

1-1-2017

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TURGUT, KENAN; ÖZYİĞİT, YAŞAR; TÜTÜNCÜ, BEGÜM; and SÖZMEN, ESRA UÇAR (2017) "Agronomic and chemical performance of selected *Origanum dubium* Boiss. clones for industrial use," *Turkish Journal of Agriculture and Forestry*. Vol. 41: No. 4, Article 4. <https://doi.org/10.3906/tar-1612-75>
Available at: <https://journals.tubitak.gov.tr/agriculture/vol41/iss4/4>

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Agronomic and chemical performance of selected *Origanum dubium* Boiss. clones for industrial use

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Received: 19.12.2016 • Accepted/Published Online: 01.04.2017 • Final Version: 25.08.2017

Abstract: *Origanum dubium* Boiss. is an economically important wild oregano species that is intensely collected from the natural flora of Antalya, Turkey. This herb is used mainly to produce an essential oil due to its high yield and high carvacrol content. The aim of this study was to improve the biomass yield and essential oil and carvacrol content of *O. dubium* for industrial use. In preliminary studies, a basic population was established using seeds collected from wild plants and then trials of A and B clones were run. Dry biomass yield per A clone plant and essential oil content varied at 5.05–40.86 g and 5%–14%, respectively. Carvacrol was the main constituent in all samples and varied from 72.26% to 88.21%. Dry biomass yield per B clone plant was 21.64–95.52 g, dry leaf yield per plant was 14.80–48.77 g, essential oil content was 6%–9%, and carvacrol rate was 80.99%–88.46%. After evaluating the B clones, 10 clones were selected and they were planted in a plot with a randomized block design and three replications in Antalya in 2014. In this trial, clone 7 produced the highest biomass yields per plot (2874.33 g fresh and 1185.00 g dry) and the highest fresh (2379.67 g) and dry (795.33 g) leaf yields per plot. Essential oil content varied from 7.97% to 11.16%. Clone 137 had the best essential oil content (11.16%), followed by clone 7 (10.76%) and clone 119 (10.59%). Clone 7 appeared to be superior to the others in terms of agronomic features, followed by clone 119. Essential oil and carvacrol rates were 10.76% and 85.02% in clone 7 and 10.59% and 91.04% in clone 119, respectively. Consequently, clones 7 and 119 appeared to be superior to the others.

Key words: Agronomic features, carvacrol, clone selection, essential oil, *Origanum dubium*

1. Introduction

Origanum dubium Boiss. is an economically important wild oregano species in Turkey. The most important characteristic of this oregano is its high essential oil content (6%–8%). This herb is intensely harvested from wild uplands near the towns of Alanya and Gazipaşa in Antalya Province and is used to produce essential oil. Thus, natural populations have been decreasing annually. Various genotypes and chemotypes have been reported within wild populations, such as high carvacrol and high linalool types (Turgut et al., 2013). Therefore, cultivating *O. dubium* seems to be the most convenient way to conserve wild populations and produce stable quantities of drugs. *O. dubium* grows wild in Turkey, Greece, and Cyprus. *O. dubium* is composed of carvacrol, linalool, and linalool-carvacrol chemotypes, as well as the thymol chemotype. The thymol, linalool, and carvacrol chemotypes have been reported in Turkish *O. dubium* populations, whereas those in Cyprus are only the carvacrol chemotype (Figuéredo et al., 2006). Lukas et al. (2013) reported taxonomic

uncertainties concerning the section *Majorana*. They assessed the taxonomic status of *O. onites*, *O. syriacum*, *O. dubium*, and *O. majorana* and discussed the evolutionary relationships in the section *Majorana* after considering molecular, morphological, and phytochemical data. According to their results, the 'cymyl' chemotype of *O. majorana* L. is classified as *O. dubium*. The leaves and flowers of this species are used to treat gastrointestinal problems and as a diuretic, and the essential oil is used as an antirheumatic (Arnold et al., 1993). In addition, the essential oil of *O. dubium* has been evaluated as a pharmaceutical and industrial product (Baser et al., 1993; Vera and Chane-Ming, 1999) due to its antimicrobial and potential antioxidant activity (Karioti et al., 2006). The biological activities of *O. dubium* Boiss. essential oil, such as anticarcinogenic (Koparal and Zeytinoglu, 2003), antioxidant (Mezzoug et al., 2007), fungicidal (Ahmad et al., 2011; Dambolena et al., 2011), insecticidal (Tang et al., 2011), and antimicrobial properties (Nostro and Papalia, 2012) are associated with carvacrol content.

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Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is a natural monoterpene phenol. Use of oregano essential oils has increased significantly in various sectors (food, health, agriculture, cosmetics etc.). Thus, the contents of essential oil and carvacrol from oregano are very important due to their biological activities. Selection breeding is an efficient tool for agronomic and chemical improvement of *Origanum* species due to their high genetic diversities (Franz and Novak, 1996). Clone selection appears to be suitable for *O. dubium* since it is cross-pollinated and propagated clonally. Clone selection was used to improve biomass yield and essential oil content of *O. onites* (Ceylan et al., 2003). *O. dubium* is used mainly for its essential oil and not as a culinary herb or for herbal tea production because of the high essential oil content. Therefore, development of new genotypes with much higher essential oil and carvacrol content will be very valuable for the industry. The aim of the present study was to improve the biomass yield and essential oil and carvacrol contents of cultivated *O. dubium* for industrial use.

2. Materials and methods

2.1. Plant material

This study was conducted in Antalya, located in the Mediterranean Region of Turkey (33 m above sea level and 36°53'N; 30°38'E). This location is characterized by a Mediterranean climate with 1068 mm total rainfall and mean air temperature of 19.7 °C (range, 13.6–24.2 °C). The terra-rossa-type soil characteristics of the experimental field were clay loam, very high in lime, low in salt, and slightly alkaline (pH 7.7). The 0–30-cm soil layer had low concentrations of organic material and sufficient nitrogen. Available soil phosphorus content was low but useful potassium content was high.

High essential oil content and carvacrol-type *O. dubium* Boiss. populations were identified in preliminary studies, and their seeds were collected from the natural flora of Gazipaşa in Antalya Province, Turkey (1372 m above sea level and N36 26.749 E32 28.266). The seeds were germinated in a greenhouse in April 2011, and 2200 healthy seedlings were transferred to an experimental plot for individual plant selection in May 2011. One hundred plants (genotypes) were selected according to their agronomic features and essential oil content. Then 10 stem cuttings from each plant (genotype) were rooted under greenhouse conditions and planted in rows; thus, a total of 100 rows of 1000 plants per row were established in the open field and called clone A in 2012. Each row was harvested separately for observations and analyses. After drying the plants, the essential oil was obtained for gas chromatography–mass spectroscopy (GC-MS) analyses. According to observations and all parameters, 30 rows (clones) were selected for establishing the B clone. Fifty

stem cuttings from each row were rooted and planted in the plots in May 2013. After evaluating the agronomic and chemical data of 30 clones, 10 (plots) were selected for the replicated plot trial.

2.2. Experimental design

Stem cuttings (minimum of 200 each) from 10 selected plots (clones) were rooted under a mist irrigation system in a greenhouse for the replicated plot trial. Rooted seedlings were planted in the plots in a randomized block design with three replications in May 2014. Row and intrarow spacing were 50 cm × 25 cm with 50 plants per plot. The plants were irrigated with drip irrigation and no fertilizers were applied. Weeds were controlled manually with garden tools. Thirty plants from each plot were used for observations and analyses, excluding side plants. After collecting data from the plants, each plot was harvested separately at the inflorescence stage in August 2014 and fresh biomass and leaf yields were obtained. Then 200 g of aerial parts of the plants from each plot were dried at 35 °C in an oven to calculate dry biomass and dry leaf yields. In addition, essential oil was extracted, and the GC-MS analysis was performed using dried samples.

2.3. Essential oil isolation

Twenty-gram dried samples (aerial parts of the plants) from each plot were subjected to water (250 mL) distillation for 3 h using a Clevenger-type apparatus. As a result, the percentage of essential oil was measured by the volumetric method (v/w) in each sample.

2.4. GC-MS analysis of the essential oil

Samples were diluted 1:50 with hexane for analyses. GC-MS analyses were performed on a gas chromatography (7890A; Agilent Technologies Inc., Palo Alto, CA, USA) mass detector (Agilent 5975C) GC-MS system operating in the electrospray ionization mode at 70 eV, equipped with a split/splitless injector (250 °C). Temperature was programmed from 60 °C (10 min) to 250 °C (10.5 min) and the analysis was carried out in a total time of 60 min. Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The HP Innovax Capillary column (60.0 m × 0.25 mm × 0.25 µm) was used. A 1-µL sample was injected at a split rate of 50:1. The components of the *O. dubium* essential oil were identified by comparison of their relative retention times and mass spectra with the OIL ADAMS, NIST, and Wiley libraries. Retention indices of all the components were determined by the Kovats method.

2.5. Statistical analysis

SAS statistical software (SAS Institute, Cary, NC, USA) was used for the data analysis. Analysis of variance was used to detect differences followed by Duncan's multiple comparison test. A P-value < 0.05 was considered significant.

3. Results and discussion

The aim of this study was to select *O. dubium* genotypes with high essential oil and carvacrol contents using the clone selection method. Seeds were collected from wild carvacrol-type *O. dubium* plant populations growing in the flora of Gazipaşa, Antalya, in 2011 and a basic population (2100 plants) was established to select the clones. Essential oil rates in this population varied from 2.07% to 5.65%. Ceylan et al. (2003) also found significant variations among individual *Origanum onites* plants. A total of 100 plants containing essential oil rates >4% were selected and propagated using stem cuttings, and the A clone was established in 2012. Each row (clone) was harvested separately for observations and analyses. Dry biomass yield per A clone plant was 5.05–40.86 g, dry leaf yield per plant was 4.98–27.75 g, and the essential oil rate was 5%–14%. A total of 25–35 components were found in the essential oil samples and carvacrol was quantitatively the main component, followed by p-cymene, γ -terpinene, β -myrcene, α -thujene, and trans-sabinene. Carvacrol rates in clones were 72.26%–88.21%. Ceylan et al. (2003) reported significant differences in the agronomic traits of clones and variations in essential oil content of *O. onites*. According to our results, 30 rows (clones) were selected to establish the B clone. Plant height of the B clone was 37.18–72.60 cm (mean, 51.27 cm), with 12.9–54.67 stems per plant (mean, 31.73); fresh biomass yield per plant was 41.93–185.44 g (mean, 94.05 g); dry biomass yield per plant was 21.64–95.52 g (mean, 50.39 g); fresh leaf yield per plant was 22.31–70.74 g (mean, 39.13 g); dry leaf yield per plant was 14.80–48.77 g (mean, 26.99 g); leaf/stem ratio was 0.58–1.97 (mean, 1.34); essential oil rate was 6%–9% (mean, 7.36%); and carvacrol rate was 80.99%–

88.46% (mean, 84.99%). Essential oil composition appears to differ between plant populations and localities because the chemical profile is highly affected by genetic and environmental factors (Ceylan et al., 2003).

After evaluating the agronomic and chemical data of 30 clones, 10 (rows) were selected for the replicated plot trial and 200 stem cuttings from each were rooted. They were planted in plots in a randomized block design with three replicates. In this trial, plant heights were significantly different among the clones and clone 7 was the tallest (50 cm), followed by clones 16 and 123 (Table 1). The number of stems per plant varied significantly between clones and the highest number was from clone 119 (four stems). Fresh and dry biomass yields of the selected clones were significantly different (Table 1). Clone 7 produced the highest biomass yields (2874.33 g/plot fresh and 1185.00 g/plot dry) followed by clone 4 (2269.67 g/plot fresh and 754.00 g/plot dry) and clone 119 (2,148.67 g/plot fresh and 757.00 g/plot dry) (Table 1, Figure 1). On the other hand, clones 19, 62 and 137 produced the lowest fresh and dry biomass yields per plot (Table 1). Clone 7 was superior in terms of fresh (2379.67 g/plot) and dry (795.33 g/plot) leaf yields, followed by clone 119 (1650.67 g/plot fresh and 557.67 g/plot dry) and clone 4 (1590.00 g/plot fresh and 493.33 g/plot dry) (Table 1; Figure 1). Similar to biomass yields, clones 19, 62, and 137 produced the lowest fresh and dry leaf yields per plot. According to the results, clone 7 showed the highest agronomic performance, followed by clones 119 and 4. Clones 7, 19, 62, 119, and 137 produced >10% essential oil content. Essential oil contents of the selected clones varied from 7.97% to 11.16% (Table 1). Clones 6, 7, 16, 19, 62, 119, and 137 were placed in the same statistical group with higher essential oil content. On

Table 1. Agronomic features and essential oil rates of selected clones.

Clone no.	Plant height (cm)**		Number of stems (no.)**		Fresh biomass yield (g/plot)**		Dry biomass yield (g/plot)**		Fresh leaf yield (g/plot)**		Dry leaf yield (g/plot)**		Essential oil content (%)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4	39.67	C	3	BC	2269.67	B	754.00	B	1590.00	B	493.33	C	7.98	B
6	39.00	C	2	BC	1811.00	D	536.33	C	1439.33	C	449.67	CD	9.99	A
7	50.00	A	2	BC	2874.33	A	1185.00	A	2379.67	A	795.33	A	10.76	A
16	46.00	B	2	BC	1524.00	E	561.33	C	1184.00	D	416.67	D	9.94	A
19	35.67	D	2	C	1013.67	G	349.67	D	788.00	G	297.67	EF	10.12	A
58	27.00	F	3	AB	1241.67	F	400.00	D	997.67	E	327.00	E	8.12	B
62	32.00	E	2	BC	929.00	G	340.67	D	741.67	G	254.67	FG	10.23	A
119	35.67	D	4	A	2148.67	C	757.00	B	1650.67	B	557.67	B	10.59	A
123	44.67	B	3	BC	1764.67	D	574.00	C	1394.00	C	461.33	CD	7.97	B
137	29.00	F	3	AB	1003.33	G	354.00	D	884.00	F	241.67	G	11.16	A
F value	87.06		3.86		365.71		118.39		315.49		126.36		7.78	

**Means within a column followed by different letters are different at the $P \leq 0.01$ level

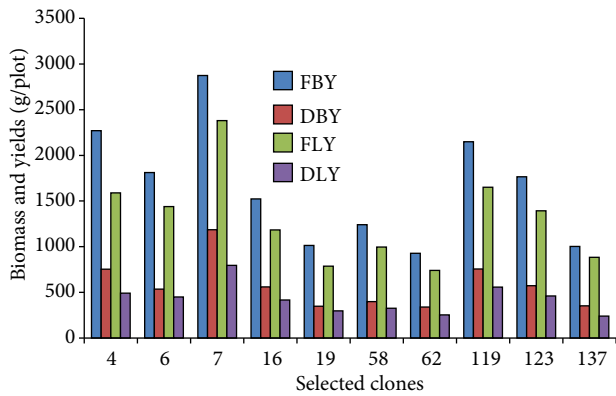


Figure 1. Biomass and leaf yields of selected clones (FBY: fresh biomass yield, DBY: dry biomass yield, FLY: fresh leaf yield, DLY: dry leaf yield).

the other hand, clones 4, 58, and 123 produced the lowest values (Table 1). Although clone 137 produced the highest essential oil content (11.16%), with an 86.87% of carvacrol rate, its agronomic performance was unsatisfactory. These essential oil contents were much higher than those reported previously, such as 7.6% (Sarer et al., 1982), 6.5%–7.7% (Baser et al., 1993), and 5.0%–8.2% (Turgut et al., 2013).

In total, 15 different components were identified by GC-MS analysis, representing 98.46%–99.68% of the essential oil. Carvacrol was the largest quantitative component, followed by p-cymene, γ -terpinene, β -myrcene, α -thujene, and trans-sabinene (Table 2). The plants were rich in

active monoterpene phenols, such as carvacrol, and monoterpene hydrocarbon precursors, such as p-cymene and γ -terpinene. The ‘cymyl’ pathway accumulates γ -terpinene, p-cymene, carvacrol, and thymol, and these compounds are characteristic for a number of *Origanum* species, e.g., *O. dubium* Boiss., *Origanum onites* L., and *Origanum syriacum* L. (Skoula and Harborne, 2002; Lukas et al., 2010). Figuerédo et al. (2006) studied the composition of oils from six species of carvacrol-rich Mediterranean oregano and found that the amounts of γ -terpinene (1%–15%) and p-cymene (1%–9%) were more abundant in *O. compactum*, *O. dictamnus*, *O. dubium*, *O. minutiflorum*, *O. onites*, and *O. vulgare* subsp. *hirtum* than those in the other species studied. Carvacrol rates in the essential oils obtained from the selected clones were 81.85%–91.04%, and clone 119 was the highest (Table 2). Clones 7, 58, 62, 119, 123, and 137 gave carvacrol rates >85% (Table 2). Carvacrol contents of the different genotypes were higher than those previously reported for *O. majorana* from Turkey, such as 78.27%–79.46% (Baser et al., 1993), and *O. dubium* from Cyprus at 69.5%–71.3% (Karioti et al., 2006).

Essential oil and carvacrol rates were 10.76% and 85.02% in clone 7 and 10.59% and 91.04% in clone 119, respectively. Clones 7 and 119 appeared to be superior to the others when agronomic features, essential oil, and carvacrol rates were evaluated together (Figure 2). Therefore, both clones were determined to be candidates for cultivar registration.

O. dubium is one of the most economically important wild oregano species in Turkey and is collected from the

Table 2. Carvacrol and other major components of the essential oils isolated from selected clones (%).

Clone no.	4	6	7	16	19	58	62	119	123	137
Carvacrol	83.30	81.85	85.02	84.87	84.34	87.88	87.52	91.04	86.48	86.87
γ -terpinene	5.06	6.25	3.60	4.40	3.84	2.41	2.02	1.77	2.57	3.00
p-cymene	3.55	3.27	3.04	3.35	3.00	2.73	3.45	2.40	3.00	2.51
β -myrcene	1.58	1.65	1.87	1.80	1.80	1.75	1.65	1.05	1.74	1.89
α -thujene	1.10	1.63	1.51	1.46	1.47	1.43	1.23	0.45	1.45	1.58
trans-sabinene	1.32	0.97	1.07	1.08	1.12	0.99	1.00	0.98	1.06	0.93

Table 3. Essential oil and carvacrol contents per plot of selected clones (g/plot).

Clone no.	4	6	7	16	19	58	62	119	123	137
EOY/P (g/plot)	60.17	53.54	127.50	55.76	35.31	32.48	34.78	80.16	45.74	39.50
CY/P (g/plot) * ζ ***	50.12	43.82	108.40	47.32	29.78	28.54	30.44	72.97	39.55	34.31

EOY/P: essential oil yield per plot, CY/P: carvacrol yield per plot.

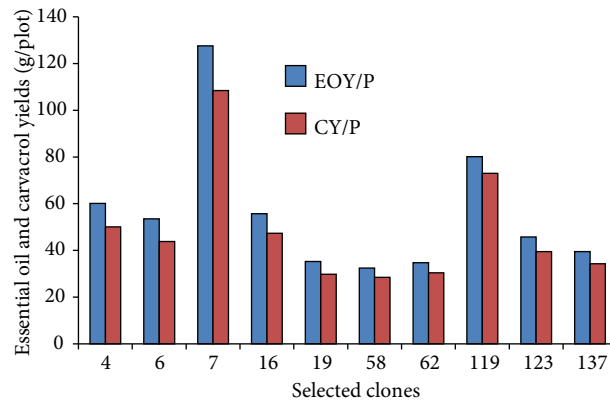


Figure 2. Essential oil and carvacrol yields per plot (EOY: EOY/P: essential oil yield per plot, CY/P: carvacrol yield per plot).

natural flora of Antalya. This species is used mainly for essential oil due to its high essential oil and carvacrol contents. The essential oil industry demands high essential oil content of high quality but growers prefer high biomass yield. Therefore, developing new genotypes with high essential oil yields per hectare is very important to satisfy both the industry and growers. The carvacrol rate in oregano species is also important because the essential oil is the biologically most active component. As seen in Table 3 and Figure 2, clone 7 produced the highest yields of

essential oil (127.50 g/plot) and carvacrol (108.40 g/plot) per plot, followed by clone 119 (80.16 g/plot and 72.97 g/plot, respectively). On the other hand, clones 19, 58, and 62 produced the lowest essential oil and carvacrol yields.

Acknowledgment

This study was supported by the Scientific and Technological Research Council of Turkey (project no. 110O702).

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