reported by other researchers, such as 0.31% (Toncer and Kizil, 2004), 0.68 to 1.28% (Hosseini et al., 2018), 0.95 to 1.24% (Rezaei-Chiyaneh et al., 2018), 0.23 to 0.26 (Bayati et al., 2020), 0.04 to 0.19% (Ali et al., 2020), and 0.09 to 0.10% (Kara et al., 2021). The emission of volatiles and the yield and composition of essential oils are significantly influenced by biotic factors such as insects and abiotic factors such as light, temperature, and precipitation.

Significant differences were found in the SOC values between years and genotypes. It is observed that 7% higher SOC value (27.6%) was obtained in the 1st year compared to the SOC value (24.5%) in the 2nd year. The increased SOC in the first year may be a result of improved dry matter production and increased source capacity, which may have enhanced the translocation of photo-assimilates to the sink, thus increasing oil biosynthesis. In terms of genotypes, the highest SOC was obtained from G1 (26.9%) and G2 (26.8%) genotypes, while the lowest was obtained from G3 genotype (24.5%). Variations in the SOC of black cumin have also been reported by other researchers; 25 to 35% (Toncer and Kizil, 2004), 28 to 33% (Hosseini et al., 2018), 8 to 40% (Beyzi, 2020), 32 to 37% (Ozer et al., 2020), 7 to 19% (Bayati et al., 2020), 31 to 33% (Kara et al., 2021), and 34 to 46% (Moradzadeh et al., 2021). There are low, high, and similar values when comparing the SOC results obtained by the researchers with the results of this study. The variation in SOC observed in these different studies may reflect of the genotype of the plant as well as environmental influences.

Significant differences were found in terms of SOY values between years and genotypes. It is observed the SOY value obtained in the second year (262.1 kg ha^{-1}) was %58 higher than the SOY value obtained in the first year (152.0 kg ha^{-1}). Among the genotypes, the highest SOY was obtained from the G1 (263.1 kg ha^{-1}) and G3 (224.1 kg ha^{-1}) genotypes, while the lowest SOY was obtained from the G2 genotype (144.2 kg ha^{-1}). Variations in the SOY of black cumin have also been reported by other researchers; 242 to 568 kg ha^{-1} (Hosseini et al., 2018), 412 to 428 kg ha^{-1} (Ozer et al., 2020), 27 to 133 kg ha^{-1} (Bayati et al., 2020), and 190 to 392 kg ha^{-1} (Moradzadeh et al., 2021). Regarding the subject, it is thought that the differences in the seed oil yield may be due to the genetic differences of the genotypes.

Essential Oil Composition of Black Cumin

The chemical composition of black cumin plant's essential oils was examined using GC/MS analysis, and the results are presented in Table 2. Figure 3 shows a representative GC/MS chromatogram illustrating the essential oil and fatty oil composition. 23 components were identified, representing 89.48%-97.15% of the total essential oil. The major components were *p*-cymene (51.45%-66.33%), trans-4-Methoxythujane (8.40%-11.90%), Thymoquinone (0.11%-19.26%), γ-Terpinene (1.28%-9.09%), and Limonene (2.93%-4.50%), respectively. Based on the heat map shown in Figure 4, the visualization of essential oil components was conducted considering different genotypes and sowing dates.

Compared to the genotypes for *p*-cymene, the highest amounts were found in the G3 genotype. When comparing the p-cymene content with previous studies, a range of diverse results were observed, varying from 25.00% to 25.90% (Botnick et al., 2012), 22.05% (Kazemi, 2015), 34.10% to 39.90% (Al Turkmani et al., 2015), 59.50% (Khalid and Shedeed, 2016), 34.67% (Ndirangu et al., 2020), 25.01% to 26.90% (Kara et al., 2021), 60.20% (Sakdasri et al., 2021), 27.70% to 38.10% (Ghanavi et al., 2022), 49.27% (Ciesielska-Figlon et al., 2022). These variations may have resulted from a range of ecological factors, including as soil fertility, air temperature, radiation, and precipitation, which all affected the formation of components in black cumin. Also, in medicinal and aromatic plants, the composition of essential oil is a key marker that has been altered by a variety of factors, including plant species, plant age, agricultural techniques, extraction methods, plant growth stages, and post-harvest processing (Barut et al., 2021).

Table 2. The essential oil composition of black cumin based on the different sowing dates and genotypes

Components	RT (min)	S1 G1	S1 G2	S1 G3	S2 G1	S2 G2	S2 G3	S3 G1	S3 G2	S3 G3
		Relative Peak Area (%)								
Limonene	11.615	4.00	4.50	3.26	2.93	4.11	3.42	2.96	3.90	3.62
γ -Terpinene	12.143	9.09	7.23	1.28	3.52	6.52	2.34	1.41	5.90	3.53
Terpinolene	12.494	0.28	0.20	0.13	0.23	0.21	0.12	0.12	0.20	0.10
p-Cymene	13.099	55.28	62.78	66.28	51.45	58.97	66.10	51.76	58.10	66.33
trans-4-Methoxythujane	13.675	8.40	10.35	10.10	11.90	9.67	9.90	8.82	9.30	10.20
α-Longipinene	14.838	-	0.76	-	0.76	0.62	2.05	1.22	0.70	2.09
Linalol	16.286	0.20	0.09	-	0.56	0.09	0.11	0.20	0.20	0.11
Valencene	16.624	1.94	2.60	1.35	3.87	3.10	2.45	3.15	3.20	3.95
Longifolene	17.046	0.04	0.05	-	0.04	0.05	-	0.05	0.05	-
2-Caren-4-ol	17.301	0.69	0.65	0.62	1.05	0.69	0.59	0.80	0.53	0.56
3-p-Menthen-7-al	17.598	2.03	1.75	1.84	2.50	1.46	1.87	2.16	1.53	2.02
4-Carvomenthenol	17.877	1.64	0.98	0.88	2.33	0.87	0.88	1.39	0.91	-
Carane	19.509	0.05	0.07	-	0.04	0.08	0.09	0.06	0.09	0.09
Carvone	21.011	-	-	-	0.33	-	-	0.09	-	-
Thymoquinone	21.355	4.88	0.11	-	10.14	0.15	-	19.26	0.18	-
Cyclododecane	21.503	-	0.05	-	-	-	-	-	-	-
cis-cyclooctene oxide	22.079	-	0.38	0.47	-	0.31	0.40	-	0.29	0.32
7-Heptadecene, 1-chloro-	22.328	-	0.06	-	-	0.04	-	-	0.05	-
α-Gurgujene	22.833	0.15	0.19	-	0.13	0.18	-	0.18	0.20	-
Pimaradiene	23.343	-	0.85	0.24	0.36	0.64	0.24	0.13	0.91	0.23
1,7-Dodecadiene	23.860	0.16	0.38	0.21	0.19	0.29	0.29	0.32	0.36	0.36
Wine lactone	24.121	0.21	-	-	0.22	-	-	-	-	-
Carvacrol	24.667	4.07	3.12	2.82	4.39	3.15	2.70	2.72	3.09	2.58
	Total	93.11	97.15	89.48	96.94	91.20	93.55	96.80	89.69	96.09

S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype

Table 3. The fatty acid composition according to different sowing dates and genotypes

Components	C 16:0 Palmitic acid	C 16:1 Palmitoleic acid	C 18:0 Stearic acid	C 18:1 Oleic acid	C 18:2 Linoleic acid	C 18:3 Linolenic acid	C 20:2 Eicosadienoic acid	Total
RT (min)	11.876	12.535	13.811	14.541	15.639	17.081	18.494	
S1 G1	14.71	0.16	3.16	20.98	57.18	0.55	3.16	99.90
S1 G2	14.41	0.05	2.95	22.42	57.16	0.60	2.41	100.00
S1 G3	13.89	0.18	3.24	23.52	55.98	0.46	2.70	99.97
S2 G1	13.92	0.19	3.02	24.46	54.94	-	2.63	99.16
S2 G2	14.50	0.22	3.53	24.14	54.53	0.67	2.27	99.86
S2 G3	15.02	0.15	3.34	24.34	53.97	-	2.31	99.13
S3 G1	13.73	-	2.31	26.40	57.56	-	-	100.00
S3 S2	14.96	0.21	2.95	22.03	56.64	-	2.55	99.34
S3 G3	14.00	-	3.47	25.19	54.40	-	2.14	99.20
Mean	14.35	0.17	3.11	23.72	55.82	0.57	2.52	

S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype

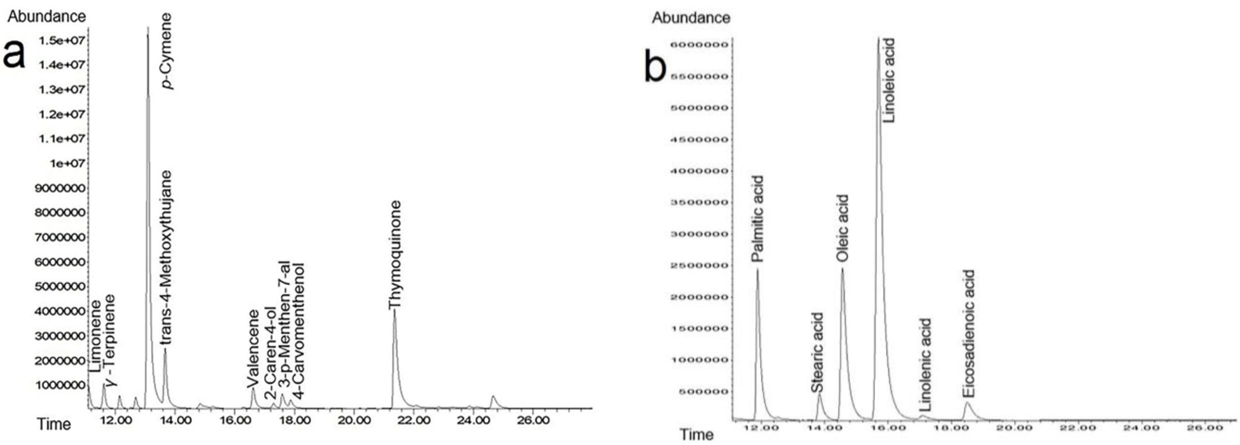

Figure 3. The representative GC/MS chromatogram of essential oil components and fatty acids (a: Essential oil components b: Fatty acids).

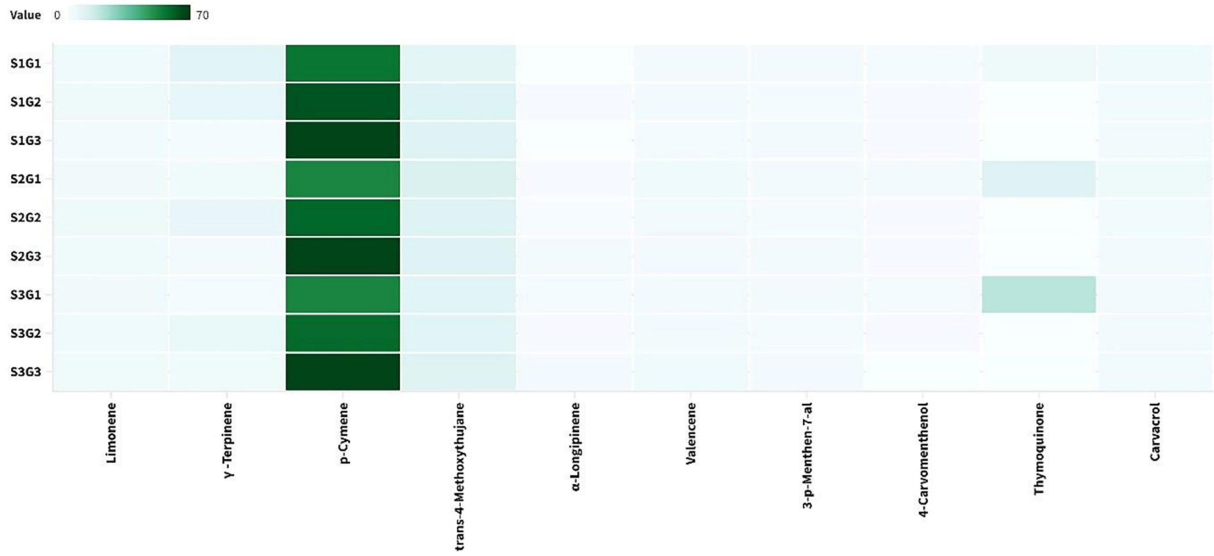

Figure 4. Heat map of main essential oil components according to different sowing dates and genotypes (S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype).

p-Cymene, also known as p-cymol or p-isopropyltoluene, is an aromatic chemical with alkyl substitution that occurs naturally in the essential oils of many aromatic plant species.

It is a precursor of the main volatile components like thymol and carvacrol, which are well-known for their broad bioactivity (Bouyahya et al., 2017). Numerous types of studies have revealed the pharmacological effects of *p*-cymene, including anti-inflammatory, antiparasitic, antioxidant, antidiabetic, anticancer, antibacterial, antiviral, and antifungal activities (Balahbib et al., 2021). It has been reported that the anti-inflammatory property of black cumin essential oil is mainly due to *p*-cymene (Ciesielska-Figlon et al., 2022). Similar to the p-cymene content, previous studies have reported varying trans-4-Methoxythujane content; %4.00 (Wajs et al., 2008), 3.80% to 4.00% (Botnick et al., 2012), 5.81% (Ndirangu et al., 2020), 3.40% to 4.80% (Ghanavi et al., 2022), 5.83% (Ciesielska-Figlon et al., 2022). Researchers reported that the aroma of trans-4-methylthujane is intensely camphoric, and woody, with a soil scent (Wajs et al., 2008). The highest amounts of thymoquinone were found in the G1 genotype. Investigations by other researchers were revealed differences in the thymoquinone content of black cumin, with recorded values such as 20.32% (Kazemi, 2015), 17.20% to 25.90% (Al Turkmani et al., 2015), 3.00% (Khalid and Shedeed, 2016), 67.70% (Palabiyik and Aytaç, 2018), 2.39% to 4.41% (Kara et al., 2021), 4.00% (Sakdasri et al., 2021), 19.50% to 40.90% (Ghanavi et al., 2022), 2.26% (Ciesielska-Figlon et al., 2022). Thymoquinone has antioxidant properties and has been shown to induce apoptosis and inhibit cell division in cancer cells, thus supporting its therapeutic potential (Burits and Bucar, 2000; Chehl et al., 2009; Banerjee et al., 2010; Zaid et al., 2012). It is a potent prospective therapeutic candidate for cancer treatment because it successfully suppresses tumor angiogenesis and tumor growth (Yi et al., 2008). Furthermore, the significant negative correlation between *p*-cymene and thymoquinone, which are the main components, was revealed for the first time (Figure 6).

Fatty Acid Composition of Black Cumin

The chemical composition of the fatty oil was examined using GC/MS analysis, and the results are presented in Table 3. The chemical composition of the fatty oil was C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 18:3 (Linolenic acid), and C 20:2 (Eicosadienoic acid). Six fatty acids were identified, representing 99.13%-100% of the total fatty seed oils. Palmitic and stearic acids are the most common saturated fatty acids found in plant oils, while linoleic and oleic acids are unsaturated fatty acids. The main fatty acids were determined as C 18:2 Linoleic acid (53.97%–57.56%), C 18:1 Oleic acid (20.98–26.40), and C 16:0 Palmitic acid (13.73%–15.02%). Based on the heat map illustrated in Figure 5, the visualization of fatty acids was performed considering various genotypes and sowing dates.

When comparing the linoleic acid content with previous studies, a range of diverse results were observed, varying from 39% to 44% (Palabiyik and Aytaç, 2018), 53% to 58% (Beyzi, 2020), and 50% (Bayati et al., 2020). The results presented in this study were either comparable or higher than the results reported in previous studies, with a mean value of 56%. Linoleic acid was the main fatty acid in all of these studies. In terms of the nutritional advantages offered by plant oils, a higher concentration of linoleic acid is considered beneficial (Al-Jassir, 1992). When comparing oleic acid with previous studies, varying results were found, such as 33%–38% (Palabiyik and Aytaç, 2018), 19%–31% (Beyzi, 2020), 22% (Bayati et al., 2020) and 24%–28% (Moradzadeh et al., 2021). Upon comparing the palmitic acid content with previous studies, a range of different results was observed, ranging from 10%–20% (Beyzi, 2020), and 16% (Bayati et al., 2020). In the present study, several fatty acids were identified with relatively low levels, including 16:1 (Palmitoleic acid), C 18:0 (Stearic acid), C 18:3 (Linolenic acid), and C 20:2 (Eicosadienoic acid). The reduction in temperature induces changes in the ratio of saturated to unsaturated fatty acids, leading to a transition of plant membrane lipids from a liquid to a solid state (Steponkus, 1984; Murata and Los, 1997).

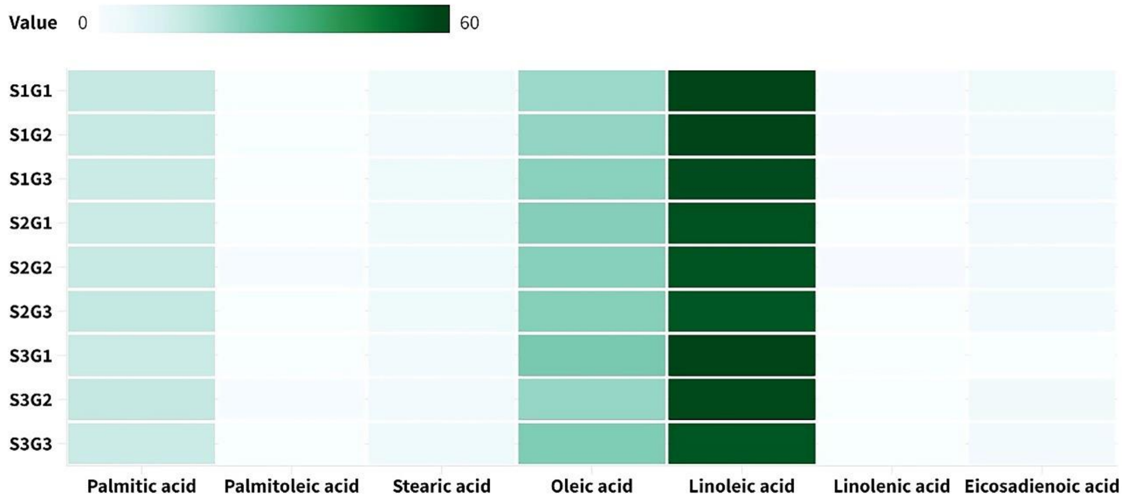

Figure 5. Heatmap of fatty oil composition according to different sowing dates and cultivars (S1: October 15th, S2: November 1st, S3: November 15th, G1: Çameli cultivar, G2: Adana genotype, G3: Iraq genotype).

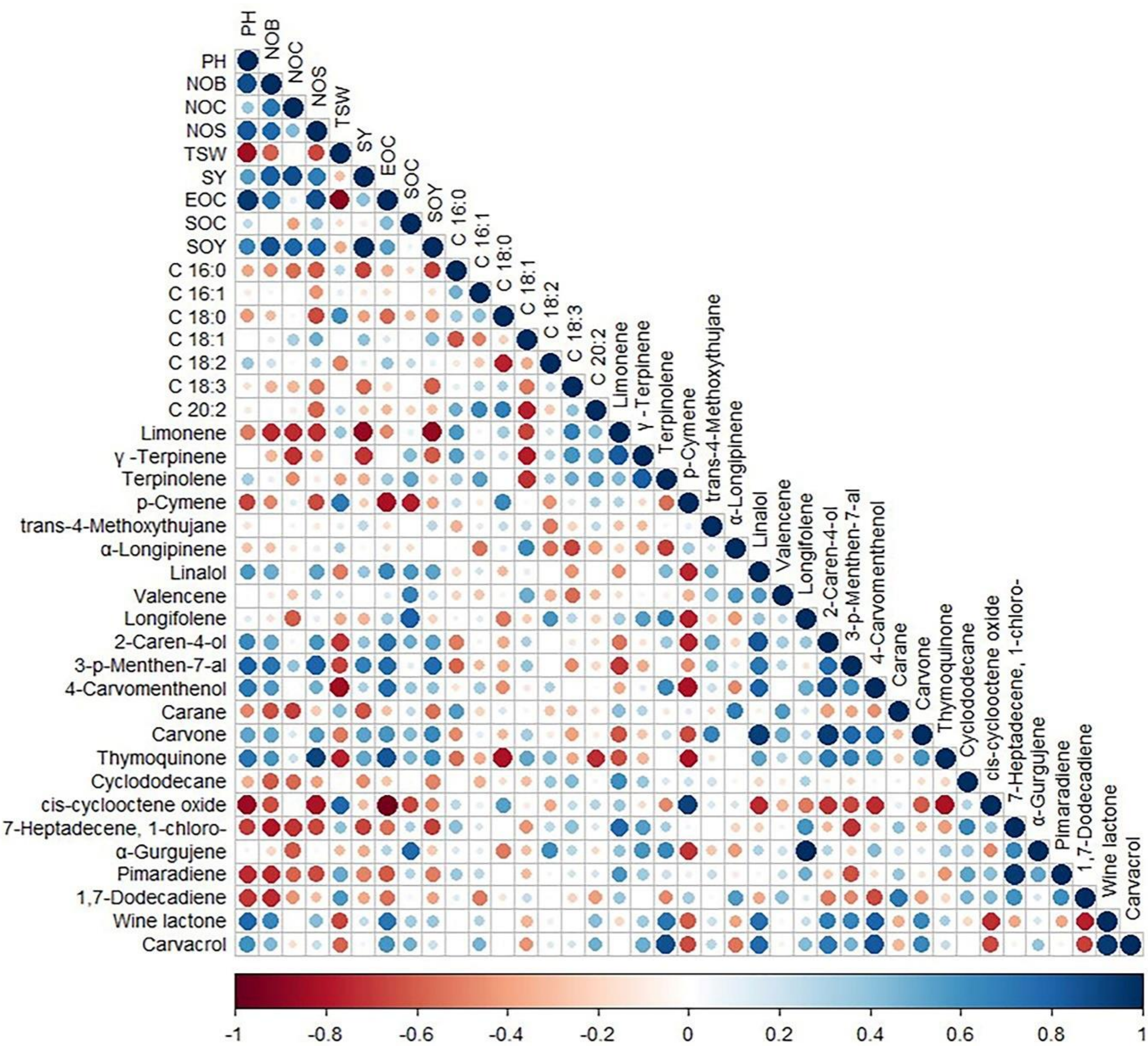

Figure 6. Correlation graph in terms of agronomic and quality traits (S1: October 15th sowing date, S2: November 1st sowing date, S3: November 15th sowing date, G1: Çameli cultivar, G2: Adana genotype, G3: Irak genotype, PH: Plant height, NOB: Number of branches, NOC: Number of capsules, NOS: Number of seeds, TSW: Thousand seed weight, SY: Seed yield, EOC: Essential oil content, SOC: Seed oil content, SOY: Seed fatty oil yield, C 16:0: Palmitic acid, C 16:1: Palmitoleic acid, C 18:0: Stearic acid, C 18:1: Oleic acid, C 18:2: Linoleic acid, C 18:3: Linolenic acid, C 20:2: Eicosadienoic acid.).

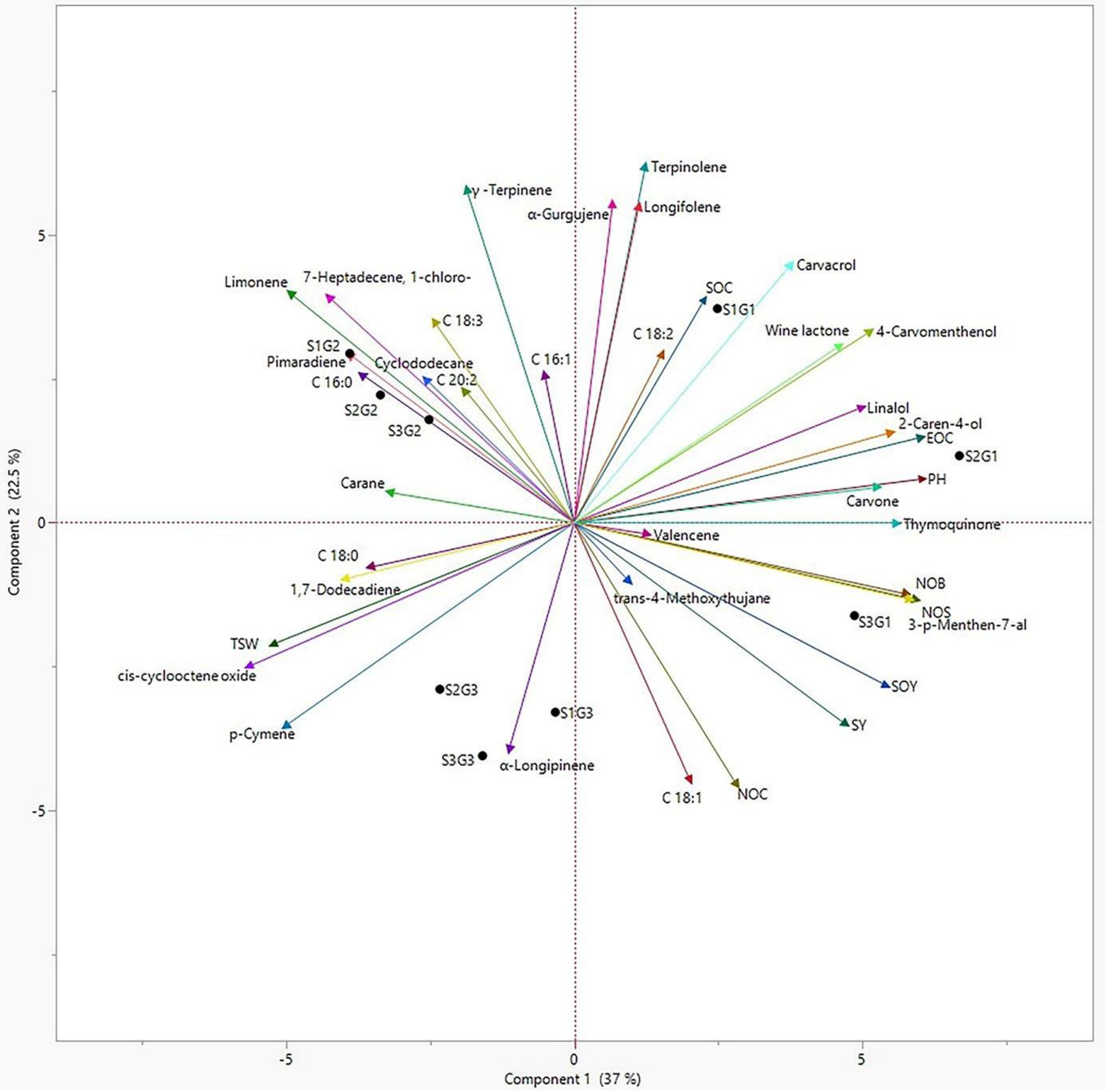

Figure 7. PCA on correlations of the agronomic traits, essential oil components, and fatty acids (S1: October 15th sowing date, S2: November 1st sowing date, S3: November 15th sowing date, G1: Çameli cultivar, G2: Adana genotype, G3: Irak genotype, PH: Plant Height, NOB: Number of Branches, NOC: Number of Capsules, NOS: Number of seeds, TSW: Thousand seed weight, SY: Seed yield, EOC: Essential oil content, SOC: Seed fatty oil content, SOY: Seed fatty oil yield, C 16:0: Palmitic acid, C 16:1: Palmitoleic acid, C 18:0: Stearic acid, C 18:1: Oleic acid, C 18:2: Linoleic acid, C 18:3: Linolenic acid, C 20:2: Eicosadienoic acid).

Moreover, genetic variations, seed production and storage conditions, soil fertility, oil extraction and detection methods, and quantitative methods can all contribute to changes in fatty acid profiles (Barut et al., 2022).

Correlation and Pcabiplot Analysis of the Treatments Based on the Important Traits

The correlation between agronomic traits and quality characteristics is given in Figure 6. Figure 6 shows the correlation of the traits, where the colours represent negative or positive correlations, and the size of the circles signifies the level of importance. The fatty acid composition of plant fatty oils is not always under standard conditions, there are characteristic differences depending on species and varieties, and it may vary depending on many other factors. The distribution and position of the fatty acids in the oil directly affect the quality of the oil, its importance in nutrition, and its technological values. For this reason, it is important in terms of oil quality to know how the fatty acid components of oil plants will change as a result of which conditions and what effect. When Figure 6 is examined, it is observed that there is a negative correlation between TSW and PH, NOB, and NOS, as well as between linoleic acid and stearic acid, which are fatty oil components. In order to evaluate the obtained data as a whole, the data were subjected to principal component analysis (Figure 7). An important part of the variability constituting 59.50% of the total variation, it is observed that they are in a relationship on the Component 1 and Component 2 axes as shown in Figure 7.

Conclusion

The aim of the study was to evaluate the yield and quality characteristics of black cumin, which is a cool season plant, at different sowing dates and in different genotypes in the Mediterranean region, which is highly impacted by global warming and climate change. Differences were observed in the adaptation of the genotypes based on the changing climatic conditions in both years, and Adana and Iraq genotypes showed weak growth compared to Çameli cultivar. Seed yields were adversely affected due to the low number of seed formations and the inability to mature some of the seeds formed due to the low number of flowers with the late spring frosts and the high temperatures observed in May negatively affecting the fertilization of the flowers. While the sowing date had no effect on seed yield and seed oil yield, it was determined that Çameli (G1) and Iraq (G3) genotypes performed better. The expansion of black cumin cultivation in our country is facilitated by the development of improved varieties that exhibit high yield and superior quality traits well-suited to the regional conditions. Black cumin, which occupies an important place among medicinal and aromatic plants obtained from different regions of Türkiye, is compatible with the ecological conditions of our region and resistant to adverse factors, determining the most suitable sowing date and variety recommended below can the conditions of our country and in particular the province of Adana. Identifying resistant, productive, and high-quality genotypes, can contribute to the region's and country's economy. Recognizing that black seed quality is as important as yield, quality criteria for both fatty oil and essential oil components have been proposed. In future studies, it is recommended to conduct appropriate breeding studies to improve high-yielding, region-adapted cultivars and increase insect populations that increase pollination in flowering flowers.

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