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Agronomical and technological researches on oregano (*Origanum onites* l.) in Diyarbakir ecological conditions

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Abstract

Origanum onites L. is one of the most important medicinal and aromatic plant grown in the Mediterranean area and extensively using in many traditional medicinal and culinary since ancient times. This study aims to determine the agronomical and technological characteristics of oregano at four different plant densities (70x20, 70x30, 70x40, 70x50 cm) and two harvests. The effects of this application was noticed on the fifteen flowing parameters; plant height, fresh and dry herb yield, dry leaf yield, canopy diameter, essential oil content and essential oil composition etc. According to the results; significant differences were found statistically between harvests, in all properties being studied; except dry herb yield. Fresh and dry herb yield varied from 11.1 to 13.7 t ha^{-1} and from 3.8 to 5.0 t ha^{-1} per harvest, respectively in a year. Dry leaf yield varied between 1.7–2.9 t ha^{-1} per harvest in a year. Carvacrol, 1,8 cineole and γ-terpinene were found as major components of oreganos. Mean Carvacrol percentage determined as 53.9%, 1,8 cineole 9.7% and γ-terpinene as 5.7% respectively. The experimental was randomized complete block design with four replications. The extraction of the samples were performed using a Clevenger-type apparatus for steam distillation. The essential oil components of Oregano were determined by gas chromatography (GC). According to the results of this study, differences between two cuttings except drog yield were found to be significant for all properties studied. As a conclusion, Oregano cultivation can be practicable in Diyarbakır ecological conditions in terms of drog herb and leaf yields and essential oil constitutions.

Key Words: Oregano, Plant Density, Harvest Time, Essential oil

Introduction

In recent years, demand for natural products from aromatic and medicinal plants has increased considerably as substitutes for artificial products, in terms of pharmacological properties (Atanasov et al. 2015). Among the various natural products, essential oils have gained great popularity in different industries, including the cosmetics, food, and pharmaceutical industries, due to their valuable characteristics such as unique colors, strong odor, and high volatility (Carvalho et al. 2016; Maggio et al. 2016). Particularly, essential oils have an important role in the health care due to their remarkable biological features (Raut and Karuppayil 2014).

Turkey is the most exporting country, among countries the thyme exporting. In terms of essential oil and exporting product, *Origanum onites* L. has an important medicinal and aromatic plant. There are about 30 species of *Origanum onites* L. Twenty-one of them exist in Turkey. They are grown naturally in stony, rocky and slope areas (Ietswaart, 1980; Baytop, 1984; Ceylan, 1997; Padulosi, 1996).

The thyme herb includes from 2% to 8% essential oil (Başer, 2001). This oil has antibacterial and antifungal properties (Özgüven et al. 1987, Kızıl and Uyar, 2005). After extracting essential oil, remaining as known hydrosol (thyme’s water) has been selling. Recently, consumption of hydrosol is become widespread. It is claimed that hydrosol has beneficial properties for treatment of stomach and intestine ailments (Aydın, 1996, Yılmaz et al., 2017).

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When thyme used as tea, it has a good effect in digesting and as an antioxidant (Başer, 2001). The results of some investigations showed that thyme's products could affect insect (Kesdek et al., 2013) and funguses (Özgüven andnokay, 1986; Ünlü, 1995, Kordali et al., 2008), and overuse of the thyme did not cause toxicity. It is scientifically approved that thyme mostly used as expectorant and its product (carvacrol) has also strong effect as analgesic and curative (Aydın, 1996, Chishti et al., 2013). Thyme contains plentiful pollen for bees and useful grass for animals. Therefore, bees fed with thyme produce more quality honey and animals fed on thyme gave beter quality milks and milk's products (Ortiz and Fernandez, 1992). Moreover, It was reported that thyme and thyme products was being used as food preservative (Er, 1994).

Studies on oregano species are inadequate, especially in our region. There are no standard on products collected from wild-area in Turkey. Importing countries bring a standard for any medicinal plants such as esential oil content should be at least 3%, carvacrol and thymol content should be high. The objective of this study was to determine technological and agronomical properties of *Origanum onites* L. at four densities and two harvests in a year from 2002 to 2004, under Diyarbakır ecological conditions.

Materials and Methods

Field trial was carried out between 2002 and 2004 growing season at Southeast Anatolia Agricultural Research Institute (latitude 37º 53 N longitudes 40º 16 E, 680 m above sea level). The soil of research place is zonal soil being generally red-brown and included in the big soil group having a clayish nature, flat or about-to-be flat, and having very small erosion and deep or medium deep. The region climate belongs to Mediterranean climate. Generally, summer is hot and drought; but winter is cold and rainfall. Long year's climatically findings showed that there were 454 mm total rainfall and average temperature was 15.8 ˚C every year (Meteorology Directorship's 2003–04 data of Diyarbakir)

Cuttings of *Origanum onites* L. were taken from healthy plants, obtained from Department of Field Crops, Agriculture Faculty of Çukurova University, Adana, Turkey, at 12 December 2001. Cuttings planted in sandy pool at green house for forming root. When plants reached at 10–15 cm plant height, they were transplanted to field on May 2002. Field trial was conducted according to randomized four density design with four reapplications. Each block area was 3m x 2.8m (8.4 m^2). The interval between two rows was 70 cm for four densities. On the other hand; the spacing between two plants on the same row was 20 cm for first density, 30 cm for second density, 40 cm for third denisty, 50 cm for fourth denisty; respectively. During the vegetation period, the plots were irrigated and weeded when required. No harvesting was done during the first year, however, the second and third year harvest was done two times; first harvest was done on May and second was done on November. Harvest times were determined according to beginning of blooming for each harvest in two years.

Each year, a basal dose of 60 kg ha^{-1} N and 60 kg ha^{-1} phosphor (20–20–0 compose) and upper dose of 60 kg ha^{-1} N (form of CAN) were applied. Basal dose of fertilizer was applied at October and November, and upper doses applied after each harvest as 30 kg ha^{-1}.

Plants, after removing border effects, were cut at height of 10 cm above soil and weighed to determine fresh herb yield, whereas dry herb yield was determined by drying fresh herb samples from each plot in a shadow and airy place during one-two week. Dry leaf yields were determined after separating the leaves and steam in the dry herb samples. Essential oil content was measured volumetrically, by hydro distillation using a Clevenger apparatus, in 20 g samples taken from each plot (mL/100g-v/w).

Data were analyzed statistically, using TOTEMSTAT and JMP ha^{-1}computer program, and means were grouped, using LSD values at significance level of 5%. The essential oil were analyzed by GC (Hewlett Packard 6890 Gas Chromatograph) Chromatographic separations were accomplished with a CAT capillary column (0,32 mm i.d.x30 m film thickness 1 µm) with injections in the spilt mode. The temperature was set to 50 ºC for 5 min initially, and increased to 180 ºC at a rate of 4 ºC per minute, then again increased 260 ºC. Nitrogen was used as a carrier gas at a flow rate of 30 mL/min. Each sample was analyzed four times every year. The identification of components was based on comparison of their relative RF values with those of authentic standards. Authentic standards were purchased from Sigma-Aldrich chemises (USA).

RESULT AND DISCUSSION

Agronomical Characteristics

Analysis of variance and average values for all agronomical characteristics (plant height, plant canopy diameter, fresh and dry herb yield, dry leaf yield and dry leaf/stem rate) are shown in Table 1. The results shows that all investigated characteristics were influenced statistically by harvests done in one year, except dry herb yield. Different plant densities affected none of characteristics significantly. Plant density x harvest interaction was found important significantly only on plant canopy diameter in 2004.

The plant height obtained from first harvests (47.9-47.4 cm) was higher than second harvests (21.0-30.8 cm) for both years. Our results of plant height are in agreement with Kırman (1993), who reported plant height as 29.7 cm for first harvest and 22.7 cm for second harvest in a year. It is estimated that the differences between harvests is due to harvest time and environmental factors of that time.

The plant canopy diameter from second harvest (50.9 cm) was higher than first harvest (38.8 cm) in 2003. But the plant canopy diameter from first harvests (54.1 cm) was higher than second harvest (43.9 cm) in 2004.Our findings are supported by Kırıcı and İnan (2001), who reported as number of plant's branchs. They reported 14.3 branch/plant for first harvest and 26.3 branch/plant for second harvest in a first year. On the other hand, they reported 23.1 branch/plant for first harvest, and 22.8 branch/plant for second harvest in a second year. The greatest plant canopy diameter (57.1 cm) was determined from 70x20 cm plant density and first harvest in 2004. The least interaction values (40.5, 43.0 and 44.1 cm) were obtained from 70x20; 70x30 and 70x40 cm plant densities and second harvest in 2004.

The fresh herb yield of first harvest (15.2 t ha^{-1}) was determined higher than second harvest (11.3 t ha^{-1}) in 2004. On the other hand, the dry herb yield obtained from first harvests (4.8, 4.7 t ha^{-1}) was higher than second harvests (4.3, 4.3 t ha^{-1}), in both 2003 and 2004. This might be due to the effects of environmental conditions. Similar findings were reported by Kıryaman (1988), who reported fresh herba as 4.4 t ha^{-1}for first harvest and 3.4 t ha^{-1}for second harvest. And, He reported dry herb as 1.5 t ha^{-1} for firs harvest and 1.0 t ha^{-1} for second harvest in a year.

The dry leaf yield of second harvest (3.4 t ha^{-1}) was higher than the first harvest (1.8 t ha^{-1}) in 2003. Also, the dry leaf/the stem rate from second harvests (80%, 66%) were higher than the first harvests (39%, 56%) respectively in both years. This variation might be because of the differences in the percentage of dry matter of plants and the enviromental conditions. The results obtained from the study are in agreement with Kırman (1993), who reported dry leaf as 1.4 t ha^{-1} for second and 0.6 t ha^{-1} for the first harvest, but they are in conflict with Kıryaman (1988), who reported dry leaf yield as 1.1 t ha^{-1} for the first harvest and 0.8 t ha^{-1} for the second harvest. The variation might be resulted from the differences of environmental conditions and agronomical applications.

Technological Characteristics

Analysis of variance and average values for all technological characteristics (essential oil content, α-pinene, β-pinene, α-terpinene, 1,8 cineole, γ-terpinene, linalool, borneol and carvacrol constituents) are shown in Table 2. According to the results, between two harvests in a year for all characteristics was determined statistically significant. Plant densities affected none of technological characteristics, except 1,8 cineole and γ-terpinene, Plant density x harvest interaction was determined statistically significant only on carvacrol component.

The essential oil content of oregano was found statistically significant between harvests. The oil content of the first harvests was higher than the second harvests' in both years. Oil content of the first year and the second year at the first and second harvest were determined as 1.8%, 1.4%, and 1.9, 1.7%, respectively. Our findings' relation with essential oil content is in agreement with Kırman (1993), who reported essential oil content of the first harvest higher (2.0%) than the second harvest (1.8%).

β-Pinene, α-terpinene, γ-terpinene, linalool and carvacrol contents from the first harvest (1.6, 1.8, 6.5, 3.0 and 61.7%, respectively) were found higher than that of the second harvest (1.3, 1.3, 4.6, 2.5 and 42.4%, respectively) in the first year. Hovewer, the α-pinene (1.0%-1.1%) and 1,8 cineole (14.7%-12.1%) contents obtained from the second harvest were higher than those of the first harvest (0,7%,0,5%-7,7%, 4.4%) respectively) in both years. Our results are in agreement with Kıryaman (1988) and Kırman (1993). Kıryaman (1988), who reported α-pinene content as 6.5% for second harvest and 4.0% for first harvest; cineol content as 8.0% for the second harvest, 7.7% for the first harvest, terpinene contrnt as 4.0% for first harvest and as 3.5% for second harvest, as borneol content 12.1% for first harvest and as 11.6% for second harvest, carvacrol content as 24.9% for first harvest and as 20.6% for second harvest. Kırman (1993), Who reported β-Pinene content as 3.7% for first harvest, 3.0% for second harvest, α-terpinene content as 9.1% for firs harvest, 6.6% for second harvest, linalool content as 8.8% for fist harvest, 8.5% for second harvest.

The borneol content (1.6%) of second harvest was higher than first harvest (1.2%), only in 2004. The results obtained from our study are in agreement with Kırman (1993), who reported borneol content as 2.3% for the second harvest, 2.0% for the first harvest. It is thought that environmental affects are the reason of this case.

γ-Terpinene content from the first harvest (6.5%) was higher than that of the second harvest (4.6%) for 2003 year. But in 2004, γ-terpinene content obtained from second harvest (7.3%) was higher than the first harvest (4.5%). It is also estimated that this contradiction resulted from the plant age, environmental conditions and plant ability to adapt to environmental conditions.

The highest carvacrol contents (65,0%; 63,3%; 63,0%) were obtained from 70x40, 70x50 and 70x30 cm plant densities at first harvest. But the lowest carvacrol contents (40.1%, 40.3%, 44.4% and 44.7%) were obtained from 40x70, 70x50, 70x20 and 70x30 applications at the second harvest, respectively in 2003. The applications mentioned above are also affective and give positive results on fresh and dry herb. Therefore, it is understandable that the environmental conditions and growing applications are very important parameters on carvacrol content (Naghdi Badi et al. 2004).

Table 1. Effect Of Plant Densities And Harvests On Plant Height, Plant Diameter, Fresh And Dry Yield And Drug Leaf Yield And Dry Leaf/Stem Rate

Density (cm)	Plant height (cm)						Plant Canopy dimater (cm)						Fresh herbage yield (t ha-1)					
	2003			2004			2003			2004			2003			2004		
	Fir. h.	sec.h.	Mean	Fir. h.	sec.h.	Mean	Fir. h.	sec. h.	Mean	Fir. h.	sec. h.	Mean	Fir. h.	sec. h.	Mean	Fir. h.	sec. h.	Mean
70x20	46.1	21.6	33.8	45.9	31.1	38.5	38.1	51.0	22.3	57.1a	40.5e	48.8	12.7	13.2	12.9	14.9	11.1	13.0
70x30	47.6	20.9	34.3	47.1	30.7	38.9	38.5	51.1	22.4	51.8 bc	43.0e	47.4	12. 0	13.2	12.6	15.7	11.5	13.6
70x40	49.7	22.1	35.9	47.2	31.0	39.1	39.9	50.8	22.7	54.4 ab	44.1de	49.2	13.7	13.3	13.5	15.3	11.4	13.4
70x50	48.3	19.8	34.0	49.7	30.8	40.2	38.9	50.9	22.5	53.2 ab	48.4 cd	50.8	11.4	11.1	11.2	15.0	11.1	13.0
Mean	47.9 a	21.0 b	34.5	47.4 a	30.8 b	39.2	38.8 b	50.9a	22.4	54.1a	43.9b	49.0	12.4	12.7	12.6	15.2 a	11.3 b	13.2
LSD(%5)	Harvest: 3.0**			Harvest: 1.9**			Harvest: 4.0**			Int.: 4.7*			n.s.			Harvest: 2.2**		

Density (cm)	Dry herbage yield (t ha-1)						Dry leaf yield (t ha-1)						Dry leaf/ stem rate (%)					
	2003			2004			2003			2004			2003			2004		
	Fir. h.	sec.h.	Mean	fir.h.	sec.h.	Mean	fir.h.	sec. h	Mean	Fir. h.	sec. h.	Mean	fir. h.	sec.h.	ave.	fir. h.	sec. h.	ave.
70x20	5.0	4.5	4.8	4.6	4.3	4.5	2.0	3.5	2.8	2.7	2.9	2.8	40	79	59	58	67	62
70x30	4.3	4.5	4.4	5.0	4.6	4.8	1.7	3.7	2.7	2.7	3.1	2.9	39	82	60	56	67	61
70x40	5.2	4.3	4.8	4.8	4.4	4.6	1.9	3.5	2.7	2.6	2.9	2.8	36	83	59	55	63	59
70x50	4.4	3.8	4.1	4.5	3.8	4.2	1.8	2.9	2.3	2.4	2.6	2.5	39	76	57	54	67	61
Mean	4.8	4.3	4.5	4.7	4.3	4.5	1.8b	3.4a	2.6	2.6	2.9	2.7	39 b	80a	59	56 b	66 a	61
LSD (5%)	ns			ns			Harvest: 0.4**			ns			Harvest: 2.8**			Harvest:3.7**		

*p>0.05, **p>0.01, Int: İnteraction, ns: No significant, Fir.: First, Sec.: Second, h.: Harvest

Table 2. Effect of Plant Densities and Harvest On *α–Pinene*, *β–Pinene*, *α-Terpinene*, *1,8 cineole*, *γ-Terpinene*, *Linalool*, *Borneol*, and *Carvacrol* Components of *Origanum onites* L.

Density (cm)	**Essential oil content (%)**						**α-pinene content (%)**						**β-pinene content (%)**					
	2003			**2004**			**2003**			**2004**			**2003**			**2004**		
	fir.h.	**sec. h.**	**Mean**	**Fir. h.**	**sec. h.**	**Mean**	**fir.h.**	**sec. h.**	**Mean**	**fir.h c.**	**sec. h.**	**Mean**	**Fir h.**	**sec. h.**	**Mean**	**fir. h.**	**sec.h c.**	**Mean**
70x20	1.9	1.4	1.7	1.8	1.7	1.8	1.0	1.2	1.1	0.5	1.1	0.8	1.8	1.4	1.6	1.5	1.4	1.4
70x30	2.0	1.3	1.6	2.1	1.9	1.9	0.7	1.2	1.0	0.6	1.1	0.9	1.7	1.2	1.4	1.7	1.5	1.6
70x40	1.7	1.6	1.6	2.1	1.8	1.9	0.7	1.1	0.9	0.7	1.1	0.9	1.4	1.4	1.4	1.8	1.4	1.6
70x50	1.7	1.4	1.5	1.9	1.7	1.8	0.7	0.8	0.8	0.5	1.1	0.8	1.7	1.1	1.4	1.6	1.4	1.5
Mean	1.8a	1.4b	1.62	1.9a	1.7b	1.9	0.7b	1.0a	0.92	0.5b	1.1a	0.84	1.6a	1.3b	1.45	1.6a	1.4b	1.53
LSd (%5)	Harvest: 0.2**			Harvesr: 0.1*			Harvest: 0.2**			Harvest: 02**			Harvest: 0.3**			Harvest: 0.3**		

Density (cm)	α-Terpinene (%)						1,8 Cineole (%)						γ-Terpinene (%)					
	2003			2004			2003			2004			2003			2004		
	Fir h.	sec.hc.	Mean	fir.hc.	sec. h.	Mean	Fir. h.	sec. h.	Mean	Fir. h.	sec. h.	ave.	Fir. h.	sec. h.	Mean	fir. h.	sec.hc.	Mean
70x20	2.1	1.3	1.7	1.3	1.3	1.3	9.2	14.5	11.9a	4	11.4	7.7	8	4.8	6.4a	4.4	7.2	5.8
70x30	1.7	1.2	1.5	1.3	1.4	1.4	7.9	15.8	11.9a	4.4	12.1	8.3	6.5	4.8	5.6ab	4.5	7.2	5.8
70x40	1.9	1.5	1.7	1.3	1.5	1.4	6	13.1	9.5b	4.6	12.1	8.3	5.8	4.6	5.2b	4.5	7.5	6
70x50	1.7	1.3	1.5	1.3	1.4	1.4	7.4	15.2	11.3a	4.6	12.7	8.6	5.8	4.2	5.0b	4.4	7.2	5.8
Mean	1.8a	1.3b	1.6	1.3	1.4	1.4	7.7b	14.7a	11.2	4.4b	12.10a	8.2	6.5a	4.6b	5.6	4.5b	7.3a	5.85
LSd (%5)	Harvest: 0.3**			ns			Harvest:1.1**		D:1.6**	Harvest: 1.0**			Harvest: 0.6**		D: 0.9*	Harvest: **0.7		

Density (cm)	Linalool (%)						Borneol (%)						Carvacrol (%)					
	2003			2004			2003			2004			2003			2004		
	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean	fir. h.	sec. h.	Mean
70x20	3.0	2.5	2.8	4.1	2	3.1	1.9	2.5	2.2	1.1	1.5	1.3	55.5b	44.4c.	50	67.5	41.9	54.7
70x30	3.0	2.4	2.7	4.8	2.4	3.6	1.8	1.9	1.8	1.1	1.6	1.4	63.0a	44.7c.	53.8	58.7	50.2	54.5
70x40	2.6	2.4	2.5	5	2.3	3.6	2.1	2	2.1	1.2	1.7	1.4	65.0a	40.1c.	52.6	62.1	50	56.1
70x50	3.3	2.6	3.0	5.2	2.4	3.8	2.3	2.4	2.3	1.3	1.7	1.5	63.3a	40.3c.	51.8	66.7	49.4	58.0
Mean	3.0a	2.5b	2.7	4.8a	2.3b	3.5	2.0	2.2	2.1	1.2b	1.6a	1.4	61.7a	42.4b	52.0	63.8a	47.9b	55.8
LSd (%5)	Harvest: 0.3**			Harvest: 0.4**			ns			Harvest: 0.1**			Int: 7.0*			Harvest:5.7**		

*p>0.05, **p>0.01, Int: İnteraction, ns: No significant, D: Density, Fir.: First, Sec.: Second, h.: Harvest

Conclusions

According to the results of these study suggestions can be as following;

1. If the first year of experiment was establishment year of oregano and there was not any harvest, 70x30 cm plant density can be suggested in terms of herb yield. This plant density is also its suitable for mechanization.
2. In the terms of essential oil, the first harvest is more suitable than the second harvest. As result, the first harvest can be suggested for obtaining the essential oil, but the second harvest is offered for obtaining high dry leaf yield.
3. As the components of essential oil; β-pinene, α-terpinene, linelool and carvacrol are rich in the first harvest, but α-pinene, 1,8 cineole and borneol are rich in second harvest. γ-terpinene is rich in both harvests in a year.
4. For fresh and dry herb yields, the first harvest is more suitable. On the other hand, the second harvest is more suitable for dry leaf yield.
5. As a conclusion, the ecological conditions of Diyarbakır are suitable for agriculture of oregano for production of essential oil and herb yield.

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