



Antibacterial Activity of Essential Oil and Extract of *Origanum onites* L. Against Bacterial Speck of Tomato and Tomato Bacterial Wilt Disease

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Abstract: This study was carried out to determine antibacterial activity of *Origanum onites* L., essential oil and ethanol extract, against *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). Plant material was collected from Tokat province in 2016. The study was carried under *in vitro* conditions and used King B medium. At concentrations, 10, 20, 30, 40 and 50% of the essential oil of *O. onites* was applied with method filter paper impregnation. The extract of *O. onites* was mixed with autoclaved King B medium to obtain final concentrations 1, 2, 3, 4 and 8%. And then 10⁶ cell/ml of *Pst* and *Cmm* suspensions were placed on the medium containing the essential oil and extract. The media without essential oil and extract were used as the negative controls. Based on the results of the study, increase in the essential oil and extract concentration resulted in increase of the efficacy on pathogens. At concentration, of 50% essential oil of *O. onites* inhibited the growth of *Pst* and *Cmm* at a rate of 73% and 93%, respectively. At concentration of 8% extract of *O. onites* inhibited the growth of *Pst* and *Cmm* at a rate of 97% and 99%, respectively. In conclusion, it has been observed *O. onites* extract has higher antibacterial activity than essential oil.

Keywords: Essential oil, Plant extract, *Origanum onites*, Antibacterial activity, *Pseudomonas syringae* pv. *tomato*, *Clavibacter michiganensis* subsp. *michiganensis*

Domates Bakteriyel Benek ve Domates Bakteriyel Solgunluk Hastalıklarına Karşı *Origanum onites* L.'in Antibakteriyel Etkisi

Özet: Bu çalışma, *Origanum onites* L.'in uçucu yağ ve etanol ekstraktının *Pseudomonas syringae* pv. *tomato* (*Pst*) ve *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) hastalık etmenlerine karşı antibakteriyel etkisini belirlemek amacıyla yürütülmüştür. Bitki materyali 2016 yılında Tokat ilinden toplanmıştır. *In vitro* koşullar altında yürütülen çalışmada King B besi yeri kullanılmıştır. *O. onites*'in %10, 20, 30, 40 ve 50'lik uçucu yağ konsantrasyonları filtre kağına emdirme yöntemi kullanılarak uygulanmıştır. Son konsantrasyon %1, 2, 3, 4 ve 8 olacak şekilde *O. onites* etanol ekstraktı otoklav edilmiş King B besi yerine karıştırılmıştır. Daha sonra 10⁶ hücre/ml oranında hazırlanan *Pst* ve *Cmm* süspansiyonu uçucu yağ ve ekstrakt içeren besi yerlerine ekilmiştir. Uçucu yağ ve ekstraktın olmadığı besi yerleri negatif kontrol olarak kullanılmıştır. Çalışma sonuçlarına göre, uçucu yağ ve ekstrakt konsantrasyonundaki artışa bağlı olarak *O. onites*'in patojenler üzerindeki etkisi de artmıştır. %50'lik *O. onites* uçucu yağ konsantrasyonu *Pst* ve *Cmm* gelişimini sırasıyla %73 ve %93 oranında engellemiştir. %8 konsantrasyondaki *O. onites* etanol ekstraktı ise *Pst* ve *Cmm* gelişimini sırasıyla %97 ve %99 oranında engellemiştir. Sonuç olarak, *O. onites* ekstraktının patojenler üzerindeki antibakteriyel etkisi, uçucu yağın etkisinden daha yüksek olarak belirlenmiştir.

Anahtar Kelimeler: Uçucu yağ, Bitki ekstraktı, *Origanum onites*, Antibakteriyel etki, *Pseudomonas syringae* pv. *tomato*, *Clavibacter michiganensis* subsp. *michiganensis*

1. Introduction

One of the major problems in agriculture today is due to pathogenic microorganisms by this way the quantity and quality of the crop products. *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) and *Pseudomonas syringae* pv. *tomato* (*Pst*) are important bacterial pathogens of tomato and cause economic losses in Turkey and world (Smitley and Mc Carter, 1982; Sherf and Macnab, 1986; Gleason *et al.*, 1993; Ricker and Riedel, 1993; Aysan *et al.*, 2005; EPPO, 2017; Aysan and Saygılı, 2008; Çetinkaya-Yıldız and Aysan, 2008). Tomato bacterial speck disease (*Pseudomonas syringae* pv. *tomato*) causes local infection (Jones *et al.*, 1991) while tomato bacterial wilt disease (*Clavibacter michiganensis* subsp. *michiganensis*) causes systemic infection on the plant (Gleason *et al.*, 1993).

Control of bacterial diseases in culture plants is difficult. In generally producers have used chemicals for plant diseases but most of the chemicals used causes several negative effects such as development of pathogenic resistance, residue problems in foodstuffs and their non-target environmental impacts (Beever and Brien, 1983; Yiğit, 1993; Baroffio *et al.*, 2003; Milijasevic *et al.*, 2009, Sheikh *et al.*, 2013).

Using of plant extract and essential oils for control of plant diseases is attract attention of investigators recently. Lamiaceae family is initial array among using plants with this objectives. The family includes many aromatic plants used spice, medicinal drugs and as a parfume. There are 200 species and 3.300 plant species in this family. The genus *Origanum*, which is semi-shrub or shrub herb that is native to the Mediterranean, Euro-Siberian and Irano-Siberian regions perennial plant, and shrubby, is included in Lamiaceae and (Aligiannis *et al.*, 2001). This plant species are recognized in the World. Most of the *Origanum* species (over 75%), are concentrated in the East Mediterranean subregion (Ietswaart, 1980). Of them, 16 species are considered as endemic for the flora of Turkey and they are composed 38

kinds and 17 hybrids (Güner *et al.*, 2000). In Turkey, 21 *Origanum* species grow, naturally. These species are used in various commercial preparations, mainly as antimicrobial and antioxidant agents (Cosentino *et al.*, 1999; Aligiannis *et al.*, 2001; Baydar *et al.*, 2004).

Oregano is one of the most potent essential oils and has been used for centuries in traditional health practices for its cleansing, as an antiviral, antibacterial, antifungal, herbicidal, antiparasitic, antioxidant, anti-inflammatory, digestive, emenagogue and anti-allergenic (Yılar *et al.*, 2013; Onaran *et al.*, 2014) substance and immune-boosting properties. Oregano essential oil was first recognized in ancient Greece. They were used for treating bacterial infections on the skin or in wounds, and it was also employed to protect food from bacteria. Its constituents are Carvacrol, Thymol, Cymene, Caryophyllene, Pinene, Bisabolene, Linalool, Borneol, Geranyl Acetate (Erdoğan *et al.*, 2012; Anonym, 2017). In addition *Origanum* species are reported to posses antibacterial activity (Ben *et al.*, 2001; Farooqi and Sreeramu, 2004). For example, Daferera *et al.*, (2003) were reported essential oils obtained from plant species of *Origanum vulgare*, *O. dictamnus*, *O. majorana*, *Thymus vulgaris*, *Lavandula angustifolia*, *Rosmarinus officinalis*, *Salvia fruticosa* effects of against, *Botrytis cinerea*, *Fusarium* sp. and *Cmm*. They have found that prevent to development this bacteria.

This study was carried out to determine antibacterial activity of *Origanum onites* L., essential oil and extract, against *Pseudomonas syringae* pv. *tomato* and *Clavibacter michiganensis* subsp. *michiganensis*.

2. Materials and Methods

2.1. Plant material

Origanum onites L. was collected from Tokat Gaziosmanpasa University land in Tokat in Turkey in June-September 2016. *Oregano* plants during the flowering period were dried ten days in a room avoiding the sun. Then dried flower,

leaves, stem and root was grinding and they were put the polyethylene bags to keep in dark.

2.2. Bacterial cultures

Bacterial isolates (*Pseudomonas syringae* pv. *tomato* and *Clavibacter michiganensis* subsp. *michiganensis*) were obtained from culture collection at Department of Plant Protection, Agricultural Faculty, Tokat Gaziosmanpaşa University, Tokat, (Turkey). The bacterial isolates were maintained at 4°C in Nutrient Broth and Glycerol.

2.3. Plant Extract

The air-dried plants of *O. onites* (100 g) were extracted three times with 95% ethanol at room temperature. The volume of 95% ethanol used in each extraction was 500 ml. The extract was concentrated under reduced pressure by a vacuum rotary evaporator to yield an ethanol extract (Gökçe *et al.*, 2007).

2.4. Extraction of essential oils

Plant materials were dried in the shade at room temperature for 10 days. The air-dried flower parts (300 g) of *O. onites* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na₂SO₄ and preserved in a sealed vial at 4 °C until further analysis (Telci *et al.*, 2006).

2.5. Antibacterial Activity of Extract

This study was carried *in vitro* and King B medium used in the study. The extract of *O. onites* was mixed with autoclaved King B medium to obtain final concentrations of 1%, 2%, 3%, 4% and 8%. The amount of 10⁶ cell/ml of *Pst* and *Cmm* suspension was placed on the medium containing the essential oil and extract. The media without extract were used as the negative controls. Three replicates were used for each treatment. King B plates were then incubated at 28 °C.

Colony density of the bacterium was measured at the end of two days incubation periods for *Pst* and at the end of three days for *Cmm*.

2.6. Antibacterial Activity of Essential oil

10%, 20%, 30%, 40% and 50% of the essential oil of *O. onites* was applied with filter paper impregnation method. The amount of 10⁶ cell/ml of *Pst* and *Cmm* suspension was plated on the medium containing the essential oil and extract. The media without essential oil were used as the negative controls. Three replicates were used for each treatment. King B plates were then incubated at 28 °C. Colony density of the bacterium was measured at the end of two days incubation periods for *Pst* and at the end of three days for *Cmm*.

$$E = (K - M / K) \times 100$$

E= inhibition rate (%)

K= density of bacteria in control petri dish

M= density of bacteria in treatment petri dish

2.7. Statistical Analysis

The results were analyzed using an Analysis of Variance (One-Way ANOVA), and the comparisons between the means were performed using the multiple comparison test of Tukey, with a significance level of 5% (P ≤ 0.05). The statistical program SPSS was used.

3. Results and Discussion

Based on the results of the study, increase in extract concentration resulted in increase of the extract efficacy. *Origanum onites* extract at 8% concentration inhibited the growths of *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) at the rates of 97,03% and 99,65% respectively. 1% extract concentration was not effective on the both pathogens (Table 1). And other concentrations had the lowest effect on the growth *Pst* and *Cmm*.

Table 1. Antibacterial activity of ethanol extract of *Origanum onites*
Çizelge 1. *Origanum onites* ethanol ekstraktının antimikrobiyal aktivitesi

Concentration (ml/l)	Inhibition rates (%)	
	<i>Cmm</i>	<i>Pst</i>
%1	15,80±5,58a ^a	07,97±0,06a
%2	37,14±5,81b	17,21±4,77ab
%3	38,74±4,73b	25,64±4,19b
%4	99,53±0,11c	32,38±3,37b
%8	99,65±0,09c	97,03± 1,87c

Inhibition rate±Standard deviation. *Cmm*=*Clavibacter michiganensis* subsp. *michiganensis*, *Pst*=*Pseudomonas syringae* pv. *tomato*,
^aThe means in the same column indicated with different letters are significantly different. (P<0.05).

Inhibited growth of essential oil on *Cmm* and *Pst* are shown in Table 2. After the treatment, the greatest effect was at 50%,

which caused 93,25% and 73,11 % inhibited respectively and was significantly different from the other concentrations ($p < 0.05$).

Table 2. Antibacterial activity of essential oil of *Origanum onites*
Çizelge 2. *Origanum onites* uçucu yağının antimikrobiyal aktivitesi

Concentration (ml/l)	Inhibition rates (%)	
	<i>Cmm</i>	<i>Pst</i>
%10	36,74±6,08a	30,69±0,60a
%20	65,01±5,14b	35,20±2,68a
%30	73,93±8,33bc	48,31±2,15b
%40	89,41±2,32bc	52,32±2,42b
%50	93,25±3,24c	73,11± 2,34c

Inhibition rate±Standard deviation. *Cmm*=*Clavibacter michiganensis* subsp. *michiganensis*, *Pst*=*Pseudomonas syringae* pv. *tomato*,
^aThe means in the same column indicated with different letters are significantly different. (P<0.05).

Similarly, increase in essential oil concentration resulted in increase of the efficacy, but *O. onites* essential oil was the more effective than *O. onites* extract. On the growth *Cmm*, 10% concentration was not effective, but other concentrations had effect at the rate of 65-93%. On the growth of *Pst*, 50% concentration was effective, merely.

Plant extracts and essential oils of the development of disease in the bodies of the suppression effect arises from factors such as phenols because of they contain secondary metabolites (Yonucu, 1997). "Carvacrol", is comed into prominence as essential component to obtain *Origanum*, *Satureja*, *Thymbra*, *Thymus*

and *Corydothymus* species, is determined act as antifungal, antibacterial, insecticidal, antihelmintic, analgesic, antioxidant.

Some studies have shown that antimicrobial effect of essential oils of *Origanum* species is related to its content the high amount of carvacrol (Baydar *et al.*, 2004, (Panizi *et al.*, 1993; Mullerriebau *et al.*, 1995; Sivropoulou *et al.*, 1996; Aliannis *et al.*, 2001).

Similarly, in another study, *Thymbra spicata* subsp. *spicata*, *Origanum syriacum* var. *beanii*, *Mentha spicata* and *Lavandula stoechas* subsp. *stoechas* essential oils were used against *Cmm*. It has been found most effect essential oil was *Thymbra spicata* subsp. *spicata* on *Cmm* (Soylu

et al., 2007). According to Yanar *et al.* (2016) investigated antimicrobial effect of *Origanum* spp., *Mentha* spp. and *Lippia* sp. of essential oils against the *Cmm* and *Botrytis cinerea*. They were obtained 0.2, 0.3, 0.4 concentrations of *O. vulgare* and *L. citriodora* species and 0.5 µl / ml, 0.3, 0.4 and 0.5 µl / ml concentrations of *O. syriacum* and *O. onites*, completely inhibited the development of *Cmm* respectively. In the second part of the study, concentration measurement tests were performed with *O. vulgare*, *O. syriacum*, *O. onites*, and *L. citriodora* essential oils. It was observed LC50 values for *Cmm* of these essential oils were 0.163, 0.122, 0.122 and 0.07 µl / ml, respectively.

4. Conclusion

In this study, it was determined that *Origanum onites* L. extract and essential oil have different proportion antibacterial activities on the some plant pathogens. Particularly, the extract have the more effective than essential oil. These studies were conducted to an inhibition activity of carvacrol depending on factors such as, the area geographic and concentration of the collected plant. In addition, plant content composition varies from region to region and therefore, the difference in the composition of the extract may lead to the inhibition of a difference in percentage from plant extracted from one area to another (Shehu *et al.*, 2015).

Determined effects of *Origanum onites* essential oil in our study are similar with those from the literature (Sarer, 1996; Tabanca *et al.*, 2001; Demirci *et al.*, 2002). Antimicrobial activity of these essential oils seems to be associated with phenolic compounds. Many compounds purified from essential oils such as carvacrol, eugenol and thymol inhibit the growth of a variety of microorganism (Bagamboula *et al.*, 2004).

As a result of, it was found that both the plant extract and the essential oil of *Origanum onites* had different inhibitory effects on antibacterial activity of *Cmm* and *Pst*. This study should also be tested in field conditions. Fractionation and characterization of these

compounds will be bioalternatives by agricultural science in the future.

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