THE REPELLENCY EFFECTS OF THREE PLANT ESSENTIAL OILS AGAINST THE TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE*

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Abstract. Many spider mite species are polyphagous and live together. They are significantly harmful pests for agricultural crops. Acaricides and insecticides are commonly used to control *T. urticae*. Spider mites can rapidly develop resistance to pesticides. Organic control may be considered as an alternative and environment-friendly approach. Three native plants essential oils were isolated by hydrodistillation technique hydro distillation in a Clevenger type apparatus. In this study, *Thymbra spicat*a L. (Labiatae), *Laurus nobilis* L. (Lauraceae) and *Myrtus communis* L. (Myrtaceae) plants have been tested for their essential oil's repellency effect. Essential oils of these three plants were compared for 48 h at four different doses (0.1, 0.5, 1 and 2 ml/l). We observed repellency effect all does and for all essential oils, whereas *M. communis* was proven to be a stronger repellent compared to *L. nobilis* at 2 ml/l.
Keywords: *control, essential oil, repellency effect, Tetranychus urticae, Tokat, Turkey*

Introduction

The two-spotted spider mites, *Tetranychus urticae* Koch. (Acarina: Tetranychidae), are about 0.5 mm in size, with reddish or greenish body color. The adult female body bears 12 pairs of dorsal seta. Two-spotted spider mites (TSSM) were initially described as a European species by Tuttle and Baker in 1968. *T. urticae* is a significant proliferous polyphagous pest, commonly controlled by pesticides in Turkey and other countries. This mite has more than 1200 known host plants (Zhang, 2003). Pesticides pose serious risks for the environment, non-target-organisms and also human life. Tetranychidae has developed resistance against 80 different acaricides to date (Darp, 2004; Miresmailli et al., 2006; Vanpottelberge et al., 2009). *T. urticae* has developed resistance to many insecticides and acaricides (Ay and Gürkan, 2005; Dermauw et al., 2013). Eventually, its control has become problematic in many regions of the world. Several researchers have focused on alternative methods to overcome the negative impacts of conventional pesticides. Essential oils and plant extracts are among the alternative methods against pests and spider mites. Plant essential oils have toxic effects on pests through contact, ingestion and fumigation; in addition, plant essential oils or their constituents have significant behavioral effects on pests, especially as repellents (Akhtar et al., 2010). Another study investigated repellency effects of plant extracts obtained from weeds and essential oils of 53 different plants on *T. urticae* (Kawka and Tomczyk, 2002). The use of plant extracts and essential oils in pest control could be an effective alternative compared to pesticides. Essential oils and their contents such as terpenoids, alkaloids and flavonoids are effective in pest control (Grodnitzky and Coats, 2002). EOs are used antibacterial, antifungal and insecticidal (Chang and Cheng, 2002). Furthermore, plant essential oils have minimal direct and/or indirect effect on predators or the ecosystem (Isman, 2006). Black thyme (*Tthymus spicata*) grows wild in some Eastern

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Mediterranean countries and Turkey (Kizil, 2010); its essential oil contains 60-80% of carvacrole. It is belongs to the Lamiaceae family. Thyme has been studied as repellents (Rajkumar and Jebanesan, 2005). *Laurus nobilis* is also a major source of essential oil and its leaf essential oil was reported to be rich in 1.8-cineole (Baydar, 2009). *L. nobilis* is a important plant of used in foods, drugs, and cosmetics. Laurus leaves and essential oils are used for antimicrobial and insecticidal activities (Patrakar et al., 2012). *Myrtus communis* (myrtle) is grows wild and cultivated. It is widespread throughout the Mediterrian region of Anatoloia-Turkey. *Myrtus* leaves and fruit of the plant contain mirtenol, cineol and terpenes. The main constituents of *M. communis* oil are 1.8-cineole, myrtenyl acetate, myrtenol and limonene (Traveset et al., 2001). The myrtle essential oil observed toxicity fumigation of lepidoptera and coleoptera species (Ayvaz et al., 2010; Tayoub et al., 2012).

This article reports repellency effects of essential oils obtained from flowers, stems and leaves of *T. spicata*, *L. nobilis* and *M. communis* against two-spotted spider mites, and positions them as an alternative to pesticides.

Material and methods

Mites culture

Two-spotted spider mites were obtained from a colony maintained at the Plant Protection Department, Tokat Gaziosmanpaşa University (GOP), Turkey. Mites were grown in a climate chamber at 26 ± 1 °C, 60-80% of relative humidity (RH) and 16 h of light and 8 h of dark (LD) photoperiod.

Material collection and processing

T. spicata, *L. nobilis* and *M. communis* were collected from around the campus area of GOP University during the summer season in June-August 2016 (Location 40.3339, 36.4769). Fresh plant parts were air-dried in the shade at room temperature and then grounded. Essential oils were extracted from a 100 g mixture of stems, flowers and leaves using a Clevenger-type apparatus within 2 h. The essential oils were later stored in sealed vials at 4 °C until the time of analyses (as previously performed by Cosimi et al., 2009).

Repellency effect

During the trial, same age ten adult female mites were transferred into 3 cm bean leaf disks as nutriment and then placed in 9 cm Petri dishes. We tested four different concentrations (0.1, 0.5, 1 and 2 ml/l) of essential oils of *T. spicata*, *L. nobilis* and *M. communis*. They were dissolved in water containing 0.3% Tween 20 and were diluted to the targeted doses. Leaf discs (30 mm diameter) were cut from *P. vulgaris* leaves. In the repellency assay, half of leaf discs were immersed in water containing 0.3% Tween 20, which served as control and the other half of the leaf discs were immersed in each essential oil solution for 20 s before drying in room temperature for 30 s. Afterwards, leaf discs were put in Petri dishes; ten adult females (1-2 day old) of *T. urticae* were placed on the center of each leaf disc. Cotton saturated with water was added in the Petri dishes in order to maintain the leaf turgor and avoid escape of mites.

Plates were then sealed with parafilm to prevent any loss of essential oils. Each treatment was repeated for three times. The number of mites on treated leaves (half of

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the leaf) and untreated leaves (the other half of the leaf) were counted at 2, 24 and 48 h post treatment respectively and repellency index was calculated by RI = (C – T / C + T) × 100 (Pascual-Villalobos and Robledo, 1998). In this formula RI stands for repellency index, T is the number of mites in treatment group and C is the number of mites in the control group.

Phytotoxicty test

Based on a study by Miresmailli and Isman (2006), essential oils of three test plants were tested on rose shoots (*Rosa damascena* Mill) using 1% solution (300 ml) consisting of essential oil, distilled water and Triton X. Each plant's essential oil (30 ml solution) was sprayed on rose shoots by using a handle sprayer, while control rose shoots remained unsprayed. Shoots were prepared in a glass flask filled with tap water (500 ml) under laboratory condition maintained at 26 ± 1 °C and 16 h of light and 8 h of dark (LD) photoperiod. Results were recorded after a treatment 24 using a scale between 0 to 4. Each treatment was replicated three times.

Data analysis

The repellency effects were analyzed by one-way analysis of variance (ANOVA) and significant differences between mean values were found by Tukey test using SPSS version 19.0. P-value < 0.05 was regarded as a significant difference.

Results and discussion

The repellent activity of three different plant essential oils tested at various concentrations after 2, 24 and 48 h post exposure period are presented in *Table 1*. *T. spicata* was found to have the lowest repellency effect at 49.9% in 2 h and 45.8% after 24 and 48 h. We also observed that the repellency effects of essential oils on adult mites would increase with time regardless of the concentration level.

Table 1. *Repellency effects of three plants' essential oil on Tetranychus urticae*

Time (h)	Concentration (ml/l)	Repellency effect (% ± SE)		
		Laurus nobilis	***Myrtus communis***	***Thymbra spicata***
2	0.1	76.9±11.98a	79.16±12.50a	49.99±08.90a
	0.5	75.00±10.44a	79.16±16.59a	70.83±11.67 a
	1	80.95±12.29a	83.33±06.29a	79.16±10.79a
	2	87.50±8.76a	95.83±04.16b	83.33±08.90a
24	0.1	58.33±08.33a	79.16±12.50a	45.83±06.09a
	0.5	66.66±08.90a	75.00±12.19ab	75.01±10.44ab
	1	70.83±07.55a	83.33±06.29a	79.16±08.76b
	2	79.16±08.76b	91.66±05.45 b	83.33±06.29a
48	0.1	50.00±06.29a	66.66±08.90a	45.83±06.09a
	0.5	58.33±08.33ab	70.83±13.26a	75.00±10.44ab
	1	66.66±06.29ab	79.16±06.09a	79.16±08.76ab
	2	75.00±04.35a	95.83±11.78 b	91.66±05.45 b

Different letters within the same rows and columns indicate significant differences between essential oils and treatment concentrations, respectively ($p \leq 0.05$) (Tukey test)

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24-h repellency effects of *L. nobilis* and *M. communis* at 0.5 ml/l concentration on adult females of two-spotted spider mites were recorded as 66.6% and 75% respectively, which was statically significant ($P < 0.05$). The repellency effect of *T. spicata* at the lowest concentration (0.1 ml/l) was found to be lower than *L. nobilis* and *M. communis* at the end of 2, 24 and 48 h and there is no difference statistically. In addition, no significant difference was found between the repellency effects at 2 h and 48 h, when applying 0.1 ml/l concentration. At 2 h, in all three plant essential oil treatments, the effect of 0.1 and 2 ml/l concentrations were statistically within the same range. The highest repellency effects were observed at the and of 48 h with *M. communis* and *T. spicata* essential oil on *T. urticae* adults as 95.8% and 91.6% respectively. Furthermore, the highest repellency effect on adult mites identified as 95.8% with 2 ml/l concentration of *M. communis* at the 48 was found to be statistically different from other groups ($F = 01.50$, d.f. = 3, $P < 0.05$). Regardless of duration, the repellency effects of all three tested essential oils on *T. urticae* adults changed with the changing concentration. Eventually, *M. communis* essential oil was observed to have highest repellency effects as compared to other test plant essential oils on *T. urticae* in 24 and 48 h exposure ($P < 0.05$).

Essential oils contain several biochemicals including acids, alcohols, aldehydes, ketones, esters, phenols, terpenes and sesquiter penes. Essential oils are used as antifeedant, antiviral, acaricidal, antimicrobial, antioxidant, antifungal, contact and repellent (Isman, 2000, 2006). In this study, we demonstrated repellency effects of essential oils from three plants belonging to Lamiaceae family against two-spotted spider mite. There is no observed any phytotoxic effect on bean plants.

Previously, different concentrations of essential oils from 14 species of Lamiaceae were shown to cause mortality and induce repellency in adult females of the carmine spider mite, *T. cinnabarinus*. (Mansour et al., 1986). Other studies also confirm repellency effect of *M. communis* essential oil against two spotted spider mites (El-Zemmity et al., 2006). In another repellency effect study, four different concentrations of rose essential oil, geraniol and citronellol were applied to *T. urticae* and results confirmed repellency effects of essential oils on the mites (Yorulmaz-Salman and Erbaş, 2014). In a repellency trial on *T. urticae* with essential oils of *Origanum vulgare* L, *Micromeria fruticosa* L. And *Nepeta racemosa* L. of the Lamiacea (mint) family, highest motalily rate was observed at 2 µl/l concetration, and it also increased with time. (Çalmaşur et al., 2006). Another study in greenhouse conditions with essential oils obtained from *Cuminum cyminum* and *Origanum syriacum* var *bevanii* revelaed fumigant effects on *T. cinnabarinus* and *Aphis gossypii* (Tuni and Sahinkaya, 1998).

Conclusion

In conclusion *M. communis* essential oil showed stronger repellency effect on TSSM adults at the highest concentration (2 ml/l) in comparison to *L. nobilis* and *T. spicata* essential oils. We concluded that all three plant essential oils can be used to control *T. urticae*. The repellency effects of *T. spicata*, *L. nobilis* and *M. communis* essential oils on TSSM adults in all concentrations increased with time. However, it is necessary to perform experiments with these three plant essential oils under the greenhouse conditions and compare results with laboratory results. As a final note, at given concentrations, the tested essential oils were not detected to have any phytotoxic effect on the plants.

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