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Changes in fatty acid and essential oil composition of sage (*Salvia officinalis* L.) leaves under NaCl stress

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ABSTRACT

Fatty acids and essential oils from hydroponically cultivated *Salvia officinalis* leaves were analyzed by GC and GC–MS. Four different levels of NaCl (25, 50, 75 and 100 mM) were applied. The first results showed that salt treatment reduced significantly the plant growth by 61% and the total fatty acids (TFA) content by 32% at 100 mM NaCl. Alpha-linolenic, gadoleic, palmitic and oleic acids were the major fatty acids. Moreover, the polyunsaturated fatty acids decreased, while the monounsaturated ones increased with respect to increasing salinity. Regarding the essential oil composition, the main compounds were α- and β-thujone, 1,8-cineole, camphor, α-humulene, viridiflorol and manool at all salt treatments. The yield had a maximum increase at 75 mM NaCl. Hence, sage can be considered as moderately salt sensitive.

1. Introduction

Sage (*Salvia officinalis* L.) is one of the most appreciate herbs for its essential oil richness and its plethora of biologically active compounds extensively used in folk medicine (Penso, 1983). Essential oil of sage is applied in the treatment of a range of diseases and has been shown to possess antimicrobial, viricidal, cytotoxic, antimutagenic and antifungal activities (Jalsenjak, Peljnajk, & Kustrak, 1987).

In Tunisia, salinity affects about 10% of the whole territory. Crops are more and more exposed to this problem accentuated by increasing climate aridity (Hachicha, Job, & Mtimet, 1994). Plants subjected to high salinity levels undergo various physiological and biochemical changes leading to numerous changes in the structure and function of cells membranes. Fatty acids are among the most prominent constituents of cell membrane lipids which play a fundamental role in permeability (Schuler et al., 1991) and fluidity (Kerkeb, Donnaire, Venema, & Rodriguez-Rosales, 2001) regulation.

Increasing soil salinity level strongly influences the essential oil biosynthesis (Solinas & Deiana, 1996). Nevertheless, investigations dealing with the effect of this stress on essential oil production are scarce. Generally, this constraint affects the composition and causes a yield reduction in Lamiaceae species (Dow, Cline, & Horning, 1981; El-Keltawi & Croteau, 1987).

This study for the first time reports the impact of salinity on plant growth, fatty acids and essential oil contents and composition of *S. officinalis* leaves.

2. Material and methods

2.1. Plant material

The present study was carried out in a greenhouse of the Biotechnology Center in Borj-Cedria Technopark (36°42′57.63″N lat, 10°25′42.23″E long, 2 m, elevation) in Borj-Cedria. *S. officinalis* was propagated by cuttings supplied from a plant nursery from Cap Bon located in North-East of Tunisia.

2.1.1. Cutting propagation

S. officinalis cuttings was propagated in 5 L containers filled with distilled water in controlled greenhouse conditions at an approximate thermo-period of 30/20 °C (day/night), 60–80% air humidity under aeration and artificial light of 282 μmol m^{-2} s^{-1} (12,000 lux) with 16/8 h photoperiod (day/night). Fifteen days later, adventives roots emerged and transferred to the culture medium as follows.

2.1.2. Salt treatment

The culture was conducted hydroponically in the previously described greenhouse conditions (see cutting propagation). Fifty

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rooted-cuttings were used for each salt treatment distributed in 5 L containers, each one containing 10 rooted-cuttings grown in quarter-strength Hoagland's solution (Hoagland & Arnon, 1950) for 15 days. Then, the growth medium was laced progressively with NaCl during 7 days to reach the following five concentrations: 0, 25, 50, 75 and 100 mM. These concentrations were kept constant during the experiment by renewing the growth medium each week. The salt treatment was applied during one month, at the end of this period salt stress symptoms (leaves chlorosis and necrosis) appeared and plants were harvested. The leaves were subjected to essential oil and total lipid extraction.

2.1.3. Dry biomass determination

At the end of the experiment three plants were harvested from each container. After recording their fresh biomass, they were oven-dried at 65 °C for one week and dry biomass was recorded. Plant growth was determined by measuring the dry weight.

2.2. Total lipid extraction and fatty acids methyl esters preparation

Triplicate subsamples of 1 g of *S. officinalis* leaves were subjected to lipid extraction using a modified version of the Bligh and Dyer (1959) method. Thus, leaf samples were kept in boiling water for 5 min and then ground manually using a mortar and pestle. Chloroform/methanol mixture (2:1, v/v) was used for lipid extraction. After washing by fixation water, the organic layer containing lipids was recovered and dried under a nitrogen stream. Total fatty acids (TFAs) of total lipids were transmethylated using sodium methoxide solution (3% in methanol) according to the method reported by Cecchi, Biasini, and Castano (1985). Methyl heptadecanoate (C17:0) was used as an internal standard. The fatty acids methyl esters (FAMEs) obtained were subjected to GC analyses.

2.3. Essential oil isolation

S. officinalis leaves were cut into small pieces. Triplicate samples of 50 g were subjected to conventional hydrodistillation for 3 h followed by a liquid–liquid extraction using diethyl ether and n-pentane as solvent ratio (1:1, v/v). In order to quantify the essential oil yield, 6-methylhept-5-en-2-one was added as internal standard. The concentration step was carried out at 35 °C using a Vigreux column under atmospheric pressure. Essential oils obtained were dried over anhydrous sodium sulphate and stored in amber vials at −20 °C until analyzed.

2.4. Chromatographic analysis

2.4.1. Gas chromatography (GC-FID)

FAMEs were analyzed by gas chromatography using a Hewlett–Packard 6890 gas chromatograph series II (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. They were separated on a polar HP Innowax capillary column (30 m × 0.25 mm coated with polyethylene glycol 0.25 μm film thickness; Hewlett–Packard, Palo Alto, CA, USA). The oven temperature was kept 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. Nitrogen was used as carrier gas at a flow rate of 1.5 mL/min; the split ratio was 60:1. The injector and detector temperatures were set at 250 and 275 °C, respectively.

Essential oils were analyzed with the same apparatus described above. A polar HP Innowax (PEG) column (30 m × 0.25 mm, 0.25 μm film thickness; Hewlett–Packard, Palo Alto, CA, USA) and a 5% diphenyl, 95% dimethylpolysiloxane capillary column (HP-5: 30 m × 0.25 mm, 0.52 μm film thickness; Hewlett–Packard, Palo Alto, CA, USA) were used. The carrier gas was nitrogen (U) with a flow rate of 1.6 mL/min. The split ratio was 60:1 and the injected volume was 1 μL of neat oil. Analysis were performed using the following temperature program: oven kept isothermally at 35 °C for 10 min, increased from 35 to 205 °C at the rate of 3 °C/min and kept isothermally at 205 °C during 10 min. Injector and detector temperatures were held at 250 and 300 °C, respectively. Relative percentages of the identified compounds were obtained from the electronic integration of the FID peak areas.

2.4.2. Gas chromatography–mass spectrometry (GC–MS)

The GC–MS analysis were performed on a gas chromatograph HP 5890 (II) interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m × 0.25 mm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 μm film thickness, Hewlett–Packard, Palo Alto, CA, USA) was used. The column temperature was programmed to rise from 40 to 280 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 mL/min. Scan time and mass range were 1 s and 50–550 *m*/*z*, respectively.

2.5. Compounds identification

The identification of FAMEs was achieved by comparing their retention times with those of authentic standards. The identification of essential oil constituents was based on the 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 (Adams, 2001).

2.6. Statistical analyses

All extractions and determinations were conducted in triplicate. Data are expressed as mean ± S.D. The means were compared by using the one-way and multivariate analysis of variance (ANOVA) followed by Duncan's multiple range tests. The differences between individual means were deemed to be significant at $P < 0.05$. A cluster analysis (CA) was performed in order to discriminate between different NaCl levels on the basis of essential oil composition. Correlation coefficients were also calculated based on fatty acid composition at different levels of NaCl. All analyses were performed by using the "Statistica v 5.1" software (Statsoft, 1998).

3. Results and discussion

3.1. Plant growth

As shown in Fig. 1, a significant ($P < 0.05$) decrease of the plant dry biomass was observed following the application of the different NaCl levels. The application of 25 mM caused a light drop (8%) in dry biomass production, while concentrations of 50 and 75 mM NaCl reduced plant biomass production by 25% and 38%, respectively, as compared to control plants. A consistent decrease by 61% in dry biomass was observed at 100 mM NaCl. Salt stress, like many other abiotic stresses, inhibits plant growth. In accordance with our findings, Hendawy and Khalid (2005) found a significant decrease in sage dry weight at 50 mM NaCl ranging between 34% and 48%. One cause of growth rate reduction under salt stress is inadequate photosynthesis owing to stomatal closure and consequently limited carbon dioxide uptake (Zhu, 2001). In

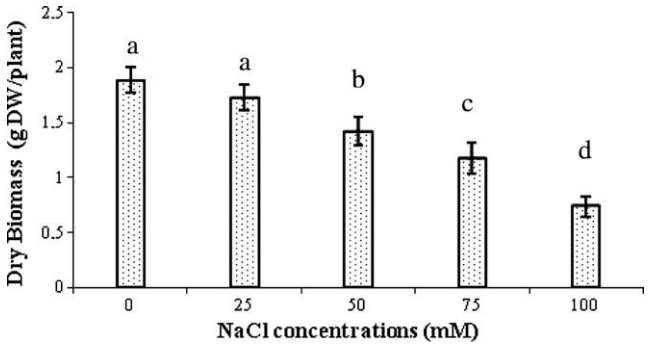

Fig. 1. Dry biomass (g DW/plant) of *Salvia officinalis* influenced by different NaCl levels. Values with different superscripts (a–d) are significantly different at $P < 0.05$.

view of Maas and Hoffman (1977), when 50% of reduction in biomass occurred at 90 mM of NaCl, the crop can be categorized as moderately salt sensitive. According to these authors, sage can be considered as moderately salt sensitive since more than 50% of biomass reduction occurred after the application of 100 mM NaCl.

3.2. Total fatty acids contents

The evolution of the total fatty acids (TFA) content in *S. officinalis* leaves subjected to increasing NaCl levels (25, 50, 75 and 100 mM) is reported in Table 1. The application of 25 and 50 mM NaCl maintained the TFA contents close to the control level. Statistically significant ($P < 0.05$) reductions of ≈16% and ≈32% in TFA were observed at the highest salinity levels namely 75 and 100 mM, respectively (Table 1). These reductions in TFA content are thought to be attributed to membrane lipids degradation under high salinity (Halliwell, 1987).

3.3. Fatty acids composition

Sage leaves in control plants were characterised by a high proportion of polyunsaturated fatty acids (PUFA) (59.78%) versus 20.55% of monounsaturated (MUFA) and 19.67% of saturated ones (SFA) (Table 1). Alpha-linolenic acid is the major foliar compound reaching over 45% of TFA, followed by linoleic (13.96%), palmitic (12.43%) and gadoleic (10.85%) acids. To the best of our knowledge, the foliar fatty acids composition of sage leaves is reported herein for the first time.

NaCl treatments induced marked changes in fatty acids composition of leaves as shown in Table 1. Varying levels of NaCl (25 to 75 mM) in the growth medium decreased the SFA and the PUFA and increased the MUFA fraction due to gadoleic acid increase. It is noteworthy to mention a possible stimulation effect of these NaCl concentrations on the eicosanoyl desaturase activity.

Besides, it is worth to highlight the relatively opposite evolution in the percentages of the linoleic and α-linolenic acids at all NaCl levels in comparison to the control. Moreover, correlation analysis was done to explore the trend of association between individual fatty acids (Table 2). Analysis using combined data from all the salt levels revealed no significant correlation between all individual fatty acids except gadoleic and oleic acids that had a significant correlation ($r = -0.90^{*}$). Furthermore, the DBIs (double bound indices), assessed in order to evaluate the unsaturation degree of the fatty acids pool, decreased in comparison to the control (Table 1).

In summary, low (25 mM), moderate (50 mM) and high (75 and 100 mM) NaCl levels decreased the degree of fatty acids unsaturation. This fact could be explained by a possible reduction of the desaturase activity which appeared as an adaptative feature to salinity (Kuiper, 1984). Since some plants could be protected against the oxidative effects of salt ions through restructuring membranes with less polyunsaturated fatty acids (Francois & Kleiman, 1990). Moreover, this low unsaturation degree limited the membrane fluidity (Kerkeb et al., 2001) and so restricted its permeability to Na^+ and Cl^- ions (Schuler et al., 1991).

3.4. Essential oil yield

Hydrodistillation of *S. officinalis* leaves (control) offered an essential oil with an average yield of 0.66% (w/w on a dry weight basis). This yield is higher than that reported for the same species from Egypt (Edris et al., 2007), but was lower than different populations cloned by cuttings from France, Czech Republic, Romania, Hungary and Portugal with respective yields of 2.05%, 2.20%,

Table 2
Correlation coefficients between fatty acids of *Salvia officinalis* leaves under saline conditions.

Variables	C16:0	[1]	[2]	[3]	[4]	[5]	[6]
[1] C16:1	0.45						
[2] C18:0	−0.01	−0.74					
[3] C18:1*n* − 9	0.69	0.46	−0.47				
[4] C18:2*n* − 6	0.76	0.70	−0.12	0.49			
[5] C18:3*n* − 3	−0.30	−0.32	−0.20	0.41	−0.37		
[6] C20:0	−0.71	−0.15	−0.53	−0.07	−0.73	0.62	
C20:1*n* − 9	−0.31	−0.25	0.53	**−0.90***	−0.16	−0.75	−0.32

* Significant at $P < 0.05$.

Table 1
Fatty acids percentages, DBI and TFA content of *Salvia officinalis* leaves cultured under different NaCl concentrations.

Fatty acids	NaCl (mM)				
	0	25	50	75	100
C16:0 (palmitic acid)	12.4 ± 0.09^{d}	13.0 ± 0.07^{c}	13.9 ± 0.1^{b}	11.9 ± 0.04^{e}	15.1 ± 0.06^{a}
C16:1 (palmitoleic acid)	0.29 ± 0.02^{e}	0.60 ± 0.03^{d}	1.19 ± 0.01^{c}	1.30 ± 0.03^{b}	1.40 ± 0.04^{a}
C18:0 (stearic acid)	1.57 ± 0.06^{b}	1.98 ± 0.05^{a}	1.09 ± 0.04^{d}	0.95 ± 0.02^{e}	1.30 ± 0.05^{c}
C18:1*n* − 9 (oleic acid)	9.41 ± 0.08^{b}	6.94 ± 0.09^{d}	9.55 ± 0.12^{b}	8.43 ± 0.01^{c}	11.4 ± 0.15^{a}
C18:2*n* − 6 (linoleic acid)	14.0 ± 0.13^{c}	14.7 ± 0.15^{b}	14.5 ± 0.3^{b}	14.6 ± 0.16^{b}	15.7 ± 0.1^{a}
C18:3*n* − 3 (α-linolenic acid)	45.8 ± 0.4^{a}	40.1 ± 0.2^{d}	41.3 ± 0.18^{c}	43.1 ± 0.35^{b}	42.8 ± 0.25^{b}
C20:0 (arachidic acid)	5.67 ± 0.07^{b}	2.40 ± 0.08^{d}	4.66 ± 0.01^{c}	5.80 ± 0.07^{a}	2.36 ± 0.08^{d}
C20:1*n* − 9 (gadoleic acid)	10.9 ± 0.02^{c}	20.2 ± 0.25^{a}	13.9 ± 0.08^{b}	13.9 ± 0.06^{b}	9.99 ± 0.03^{d}
SFA	19.7 ± 0.05^{a}	17.4 ± 0.07^{c}	19.7 ± 0.1^{a}	18.7 ± 0.14^{b}	18.8 ± 0.16^{b}
MUFA	20.6 ± 0.11^{e}	27.7 ± 0.12^{a}	24.6 ± 0.3^{b}	23.6 ± 0.19^{c}	22.8 ± 0.25^{d}
PUFA	59.8 ± 0.35^{a}	54.8 ± 0.40^{d}	55.8 ± 0.38^{c}	57.7 ± 0.41^{b}	58.5 ± 0.41^{b}
Linoleic/α-linolenic	0.30 ± 0.03^{c}	0.36 ± 0.02^{b}	0.35 ± 0.03^{b}	0.34 ± 0.01^{b}	0.37 ± 0.04^{a}
DBI	1.86 ± 0.05^{a}	1.77 ± 0.02^{c}	1.77 ± 0.03^{c}	1.82 ± 0.02^{b}	1.82 ± 0.03^{b}
TFA (mg/g DW)	17.7 ± 0.43^{a}	17.1 ± 0.69^{a}	17.5 ± 0.55^{a}	14.8 ± 0.41^{b}	12.1 ± 0.50^{c}

Values (means of three replicates ± SD) with different superscripts (a–e) are significantly different at $P < 0.05$. (SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; DBI: double bound index).

Table 3
Essential oil composition (%, w/w) of *Salvia officinalis* leaves influenced by different NaCl levels.

No.	Compounds*	RI[a]	RI[b]	NaCl (mM)					Identification
				0 (control)	25	50	75	100	
1	Tricyclene	927	1014	0.57 ± 0.05[a]	tr	0.19 ± 0.04[b]	tr	tr	GC–MS
2	α-Pinene	931	1035	0.10 ± 0.01[c]	0.10 ± 0.02[c]	0.20 ± 0.04[b]	0.20 ± 0.05[b]	0.41 ± 0.02[a]	GC–MS, Co-GC
3	α-Thujene	939	1032	0.13 ± 0.03[c]	0.13 ± 0.05[c]	0.57 ± 0.04[a]	0.33 ± 0.03[b]	tr	GC–MS
4	α-Fenchene	948	1072	tr	tr	0.40 ± 0.02[a]	0.11 ± 0.01[b]	nd	GC–MS
5	Camphene	950	1076	0.36 ± 0.05[b]	0.36 ± 0.04[b]	0.92 ± 0.03[a]	0.92 ± 0.04[a]	0.10 ± 0.01[c]	GC–MS
6	Hexanol	870	1360	nd	nd	nd	tr	3.13 ± 0.02	GC–MS, Co-GC
7	Sabinene	976	1132	0.26 ± 0.03[c]	0.18 ± 0.03[d]	0.66 ± 0.04[a]	0.39 ± 0.07[b]	0.11 ± 0.02[e]	GC–MS
8	β-Pinene	980	1118	0.69 ± 0.06[c]	0.49 ± 0.03[d]	1.57 ± 0.1[a]	1.21 ± 0.09[b]	0.46 ± 0.08[d]	GC–MS, Co-GC
9	Myrcene	988	1176	1.15 ± 0.02[c]	0.66 ± 0.02[d]	2.07 ± 0.01[a]	1.81 ± 0.02[b]	0.32 ± 0.03[e]	GC–MS
10	p-Cymene	1026	1280	tr	tr	tr	0.14 ± 0.02	tr	GC–MS, Co-GC
11	Limonene	1030	1203	nd	nd	nd	nd	7.21 ± 0.03	GC–MS, Co-GC
12	**1,8-Cineole**	**1033**	**1213**	**13.8 ± 0.03[d]**	**16.7 ± 0.04[a]**	**14.2 ± 0.04[c]**	**14.9 ± 0.04[b]**	**3.49 ± 0.02[e]**	**GC–MS, Co-GC**
13	(E)-β-Ocimene	1041	1266	0.43 ± 0.03[b]	0.24 ± 0.02[c]	0.66 ± 0.02[a]	0.60 ± 0.05[a]	0.20 ± 0.01[d]	GC–MS
14	trans-Sabinene Hydrate	1053	1474	tr	nd	tr	tr	tr	GC–MS
15	γ-Terpinene	1062	1266	0.16 ± 0.01[b]	0.16 ± 0.02[b]	0.22 ± 0.02[a]	nd	nd	GC–MS
16	cis-Sabinene Hydrate	1082	1556	tr	0.29 ± 0.03[a]	0.26 ± 0.03[a]	tr	tr	GC–MS
17	α-Fenchone	1087	1406	0.13 ± 0.03[b]	0.17 ± 0.04[a]	0.12 ± 0.02[b]	nd	nd	GC–MS
18	Terpinolene	1088	1290	0.20 ± 0.02[c]	0.10 ± 0.01[d]	0.27 ± 0.02[b]	0.37 ± 0.03[a]	tr	GC–MS, Co-GC
19	**α-Thujone**	**1089**	**1430**	**23.4 ± 0.04[b]**	**22.2 ± 0.04[c]**	**21.9 ± 0.05[d]**	**25.7 ± 0.05[a]**	**20.9 ± 0.10[e]**	**GC–MS**
20	Linalool	1098	1553	0.27 ± 0.03[c]	0.52 ± 0.02[a]	0.38 ± 0.01[b]	0.20 ± 0.02[d]	0.23 ± 0.01[d]	GC–MS, Co-GC
21	n-Undecane	1100	1100	nd	tr	tr	nd	0.48 ± 0.02	GC–MS
22	**β-Thujone**	**1103**	**1451**	**6.35 ± 0.10[b]**	**6.02 ± 0.02[c]**	**5.98 ± 0.02[d]**	**7.46 ± 0.05[a]**	**6.37 ± 0.10[b]**	**GC–MS**
23	allo-Ocimene	1113	1380	tr	0.12 ± 0.01[b]	1.01 ± 0.01[a]	tr	tr	GC–MS
24	**Camphor**	**1143**	**1532**	**17.6 ± 0.10[c]**	**25.4 ± 0.09[a]**	**17.4 ± 0.08[c]**	**16.3 ± 0.1[d]**	**19.2 ± 0.11[b]**	**GC–MS**
25	Borneol	1165	1719	1.23 ± 0.02[b]	1.76 ± 0.03[a]	0.98 ± 0.02[c]	0.48 ± 0.03[d]	0.98 ± 0.02[c]	GC–MS
26	cis-Pinocamphone	1173	–	tr	tr	tr	0.22 ± 0.03	tr	GC–MS
27	Terpinen-4-ol	1176	1611	tr	tr	0.37 ± 0.02[a]	tr	0.31 ± 0.03[a]	GC–MS
28	p-Cymen-8-ol	1181	1864	tr	tr	tr	tr	tr	GC–MS, Co-GC
29	α-Terpineol	1189	1706	0.13 ± 0.02[a]	nd	0.13 ± 0.02[a]	0.14 ± 0.01[a]	0.10 ± 0.01[a]	GC–MS
30	Myrtenol	1191	1804	0.26 ± 0.02[a]	tr	0.18 ± 0.01[b]	tr	0.18 ± 0.01[b]	GC–MS
31	Nerol	1228	1797	tr	tr	tr	tr	tr	GC–MS, Co-GC
32	Linalyl acetate	1239	1565	0.35 ± 0.03[c]	tr	0.37 ± 0.03[c]	0.60 ± 0.02[a]	0.44 ± 0.02[b]	GC–MS
33	Geraniol	1255	1857	tr	tr	tr	tr	tr	GC–MS, Co-GC
34	Bornyl acetate	1270	1590	3.93 ± 0.03[a]	0.43 ± 0.03[c]	3.44 ± 0.02[a]	3.92 ± 0.02[a]	1.18 ± 0.02[b]	GC–MS
35	Thymol	1290	–	tr	tr	tr	tr	0.20 ± 0.01	GC–MS, Co-GC
36	Carvacrol	1292	–	nd	tr	tr	tr	tr	GC–MS
37	Eugenol	1356	–	tr	tr	tr	tr	0.11 ± 0.02	GC–MS, Co-GC
38	α-Copaene	1379	1497	0.45 ± 0.03[a]	0.40 ± 0.02[a]	0.43 ± 0.02[a]	0.21 ± 0.01[b]	0.39 ± 0.02[a]	GC–MS
39	Geranyl acetate	1383	1765	tr	tr	tr	tr	tr	GC–MS
40	β-Bourbonene	1386	1535	0.24 ± 0.02[b]	0.22 ± 0.02[b]	0.18 ± 0.03[c]	nd	0.28 ± 0.04[a]	GC–MS
41	Methyl eugenol	1402	2028	0.52 ± 0.10[b]	tr	0.43 ± 0.09[c]	0.53 ± 0.10[b]	0.88 ± 0.09[a]	GC–MS, Co-GC
42	β-Caryophyllene	1418	1612	tr	tr	tr	tr	1.43 ± 0.37	GC–MS
43	β-Cubebene	1419	1552	0.49 ± 0.01[a]	tr	tr	tr	0.20 ± 0.02[b]	GC–MS
44	Aromadendrene	1443	1628	tr	tr	tr	tr	0.16 ± 0.02	GC–MS
45	**α-Humulene**	**1454**	**1687**	**4.77 ± 0.03[a]**	**2.72 ± 0.02[d]**	**4.50 ± 0.02[b]**	**4.86 ± 0.03[a]**	**3.67 ± 0.02[c]**	**GC–MS, Co-GC**
46	allo-Aromadendrene	1462	1661	0.10 ± 0.01[b]	tr	0.11 ± 0.01[b]	0.14 ± 0.02[a]	0.10 ± 0.01[b]	GC–MS
47	α-Amorphene	1474	–	0.22 ± 0.02[a]	tr	0.18 ± 0.01[b]	0.11 ± 0.01[c]	0.20 ± 0.02[a]	GC–MS
48	Germacrene-D	1480	1726	0.75 ± 0.01[a]	0.30 ± 0.02[b]	0.62 ± 0.01[a]	0.29 ± 0.02[b]	0.26 ± 0.02[b]	GC–MS
49	Bicyclogermacrene	1494	1755	tr	tr	tr	tr	0.16 ± 0.03	GC–MS
50	β-Bisabolene	1503	1741	tr	tr	0.13 ± 0.01[b]	tr	0.36 ± 0.03[a]	GC–MS
51	δ-Cadinene	1513	1773	0.18 ± 0.02[a]	0.10 ± 0.04[c]	0.19 ± 0.03[a]	0.22 ± 0.04[a]	0.16 ± 0.05[b]	GC–MS
52	Spathulenol	1572	–	tr	tr	tr	tr	tr	GC–MS
53	Caryophyllene oxide	1580	2008	tr	0.46 ± 0.04	tr	tr	tr	GC–MS
54	**Viridiflorol**	**1592**	**2104**	**9.36 ± 0.10[b]**	**6.42 ± 0.20[d]**	**9.45 ± 0.10[b]**	**8.59 ± 0.09[c]**	**11.3 ± 0.10[a]**	**GC–MS**
55	Humulene epoxide II	1606	2071	tr	tr	tr	tr	tr	GC–MS
56	β-Eudesmol	1650	–	tr	tr	tr	tr	0.11 ± 0.01	GC–MS
57	α-Bisabolol	1673	–	tr	tr	tr	tr	tr	GC–MS
58	**Manool**	**1967**	–	**6.39 ± 0.02[a]**	**3.36 ± 0.02[d]**	**5.14 ± 0.03[b]**	**4.82 ± 0.02[c]**	**6.16 ± 0.03[a]**	**GC–MS**
Total identified				95.0 ± 11.2[b]	90.0 ± 10.9[d]	95.8 ± 13.5[a]	95.8 ± 12.4[a]	91.9 ± 11.0[c]	
Essential oil yield (%, w/w)				0.66 ± 0.01[d]	0.67 ± 0.02[d]	1.02 ± 0.04[c]	2.09 ± 0.03[a]	1.68 ± 0.02[b]	

* Order of elution in HP-5 column, values with different superscripts (a–e) are significantly different at P < 0.05. *Note:* Retention indices relative to *n*-alkanes on (a) apolar column HP-5MS and (b) polar column HP Innowax. nd: Not detected, tr: trace (<0.01%). Bold values have been used for showing the main representative compounds.

2.30%, 2.50% and 2.90% (Chalchat, Michet, & Pasquier, 1998). Mirjalili, Salehi, Sonboli, and Vala (2006) showed a variation in essential oil yield ranging from 0.90% to 0.20% according to the phenological stage of *S. officinalis* cultivated in Iran. Many other factors namely material origin, extraction process and several environmental factors such as salinity are known to induce profound variations in essential oil yields (Hendawy & Khalid, 2005; Mirjalili et al., 2006).

The application of increasing NaCl concentrations (25, 50, 75 and 100 mM) gave essential oils with a yield of 0.67%, 1.02%, 2.09% and 1.68%, respectively. Essential oil yield did not vary at 25 mM, however, a significant ($P < 0.05$) increase occurred at the moderate (50 mM) and the high salt concentrations (75 and 100 mM NaCl) (Table 3). Our results showed that NaCl enhances essential oil production in *S. officinalis* and this effect is dose-dependant. Since, a level of 75 mM NaCl increased the essential

Table 4
Content of the main essential oil compounds (mg/g DW) of *Salvia officinalis* leaves subjected to different NaCl concentrations.

Main compounds	NaCl (mM)				
	0	25	50	75	100
1,8-Cineole	0.85 ± 0.09^{d}	1.14 ± 0.10^{c}	1.83 ± 0.20^{b}	3.53 ± 0.19^{a}	0.59 ± 0.05^{e}
α-Thujone	1.45 ± 0.20^{d}	1.53 ± 0.22^{d}	2.82 ± 0.19^{c}	6.08 ± 0.17^{a}	3.51 ± 0.20^{b}
β-Thujone	0.39 ± 0.08^{d}	0.41 ± 0.06^{d}	0.76 ± 0.06^{c}	1.77 ± 0.12^{a}	1.07 ± 0.11^{b}
Camphor	1.09 ± 0.19^{e}	1.69 ± 0.24^{d}	2.24 ± 0.23^{c}	3.85 ± 0.22^{a}	3.24 ± 0.22^{b}
α-Humulene	0.29 ± 0.08^{c}	1.71 ± 0.05^{d}	0.58 ± 0.05^{b}	1.15 ± 0.07^{a}	0.62 ± 0.04^{b}
Viridiflorol	0.58 ± 0.08^{c}	0.41 ± 0.08^{d}	1.22 ± 0.09^{b}	2.03 ± 0.09^{a}	1.90 ± 0.09^{a}
Manool	0.39 ± 0.07^{d}	0.22 ± 0.02^{e}	0.67 ± 0.02^{c}	1.14 ± 0.02^{a}	1.04 ± 0.03^{b}

Values (means of three replicates ± SD) with different superscripts (a–e) are significantly different at $P < 0.05$.

oil production by threefold compared to the control. In agreement with our findings, this increase is also observed in *S. officinalis* from Egypt rising from 0.47–0.48% for control plants to 0.65–0.76% for plants treated with 50 mM NaCl (Hendawy & Khalid, 2005). This trend is detected in others species of Lamiaceae family such as peppermint grown under osmotic stress (Charles, Joly, & Simon, 1990). According to these authors, the reduction in growth induced by the osmotic stress may have resulted in a new pattern of resource partitioning providing additional carbon skeletons for terpene biosynthesis and accumulation. This hypothesis is supported by Loomis (1932) suggesting a competitive sinks for photosynthesis between growth and secondary metabolism processes. In *Pinus pinea*, Ross and Sombrero (1991) showed that between 5% and 40% of fixed carbon may be allocated into the biosynthesis of essential oils in extreme conditions (Ross & Sombrero, 1991).

3.5. Essential oil composition

Essential oil compounds identified in *S. officinalis* leaves are listed in Table 3 following their elution order on the HP-5 column. In control plants, 58 compounds were identified among them α-thujone (23.43%), camphor (17.60%), 1.8-cineole (13.83%), viridiflorol (9.36%) and α-humulene (4.77%). In addition, Table 3 shows the occurrence of manool, a labdane type diterpene, at 6.39%. These compounds were commonly found in the essential oil of *S. officinalis* leaves, but with different percentages depending on season, geographic origin, environmental factors, extraction methods, plant organ (Santos-Gomes & Fernandes-Ferreira, 2001), phenological stage (Mirjalili et al., 2006), sampling techniques (Putievsky, Ravid, & Dudai, 1986) and genetic differences (Perry et al., 1999).

The application of 25 mM NaCl induced a qualitative increase of the camphor percentage. Quantitatively, an increase of 1,8-cineole and camphor was observed, but α-thujone remained the major compound (Table 4). The content increment of these main monoterpenes was concomitant with the decrease of the diterpene manool and the two prominent sesquiterpenes: viridiflorol and α-humulene. However, a level of 50 mM NaCl did not induce notable qualitative changes in essential oil composition compared to the control, but increased the content of the main compounds (Table 4). Increasing salinity to 75 mM NaCl stimulated the biosynthesis of all the compounds mainly α-thujone (Table 4). Such increase could be the carrier of the antimicrobial activity to this essential oil (Jalsenjak et al., 1987). The content increment occurring at this salt level is likely due to the enhancement of the activity of related biosynthesis enzymes. At 100 mM NaCl, major changes in the percentages of the main essential oil compounds occurred (Table 3). Hence, α-thujone and camphor have close levels (20.86% and 19.24%, respectively), whereas viridiflorol (11.28%), limonene (7.21%) increased and 1,8-cineole percentage was subjected to a significant decrease from 13.83% in the control plant to 3.49% at 100 mM NaCl. Concerning the content of the main essential oil compounds, a marked decrease mainly in 1,8-cineole was observed (Table 4). This decrease could be explained by the foliar necrosis (data not shown) leading to a senescence of some secretory gland sites of the monoterpenes accumulation.

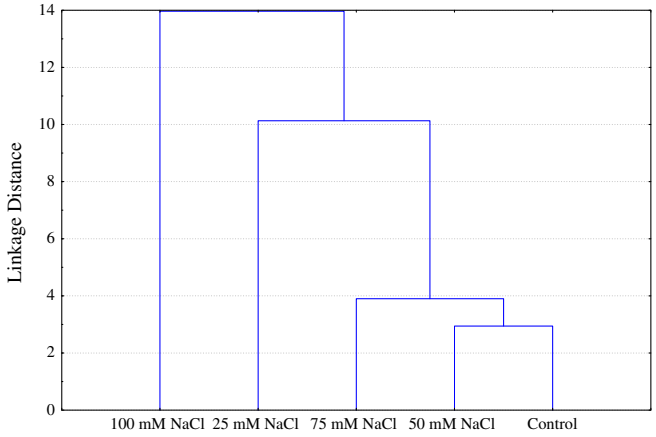

Fig. 2. Two dimensional dendrogram obtained in the cluster analysis of the essential oils treated with different NaCl levels of *Salvia officinalis* leaves based on the data from Table 3: horizontal; samples analyzed; vertical; differentiation levels between samples.

Results obtained from the cluster analysis (Fig. 2) showed the existence of one well-defined group represented by the salt level at 0 (control), 50 and 75 mM. Other NaCl levels represented by 25 and 100 mM were distinguished from the latter group both in quality and in quantity.

4. Conclusions

The application of NaCl concentrations reduced the plant growth and caused a subsequent gradual decrease in TFA content. Moreover, a reduction in PUFA in favour of MUFA was noticed. This fact could be considered as one of the aspect of *S. officinalis* adaptation in saline conditions. Furthermore, the essential oil production was stimulated in response to salt constraint, and this effect was dose-dependant. Since, at 75 mM the yield had a maximum increase but declined at 100 mM NaCl. In summary, the reduction in plant growth and total fatty acids content and the increase of essential oil production induced by the salt constraint may be a result of a new pattern of resource partitioning providing more carbon skeletons for terpene biosynthesis and accumulation.

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