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Original Article

Control of *Tetranychus urticae* Koch by Thyme, Lavender and Eucalyptus Essential Oils

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Abstract

Two spotted spider mite (TSSM), *Tetranychus urticae* Koch, is an important pest on bean in Markazi Province (center of Iran). To evaluate the bioacaricidal activity of extracts of three essential oils of thyme (*Thymus vulgaris*), lavender (*Lavandula officinalis*) and eucalyptus (*Eucalyptus camaldulensis*) against *T. urticae* on cherry bean (*Vigna unguiculta* subsp. *sinensis*), five different concentrations (0.5%, 1%, 2%, 3% and 4%) were used, during 2009-2011. The type of design was randomized complete block design with four replications. The results showed that lavender revealed the most acaricidal properties against *T. urticae* followed by thyme and eucalyptus. The LC_{50} values of lavender, thyme and eucalyptus for adult mites were 0.65, 1.84 and 2.18, respectively. Our findings revealed that essential oils of thyme, lavender and eucalyptus possess acaricidal activity against two spotted spider mite on cherry bean.

Key words*:* Eucalyptus, Lavender, Plant essential oils, *Tetranychus urticae*, Thyme

Introduction

The two-spotted spider mite, *Tetranychus urticae* is one of the most important pests responsible for yielding losses to many horticultural ornamental and agronomic crops. For several years, chemical control of mites has been extensively practiced [27, 29]. A major problem in the control of *T. urticae* is its response to develop resistance to many acaricides due to an approximate 5-fold increase in the mixed function of oxidase activity [23].
In Egypt, different extracts from *Syzygium cumini* L. against *T. urticae* were used to control mite population and its ethanol extract showed the most potent acaricidal activity [3]. Since resistance problems and high residual levels of botanical insecticides make bioacaricides as attractive alternatives for pest management, whereas botanicals reputedly pose little threat to the environment or human health [19].
Therefore, methods used to detect and determine these multiresidues in food products by LC-MS-MS tandem spectroscopy may hinder its marketing [2]. Two spotted spider mite females were repelled by spinosad and largely oviposited and fed on nonspinosad treated areas. Spinosad did not affect the behavior of *Panonychus ulmi* females. When *T. urticae* females were released on potted bean plants (two-leaf stage) in which leaves received spinosad sprays on the adaxial or abaxial leaf surfaces, or complete spinosad coverage on one or two of the leaves, mite population increase lagged significantly behind those released on control plants. These results indicate that *Syzygium cumini* and spinosad have significant acaricidal effects against *T. urticae* but not *P. ulmi* [2, 31].
Therefore, the use of essential oils of plant extracts in pest management programs has recently attracted the attention of many scientists. Pesticides based on plant origin seem to be recommended as they generally have a very short persistence in the plant [19, 24]. However, the selectivity of these products has to be strictly evaluated for different species of natural enemies as deleterious or sometimes positive effects wereecorded among the natural enemies complex [14, 17].
In insects, glutathione-S-transferase represents a very interesting enzyme carrying out detoxification mechanism due to their involvement in tolerance to acaricides [3. 13]. It is reported that most xenobiotics are subjected to enzymatic modification after penetration through protein binding and transportation in biological system like insects and acaricide. It had been clearly demonstrated that several enzymatic system such as esterase (α and β), and phosphatase (alkaline and acid) can play a vital role in the detoxification of xenobiotics to nontoxic materials [1].

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Herein, this study was aimed to evaluate the acaricidal activity (LC_{50} value) of extracts of essential oils of lavender, Thyme and Eucalyptus against *T. urticae*.

Materials and Methods

Mite colony

Adults of TSSM were originally collected from common bean (*Phaseolus vulgaris*) fields of the Khomein region, Iran in May 2009. These mites were reared on cherry bean (*Vigna unguiculta* subsp. *sinensis*) plants that cultivated on plastic pots (20cm diameter × 25 cm height) in a growth chamber (27±2 ºC, 70 ± 5% RH and a photoperiod of 16 L:8 D h.) for at least two months (several generations) before conducting the experiments. All experiments were performed at the some above-mentioned conditions in growth chambers. Required cultivars were obtained from the Bean Research Institute of Khomein, Iran. The seeds were planted in plastic pots (20cm diameter × 25 cm height) filled with fertilized field soil. Cherry bean was planted in 20 replications and maintained in a greenhouse after four weeks; bean leaves were detached and used to for leaf discs preparation. During the experiments, all plants were irrigated at the same time and no fertilizers or pesticides were used.

Leaf discs

To perform the experiments, the leaf disc method was used [20, 21]. Each leaf disc separated by plastic padding 2 ×2 cm of area center of cherry bean leaves. Each leaf disc was placed on plastic Petri dishes (8cm diameter × 1.5cm height with a hole in its center). Thereafter, one fully expanded young leaf (third leaf below the apical meristem of one month-old plants) was randomly collected and used for the leaf disc preparation. The leaves of bean plant were selected from all replications and cut into a leaf disc (2cm×2cm) and then placed on water-saturated cotton in the Petri dish with the underside facing upward. During the experiments, bean seeds were periodically planted in the greenhouse (every 10 days), and to reduce the effects of plant age on mite development and fecundity, the new leaf discs separated and the mites transferred on them.

Plant materials

Common thyme (*Thymus vulgaris* Sibth & Sm), Common lavender *(Lavandula officinalis* Chaix) and River Red Gum, (*Eucalyptus camaldulensis* Dehnh.) were collected from the Medicine Plants Research Institute of Arak, Iran.

Preparation of essential oils

The whole plants (herbs) of Thyme and Lavender and leaves of Eucalyptus were dried for a week at room temperature, and then crushed according to the method of Calmasur *et al*. [7]. Essential oils were obtained by hydro distillation (deionized water for 4 h) under vacuum according to the method of Aroiee *et al*. [4]. After decanting and drying over anhydrous sodium sulfate, essential oils and components, were kept under low temperature (4 ºC) until used. Series of aqueous concentrations of each essential oil were prepared with Triton X-100 as surfactant at a rate of 0.1%.

Treatment of eggs

Leaf discs (4 cm^2) of bean leaves were used as substrate to ovipositor. Four leaf discs were used for each treatment and five mite females were transferred to each disc and left 24 h to lay eggs, then females were removed. Thereafter, forty eggs, on four discs, were treated with one of the five concentrations (0.5%, 1.0%, 2.0%, 3.0% and 4.0%). The study was initiated with cohort eggs of the *T. urticae*. In this regards, 10 pairs of *T. urticae* were transferred into new leaf discs and 12 hours later, the laid eggs were collected from these leaf discs and individually transferred onto new ones (all transferring were performed using a fine camel's hair brush).

Each of these concentrations was prepared with distilled water (for example: 0.5 ml of the plant extract per 100 ml of the distilled water). Eggs were sprayed by a glass atomizer, with a serial of concentrations for each essential oil. Sprayer calibration was 1mL of the solution per 2 cm^2 of the leaf area. Eggs were incubated at (27 ± 2 ºC, 70 ± 5% RH) for seven days till hatching. The numbers of hatching and non hatching eggs were recorded.

Treatment of adult females

3 days old *T. urticae* females were obtained by placing 100 nymphs onto the culture, and wet cotton pads in Petri dishes were placed on excised bean leaves. The emerged females and males were transferred to new bean leaves for 2-3 days and allowed to mate. Afterwards, forty females were transferred equally to four discs (4 cm^2), and then treated with one of the previous treatments (in the Tetranychid mites, females and males are easily separated: end of body in the male mites is the V shape and end of body in the female mites is the U shape). Control treatment was operated by Triton X-100 at a rate of 0.1%. Mortality was estimated for the adult females after 24 h of spraying and estimated by Abbot's formula and LC_{50}, LC_{90} and slope values were estimated according to Finney [12].

Statistical analysis

Experimental data were statistically analyzed by using Costa software (cohort software, Berkeley). Significance of results was obtained by randomized one way ANOVA, and the means were separated using the Duncan's multiple range test at $P<0.01$ [11].

Results

Essential oils of plants are promising alternative natural products for the control of *T. urticae*. These oils facilitate the handling and its application, besides they can be an option of lower cost in relation to the studies of chemical control. Data presented in Table 1 demonstrated that lavender essential oil was the most potent effective acaricidal agent against *T. urticae*, which enhanced the highest adult female mortality and lowest egg hatchability. Adult mortality percentages after 24 h were 42.50%, 75%, 90%, 95% and 100% for lavender by spraying the different concentrations of 0.5%, 1%, 2%, 3% and 4%, respectively. The percentages of corresponding mortalities for thyme was 20%, 30%, 42.50%, 72.50%, and 85%, while 17.50%, 27.50%, 40%, 70%, and 80% was reported for Eucalyptus, respectively.

Hatchability percentages after six days were 75%, 55%, 30%, 16% and 10% for lavender; 95%, 87.50%, 80%, 72.50%, 57.50% for thyme and 95%, 92.50%, 82.50%, 77.50% and 67.50% for Eucalyptus, respectively, for control treatment (Triton X-100 at a rate of 0.1%), adult mortality was 10% and egg hatchability was 95%.

Table 2 shows that essential oil of lavender represented the most potent acaricidal activities followed by thyme and eucalyptus. The LC_{50} values after 24 h for adults were 0.65%, 1.84% and 2.18%, respectively, while for eggs 1.17 %, 6.26 % and 7.33% were recorded after seven days. The slope values of the regression line were 2.41, 2.53 and 2.49 for adults and 2.28, 1.89 and 2.15 for eggs, respectively. LC_{90} values were 2.27%, 5.91% and 7.13% for adults and 4.34%, 9.81% and 28.95% for eggs, respectively (Dead mites and dead eggs can easily be identified. Dead mites with wrinkled body and without mobility are on the leaves and also, dead eggs don't hatch).

Table 1 Effect of plants essential oils against egg hatchability and adult mortality of *T. urticae* (mean ± SD) (%)

Concentration (%)	*Lavandula officinalis*		*Thymus vulgaris*		*Eucalyptus camaldulensis*	
	Adult mortality	Egg hatchability	Adult mortality	Egg hatchability	Adult mortality	Egg hatchability
Control	10.00 ± 1.29	95.00 ± 0.58	10.00 ± 1.14	95.00 ± 0.58	10.00 ± 1.29	95.00 ± 0.58
0.5	42.50 ± 1.71	75.00 ± 1.29	20.00 ± 1.29	95.00 ± 0.58	17.50 ± 0.96	95.00 ± 0.58
1.0	75.00 ± 1.70	55.00 ± 2.38	30.00 ± 0.82	87.50 ± 0.50	27.50 ± 1.71	92.50 ± 0.96
2.0	90.00 ± 0.82	30.00 ± 0.58	45.50 ± 2.06	80.00 ± 1.15	40.00 ± 1.41	82.50 ± 1.26
3.0	95.00 ± 0.58	16.00 ± 0.10	75.50 ± 1.50	72.50 ± 1.50	70.00 ± 2.24	77.50 ± 1.71
4.0	100.00 ± 0.00	10.00 ± 1.41	85.00 ± 0.82	57.50 ± 2.36	80.00 ± 0.82	67.50 ± 0.58

Table 2 Accaricidal effect of plants essential oils against *T. urticae* (mean ± SD) (%)

Concentration (%)	*Lavandula officinalis*		*Thymus vulgaris*		*Eucalyptus camaldulensis*	
	Adults	Eggs	Adults	Eggs	Adults	Eggs
LC_{50} (%)	0.65	1.17	1.84	6.26	2.18	7.33
Lower limit	0.46	0.94	1.53	4.18	1.82	4.74
Upper limit	0.82	1.45	2.21	25.40	2.67	39.05
Index	100.00	100.00	35.44	19.11	29.82	16.31
Slope	2.41	2.28	2.53	1.89	2.49	2.15
LC_{99} (%)	2.27	4.34	5.91	9.81	7.13	28.95

Discussion

The present results of lavender are in agreement with those documented by Ma *et al.* [18],who found that the highest effect of terpinene-4-ol on esterase activity was noted during recover adult stage of housefly (*Musca domestica*). The activities of both acid phosphatase and alkaline phosphatase in insects were induced by terpinen-4-ol. The activities of glutathione-S-transferase were inhibited at exciting, convulsing and paralysis stages, but gradually recovered at recovering stage. The activities of glutathione-S-transferase probably had relations with toxicity of terpinen-4-ol against larvae of the *Mythimna separate* [18].This point will be taken in our consideration in the near future to clarify inhibition of both phosphatase and its individual one. The activity of glutathione S-transferase was inhibited in exciting, convulsing and paralysis stages of the 5th star larvae of *Mythimna separata*, but it gradually recovered in the recovery stage. This affected the metabolism and activity of phosphatase and esterase enzymes. On the other hand, the inhibited insect glutathione-S-transferase will inhibit normal metabolism. The activity of glutathione-S-transferase at LC50 of the essential oil indicates that the activities of this enzyme were recovered and could defend against free radical and it showed more activity when it could be detected at specific LC50 of essential oils extract in recovered mite.

Acaricidal activities of three essential oils (lavender, thyme and eucalyptus) against *T. urticae* Koch have been approved that lavender is the most efficient one [28]. Lavender and thyme essential oils showed relationship between essential oil contents and activity of enzyme glutathione-S-transferase, non specific esterase and alkaline phosphatase as well as inhibition of protease enzyme in two spotted spider mite. The major essential oil contents of lavender are α-bisabolol oxide A (35.251%), and trans-α-farersene (7.758%), while the main components of thyme are terpinen-4-ol (23.860%), p-cymene (23.404%) and sabinene (10.904%). The major components of both plant oils may be responsible for the changes in enzyme activities of *T. urticae*. The present results are in agreement with the data cited by Kawka [16], who studied the effect of lavender oil from fresh and dry flowers on *T. urticae*. Leaves extract showed greater mortality. It has been claimed that increased activities of detoxifying and antioxidant enzyme systems in acaricides had been responsible for the resistance [3].

The decrease in proteinase enzyme which is involved in the biological system of defense proves the presence of proteinase inhibitor in the extracts as cited by Born *et al.* [6], Kant *et al.* [15] and Azzouz *et al.* [5], who suggest that the extracts can induce defense gene expression of proteinase inhibitor activity. Proteinase inhibitors are proteins that inhibit digestive enzymes in the gut of arthropod herbivores, which can reduce their growth, reproduction. Glutathione-S-transferases are major enzymes involved in metabolic resistance to insecticides, as well as in the detoxification mechanisms of many molecules and, probably, in the transport of physiologically important lipophilic compounds. Glutathione-S-transferases play an important role in protecting tissues from oxidative damage and stress [13, 30].

The changes in the activity of α, βesterase, glutathione-S-transferase and alkaline phosphatase and protease enzymes in target site susceptibility are key biochemical mechanisms of development of active component of essential oils which show more potency against Tetranychidae. These studies laid a solid foundation for further studies on the biochemical mechanisms of resistance in *Tetranychus cinnabarinus* and other spider mites. Even this suggestion was approved by Ma *et al*. [18], who determined the bioactivity and effect of terpinen-4-ol on activities of some enzymes in adult housefly (*Musca domestica*). The results showed that the LD50 of terpinen-4-ol was 23.91μg/insect. The poisoning symptom of terpinen-4-ol could be divided into four stages i.e. excitation, convulsion, paralysis and recover stages. The highest effect of terpinen-4-ol on esterase activity was measured during recover stage (8±0.009 μmol/20 min). Glutathione-S-transferase, monooxygenase (P450) and esterases activity were detected in resistance in two spotted spider mite [23]. In contrast, no sesquiterpenes were detected in the fresh resin oil and it was constituted basically by monoterpenes hydrocarbons (42.4%) and oxygen-containing monoterpenes (27.7%), of which α-phellandrene (13.9%) and terpinen-4-ol (7.4%) were the major components, respectively [35]. Conceivably, such challenge has forced the development of mechanisms for survival and adaptation throughout evolution and insecticides activity of these essential oils against *Anopheles stephensi* [22]. Furthermore, and in the above context, induction of detoxifying enzymes by a large number of toxicants has been observed in arthropods [9]. The present results are in agreement with that of Wendel *et al.* [35], who studied the evaluation of the acaricidal activity of some essential oils against TSSM, such as fresh and aged resin (*Protium bahianum*) showed higher oil yield 4.6 % and 3.2%, respectively. About 22 and 13 components were identified in the oils from the fresh and aged resins, comprising 95.8% and 98.6%, respectively. In the fresh resin oil, monoterpenes (70%) were the major group of constituents, mainly p-cymene (18.3%) followed by hydrocarbons, such as α-phellandrene (14.0%), tricyclene (11.4%) and β-phellandrene (9.1%), while the aged resin oil contained sesquiterpenes as the major group with santalol acetate (83.1%) as the principal component.

Treatment with chloroform extract from *Kochia scoparia* (L.) Schrad enhanced SOD, POD and CAT activities during the 24 hour after treatment [8, 33] and traditional Chinese plant can cause toxicity to *Tetranychus cinnabarinus* [26, 36] and even glucoside had acaricial effect [25]. Acaricidal activities of *Wikstroemia chamaedaphne* Meisn extracts against *Tetranychus* were also reported. Twenty-nine compounds were identified with potential acaricidal activity against *Tetranychus cinnabarinus* [34] and had effect on the activity of *Tetranychus* enzymes [32]. The essential oils from accessions of *Lippia sidoides* Cham. (Verbenaceae) were characterized by GC and GC/MS and investigated for their acaricidal activity against the two-spotted spider mite *T. urticae* Koch [10].

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