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Chemical composition and allelopathic effect of *Origanum onites* L. essential oil

Origanum onites L. uçucu yağıının kimyasal bileşenleri ve allelopatik etkisi

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ABSTRACT

In this study, chemical composition and allelopathic effect of essential oil obtained from ground parts (shoots+leaves+flowers) of *Origanum onites* L. plant on seed germination and seedling growth of different plant species were investigated. Essential oil was obtained with the use of the hydro-distillation method from *O. onites* plant collected from Mersin province. It was identified 24 different compounds by GC/MS analysis in *O. onites* essential oil, while the main compounds were determined as carvacrol (59.87%), γ -terpinene (17.08%) and β -cymene (8.83%). The allelopathic effect of the essential oil, two layers of filter paper were placed bottom of 9 cm diameter disposable Petri dishes then seeds of *Amaranthus retroflexus* L., *Triticum aestivum* L. and *Lepidium sativum* L. were homogeneously distributed on filter paper. Filter papers were thoroughly moistened using distilled water. The filter paper was glued to the center of the lid of each Petri dish. The different concentrations (0, 0.5, 1, 1.5, 5, 15 μ l/Petri dishes) of the essential oil were applied to the filter paper pieces. Then lid of each Petri dish was closed and sealed with Parafilm. Petri dishes were incubation at 12 h dark-12 h light periods with an average temperature of 24 ± 1 °C for 1-4 weeks. At the end of incubation period, germination rates, root and shoot lengths of the test plants were determined. The 15 μ l/Petri concentration of *O. onites* essential oil were completely inhibited seed germination, root and shoot growth of *A. retroflexus* L., *T. aestivum* L. and *L. sativum* L. plants. *A. retroflexus* L. was found to be more sensitive to essential oil. The results show that *O. onites* essential oil has potential to be used in the control of *A. retroflexus* L., which has high allelopathic effect on seed germination and seedling development of *L. sativum* L., *T. aestivum* L., *A. retroflexus* L.

INTRODUCTION

Biotic and abiotic stress factors in the agricultural lands cause significant yield and quality loss in agricultural crops.

Weeds, one of these biotic factors, cause yield and quality losses through allelopathy as well as competing against

cultivated plants for light, food stuffs in the environment and water (Joshi and Joshi 2016, Kong et al. 2007). In allelopathy, a plant shows phytotoxic effects by affecting the growth of other plant/plants positively or negatively due to the compounds called as allelochemicals that it releases (Alam and Islam 2002, Bajalan et al. 2013). The compounds leading to this phytotoxic effect may be present in different parts of plant/plants (Zeng et al. 2008) and they may cause negative effects in other plants in a production areas or environments. The cultivated plant having this property may be used in weed control in the agriculture production systems. However, the weeds with these compounds cause phytotoxic effects in cultivated plants. The plants contain these secondary compounds by producing them throughout their lives. Their amount and composition vary based on plants. Different researchers have conducted studies on potential of using these different compounds in the control of weeds in agriculture. These studies are also conducted on the other family members, especially Lamiaceae and Asteraceae families.

Lamiaceae family with its 250 genus and 7133 species has a wide spreading area in the world (Harley et al. 2004, Kaya et al. 2017). The members of this family spread in plains with high altitude in the tropical and temperate regions (Cantino et al. 1992). Lamiaceae, which is the largest 3rd family in the flora of Turkey, are represented by 45 genera, 558 species and 742 taxa and its endemism rate is 42.2% (Belen 2012, Kaya et al. 2017, Koyuncu et al. 2010). As the plants included in this family are rich in essential oils and secondary compounds, they have an important place in many fields such as medicine industry (Kahraman et al. 2009).

The genus *Origanum* included in the Lamiaceae family is represented by 22 species and 32 taxa in Turkey. 21 of these species are endemic and the endemism rate of the genus is 65.2% (Aydin et al. 1998, Dundar et al. 2008). The torrid and temperate southwest Eurasia and Mediterranean regions have the suitable climatic conditions for the *Origanum* species to grow (Kokkini 1996). Thyme has a primary role among the spices used in kitchens in the world trade. It is produced in France, Greece, Spain and Turkey in Europe and Mexico and Chile, and Peru in South America (Barreyro et al. 2005, Olivier 1996). Turkey also exports many thyme species and these species are *O. onites*, *O. minutiflorum*, *O. majorana*, *O. syriacum* var. *bevanii* and *O. vulgare* (Baser et al. 1993, Kirimer et al. 2003, Toncer et al. 2009). Among these species, the most exported species is *O. onites* in the world (Yaldız et al. 2005).

Essential oils are an alternative potential source against the pathogen organisms causing infection due to the

antimicrobial substances. It is accepted throughout the world that the herbal products are less toxic effect for environment and human health in the control of diseases (Lee et al. 2007). It has been reported that especially essential oils show effective antifungal activities against various fungal pathogens under both *in vitro* and *in vivo* conditions (Baruah et al. 1996). It has been reported that the *Origanum* essential oil has anti-bacterial, anti-oxidant, anti-fungal cytotoxic and insecticidal activity (Muller et al. 1995, Tepe et al. 2004, Traboulsi et al. 2002, Wilson et al. 1997, Vagi et al. 2005). Also in a similar study, it has been reported that the members of the Lamiaceae family have antifungal activity on *Verticillium dahliae* pathogen (Rus et al. 2015). It has been reported that *Origanum* species have biological activity (García-Beltrán and Esteban 2015) and *O. onites* has bio-herbicidal activity on five wheat genotypes and weeds (Atak et al. 2016).

The aim of this study was to investigate the chemical composition and herbicidal activity of *Origanum onites* plant essential oil on *Amaranthus retroflexus*, *Triticum aestivum*, and *Lepidium sativum* seed germination and root-shoot growth.

MATERIALS AND METHODS

Plant material and extraction of essential oils

O. onites plants were collected during the flowering stage in vegetation period of 2017 in Mersin province. Essential oils were obtained from the plants dried in shade by using the Neo-Clevenger apparatus and hydro-distillation method. The obtained essential oils were kept in dark bottle at 4 °C until the activity studies.

The effect of essential oils on the seed germination and seedling development of the test plants

The studies investigating the effect of the plant essential oil on the seed germination of the test plants were conducted in the sterile Petri dishes with 9 cm diameter. The seeds were homogeneously placed into the sterile Petri dishes with 2-layer filter paper and the filter papers were humidified properly using distilled water. As the water solubility of the essential oils is low, it was used in the form of gas in the test. For this purpose, a piece of blotting paper was glued from their corners to the lids of the sterile Petri dishes and then the essential oils were dripped on this piece of paper using a micropipette and the lids of the up parts Petri dishes were closed immediately and wrapped tightly by the Parafilm (Önen et al. 2002). In the test, the essential oils were applied in 0 (control), 0.5, 1, 1.5, 5, 15 µl/Petri doses. The Petri dishes were placed to incubation at 24±1 °C under 12 h light and 12 h dark conditions for 1-4 weeks (Kadioglu 2004, Kordali

et al. 2009, Sadeghi et al. 2010). At the end of the period, the germination rate (%) and root and shoot length (cm) were determined. The experiments were carried out as 4 replicates and repeated 2 times.

Gas chromatography and gas chromatography-mass spectrometry (GC-MS)

20 mg of essential oil was dissolved in acetone of 1.2 ml and it was made prepared for the analysis. The analysis was performed with BPX5 (0.25 mm ID, film thickness 0.25 μ m) 30 m capillary column and Perkin Elmer Clarus 500 GC-MS. While the injection volume was determined as 2 μ l, the injection port temperature was determined as 250 °C. As the carrier gas, helium was used with a 50:1 split rate and 1 ml/minute flow rate. In the oven program, the oven was increased up to 100 °C with a 5 °C/min heating rate, at the starting temperature of 50 °C and it was kept for 2 min at this temperature. Then, it was increased to 220 °C with a 3 °C/min heating rate and was kept for 2 min at this temperature. The total program period was determined as 30 min. MS

parameters; ionizer: EI, ionizing energy: 70 eV; and ion source transfer temperature was set to 250 °C.

The compounds were clarified by comparing the retention times of the current standard compounds in the column with the retention time of the compounds of the samples (co-injection), comparing the Kovats index or retention index (RI) values given in the literature and comparing the specific mass spectra of the compounds with the data (NIST, Willey and Pfleger) in the MS libraries present in the digital environment. And the relative percentages of the compounds in the essential oil were calculated with Turbomass ver 5.4.2 software, by multiplying the ratio of peak areas of each compound to total peak area by 100.

Statistical analysis

The analysis of variance (ANOVA) was used to determine the significant of differences between experiment treatments, and averages were compared using the DUNCAN test. Statistical analyses were carried out using the SPSS 15.0 software.

Table 1. The chemical composition of the *Origanum onites* essential oil (GC/MS)

Compound number	RT* (min)	RI**	%	Name
1	11.454	896	0.31	2-Thujene
2	11.734	906	0.13	α -Pinene
3	12.914	946	0.20	1-Octen-3-ol
4	13.308	958	0.43	β -Pinene
5	13.918	977	0.08	α -Phellandrene
6	14.309	988	1.22	α -Terpinene
7	14.576	996	8.83	p-Cymene
8	15.212	1016	0.10	3-Carene
9	15.726	1031	17.08	γ -Terpinene
10	16.059	1041	0.37	4-Thujanol
11	17.000	1068	0.56	Linalool
12	17.146	1072	0.20	p-Mentha-2-en-1-ol
13	19.593	1143	0.44	Borneol
14	19.936	1153	0.28	4-Terpineol
15	20.135	1158	0.15	p-Cymene-8-ol
16	20.369	1165	0.10	α -Terpineol
17	23.366	1252	0.15	Phenol, 2,3,5,6-tetramethyl
18	23.593	1259	2.77	Thymol
19	24.072	1272	59.87	Carvacrol
20	28.155	1395	4.98	Caryophyllene
21	29.206	1430	0.22	Humulene
22	30.480	1471	0.43	γ -Elemene
23	32.941	1553	0.20	Spathulenol
24	33.158	1561	0.91	Caryophyllene oxide
Total			100	

*RT:Retention time,** RI:Retention index

RESULTS AND DISCUSSION

Tables 1, 2, 3, and 4 and Figures 1, 2, and 3 show the results concerning chemical composition of *O. onites* essential oil and its effect on seed germination and root-shoot growth of *A. retroflexus*, *T. aestivum*, and *L. sativum*.

According to the results of GC/MS, a total of 24 compounds, including carvacrol (59.87%), γ -terpinene (17.08%), and p-cymene (8.83%) as the main compounds, were identified in *O. onites* essential oil (Table 1). The composition of the essential oil compound of the plants may vary based on the location where they are collected. In the previous studies, it was determined that carvacrol was 77.2% and p-cymene was 10.9% in Balikesir (Kazdağı) sample of *O. onites* essential oil and linalool was 90.9% and carvacrol was 1.8% in the Antalya (Beycik) sample (Baser et al. 1993). In the other studies conducted on *O. onites* essential oil, 12 compounds, as linalool (50.53%) being the main component, were identified (Özkan and Erdoğan 2011); and 53 components together with carvacrol (79.2%) and thymol (4.4%) were identified

(Altintas et al. 2013). Also, it was (Copur et al. 2010) determined carvacrol (57.01%) as the principal component in *O. onites* essential oil. The main components of the seven samples of *O. onites* essential oil collected in Greek island of Ikaria were determined as carvacrol (72.25-89.22), p-cymene (1.43-6.00), and γ -terpinene (1.37-6.51) (Economou et al. 2011). *O. onites* essential oils collected from different locations are different the main components and number of components. The factors such as environmental conditions, development period, harvest season, soil conditions and the obtainment of the essential oil are effective on secondary metabolites in plants (Toncer et al. 2009).

The essential oils have different effects on plant developments such as seed germination (Barney et al. 2005). The *O. onites* essential oil had effects of different levels on the seed germination and the root and shoot growths in the test plants. The essential oil *A. retroflexus* completely inhibited the seed germination, root and shoot development in the doses of 1 μ l compared to the control (Table 2, Figure 1).

Table 2. The effect of *Origanum onites* essential oil on seed germination and growth of *Amaranthus retroflexus*

Doses (μ l)	GR* (%)	RL** (mm)	SL*** (mm)
Control	70.66a \pm 5.81	8.99a \pm 0.69	8.81a \pm 0.80
0.5	13.33b \pm 3.52	2.07b \pm 0.24	1.9b \pm 0.08
1	0.00c \pm 0.0	0.00c \pm 0.0	0.00c \pm 0.0
1.5	0.00c \pm 0.0	0.00c \pm 0.0	0.00c \pm 0.0
5	0.00c \pm 0.0	0.00c \pm 0.0	0.00c \pm 0.0
15	0.00c \pm 0.0	0.00c \pm 0.0	0.00c \pm 0.0

*GR:Germination; **RL:Root length; ***SL:Shoot length *The means with different letters in the same column are different at the significance level of $p<0.05$ according to DUNCAN

Figure 1. The inhibition rate of *Origanum onites* essential oil on seed germination and growth of *Amaranthus retroflexus* (GR:Germination; RL:Root length; SL:Shoot length)

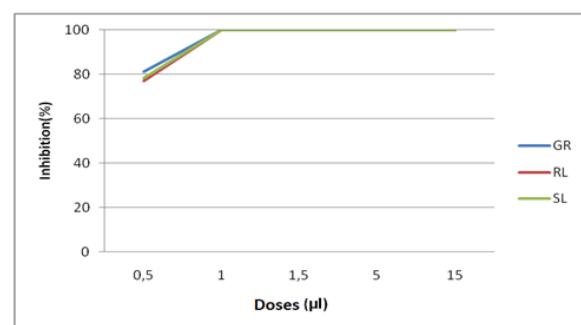


Table 3. The effect of *Origanum onites* essential oil on seed germination and growth of *Triticum aestivum*

Doses (μ l)	GR (%)	RL (mm)	SL (mm)
Control	100.0a \pm 0.0	86.13a \pm 8.13	60.46a \pm 6.22
0.5	96.0a \pm 2.30	56.57b \pm 0.30	37.75b \pm 0.68
1	78.66b \pm 11.39	53.48b \pm 0.36	36.29b \pm 0.49
1.5	76.00b \pm 2.30	40.51c \pm 0.31	33.13b \pm 0.51
5	24.00c \pm 6.11	3.44d \pm 0.03	10.19c \pm 0.54
15	0.00d \pm 0.0	0.00d \pm 0.0	0.00d \pm 0.0

GR:Germination; RL:Root length; SL:Shoot length *The means with different letters in the same column are different at the significance level of $p<0.05$ according to DUNCAN

Figure 2. The inhibition rate of *Origanum onites* essential oil on seed germination and growth of *Triticum aestivum* (GR:Germination; RL:Root length; SL:Shoot length)

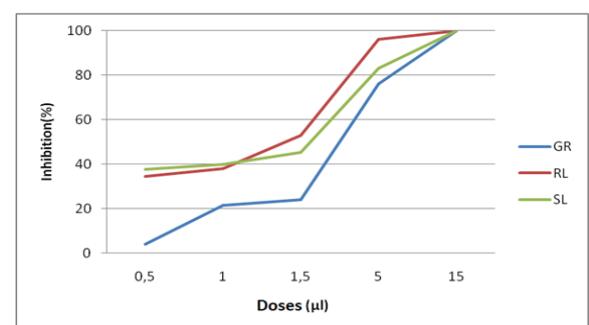
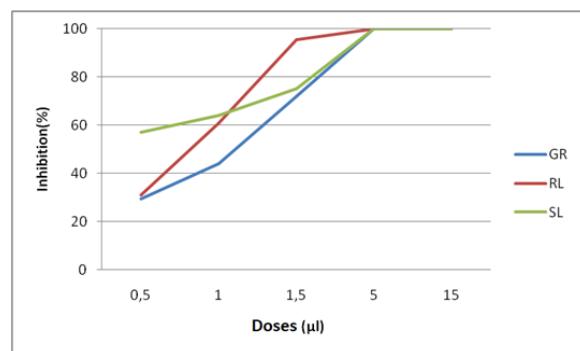


Table 4. The effect of *Origanum onites* essential oil on seed germination and growth of *Lepidium sativum*

Doses (μl)	GR (%)	RL (mm)	SL (mm)
Control	100.0a [*] ±0.0	97.45a±8.65	14.09a±1.45
0.5	70.66b±10.66	67.03b±0.03	6.06b±0.0
1	56.0b±4.0	38.04c±0.03	5.07b±0.02
1.5	28.0c±8.32	4.44d±0.02	3.49cb±0.0
5	0.0d±0.0	0.0d±0.0	0.0d±0.0
15	0.0d±0.0	0.0d±0.0	0.0d±0.0

GR:Germination; RL:Root length; SL:Shoot length *The means with different letters in the same column are different at the significance level of $p<0.05$ according to DUNCAN test

Figure 3. The inhibition rate of *Origanum onites* essential oil on seed germination and growth of *Lepidium sativum* (GR:Germination; RL:Root length; SL:Shoot length)

O. onites essential oil was more effective against weeds and the phytotoxic effect occurred in cultivated plants in high doses. Essential oils may have effects in different levels on different plants and even on the genotypes of the same species (Atak et al. 2016, Erbaş et al. 2015).

Origanum species have a rich biological activity such as antibacterial, antifungal, antioxidant, anti-inflammatory, antitumor, antiviral and antiparasitic activities (García-Beltrán and Esteban 2015). Also, it has been reported that *Origanum onites* essential oil has allelopathic activity on the seed germination, plant height, root length and root number of *Onobrychis viciifolia* under *in vitro* conditions (Altindal and Altindal 2013). *O. onites* essential oil has had a high inhibiting effect on the seed germinations of *A. retroflexus*, *Centaurea solstitialis*, *Sinapis arvensis*, *Sonchus oleraceus*, *Raphanus raphanistrum* and *Rumex nepalensis* (Azirak and Karaman 2008). In the studies conducted on the other plant species, it has been reported that *A. vulgaris* essential oil affects *Lepidium sativum* seed germination and growth negatively (Barney et al. 2005) and monoterpenes hydrocarbons and

oxygenated monoterpenes have an allelopathic effect on *A. retroflexus* seed germination and seedling development (Kordali et al. 2007).

It has been reported in the previous applications that different allelochemicals and their different doses show different reactions (Atak et al. 2016). It has been revealed that some essential oils have an inhibiting effect on the wheat seed generation (Dudai et al. 1999) and *Lavandula x hybrida* Rev essential oil has different effects on cereal products (Gitsopoulus et al. 2013). Also in the present study, it was revealed that *O. onites* essential oil had an allelopathic effect depending on dose and test plant. This result is similarly with the ongoing studies.

The present study revealed that *O. onites* essential oil had a phytotoxic effect on seed germination and root and shoot lengths of *A. retroflexus*, *T. aestivum*, and *L. sativum* in Petri dishes conditions. Today, it has been revealed that the herbicides, that have the highest usage rate in pesticides used against the plant diseases and insects in the agricultural productions, have negative effects on the environment, human and the non-targeted plants/other organisms. Therefore, the results of this study and similar studies have become more important. It is important that the results of this study are utilizable in the agricultural field.

ÖZET

Bu çalışmada, *Origanum onites* L. bitkisinin toprak üstü (sürgün+yatprak+çicek) aksamından elde edilen uçucu yağın kimyasal bileşenleri ve farklı bitkilerin çimlenme ve fide gelişimine olan allelopatik etkileri araştırılmıştır. Bu amaçla Mersin ilinden toplanan *O. onites* bitkisinden hidro-distilasyon yöntemi kullanılarak uçucu yağ elde edilmiştir. GC/MS analizi sonucunda *O. onites* uçucu yağında carvacrol (%59.87), γ -terpinene (%17.08) ve β -cymene (%8.83) temel bileşenler olmak üzere 24 bileşen belirlenmiştir. Allelopatik etki 9 cm çaplı Petri kaplarında yürütülmüştür. Petri kaplarına 2 kat halinde kurutma kağıdı yerleştirilmiş ve *Amaranthus retroflexus* L., *Lepidium sativum* L., *Triticum aestivum* L. tohumları homojen olarak dağıtılmıştır. Kurutma kağıtları distile su kullanılarak iyice nemlendirilmiştir. Her bir Petri kabının kapağının merkezine zamka yapıştırılmış kurutma kağısına uçucu yağlar farklı konsantrasyonlarda (0, 0.5, 1, 1.5, 5, 15 μ l/Petri) bir mikropipet yardımcı ile damlatılarak, Petri kapları örtüerek Parafilm ile sıkıca sarılmıştır. Petri kapları 12 saat aydınlatık-12 saat karanlık ve ortalama 24 ± 1 °C koşullarda 1-4 hafta boyunca inkübasyona bırakılmıştır. Sürenin sonunda çimlenme oranı, kök ve sürgün boyları belirlenmiştir. *O. onites* uçucu yağı *A. retroflexus* L., *L. sativum* L., *T. aestivum* L. bitkilerinin tohum çimlenmesi ile kök ve

sürgün gelişimini 15 μ l/Petri dozda tamamen engellemiştir. Uçucu yağı karşı *A. retroflexus* L.'un daha hassas olduğu tespit edilmiştir. Elde edilen sonuçlara göre, *O. onites* uçucu yağıının yüksek seviyede *L. sativum* L., *T. aestivum* L., *A. retroflexus* L. tohum çimlenmesi ve fide gelişimleri üzerine allelopatik etki gösterdiği ve *A. retroflexus* L. kontrolünde kullanılabılır potansiyele sahip olduğu belirlenmiştir.

Anahtar kelimeler: allelopatik etki, *Origanum onites*, uçucu yağı, tohum çimlenmesi

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