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Effect of Solution Conductivity on the Volatile Constituents of *Origanum dictamnus* L. in Nutrient Film Culture

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The chemical composition of the essential oils obtained from leaves and bracts of hydroponically cultivated *Origanum dictamnus* L. (Cretan dittany), growing under various electrical conductivity (EC) levels (2.0, 4.0, and 6.0 mS/cm), was studied, using the nutrient film technique (NFT). The analysis of the essential oil content was achieved by GC-MS technique, and totals of 41 and 38 different compounds were identified in both cases of large-leaved and narrow-leaved samples of leaves and bracts, respectively. Differences in the composition content and of the percentage of each of the constituents in the two studied samples (i.e., large-leaved and narrow-leaved) and within the essential oils of leaves and bracts in both samples were observed. Carvacrol and *p*-cymene were identified as the main constituents in all essential oils, whereas thymoquinone was found in higher percentage in the essential oils of large-leaved than in narrow-leaved plants. The results obtained from GC-MS analysis were submitted to chemometric analysis, and a phenotypic similarity of the essential oils of narrow-leaved *O. dictamnus* was observed, whereas the essential oils of large-leaved O*. dictamnus* showed two separate subgroups

KEYWORDS: ***Origanum dictamnus*** **essential oil; hydroponic cultivation; electrical conductivity; GC-MS**

INTRODUCTION

Origanum dictamnus L. (Lamiaceae) or Cretan dittany is a white-woolly subshrub up to 25 cm high, endemic to Crete (Greece), with egg-shaped or rounded leaves, loose terminals of pink flowers, and large purple bracts larger than calyx. The plant grows in the wild on the rocky slopes of mountainous Crete (*1*, *2*). It is also cultivated because of its therapeutic properties (*3*), which have been known since ancient times. It has been used as a remedy for ailments of the stomach and intestinal tract, rheumatism, and is especially good for difficulties of childbirth, as an antihemorrhaging agent, and as a stimulant of the nervous system (*4*, *5*). In Crete (Greece) it is well-known as "erontas," which means "love" and as "dictamnos".

The chemical composition of the essential oil of leaves and bracts as a mixture has been studied (*6*), and recently have been published papers on the volatiles of bracts and leaves of wild and hydroponically cultivated plants using different concentrations of nitrogen and phosphorus (*3*, *7*) in the nutrient solution.

The information available on the hydroponic cultivation of aromatic and medicinal plants is limited (*7*). The nutrient film technique (NFT) in which the plants are grown in shallow channels containing a flowing nutrient solution continuously recirculated is used for commercial vegetable production (*8*) and has been applied in plant nutrition studies. Economakis (*8*) reported a series of experiments to establish that dittany plants could be successfully grown with the NFT, which can improve the yield and affect the chemical composition of the essential oil.

Continuing our experiments on the hydroponically cultivated *O. dictamnus* (*3*, *7*) and in order to better our understanding of how the NFT affects the growth and composition of dittany plants, we undertook a study concerning the electrical conductivity (EC) in the nutrient solution at three different levels. The results were compared with those recently published by us (*3*, *7*) when different concentrations of nitrogen and of phosphorus

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were used in the nutrient solution for the cultivation of *O. dictamnus*. However, the aim of our current study was to investigate the effect of the EC on the yield and chemical composition of the essential oils of large-leaved and narrow-leaved samples of leaves and bracts of *O. dictamnus* L., using the NFT.

MATERIALS AND METHODS

Chemometric Statistical Analysis. The data of **Tables 1** and **2** were submitted to multivariate statistical analysis to clarify the phenotypic relationships of the two samples (i.e., *O. dictamnus* large-leaved and narrow-leaved). Prior to principal component analysis (PCA), the variables were standardized for a normalized PCA. The data set was processed through a Statistica commercial package. City-block (Manhattan, method to measure the distance among the samples) was used to measure the similarity between samples of large-leaved and narrow-leaved of *O. dictamnus*, and Ward's linkage method was used as an agglomerative algorithm (amalgamation joining rule). Moreover, we applied the test of correlation coefficient to the data of **Tables 1** and **2** to clarify the possible linear correlation between the studied samples. To determine various correlations among the studied samples, a Pearson product moment was applied to the whole set of data (**Tables 1** and **2**). Correlations are significant if $p < 0.05$.

Plant Material and Experimental Conditions. Shoot cuttings from a wild population of *O. dictamnus* were used as plant materials, and the experiment was carried out in an unheated glasshouse at the Subtropical Plants and Olive Trees Institute, Chania, Creece. The NFT is a solution culture method, in which plants have their roots in a shallow stream of recirculating nutrient solution. The nutrient solution required for plant growth is discharged into the upper (inlet) end of a sloping watertight channel containing the plants and flows as a shallow stream through the root system. At the lower end of the channel (outlet), the solution is collected in a catchment tank and pumped back to the inlet for recirculation (*8*). The NFT system has been designed by one of us (C. Economakis), and each treatment occupied one channel and had separate "header" and "catchment" tanks. The nutrient solution was flowing by gravity at a rate of 800 mL/min for each channel and was complete with nitrogen (150 mg/L) and phosphorus (32 mg/L). To maintain constant the ratio of the nutrient elements, the solution was analyzed weekly for nitrogen and phosphorus, with the appropriate adjustments made when necessary. The volume of the nutrient solution was kept constant, the pH value was 6, and the EC varied according to treatment level (i.e., 2.0, 4.0, and 6.0 mS/cm). The pH and EC values were monitored and adjusted daily by adding 5% HNO_3 and complete stock nutrient solution, respectively.

The plant material used was produced by using single plant shoot cuttings from a wild population of *O. dictamnus*, which were placed under mist propagation conditions, and after 3 months, 32 rooted cuttings were placed, 25 cm apart, to each channel of the NFT system. Two channels, as replicates, were used for each of the three EC levels examined (i.e., 2.0, 4.0, and 6.0 mS/cm). After 7 months, 10 plants from each treatment, being at the start of flowering stage (during the middle of June), were sampled and their shoots weighed and placed in oven at 40 ° C until constant weight. The dried shoots of plants belonging to the same treatment were mixed and separated into leaves and bracts.

Isolation of Essential Oils. Samples from leaves and bracts were subjected to separate hydrodistillation for 3 h. All oils obtained were dried over anhydrous Na_2SO_4 and stored under refrigeration (4 °C).

Gas Chromatography−Mass Spectrometry (GC-MS). The essential oils were analyzed using a capillary GC-MS system operating in the EI mode. The GC-MS analysis was carried out using a Hewlett-Packard (HP) 5973 mass selective detector and a DB-5 MS fused silica capillary column of 30 m × 0.25 mm (0.25 μm film thickness). The column was temperature programmed as follows: 50 °C for 5 min, and then the temperature was increased to 280 °C, at a rate of 3 °C/min. Mass unit conditions were as follows: ion source, 230 °C; ionization energy, 70 eV; electron current, 1435 μA. Identification of constituents was based on comparison of their mass spectra with those of Wiley 275 and NBS libraries (*7*) and those described by Adams (*9*), as well as by comparison of their retention indices with literature data (*7*).

Table 1. Chemical and Percentage Composition of the Essential Oils of Leaves and Bracts of Large-Leaved Cultivated *O. dictamnus*

			leaves[a]			bracts		
	compound[b]	RI[c]	1	2	3	4	5	6
1	α-thujene[d]	931	1.3		1.4	1.4		1.0
2	α-pinene	939	0.8	0.7	0.9	1.0		0.6
3	camphene	953	0.1		0.1	0.2		0.1
4	2,4(10)-thujadiene	960				tr[e]		
5	sabinene	975			0.2			
6	*β*-pinene	980	0.2		0.2	0.4		
7	1-octen-3-ol	978	0.2			0.1		
8	3-octanol	993	0.2		0.1	0.1		
9	*δ*–3-carene	1011	0.1		0.1	0.1		
10	α-terpinene	1018	0.2					
11	*p*-cymene	1026	45.5		45.3	17.5		20.2
12	*o*-cymene	1028			0.1	0.1		
13	*β*-phellandrene	1031	0.8		0.7			
14	*cis*-sabinene hydrate	1068	1.6	1.9	1.5	0.9	1.6	1.2
15	*trans*-sabinene hydrate	1097	0.4		0.4	0.1		
16	borneol	1165	0.1		0.2	0.1		
17	terpine-4-ol	1177	0.6		0.4	0.3		
18	*p*-cymen-8-ol	1183	0.1		0.1			
19	α-terpineol	1189	0.1		0.1			
20	*cis*-dihydrocarvone	1193			tr			
21	*trans*-dihydrocarvone	1201			tr			
22	carvone	1242			tr			
23	carvacrol methyl ether	1245				tr	tr	
24	thymoquinone	1249	10.9	11.2	11.6	10.1	10.1	4.7
25	thymol	1290	0.3		0.4			
26	carvacrol	1298	34.2	58.7	33.5	54.5	85.2	67.1
27	α-cubebene	1351			tr			
28	eugenol	1356	0.2					
29	*cis*-carvacrol acetate	1372				0.2		
30	α-copaene	1376	0.5	1.0	0.5	0.1		
31	*β*-cubebene	1390		1.5				
32	*β*-caryophyllene	1418	0.3	0.9	tr			
33	α-humulene	1454		0.7				
34	*γ*-muurolene	1477		2.1				0.4
35	germacrene D	1480		1.2		tr		
36	*β*-bisabolene	1509	0.1	1.0	0.1	0.2		
37	*δ*-cadinene	1524		2.0	tr	0.1		
38	caryophyllene oxide	1580	0.5	2.2	0.8	1.9	1.3	1.9
39	6,10,14-trimethyl-2-pentadecanone					tr		
40	kaurene	2043	0.2		0.2			
41	abietatriene	2054						
total identified (%)			99.5	85.1	98.9	89.4	98.2	97.2

[a] 1–3 and 4–6, essential oils of leaves and bracts of cultivated large-leaved *O. dictamnus* using 6, 4, and 2 mS/cm electrical conductivity, respectively. [b] Compounds are listed according to their t_R to the DB-5 column. [c] Retention indices on DB-5 column were calculated according to the method of Van den Dool and Kratz (*13*). [d] Retention indices and mass spectra fragmentation pattern have been used to tentatively identify the compounds in **Table 1**. [e] Trace.

RESULTS AND DISCUSSION

Chemical Composition and Yield of Essential Oils. The yields (v/w) of the essential oils of leaves and bracts of the cultivated large-leaved and narrow-leaved *O. dictamnus* are shown in **Table 3**.

The chemical composition of the essential oils was analyzed by GC(EI)-MS, and the qualitative and quantitative results are shown in **Tables 1** and **2**. **Figures 1−3** summarize the data in **Tables 1** and **2**. The analysis of the compounds of the oils of the leaves and bracts in the case of large-leaved *O. dictamnus* showed qualitative and quantitave differences due to the different ECs used in the nutrient solution. We noted that the constituents

Table 2. Chemical and Percentage Composition of the Essential Oils of Leaves and Bracts of Narrow-Leaved Cultivated *O. dictamnus*

			leaves[a]			bracts		
	compound[b]	RI[c]	1	2	3	4	5	6
1	α-thujene[d]	931	0.9	0.5	0.3			
2	α-pinene	939	0.8	0.6	0.3			0.1
3	camphene	953	0.2	0.2	0.1			
4	β-pinene	980	0.3	0.4				
5	1-octen-3-ol	978			0.3	0.2	0.3	
6	myrcene	991	0.3					
7	3-octanol	993			0.1	tr[e]	0.1	
8	α-phellandrene	1005	0.1					
9	δ-3-carene	1011	0.1		0.1			
10	α-terpinene	1018	0.2		tr			
11	*p*-cymene	1026	15.1	27.8	14.1		5.5	
12	1,8-cineole	1031				tr		
13	γ-terpinene	1062	0.1					
14	*cis*-sabinene hydrate	1068	0.4	1.1	1.2	0.1	1.5	0.5
15	terpinolene	1088	0.1	0.1	0.1			
16	*p*-cymenene	1089		0.1	0.1			
17	*trans*-sabinene hydrate	1097	0.1	0.4	0.3	1.2	0.7	0.5
18	*trans*-pinocarveol	1139	tr					
19	*trans*-sabinol	1140					tr	
20	borneol	1165		0.3	0.4	0.9	0.7	0.7
21	terpinen-4-ol	1177	0.1	0.4	0.7	0.6	1.0	0.5
22	*p*-cymen-8-ol	1183			0.1	0.4	0.3	0.2
23	*cis*-dihydrocarvone	1193			tr	0.1	0.1	
24	carvone	1242		0.1		0.1	0.2	
25	carvacrol methyl ether	1245	tr		0.1	0.1		0.2
26	thymoquinone	1249		2.1	1.5	3.9	2.2	1.9
27	thymol	1290				0.5	0.3	0.5
28	carvacrol	1298	70.6	61.3	69.7	85.8	79.9	87.3
29	carvacrol acetate	1372	tr		0.1			
30	α-copaene	1376		0.2	0.1	0.1	0.2	
31	β-caryophyllene	1418	0.1		0.1		0.1	
32	β-bisabolene	1509	0.1	0.1	0.1		0.2	
33	γ-cadinene	1513			tr			
34	δ-cadinene	1524	tr		tr		0.1	
35	caryophyllene oxide	1580	1.2	1.1	1.1		1.2	
36	manoyl oxide	1997			tr			
37	6,10,14-trimethyl-2-pentadecanone		0.1		0.1		tr	
38	abietatriene	2054			0.1		0.1	
total identified (%)			90.9	96.8	91.3	94.0	94.7	92.4

[a] 1–3 and 4–6: essential oils of leaves and bracts of cultivated narrow-leaved *O. dictamnus* using 6, 4, and 2 mS/cm electrical conductivity, respectively. [b] Compounds are listed according to their t_R to the DB-5 column. [c] Retention indices on DB-5 column were calculated according to the method of Van den Dool and Kratz (*13*). [d] Retention indices and mass spectra fragmentation pattern have been used to tentatively identify the compounds in **Table 2**. [e] Trace.

Table 3. Yield (Percent v/w) of the Essential Oils of Leaves and Bracts of Large-Leaved and Narrow-Leaved Cultivated *O. dictamnus*[a]

electrical conductivity,	larged-leaved		narrow-leaved	
mS/cm	leaves	bracts	leaves	bracts
2	3.22a	4.16c	2.65b	3.26c
4	2.98a	4.76b	3.03a	4.08b
6	2.90a	5.30a	3.00a	4.80a

[a] Values of each column followed by different letters are significantly different ($p = 0.05$) according to Duncan's test.

α-thujene, camphene, β-pinene, 1-octen-3-ol, 3-octanol, δ-3-carene, α-terpinene, *p*-cymene, β-phellandrene, *trans*-sabinene hydrate, borneol, terpine-4-ol, *p*-cymen-8-ol, α-terpineol, thymol, and kaurene identified in leaves using ECs of 6 and 2 mS/cm were not present at all in the essential oil of leaves using an EC of 4 mS/cm. In the case of bracts it is of interest that with cultivation of *O. dictamnus* using the same EC (i.e., 4 mS/cm) the essential oil was found to be very poor in its composition,

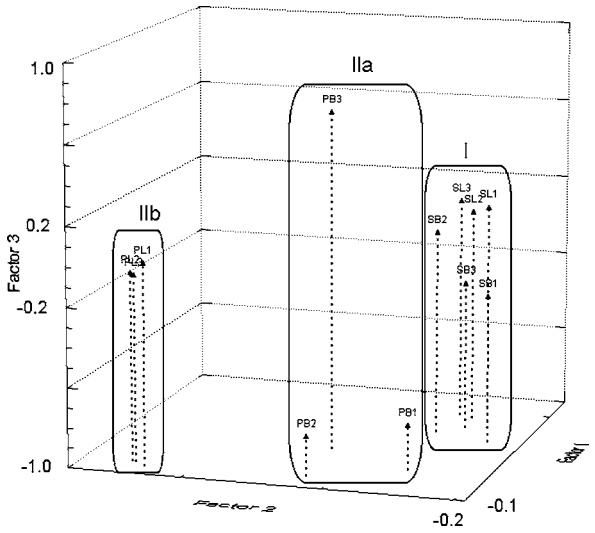

Figure 1. Relative positions of samples in the space defined by the first three principal components. I, IIa, and IIb represent the groups obtained from the correlation analysis (**Table 4**). PL1–PL3 represent samples of the leaves of large-leaved cultivated *O. dictamnus* using 6, 4, and 2 mS/cm EC, respectively. PB1–PB3 represent samples of the bracts of large-leaved cultivated *O. dictamnus* using 6, 4, and 2 mS/cm EC, respectively (**Table 1**). SL1–SL3 represent samples of the leaves of narrow-leaved cultivated *O. dictamnus* using 6, 4, and 2 mS/cm EC, respectively. SB1–SB3 represent samples of the bracts of narrow-leaved cultivated *O. dictamnus* using 6, 4, and 2 m S/cm EC, respectively (**Table 2**).

having as the main constituents carvacrol (85.19%) and thymoquinone (10.07%), whereas caryophyllene oxide was found in low percentage (1.3%). Carvacrol was present in high percentage (33.49−85.19%) in all essential oils of leaves and bracts of the large-leaved sample, followed by *p*-cymene, which was found to be in sufficient percentages (from 45.49 to 17.45%) in the essential oils of leaves and bracts using ECs of 6 and 2 mS/cm. *p*-Cymene was totally absent from the composition of the essential oil of leaves and bracts when the EC was 4 mS/cm (**Table 1**). The essential oils of leaves and bracts of the narrow-leaved sample of *O. dictamnus* were also characterized by the presence of carvacrol and *p*-cymene, which were the predominant compounds (61.33−85.82 and 5.53−27.83%, respectively). *p*-Cymene was totally absent in the case of the essential oils of bracts in which the ECs used were 6 and 2 mS/cm. Analysis of the essential oils of *O. dictamnus* cultivated using different ECs showed 41 and 38 identified constituents, in contrast to the 25 constituents identified in the cultivated and wild *O. dictamnus* (*3*). Cultivation of *O. dictamnus* using different concentrations of phosphorus in the nutrient solution led to an increasing composition of constituents up to 46 identified in the previous analysis of the essential oils of leaves and bracts (*7*). Carvacrol was identified as predominant in all essential oils that have been studied previously and remained higher in the bracts than in leaves (*3*, *7*); its percentage content was comparable to that determined in our study in all essential oils. The percentage content of *p*-cymene remained high in the leaves of large-leaved and narrow-leaved *O. dictamnus* and was comparable to that determined in *O. dictamnus* hydroponically cultivated using phosphorus in the nutrient solution (*7*). On the contrary, the percentage content of *p*-cymene was lower in the wild plant as well as in the plant hydroponically cultivated using different concentrations of nitrogen in the nutrient solution (*3*) than in the plant cultivated using phosphorus in the nutrient solution (*7*) and than that determined in the leaves and bracts of large-leaved and narrow-leaved *O. dictamnus*. In our study,

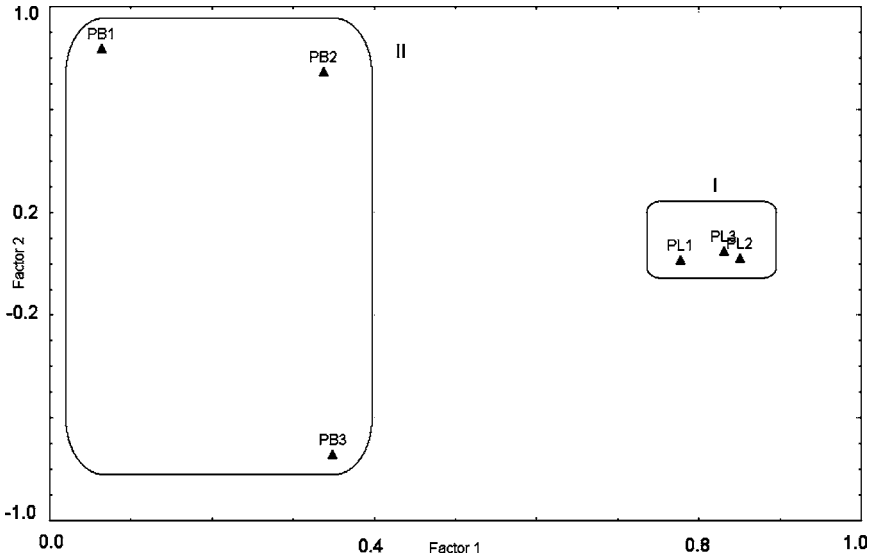

Figure 2. Two-dimensional graph obtained in the PCA of the data set of **Table 1**. I and II represent the groups obtained from the correlation analysis (**Table 4**). PL1–PL3 represent samples of the leaves of large-leaved cultivated *O. dictamnus* using 6, 4, and 2 m S/cm EC, respectively. PB1–PB3 represent samples of the bracts of large-leaved cultivated *O. dictamnus* using 6, 4, and 2 mS/cm EC, respectively (**Table 1**).

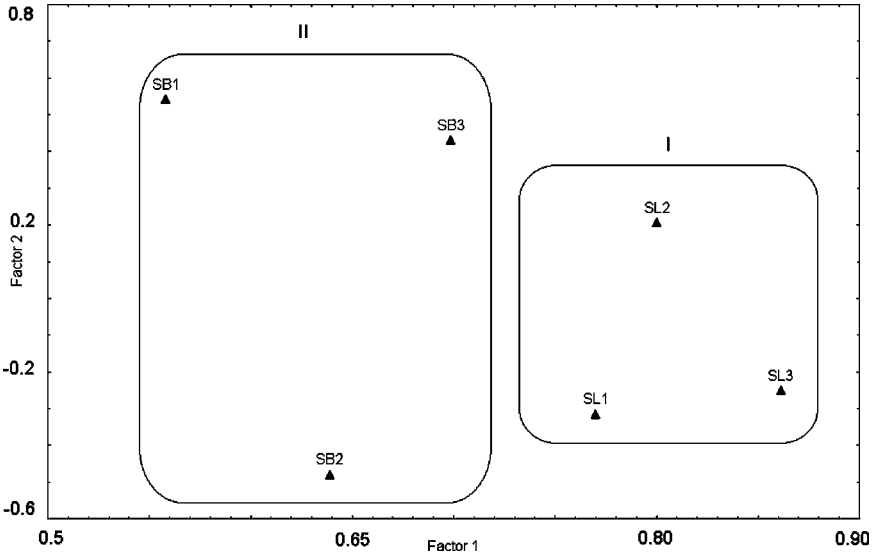

Figure 3. Two-dimensional graph obtained in the PCA of the data set of **Table 2.** I and II represent the groups obtained from the correlation analysis (**Table 4**). SL1–SL3 represent samples of the leaves of narrow-leaved cultivated *O. dictamnus* using 6, 4, and 2 mS/cm EC, respectively. SB1–SB3 represent samples of the bracts of narrow-leaved cultivated *O. dictamnus* using 6, 4, and 2 m S/cm EC, respectively (**Table 2**).

Table 4. Pearson's Correlation Coefficient[a,b]

variable	1P[a]	2P	3P	4P	5P	6P	1S	2S	3S	4S	5S	6S
1P	1.00	0.53*[b]	0.52*	0.11	0.11	0.16	0.07	–0.04	0.05	–0.15	0.09	–0.01
2P	0.53	1.00	0.57*	0.00	0.32*	0.23	–0.07	0.03	0.12	0.06	0.07	0.00
3P	0.52	0.57	1.00	0.08	0.26	0.20	–0.03	0.05	0.05	–0.06	0.07	0.07
4P	0.11	0.00	0.08	1.00	0.48*	–0.43*	0.22	0.09	0.04	0.05	0.13	0.17
5P	0.11	0.32	0.26	0.48	1.00	–0.30	–0.15	0.01	–0.07	0.24	–0.08	0.24
6P	0.16	0.23	0.20	–0.43	–0.30	1.00	0.27	0.41*	0.28	0.03	0.12	0.12
1S	0.07	–0.07	–0.03	0.22	–0.15	0.27	1.00	0.62*	0.68*	0.18	0.38*	0.36*
2S	–0.04	0.03	0.05	0.09	0.01	0.41	0.62	1.00	0.54*	0.34*	0.29	0.62*
3S	0.05	0.12	0.05	0.04	–0.07	0.28	0.68	0.54	1.00	0.46*	0.61*	0.39*
4S	–0.15	0.06	–0.06	0.05	0.24	0.03	0.18	0.34	0.46	1.00	0.22	0.38*
5S	0.09	0.07	0.07	0.13	–0.08	0.12	0.38	0.29	0.61	0.22	1.00	0.32*
6S	–0.01	0.00	0.07	0.17	0.24	0.12	0.36	0.62	0.39	0.38	0.32	1.00

[a] 1P–3P and 4P–6P: essential oils of leaves and bracts of cultivated large-leaved *O. dictamnus* using 6, 4, and 2 mS/cm electrical conductivity, respectively; 1S–3S and 4S–6S: essential oils of leaves and bracts of cultivated narrow-leaved *O. dictamnus* using 6, 4, and 2 mS/cm electrical conductivity, respectively. [b] Correlations marked * are significant at $p < 0.05$.

the results concerning the identification of the constituents limonene, linalool, tricyclene, and camphor are in accordance with those recently published from the analysis of hydroponically cultivated *O. dictamnus* using different phosphorus

concentrations in the nutrient solution (*7*) in which the above constituents were not identified, contrary to the results published from the analysis of the wild and cultivated *O. dictamnus* using different nitrogen concentrations in the nutrient solution (*3*) in which the above constituents were identified. It is noteworthy that an increase in the percentage of *p*-cymene in the case of large-leaved *O. dictamnus* was accompanied by a decrease in the percentage of carvacrol (**Table 1**), whereas this phenomenon is opposite in the case of narrow-leaved *O. dictamnus* (**Table 2**). This phenomenon is in accordance with that observed when phosphorus was used in the nutrient solution, especially in the case of leaves (*7*). It seems likely that carvacrol might be synthesized by the aromatization of γ-terpinene to *p*-cymene followed by hydroxylation of *p*-cymene to produce carvacrol. However, γ-terpinene seems to play a role in this biosynthetic pathway, and γ-terpinene synthase is an enzyme that is involved in the biosynthesis of aromatic monoterpenes. This was detected in cell-free extracts of the aromatic plant *Origanum vulgare*, which produces carvacrol, and assumes a key role for this enzyme (*10*, *11*).

The results from our analysis as well as from that previously reported concerning hydroponic cultivation of *O. dictamnus* using different phosphorus concentrations in the nutrient solution showed newly identified constituents (**Tables 1** and **2**) (*7*), which have not been reported in previous studies (*3*, *6*).

From the above results and from those recently reported by us (*3*, *7*, *12*) we can conclude that hydroponic cultivation using the NFT is a suitable methodology for growing aromatic and medicinal plants and seems to be a potent methodology for cultivating *O. dictamnus* successfully for its chemical composition and essential oil yield.

Results obtained from the correlation analysis (**Table 4**) showed the existence of three well-defined groups (I, IIa, IIb). According to **Figure 1**, the phenotypic similarity of the samples of narrow-leaved *O. dictamnus* is very high, forming a completely separate group (i.e., I). On the other hand, the samples of large-leaved *O. dictamnus*, according to PCA, showed two separate subgroups. The first one (i.e., IIa), with the samples of the bracts, and the second one (i.e., IIb) consisting of the samples of the leaves, have higher phenotypic similarity than that of the bracts. The same results are obtained if PCA is applied within the group of narrow-leaved (**Figure 2**) as well as the group of large-leaved palnts (**Figure 3**). From the data matrix of **Table 4**, we can conclude that many significant positive correlations are observed between the pairs of the narrow-leaved samples. With the exception of the pair of correlation between 6P and 2S (**Table 4**), there is no other significant correlation observed between the studied samples of large-leaved and narrow-leaved plants. Moreover, it is important that the only significant negative correlation observed is that of the pair of 4P and 6P (**Table 4**).

In conclusion, we can say that the NFT seems to be excellent for hydroponically cultivated plants to produce a much higher yield (percent) of essential oil than that of plants from the same population grown in the wild. On the basis of the results from this study and results recently published by us on the same subject, Cretan dittany seems to have great potential in NFT cultivation, and by controlling the nutrient solution elements and EC, it could be possible to improve the yield and quality of the essential oil.

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