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Article

Essential oils of some Lamiaceae plants: analysis and their activity against *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT
The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a significant pest that impacts greenhouse, vegetable, and ornamental crops globally. Historically, pesticides have been utilized to control this pest, however there are apprehensions regarding their frequent use. Consequently, plant essential oils have emerged as a potential pesticide substitute, generating considerable attention in pest management. The essential oils were obtained via water distillation using a Clevenger apparatus. Their efficacy against different developmental stages of *T. urticae* was assessed using a vapor-phase mortality method that did not require direct contact. The repellent and fumigant toxicity of the essential oils were also examined on adult mites. Based on GC-MS analysis the main components of *Perovskia abrotanoides*, *Rosmarinus officinalis*, *Salvia sahendica*, *Satureja hortensis*, and *Thymus daenensis* essential oils were menthol, verbenone, α-pinene, carvacrol, and thymol, respectively. The acaricidal activity of the vital oils followed a dose-dependent manner. Essential oil from *T. daenensis* was more toxic against adults of *T. urticae* (LC_{50} = 1.21µl/L air and essential oil from *S. hortensis* was more toxic against larvae (LC_{50} = 2.48µl/L air) and eggs (LC_{50} = 6.48 µl/L air) of *T. urticae*, respectively. Results of the repellency assay showed that essential oils of *S. hortensis* had the highest repellency against adults of *T. urticae*.

KEYWORDS: Essential oil, fumigant toxicity, medicinal plants, repellency, two-spotted spider mite.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a serious pest that can cause damage to greenhouse and field vegetables and crops. This damage can lead to the death of the entire plant, both directly and indirectly (Badawy *et al*. 2010; Roh *et al*. 2011). Management of this pest often relies on synthetic pesticides or the introduction of natural enemies. However, the primary challenge in controlling this pest is its increasing resistance to most synthetic pesticides. Additionally, the limited effectiveness of biological control agents against the large pest population,

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combined with their high susceptibility to most pesticides, further complicates the pest control (Stumpf *et al*. 2001; Miresmailli *et al*. 2006; Laborda *et al*. 2013). To address these inefficiencies, plant essential oils with insecticidal, acaricidal, repellent, and antifeedant properties have gained significant interest as a potential substitute for synthetic pesticides. Essential oils are natural plant products that consist of a blend of terpenoids.

Terpenoids (isoprenoids) are a vast and varied lipid group. These compounds occur naturally and are the primary components of essential oils in plants. They contribute to the plant's scent and serve as a defense mechanism against both living organisms and environmental factors (Singh and Sharma 2015). Most of these secondary metabolites e.g., eugenol, thymol, carvacrol, menthol, citronellal, citral, pulegone, cinnamaldehyde, and 1,8-cineole offer potent bioactive properties (Isman and Machial 2006; Pérez-Bermúdez *et al*. 2010; Laborda *et al*. 2013).

Essential oils from plants in the Lamiaceae family, such as *Lavandula*, *Mentha*, *Origanum*, *Satureja*, and *Salvia*, have shown strong acaricidal, insecticidal, and ovicidal properties against *T. urticae*, making them promising alternatives to synthetic pesticides. Their natural origin and multifaceted modes of action, including fumigant and contact toxicity, make them a valuable resource for pest control in both agricultural and greenhouse settings. Several studies highlight the efficacy of essential oils derived from Lamiaceae family members in managing *T. urticae*, a common and harmful agricultural pest. Essential oil vapors from *Nepeta racemosa*, *Origanum vulgare*, and *Micromeria fruticosa* plants were effective against both *T. urticae* and *Bemisia tabaci* (Gennadius). The highest mortality occurred at 2 µL/L air concentration with extended exposure time. *Tetranychus urticae* showed more tolerance compared to *B. tabaci*, but the oils from these Lamiaceae plants demonstrated strong potential in pest management under greenhouse conditions (Çalmaşur *et al*. 2006). *Lavandula intermedia, Salvia officinalis, R. officinalis,* and *Hyssopus officinalis* essential oils demonstrated contact toxicity, repellency, and ovicidal activity against *T. urticae*, with a greater efficacy in repelling nymphs than adults. Lavandin exhibited the strongest ovicidal effect, while sage showed the weakest. These findings suggest that these essential oils are suitable for integrated pest management programs targeting *T. urticae* (Salman *et al*. 2015). The oil from *M. piperita* and its main compound, menthol, were found to be highly toxic to *T. urticae*, especially through fumigation. The oil was significantly more toxic than menthol alone, reducing the mite's ability to produce offspring. The way the oil works suggests that it has potential as an acaricide by being absorbed through the mite's respiratory system (Souza *et al*. 2022). The essential oils of *S. hortensis*, *Ocimum basilicum*, and *T. vulgaris* have demonstrated acaricidal and insecticidal activity, with *S. hortensis* being the most effective. Significant mortality rates were achieved at concentrations of 0.39 to 3.125 µL/L air, suggesting that these oils have the potential to be used as alternatives to chemical pesticides for controlling *T. urticae* under greenhouse conditions (Aslan *et al*. 2004). Essential oil from *R. officinalis* demonstrates synergistic toxicity when combined with its major constituents, indicating the necessity of all components for maximum efficacy. This characteristic makes *R. officinalis* oil a promising option for biopesticides targeting *T. urticae* (Miresmailli *et al*. 2006).

The Lamiaceae is a plant family with numerous species globally (Laborda *et al*. 2013). Essential oils of five species of this family i.e., *Perovskia abrotanoides* Kar., *Rosmarinus officinalis* L., *Salvia sahendica* Boiss. & Buhse, *Satureja hortensis* L. and *Thymus daenensis* Celak were extracted, analyzed, and assayed against *T. urticae*, a highly destructive agricultural pest, at different developmental stages. Despite extensive research on the insecticidal properties of essential oils, their specific effects on *T. urticae* at different developmental stages remain inadequately explored. This study addresses this gap by analyzing the chemical composition of these oils and testing their effectiveness on various life stages of the pest. The need for this research arises from the increasing demand for eco-friendly alternatives to synthetic pesticides, which carry environmental and health risks. Identifying natural, plant-based solutions could enhance sustainable pest control strategies in agriculture.

MATERIALS AND METHODS

Plant material and preparation of essential oil

At their flowering stage, *P. abrotanoides*, *R. officinalis*, *S. sahendica*, *S. hortensis*, *and T. daenensis* were collected from Shiraz and Dezfoul, Iran, shed dried, and then their aerial parts were ground. Two hours of simultaneous hydrodistillation using the Clevenger-type apparatus was used to isolate the essential oils. Different layers of the oils were separated, kept in airtight sealed glass containers, and stored at +4 °C before the gas chromatography/mass spectrometry (GC-MS analysis).

Rearing, maintenance, and developmental synchronization of the mites

The colony of *T. urticae* was obtained from infested plants in an Isfahan greenhouse. The mites were maintained at 25 ± 2 °C, 60 ± 10% RH, and 16:8 L/D on kidney beans (*Phaseolus vulgaris*) seedlings in the Laboratory of Entomology, Isfahan University of Technology, Isfahan, Iran. To assess the acaricidal effects of the essential oils 2- to 3-days-old female adults were used. To this purpose, fresh seedlings with 3–4 leaves of kidney beans were placed between the mite-infested bean plants for 24–48 hours. The adult females of the mite that moved onto the fresh leaves were used for experiments. For ovicidal bioassays, the adult mites were transferred to seedlings of kidney beans using a fine brush. After 24 h the laid eggs were separated and treated with the essential oils. To obtain the same-aged larvae, adults were placed on kidney bean plants and allowed to lay eggs. After five days eggs hatched and the effects of essential oils were evaluated against the larval stage.

Chemical analysis of essential oils

To isolate and identify major constituents of the essential oils, extracted oils were subjected to GC-MS (model Agilent 7890A) equipped with an analytical column (30M × 0.25µm, Agilent 5975C, Mass. The carrier gas was Helium and the oven temperature was set at an initial temperature of 60–280 °C (Baharum *et al.* 2010). The identities of the components of the essential oils were confirmed based on GC retention indices regarding a homologous series of C11-C24 *n*-alkanes and the equation from Mitić *et al.* (2011) as well as by computer matching against the mass spectral library of the GC/MS data along with other published mass spectra (Adams 1995).

Fumigant activity assay of the essential oils

A vapor-phase mortality method assessed the fumigant toxicity of the essential oils against adults, larvae, and eggs of *T. urticae* without direct contact with the specimens with the toxicants (Choi *et al*. 2004; Roh *et al*. 2013; Souza *et al*. 2022). Three centimeters diameter discs of kidney bean leaves, two weeks old were punched and placed on water-soaked cotton pads in plastic Petri dishes (2 × 9 cm). Bioassays were conducted using five concentrations (0.25, 0.5, 1, 2, and 4 µl/l air) of essential oils from *M. piperita*, *M. pulegium*, and *M. spicata* against adult *T. urticae*. For *M. longifolia*, four concentrations (2.5, 5, 10, and 20 µl/l air) were used against adults and (1.25, 2.5, 5, and 10 µl/l air) for larvae, while egg bioassays involved four concentrations (5, 25, 50, and 75 µl/l air). Additionally, bioassays for larvae and eggs employed five concentrations (1.25, 2.5, 5, 10, and 20 µl/l air) and (2.5, 5, 10, 20, and 25 µl/l air), respectively, for the essential oils of *M. piperita*, *M. pulegium*, and *M. spicata*. Acetone served as the control. Leaf discs were placed on water-soaked cotton pads in 2 × 9 cm plastic Petri dishes, with acetone used to create different concentrations. One µl of essential oil was applied to the inner surface of the dishes, while control filter paper received 1 µl of acetone. The dishes were maintained at 26 ± 2 °C, 55–60% RH, with a 16:8 (L:D) photoperiod. Mortality rates for adults, larvae, and eggs were assessed 1, 1, and 5 days post-treatment, respectively.

Repellent activity assay of the essential oils

To evaluate the repellent activity of the essential oils against adults of the mite, the discs of the kidney bean leaves, six cm in diameter were cut in half, and three concentrations, 10, 100, and 1000 mg/L of the solutions (prepared by diluting the essential oil in acetone) were thoroughly applied on the surface of the half-leaf discs using a micropipette. The other half of the leaf was treated with acetone alone and used as a control. The discs were air-dried for 5 minutes to evaporate the solvent and placed into 10 cm Petri dishes. Adult mites (20 per replication) were released at the center of the disc. The experiment was repeated 4 times. After two hours, the number of mites on the discs was counted. The percentage of each essential oil repellency (%R) was calculated according to the following equation:

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

where, T is the number of mites in the treatment, C is the number of mites in the control and N is the number of used mites in the test (Nerio *et al*. 2009).

Statistical analysis

Analysis of variance (ANOVA) was used to find variability inside the repellency data. The mean values were compared by LSD test ($P < 0.05$) using SAS software. Corrected mortality (% of adults, larvae, and eggs) was transformed by Abbott's formula. The mean lethal concentrations. The LC_{50} values of mortality were assessed by probit analysis using POLO-PC with 95% confidence (Robertson *et al*. 1980). The comparison of the values LC_{50} was done by calculating the 95% confidence interval for the LC_{50} for each developmental stage, concluding that no significant difference exists if the two confidence intervals overlap or by comparing the ratio of the LC_{50}s to 1 (Wheeler *et al*. 2006).

RESULTS

Analysis of the essential oils

The chemical composition of the essential oils is represented in Table 1. This table shows an interspecific difference among the essential oils of the plants. A total of 37 compounds are presented. Out of these 37, the detected compounds of *P. abrotanoides, R. officinalis, S. sahendica, S. hortensis*, and *T. daenensis* were 19, 23, 25, 22, and 23, respectively. However, nine compounds i.e., α-thujan, α-pinene, camphene, β-pinene, β-myrcene, α-terpinene, 1,8-cineole, γ-terpinene, and thymol were common among the species. The predominant component of each species was menthol (29.3%), verbenone (19.0%), α-pinene (15.2%), carvacrol (57.0%), and thymol (65.2%), respectively.

Fumigant toxicity assay

The vapors of the essential oils were almost toxic to *T. urticae* adults and caused approximately 80% mortality in high doses (Table 2). The LC_{50} values of fumigant toxicity of the essential oils against *T. urticae* eggs, larvae, and adults were calculated. The essential oils showed acaricidal activities in a dose-dependent manner. Essential oil from *T. daenensis* was more toxic against adults of *T. urticae* (LC_{50} = 1.21µl/L air) and essential oil from *S. hortensis* was more toxic against larvae (LC_{50} = 2.48µl/L air) and eggs (LC_{50} = 6.48 µl/L air) of *T. urticae* respectively. The high χ^2 value (e.g., the eggs treated with essential oil of *T. daenensis*) indicates a significant deviation from the probit model. The highest value for slope (e.g., the adults and larvae treated with essential oil of *T. daenensis*) indicates the least heterogeneity in the population.

Based on the LC_{50} values and their 95% fiducial limits, it is evident that the larvae were significantly more sensitive to *P. abrotanoides* essential oil compared to the adults and eggs. There was no significant difference in sensitivity between the adults and the eggs. In *R. officinalis*, the larval stage showed greater sensitivity than the other stages, while the adult LC50 value was notably lower than that of the eggs. In *S. sahendica*, the LC_{50} values for adult and larval stages were similar, but the LC_{50} value for eggs was significantly higher. All developmental stages of the mite showed similar sensitivity to *S. hortensis*, with no significant differences observed. The bioassay of *T. daenensis* essential oil revealed that adult mites are significantly more sensitive than other developmental stages, with larvae also exhibiting greater sensitivity than eggs (Table 2). Our results indicate that mite eggs are the least sensitive stage to the essential oils tested in this research. By examining the toxicity ratios of various essential oils at different developmental stages of the spider mite, it is evident that the differences in toxicity among these oils are significant across the pest's life cycle (Table 3).

Table 1. Main components of essential oils from five species of Lamiaceae determined by GC-MS.

Compound	RI	% Composition				
		Perovskia abrotanoides	***Rosmarinus officinalis***	***Salvia sahendica***	***Satureja hortensis***	***Thymus daenensis***
α-Thujan	930	0.03	0.18	1.19	0.6	1.23
α-Pinene	940	0.65	11.82	**15.21**	0.6	1.01
Camphene	951	0.07	3.55	2.12	0.1	0.49
Sabinene	976	0.52	n.d.	8.96	n.d.	n.d.
3-Octanone	978	n.d.	2.54	n.d.	n.d.	n.d.
β-Pinene	979	0.97	0.52	**14.57**	0.11	0.35
β-Myrcene	992	0.17	2.17	0.8	1.04	1.54
3-Octanol	997	n.d.	0.5	n.d.	n.d.	n.d.
α-Phellandrene	1006	n.d.	0.13	0.09	0.22	0.32
Δ-3-Carene	1011	n.d.	n.d.	0.06	0.05	0.12
α-Terpinene	1017	0.16	0.33	0.38	2.99	1.78
P-Cymene	1025	n.d.	1.52	1.23	15.47	5.17
β-Phellandrene	1030	n.d.	n.d.	n.d.	n.d.	0.53
Limonene	1031	5.36	n.d.	2.38	0.41	n.d.
1,8-Cineole	1033	5.85	12.20	2.26	0.15	1.87
β-Ocymene Z	1036	n.d	n.d.	0.16	n.d.	n.d.
β-Ocymene Y	1046	n.d.	n.d.	n.d.	n.d.	0.08
γ-Terpinene	1057	0.28	0.34	0.65	18.27	8.02
Cis- Sabinenhydrate	1066	0.34	n.d.	0.17	0.29	0.16
α-Terpinolene	1086	n.d.	0.68	1.59	0.11	0.17
Linalool	1102	n.d.	2.99	4.93	0.29	0.58
Borneol	1163	n.d.	10.81	3.34	0.14	1.32
Terpinene-4-ol	1173	n.d.	1.51	2.73	0.28	0.37
Menthol	1180	**29.28**	n.d.	n.d.	n.d.	n.d.
α-Terpineol	1188	n.d.	3.11	2.8	n.d.	n.d.
Myrtenol	1194	n.d.	0.41	1.69	n.d.	n.d.
Verbenone	1209	n.d.	**19.05**	n.d.	n.d.	n.d.
Trans-(+)-Carveol	1215	n.d.	n.d.	0.32	n.d.	n.d.
Pulegone	1237	4.27	n.d.	n.d.	0.16	n.d.
Linalyl acetate	1252	n.d.	n.d.	11.61	n.d.	n.d.
Thymol	1291	1.26	0.20	0.99	0.09	**65.23**
Menthyl acetate	1292	10.6	n.d.	n.d.	n.d.	n.d.
Carvacrol	1296	0.19	0.08	n.d.	**57.01**	4.62
α-Terpinenyl acetate	1343	n.d.	n.d.	4.19	n.d.	n.d.
Trans-Caryophyllene	1412	1.4	0.87	n.d.	0.21	2
α-Humulene	1446	n.d.	0.27	n.d.	n.d.	0.07
Spathulenol	1520	0.13	n.d.	n.d.	0.06	0.07

n.d.= no detected, RI = Retention index

Table 2. The LC_{50} (fumigant toxicity) of essential oils from Lamiaceae plants against adults, larvae, and eggs of *Tetranychus urticae*.

Essential oils	Pest developmental stage	n	LC_{50} (Fiducial limits 95%) (μL/L air)	Slope ± SE	χ^2	df
Perovskia abrotanoides	adult	400	15.4 (12.8–18.4)	1.0 ± 0.2	5.5	18
	larva	885	2.9 (3.7–5.0)	1.3 ± 0.1	8.9	18
	egg	1380	10.5 (8.1–13.1)	1.5 ± 0.1	20.2	18
Rosmarinus officinalis	adult	320	7.5 (6.1–9.0)	1.9 ± 0.2	2.5	14
	larva	932	3.6 (3.2–4.0)	1.5 ± 0.1	3.6	14
	egg	1080	16.2 (13.7–18.6)	1. 6 ± 0.1	3.8	14
Salvia sahendica	adult	370	6.7 (5.3–8.3)	1.5 ± 0.2	10.1	17
	larva	1120	5.3 (4.6–6.1)	1.4 ± 0.1	18.8	18
	egg	900	15.7 (13.4–18.2)	1.4 ± 0.1	4.9	10
Satureja hortensis	adult	320	4.8 (3.9–5.7)	2.0 ± 0.2	1.7	14
	larva	800	2.5 (3.1–2.8)	1.7 ± 0.1	5.4	14
	egg	1400	6.5 (5.6–8.2)	1.4 ± 0.1	31.2	18
Thymus daenensis	adult	320	1.2 (0.9–1.7)	2.0 ± 0.2	1.6	14
	larva	948	4.3 (3.7–5.0)	2.0 ± 0.1	19.7	14
	egg	1400	7.2 (6.3–8.1)	1.3 ± 0.1	36.8	18

N = number of specimens, $\chi 2$ = chi-square, df = degree of freedom

Table 3. Toxicity ratio of different essential oils in various developmental stages of the spider mite, *Tetranychus urticae*.

Essential oil	Developmental stage	Toxicity ratio
Perovskia abrotanoides* vs. *Rosmarinus officinalis	adult	2.05 > 1*
	larva	1.2 > 1*
	egg	1.5 > 1*
P. abrotanoides* vs. *Salvia sahendica	adult	2.3 > 1*
	larva	1.8 > 1*
	egg	1.5 > 1*
P. abrotanoides* vs. *Satureja hortensis	adult	3.2 > 1*
	larva	1.2 > 1*
	egg	1.6 > 1*
P. abrotanoides* vs. *Thymus daenensis	adult	12.8 > 1*
	larva	1.5 > 1*
	egg	1.5 > 1*
R. officinalis* vs. *S. sahendica	adult	3.2 > 1*
	larva	1.2 > 1*
	egg	1.6 > 1*
R. officinalis* vs. *S. hortensis	adult	1.6 > 1*
	larva	1.4 > 1*
	egg	2.5 > 1*
R. officinalis* vs. *T. daenensis	adult	6.6 > 1*
	larva	1.2 > 1*
	egg	2.2 > 1*
S. sahendica* vs. *S. hortensis	adult	1.4 > 1*
	larva	2.1 > 1*
	egg	2.4 > 1*
S. sahendica* vs. *T. daenensis	adult	5.6 > 1*
	larva	1.2 > 1*
	egg	2.2 > 1*
S. hortensis* vs. *T. daenensis	adult	4.0 > 1*
	larva	1.7 > 1*
	egg	1.1 > 1*

* The toxicity level is significantly different.

Repellency assay

The three concentrations (10, 100, and 1000 mg/l) were used for the repellency assay. The highest concentration had 60–87.5% repellency (Table 4) Results of the repellency assay revealed that the highest dose (1000 mg/l) of essential oils of *S. hortensis* and *P. abrotanoides* had the highest and lowest repellency on *T. urticae* adults, respectively.

Table 4. Repellency percent (± SE) for selected essential oils from Lamiaceae.

Essential oils	Concentration (mg/l)		
	10	100	1000
Perovskia abrotanoides	12.5 ± 4.8^{a}	32.0 ± 10.3^{b}	35.0 ± 11.9^{b}
Rosmarinus officinalis	10.0 ± 4.1^{a}	35.0 ± 13.2^{b}	60.0 ± 7.1^{c}
Satureja hortensis	30.0 ± 5.8^{a}	50.0 ± 14.1^{b}	87.5 ± 4.8^{c}
Salvia sahendica	25.0 ± 9.6^{a}	40.0 ± 18.3^{b}	72.5 ± 8.5^{c}
Thymus daenensis	12.5 ± 9.5^{a}	42.5 ± 8.5^{b}	80.0 ± 8.2^{c}

Means with the same letters in each row indicate no significant differences (LSD test at $P < 0.05$).

DISCUSSION

Our results indicate that the essential oils' predominant terpenoids are menthol, verbenone, α-pinene, carvacrol, and thymol. Monoterpenoids, natural products with a 10-carbon backbone structure, serve as the primary insecticidal component in numerous plant essential oils.

α-pinene and β-pinene are the alkenes of monoterpenoids which are found at the highest levels in the essential oils of *S. sahendica*. α-pinene was also a predominant compound of *R. officinalis* whereas, β-pinene was found at a high level in *S. sahendica*. Thymol and carvacrol are phenolic monoterpenoids found at the highest levels in *T. daenensis* and *S. hortensis*, respectively. Menthyl acetate, the acetate ester of menthol, is another natural monoterpene that contributes as the main substituent of *P. abrotanoides* essential oil. Verbenone is a bicyclic ketone of monoterpenoids, a natural constituent of plant essential oils and insect pheromones. This chemical serves as a natural repellent for bark beetles. This monoterpene was at the highest level in the essential oil of *R. officinalis*. However, as evident from our results, monoterpenoids are the predominant constituents of the Lamiaceae essential oils. These terpenoids are non-polar, high lipophilic molecules that can rapidly penetrate the insect cuticle and interfere with their physiological functions(Regnault-Roger and Hamraoui 1995; Moazeni *et al*. 2014).

In *P. abrotanoides*, menthol, a cyclic monoterpene alcohol, was the major component of the essential oil, comprising 29.3%. Interestingly, in some previous studies, the concentration of this chemical in the essential oil of *P. abrotanoides* was almost negligible (Morteza-Semnani 2004; Kolbady Nejad *et al.*, 2013). In other studies, camphor, 1,8-cineole, and α-pinene were found as the predominant molecules of *P. abrotanoides* essential oil (Sajjadi *et al*. 2005; Ghaffari *et al*. 2018). In a study done by (Ashraf *et al*. 2014)) (E-9-dodecenal, octadecanoic acid, methyl ester, 2,2,5,5-tetramethylhexane were reported as the main constituents of the essential oil of stem whereas, hexadecanoic acid, methyl ester, lupeol, octadecenoic acid, methyl ester, eicosane, and tetradecane were the major components of leaves of *P. abrotanoides*.

Previous studies demonstrated 1,8-cineol, camphor, α-pinene, limonene, and camphene as the major components in the essential oil of *R. officinalis* (Miresmailli *et al*. 2006; Hussain *et al*. 2010; Laborda *et al*. 2013; Takayama *et al*. 2016). However, in the above-mentioned studies 1,8-cineol was the predominant molecule of the essential oils but in our study verbenone with more than 19.0% was found to be the major constituent of the oil. In a review, (Karpiński 2020) concentration of verbenone in the essential oil of *R. officinalis* varied from 1.36 to 12.0%. In the current study, analysis of the essential oil of *S. sahendica* showed that α-pinene with 15.2% and β-pinene with 14.6% concentration were the major constituents. The same results were reported by (Salehi *et al*. 2007) and (Dehghan *et al*. 2018).

By analysis of the essential oil of *S. hortensis*, we concluded that carvacrol with a concentration of 57.0% is the main component of the essential oil. Previous studies (Mihajilov-Krstev *et al*. 2009; Farzaneh *et al*. 2015; Farmanpour-Kalalagh *et al*. 2020) also demonstrated carvacrol as the main molecule of essential oil but in another study, ((Mahboubi and Kazempour 2011) thymol was reported as the major constituent of the essential oil. Moreover, P-cymene (29.47%), γ-terpinene (28.02%), and carvacrol (25.97%) were reported as the major molecules of the essential oil (Azimi *et al*. 2018). Our results showed that thymol with a concentration of 65.2% is the major component of *T. daenensis*. In earlier studies (Sajjadi *et al*. 2005; Bahreininejad *et al*. 2010; Moazeni *et al*. 2014), carvacrol and thymol, were reported as the main constituents of the essential oil. However, different factors e.g., geographical area, collecting season, distillation technique, stage, and part of the plant used for distillation, and the presence of chemotypes and chemical races within the same species could substantially affect the chemical composition of essential oils (Chaudhary *et al.* 2011). In agreement with the above, it was concluded that in the full flowering stage of *S. sahendica* the content of some main molecules reached the highest level (Salehi *et al*. 2007). Studies by (Bahreininejad *et al*. 2010) indicate a population-dependent difference in the major constituents of essential oil of *T. daenensis.* In another research (Reegan *et al*. 2013) larvicidal effects of three extractions i.e., hexane, ethyl acetate, and methanol extract of *Cliona celata* Grant on *Culex quinquefasciatus* Say and *Aedes aegypti* (L.) (Diptera: Culicidae), was examined and the methanol extract showed the highest activity.

The present study revealed that essential oils of selected Lamiaceae plants possess fumigant as well as repellent activities against the two-spotted spider mite, *T. urticae*. By comparing the LC_{50} values of different essential oils, the essential oil of *T. daenensis* was more toxic against adults whereas the essential oil of *S. hortensis* was more toxic against larvae and eggs of *T. urticae*. Overall, the larvicidal effects of the tested essential oils were greatly more than their ovicidal effects. This is evident in *R. officinalis* (LC_{50} = 3.6 µl/L for larvae vs. LC_{50} = 16.2 µl/L for eggs). The toxicity of essential oils is influenced by several factors, such as the specific essential oil used, the target species, and the developmental stage of the target species. Larvae are generally more active than eggs, leading to higher exposure to essential oil vapors. This increased activity enhances the interaction between the essential oil and the larvae, resulting in greater larvicidal effects. Larvae also have a higher metabolic rate compared to eggs, meaning they consume more oxygen, which is crucial for the toxicity of fumigants. As a result, larvae may absorb more of the essential oil, leading to higher mortality rates. (Alzahrani and Ebert 2019; Kamaraj *et al*. 2023). The chemical composition of essential oils plays a significant role in their toxicity. Some components may be more effective against larvae, while others might be more potent against eggs. For instance, essential oils from garlic and asafoetida have shown strong ovicidal and larvicidal activity due to compounds like allyl disulfide (Muturi *et al*. 2018). Additionally, essential oils can affect insects through various mechanisms, such as disrupting cellular membranes, inhibiting enzymatic activity, or interfering with hormonal regulation. These mechanisms might be more effective at certain developmental stages, leading to differences in ovicidal and larvicidal effects (Sangha *et al*. 2017; Kamaraj *et al*. 2023). However, different biotic and abiotic modifying factors that may pertain to chemicals, exposure, surrounding medium, and the organisms can substantially influence the toxicity of xenobiotics (Mance 1987). Different studies indicated the acaricidal effects of some plant essential oils (Choi *et al*. 2004; Miresmailli *et al*. 2006; Tomczyk and Suszko 2011; Araújo *et al*. 2012; Laborda *et al*. 2013). A study on the fumigant and contact activity of monoterpenes against *T. urticae* demonstrated the acetylcholinesterase (AChE) inhibitory effect of the tested compounds (cuminaldehyde, linalool, and menthol). However, other compounds such as carvone showed a significant fumigant but a weak AChE inhibitory activity (Badawy *et al*. 2010)). Among 29 compounds of the essential oil of *Lippa sidoides* (Verbenaceae), thymol and carvacrol exhibited potent acaricidal activity against *T. urticae* (Cavalcanti *et al*. 2010). The study investigated the contact toxicity of essential oils from 10

Lamiaceae plants against *T. urticae*. Bioassays were conducted at varying concentrations (200, 400, 800, and 160 ppm) using hydrodistillation to obtain the oils. Results indicated that essential oils from *Zataria multiflora* and *M. piperita* demonstrated the highest toxicity, with LC_{50} values of 419.44 mg/L and 425.42 mg/L, respectively. The essential oils showed acaricidal effects in a dose-dependent manner and exhibited minimal phytotoxicity at concentrations below 160 ppm (Kaveh *et al*. 2014). By testing the essential oil of *S. hortensis*, *Ocimum basilicum* L. (Lamiaceae), and *T. vulgaris* against the nymphs and adults of *T. urticae* although desirable acaricidal activities were achieved, the acaricidal activity of *S. hortensis* was significantly higher than that of other essential oils (Aslan *et al*. 2004). Essential oils from Lamiaceae plants demonstrate various biological activities, largely shaped by their complex chemical compositions. These activities depend on specific phytochemical profiles, including terpenoids, phenolic compounds, and other secondary metabolites. These properties lend themselves to significant medicinal applications, including antioxidant, antifungal, antibacterial, and anti-inflammatory effects. Furthermore, some Lamiaceae species show efficacy against insects and potential in environmental remediation and thermal insulation. Plant species, environmental conditions, and extraction methods influence variations in these compounds (Ramos da Silva *et al*. 2021; Spréa *et al*. 2024). However, the essential oils may function by inhibiting acetylcholinesterase and cytochrome P_{450} monooxygenases, as well as affecting the lipid membranes (Badawy *et al*. 2010). Essential oils can inhibit acetylcholinesterase, an enzyme responsible for breaking down the neurotransmitter acetylcholine. This inhibition can result in increased levels of acetylcholine in synaptic clefts, potentially enhancing neurotransmission and exhibiting neuroprotective effects. Cytochrome monooxygenases are involved in the metabolism of various substances, including pesticides. Essential oils may inhibit cytochrome enzymes, affecting pesticide metabolism and potentially leading to interactions with exotic compounds. Essential oils can disrupt the integrity of lipid membranes in microbial cells. For example, studies have shown that essential oils increase membrane permeability, leading to leakage of intracellular contents such as proteins and nucleic acids. This disruption is often irreversible and can result in cell death. The components of essential oils, such as terpenoids and phenolic compounds, can interact with phospholipid membranes (Yap *et al*. 2021; Li *et al*. 2022). These interactions may alter membrane fluidity and permeability, impacting cellular functions. The multifaceted actions of essential oils through enzyme inhibition and membrane disruption make them valuable in various applications, including antimicrobial treatments and potential therapeutic agents. Their ability to modulate critical biological processes highlights their significance in pharmacology and natural medicine.

Among the tested essential oils, the essential oil of *S. hortensis* showed the highest repellency at all three concentrations whereas, this property was at the lowest level in the case of *R. officinalis* essential oil. Comparing the LC_{50} values of different essential oils, it is clear that *S. hortensis* exhibited the highest mortality, which is positively correlated with its repellent activity. Essential oils with high toxicity also tend to have strong repellent properties, making them valuable for integrated pest management strategies. Understanding this relationship helps in choosing the right essential oils for effective pest control while reducing reliance on synthetic chemicals. Studies by (Maedeh *et al*. 2011) showed a high level of repellency of *S. hortensis* essential oil against three stored product pests. The essential oil of *S. bachtiarica* was also found to be repellent to the adult females of *T. urticae* (Farahani *et al*. 2020). Similarly, essential oils of *Curcuma longa* demonstrated varying LC_{50} values against different ant species, while also showing significant repellency at specific concentrations. This reinforces the idea that effective insecticides often serve dual roles as a repellent.

CONCLUSION

This study demonstrates the potential of essential oils from various Lamiaceae species as effective bio-pesticides against the two-spotted spider mite, *T. urticae*. Key active components, including monoterpenes and phenolic compounds, were identified through chemical analysis, showing significant acaricidal activity. These results indicate that essential oils, particularly from species like

S. hortensis, can be a natural, eco-friendly alternative to synthetic pesticides for managing *T. urticae* infestations in agriculture. Understanding both LC_{50} values and repellency is essential for developing effective insect control strategies with essential oils, as combining these measures can identify oils that kill insects and prevent contact with treated areas. However, further research is required to refine application methods and dosages for field use and evaluate potential non-target effects on beneficial arthropods and the environment. Integrating these essential oils into pest management strategies offers promise for sustainable crop protection.

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اسانس برخی از گیاهان Lamiaceae: تجزیه و تحلیل و فعالیت آنها در برابر *Tetranychus urticae* (Acari: Tetranychidae)

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چکیده

کنهٔ تارتن دولکه‌ای، *Tetranychus urticae* Koch (Acari: Tetranychidae)، آفت مهمی است که محصولات گلخانه‌ای، سبزیجات و زینتی را در سطح جهانی تحت تاثیر قرار می‌دهد. به‌طور معمول از سموم دفع آفات برای کنترل این آفت استفاده شده است، اما نگرانی‌هایی در مورد استفاده مکرر از آنها وجود دارد. در نتیجه، اسانس‌های گیاهی به‌عنوان جایگزینی بالقوه آفت‌کش ظاهر شده‌اند و توجه بسیاری را در مدیریت آفات به خود جلب کرده‌اند. اسانس‌ها از طریق تقطیر آب با استفاده از دستگاه کلونجر به دست آمدند. اثربخشی آنها در برابر مراحل مختلف رشدی *T. urticae* با استفاده از روش مرگ و میر فاز بخار که نیازی به تماس مستقیم نداشت، ارزیابی شد. سمیت تنفسی و دورکنندگی اسانس‌ها نیز بر روی کنه‌های کامل مورد بررسی قرار گرفت. بر اساس تجزیه و تحلیل GC-MS اجزای اصلی اسانس‌های *Perovskia abrotanoides*، *Rosmarinus officinalis*، *Salvia sahendica*، *Satureja hortensis* و *Thymus daenensis* به ترتیب منتول، وربنون، آلفا-پینن، کارواکرول و تیمول بودند. فعالیت کنه‌کشی روغن‌های گیاهی با دُز رابطه مستقیم داشت. اسانس *T. daenensis* برای کنه‌های کامل *T. urticae* سمیت بیشتری داشت (LC_{50} = 1.21 µl/L) و اسانس *S. hortensis* برای لارو (LC_{50} = 2.48µl/L) و تخم‌های *T. urticae* (LC_{50} = 6.48 µl /L) سمیت بیشتری داشت. نتایج آزمایش نشان داد که اسانس *S. hortensis* بیشترین دورکنندگی را برای کنه‌های بالغ *T. urticae* دارد.

واژگان کلیدی: اسانس، سمیت تنفسی، گیاهان دارویی، دورکنندگی، کنهٔ تارتن دولکه‌ای.

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