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Volatile Oil Components of Laurel (*Laurus nobilis* L.) Leaves Obtained from Plants Cultivated under Salinity Stress Conditions

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Abstract: Laurel (*Laurus nobilis* L.) is a strict endemic species of natural vegetation of the Mediterranean region, which is known for its medicinal, aromatic, forestry, ornamental and culinary properties. This species produces valuable essential oil (EO). The content of EO and its composition depend on different factors, e.g., genetic, cultural practices and environmental conditions. Among these, salt stress is a major limiting factor, which affects almost all plant functions. Similar to essential oils (EOs), biotic and abiotic stresses may stimulate or inhibit the emission of volatile compounds (VCs) in plant materials, suggesting that these substances can be responsible on stress defense strategies. Therefore, an experiment was conducted to assess the effect of different NaCl concentrations (0, 50, 100 and 150 mM) of the irrigation water on VCs of laurel leaves. Our results showed that salt stress affected the volatile metabolites compounds, mainly the major ones. For instance, 1,8-cineole and linalool were negatively affected by high salinity levels, while the opposite was observed for α-terpenyl acetate and methyl eugenol. The proportion of grouped compounds of laurel VCs also differed among the studied treatments. The relative content of oxygenated monoterpenes and monoterpene hydrocarbons, respectively the first and the second largest groups, decreased with increasing NaCl concentration. Differently, the relative amount of sesquiterpene hydrocarbon group increased, especially at 100 mM NaCl. These findings indicate that the cultivation of laurel in marginal lands, characterized by high salinity or low-quality water, must be carefully evaluated because it significantly varies the quality of its products.

Keywords: salinity; NaCl; 1,8-cineole; α-terpenyl acetate; methyl eugenol

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1. Introduction

Since ancient times, essential oils (EOs) have been recognized for their medicinal value; they are very interesting and powerful natural plant products that continue to be of paramount importance at present day [1]. Carvalho et al. [2] reported that, nowadays, consumers around the world are increasingly focused on health and beauty and the renewed consumer interest in natural cosmetic products creates the demand for new products and reformulation of others with botanical and functional ingredients. In cosmetic products, EOs play a major role as fragrance ingredients, then they can optimize its proprieties and preservation, as well as the marketing image of the final product [2]. EOs have been used as perfumes, flavors for foods and beverages or to heal both body and mind for thousands of years [3–6]. Zouari et al. [7] showed that EOs are a very heterogeneous mixture of many compounds at various concentrations. In fact, each EO is characterized by some major compounds which can reach high levels, as compared to other compounds present in trace amounts [8].

Multiple properties of secondary metabolites, such as drugs, herbicides, biological, flavoring agents, colors, poisons, hallucinogenic substances and perfumes, make them interesting for the use in biotechnology [9]. Factors such as climatic and geographic conditions, as well as ontogeny of collected plants, can affect production of EO, composition and biological activities [10]. EOs, as well as their isolated compounds, are widely used in cosmetic products, as they offer a variety of benefits. Their biological activities range from analgesic, antiseptic, antimicrobial, carminative, diuretic, spasmolytic to hyperemic and stimulatory [11]. Additionally, extracts obtained from valuable plants have gained scientific interest for having an antifungal activity [12–14].

Many studies have reported that EOs have an antifungal effect [15–17], can act as botanical insecticides [18] and it could be utilized to protect body organs against carcinogenesis [19–21].

Laurel belongs to the Lauraceae family, which includes 32 genera and about 2000–2500 species and represents one of the most appreciated aromatic herbs with multiple properties [22]. EO of laurel has been reported also as a powerful analgesic product indicated in arthritis, polyarthritis, in osteomuscular rheumatism and sprain problems [23]. In the same context, EO of laurel can be used on the treatment of diabetes and the prevention of migraines [24]. Furthermore, it has antibacterial and antimicrobial properties [25,26]. 1,8-Cineole, sabinene and α-terpinyl acetate have been identified as the major component of EOs of many plants, including laurel [27–29].

Sarmoum et al. [30] showed that plant growth and development are adversely affected by many environmental stresses, such as drought, low temperature, humidity, wind, salinity, flooding, heat, drought, oxidative stress and heavy metal toxicity. Salinity is the one of the major environmental stress factors limiting crop growth and productivity in many arid and semiarid regions [31]. It certainly affects EOs' biosynthesis and secretion [32]. Hendawy and Khalid [33] reported the stimulating effects of abiotic stresses on the synthesis of secondary metabolites in plants. Both quantity and quality of EOs can be altered by environmental factors. Salinity represents a strong restriction in increasing crop productivity and affects the secondary metabolites of plants. This result has been observed, for instance, by Akula et al. [34], who reported that salt stress creates both ionic and osmotic stress in plants, resulting in either an increase or decrease of specific secondary metabolites in plants.

Salinity is, at present, one of the most serious environmental problems influencing especially arid climates. In Tunisia, more than 47% of water used for irrigation has a salt content between 1.5 and 3 g L^{-1} [35]. In the semi-arid and arid climate of Tunisia, shortage and variability of rainfall and high evaporation affect the water and the balance of salts in the soil [36]. Additionally, the important quantities of saline water used to irrigate crops lead to soil quality deterioration and a sharp decrease in agricultural productivity [37]. In addition, Louati et al. [38] have noticed that irrigating with saline water further degrades natural water resources.

The performance of laurel in drought stress and the effect of this in EOs production and composition have been studied by Matallah et al. [39]. They found that water stress had a significant effect on the constituents of laurel EO; this influence depends on the treatment levels and varieties. Among secondary metabolites, and similar to EOs, a wide range of volatile compounds (VCs) are also emitted by plants under different stress conditions. Although there have been reports on the various virtues and especially the medicinal properties of laurel, a review of the literature did not reveal any previous research on the effects of abiotic stresses, more precisely salinity, on the VCs of this plant. Therefore, the aim of the present work was to test the effect of different concentration of NaCl on the VCs of laurel leaves.

2. Materials and Methods

2.1. Plant Materials

The study was performed at the department of Agronomy and Biotechnology (National Agronomic Institute of Tunisia–INAT) in Tunis (36°49′ N, 10°10′ E, 8 m a.s.l.). The

climate, of the Mediterranean type, has a minimum average temperature of 7.5 °C and a maximum average temperature of 31.7 °C.

In March 2014, a total number of 200 young plants (2 years old) of laurel ecotype Sousse (a city in the East-Center of Tunisia) was randomly selected from a commercial nursery. Each plant was transplanted in plastic pot (17 cm diameter). The substrate used is a mixture of equal parts of soil and peat. The physical-chemical characteristics of the soil were: 49% sand, 32% silt, 26% clay, pH 7.9, 0.9% organic matter, 25% total $CaCO_3$ and 0.152 dS m^{-1} electrical conductivity. The peat used had a dry matter content of 25%, an organic matter content of 90%, a pH of 6.4 and an electrical conductivity of 0.4 dS m^{-1}.

After three months of plant establishment, plants were subjected to irrigation with saline solutions having different NaCl concentrations (50, 100 and 150 mM NaCl). The control was irrigated with tap water (7.93 meq L^{-1} NaCl). The different irrigation waters had electrical conductivities increasing from 1.4 (control), 5.3, 9.7 and 14.1 dS m^{-1} (150 mM NaCl). Irrigations were conducted to maintain soil moisture near maximum water-holding capacity. Water was accurately provided to obtain approximately 15% leaching fraction to prevent salinity to buildup in the substrate. In order to save plants from rainfall, plastic films were used during the rainy season. Plants were kept outside and positioned in the experimental site according to a completely randomized design, with 50 plants for each treatment. Fertilization occurred monthly with a modified half-strength Hoagland solution (pH 6.3; N 7.5 mM, P 0.5 mM, Ca 2.0 mM, Mg 1.0 mM, S 1.0 mM, B 23.0 µM, Mn 4.6 µM, Zn 0.38 µM, Cu 0.14 µM, Mo 0.05 µM, Fe 10.0 µM).

Leaves from cultivated plants, which were irrigated for 9 months with the different concentrations of NaCl studies, were harvested on 1 March 2015 and dried at a temperature of 30 °C until constant weight. Major details on the experiment and the results concerning plant growth response are reported in Ben Ayed et al. [40].

2.2. Analysis of Volatile Compounds

2.2.1. Head Space-Solid Phase Microextraction (HS-SPME)

The shade-dried leaves of laurel were used for head space solid phase microextraction (HS-SPME) coupled with gas chromatograph (GC×GC-FID). Leaves were randomly harvested from 12 selected plants of each treatment for the extraction of EOs; leaves from two plants were pulled together to obtain six repetitions per treatment, for a total of 24 samples. A disc of 6 mm of diameter was taken from each leaf using a perforator, and then placed in a 10 mL volume vial previously cleaned with nitrogen. A number of discs equal to about 25 mg by weight was used. Flasks were then placed in a water bath at 50 °C for 15 min. After this conditioning time, with the aid of a manual holder, the SPME fiber was inserted into the vial and exposed for 10 min. After the exposed time, the fiber was then retracted and inserted into the injector of the gas chromatograph. The SPME fiber used to collect volatile fraction was DVB/CAR/PDMS, STABLEFLEX (2 cm) (Supelco Corp., Bellefonte, PA, USA).

2.2.2. Gas Chromatography (GC×GC-FID) Analysis

The leaf VCs were analyzed using Agilent model 7890-A series gas chromatography equipped with a modulator (Agilent G3486A CFT, Santa Clara, CA, USA), and a flame ionization detector (FID). The operative conditions were: first column CP-SIL 8 CB LOW Bleed/MS (30 m × 0.25 mm, 0.25 µm film thickness Agilent Technologies, Santa Clara, CA, USA), with a flow of 0.25 mL min^{-1}; second column CP WAX 52 CB (5 m × 0.25 mm, 0.50 µm film thickness; Agilent Technologies, Santa Clara, CA, USA) with a flow of 29 mL min^{-1}. The carrier gas was hydrogen. Analysis was performed using the following oven temperature program: initial 40 °C, increased to 190 °C, rate of 3 °C min^{-1}, increase to 240 °C, rate 5 °C min^{-1} (hold time for 5 min). Detector FID: 250 °C, hydrogen flow, 20 mL min^{-1}; air flow 450 mL min^{-1}. Inlet 250 °C in splitless mode. SPME fiber GC injection for 0.2 min.

The amount of the single substances identified are expressed as a percentage of the total amount of the substances identified corrected for the total weight of the discs of the

single sample. Substances were identified by comparison with a GC standards or literature retention time indexes.

3. Statistical Analysis

The VCs of laurel leaves grown with different level of NaCl concentration were visualized by non-metric multidimensional scaling (NMDS) of Bray–Curtis distances of leaf samples. To further test the effect of NaCl concentration volatile compounds, permutational multivariate analyses of variance (PERMANOVA) were carried out. An analysis of variance (ANOVA) was also performed to test the effect of NaCl concentration on the single compound, or grouped compounds, followed by a post hoc Tukey test to discriminate the means.

4. Results

The graphical visualization of NMDS (stress value = 0.06) displayed a good separation of treatments (Figure 1) and this was statistically supported by PERMANOVA, which revealed a high significance of treatments ($p < 0.001$). The further analysis performed with ANOVA, followed by a Tukey test on the single or grouped compounds, revealed a significant effect of the NaCl concentration on most of the components (Tables 1 and 2).

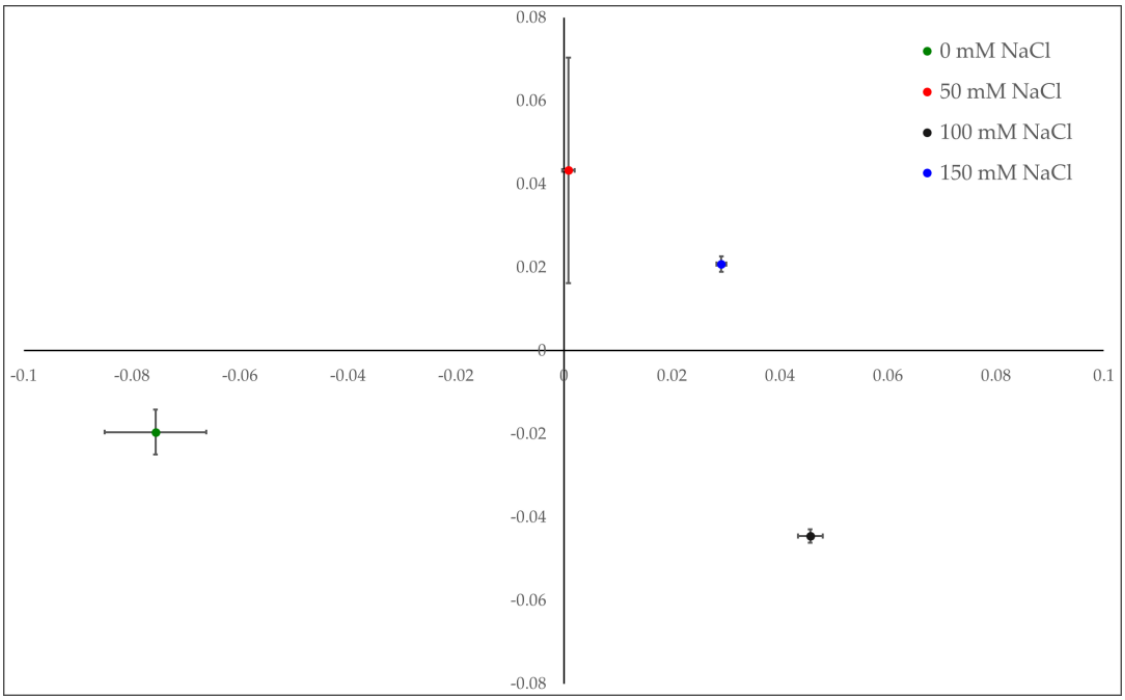

Figure 1. Graphical visualization of NMDS of the effects of different levels of NaCl concentration on volatile components of *Laurus nobilis* leaves. Centroids, indicating the mean value, are presented with their standard errors in two dimensions.

Table 1. Salinity effect on the composition (%) of the volatile components of *Laurus nobilis* leaves. Data are presented as mean ± SE.

			NaCl (mM)			
No	**Compounds**	**Kavats Index**	**0**	**50**	**100**	**150**
1	α-Thujene	930	0.24 ± 0.01 ^{b}	0.36 ± 0.08 ^{a}	0.38 ± 0.1 ^{a}	0.26 ± 0.03 ^{b}
2	α-Pinene	941	2.22 ± 0.25 ^{a}	1.26 ± 0.21 ^{b}	1.18 ± 0.11 ^{b}	1.20 ± 0.17 ^{b}
3	β-Pinene	986	2.10 ± 0.31 ^{a}	0.91 ± 0.36 ^{b}	0.7 ± 0.18 ^{c}	0.62 ± 0.11 ^{c}

Table 1. *Cont.*

No	Compounds	Kavats Index	NaCl (mM)			
			0	50	100	150
4	Sabinene	979	0.85 ± 0.3 ^{c}	0.90 ± 0.07 ^{b}	0.93 ± 0.36 ^{b}	1.20 ± 0.5 ^{a}
5	α-Terpenyl acetate	1357	5.70 ± 0.13 ^{b}	6.74 ± 0.63 ^{ab}	7.63 ± 0.7 ^{a}	7.98 ± 0.55 ^{a}
6	1,8-Cineole	1045	18.43 ± 0.18 ^{a}	16.83 ± 0.22 ^{b}	12.67 ± 0.03 ^{c}	15.42 ± 0.09 ^{b}
7	Camphene	964	0.13 ± 0.07 ^{a}	0.11 ± 0.02 ^{ab}	0.10 ± 0.05 ^{ab}	0.07 ± 0.01 ^{b}
8	Linalool	1201	4.74 ± 0.12 ^{a}	4.60 ± 0.23 ^{a}	4.55 ± 0.09 ^{a}	3.08 ± 0.01 ^{b}
9	α-Terpineol	1208	2.00 ± 1.01 ^{a}	1.24 ± 0.89 ^{b}	1.25 ± 0.4 ^{b}	1.20 ± 0.19 ^{b}
10	Bornyl acetate	1294	0.63 ± 0.01 ^{ab}	0.65 ± 0.02 ^{ab}	0.78 ± 0.06 ^{a}	0.57 ± 0.02 ^{b}
11	γ-Terpinene	1064	0.22 ± 0.8 ^{a}	0.22 ± 0.06 ^{a}	0.21 ± 0.01 ^{a}	0.24 ± 0.1 ^{a}
12	*p*-Cymene	1035	2.61 ± 0.25 ^{a}	2.06 ± 0.11 ^{b}	1.96 ± 0.63 ^{b}	1.94 ± 0.14 ^{b}
13	Borneol	1189	0.31 ± 0.14 ^{a}	0.22 ± 0.06 ^{ab}	0.12 ± 0.01 ^{b}	0.10 ± 0.21 ^{b}
14	Methyl eugenol	1412	5.13 ± 0.13 ^{b}	5.14 ± 0.11 ^{b}	6.55 ± 0.26 ^{a}	5.90 ± 0.19 ^{ab}
15	α-Terpinolene	1093	0.45 ± 0.01 ^{a}	0.44 ± 0.03 ^{a}	0.41 ± 0.09 ^{a}	0.45 ± 0.02 ^{a}
16	Limonene	1039	1.99 ± 0.07 ^{a}	2.00 ± 0.18 ^{a}	2.00 ± 0.26 ^{a}	2.10 ± 0.32 ^{a}
17	α-Terpinene	1025	0.81 ± 0.11 ^{a}	0.74 ± 0.08 ^{a}	0.70 ± 0.04 ^{a}	0.70 ± 0.02 ^{a}
18	Terpinen-4-ol	1196	1.50 ± 0.07 ^{a}	0.73 ± 0.21 ^{b}	0.69 ± 0.05 ^{b}	0.86 ± 0.1 ^{b}
19	*neo*-Isopulegol	1306	2.67 ± 0.03 ^{a}	2.35 ± 0.12 ^{b}	2.72 ± 0.09 ^{a}	2.62 ± 0.1 ^{a}
20	α-Phellandrene	1013	0.21 ± 0.15 ^{a}	0.11 ± 0.03 ^{b}	0.18 ± 0.06 ^{a}	0.10 ± 0.09 ^{b}
21	*trans*-Sabinene hydrate	1111	0.18 ± 0.23 ^{a}	0.09 ± 0.08 ^{b}	0.15 ± 0.06 ^{a}	0.13 ± 0.05 ^{ab}
22	*trans*-Pinocarveol	1139	3.35 ± 0.22 ^{a}	3.43 ± 0.09 ^{a}	3.86 ± 0.13 ^{a}	3.05 ± 0.41 ^{b}
24	γ-Cadinene	1491	0.24 ± 0.11 ^{b}	0.43 ± 0.10 ^{a}	0.32 ± 0.09 ^{ab}	0.48 ± 0.31 ^{a}
23	Valencene	1490	0.10 ± 0.02 ^{a}	0.13 ± 0.06 ^{a}	0.09 ± 0.02 ^{a}	0.11 ± 0.03 ^{a}
24	*trans*-Piperitol	1225	0.20 ± 0.01 ^{ab}	0.22 ± 0.09 ^{ab}	0.27 ± 0.18 ^{a}	0.15 ± 0.06 ^{b}
25	Zingiberene	1497	0.21 ± 0.11 ^{b}	0.25 ± 0.02 ^{b}	0.29 ± 0.01 ^{b}	0.36 ± 0.16 ^{a}
26	Tricyclene	934	0.93 ± 0.07 ^{b}	1.06 ± 0.28 ^{a}	0.92 ± 0.13 ^{b}	0.93 ± 0.03 ^{b}
27	*E*-Linalool oxide	1079	0.24 ± 0.12 ^{b}	0.24 ± 0.03 ^{b}	0.31 ± 0.01 ^{a}	0.26 ± 0.10 ^{b}
28	(*Z*)-β-Ocimene	1034	0.33 ± 0.16 ^{a}	0.31 ± 0.09 ^{a}	0.3 ± 0.22 ^{a}	0.31 ± 0.03 ^{a}
29	*o*-Cymene	1024	0.09 ± 0.07 ^{a}	0.09 ± 0.01 ^{a}	0.11 ± 0.25 ^{a}	0.14 ± 0.03 ^{a}
30	Myrcene	989	0.16 ± 0.1 ^{b}	0.33 ± 0.20 ^{a}	0.33 ± 0.12 ^{a}	0.26 ± 0.07 ^{ab}
31	*cis*-p-Mentha-1,3,8-triene	1121	0.27 ± 0.11 ^{b}	0.25 ± 0.22 ^{b}	0.31 ± 0.41 ^{a}	0.25 ± 0.17 ^{b}
32	Bicyclogermacrene	1516	0.44 ± 0.14 ^{b}	0.76 ± 0.28 ^{a}	0.64 ± 0.09 ^{ab}	0.74 ± 0.19 ^{a}
33	β-Elemene	1401	0.54 ± 0.21 ^{b}	0.63 ± 0.54 ^{a}	0.49 ± 0.18 ^{b}	0.61 ± 0.24 ^{a}
34	*trans*-Caryophyllene	1445	0.74 ± 0.13 ^{a}	0.60 ± 0.03 ^{a}	0.75 ± 0.06 ^{a}	0.66 ± 0.17 ^{a}
35	δ-Terpinyl acetate	1322	1.39 ± 0.12 ^{a}	1.40 ± 0.09 ^{a}	1.48 ± 0.03 ^{a}	1.44 ± 0.02 ^{a}
36	*m*-Cymene	1031	0.10 ± 0.13 ^{b}	0.06 ± 0.22 ^{b}	0.12 ± 0.07 ^{a}	0.06 ± 0.02 ^{b}
37	3-Ethyl-2,5-dimethylhexane	981	1.25 ± 0.02 ^{a}	1.09 ± 0.04 ^{b}	1.00 ± 0.11 ^{b}	1.05 ± 0.01 ^{b}
38	β-Bisabolene	1443	0.19 ± 0.14 ^{b}	0.28 ± 0.06 ^{a}	0.16 ± 0.11 ^{b}	0.19 ± 0.07 ^{b}
39	(*E*)-α-Bergamotene	1447	0.15 ± 0.13 ^{a}	0.14 ± 0.09 ^{a}	0.17 ± 0.06 ^{a}	0.14 ± 0.18 ^{a}
40	Aromadendrene	1456	0.19 ± 0.12 ^{a}	0.11 ± 0.02 ^{b}	0.13 ± 0.07 ^{b}	0.21 ± 0.05 ^{a}
41	2,5-Dimethoxy-*p*-cymene	1424	0.87 ± 0.11 ^{c}	1.30 ± 0.33 ^{b}	4.02 ± 0.23 ^{a}	1.05 ± 0.18 ^{c}
42	Valencene	1490	0.10 ± 0.06 ^{a}	0.13 ± 0.18 ^{a}	0.09 ± 0.01 ^{a}	0.11 ± 0.03 ^{a}
43	α-Copaene	1391	0.36 ± 0.66 ^{b}	0.57 ± 0.17 ^{a}	0.53 ± 1.01 ^{a}	0.62 ± 0.09 ^{a}
44	α-Humulene	1476	0.12 ± 0.03 ^{a}	0.14 ± 0.01 ^{a}	0.14 ± 0.08 ^{a}	0.16 ± 0.25 ^{a}
45	γ-Curcumene	1487	0.08 ± 0.11 ^{a}	0.10 ± 0.04 ^{a}	0.08 ± 0.1 ^{a}	0.04 ± 0.18 ^{a}
46	δ-Cadinene	1513	0.24 ± 0.13 ^{b}	0.43 ± 0.03 ^{a}	0.32 ± 0.09 ^{b}	0.47 ± 0.09 ^{a}
47	Safrole	1358	0.30 ± 0.10 ^{b}	0.35 ± 0.18 ^{b}	0.41 ± 0.09 ^{a}	0.46 ± 0.15 ^{a}
48	(E)-γ-bisabolene	1443	0.26 ± 0.11 ^{a}	0.27 ± 0.22 ^{a}	0.28 ± 0.09 ^{a}	0.27 ± 0.14 ^{a}
49	Sabina ketone	1177	0.42 ± 0.13 ^{a}	0.27 ± 0.07 ^{b}	0.25 ± 0.10 ^{b}	0.27 ± 0.11 ^{b}
50	*cis-p-M*entha-1,5-dien-8-ol	1171	0.18 ± 0.22 ^{a}	0.08 ± 0.13 ^{b}	0.08 ± 0.25 ^{b}	0.09 ± 0.11 ^{b}
51	Neryl propionate	1424	0.23 ± 0.64 ^{a}	0.25 ± 0.10 ^{a}	0.20 ± 0.06 ^{a}	0.23 ± 0.64 ^{a}
52	6-Methyl-hept-5-en-2-one	992	0.49 ± 0.08 ^{a}	0.50 ± 0.03 ^{a}	0.54 ± 0.01 ^{a}	0.55 ± 0.10 ^{a}
53	*E*-Cinnamaldehyde	1268	0.10 ± 0.08 ^{b}	0.11 ± 0.15 ^{b}	0.16 ± 0.32 ^{a}	0.08 ± 0.08 ^{b}
	Total		67.98	66.29	65.01	62.67
	Other identified compounds		7.97	4.57	7.87	8.14
	Unidentified compounds		24.05	29.14	27.12	29.19

Values followed by different letters are significantly different at $p < 0.05$.

Table 2. Percentage of the different grouped compounds of *Laurus nobilis* volatile compound extracted from leaves under salt stress. Data are presented as mean ± SE.

	NaCl (mM)			
Grouped Compounds	**0**	**50**	**100**	**150**
Monoterpene hydrocarbons	13.71 ± 0.61 ^{a}	12.21 ± 0.55 ^{ab}	11.84 ± 0.41 ^{b}	10.83 ± 0.36 ^{b}
Oxygenated monoterpenes	41.52 ± 0.32 ^{a}	40.82 ± 0.25 ^{ab}	39.56 ± 0.21 ^{b}	38.95 ± 0.1 ^{b}
Sesquiterpene hydrocarbons	3.92 ± 1.02 ^{c}	5.92 ± 1.51 ^{b}	7.45 ± 1.78 ^{a}	7.16 ± 1.65 ^{a}
Oxygenated sesquiterpenes	0.13 ± 0.04 ^{a}	0.15 ± 0.02 ^{a}	0.12 ± 0.23 ^{a}	0.13 ± 0.2 ^{a}
Aromatic compound	5.23 ± 0.24 ^{a}	5.25 ± 0.06 ^{a}	6.68 ± 0.14 ^{a}	6.05 ± 0.16 ^{a}
Alcohol	0.23 ± 0.02 ^{a}	0.25 ± 0.07 ^{a}	0.21± 0.15 ^{a}	0.26 ± 0.20 ^{a}
Aliphatic aldehydes	1.35 ± 0.01 ^{a}	1.20 ± 0.04 ^{b}	1.16 ± 0.00 ^{b}	1.13 ± 0.02 ^{b}

Values followed by different letter are significantly different at $p < 0.05$.

The component percentages of the leaf VCs changed with the change of the salinity concentration, as reported in the Table 1. In fact, although the instrumentation detected and identified 181 compounds, many of with at very low level, we did not include in the table compounds present at a relative ratio lower than 0.1% (as an average of all treatments and replication), whose analysis did not reveal statistical significance of treatments; thus, only 53 of the 181 compounds are listed. The unidentified compounds ranged between 24.05 and 29.19% (Table 1).

Results show that among the many compounds detected in the VCs occurred of laurel the most represented was 1,8-cineole (ranging from 12.6 to 18.4%), followed by α-terpenyl acetate (from 5.7 to 7.9%) and methyl eugenol (from 5.1 to 6.5%). These and other compounds were commonly found with plants treated with salt stress but their relative amount increased, decreased or did not change under salt stress as compared with untreated plants. In fact, sabinene, α-terpenyl acetate, eugenol, zingiberene and α-copaene increased considerably with an increasing NaCl level. In contrast, α- and β-pinene, 1,8-cineole, camphene, linalool, *p*-cymene, borneol, terpinen-4-ol and 3-ethyl-2,5-dimethylhexane were reduced with increasing NaCl concentration. It is worthy to note, in particular, that the relative amounts of 1,8-cineole (the major compound of laurel EO), was significantly affected by salinity, it decreased gradually with increasing NaCl level and achieved the lowest percentage at 100 NaCl, then at 150 mM NaCl it slightly but significantly increased again. Furthermore, as presented in the Table 1, the relative amount of α-thujene, *m*-cymene, *trans*-pinocarveol, *cis*-*p*-mentha-1,3,8-triene, *E*-linalool oxide and 2,5-dimethoxy-*p*-cymene increased gradually with increasing salt stress and reached the highest values at 100 mM and then decreased at the highest level of NaCl (150 mM NaCl). Additionally, myrcene, bornyl acetate and methyl eugenol have, in absolute terms, the highest values with 100 mM NaCl. Finally, the relative amount of many other compounds, e.g., α-terpinolene, limonene, α-terpinene, *trans*-caryophyllene and δ-terpinyl acetate, did not change.

Grouped compounds of laurel VC are summarized in Table 2. The composition of laurel VC is primarily made up of oxygenated monoterpenes. This group was the main group at different salt levels (about 40%) and with increasing NaCl concentration, the values for this group decreased. Monoterpene hydrocarbons were the second group for importance, producing the 13.71% of the total EO in the control plants and, also for this group, the relative EO amounts decreased with increasing NaCl levels. Differently, along with the increase of NaCl level, the relative amount of sesquiterpene hydrocarbons group increased reaching the highest values at 100 mM NaCl (7.17%). The remaining fractions, i.e., oxygenated sesquiterpenes, aromatic compounds, alcohol and aliphatic aldehydes, formed the minor classes, as shown in Table 2, and, with the exception of the latter, they were not affected by treatments.

5. Discussion

The VCs of laurel are a mixture of various compounds, also known as secondary metabolites, with a peculiar taste, useful in modern industry including the pharmaceutical, tobacco, food industries [41] and plant protection against insects and pathogens [42]. EO content and composition are known to be affected by environmental conditions including physical and chemical characteristics of soils [43–45], and macronutrient and micronutrient contents of soils [46–48]. In this study, the effects of different concentration of salt on the composition of the VC extracted from the leaves of laurel were investigated.

The analysis revealed that 1,8-cineole, α-terpenyl acetate and methyl eugenol are the main components, with other compounds being present at lower and different percentages or even in traces. This is in accordance with various experimental studies: Sangun et al. [29], for instance, found that 1,8-cineole is the major component in the leaves EO of laurel. Many constituents of laurel EOs have different and important utilizations. 1,8-Cineole, in particular, is widely used in the preparation of pharmaceuticals. It is also used as a local anesthetic and disinfectant in the treatment of inflammation and for inducing sputum and lowering blood pressure [49].

Under salt stress, the composition of VC of several species was modified. Heidari et al. [50] demonstrated that stresses caused by abiotic environmental factors such as salinity as well as drought can have a prominent effect on the chemical plasticity of the EO composition of medicinal herbs. In fact, salt stress affected EO composition of *Matricaria chamomila* [51], *Melissa officinalis* [52], *Calendula officinalis* [53] and *Mentha piperita* [54]. Significant correlations were observed between salt concentrations and major EO components of *Mentha piperita* [10].

Accordingly, our results indicate that the compositions of VC are modified in moderate and high salinity. Particularly, we noted that major compounds are significantly affected by salinity (Table 1). The relative amount of 1,8-cineole decreased with increasing salt concentration and achieved lower values at 100 mM NaCl. Similar results were observed in *Rosmarinus officinalis* by Tounekti et al. [55], as the relative content of 1,8-cineole decreased with the increase of the NaCl level. Additionally, Alaei et al. [56] showed that geranyl acetate and geraniol were the main components of *Dracocephalum moldavica* EO and with increasing salinity, the relative amount of these two compounds decreased significantly. Comparable effects were reported in *Mentha Piperita* grown under salt stress by Del Rosario et al. [57], who found a decrease in its VCs.

As can be seen in Table 1, for the others predominant components, we observed that with increasing salinity their relative amount was also significantly affected. α-Terpenyl acetate increased in percentage with increasing NaCl concentration. We also noted that salt stress increased methyl eugenol in comparison to the control and this was more pronounced at 100 mM NaCl. According to Ben Taarit et al. [58], these variations may be explained by the induction of the specific enzymes involved in the biosynthesis of the later compounds by salt stress. Accordingly, Hendawy and Khalid [33] revealed that variations in EO composition could be due to its effect on enzyme activity and metabolism improvement.

It has been highlighted the important biological role of EOs against plant pathogens and even in plant-to-plant interaction [59–61], but it is not clear if they are involved in plant adaptation to environmental abiotic stresses. Various studies have suggested that 1,8-cineole has an inhibitory effect on germination and early development of plants [29,62]. It has been also hypotized that an increase of these substances might reduce water losses and leaf temperature [59].

Apart from that, it is also possible that the change in both the number and composition of VCs is a mere consequence of the change in oil gland (or idioblasts in the case of laurel) density due to a lower leaf expansion. Furthermore, the increase of EO content can also be related to the decline of photosynthesis, leading to an oversupply of energy that is redirected toward the production of highly reduced secondary metabolites. On the other hand, abiotic stresses can reduce EOs because of a decline of plants anabolism or alteration of enzyme activity in general [63,64]. This would explain the lack of consistency in EOs'

yield and composition in plants subjected to abiotic stresses, and the fact that the highest or the lowest values were often observed at an intermediate salinity level (i.e., 100 mM NaCl).

In our study, salt stress had a significant effect on the groups of compounds, and this influence depends on the treatment levels. Indeed, we noted that a high level of NaCl stimulated sesquiterpene hydrocarbons content in comparison to the control. Their relative contents were the highest already at 100 mM NaCl. A reduction in monoterpene hydrocarbons and oxygenated monoterpenes was pronounced in salt stress condition. Indeed, for laurel, Maatallah et al. [39] showed that the chemical groups of the EO varied in water stress condition. The change of different groups of VC, as a consequence of salinity, have also been elucidated in other many species. In fact, a reduction of monoterpenes hydrocarbons has been previously observed in *Lawsonia inermis* [65], and in *Schizonepeta tenuifolia* subjected to increasing salt stress [66]. Conversely, in *Salvia mirzayanii,* Valifard et al. [67] showed that salt stress stimulates the secretion of monoterpenes hydrocarbons and oxygenated monoterpenes and inhibits the sesquiterpenes hydrocarbons and oxygenated sesquiterpene. As previously stated, these fluctuations could be explained by several possible causes, not least the induction of specific enzymes involved in the production of EOs at each level of salt stress [64].

6. Conclusions

In response to increasing salinity, laurel leaf VCs were strongly affected by increasing concentrations of NaCl in the irrigation water. For instance, concentrations of 50 and 100 mM NaCl stimulated a higher relative amount of sesquiterpene hydrocarbons families. Differently, NaCl decreased the relative content of oxygenated monoterpenes, monoterpene hydrocarbons and aliphatic aldehydes.

The single VC of laurel leaves also varied with the different level of NaCl used. This was more marked for the major element (1,8-cineole), which decreases with increasing salt, more particularly at 100 mM NaCl. Thus, owing to its wild occurrence in various environments and because of the high curing value and the various cosmetic and medicinal virtues of its EOs, future studies should consider *Laurus nobilis* as promising plants for marginal lands in arid and semi-arid regions characterized by high salinity or low-quality water.

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