AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic ISSN 1842-4309

Not. Bot. Hort. Agrobot. Cluj 37 (2) 2009, 177-181

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Changes in Essential Oil Composition of Oregano (*Origanum onites* L.) due to Diurnal Variations at Different Development Stages

Ozlem TONCER[1)], Sengul KARAMAN[2)], Suleyman KIZIL[1)], Emel DIRAZ[2)]

[1)] *Dicle University, Department of Field Crops, Faculty of Agriculture, 21280 Diyarbakir, Turkey; toncer@dicle.edu.tr.*
[2)] *Kahramanmaras Sutcu Imam University, Department of Biology, Faculty of Science and Letters, Turkey; sengulk@mail.ksu.edu.tr.*

Abstract

The composition of essential oil in plants was affected by genetical and environmental conditions, which is determined by growth region and harvesting time in terms of ontogenetical and diurnal variability. In the present study, aerial parts of *Origanum onites* were harvested at three different development stages (pre, full and post flowering) and six different times of the day (06:00, 10:00, 12:00, 16:00, 20:00 and 24:00 h). A total of twenty-six components were identified by GC-MS,The major component was carvacrol, followed by thymol, p-cymene and γ-terpinene. The content of carvacrol in the essential oil varied between 24.66 and 52.58% and the highest carvacrol content was obtained from 10:00 h at pre-flowering stages, thymol content changed between 2.80 and 23.77% and the highest thymol content was also obtained from 24:00 h at pre-flowering stages.

Keywords: *Origanum onites*, oregano, development stages, diurnal variability, essential oil composition

Introduction

The genus *Origanum (Labiatae)* is represented in Turkey by 22 species or 32 taxa, 21 being endemic (Dundar *et al.,* 2008) and the ratio of endemism in the genus is 65.2 % (Aydin *et al.,* 1998). Oregano plays a primary role among culinary herbs in world trade (Olivier, 1996) and it is produced mainly in France, Greece, Spain, and Turkey in Europe, and Chile, Mexico and Peru in America (Barreyro *et. al.*, 2005). *Origanum onites, O. minutiflorum, O. majorana, O. syriacum var. bevanii, O. vulgare var. hirtum* are exported from Turkey (Baser *et al.,* 1993; Kirimer *et al.,* 2003). Turkish Oregano (*O. onites*) is the most exported *Origanum* species from Turkey to the entire world (Yaldiz *et al.,* 2005) and is commonly known as thyme, "Izmir kekiği", "Bilyalı kekik" or White thyme and it includes 2-3 % of essential oil (Gonuz and Ozorgucu, 1999). Oregano has been used as a stimulant, analgesic, antitussive, expectorant, sedative, antiparasitic, and antihelminthic in Turkish folk medicine, and it is mostly used for gastrointestinal complaints (Dundar *et al.*, 2008). The main components of essential oil of *O. onites* are carvacrol, thymol, p-cymene, and gamma-terpinene, borneol, linalool, alpha-terpinene (Yaldiz *et al.,* 2005; Ceylan *et al.,* 1999; Kacar *et al.,* 2006; Baydar *et al.,* 2004; Demirci *et al.,* 2004). However Kokkini *et al.* (2004) stated that Turkish oregano had a higher amounts of sabinene; a monoterpene that is used in manufacturing of fragrance and flavor concentrates of all types. Biological activities of *Oregano* depended mainly on carvacrol and thymol. Carvacrol is an oxygenated monoterpene with multiple pharmacological actions (Baser, 2008).

The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by the environmental influences of a particular growing region, and also by the agronomic conditions, harvesting time and the type of processing (Miguel *et al.*, 2004; Baydar *et al.,* 2004). One of the most important characteristics of oil accumulation is its dependence on the developmental stage of the plant per se as well as its concerned part (Sangwan *et al.*, 2001).

Studies made on the effects of ontogenetic and diurnal variations in the essential oil composition of *O. onites* (Kizil *et al.,* 2008; Yaldiz *et al.,* 2005) and the other Origanum species (Gumuscu *et al.*, 2008; Ceylan, 1976) have been investigated. These reports showed that the variations depending on different times of the day and development period can influence the active substance and the herbal productivity of the plant.

In this paper, we report about essential oil content and composition of *O. onites* at different development stages and diurnal variations in each development stages under semi-arid conditions of the Southeast Anatolia, Turkey.

Materials and methods

Plant materials

This study was carried out during 2007 growing season (from May to July) at Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir, Turkey (37^0 55' N; 40^0 12' E; 660 m above sea level). Monthly mean temperatures, relative humidity and total precipitation of

Diyarbakır climatic conditions from May to July are presented in Tab. 1.

O. onites grows natively in the Aegean and western Mediterranean costal line of Turkey (Baser *et al.*, 1993). In this study, oregano plants of four years old, was collected from Medicinal and Aromatic Plants collection garden of Department of Field Crops, Faculty of Agriculture, Dicle University, Turkey. Plants were harvested in three developing moments, i.e. pre-flowering (28 May 2007), full-flowering (13 June 2007) and post-flowering (02 July 2007) , six times a day (06:00, 10:00, 12:00, 16:00, 20:00 and 24:00 h) by a randomized collection of 10 individuals for each development stage. Plants were cut at 10 cm above soil levels, air-drying of the plant was performed in a shady place at room temperature. Then they were used for the analysis of essential oil composition.

Essential oil Analysis

Dried plant material (20 g of each *O. onites* applications) was subjected to hydrodistillation for 3h using a Clevenger type apparatus for determining the oil content (v/w). The oil composition was determined with GC-MS. The yields of oil ranged from 2.20 to 4.70% depend of different harvest stages and time of the day.

Tab. 1. Climatic conditions of months during development stages in 2007

Months	Mean Temperature (°C)		Mean Rainfall (mm)	Relative Humidity (%)
	Min	Max		
May	4.9	33.8	45.5	75.5
June	12.1	39.5	19.5	52.0
July	19.4	41.9	0.0	44.0

GC-MS analyses were conducted in the Plant Physiology Laboratory in Biology Dept. of Kahramanmaras Sutcu Imam University. Qualification of the oil was analyzed on an Agilent 5975C Mass Spectrometer coupled with a Agilent GC-6890II series. The GC was equipped with a HP-88 capillary column (100 m x 250 μm x 0.20 μm film thickness) . He has used carrier gas with flow rate of 1.0 mL/min. The GC oven temperature was programmed as follows: 70 oC (1 min), 230 oC at of 10 oC/min and then kept at 230 oC at 20 min. The injector temperature was 250 oC. The mass spectrometer was operating in EI mode at 70 eV. Split ratio was 20:1. Mass range 35-400m/z; scan speed (amu/s): 1000.

10μL of the oil was mixed 0.5 ml diethyl ether and 1 μL of the concentrations injected into the column. The components of the oil were identified by comparing their retention indices and mass spectra with those of pure authentic samples and NIST98, Willey7n.1 and Flavor2 libraries reference compounds.

Data on the mean thymol and carvacrol contents were analysed statistically using MSTAT-C (Michigan State University) computer program, and means were grouped using the least significant difference (LSD) test ($p<0.05$).

Results and discussion

Our research gives comparative GC/MS analyses of the *O. onites* essential oils obtained at three different development stages and six different time of the day of the vegetative cycle. Twenty-six components were identified in *O. onites* oil. These components constituted 81.74–98.84% of the total oil. The chemical components of O.onites oil due to harvest stages and time of the day are presented in Tab. 2. The main components of the oil was carvacrol (24.66-52.58%) followed by thymol (2.80-23.77%), p-cymene (4.44-7.97%) and γ-terpinene (7.02-11.16%) which was the most abundant components during the vegetative cycle. Many of the studies reported that carvacrol, thymol, p-cymene, and γ-terpinene were found as major components in oils of O onites. (Baser *et al.,* 1993; Ruberto *et al.*, 1993; Baydar 2002; Ceylan *et. al.,* 2003; Demirci *et. al.,* 2004). The samples of different development stages and time of the day exhibited an appreciable percent as well: myrecene (1.68-2.79%), γ-terpinene (2.56-4.56%), linalool (0.14-13.93%), cis-sabinen hydrate (0.81-5.63%), terpinen-4-ol (1.10-6.16%) and isoborneol (0.99 - 3.24%). The results of Kizil et. al. (2008) showed that *O. onites* essential oils ranged from 42.12 to 57.0% for carvacrol, from 13.21 to 21.88% for thymol, and from 8.23 to 20.28% for linalool. Also Oflaz et al (2002) reported that carvacrol content of the oil ranged from 0.9 to 80%, thymol content varied between 0.5% and 21%.

In terms of γ-terpinene, the high content (11.16%) was obtained from 12: 00 h at post-flowering stages, the lowest content (7.02%) was obtained from 12:00 h at pre-flowering stage. The highest p-cymene content (7.97%) was obtained from 10:00 h at pre-flowering; 16:00 h at pre-flowering had the lowest p-cymene content (5.24%). Linalool showed dramatically changes on concentrations during the development stages and in terms of time of the day, the highest linalool content (13.93%) was observed at 06:00 h from full-flowering, the lowest content of linalool (0.14%) was obtained at 10:00 h from pre-flowering. Baydar reported that *Origanum* oil contain linalool %11.91 - 32.50, p-cymene 1.90 - 6.38%, γ-terpinene 0.00 - 3.99% ve borneol 0.35 - 3.27%. Oflaz *et al.* (2002) stated that terpinene content of the oregano oil between 0.1 - 4.8%, p-cymene content between 0.1%- 4.8% and linalool content between 0.2% - 90.3%. Yaldiz *et al.* (2005) also reported that γ-terpinene and linalool ranged from 2.95 to 9.43 % and from 2.43 to 17.51%. Kizil *et al.* (2008) determined that the content of p-cymene, γ-terpinene and linalool ranged 1.05 - 5.78%, 1.43 - 8.10% and 8.23 - 20.28%, re-

Tab. 2. Essential oil composition (%) of *O. onites* obtained from six different times of the day at three different development stages

Development stages	RT* (min)	Pre-Flowering						Full-Flowering						Post-Flowering					
Compounds/Hours		06:00	10:00	12:00	16:00	20:00	24:00	06:00	10:00	12:00	16:00	20:00	24:00	06:00	10:00	12:00	16:00	20:00	24:00
α-pinene	10.91	-	-	-	-	-	-	2.28	-	-	1.75	0.11	0.96	4.92	2.23	0.21	1.76	-	-
Camphene	11.40	0.37	0.25	0.21	0.15	0.23	0.15	0.42	0.34	0.31	0.39	0.52	0.37	0.63	0.55	0.17	0.40	0.39	0.66
Myrecene	11.83	2.18	2.52	1.77	1.57	2.47	1.79	2.06	2.20	2.24	2.79	2.85	2.27	2.66	2.13	1.68	2.19	2.16	2.61
α-phellandrane	12.05	0.41	0.47	0.33	0.28	0.42	0.29	0.36	0.34	0.42	0.38	0.46	0.45	0.49-	0.37	0.32	0.38	0.32	0.53
α-terpinene	12.20	3.47	3.80	2.65	2.56	3.75	3.14	2.76	3.04	3.21	4.39	4.56	3.66	3.82	2.88	2.76	3.08	2.76	4.31
Ocimene	12.42	0.55	0.38	0.09	0.09	0.13	0.12	0.37	0.30	0.16	0.09	0.09	0.13	0.07	0.16	0.10	0.36	0.05	0.14
γ-terpinene	12.66	9.18	9.70	7.02	7.80	9.55	7.86	7.78	7.04	8.64	8.78	8.19	8.55	9.86	8.40	7.77	9.82	7.22	11.16
Eucalyptol	12.96	0.44	0.36	0.28	0.28	0.43	0.47	0.25	0.49	0.30	0.90	0.77	0.31	0.47	0.42	0.31	0.39	0.52	0.49
p-cymene	13.40	7.32	7.97	6.58	5.24	6.02	6.51	5.74	5.67	6.98	5.56	6.33	6.08	6.51	5.43	5.44	6.21	4.44	7.04
1-octen-3 ol	14.84	0.51	0.32	0.29	0.22	0.22	0.26	0.21	0.26	0.23	0.21	0.27	0.21	0.20	0.21	0.26	0.25	0.25	0.24
Tran sabinen hydrate	15.70	0.63	0.28	0.40	0.40	0.56	0.94	0.28	0.76	0.30	0.77	0.51	0.37	0.61	0.47	0.43	0.36	0.66	0.30
Linalool	15.92	0.80	0.14	1.47	9.91	1.20	5.47	13.93	0.32	7.32	6.20	0.16	3.78	0.30	12.12	9.48	1.81	4.51	0.45
cis- sabinen hydrate	16.74	3.35	1.73	1.86	2.13	3.00	5.63	0.81	3.97	1.17	4.59	3.42	1.16	2.68	1.89	2.19	1.83	3.49	1.65
α-campholena	16.93	0.55	0.38	0.32	0.30	0.31	0.34	0.28	0.35	0.36	0.22	0.36	0.39	0.87	1.40	0.29	0.59	0.98	0.65
terpinen-4—ol	17.17	3.07	1.55	1.48	1.80	2.65	3.26	1.10	3.53	1.41	6.16	4.97	1.24	2.36	1.52	1.85	2.17	4.17	2.09
Germacrene-D	17.75	0.87	0.15	0.13	0.13	0.12	0.38	0.12	0.19	0.33	0.11	0.35	0.36	0.80	0.69	0.10	0.48	0.41	0.44
α- terpineol	18.01	1.27	0.54	0.49	0.57	0.65	0.96	0.53	0.96	0.53	1.22	1.12	0.29	0.68	0.55	0.52	0.53	1.12	0.78
Isoborneol	18.38	2.59	1.26	1.10	1.00	1.06	1.15	1.69	2.36	1.49	1.83	2.40	1.75	2.47	2.39	0.99	3.24	2.93	2.87
Cyclohexane	18.58	0.13	0.15	0.10	0.12	0.11	0.15	0.12	0.14	0.12	0.15	0.14	0.16	0.11	0.15	0.09	0.15	0.11	0.13
Trans carveol	19.25	1.05	0.15	0.11	0.09	0.10	0.11	0.08	0.12	0.13	0.15	0.11	0.08	0.09	0.09	0.22	0.17	0.12	0.15
Carvone	19.64	0.60	0.24	0.23	0.15	0.21	0.15	0.18	0.36	0.21	0.35	0.31	0.21	0.31	0.24	0.19	0.31	0.46	0.22
Carvenone	20.23	0.75	0.73	0.18	0.21	0.21	0.36	0.12	0.11	0.16	0.29	0.17	0.15	0.13	0.12	0.22	0.18	0.16	0.18
Methyl cinnamate	21.58	1.56	0.06	0.09	0.02	0.06	0.05	0.28	0.03	0.04	0.05	0.07	0.08	0.03	0.16	0.12	0.04	0.05	0.05
Thymol	22.22	14.31	8.25	13.63	17.92	17.05	23.77	17.69	9.17	13.14	10.49	18.36	22.86	6.97	5.33	16.97	10.83	2.80	18.05
Carvacrol	22.79	24.66	52.58	41.82	37.17	42.02	30.97	38.83	51.06	43.63	32.78	40.83	41.00	50.42	40.76	39.64	44.62	51.60	37.56
α- cadinol	24.90	1.12	0.27	0.76	0.26	0.33	0.35	0.25	0.40	0.39	0.40	0.55	0.43	0.38	0.36	0.25	0.34	0.61	0.30
Total		81.74	94.23	83.39	90.37	89.58	94.63	98.52	93.53	93.22	91.00	97.98	97.30	98.84	91.02	92.57	92.49	92.29	93.01

*Retention time

spectively. The differences in the reports from the previously literatures may be associated with the variances in the factors including genetic, seasonal, temperature, moisture, soil, day length changes on oil production and quality (Farooqi *et al.*, 1999; Ceylan et. al., 2003; Russo et. al., 1998; Ruberto *et al.*, 1993; Yaldiz *et al.*, 2005; Baydar *et al.*, 2004; Gonuz and Ozorgucu, 2003).

Because of high content of carvacrol and thymol in the oil samples, these two major components were analyzed statistically. The interaction between harvest time and collecting hours on carvacrol and thymol was found statistically significant (P<0.05) (Tab. 3. and 4.). The highest carvacrol content (52.58%) was obtained from pre-flowering period and at 10:00 h and the lowest one (24.66%) had at the same stage but at 06:00 h (Tab. 3.). Carvacrol generally showed higher mean percentage at post-flowering stages than the others. Thymol content of oregano oil was high at pre-flowering at 12:00 h (23.76%) and it was low post-flowering at 20:00 h (2.79%) (Tab. 4). In general, carvacrol percentage of oregano oil was high at the mornings, whereas thymol content was low at the same time. Also the observed increase in carvacrol percentage with decreasing thymol content due to the development stage indicates a biosynthetic correlation between the two compounds. Baydar (2002) reported that major components of oregano, between carvacrol and thymol show inverse ratio. Similarly, Russo *et al.* (1998) also reported that chemotypes form in botanical species, genetically codified enzymatic equipment directs biosynthesis to the preferential formation of definite compounds.

Due to collecting hours, significant changes have shown in thymol and carvacrol content, but it was a non-linear correlation between collecting hours and flowering period for these compounds. It may be that the seeds used have a different genetic structure, because it is known that synthesis of essential oils may change plant's genetic structure (Ceylan *et al.*, 2005; Baser *et al.*, 1993). The more uniform chemical content (thymol, carvacrol, linalool) can be determined via plant breeding to be high desirable chemical content in the next studies.

Tab.3. Content of Carvacrol (%) at different development stages and hours in day

Collecting times (h)	Development Stages			Mean
	Pre-Flowering	Full-Flowering	Post-Flowering	
6^{00}	24.66^{h}	38.83^{def}	50.42^{abc}	37.97
10^{00}	52.58^{a}	51.06^{ab}	40.76^{de}	48.13
12^{00}	41.81^{de}	43.63^{cde}	39.64^{def}	41.69
16^{00}	37.17^{efg}	32.78^{fg}	44.62^{bcd}	38.19
20^{00}	42.02^{de}	40.83^{de}	51.60^{ab}	44.82
24^{00}	30.97^{gh}	40.94^{de}	37.56^{defg}	36.99
Mean	38.20	41.34	44.10	
LSD (0.05)*	Harvest stages* hours of day = 7.24			

*Values fallowed by the same letter in a column do not differ significantly according to LSD.

Tab. 4. Content of Thymol (%) at different development stages and hours in day.

Collecting times (h)	Development Stages			Mean
	Pre-Flowering	Full-Flowering	Post-Flowering	
6^{00}	14.31^{cde}	17.53^{cd}	6.97^{hij}	12.94
10^{00}	8.250^{ghi}	9.17^{fghi}	5.33^{ij}	7.58
12^{00}	13.64^{cdef}	13.14^{defg}	16.97^{cd}	14.58
16^{00}	17.92^{bcd}	10.49^{efgh}	10.82^{efgh}	13.08
20^{00}	13.77^{cdef}	18.36^{bc}	2.79^{j}	11.64
24^{00}	23.76^{a}	22.85^{ab}	18.05^{bcd}	21.56
Mean	15.27	15.28	10.16	
LSD (0.05)*	Harvest stages* hours of day = 5.12			

*Values fallowed by the same letter in a column do not differ significantly according to LSD.

As a result, O.onites growing in Southeastern Anatolia of Turkey biosynthesized essential oils with carvacrol followed by thymol, p-cymene and γ-terpinene as the main constituents. The highest carvacrol content (52.58%) was obtained from 10:00 h at pre-flowering stages, the highest thymol content was obtained from 24:00 h at pre-flowering stages as 23.77 %.

References

Aydin, S., Y. Ozturk, R. Beis and K. H. C. Baser (1998). Investigation of *Origanum onites*, *Sideritis congesta* and *Satureja cuneifolia* essential oils for analgesic activity. Phytotherapy Research. 10(4):342-344.

Barreyro, R, J. Ringuelet and S. Agrícola (2005). Nitrogen fertilization and yield in oregano (*Origanum x applii*) Cien.. Inv. Agr. 32(1):34-38.

Baser, K .H. C., T. Ozek, G. Tumen and E. Sezik (1993). Composition of the essential oils of Turkish *Origanum* species with commercial importance. Journal of Essential Oil Research. 5(6):619-623.

Baser, K. H. C. (2008). Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Current Pharmaceutical Design. 14(29):3106-3119.

Baydar, H, O. Sagdic, G. Ozkan and T. Karadogan (2004). Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. Food Control. 15:169–172.

Baydar, H. (2002). Researches on the yield and essential oil quality of oregano (Origanum onites L.) under the condition of Isparta Province. Journal of Basic and Applied Siences of Suleyman Demirel University. 6(2):17-24.

Ceylan, A. (1976). Yield and ontogenetic variability in Origanum smyrnaeum L.. Journal of Agricultural Faculty of Ege University. 13:139-143.

Ceylan, A., E. Bayram and H. Geren (19990. Investigation

on agronomical and quality characteristics of improved clonnes in Origanum (*Origanum onites* L.) breeding. Tr. J. of Agriculture and Forestry. 23(5):1163-1168.

Ceylan, A., E. Bayram, N. Sahbaz, H. Otan and S. Karaman (2003). Yield Performance and Essential Oil Composition of Individual Plants and Improved Clones of *Origanum onites* L. Grown in The Aegean Region of Turkey. Israel Journal of Plant Sciences. 51(4):285-290.

Demirci, F., D. H. Paper, G. Franz and K. H. C. Baser (2004). Investigation of the *Origanum onites* L. essential oil using the chorioallantoic membrane (CAM) assay. *J. Agric. Food Chem. 52(*2):251–254.

Dundar, E, E. G. Olgun, S. Isiksoy, M. Kurkcuoglu, K. H. C. Baser and C. Bal (2008). The effects of intra-rectal and intra-peritoneal application of *Origanum onites* L. essential oil on 2,4,6-trinitrobenzenesulfonic acid-induced colitis in the rat. Experimental and Toxicologic Pathology. 59:399–408.

Farooqi, A. H. A., N. S. Sangwan and R. S. Sangwan (1999). Effect of different photoperiodic regimes on growth, flowering and essential oil in *Mentha* species Plant Growth Regulation. 29:181–187.

Gumuscu, A., A. Ipek, E. O. Sarihan, B. Gurbuz., M. D. Kaya and N. Arslan (2008). Effects of diurnal and ontogenetic variability on essential oil composition of oregano (*Origanum vulgare* var. *hirtum*). Asian Journal of Chemistry. 20(2): 1290-1294.

Gonuz, A and B. Ozorgucu (1999). An investigation on the morphology, anatomy and ecology of *Origanum onites* L.. Tr. J. of Botany. 23:19-32.

Kacar, O, E. Goksu and N. Azkan (2006). The effect of different plant densities on some agronomic and quality characteristics of oregano (*Origanum onites* L.). Journal of Agricultural Faculty of Uludag University. 2(21):51-60.

Kirimer, N., I. Boydag, N. Sargin and O. Arslandere (2003). Trading Configuration of Origanum Species in Nature. TUBITAK-TBAG-C. Sek. 10 Project (101T012).

Kizil, S, A. Ipek, N. Arslan and K. M. Khawar (2008). Effect of different developing stages on some agronomical characteristics and essential oil composition of oregano (*Origanum onites*). New Zealand Journal of Crop and Horticultural Science. 36(1):71-76.

Kokkini, S., R. Karousou, E. Hanlidou and T. Lanaras (2004). Essential oil composition of Greek (*Origanum vulgare* ssp. *hirtum*) and Turkish (*O. onites*) oregano: a tool for their distinction. Journal of Essential Oil Research. 16(4):334-338.

Miguel, M., C. Guerrero, H. Rodrigues and J. C. Brito (2004). Main components of the essential oils from wild Portuguese *Thymus mastichina* (L.) L. ssp. *mastichina* in different developmental stages or under culture conditions. http://findarticles.com/p/articles/mi_qa4091.

Oflaz, S., M. Kurkcuoglu and K. H. C. Baser (2002). Pharmacognostic studies on *Origanum onites* ve *Origanum vulgare* Subsp. H*irtum.* Proceedings of 14th International Symposium Plant Originated Crude Drugs, 29-31 May 2002, Eskişehir Publication Date On Web: June 2004 (In Turkish).

Olivier, G. W. (19960. The world market of oregano. p. 141–145. In: S. Padulosi, (Eds.). Oregano. Promoting the conservation and use of underutilized and neglected crops. Proceedings of the IPGRI International Workshop on Oregano. Institute of Plant Genetics and Crop Plant Research, Gater-sleben/ International Plant Genetic Resources Institute, Valenzano, Italy.

Ruberto, G., D. Biondi, R. Meli and M. Piattelli (1993). Volatile flavour components of Sicilian *Origanum onites* L.. Flavour and Fragrance Journal. 8(4):197-200.

Russo, M., G. C. Galletti, P. Bocchini and A. Carnacini (1998). Essential Oil Chemical Composition of Wild Populations of Italian Oregano Spice (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart): A Preliminary Evaluation Of Their Use in Chemotaxonomy By Cluster Analysis. 1. Inflorescences J. Agric. Food Chem. 46(9):3741-3746.

Sangwan, N. S., A. H. A. Farooqi, F. Shabih and R. S. Sangwan (2001). Regulation of essential oil production in plants. Plant Growth Regul. 34:3–21.

Yaldiz, G, N. Sekeroglu,. M. Ozgüven, M. Kirpik (2005). Seasonal and diurnal variability of essential oil and its components in Origanum onites L. grown in the ecological conditions of Cukurova. Grasas y Aceites. 56(4):254-258.