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Evaluation of the *In Vitro* Fungicidal Activity of Summer Savory and Lavender Essential Oils Against *Fusarium solani*

Abstract

Fusarium spp is one of the major phytopathogenic microfungus strains causing severe losses in many economically cultivated crops. The soil-borne pathogen *Fusarium solani* has historically been considered a serious agent across the globe, causing vascular wilt and root rot in agroeconomic crops and eventually leading to plant death. Three different concentrations (1 µl, 2 µl, and 4 µl) of essential oils (EO) extracted from lavender (*Lavandula officinalis* L.) and summer savory (*Satureja hortensis* L.) plants were mixed separately with PDA medium, and their antifungal effect against *F. solani* was investigated *in vitro*. When the results of the experiment were evaluated statistically, it was determined that the increasing concentrations of summer savory essential oil repressed the mycelial growth of the fungus, while lavender oil did not have any positive or negative effects. The inhibition activity of summer savory EO on *F. solani* was calculated as 43, 53, and 90% at the concentrations of 1, 2, and 4 µl, respectively. In this study, it was found that summer savory EO, even at a minimum dose, had a negative effect on agriculturally important wilt agent. In this context, it can be asserted that summer savory EO is a promising natural substance for the development of various fungicide solutions to prevent fungal diseases caused by vascular origin.

1. INTRODUCTION

Fusarium solani, a well-known opportunistic agent that can be found in every part of the world with varying subspecies in saprophytic and parasitic forms, is a cause of fungal infection in animals and humans (Brayford, 1993; O'Donnell et al., 2015; Tuxbury et al., 2014). Surviving in the soil for years, the agent can be spread over long distances through wind, agricultural equipment, and irrigation. Host plants infected with different strains of fungus often exhibit irreversible severe symptoms such as wilting, die back, root and fruit rot (Al- Sadi et al., 2014; Bueno et al., 2014; Yaseen and D'Onghia, 2012). With its wide host range on a global scale, *F. solani* has been reported in many countries including Turkey, Czech Republic, Japan, India, Iran, the United States, Iraq, Malaysia, and Germany (Kurt et al., 2020; Ondřej et al., 2008; Sugiura et al., 2003; Ramteke et al., 2019; Abedi-Tizaki et al., 2016; Romberg and Davis, 2007; Hafizi et al., 2013; Farahani-Kofoet et al., 2020). Essential oils, also known as volatile oils, are natural oily substances that are obtained from the leaves, fruits, barks or roots of aromatic plants. They are liquid at room temperature, easily crystallized, usually colorless or light yellow and have a strong odor. Distillation, extraction, and pressing are commonly used methods to obtain these oils. Until today, more than 2000 chemical components have been reported to be present in EOs, mainly terpenes, phenylpropanes and compounds containing small amounts of alcohols, aldehydes, esters, phenols, nitrogen, and sulfur (Çelik and Çelik, 2007; Kılıç, 2008). Due to the physiological effects of the active compounds they have at the cellular level, EOs are widely used singly or in a mixed manner in the fields of aromatherapy and industry. One of the most researched aspects of EOs is related to their antimicrobial and insect-repellent activities. The effectiveness of these oils varies widely depending on the type and amount of the active ingredients they contain (Ceylan, 1983; 1987; Linskens and Jackson, 1997; Bayaz, 2014; Limoncu et al., 2017). Previous studies in the literature provide a wide-ranging documentation on the antimicrobial functionality of EOs and their contents. The antagonistic effect of EOs against many viral, bacterial, and fungal pathogens of plant and animal origin has been well-investigated. The antibacterial effect of EOs on many microorganisms, including Gram (-) and Gram (+) bacteria, has been extensively covered in the literature (Nostro et al., 2000; El-Shazly et al., 2002; Al-Howiriny, 2003; Sartoratto et al., 2004; Chouhan et al., 2017; Gadisa et al., 2019; Man et al., 2019; György et al., 2020). Some other studies have focused on the antiviral activities of EOs. Bammi et al. (1997) and Bishop (1995) reported that EOs were effective inhibitors against the replication of Epstein-Barr virus (EBV) and *Tobacco mosaic virus* (TMV), respectively. Today, there is a comprehensive documentation on the inhibitory effect of EOs against SARS-CoV-2, Influenza A/WS/33, and herpes simplex virus type 1 (HSV-1) infections. The antifungal properties of EOs vary depending on the source and part of the plant, growing environment, climate, and quantity. Many plants in a broad spectrum, including sage, laurel, cinnamon, thyme, lemongrass, nettle, basil and geranium, have natural fungistatic and/or fungicidally effective essential oil compounds that tend to reduce the growth of various fungal pathogens (Angioni et al., 2006; Pinto et al., 2007; Guynot et al., 2003; Wang et al., 2009; Rasooli et al., 2006; Tzortzakis et al., 2007; Sahin et al., 2004; Nguefack et al., 2009). Increasing resistance to fungicides in plants has led to the need for the development of new antifungal strategies. Thanks to their content of terpenoids, terpenes, and aromatic compounds, EOs are one of the main candidates for the development of antifungal preparations (Bassole and Juliani, 2012). In this study, we investigated the anti-fungal activities of lavender and summer savory essential oils against *F.*

solani, one of the main fungal agents that infect economically important crop plants, in increasing doses under laboratory conditions.

2. MATERIALS and METHODS

2.1. Experimental fungal isolate and source of essential oils

From previous studies, a fungus (*F. solani*) isolated from the diseased parts of bean plants grown in Bingol province was used as the experimental pathogen (Sarac Sivrikaya et al. 2021). The essential oils of lavender and summer savory plants used in the experiments were extracted by a commercial enterprise through the water vapor distillation method using the Clevenger apparatus. The essential oils were kept in dark-colored and tightly closed bottles at + 4 °C until use.

2.2. Culture media for inhibition tests

A common medium Potato Dextrose Agar (PDA) was used for the purification and growth of the fungal pathogen. The prepared medium was sterilized, and approximately 20 ml of liquid medium was transferred to glass petri dishes. Immediately, 1 µl, 2 µl, and 4 µl of essential oils were added to the PDA media to form application groups. Each application was carried out in 3 repetitions, as well as the control groups with only the pathogen and PDA medium without essential oil.

2.3. Preparation of pathogen fungus for inoculum

F. solani was inoculated into the prepared media and incubated for 7 days at 25 ° C for growth. After fungal growth, 8 mm diameter fungal discs were cut out from the culture medium to test the antifungal activity of the oils.

2.4. Assessment of antifungal activity and statistical analysis

To determine fungal inhibition, the removed fungal discs were placed in the center of the petri dishes containing PDA medium, and the petri dishes were covered with parafilm to prevent a possible contamination. After 7 days of incubation at 24 ± 1°C, the diameters of fungal colonies were measured vertically and in separate directions ignoring 8 mm fungal disc diameters, photographed and recorded (Benjilali *et al.*, 1984). Percent inhibition was calculated according to the formula reported Deans and Svoboda (1990).

$$\text{Inhibition rates } (\%) = \frac{\mathrm{gc-gt}}{gc} \times 100$$

Analysis of variance (ANOVA) was used to determine the differences between the treatment groups. It was tested whether the variances of the data were homogeneous, and the means were compared using the DUNCAN test. The statistical significance was set at P<0.05.

3. RESULTS and DISCUSSION

F. solani is one of the most common fungus species in agricultural production areas worldwide (Saremi et al., 2011). Soil-borne pathogen attack causes plant death due to the development of root rot complex on field and horticultural crops, resulting in serious yield losses. Recently, it has been reported by most researchers that essential oils obtained from various medicinal and aromatic plants can be used as natural fungicides in the control of some plant and bee diseases (Djihane et al., 2017; Tutun et al., 2018). Many plant species can synthesize species-specific EOs in different amounts at different developmental stages in different tissues. The biological activities of essential oils vary considerably depending on the genotype and ecological environment of the plant and the test method and microorganism used (Rota et al., 2004; Yeşil Çeliktaş et al., 2007). Thanks to their chemical composition, essential oils obtained from plants such as sage, rosemary, laurel, oregano, clove, thyme, cumin, lavender, lemongrass, marjoram, mint, lemon balm, eucalyptus, fennel, and cinnamon have been previously used to control plant fungal diseases (Soylu et al., 2005; Lee et al., 2007). In this study, we investigated the antifungal effects of lavender and summer savory EOs containing chemical aromatic compounds with antimicrobial properties. Under *in vitro* conditions, the inhibitory effect of three different doses (1 µl, 2 µl, 4 µl) was examined against mycelial growth of *F. solani*, and their percentage inhibition values were determined (Table 1). Summer savory EOs clearly suppressed the mycelial growth of the test pathogen *F. solani*, but lavender EOs did not generate any noticeable results. Summer savory oil showed a biological activity at increasing doses, which was confirmed both statistically and by the radial inhibition zone (Figure 1).

Table 1. *In vitro* antifungal activity of *S. hortensis* and *L. officinalis* essential oils against *F. solani*

Extracts types	Dose	Inhibition (%)	Mean growth diameter (mm)
S. hortensis	4 µl	90.0	10,75[a*]
	2 µl	53.0	33,25[b]
	1 µl	43.0	39,00[c]
L. officinalis	4 µl	3.0	60,75[d]
	2 µl	3.0	61,00[d]
	1 µl	3.0	62,00[de]
NC**	-	0.0	62,75[e]

*The difference between the means with the same letters in the same column is not statistically significant ($p<0.05$) **NC: no-treatment control

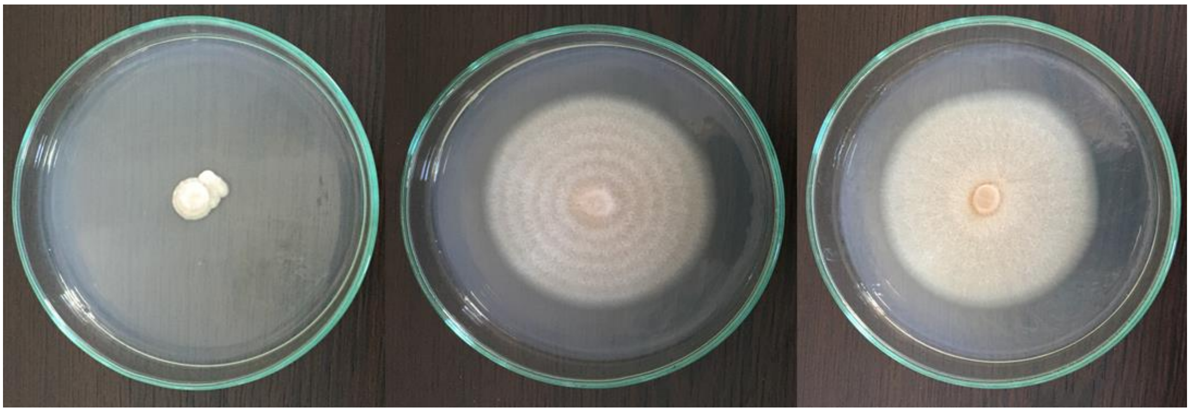

Figure 1. The anttifungal activities of 4 µl of *S. hortensis* and *L. officinalis* EOs against *F. solani* isolated from *P. vulgaris* in glass petri dishes containing PDA medium. A: *S. hortensis* EOs treatment, B: *L. officinalis* EOs treatment; C: NC (No-treatment control)

Lavender, belonging to the Lamiaceae family, has two main important antimicrobial chemical compounds: linalyl acetate and linalool (Śmigielski et al., 2013). Due to their antibacterial, antifungal, and healing properties, EOs extracted from some species of genus Lavandula are one of today's popular topics. Lavender EOs have been extensively used as a strong antimicrobial agent to eliminate foodborne pathogens, such as *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus*, and *Listeria monocytogenes*, and *L. innocua* as well as other bacteria (*Clostridium perfringens, Pseudomonas aeruginosa, Proteus vulgaris, Enterobacter aerogenes*) (Dadalioglu and Evrendilek, 2004; Nikolis et al., 2014; Djenane et al., 2012; Varona et al., 2013; Viuda-Martos et al., 2011). Furthermore, some previous studies on lavandula EO focused on its inhibitory effect against human fungal strains including *Candida albicans* and *Aspergillus niger*, and while some others focused on controlling various phytopathogenic fungi (Tarek et al., 2014). Yohalem and Passey, (2011) reported that *L. angustifolia* and *L. x* *intermedia* EOs were toxic to the wilting agent *Verticillium dahlia* in the strawberry plant. The essential oil from *L. stoechas*, another species of the genus Lavender, was reported to have an effect as a biofungicide by negatively affecting the membrane integrity of the gray mold (*Botrytis cinerea*) and wilting pathogen (*V. dahlia*) in tomato plants (Kadoglidou et al., 2011; Soylu et al., 2010). Additionally, a different study showed that EO from *L. stoechas* was effective against *Phytophthora infestans*, which is responsible for late blight disease in tomato (Soylu et al., 2006). In contrast, other reports showed that EO extracted from *L. angustifolia* had a moderate antifungal effect against *B. cinerea*, *Rhizopus stolonifer*, and *A. niger*, which are critical fungal pathogens in the postharvest stage of strawberries (Hadian et al., 2008). Similarly, Lopez-Reyes et al. (2010) showed that essential oil from *L. officinalis* exhibited a low toxicity against *B. cinerea* and *Penicillium expansum* fungal infection in postharvest apples, which is consistent with the results obtained for *L. officinalis* EO against *F. solani* in the present study. As can be seen in the abovementioned studies, lavender essential oils were reported to have a unique fungicidal activity against different fungi species depend on the lavender species from which they were extracted. But, the results of our study showed that lavender oil did not exhibit any statistically significant toxicity against soil-borne fungus *F. solani*, even in the increasing doses. In a previous study, the low antifungal efficacy of *L. hybrida* and *L. angustifolia* essential oils was attributed to the insufficiency of thymol and carvacrol components (Giordani et al., 2004). Possibly, the same applies to our study, and therefore, antifungal activity could not be determined at the doses used in the present study. A striking point to note in Figure 1 is that there is a minimal difference between the inhibition effects of the lavender essential oil applications. In this context, it is necessary to apply a higher concentration than we used in order to observe the inhibition zone against *F. solani* fungus. Al –Naser and Al- Abrass (2014) support this idea in that they used the same essential oil, but obtained by the GC-MC method, and found a broad spectrum fungistatic activity against *F. solani*, *F. oxysporum*, and *A. niger*, possibly due to the fact that they used higher amounts of essential oil (500, 1000, and 1500 µl) than ours (1, 2, and 4 µl). As a result, most reports have supported that lavandula EOs can be used, to some extent, as an alternative to the synthetic fungicides being used currently. The summer savory-associated antifungal outputs were reported to be more remarkable than the others, and several previous studies supported the potential antifungal activity of *Satureja hortensis*. We found that the test pathogen was more susceptible to summer savory EOs compared to lavender oil, even in minimal doses. Summer savory essential oil contains active ingredients with antimicrobial and food preservative properties, mainly phenol derivative carvacrol, cyclohexone, cymene, and thymol (Özkalp and Özcan, 2009). Şahin et al. (2003) tested hexane and methanol extracts of summer savory against 55 bacterial species, and 31 isolates of 1 yeast and 4 fungus species and reported that Hexane extracts were toxic to a wide range of test microorganisms, but both extracts did not display inhibition against phytopathogenic fungi *Alternaria alternata*, *A. flavus*, *Fusarium oxysporum*, and *Penicillium* spp, suggesting that EOs have more fungicidal activity than extracts. Like our study, Dikbaş et al. (2008) supported these results and reported that summer savory EOs reduced the development of fungal pathogens (*A. flavus*) *in vitro* even at low concentrations (25, 12.5, and 6.25 µl/mL). The results of our study for inhibition effect are supported by several previous studies, such as Özcan and Boyraz (2000) for six moulds (*F. oxysporum* f. sp. *phaseoli*, *Macrophomina phaseoli*, *B. cinerea*, *Rhizoctonia solani*, *A. solani*, and *A. parasiticus*), Usanmaz Bozhüyük *et al.* (2019) for eight *Fusarium* species (*F.*

avenaceaum, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. sambucinum*, *F. semitectum*, and *F. solani*), and Usanmaz Bozhüyük et al. (2015) for agricultural pathogenic fungus group (*Botrytis* sp., *F. equiseti*, *Nigrospora oryzae*, *P. capsici*, and *R. solani*). The fungicidal potential of essential oils has also been showed against human and plant fungal pathogens such as *A. citri*, *Alternaria* sp., *Bipolaris sorokiniana*, and *Acremonium sclerotigenum* (Yazdanpanah and Mohamadi, 2014; Mafakheri and Mirghazanfari, 2018).

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In conclusion, first of all, EOs are organic and eco-friendly substances compared to antifungal pesticides. Both our study and the current literature have proven that essential oils from lavender and summer savory have an antimicrobial efficacy and can be used as an anti-*fusarium* agent as an alternative to synthetic fungicides in the future. Therefore, in order to achieve a sustainable agriculture, these two effective essential oils could be evaluated as a potential resource in the management of main infectious fungal pathogens in plants causing damage to the agricultural ecosystem and economic loss.

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