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INVESTIGATION OF THE ANTIFUNGAL EFFECT OF *THYMBRA SPICATA* L. AND *ROSMARINUS OFFICINALIS* L. ESSENTIAL OILS ON *FUSARIUM OXYSPORUM* F.SP. *CUCUMERINUM* AND *FUSARIUM OXYSPORUM* F.SP. *MELONIS*

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Abstract

This study was carried out to determine the antifungal activity of *Thymbra spicata* L. and *Rosmarinus officinalis* L. plant essential oils. In this context, the antifungal effect of 0 (Control), 0.5, 1, 2, 4, 8 µlpetri^{-1} doses of plant essential oils on *Fusarium oxysporum* f. sp. *cucumerinum* (FOC) and *Fusarium oxysporum* f. sp. *melonis* (FOM) was identified. In order to determine the antifungal effect of essential oils, the filter paper adhered to the covers of the petri dishes was impregnated with the micropipette. Additionally, it was suggested that the plant essential oils inhibited the mycelial growth of FOM and FOC compared to the control. As a result of the study, 8 µlpetri^{-1} dose of *T. spicata* essential oil inhibited the mycelium growth of FOC and FOM by 100%. On the other hand, it was determined that 8 µlpetri^{-1} dose of *R. officinalis* essential oil inhibited the mycelium growth of FOC and FOM by 61.32% and 71.97%, respectively. In the study, LC_{50} and LC_{90} values were also calculated by carrying out a dose-effect study. The LC_{50} and LC_{90} values of FOM and FOC for *T. spicata*'nın essential oil were calculated as 0.58-1.26 µL and 0.46-1.07 µL, respectively, while the LC_{50} and LC_{90} values, of FOM and FOC for *R. officinalis* essential oil were calculated as 4.04-37.56 µL and 3.92-95.84 µL respectively. The *T spicata*, *R. officinalis* essential oils used in the experiment have a potential to be an alternative to fungus in controlling FOC and FOM diseases. It was concluded that the essential oils used in the experiment have a potential to be an alternative to synthetic pesticides in controlling diseases.

Keywords: Antifungal effect, *Thymbra spicata*, *Rosmarinus officinalis*, Essential oil

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1. Introduction

Fusarium oxysporum is a soil-based fungal pathogen. This disease causes vascular paleness and root rot in host plant by invading plant roots. The disease has more than 120 species-specific hosts. Two of the most important disease types of *Fusarium oxysporum* are F. *oxysporum* f. sp. *cucumerinum* Schlecht and *Fusarium oxysporum* f. sp. m*elonis* W.C. Snyder & H.N. Hans. An important disease in cucumber plant is Fusarium paleness caused by FOC. This fungus, which has the ability to infect the cucumber plant at any stage of growth, causes serious loss of crops in open field and greenhouse condition cucumber growing cultivation areas both Turkey and in the World (Yıldız and Delen, 1977; Jenkins, 1983; Vakalounakis, 1988; Yucel, 1994; Ozan and Askın, 2006). FOM is an important soil-borne fungal disease that causes large yield losses in melon production areas in the world and Turkey. FOM infects the root and the root throat of the melon plant, causing the plant to have paleness (Yucel et al., 1994; Erzurum et al., 1999; Schreude et al., 2000).

Fungicides have been used to control FOM and FOC diseases. The resistance of diseases against fungicides used, the negative effect by fungicides used for human and environment health have created important problems. For this reason, biological control methods of diseases seems alternative methods chemistry (Mares et al., 2004). It is acknowledged by the whole world that herbal products are less toxic to the environment and human health in controlling diseases (Lee et al., 2007). In particular, it was reported that essential oils exhibit good antifungal activity against various pathogens, both in vitro and in vivo conditions (Baruah et al., 1996; Sharma and Verma, 2004; Bayan et al., 2017).

T. spicata and *R. officinalis* are medical aromatic plants included in the Lamiaceae family. *T. spicata*, which is naturally occurring in Turkey, is a plant known by the local names of Karabas oregano, Black thyme, Zahter and its essential oil has antifungal and antibacterial effect (Bayan et al., 2017). *R. officinalis*, which is naturally occurring in Turkey, and local name called Kusdili, Hasalbal and Akpuren among the people is a 50-100 cm high perennial rosemary plant having a bushy appearance (Baytop, 1984). Scientific studies have revealed the antibacterial, antifungal and antiviral and immune system healing effects of rosemary (Centeno et al., 2010).

This study was carried out to determine the antifungal effect of *T. spicata* and *R. officinalis* essential oils under in vitro conditions against FOC and FOM diseases fungal pathogens agents that cause significant yield losses in cucumber and melon growing areas.

2. Materials and Methods

2.1. Collecting Plant Samples

T. spicata plant was collected from Kahramanmaraş province (Turkey), while *R. officinalis* was collected from Mersin (Turkey) from the naturally distributed areas.

2.2. Obtaining Essential Oils

Essential oils of plants were obtained by hydro-distillation using a Schilcher apparatus. The plant samples, purified water (1:10 w/v) was added and boiled for 2 hours (Telci et al., 2006). The obtained essential oils were kept at +4 ºC until used in the experiments.

2.3. Obtaining Fungus Cultures

The *Fusarium oxysporum* f.sp. *cucumerinum* and *Fusarium oxysporum* f. sp. *melonis* plant fungal pathogens, which were used in the experiments, were obtained from the stock culture in Phytopathology Laboratories of Ahi Evran University, Faculty of Agriculture Department of Plant Protection. Among these stock cultures, 7-day cultures developed in 90 mm-petri dishes containing Potato dextrose agar (PDA) at 25 ± 2 °C were used.

2.4. In Vitro Antifungal Effect of Essential Oils

Potato dextrose agar (PDA) prepared in 500 ml erlenmeyer were autoclaved and cooled to 40°C and transferred to 60 mm diameter petri dishes (10 ml each petri dishes). A piece of gum drying paper (5-mm-diameter) was glued on the covers of petri dishes to the center of the cover, then essential oils were dropped onto this piece of paper using a micropipette and the petri dishes were immediately covered and wrapped tightly with a parafilm (Önen et al., 2002). In the experiment, essential oils were administered at 0 (Control group), 0.5, 1, 2, 4 and 8 µlpetri^{-1} doses. Petri dishes were left for incubation for 1 week under 25 ± 2 °C conditions. Researches were carried out replicates 3 times with 2 repetitions. At the end of the period, mycelium growth values of diseases in petri dishes were measured with a compass. The inhibition percentages of the essential oil at different doses was calculated by comparing mycelium growth between control and administration doses (Pandey et al., 1982).

$$MGI = 100 \times (C - T)/C$$

MGI: Mycelium Growth Inhibition rate (%)

C: Radial mycelium growth in control

T: Radial mycelium growth in treatments

2.5. Statistical analysis

The LC_{50} and LC_{90} values of the essential oil were calculated by using SPSS 15 Probit in the experiment as well. The significance of the differences between the treatments in the experiments was determined by the analysis of variance (ANOVA) and the means were compared using the DUNCAN test. Statistical analyzes were carried out using SPSS program.

3. Results and Discussions

The antifungal effect of plant essential oils on the mycelium growth of FOM and FOC was determined. The effect of plant essential oils on mycelium growth of the pathogen increased depending on the increased dose. The mycelium growth of plant pathogenic fungi is given in Figure 1.

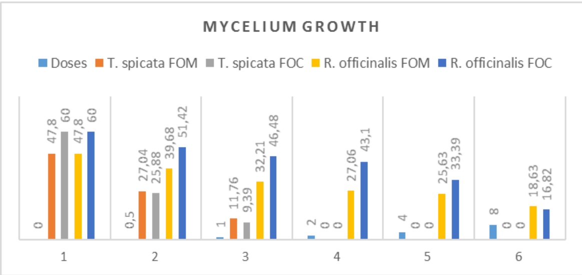

Figure 1. Dose-dependent mycelium growth of FOM and FOC.

Considering the percentage effect of the essential oils at 2 µlpetri-1doses on mycelium growthof fungi, it was determined that *T. spicata* essential oil inhibited the mycelium growth of FOM and FOC by 84.35% and 75.38%, respectively, while the same dose of *R. officinalis* essential oil inhibited the mycelium growth of the diseases by 25.53% and 32.60%, respectively (Table 1).

Table 1. The effect of *T. spicata* and *R. officinalis* essential oil on the mycelium growth of FOM (%) and FOC (%).

Doses	*Thymbra spicata*		*Rosmarinus officinalis*	
	FOC	FOM	FOC	FOM
Control	0.00±0.00^{d*}	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{f}
0,5 (µlpetri^{-1})	43.43±4.31^{c}	56.86±1.31^{c}	16.99±0.93^{d}	14.30±0.69^{e}
1	75.38±4.43^{b}	84.35±2.39^{b}	32.60±2.60^{c}	25.53±0.97^{d}
2	100.00±0.00^{a}	100.00±0.00^{a}	43.40±1.83^{b}	28.18±1.00^{c}
4	100.00±0.00^{a}	100.00±0.00^{a}	46.38±2.21^{b}	44.36±1.23^{b}
8	100.00±0.00^{a}	100.00±0.00^{a}	61.32±3.21^{a}	71.97±3.80^{a}

FOC = *Fusarium oxysporum* f. sp. *cucumerinum*, FOM = *Fusarium oxysporum* f. sp. *melonis*

*Averages with different letters in the same column differ in significance level $p < 0.05$ according to DUNCAN

Considering 2 µlpetri-1 doses of the essential oils, it was found that *T. spicata* inhibited the mycelium growth of FOM and FOC by 100%, while *R. officinalis* essential oil inhibited the mycelium growth of the plant pathogens by 28.18%and 43.40%, respectively. The highest dose of *R. officinalis* inhibited the mycelium growth of FOM and FOC plant pathogens by 61.32% and 71.97%, respectively. As a result of the study, it was determined that the effect of *T. spicata* essential oil on the mycelium growth of FOM and FOC was more than that of the *R. officinalis* essential oil. In addition, the dose-effect study was performed and the LC50 and LC90 values of the essential oils were calculated in the study. As a result of the dose-effect study, the LC50 and LC90 values of *T. spicata* essential oil on FOM and FOC were calculated as 0.58-1.26 µl and 0.46-1.07 µl, respectively, while the LC50 and LC90 values of *R. officinalis* essential oil on FOM and FOC were calculated as 4.04-37.56 µl and 3.92-95.84 µl, respectively (Table 2).

Table 2. Results of dose-effect analysis of essential oils on FOM and FOC.

Plant Pathogens	*Rosmarinus officinalis*			
	LC_{50}(µl)	LC_{90}(µl)	Slope ± Standard error	X^2
FOM	4.04 (3.05-5.91)	37.56 (19.31-120.16)	1.32±0.19	3.43
FOC	3.92(2.58-7.85)	95.84(29.03-1798.33)	0.92±0.20	1.25
	Thymbra spicata			
	LC_{50}(µl)	LC_{90}(µl)	Slope ± Standard error	X^2
FOM	0.58 (0.47-0.68)	1.26 (1.05-1.73)	3.82±0.64	2.45
FOC	0.46 (0.34-0.55)	1.07 (0.90-1.43)	3.53±0.63	1.70

FOC = *Fusarium oxysporum* f. sp. *cucumerinum*, FOM = *Fusarium oxysporum* f. sp. *melonis*

The efficiency of the essential oils on plant pathogenic fungi has been investigated by many researchers (Yılar et al., 2013). The antifungal activity of *T. spicata* essential oils was reported on Candida species (Kılıç, 2006). It was reported that *T. spicata* essential oil exhibits significant antifungal activity against plant pathogens, such as *Fusarium moniliforme*, *Phytophthora capsici* and *Sclerotinia sclerotiorum* (Muller-Riebau et al., 1997). On the other hand, in another study conducted earlier, Bayan et al (2017) reported that *T. spicata* essential oil exhibits high levels of antifungal activity against *F. oxysporum* f. sp. *lycopersici*, *A. solani*, *V. dahlia* and *R. solani*. Similar studies showed that rosemary plants have biological activity. It was suggested that *Rosmarinus officinalis* showed antifungal activity against *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum* pathogen (Ozcan and Chalchat, 2008). Furthermore, it was reported that a 1% volatile emulsion of rosemary essential oil has a high antifungal activity against *Botrytis cinerea* and *Penicillum expansum* pathogens, which are important disease agents for apples (Lopez-Reyeset al., 2010). It was also reported that rosemary essential oil at 25.6 µgml-1 dose inhibited fungus growth against *Botrytis cinerea* (Soylu et al., 2010).

As a result of the study conducted, it was determined that *T. spicata* and *R. officinalis* essential oils are highly effective in the mycelium growth inhibition of FOM and FOC. The previously performed studies support our findings in petri dishes. The results of this research are of great importance today in which it is known that fungal diseases gain resistance against the fungicides used intensively in controlling diseases, their harmful effects on human and environment, as well as residual problems.

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