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Araştırma Makalesi (Research Article)

The Effect of Photoperiod on the Biomass and Quality Variables of Certain *Origanum* spp.

*Reyhan BAHTİYARCA BAĞDAT[1], Aditya VYAS[2], Lyle E. CRAKER[3]

[1]Central Research Institute for Field Crops, Ankara, Turkey
[2]Department of Pharmacy, BITS Pilani Hyderabad Campus, Hyderabad, India.
[3]University of Massachusetts, Stockbridge School of Agriculture, Amherst, MA, U.S.A
*Corresponding author e-mail (Sorumlu yazar e-posta): reyhanbagdat@yahoo.com

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Abstract

The biomass and essential oil yield of *Origanum vulgare* var. *hirtum* and *O. syriacum* were investigated under 10 h, 13 h and 16 h fluorescent light with a temperature of 25°C (77 F) in the growth chamber. The effect of different photoperiods to the yield and quality variables of both species were recorded. The observations were taken from early flowering stage of the plants. The highest plant height was obtained from *O. syriacum* with 13h photoperiod as 32.78 cm. The maximum number of shoot was recorded at *O. vulgare* var. *hirtum* as 14.3 per plant from 13h. The maximum canopy diameter was received from *O. syriacum* at 10h, and the measurements in horizontal and vertical directions were 28.63 cm and 25.73 cm, respectively. This application also had the highest essential oil content with 2.09%. After transplanting these both species' pots to the fields (ALC, Agricultural Learning Center, UMass, Amherst) three different cuttings were carried out, the highest carvacrol yield was determined from the late fall harvest of *O. vulgare* var. *hirtum* with 74.55%, and *O. syriacum* with 33.35%.

Keywords: *Origanum vulgare* var. *hirtum*, *Origanum syriacum*, photoperiodism, carvacrol

Gün Uzunluklarının Bazı Kekik (*Origanum* spp.)Türlerinde Biyolojik Kütle ve Kalite Özelliklerine Etkisinin Araştırılması

Öz

Origanum vulgare var. *hirtum* (İstanbul kekiği) ve *O. syriacum* (Suriye kekiği) olarak ta adlandırılan iki kekik türünün 10, 13 ve 16 saat süreyle iklim dolabında 25°C'de floresan ışığına maruz bırakılmalarının türlerin bazı büyüme parametreleri ile herba verimleri ve uçucu yağ özellikleri üzerine olan etkileri araştırılmıştır. Farklı fotoperiyodik muamelelerin verim ve kalite üzerine etkileri kaydedilmiştir. Gözlemler bitkilerin ilk çiçeklenme dönemlerinden alınmıştır. En yüksek bitki boyu *O. syriacum*' da 13 saatlik gün uzunluğundan 32.78 cm olarak elde edilmiştir. En fazla sürgün sayısı bitki başına ortalama 14.3 ile *O. vulgare* var. *hirtum*'dan 13 saatlik fotoperiyodizm den elde edilmiştir. Kanopi çapı en geniş tür *O. syriacum* olmuş, yatay ve dikey genişliği en yüksek çapı 28.63-25.73 cm ile 10 saat ışıklandırılan saksılardan elde edilmiştir. En yüksek uçucu yağ verimi %2.09 ile yine bu muameleden elde edilmiştir. Her iki türe ait saksıdaki bitkiler, Masachusetts Üniversitesi'nin araştırma Uygulama çiftliği'nde tarla denemelerine alınmışlar ve sonrasında bu bitkilerden üç farklı tarihte hasat gerçekleştirilmiştir. Bu biçimler göz önüne alındığında en yüksek karvakrol oranına sahip tür %74.55 ile geç sonbahar hasatından Origanum vulgare var. hirtum' dan, %33.35 ile de *O. syriacum*' dan elde edilmiştir.

Anahtar Kelimeler: *Origanum vulgare* var. *hirtum*, *Origanum syriacum*, fotoperiyodizm- gün uzunluğu, karvakrol

Introduction

Oregano with many different species, mainly collected from natural flora is, very rich in genetic diversity and characterized by high morphological variability. *Origanum subshrubs*, *spontaneous* in many regions of the Mediterranean area, are increasingly becoming a popular cultivated herb for industrial purposes. The flavor of these species is

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generally strong phenolic due to the presence of thymol, carvacrol or a mixture of the two as main compounds in their essential oil (Fleisher and Sneer 1982; Marzi 1997). According to the flora Europaea (Tutin 1972) *Origanum heracleoticum* L. (synonyms *Origanum vulgare* var. *hirtum*) is distributed in the SE Europe from Sardinia to the Aegean Region. Fleisher and Fleisher (1991) determined carvacrol and thymol in *Origanum syriacum* and carvacrol, geraniol, geranyl esters, ethyl cinnamate from another sample of *O. syriacum*.

O. syriacum commonly known as 'Syrian marjoram' is an aromatic, herbaceous and perennial plant growing wild in the Sinai desert of Egypt (Täckholm, 1974). Essential oils obtained from *Origanum vulgare* subsp. *hirtum* and *Origanum syriacum* are documented to contain a high level of the monoterpene phenol carvacrol, which is a hydrophobic compound that exhibits high antimicrobial and antioxidant activities responsible for, anti-inflammatory, analgesic activities of the essential oil (Kamimura et al. 2014). The variation of the photoperiod has an effect on the plant growth, quality and mass and should also affect the yield and composition of the essential oil extract. Origanum is a long-day plant. The photoperiod does influence the growth of the plant and the floral differentiation. Plants grown under conditions of 16-12 light- hours/day enter the full floral differentiation stage around the sixtieth and the ninetieth day of cultivation, respectively. Plants grown in 12 hours day length conditions are more vigorous, with a larger leaf area and a greater plant total dry weight (Marzi 1997). The main chemical compounds in *O. heracleoticum* were carvacrol, γ-terpinene and p-cymene (Fleisher and Sneer, 1982) and in *O. vulgare* subsp. hirtum were carvacrol, terpene and p-cymole (Hoppe 1958). This present work aims to investigate the differences in growth rates and total biomass of the two species under the different photoperiods and find the variation in carvacrol content in the essential oil of the two species of oregano due to the variation of photoperiod. This may be helpful for inferring the economic viability of using artificial lighting by farmers or pharmaceutical companies to extend the day length in order to increase growth or essential oil yield.

Material and Method

Seeds of *Origanum vulgare* spp. *hirtum* and *Origanum syriacum* were purchased from Johnny’s Selected Seeds and Horizon Herbs Companies, US, respectively. The research was conducted in CNS Greenhouses of UMass (University of Massachusetts, Amherst, MA,US) having an automated building systems control lighting, temperature, humidity and irrigation adapting the interior environment in response to the sun, the wind, and the weather.

The seeds were sown on February 10th, 2014 to the plastic seedling trays (65 mm cell dimension and 70 mm depth, with 530x340 mm external dimension) full of Sun Gro LC1 growing mix (formulated with Canadian sphagnum, peat moss, coarse pearlite, starter nutrient charge with gypsum and dolomite limestone) and kept grown in this medium. They were germinated in 13 days and reached an optimum size of about 2 inches (5.08 cm) in 6 weeks after germination. In that stage the seedlings were transplanted into 6 inches (15.24 cm) pots full of LC1 growing mix. The pots were placed into the growth chambers (E 7/2 dual compartment units having 0.75 m2x 64 cm of growing space and lighting delivers up to 33 umoles/m^2/sec). The greenhouse experiments were designed at completely randomized design technique with three replicate. Each replicate consists of 6 pots and subjected to a photoperiod of 10 hours, 13 hours, and 16 hours of artificial fluorescent light within a 24h cycle. The temperature was maintained constant at 25°C (77 F) in the growth chamber. The plants were harvested at flowering stage in the recommended period when the essential oil ratio was the highest amount, June 26th 2014. Plant height (cm/per plant), number of shoots, horizontal and vertical canopy widths (cm), fresh herb yield (g/per plant), dry herb yield (g/per plant) and leaf area (cm^2/per plant) were measured and recorded. After the first pot trim in late June (26th 2014, the pots were transferred to the experimental field. Three more harvests were recorded at successive dates: August 14th, September 24th and October 28th, 2014. The leaf areas were determined by LI-3100C Area meter having quiet belt system and press roller to flatten curled leaves in cm^2/cm^2 per plant.

Essential oil analyses were conducted both in pot and field experiments by using steam distillation apparatus (neo-clevenger) in the laboratory of Medicinal and Aromatic Plants Program, Stockbridge Agriculture, UMass. Fresh material was kept to dry 3 days in a 35°C incubator, inside paper lunch bags. Dry leaves were placed in a distillation apparatus with 2 L of distilled water and vapor distilled for 3 h steam distillation of MAPs were recommended by several previous studies for lavender and rosemary (Tannous et al. 2004; Baydar and Kineci 2009; Boutekedjiret et al. 2003).

The essential oil components were isolated in Western Mediterranean Agricultural Research Institute, Antalya, Turkey. The samples were diluted by 1:100 hexane. The GC/MS analysis was carried out with an Agilent 7890A system and with an Agilent 5975C Mass detector. HP Innowax Capillary column (60.0 m x 0.25 mm x 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). Split ratio was adjusted to 40:1. GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min. The injector temperature was at 250°C. Scanning range for the mass detector was (*m/z*) 35-450 atomic mass unit and the electron bombardment ionization energy was 70 eV.

The components of essential oils were identified by comparison of their mass spectra with those in the Adams Library, Wiley GC/MS Library, Mass Finder Library, and confirmed by comparison of their retention indices (RRI). The percentages of the components were determined by FID detector and identification of the compounds was performed using MS detection. The results were analyzed by analyses of variance and ranged by Duncan's multiple range tests (Adams 2007; Davies 1990; Jennings and Shibamoto 1980; Düzgüneş 1987).

Results and Discussions

Following yield and quality parameters of *O. syriacum* and *O. vulgare* var. *hirtum* were recorded from greenhouse experiment of these exposed to three different photoperiod of 10, 13 and 16h. The highest plant height was recorded at 16h photoperiod, and no significant differences were found between the species and among the applications. The highest number of shoots recorded was 14.3 in *O. syriacum* at 13h daylight, and 11.2 in O. *vulgare* var. *hirtum* at 16h daylight (Table 1). The main yield parameters were found statistically significant (fresh herb, dry herb, leaf area index and essential oil) considering plant species. The highest leaf area index was 113.3 cm^2 in *O. syriacum* at 16h daylight, and 196.5 cm^2 in *O. vulgare* var. *hirtum* at 10h. The mean essential oil ratio was found statistically significant among the species and applications at the 0.01 level. The highest amount of essential oil yield was obtained from 10 h photoperiod with 2.65% and 1.30% respectively (Table 2).

Table1. The mean plant height (cm/per plant), number of shoot and canopy widths (cm/per plant) of *Origanum syriacum* (1st) and *O. vulgare* var. *hirtum* (2nd) species grown into 10, 13 and 16 h daylights

Çizelge 1. Origanum syriacum (1nci) ve O. vulgare var. hirtum (2nci) türlerinde farklı gün ışığında (10,13 ve 16 saat) yetiştirilen bitkilerin bitki başına ortalama bitki boyu (cm), sürgün sayısı ve kanopi çapları (cm)

Photoperiods	Plant height		Number of Shoot		Canopy Width		Canopy Width 90	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
10 h	24.9	29.9	13.0	6.1	24.1	28.6	20.0	25.7
13 h	28.1	32.7	14.3	10.7	22.3	27.0	14.4	18.8
16 h	28.7	30.4	10.7	11.2	19.5	23.6	19.2	16.8
F_1	3.29		7.83*		3.69		2.68	
F_2	0.77		2.0		1.51		6.03*	

*: 1% ,**: 5% statistically significant F_1= F species, F_2= F applications

: 1% ,: 5% istatistiki olarak önemli F_1= F türler arası, F_2= F muameleler arası*

Table 2. The mean fresh herb (g/per plant) and dry herb (g/per plant) yield and leaf area (cm^2/cm^2) and essential oil yield of (%) *Origanum syriacum* (1st) and *O. vulgare* var. *hirtum* (2nd) species growing into 10, 13 and 16 h daylights

Çizelge 2. Origanum syriacum (1nci) ve O. vulgare var. hirtum (2nci) türlerinde farklı gün ışığında (10,13 ve 16 saat) yetiştirilen bitkilerin bitki başına ortalama yeşil herba (g), drog herba(g), yaprak alanı (cm^2) ve uçucu yağ oranları (%)

Photoperiods	Fresh Herb		Dry Herb		Leaf Area		Essential Oil ratio	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
10 h	4.2	7.8	1.4	2.5	103.6	196.5	2.65	1.30
13 h	4.8	4.6	1.6	1.6	108.2	127.7	1.23	0.73
16 h	4.0	4.7	1.3	1.7	113.3	133.5	2.17	1.18
F_1	5.02*		5.01*		6.07*		268.91**	
F_2	2.90		1.45		1.22		104.22**	

*: 1% ,**: 5% statistically significant F_1= F species, F_2= F applications

: 1% ,: 5% istatistiki olarak önemli F_1= F türler arası, F_2= F muameleler arası*

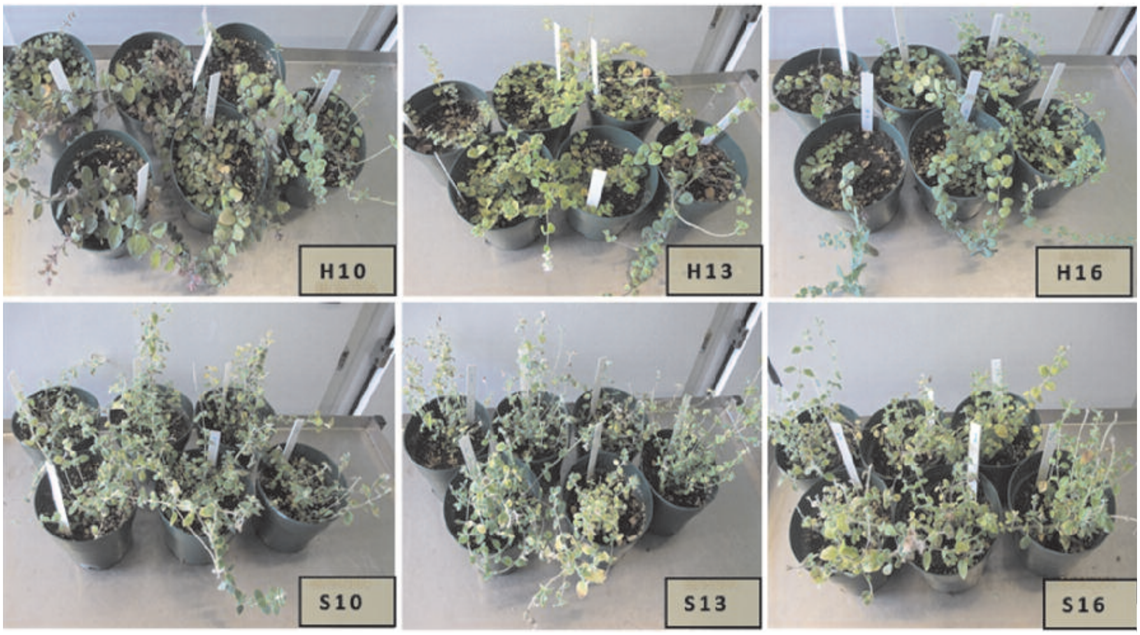

Figure 1. *O. vulgare* var. *hirtum* and *O. syriacum* plants in the pots subjected to 10h, 13h and 16h daylight in the growth camber

Şekil 1. 10, 13 ve 16 saat süreyle gün ışığına maruz kalan iklim dolabındaki O. vulgare var. hirtum ve O. syriacum bitkileri

Table 3. The essential oil composition of *Origanum vulgare* var. *hirtum* harvested successively on August 14th, September 24th and October 28th, 2014.

Çizelge 3. 14 Ağustos, 24 Eylül ve 28 Ekim tarihlerinde hasat edilen Origanum vulgare var. hirtum türünün uçucu yağ kompozisyonu

Peak	R.T.	Compounds	August 14th	September 24th	October 28th
1	12.8	alpha-pinene	0.77	0.41	0.44
2	13	alpha-thujene	1.69	0.99	0.61
3	16.6	beta-pinene	0.19	NF	NF
4	19	beta-myrcene	2.13	1.36	NF
5	19.2	alpha-phellandrene	0.23	0.15	NF
6	19.9	alpha-terpinene	1.93	1.26	NF
7	20.7	limonene	0.28	0.23	NF
8	21.2	beta-phellandrene	0.27	0.24	NF
9	22.2	*cis-ocimene*	0.96	0.53	NF
10	22.8	gamma-terpinene	13.2	9.55	NF
11	22.9	beta-ocimene	0.34	1.61	NF
12	23.1	3-octanon	0.33	0.53	0.47
13	23.9	cymene	9.54	8.66	15.64
14	30.3	1-octen-3-ol	1.99	3.71	2.22
15	31.0	cis-sabinene hydrate acetate	0.22	0.34	0.43
16	35.7	carvacrol methyl ether	0.63	0.47	0.41
17	35.8	beta-caryophyllene	1.28	0.5	0.62
18	38.1	alpha-humulene	0.18	NF	NF
19	38.7	borneol	0.34	NF	0.56
20	39.4	beta-bisabolene	0.54	1.29	0.76
21	50.9	thymol	4.50	0.36	0.71
22	51.7	carvacrol	58.3	65.6	74.55
23	17.2	sabinene	NF	0.83	NF
24	33.6	trans-sabinene hydrate acetate	NF	0.37	0.49
25	39.2	germacrene	NF	0.17	NF
26	19.9	alpha-terpinene	NF	NF	0.36
27	20.7	limonene	NF	NF	0.17
28	42.7	alpha-thujaplicin	NF	NF	0.18
29	45.1	Not Identified	0.17	0.89	0.85

Marzi (1997) recorded the highest plant height from 16h daylight as 73.3 cm compared to 8h (35.1 cm) and 12h (60.5 cm) from O. *vulgare* var. *hirtum* under the field experiment conditions.

In the same research conducted by Marzi, the highest dry weight was obtained from 12h daylight as 7.3 g. While the oil glands' density wasn’t affected by different photoperiodic conditions, a slight increase was observed when moving from short to longer days (e.g. 7.0 to 8.6 and 9.7 glands/mm^2 have been recorded from 12 to 16 light-hours/day). The root system showed a better growing and dry weight, in 12h daylight. In the present research the highest dry herb yield in *O. vulgare* var. *hirtum* was recorded at 13h daylight and this result was consistent with Marzi’s (1997) findings.

Tınmaz et al (2002) evaluated a large number of *Origanum vulgare* var. *hirtum* wild accession collected from the Marmara Region of Turkey regarding their essential oil yield and components. Of the 61 samples tested, 45 accession had more than 3% essential oil yield. The carvacrol ratio of wild plants ranged between 7.5-82.9% and the thymol 0.3-60.1%. After cultivation, the carvacrol and thymol ratios were increased up to 82.9% and 68.0%, respectively. A slight increase of essential oil was recorded from 56 accession when they were growing under field conditions.

According to Table 3. the main essential oil component of *O. vulgare* var. *hirtum* was carvacrol which increased with the seasonal progress as 58.3%, 65.6% and 74.55%. Unlike carvacrol, thymol content was decreased when the harvest delayed; as 4.50%, 0.36% and 0.71%. Cymene, gamma-terpinene and 1-octen-3-ol were the followings coming after that by 9.54%, 8.66% and 15.64%; 13.2%, 9.55%; 1.99%, 3.71% and 2.22%, respectively.

Tonçer et al. (2010) searched the seasonal variations of *Origanum syriacum* and identified twenty-five components from its essential oil. Thymol was the main component with 46.70% in the summer and p-cymene was another with 62.18% in the early spring. The components γ–Terpinene ranged from 0.63 - 19.08%, α–terpinene from 0.67 - 4.85% and carvacrol from 0.73 to 8.90%.

Consistent with the findings of Tonçer et al. (2010) in the present study the main essential oil components of *O. syriacum* were thymol and carvacrol; and ranged between 54.83% to 52.31 and 33.35% to 22.26%, seasonally (Table 4). Unlike the both study, Fleisher and Fleisher (1991) determined carvacrol and thymol in *O. syriacum*, and carvacrol, geraniol, geranyl esters, ethyl cinnamate from another sample of *O. syriacum*.

Table 4. The essential oil composition of *Origanum syriacum* harvested successively at August 14th, September 24th and October 28th, 2014.

Çizelge 4. 14 Ağustos, 24 Eylül ve 28 Ekim tarihlerinde hasat edilen Origanum syriacum türünün uçucu yağ kompozisyonu

Peak	R.T.	Compounds	August 14th	September 24th	October 28th
1	12.8	alpha-pinene	NF	0.41	0.41
2	12.9	alpha-thujene	0.18	0.92	0.65
3	17.1	sabinene	NF	0.49	NF
4	19.0	beta-myrcene	0.35	1.64	0.89
5	19.1	alpha-phellandrene	NF	0.22	NF
6	19.8	alpha-terpinene	0.47	1.65	0.94
7	20.7	limonene	NF	0.34	0.24
8	21.1	beta-phellandrene	NF	0.27	NF
9	22.7	gamma-terpinene	1.04	7.62	2.89
10	23.9	cymene cis-sabinene hydrate	3.33	5.74	NF
11	28.2	3-octanol	NF	0.31	0.27
12	30.3	1-Octen-3-ol cis sabinene	NF	0.33	0.37
13	31.0	Acetate trans-sabinene	NF	1.21	0.80
14	33.8	hydrate acetate	NF	4.75	4.96
15	35.7	carvacrol methyl ether	NF	0.74	0.44
16	35.8	beta-caryophyllene	0.58	1.09	2.00
17	38.0	alpha-humulene	NF	0.17	NF
18	38.7	borneol	0.23	NF	0.53
19	50.9	thymol	54.83	50.18	52.31
20	51.7	carvacrol	33.35	21.36	22.26
21		Not Identified	1.06	0.57	0.78

Conclusions

For many floriculture crops, photoperiod controls growth and flowering, and a small change in photoperiod can mean the difference between vegetative growth and rapid flowering. Many herbaceous perennials for example, flower when the photoperiod, or daylength, exceeds a set duration. This may help us to manipulate the natural photoperiod to promote vegetative growth (such as to bulk up plant size or for cutting production) or flowering, whichever is desired. Therefore, successful production of many crops requires an understanding of how plants respond to photoperiod, how photoperiod changes during the year and how to modify the photoperiod to control growth and development (Runkle 2002). *Origanum* species are considered as long day plants (Davidenco et al. 2014) which only flower, or flower more rapidly, when the photoperiod is longer than a critical duration.

In this research the influence of the daylights on the biomass and essential oil yield of *O. vulgare* var. *hirtum* and *O. syriacum* were observed and determined. When the daylight increased it enhanced the plant height and number of shoot in both subspecies. The highest essential oil yield was recorded at 10h daylight in both. The main essential oil component of *O. vulgare* var. *hirtum* was carvacrol which increased by the seasonal progress as 58.3%, 65.6% and 74.55%. Unlike carvacrol, thymol content was decreased when the harvest delayed; as 4.50%, 0.36% and 0.71%. Cymene, gamma-terpinene and 1-octen-3-ol were the followings coming after that by 9.54%, 8.66% and 15.64%; 13.2%, 9.55%; 1.99%, 3.71% and 2.22%, respectively. The main essential oil components of *O. syriacum* were thymol and carvacrol; ranged 54.83% to 52.31 and 33.35% to 22.26%, seasonally.

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