

Original Article

Comparative Study on the Acaricidal Activities of Essential Oils from *Ziziphora clinopodioides*, *Thymus vulgaris*, *Rosmarinus officinalis* and *Lavandula angustifolia* against *Tetranychus cinnabarinus* Boisduval, on Cut Roses

Seyed Saeid Modarres Najafabadi

Department of Entomology, Agriculture and Natural Resources Research Center, Arak, Iran

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Abstract

A comparative study was made between the acaricidal activities of some essential oils: rosemary (*Rosmarinus officinalis* L.), lavender (*Lavandula angustifolia* Mill.), thyme (*Thymus vulgaris* L.) and ziziphora (*Ziziphora clinopodioides* Lam.) on the important mite pest, *Tetranychus cinnabarinus* Boisduval, the carmine spider mite, on cut roses during 2010-2012. The sublethal concentrations of ziziphora oil (0.125%), thyme oil (0.5%), rosemary oil (0.25%) and lavender oil (0.125%) were used. Biological tests were carried out for a susceptible laboratory carmine spider mite strain reared under controlled conditions of 27 ± 2 °C; 60% R.H. and 16L:8D photoperiod on rose leaves in greenhouse. The results showed that thyme oil causes the highest significant reduction in the fecundity and fertility as compared to the other tested oils. Thyme and ziziphora oil greatly affected the number of larvae and nymphs (for thyme oil 70.7 ± 2.8 and 7.3 ± 0.3 , for ziziphora oil 109.1 ± 4.7 and 17.7 ± 1.8 , respectively) that reached next biological stages. Results were tabulated, analyzed, discussed and prepared to be fit in any IPM program for combating these pest animals.

Key words: *Tetranychus cinnabarinus*, Acaricidal activities, Essential oils, Roses

Introduction

A well known fact is that the acarid family (Tetranychidae), contains more than 1,200 polyphagous mite pest species which might be considered the most important one [1]. It attacks over 300 host plants including vegetable, fruit and ornamental plants [2]. Defoliation, leaf burning, and even plant death can occur due to direct damages caused by feeding habits. Indirect effects of feeding may include decrease in photosynthesis, transpiration and can lead to yellow to white discoloration of the leaf often referred to as bronzing, causing loss of quality and yield or the death of the host plants [3].

Interestingly, visiting Canadian scientists recently identified the mite as *T. cinnabarinus* Boisduval, the carmine spider mite, a red form of the two-

spotted spider mite, *T. urticae* Koch. The latest scientific literature suggests that many of the mites closely related to the two-spotted spider mite cannot be separated by any simple means and collectively are referred to as the two-spotted spider mite complex. Needless to say, despite much research, there is confusion among acarologists whether *T. cinnabarinus* is actually a distinct species [4,5].

Unfortunately, members of this mite pest family have been found to be resistant against synthetic chemical acaricides. The reasons for this may be their short developmental period and high fecundity enforced by life-history characteristics to produce successive generations. Hence, there is a real need to find other effective and safer approaches like natural products. Natural products are excellent alternative to synthetic pesticides, as a mean to

reduce negative impacts to human health and the environment. They are more compatible with the environmental components than synthetic pesticides. At present, the plant derived essential oils are attracting interests of the scientists, as these are environmentally safe and non-toxic to human [6]. Many plant-derived essential oils like rosemary, lavender, thyme and ziziphora have insecticidal and miticidal properties, with a broad-spectrum of activity against many different types of soft-bodied arthropod pests [7]. This is mainly due to multiple modes of action, including antifeedant and repellent activity, molting, and respiration inhibition, growth, and fecundity reduction, cuticle disruption, and activity on the octopamine pathway in the central nervous system [8].

Under natural conditions, organisms are subject to a combination of environmental factors, both biotic and abiotic. It is this combination that ultimately determines the distribution and abundance of a species. The relationship between temperature and insecticide toxicity in insects has been studied widely. Although this phenomenon has been examined extensively in many insect species, few studies have compared the responses of insecticide susceptible with insecticide-resistant strains at different temperatures [9].

The aim of this study was to find out the efficacy of some plant-derived essential oils in controlling the two-spotted spider mite and to determine how they would affect the reproductive rates, under laboratory conditions.

Material and Methods

Rearing of carmine spider mite strain

The carmine spider mites were collected from infested leaves of the Rose trees grown in the experimental greenhouse of National Research Center of Ornamental Plants in Mahallat-Iran. The adult females of mites were transferred with a brush to disks of rose leaves kept on moist cotton wool pads in Petri-dishes for 24 hours. The deposited eggs were kept under constant temperature of 27 ± 2 °C; 60% R.H. and 16L:8D photoperiod until hatched. The newly hatched larvae were then transferred to fresh leaves. Rose cutting holding about 8 leaves each was placed in glass jars containing tap water which was changed every 48 hours. The Rose cuttings were changed twice a week in summer and weekly in winter. The colony was bred in a climatically controlled room at 27 ± 2 °C; 60% R.H. and 16L:8D photoperiod for one year [10].

The compounds used and preparation of essential oils

Rosemary (*Rosmarinus officinalis* L.), lavender (*Lavandula angustifolia* Mill.), thyme (*Thymus vulgaris* L.) and ziziphora (*Ziziphora clinopodioides* Lam.) were collected from the Medicinal Plants Research Station of Arak-Iran.

The whole plants (herbs) were dried at room temperature for a week, and then crushed according to the method of Calmasur *et al.* [11]. Essential oils were obtained by hydro-distillation (deionized water for 4h) under vacuum according to the method of Aroiee *et al.* [12]. After decanting and drying over anhydrous sodium sulfate, essential oils and components, were kept under low temperature (4 °C) until used.

Calculating the LC_{50} of the compounds under investigation

Five serially diluted concentrations covering the range of 0 to 100% mortality were used and 5 replicates per concentration were prepared to calculate the LC_{50} of the essential oils used. Series of aqueous concentrations of essential oils were prepared using Triton X-100 as surfactant at the rate of 0.1% and Triton X-100 alone as the control treatment [13]. According to the method of Mailloux and Morrison [14], discs of Rose leaves (1cm in diameter) were placed in Petri-dishes lined with water-saturated cotton wool. The cotton should be wet to avoid migration of the mites to the lower leaf surface. With the aid of a binocular-microscope and with a fine paintbrush, 60 adult female mites were introduced on the lower surface of the discs in triplicates per treatment. Each Petri-dish was sprayed with a constant amount of the tested solutions for 5 seconds using a glass manual atomizer (Sigma glass spray unit No. S 3135). They were left to dry for 30 min, then subsequently placed in a climatically controlled room at 27 ± 2 °C; 60% R.H. and 16L:8D photoperiod [10]. The survivors were counted after 24 hours and the mean mortality rates were calculated.

Evaluation of female mite fecundity, fertility and offspring development

To evaluate the female fecundity, fertility and offspring development the following sublethal concentration for each oil, rosemary (0.125%), lavender (0.5%), thyme (0.25%) and ziziphora (0.125%), was used. Discs of rose leaves (1 inch in diameter) were dipped in the tested solutions and gently agitated for 5 seconds, then gently blotted to

remove excess liquid and allowed to dry for 30-45 minutes. Then placed in petri-dishes with water-saturated cotton wool in triplicates per treatment. Control individuals were sprayed with Triton X-100. With the aid of a binocular-microscope, 20 quiescent female deutonymphs (after mating) were transferred using fine paintbrush to the lower surface of leaf discs and were allowed to deposit eggs for 4 days. All Petri-dishes were then placed under constant temperature at 27 ± 2 °C; 60% R.H. and 16L: 8D photoperiod. After 4 days, number of live females and eggs laid were monitored [15]. All the females were then removed and the survivors were counted, then the mean mortality rates were calculated. Daily observations during the incubation period up to emergence of the larvae and after eclosion of the larvae till adulthood were made with a stereomicroscope to determine the developmental periods [16].

Statistical analysis

All data concerning mortality, fertility and hatchability rates were presented as arithmetic means (\pm SE). To determine the differences between groups, data were analyzed by student t-test or analysis of variance (ANOVA). All the statistical tests were performed by using SPSS 15.0.0 software packages (USA). The LC_{50} values of rosemary, lavender, thyme and ziziphora essential oils calculate by probit analysis.

Results

LC_{50} values of tested compounds

High and low levels of lethal concentrations (LC_{25} , LC_{50} and LC_{90}) of the essential oils of rosemary, lavender, thyme and ziziphora on *T. cinnabarinus* on cut roses in greenhouse are given in Table 1. The LC_{50} values of rosemary, lavender, thyme and ziziphora essential oils calculate by probit analysis for adult females 24 hours post-treatment were 2.4, 1.8, 0.7, and 1.1%, respectively. Hence, The LC_{50} values of thyme and rosemary oils on *T. cinnabarinus*, were significantly the lowest and the highest, respectively. The LC_{25} values of tested compounds were 1.3, 1.00, 0.3 and 0.7% and LC_{90} were 4.7, 3.1, 2.1 and 2.8%, respectively. So, the results showed that the thyme oil had the highest acaricidal activity against *T. cinnabarinus* in comparison with other essential oils.

Fecundity and fertility of *T. cinnabarinus*

Fecundity and fertility of *T. cinnabarinus* adult females treated with various concentrations of ziziphora oil (0.125%), thyme oil (0.5%), rosemary oil (0.25%) and lavender oil (0.125%) at 27 ± 2 °C, are shown in Table 2. Fecundity and fertility of *T. cinnabarinus* adult females, treated with these concentrations of essential oils, showed significant differences. The lowest and the highest fecundity values, measured were for thyme (3.7 ± 0.2) and lavender oil (5.9 ± 0.5), respectively and the highest and the lowest fertility values obtained were of thyme (92.3 ± 2.2) and rosemary oil (141.3 ± 2.1), respectively. The highest number of larvae developed to nymphs (127.7 ± 4.8) and nymphs developed to adults (43.3 ± 1.3), were observed when rosemary oil was used and the lowest numbers seen were 70.7 ± 2.8 and 7.3 ± 0.3 when thyme oil was used, respectively.

Offspring development of *T. cinnabarinus*

Offspring development of *T. cinnabarinus* treated with concentrations of ziziphora oil (0.125%), thyme oil (0.5%), rosemary oil (0.25%) and lavender oil (0.125%) at 27 ± 2 °C, are shown in Table 3. The results show that all the essential oils affected the development of the offspring of *T. cinnabarinus* and showed reduction in the developmental periods in comparison with control treatment. The total time required till the egg deposition, for ziziphora, thyme, rosemary, lavender oil and control treatments, were 15, 15, 19, 17 and 20 days, respectively.

Discussion

Over 120 plants and plant products have been demonstrated to have insecticidal or deterrent activity against stored product pests [17]. Many producers in parts of Asia and Africa have used some of these botanicals to protect their legumes from attack by bruchids, with varying degrees of success [18, 19]. The essential oils extracted from many species of Apiaceae and Lamiaceae have shown strong insecticidal effects on stored-product pests [8,20-22]. It has been reported that essential oils extracted from *Thymus persicus* (Ronniger ex Rech. f.) Jalas [22], *Ziziphora clinopodioides* Lam. [23] and *Hyptis spicigera* Lam. [24] from Lamiaceae and *Trachyspermum ammi* Sprague, *Anethum graveolens* L. and *Cuminum cyminum* L. [8,20] from Apiaceae, were relatively effective against several stored product insects.

Table 1 High and low levels of LC percent Of essential oils on *T. cinnabarinus* on cut roses in greenhouse.

Essential oils	Number	LC percent			a ± SE	b ± SE	Chi-Square (df)
		95% Confidence interval					
		LC ₂₅	LC ₅₀	LC ₉₀			
<i>Rosmarinus officinalis</i>	150	1.3 0.9 -3.1	2.4 1.8 – 6.4	4.7 3/00 6.4	0.310 – 0.102	± 1.943 0.195	± 1.54 (6)
<i>Lavandula angustifolia</i>	150	1.00 0.2 – 2.6	1.8 0.9 – 4.1	3.1 2.3 – 5.1	0.477 0.011	± 2.269 0.147	± 5.61 (6)
<i>Thymus vulgaris</i>	150	0.3 0.01 – 1.00	0.7 0.1 – 1.9	2.1 1/00 3.6	0.796 – 0.147	± 2.609 0.175	± 10.87 (6)
<i>Ziziphora clinopodioides</i>	150	0.7 0.1 – 2.1	1.1 0.3 – 2.4	2.8 1.9 – 4.3	0.522 0.248	± 3.201 0.221	± 8.25 (6)

Table 2 Fecundity and fertility of *T. cinnabarinus* adult females treated with concentrations of ziziphora, thyme, rosemary and lavandula oil.

Parameters	Control	<i>Z. clinopodioides</i>	<i>T. vulgaris</i>	<i>R. officinalis</i>	<i>L. angustifolia</i>
No. of eggs deposited/females (Fecundity Mean ± SE)	9.3 ± 0.2a	4.5 ± 0.6bc	3.7 ± 0.2c	5.8 ± 0.5b	5.9 ± 0.5b
No. of Hatched eggs (Fertility Mean ± SE)	157.3 ± 5.3a	118.3 ± 3.4ab	92.3 ± 2.2b	141.3 ± 2.1a	138.7 ± 2.1a
No. of Larvae developed to nymphus (Mean ± SE)	145.7 ± 3.1a	109.1 ± 4.7c	70.7 ± 2.8d	127.7 ± 4.8b	117.2 ± 2.6c
No. of Nymphus developed to adults (Mean ± SE)	104.3 ± 5.4a	17.7 ± 1.8c	7.3 ± 0.3d	43.3 ± 1.3b	23.3 ± 1.7bc

Means followed by the same letters within rows are not significantly different (one-way ANOVA, =0.05).

Table 3 Offspring development of *T. cinnabarinus* treated with concentrations of ziziphora, thyme, rosemary and lavandula oil.

Treatments	Parameters	Developmental period in days					
		Egg to larva	Larva to nymph	Nymph to adult	Total days	Days for eggs deposition	Total time till eggs deposition
Control		4	3	6	13	7	20
<i>Ziziphora clinopodioides</i>		2	2	5	9	6	15
<i>Thymus vulgaris</i>		2	2	4	8	7	15
<i>Rosmarinus officinalis</i>		4	3	6	13	6	19
<i>Lavandula angustifolia</i>		3	2	6	11	4	17

The present study showed that the LC₅₀ values of rosemary, lavandula, thyme and ziziphora oils were 2.4, 1.8, 0.7, and 1.1%, respectively. The LC₅₀ of rosemary oil was higher than that reported by Miresmailli *et al.* [25] who recorded the LC₅₀ of 1% for rosemary oil against adult female spider mites reared on bean plants and 1.3% for those reared on tomato plants. By using the leaf disc painting method, the LC₅₀ of pure rosemary oil as 1.3%, Hexacide (containing 5% rosemary oil) as 0.4%, EcoTrol (containing 10% rosemary oil) as 0.6% and Sporan (containing 18% rosemary oil) as 1.1% were recorded for spider mites [26].

In the present research, thyme oil showed the lowest LC₅₀ against *T. cinnabarinus* under laboratory conditions in comparison with the other extracts while rosemary oil showed the highest LC₅₀. However, the differences between LC₅₀ values of the oils examined in this study and other studies may be due to the formulation of the oils, the strain of spider mites, laboratory condition and extraction methods. The lavender oil represented the most potent and efficient acaricidal agent against *Tetranychus* followed by thyme and eucalyptus. The LC₅₀ values of lavender, thyme and eucalyptus essential oils for adult mites were 0.65, 1.84 and

2.18, respectively [27].

In addition, for thyme and rosemary oil, developmental period of carmine spider mite completed in about 15 and 19 days respectively, against 20 days in the control as shown in Table (3). It clearly indicates that rosemary oil can be considered as an efficient acaricide against TSSM, causing complete mortality in the laboratory at concentrations that cause no phytotoxicity to host plants and can affect oviposition behavior. Moreover, another important characteristic of rosemary oil is its complex chemical composition; it is a mixture of terpenoids. Complete mortality (100%) of mites was obtained with a 2% concentration of the oil on bean plants and 4% on tomato plants in greenhouses [25,28].

Acaricidal activities of three essential oil extracts (lavender, thyme and eucalyptus) against *T. urticae* Koch prove that lavender is the most efficient one [1, 29]. 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 oils content 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 extracts may be responsible for the changes in enzyme activities of *T. urticae* [30, 29]. The present results are in agreement with the data cited by Kawka [31], who studied the effect of lavender extracts from fresh and dry flowers on *T. urticae*. Leaf 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 [32].

The decrease in proteinase enzyme which is involved in the biological defence system proves the presence of proteinase inhibitor in the extracts as cited by Azzouz *et al.* [33], Born *et al.* [34] and Kant *et al.* [35], who suggested 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 and 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 [36,37].

According to Miresmaili [26], Rosemary oil was found to be more toxic to spider mites as a contact toxicant while it was more effective against whiteflies as fumigant. Differences between the present results and that of other researches [25-26,28] may be attributed to the differences in the concentrations used and the method they adopt for a low concentration of rosemary oil. Choi *et al.* [38] evaluated the toxicity of 53 essential oils including rosemary against eggs and adults of two-spotted spider mites as fumigant. Rosemary oil was not very toxic (mortality<60%) comparing to caraway seed, citronella java, lemon, eucalyptus, pennyroyal and peppermint oil, which were highly toxic (mortality>90%) to the tested mites. Our results clearly indicate that rosemary oil based pesticides are not environmentally persistent. In all experiments, toxicity of residues significantly declined after 24 hours. All these conflicting results indicate that rosemary oil activity may be affected by its volatilization, concentration, formulation and technique used.

Regarding to Miresmaili and Isman [28] choice tests results, rosemary oil is significantly repellent to the two-spotted spider mite. It repelled mites for \approx 6 hours and then mites gradually started to move toward treated discs. However, mites preferred untreated leaves for oviposition. Repellent effects of rosemary oil cannot be considered as a stand-alone control method, but they can be combined with other methods to improve pest management strategies. Regarding rosemary essential oil, several bioactivities have been documented, including insecticidal properties [39,40-41]. An acaricidal effect against *R. microplus* was found (88.98 and 100%) at concentrations of 10 and 20% rosemary essential oil, respectively. This activity, however, decreased at lower concentrations. The main chemical constituents of this oil, which are probably responsible for acaricidal activity, are α -pinene, verbenone, and 1,8-cineol, compounds whose insecticidal activities have been previously demonstrated [39].

Momen *et al.* [42] studied the deterrent and toxic effect of *R. officinalis* on the two tetranychid mites *T. urticae* Koch and *E. orientalis* Klein under laboratory conditions. Leaf discs treated with increasing concentrations of the two oils showed increased mortality of both spider mites and reduction in the total numbers of laying eggs.

These results could be due to the oil of the higher oxygenated compounds content that was more effective in this respect. Little is known about the exact site of action of rosemary oil and other plant essential oils on the two-spotted spider mites. The octopaminergic nervous system is considered to be the site of action of essential oils in the American cockroach [43], but this may not be the case for the two-spotted spider mite and there is the possibility that the essential oils have more than one site of action since they are complex mixtures.

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