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# Fatty Acids, Essential Oil, And Phenolics Modifications of Black Cumin Fruit under NaCl Stress Conditions

Soumaya Bourgou,* Iness Bettaieb, Moufida Saidani, and Brahim Marzouk

Laboratoire des Substances Bioactives Centre de Biotechnologie à la Technopole de Borj-Cédria (CBBC), BP 901, Hammam-Lif, Tunisia

This research evaluated the effect of saline conditions on fruit yield, fatty acids, and essential oils compositions and phenolics content of black cumin (*Nigella sativa*). This plant is one of the most commonly found aromatics in the Mediterranean kitchen. Increasing NaCl levels to 60 mM decreased significantly the fruits yield by 58% and the total fatty acids amount by 35%. Fatty acids composition analysis indicated that linoleic acid was the major fatty acid (58.09%) followed by oleic (19.21%) and palmitic (14.77%) acids. Salinity enhanced the linoleic acid percentage but did not affect the unsaturation degree of the fatty acids pool and thus the oil quality. The essential oil yield was 0.39% based on the dry weight and increased to 0.53, 0.56, and 0.72% at 20, 40, and 60 mM NaCl. Salinity results on the modification of the essential oil chemotype from *p*-cymene in controls to $\gamma$-terpinene/*p*-cymene in salt-stressed plants. The amounts of total phenolics were lower in the treated plants. Salinity decreased mainly the amount of the major class, benzoics acids, by 24, 29, and 44% at 20, 40, and 60 mM NaCl. The results suggest that salt treatment may regulate bioactive compounds production in black cumin fruits, influencing their nutritional and industrial values.

**KEYWORDS: Black cumin; fruits yield; fatty acids; essential oil; phenolics; NaCl stress**

## INTRODUCTION

Among the different environmental constraints, salinity is the most abiotic factor limiting plant growth and productivity (*1*). The deleterious effects of salinity on plant growth are associated with low water potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress), or a combination of these different factors (*2*). During the onset and development of salt stress within a plant, all of the major processes are affected including lipid metabolism (*3*) Membranes are the primary targets of salt injury (*4*), and the fatty acid composition of membranes has been found to change in response to salinity, which may contribute to the control of membrane fluidity and functionality (*5*). In the case of aromatic and medicinal plants, abiotic stresses may also cause significant changes in the yield and composition of secondary metabolites. For example, drought, heavy metals, and nitrogen deficiency have been found to influence essential oil and polyphenols biosynthesis (*6*−*11*). However, investigations dealing with the effect of salinity on essential oil and phenolics production are scarce (*12*−*14*).

Black cumin (*Nigella sativa* L.) is an aromatic and medicinal plant that belongs to the Ranunculacea family. The seeds are a valuable organ used traditionally in several countries for culinary and medicinal purposes. In fact, they are used as a spice in the flavoring of foods, especially bakery products and cheese, and in the preparation of a traditional sweet dish, composed of black cumin paste, which is sweetened with honey or syrup (*15*). They are also used as a natural remedy for the treatment of several illnesses including asthma, cough, influenza, and eczema and as a diuretic, lactagogue, and vermifuge (*16*).

Black cumin seeds are of considerable phytochemical interest as they are a source of bioactive compounds with nutritional and therapeutic values. In fact, the seed is oleaginous and is rich in polyunsaturated fatty acids (PUFAs), mainly linoleic acid (*15*,*17*). PUFAs are valuable products because of their involvement in several aspects of human health. They are biosynthetic precursors of the eicosanoids (i.e., prostaglandins). These are signaling molecules with complex control over many body systems, having effects on cardiovascular diseases, triglycerides levels, blood pressure, and arthritis (*18*). Linoleic acid is an essential fatty acid, as it cannot be synthesized by the human organism, so linoleic oil types are a good source of essential fatty acids for human nutrition and also could be of interest in the cosmetic industry due to the effect of linoleic acid on the skin's moisture balance (*19*).

The seeds also contain medicinally important secondary metabolites including essential oils (*20*,*21*). Our previous study demonstrated that Tunisian seeds essential oil displayed a *p*-cymene chemotype distinct from other origins (*21*). This hydrocarbon monoterpene is important for industry because of its use as an intermediate in industrial fine chemicals syntheses for fragrances, flavorings, herbicides, pharmaceuticals, *p*-cresol production, syntheses of non-nitrated musks (i.e., tonalide), etc. (*22*). Moreover, black cumin essential oil exhibited antioxidant, anticancer, anti-inflammatory, antibacterial, and antimutagenic activities (*21*,*23*) as well as analgesic (*24*) and antiulcer (*25*) properties. Furthermore, the seeds are rich in polyphenol compounds, mainly phenolic acids (*21*). Phenolics display a wide range of

*To whom correspondence should be addressed. Tel: +21671430855. Fax: +21679412638. E-mail: soumaya_swiss@yahoo.ca.


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physiological properties such as antiallergic, antiatherogenic, anti-inflammatory, antimicrobial, and antithrombotic ones, but they are especially powerful antioxidants (*26*).

Studies regarding the impact of abiotic stress on bioactive compounds of black cumin are very scarce and limited to that of Mozaffari et al. (*27*), who found that the water stress greatly influenced the composition of black cumin essential oil. To the best of our knowledge, no study has been conducted concerning black cumin lipids, essential oil, and phenolics changes under salinity. So, the present work aims to determine salinity effects on essential oil and fatty acid *N. sativa* composition as well as on polyphenol contents.

## MATERIALS AND METHODS

**Plant Material and Growth Conditions.** Black cumin seeds were collected from cultivated plants in the region of Menzel Temime (Northeastern Tunisia). Botanical identification of this species was carried out by Prof. A. Smaoui (Biotechnologic Center in Borj-Cedria Technopark, Tunisia), and a voucher specimen has been kept in our laboratory for future reference. Seeds were sown in pots (two seeds per pot) filled with inert sand and irrigated with distilled water until germination (four weeks). Seedlings were then irrigated with quarter-strength Hoagland's solution (*28*). After 20 days, pots were partitioned into four lots of 100 individuals each and irrigated with the culture medium supplemented with 0, 20, 40, and 60 mM NaCl. The experiment was performed in a greenhouse [with a 14 h photoperiod; photosynthetic photon flux density (PPFD): 141 $\mu$mol m/2 s/1; mean temperature and relative humidity were, respectively, $30 \pm 5$ °C, $55 \pm 5$% day and $16 \pm 2$ °C, $90 \pm 5$% night]. After 12 weeks, seeds were harvested and weighted.

**Extraction of Total Lipids.** Triplicate subsamples of 1 g were extracted using the method of Marzouk and Cherif (*29*). Thus, samples were fixed in boiling water for 5 min and then ground manually in a china mortar using a mixture of chloroform/methanol/hexane (3:2:1, v/v/v). After it was washed with water for fixation and decantation at +4 °C, the organic phase containing total lipids was recovered and dried under a nitrogen stream.

**Fatty Acids Methylation and Analysis.** Total fatty acids (TFA) of total lipids were transformed into their corresponding methyl esters as described by Cecchi et al. (*30*). Transmethylation was made using 3% sodium methylate. Heptadecanoic acid methyl ester (C17:0) was used as an internal standard. The fatty acid methyl esters (FAMEs) obtained were analyzed by gas chromatography (GC) using a Hewlett Packard 6890 apparatus (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A HP-Innowax capillary column (polyethylene glycol: 30 m, 0.25 mm i.d, 0.25 $\mu$m film thickness; Agilent Technologies, Hewlett Packard) was used; the flow of the carrier gas ($N_2$, U) was 1.6 mL/min, and the split ratio was 60:1. The initial oven temperature was held at 150 °C for 1 min, increased at a rate of 15 °C/min to 200 °C, and then held there for 3 min and finally ramped at 2 °C/min to 242 °C. The detector and injector temperatures were set at 275 and 250 °C, respectively. FAMEs were identified by comparison of their retention times with those of pure reference standards.

**Essential Oil Isolation.** Black cumin seeds were ground finely. Triplicate samples of 20 g were subjected to hydrodistillation for 90 min. To quantify the essential oil yield, 6-methyl-5-hepten-2-one was added as an internal standard. The oil was then dried with anhydrous sodium sulfate and stored at −20 °C in darkness until used.

**GC-FID.** Essential oils were analyzed by GC using the same apparatus previously described. A HP-Innowax capillary column (polyethylene glycol: 30 m, 0.25 mm i.d, 0.25 $\mu$m film thickness; Agilent Technologies, Hewlett Packard) was used, and analyses were performed using the following temperature program: oven temperature isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C/min, and isotherm at 205 °C over 10 min. Injector and detector temperatures were held, respectively, at 250 and 300 °C.

**Gas Chromatography−Mass Spectrometry (GC-MS).** GC-MS analyses of essential oil volatile components were carried out on a gas chromatograph HP 5890 (II) coupled to a HP 5972 mass spectrometer (Agilent Technologies) with electron impact ionization (70 eV). A HP-5MS capillary column (30 m × 0.25 mm, 0.25 $\mu$m film thickness; Agilent Technologies, Hewlett Packard) was used. The oven temperature was programmed to rise from 50 to 240 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 mL/min; the split ratio was 60:1. The scan time and mass range were 1 s and 40−300 $m/z$, respectively.

**Compounds Identification.** Identification of essential oil volatile compounds was based on the calculation of their retention indices (RI) relative to ($C_8$−$C_{22}$) *n*-alkanes with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC-MS data system and other published mass spectra (*31*).

**Polyphenols Extraction.** Methanol seed extracts were obtained by stirring 3 g of dry seeds powder with 30 mL of pure methanol for 30 min. The extracts were then kept for 24 h at 4 °C, filtred through Whatman #4 filter paper, and evaporated under vacuum to dryness.

**Total Polyphenols Amounts.** Total polyphenols of the methanol extracts were assayed using the Folin−Ciocalteu reagent, following the Dewanto et al. (*32*) method. An aliquot of 0.125 mL of diluted sample extract was added to 0.5 mL of distilled water and 0.125 mL of Folin−Ciocalteu reagent. The mixture was shaken and allowed to stand for 3 min, before addition of 1.25 mL of 7% $Na_2CO_3$. The solution was then adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark for 90 min, the absorbance at 760 nm was read versus the prepared blank. Total phenolics content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. All samples were analyzed in three replications.

**Hydrolysis and Analysis of Phenolic Compounds Using RP-HPLC.** Dried samples from seeds were hydrolyzed according to the slightly modified method of Proestos et al. (*33*). The acidic hydrolysis was used to release the aglycones to simplify the identification process since the free forms of phenolic compounds are rarely present in plants and they occur as esters, glycosides, or bound to the cell wall (*34*). Twenty milliliters of methanol was added to 0.5 g of a dried sample. Then, 10 mL of 1 M HCl was added. The mixture was stirred carefully and sonicated for 15 min and refluxed in a water bath at 90 °C for 2 h. The obtained mixture was injected to HPLC. The phenolic compounds analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC) coupled with an UV−vis multiwavelength detector. The separation was carried out on a 250 mm × 4.6 mm, 4 $\mu$m Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulfuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program was as follows: 15% A/85% B, 0−12 min; 40% A/60% B, 12−14 min; 60% A/40% B, 14−18 min; 80% A/20% B, 18−20 min; 90% A/10% B, 20−24 min; and 100% A, 24−28 min (*21*). The injection volume was 20 $\mu$L, and peaks were monitored at 280 nm. Samples were filtered through a 0.45 $\mu$m membrane filter before injection. Phenolic compounds were identified by congruent retention times compared with standards. Analyses were performed in triplicate.

**Statistics Analysis.** Data were subjected to statistical analysis using statistical program package STATISTICA (*35*). The one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was employed, and the differences between individual means were deemed to be significant at $p < 0.05$.

## RESULTS AND DISCUSSION

**Effect of Salinity on Seeds Yield.** As it is shown in **Figure 1**, salinity affected significantly the seeds production of black cumin, and the reduction was parallel to stress intensity. In fact, the yield decreased by 12, 21, and 58% in response to 20, 40, and 60 mM NaCl. The depressive effect of salt stress on fruit yield has been reported earlier in several aromatic and medicinal plants including *Foeniculum vulgare* (*36*) and *Trachyspermum ammi* (*37*). A decrease of seeds yield might arise from a reduction of flower production and/or a decrease of their fertility (*38*). The accumulation of $Na^+$ and $Cl^-$ in pollen and stigma is known to be strongly implied in salt-induced sterility (*39*).

**Effect of Salinity on TFAs Amount.** In the control, TFAs account for 383.21 mg/g DW. As shown in **Figure 2**, TFAs

remained unchanged under 20 and 40 mM NaCl; however, a statistically significant ($P < 0.05$) reduction of 35% of TFA was observed at the highest salinity level (60 mM). The effect of salinity on seeds oil has been found to depend on the species and the stress intensity. Salinity decreased the oil yield of *Salvia hispanica* (*40*) and *Helianthus annuus* (*41*), increased it in *Oenothera biennis*, and had no effect on *Matthiola tricuspidata* (*40*). According to Smaoui and Chereif (*42*), the drastic effect of salinity on cotton seeds lipids may be due to the inhibition of photosynthetic activity in leaves induced by high NaCl concentrations, leading to less transport of photosynthetates from leaves to seeds.

**Effect of Salinity on Fatty Acids Composition.** Analysis of the fatty acids composition indicated that in control plants, linoleic acid (C18:2) was the major compound (58.09% of TFA) followed by oleic (C18:1) and palmitic (C16:0) acids, which constitute 19.21 and 14.77% of TFA, respectively (**Table 1**). The seeds were characterized by the presence of a high proportion of PUFAs (58.50% of TFA). Monounsaturated (MUFA) and saturated fatty acids (SFA) represented 19.49 and 22.01%, respectively.

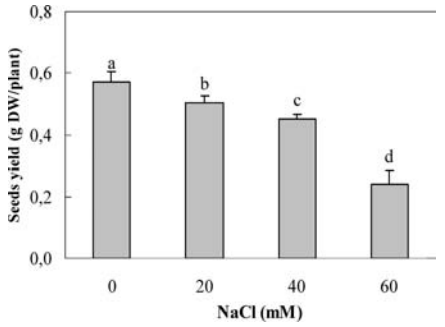


**Figure 1.** Effect of NaCl concentrations on the seeds yield of black cumin. Means of three replicates. Values with different superscripts are significantly different at $p < 0.05$.

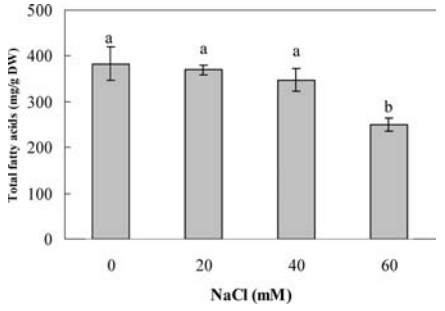


**Figure 2.** Effect of NaCl concentrations on the TFAs content of black cumin seeds. Means of three replicates. Values with different superscripts are significantly different at $p < 0.05$.

Salinity influenced the fatty acids composition of the seeds as is shown in **Table 1**. Treatments with 20, 40, and 60 mM NaCl increased the PUFA fraction due to the increase of linoleic acid proportion. However, salinity affected the MUFA fraction, which decreased by 10, 13, and 12% in response to 20, 40, and 60 mM NaCl and which was mainly mediated by the reduction of the oleic acid proportion. Salt treatments did not elicit changes on the SFA fraction as well as the proportion of the major SFA, palmitic acid. Moreover, the linoleic/oleic acid ratio was found to increase in comparison to control under salinity (**Table 1**), which suggests the enhancement of Δ12-oleoyl desaturase responsible for linoleic acid biosynthesis. Abiotic stresses may cause accelerated and earlier embryo development, stimulating enzymatic activities of FA biosynthesis, including those of oleoyl Δ-12 desaturase (*43*).

On the other hand, the effect of salinity on the degree of fatty acids unsaturation was assessed using the double bond index (DBI) calculated according to Rie De Vos et al. (*44*). Results showed that this parameter remained unchanged in response to salt treatments. This result indicates that salinity does not affect the unsaturation degree of the fatty acids pool and thus the quality and the stability of the black cumin oil. In addition, the degree of fatty acid unsaturation is an important factor intervening in the maintenance of membrane fluidity and in providing the appropriate environment for membrane function and in the processes of plant adaptation in front of constraining conditions (*5*).

**Effect of Salinity on Essential Oil Yield.** The essential oils of nontreated and treated seeds of black cumin were obtained as yellow oils. In the control seeds, the yield was 0.39% (expressed as g/100 g DW, **Figure 3**). Under salinity, the oil yield increased to 0.53, 0.56, and 0.72% at 20, 40, and 60 mM NaCl, respectively. Thus, salinity improved the black cumin essential oil yield, which was proportional to the stress intensity. Earlier studies reported an increase of black cumin essential oil yield in response to water stress (*27*), which confirms, in addition to our results, the sensitivity of the oil production in this plant to environmental constrains. Stress-induced alterations in essential oil accumulation are considered to be mainly due to its effect on plant growth

**Table 1.** Effect of NaCl Concentrations on Fatty Acids Composition (%) of Black Cumin Seeds[a]

| | NaCl (mM) | | | |
|---|---|---|---|---|
| fatty acids | 0 | 20 | 40 | 60 |
| C14:0 (myristic acid) | 0.40 ± 0.03 a | 0.45 ± 0.01 a | 0.40 ± 0.02 a | 0.42 ± 0.07 a |
| C16:0 (palmitic acid) | 14.77 ± 0.47 a | 15.43 ± 0.24 a | 14.80 ± 0.08 a | 15.35 ± 0.69 a |
| C16:1 (palmitoleic acid) | 0.28 ± 0.05 a | 0.30 ± 0.02 a | 0.28 ± 0.02 a | 0.28 ± 0.01 a |
| C18:0 (stearic acid) | 2.90 ± 0.01 a | 2.59 ± 0.04 c | 2.60 ± 0.00 c | 2.77 ± 0.05 b |
| C18:1 (oleic acid) | 19.21 ± 0.13 a | 17.25 ± 0.23 b | 16.60 ± 0.21 c | 16.81 ± 0.23 c |
| C18:2 (linoleic acid) | 58.09 ± 0.54 b | 60.00 ± 0.10 a | 60.31 ± 0.79 a | 60.53 ± 0.36 a |
| C18:3 (linolenic acid) | 0.41 ± 0.06 a | 0.42 ± 0.09 a | 0.36 ± 0.05 a | 0.39 ± 0.01 a |
| C20:0 (arachidic acid) | 1.05 ± 0.43 ab | 0.70 ± 0.02 ab | 1.54 ± 0.89 a | 0.52 ± 0.02 b |
| C22:0 (behenic acid) | 2.89 ± 0.34 a | 2.87 ± 0.07 a | 3.11 ± 0.07 a | 2.93 ± 0.15 a |
| SFA | 22.01 ± 0.60 a | 22.03 ± 0.23 a | 22.45 ± 1.06 a | 21.99 ± 0.96 a |
| MUFA | 19.49 ± 0.20 a | 17.55 ± 0.24 b | 16.88 ± 0.20 c | 17.09 ± 0.21 c |
| PUFA | 58.50 ± 0.64 b | 60.41 ± 0.01 a | 60.67 ± 0.74 a | 60.92 ± 0.35 a |
| linoleic/oleic | 3.02 ± 0.01 c | 3.48 ± 0.04 b | 3.63 ± 0.08 a | 3.60 ± 0.03 a |
| DBI | 1.37 ± 0.01 a | 1.39 ± 0.00 a | 1.38 ± 0.01 a | 1.39 ± 0.01 a |

[a] Values (means of three replicates ± SDs) with different letters (a−c) are significantly different at $P < 0.05$.

and differentiation (*45*). The stimulation of essential oil production could be related to a higher density of the oil glands and an increase in the absolute number of glands produced under osmotic stress (*45*).

**Effect of Salinity on Essential Oil Composition.** Variations of the composition of the black cumin essential oil under stress are illustrated in the **Table 2**. In the control, 21 compounds were identified, accounting for 93.07% of total constituents. The essential oil was characterized by the dominance of monoterpenes hydrocarbons, which constituted the main class (84.26%) followed by the oxygenated monoterpenes (7.54%), while sesquiterpenes were weakly represented (1.27%). The essential oil was of the *p*-cymene chemotype since it was the major compound with a relative percentage of 43.65%. Other main compounds were

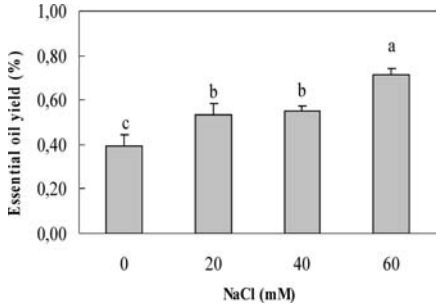


**Figure 3.** Effect of NaCl concentrations on the essential oil yield (%) of black cumin seeds. Means of three replicates. Values with different superscripts are significantly different at $p < 0.05$.

$\gamma$-terpinene (15.20%), α-thujene (14.88%), and carvacrol (5.30%). The ketone thymoquinone, which is a typical compound of black cumin essential oil (*20*), was present at a low percentage of 0.12%.

Application of salinity induced marked changes on the essential oil quality. The percentage of the *p*-cymene decreased under the different treatments by about 25% under 20 and 60 mM NaCl and 28% under 40 mM NaCl. In fact, the percentage decreased to 32.77, 31.41, and 32.93 under 20, 40, and 60 mM NaCl, respectively. However, the salt stress increased the level of the second main compound, $\gamma$-terpinene, by about 2-fold under the different NaCl levels. The latter became the most abundant compound in the oil. Indeed, its percentage reached 33.87, 34.80, and 39.32% under 20, 40, and 60 mM NaCl, respectively. Thus, the salinity induced the modification of the essential oil chemotype from *p*-cymene in the control seeds to $\gamma$-terpinene/*p*-cymene in salt-stressed seeds. This change will probably result in the modification of the essential oil odor. It has been shown that the aroma property of *p*-cymene was dependent on its concentration; *p*-cymene has a kerosene-like aroma note at relatively high concentrations but changed to a citrus and greenlike aroma note at low concentrations, while $\gamma$-terpinene has a citrus aroma (*46*). Similarly to us, Mozaffari et al. (*27*) reported a significant water stress effect on the essential oil quality of the Iranian black cumin; in fact, the chemotype was modified from *p*-cymene/thymoquinone in controls to thymquinone under sever water stress.

Concerning the other main essential oil compounds, salinity was found to greatly decrease the percentage of carvacrol, while the level of α-thujene was only reduced under 20 and 60 mM NaCl. The decrease of *p*-cymene and carvacrol accompanied by the increase of $\gamma$-terpinene is in accordance with their biosynthetic

**Table 2.** Proportions (%) of the Essential Oil Compounds of Black Cumin Seeds Subjected to Different NaCl Concentrations[a]

| | | | NaCl | | | |
|---|---|---|---|---|---|---|
| compounds[b] | RI[c] | RI[c] | 0 | 20 | 40 | 60 |
| α-thujene | 928 | 1035 | 14.88 ± 2.05 a | 10.45 ± 1.03 b | 14.50 ± 0.36 a | 9.11 ± 0.70 b |
| α-pinene | 939 | 1032 | 2.60 ± 0.33 a | 1.59 ± 0.50 b | 2.81 ± 0.35 a | 1.77 ± 0.22 b |
| camphene | 954 | 1076 | 0.03 ± 0.00 a | 0.04 ± 0.00 a | 0.04 ± 0.01 a | 0.03 ± 0.01 a |
| sabinene | 975 | 1132 | 1.07 ± 0.04 b | 1.16 ± 0.06 ab | 1.31 ± 0.11 a | 0.90 ± 0.01 c |
| *β*-pinene | 980 | 1118 | 2.52 ± 0.08 a | 2.36 ± 0.22 ab | 2.73 ± 0.11 a | 2.10 ± 0.33 b |
| myrcene | 991 | 1174 | 0.14 ± 0.00 b | 0.22 ± 0.04 ab | 0.25 ± 0.03 a | 0.23 ± 0.08 ab |
| α-phellandrene | 1006 | 1176 | 0.29 ± 0.01 b | 0.50 ± 0.09 a | 0.62 ± 0.11 a | 0.56 ± 0.15 a |
| α-terpinene | 1018 | 1188 | 2.06 ± 0.28 b | 2.62 ± 0.68 ab | 3.09 ± 0.00 a | 2.88 ± 0.28 a |
| *p*-cymene | 1026 | 1280 | 43.65 ± 0.61 a | 32.77 ± 0.52 b | 31.41 ± 0.11 b | 32.93 ± 2.17 b |
| limonene | 1030 | 1203 | 1.27 ± 0.11 a | 1.59 ± 0.01 a | 1.38 ± 0.12 a | 1.40 ± 0.45 a |
| 1,8-cineole | 1033 | 1213 | 0.40 ± 0.28 a | 0.05 ± 0.00 b | 0.03 ± 0.01 b | 0.05 ± 0.00 b |
| *trans*-sabinene hydrate | 1053 | 1474 | 0.05 ± 0.03 b | 0.11 ± 0.02 a | 0.04 ± 0.01 b | 0.11 ± 0.02 a |
| $\gamma$-terpinene | 1062 | 1255 | 15.20 ± 3.40 c | 33.87 ± 0.10 b | 34.80 ± 0.11 b | 39.32 ± 0.73 a |
| terpinolene | 1092 | 1290 | 0.49 ± 0.19 a | 0.27 ± 0.01 b | 0.26 ± 0.05 b | ND |
| terpinen-4-ol | 1178 | 1611 | 1.58 ± 0.50 a | 0.61 ± 0.15 b | 0.34 ± 0.12 b | 0.66 ± 0.09 b |
| *p*-cymen-8-ol | 1183 | 1864 | 0.08 ± 0.02 a | 0.03 ± 0.00 b | 0.02 ± 0.01 b | 0.09 ± 001 a |
| thymoquinone | 1252 | 1758 | 0.12 ± 0.00 a | 0.07 ± 0.02 b | 0.06 ± 0.01 b | 0.12 ± 0.02 a |
| bornyl acetate | 1270 | 1590 | 0.01 ± 0.00 b | 0.08 ± 0.05 a | 0.04 ± 0.01 ab | 0.05 ± 0.00 ab |
| thymol | 1290 | 2198 | 0.05 ± 0.00 b | 0.02 ± 0.01 c | 0.02 ± 0.01 c | 0.08 ± 0.01 a |
| carvacrol | 1292 | 2239 | 5.30 ± 0.03 a | 1.61 ± 0.47 b | 0.27 ± 0.02 c | 0.45 ± 0.11 c |
| longifolene | 1405 | 1565 | 1.27 ± 0.08 a | 0.17 ± 0.11 b | 0.13 ± 0.01 b | 0.20 ± 0.01 b |
| | | | chemical classes | | | |
| monoterpenes hydrocarbons | | | 84.26 ± 2.41 c | 87.55 ± 5.12 bc | 93.24 ± 0.20 a | 91.34 ± 0.82 ab |
| phenols | | | 5. 35 ± 0.02 a | 1.64 ± 0.68 b | 0.29 ± 0.03 c | 0.53 ± 0.12 c |
| alcools | | | 1.66 ± 0.49 a | 0.64 ± 0.15 b | 0.36 ± 0.03 b | 0.75 ± 0.10 b |
| ketones | | | 0.12 ± 0.00 a | 0.07 ± 0.02 b | 0.06 ± 0.01 b | 0.12 ± 0.02 a |
| esters | | | 0.01 ± 0.00 b | 0.08 ± 0.05 a | 0.04 ± 0.01 ab | 0.05 ± 0.00 ab |
| ethers | | | 0.40 ± 0.28 a | 0.05 ± 0.00 b | 0.03 ± 0.01 b | 0.05 ± 0.00 b |
| sesquiterpenes | | | 1.27 ± 0.08 a | 0.17 ± 0.11 b | 0.13 ± 0.01 b | 0.20 ± 0.01 b |

[a] Values with different letters (a−c) are significantly different at $P < 0.05$. ND, not detected. [b] Components are listed in order of elution in apolar column (HP-5). [c] RI calculated using, respectively, an apolar column HP-5MS and polar column HP-Innowax.

**Table 3.** Amounts ($\mu$g/g DW) of the Essential Oil Compounds of Black Cumin Seeds Subjected to Different NaCl Concentrations[a]

| | NaCl (mM) | | | |
|---|---|---|---|---|
| compounds | 0 | 20 | 40 | 60 |
| α-thujene | 569.46 ± 25.41 bc | 536.22 ± 90.02 c | 798.57 ± 27.15 a | 638.98 ± 23.76 b |
| α-pinene | 100.11 ± 8.65 c | 70.88 ± 1.17 d | 154.54 ± 27.16 a | 124.08 ± 5.68 b |
| camphene | 1.09 ± 0.04 b | 1.9 ± 0.94 ab | 2.26 ± 0.18 a | 1.85 ± 0.29 ab |
| sabinene | 42.01 ± 7.14 b | 60.34 ± 13.72 a | 72.35 ± 1.70 a | 63.30 ± 1.22 a |
| β-pinene | 98.65 ± 17.53 c | 121.33 ± 12.76 b | 150.34 ± 1.85 a | 147.25 ± 2.83 a |
| myrcene | 5.73 ± 1.48 c | 11.13 ± 3.31 b | 14.03 ± 0.77 a | 16.07 ± 1.02 a |
| α-phellandrene | 11.65 ± 3.16 c | 24.67 ± 5.05 b | 34.09 ± 1.62 a | 39.59 ± 1.16 a |
| α-terpinene | 82.88 ± 9.05 c | 125.96 ± 28.44 b | 170.33 ± 13.03 a | 202.23 ± 28.34 a |
| *p*-cymene | 1722.56 ± 38.70 b | 1733.27 ± 81 b | 1729.53 ± 43.34 b | 2309.13 ± 45.26 a |
| limonene | 50.63 ± 15.12 c | 85.19 ± 17.85 ab | 75.76 ± 1.23 b | 97.83 ± 1.25 a |
| 1,8-cineole | 13.12 ± 3.26 a | 2.44 ± 1.17 b | 1.84 ± 0.24 b | 3.40 ± 0.51 b |
| *trans*-sabinene hydrate | 2.30 ± 1.73 b | 5.99 ± 2.33 a | 2.41 ± 0.17 b | 7.61 ± 0.51 a |
| γ-terpinene | 629.76 ± 57.64 c | 1757.57 ± 139 b | 1916.58 ± 35.75 b | 2757.67 ± 10.38 a |
| terpinolene | 16.48 ± 1.00 a | 14.09 ± 1.57 b | 14.12 ± 0.48 b | |
| terpinen-4-ol | 58.92 ± 6.67 a | 35.65 ± 12.27 b | 18.66 ± 0.48 c | 46.43 ± 7.35 ab |
| *p*-cymen-8-ol | 3.04 ± 0.47 b | 1.52 ± 0.87 c | 0.97 ± 0.09 c | 6.32 ± 0.68 a |
| thymoquinone | 4.68 ± 0.83 b | 4.39 ± 2.63 b | 3.09 ± 0.24 b | 8.75 ± 0.28 a |
| bornyle acetate | 0.28 ± 0.05 c | 5.25 ± 2.92 a | 1.97 ± 0.18 bc | 3.23 ± 0.43 ab |
| thymol | 2.0 ± 0.63 b | 1.52 ± 0.61 b | 1.34 ± 0.35 b | 5.48 ± 0.56 a |
| carvacrol | 208.32 ± 42.80 a | 95.63 ± 19.84 b | 14.99 ± 0.65 c | 31.34 ± 1.21 c |
| longifolene | 42.55 ± 3.72 a | 11.09 ± 3.82 bc | 7.10 ± 0.49 c | 13.77 ± 0.59 b |
| | | chemical classes | | |
| monoterpenes hydrocarbons | 3333.32 ± 70.72 d | 4548.54 ± 110.67 c | 5134.89 ± 100.19 b | 6405.58 ± 71.06 a |
| phenols | 210.32 ± 42.21 a | 97.15 ± 28.05 b | 16.33 ± 0.93 c | 36.82 ± 1.75 c |
| alcools | 61.96 ± 7.15 a | 37.17 ± 11.39 b | 19.63 ± 0.44 c | 52.75 ± 8.03 a |
| ketones | 4.68 ± 0.83 b | 4.39 ± 2.63 b | 3.09 ± 0.24 b | 8.75 ± 0.28 a |
| esters | 0.28 ± 0.05 c | 5.25 ± 2.92 a | 1.97 ± 0.18 bc | 3.23 ± 0.43 ab |
| ethers | 13.12 ± 3.26 a | 2.44 ± 1.17 b | 1.84 ± 0.24 b | 3.40 ± 0.51 b |
| sesquiterpenes | 42.55 ± 3.72 a | 11.09 ± 3.82 bc | 7.10 ± 0.49 c | 13.77 ± 0.59 b |

[a] Values (means of three replicates ± SDs) with different letters (a−d) are significantly different at $P < 0.05$.

pathway, in which γ-terpinene represents their common biosynthesis precursor (*47*).

Investigation of the effect of salinity on essential oil classes showed that the proportion of monoterpenes hydrocarbon class enhanced under salinity; however, NaCl treatments reduced the percentage of the sesquiterpenes and oxygenated monoterpenes (phenols, alcools, ketones, esters, and ethers) classes. The latter was mainly induced by the drastic decrease of the phenols fraction by 69, 95, and 90% under 20, 40, and 60 mM NaCl, respectively.

Quantitatively, salinity influenced the amounts of the essential oil compounds, and changes were dose-dependent. The amount of the major class, monoterpenes hydrocarbons, was positively correlated with increasing NaCl concentrations; an enhancement of 36, 54, and 92% with respect to control was found at 20, 40, and 60 mM NaCl. This raise was mainly mediated by the increase of the content of the majors compounds, especially γ-terpinene. Indeed, the amount of the later compound was enhanced by about three times at 20 and 40 mM NaCl and four times under 60 mM NaCl. The *p*-cymene content was not significantly influenced by 20 and 40 mM NaCl but increased by 34% under 60 mM NaCl. Moreover, NaCl treatments were found to enhance the contents of rest of monoterpenes hydrocarbons at the exception of α-thujene and α-pinene, which were reduced under 20 mM NaCl, and terpinolene, whose biosynthesis was completely inhibited by 60 mM NaCl (**Table 3**). The changes of compounds amounts occurring at the different salt levels are likely due to the changes of the activity of the related biosynthesis enzymes in response to the stress (*48*, *49*). The formation of monoterpenes is catalyzed by terpenes synthases, the activity of which is mediated by developmental and stress-related programs (*49*). Terpinolene is structurally consistent with the formation as a deprotonation product of the terpinen-8-yl cation, while other similarly derived *p*-menthanes (e.g., α- and γ-terpinene) are produced from terpinen-4-yl (*50*). The alteration of terpinolene amount and the increase of γ-terpinene suggest the inhibition of terpinolene synthase by salinity, while γ-terpinene synthase was activated.

Moreover, salinity reduced the oxygenated monoterpenes class essentially due to the decrease of the content of the most represented compounds, carvacrol and terpinen-4-ol. However, the content of several minor compounds including thymol, *p*-cymen-8-ol, and thymoquinone increased especially at 60 mM NaCl. Concerning the sesquiterpenes content, this class was reduced by salinity (**Table 3**).

Thus, the results showed that salinity improved the production of several valuable volatiles, which have potential use in pharmaceutical, food, and chemical industries; among the bioactive compounds, *p*-cymene and γ-terpinene have been reported as strong anti-inflammatory, insecticidal, and antispasmodic compounds (*23*, *51*, *52*). Moreover, thymol is well-known as a powerful antioxidant (*53*). Thymoquinone has a wide spectrum of activities and acts at very low concentrations; it was ascribed to possess anticancer, antibacterial, anti-inflammatory, and antioxidant properties, to decrease the nephrotoxicity of some chemotherapeutic agents, and to possess protective effects against gastric lesions (*23*, *54*, *55*). In addition, *p*-cymene is an important product and valuable intermediate in the chemical industry. Among others, it is used as a solvent for dyes and varnishes, as a heat transfer medium, as an additive in fragrances and musk perfumes, and as a masking odor for industrial products (*22*).

**Effect of Salinity on Phenolics Amounts.** In control, total polyphenols of black cumin seeds assessed by Folin−Ciocalteu reagent was 11.69 mg GAE/g DW. Salt treatments were found to

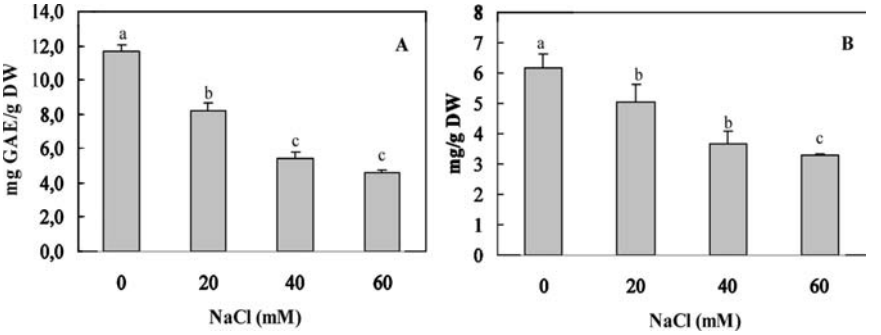


**Figure 4.** Effect of NaCl concentrations on the total phenolics quantified by Folin−Ciocalteu (**A**) and HPLC (**B**) of black cumin seeds. Means of three replicates. Values with different superscripts are significantly different at $p < 0.05$.

affect polyphenol contents, which decreased by 30, 54, and 61% in response to 20, 40, and 60 mM NaCl (**Figure 4**). Phenolics were further analyzed using RP-HPLC. In controls, total phenolics represented 5.63 mg/g DW and decreased by 20, 28, and 43% in response to 20, 40, and 60 mM NaCl. On the other hand, the amounts of total phenolic compounds assessed by HPLC were significantly lesser than the ones obtained by the Folin−Ciocalteu method. The latter was a predictable method due to the weak selectivity of the Folin−Ciocalteu reagent, as it reacts positively with different antioxidant compounds [phenolic and nonphenolic substances (*56*)]. However, these two methods confirmed that the total phenolics content of black cumin was altered proportionally to the salt stress intensity. Earlier studies reported that total polyphenols content decreased in coriander fruits cultured under salinity (*12*) and varied depending on maturity stage in salt-stressed pepper fruits (*57*). However, salt stress was found to increase total polyphenols content in the medicinal halophyte *Cakile maritima* (*13*). The enhancement of the phenolics metabolism is one of the responses to biotic and abiotic stresses (*58*). Environmental stresses such as salinity stimulate reactive oxygen species (ROS) production (*59*). Phenolics are well-known antioxidants acting as powerful radical scavengers and ions chelators (*26*), and their content increment under salinity is thought to be involved in the prevention of stress-induced oxidative damage or in maintenance of osmotic balance (*60*).

Furthermore, RP-HPLC analysis indicated that the decrease of black cumin phenolics amount was mainly mediated by the reduction of the most represented class, benzoates (**Figure 5**). In fact, the latter was reduced proportionally to the stress by 24, 29, and 44% at 20, 40, and 60 mM NaCl. Benzoic acids are strong antioxidant compounds; they are capable of reacting with and quenching radicals and forming chelating complexes with transition metals, and theses abilities are attributed to their structure, namely, the substituents of the phenyl ring (*61*, *62*). The decrease of benzoic acids content may affect the nutritional value of black cumin seeds, widely consumed in food, as a source of antioxidant compounds with high benefits for human health. Moreover, in control, cinnamates and flavonoids were weakly represented, and the effects of salinity on these classes were dose-dependent (**Figure 4**); cinnamates were significantly influenced only at 40 and 60 mM NaCl, while flavonoids decreased in response to the different NaCl treatments. Besides, both classes were found to be mostly reduced at 40 mM NaCl.

In conclusion, the investigation presented here revealed that although NaCl treatments decreased the seeds yield of black cumin, they do not alter the quality of the oil; in fact, the unsaturation degree remained stable. Moreover, salt treatments enhanced the proportion of the major fatty acid, linoleic acid, which is an essential fatty acid of great importance for human nutrition and industry. Salinity was found to significantly influence

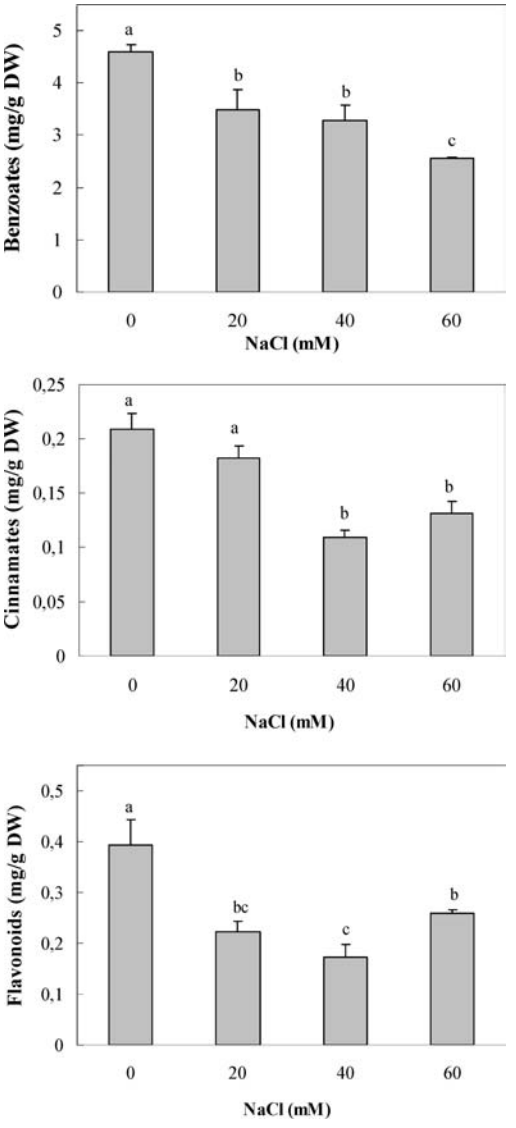


**Figure 5.** Effect of NaCl concentrations on benzoates, cinnamates, and flavonoids contents of black cumin seeds. Means of three replicates. Values with different superscripts are significantly different at $p < 0.05$.

the secondary metabolites production. It stimulates the essential oil production, especially monoterpenes hydrocarbons biosynthesis. Furthermore, stress influenced the quality of the oil by changing the chemotype from *p*-cymene in controls to $\gamma$-terpinene/*p*-cymene in stressed plants. However, salinity reduced phenolics biosynthesis, especially benzoic acids production. These results suggest the stimulation of isoprenoids

pathway by NaCl stress, while shikimate and phenypropanoid pathways were inhibited. Thus, black cumin seeds produced under saline conditions may function as a potential oil and essential oil source.

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