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Exploring the Antifungal Efficacy of Essential Oils against Alternaria solani, the Causative Pathogen of Early Leaf Blight in Tomato Plants

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Alternaria solani, Antifungal, Early blight disease, Essential oils, Tomato

Abstract

Alternaria solani is an important necrotrophic fungus that is responsible for the early blight disease which is a common disease in tomato fields. In this research, the antifungal activity of essential oils extracted from Satureja hortensis L., Thymbra spicata L., Thymus sipyleus Boiss, Origanum majorana L., O. syriacium L., O. onites L., O. vulgare L., Artemisia absinthium L., A. santonicum L. and A. spicigera C. koch were tested against Alternaria solani the causative agent of tomato early blight disease. The study was carried out in two different ways, in vitro and in vivo. In vitro studies were conducted on media mixed with essential oils, and different concentrations of oils were examined on fungal growth in petri dishes. In in vivo studies, the effects of essential oils on the development of disease lesions were evaluated by infecting healthy tomato fruits. The concentrations used in the study were 140, 280, 560 and 1000 µl/L in the in vitro and 2.5, 5, 10 and 20 µl/fruit under in vivo conditions. Among the in vitro trials, the essential oils extracted from Thymus sipyleus and O. onites exhibited the most pronounced antifungal efficacy, achieving complete growth inhibition of the fungal pathogen across all concentrations whereas the essential oils from Artemisia spicigera and A. santonicum dis-played relatively lower efficacy compared to the other oils. Conversely, under in vivo condi-tions, the essential oils derived from O. majorana and A. absinthium demonstrated the great-est capacity to impede the development of disease lesions on the fruit, yielding inhibition rates of 84% and 64% at 2.5 µl per fruit concentrations, respectively. In contrast the essential oils from Artemisia spicigera and A. santonicum had the least effect on the development of the disease, as there was disease lesions appeared even on the fruits that were applied at 20µl/fruit doses. Although most of the essential oils had some degree of antifungal action, still much effort is needed to put on both in vitro and in vivo trials to strength the reliability and con-sistency of the future related researches.

Uçucu Yağların Domates Bitkilerinde Erken Yaprak Yanıklığına Neden Olan Patojen Alternaria solani'ye Karşı Antifungal Etkinliğinin Araştırılması

Anahtar Kelimeler

Alternaria solani, Antifungal, Domates, Erken yanıklık hastalığı, Uçucu yağlar

Özet

Alternaria solani, domates tarlalarında yaygın olarak görülen erken solgunluk hastalığının sorumlusu olan önemli bir nekrotrofik fungus türüdür. Bu araştırmada, Satureja hortensis L, Thymbra spicata L, Thymus sipyleus Boiss, Origanum majorana L, O. syriacium L, O. onites L, O. vulgare L, Artemisia absinthium L, A. santonicum L. ve A. spicigera C. koch bitkilerin-den elde edilen uçucu yağların Alternaria solani'ye karşı antifungal aktivitesi test edilmiştir. Çalışma iki farklı şekilde invitro ve in vivo olarak gerçekleştirilmiştir. İn vitro çalışmalarında uçucu yağlarla karıştırılmış besi yerleri üzerinde yapılmıştır ve yağların farklı konsantrasyonu petri kaplarında büyütülmüş fungusların üzerine bakılmıştır. İn vivo çalışmalarda ise sağlıklı domates meyveleri üzerine hastalık bulaştırarak uçucu yağların hastalık lezyonların gelişmesini etkileri değerlendirilmiştir. Çalışmada kullanılan konsantrasyonlar in vitro koşullarda 140, 280, 560 ve 1000 µl/L, in vivo koşullarda ise 2.5, 5, 10 ve 20 µl/meyve olarak belirlenmiştir. İn vitro deneyler arasında Thymus sipyleus ve O. onites'ten elde edilen uçucu yağlar, tüm konsantra-syonlarda fungal patojenin tamamen büyümesini engelleyen en belirgin antifungal etkinliği sergilemiştir. Artemisia spicigera ve A. santonicum'un uçucu yağları diğer yağlara göre daha düşük etkinlik göstermiştir. Öte yandan, in vivo koşullarda O. majorana ve A. absinthium'dan elde edilen uçucu yağlar, meyve üzerinde hastalık lezyonlarının gelişimini en çok engelleyenler olmuş ve 2.5 µl/meyve konsantrasyonunda sırasıyla %84 ve %64 inhibitasyon oranlarına neden olmuştur. Buna karşılık, Artemisia spicigera ve A. santonicum'un uçucu yağları has-talığın gelişimine en az etki etmiş ve 20 µl/meyve dozunda bile meyvelerde hastalık lezyonları görülmüştür. Uçucu yağların çoğu bir dereceye kadar antifungal etkiye sahip olsa da, gelecekle ilgili araştırmaların güvenilirliğini ve tutarlılığını artırmak için hem in vitro hem de in vivo denemelere daha fazla çaba harcanması gerekmektedir.

1. INTRODUCTİON

Tomato plant is considered the second largest produced and consumed crop in the world after potatoes. Due to its value and production volume, it has high economic position in the global agriculture sector (Heuvelink 1996, Anonim 2013). With the abiotic factors that affect the yield and quality of tomatoes there are also bacterial, phytoplazma, fungal and viral dis-eases. Alternaria solani (Ell. & Mart.) Jones and Grout. Is the responsible pathogen of early blight disease in tomato. It is an important necrotrophic fungus and it is a common disease of tomatoes grown in areas with frequent rain, high humidity and dense dew (Agrios 2005). Un-der favorable conditions, the disease develops in the leaves, stem, branches and fruits, and causes leaf shedding, early drying of branches and fruits which results yield loss of up to %50-86 (Mathur and Shekhawat 1986).

It is very difficult to manage early blight disease of tomato using cultural practices (Smith and Kotcon 2002), therefore this makes use chemical control unavoidable. However fungal patho-gen's resistance to fungicides (Herriot et al. 1986) and the fungicide's negative effect on the environment and human health encourages seeking alternative methods to control the disease both in the fields and storage houses and one of these methods is the use of plant based pesti-cides (Abeysinghe 2009, Jegathambigai et al. 2010). Essential oils, which originate from or-ganic hydrocarpons (oxygenated compounds, sesquiterpenes, and monoterpenes) that are re-sponsible for their antimicrobial effect, have lipophilic properties (Regnault-Roger et al. 2012, Üstüner et al., 2018a) and they have been employed in various scientific and commercial ap-plications for an extended period (Kara 2020). Compounds in the essential oils with the anti-microbial properties cause antimicrobial effect by losing the energy of the microbial cell (Feng and Zheng 2007, Nerio et al. 2010, Tian et al. 2012). Due to these effects, essential oils are used in common in folk medicine, food preservation and pharmaceutical industries (Kumar et al. 2008).

Significant increment of the researches in plant based essential oils and extracts which are aimed to develop highly secure antifungal products that can be used in the control of plant pathogens has been seen made in the last 2 decades (Duke 1990, Simmonds et al. 1992, Ben-ner 1993, Gorris and Smid 1995, Isman 2000, Ustuner et al., 2019). Until today, many plant species that are rich in secondary metabolites such as tannins, terpenoids, alkaloids and flavo-noids have been reported to have antimicrobial properties (Cowan 1999, Lyr et al. 1999, Law-less 2002, Pretorius et al. 2002, Üstüner et al., 2018b).

It has been reported that essential oils and their components inhibit mycelial development and spore germination by causing; detachment of lipid layers from the cell membranes of fun-gi, metabolic disorders in cytoplasmic and mitochondrial membranes and degradation of membrane structures and permeability by affecting cell membrane integrity (Bakkali et al. 2008, Iscan et al., 2016, Sivakumar and Bautista-Bañosö 2014). In a research done by Mo-hammadi et al. (2012) to determine the effectiveness of essential oils obtained from plants in order to control the post-harvest fruit decay in peach fruits, they found that the weight loss of fruits were decreased and storage periods prolonged. In the present study the effect of differ-ent essential oils on the growth and development of Alternaria solani; the agent of early blight of tomato, were examined using in vitro and in vivo (Tomato fruits) bioassays.

2. MATERİALS AND METHODS

2.1. Materials

Materials employed in the study were ten different plant species which were used as essential oil source namely; Satureja hortensis L., Thymbra spicata L., Origanum majorana L., O. syri-acum L., O. vulgare L., O. onites L., Artemisia santonicum L., A. absinthium L., A. spicigera C. Koch. Thymus sipyleus Boiss; these plant species were collected from various regions in Turkey at the flowering stage. Tomato fruits, which also were part of the study, were handled carefully from local markets selecting the healthy fruits and considering the homogeneity. Additionally, Alternaria solani pathogen was isolated from diseased tomatoes.

2.2. Essential oil extraction and their chemical composition

Essential oils were extracted from plant species that were dried under shade and powdered using grinder. Then powdered materials were subjected to hydro-distillation process for 4-6 hours using a Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulfate and stored under N2 in a sealed vial at 4°C until used for bioassays. The chemical compositions of the essential oils in this study was cited from different sources and presented in the table below (Table 1) with their reference.

Table 1. Chemical compositions of the used essential oils

Essential oil plant	Components	Relative percentage (%)	Literature
Origanum vulgare L.	*Carvacrol* *p-cymene* *linalool* *α-terpeniol* *(-)-terpinen-4-ol*	63,97 12,63 3,67 2,54 2,24	Özkan et al. (2017)
O. onites L.	*Carvacrol* *Linalool* *p-cymene* *gamma-terpinene* *thymol*	70,0 9,7 7 2,0 1,7	Tasdemir et al. (2017)
O. majorana L.	*Carvacrol* *terpinen-4-ol* *cis-sabinene hydrate* *sabinene* *p-cymene* *trans-sabinene hydrate* *α-terpineol*	65 31,15-15,76 6,91 6,83 3,86 3,71 4,0	Prerna and Vasudeva (2015)
O. syriacium L.	*Thymol* *carvacrol* *p-cymene* *γ-terpinene* *β-caryophyllene*	37,8-56,3 10,3-35,8 4,2-14,8 1,5-10,6 1,2-2,1	El-Alam et al. (2019)
Artemisia spicigera C. koch	*Camphor* *1,8-cineole* *β-Eudesmol* *borneol* *cubenol* *terpinen-4-ol* *α-terpineol* *spathulenol* *caryophyllene oxide*	34,9 9,5 7,2 5,1 4,2 1,2 1,6 3,7 1,8	Kordali et al. (2005)
A. santonicum L.	*Camphor* *1,8-cineole* *borneol* *terpinen-4-ol* *α-terpineol* *spathulenol* *caryophyllene oxide*	18,2 9,5 4,0 3,5 4,1 1,3 1,7	Kordali et al. (2005)
A. absinthium L.	*Chamazulene* *nuciferol butanoate* *nuciferol propionate* *caryophyllene oxide* *terpinen-4-ol* *(Z)-sesquisabinenehydrate* *spathulenol*	17,8 8,2 5,1 4,3 1,8 2,7 1,8	Kordali et al. (2005)
Satureja hortensis L.	*Carvacrol* *γ-terpinene* *β-cymene* *α-terpinene* *thymol*	54,74 20,94 12,30 2,04 1,97	Tozlu et al. (2011)
Thymbra spicata L.	*Carvacrol* *γ -terpinene* *β-cymene* *α-terpinene* *thujene* *trans-caryophyllene* *β-myrcene*	34,9 25,6 9,1 6,9 5,2 5,1 4,8	Kılıç (2015)
Thymus sipyleus Boiss.	*Thymol* *Carvacrol*	38,31 37,95	Ceylan and Ugur (2015)

γ-terpinene	7,28
p-cymene	4,16
borneol	3,83
bisabolene	2,32
trans-caryophyllene	1,83
α-pinene	1,0

2.3. Pathogen isolation

The fungus were isolated from diseased tomato fruits and identified under microscope. The isolates were around forty, and then they were cultured to select the best-developed ones in a period of one week. From these, eleven isolates were selected and used in the study.

2.4. Experimental Assays

2.4.1. In vitro experiment

To evaluate the in vitro impact of essential oils, a culture medium comprising Potatoes Dextrose Agar (PDA) was prepared and subsequently cooled to a temperature of 40°C. Simultaneously, varying concentrations of essential oils (140, 180, 560, and 1000 µl/L) were individually mixed with Dimethyl Sulfoxide (DMSO) to ensure the creation of a homogeneous mixture. These essential oil concentrations were then introduced into Erlenmeyer flasks containing the agar and were thoroughly mixed through manual agitation. Subsequently, the medium was poured into Petri dishes and allowed to solidify before the placement of fungal discs.

Fungal discs measuring 5mm in diameter, obtained from fungal cultures aged 7 days, were positioned at the center of each Petri dish, ensuring direct contact between the mycelia and the growth medium by placing the mycelial surface of the disc facing downward. The Petri plates were sealed with Parafilm and maintained at a constant temperature of 25±2°C within an incubator. Over a span of 7 days, the diameters of the fungal mycelia were measured at 24-hour intervals. Negative control samples consisted of PDA plates devoid of essential oils and containing only a DMSO-water solution (1%, v/v), while positive control samples were PDA plates treated with Captan WP fungicide (20.0 µg/cm²). Four replications were performed for each isolate, and the mean of these replicates was calculated after 7 days of measurements.

Ultimately, the percentage of mycelial growth inhibition (GI) was computed using the following formula:

$$GI= \frac{(C-T)}{C} x100$$

Where C is the mean of the negative control and T is the mean of the treated petri plates.

2.4.2. Tomatoes fruit experiment (In vivo)

To make surface disinfection of tomato fruits, fruits were put in 1% sodium hypochlorite solu-tion for 1 minute, washed with pure water, dried using blotting paper, and then placed in ster-ile plastic boxes. Surface-sterilized fruits were wounded with a sterilized scalpel and a 6 mm diameter and 3 mm deep holes were opened in each fruit (Lopez-Reyes et al. 2010). Fungal discs with a diameter of 5.0 mm were cut from the 7 days old fungus culture, placed at the opened holes on the fruits. After 24 hours, different essential oil concentrations (2.5, 5, 10 and 20 µl/fruit) were applied on the areas where the inoculation was made. Then, the plastic boxes were closed with their lids and left in the room temperature. A week later, the developed dis-ease lesions were measured using ruler. Each treatment had three replications and the entire experiment was repeated twice. Fruits that were applied sterile water after the inoculation were used as a control. The disease development inhibition percentage was calculated using the following formula;

$GI= \frac{(C-T)}{C} x100$ where C representing the disease development recorded from the control and T representing the disease development which was recorded from treatment groups.

2.5. Statistical analysis

The data recorded from both experiments were analyzed separately using SPSS 20 software package. To check the statistical significance of the results one way Anova test was used and Duncan multiple test was applied for mean separation.

3. RESULTS AND DİSCUSSİON

3.1. In vitro antifungal effects of the essential oils on Alternaria solani

The essential oils derived from ten distinct plant species exhibited varying effects on the growth of Alternaria solani within the PDA medium. When evaluating the antifungal proper-ties of these essential oils, it was observed that their inhibitory effects on the pathogen's growth were relatively consistent, albeit with minor discrepancies. Notably, essential oils ex-tracted from Thymus sipyleus and Origanum onites proved to be the most potent, resulting in complete growth inhibition akin to that achieved by the commercially employed fungicide, which served as the positive control. In contrast, the essential oils from Artemisia spicigera and A. santonicum displayed relatively lower efficacy compared to the other oils.

Significant antifungal effects were also observed when employing the essential oils of Origa-num majorana in Petri plates (refer to Table 2). Among the four distinct essential oil concen-trations employed, only the lowest concentration, which amounted to 140 µl/L, allowed for some degree of growth in A. solani isolates. Conversely, at the three higher concentrations (280, 560, and 1000 µl/L), fungal growth was entirely suppressed. Even at the lowest concen-tration of the oil (140 µl/L), a notable degree of fungal growth inhibition, ranging from 7% to 81%, was still evident.

Table 2. Antifungal activitity of Origanum majorana essential oils against Alternaria solani isolates

	Essential oil doses (µl/L)									
	Mycelium development(mm)									
***A. solani* İsolates**	**140 µl/L**	**Inhibition rate (%)**	**280 µl/L**	**Inhibition rate (%)**	**560 µl/L**	**Inhibition rate (%)**	**1000 µl/L**	**Engelleme oranı (%)**	**Positive Control 1.25g/L**	**Negative Control**
D10D	13.1±1.0a	66	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	38.7±1.1
D10F	9.9±0.7b	74	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	38.1±0.9
D10G	13.4±1.5a	57.7	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.7±0.8
D13A	10.1±0.4a	69.5	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	33.2±0.5
D14A	8.7±0.8b	62.9	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	23.5±15.7
D18A	7.3±0.3b	74	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	28.1±4.1
D4E	9.1±0.1b	71	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.8±3.0
D6B	13.3±0.4a	57	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.1±1.0
D6D	5.9±1.0b	81	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.2±2.2
D7A	10.5±1.1a	63.5	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	28.8±2.0
D7B	11.1±0.8a	66.7	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	33.4±0.7

****Values in the same column with different letters are significantly different (P <0.05)***

Origanum vulgare essential oils also showed 100% growth inhibition at the concentrations of 280, 560 and 1000 µl/L. However, this essential oil imposed great impact on the fungal iso-lates in their lowest concentration (140 µl/L) (Table 3). The recorded inhibitions in this con-centration were between 88.6% and 98%. Nevertheless, essential oils extracted from Origa-num syriacium had nearly similar effect with the above-discussed two species with a little higher antifungal effect. In this oil, at the lowest concentration used in the experiment, it has been seen that the growth of six of the eleven A. solani isolates were 100% inhibited (Table 4). The most active Origanum species used in the experiment was O. onites. The oil from this spe-cies 100% inhibited the growth of the pathogen in all the concentrations.

Table 3. Antifungal activitity of Origanum vulgare essential oils against Alternaria solani isolates

	Essential oil doses (µl/L)									
	Mycelium development(mm)									
***A. solani* İsolates**	**140 µl/L**	**Inhibition rate (%)**	**280 µl/L**	**Inhibition rate (%)**	**560 µl/L**	**Inhibition rate (%)**	**1000 µl/L**	**Inhibition rate (%)**	**Positive Control 1.25g/L**	**Negative Control**
D10D	0.0±0.0b	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	38.7±1.1

D10F	2.4±0.4a	93.7	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	38.1±0.9
D10G	0.5±0.4a	98	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.7±0.8
D13A	2.6±0.3a	92	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	33.2±0.5
D14A	1.1±0.2a	95	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	23.5±15.7
D18A	2.1±0.5a	92.5	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	28.1±4.1
D4E	3.6±0.7a	88.6	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.8±3.0
D6B	0.0±0.0b	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.1±1.0
D6D	0.0±0.0b	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.2±2.2
D7A	0.9±0.6a	96.8	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	28.8±2.0
D7B	2.1±1.1a	93.7	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	33.4±0.7

*Values in the same column with different letters are significantly different (P <0.05)

Table 4. Antifungal activitity of Origanum syriacium essential oils against Alternaria solani isolates

	Essential oil doses (µl/L)									
	Mycelium development(mm)									
***A. solani* İsolates**	**140 µl/L**	**Inhibition rate (%)**	**280 µl/L**	**Inhibition rate (%)**	**560 µl/L**	**Inhibition rate (%)**	**1000 µl/L**	**Inhibition rate (%)**	**Positive Kontrol 1.25g/L**	**Negative Control (Only PDA)**
D10D	0.8±0.2a	97.9	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	38.7±1.1
D10F	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	38.1±0.9
D10G	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	31.7±0.8
D13A	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	33.2±0.5
D14A	4.6±0.3a	80	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	23.5±15.7
D18A	3.8±0.6a	86	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	28.1±4.1
D4E	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	31.8±3.0
D6B	2.2±1.5a	92.9	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	31.1±1.0
D6D	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	31.2±2.2
D7A	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	28.8±2.0
D7B	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	100	0.0±0.0b	33.4±0.7

*Values in the same column with different letters are significantly different (P <0.05)

The essential oils from Artemisia absinthium caused complete growth inhibition at 280, 560 and 1000 µl/L doses. Whereas at the 140 µl/L dose of the oil, Recorded inhibition per-centage was between 46.6 and 89%. A. spicigera and A. santonicum were not effective against Alternaria solani isolates as A. absinthium (Tables 5-7). These two essential oils did not show good antifungal effect because the fungal isolates has grown in all the concentrations. However, the growth of the fungal isolates decreased with the increase of the oil dose. At the smallest dose (140 µl/L), the oil had no remarkable effect on the growth of the fungi as there were no much differences in the growth with the negative control plates. In this dose, all the fungal isolates revealed mycelial growth. Considering the presence of A. spicigera essential oil at the concentration of 560 µl/L, five of the eleven isolates were completely inhibited by the oil whereas 55.8-100% growth inhibition was recorded from the remaining isolates. In the highest concentration (1000 µl/L), the oil of A. spicigera prevented the growth of all the iso-lates except two isolates. Considering A. santonicun at the concentration

of 560 µl/L, an inhi-bition percentage changing between 69 to 90.6% were obtained from the isolates but at the biggest concentration (1000 µl/L), it was confirmed that all the isolates were inhibited.

Table 5. Antifungal activitity of Artemesia absinthium essential oils against Alternaria solani isolates

	Essential oil doses (µl/L)									
	Mycelium development(mm)									
***A. solani* İsolates**	**140 µl/L**	**Inhibition rate (%)**	**280 µl/L**	**Inhibition rate (%)**	**560 µl/L**	**Inhibition rate (%)**	**1000 µl/L**	**Inhibition rate (%)**	**Positive Control 1.25g/L**	**Negative Control (Only PDA)**
D10D	10±0.5a	74	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	38.7±1.1
D10F	12.6±3.0a	66.9	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	38.1±0.9
D10G	7.3±0.4b	76.9	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	31.7±0.8
D13A	7.1±0.2b	78.6	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	33.2±0.5
D14A	5.1±6.0b	78	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	23.5±1.5
D18A	15±1.1a	46.6	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	28.1±4.1
D4E	8.3±0.08b	73.8	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	31.8±3.0
D6B	10±1.3a	67.8	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	31.1±1.0
D6D	10±1.1a	67.9	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	31.2±2.2
D7A	11.5±1.8a	60	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	28.8±2.0
D7B	3.6±4.8c	89	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	33.4±0.7

*Values in the same column with different letters are significantly different (P <0.05)

Table 6. Antifungal activitity of Artemesia spicigera essential oils against Alternaria solani isolates

	Essential oil doses (µl/L)									
	Mycelium development(mm)									
***A. solani* İsolates**	**140 µl/L**	**Inhibition rate (%) (%)**	**280 µl/L**	**Inhibition rate (%) (%)**	**560 µl/L**	**Inhibition rate (%) (%)**	**1000 µl/L**	**Inhibition rate (%) (%)**	**Positive Control 1.25g/L**	**Negative. Control (Only PDA)**
D10D	18.6±1.6b	51.7	8.2±3.3d	78.8	0.0±0.0f	100	0.0±0.0f	100	0.0±0.0f	38.7±1.1
D10F	9.0±1.3d	76	4.7±1.0e	87.6	0.0±0.0f	100	0.0±0.0f	100	0.0±0.0f	38.1±0.9
D10G	21±3.9a	33.7	20±3.5a	36.9	14±0.2c	55.8	9.2±0.6d	70.9	0.0±0.0f	31.7±0.8
D13A	20.3±1.8a	38.8	14.6±2.4c	56	4.8±0.2e	85.5	0.0±0.0f	100	0.0±0.0f	33.2±0.5
D14A	22.8±0.8a	2.97	20.1±2.9a	14	10±0.8c	57	4.7±0.2e	80	0.0±0.0f	23.5±15.7
D18A	8.9±3.3d	68	3.7±0.4d	86.6	0.0±0.0f	100	0.0±0.0f	100	0.0±0.0f	28.1±4.1
D4E	23.5±0.3a	23.8	19.3±0.6b	39	7.0±0.4d	77.9	0.0±0.0f	100	0.0±0.0f	31.8±3.0
D6B	13±1.3c	58	12±0.0c	61	0.0±0.0f	100	0.0±0.0f	100	0.0±0.0f	31.1±1.0

D6D	13.3±1.2c	57	4.1±0.8e	86.8	0.0±0.0f	100	0.0±0.0f	100	0.0±0.0f	31.2±2.2
D7A	21.3±0.4a	26	17.3±4.2b	39.9	7.5±0.4d	73.9	0.0±0.0f	100	0.0±0.0f	28.8±2.0
D7B	13.3±0.05c	60	10.4±1.0a	68.8	4.7±0.2e	85.9	0.0±0.0f	100	0.0±0.0f	33.4±0.7

*Values in the same column with different letters are significantly different (P <0.05)

Table 7. Antifungal activitity of Artemesia santonicum essential oils against Alternaria solani isolates

	Essential oil doses (µl/L)									
	Mycelium development(mm)									
***A. solani* İsolates**	**140 µl/L**	**Inhibition rate (%)**	**280 µl/L**	**Inhibition rate (%)**	**560 µl/L**	**Inhibition rate (%)**	**1000 µl/L**	**Inhibition rate (%)**	**Positive Control 1.25g/L**	**Negative Control (only PDA)**
D10D	23.4±1.1a	39.5	14.9±0.1c	25.8	3.6±0.0e	90.6	0.0±0.0f	100	0.0±0.0f	38.7±1.1
D10F	18.2±0.4b	52	17.2±0.8b	54	7.1±0.8d	81	1.0±0.8e	97	0.0±0.0f	38.1±0.9
D10G	23.6±0.6a	25.5	16.8±1.2b	47	6.8±0.8d	78.5	0.0±0.0f	100	0.0±0.0f	31.7±0.8
D13A	17.9±0.4b	46	16±0.8b	51	6.3±0.6d	81	0.0±0.0f	100	0.0±0.0f	33.2±0.5
D14A	22.1±0.6a	5.9	16.4±1.1b	30	6.6±0.4d	71.9	1.6±0.4e	93	0.0±0.0f	23.5±15.
D18A	16.2±2.8b	42	8.9±2.3d	68	0.0±0.0f	100	0.0±0.0f	100	0.0±0.0f	28.1±4.1
D4E	20.2±2.3a	36	14.7±2.4c	53.7	4.6±0.2e	85.5	0.0±0.0f	100	0.0±0.0f	31.8±3.0
D6B	19.9±0.1b	36	14.5±0.3c	53	9.6±0.4d	69	0.0±0.0f	100	0.0±0.0f	31.1±1.0
D6D	23.4±1.3a	25	14.2±0.8c	54	8.6±1.0d	72	0.0±0.0f	100	0.0±0.0f	31.2±2.2
D7A	22.6±1.7a	21.5	16.5±0.8b	42.7	4.5±0.4e	84	1.0±0.1e	96.5	0.0±0.0f	28.8±2.0
D7B	15.4±0.7b	53.8	12.8±2.2c	61.6	4.0±0.1e	88	0.0±0.0f	100	0.0±0.0f	33.4±0.7

*Values in the same column with different letters are significantly different (P <0.05)

The essential oils from Satureja hortensis had also great inhibitory effect. The antifungal activ-ity of the oil of this plant was more or less similar to the most of the used plants in the experi-ment as there were slight growths of the fungal isolates only at the lowest concentration (Ta-ble 8). The calculated percent of inhibition caused by this oil in the 140 µl/L concentration was stated between 77.6% and 88%. Comparatively, the essential oil obtained from Thymbra spicata had good antifungal effect, but the results showed that complete inhibition of growth of the all isolates were recorded only at the highest two concentrations in the study (560 and 1000 µl/L) (Table 9) . The last tested essential oil was Thymus sipyleus, in this plant as men-tioned earlier; excellent antifungal effect was seen against all the used A.solani isolates and completely inhibited the mycelial growth of the fungus in all concentrations.

Table 8. Antifungal activitity of Satureja hortensis essential oils against Alternaria solani isolates

	Essential oil doses (µl/L)									
	Mycelium development(mm)									
***A. solani* İsolates**	**140 µl/L**	**Inhibition rate (%)**	**280 µl/L**	**Inhibition rate (%)**	**560 µl/L**	**Inhibition rate (%)**	**1000 µl/L**	**Inhibition rate (%)**	**Positive Control 1.25g/L**	**Negative Control (Only PDA)**
D10D	4.9±0.5a	87	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	38.7±1.1
D10F	8.5±1.1a	77.6	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	38.1±0.9
D10G	4.5±0.4b	85.8	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.7±0.8

D13A	5.7±0.1a	82.8	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	33.2±0.5a
D14A	4.4±0.1b	81	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	23.5±15.7
D18A	5.3±0.5a	81	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	28.1±4.1
D4E	6.5±0.6a	79.5	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.8±3.0
D6B	4.0±0.9b	87	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.1±1.0
D6D	3.7±0.1b	88	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	31.2±2.2
D7A	3.4±0.4b	88	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	28.8±2.0
D7B	5.5±1.4a	83.5	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	33.4±0.7

*Values in the same column with different letters are significantly different (P <0.05)

Table 9. Antifungal activitity of Thymbra spicata essential oils against Alternaria solani isolates

	Essential oil doses (µl/L)									
	Mycelium development(mm)									
***A. solani* İsolates**	**140 µl/L**	**Inhibition rate (%)**	**280 µl/L**	**Inhibition rate (%)**	**560 µl/L**	**Inhibition rate (%)**	**1000 µl/L**	**Inhibition rate (%)**	**Positive Control 1.25g/L**	**Negative Control (Only PDA)**
D10D	12.8±2.1a	66.9	7.6±5.1b	80	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	38.7±1.1
D10F	12.8±2.4a	66	7.6±0.9b	80	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	38.1±0.9
D10G	10.8±0.3a	65.9	3.4±0.3c	89	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	31.7±0.8
D13A	7.5±0.6b	77	4.0±0.5c	87.9	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	33.2±0.5
D14A	5.9±1.2b	74.8	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	23.5±15.7
D18A	4.9±1.1c	82.5	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	28.1±4.1
D4E	7.5±1.0b	76	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	31.8±3.0
D6B	5.4±0.9a	82.6	4.1±0.6c	86.8	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	31.1±1.0
D6D	4.6±1.1c	85	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	31.2±2.2
D7A	5.6±0.2b	80.5	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	28.8±2.0
D7B	13.8±1.4a	58.6	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	100	0.0±0.0d	33.4±0.7

*Values in the same column with different letters are significantly different (P <0.05)

3.2. In vivo antifungal activity of the essential oils

The effect of the essential oils in the in vivo conditions was evaluated on the pathogen inocu-lated-tomato fruits. All the oils used in the petri plate assay were again tested in this experi-ment, regardless of their effectiveness in the petri plates. Seven days after treatment the rec-orded disease lesions on the tomatoes were significantly different from that in the control fruits. Most of the essential oils showed promising antifungal effect against the development of the disease on tomato fruits as illustrated in Figure 1. Especially the essential oils that ex-hibited high activity in the in vitro showed good antifungal activity in the in vivo conditions too.

The essential oil from Artemisia spicigera and A. santonicum had the least effect on the devel-opment of the disease, as there was disease lesions appeared even on the fruits that were ap-plied at 20µl/fruit doses. In this two plant essential oils, their inhibitory percentage was 0.6, 19.0, 53.8 and 71.0 % in A. santanonicum and 12.9, 30.7, 53.8 and 69.0 % in A. spicigera at 2.5, 5, 10 and 20 µl/fruit concentrations respectively. In contrast, all the other essential oils inhibited disease lesions development at 10 and 20 µl/fruit concentrations. However, essential oils from Origanum

majorana and A. absinthium essential oils has prevented disease lesion development on the fruit at all concentrations except the lowest concentration (2.5 µl/fruit).

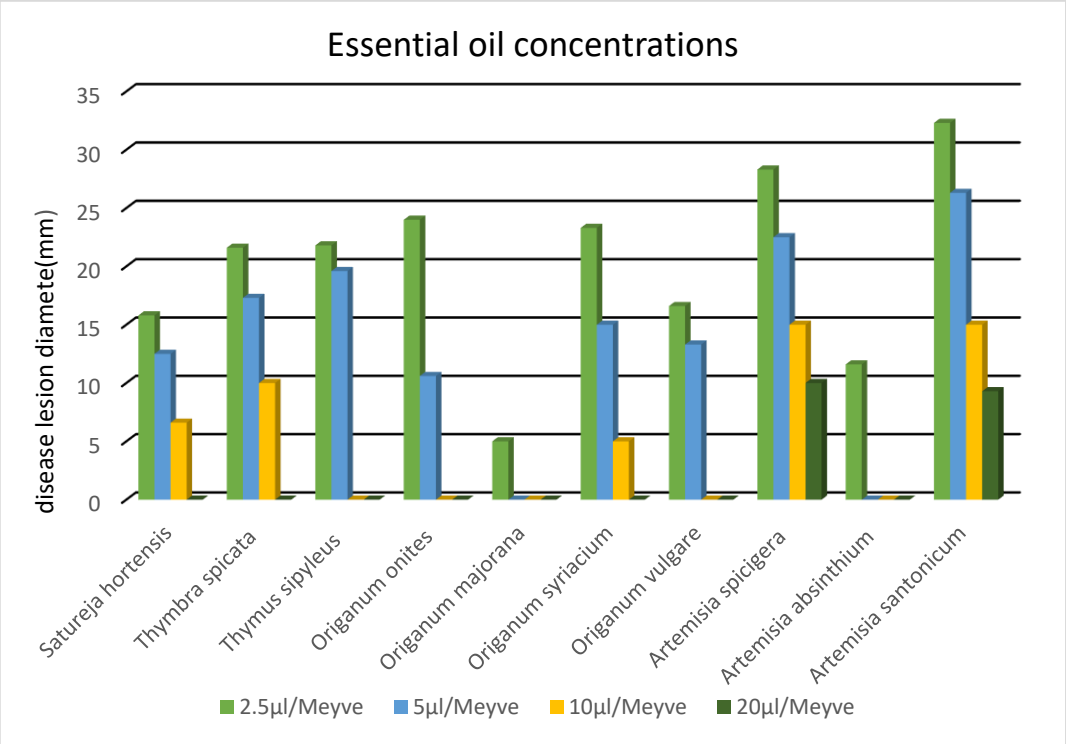

Figure. 1. Effect of the essential oils on disease lesion development on the tomatoes fruits

Essential oils, derived from plants, possess a rich historical tradition of utilization and are recognized for their potential antimicrobial and insecticidal properties. This has spurred extensive research endeavors aimed at exploring their suitability as agents for controlling plant diseases. While the precise mechanisms underpinning the antimicrobial attributes of essential oils remain partially understood, various hypothetical mechanisms have been proposed. Sever-al investigations have elucidated that essential oils may operate through mechanisms such as cellular accumulation, modulation of cell permeability, disruption of vital organelle mem-branes, and alteration of overall cellular morphology (Hue et al., 2017; Bajpai et al., 2013; Tian et al., 2012). These processes, in turn, can lead to cellular leakage and demise of the targeted organism.

Essential oils from ten different plant species were used in the study both in vitro and in in vivo bioassays. Most of them exhibited high antifungal activity against the growth and development of plant pathogenic Alternaria solani fungus. When the antifungal effects of most essential oils are analyzed, it was determined that the rates of inhibition on the growth and development of the pathogen caused by the used essential oils are close to each other with little differences. In Petri dish trials, Thymus sipyleus and Origanum onites essential oils has caused the most pronounce antifungal impact by 100% inhibiting the mycelial development of all the fungal isolates at all concentrations. In contrast, Artemisia spicigera and A. santonicum essential oils have been seen to have a low antifungal effect compared to other essential oils in the petri dish bioassay. In the other hand, it was found that essential oils applied on tomato fruits, completely stopped the disease lesion formation on the fruits at the higher concentra-tions. However, it has been confirmed that A. spicigera and A. santonicum essential oils had low antifungal activity on the tomato fruits too.

Study done by Tomazoni et al (2017) reported that essential oils from Eucalyptus staigeriana, Eucalyptus globulus and Cinnamomum camphora had antifungal effects both in vitro and in vivo on early blight disease agent. However, some studies did not report antifungal activity of the essential oils. Philips et al. (2012), researched the antifungal effect of 50:50 mixture of essential oils (orange: bergamot) on Alternaria alternata inoculated tomatoes and found no effective antifungal activity caused by oils. Another study reported that the essential oils ob-tained from Cuminum cyminum L., Mentha longifolia L. and Allium sativum L. were found to be 100% effective on Verticilium dahlia Kleb. mycelium growth in all concentrations exam-ined (Üstüner et al., 2018b). Many studies have substantiated the antimicrobial efficacy of essential oils, yet their prevalent issues, such as their pronounced volatility, odor, cost, and potential impact on fruit flavor, are widely acknowledged (Bakkali et al., 2008). Aktepe et al (2019), underlined that the active ingredients of these essential oils contribute to the emer-gence of novel chemicals in agriculture. It is well-known that the mechanisms underlying the antimicrobial activity of essential oils differ contingent upon the specific type of essential oil and the microbial strain employed (Chouhan et al., 2017). This variability in factors could ac-count for the disparate findings observed among various research investigations.

CONCLUSİON

In conclusion, the investigation found that a majority of the essential oils examined in this research exhibited favorable antifungal properties against Alternaria solani. Nevertheless, it is imperative to emphasize the necessity for further research efforts aimed at facilitating the po-tential development of fungicides based on essential oils. Ensuring the reliability and con-sistency of such research requires additional experimentation in both controlled laboratory settings (in vitro) and real-world conditions (in vivo).

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