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Araştırma Makalesi

Domateste Bakteriyel Leke Hastalığına Neden Olan *Pseudomonas syringae* pv. *tomato*'nun Farklı Bitki Uçucu Yağları ile Kontrolü

Mesude Figen DÖNMEZ[1*], Işıl TEMEL[1], Esat TEMTEK[1]

[1:]Iğdır Üniversitesi, Iğdır Ziraat Fakültesi

* Corresponding Author:sudefigen@hotmail.com

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ÖZ

Tohum kaynaklı *Pseudomonas syringae* pv. *tomato* (Pst 76) domateste bakteriyel leke hastalığına neden olmakta ve domates yetiştiriciliğinde verim ve kaliteyi etkileyerek ciddi ekonomik kayıplara yol açmaktadır. Bu çalışmada, *Mentha longifolia, Thymus migricus, Salvia hydrangea, Artemisia absinthium* ve *Achillea arabica* bitkilerinden distilasyon yoluyla elde edilen uçucu yağların *P. syringae* pv. *tomato*'nun kontrolü üzerindeki etkisi araştırılmıştır. Bitki uçucu yağlarının *P. syringae* pv. *tomato*'ya karşı antibakteriyel aktiviteleri in vitro agar disk difüzyon yöntemi ile test edilmiştir. Ayrıca, bitki uçucu yağlarının Pst 76'nın büyümesini engellediği minimum inhibitör konsantrasyonlar belirlenmiştir. Tüm yağların hastalık etmenine karşı 4.0-30.0 mm arasında bir inhibisyon zonu oluşturduğu ve en yüksek aktivitenin *T. migricus* bitkisinin yağı ile yapılan uygulamadan elde edildiği belirlenmiştir. Kontrol grubunda *T. migricus* ve *M. longifolia* bitkilerinden elde edilen yağların metilmisine göre daha yüksek antimikrobiyal aktiviteye sahip olduğu tespit edilmiştir. Elde edilen sonuçlar doğrultusunda, uçucu yağlar ile hastalık kontrolü, entegre mücadele programlarına dahil edilebilecek alternatif bir yöntem olarak görülmüştür.

Anahtar kelimeler: Antibakteriyel aktivite, Bakteriyel leke. Uçucu yağ, Pseudomonas syringae pv. tomato

The Control of *Pseudomonas syringae* pv. *tomato*, Causing Bacterial Spot Disease in Tomato, by Different Plant Essential Oils

ABSTRACT

Seed-borne *Pseudomonas syringae* pv. *tomato* (Pst 76) causes bacterial spot disease in tomatoes and causes serious economic losses by affecting yield and quality in tomato cultivation. In this study, the effect of essential oils obtained by distillation from *Mentha longifolia*, *Thymus migricus*, *Salvia hydrangea* and *Artemisia absinthium* and *Achillea arabica* plants on the control of *P. syringae* pv. *tomato* was investigated. The antibacterial activities of plant essential oils against *P. syringae* pv. *tomato* were tested by the *in vitro* agar disk diffusion method. Additionally, minimum inhibitory concentrations at which plant essential oils inhibit the growth of Pst 76 were determined. It was determined that all oils created an inhibition zone between 4.0-30.0 mm against the disease agent and the highest activity was obtained from application with the oil of the *T. migricus* plant. It was determined that the oils obtained from *T. migricus* and *M. longifolia* plants had higher antimicrobial activity than methylmycin in the control group. In line with the results obtained, disease control with essential oils has been seen as an alternative method that can be included in integrated control programs.

Key words: Antibacterial activity, Bacterial spot, Essential oil, *Pseudomonas syringae* pv. *tomato*

INTRODUCTION

Bacterial spot disease caused by *Pseudomonas syringae* pv. *tomato* (Okabe) is one of the most devastating diseases of tomato (*Solanum lycopersicon* L.), a food-valuable agricultural crop (Regassa et al., 2016). *Pseudomonas syringae* pv. *tomato* (Pst 76) spreads rapidly to other plants in the field when suitable climatic conditions occur (McCarter et al., 1983) and causes significant economic damages by causing great losses in tomato production (Uppalapati et al., 2008). When the pathogen enters the plant, it exhibits a very aggressive behavior and reduces the market value of the product by infecting all above-ground organs of the plant (Xin and He, 2013).

In recent times, the use of chemicals in the control of phytopathogens has increased considerably (Farimaz et al., 2014). However, problems such as the fact that the intensive use of chemicals leads to phytotoxicity in plants, causes accumulation in the soil (Balestra et al., 2009), poses a significant risk to human health (Granata et al., 2011) and leads to resistance problem in microorganisms (Chacón-Hernández et al., 2020) have directed the producers to alternative control methods. Therefore, the study of compounds derived from plants has recently been among the popular topics. Türkiye, which has a rich flora, is home to many plants with antimicrobial properties in its geography and is an important source for the investigation of essential oils with antimicrobial activity (Nazzaro et al., 2017). The use of antimicrobial substances obtained from plant essential oils draws attention as they are easily found in nature, are inexpensive, do not emit toxic substances, do not cause soil and water pollution, do not create residues that threaten human health, and are specific to disease agents (Erler, 2000). In different studies, it was revealed that essential oil treatments affected the resistance mechanism by showing positive effects on the signaling network in plants (Kokoskova et al., 2011; Umarusman, 2018). Furthermore, it has been reported that the components such as carvacrol, thymol, menthol, campor and linalool possessed by these essential oils have antimicrobial activity against microorganisms (Liu et al., 2019; Temtek, 2021). Various studies are showing that essential oils obtained from *Achilla, Artemisia, Thymus*, *Salvia* and *Mentha* species have antibacterial activity against different pathogens that cause disease in plants. (Mengulluoglu and Soylu, 2012; Ghavam et al., 2020; Bozkurt et al., 2020).

In previous studies conducted by us, has been detected showed high antibacterial activity against *Clavibacter michiganensis* subsp. *michiganensis* the agent of tomato bacterial wilt and cancer (Temel et al., 2023), and three different strains of *Acidovorax citrulli* agent of the bacterial fruit spot (Temtek, 2021) of essential oils obtained from *Thymus migricus* Klokov & Des. Shost, *Salvia hydrangea* DC Ex Bentham*, Mentha longifolia* L., *Artemisia absinthium* L. and *Achillea arabica* Kotschy. This study was aimed to determine the antibacterial activities of essential oils obtained from the mentioned plants against the Pst 76, which causes problems in tomato production, under in vitro conditions and to determine the minimum inhibitory concentration (MIC) values that inhibit the development of pathogens.

MATERIAL AND METHOD

Pathogenic bacterial strain and plants from which essential oils are obtained

The pathogenic bacterial strain (Pst 76) was taken from the culture collection of Assoc. Prof. Mesude Figen DÖNMEZ. In this study, the oils whose antimicrobial activity was tested were obtained from *T. migricus*, *S. hydrangea*, *M longifolia* plants in the Lamiaceae family and *A. absinthium* and *A. arabica* in the Asteraceae family (Temtek, 2021). Detailed information about plant essential oils is presented in Table 1.

Table 1. Some information about the essential oils used in the study (Temtek, 2021; Temel et al., 2023)

Essential oils	The Essential Oils Yield (%)	Number of Components	Main Components and amount (%) Essential Oils
Thymus migricus	0,7	30	á-Pinene, (-) (4.96%), Ç-Terpinene (9.82%), P-Cymene (27.27%), Thymol (32.75%)
Salvia hydrangea	0,25	37	(-)-á-Elemene (7.01%), á-Selinene (13.38%), Camphor (13.34%), L-4-Terpineneol (8.4%),
Mentha longifolia	0,3	40	1-Menthone (5.75%), Pulegone (12.47%), Piperitone Oxide (15.55%), Piperitenone Oxide (13.61%)
Artemisia absinthium	0,7	32	1,8-Cineole (19.54%), Camphene (5.31%), Pulegone (7.86%), á-Thujone (34.64%)
Achillea arabica	0,2	43	(-)-á-Elemene (7.01%), á-Selinene (13.38%), Camphor (13.34%), L-4-Terpineneol (8.4%)

Antibacterial activity assay

Pst 76 strain kept at -80°C was grown in Nutrient agar (NA) broth, and then taken with a sterile loop, transferred to Nutrient Broth (NB) and then incubated for 12 hours in a shaker set at 27°C at 150 rpm/min.

Antibacterial activities of essential oils were determined according to the method reported by Eriş (2006). For this purpose, 100 µl of the pathogen solution prepared at a density of 10^8 CFU/ml (turbidimeter) was taken and spread on the petri surface. Then, three empty discs were placed at equal distances from each other on the petri dishes and 10 µl of essential oil was dripped on the discs. The petri dishes were covered with parafilm and incubated for five days. At the end of the incubation, the clean zone area around the discs was measured. The experiment was carried out in three replicates. Methylmicin disc (30 mg) was used as the positive control and NB was used as negative control in the study.

Determination of Minimum Inhibitory Concentrations of plant essential oils

Serial dilutions (7.8-15.63-31.25-62.5-125-250-500-1000 µl) were prepared with 10% dimethyl sulfoxide to determine the minimum inhibition concentration values of the essential oils showing antibacterial activity against the pathogen. Pst 76 was grown in NA, then taken with a loop, transferred to NB, and incubated for 12 hours in a shaker set at 27 ^{o}C at 150 rpm/min. After incubation, the pathogen solution (10^8 cfu/ml) was spread on petri dishes containing NA. Then 10 µl of essential oils prepared at different concentrations were dripped into the petri dishes. The smallest concentration that inhibited pathogen growth in the petri dish was determined as the minimum inhibition concentration of the oils (Dönmez et al., 2020).

Analysis of data

The zone values obtained as a result of the antibacterial activity test were subjected to a one-way analysis of variance (ANOVA) in the SPSS (17.0) statistical package program. The values that were found to be significant were grouped at the probability level of $P \leq 0.01$ according to the Duncan's test. Depending on the differences in the major components and amounts of essential oils, a heat map was created using the "heat map.2" command in the "glots" library in the R package programme.

RESULTS AND DISCUSSION

Antibacterial activity assay

All of the essential oils tested in the study showed antibacterial activity against Pst 76 and inhibited the growth of pathogens at different rates. As a result of statistical analysis, a significant difference ($p \leq 0.01$) was found between the zone values obtained due to the applications. It was determined that the application with the essential oil obtained from the *T. migricus* plant had the highest antibacterial activity (Table 2).

Table 2. Inhibition diameters formed by plant essential oils against Pst 76

Treatments (Essential oils)	Zone (Pathogen growth inhibition values;mm)
Salvia hydrangea	7.3 ± 0.05^{cd}*
Thymus migricus	30.0 ± 0.45^{a}
Mentha longifolia	16.0 ± 0.36^{b}
Achillea arabica	10.3 ± 0.15b^{cd}
Artemisia absinthium	4.0 ± 0.34^{de}
Methylmicin (30 mg)	13.3 ± 0.05^{bc}
Negative control	0.0 ± 0.0^{e}

*There is a statistical difference between the values shown with different letters in the same column ($p \leq 0.01$).

Considering the values in Table 2, it was observed that the highest antibacterial activity (30 mm) against Pst 76 was obtained from *T. migricus* essential oil and the lowest inhibition zone value (4 mm) was obtained from the application with *A absinthium* essential oil. Except for the applications with *S. hydrangae* and *A. absinthium* essential oils, all other applications suppressed pathogen growth at a higher rate than methylmicin antibiotic discs were determined.

Minimum Inhibitory Concentrations (MIC)

The minimum inhibitory concentrations of plant essential oils tested for antibacterial effect against Pst 76 were determined. The concentration values preventing the development of Pst 76 are presented in Table 3.

Table 3. MIC values formed by essential oils against Pst 76

***Pseudomonas syringae pv. tomato* strain 76 (mm)**								
	1000	**500**	**125**	**62.5**	**31.25**	**15.63**	**7.8**	**MIC (µl/ml)**
Salvia hydrangea	**0.8**							**1000**
Thymus migricus	**30.8**	**24.7**	**19.3**	**17.0**	**14.2**	**10.9**		**15.63**
Mentha longifolia	**16.9**	**11.2**	**9.3**					**125**
Achillea arabica	**10.4**							**1000**
Artemisia absinthium	**0.2**							**1000**

*MIC; Minimum inhibitory concentrations

Upon examining the values in Table 3, while the minimum inhibitory concentration at which *Thymus migricus* essential oil prevented the development of Pst 76 is 15.63 µl ml^{-1}, this value was 125 µl ml^{-1}in *Mentha longifolia* essential oil and 1000 µl ml^{-1}in *Salvia hydrangea*, *Achillea arabica* and *Artemisia absinthium* essential oils. It was pointed out that the data from the agar disc diffusion test and the study's findings supported one another.

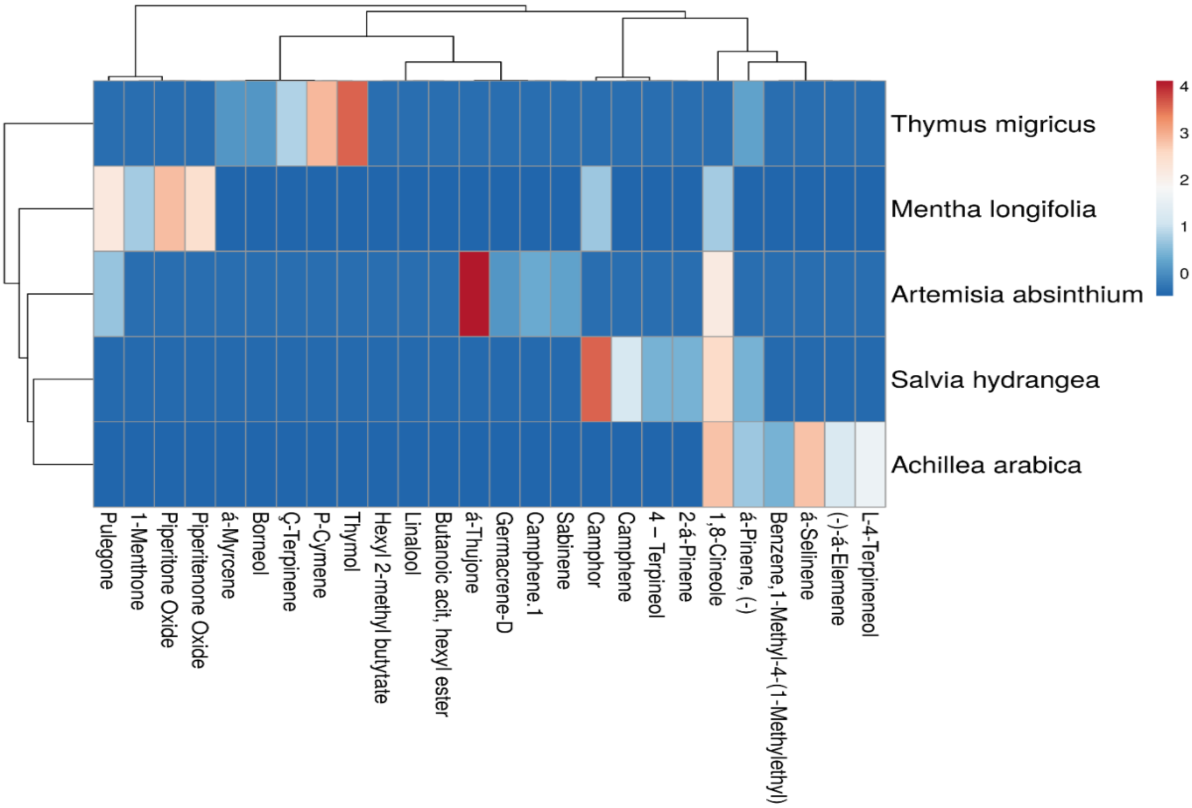

Figure 1. Heat map with classification of essential oils according to their main components

In order to visualise, clarify, cluster, and associate the parameters considered for the present study, we have constructed a heat-map. In this regard, essential oil compounds identified corresponding to the diverse plant species were used for the construction. Accordingly, two major clusters were observed for each dependent (essential oil compounds) and independent variables (plant species). Regarding plant species, *Thymus migricus* was clearly discriminated from other plant species. The other species were grouped into another major cluster. Concerning the compounds identified, two major clusters were also revealed. A clear clustering was not observed. The compounds of *Mentha longifolia* were separately grouped.

Upon examining the studies on the same species, it was observed that there were differences in the content and component amounts of essential oils. In our study, Camphene was found as the main component in *S. hydrangea* essential oil, however, in the study of Rustaiyan et al., (1997), it was observed that this component was not included in the essential oil of the same plant. In the study in which the chemical components of the essential oil obtained from the *T migricus* plant were determined, thymol was determined as the main component in parallel with the results of this study (Başer et al., 2002). In another study, 1.8 cineole, ascaridol, isoascaridol and camphor were determined among the chemical components of essential oils of *Achillea* species collected from different cities in Türkiye (Toncer et al., 2010). It was determined that the essential oil components of *Artemisia* species, which were determined to show a significant chemical variation within the species (Nguyen and Németh, 2016), could vary depending on the differences in environmental conditions (Bezić et al., 2003), and differ according to the soil composition and the countries where they were grown (Morteza-Semnani and Akbarzadeh, 2005).

Different studies have reported that the region where the plant grows (Sonbali et al., 2009), climate, altitude and soil characteristics (Senatore et al., 1997; Ebrahimi and Ranjbar, 2016) and hereditary characteristics of the plant (Sagnard et al., 2002) have a significant effect on the chemical components of essential oil. It has also been reported that harvest time, drying, extraction method and physiological factors cause variations in the components of essential oils (Figueiredo et al., 2008). Moreover, it was also reported that the yield and components of the essential oil changed depending on the development period and the collected part of the plant (Rahimmalek et al., 2009). In the study conducted by Loziene and Venskutonis (2005), it was reported that sudden changes in environmental factors affected essential oil components.

In this study, the essential oil obtained from *T migricus* was found to have the highest antibacterial activity against Pst 76. In many studies conducted in the past years, it was demonstrated that essential oils had antimicrobial activity against plant pathogenic microorganisms that cause damage to the yield and quality of agricultural products (Radaellie et al., 2016)· It has been determined that essential oils obtained from plants in the Lamiaceae family are rich in phenolic compounds responsible for significant herbicidal, insecticidal and antimicrobial activity (Mamadalieva et al., 2017; Kaya et al., 2018; Kachur et al., 2019; Bozkurt et al., 2020). It has been reported that Thymol, which gives thyme its scent, is a strong antibiotic (Akgül, 1993) and that thyme essential oil and its components cause cell membrane deterioration of some fungal and bacterial disease agents (da Silva et al., 2019; Liu et al., 2019; Churklam et al., 2020).

When the MIC values of essential oils were analysed, it was found that *T migricus* was effective against Pst 76 at the lowest concentration. In a study, the effect of different essential oils and chemical compounds against Pst 76 was tested and it was reported that the oil obtained from thyme inhibited the growth of the pathogen at a concentration of 10%. (da Silva et al., 2014). Various studies have shown that the antimicrobial activity of essential oils may be due to their hydrophobic structure, which allows these compounds to easily penetrate microbial cells and cause changes in their structure and functionality (Lucas et al., 2012; Yong et al., 2015; da Silva et al., 2019). Furthermore, it has been revealed by the studies that the extraction technique of the essential oil, the amount of antimicrobial substance absorbed into the disc, the incubation time and temperature, the microorganism studied and the amount of inoculum lead to differences in the antimicrobial activity of the essential oil (Dorman and Deans 2000; Çopuroğlu, 2013; Turhan, 2015).

CONCLUSION

The popularity of sustainability in agriculture in recent years has contributed to producers' awareness. So, the interest in alternative applications to the use of chemicals to control diseases that cause significant losses in the yield and quality of agricultural products has also increased. Considering the factors such as the fact that essential oils obtained from medicinal and aromatic plants do not have negative effects on human and environmental health, do not cause residues in agricultural products and are easy to obtain, many studies have shown that these oils can be used in the control of plant diseases. When the results obtained from the present study were evaluated, it was seen that the use of plant essential oils could be a highly effective method in the control of Pst 76. Various studies are revealing the antibacterial effect of these essential oils. However, there are significant differences in the antibacterial properties of the oils obtained from the plants collected from different localities. Therefore, it is important to investigate the components of essential oils and evaluate their antibacterial effect against pathogenic microorganisms. In this study, it was determined that the essential oils of *T. migricus* and *M. longifolia* had high antibacterial activity against Pst 76. Although promising results are obtained in this regard, the applicability of plant essential oils to tomato seeds, roots and green parts of the plant should also be tested in *in vivo* studies. In this respect, the results of the study will contribute to the development of antibacterial substances, which is an alternative method in the control of plant diseases and will constitute a step for future studies.

Conflict of Interest Declaration: The authors of the article declare that they do not have any conflict of interest.

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YAZAR ORCID NUMARALARI

Mesude Figen DÖNMEZ https://orcid.org/ 0000-0002-7992-8252

Işıl Temel https://orcid.org/ 0000-0001-5968-3609

Esat Temtek https://orcid.org/ 0000-0002-0149-129X

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