

<sup>1</sup>Department of Field Crops, Faculty of Agriculture, Tekirdağ Namık Kemal University, Tekirdağ, Türkiye

<sup>2</sup>Department of Food Technology, Corlu Vocational School, Tekirdağ Namık Kemal University, Tekirdağ, Türkiye

<sup>3</sup>Department of Biosystem Engineering, Faculty of Agriculture Tekirdağ Namık Kemal University Tekirdağ, Türkiye

## Changes in secondary metabolites and essential oil contents in black cumin (*Nigella sativa* L.) in response to drought stress

Yasemin Erdoğan<sup>1\*</sup>, Sıla Barut Gök<sup>2</sup> and Erhan Göçmen<sup>3</sup>

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### Summary

Water stress is one of the foremost stress factors affecting the production of secondary metabolites in plants. The aim of the research was to evaluate the response of black cumin (*Nigella sativa* L.) to water stress in terms of bioactive compounds and essential oil constituents in a semi-arid region (Thrace region, Türkiye). Black cumin seeds were subjected to six irrigation regimes ( $I_0$ ,  $I_{25}$ ,  $I_{50}$ ,  $I_{75}$ ,  $I_{100}$ ,  $I_{125}$ ). The total phenolic contents of extracts decreased significantly by about 0.39-0.59-fold under irrigation regimes from  $I_{75}$  to  $I_{125}$ , compared to the drought stress conditions ( $I_0$ ). Irrigation regimes, except over-irrigation ( $I_{125}$ ) and low water deficit ( $I_{75}$ ) conditions, had no significant effect on the antioxidant activity. The highest flavonoid content recorded under the over-irrigation regime was  $406.6 \pm 4.6$  mg QE g<sup>-1</sup>. The effect of irrigation on essential oil content was statistically significant. The lowest thymoquinone content was obtained under the drought stress conditions ( $I_0$ ), while the highest thymoquinone content was obtained from the high-water deficit conditions ( $I_{25}$ ). Based on principal component analysis, carvacrol and 4-terpineol were the most determinant compounds involved in adaptation to drought stress, and the most determinant component for low water stress was thymoquinone, which had the highest rate in the study.

**Keywords:** *Nigella sativa* L.; drought stress; bioactive constituents; thymoquinone; essential oil

### Introduction

Plants produce primary and secondary metabolites. While primary metabolites are directly involved in plant development and growth, secondary metabolites are multifunctional ones that are typically involved in plant defense and environmental communication (HARTMAN, 2007). Plant secondary metabolites are important sources for pharmaceuticals, food additives, flavors and other industrial materials (ELSHAFIE et al., 2023).

The biosynthesis of secondary metabolites can be influenced by several factors, including genotype, environmental conditions, and agronomic practices. Multiple environment stress conditions, both biotic (fungi, insects, viruses, etc.) or abiotic (drought, salinity, temperature, UV etc.) factors may occur in plants. The secondary metabolism in plants is a mechanism of adaptation and evolution as a defense to harsh environmental factors that induce stress (AGUIRRE-BECERRA et al., 2021; JAN et al., 2021). Drought is an important abiotic stress factor affecting agricultural production. Therefore, plants synthesize various metabolites to mitigate drought stress (CHAN et al., 2013). Recent studies of the effects of drought stress on plant secondary metabolites such as phenolics, terpenoids, nitrogen-containing compounds, etc. showed that several compounds

increased under drought stress, some of them decreased and some were not affected (SELMAR and KLEINWÄCHTER, 2013; QADERI et al., 2023).

Recent studies showed that black cumin seeds have a high potential in terms of modern medicine. Black cumin genotypes have been evaluated separately for nutritional and medicinal usage. The genotypes with low thymoquinone content are commercially classified as “sweet black cumin”, while those with high thymoquinone ratio are classified as “bitter black cumin” (TELCI et al., 2023). TELCI et al. (2024) reported that *N. sativa* genotypes with thymoquinone content > 4% are exploitable for medicinal purposes. Black cumin seeds can contain between 16% to 52.5% fixed oil (BAYTORE and YAVER, 2014; AL JASSIR, 1992) and 0.1% to 1.7% essential oil (ASHRAF et al., 2006; WAJS et al., 2008; TELCI et al., 2022). The fatty acid profile of the fixed oil consists of linoleic acid (50.3-49.2%), oleic acid (25.0-23.7%), and palmitic acid (17.2-18.4%) (CHEIKH-ROUHOU et al., 2007). The major components of black cumin essential oil have been reported as nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol,  $\alpha$  and  $\beta$ -pinene, d-limonene, d-citronellol, and p-cymene (DATTA et al., 2012). In addition to these, black cumin seeds are rich in further bioactive compounds such as steroids, triterpenoids, flavonoids, phenols, proteins, alkaloids, tannin, sesquiterpenoid hydrocarbons, monoterpene alcohol, and monoterpene ketone (thymoquinone) (BHAVIKATTI et al., 2024). MARIOD et al. (2009) investigated the antioxidant activities of the crude hexane of black cumin seed cake and reported that total phenolics were 12.1 mg gallic acid equivalent (GAE g<sup>-1</sup>). THIPPESWAMY et al. (2005) reported that the IC<sub>50</sub> values for the methanolic extract of black cumin were 1.24 mg dry weight of seeds with the DPPH radical scavenging system. Moreover, high amounts of epicatechin (1.88-2.37 mg g<sup>-1</sup>) and rutin (0.96-1.21 mg g<sup>-1</sup>) were detected in black cumin seed extracts (GUEFFAI et al., 2022).

Water is one of the most important limiting factors for optimal plant growth and development. Agriculture is the largest water-consuming activity worldwide, accounting for 70% of total freshwater withdrawals on average (JAYASIRI et al., 2022). Fresh water is a resource that must be carefully managed for global food security. The need for water for sustainable agricultural production is increasing, and the availability of sufficient high quality water is decreasing due to climate change. One-third of the world's lands are classified as arid and semi-arid regions (LANGE, 2012). Optimization of irrigation has become necessary to increase the yield per unit water, especially in these climates (OZER et al., 2020). Black cumin is mainly produced in arid regions. However, black cumin was stated to be sensitive to water stress. It was reported that the yield, yield components and quality of the plant change with the amount of irrigation water (REZAEI-CHIYANEH et al., 2018; BAYATI et al., 2020; OZER et al., 2020). Moreover, alterations in the amount of essential oil components such as thymoquinone,  $\alpha$ -terpinene and p-cymene were reported

\* Corresponding author

ed as a response of black cumin to water stress (MOZAFFARI et al., 2000). Irrigation studies about black cumin are mostly related to yield and yield components, studies investigating the effects on secondary metabolite components are limited.

The aim of the study was to evaluate the effects of different irrigation treatments on bioactive compounds in terms of antioxidant capacity, total phenolic and total flavonoid content in addition to the yield and essential oil profile constituent profile of the essential oil of black cumin (*Nigella sativa* L.) grown in Thrace Region (Türkiye).

## Material and methods

### Plant material and growth conditions

Çameli black cumin variety was used as the plant material. Çameli variety can be considered as «spicy black cumin» due to its thymoquinone content of 2.15% stated as previously reported by TELCI et al. (2024). The experiment was conducted at Silivri Agricultural Research and Production Center in Istanbul (Thrace region), Türkiye in 2021. The site is located at N 41°03'; E 28°0', 53 m above sea level. The climate at the experimental location is semi-arid. The averages for annual temperature, relative humidity, wind speed, sunshine duration per day, and total annual precipitation are 14.1 °C, 76.9%, 2.90 m s<sup>-1</sup>, 6.5 h and 580.8 mm, respectively.

Soil properties were as follows: organic matter 1.69-1.50%; calcareous CaCO<sub>3</sub> 0.86-0.78%; pH 6.42-6.52; electrical conductivity (EC) 0.96-0.71 dS m<sup>-1</sup>; total P<sub>2</sub>O<sub>5</sub> 111.70-687.00 kg ha<sup>-1</sup>; and total K<sub>2</sub>O 972.80-788.70 kg ha<sup>-1</sup>.

### Experimental design, irrigation treatments and precipitation

The experiment was conducted using a randomized complete block design with three replicates. Black cumin seeds were sowed on April 13, 2021. The plots were 1.2 m wide and 5 m long, consisting of six rows spaced 20 cm apart. Seeds were sown at 2-3 cm depth (DATTA et al., 2012). Planting density was 150 plants m<sup>-2</sup>. Fertilizer application was 50 kg ha<sup>-1</sup> N and 50 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> at planting (OEZGUEVEN and SEKEROGLU, 2007). Weeding control of the plots was done by hoeing. The plants were harvested manually at full seed maturity.

Irrigation treatments were designed by applying different levels of the ET<sub>0</sub> value calculated by considering the reference evapotranspiration equation given in ALLEN (1998). The treatment coefficients (0, 0.25, 0.50, 0.75, 1.00, 1.25) were at 6 different levels.

These six irrigation regimes were over-irrigation (I<sub>125</sub>), full irrigation (I<sub>100</sub>), low water deficit (I<sub>75</sub>), moderate water deficit (I<sub>50</sub>), high water deficit (I<sub>25</sub>) and control (non-irrigation) or rain-fed (I<sub>0</sub>).

The amount of irrigation water to be applied was determined by the following equation (1).

$$I = ET_0 * k * P * A \quad (1)$$

Where I is the irrigation amount (mm), ET<sub>0</sub> is the reference evapotranspiration for a 7-day interval (mm), k is the coefficient, P is the percentage of wetted area and A is the parcel area.

The soil moisture measurements initiated on 13 April 2021 with planting and finalized on 5 August 2021 with harvesting. Irrigation applications started on 4 June 2021 and the irrigation application finalized on 29 July 2021. A total of 9 irrigations were applied to the experimental treatments. In the experiment, amounts of 83.74 mm, 167.40 mm, 251.14 mm, 334.80 mm and 418.54 mm irrigation water were applied for the treatments in which 25%, 50%, 75%, 100% and 125% of the ET<sub>0</sub> value were used, respectively. In addition, 114.6 mm of precipitation was measured between 13 April and 5 August 2021. The amount of precipitation in the period after planting the plant was 13 mm in April, 32.2 mm in May, 57.8 mm in June, and 11.6 mm in July. There was no precipitation in August. The rainfall measured during the irrigation period was 79 mm. In the experiment, the evapo-

transpiration amounts (0%, 25%, 50%, 75%, 100% and 125%) in the six different treatments were 209.90 mm, 287.03 mm, 361.85 mm, 441.98 mm, 514.7 mm and 611.73 mm, respectively.

### Essential oil distillation and GC/MS analysis

Essential oil distillation was performed using the Clevenger-type apparatus according to ASHRAF et al. (2006). For this, 20 g of dried and ground seeds were hydro-distilled for 3 h with 250 ml of deionized water using the boiling stone during the process. The hydro-distilled oils were collected and stored in amber airtight vials at 4 ± 1 °C until analysis.

Gas chromatography/mass spectrometry (GC/MS) analyses of black cumin essential oils were performed using GC-MS (Hewlett Packard 6890) with a Rtx-5MS (coated with 5%-phenyl-methyl polysiloxane) capillary column (30 m x d = 250 0.25 µm, film thickness: 0.25 µm), programmed as follows: initial temperature 100 °C (2 min), from 100 to 200 °C at a rate of 8 °C min<sup>-1</sup> and maintained at 200 °C (2 min). Helium was used as carrier gas at a constant flow rate of 1.0 ml/min. The injector temperature was 200 °C. The samples were injected with a splitting ratio of 10:1. The injected quantity was 0.2 µl. The GC/MS interface temperatures were maintained at 250 °C. The ion source and the detector temperatures were maintained at 250 °C. Ionization energy was 70 eV. The mass spectra (MS) were recorded in full scan mode (mass range m/z 30 to 450 uma). The components were identified by comparing the retention index (RI) obtained on a nonpolar HP-5MS column with the RI in the literature, matching the recorded mass spectra with those in mass spectral library references (NIST 08, National Institute of Standards and Technology, Wiley) (ASHRAF et al., 2006).

### Preparation of seed extracts

The extraction procedure was performed according to the method of KHATTAK et al. (2007) with some modifications. The seeds were ground in a laboratory mill (Analysenmühle A10, IKA-Werke GmbH, Germany) and defatted with hexane using a stirrer for 24 hours. A 5 g portion of the defatted flour was weighed into dark screw-capped glass bottles and extracted at room temperature (25 ± 1 °C) for 24 hours at 140 rpm by shaking (Edmund Bühler GmbH KS-15) with 200 ml of 80% (v/v) aqueous MeOH. The extracts were centrifuged at 10,000 rpm for 30 min at 15 °C and the supernatant was collected. The extraction procedure was carried out in triplicate.

### Total phenolic content (TPC)

Total phenolic content (TPC) in black cumin was determined by modifying the method based on the reaction occurring between Folin-Ciocalteu reagent and phenolic compounds (FRANKE et al., 2004). A 500 µl aliquot of methanolic extract was diluted with deionized water (2.0 ml), and the obtained solution was mixed with Folin-Ciocalteu reagent (2.5 ml). The mixture was left for 3 min. Then, 2 ml of sodium carbonate solution (20%) was added. The absorption of the mixture was read at 720 nm wavelength after 1 hour incubation and calculated with the standard curve for gallic acid (SINGLETON and ROSSI, 1965). The TPC results are given as mg gallic acid equivalent/g dry matter (mg GAE g<sup>-1</sup> dry matter) of sample.

### Total flavonoid content (TFC)

The total flavonoid content (TFC) in black cumin was analyzed by a spectrophotometric method described by DRAGOVIC-UZELAC et al. (2010) based on the reaction between aluminum chloride and the flavonoids.

Deionized water (4 ml) was added into the methanolic extract (1 ml). After 5 min, sodium nitrate (NaNO<sub>2</sub>) (1:20 w/v) solution (0.3 ml) and

10% aluminum chloride ( $\text{AlCl}_3$ ) (1:10) (3 ml) was placed into the flask and followed by sodium hydroxide (NaOH) 1.0 M (2 ml) after six min. After mixing again, the solution was centrifuged at  $2.236 \times g$  for 5 minutes. Absorbance of the mixture was read at 510 nm. TFC is expressed as mg quercetin equivalent (QE) per g of extract weight. All samples were analyzed in triplicate.

### Radical scavenging activity (RSA)

Antioxidant capacity (AC) of black cumin was examined according to the method of BRAND-WILLIAMS et al. (1995). For this, 600  $\mu\text{l}$  of DPPH\* radical (1M) and different concentrations of seed extracts were mixed in tubes. The total volume was completed to 6 ml with MeOH. The resulting absorbance was recorded at 517 nm after 30 min incubation in darkness and at ambient temperature (25 °C). Absorbance was detected at 517 nm against the blank. AC for each of the samples was estimated (I%) by using equation (2).

$$\text{Inhibition \%} = ((A_{\text{blank}} - A_{\text{extract}}) / A_{\text{blank}}) \times 100 \quad (2)$$

In Eq (3), the absorption of the extract is expressed as  $A_{\text{extract}}$  and absorption of the control is expressed as  $A_{\text{blank}}$ . %I values were graphed against each concentration for methanolic extract and linear regression analysis was implemented in order to calculate the equation defining the curve. The  $\text{EC}_{50}$  value, which is the amount of antioxidant necessary to decrease the initial DPPH\* concentration by 50%, was calculated by using Eq (3).

### Statistical methods

Data were statistically analyzed using analysis of variance with JMP Pro version 16 (SAS Institute Inc.). The differences between means were analyzed by least significant difference (LSD) test ( $P \leq 0.05$ ) (STELL and TORRIE, 1986).

Principal component analysis (PCA) was applied to the experimental data in order to visualize the differences among the black cumin samples. TPC, TFC and  $\text{EC}_{50}$ , essential oil content and profile, as well as different irrigation treatments were used in PCA. The default graphical view of a PCA is a plot of the data with respect to the first two component axes (JONES and SALL, 2011). Data was analyzed and constructed as a  $p \times q$  matrix, where  $p$  is the number of attributes and  $q$  is the number of samples (DE-SILVA et al., 2019). On PCA-a,  $3 \times 6$  (3 variables and 6 samples) and on PCA-b,  $7 \times 6$  (7 variables and 6 samples) matrices were generated (GEZEK et al., 2019). The JMP Pro version 16 (SAS Institute Inc.) was used for the analysis.

## Results and discussion

### Total phenolic content (TPC)

The analysis of variance results for the total phenolic content (TPC) of seeds under the six irrigation regimes are presented in Tab. 1. TPC of non-irrigation regime black cumin extract estimated by the Folin-Ciocalteu method was  $1292.9 \pm 11.4$  mg of GAE  $\text{g}^{-1}$  DW. The phenolic content of black cumin significantly decreased when seeds were subjected to different irrigation regimes compared to drought stress conditions. The low water deficit ( $I_{75}$ ) recorded the highest and over-irrigation ( $I_{125}$ ) conditions had the lowest change in the phenolic content. Thus, the total phenolic contents of black cumin extracts decreased significantly by about 0.39-0.59-fold under three irrigation regimes from  $I_{75}$  to  $I_{125}$ , compared to the phenolic content observed under drought stress conditions ( $I_0$ ). However, there was no significant difference between the effect of high stress or high-water deficit ( $I_{25}$ ) and the over-irrigation regime ( $I_{125}$ ) on the phenolic content of the seeds.

The results for phenolic content correlate with the findings of REBEY et al. (2012), who reported a significant increase of 15.3% in the total

**Tab. 1:** Mean comparisons of irrigation regimes in terms of TPC, TFC and  $\text{EC}_{50}$  of *Nigella sativa* L. seed extract

IR	TPC (mg GAE $\text{g}^{-1}$ DW)	TFC (mg quercetin $\text{g}^{-1}$ DW)	$\text{EC}_{50}$ ( $\mu\text{g/ml}$ ) DPPH
$I_0$	$1292.9 \pm 11.4^a$	$209.6 \pm 1.2^d$	$776.8 \pm 10.0^a$
$I_{25}$	$759.2 \pm 6.66^b$	$251.9 \pm 8.9^c$	$696.0 \pm 38.7^{ab}$
$I_{50}$	$640.6 \pm 20.13^c$	$274.6 \pm 5.5^b$	$637.0 \pm 55.1^{ab}$
$I_{75}$	$509.6 \pm 19.09^d$	$275.2 \pm 7.8^b$	$516.7 \pm 10.4^{bc}$
$I_{100}$	$632.6 \pm 3.21^c$	$239.6 \pm 13.6^c$	$701.8 \pm 70.1^{ab}$
$I_{125}$	$769.2 \pm 9.61^b$	$406.6 \pm 4.6^a$	$263.8 \pm 2.7^c$
LSD	21.03	12.76	207.08
Prob F	<.0001**	<.0001**	<.0274*
CV.	1.5	2.53	18.15

\*: significant  $p < 0.05$ , \*\*: non-significant  $p < 0.01$ .

phenolic content of cumin seeds under severe drought. Also SZABO et al. (2022) found that drought stress increased phenol content in thyme. The high phenolic content observed in the full stress conditions ( $I_0$ ) could be attributed to the response of cumin to drought (REBEY et al., 2012; ALINIAN et al., 2016). BOURGOU et al. (2010) reported an increase in phenolic content of the black cumin under NaCl stress conditions. ALBERGARIA et al. (2020) stated that the common idea that the response to water stress in terms of the amount of phenolic compounds in plants will be an increase, in any case, is mostly wrong. It was reported that the response to the water stress concentration depends on many factors, and that the response can be a decrease or maintenance of the current situation in the plant. It was emphasized that alteration of secondary metabolite concentrations in the plant depends on the conditions in which the plant grows and is also affected by the metabolic pathways responsible for accumulation in the plant (AKULA and RAVISHANKAR, 2011). Increase in the accumulation of phenolics could result from the accumulation of soluble carbohydrates in plants due to the reduction in the transportation of soluble sugar (ALINIAN et al., 2016). Moreover, it also could be ascribed to enhanced phenylalanine ammonia-lyase enzyme activity under water stress conditions (OH et al., 2009).

### Total flavonoid content (TFC)

The analysis of variance results for the total flavonoid content (TFC) of black cumin seeds under the six irrigation regimes are presented in Tab. 1. Unlike the phenolic content of the seeds, total flavonoid content increased significantly in all irrigation regimes compared to the drought stress conditions ( $I_0$ ). The lowest and the highest flavonoid contents were recorded full stress conditions as  $209.6 \pm 1.2$  mg QE  $\text{g}^{-1}$  and the over irrigation regime as  $406.6 \pm 4.6$  mg QE  $\text{g}^{-1}$ , respectively. Thus, the total flavonoid contents of black cumin extracts increased significantly by 1.20-, 1.31-, 1.31-, 1.14- and 1.94-fold under irrigation regimes from  $I_{25}$  to  $I_{125}$ , respectively, compared to the flavonoid content observed under drought stress conditions ( $I_0$ ). Consistent with our findings, ALINIAN et al. (2016) showed that the flavonoid content of cumin seeds increased significantly under water deficit stress. It was reported by EMAMIBISTGANI et al. (2017) that the flavonoid content of Chinese skullcap increased under water stress conditions and a declining trend was observed in leaf flavonoids of thyme under drought stress conditions, respectively. Similar to the results, YELOOJEH et al. (2020) stated that the TFC of safflower seeds decreased by 37.3% under water deficit conditions. Contrary to these results, it was observed that the total flavonoid content of *Labisia pumila* increased under low level of irrigation. JAAFAR et al. (2012) also reported that the change in flavonoids depended on the cultivar

and the irrigation level applied. Previous studies show that the response of the plant due to drought stress can vary among and within species and plant parts in terms of flavonoid and phenolic contents (YELOOJEH et al., 2020).

### Radical scavenging activity (RSA) assay

The analysis of variance results for the radical scavenging activity (RSA) of *Nigella sativa* L. seeds under six irrigation regimes are presented in Tab. 1. The DPPH radical scavenging activities of methanol extracts from over-irrigated plants ( $I_{125}$ ) showed the highest activity to neutralize the DPPH radical compared to the extract procured under drought stress conditions ( $I_0$ ). Irrigation regimes except over-irrigation ( $I_{125}$ ) and low water deficit ( $I_{75}$ ) conditions had no significant effect on the RSA of black cumin compared to the drought stress conditions ( $I_0$ ). The RSA of the seeds were 33.5 and 66.0% higher, correspondingly, in low stress ( $I_{75}$ ) and over-irrigation ( $I_{125}$ ) conditions than under full stress conditions ( $I_0$ ). In accordance with our findings, REZAEI-CHIYANEH et al. (2018) reported an increase in the antioxidant enzyme activity with increasing water deficit stress in black cumin. A previous study performed in the same climate (Thrace region) showed that water stress had a significant ( $P<0.01$ ) impact on antioxidant activity of sunflower seeds (BARUT GOK et al., 2022). In a similar manner, the highest antioxidant activity was obtained with the low water deficit conditions ( $I_{75}$ ). Contrarily, REBEY et al. (2012) determined that drought stress conditions increased the antioxidant activity of cumin seeds. MITTLER (2002) stated that environmental stresses stimulate reactive oxygen species production. ALOTHMAN et al. (2009) reported that the behavior characteristics of antioxidants in plants may differ widely and have not yet been fully elucidated. REBEY et al. (2012) stated that the antioxidant activity was significantly affected by the plant variety. The RSA obtained in some irrigation regimes is not in parallel with the total phenolic content, which is mostly due to the fact that the antioxidant activity of phenolic compounds depends on their structure (BARUT GOK, 2021). Furthermore, non-phenolic compounds such as L-ascorbic acid and tocopherols might be acted as potential scavengers of DPPH radicals (PAJAK et al., 2014).

### Essential Oil

The essential oil content of *Nigella sativa* L. seeds under six irrigation regimes are presented in Tab. 2. The effects of irrigation treatments on essential oil content were found to be statistically significant ( $p<0.0001$ ). The essential oil content of black cumin seeds varied between 0.54-0.77%. The lowest essential oil content was obtained from  $I_{50}$ , while the highest essential oil content was obtained from  $I_0$ . In plants from which essential oil content is obtained, stress con-

ditions increased the percentage of essential oil because more metabolites are produced in plants under stress conditions. The findings correspond with those reported earlier about black cumin, which stated there was an increase in essential oil content under water stress (BAYATI et al., 2020). In addition to this, the results are in agreement with findings of PETROPOULOS et al. (2008) studying parsley, and BETTAIEB et al. (2009) experimenting with *Salvia officinalis*, both of whom recorded higher levels of essential oil content under water deficit. The increase in the essential oil accumulation may be due to insufficient accumulation of primary metabolites under drought stress conditions. Biomass yield of the plant exposed to drought may affect the total content of secondary metabolites. Previous studies reported that the accumulation of mostly naturally occurring plant products (per unit biomass weight) increases significantly under drought stress conditions (MAHAJAN et al. 2020).

The major chemical compounds of *Nigella sativa* L. essential oil resulting from irrigation treatments were thymoquinone (59.08-62.38%), carvacrol (13.35-16.34%) and longifolene (5.85-12.50%), while the minor compounds were limonene (2.43-2.84 %) and 4-terpineol (1.12-2.35) (Tab. 2). The remaining constituents were micro components with concentrations in the essential oil <2% (Tab. 2). The lowest thymoquinone content was obtained under the drought stress conditions ( $I_0$ ), while the highest thymoquinone content was obtained from the high-water deficit conditions ( $I_{25}$ ). Similar to our results, MOZAFFARI et al. (2000) reported that the thymoquinone compound decreased with the application of water restrictions. Our results are in accordance with previous reports which show that black cumin is a rich source of thymoquinone (BURITS and BUCAR, 2000; MOZAFFARI et al., 2000). The main pharmacologically active component in the essential oil of *N. sativa* is thymoquinone (ERDOGAN et al., 2020). The essential oil of black cumin contains not only high levels of thymoquinone or p-cymene but also various levels of carvacrol,  $\alpha$ -thujene, longifolene,  $\beta$ -pinene,  $\alpha$ -pinene,  $\gamma$ -terpinene, 4-terpineol, thymohydroquinone, dihydro thymoquinone and t-anethole (BURITS and BUCAR, 2000; ERDOGAN et al., 2020). The highest carvacrol and 4-terpineol contents were detected under full stress conditions ( $I_0$ ). Thymoquinone belongs to a class of compounds known as terpenoids (BURITS and BUCAR, 2000), which were reported to have high anti-inflammatory effects (SALEM, 2005). Besides thymoquinone, another oxygenated terpene, 4-terpineol, contributes to the anti-inflammatory potential of black cumin essential oil (BOURGOU et al., 2010). In addition to this, it was reported that thymoquinone and carvacrol promote the *in vitro* antioxidant activity of *N. sativa* essential oil (BURITS and BUCAR, 2000). In addition to this, BOURGOU et al. (2010) reported that the antibacterial and anticancer effects of black cumin essential oil could be mainly due to the action of thymoquinone and longifolene. However, there are several studies reporting the major component of black cumin essential oil as p-cymene (ASHRAF et al.,

**Tab. 2:** Essential oil content and % composition of *Nigella sativa* L. seed extract

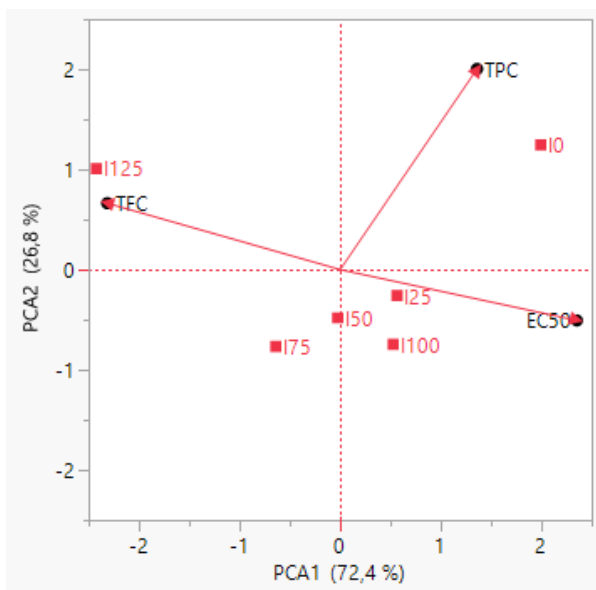
Treatments	Thymoquinone	Carvacrol	Longifolene	Limonene	4-Terpineol	Other	EO (%)
$I_0$	59.08	16.34	5.85	2.56	2.35	13.10	0.77±0.04 <sup>a</sup>
$I_{25}$	62.38	14.53	8.41	2.84	1.90	9.94	0.67±0.02 <sup>bc</sup>
$I_{50}$	59.38	11.25	12.50	2.43	1.12	13.32	0.59±0.05 <sup>cd</sup>
$I_{75}$	62.24	13.35	10.06	2.61	1.54	9.60	0.54±0.02 <sup>d</sup>
$I_{100}$	60.79	13.46	8.66	2.71	1.55	12.83	0.59±0.05 <sup>cd</sup>
$I_{125}$	59.73	15.61	8.46	2.82	1.79	11.59	0.68±0.06 <sup>b</sup>
LSD							0.07
ANOVA							**
CV							6.70

\*, significant  $p<0.05$ , \*\*, non-significant  $p<0.01$ .

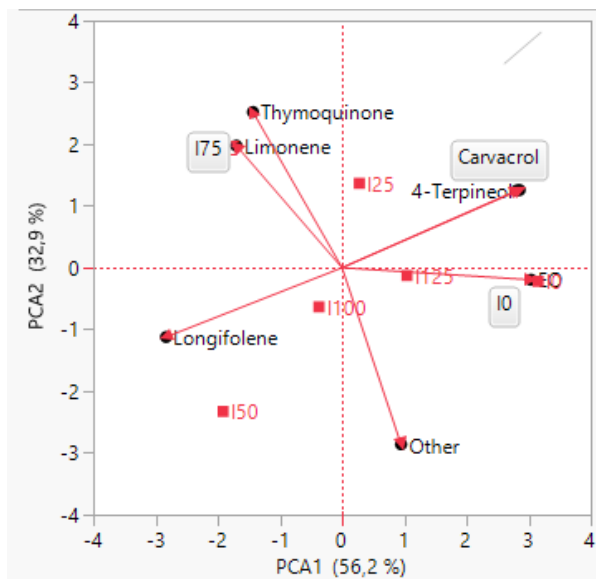
2006; BOURGOU et al., 2010). The alteration in the results might be attributed to many factors, such as genetic differences in the plants, environmental conditions, cultivation techniques and essential oil extraction methods (SAKAR et al., 2023).

### Principal components analysis (PCA)

TPC, TFC and EC<sub>50</sub> for six different irrigation treatments of *Nigella sativa* L. seed extract were analyzed using principal component analysis (PCA-a analysis) (Fig. 1). The first two principal components (PC1 72.4% and PC2 26.8%) explained 99.2% of the variation. The biplot generated from PC1 and PC2 indicated that TPC, TFC and EC<sub>50</sub> from different irrigation treatments were classified under three subgroups. The 1st subgroup of EC<sub>50</sub> was characterized by high-water deficit (I<sub>25</sub>) and was situated in the low right quadrant of the plot. The 2nd subgroup of the TPC was characterized by drought



**Fig. 1:** PCA-a for TPC, TFC and EC<sub>50</sub> of different irrigation treatments for black cumin.



**Fig. 2:** PCA-b for essential oil and compounds of different irrigation treatments for black cumin.

stress conditions (I<sub>0</sub>) and was situated in the high right quadrant of the plot. The 3rd subgroup of the TFC was characterized by over-irrigation (I<sub>125</sub>) and was situated in the high left quadrant of the plot. Essential oil content and components (%) of *Nigella sativa* L. seed extract in six different irrigation treatments were analyzed using PCA-b analysis (Fig. 2). The first two principal components (PC1 56.2% and PC2 32.9%) explained 89.1% of the variation. The biplot achieved from PC1 and PC2 indicated that essential oil content and % components in different irrigation treatments were classified under five subgroups: The 1st subgroup of other essential oil components was characterized by full irrigation (I<sub>100</sub>) and was situated in the low right quadrant of the plot. The 2nd subgroup of the EO was characterized by drought stress conditions and over irrigation (I<sub>0</sub> and I<sub>125</sub>) and was situated in the low right quadrant of the plot. The 3rd subgroup of the carvacrol and 4-terpineol were characterized by high water deficit (I<sub>25</sub>) and was situated in the high left quadrant of the plot. The 4th subgroup of thymoquinone and limonene was characterized by low water deficit (I<sub>75</sub>) and was situated in the high left quadrant of the plot. The 5th subgroup of longifolene was characterized by moderate water deficit (I<sub>50</sub>) and was situated in the low left quadrant of the plot.

### Conclusion

Black cumin has nutraceutical potential against various physiological threats, not only due to its fertile phytochemistry in essential oil, but also due to its bioactive components, such as phenolics and antioxidants in the non-fat extract. The valuable content of phenolics, antioxidants and essential oil components makes black cumin a valuable product for the pharmaceutical, food flavoring and agrochemical industries. Water is one of the most important limiting factors for optimal plant growth. However, fresh water is an essential source that has to be carefully managed for sustainable agricultural production. In the study, the total phenolic content of *Nigella sativa* L. seed extracts decreased under water deficit compared to drought stress conditions. Moreover, irrigation regimes, except for over-irrigation and low water stress conditions, positively influenced the antioxidant potential of seed extracts. However, the induced water deficit stress affected one of the major compounds, thymoquinone, a terpenoid that promotes the anti-inflammatory effect and *in vitro* antioxidant activity of *N. sativa* essential oil.

Irrigation should be maintained at an economic level to enhance the bioactive constituents of the plant. There is a lack of studies on the metabolic responses to water stress in different varieties of seeds. The present research ascertains that as a natural source of phenolics, antioxidant and essential oil, black cumin could be produced effectively by manipulating the irrigation regime. Further investigation regarding the interaction between the phenolic is needed.

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### Conflict of interest

No potential conflict of interest was reported by the author.


### References

- AGUIRRE-BECERRA, H., VAZQUEZ-HERNANDEZ, M.C., SAENZ DE LA O.D., ALVARADO-MARIANA, A., GUEVARA-GONZALEZ, R.G., GARCIA-TREJO, J.F., FERERINO-PEREZ, A.A., 2021: Role of stress and defense in plant

- secondary metabolites production. *Bioactive Natural Prod. Pharma. Appl.* 151-195. DOI: [10.1007/978-3-030-54027-2\\_5](https://doi.org/10.1007/978-3-030-54027-2_5)
- AKULA, R., RAVISHANKAR, G.A., 2011: Influence of abiotic stress signals on secondary metabolites in Plants. *Plant Signaling and Behaviour.* 6, 1720-1731. DOI: [10.4161/psb.6.11.17613](https://doi.org/10.4161/psb.6.11.17613)
- ALBERGARIA, E.T., OLIVEIRA, A.F.M., ALBUQUERQUE, U.P., 2020: The effect of water deficit stress on the composition of phenolic compounds in medicinal plants. *African J. Bot.* 131,12-17. DOI: [10.1016/j.sajb.2020.02.002](https://doi.org/10.1016/j.sajb.2020.02.002)
- ALINIAN, S., RAZMJOO, J., ZEINALI, H., 2016: Flavonoids, anthocyanins, phenolics and essential oil produced in cumin (*Cuminum cyminum* L.) accessions under different irrigation regimes. *Ind. Crops Prod.* 81, 49-55. DOI: [10.1016/j.indcrop.2015.11.040](https://doi.org/10.1016/j.indcrop.2015.11.040)
- ALLEN, R.G., PEREIRA, L.S., RAES, D., SMITH, M., 1998: Crop evapotranspiration-guidelines for computing crop water requirements-FAO irrigation and drainage paper 56. Fao, Rome. ISBN 92-5-104219-5.
- ALOTHMAN, M., BHAT, R. KARIM, A.A., 2009: Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Trends Food Sci Technol.* 20(5), 201-212. DOI: [10.1016/j.tifs.2009.02.003](https://doi.org/10.1016/j.tifs.2009.02.003)
- ASHRAF, M., ALI, Q., IQBAL, Z., 2006: Effect of nitrogen application rate on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa* L.) seeds. *J. Sci. Food Agricult.* 86(6), 871-876. DOI: [10.1002/jsfa.2426](https://doi.org/10.1002/jsfa.2426)
- BARUT GÖK, S., 2021: UV-C treatment of apple and grape juices by modified UV-C reactor based on dean vortex technology: microbial, physicochemical and sensorial parameters evaluation. *Food Bioprocess Technol.* 14, 1055-1066. DOI: [10.1007/s11947-021-02624-z](https://doi.org/10.1007/s11947-021-02624-z)
- BARUT GÖK, S., ERDOĐDU, Y., GÖÇMEN, E., SALBAŞ, B., ERDEM, T., 2022: Evaluation of the phenolic content and antioxidant activity of sunflower seeds under deficit irrigation conditions. *Rivista Italiana Delle Sostanze Grasse.* 99(4), 285-294.
- BAYATI, P., KARIMMOJENI, H., RAZMJOO, J., 2020: Changes in essential oil yield and fatty acid contents in black cumin (*Nigella sativa* L.) genotypes in response to drought stress. *Ind. Crops Prod.* 155, 112764. DOI: [10.1016/j.indcrop.2020.112764](https://doi.org/10.1016/j.indcrop.2020.112764)
- BAYTORE F., YAVER S., 2014: Bazı çörek otu (*Nigella sativa* L.) popülasyonlarının verim ve verim kriterlerinin belirlenmesi. II, Tıbbi Aromatik Bitkiler Sempozyumu; 23-25 September 2014, Yalova.
- BETTAIEB, I., ZAKHAMA, N., AIDI WANNES, W., MARZOUK, B., 2009: Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. *Scientia Horticult.* 120, 271-275. DOI: [10.1016/j.scienta.2008.10.016](https://doi.org/10.1016/j.scienta.2008.10.016)
- BHAVIKATTI, S.K., ZAINUDDIN, S.L.A., RAMLI, R.B., NADAF, S.J., DANDGE, P.B., KHALATE, M., KAROBARI, M.I., 2024: Insights into the antioxidant, anti-inflammatory and anti-microbial potential of *Nigella sativa* essential oil against oral pathogens. *Sci Reports.* 14(1), 11878. DOI: [10.1038/s41598-024-62915-1](https://doi.org/10.1038/s41598-024-62915-1)
- BOURGOU, S., PICHETTE, A., MARZOUK, B., LEGAULT, J., 2010: Bioactivities of black cumin essential oil and its main terpenes from Tunisia. *South African J. Bot.* 76(2), 210-216. DOI: [10.1016/j.sajb.2009.10.009](https://doi.org/10.1016/j.sajb.2009.10.009)
- BRAND-WILLIAMS, W., CUVELIER, M.E., BERSET, C.L.W.T., 1995: Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* 28(1), 25-30. DOI: [10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- BURITS, M., BUCAR, F., 2000: Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research.* 14, 323-328. DOI: [10.1002/1099-1573\(200008\)14:5%3C323::AID-PTR621%3E3.0.CO;2-Q](https://doi.org/10.1002/1099-1573(200008)14:5%3C323::AID-PTR621%3E3.0.CO;2-Q)
- CHAN, K.X., WIRTZ, M., PHUA, S.Y., ESTAVILLO, G.M., POGSON, B.J., 2013: Balancing metabolites in drought: the sulfur assimilation conundrum. *Trends Plant Sci.* 18(1), 18-29. DOI: [10.1016/j.tplants.2012.07.005](https://doi.org/10.1016/j.tplants.2012.07.005)
- CHEIKH-ROUHO, S., BESBES, S., HENTATI, B., BLECKER, C., DEROANNE, C., ATTIA, H., 2007: *Nigella sativa* L.: Chemical composition and physicochemical characteristics of lipid fraction. *Food Chem.* 101(2), 673-681. DOI: [10.1016/j.foodchem.2006.02.022](https://doi.org/10.1016/j.foodchem.2006.02.022)
- DATTA, A.K., SAHA, A., BHATTACHARYA, A., MANDAL, A., PAUL, R., SENGUPTA, S., 2012: Black cumin (*Nigella sativa* L.) – a review. *J. Plant Develop. Sci.* 4(1), 1-43.
- DE SILVA, C.C., BECKMAN, S.P., LIU, S., BOWLER, N., 2019: Principal component analysis (PCA) as a statistical tool for identifying key indicators of nuclear power plant cable insulation degradation. In: *Proceedings of the 18<sup>th</sup> International Conference on Environmental Degradation of Materials in Nuclear Power Systems – Water Reactors*, 1227-1239. Springer International Publishing. DOI: [10.1007/978-3-030-04639-2](https://doi.org/10.1007/978-3-030-04639-2)
- DRAGOVIC-UZELAC, V., SAVIC, Z., BRALA, A., LEVAJ, B., BURSAC KOVACEVIC, D., BISKO, A., 2010: Evaluation of phenolic content and antioxidant capacity of blueberry cultivars (*Vaccinium corymbosum* L.) grown in northwest Croatia. *Food Technol Biotechnol.* 48, 214-221. DOI: [10.1080/15538362.2018.1425653](https://doi.org/10.1080/15538362.2018.1425653)
- ELSHAFIE, H.S., CAMELE, I., MOHAMED, A.A., 2023: A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. *Int. J. Molecular Sci.* 24(4), 3266. DOI: [10.3390/ijms24043266](https://doi.org/10.3390/ijms24043266)
- EMAMI BISTGANI, Z., SIADAT, S.A., BAKHSHANDEH, A., GHASEMI PIRBALOUTI, A., HASHEMI, M., 2017: Morpho-physiological and phytochemical traits of (*Thymus daenesis* Celark.) in responses to deficit irrigation and chitosan application. *Acta Physiol. Plant.* 39, 231. DOI: [10.1007/s11738-017-2526-2](https://doi.org/10.1007/s11738-017-2526-2)
- ERDOĐAN, Ü., YILMAZER, M., ERBAŞ, S., 2020: Hydrodistillation of *Nigella sativa* seed and analysis of thymoquinone with HPLC and GC-MS. *Bilge Int. J. Sci. Technol. Res.* 4(1), 27-30. DOI: [10.30516/bilgesci.688845](https://doi.org/10.30516/bilgesci.688845)
- FRANKE, S.I.R., CKLESS, K., SILVEIRA, J.D., RUBENSAM, G., BRENDDEL, M., ERDTMANN, B., HENRIQUES, J.A.P., 2004: Study of antioxidant and mutagenic activity of different orange juices. *Food Chem.* 88(1), 45-55. DOI: [10.1016/j.foodchem.2004.01.021](https://doi.org/10.1016/j.foodchem.2004.01.021)
- GEZEK, G., HASHEMI, P., KALAYCIOĐLU, Z., KAYGUSUZ, H., SARIOĐLU, G., DÖKER, S., ERIM, F.B., 2019: Evaluation of some Turkish *Salvia* species by principal component analysis based on their vitamin B2, mineral composition, and antioxidant properties. *LWT.* 100, 287-293. DOI: [10.1016/j.lwt.2018.10.066](https://doi.org/10.1016/j.lwt.2018.10.066)
- GUEFFAI, A., GONZALEZ-SERRANO, D.J., CHRISTODOULOU, M.C., ORELLANA-PALACIOS, J.C., ORTEGA, M.L.S., OULDMOUMNA, A., 2022: Phenolics from defatted black cumin seeds (*Nigella sativa* L.): Ultrasound-assisted extraction optimization, comparison, and antioxidant activity. *Biomolecules* 12(9), 1311. DOI: [10.3390/biom12091311](https://doi.org/10.3390/biom12091311)
- HARTMANN, T., 2007: From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry* 68(22-24), 2831-2846. DOI: [10.1016/j.phytochem.2007.09.017](https://doi.org/10.1016/j.phytochem.2007.09.017)
- JAAFAR, H.Z., IBRAHIM, M.H., FAKRI, N.F.M., 2012: Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), malondialdehyde (MDA) and photosynthetic responses of Malaysian Kacip Fatimah (*Labisia pumila* Benth). *Molecules* 17(6), 7305-7322. DOI: [10.3390/molecules17067305](https://doi.org/10.3390/molecules17067305)
- JAN, R., ASAF, S., NUMAN, M., LUBNA, KIM, K.M., 2021: Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy* 11(5), 968. DOI: [10.3390/agronomy11050968](https://doi.org/10.3390/agronomy11050968)
- JAYASIRI, M.M.J.G.C.N., YADAV, S., DAYAWANSA, N.D.K., INGOLD, K., 2022: Managing agricultural water resources: Addressing the complexity of innovation, social perspectives, and water governance in Sri Lanka. *Irrig. Drain.* 71, 71-85. DOI: [10.1002/ird.2693](https://doi.org/10.1002/ird.2693)
- KHATTAK, A.B., ZEB, A., BIBI, N., KHALIL, S.A., KHATTAK, M.S., 2007: Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum* L.) sprouts. *Food Chem.* 104(3), 1074-1079. DOI: [10.1016/j.foodchem.2007.01.022](https://doi.org/10.1016/j.foodchem.2007.01.022)
- LANGE, O.L., 2012: *Physiological plant ecology II: water relations and carbon assimilation* (Vol. 12). Springer Science and Business Media. DOI: [10.1007/978-3-642-68150-9](https://doi.org/10.1007/978-3-642-68150-9)
- MAHAJAN, M., KUIRY, R., PAL, P.K., 2020: Understanding the consequence of environmental stress for accumulation of secondary metabolites in medicinal and aromatic plants. *Journal of Applied Research on Medicinal and Aromatic Plants* 18, 100255. DOI: [10.1016/j.jarmap.2020.100255](https://doi.org/10.1016/j.jarmap.2020.100255)
- MARIOD, A.A., IBRAHIM, R.M., ISMAIL, M., ISMAIL, N., 2009: Antioxidant activity and phenolic content of phenolic rich fractions obtained from

- black cumin (*Nigella sativa*) seed cake. *Food Chem.* 116(1), 306-312. DOI: [10.1016/j.foodchem.2009.02.051](https://doi.org/10.1016/j.foodchem.2009.02.051)
- MOZAFFARI, F., GHORBANLI, S., BABAI, M., FARZAMI, M., 2000: The effect of water stress on the seed oil of black cumin (*Nigella sativa* L.). *J. Essent. Oil Res.* 12, 36-38. DOI: [10.1080/10412905.2000.9712036](https://doi.org/10.1080/10412905.2000.9712036)
- OEZGUEVEN, M., ŞEKEROĞLU, N., 2007, March: Agricultural practices for high yield and quality of black cumin (*Nigella sativa* L.) cultivated in Turkey. In *International Symposium on Medicinal and Nutraceuical Plants 756*, 329-338. DOI: [10.17660/ActaHortic.2007.756.35](https://doi.org/10.17660/ActaHortic.2007.756.35)
- OH, M.M., TRICK, H.N., RAJASHEKARA, C.B., 2009: Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. *J. Plant Physiol.* 166, 180-191. DOI: [10.1016/j.jplph.2008.04.015](https://doi.org/10.1016/j.jplph.2008.04.015)
- OZER, H., COBAN, F., SAHIN, U., ORS, S., 2020: Response of black cumin (*Nigella sativa* L.) to deficit irrigation in a semi-arid region: Growth, yield, quality, and water productivity. *Ind. Crops Prod.* 144, 112048. DOI: [10.1016/j.indcrop.2019.112048](https://doi.org/10.1016/j.indcrop.2019.112048)
- PAJAŁ, P., SOCHA, R., GAŁKOWSKA, D., ROŻNOWSKI, J., FORTUNA, T., 2014: Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chem.* 143, 300-306. DOI: [10.1016/j.foodchem.2013.07.064](https://doi.org/10.1016/j.foodchem.2013.07.064)
- PETROPOULOS, S.A., DIMITRA, D., POLISSIOU, M.G., PASSAM, H.C., 2008: The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. *Scientia Horticult.* 15, 393-397. DOI: [10.1016/j.scienta.2007.10.008](https://doi.org/10.1016/j.scienta.2007.10.008)
- QADERI, M.M., MARTEL, A.B., STRUGNELL, C.A., 2023: Environmental factors regulate plant secondary metabolites. *Plants* 12(3), 447. DOI: [10.3390/plants12030447](https://doi.org/10.3390/plants12030447)
- REBEY, I.B., ZAKHAMA, N., KAROUI, I.J., MARZOUK, B., 2012: Polyphenol composition and antioxidant activity of cumin (*Cuminum cyminum* L.) seed extract under drought. *J. Food Sci.* 77(6), 734-739. DOI: [10.1111/j.1750-3841.2012.02731.x](https://doi.org/10.1111/j.1750-3841.2012.02731.x)
- REZAEI-CHIYANEH, E., SEYYEDI, S.M., EBRAHIMIAN, E., MOGHADDAM, S.S., DAMALAS, C.A., 2018: Exogenous application of gamma-aminobutyric acid (GABA) alleviates the effect of water deficit stress in black cumin (*Nigella sativa* L.). *Ind. Crops Prod.* 112, 741-748. DOI: [10.1016/j.indcrop.2017.12.067](https://doi.org/10.1016/j.indcrop.2017.12.067)
- SALEM, M.L., 2005: Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *Int Immunopharmacol.* 5(13-14), 1749-1770. DOI: [10.1016/j.intimp.2005.06.008](https://doi.org/10.1016/j.intimp.2005.06.008)
- SAKAR, E.H., ZEROUAL, A., KASRATI, A., GHARBY, S., 2023: Combined effects of domestication and extraction technique on essential oil yield, chemical profiling, and antioxidant and antimicrobial activities of rosemary (*Rosmarinus officinalis* L.). *J. Food Biochem.* 2023, 6308773. DOI: [10.1155/2023/6308773](https://doi.org/10.1155/2023/6308773)
- SELMAR, D., KLEINWÄCHTER, M., 2013: Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products. *Plant Cell Physiol.* 54(6), 817-826. DOI: [10.1093/pcp/pct054](https://doi.org/10.1093/pcp/pct054)
- SINGLETON, V.L., ROSSI, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Viticult.* 16, 144-158. DOI: [10.5344/ajev.1965.16.3.144](https://doi.org/10.5344/ajev.1965.16.3.144)
- STEEL, R.G.D., TORRIE, J.H., 1986: Principles and procedures of statistics: A biometrical approach, 2<sup>nd</sup> ed. McGraw Hill Co. Inc., New York. ISBN: 978-0-07-060926-6.
- TELCI, I., IZGI, M.N., OZEK, T., YASAK, S., YUR, S., OZEK, G., 2022: Effects of different nitrogen doses on thymoquinone and fatty acid composition in seed oil of black cumin (*Nigella sativa* L.). *J. Am. Oil Chemists' Soc.* 99(3), 229-237. DOI: [10.1002/aocs.12570](https://doi.org/10.1002/aocs.12570)
- TELCI, İ., ÖZEK, T., DEMİRTAŞ, İ., ÖZEK, G., YUR, S., ERSOY, S., KARAKURT, Y., 2023: Studies on black cumin genotypes of Türkiye: Agronomy, seed and thymoquinone yields. *J. Appl. Res. Med. Arom. Plants.* 35, 100494. DOI: [10.1016/j.jarmap.2023.100494](https://doi.org/10.1016/j.jarmap.2023.100494)
- TELCI, İ., ÖZEK, T., GÜL, F., YUR, S., ÖZEK, G., DEMİRTAŞ, İ., KACAR, O., 2024: Diversity of black cumin genotypes and their classification based on functional properties. *Biochem. System Ecol.* 113, 104802. DOI: [10.1016/j.bse.2024.104802](https://doi.org/10.1016/j.bse.2024.104802)
- THIPPESWAMY, N.B., NAIDU, K.A., 2005: Antioxidant potency of cumin varieties - cumin, black cumin and bitter cumin on antioxidant systems. *Europ. Food Res. Technol.* 220, 472-476. DOI: [10.1007/s00217-004-1087-y](https://doi.org/10.1007/s00217-004-1087-y)
- WAJS, A., BONIKOWSKI, R., KALEMBA, D., 2008: Composition of essential oil from seeds of *Nigella sativa* L. cultivated in Poland. *Flavour Frag. J.* 23(2), 126-132. DOI: [10.1002/ffj.1866](https://doi.org/10.1002/ffj.1866)
- YELOOJEH, A.K., SAEIDI, G., SABZALIAN, M.R., 2020: Drought stress improves the composition of secondary metabolites in safflower flower at the expense of reduction in seed yield and oil content. *Industr. Crops Prod.* 154, 112496. DOI: [10.1016/j.indcrop.2020.112496](https://doi.org/10.1016/j.indcrop.2020.112496)

## ORCID


Yasemin Erdoğan  <https://orcid.org/0000-0001-8393-3444>Sıla Barut Gök  <https://orcid.org/0000-0001-7666-9630>Erhan Göçmen  <https://orcid.org/0000-0001-6199-7842>

Address of the corresponding author

Yasemin Erdoğan, Department of Field Crops, Faculty of Agriculture, Tekirdağ Namık Kemal University, Tekirdağ, Türkiye

E-mail: yerdogdu@nku.edu.tr

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