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Research Article

Antifungal Activity of *Thymbra spicata* L. and *Rosmarinus officinalis* L. Essential Oils against *Monilinia fructigena* Honey in Whetze

Melih YILAR*, Yusuf BAYAR

Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, 40100 Kırşehir

*Corresponding author:melih.yilar@ahievran.edu.tr

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Abstract

This study was carried out to determine the antifungal potential of *Thymbra spicata* L. and *Rosmarinus officinalis* L. (Lamiaceae) plant essential oils against the *Monilinia fructigena* Honey in Whetze pathogen which is one of the major disease agents of soft seeds. The essential oils were dropped on a filter paper adhered to the covers of petri dishes with a micropipette at the doses of 0 (Control), 0.5, 0.8, 1, 1.5, 2, 4, 8, 16 µl/petri dish. At the end of the seven days incubation period, the diameters of the mycelium growth in petri dishes were measured with an automatic caliper. As a result, *T. sp*icata and *R. officinalis* plant essential oils were found to be statistically significant on the pathogen. Depending on the increased dose, *R. officinalis* essential oil inhibited the development of the *M. fructigena* mycelium and inhibited by 100% in 16 µl/petri dish. On the other hand, *T. spicata* essential oil influenced the mycelium development of the pathogen more and inhibited it by 100% at the dose of 2µl/petri dish. As a result ofdose-response study regarding *T. spicata* and *R. officinalis* essential oils against *M. fructigena* the LC_{50} and LC_{90} values were found to be 0.80-1.63 and 5.36-12.17, respectively. These findings showed that *T. spicata* and *R. officinalis* essential oils may be an alternative to synthetic fungicides for controlling *M. fructigena*.

Keywords:*Thymbra spicata*, *Rosmarinus officinalis*,*Monilinia fructigena*, essential oil, antifungal activity, biopescticide

Thymbra spicata L. ve *Rosmarinus officinalis* L. Uçucu Yağlarının *Monilinia fructigena* Honey in Whetze'ya Karşı Antifungal Aktivitesi

Özet

Bu çalışma, yumuşak çekirdeklilerin önemli hastalık etmenlerden biri olan *Monilinia fructigena* patojenine karşı *Thymbra spicata* L. ve *Rosmarinus officinalis* L. (Lamiaceae) bitki uçucu yağlarının antifungal potansiyelinin belirlenmesi amacıyla yürütülmüştür. Uçucu yağlar petri kaplarının kapaklarına yapıştırılan filtre kağıdına 0 (Kontrol),0.5, 0.8, 1, 1.5, 2, 4, 8, 16 µl/petri dozda mikropipetle damlatılmıştır. Yedi günlük inkubasyon süresi sonunda petrilerdeki miselyum çapları otomatik kumpas ile ölçülmüştür. Sonuçta *T. spicata* ve *R. officinalis* bitki uçucu yağları patojen üzerine istatistiki olarak önemli derece etkili bulunmuştur. *R. officinalis* uçucu yağı artan doza bağlı olarak *M. fructigena* miselyum gelişimini engellemiş olup 16 µl/petri kabı dozda %100 engellemiştir. *T. spicata* uçucu yağı ise patojenin miselyum gelişimi üzerine daha fazla etki göstermiş; 2µl/petri kabı dozda %100 inhibe etmiştir. *T. spicata* ve *R. officinalis* uçucu yağlarının *M. fructigena*karşı doz-etki çalışması sonucundaLC_{50} ve LC_{90} değerleri sırasıyla, 0.80-1.63 ve 5.36-12.17 olarak bulunmuştur. Bu bulgular, *T. spicata* ve *R. officinalis* uçucu yağlarının, *M. fructigena* kontrolünde sentetik fungusitlere alternatif olabileceğini göstermiştir.

Anahtar kelimeler:*Thymbra spicata*, *Rosmarinus officinalis*, *Monilinia fructigena*, uçucu yağ, antifungal aktivite, biyopestisit

Introduction

In agricultural areas, diseases, pests and weeds that invade crops cause significant losses. The plant pathogenic fungal diseases lead to crop losses both in land and depot. Synthetic fungicides are used to control diseases to prevent these losses. The unconscious use of these fungicides for many years has brought about the endurance problem in pathogens as well as the problem of the accumulation of toxic substances in water, air, soil and food (Arslan and Karabulut, 2005; Işık et al., 2016). These adverse effects of pesticides have led investigators to seek new alternatives.

One of these alternative methods is to use herbal products that are environmentally friendly and without residue problem and that can be used to control diseases and pests. The plants growing in nature have very rich contents. These compounds that they contain have quite different biological activities. It has been revealed by the work of various researchers that plant essential oils and extracts can be used to control diseases, pests and weeds (Kaur et al., 2010; Hassannejad and Ghafarbi, 2013; Douiri et al., 2014; Carvalho et al., 2016).

The *Monilinia fructigena* pathogen is an important disease factor causing brown rot in soft seeds before and after harvesting. The disease spreads through the contact of infected fruits with healthy fruits (Anonymous, 2017). Starting from the field and continuing in the depot, it limits the synthetic drugs to be used in fighting against disease due to the residual problem. For this reason, the studies on the biological activities of the plant-derived compounds, which are environmentally friendly, not harmful to human health and have no residual problems, have recently gained pace. In this study, it is aimed to determine the antifungal activity of *Rosmarinus officinalis* and *Thymbra spicata* plant essential oils on *M. fructigena* disease factor.

Materials and Methods

Plant materials

In the studies, the plant material of *Rosmarinus officinalis* was collected from İçel province andthe plant material of *Thymbra spicata* was collected from Kahramanmaraş province. The harvested plant material was dried in the dark at room temperature and made ready for experiments by grinding.

The extraction of essential oils

The essential oils of plants were obtained by means of the hydro-distillation method using a Schilcher apparatus. The plant samples were boiled for 2 hours by adding pure water (1:10 w/v). The essential oils obtained were kept at +4 ^{0}C until they are used in the experiments.

Fungus culture

The plant pathogen of *M. fructigena* used in the experiments was obtained from the stock culture in the Phytopathology laboratories of the Department of Plant Protection, Ahi Evran University, Faculty of Agriculture. Among these stock cultures, 7-day-old cultures developed in petri dishes (90 mm) containing PDA at 25±2 ^{o}C were used.

The antifungal activity of essential oils in vitro on M. fructigena

The PDA prepared in 500-ml erlenmeyer to be used in the experiments were autoclaved and cooled down to 40 ^{o}C and transferred to 60-mm-diameter petri dishes (10 ml/petri dishes). Sterile blotting papers adhered to the covers of petri dishes and the essential oils were impregnated with the blotting papers at doses (0 (Control), 0.5, 0.8, 1, 1.5, 2, 4, 8, 16 µl/petri dishes) determined by means of a micropipette. As a control group, sterile pure water was impregnated into the blotting paper. The petri dishes were covered with a parafilm and left to incubate at 25^{o}C. Seven days after the treatment, the mycelial growth of diseases was measured with a digital caliper. The treatments were done with four replications and two repetitions. The inhibition rate (%) of essential oils is calculated according to the following formula (Pandey et al., 1982):

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

MGI: Mycelial Growth Inhibition rate (%)
C: Radial mycelium development in the control
T: Radial mycelium development in the treatments

Statistical analysis

The differences between the treatments in the tests were determined by the significance analysis of variance (ANOVA) and the means were compared by using the DUNCAN test. The statistical analyzes were performed using the SPSS computer program. The data obtained in the experiment were analyzed by Probit analysis in the SPSS 15 analysis program and the LC_{50}, LC_{90} values were calculated.

Result and Discussion

In many similar studies, it has been reported that the extracts and especially essential oils of plants have a significant effect on plant pathogens (Cakır et al., 2005; Mahilrajan et al., 2014). It has been again suggested by different researchers that essential oils exhibit antimicrobial activity effects when used as a phase against plant pathogens, fungus and bacteria (Edris and Farrag, 2003; Soylu et al., 2005).

The antifungal activity of the essential oils of *Rosmarinus officinalis* and *Thymbra spicata* plants by using the treatment doses of 0 (Control), 0.5, 0.8, 1, 1.5, 2, 4, 8, 16 µl/petri against *M. fructigena* mycelium development was determined by this study. The effect of *R. officinalis* and *T. spicata* essential oils on the pathogen differed depending on the essential oil and treatment doses (Table 1).

Table 1. The effect of *Rosmarinus officinalis* and *Thymbra spicata* essential oil on the mycelium development of *Monilinia fructigena*

Doses(µl/petri)	*Rosmarinus officinalis*		*Thymbra spicata*	
	MG(mm)	MGI(%)	MG(mm)	MGI(%)
Control	47.40±1.29 a*	0.00	47.40±1.29a	0.00
0.5 µl	41.43±1.43b	12.58	22.91±2.12b	51.65
0.8 µl	37.03±0.85c	21.88	20.78±1.24b	56.15
1 µl	33.58±0.32d	29.15	20.32±0.40b	57.11
1.5 µl	32.74±0.14d	30.92	11.92±1.41c	74.83
2 µl	29.68±0.15e	37.38	0.00±0.00d	100
4 µl	27.57±0.08e	41.82	0.00±0.00d	100
8 µl	20.81±0.25f	56.10	0.00±0.00d	100
16 µl	0.00±0.00g	100	0.00±0.00d	100

*The means in the same column with different letters are different at $p<0.05$ significance level according to the DUNCAN's test. MGI: Mycelial growth inhibition, MG: Mycelial growth.

The effect of *R. officinalis* essential oil on *M. fuctigena* was found statistically significant at $P<0.05$ significance level.

There were increases in adverse effect due to the increased dose. Accordingly, *R. officinalis* essential oil exhibits 12.58% inhibition of the mycelium development of the pathogen at 0.5µl/petri dose, whereas a 100% effect occurred at 16 µl/petri dose (Table 1, Figure 1).

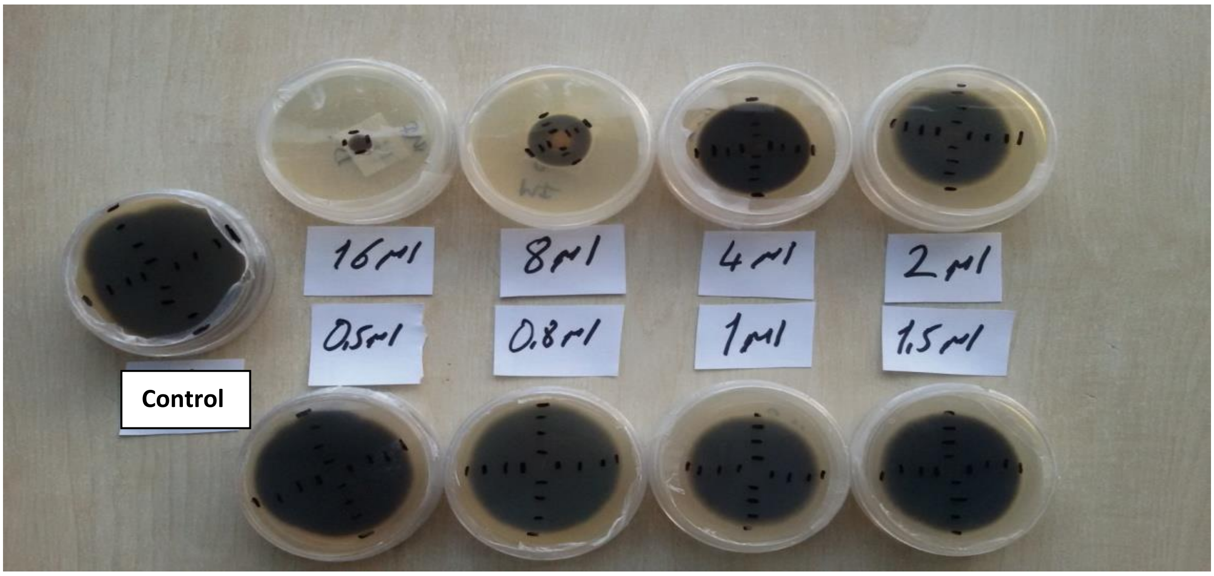

Figure 1. The effect of *Rosmarinus officinalis* essential oil on the mycelium development of *Monilinia fructigena*

Table 2. The dose-response experiment results between *Rosmarinus officinalis* and *Thymbra spicata* essential oils and *Monilinia fructigena* fungi

Test Plants	LC_{50}(µl/ml)	LC_{90}(µl/ml)	Slope ± Standard error	X^2
Rosmarinus officinalis	5.36 (3.77-8.33)	12.17 (8.95-20.49)	0.188±0.015	45.16
Thymbra spicata	0.80 (0.51-1.05)	1.63 (1.31-2.38)	1.54±0.0.12	45.80

The dose-response study results of *R. officinalis* essential oil on *M. fuctigena* are given in Table 2. Accordingly, it is seen that the LC_{50} and LC_{90} values are 5.36 and 12.17, respectively (Table 2). Similar studies have also shown that rosemary plants have biological activity. It has been suggested that rosemary chemotypes exhibit antifungal activity against *Candida albicans* pathogen (Matsuzaki et al., 2013; Ksouri et al., 2017). It has also been reported that the 1% emulsion of rosemary essential oil has a high antifungal activity against *Botrytis cinerea* and *Penicillum expansum* pathogens, which are important disease agents in apples (Lopez-Reyes et al., 2010). It was determined that rosemary essential oil inhibits the fungus development against *Botrytis cinerea* at 25.6 µg/ml dose (Soylu et al., 2010). It was reported by Mancini et al. (2014) that a dose of 1000 ppm *Origanum vulgar e*essential oil inhibits the mycelial development of the pathogens completely in the study carried out against *Monilinia laxa, M. fructigena and M. fructicola*, which are three important factors of field and storage diseases.

T. spicata essential oil was found to be more effective on *M. fructigena* pathogen than *R. officinalis* essential oil. *T. spicata* essential oil inhibited the mycelium development of the pathogen at 0.5 µl/petri-dish dose by 74.83%, whereas a 100% inhibition occurred at 2 µl/petri-dish dose (Table 1, Figure 2).

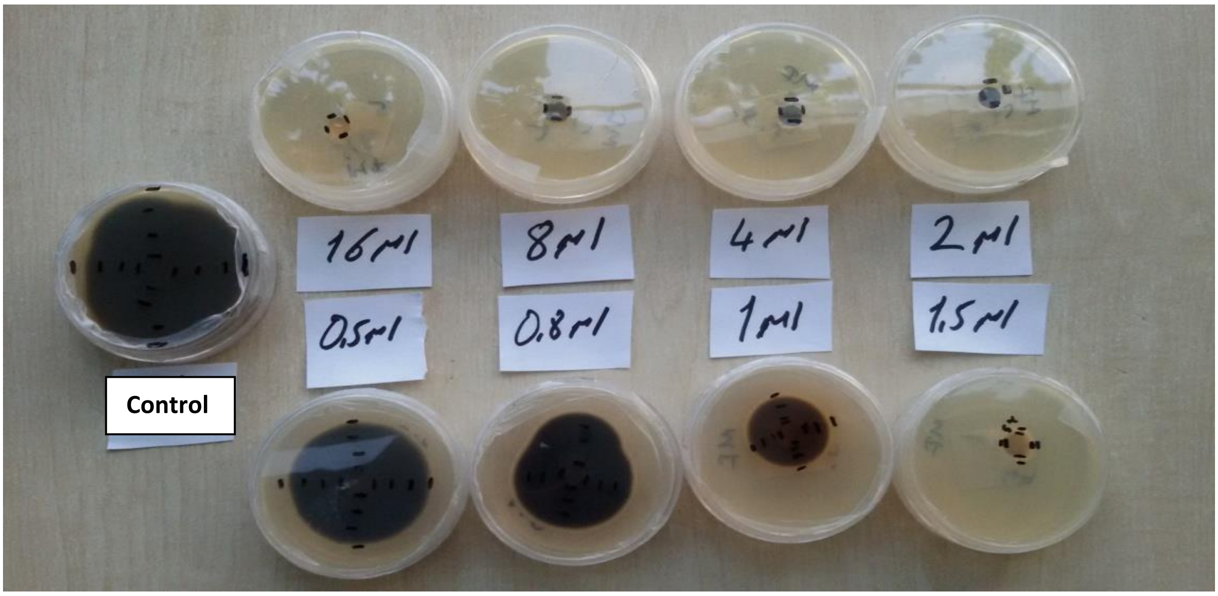

Figure 2. The effect of *Thymbra spicata* essential oil on the mycelium development of *M. fructigena*

The results of the dose-response studies regarding the effect of *T. spicata* essential oil on *M. fuctigena* are given in Table 2. Accordingly, the LC_{50} and LC_{90} values were found to be 0.80 and 1.63. The biological activity of *T. spicata* essential oil has been suggested by other researchers. In this context, it has been reported that a 10 µl of *T. spicata* essential oil (at $142x10^{-3}$µl/ml air concentration) inhibits the mycelium development of *B. cinerea*, *M. fructigena* and *M. laxa* on the 3rd day by 100%, while it inhibits the mycelium development of *Penicillium expansum* by 36.4% (Ünlü et al., 2009). In the study stating that 8µl/ml dose of *T. spicata* essential oil inhibits the mycelium development of *Sclerotinia sclerotiorum* by 98.64% and that of *Penicillium spp.* by 100%, it has been reported that the LC_{50}-LC_{90} values for *S. sclerotiorum* and *Penicillium spp.* were 0.834-3.771 and 0.602-0.953, respectively (Kadıoğlu et al., 2013). In this context, it has been reported by researchers that the main component of *T. spicata* essential oil is carvacrol (16.1–62.9%) (Barakat et al., 2013) and the carvacrol component has a high antimicrobial activity (Panizi et al., 1993; Belgüzar et al., 2013; Bagamboula et al., 2004).

Conclusion

Consequently, in this study where antifungal effect of the essential oils of *R. officinalis* and *T. spicata* plants on *M. fructigena* fungus was revealed, the pathogen reacted to plant essential oils at different levels depending on the increased dose. The pathogen was found to be more sensitive to *T. spicata* essential oil. The usability potential of both of the essential oils was determined to be high for controlling *M. fructigena* pathogen. Today, in which fungicides that are used intensively in controlling of these disease and are known to have harmful effects on human health and the environment as well as their residual problems, the findings of this study are of importance.

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