Toxicity and Oviposition Deterrence and Repellency of *Mentha pulegium* (Lamiaceae) Essential Oils against *Tetranychus urticae* Koch (Tetranychidae)

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Abstract: The two-spotted spider mite, *Tetranychus urticae* Koch, one of the major pests of various agricultural plants, causes many problems on agricultural, horticultural and ornamental plants. In recent years, using plant-derived compounds such as essential oils and extracts was attended for their insecticidal and acaricidal properties. *Mentha pulegium* is a native plant of temperate Asia, was identified as an acaricidal plant for the first time in this study. The impact of essential oils of this plant on fumigant toxicity, LT50, oviposition deterrent activity and repellency against *Tetranychus urticae* was studied at different concentrations. The results stated that *M. pulegium* oils had high toxicity on eggs and adults of two-spotted spider mite (LC50 was 2.57 and 2.25 µL L\(^{-1}\) for eggs and adults, respectively). In addition, all tested concentrations had oviposition deterrent activity and repellency on adults of this mite.

Key words: Essential oils, Fumigant toxicity, *Tetranychus urticae*, repellency, oviposition deterrence, *Mentha pulegium*.

Introduction

A polyphagous and major pest, the two-spotted spider mite, *Tetranychus urticae* Koch (Acarina: Tetranychidae) affects crops by direct feeding and reducing the area of photosynthetic activity and causing leaf abscission in severe infestations 1. *T. urticae* is a major pest on crops of field, glasshouse, horticultural, ornamentals and fruit trees and has been reported to attack about 1,200 plant species 2,3. A major problem in controlling spider mites resides in their ability to rapidly develop resistance to acaricides because of their high reproductive potential and short life cycle 4.

It is important to reduce the use of synthetic acaricides and replace them with compounds having different modes of action for resistance management 5. Because of innately biodegradable and less toxic to non-target organisms, natural products are generally preferred 6. In the recent years, research has been turning to selective biorational pesticides such as plant-derived compounds, because they are safe to mammals and the environment, compared to chemicals 7. Essential oils are usually extracted from various parts of plants 8,9 and have been evaluated for their acaricidal and insecticidal activity 10,11,12,13, 14,15,16. Pumnuan et al. studied the effect of *Litsea cubeba*, *L. salicifolia* and *Melaleuca cajuputi* on...
the Mushroom mite, *Luciaphorus perniciosus* (Acari: Pygmephoridae) and found these plants effective on this acari pest 17. In another study, Kim *et al.* demonstrated that various essential oils have high toxicity to adults of *Dermatophagoides gallinae* De Geer through contact 13. Also, *Mentha microphylla* K. oil caused 56 to 100 % mortality of *T. urticae* in fumigation bioassay 18.

The Lamiaceae family is recognized for its vital oils, usages for medicinal purposes and antimicrobial activity of different species 19. One of the important genera of the Lamiaceae is *Mentha* sp, with approximately 19 species and 13 natural hybrids. *Mentha* plants are fast growing and usually tolerate a wide range of agro-climatic conditions 20. *Mentha pulegium* L. (Syn. *Pulegium vulgar* Mill.), is native to temperate Asia, Africa and Europe. This plant grows along the ground and spreads through its underground root system 21. It is used as an insect repellent. Also it is used for its antispasmodic, diaphoretic and anti-inflammatory properties in Iran 22. In some papers, tests of *Mentha* species on different properties of insects and mites were reported. Rim and Jee tested *M. pulegium* L. oil on *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* through fumigation and contact bioassays 23. Mahmoudvand *et al.*, stated that essential oil of *M. pulegium* has fumigant toxicity on *Sitophilus granaries* 15. Another species of *Mentha, M. piperita* had high fumigant toxicity on *Callosobruchus maculatus* 24.

Based on author knowledge, there are no published reports on the acaricidal activity of the essential oil of *M. pulegium*, up to now. The objective of this work was to investigate the acaricidal activity of *M. pulegium* against *T. urticae* including fumigation to eggs and adults, oviposition deterrent activity, and repellency.

Materials and methods

**Essential oil extraction**

The leaves of *M. pulegium* were collected for the extraction of the essential oil. All the leaves were dried in shade under indoor conditions and were subjected to hydrodistillation using Clevenger type distiller 25. For the extraction, 50 g of dried leaves were put in water (1:12 w:v) for distillation during 4 h. Extracted essential oils were dried via anhydrous sodium sulphate and stored at 4°C in darkness. Pure essential oil was used for fumigant bioassay tests.

**Acaries rearing**

The original colony of *T. urticae* was supplied by the Acarology laboratory of the Faculty of Agriculture, University of Tehran, Karaj, Iran. The pest rearing was conducted as follows: lima bean (*Phaseolus vulgaris*) seeds were planted in plastic jars (15 cm in diameter) at a rate of 4-5 seeds per jar, and incubated under muslin cage to prevent any infestation. Jars containing lima bean seedlings (15-20 cm long) were taken to the laboratory, then infested leaves of *T. urticae* were transferred to these plants and left to reproduce under the laboratory conditions maintained at 25 ± 1°C. This colony was supplied with fresh lima bean plants from time to time when it was necessary.

**Toxicity of the oil on adults**

**Calculating LC₉₀ and LT₅₀ values of the oil**

The fumigant toxicity of the essential oil on *T. urticae* adults was tested in plastic vials (140 ml). In each vial, 10 adults (1-3 days old) were released on lima bean leaves. Filter paper disks (Whatman No. 1), cut into 2 cm in diameter and impregnated with the series of concentrations of *M. pulegium* essential oil, were attached to undersurface of screw caps of plastic vials. After 24 h, the number of dead mites were recorded. Four replications were run for each concentration and the control. All tests were carried out in a growth chamber set at 25 ± 1°C and 70 ± 5 % RH with a 16/8 h light/dark photoperiod.

To investigate the lethal time (LT₅₀) of *M. pulegium* against the mites, the essential oil on this pest was used from high concentrations those obtained from bioassay tests (10 and 100 μL L⁻¹ air). Selected concentrations similar to mentioned method for bioassay test was tested and for each dose, four replications were performed. The mortality of subjects was counted at 3 h intervals until death of all mites.

**Toxicity of the oil on eggs**

For contact toxicity assay with eggs, five individuals of *T. urticae* females were allowed
to oviposit for 24 h on 3 cm diameter lima bean leaf discs resting on wet cotton pads in a Petri dish (9 cm diameter). Then they were removed and the eggs were counted. The leaf discs containing 10 eggs were put in plastic vials and exposed 24 h to different concentrations. The method of this test was similar to that for adulticidal assay with different subject.

Oviposition deterrence effect of the oil
To investigate the effect of the essential oil on the oviposition reduction, four concentrations (50, 250, 500 and 1000 mg L$^{-1}$) of the essential oil of *M. pulegium* were prepared (mixed with water and ethanol (70:30)). For each treatment, 20 µL of various ethanol solutions were released on the surface of leaves (3 cm in diameter). After evaporation of the water and ethanol, two pairs (2 males and 2 females) of adults (3-days-old) of *T. urticae* for each concentration and the control were selected and placed in a plastic vial (140 ml). For the control, only ethanol and water were used. For each concentration, 4 replications were performed. The number of eggs laid was recorded after 24 h and oviposition deterrence was calculated using the following formula:

$$\text{Oviposition deterrence} = \left[1 - \frac{\text{NE}_t}{\text{NE}_c}\right] \times 100$$

Where, **NE**$_t$=Number of eggs in treatment, **NE**$_c$=Number of eggs in control

Repellency test of the oil
The repellency test of *M. pulegium* oil was made according to the method described by Kogan and Goeden. Leaf disks of lima bean of 3 cm diameter were used to evaluate the repellency of the oils. Half of the disk was immersed into an ethanolic solution of the oil in four concentrations (500, 2500, 5000 and 10000 mg L$^{-1}$) for 5 sec and after drying, the other half of the disk was immersed in ethanol and water solution, which served as control. Ten male and female mites were placed on each disk (five mites on each half leaf disk). Each treatment was repeated 4 times. The evaluation was made after 24 h where the numbers of mites present in each half of the leaf were counted. The repellency percentage (% R) of the oils was calculated according to the equation:

$$\% \text{ R} = \frac{C - T}{N} \times 100$$

Where, **T** = number of mites in the treatment, **C** = number of mites in the control, **N** = Number of mites used in the test.

Statistical analysis
The lethal concentrations (LC$_{50}$ and LC$_{90}$) and the lethal time (LT$_{50}$) values of mortality were assessed by Probit analysis using SAS software. The data from the oviposition, deterrence and repellency percentage were subjected to one-way ANOVA ($P<0.05$) after checking for normality. Means were compared by Duncan’s new multiple Range Test, admitting significant differences at $P<0.05$. The SAS software was used for all analyses.

Results
**LC$_{50}$ and LT$_{50}$ values of essential oil**
**LC$_{50}$** and **LC$_{90}$** values of the essential oil of *M. pulegium* on eggs and adults of the two-spotted spider mite after 24 h are shown in Table 1. Values of fumigant LC$_{50}$ of essential oil on eggs and adults were 2.57 and 2.25 µL L$^{-1}$ air, respectively. The lethal mortality time (LT$_{50}$) of essential oil of *M. pulegium* on adults of *T. urticae* was 22.35 and 5.81 h for 2.8 and 28 µL L$^{-1}$ air, respectively. All of the treated acaries by 100 µL L$^{-1}$ air concentration were killed after a few minutes from the beginning of test (Table 2).

| Table 1. Fumigant toxicity of essential oil of *M. pulegium* against eggs and adults of *T. urticae* after 24 h. |
|---------------------------|-----------------|-----------------|-----------------|------------------|-----------------|
| n | Df | LC$_{50}$ (µL L$^{-1}$ air) | LC$_{90}$ (µL L$^{-1}$ air) | Slope ± SE | X$^2$ |
| Adult | 240 | 4 | 2.57 (2.01-2.97) | 5.73 (4.53-10.57) | 3.66 ± 2.84 | 4.5 |
| Eggs | 160 | 2 | 2.25 (1.71-8.4) | 5.04 (3.89-6.55) | 0.02 ± 0.01 | 1.54 |
Repellency of *M. pulegium* essential oil of acari

Although all concentrations tested had repellency on the mite, there weren’t any significant differences among various concentrations of *M. pulegium* (df=3, 13, F=0.74, P=0.54). The highest repellency was observed at 500 and 1000 mg L\(^{-1}\) (Fig. 1) and [Fig. 1]

*all of the treated acaries died a few minutes after the beginning of the test

**Repellency of *M. pulegium* essential oil of acari**

<table>
<thead>
<tr>
<th>Concentration (µL L(^{-1}) air)</th>
<th>df</th>
<th>LT(_{50})</th>
<th>LT(_{90})</th>
<th>Slope ± SE</th>
<th>X(^2)</th>
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<td>2.8</td>
<td>6</td>
<td>22.35 (19.75-26.81)</td>
<td>37.61 (31.64-49.06)</td>
<td>0.08 ± 0.01</td>
<td>2.63</td>
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<td>28</td>
<td>3</td>
<td>5.81 (4.54-6.83)</td>
<td>10.98 (9.68-13.09)</td>
<td>0.24 ± 0.03</td>
<td>0.55</td>
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Table 2. Values of lethal time of mortality (LT\(_{50}\)) of *M. pulegium* essential oil on adults of *T. urticae* after 24 h.

*all of the treated acaries died a few minutes after the beginning of the test

**Repellency of *M. pulegium* essential oil of acari**

Fig. 1. Percentage (±SE) of oviposition deterrence of *M. pulegium* essential oil on adults of *T. urticae*. Means marked with different letters are significantly different (P<0.05; Duncan). (df=3, 13, F=0.74, P=0.54)

Fig. 2. Repellency (%) effect of *M. pulegium* essential oil on adults of *T. urticae*. Means marked with different letters are significantly different (P<0.05; Duncan). (df=3, 13, F=0.44, P=0.73)
Oviposition deterrence of the essential oil on acari

Figure 2 reports the percentage of deterrence of various concentrations of the essential oil of *M. pulegium* on oviposition of *T. urticae*. The results indicated that all concentrations were significantly different for this parameter (>85%) (df=3, 13, F=0.44, P=0.73). The perfect deterrence (100%) was seen in 500 and 1000 mg L$^{-1}$ concentrations.

**Discussion and conclusion**

The current study reported the effect of the essential oil of *M. pulegium* on various biological parameters of *T. urticae*. The results showed that this oil had high toxicity on two life stages of this important pest (LC$_{50}$ = 2.25 and 2.75 µl L$^{-1}$ air on eggs and adults, respectively). Susceptibility of eggs was higher than that of adults in our study. In some previous studies, impacts of plant oils was demonstrated on insecticidal, acaricidal, oviposition deterrence, repellent and antifeedant of mites. Mahmoudvand et al. stated that the oil of *M. pulegium* had high fumigant toxicity on *Sittophilus granarius* L. (Coleoptera: Curculionidae); the LC$_{50}$ value was 0.038 µl L$^{-1}$ air. The LC$_{50}$ values of the oil observed in this study and the results of Mahmoudvand et al., demonstrated that the fumigant toxicity of this plant oil is very high as compared with other oils. Lamiri et al., found that the essential oil of *M. pulegium* caused 100% mortality of *Mayetiola destructor* Say (Diptera: Cecidomyiidae). Similarly, Aroiee et al., stated that *Mentha piperita* oil caused 52-62% mortality of green house whiteflies, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). In another study, Konstantopoulou et al., investigated the insecticidal impact of *M. pulegium* essential oil on *Drosophila auraria* Complex (Diptera: Drosophilidae) and reported that this oil could kill 100% of tested insects after 30 min. Pavela evaluated fumigant activity (similar to our method) and topical bioassay of different oils such as *M. pulegium* against *Musca domestica* L. (Diptera: Muscidae) under laboratory conditions. The values of LC$_{50}$ were 4.7 µg/cm$^2$ and 13 µg/fly in fumigation and topical tests. Also, *M. piperita* and *M. spicata* L. oils were tested as fumigant against adults of two-spotted spider mite, *T. urticae*. *M. piperita* caused more than 90% and *M. spicata* 81 to 82% mortality of mite colonies. Sertkaya et al., tested *Mentha spicata* L. oil vapor against females of the carmine spider mite, *Tetranychus cinnabarinus* and recorded LC$_{50}$ of 1.8 µg/ml. Konstantopoulou et al., investigated the essential oil of *M. pulegium* against 3rd instar larvae of *D. auraria* and obtained 80% mortality after 48 h.

In our study, LT$_{50}$ values of three oil concentrations (2.8, 28 and 100 µL L$^{-1}$ air) of *M. pulegium* on *T. urticae* were recorded. Results showed that the time of mortality was shortened with increasing concentrations. All subjects died in 100 µL L$^{-1}$ air group, a few minutes after exposure. In addition, oviposition deterrent activity of *M. pulegium* oil on this mite was investigated. All the concentrations (500, 2500, 5000 and 10000 mg L$^{-1}$) decreased the oviposition of acari females after 24 h. However, the mean comparison showed there wasn’t any significant difference between all concentrations. Aziz and Abbass investigated the effect of different concentrations of *Mentha rotundifolia* L. Hudson and *M. pulegium* on oviposition of *Callosobruchus maculatus*. Kumar et al., evaluated oviposition deterrence and ovicidal activity of *Mentha* oils for *Callosobruchus chinensis*. The study showed that this oil had oviposition deterrence on *C. chinensis*. The results indicated that these oils had significant reduction of fecundity of this coleopteran pest. The results of Aziz and Abbass and Kumar et al., corroborated our results on the effect of *M. pulegium* on oviposition deterrence of arthropod animals using different subjects.

Repellent activity of *M. pulegium* essential oils on adults of *T. urticae* was evaluated in our study. Used concentrations in this test (50, 250, 500 and 1000 mg L$^{-1}$) were lower than those for oviposition deterrence test. All these concentrations significantly affected *T. urticae*. The repellency percentage of all concentrations was more than 85%. The two highest concentrations had 100% repellent effect on adult mites.

Erler et al., reported that *M. piperita* had repellent effects on female adults of *Culex pipiens* L. (Diptera: Culicidae). Pontes et al., found that...
essential oils extracted from Protium heptaphyllum (Aubl.) (Burseraceae) leaves and fruits had repellent effects on adults of the two spotted spider mite. Therefore, the results observed in this study demonstrate that M. pulegium essential oil would be a good choice for replacing chemical insecticides.

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References