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Research Article/Araştırma Makalesi

Isolation and identification of *Xanthomonas citri subsp. malvacearum*, cotton bacterial blight disease agent and determination of the antibacterial activity of various plant essential oils

Pamuk bakteriyel yanıklık etmeni Xanthomonas citri subsp. malvacearum'un izolasyonu, tanılanması ve farklı bitki uçucu yağlarının antibakteriyel etkinliğinin belirlenmesi

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ABSTRACT

Cotton is one of the most important industrial crops produced in large areas in Turkey. Cotton bacterial blight disease agent *Xanthomonas citri* subsp. *malvacearum* (Syn. *Xanthomonas axonopodis* pv. *malvacearum*) is a seed-borne pathogen. Plant essential oils are natural antimicrobial compounds that have the potential to be used as an alternative to chemical control of seed-borne phytopathogens. In this study, the bacterial pathogen *X. citri* subsp. *malvacearum* was isolated from cotton plants showing typical disease symptoms during the 2023 growing season in Diyarbakır provinces and the antibacterial effect of essential oils obtained from 11 different medicinal plants (*Thymus serpyllum, Origanum syriacum, Thymus syriacus, Origanum onites, Cistus laden, Salvia aramiensis, Laurus nobilis, Hypericum perforatum, Rosmarinus officinalis, Origanum majorana* and *Thymbra spicata*) were determined by disk diffusion method. Bacterial isolates, obtained from cotton plants showing typical disease symptoms, were identified as *X. citri* subsp. *malvacearum* by biochemical and pathogenicity tests and also MALDI-TOF analysis. The essential oils used in the study showed antibacterial activity against the bacterial agent by forming an inhibition zone with a diameter of 6.33-46.33 mm in the nutrient media. *T. serpyllum* and *T. spicata* (43.33 mm), *O. syriacum* (43.00 mm), *T. syriacus* (38.33 mm) and *O. onites* (37.3 mm), were identified as the most effective essential oils respectively. The essential oils of *H. perforatum* (9.67 mm), *L. nobilis* (9.0 mm) and *S. aramiensis* (6.33 mm) showed relatively lower antibacterial activity against the disease agent. The results showed that essential oils of *Thymus* spp., *Thymbra* spp. and *Origanum* spp. have the potential to be used as an alternative to chemical control against seed-borne bacterial plant pathogens.

Key Words: Cotton, bacterial blight, *Xanthomonas,* essential oil, antibacterial

ÖZ

Pamuk Türkiye'de geniş alanlarda üretimi yapılan önemli endüstri bitkilerin başında gelir. Pamuk bakteriyel yanıklık hastalığı etmeni *Xanthomonas citri* subsp. *malvacearum* (Syn. *Xanthomonas axonopodis* pv. *malvacearum*) tohum kökenli bir patojendir. Bitki uçucu yağları tohum kökenli fitopatojen türlere karşı kimyasal mücadeleye alternatif olarak kullanım potansiyeline sahip doğal antimikrobiyal bileşiklerdir. Bu çalışmada, Diyarbakır ilinde 2023 yetiştirme sezonunda tipik hastalık belirtileri gösteren pamuk bitkilerinden *X. citri* subsp. *malvacearum* bakteriyel etmenin izolasyonu, tanılanması ve 11 farklı tıbbi bitkiden (*Thymus serpyllum, Origanum syriacum, Thymus syriacus, Origanum onites, Cistus laden, Salvia aramiensis, Laurus nobilis, Hypericum perforatum, Rosmarinus officinalis, Origanum majorana* ve *Thymbra spicata*) elde edilen uçucu yağların antibakteriyel etkisi disk difüzyon yöntemi ile belirlenmiştir. Tipik hastalık belirtileri gösteren pamuk bitkilerinden izole edilen izolatlar biyokimyasal ve patojenite testleri ve MALDI-TOF ile tür düzeyinde *X. citri* subsp. *malvacearum* olarak tanılanmıştır. Çalışmada kullanılan bitki uçucu yağları besi yerinde 6.33-46.33 mm çapında engelleme zonu oluşturmak suretiyle bakteriyel etmene karşı antibakteriyel etkinlik göstermiştir. En etkili uçucun yağlar sırasıyla *T. serpyllum* ve *T. spicata* (43.33 mm), *O. syriacum* (43.00 mm), *T. syriacus* (38.33 mm) ve *O. onites* (37.3 mm) olarak belirlenmiştir. *H. perforatum* (9.67 mm), *L. nobilis* (9.0 mm) ve *S. aramiensis* (6.33 mm) bitki uçucu yağları hastalık etmene karşı nispeten daha düşük oranlarda antibakteriyel etkinlik göstermiştir. Elde edilen sonuçlar *Thymus* spp., *Thymbra* spp. ve *Origanum* spp.'ye ait bitki uçucu yağlarının tohum kökenli bitki patojeni bakteriyel hastalık etmenine karşı kimyasal mücadeleye alternatif mücadele yöntemi olarak uygulanma potansiyeline sahip olduğunu göstermiştir.

Anahtar Kelimeler: Pamuk, bakteriyel yanıklık, *Xanthomonas,* uçucu yağ, antibakteriyel

Introduction

Cotton (*Gossypium hirsutum*) is a significant industrial crop cultivated in various regions worldwide, contributing to substantial agricultural and economic potential in its producing countries. In Turkey, extensive cultivation of cotton plants in the Aegean, Mediterranean, South-eastern, and Eastern Anatolian regions is often plagued by fungal and bacterial diseases, leading to considerable losses in yield and quality. Bacterial angular leaf spot and boll rot, caused by *Xanthomonas citri* subsp. *malvacearum* (ex Smith) Schaad *et al*. (formerly known as *Xanthomonas axonopodis* pv. *malvacearum*) is one of the prominent diseases that affect cotton plants (Verma and Singh, 1974). The pathogen typically spreads through infected seeds in cotton bolls, giving rise to various disease symptoms in the affected plants, including seedling blight, angular leaf spots, vein necrosis, black arm, and boll rot (Hillocks, 1981; Shenge 2001; Delannoy et al., 2005; Anmod et al., 2022). The manifestation of disease symptoms leads to a reduction in chlorophyll content in leaves and stems, ultimately causing significant damage to photosynthetic activity, yield, and fibre quality (Meshram and Raj, 1988). Yield losses in cotton were previously reported to range from 10% to 30%, reaching 50% or more in cotton grown in Africa or Asia (Verma, 1986; Thaxton and El-Zik, 2001; Mishra and Krishna, 2001; Delannoy et al., 2005; Raghavendra et al., 2009). While losses are less pronounced when disease symptoms are confined to leaves, they can escalate to 90% when stem lesions develop (Singh et al., 2007). Under field conditions, bacterial blight can cause yield losses up to 50% (Watkins, 1981).

The bacterial blight disease caused by *X. citri* subsp. *malvacearum* has increasingly become a global impediment to cotton production. The most effective control approach against this disease is generally acknowledged to be the utilization of resistant cotton varieties. Geographical origins of cotton species exhibit variations in susceptibility to *X. citri* subsp. *malvacearum*, ranging from highly susceptible to highly resistant. It has been reported that resistance in cotton plants against *X. citri* subsp. *malvacearum*, with its broad host range, can be rapidly overcome (Verma, 1986; Wallace and El Zik, 1990; Hillocks, 1992; Shelke et al., 2012). Hunter et al. (1968) identified 19 physiological races of the pathogen using ten different cotton varieties, while Verma and Singh (1974) expanded this number to 32 races using only seven cotton varieties. The distribution of races varies from country to country (Oliveira et al., 2011). Different races of the pathogen (such as races 1, 2, 5, 6, 8, 12, 13, 16, and 18) have been reported to cause diseases in various cotton varieties grown in Australia, India, the United States, Nigeria, Zimbabwe, Africa, and Nicaragua (Ajene et al., 2014; Anmod et al., 2022). In addition to the six previously identified races (race 1, 2, 8, 21, 26, and 32) on different cotton varieties grown in Syria, four new races (race 3, 4, 11, 28) have been described (Abdo-Hasan, 2008). Epidemiological studies conducted in Africa and other regions of the world have reported that some highly virulent (HV) races of the pathogen can overcome resistance in cotton varieties (El-Zik and Thaxton, 1994). Regarding the presence of the pathogen, limited studies have been conducted in Turkey on race characteristics, and the reactions of cotton varieties to the pathogen (Türkmenoğlu, 1969; Zachowski et al., 1989). The disease has recently re-emerged in the cotton cultivation areas in the South-eastern Anatolian Region of Turkey.

The utilization of resistant varieties against seed-borne bacterial pathogens presents a practical, environmentally friendly, and economical solution. However, there is a lack of highly resistant cotton varieties with high yield against cotton bacterial blight disease (Khan, 1996; Mondal et al., 2001). The most common method in combating plant diseases involves the use of chemical pesticides (Burr, 2001). In the control of cotton bacterial blight disease, some countries widely employ fungicides such as copper, Dithane M-45, and sulphur. However, it has been reported that systemic infection by the seed-borne pathogen does not yield the expected success (Sarker et al., 2017). The extended utilization of chemical pesticides may culminate in the attainment of effectiveness thresholds for these products, thereby yielding inadequate improvements in crop yield. Additionally, the over application of chemical pesticides can engender adverse, and frequently irreversible, alterations in agricultural ecosystems. (Zenelt et al., 2021). In the current era of heightened awareness of sustainability and the realization that soil is not an unlimited production resource, the adoption of good agricultural practices minimizing chemical use has become crucial (Eryılmaz et al., 2019). In this context, the use of volatile oils and extracts obtained from plants, which are naturally present, do not pose a risk of spreading toxic substances, rapidly decompose without causing environmental pollution, and do not carry residue risks, is seen as a promising alternative in the fight against plant diseases (Raghavendra et al., 2009; Sertkaya et al., 2010; Mbega et al., 2012; Atay and Soylu, 2022; Atay and Soylu, 2023).

The antibacterial potentials of essential oils of medicinal plant species, which belong to *Thymus, Mentha, Achilla, Artemisia*, and *Salvia* genus, were previously reported to poses against certain phytopathogenic bacterial disease agents (Daferera et al., 2003; Soylu et al., 2009; Mengulluoglu and Soylu, 2012; Küçükbay et al., 2014; Ünlü and Elçi, 2019; Bozkurt et al., 2020; Ghavam et al., 2020; Dönmez et al., 2020; Orzali et al., 2020; Temtek, 2021). Among the plants used in antimicrobial studies, Thyme species stand out with their widespread distribution, economic significance, and health-related properties. The antimicrobial activities of plant extracts and volatile oils from these species are economically important and play a crucial role in disease control methods against important disease agents (Regnault-Roger et al., 2004; Pavela, 2006; Türkmen et al., 2022). Literature reviews have indicated that very few studies were conducted on the antibacterial activity of plant essential oils and extracts against the cotton bacterial blight disease agent (Kızıl et al., 2005; Naqvi et al., 2022).

In this study, the essential oils of different plant species (*Thymus serpyllum, Origanum syriacum, Thymus syriacus, Origanum onites, Cistus laden, Salvia aramiensis, Laurus nobilis, Hypericum perforatum, Rosmarinus officinalis, Origanum majorana,* and *Thymbra spicata*) were investigated for their antibacterial effects against the seed-borne plant pathogen *Xanthomonas citri* subsp. *malvacearum*.

Materials and Methods

Isolation and identification of the bacterial pathogen

The bacterial pathogen responsible for the disease was isolated from the leaves and unopened cotton bolls exhibiting typical disease symptoms, sent for diagnosis from Bismil district of Diyarbakır province to the Bacteriology Laboratory of the Department of Plant Protection at Mustafa Kemal University, Faculty of

Agriculture, Hatay (Figure 1). Visibly water-soaked lesion bearing green leaves were initially subjected to surface disinfection with 5% sodium hypochlorite for 2-3 minutes, followed by three rinses with sterile water. After surface disinfection, the tissues were macerated in sterile 50 mM $MgCl_2$ solution for 30 minutes and then spread onto King B (KB) Agar medium. The petri dishes were incubated at 28°C for 2 days. Bacterial isolates were also obtained directly from the water-soaked lesions on bolls, after surface disinfection with 70% ethanol, using the tissue imprinting method on King B (KB) agar medium (Aktan and Soylu, 2020). Pure isolates were obtained by transferring bacterial isolates from typical yellow colonies grown on KB agar to fresh KB agar plates. After applying classical biochemical tests (gram staining, oxidase test, motility, sporulation, pectolytic activity, gelatine liquefaction, KOH test, catalase tests, etc.), the species identification of the isolates was confirmed using MALDI-TOF MS (Bruker Daltonics GmbH, Bremen, Germany) methods (Lelliot and Stead, 1987; Schaad et al., 2001; Aktan and Soylu, 2020). The concentration of the bacterial suspension of obtained isolates was adjusted to 10^6 cfu ml^{-1} by dilution, and pathogenicity tests were conducted on cotyledon leaves of three-week-old healthy cotton seedlings, including hypersensitive reaction (HR) tests on tobacco leaves. As a control, the cotyledon leaves of healthy seedlings were treated with sterile water. Inoculated plants were covered with transparent bags for 1 day in growth chambers at 26°C with high relative humidity, and then the covers were opened, and disease symptoms were daily monitored for 5 days.

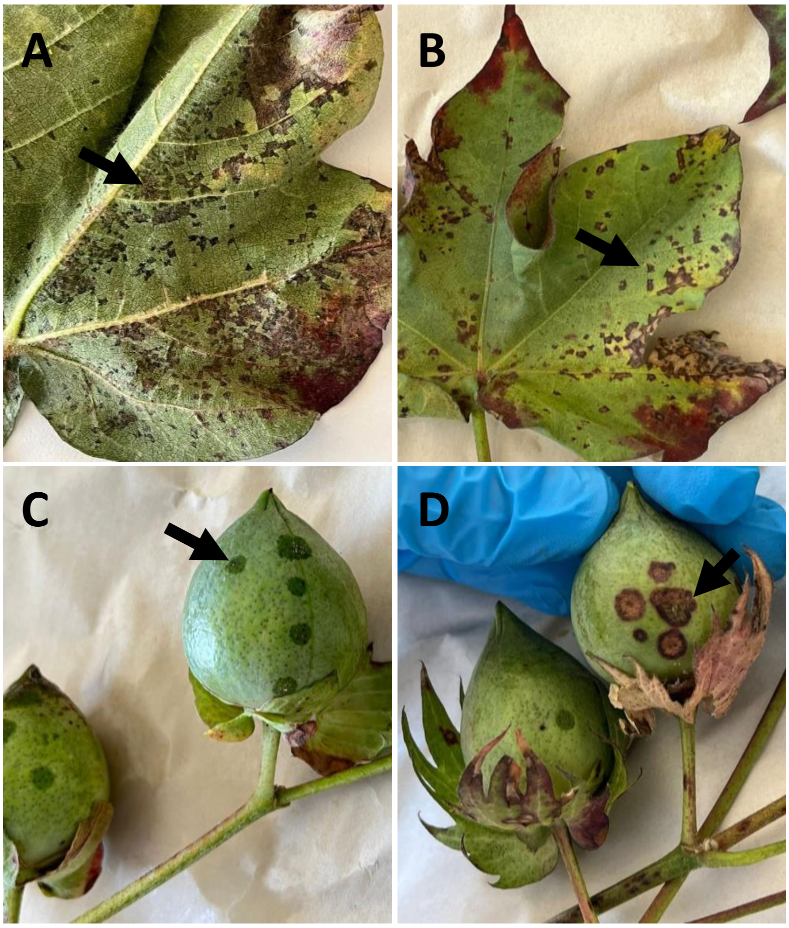

Figure 1. Typical symptoms (arrow) of *Xanthomonas citri subsp. malvacearum* on cotton leaf (A,B) and boll (C,D)

Determination of the antibacterial activity of essential oils

The essential oils used in this study were obtained from existing essential oil stocks with previously demonstrated antimicrobial efficacy against various plant pathogenic bacteria and fungal agents in the Phytopathology Laboratory of the Department of Plant Protection at Mustafa Kemal University Faculty of Agriculture. The antibacterial effect of the essential oils used in the study was assessed using the disk diffusion method (Soylu et al., 2022). A bacterial suspension of the virulent bacterial isolate, prepared in sterile water with a concentration of OD_{620}=0.12, was spread on King B (KB) agar medium using a spreader at a concentration of 10^8 cells/mL. Subsequently, sterile antimicrobial sensitivity test disks (6 mm diameter) were placed at the centre of each petri dish, and 5 µl of essential oil was added to each disk. To prevent the evaporation of essential oils from the edges of petri dishes, dishes were wrapped with double-layered Parafilm, followed by incubation at 26°C for 2 days. Control plates were prepared using disks with sterile distilled water (Bozkurt et al., 2020). The antibacterial effect of the essential oils was evaluated by measuring the diameters of the inhibition zones formed beneath and around the disks after the removal of the disks (Figure 2).

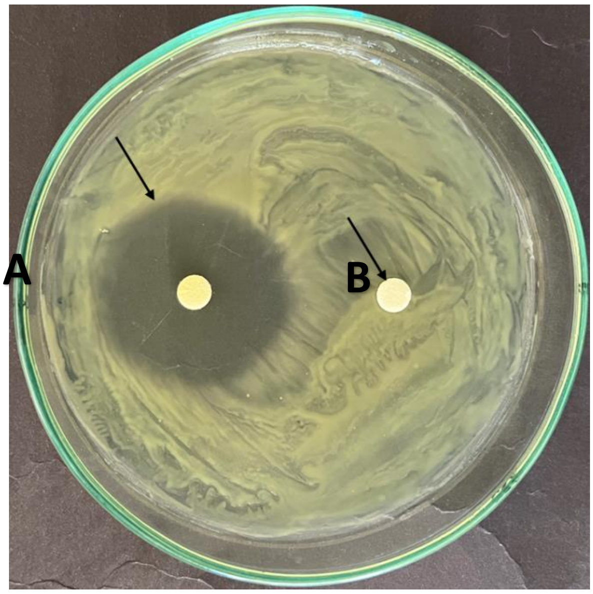

Figure 2. (A) Inhibitory effect of *Thymus serpyllum* essential oil, the most effective of the essential oils tested, on the growth of *Xanthomonas citri* subsp. *malvacearum*. The transparent inhibition zone (arrow) around the antimicrobial susceptibility test disk containing 5 µl of the essential oil is clearly visible. (B) Indicates the absence of any inhibition zone around the disk containing sterile medium.

Statistical analysis

In vitro antibacterial efficacy experiments were conducted for each essential oil according to a Randomized Complete Block Design in at least three different petri dishes, and the trial was repeated at two distinct time points. The values obtained in the experiments were subjected to analysis of variance (ANOVA) using the SPSS statistical program (SPSS Statistics 17.0). The differences between applications were compared using the Duncan Multiple Comparison Test (P≤0.05).

Results and Discussions

Isolation and identification of the pathogen

The causal agent of the disease was isolated from diseased plants sent for diagnosis by cotton growers in Bismil district of Diyarbakır province in September 2023 to the Department of Plant Protection, Faculty of Agriculture, Hatay Mustafa Kemal University (Figure 1). Isolates, exhibiting clear yellow-coloured, sharply defined, nonfluorescent colonies on King B medium, were purified from these colonies. Biochemical tests confirmed the isolates as gram-negative with positive reactions in starch hydrolysis (pectolytic activity), catalase, oxidase, and gelatine liquefaction tests. All representative isolates demonstrated an extreme hypersensitivity reaction (HR) in the form of tissue collapse 48 hours post-inoculation on tobacco leaves. Sprayed cotton cotyledon leaves showed water-soaked symptoms with a diameter of 2-4 mm five days after inoculation. The results of biochemical and pathogenic tests for the pathogen were consistent with previous study findings (Schaad et al., 2001).

Recent advancements in bacterial diagnostics have seen the increasing use of MALDI-TOF MS, a next-generation method, as an alternative to chromatographic and DNA-based molecular methods for identification of plant pathogenic bacterial and fungal disease agents (Pulcrano et al., 2012; Uysal et al., 2019; Aktan and Soylu, 2020; Bozkurt et al., 2020; Soylu et al., 2023). The diagnostic results obtained through biochemical tests were also confirmed by MALDI-TOF MS, which has become a widely adopted method in recent years. The bacterium isolates obtained from infected cotton leaves and bolls, as well as those from pathogenicity tests, exhibited high similarity index values, matching reference isolates in the MALDI-TOF database such as *X. citri* pv. *malvacearum* DSM 3489, 1220, and *X. axonopodis* pv. *malvacearum* CFBP-2530 with values ranging from 2.066 to 2.175 (Figure 3).

Analyte Name: E1
Analyte Description:
Analyte ID: YY
Analyte Creation Date/Time: 2023-09-08T03:48:21.142
Applied MSP Library(ies):
Applied Taxonomy Tree: Projects, Bruker Taxonomy, Taxonomy

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Xanthomonas citri pv malvacearum DSM 3849 DSM	2.175	346
2 (++)	Xanthomonas citri pv malvacearum DSM 1220 DSM	2.149	346
3 (++)	CFBP-2530_Xanthomonas axonopodis pv. malvacearum_20160825	2.066	100134258

Figure 3. MALDI-TOF identification result of *X. citri* subsp. *malvacearum* MKUBK-Xcm YY isolate obtained from cotton bolls showing typical disease symptoms and used in pathogenicity test

Antibacterial activity of different plant essential oils against Xanthomonas citri pv. *malvacearum*

The antibacterial activities of essential oils were investigated by measuring the diameters of inhibition zones formed around the disks (Figure 2). The results obtained are presented in Table 1. Significant differences in the antibacterial effects of essential oils obtained from different plants against the bacterial isolate.

Essential oils from *Thymus, Thymbra*, and *Origanum* spp exhibited higher antibacterial activity compared to other essential oils used in the experiment (Table 1). *In vitro* studies demonstrated that *T. serpyllum* (43.33 mm), *T. spicata* (43.33 mm), and *O. syriacum* (43.0 mm) exhibited the highest antibacterial activity against *X. citri* pv. *malvacearum*, and due to the similarity in their antibacterial effects, these oils were appeared in the same statistical group. These essential oils were followed by *T. syriacus* (38.33 mm) and *O. onites* (37.33 mm). The lowest antibacterial activities were displayed by the essential oils of *S. aramiensis* (6.33 mm) and *L. nobilis* (9.00 mm) (Table 1).

Table 1. Antibacterial activity[a] of plant essential oils on the development of the bacterial blight disease agent *Xanthomonas citri* subsp. *malvacearum*

Plant essential oils *Bitki Uçucu Yağları*	**Diameter of inhibition zones (mm)** *Engelleme zon çapıları (mm)*
Thymus serpyllum	43.33 (±1.02)[f]
Thymbra spicata	43.33 (±0.88)[f]
Origanum syriacum	43.0 (±2.08)[f]
Thymus syriacus	38.33 (±2.02)[e]
Origanum onites	37.33 (±1.20)[e]
Rosmarinus officinalis	14.67 (±0.33)[d]
Origanum majorana	14.67 (±0.33)[d]
Cistus laden	11.67 (±0.33)[cd]
Hypericum perforatum	9.67 (±0.33)[c]
Laurus nobilis	9.0 (±0.57)[bc]
Salvia aramiensis	6.33 (±0.33)[b]
Kontrol	0.0[a]

[a] The obtained values represent the average diameter of bacterial zones (mm) measured in three different petri dishes. Similar lowercase letters next to the mean values within the column indicate that there is no statistically significant difference between the essential oils (Duncan's Multiple Range Test, $P < 0.05$).

The findings of this study clearly demonstrate the potential use of plant essential oils as antibacterial compounds against the seed borne pathogen causing leaf and boll blight in cotton. While the antibacterial effects of essential oils have been extensively researched against microorganisms causing issues in human and food contexts, limited number of studies exist in the literature regarding the determination of antibacterial activities of plant extracts and essential oils against plant pathogenic bacterial disease agents. *Thymbra spicata* var. *spicata* essential oil has been investigated for its antibacterial effects on economically important plant pathogenic bacteria (Basim et al., 2000). The antibacterial effects of the essential oil obtained from various plant species of Lamicaeae family (such as *Ocimum cillatum, Lippia gracilis* and those obtained from interspecific hybrids) were also evaluated against different plant bacterial disease agents belonging to *Ralstonia, Pseudomonas, Xanthomonas, Brenneria, Pantoea, Agrobacterium* and *Rhodococcus* genus (Moghaddam et al., 2014; Schollenberger et al., 2018; da Silva et al., 2019).

In contrast to numerous studies investigating the antibacterial activity of essential oils against different *Xanthomonas* spp., there is a limited number of studies focusing on *X. citri* subsp. *malvacearum*, the causal agent of cotton bacterial blight disease. Previous research by Kızıl et al. (2005) reported the ineffectiveness of essential oils from cumin (*Cuminum cyminum*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*), spearmint (*Mentha spicata*), and anise (*Pimpinella anisum*) against *Xanthomonas campestris* pv. *malvacearum*. They found that coriander (*Coriandrum sativum*) and hyssop (*Hyssopus officinalis*) essential oils were effective against the pathogen. Kızıl and Uyar (2006) indicated that essential oils from *Thymbra spicata, Satureja hortensis, Origanum onites,* and *Thymus kotschyanus* had lower effectiveness against *X. campestris* pv. *malvacearum* compared to other tested plant pathogenic species. Shirsat (2008) reported that extracts from *Terminalia thorelii* leaves and fruits exhibited a high inhibition zone against *X. axanopodis* pv. *malvacearum* isolated from cotton plants.

A recent study by Mangalagiri et al. (2021)

investigated the antibacterial activity of essential oils from various plant species, including *Cymbopogon flexuosus, C. martini, Eucalyptus citridora, Tagetus minuta, Pelargonium sp., C. winterianus,* and *Mentha arvensis*, against *X. citri* subsp. *malvacearum*. The results indicated varying levels of antibacterial efficacy among the tested essential oils, with *Cymbopogon flexuosus, C. martini*, and *Eucalyptus citridora* showing particularly high antibacterial activity. Similarly, Naqvi et al. (2022) investigated the antibacterial properties of essential oils from different plant species, such as *Syzygium aromaticum, Curcuma longa, Moringa oleifera, Azadirachta indica, Mangifera indica, Mentha piperita, Aloe vera, Syzygium cumini, Citrus limon*, and *Prosopis juliflora*, against *X. citri* subsp. *malvacearum*. Among the tested essential oils, *Mentha piperita, Syzygium cumini, Citrus limon, Moringa oleifera*, and *Syzygium aromaticum* were identified as the most effective essential oil bearing plants, forming inhibition zones ranging from 8.41 to 8.58 mm.

The essential oils used in this study from *Origanum, Thymbra*, and *Thymus* plant species were previously determined to contain high levels of carvacrol, thymol, and γ-terpinene (Bozkurt et al., 2020; Kara et al., 2022). Previous studies showed that these oils and their main components trigger rapid cell breakdown in fungal and bacterial pathogens (da Silva et al., 2019; Liu et al., 2019; Churklam et al., 2020; Kachur and Suntres, 2020). Antibacterial efficacies of plant extracts and essential oils from savory (*Satureja hortensis*), thyme (*Thymus spicigera*), oregano (*Origanum onites*), and round-leaved oregano (*Origanum rotundifolium*) were investigated against *Xanthomonas axonopodis* pv. *vesicatoria*. Essential oils exhibited greater antibacterial activity compared to plant extracts (Dadaşoğlu et al., 2016).

Furthermore, antibacterial activity of essential oils of *Helichrysum italicum, Inula graveolens, Cistus creticus, Echinacea purpurea*, and *Hypericum perforatum* were tested against potato soft rot disease agent *Pectobacterium carotovorum* subsp. *carotovorum* and halo blight of beans disease agent *Pseudomonas syringae* pv. *phaseolicola* (Gümüş et al., 2022). Amongst the tested essential oils, *Hypericum perforatum* essential oil demonstrated the highest antibacterial activity against *P. carotovorum* subsp. *carotovorum* in comparison to *P. syringae* pv. *phaseolicola*.

In a study conducted in Sudan, the inhibitory effects of chitosan compounds at various concentrations (1, 3, 5, and 10 mg/ml), antagonist bacterial isolate *Paenibacillus* spp., and water extracts and oils from the barks of certain plants (*Syzygium aromaticum, Nigella sativa, Trigonella foenum graecum*, and *Allium sativum*) and camel urine were evaluated for their effectiveness in inhibiting the *in vitro* growth of *X. axonopodis* pv. *malvacearum*. All tested chitosan concentrations, except 1 mg/ml, exhibited inhibitory effects against bacteria. Chitosan at 10 mg/ml showed the highest inhibitory effectiveness with a 14 mm inhibition zone, followed by 5 mg/ml and 3 mg/ml, which produced 12 mm and 11 mm inhibition zones, respectively.

Conclusions

This study focuses on isolating and identifying the cotton bacterial blight disease agent, *Xanthomonas citri* subsp. *malvacearum*, while also exploring the antibacterial activities of various plant essential oils. The investigation aims to contribute to the understanding of potential botanical agents against bacterial pathogens, thereby offering insights into novel strategies for disease management in agriculture. In consideration of the absence of cotton varieties resistant to bacterial blight disease, the potential use of plant essential oils exhibiting antibacterial activity against *Xanthomonas citri* subsp. *malvacearum* becomes particularly intriguing. The results obtained from this study demonstrate the antibacterial potential of essential oils derived from plants such as *Thymus* spp., *Thymbra* spp., and *Origanum* spp. against *X. citri* subsp. *malvacearum*. This study contributes to the growing body of research on the antibacterial activity of plant essential oils against *Xanthomonas citri* subsp. *malvacearum*, providing valuable insights into potential alternatives for the control of cotton bacterial blight disease. These findings open avenues for further research on the development of sustainable and environmentally friendly strategies for managing bacterial diseases in agriculture. However, for a more comprehensive understanding of the antibacterial effects of essential oils under *in vivo* conditions and their economic feasibility, further detailed investigations are warranted in future studies. This highlights the significance of exploring the *in vivo* antibacterial efficacy of these essential oils and assessing their economic viability.

Conflict of interest:

The authors declare that they have no conflict of interest.

Author contributions:

Setting up the experiments, methodology, interpretation of results, and writing of the original draft were provided by Soner Soylu; Merve Kara, Yusuf Gümüş performed the methodology including isolation, identification, pathogenicity and antibacterial assays; Emine Mine Soylu contributed to the methodology, reviewed and edited the manuscript. All authors critically reviewed and approved the final manuscript.

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