*Acta Pharm.* **62** (2012) 251–261 DOI: 10.2478/v10007-012-0019-9

Short communication

# Culture conditions and salt effects on essential oil composition of sweet marjoram (*Origanum majorana*) from Tunisia

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Accepted May 3, 2012

O. majorana shoots were investigated for their essential oil (EO) composition. Two experiments were carried out; the first on hydroponic medium in a culture chamber and the second on inert sand in a greenhouse for 20 days. Plants were cultivated for 17 days in hydroponic medium supplemented with NaCl 100 mmol L<sup>-1</sup>. The results showed that the O. majorana hydroponic medium offered higher essential oil yield than that from the greenhouse. The latter increased significantly in yield (by 50 %) under saline constraint while it did not change in the culture chamber. Under greenhouse conditions and in the absence of salt treatment, the major constituents were terpinen-4-ol and trans-sabinene hydrate. However, in the culture chamber, the major volatile components were cis-sabinene hydrate and terpinen-4-ol. In the presence of NaCl, new compounds appeared, such as eicosane, spathulenol, eugenol, and phenol. In addition, in the greenhouse, with or without salt, a very important change of trans-sabinene hydrate concentration in EO occurred, whereas in the culture chamber change appeared in cis--sabinene hydrate content.

Keywords: Origanum majorana, greenhouse, culture chamber, salt, yield, terpinen-4-ol

Sweet marjoram (*Origanum majorana* L., syn. *Majorana hortensis Moench*) is a herbaceous and perennial plant native to Cyprus and the Eastern Mediterranean (1). It is an appreciated herb species and its essential oil is also used in perfumery because of its spicy herbaceous notes (2). In addition, essential oil (EO) of *O. majorana* is utilized in the manufacture of fungicides, and various pharmaceutical and industrial products (2).

Two chemotypes characterize *O. majorana* essential oil: terpinen-4-ol/sabinene hydrate chemotype (3), and thymol (or carvacrol) chemotype (4). In the first chemotype,

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the two major constituents are responsible for the characteristic flavor and fragrance of marjoram oil (5).

In medicinal and aromatic plants, the biosynthesis of secondary metabolites such as essential oils and their constituents is strongly influenced by environmental factors (6). One of the most important environmental constraints that affect almost 50 % of irrigated areas is salinity (7). This constraint generally modifies essential oil biosynthesis and its secretion (8).

The essential oil yield of some species can change with age, growth cycle, climatic conditions, soil type and cropping pattern (9). Culturing conditions can affect the quality of essential oil (10). Moreover, the age of the plant has a significant effect on its essential oil composition (11). However, there is no clear information on the possible influence of culture conditions on essential oil yield and composition in *O. majorana*. In this paper, we report on combined effects of NaCl treatment and culturing conditions of Tunisian marjoram aerial parts.

#### EXPERIMENTAL

#### Plant material

Marjoram (*Origanum majorana* L., *Lamiaceae*) plants were collected at the 6-leaf stage from a nursery located in Soliman in northeastern Tunisia (latitude 36° 41′ 47 N; longitude 10° 29′ 30 E; altitude 1500 m). These 11 days old plants were divided into two lots. The first was used for a hydroponic culture in eight-strength Hoagland's solution (12). Plants were placed in a culture chamber under 16 h light/8 h dark conditions at 22/18 °C, photosynthetically active radion at 150 µmol m<sup>-2</sup> s<sup>-1</sup>. The second lot of plants was transferred to plastic pots (1 plant per pot) filled with inert sand. Plants were irrigated with the same nutrient medium and placed in a greenhouse at 35 °C (day) and 26 °C (night). After 20 days of acclimatization, individual plants were grown without salt (control) or with NaCl (100 mmol L<sup>-1</sup>) for 17 days prior to harvest.

# Essential oil isolation

Essential oil was extracted by classical hydrodistillation of fresh shoots (50 g) during 90 min according to Msaada *et al.* (13). The distillate was submitted to a liquid-liquid extraction in diethyl ether and the organic phase was concentrated at 35 °C using a Vigreux column. In order to quantify EO and its constituents, 6-methyl-5-hepten-2-one was used as an internal standard. Essential oil obtained was stored at –20 °C prior to analysis. Each extraction was made in triplicate.

#### GC-FID

Gas chromatography analysis was carried out on a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A polar column HP Innowax (PEG) and an apolar HP-5 column (30 m  $\times$  0.25 mm, 0.25 µm film thickness, Agilent Technologies) were used. The nitrogen flow rate was 1.6 mL min<sup>-1</sup> and the split ratio was 60:1. EO analysis was performed using the following temperature program: oven 35 °C for 10 min, from 35 to 205 °C at a rate of 3 °C min<sup>-1</sup>, and isotherm at 225 °C for 10 min. Injector and detector temperature, were maintained at 250 and 300 °C, respectively.

Retention indices were calculated using a homologous series of *n*-alkanes C6–C20.

# 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 (apolar, 30 m × 0.25 mm, 0.25-µm film thickness, Agilent Technologies) was used. Column temperature was programmed to rise from 50 to 240 °C at a rate of 5 °C min<sup>-1</sup>. The carrier gas was helium with a flow rate of 1.2 mL min<sup>-1</sup>; split ratio was 60:1. Scan time and mass range were 1 s and 40–300 m/z, respectively.

## Statistical analysis

All extractions and analyses were conducted in triplicate. Data were expressed as means  $\pm$  SD.

#### RESULTS AND DISCUSSION

# Essential oil yield

Plants growing under different culture conditions did not produce equal yields of EO and gave rise to different contents and compositions in plant shoots (Table I). Thus, in the culture chamber, the essential oil yield based on dry mass, was 0.8 % in the control, and in the presence of NaCl (100 mmol  $L^{-1}$ ). In the greenhouse, EO decreased by a factor of two in the presence of salt.

In our previous work (14) we showed that Canadian *O. majorana* can tolerate a moderate NaCl concentration (50 mmol L<sup>-1</sup>) without modification in EO yield and composition. However, at a high NaCl concentration (100 mmol L<sup>-1</sup>), significantly modified essential oil yield and quality occured. Similar findings were reported for *Salvia hispanica* (8). In contrast, salt addition enhanced EO yield in *Oenothera biennis* (8). In the case of

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	Essential oil yield (%)					
	NaCl <sup>a</sup> (100 mmol L <sup>-1</sup> )	NaCl (100 mmol L <sup>-1</sup> ) <sup>a</sup>				
Greenhouse	$0.4 \pm 0.1$	$0.2 \pm 0.03^{b}$				
Culture chamber	$0.8 \pm 0.0_4$	$0.8 \pm 0.02^{b}$				

<sup>a</sup> Mean  $\pm$  SD, n = 3.

 $^{\rm b}$  Significantly different after salt addition at p < 0.05.

*Mathiola tricuspidata*, salinity did not exert any effect, on EO yield (8). Diversity of the findings showed that the effect of salt on essential oil yield depends on the salt concentration, culture medium and tolerance of the species.

## Culture chamber conditions: essential oil composition of O. majorana shoots

Gas chromatography analysis of essential oils from the culture chamber showed 44 compounds accounting for 99.86 % of the total EO (Table II), as represented in Figs. 1 c,d. In control plant shoots, the main compound was *cis*-sabinene hydrate (43.66 %) followed by terpinen-4-ol (23.21 %), *trans*-sabinene hydrate (8.17 %) and  $\alpha$ -humulene (7.30 %). Salt addition induced a decrease estimated at 4.40 % for *cis*-sabinene hydrate, 5.66 % terpinen-4-ol, 4.83 % *trans*-sabinene hydrate and 2.89 %  $\alpha$ -humulene as compared to the control. In that case, EO was of *cis*-sabinene hydrate/terpinen-4-ol chemotype, which is responsible for the characteristic flavor and fragrance of marjoram.

Our results are in accord with those of Bronchio *et al.* (3). Our previous data (14) showed that the essential oil of Canadian *Origanum* cultivated under the same conditions as the Tunisian one was found to be rich in *trans*-sabinene hydrate (47.67 %), and terpinen-4-ol (20.82 %).

Salinity had a significant effect (p < 0.05) on the composition and content of the major EO. In the culture camber *cis*-sabinene hydrate and terpinen-4-ol proportions were decreased, while their concentrations increased. In fact, NaCl (100 mmol L<sup>-1</sup>) increased *cis*-sabinene hydrate and terpinen-4-ol contents significantly (125 times to reach 20.60 mg g<sup>-1</sup> and 6 times to reach 0.556 mg g<sup>-1</sup>) respectively. Despite these changes, *O. majorana* retained the same chemotype after salt addition.

In the culture chamber, an important bioconversion of EO composition of *O. majorana* shoots from *trans*-sabinene hydrate into *cis*-sabinene hydrate was detected (Table II). These two isomers are biosynthesized by the same enzyme, sabinene hydrate synthase (1). In fact, these culture conditions activate this enzyme, favouring the biosynthesis of *cis*-sabinene hydrate. Indeed, this is an intensive and spic component of marjoram, whereas the *trans*-sabinene hydrate does not have any typical properties of marjoram (1).

Essential oil composition of shoots was characterized by the prevalence of terpenic alcohols in a proportion of 86.20 %, standing for *cis*-sabinene hydrate, *trans*-sabinene hydrate, and terpinen-4-ol, and 8.48 % for monoterpene hydrocarbons (Table II). In the same context, Baatour *et al.* (14) indicated that the most represented class of EO components of *O. majorana* native to Canada was that of oxygenated monoterpenes, followed by monoterpene hydrocarbons and esters in control plants. A decrease in these different compound proportions was observed after salt addition, except for monoterpene hydrocarbons and phenols.

#### Greenhouse conditions: Essential oil composition O. majorana shoots

Thirty-two compounds were found to represent 99.55 % of EO (Table III), as shown in Figs. 1 a,b. Independent of the applied treatment, terpinen-4-ol was detected as the major compound (31.77 %), followed by *trans*-sabinene hydrate (24.18 %) and  $\alpha$ -ter-

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Fig. 1. Chromatogram of essential oil compounds from Origanum majorana.

a) 100 mmol L<sup>-1</sup> NaCl in the greenhouse after 17 days of treatment; b) 100 mmol L<sup>-1</sup> NaCl in the greenhouse, after 17 days of treatment; c) 100 mmol L<sup>-1</sup> NaCl, in the culture chamber, after 17 days of treatment; d) 75 mmol L<sup>-1</sup> NaCl, in the culture chamber, after 17 days of treatment.

SI: internal standard (6-methyl 5 heptene 2 one). 1: Tricyclene, 2: α-pinene; 3: α-tujene; 4: sabinene; 5: β-myrcene; 6: α-phellandrene; 7: α-terpinene; 8: limonene; 8': 1,8-cineole; 9: γ-terpinene; 10: *p*-cymene; 11: terpinolene; 11': octanol; 12: *cis*-3-hexanol; 12': hexanol; 13: *trans*-sabinene hydrate; 13': linalyl acetate; 13'': bornyl acetate; 14: linalool, 15: *cis*-sabinene hydrate; 15': *cis*-*p*-menth-en-1-ol; 16: β-elemene, 17: terpinen-4-ol, 18: β-caryophyllene, 18': α-humulene; 19: aromadendrene; 20: bicyclogermacrene; 21: α-terpenyl acetate; 22: α-terpineol; 23: borneol; 24: neryl acetate, 25: geranyl acetate, 26: nerol, 27: myrthenol; 27'': *cis*-caveol; 28: nonadecane; 29: eiocasane; 30: spathuneol; 31: eugenol; 32: phenol; 32': thymol; 33: carvacrol.

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	NaCl						
				0 (mmol L <sup>-1</sup> )	100 (mmol L <sup>-1</sup> )	0 (mmol L <sup>-1</sup> )	100 (mmol L <sup>-1</sup> )
Compound	$t_{\rm R}$ (min)	KIa	KIb	(%) <sup>c</sup>	(%) <sup>c</sup>	(mg g <sup>-1</sup> ) <sup>c,d</sup>	(mg g <sup>-1</sup> ) <sup>c,d</sup>
Tricyclene	5.32	927	1014	0.142	0.193	0.045	0.409 <sup>e</sup>
α-Pinene	5.54	931	1035	0.07	0.106 <sup>e</sup>	0.532	0.011 <sup>e</sup>
α-Thujene	5.66	939	1032	0.339	0.106 <sup>e</sup>	0.265	0.021 <sup>e</sup>
Sabinene	6.11	976	1132	0.094	0.134 <sup>e</sup>	0.352	0.290 <sup>e</sup>
β-myrcene	8.74	991	1174	ND	0.016	ND	0.138
α-Phellandrene	9.7	1006	1176	0.562	0.787 <sup>e</sup>	2.106	1.041 <sup>e</sup>
α-Terpinene	13.04	1016	1188	1.292	1.730 <sup>e</sup>	4.839	0.046 <sup>e</sup>
Limonene	13.43	1030	1203	0.517	0.742 <sup>e</sup>	1.937	0.091 <sup>e</sup>
1.8-Cineole	14.56	1033	1213	0.582	0.782 <sup>e</sup>	2.179	0.022 <sup>e</sup>
γ-Terpinene	14.99	1062	1266	0.062	0.030 <sup>e</sup>	0.235	0.843 <sup>e</sup>
<i>p</i> -Cymene	16.12	1026	1280	0.050	0.054	0.187	0.334 <sup>e</sup>
Terpinolene	17.33	1088	1290	0.069	0.107 <sup>e</sup>	0.261	0.042 <sup>e</sup>
Octanal	19.39	-	-	0.474	0.725 <sup>e</sup>	1.777	0.144 <sup>e</sup>
Hexanol	25.12	-	-	0.210	0.020 <sup>e</sup>	0.789	0.592 <sup>e</sup>
cis-3-Hexanol	26.95	-	1385	ND	0.010	ND	0.010
trans-Sabinene hydrate	29.74	1053	1474	8.178	7.783 <sup>e</sup>	0.030	1.600 <sup>e</sup>
Linalool	29.89	1098	1553	ND	0.043	0.289	0.084 <sup>e</sup>
cis-Sabinene hydrate	32.06	1082	1556	43.667	41.745 <sup>e</sup>	0.163	20.595 <sup>e</sup>
cis-p-Menthen-1-ol	32.34	1129	1562	4.892	3.544 <sup>e</sup>	0.018	1.808 <sup>e</sup>
Linalyl acetate	32.49	1257	1556	0.993	1.472 <sup>e</sup>	3.720	8.845 <sup>e</sup>
Bornyl acetate	32.58	1295	1597	2.272	1.974 <sup>e</sup>	8.507	8.845 <sup>e</sup>
β-Elemene	33.33	1391	1601	0.329	0.837 <sup>e</sup>	1.234	0.289 <sup>e</sup>
Terpinene-4-ol	34.11	1176	1611	23.209	21.894 <sup>e</sup>	0.086	0.556 <sup>e</sup>
B-Caryophyllene	35.19	1419	1612	0.450	0.507 <sup>e</sup>	1.688	58.240 <sup>e</sup>
Aromadendrene	36.07	1443	1628	ND	0.0169	ND	3.834
α-Humulene	36.85	1454	1687	7.300	7.089 <sup>e</sup>	1.301	1.160 <sup>e</sup>
Bicyclogermacrene	37.06	1344	1705	0.199	0.227 <sup>e</sup>	0.746	0.285 <sup>e</sup>
α-Terpenyl acetate	37.43	1494	1755	ND	0.021	ND	2.049
Myrtenyl acetate	37.43	1335	1701	ND	0.021	ND	2.049
α-Terpineol	40.08	1189	1713	4.619	4.995 <sup>e</sup>	0.017	9.820 <sup>e</sup>
Borneol	39.60	1165	1702	0.203	0.241 <sup>e</sup>	0.763	41.500 <sup>e</sup>
Neryl acetate	40.08	1385	1733	0.133	0.193 <sup>e</sup>	0.499	1.740 <sup>e</sup>
Geranyl acetate	40.35	1383	1765	ND	0.007	ND	0.811
Nerol	41.68	1228	1797	0.170	0.161 <sup>e</sup>	0.638	0.468 <sup>e</sup>

Table II. Essential oil composition of Origanum majorana in the culture chamber by GC-MS

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Table II. continued								
Myrtenol	42.61 1194 1804			0.027	0.024 <sup>e</sup>	0.104	3.253 <sup>e</sup>	
Geraniol		42.80	1255	1857	ND	0.025	ND	0.021
cis-Caveol		43.37	1247	1804	0.449	0.462 <sup>e</sup>	1.683	0.392 <sup>e</sup>
Nonadecane		48.81	1581	1900	ND	0.011	ND	0.010
Eicosane		49.56	1996	2000	0.036	0.034 <sup>e</sup>	0.138	0.065 <sup>e</sup>
Spathulenol		50.26	_	2144	ND	0.012	ND	0.008
Phenol		51.85	-	_	ND	0.056	ND	0.093
Eugenol		52.40	1401	2030	ND	0.013	ND	5.186
Thymol		55.08	1295	-	ND	0.041	ND	0.063
Carvacrol		57.65	1302	_	0.025	0.045 <sup>e</sup>	0.096	0.023 <sup>e</sup>
	Classes							
	Monoterpene hydrocarbons				8.483	11.126 <sup>e</sup>	31.766	4.744 <sup>e</sup>
	Terpenic a	lcohols			86.207	81.671 <sup>e</sup>	320.141	48.012 <sup>e</sup>
	Sesquiterp	ene hyd	lrocarbo	ns	0.714	0.156 <sup>e</sup>	2.675	0.162 <sup>e</sup>
	Terpenic e	esters			3.265	3.306 <sup>e</sup>	12.228	0.048 <sup>e</sup>
	Aliphatic hydrocarbons Phenols				0.003	0.004 <sup>e</sup>	0.138	0.076 <sup>e</sup>
					0.025	0.156 <sup>e</sup>	0.964	0.193 <sup>e</sup>

 $t_{\rm R}$  – retention time, KI – Kovat's index on: a apolar column HP-5MS, b polar column HP-Innowax (relative to n-alkane), ND – not detected.

<sup>c</sup> Mean of 3 replicates.

<sup>d</sup> Dry mass basis.

<sup>e</sup> Significantly different after salt addition at p < 0.05.

pineol (7.30 %). In the presence of NaCl (100 mmol L<sup>-1</sup>), a small increase was observed in terpinen-4-ol percentage, while percentages of *trans*-sabinene hydrate and  $\alpha$ -terpineol were not affected.

In the absence or presence of salt, *O. majorana* EO was of *trans*-sabinene hydrate/ terpinen-4-ol chemotype. These results are in agreement with those of Hamrouni *et al.* (9), who reported that the major constituents were terpinen-4-ol, *cis*-sabinene hydrate, and *trans*-sabinene hydrate. Later investigations showed that some of the above main constituents were found in numerous EO samples of *O. majorana*. Novak *et al.* (1) mentioned that the main compounds of marjoram EO were the epimeric monoterpene alcohols, *trans*-sabinene hydrate, *cis*-sabinene hydrate, and *cis*-sabinene hydrate acetate. In fact, according to Vera and Chane-Ming (2), the main components were terpinen-4-ol and *cis*-sabinene hydrate in EO of *O. majorana* originating from Island.

Our results showed that NaCl modified the essential oil composition. In fact, new compounds appeared: eicosane, spathulenol, eugenol, and phenol (Table III). However, myrcene, present in the control, disappeared under saline conditions.

Indeed, a very important modification of EO composition was bioconversion from *cis*-sabinene hydrate into *trans*-sabinene hydrate. Culture conditions had an important effect on the transformation of these two isomers: from *cis*- to *trans*-sabinene hydrate under greenhouse conditions and from *trans*- to *cis*-sabinene hydrate under culture cham-

ber conditions. These results suggest that culture conditions activated sabinene hydrate synthase in favor of *cis*-sabinene hydrate or *trans*-sabinene hydrate.

Under saline conditions, contents of all compounds evolved in the same way (Table III). The composition of EO of *O. majorana* shoots is primarily made up of terpenic alcohols (78.27 %), followed by monoterpene hydrocarbons in the control (18.74 %). Salinity decreased the content of each class, except for aliphatic hydrocarbons and terpenic alcohols (Table III).

The quality of the EO produced and its composition in *Origanum majorana* depends on culturing conditions (15). Our findings are in accord with those of Avry and Gallouin (9) and Kotan *et al.* (16). Essential oil of *O. majorana* plants cultivated in a greenhouse had lower level of flavor than EO of plants cultivated in the culture chamber due to the great difference in the *cis*-sabinene hydrate production. Therefore marjoram cultivated

				NaCl (m	nmol L <sup>-1</sup> )		
				0	100	0	100
Compound	$t_{\rm R}$ (min)	RIa	RI <sup>b</sup>	(%) <sup>c</sup>	(%) <sup>c</sup>	(mg g <sup>-1</sup> ) <sup>c,d</sup>	(mg g <sup>-1</sup> ) <sup>c,d</sup>
Tricyclene	3.28	-	_	0.252	ND	0.045	ND
α-Pinene	3.55	931	1035	0.316	0.121 <sup>e</sup>	0.056	0.019 <sup>e</sup>
α-Thujene	3.69	939	1032	0.506	0.430 <sup>e</sup>	0.090	0.070 <sup>e</sup>
Sabinene	5.74	976	1132	0.181	2.067 <sup>e</sup>	0.032	0.338 <sup>e</sup>
β-Myrcene	6.45	991	1174	3.807	ND	0.678	ND
α-Phellandrene	9.84	1006	1176	4.145	3.602 <sup>e</sup>	0.738	0.589 <sup>e</sup>
α-Terpinene	11.39	1016	1188	0.710	0.724 <sup>e</sup>	0.126	0.118 <sup>e</sup>
Limonene	11.49	1030	1203	0.162	2.205 <sup>e</sup>	0.028	0.361 <sup>e</sup>
γ-Terpinene	11.61	1033	1213	1.448	1.903 <sup>e</sup>	0.258	0.311 <sup>e</sup>
<i>p</i> -Cymene	14.25	1062	1266	5.883	3.215 <sup>e</sup>	1.048	0.526 <sup>e</sup>
Terpinolene	16.62	1026	1280	1.183	1.081 <sup>e</sup>	0.210	0.176 <sup>e</sup>
cis-3-Hexanol	26.62	1088	1290	7.428	10.650 <sup>e</sup>	1.323	1.743 <sup>e</sup>
trans-Sabinene hydrate	29.74	1053	1474	24.181	24.541 <sup>e</sup>	4.309	4.017
Linalool	29.89	1098	1553	3.994	3.724 <sup>e</sup>	0.711	0.609 <sup>e</sup>
cis-Sabinene hydrate	30.283	1082	1556	2.235	2.492 <sup>e</sup>	0.398	0.408 <sup>e</sup>
β-Elemene	31.729	1053	1474	0.197	0.225 <sup>e</sup>	0.035	0.036
Terpinen-4-ol	31.91	1098	1553	31.77	29.050 <sup>e</sup>	5.662	4.756 <sup>e</sup>
β-Caryophyllene	32.47	1082	1556	0.210	0.311 <sup>e</sup>	0.037	0.051 <sup>e</sup>
Aromadendrene	33.00	1129	1562	0.864	0.545 <sup>e</sup>	0.154	0.089 <sup>e</sup>
Bicyclogermacrene	34.86	1257	1556	0.335	0.223 <sup>e</sup>	0.059	0.036 <sup>e</sup>
$\alpha$ -Terpenyl acetate	35.40	1295	1597	0.320	0.238 <sup>e</sup>	0.057	0.039 <sup>e</sup>
α-Terpineol	35.78	1391	1601	7.300	7.089 <sup>e</sup>	1.301	1.160 <sup>e</sup>

Table III. Essential oil composition of Origanum majorana in the greenhouse by GC-MS.

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Table III. continued								
Borneol	37.19	1176	1611	0.885	0.786 <sup>e</sup>	0.157	0.128 <sup>e</sup>	
Neryl acetate	37.49	1385	1733	0.495	0.289 <sup>e</sup>	0.088	0.047 <sup>e</sup>	
Geranyl acetate	38.13	1419	1612	0.145	0.147	0.025	0.024	
Nerol	39.49	1228	1797	0.112	ND	0.020	0.037 <sup>e</sup>	
Myrtenol	41.27	1191	1804	0.361	ND	0.064	0.053 <sup>e</sup>	
Nonadecane	43.61	1454	1687	0.123	0.147	0.025	0.024	
Eicosane	52.95	1344	1705	ND	0.229	ND	0.039	
Spathulenol	54.162	1494	1755	ND	0.329	ND	0.023	
Eugenol	55.482	1335	1701	ND	0.142	ND	0.018	
Phenol	61.071	-	-	ND	0.115	ND	0.020	
Classes							ND	
Monoterpene hydrocarbons			18.749	15.577 <sup>e</sup>	3.313	2.550 <sup>e</sup>	ND	
Terpenic alcohols			78.270	79.365 <sup>e</sup>	1.395	1.195 <sup>e</sup>	ND	
Sesquiterpene hydrocarbons			1.410	1.081 <sup>e</sup>	0.251	0.177 <sup>e</sup>		
Terpenic esters			1.320	1.005 <sup>e</sup>	0.235	0.110 <sup>e</sup>		
Aliphatic hydrocarbons			0.123	0.377 <sup>e</sup>	0.021	0.059 <sup>e</sup>		
Phenols			0	0.115	0	0.044		

 $t_R$  – Retention time.

KI – Kovat's index on: <sup>a</sup> apolar column HP-5MS, <sup>b</sup> polar column HP-Innowax (relative to n-alkane). ND – not detected.

<sup>c</sup> Mean of 3 replicates.

<sup>d</sup> Drug mass basis.

<sup>e</sup> Significantly, different after salt addition at p < 0.05.

in the greenhouse was of higher quality than that cultivated in the culture chamber because of its high terpinen-4-ol concentration (Tables II, III). The EO is believed to have had medicinal properties because of the presence of biologically active compounds such as terpinen-4-ol (5). In fact, terpinen-4-ol,  $\alpha$ -terpineol, and linalool exhibited high antibacterial activity. *O. majorana* EO is known for its strong antimicrobial activity and could therefore be used in food applications.

#### CONCLUSIONS

Our data indicate high variations in quantitative and qualitative composition of *O. majorana* EOs produced under two conditions: greenhouse and culture chamber. The study showed that EO yield and composition depend on the culturing and climatic conditions. We observed an important modification in EO composition of *O. majorana* shoots by converting *trans*-sabinene hydrate into *cis*-sabinene hydrate in the culture chamber and *cis*- to *trans*-sabinene hydrate in the greenhouse. The EO of the latter was considered to be of higher quality than that cultivated in the culture chamber, because of its higher concentration of oxygenated compounds, particularly bioactive compounds such as terpinen-4-ol.

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### SAŽETAK

### Utjecaj uvjeta uzgoja i dodatka soli na sastav eteričnog ulja slatkog mažurana (*Origanum majorana*) iz Tunisa

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U radu je opisano ispitivanje sastava eteričnog ulja izdanaka biljke *O. majorana*. Provedena su dva eksperimenta: prvi na hidroponom mediju u komorama za uzgoj, a drugi na inertnom pijesku u stakleniku tijekom 20 dana. Biljke su uzgajane 17 dana u hidroponom mediju u koji je dodan NaCl 100 mmol L<sup>-1</sup>. Rezultati ukazuju na to da hidroponi medij *O. majorana* osigurava veće prinose eteričnog ulja nego staklenik. U stakleniku se prinos ulja značajno povećao dodavanjem 50 % soli dok u uzgoju u uzgojnoj komori nije bilo promjene. U uvjetima u stakleniku i u odsutnosti soli, najvažniji sastojci ulja bili su terpinen-4-ol i *trans*-sabinen hidrat, dok su u uvjetima uzgojne komore najvažnije hlapljive komponente bile *cis*-sabinen hidrat i terpinen-4-ol. U prisutnosti NaCl-a, pojavili su se novi sastojci, kao što su eikozan, spatulenol, eugenol i fenol. Dodatno je uz stakleničke uvjete, sa i bez soli, došlo do važne promjene u količini *trans*-sabinen hidrata u eteričnom ulju, dok se u komorama promijenio sadržaj *cis*-sabinen hidrata.

Ključne riječi: Origanum majorana, staklenik, uzgojna komora, sol, prinos, terpinen-4-ol

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