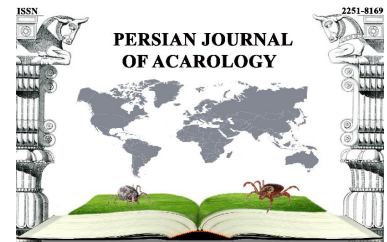




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Article

Toxicity and repellency effects of three essential oils on two populations of *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT

Essential oils are environmentally benign agents which are used as alternative compounds for chemical pesticides in order to control pests. Thus, in the current study, the toxicity and repellency effects of essential oils, extracted from three medicinal plants of the Lamiaceae family, including *Thymus daenensis*, *Satureja khuzestanica*, and *S. bakhtiarica*, were evaluated against a resistant (MhR) and a susceptible (KrS) population of *Tetranychus urticae*. Results based on probit analysis revealed that all extracted essential oils to some extent showed both repellency and toxicity effects on both populations of the mite. The LC₅₀ values of *S. khuzestanica* extract were the lowest against the both populations (31.16 $\mu\text{L} \cdot \text{L}^{-1}$ air for KrS population and 56.29 $\mu\text{L} \cdot \text{L}^{-1}$ air for MhR population). Also, all extracted essential oils were found to be repellent to the adult females of both MhR and KrS populations, with higher repellency effect of *S. khuzestanica* oil. The effect of the LC₂₀ of the essential oils on detoxifying enzymes including Glutathione S-transferases (GSTs), esterases (ESTs) and cytochrome P450 monooxygenases (P450) was tested against both populations. Significant higher activity of all enzymes was detected in MhR population regardless of essential oils treatment. The activity of GSTs and esterases increased significantly in mites treated with all essential oils, while a tendency for a decrease in P450 activity was detected following essential oils treatment. These results indicate that the essential oils of the all selected plants, especially *S. khuzestanica*, have a good potential to be used in integrated management programs of *T. urticae* which is a serious pest in the greenhouses.

KEY WORDS: Detoxification enzymes; IPM; *Satureja bakhtiarica*; *S. khuzestanica*; *Thymus daenensis*; Two-spotted spider mite.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a serious pest of crops worldwide with a host range of about 1200 plant species in more than 250 families. Thus, in the area with high infestation levels, economic damage occurs on a wide range of fruit trees, vegetables, field crops, and ornamental plants in both open fields and greenhouses (Gorman *et al.* 2002; Vassiliou and Kitsis 2013). Moreover, nowadays that cultivation of vegetables in the greenhouses has become a common practice in many areas of the world, importance of this pest has become more evident since both immature and adult stages of the mite feed from cell content using their cheliceral stylets; as a result, they initially produce yellowish chlorotic spots on the upper leaf

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surface and as feeding progresses, eventually the leaves become necrotic and die (Aucejo-Romero *et al.* 2004).

Despite the development of several methods for successful control of *T. urticae*, the use of synthetic acaricides is the major means of keeping the mite population below an economic injury level (Van Leeuwen *et al.* 2015). However, *T. urticae* is notorious for its ability to develop rapid resistance to pesticides due to having a variety of features such as high fecundity, short generation time, inbreeding, and arrhenotokous reproduction (Stumpf and Nauen 2002; Van Leeuwen *et al.* 2009; Grbić *et al.* 2011). *T. urticae* has the greatest resistance capability to chemical pesticides among all arthropods; over 500 cases of resistance to almost 100 active ingredients have been reported by Arthropod Pesticide Resistance Database (APRD, 2019) (<http://www.pesticideresistance.com/search.php>). Also, it should be born in mind that the mite resistance to newly developed pesticides within a short time is a sign of indiscriminate use of pesticides resulting in cross-resistance within and between various classes of pesticides (Grbić *et al.* 2011) and that they could be reasons for control failures of resistant populations (Herron and Rophail 1998; Nauen *et al.* 2001; Ay and Kara 2011; Vassiliou and Kitsis 2013; Piraneo *et al.* 2015; Khalighi *et al.* 2016).

It is needless to say that pesticides can negatively affect non-target organisms, especially pollinators and natural enemies thus disrupting the natural processes of pollination and biological control. In addition, pesticide residues on agricultural products such as freshly consumed fruits and vegetables are major health concerns. Therefore, the combination of the above-mentioned issues create tremendous pressure on scientists to put extra efforts in order to develop novel control agents with high efficiency and specificity against the target pest with lower detrimental effects on the environment and non-target organisms.

Plants are a major source of secondary metabolites, most of which are believed to be involved in defense response against herbivores (Guleria and Tiku 2009; Rana *et al.* 2016). Therefore, plants provide a large and diverse reservoir of natural compounds with the pesticidal activity known as botanicals (Klocke 1987; Koul *et al.* 2008). In recent years, botanicals have received increasing attention in pest management programs with respect to their lower harmful effects on non-target organisms, safety to human and easy biodegradability (Simmonds *et al.* 2002; Guleria and Tiku 2009). Currently, several plant-derived pesticides are commercially available for control of arthropod pests in agriculture and household pests, and thus botanicals are emerging to be a major focal point in the integrated pest management programs in agriculture and household pests (Guleria and Tiku 2009; Jindal *et al.* 2013).

The essential oils taken from aromatic plants contain volatile compounds with pesticide, repellent, and antifeedant activities against a wide range of arthropods (Kéita *et al.* 2000; Nenaah and Ibrahim 2011; Reichert *et al.* 2019). Several species of Lamiaceae family are grown as ornamental plants; oils taken from these plant species have extensive uses in perfumery, cosmetics, pharmaceutical, and food industries (Khoury *et al.* 2016). The pesticidal activity of essential oils extracted from these plants against several stored product and greenhouse pests has been reported (El-Gengaihi *et al.* 1996; Michaelakis *et al.* 2007; Görür *et al.* 2008; Ayvaz *et al.* 2010; Kumar *et al.* 2011). da Silva Moura *et al.* (2019) studied the insecticidal activity of *Vanillosmopsis arborea* essential oil and its major constituent α -bisabolol against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) and found that the LC50 and LC95 of the essential oil of *V. arborea* and α -bisabolol were 5.23 and 12.97 $\mu\text{L L}^{-1}$ of air and 2.47 and 8.82 $\mu\text{L L}^{-1}$ of air, respectively. They also indicated that treatment with different concentrations of *V. arborea* essential oil and α -bisabolol led to a decrease in oviposition and population growth rate. Despite the high potential of plant essential oils in pest control, one drawback is their low stability which can be solved by nanoparticle formulation (Kumar *et al.* 2018; Sutthanont *et al.* 2019). Therefore, it seems that the use of essential oils as pest control agents is promising.

Thus, the aims of the current study were to evaluate the fumigant and repellency activity of essential oils taken from three species of Lamiaceae family (*T. daenensis* Celak, *S. bakhtiarica* Bung,

and *S. khuzestanica* Jamzad) against two susceptible and resistant populations of *T. urticae*, using two methods, in vivo bioassays and enzyme assays hoping these findings could help farmers tackle a major obstacle of growing ornamental and vegetable plants in the greenhouses.

MATERIALS AND METHODS

Spider mites

A resistant population of *T. urticae* (MhR) was collected from infested greenhouses of ornamental plants of Mahallat city (Markazi Province, Iran) in 2018. This line has been exposed to a wide range of acaricides for several generations according to direct interviews with local growers. A susceptible population (KrS) was obtained from the Acarology Laboratory of (University of Tehran, Karaj, Iran), which had been reared in the greenhouse on kidney bean, *Phaseolus vulgaris* L. (Fabaceae) at least for three years (2015–2018) with no history of exposure to any pesticide. A stock colony of each population was established on kidney bean plants in a research greenhouse under controlled conditions (25 ± 3 °C, $60 \pm 10\%$ RH, and a 16L: 8D photoperiod).

Essential oil extraction

The aerial parts of plant species including *T. daenensis*, *S. bakhtiarica*, and *S. khuzestanica* were collected from their natural habitats in Iran (Chaharmahal and Bakhtiari Province). The materials were air-dried in shadow for one week and used to extract the essential oils using the hydrodistillation method (Aslan *et al.* 2004). Briefly, the dried materials (100 g) were finely milled and the powder was subjected to hydrodistillation for 3 h with distilled water (as solvent) using a Clevenger apparatus. The materials yielded a pale yellowish oil (1.18% w/w), which was dehydrated with anhydrous sodium sulfate, and then stored in sealed vials at 4 °C.

Fumigant toxicity

The fumigant toxicity of essential oils taken from three plant species was evaluated against the adult stage of *T. urticae* (< 24-h old) using fumigation method (Choi *et al.* 2004). Filter papers (1 × 1 cm) were treated with different volumes of the essential oils using micro-pipettes and pasted immediately on internal surfaces of the door of Petri dishes (6 cm diameter). In the control group, the filter papers were treated with the same volume of distilled water.

At first the bean leaves (the same age) were selected, cut, and put on wet cotton which covered a Petri dish of 9 cm diameter and a height of 1.5 cm. Twenty adult females of *T. urticae* were transferred on leaf disc using a soft paint brush. Oviposition was terminated by removing all females after 24 h.

Twenty newly emerged (0–24 h) adult females of *T. urticae* were released on kidney bean leaf disc inside each Petri dish. The dishes were then sealed with parafilm to prevent the escape of mites. Treated mites were maintained in a growth chamber (25 ± 1 °C, $60 \pm 10\%$ RH, and 16L:8D h photoperiod) and after 24-hour exposure, mortality was recorded. The mites were considered as dead when did not move their appendages following stimulation with a fine brush. Mortality data were used for estimation of lethal concentrations (LCs). All bioassays were replicated at least three times and each replicate contained five concentrations and each concentration 120 mites.

Repellency

The repellent properties of essential oils against adult spider mites were studied using Miresmailli *et al.* (2006) method with slight modifications. Different lethal concentrations (LC₂₀, LC₅₀, and LC₇₀) of each essential oil, estimated from previous assays, were applied over kidney bean leaf discs (5 cm diameter) using micro-pipette. The same volume of distilled water was applied in a similar fashion for control. After drying at room temperature for 1 h, treated and control leaf discs were placed on a wet filter paper, with a distance of 3 cm from each other, inside a Petri dish (12 cm diameter). The

Petri dish lid was covered with a fine net (100-mm mesh) to exclude the fumigant effects of the essential oils. 50 adult females of *T. urticae* (< 24-h old) were released on an assumed line located at the middle of the filter paper with an equal distance from treated and control leaf discs. After 24-hour exposure, the numbers of spider mites on each leaf disc was recorded. This experiment was carried out with six replicates for each essential oil. The repellent index (RI) was calculated using the Kogan and Goeden (1970) equation:

$$RI = \frac{2G}{G + P}$$

where, *G* and *P* are the number of mites on treated and control leaf discs, respectively. The mean and standard deviation were determined for each calculated RI. A mean value < 1–SD shows repellent property, while a mean value > 1+SD represents the attraction effect of the essential oils. A mean value between 1 – SD and 1 + SD show neutral effects of essential oil (Kogan and Goeden 1970).

Enzyme assay

The activity of detoxifying enzymes was assayed in the resistant and the susceptible populations of *T. urticae* (< 24-h old) surviving from 24 hours of exposure to sublethal concentration (LC₂₀) of the essential oils. All enzyme assays were done in triplicates.

Protein concentration

The total protein content was measured based on the Bradford (1976) method, using bovine serum albumin as a standard.

Enzyme preparation

Preparation of glutathione S-transferase (GST) and cytochrome monooxygenase P₄₅₀ (P450) samples was performed by homogenizing 100 adult females of *T. urticae* in cold sodium phosphate (0.1 M, pH 7) buffer on ice using a Potter–Elvehjem homogenizer, while the source of esterases was prepared by homogenizing 50 female mites in chilled sodium phosphate buffer (0.1 M, pH 7) containing 0.1% (w/v) Triton X-100. The homogenized samples were then centrifuged at 10,000 g for 15 min and the supernatant was used for evaluation of the enzyme activities (Kwon *et al.* 2010).

GST

The activity of GSTs was quantified according to the method of Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates. The reaction mixture in wells of a 96-well microplate consisted of 15 µl supernatant, 50 µl CDNB (63 mM), 100 µl GSH (10 mM), and 150 µl phosphate buffer (0.1 M and pH 7.5). Enzyme activity was determined by the change in absorbance as measured every 30 s for 5 min at 340 nm using microplate reader (ELX808 Bio-Tek).

P450

The amount of P450 was measured based on the method of Brogdon *et al.* (1997) using 3, 3', 5, 5', tetramethylbenzidine (TMBZ) as substrate. The reaction mixture, containing 20 µl supernatant, 80 µl phosphate buffer (0.625 M, pH 7.2), 200 µl TMBZ (0.5 mg. mL⁻¹), and 25 µl H₂O₂ (3%) per well of a 96-well microplate, were incubated at room temperature for 2 h, after which the absorbance was measured at 450 nm as an end-point. A standard curve of absorbance against the amount of purified cytochrome C was constructed to calculate the amount of cytochrome P₄₅₀ per milligram of protein (Brogdon *et al.* 1997).

ESTs

Esterase (EST) activity was measured using α-naphthyl acetate (α-Na) and β-naphthyl acetate (β-

Na) as substrates (Van Asperen, 1962). The reaction mixture, containing 20 μL supernatant, 70 μL phosphate buffer (0.1 M, pH 7), and 90 μL substrate (30 mM in acetone) per well of a 96-well microplate, were incubated at room temperature for 30 min followed by the addition of 90 μL fast blue RR. The absorbance was read at 450 nm for α -Na and at 540 nm for β -Na every 2 min for 20 min using a microplate reader (ELX808 Bio-Tek).

Statistical analysis

The percentages of mortality were corrected using Abbott's formula:

$$\text{Corrected mortality} = (T-C/100-C) \times 100$$

where, T and C are the number of dead mites in treatment and control groups, respectively (Abbott 1925). The corrected mortality data were used to estimate the lethal concentrations (LC₂₀, LC₅₀, and LC₉₀) for each essential oil using Probit analysis in POLO-Plus 1.0 software (LeOra 1994). The mean values of repellency indices and enzyme activities among resistant (MhR) and susceptible (KrS) populations, treated with three essential oils, were exposed to Analysis of Variance (ANOVA) in SPSS software (version 22). Significant differences were evaluated using Tukey's post hoc test at $P < 0.05$ level.

RESULTS

Fumigant toxicity

The median lethal concentrations (LC₅₀) of *S. khuzestanica*, *S. bakhtiarica*, and *T. daenensis* essential oils against KrS population of *T. urticae* were 31.16, 44.06, and 41.68 $\mu\text{L. L}^{-1}$ air, respectively (Table 1). The order of toxicity of the three essential oils was *S. khuzestanica* > *T. daenensis* > *S. bakhtiarica* indicating higher toxicity of *S. khuzestanica* essential oil against KrS population of *T. urticae* in comparison with the other two oils.

Table 1. Probit analysis of estimation of median lethal concentration (LC₅₀) ($\mu\text{L. L}^{-1}$ air) of three essential oils against a susceptible (KrS) and a resistant (MhR) population of *T. urticae*.

Essential oils	N	LC50 (95% CI) $\mu\text{L. L}^{-1}$ air	χ^2 (df)	Slope \pm SE
Susceptible population (KrS)				
<i>T. daenensis</i>	360	41.68 (37.70–45.63)	2.36 (13)	4.49 \pm 0.550
<i>S. khuzestanica</i>	360	31.16 (27.38–34.73)	3.27 (13)	3.86 \pm 0.508
<i>S. bakhtiarica</i>	360	44.06 (39.92–48.75)	4.66 (13)	3.70 \pm 0.506
Resistant population (MhR)				
<i>T. daenensis</i>	360	124.47 (114.21–134.49)	2.14(13)	5.36 \pm 0.652
<i>S. khuzestanica</i>	360	56.29 (49.99–62.58)	3.92 (13)	3.59 \pm 0.446
<i>S. bakhtiarica</i>	360	105.39 (93.72–116.14)	2.51 (13)	3.70 \pm 0.621

The LC₅₀ values of *S. khuzestanica*, *S. bakhtiarica*, and *T. daenensis* essential oils against MhR population of *T. urticae* were 56.29, 105.39, and 124.47 $\mu\text{L. L}^{-1}$ air, respectively (Table 1) showing the order of toxicity of *S. khuzestanica* > *S. bakhtiarica* > *T. daenensis*. There was a significant difference in the toxicity of three essential oils to MhR population ($P < 0.05$). Probit analysis revealed a significant increase in LC₅₀ of all essential oils when applied against the resistant population (MhR) as evidenced by a comparison of the 95% confidence interval (Table 1). The MhR to KrS LC₅₀ ratios were 1.80, 2.39, and 2.98 for *S. khuzestanica*, *S. bakhtiarica*, and *T. daenensis* essential oils, respectively (Table 2) showing resistant strains of the mite against synthetic pesticides are more

resistant to essential oils in comparison to the susceptible mites. Complete mortality (LC₉₉) of adult spider mites was obtained with 338.15, 345.19, and 250.35 $\mu\text{L.L}^{-1}$ air of *T. daenensis*, *S. bakhtiarica*, and *S. khuzestanica* essential oils in MhR population and 137.26, 187.58, and 125.02 $\mu\text{L.L}^{-1}$ air in KrS population, respectively.

Table 2. The ratio of the median lethal concentrations (LC₅₀) of three essential oils when applied against a susceptible (KrS) and a resistant (MhR) population of *T. urticae*.

Essential oil	LC ₅₀ (95% CI) $\mu\text{L.L}^{-1}$ air		MhR/KrS LC ₅₀ ratio (lower-upper)
	MhR	KrS	
<i>T. daenensis</i>	124.47 (114.21–134.49)	41.68 (37.70–45.63)	2.99 (2.64–3.38)
<i>S. khuzestanica</i>	56.29 (49.99–62.58)	31.16 (27.38–34.73)	1.81 (1.54–2.12)
<i>S. bakhtiarica</i>	105.39 (93.72–116.14)	44.06 (39.92–48.75)	2.39 (2.06–2.78)

Repellent activity

The repellent properties of three concentrations (LC₁₀, LC₂₀, and LC₅₀) of each essential oil on MhR and KrS populations were also studied. Results indicated that there were significant differences in repellency indices (RI) among different concentrations of essential oils on both MhR (ANOVA: df = 8, F = 24.43, $P < 0.001$) and KrS (ANOVA: df = 8, F = 26.16, $P < 0.001$) populations, with diverse effects ranging from repellency to attractiveness (Tables 3, 4). The LC₁₀ of all essential oils was found to be an attractant to adult mites; the highest and the lowest repellency indices (RI) on both mite populations were recorded for essential oils of *S. bakhtiarica* and *S. khuzestanica*, respectively (Tables 3, 4). No significant repellency or attractiveness of LC₂₀ of *T. daenensis* extract was observed against MhR and KrS populations. However, the LC₂₀ of *S. bakhtiarica* essential oil was found to be attractive to both populations but the LC₂₀ of *S. khuzestanica* extract was repellent to KrS population and neutral to MhR population (Tables 3, 4). The LC₅₀ of all essential oils was found to be repellent to MhR and KrS populations, with higher repellency of *S. khuzestanica* essential oil on both populations (Tables 3, 4).

Table 3. Repellent effects of different lethal concentrations (LC₁₀, LC₂₀, and LC₅₀) ($\mu\text{L.L}^{-1}$ air) of three essential oils on a susceptible population (KrS) of *T. urticae*. Different letters denote significantly different values from one another (Tukey).

Essential oil	N	Lethal Concentration	Concentration $\mu\text{L.L}^{-1}$ air	RI ¹	SD ²	Effect
<i>T. daenensis</i>	300	LC ₁₀	21.61	1.33 ^{ab}	0.12	attractant
	300	LC ₂₀	27.08	0.99 ^{cd}	0.12	neutral
	300	LC ₅₀	41.68	0.48 ^f	0.08	repellent
<i>S. bakhtiarica</i>	300	LC ₁₀	19.84	1.52 ^a	0.16	attractant
	300	LC ₂₀	26.09	1.15 ^{bc}	0.12	attractant
	300	LC ₅₀	44.06	0.59 ^{ef}	0.12	repellent
<i>S. khuzestanica</i>	300	LC ₁₀	14.50	1.25 ^b	0.12	attractant
	300	LC ₂₀	18.85	0.77 ^{de}	0.18	repellent
	300	LC ₅₀	31.16	0.4 ^f	0.16	repellent

¹ Repellence Index

² Standard deviation

Table 4. Repellent effects of different lethal concentrations (LC10, LC20, and LC50) ($\mu\text{L. L}^{-1}$ air) of three essential oils on a resistant population (MhR) of *T. urticae*. Different letters denote significantly different values from one another (Tukey).

Essential oil	N	Lethal dose	Concentration	RI ¹	SD ²	Effect
<i>T. daenensis</i>	300	LC ₁₀	71.77	1.52 ^{ab}	0.16	attractant
	300	LC ₂₀	86.70	1.23 ^{cd}	0.12	neutral
	300	LC ₅₀	124.47	0.69 ^{ef}	0.12	repellent
<i>S. bakhtiarica</i>	300	LC ₁₀	54.82	1.63 ^a	0.12	attractant
	300	LC ₂₀	68.61	1.41 ^{abc}	0.12	attractant
	300	LC ₅₀	105.39	0.77 ^e	0.12	repellent
<i>S. khuzestanica</i>	300	LC ₁₀	24.74	1.33 ^{bc}	0.17	attractant
	300	LC ₂₀	32.81	1.04 ^d	0.16	neutral
	300	LC ₅₀	56.29	0.45 ^f	0.16	repellent

¹ Repellence Index

² Standard deviation

GST

The activity of major detoxifying enzymes in response to exposure to LC₂₀ of essential oils was studied on both MhR and KrS populations.

Impact of essential oils on GST activity in *T. urticae* showed that essential oils led to an increased level of GST in treated mites compared to control. So that GST activity in MhR population treated with *S. khuzestanica*, *S. bakhtiarica*, and *T. daenensis* increased significantly approximately 1.52, 1.71, and 1.42-fold in comparison with Control (ANOVA: $F_{3,8} = 79.95$, $P < 0.001$). Whilst, in KrS population, GST activity went up nearly 1.77, 1.65, and 1.39-fold compared to control (ANOVA: $F_{3,8} = 28.73$, $P < 0.001$), respectively (Fig. 1). According to Fig. 1, In MhR population, treated mite with *S. bakhtiarica* had the highest GST activity among other essential oils whereas, in KrS population, the highest GST activity was observed in mites which were treated with LC₂₀ of *S. khuzestanica*. There were significant differences between GST activity in KrS and MhR populations. (ANOVA: $F_{7,16} = 68.83$, $P < 0.001$).

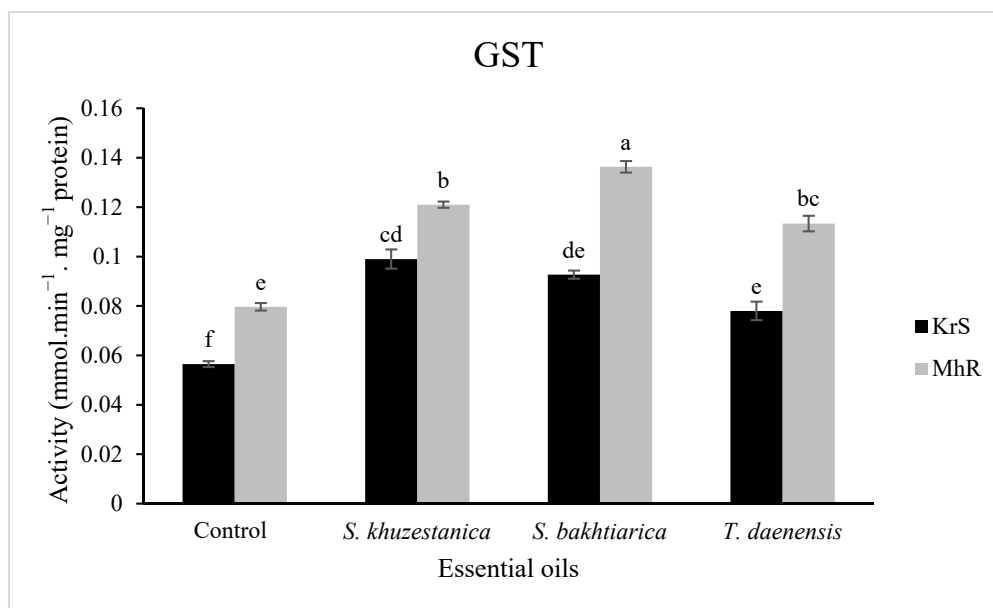


Figure 1. Glutathione S-transferases activity of a susceptible (KrS) and a resistant (MhR) population of *T. urticae* exposed to LC₂₀ of three essential oils. The values represent the means and standard errors for three replicates of 50 individuals. Different letters denote significantly different values from one another (Tukey).

P450

The amounts of P450 were measured in essential oil-treated populations of *T. urticae*. The results revealed that the amount of P450 was suppressed significantly in MhR treated with *S. bakhtiarica* and *T. daenensis* essential oils in comparison with control. Whilst, in the *S. khuzestanica* essential oil increased P450 values (ANOVA: $F_{3,8} = 7.49$, $P < 0.01$) (Fig. 2). In KrS population, a significant decrease in P450 activity was observed in mites treated with *S. khuzestanica* essential oil (ANOVA: $F_{3,8} = 5.72$, $P < 0.05$) (Fig. 2). The cytochrome P450 content in untreated KrS and MhR populations were 0.042 and 0.061 $\mu\text{g}/\text{mL}$, respectively suggesting that the amount of P450 in MhR population was 1.46-fold higher than KrS population. Based on statistical analyses there was a significant difference between the amounts of this protein in two populations ($P < 0.01$).

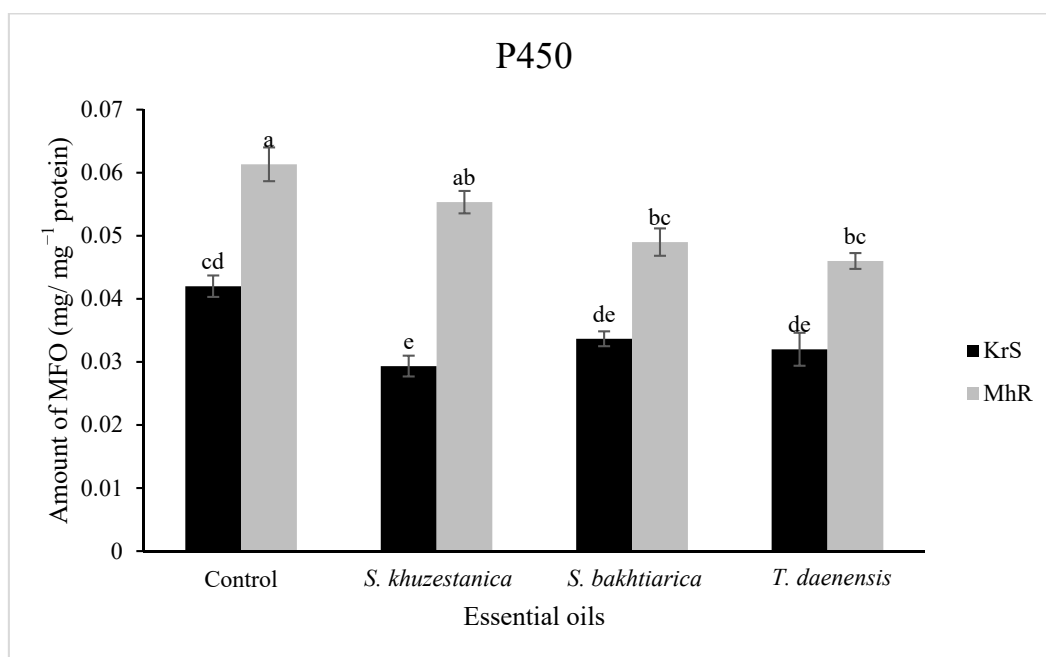


Figure 2. Cytochrome P450 monooxygenase amount of a susceptible (KrS) and a resistant (MhR) population of *T. urticae* exposed to LC_{20} of three essential oils. The values represent the means and standard errors for three replicates of 50 individuals. Different letters denote significantly different values from one another (Tukey).

EST

Treatment of both populations with the essential oils resulted in significant increase in EST activity with NA as substrate (for KrS population: ANOVA: $F_{3,8} = 53.43$, $P < 0.01$; and for MhR population: ANOVA: $F_{3,8} = 121.98$, $P < 0.001$) (Fig. 3). The highest activity of esterases (α -Na) was detected in *S. bakhtiarica* and *T. daenensis* treated in KrS and MhR populations, respectively. Also, there were significant differences in EST activity between two populations (ANOVA: $F_{7,16} = 417.61$, $P < 0.001$). By contrast, no significant change in esterase activity of mites was detected following treatment of essential oils when β -Na was used as a substrate (ANOVA: $F_{7,16} = 2.16$, $P = 0.96$) (Fig. 4).

The activity of GST, P450, and esterase (α -Na) in MhR population was significantly greater than those of KrS population in the controls (t-test, $P < 0.01$) (Figs. 1–3). However, there were no significant differences in the esterase activity of MhR and KrS populations when β -Na was used as substrate (t-test, $P > 0.05$) (Fig. 4).

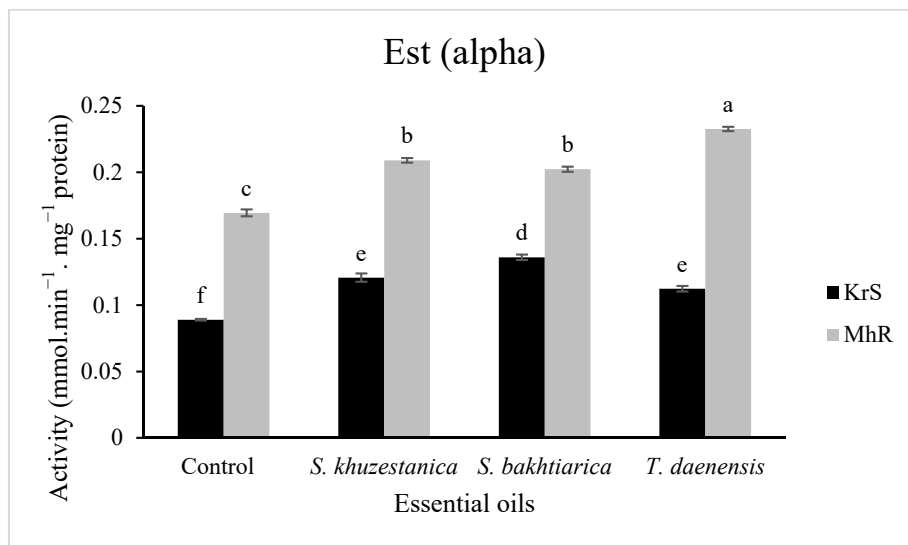


Figure 3. Esterase activity of a susceptible (KrS) and a resistant (MhR) population of *T. urticae* exposed to LC₂₀ of three essential oils using α-naphthyl acetate as substrate. The values represent the means and standard errors for three replicates of 50 individuals. Different letters denote significantly different values from one another (Tukey).

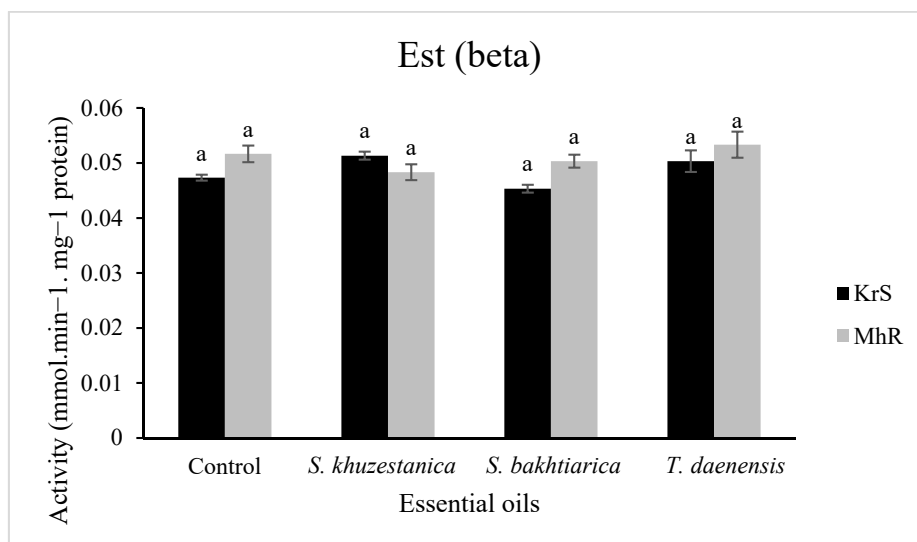


Figure 4. Esterase activity of a susceptible (KrS) and a resistant (MhR) population of *T. urticae* exposed to LC₂₀ of three essential oils using β-naphthyl acetate as substrate. The values represent the means and standard errors for three replicates of 50 individuals. Different letters denote significantly different values from one another (Tukey).

DISCUSSION

The essential oils extracted from plants have long been suggested as a promising alternative to synthetic chemical pesticides for pest control due to their little threats to the environment and human health (Isman 2006). Therefore, using plant secondary metabolites for pest management seems to be economically and environmentally profitable. In the current study, we evaluated the toxicity of essential oils extracted from three medicinal plants against two populations of *T. urticae* with different rates of resistance to common pesticides. The MhR population originated from ornamental greenhouses which have been exposed to pesticide pressure for many generations with many reports of control failure using common pesticides, such as propargite, abamectin, chlorpyrifos, and spiroadiclofen due to resistance development (Mohammadzadeh *et al.* 2014; Farahani *et al.* 2016,

2018). The susceptible population (KrS) was obtained from a research laboratory with no history of exposure to pesticides.

Although laboratory bioassays showed that the essential oil of *S. khuzestanica* was toxic to both MhR and KrS populations, there was a significant difference in LC₅₀ values of these essential oils between MhR and KrS populations. The lethal dose ratios (LC₅₀ value of essential oil in MhR/LC₅₀ value of essential oil in KrS) were estimated to be 2.99, 2.39, and 1.81 for *T. daenensis*, *S. bakhtiarica*, and *S. khuzestanica*, respectively (Table 2). Given that these populations have never before been exposed to these essential oils, these findings highlight the occurrence of cross-resistance in MhR population, resulting from resistance to other common pesticides.

The observed resistance in this study falls into the first group of resistance (low-level resistance class (RR ≤ 10)) based on Georghiou and Saito (1983) Resistance classification. The results of complete mortality (LC₁₀₀) indicate that the essential oils can be considered an acaricide against *T. urticae* in an area where conventional pesticides can no longer be effectively used since they cause 100% mortality in concentrations that are very unlikely to be harmful to host plants and non-target organisms. In agreement with these findings, the insecticidal and acaricidal properties of essential oils and their potential use in integrated management programs of pests have been documented in many studies (Ayvaz *et al.* 2010). de Melo *et al.* (2018) reported that *Aristolochia trilobata* essential oil displayed significant acaricidal activity against the pest *T. urticae* by both fumigant and residual contact assays. Furthermore, *Lavandula latifolia* essential oil significantly reduced the survival rate and fecundity of *T. urticae* (Laborda *et al.* 2018).

The repellency effect of three different concentrations of essential oils to adult *T. urticae* showed that the LC₅₀ of all essential oils were proved to be repellent to both MhR and KrS populations, while the repellency effect of LC₂₀ was observed only by *S. khuzestanica* essential oil. Similarly, Kheradmand *et al.* (2015) reported that the LC₂₀ and LC₂₅ of the essential oils taken from *Cuminum cyminum* (Apiaceae) and *Mentha spicata* (Lamiaceae) were repellents to *T. urticae*, while lower concentrations are neutral or even may act as an attractant to adult mites.

A set of physiological assays was carried out to measure the activity of major detoxifying enzymes in resistant and susceptible populations of *T. urticae* in response to exposure to essential oils. Based on our findings, the activity of GSTs and esterase (α -Na) increased significantly in treated MhR and KrS populations compared with control group, indicating the treated mites react physiologically i.e. increase detoxifying enzyme to toxic chemicals in order to metabolize them. Carreño Otero *et al.* (2018) studied the effects of *Cymbopogon flexuosus* essential oil, on GST and nonspecific esterases (α - and β -) on two populations (Rock and WSant) of *Aedes aegypti* L. They found that high concentrations of essential oil reduced GST, α - and β -esterase activities of Rock population. Also, protein, esterase, and GST of *Callosobruchus maculatus* F. and *Sitophilus oryzae* L. were affected significantly by the essential oil of *Atalantia monophylla* (Nattudurai *et al.* 2016). Moreover, there are many reports showing the effects of essential oils on insect detoxifying enzymes (Liao *et al.* 2017; Gao *et al.* 2019). These results imply that GSTs, and to a lesser extent esterases, are the major enzymes involved in metabolic resistance of the mites to plant essential oils. Esterases are an important group of metabolizing enzymes participating in xenobiotic detoxification or sequestration, a process that surrounds toxic compounds thus preventing them from gaining access to the target sites. GSTs, on the other hand, belong to a superfamily of enzymes that are involved in phase II detoxification of xenobiotics. These enzymes catalyze the conjugation of the tripeptide glutathione to the electrophilic center of lipophilic compounds, thereby increasing their solubility and aiding excretion from the organisms (Hayes *et al.* 2005). Given the significant increase of GST and esterase (α -Na) activity following exposure to essential oils in both MhR and KrS populations, we hypothesized that these enzyme activities increase in resistant (MhR) population following exposure to pesticides which was confirmed by calculation of the GST and esterase (α -Na) ratio of MhR to KrS population. By contrast, our findings showed that a significant decrease in P450 activity was detected in treated mites, while the activity of esterase (β -Na) did not change in response to essential

oil treatment. Cytochrome P₄₅₀ monooxygenases (CYPs) are important metabolic systems that contribute to catabolism and anabolism of endogenous compounds such as hormones and pheromones and also in detoxification or activation of xenobiotics (Brown *et al.* 2003).

It is concluded that results of the current study indicate that there is cross-resistance to the applied essential oils in MhR population of *T. urticae*. Also, essential oils taken from some medicinal plants of Lamiaceae family have the potential to be used as ecofriendly acaricides for management of *T. urticae* in agricultural ecosystem. These plant-derived pesticides have lower harmful effects on non-target organisms and human and are easily biodegradable, thus are suitable alternatives for synthetic pesticides in agricultural fields. With respect to their diverse modes of action, these compounds can be efficiently included in pest control programs for management of resistance development by the pests.

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اثر کنه‌کشی و دورکنندگی سه اسانس گیاهی روی دو جمعیت کنه تارتن دو لکه‌ای *Tetranychus urticae* (Acari: Tetranychidae)

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چکیده

اسانس‌های گیاهی جزء ترکیبات ثانویه گیاهی‌اند که توانایی زیاد حشره‌کشی و کنه‌کشی دارند و به عنوان جایگزین آفت‌کش‌های شیمیایی در مهار آفات مورد استفاده قرار می‌گیرند. در این پژوهش، اثر کنه‌کشی و دورکنندگی سه اسانس بومی استخراج شده از گیاهان خانواده *Lamiaceae* شامل آویشن دنايي (*Thymus daenensis*)، مرزه خوزستانی (*Satureja khuzestanica*) و مرزه بختیاری (*S. bakhtiarica*) روی دو جمعیت حساس (KrS) و مقاوم (MhR) کنه تارتن دو لکه‌ای *Tetranychus urticae* که به ترتیب از کرج و محلات جمع آوری شده بودند مورد بررسی قرار گرفت. نتایج نشان داد که بیشترین میزان کشندگی در هر دو جمعیت کرج و محلات مربوط به اسانس مرزه خوزستانی به ترتیب با LC₅₀ برابر با ۳۱/۱۶ و ۵۶/۲۹ میکرولیتر بر لیتر هوا بود. تمامی اسانس‌های گیاهی مورد استفاده دارای اثر دورکنندگی روی کنه ماده بالغ در هر دو جمعیت بودند به طوری که مرزه خوزستانی بیشترین اثر دورکنندگی را در بین دیگر اسانس‌ها داشت. نتایج اثر غلظت کشنده ۲۰ درصد سه اسانس فوق روی فعالیت آنزیم‌های سیتوکروم P450، گلوکوتایون اس-ترانسفراز و استراز نشان داد میزان فعالیت هر سه آنزیم مذکور در جمعیت محلات به طور معنی‌داری بیشتر از جمعیت کرج است. همچنین تیمار کنه ماده بالغ با غلظت کشنده ۲۰ درصد هر یک از اسانس‌ها منجر به افزایش فعالیت آنزیم‌های استراز و گلوکوتایون اس-ترانسفراز در هر دو جمعیت شد در حالی که میزان مونواکسیژنازهای سیتوکروم P450 در هر دو جمعیت کاهش یافت. نتایج حاصل از این پژوهش نشان می‌دهد اسانس‌های گیاهی مورد استفاده، به ویژه اسانس مرزه خوزستانی، پتانسیل زیادی برای استفاده در برنامه‌های مدیریت تلفیقی کنه تارتن دو لکه‌ای دارند.

واژگان کلیدی: آنزیم‌های سم‌زدایی؛ مدیریت تلفیقی آفات؛ مرزه بختیاری؛ مرزه خوزستانی؛ آویشن دنايي؛ کنه تارتن دو لکه‌ای.

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