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Content and composition of essential oil of four *Origanum vulgare* L. accessions under reduced and normal light intensity conditions

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Summary

The variation in the chemical composition and content of the essential oil was examined in four *Origanum vulgare* accessions (Ov): 1- *Origanum vulgare* L. ssp. *vulgare* (=Ovu), 2- *Origanum vulgare* L. ssp. *hirtum* (Link) letswaart (=Ohi), 3- *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek (=Ovi) and 4- *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek × *O. majorana* L. (=Oxm), growing under reduced (26%) and normal light intensity. Altogether, 64 compounds representing 98.95% of the total oil were identified. Reduced light had a minor effect on the composition of essential oil. It decreased the content of *p*-cymene in Ohi and increased the sabinene content in Oxm herb samples. The essential oil of Ovu in both samples was mainly composed of *trans*-sabinene hydrate, *β*-caryophellene and germacrene D. The major components of essential oil of Ohi were thymol and carvacrol followed by *γ*-terpinene and *p*-cymene. Herb samples had a considerably higher amount of essential oil than leaf samples. In herb extracts of Ovi, cymyl compounds such as *p*-cymene, thymol and *γ*-terpinene were dominant. Oxm was characterized by several monoterpenes with low concentrations including *γ*-terpinene, sabinene *cis-b*-ocimene and carvacrol methyl ether. The results of the current study suggest a chemical toleration of the evaluated accessions to the applied light reduction. Furthermore, a full investigation of essential oil profiles of *Origanum vulgare* accessions is presented.

Abbreviations

EO: essential oil; NLI: normal light intensity; Ohi: *Origanum vulgare* L. ssp. *hirtum* (Link) letswaart; Ovi: *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek; Ovu: *Origanum vulgare* L. ssp. *vulgare*; Oxm: *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek × *O. majorana* L.; RLI: reduced light intensity.

Introduction

Within the genus *Origanum*, Ietswaart (1980) distinguished six subspecies of *O. vulgare* L. (Ov): 1- ssp. *hirtum* (Link) Ietswaart, 2- ssp. *vulgare*, 3- ssp. *virens* (Hoffmannsegg et Link) Ietswaart, 4- ssp. *viride* (Boissier) Hayek, 5- ssp. *gracile* (Kock) Ietswaart and 6- ssp. *glandulosum* (Desfontaines) Ietswaart based on the morphological criteria. However, as Ietswaart (1980) established the revision of *Origanum*, Ov accessions were not extensively investigated regarding their chemical characteristics. With regard to the bulk of research addressing the EO content and composition in Ov accessions, it is proposed that these parameters can also be used for the discrimination of the Ov plants (D'Antuono et al., 2000; Novak et al., 2003a; Skoula and Harborne, 2004), therefore, a full investigation about EO profiles of all Ov accessions is recommended.

Skoula et al. (1999) proposed that *Origanum* species are rich in either sabinyl compounds or cymyl compounds but never in both. Ohi is an oil-rich accession with high quality EO, due to high concentrations of carvacrol and/or thymol in its EO. Other main compounds in EO of Ohi include *γ*-terpinene, *p*-cymene (Baranauskienė et al., 2013; Johnson et al., 2004; Novak et al., 2003a). In Ohi plants two main chemotypes: carvacrol type (Baranauskienė et al., 2013; Johnson et al., 2004; Skoula et al., 1999; Tibaldi et al., 2011) and thymol type (Jerkovic et al., 2001; Russo et al., 1996) have been reported. Moreover, an intermediate type containing both thymol and carvacrol with/without high content of *p*-cymene and *γ*-terpinene has also been identified (D'Antuono et al., 2000; Milos et al., 2000). Ovu and Ovi are poor sources of volatiles (Baranauskienė et al., 2013; Lukas et al., 2013). Ovu is rich in acyclic compounds and sesquiterpenoids while the EO of Ovi is rich in either acyclic compounds (mainly linalool and linalyl acetate) and sesquiterpenoids or in cymyl-compounds (mainly *γ*-terpinene, carvacrol and/or thymol together with other related compounds). Sabinyl-compounds (mainly sabinene and *cis-*/*trans*-sabinene hydrate acetate) can be found mainly in Ovi (Skoula and Harborne, 2004). Nevertheless, the EO of Ovu plants from Lithuania have been observed with essentially high concentration of sabinene, *β*-ocimene, *β*-caryophyllene and germacrene D (Baranauskienė et al., 2013).

The expression of EO compounds in *Origanum* plants is principally influenced by the genotype (Azizi et al., 2012). Additionally, the environmental factors including season (Grausgruber-Gröger et al., 2012; Johnson et al., 2004), light intensity (Chang et al., 2008; Tibaldi et al., 2011) and photoperiod (Circella et al., 1995; Dudai et al., 1992) can influence the composition of essential oil in Lamiaceae plants. Moreover, it has been observed that the growing conditions such as harvest time (Baranauskienė et al., 2013), spatial distribution (De Falco et al., 2013) and post-harvest conditions (Tibaldi et al., 2011) changed the composition and content of EO in *Origanum* plants.

Furthermore, composition and content of EO vary noticeably among plant organs at different development stages. However, flowering parts of Dalmatian sage (*Salvia officinalis* L.) had considerably higher EO compared to leaves and stems. Moreover, the concentration of monoterpene and sesquiterpene fractions of EO varied among these organs (Perry et al., 1999). In young developing leaves of sweet basil (*Ocimum basilicum* L.) the content of EO was higher than in mature leaves. Besides, different composition of EO was observed in the young and mature leaves (Werker et al., 1993). Actually, in the Lamiaceae family the density and distribution of glandular trichomes, which are responsible for EO secretion, is higher on reproductive organs and young leaves compared to mature leaves (Werker, 1993).

The effect of light intensity on the content and composition of EO in several Lamiaceae plants was reported. Light irradiance influences the content and composition of EO through alteration in photosynthesis, physiological, and morphological processes of plants (Figueiredo et al., 2008). Sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*) under 15%, 27%, 45% and 100% of full sunlight conditions showed different content and profile of EO. The sage plants under 45% of full sunlight had the highest content of EO with higher

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level of (+)-thujanone and decreased level of camphor. In thyme the highest content of EO with thymol and myrcene concentrations occurred in full sunlight (Li et al., 1996). In the closely related species *O. syriacum* the composition of essential oil was influenced by the effects of temperature, photoperiod, and light intensity. Higher light intensity enhanced the relative content of *p*-cymene, while the content of phenolic monoterpenes, for example thymol and γ-terpinene, was generally decreased (Dudai et al., 1992).

Although Ov plants are native to the warm climate of Mediterranean regions with high solar irradiance (Ietswaart, 1980), these plants are also cultivated in temperate climate conditions with lower light intensities and sunny days similar to those in Germany (Honermeier et al., 2013). Therefore, it is important to discover if light intensity can influence the EO properties of Oregano plants. In our previous publication the effect of light reduction on the physical properties of glandular trichomes was evaluated (Shafiee-Hajiabad et al., 2015). In this paper four relevant Ov accessions were studied under reduced light intensity (RLI) and normal light intensity conditions (NLI) regarding their EO content and composition. On the other hand, a full profile of EO in two types of samples: herb and first developed leaf (third leaf) of these accessions will be presented.

Materials and methods

Experimental design

Plant material and design: In 2012, four *Origanum* accessions, provided by the National German Genebank (IPK Gatersleben), were investigated in the research station of Rauischholzhausen, Justus Liebig University of Giessen, Germany. A pot experiment was conducted with four accessions (Tab. 1) and two light intensities: reduced light and normal light intensity as the treatments including four replications. Each pot (one plant per pot) was considered as one replication.

Tab. 1: Subspecies and origin of investigated *Origanum* accessions

No.	Subspecies	Accession no.	Origin
1	*Origanum vulgare* L. ssp. *vulgare* (Ovu)	ORI 07 /79	unknown
2	*Origanum vulgare* L. ssp. *hirtum* (Link) letswaart (Ohi)	ORI 34 /03	USA
3	*Origanum vulgare* L. ssp. *viride* (Ovi)	ORI 29	Italy
4	*Origanum vulgare* L. ssp. *viride* (Boiss.) × *O. majorana* (Oxm)	ORI 35	Italy

The seeds were sown on 17th April 2012 and plantlets with three leaves were transferred to pots on April 24th 2012. The first developed leaf (third leaf from the apex) and herb were used as sample. The collection of leaf samples was before harvest on July 4th 2012. In order to take the leaf samples, four representative leaves (third leaf from the apex) from each pot were collected and mixed. In the case of herb samples, the main stems were separated and a mixture of the rest was considered as herb samples. Both sample types were dried at 39 °C for 48 hours in an air-circulating oven.

Light reduction: The pots were arranged from the north-west to south-east direction under normal light intensity (NLI) and reduced light intensity (RLI) conditions. The light reduction was applied by using a wire-house (a woven metal net with 16.7 × 16.7 mm square mesh and 1.86 mm wire diameter). The light intensity was measured daily between 9 and 11 a.m. near to the pots by the use of a light meter (EBLX4, Hartman & Braun AG, Frankfurt Germany). The mean daily light intensity during the plants growth cycle (May to August) inside and outside of the wire-house was calculated (43761 lux and 59067 respectively). The wire-house reduced the light intensity by an average of 26%. The climate parameters of the research station did not vary considerably inside and outside of the wire-house. During the growth period of *Origanum* plants a mean air temperature of 15.1 °C and a mean air humidity of 78.4% was measured. The soil used in this experiment was prepared according to the method described in Shafiee-Hajiabad et al. (2015).

GC-MS analyses

Dried plant material was immersed in dichloromethane and extracted for 30 min in an ultrasonic water bath (Lukas et al., 2009). The extracts were separately prepared from the third leaves (30 mg/ml) and the rest of herb (300 mg/10 ml). The extracts were filtered through cellulose wadding in a Pasteur pipette and analyzed by gas chromatography coupled with gas chromatography mass-spectrometer (GC/MS) [HP 6890 coupled with a HP 5972 MSD (Hewlett-Packard, Palo Alto, CA, USA)]. For quantitative analyses, biphenyl 30 (mgl^{-1}) was used as internal standard.

GC-MS was used for identification of the compounds with the following conditions: DB-5MS capillary column (30 m * 0.25 mm i.d.; 0.25 µm film thickness; Agilent); helium as carrier gas (average velocity 42 cm s^{-1}); injector temperature 250 °C, split ratio 1:1; temperature program: 60 °C for 4 min, rising to 180 °C at 3 °C min, and finally holding at 320 °C for 5 min.

Retention indices (RI) of the sample components were determined on the basis of homologous *n*-alkane hydrocarbons (Retention index standard for gas chromatography, Sigma, Vienna, Austria) under the same conditions. The composition was obtained by peak area normalization, and the response factor for each component was considered to equal 1. The compounds were identified by comparing their retention indices (Adams, 2007) and the mass spectra to published data (McLafferty, 1989).

Statistical analyses

A completely randomized experimental design was conducted as a two-factorial pot experiment. The analysis of variances was carried out using the IBM SPSS program version 20 on all parameters. A two-way between-groups analysis of variance was conducted to explore the impact of accessions and light intensity on the composition and content of EO in herb and leaf samples. Data of two different samples (herb and leaf) of the investigated accessions were analyzed separately in two-way analysis of variance (ANOVA).

Duncan's multiple-range test was applied for the calculation of the significant differences among and between the groups at a probability level ($P < 0.01$). LSD values were calculated and used for the mean comparison of different treatments within one factor. All data were reported as means (mg per g dry matter) ± standard error.

Results

Effect of light intensity on content and composition of EO

The applied treatment during the growth of Ov accessions led to alteration in the content of some chemical compounds, but many of these changes were not found to be statistically significant. In particular, the content of carvacrol in leaf samples was higher under NLI (0.2 mg/g) than RLI (0.08 mg/g) condition. Conversely, thymol content was lower under NLI in both sample types, although in both cases this effect was not statistically relevant (Tab. 2). An interaction effect between accessions and light intensity was observed on sabi-

Tab. 2: The average concentration of all detected chemical compounds (mg/g) in extracts of herb and leaf samples of *Origanum vulgare* accessions (Ovu: ssp. *vulgare*, Ohi: ssp. *hirtum*, Ovi: ssp. *viride*, Oxm: ssp. *viride* × *majorana*) by using GC/MS, RLI: reduced light intensity, NLI: normal light intensity, H: Herb sample, L: leaf sample.

			Accessions								Light condition				Sample	
			Ovu		Ohi		Ovi		Oxm		RLI		NLI			
Nr.	Compounds	KI [a]	H	L	H	L	H	L	H	L	H	L	H	L	H	L
1	Sabinene	969	0.049	0.079	-	0.002	0.003	0.007	0.095	0.181	0.024	0.053	0.049	0.082	0.036	0.067
2	1-Octen-3-ol	979	0.068	0.063	0.110	-	0.011	0.002	0.031	0.116	0.062	0.039	0.048	0.052	0.055	0.045
3	3-Octanone	983	-	-	0.031	-	0.001	0.002	-	-	0.009	0.001	0.008	-	0.008	-
4	Myrcene	990	0.006	0.003	0.207	0.006	0.022	0.019	0.016	0.047	0.065	0.023	0.060	0.014	0.063	0.019
5	α-Terpinene	1021	-	-	-	-	-	0.005	-	0.006	-	0.003	-	0.003	-	0.003
6	ϱ-Cymene	1024	0.023	0.006	1.196	0.054	0.352	0.181	0.112	0.041	0.457	0.058	0.384	0.083	0.421	0.071
7	β-Phellandrene	1029	0.021	0.003	-	-	-	-	-	0.003	0.008	0.002	0.002	0.001	0.005	0.002
8	*cis*-β-Ocimene	1037	0.060	0.143	0.017	-	0.071	0.125	0.033	0.180	0.040	0.131	0.051	0.093	0.045	0.112
9	*trans*-β-Ocimene	1050	0.019	0.051	0.066	-	0.015	0.034	0.012	0.088	0.009	0.045	0.048	0.041	0.028	0.043
10	γ-Terpinene	1059	0.036	0.019	1.645	0.063	0.193	0.293	0.141	0.388	0.537	0.185	0.471	0.196	0.504	0.191
11	*cis*-Sabinene hydrate	1070	0.016	-	0.031	-	0.005	-	0.023	0.006	0.020	0.002	0.018	0.001	0.019	0.002
12	***trans*-Sabinene hydrate**	**1098**	0.400	0.151	0.028	-	-	0.008	-	0.002	0.156	0.028	0.059	0.052	0.107	0.040
13	**Linalool**	**1096**	0.002	-	0.005	-	0.012	-	0.007	-	0.005	-	0.008	-	0.007	-
14	Octadienal <(2E.4E)>	1116	-	-	0.009	-	-	-	-	-	0.002	-	0.002	-	0.002	-
15	*allo*-Ocimene	1128	0.031	0.056	0.002	-	0.035	0.055	0.013	0.075	0.016	0.055	0.024	0.038	0.020	0.047
16	Borneol	1169	-	-	0.028	-	-	-	-	-	0.008	-	0.006	-	0.007	-
17	Terpinen-4-ol	1177	0.001	-	0.029	-	-	-	-	-	0.008	-	0.007	-	0.008	-
18	α-Terpineol	1188	0.007	-	0.006	-	-	-	0.001	0.002	0.004	0.001	0.003	0.001	0.003	0.001
19	*cis*-Dihydrocarvone	1192	-	-	0.005	-	-	-	-	-	0.001	-	0.001	-	0.001	-
20	*trans*-Dihydrocarvone	1200	-	-	0.003	-	-	-	-	-	0.001	-	-	-	0.001	-
21	Thymol methyl ether	1235	0.014	0.003	-	-	0.112	0.067	0.006	-	0.036	0.024	0.029	0.011	0.033	0.018
22	Carvacrol methyl ether	1244	0.001	-	0.026	-	0.046	0.029	0.108	0.139	0.046	0.051	0.045	0.033	0.045	0.042
23	Thymoquinone	1252	-	-	0.132	-	0.016	-	0.001	-	0.033	-	0.041	-	0.037	-
24	*trans*-Sabinene hydrate acetate	1256	-	-	0.022	-	-	-	-	-	0.011	-	-	-	0.006	-
25	*trans*-Anethole	1284	-	0.021	0.011	-	-	-	-	0.004	0.003	0.008	0.002	0.004	0.003	0.006
26	Thymol	1290	0.072	0.031	4.519	0.135	0.387	0.401	0.027	-	1.317	0.165	1.185	0.118	1.251	0.142
27	Carvacrol	1299	0.010	-	3.651	0.478	0.006	-	0.059	0.092	0.933	0.081	0.929	0.204	0.931	0.143
28	Dihydrocarveol acetate	1307	-	-	0.030	-	-	-	-	-	0.015	-	-	-	0.007	-
29	Menth-1-en-7-al <3-oxo-ϱ>	1333	-	-	0.001	-	-	-	-	-	-	-	0.001	-	trace	-
30	α-Terpinyl acetate	1346	-	-	-	-	0.001	0.002	-	-	-	0.001	-	-	trace	trace
31	Thymol acetate	1352	-	-	-	-	trace	-	-	-	-	-	0.001	-	0.001	-
32	Carvacrol acetate	1371	-	-	0.002	-	0.008	-	-	-	-	-	0.025	-	0.013	-

33	β-Bourbonene	1388	0.082	0.092	0.001	-	-	-	0.023	0.035	0.032	0.029	-	0.035	0.016	0.032
34	β-Elemene	1390	0.004	-	-	-	0.022	-	-	0.018	0.002	-	0.114	0.009	0.058	0.004
35	β-Caryophyllene	1419	0.234	0.286	0.208	0.007	-	0.027	0.033	0.073	0.135	0.117	-	0.081	0.068	0.099
36	β-Cedrene	1420	-	0.022	-	-	-	-	-	0.007	-	0.008	0.005	0.006	0.003	0.007
37	β-Copaene	1432	0.022	-	-	-	-	-	0.002	-	0.007	-	-	-	0.003	-
38	β-Humulene	1438	-	0.005	-	-	0.003	-	-	-	-	0.002	0.037	0.001	0.018	0.001
39	Aromadendrene	1441	-	0.003	0.144	-	-	-	-	-	0.037	0.001	0.002	-	0.019	0.001
40	*cis*-Muurola-3.5-diene	1450	0.008	-	-	-	0.002	-	-	-	0.002	-	0.012	-	0.007	-
41	α-Humulene	1454	0.026	0.027	0.022	-	-	0.001	0.003	0.009	0.014	0.011	0.002	0.007	0.008	0.009
42	*allo* Aromadendrene	1460	0.007	0.008	-	-	-	-	-	-	0.002	0.001	-	0.003	0.001	0.002
43	Caryophyllene 9-epi-(e)-	1466	0.001	-	-	-	0.002	-	-	-	-	-	-	-	trace	-
44	γ-Himachalene	1482	-	-	0.001	-	0.006	-	-	-	0.001	-	0.045	-	0.023	-
45	Germacrene D	1485	0.179	0.268	-	-	-	0.018	0.026	0.134	0.060	0.134	-	0.077	0.030	0.105
46	*cis*-β-Guaiene	1493	-	-	0.001	-	-	-	-	-	-	-	0.001	-	0.001	-
47	Bicyclogermacrene	1500	0.005	0.002	-	-	-	0.002	-	0.004	0.002	0.002	-	0.001	0.001	0.002
48	*trans-trans*-α-Farnesene	1505	0.002	0.003	-	-	-	0.003	0.004	0.020	-	0.008	0.003	0.005	0.002	0.006
49	β-Bisabolene	1505	0.004	-	0.088	0.004	0.022	0.017	0.010	0.007	0.029	0.008	0.033	0.006	0.031	0.007
50	γ-Cadinene	1513	-	-	0.002	-	0.002	-	-	-	0.001	-	0.001	-	0.001	-
51	endo-1-Bourbonanol	1520	-	-	-	-	-	-	0.002	-	-	-	0.001	-	0.001	-
52	γ- Cadinene	1523	-	-	0.004	-	0.002	-	-	-	0.002	-	0.001	-	0.002	-
53	*trans*-γ-Bisabolene	1531	-	-	-	-	-	-	0.002	0.004	-	-	0.001	0.002	-	0.001
54	Thymohydroquinone	1555	-	-	0.009	-	-	-	-	-	0.002	-	0.002	-	0.002	-
55	Germacrene D -4-ol	1575	0.064	0.128	-	-	0.001	0.007	0.005	0.031	0.016	0.035	0.019	0.048	0.018	0.042
56	Spathulenol	1578	-	-	0.004	-	0.002	-	0.001	-	0.002	-	0.002	-	0.002	-
57	Caryophellene oxide	1583	0.026	-	0.020	-	0.008	-	0.016	0.002	0.020	-	0.015	0.001	0.018	0.001
58	Globulol	1590	0.002	-	-	-	-	-	-	-	-	-	0.001	-	trace	-
59	Humulene epoxide II	1608	0.002	-	-	-	-	-	0.002	-	0.001	-	0.001	-	0.001	-
60	*allo*-Aromadendrene epoxide	1641	0.003	-	-	-	-	-	-	-	0.001	-	-	-	0.001	-
61	*epi*-α-Muurolol	1642	0.001	-	-	-	-	-	-	-	-	-	-	-	trace	-
62	14-hydroxy-9-epi-(e)-Caryophyllene	1669	0.001	-	-	-	-	-	-	-	-	-	-	-	trace	-
63	Germacra-4(15), 5,10(14)-trien-1-α-ol	1686	0.011	-	-	-	-	-	0.001	-	0.003	-	0.003	-	0.003	-
64	Oplopanone	1740	0.006	-	-	-	-	-	0.001	-	0.002	-	0.002	-	0.002	-
	unknown		-	-	0.119	-	0.015	0.002	0.003	0.002	0.029	-	0.040	0.002	0.034	0.001
	Total content of EO		1.528	1.474	12.435	0.749	1.384	1.307	0.817	1.719	4.231	1.312	3.851	1.312	3.996	1.312

[a] Kováts retention indices calculated against C8–C10 n-alkanes on nonpolar DB-5 column.
*: The concentration was lower than 0.0009 mg/g; -: it was not detected

nene and *p*-cymene contents in herb. RLI decreased the content of sabinene only in Oxm plants. The content of *p*-cymene increased in Ohi under RLI condition. Ovi plants, however, showed an opposite trend; although, it was not statistically significant (Fig. 1).

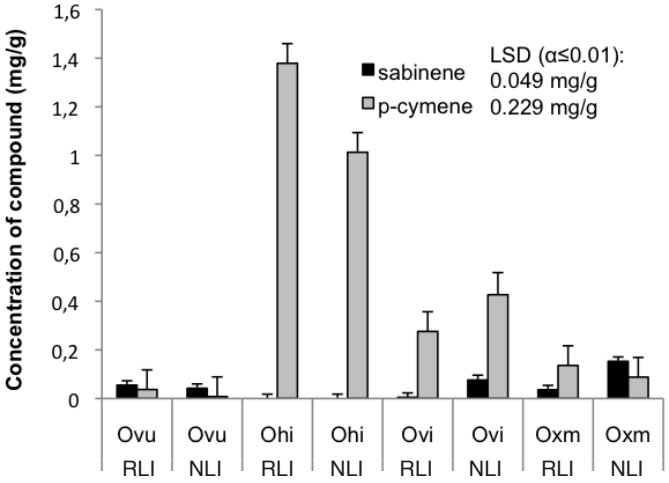

Fig. 1: Effects of accession and light intensity on the chemical compounds of essential oil in herb samples (s.e.: mean standard error of mean, Ovu: *Origanum vulgare* ssp. *vulgare*, Ohi: *Origanum vulgare* ssp. *hirtum*, Ovi: *Origanum vulgare* ssp. *viride*, Oxm: *Origanum vulgare* ssp. *viride* × *majorana*), RLI: reduced light intensity, NLI: normal light intensity

Effect of accessions on EO content and composition of herb samples

The investigated accessions showed different profiles of EO both in herb and leaf samples. In total, 64 compounds were identified in herb and leaf extracts of Ov accessions (Tab. 2). The mean concentrations of twenty compounds, with higher concentrations than 0.05 mg/g, were analyzed statistically.

The results of the mean comparisons of EO compounds in herb samples are presented in Tab. 3. In the herb Ovu, *trans*-sabinene hydrate was the main compound (26 % of the total EO content). It was followed by *β*-caryophyllene and germacrene D (15.3 % and 11.7 % of the total EO content respectively). Further six compounds namely *β*-bourbonene, thymol, 1-octen-3-ol, germacrene D-4-ol, *cis*-*β*-ocimene and sabinene were detected with relevant concentrations of more than 0.05 mg/g. Another thirty compounds were identified with a lower concentration of 0.05 mg/g in herb samples (Tab. 2). In the means comparison of chemical compounds, octen-3-ol <1->, *cis*-*β*-ocimene, *trans*-sabinene hydrate, allo-ocimene, *β*-bourbonene, germacrene D and germacrene D-4-ol were significantly higher in Ovu compared to other accessions (Tab. 3).

Compared to Ovu a different composition of EO was found in Ohi. Thymol and carvacrol together reached around 65 % of total compounds (4.5 and 3.6 mg/g representing 36.3 % and 29.4 % of total content of EO). Two further compounds namely *γ*-terpinene and *p*-cymene were detected with higher concentrations than other compounds (Tab. 2).

In herb extracts of Ovi, *p*-cymene and thymol were the dominant compounds (representing 31.3 % and 26.9 % of total content EO respectively) followed by *γ*-terpinene, thymol methyl ether, and *cis*-*β*-ocimene. In the means comparison of the main compounds of Ovi herb samples *cis*-*β*-ocimene and thymol methyl ether had higher concentrations compared to other accessions (Tab. 3).

Tab. 3: Main chemical compounds in herb samples of *Origanum vulgare* accessions detected by using GC/MS (S.E.: standard error of the mean; Ovu: ssp. *vulgare*, Ohi: ssp. *hirtum*, Ovi: ssp. *viride*, Oxm: ssp. *viride* × *majorana*)

Compounds	Mean ± S.E (mg/g)				LSD (α<0.01)
	Ovu	Ohi	Ovi	Oxm	
1-Octen-3-ol	0.068±0.13ab	0.11±0.13a	0.011±0.13b	0.031±0.13b	0.052**
Myrcene	0.006±0.016b	0.207±0.016a	0.022±0.017b	0.016±0.016b	0.62**
cis-β-Ocimene	0.060±0.014a	0.017±0.014 b	0.055±0.014a	0.033±0.014 b	0.054*
γ-Terpinene	0.036±0.155b	1.645±0.155a	0.193±0.155b	0.141±0.155b	0.61**
trans-Sabinene hydrate	0.40±0.098a	0.028±0.098b	0±0.0104b	0±0.098b	0.38*
allo Ocimene	0.031±0.007a	0.002±0.007b	0.035±0.008b	0.013±0.007b	0.029*
Thymol methyl ether	0.014±0.014b	0±0.014b	0.112±0.015a	0.006±0.014b	0.056**
Carvacrol methyl ether	0.001±0.011c	0.026±0.011bc	0.046±0.011b	0.108±0.011a	0.043**
Thymoquinone	0±0.022b	0.132±0.022a	0.016±0.023b	0.001±0.022b	0.086**
Thymol	0.072±0.665b	4.519±0.665a	0.387±0.706b	0.027±0.665b	2.65**
Carvacrol	0.010±0.666b	3.651±0.666ab	0.006±0.706b	0.059±0.666b	2.65**
β-Bourbonene	0.082±0.012a	0.001±0.012b	0.08±0.013b	0.023±0.012b	0.047**
Aromadendrene	0±0.015b	0.144±0.015a	0.003±0.016b	0±0.015b	0.058**
Germacrene D	0.179±0.011a	0±0.011b	0.013±0.011b	0.026±0.011b	0.042**
β-Bisabolene	0.004±0.01b	0.088±0.01a	0.022±0.011b	0.010±0.01b	0.041**
Germacrene D -4-ol	0.064±0.007a	0±0.007b	0.001±0.007b	0.005±0.007b	0.026**
Total content of EO	1.528±0.704b	12.435±0.704a	1.384±0.747b	0.818±0.704b	2.81**

Values within rows followed by the same letter (a–c) do not differ statistically (Duncan test).
** at α <0.01; * at α <0.05; LSD: least significant difference

No dominant compound was identified in either herb or leaf samples of Oxm. The herb samples were characterized by several monoterpenoid compounds namely γ-terpinene (22.6% of the total EO content), sabinene (10.5%), *cis*-β-ocimene (10.5%), carvacrol methyl ether (8.1%), 1-octen-3-ol (6.7%), and carvacrol (5.4%) (Tab. 3).

Effect of accessions on EO content and composition of leaf samples

The mean comparisons of the main EO compounds in leaf samples are reported in Tab. 4. The same pattern of EO composition was observed in Ovu leaf samples. However, the concentration of germacrene D-4-ol, *cis*-β-ocimen, *trans*-β-ocimen in leaf samples were about two times greater than in herb samples (Tab. 2). In Ovu plants, the total content of EO was very low in both sample types (1.53 and 1.47 mg/g respectively) (Tab. 3 and Fig. 2a).

In contrast to the herb samples of Ohi, a higher concentration of carvacrol and a lower concentration of thymol were detected in leaf samples (0.48 and 0.13 mg/g, representing 63.8% and 18% of total content of EO). The content of EO was notably higher in herb samples compared to leaf samples (12.43 and 0.79 mg/g respectively) (Tab. 2 and Fig. 2b).

In Ovi leaf samples six compounds had higher concentrations compared to other accessions: myrcene, *p*-cymene, *cis*-β-ocimene, thymol methyl ether, thymol and β-bisabolene (Tab. 4). In contrast to the herb in leaf extracts of Ovi, a lower content of *p*-cymene and a higher content of thymol (representing 19.6% and 30.7% of total EO content, respectively) was detected (Fig. 2c). In addition, γ-terpinene (20.8%) reached a relatively large concentration followed by *cis*-β-ocimene, thymol methyl ether and *allo*-ocimene (Tab. 2). Interestingly, it was observed that the concentration of thymol in Ovi leaf samples was higher even than in Ohi samples.

In leaf samples of Oxm, γ-terpinene (17.3%) had a greater concentration than *p*-cymene (13.7%). It was followed by carvacrol methyl ether (13.2%) and sabinene (7.8%) (Tab. 4). Interestingly, the leaf samples had superior EO content in comparison to herb samples (Tab. 2 and Fig. 2d).

Discussion

Effect of light intensity on content and composition of EO

Sabinene is one of the final products of the sabinyl pathway (Novak et al., 2010). Different investigations showed the effect of abiotic factors on this pathway and its final products. Novak et al. (2010) found out that by increasing the temperature from 18 to 26 °C, the concentration of sabinene increased linearly from 3.9% to 5.3%. In *Origanum majorana* L. sabinene, *cis*-sabinene hydrate and *trans*-sabinene hydrate were produced in larger quantities in the plants grown under longer days, whereas the content of terpinenes decreased with increasing day length (Circella et al., 1995). De Falco et al. (2013) observed Ovu plants grown in single-rows were rich in sabinene. As single-row plants received higher light intensities compared to binate-rows, it can be assumed that the lower light intensity in binate-rows decreased sabinene content in EO, confirming our results. Moreover, in our samples, trans-sabinene hydrate (15.02 min) appeared earlier than linalool (15.11 min) (Tab. 2). According to Adams (2007), however, trans-sabinene hydrate (KI: 1098) should appear after linalool (KI: 1096) in the chromatogram.

In the process of cymyl compounds biosynthesis, *p*-cymene is formed by aromatisation of γ-terpinene (Lukas et al., 2010; Poulose and Croteau, 1978). Afterwards, thymol and carvacrol are synthesized by hydroxylation of *p*-cymene by two hypothetical hydroxylases. It is proposed that the alteration in the composition of cymyl compounds, as a reaction to different environmental conditions, is mediated by carvacrol hydroxylase and thymol hydroxylase (Novak et al., 2010). Accordingly, when light intensity was reduced, the activity of carvacrol hydoxylase was diminished. Therefore, in Ohi under a RLI condition an accumulation of *p*-cymene and smaller concentration of carvacrol (RLI: 0.24, NLI: 0.71 mg/g, data not presented) was observed.

Confirming our results, Johnson et al. (2004) observed in Ohi plants decreased p-cymene concentration and increased carvacrol concentration during spring to summer time. Specifically, in Ohi plants the 50%-shade treatment modified the profile of EO. The EO of the plants under full light treatment was mainly composed of 4-terpineol, γ-terpinene, carvacrol, *p*-cymene and α-terpinene. Moreover,

Tab. 4: Main chemical compounds in leaf samples of *Origanum vulgare* accessions detected by using GC/MS (S.E.: standard error of the mean; Ovu: ssp. *vulgare*, Ohi: ssp. *hirtum*, Ovi: ssp. *viride*, Oxm: ssp. *viride* × *majorana*)

Compounds	Mean ± S.E (mg/g)				LSD (α<0.01
	Ovu	Ohi	Ovi	Oxm	
Sabinene	0.079±0.023b	0.002±0.025b	0.007±0.023 b	0.181±0.023a	0.094**
1-Octen-3-ol	0.063±0.017ab	0±0.018b	0.002±0.017b	0.116±0.017a	0.067**
Myrcene	0.003±0.008b	0.006±0.008b	0.019±0.008ab	0.047±0.008a	0.031**
ϱ-Cymene	0.006±0.02b	0.054±0.02b	0.181±0.02a	0.041±0.02b	0.08**
cis-β-Ocimene	0.143±0.032a	0±0.035b	0.125±0.032a	0.18±0.032a	0.098**
trans-β-Ocimen	0.051±0.014ab	0±0.015b	0.034±0.014b	0.088±0.014a	0.041**
trans-Sabinene hydrate	0.151±0.036a	0±0.039b	0.008±0.036b	0.002±0.036b	0.15*
Thymol methyl ether	0.003±0.012b	0±0.013b	0.067±0.012a	0±0.012b	0.051**
Thymol	0.031±0.062b	0.135±0.067b	0.401±0.062a	0±0.062b	0.25**
β-Bourbonene	0.092±0.009a	0±0.01b	0±0.009b	0.035±0.009b	0.036**
Germacrene D	0.268±0.033a	0±0.036b	0.018±0.033b	0.134±0.033b	0.137**
β-Bisabolene	0±0.004b	0.004±0.005ab	0.017±0.004a	0.007±0.004ab	0.017*
Germacrene D -4-ol	0.128±0.015a	0±0.016b	0.007±0.015b	0.031±0.015b	0.06**

Values within rows followed by the same letter (a–d) do not differ statistically (Duncan test)
** at α <0.01; * at α <0.05; LSD: least significant difference

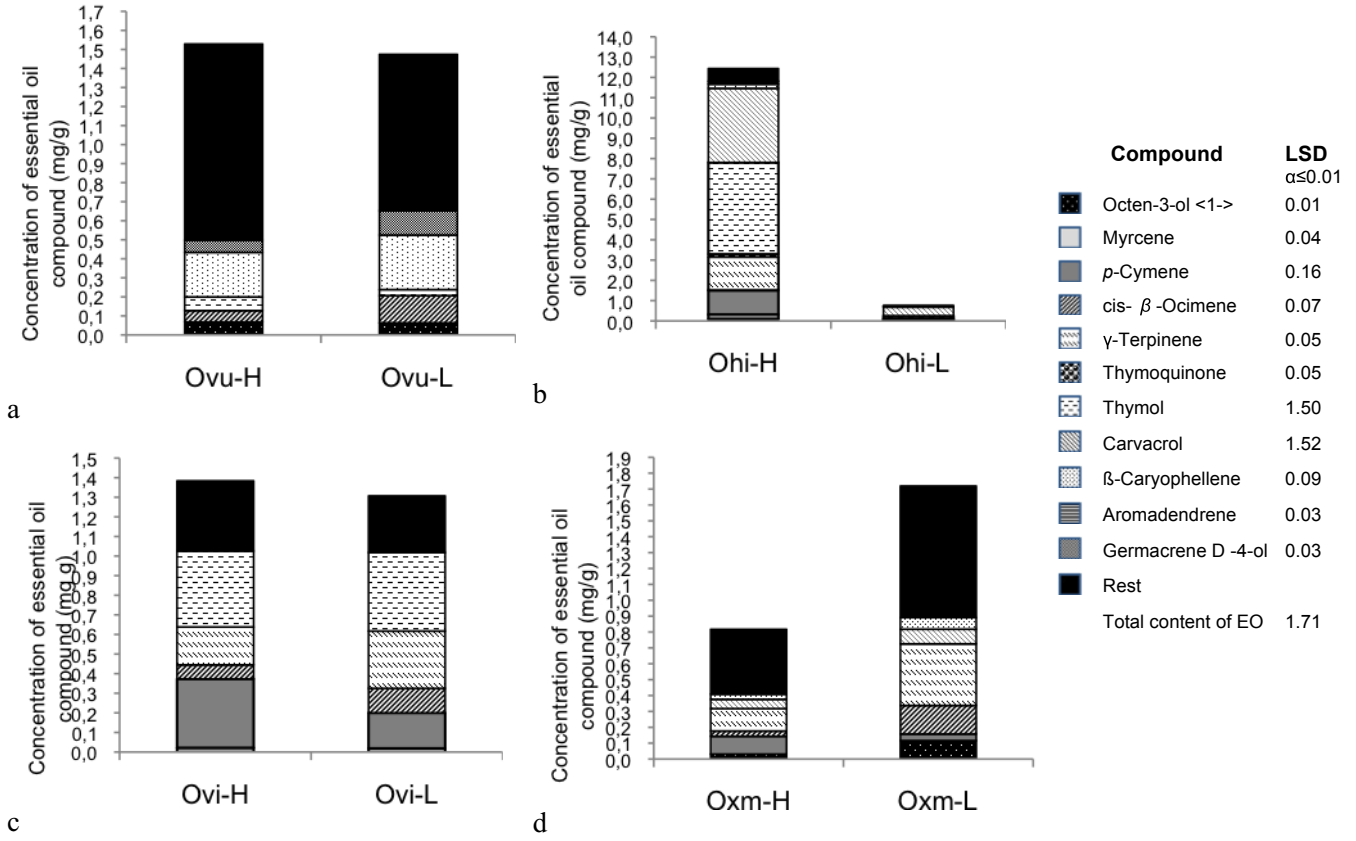

Fig. 2: Composition of essential oil in herb (H) and third leaf (L) samples of *Origanum vulgare* accessions
Ovu: *Origanum vulgare* ssp. *vulgare*
Ohi: *Origanum vulgare* ssp. *hirtum*
Ovi: *Origanum vulgare* ssp. *viride*
Oxm: *Origanum vulgare* ssp. *viride* × *majorana*

in the 50 %-shade treatment this composition changed to γ-terpinene, 4-terpineol, carvacrol, *p*-cymene and allo cymene. In the reduced light condition, the concentration of *p*-cymene increased and the concentration of sabinene decreased (TIBALDI et al., 2011).

Effect of accessions on EO content and composition of herb and leaf samples

In all accessions, the extracts of herb samples were characterized by higher biochemical diversity compared to the extracts received from the leaf samples (Tab. 2). This property refers to the presentation of different plant organs, including leaves, flowers and young stems in one herb sample. Furthermore, the ontological stage of plant organs in herb samples can influence the composition of EO (JOHNSON et al., 2004; RADUĐIENĖ et al., 2005; TIBALDI et al., 2011).

Ovu plants have been found to be rich in acyclic compounds and sesquiterpenoids (SKOULA and HARBORNE, 2004). AZIZI et al. (2012) analyzed the EO composition of Ovu and found that two sesquiterpenes: β-caryophyllene and germacrene D composed more than 60 % of EO. Other main components included terpinene-4-ol (6.7 %), spathulenol (4.4 %) and γ-terpinene (3.9 %). They could not identify any sabinene, 1-octen-3-ol, ocimene compounds or germacrene D-4-ol which were detected in the Ovu plants of the current experiment. Besides, the concentration of *trans*-sabinene hydrate was very low in their experiment. However, these compounds were identified by DE FALCO et al. (2013) and BARANAUSKIENĖ et al. (2013) who intensively investigated the EO of Ovu.

Furthermore, in 374 individuals of Ovu plants from Austria the monoterpene fraction was mainly made up of sabinyl-compounds (primarily sabinene and *cis*-sabinene hydrate) and/or cymyl-compounds (primarily *p*-cymene, γ-terpinene, and carvacrol) usually accompanied by smaller amounts of bornyl-compounds and acyclic compounds (LUKAS et al., 2013). In the current study, Ovu plants were rich in sabinyl compounds (*trans*-sabinene hydrate and *cis*-sabinene hydrate) and sesquiterpenoids (β-caryophyllene and germacrene-D). Each of the other compounds, such as 1-octan-3-ol, β-ocimene (an acyclic compound) and thymol (a cymyl-compound), had a concentration of more than 5 %. Therefore, these plants are comparable to the mixed-type of Austrian Ovu plants (LUKAS et al., 2013) and with Ovu plant from Lithuania which were analyzed by RADUĐIENĖ et al. (2005).

According to our results, it can be proposed that the investigated Ohi accession is an intermediate type with high content of *p*-cymene and γ-terpinene. These results confirm the findings of AZIZI et al. (2012) who investigated the Ohi accession from the same source. Moreover, the greater density of glandular trichomes on the inflorescences of Ov plants generates the superior EO content in this organ compared to other plant's parts (WERKER et al., 1985). The existence of flowers in herb samples should be the explanation for higher EO content in these samples. In the previous study, a higher density of peltate glandular trichomes was reported in Ohi leaf samples when compared with other accessions (SHAFIEE-HAJIABAD et al., 2015). The result of the current study showed no difference in EO content in leaf samples of all accessions. Particularly, in the case of Ohi not only the content of each compound but also the total content of EO was very low in leaf samples compared to herb samples (Tab. 4).

Since peltate glandular trichomes, the primary location for EO secretion, are formed on the epidermal surfaces of plants and because the leaves of Ohi are thicker than Ovi (SHAFIEE-HAJIABAD et al., 2014), it is possible that the leaf samples of Ohi involve more parenchyma than epidermal tissue. As a consequence, less peltate glandular trichomes were presented in the Ohi leaf samples; therefore less EO was detected in leaf samples of Ohi compared to other accessions. However, in the herb samples a considerably higher amount of EO was detected. It was also observed that the composition of EO changed among different organs (WERKER et al., 1985) as well as among different peltate glandular trichomes of one leaf (JOHNSON et al., 2004). Therefore, different profiles of EO in herb and leaf samples can be expected.

In contrast to our results, EO composition analysis of Ovi plants by AZIZI et al. (2012) showed that thymol (62.5%) was the dominant compound, and other four compounds: β-caryophyllene, thymol methyl ester, carvacrol methyl ester, as well as β-bisabolene had concentrations between 3.5 to 4.9%. Moreover, Ovi plants native to Crete were found to be rich in sabinyl compounds, acyclic compounds and sesquiterpenoids (SKOULA et al., 1999) whereas in Ovi plants from Argentina the main compound was carvacrol (FARÍAS et al., 2010). In Ovi plants from Turkey, the sesquiterpenoid compounds composed the main fraction of EO (caryophyllene oxide, caryophyllene, spathulenol and caryophyllenol II: 41.8%), followed by linalool (8.3%), 1,8-cineol (8%), while *p*-cymene had a low concentration (4.1%) (KOLDAŞ et al., 2014). In Iranian Ovi plants, the monoterpene fraction constitutes 52.4% of the oil with the main components such as linalyl acetate (20.1%), sabinene (13.4%), γ-terpinene (5.6%), *trans*-ocimene (3.6%), and cis-ocimene (3.4%) (AFSHARYPUOR et al., 1997).

In the case of Oxm, our results showed enhanced concentrations of monoterpenes and a low concentration of the sesquiterpene fraction. In contrast, AZIZI et al. (2012) found that carvacrol (22.5%), germacrene D (24.8%), β-caryophyllene (9.8%), carvacrol methyl ester (9.7%) and terpinene-4-ol (4%), as the main compounds in Oxm. The EO composition of Oxm investigated in this study is somehow similar to EO profile of "green Spanish" clone (*O. vulgare* ssp. *viridulum*) from Argentina (FARIAS et al., 2010). Generally, bicyclic sabinyl monoterpenes (*cis*-sabinene hydrate, *cis*-sabinene hydrate acetate, *trans*-sabinene hydrate and sabinene) are the main EO compounds of marjoram (*Origanum majorana* L.). While *p*-cymyl compounds are characteristic for oregano (*Origanum* spp.), and they are completely absent in the cultivated chemo variety of marjoram (NOVAK et al., 2004). This accession, which is rich both in sabinyl compounds and cymyl compounds, shows an interesting composition of EO.

Furthermore, thymoquinone was detected in the herb samples of all investigated accessions (Tab. 2). However, its concentration was considerably higher in Ohi compared to other accession (Tab. 3). This compound is an interesting consistent of EO which was reported previously in the genus *Origanum* (RUSSO et al., 1996; SKOULA et al., 1999). It exhibits promising effects against inflammatory diseases and cancer by enhancing the anticancer potential of clinical drugs, while reducing unwanted side effects (SCHNEIDER-STOCK et al., 2014).

In conclusion, the current study found that light intensity made small changes to the EO composition. Accordingly, it can propose that the biosynthetic pathway of these compounds is mostly genetically influenced and most likely in order to have relevant changes in the EO profile, more intensive light reduction or light difference is required. However, the effect of applied light reduction on macro and micromorphological parameters was previously studied (SHAFIEE-HAJIABAD et al., 2015). Besides, minor changes of EO composition under two light intensities reveal the genotype stability of these plants. Since for the industry of medicinal plants a constant quality through all environmental conditions is desirable; genotype stability of the investigated accessions can be considered as a positive characteristic (NOVAK et al., 2003b). Furthermore, the presence of thymoquinone, a pharmaceutically important compound, in the herb samples of all investigated accessions specially Ohi, is another interesting result which confirms Ov plant's consume as a natural source of thymoquinone production.

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