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Original Article

Relation between salt tolerance and biochemical changes in cumin (*Cuminum cyminum* L.) seeds

Iness Bettaieb Rebey[*], *Soumaya Bourgou*, *Fatma Zohra Rahali*, *Kamel Msaada*, *Riadh Ksouri*, *Brahim Marzouk*

Laboratoire des Plantes Aromatiques et Médicinales, Centre de Biotechnologie de Borj-Cédria, Hammem-Lif, Tunisia

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ABSTRACT

In this study, the effects of salinity on growth, fatty acid, essential oil, and phenolic composition of cumin (*Cuminum cyminum* L.) seeds as well as the antioxidant activities of their extracts were investigated. Plants were treated with different concentrations of NaCl treatment: 0, 50, 75, and 125 mmoL. Plant growth was significantly reduced with the severity of saline treatment. This also caused important reductions in the seed yield and yield components. Besides, NaCl treatments affected fatty acid composition. Petroselinic and linoleic acids proportions diminished consistently with the increase in NaCl concentration, whereas palmitic acid proportion increased. Furthermore, NaCl enhanced essential oil production in *C. cyminum* seeds and induced marked changes on the essential oil quality. Essential oil chemotype was modified from γ-terpinene/1-phenyl-1,2 ethanediol in control to γ-terpinene/β-pinene in salt stressed plants. Total polyphenol content was higher in treated seeds, and salinity improved the amount of individual phenolic compounds. Moreover, antioxidant activities of the extracts were determined by four different test systems, namely 2,2-diphenyl-1-picrylhydrazyl, β-carotene/linoleic acid chelating, and reducing power assays. The highest antioxidant activities were reveled in severe stressed plants. In this case, cumin seeds produced under saline conditions may function as a potential source of essential oil and antioxidant compounds, which could support the utilization of this plant in a large field of applications such as food industry.

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1. Introduction

In modern agriculture, there are species with secondary metabolites that have desired aromatic qualities, therapeutic specificities, or provide a generous source material for the perfume and chemical industries [1]. Natural products, especially those produced by edible and medicinal plant species, are currently under special interest as food additives due to their safety, usefulness, and accessibility. Cumin (*Cuminum*

* *Corresponding author*. Laboratoire des Plantes Aromatiques et Médicinales, Centre de Biotechnologie de Borj-Cedria, BP 901, Hammem-Lif 2050, Tunisia.
E-mail address: rosainess@yahoo.fr (I. Bettaieb Rebey).

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cyminum L.), a member of the Apiaceae family, is one of these species. It is cultivated mainly in India, China, Saudi Arabia, and in the countries adjoining the Mediterranean Sea [2]. Cumin seeds are used as popular aromatic herbs and culinary spices. It is used mainly in traditional and veterinary medicine as a stimulant, carminative, astringent, and as a remedy against indigestion, flatulence, and diarrhea [3]. All these properties make cumin a good candidate for being used as protective agent in food packaging, mainly to protect those foodstuffs that cannot be spiked or produced with additives, such as fresh products. The composition of *C. cyminum* essential oil (EO) from different regions has been the subject of previous studies [4].

Salinity is one of the main limiting factors for agricultural production. Plants subjected to high salinity levels undergo various physiological and biochemical changes, leading to numerous modifications in the structure and function of cell membranes [5]. If global food production is to be maintained, it seems reasonable to predict that enhancement of salt tolerance of crops will be increasingly important to many plant breeding programs [6]. In Tunisia, salinity affects about 10% of the whole territory. Crops are increasingly exposed to this constraint accentuated by increasing climate aridity [7]. In view of the wide extending soil salinity, the study of chemical responses of the medicinal and aromatic plants to salinity needs some focus in Tunisia. Plant responses to NaCl effects have been studied intensively using anatomical, physiological, molecular, and proteomic approaches [8]. Salinity causes an imbalance of the cellular ions resulting in osmotic stress [9] which makes water uptake difficult [10]. It also causes a limitation of nutrient uptake due to the ability of sodium to compete with the essential cations necessary for cell function [11]. Salinity impact on EO and fatty acid composition has been recently reported in different aromatic and medicinal plants, such as coriander [12], black cumin [13], sweet majorana [14], and ocimum [15].

One of the major consequences of various environmental stresses, including salinity, is oxidative stress mediated by increased levels of reactive oxygen species (ROS). ROS have the potential to interact with many cellular components, causing significant damage to membranes and other cellular structures [16]. Plants produce a large number of antioxidants aimed at scavenging or detoxifying ROS [17]. In plants, the biosynthesis of polyphenol and their accumulation are generally stimulated in response to biotic/abiotic constraints [18], such as salinity [19]. Phenolic compounds act as antioxidants that protect plants against the damaging effects of increased ROS levels due to salt stress [20]. Therefore, salt-treated plants might represent potential sources of polyphenols by increasing polyphenol concentration in the tissues and restricting biomass production. Thus, as proposed by De Abreu and Mazzafera [21], optimal polyphenol yield would be obtained using stress-tolerant species.

Whereas, to the best of our knowledge, there have been no previous reports relative to cumin bioactive compounds production and the capacity of these molecules to scavenge toxic free radicals under salinity stress. Thus, this study was undertaken to evaluate, for the first time, the effect of NaCl impact on some biochemical responses of *C. cyminum* seeds as well as the antioxidant behaviors of their extracts.

2. Materials and methods

2.1. Plant material and growth conditions

Cumin seeds were collected from cultivated plants in the region of Menzel Temime (Northeastern Tunisia). Botanical identification of this species was carried out by Professor A. Smaoui (Biotechnologic Center in Borj-Cedria Technopark, Tunisia), and a voucher specimen was kept in our laboratory for future reference. Seeds were germinated on an inert substrate at 25°C. After 10 days, seedlings were transferred to 6-L plastic pots (six plants per pot) and were hydroponically cultivated using aerated quarter-strength Hoagland's solution [22]. At two-leaf stage, plants were separated in four groups, irrigated with a nutrient solution that was supplemented with different NaCl concentrations (0, 50, 75 and 125 mmoL). To avoid osmotic shock, salt concentrations increased stepwise daily by 25 mmol NaCl. The nutrient solutions were replaced after every 4 days. The experiment was replicated six times and was performed under controlled conditions (18–25°C temperature, 16/8 hours light/darkness, 60–80% relative humidity, and under artificial light of 141 μmol/m^2/s). Seeds were harvested at the fruiting stage, 15 weeks after treatment and weighted.

2.2. Oil extraction

Cumin seeds were finely ground with a type A10 blade-carbide grinding (Ika-Werk, Staufen, Germany). Next, 10 g of each ground sample was extracted using a soxhlet apparatus with 100 mL hexane (LabScan Ltd., Dublin, Ireland) for 6 hours. The extraction was protected against light. Oil was removed after mixture filtration and solvent evaporation under reduced pressure.

2.3. Total lipid extraction

Total lipids from seeds were extracted using the modified method of Bligh and Dyer [23], according to Marzouk and Cherif [24]. Thus, 0.5 g air-dried seeds were fixed in boiling water for 5 minutes and then ground manually with chloroform–methanol–hexane mixture (1:2:1, v/v/v). After washing with water of fixation and decantation during 24 hours at 4°C, the organic phase containing total lipids was dried under a stream of nitrogen, dissolved in toluene–ethanol (4:1, v/v) mixture, and stored at −80°C for further analyses.

2.4. Fatty acid methylation and analysis

Total fatty acids were converted into their methyl esters using 3% sodium methylate in methanol, according to the method described by Cecchi et al [25]. Heptadecanoic acid (C17:0) methyl ester was used as an internal standard to quantify fatty acids. The superior phase that contains fatty acid methyl esters (FAMEs) was aspired and the solvent volume reduced under a stream of nitrogen prior to analysis. FAMEs were analyzed by gas chromatography using a Hewlett-Packard 6890 chromatograph (Agilent Technologies, Palo Alto, CA,

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USA) equipped with a flame ionization detector and an electronic pressure control injector. They were separated on a RT-2560 capillary column (100 m length, 0.25 mmoL i.d., 0.20 mmoL film thickness). The oven temperature was kept at 170°C for 2 minutes, followed by a 3°C min/min ramp to 240°C, and finally held there for additional 15 minutes. Nitrogen (U) was used as a carrier gas at a flow rate of 1.2 mmoL/min. The injector and detector temperatures were maintained at 225°C. A comparison of the retention times of the FAMEs with those of coinjected authentic standards (Analytical Reagent, Lab-Scan Ltd., Dublin, Ireland) was made to facilitate identification.

2.5. EO extraction

Samples of air-dried cumin fruits were finely ground in an electric grinder (Type A10, IKA-Werke, Staufen, Germany). Next, 50 g of each ground sample was subjected to hydrodistillation for 120 minutes. The hydrodistillation was performed by a simple laboratory Quickfit apparatus consisting of a 1000 mL steam generator flask, distillation flask, condenser, and a receiving vessel. The distillate obtained was extracted using diethyl ether as a solvent (v/v) and dried over anhydrous sodium sulphate. The organic layer was subsequently concentrated at 35°C using a Vigreux column, and the EO was stored at −20°C prior to analysis.

2.6. Gas chromatography

Analytical gas chromatography was performed on a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector and an electronic pressure control injector [12]. A polar HP Innowax (PEG) column and an apolar HP-5 column (30 m × 0.25 mmoL, 0.25 μm film thickness) were used. The flow of the carrier gas (N^2) was 1.6 mL/min. The split ratio was 60:1. The analysis was performed using the following temperature program: oven temperature isotherm at 35°C for 10 minutes, from 35°C to 205°C at the rate of 3°C/min and isotherm at 205°C during 10 minutes. Injector and detector temperatures were held at 250°C and 300°C, respectively.

2.7. Gas chromatography–Mass spectrometry (GC–MS)

GC–MS analysis was performed on a gas chromatograph HP 5890 (II) interfaced with a HP 5972 mass spectrometer with electron impact ionization (70 eV). A HP-5MS capillary column (30 m × 0.25 mmoL, 0.25 μm film thickness) was used [12]. The column temperature was programmed to rise from 50°C to 240°C at the rate of 5°C/min. The carrier gas was helium with a flow rate of 1.2 mL/min; split ratio was 60:1. Scan time and mass range were 1 second and 40–300 m/z, respectively.

2.8. Compound identification

Compound identification was assigned by comparison of their retention indices relative to (C_8–C_{22}) n-alkanes with those of literature or 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 [26]. The percentage determination was based on peak area normalization without using correction factors.

2.9. Polyphenol extraction and analyses

2.9.1. Preparation of extracts

Seed extracts were obtained by stirring 1 g of dry material powder with 10 mL of 80% acetone for 30 minutes. Extraction was carried out using maceration at room temperature for 24 hours followed by filtration through Whatman No. 4 filter paper and after evaporation to dryness. The yield (%) of evaporated dried extracts was calculated as 100 × DWextr/DWsamp, where DWextr is the weight of extract after evaporation of solvent, and DWsamp is the dry weight of the original sample. Samples were stored at 4°C until analysis.

2.9.2. Total phenolic content (TPC)

The TPC of the acetone extracts was determined using Folin–Ciocalteu reagent (Merck), according to the procedure described by Dewanto et al [27]. Briefly, 125 μL of sample extract was dissolved in 500 μL of distilled water and 125 μL of Folin–Ciocalteu reagent. The mixture was shaken, before addition of 1.25 mL of 7% Na_2CO_3, adjusting with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark for 90 minutes, the absorbance at 760 nm was measured versus the prepared blank. TPC was expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through a calibration curve with gallic acid.

2.9.3. Reverse phase high performance liquid chromatography evaluation of phenolic compounds

Dried samples from cumin seeds were hydrolyzed according to the method of Proestos et al [28]. Next, 20 mL of 80% acetone containing butylated hydroxytoluene (1 g/L) was added to 0.5 g of the dried sample. Then, 10 mL of 1M HCl was added. The mixture was stirred carefully and sonicated for 15 minutes and refluxed in a water bath at 90°C for 2 hours. The obtained mixture was injected to HPLC. The phenolic compound analysis was performed using an Agilent Technologies 1100 series liquid chromatograph (RP–HPLC) coupled with a UV-Vis multiwavelength detector. The separation was carried out on a 250 × 4.6-mmoL, 4-μm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program MOLE was as follows: 15% A/85% B 0–12 minutes, 40% A/60% B 12–14 minutes, 60% A/40% B 14–18 minutes, 80% A/20% B 18–20 minutes, 90% A/10% B 20–24 minutes, and 100% A 24–28 minutes. The injection volume was 20 μL, and peaks were monitored at 280 nm. Samples were filtered through a 0.45-μm membrane filter before injection. Peaks were identified by congruent retention times compared with standards.

2.9.4. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Radical scavenging activity was determined according to Hanato et al [29]. First, 2 mL of the extract at different

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concentrations were added to 0.5 mL of a 0.2 mmoL DPPH methanolic solution. After shaking, the mixture was incubated at room temperature in the dark for 30 minutes, and the absorbance was subsequently measured at 517 nm. Butylated hydroxyanisole (BHA) was used as a positive reference and methanol as a negative reference. DPPH radical scavenging activity was expressed as the inhibition percentage (I%) and was calculated using the following formula:

$$I\% = 100 \times (A\ blank - A\ sample)/A\ blank$$

where A blank is the absorbance of the control at 30-minute reaction (containing all reagents except the test compound), and A sample is the absorbance of the sample at 30 minutes. Antiradical activity was expressed as IC_{50} and defined as the concentration of the extract generating 50% inhibition.

2.9.5. *β-Carotene–linoleic acid bleaching assay*

Antioxidant activity was evaluated according to the β-carotene bleaching method described by Tepe et al [30]. A stock solution of β-carotene–linoleic acid mixture was prepared by dissolving 0.5 mg of β-carotene in 1 mL of chloroform and adding 40 mg of linoleic acid with 400 mg of Tween 40. Chloroform was completely evaporated using a vacuum evaporator. Subsequently, 100 mL of oxygenated distilled water was added to the residue; 3 mL of this mixture was dispensed to test tubes, and 200 μL of each extract was added. The emulsion system was incubated for 2 hours at 50°C, together with two controls, one containing BHT as a positive control and another with the same volume of distilled water instead of the extracts. In the test tube with BHT, the yellow color was maintained during the incubation period, and the absorbance was measured at 470 nm.

2.9.6. *Chelating effect on ferrous ions*

The ferrous ion chelating activity of different organ extracts and EOs was assessed as described by Zhao et al [31]. Different concentrations of the sample were added to 0.05 mL of $FeCl_2\_4H_2O$ solution (2 mmoL) and left for incubation at room temperature for 5 minutes. Next, the reaction was initiated by adding 0.1 mL of ferrozine (5 mmoL), and the mixture was adjusted to 3 mL with deionized water, shaken vigorously, and left standing at room temperature for 10 minutes. Absorbance of the solution was subsequently measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the formula given below:

$$\text{Metal chelating effect}(\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the ferrozine–Fe^{2+} complex, and A_1 is the absorbance of the test compound. Results were expressed as IC_{50}, efficient concentration corresponding to 50% ferrous iron chelating. EDTA was used as a positive control.

2.9.7. *Reducing power*

The reducing power of different seed extracts was assessed using the method of Oyaizu [32]. A volume of 1 mL of different concentrations of seed extracts in 80% acetone were mixed with 2.5 mL of 0.2 mmoL sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$], and incubated in a water bath at 50°C for 20 minutes. Next, 2.5 mL of 10% trichloroacetic acid was added to the mixture that was centrifuged at 650*g* for 10 minutes. The supernatant (2.5 mL) was subsequently mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The intensity of the blue–green color was measured at 700 nm. The EC_{50} value (mg/mL) is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as a positive control.

2.10. *Statistical analysis*

Data were subjected to statistical analysis using statistical program package STATISTICA. The percentage of each parameter was the mean of six replicates ± standard deviation, and the differences between individual means were deemed to be significant at $p < 0.05$.

3. Results and discussion

3.1. *Effect of salinity on plant growth and yield components*

As shown in Table 1, significant decreases in plant growth were observed with the severity of salt treatment. Therefore, the application of 50 mmoL NaCl caused a light drop (6.11%) in the plant height, whereas 75 mmoL and 125 mmoL of NaCl reduced the plant height by about 32.15% and 54%, respectively, compared with the controls. Also, we noted that salinity led to a substantial decline in dry matter estimated by about 18.5%, 35.61%, and 29.12% with 50, 75, and 125 mmoL of NaCl, respectively. These results indicated that salinity limited the biomass production of cumin seeds and can be explained, according to Lovelock and Ball [33] by the reduction of carbon fixation and the biomass allocation between leaf, stem, and root, which would alter the balance of photosynthesis and respiration. Other possibilities relate to osmotic adjustment: perhaps an inability to accumulate and/or distribute sufficient nutrients or synthesize sufficient organic solutes, the futile cycling of ions [34], or the energetic demands of ion compartmentation *per se* [35].

As shown in Figure 1, increasing salinity levels in the growth medium caused a marked inhibitory effect on seed yield per plant of *C. cyminum*. This decrease was observed at 50, 75, and 125 mmoL NaCl by about 29%, 39%, and 47%, respectively, compared with the controls. The depressive effect of salt on seed yield has been reported earlier in several aromatic and medicinal plants including *Foeniculum vulgare* [36] and *Trachyspermum ammoli* [37]. One cause of this yield reduction under saline constraint is an inadequate photosynthesis owing to stomatal closure limiting carbon dioxide uptake [38]. Concomitant with the decrease in seed yield, the number of umbels per plant, the 1000 seed weight as well as the number of umbellets per umbel of *C. cyminum* diminished significantly with the increasing concentration of NaCl (Figure 1). A decrease in seed yield might arise from a reduction of flower production and/or a decrease of their fertility.

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Table 1 – Effect of salinity on *Cuminum cyminum* L. plant height, fresh matter, dry matter, weight, dry matter percentage, and seed yield (per plant).

	Height (cm)	Fresh matter weight (g)	Dry matter weight (g)	Dry matter (%)	Seed yield per plant (g)
0 mmoL	8.44 ± 0.33^{a}	5.37 ± 0.10^{a}	1.75 ± 0.03^{a}	30.60 ± 0.03^{a}	3.04 ± 0.02^{a}
50 mmoL	7.92 ± 0.41^{b}	$4.16 \pm 0.05^{a,b}$	1.04 ± 0.12^{b}	25.04 ± 0.08^{b}	2.15 ± 0.04^{b}
75 mmoL	5.72 ± 0.66^{c}	3.45 ± 0.02^{b}	0.67 ± 0.01^{c}	19.70 ± 0.22^{c}	1.85 ± 0.08^{b}
125 mmoL	$3.88 \pm 0.06^{d,c}$	2.03 ± 0.02^{c}	0.44 ± 0.02^{d}	21.68 ± 0.02^{c}	1.61 ± 0.05^{b}

Values with different superscripts (a–d) are significantly different at $p < 0.05$ (means of six replicates ± SD).

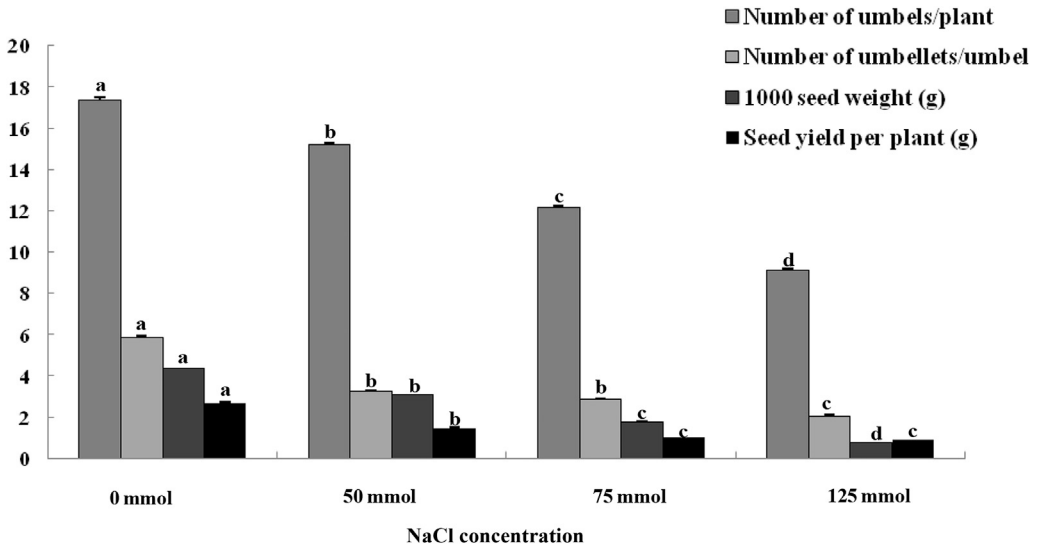

Figure 1 – Effect of salinity on yield components in *Cuminum cyminum* L. seeds. Values followed by different superscripts (a–d) in the rows are significantly different at $p < 0.05$ (means of six replicates).

The accumulation of salt in pollen and stigma is known to be strongly implied in salt-induced sterility [39].

3.2. *Salinity effect on oil yield and fatty acid composition*

Based on our experimental data (Figure 2), it was shown that the oil yield (%) of cumin seeds declined sharply with the severity of NaCl treatment. A progressive decrease with increasing salinity level was also observed. Thus, salt reduced oil yield on average 15.51%, 32.26%, and 45.23% with 50, 75, and 125 mmoL of NaCl, respectively, compared with the controls (Figure 2). In a previous works Irving et al. [40] reported, also, a decrease of oil content in safflower seeds. In cumin seeds, the reduction of oil yield could be attributed to inhibition of lipid biosynthesis and/or stimulation of lipolytic and peroxidative activities under salt effect [41]. As shown in Table 2, lipids extracted from cumin seeds are dominated by C16 and C18 fatty acids which are typical in higher plants [42]. Analysis of fatty acid composition indicated that in controlled plants, petroselinic acid (C18:1 n12) was the major compound [55.9% of total fatty acid (TFA)], followed by palmitic (C16:0) and linoleic (C18:2 n6) acids, which constitute 23.82% and 12.40% of TFA, respectively (Table 2). Cumin seeds were characterized by the presence of a high proportion of monounsaturated fatty acids (MUFAs) (58.34% of TFA). Polyunsaturated (PUFAs) and saturated fatty acids (SFAs) represented 12.61% and 27.88% of TFA, respectively. It is well known that fatty acids, the main components of plasma membrane lipids, are considered important in salt tolerance of plants by maintaining the membrane fluidity [43]. As shown in Table 2, fatty acid composition of cumin seeds was modified by NaCl treatments. Salinity reduced the percentage of petroselinic acid by 22.36, 20.72, 19.49; and that of linoleic one by

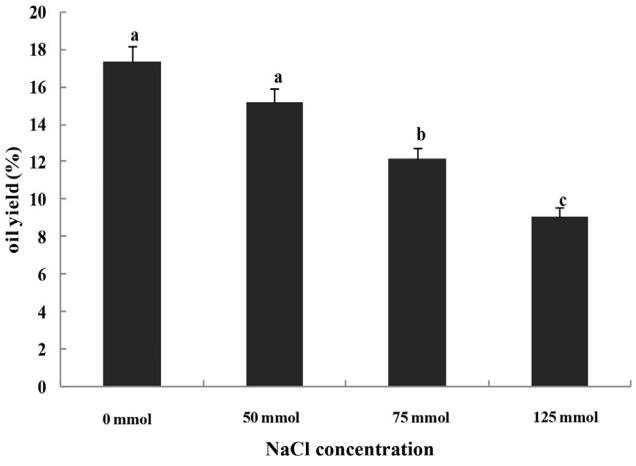

Figure 2 – Effect of salinity on oil yield (% DM) of *Cuminum cyminum* L. seeds. Values with different superscripts (a–c) are significantly different at $p < 0.05$ (means of six replicates).

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Table 2 – Effect of salinity on fatty acid composition (%) and DBI changes from *Cuminum cyminum* L. seeds.

Fatty acids (%)	0 mmoL	50 mmoL	75 mmoL	125 mmoL
C8:0 (caprylic acid)	1.63 ± 0.03^{a}	1.54 ± 0.11^{a}	1.66 ± 0.27^{a}	0.66 ± 0.27^{b}
C10:0 (capric acid)	0.92 ± 0.01^{b}	1.01 ± 0.02^{b}	0.83 ± 0.03^{b}	1.83 ± 0.03^{a}
C12:0 (lauric acid)	0.16 ± 0.01^{b}	4.12 ± 0.34^{a}	$3.10 \pm 0.65^{a,b}$	$2.10 \pm 0.65^{a,b}$
C13:0 (tridecanoic acid)	1.20 ± 0.02^{a}	1.54 ± 0.02^{a}	0.34 ± 0.00^{b}	1.34 ± 0.00^{a}
C14:0 (myristic acid)	0.15 ± 0.00^{a}	0.18 ± 0.01^{a}	0.03 ± 0.00^{b}	0.23 ± 0.00^{a}
C16:0 (palmitic acid)	23.82 ± 0.10^{c}	34.16 ± 1.45^{b}	$39.22 \pm 2.34^{a,b}$	44.02 ± 2.34^{a}
C16:1n7 (palmitoleic acid)	2.12 ± 0.01^{a}	$1.45 \pm 0.07^{a,b}$	0.63 ± 0.01^{b}	0.43 ± 0.01^{b}
C18:1n9 (oleic acid)	0.32 ± 0.09^{a}	0.34 ± 0.02^{a}	0.29 ± 0.02^{a}	0.49 ± 0.02^{a}
C18:1n12 (petroselinic acid)	55.9 ± 0.34^{a}	43.4 ± 1.88^{b}	45.66 ± 1.92^{b}	35.66 ± 1.92^{c}
C18:2n6 (linoleic acid)	12.40 ± 0.11^{a}	9.83 ± 0.54^{b}	6.43 ± 0.09^{c}	3.48 ± 0.09^{d}
C18:3n-3 (α-linolenic acid)	0.20 ± 0.02^{a}	0.24 ± 0.01^{a}	0.15 ± 0.02^{a}	0.10 ± 0.02^{a}
SFA	$27.8\ 8 \pm 0.12^{c}$	42.55 ± 1.52^{b}	45.18 ± 1.45^{b}	50.18 ± 1.45^{a}
MUFA	58.34 ± 0.87^{a}	45.19 ± 0.84^{b}	46.58 ± 0.09^{b}	36.58 ± 0.09^{c}
PUFA	12.61 ± 0.11^{a}	10.07 ± 0.26^{a}	6.58 ± 0.78^{b}	3.58 ± 0.78^{c}
UFA	70.95 ± 0.14^{a}	55.26 ± 0.89^{b}	43.16 ± 0.22^{c}	$40.16 \pm 0.22^{c,d}$
DBI	0.81 ± 0.01^{a}	$0.65 \pm 0.01^{a,b}$	$0.59 \pm 0.00^{a,b}$	0.43 ± 0.00^{c}

Values with different superscripts (a–d) are significantly different at $p < 0.05$ (means of six replicates).
DBI = double bound index; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid; UFA = unsaturated fatty acid.

48.14 and 37.38; 71.93%, respectively, at 50, 75 and 125 mmoL NaCl. In addition, the level of linoleic acid diminished especially in accordance to the degree of salt constraint. In this context, Zhang et al [44] reported that the decreased level of linoleic acid under abiotic stress reflects damage. In contrast, the decrease in the level of petroselinic acid with increasing NaCl concentrations was accompanied by a concomitant increase of palmitic acid proportion and the increases were 47%, 69%, and 91% at 50, 75, and 125 mmoL NaCl, respectively. Likewise, increasing salinity levels at 50, 75, and 125 mmoL improved lauric acid proportion by about 25-fold, 19-fold, and 13-fold, respectively, compared with the control. Similarly to our results, membrane PUFAs were decreased, whereas SFAs were increased in borage leaves under high salinity, which was considered as an adaptation to salinity [45]. The authors indicate that the decrease in fatty acid unsaturation under salt was due to a reduction in the desaturase activity, which suggested as an adaptive feature to salinity. In addition, saline stress can be toxic to lipid metabolism, due to Na^{+} and Cl^{-} accumulation. All enzymatic activities including that of desaturase might be disrupted. In developing seeds, fatty acid synthesis up to the formation of 18:1 occurs in plastids, whereas desaturation to 18:2 and 18:3 occurs in the cytosol [46]. Therefore, environmental factors may affect fatty acids not only by modifying enzyme activities but also by affecting fatty acid transport from the plastid.

The degree of fatty acid unsaturation is important to maintain the membrane fluidity and to provide the appropriate environment for membrane functions [47]. In addition, Mansour et al [48] confirmed that changes in plasma membrane lipid composition may result in plasma membrane fluidity changes, as suggested by previous studies that a less fluid plasma membrane bilayer supports reduced NaCl permeability, stimulating the formation of a more rigid membrane. In cumin seeds, under 50, 75 and 125 mmoL NaCl concentrations, the unsaturated fatty acid (UFA) to SFA ratio was reduced from 2.54 to 0.80 which leads probably to a decrease in the passive membrane permeability [49]. The effect of salinity on the degree of fatty acids unsaturation was assessed using the double bond index calculated according to Rie De Vos et al [50]. Results showed that this parameter decreased in comparison to the control. This result indicates that salinity affects the unsaturation degree of the fatty acids pool and thus the quality and the stability of the cumin oil. This fact could be explained by a possible reduction of the desaturase activity, which appeared as an adaptive feature to salinity [51]. In fact, the ability to adjust membrane lipid fluidity by changing levels of UFAs is a feature of stress acclimating plants provided mainly by the regulated activity of fatty acid desaturases. Modification of membrane fluidity results in an environment suitable for the function of critical integral proteins, such as the photosynthetic machinery, during stresses [52]. In good agreement with our results, a decrease in the unsaturation degree was observed in *Salvia officinalis* [5] and *Coriandrum sativum* [12], whereas an increase was observed in *Carthamus tinctorius* [53], indicating that the response to salt constraint depends on plant species.

3.3. *Salinity effect on EO yield and component proportions*

As shown in Figure 3, in the control seeds, EO yield was 1.80%, based on their dry weight and was significantly affected by the salt treatment. The application of increasing NaCl concentrations (50, 75, and 125 mmoL) resulted in EO yield of 1.80%, 2.22%, and 2.80%, respectively. Thus, NaCl enhances EO production of *C. cyminum*.

It is well known that EO yield is influenced by intrinsic parameters (such as growth stages) and extrinsic ones (such as pedoclimatic conditions and salinity). Significant changes of EO yield ($p < 0.05$), as calculated on the basis of dry weight, were observed among the different salt doses for EO yield (w/w). The EO yield increased significantly with the constraint severity and reached 2.80% for 75 mmoL NaCl (Figure 3). Such increase of EO yield by salinity has been reported earlier in leaves of other plant species, such as *Salvia officinalis* [5],

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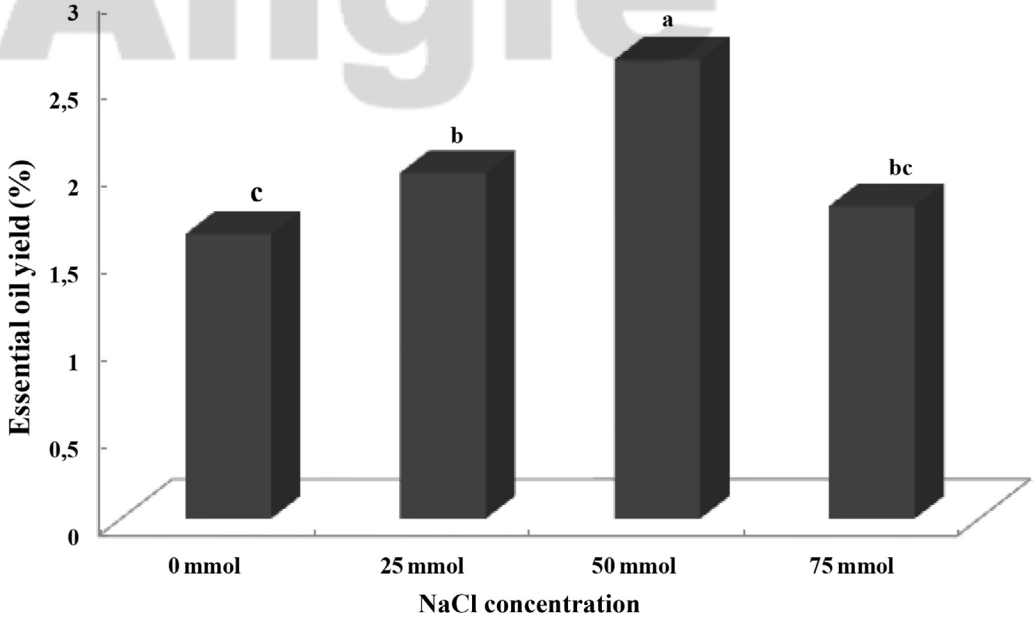

Figure 3 – Effect of salinity on essential oil yield (%) of *Cuminum cyminum* L. seeds. Values with different superscripts (a–c) are significantly different at $p < 0.05$ (means of six replicates).

Coriandrum sativum [53] and *Nigella sativa* [13]. The stimulation of EO production under salinity could be due to a higher oil gland density and an increase in the absolute number of glands produced [53]. Conversely, salt constraint decreased oil yield of *Carthamus tinctorius* [54] and *Salvia sclarea* [55].

Changes in EO composition of cumin seeds under salt are illustrated in Table 3. In the control, 40 compounds were identified, accounting for 94.67% of total constituents. The EO was characterized by the dominance of terpenic hydrocarbons, which constituted the main class (54.38%), followed by alcohols (26.90%), whereas esters were weakly represented (0.03%). The EO was of the γ-terpinene (25.58%)/1-phenyl-1,2 ethanediol (23.16%) chemotype. Other main compounds were β-pinene (15.16%), *p*-cymene (9.05%), and cuminaldheyde (15.31%). Numerous literatures concerning the EO composition of *C. cyminum* seeds exist. However, there is no information about NaCl salinity effect on this composition.

Therefore, application of salinity induced marked changes in the EO quality.

The γ-terpinene percentage increased with the different treatments by about 19%, 36.82%, and 6.84% at 50, 75, and 125 mmoL NaCl concentrations, respectively. Furthermore, salinity improved the percentage of β-pinene by about 35%, compared with the control, at 75 mmol NaCl. The latter became the second most abundant compound in the oil. Indeed, its percentage reached 18.84%, 20.37%, and 18.11% under 50, 75, and 125 mmol NaCl, respectively. Also, under the effect of salinity at 50, 75, and 125 mmol NaCl, cuminaldehyde proportions increased by about 9.87%, 17.76%, and 6.84%, respectively, compared with the control. However, the salt stress decreased the level of 1-phenyl-1,2 ethanediol and *p*-cymene under the different NaCl concentrations. Thus, salinity induced the modification of the EO chemotype from γ-terpinene/1-phenyl-1,2 ethanediol in the control seeds to γ-terpinene/β-Pinene in salt treated seeds. This change will probably result in the modification of the EO 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 changes to a citrus- and green-like aroma note at low concentrations, whereas γ-terpinene has a citrus aroma [13]. The decrease of *p*-cymene percentage accompanied by the increase of γ-terpinene one is in accordance with their biosynthetic. Major changes observed in the composition were due to the relative proportions of constituents and not due to the presence of new or the absence of particular ones. In this context, Hendawy and Khalid [56] reported that variations in EO yield and composition could be due to its effect on enzyme activity and metabolism improvements.

3.4. *Salinity effect on TPC*

In control plants, seeds extracts offered a TPC estimated for 18.35 and 14.84 mg GAE/g DW, respectively, by Folin–Ciocalteu and hydrolysis acid methods. Thus, the contents of phenolic compounds as assessed by RP-HPLC are too inferior to those obtained by the Folin–Ciocalteu method. This difference could be explained by the weak selectivity of the Folin–Ciocalteu reagent as it reacts positively with different antioxidant compounds [57].

Under salt treatment, TPC increased proportionally with the increase in salt concentration (Table 4). The increase was slight in response to low salinity (25 mmoL NaCl), but it was high under moderate and especially severe salinity; in fact, the stimulations were about 87% at 75 mmoL NaCl and 122% at 125 mmoL NaCl. These results suggest that the phenylpropanoid metabolism in cumin seems to be stimulated under salinity. Allocation of carbon to carbon-based secondary

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Table 3 – Salinity impact on essential oil composition (%) of *Cuminum cyminum* L. seeds.

Compounds*	RI[a]	RI[b]	Identification	NaCl (mmoL)			
				0	50	75	125
Terpenic hydrocarbons				54.38 ± 0.30[b]	61.17 ± 0.22[a]	62.04 ± 0.44[a]	55.59 ± 0.75[b]
α-Pinene	922	1065		0.22 ± 0.01[b]	0.20 ± 0.02[b]	0.22 ± 0.01[b]	0.81 ± 0.01[a]
α-Thujene	928	1035	MS	0.66 ± 0.03[b]	0.44 ± 0.04[b]	0.60 ± 0.04[b]	1.25 ± 0.02[a]
Camphene	954	1076	RI, MS	0.23 ± 0.01[b]	0.56 ± 0.03[a]	0.25 ± 0.02[b]	0.75 ± 0.02[a]
β-Pinene	980	1118	RI, MS, Co-GC	15.16 ± 0.32[b]	18.84 ± 0.13[a]	20.37 ± 0.52[a]	18.17 ± 0.22[a]
Sabinene	975	1132	RI, MS	0.44 ± 0.02[a]	0.22 ± 0.04[b]	0.03 ± 0.01[c]	0.45 ± 0.02[a]
α-Terpinene	1018	1188	MS, Co-GC	1.30 ± 0.04[a]	0.53 ± 0.01[b]	0.27 ± 0.32[b]	0.55 ± 0.02[b]
1-8,cineole	1033	1233	RI, MS, Co-GC	0.29 ± 0.01[b]	0.78 ± 0.04[a]	0.24 ± 0.01[b]	0.35 ± 0.05[b]
(E)-β-Ocimene	1040	1266	RI, MS	0.40 ± 0.03[a]	0.20 ± 0.01[b]	0.06 ± 0.01[c]	0.04 ± 0.01[c]
γ-Terpinene	1062	1255	RI, MS	25.58 ± 1.22[b]	30.45 ± 0.02[a]	35.74 ± 0.22[a]	27.17 ± 0.32[b]
p-Cymene	1026	1280	RI, MS, Co-GC	9.05 ± 0.17[a]	7.15 ± 0.03[b]	3.19 ± 0.03[c]	4.44 ± 0.88[c]
Terpinolene	1092	1290	RI, MS, Co-GC	0.03 ± 0.00[b]	0.25 ± 0.01[a]	0.17 ± 0.01[a]	0.12 ± 0.03[ab]
(E)-β-Farnesene	1461	1770	RI, MS, Co-GC	0.21 ± 0.01[b]	0.17 ± 0.02[b]	0.06 ± 0.01[c]	0.84 ± 0.04[a]
Diepi-α-Cedren	1450	1762	MS	0.37 ± 0.02[b]	0.80 ± 0.02[a]	0.45 ± 0.02[b]	0.64 ± 0.01[a]
α-Curcumene	1474	1786	MS	0.03 ± 0.01[b]	0.05 ± 0.01[a]	0.01 ± 0.01[b]	0.08 ± 0.01[a]
γ-Cadinene	1525	1773	RI, MS, Co-GC	0.19 ± 0.02[a]	0.16 ± 0.02[a]	0.11 ± 0.01[a]	0.09 ± 0.01[b]
Germacrene-D	1480	1715	RI, MS	0.16 ± 0.01[b]	0.37 ± 0.01[a]	0.22 ± 0.02[b]	0.46 ± 0.01[a]
Alcohols				26.90 ± 1.55[a]	18.13 ± 1.74[b]	14.22 ± 1.97[c]	20.40 ± 2.87[b]
2,Ethyl-1-hexanol	1101	1553	MS, Co-GC	0.14 ± 0.01[c]	0.50 ± 0.02[a]	0.54 ± 0.04[a]	0.30 ± 0.07[b]
p-Menth-2-en-1-ol	1130	1638	MS	0.17 ± 0.02[b]	0.30 ± 0.04[a]	0.22 ± 0.05[a]	0.09 ± 0.03[b]
1,4-*p*-Menthadien-7-ol	1315	1948	MS	0.02 ± 0.01[b]	0.06 ± 0.01[a]	0.04 ± 0.02[a]	0.01 ± 0.01[b]
p-Cymene-8-ol	1183	1864	RI, MS, Co-GC	0.06 ± 0.01[b]	0.12 ± 0.02[a]	0.17 ± 0.02[a]	0.09 ± 0.02[b]
Terpinene-4-ol	1178	1611	RI, MS, Co-GC	0.11 ± 0.02[a]	0.08 ± 0.01[a]	0.03 ± 0.01[b]	0.02 ± 0.01[b]
1-Phenyl-1-butanol	1355	1970	MS	3.17 ± 0.03[a]	1.50 ± 0.01[b]	2.37 ± 0.04[a]	0.50 ± 0.03[c]
1-Phenyl-1,2 ethanediol	1350	1973	MS	23.16 ± 2.11[a]	15.22 ± 0.07[b]	10.48 ± 0.09[c]	16.08 ± 0.08[b]
Carotol	1300	1897	RI, MS	tr	0.05 ± 0.35[b]	0.08 ± 0.03[b]	1.88 ± 0.02[a]
Geraniol	1255	1857	RI, MS, Co-GC	0.04 ± 0.01[b]	0.08 ± 0.02[b]	0.17 ± 0.01[b]	0.78 ± 0.02[a]
Eugenol	1356	2192	RI, MS, Co-GC	0.25 ± 0.02[b]	0.22 ± 0.44[b]	0.12 ± 0.01[b]	0.65 ± 0.03[a]
Aldehydes				15.83 ± 1.22[b]	14.70 ± 0.32[a]	18.53 ± 0.32[a]	16.66 ± 0.32[b]
Myrtenal	1237	1472	RI, MS, Co-GC	0.02 ± 0.01[b]	0.05 ± 0.01[b]	0.08 ± 0.01[b]	0.20 ± 0.02[a]
Safranal	1211	1460	RI, MS, Co-GC	0.05 ± 0.01[c]	0.10 ± 0.04[b]	0.18 ± 0.02[a]	0.12 ± 0.09[b]
Cuminaldheyde	1283	1785	MS	15.31 ± 2.12[ab]	13.90 ± 0.14[b]	18.03 ± 0.13[a]	16.06 ± 0.15[a]
Heptanal	902	1194	MS, Co-GC	0.15 ± 0.04[b]	0.23 ± 0.04[a]	0.28 ± 0.01[a]	0.18 ± 0.04[b]
Cinnamaldheyde	1283	1785	RI, MS, Co-GC	0.28 ± 0.02[b]	0.61 ± 0.05[a]	0.44 ± 0.11[a]	0.10 ± 0.04[b]
Ketones				0.24 ± 0.02[c]	0.38 ± 0.16[c]	1.54 ± 0.14[b]	2.30 ± 0.05[a]
Camphor	1143	1532	RI, MS, Co-GC	0.24 ± 0.02[a]	0.38 ± 0.04[a]	1.54 ± 0.05[b]	2.30 ± 0.07[a]
Epoxides				0.23 ± 0.02[a]	0.52 ± 0.01[a]	1.04 ± 0.01[a]	1.77 ± 0.01[a]
cis- Linalool oxide	1074	1478	RI, MS	tr	0.12 ± 0.02[a]	0.08 ± 0.01[a]	0.09 ± 0.01[a]
trans-Linalool oxide	1088	1475	RI, MS	0.06 ± 0.01[b]	0.24 ± 0.02[b]	0.67 ± 0.01[a]	0.94 ± 0.01[a]
Caryophyllene oxide	1596	2008	RI, MS	0.16 ± 0.02[b]	0.16 ± 0.04[b]	0.29 ± 0.03[b]	0.64 ± 0.07[a]
Phenols				0.17 ± 0.05[b]	0.09 ± 0.01[b]	0.13 ± 0.01[b]	0.57 ± 0.01[a]
Thymol	1290	2198	RI, MS	0.01 ± 0.00[b]	0.01 ± 0.02[b]	0.02 ± 0.01[b]	0.20 ± 0.01[a]
Carvacrol	1296	2215	MS	0.14 ± 0.02[a]	0.03 ± 0.01[b]	0.07 ± 0.01[b]	0.23 ± 0.01[a]
Apiole			MS	0.01 ± 0.00[b]	0.03 ± 0.07[b]	0.02 ± 0.01[b]	0.10 ± 0.04[a]
Methyl eugenol	1408	2004	RI, MS, Co-GC	0.01 ± 0.00[a]	0.02 ± 0.02[a]	0.02 ± 0.02[a]	0.04 ± 0.014[a]
Ester				0.03 ± 0.01[c]	0.10 ± 0.02[b]	0.24 ± 0.01[b]	0.44 ± 0.01[a]
Geranyl acetate	1383	1765	MS	0.03 ± 0.01[c]	0.10 ± 0.02[b]	0.24 ± 0.02[b]	0.44 ± 0.04[a]

Values with different superscripts (a–c) are significantly different at $p < 0.05$ (means of six replicates).
RI[a] = Order of elution in apolar column (HP-5); RI[b] = Order of elution in polar column (HP-Innowax); MS = mass spectrum; Co-GC = co-injection with authentic compound; RI = Retention indices relative to C8-C22 n-alkanes on the (HP-Innowax); tr = trace.

compounds is under genetic control and environmental conditions [58]. Literature survey indicates that plants vary widely in their response to environmental constraints. TPC of coriander seeds (*Coriandrum sativum* L.) treated with NaCl was significantly reduced [59]. Moreover, TPC varied in salt-treated pepper fruit depending on the maturation stage [19], whereas a stimulation effect of 50 and 75 mmoL NaCl on the production of polyphenols was reported by Agastian et al [60] in different genotypes of *Morus alba*.

3.5. *Salinity effect on phenolic composition*

Phenolic compounds are constituents of all higher plants. However, their biosynthesis is often induced when plants are exposed to environmental stresses, such as salinity. In this research, RP-HPLC analysis was used to identify the phenolic compounds of cumin seed by comparing with standard compounds. In the samples analyzed, it was possible to identify four hydrobenzoic acids: gallic acid, syringic acid,

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Table 4 – Quantitative (mg/g DW) changes of phenolic compounds in cumin seed extracts as influenced by salinity.

	0 mmoL	50 mmoL	75 mmoL	125 mmoL
Phenolic acids	10.49^{d}	11.83^{c}	19.68^{b}	21.26^{a}
Gallic acid	0.09 ± 0.02^{b}	0.20 ± 0.01^{b}	0.17 ± 0.02^{b}	1.12 ± 0.02^{a}
Cafeic acid	0.07 ± 0.00^{a}	0.04 ± 0.00^{a}	0.07 ± 0.04^{a}	0.05 ± 0.04^{a}
Dihydroxyphenolic acid	0.02 ± 0.00^{c}	0.07 ± 0.01^{c}	1.03 ± 0.01^{b}	2.05 ± 0.01^{a}
Dihydroxybenzoic acid	0.39 ± 0.02^{a}	0.12 ± 0.02^{b}	0.06 ± 0.01^{c}	0.03 ± 0.01^{c}
Chlorogenic acid	0.22 ± 0.01^{a}	0.18 ± 0.04^{a}	0.04 ± 0.01^{b}	0.07 ± 0.01^{b}
Syringic acid	0.64 ± 0.02^{a}	0.20 ± 0.01^{b}	0.22 ± 0.12^{b}	0.27 ± 0.12^{b}
Vanillic acid	0.03 ± 0.01^{b}	0.19 ± 0.03^{a}	0.15 ± 0.01^{a}	0.10 ± 0.01^{a}
p-Coumaric acid	4.83 ± 0.11^{b}	6.27 ± 0.09^{b}	9.03 ± 0.44^{a}	12.10 ± 0.44^{a}
Ferrulic acid	$0.47 \pm 0.03^{b,c}$	0.80 ± 0.05^{b}	3.10 ± 0.05^{a}	0.53 ± 0.05^{a}
Rosmarinic acid	$0.70 \pm 0.04^{a,b}$	1.96 ± 0.14^{a}	3.01 ± 0.02^{a}	2.51 ± 0.02^{b}
trans-2-Dihydrocinnamic acid	1.09 ± 0.41^{a}	0.60 ± 0.02^{b}	0.72 ± 0.04^{b}	0.22 ± 0.04^{b}
Cinnamic acid	0.94 ± 0.02^{b}	0.90 ± 0.06^{b}	2.08 ± 0.03^{a}	2.13 ± 0.03^{a}
Flavonoids	3.21^{d}	5.20^{c}	8.18^{b}	10.91^{a}
Luteolin	2.59 ± 0.24^{b}	2.14 ± 0.03^{b}	3.48 ± 0.11^{b}	6.28 ± 0.11^{a}
Catechin	0.23 ± 0.02^{a}	0.16 ± 0.01^{a}	0.15 ± 0.01^{a}	0.10 ± 0.01^{a}
Coumarin	0.21 ± 0.01^{b}	1.68 ± 0.04^{a}	0.04 ± 0.03^{c}	0.02 ± 0.03^{c}
Quercetin	0.02 ± 0.01^{b}	0.20 ± 0.03^{b}	1.10 ± 0.01^{a}	2.17 ± 0.01^{a}
Apigenin	0.03 ± 0.00^{a}	0.03 ± 0.01^{a}	0.02 ± 0.02^{a}	0.01 ± 0.02^{a}
Amentoflavone	0.01 ± 0.01^{c}	0.17 ± 0.01^{b}	1.26 ± 0.04^{a}	2.24 ± 0.04^{a}
Flavone	0.12 ± 0.02^{a}	0.02 ± 0.05^{b}	0.13 ± 0.01^{a}	0.09 ± 0.01^{a}
Unknown	1.14 ± 0.32^{a}	0.77 ± 0.03^{b}	2.08 ± 0.03^{a}	0.88 ± 0.03^{b}
Total	14.84	17.03	27.86	34.04

Values are means of six replications ($N \pm SD$). The data marked with the different letter, in the table, share significant differences at $p < 0.05$ (Duncan test).

dihydroxybenzoic acid, and vanillic acid; seven hydroxycinnamic acids: chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, cinnamic acid, *trans*-2-hydroxycinnamic acid, and rosmarinic acid; and seven flavonoids (Table 4). The most abundant phenolics were *p*-coumaric acid and luteolin. The results showed that the salinity enhanced the content of phenolic compounds. The accumulation of phenolic compounds in response to NaCl treatment was primarily caused by an increase in the concentrations of the two major compounds, *p*-coumaric acid and luteolin, despite the fact that the profile of minor compounds was changed (Table 4). Hence, *p*-coumaric acid content augmented with severity of salt stress. Thus, the increase was slight at 50 mmoL and abrupt at 75 and 125 mmoL NaCl. Especially, at the level of 125 mmoL, *p*-coumaric acid content was more than double compared with that of control seeds. Besides, luteolin biosynthesis seems to be enhanced by salinity as the content of this flavonoid showed an increasing trend with the increasing NaCl concentration.

Furthermore, the biosynthesis of several minor compounds was found to be significantly stimulated by salinity treatment. As shown in Table 4, rosmarinic acid content increased by about twofold, fourfold, and threefold in response to 50, 75, and 125 mmoL NaCl. Moreover, cinnamic acid biosynthesis was significantly stimulated under middle and high NaCl levels by 2.21-fold and 2.39-fold, respectively. The enhancement of amentoflavone content in treated seeds was significant; 75 and 125 mmoL NaCl induced the accumulation of these compounds 126 times (1.26 mg/g DW) and 224 times (2.24 mg/g DW), respectively, compared with that in the control (0.01 mg/g DW). On the other hand, ferulic acid content

Table 5 – Effect of salinity on antioxidant activities of cumin seed extracts.

	DPPH (IC_{50}, μg/ml)	β-Carotene bleaching (IC_{50}, μg/mL)	Chelating ability (IC_{50}, mg/mL)	Reducing power (EC_{50}, μg/mL)
0 mmoL	$16.24 \pm 0.64^{c,d}$	165.86 ± 0.23^{c}	23.65 ± 0.87^{c}	110.34 ± 3.74^{d}
50 mmoL	14.75 ± 0.31^{c}	111.72 ± 0.47^{b}	12.55 ± 0.09^{b}	65.82 ± 0.05^{b}
75 mmoL	7.16 ± 0.09^{b}	99.11 ± 0.09^{b}	4.89 ± 0.05^{a}	87.93 ± 0.21^{c}
125 mmoL	3.18 ± 0.02^{a}	47.79 ± 0.12^{a}	4.22 ± 0.35^{a}	25.99 ± 0.55^{a}
EDTA			0.03 ± 0.01	
Ascorbic acid				40 ± 0.84
BHT	0.18 ± 0.01	43 ± 0.56		

The data marked with the different letter in the table of each IC_{50} or EC_{50} value share significant differences at $p < 0.05$ (Duncan test). Each value in the table was obtained by calculating the average of six experiments.
BHT = butylated hydroxytoluene; DPPH = α-diphenyl-β-picrylhydrazyl; EC_{50} = the effective concentration at which the absorbance was 0.5; EDTA= ethylenediaminetetraacetic acid; GAE = gallic acid equivalent; IC_{50} = the concentration of the extract generating 50% inhibition.

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increased under 50 and 75 mmoL NaCl whereas the content was depressed by high salinity. In accordance with our results, salt stress was found to induce an increase in the concentration of ferulic acid in maize shoots, which was involved in reducing cell elongation and thereby shoot fresh mass [61]. Besides, Hura et al [62] reported that the accumulation of ferulic acid was a reliable biochemical parameter in drought resistance of triticale and hypothesized that this phenolic acid can support adaptation to osmotic stress conditions. Moreover, Ozfidan-Konakci et al [63] showed that the exogenous application of gallic acid enhanced the tolerance of rice cultivars to osmotic stress. Exposure of plants to osmotic stress is known to cause changes in metabolism and results in oxidative stress. Salinity is a hard environmental factor that has a major effect on plant quantity and quality [64]. Phenolics are known to be involved in the defense against biotic and abiotic factors and to contribute significantly to the antioxidant activity of plant tissues [65]. In response to stress, plants induce endogenous plant hormones, including jasmonic acid and its methylated derivate (methyl jasmonic acid), which in turn induce enzymes involved in the phenylpropanoid pathway, including phenylalanine ammonia lyase (PAL), thereby resulting in the accumulation of phenolic compounds. Similarly, salinity stress also induces the phenylpropanoid pathway via the accumulation of endogenous jasmonic acid [66] and the stimulation of PAL activation [67]. However, accumulation of phenolic compounds in plants by salinity stress may depend on the plant species; phenolic compounds failed to accumulate in broccoli [68] or lettuce [69] in response to NaCl treatment, whereas NaCl treatment increased the phenolic content of maize [70] and red pepper [19].

3.6. *Effect of salinity on antioxidant activity of cumin seed extracts*

The measurement of the antioxidant capacity of food extracts is commonly performed using several methods. Each method relates to the generation or use of a different radical that is directly involved in the oxidative process acting through a variety of mechanisms. No single assay can represent total antioxidant capacity [71]; therefore, four different and complementary assays were used to evaluate extract antioxidant activities namely DPPH-free radical scavenging, β-carotene/linoleic acid systems, the chelating ability as well as the reducing power. The results shown in Table 5 indicate that the antioxidant power of cumin seeds is significantly improved by the different salt treatments. In fact, the antiradical power as well as the aptitude to prevent the bleaching of β-carotene of cumin extracts were found to be enhanced under different salinity concentrations. The chelating ability and the reducing power were also increased in the extracts obtained from salt-treated plants compared with the control. The antioxidant response of plants to abiotic factors depends on the applied stress type and the considered organ [72]. In our experiment, the enhancement on the antioxidant capacity of cumin exposed to salinity could be due to the already reported improvement to its TPC (Table 4). Indeed, a positive correlation is always established between antioxidant capacity and phenolic content of extracts since phenolic compounds contribute directly to antioxidant activity [73].

4. Conclusion

The present work has extended our knowledge on the effect of NaCl on biochemical composition of *C. cyminum* seeds, which provoked much interest as sources of natural products, mainly in food industry due to the presence of many useful compounds. From practical standpoint, our results revealed that NaCl treatments decreased the seed yield of cumin and altered the quality and stability of the oil; in fact, the unsaturation degree of the fatty acid pool diminished. Moreover, NaCl treatment may represent an effective method to improve the nutritional quality of cumin seeds i.e., the concentration of secondary metabolites. It stimulates the EO yield and influenced the quality of the oil by changing the chemotype from γ-terpinene/1-phenyl-1,2 ethanediol to γ-terpinene/β-pinene. In addition, salinity improved phenolic biosynthesis, especially *p*-coumaric acid and luteolin production. These results suggest the stimulation of isoprenoids, shikimate, and phenypropanoid pathways by NaCl. These biochemical changes induced by salinity could reflect an adaptation response to this factor. In this case, cumin seeds produced under saline conditions may function as a potential source of EO and antioxidant compounds, which could support the utilization of this plant in a large field of applications, including agroalimentary and biological defense.

Conflicts of interest

The authors declare no conflicts of interest.

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