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Toxicity and Repellency Effects of Three Plant Essential Oils Against Two-spotted Spider Mite, *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT

To introduce ecologically safe acaricide, effects of essential oils derived from *Cuminum cyminum* (Cumin), *Syzygium aromaticum* (Clove), and *Mentha spicata* (Spearmint) were determined on *Tetranychus urticae* at 25±1°C, 65±5% RH and a photoperiod of 16:8 (L:D) hour. The essential oils extracted by hydro-distillation were characterized by means of GC and GC–MS. Bioassays were performed by filter paper diffusion without allowing direct contact. Cumin, clove, and spearmint oils contained α- Pinene (29.1%), eugenol (78.5%) and carvone (59.4%), respectively, as the major compounds. The lowest LC_{50} value for adults was recorded for cumin oil (3.74 µL L^{-1} air) followed by clove (6.13 µL L^{-1} air) and spearmint (7.53 µL L^{-1} air). The highest ovicidal activity was recorded for cumin oil (LC_{50}= 7.65 µL L^{-1} air) followed by clove (LC_{50}= 8.73 µL L^{-1} air) and spearmint (LC_{50}= 9.01 µL L^{-1} air). According to repellency tests, by increasing concentration of oils, the repellency effects were increased. The most potent repellency effect was recorded for clove, followed by spearmint and cumin oils. The three extracted essential oils seem to be suitable sources of active vapors that can be used as alternatives for chemical pesticides for controlling this pest.

Keywords: Clove, Cumin, Ovicidal activity, Spearmint.

INTRODUCTION

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a ubiquitous species, present worldwide on a wide variety of plants (Helle and Sabelis 1985). This pest has more than 1200 species of host plants (Zhang, 2003) including 150 economically important species. Often, mite-susceptible crops are protected by synthetic acaricides during the hot and dry seasons that severe outbreaks of spider mites may occur (Antonious and Snyder, 2006; Riahi *et al.*, 2013). Nevertheless, chemical control of this pest has created problems (Chueca *et al.*, 2010; Han *et al.*, 2010). Unfortunately, spider mites have been resistant to most available pesticides and the loss of acaricidal efficacy as a result of resistant mite populations is the major problem encountered (Yorulmaz and Ay, 2009; Van Pottelberge *et al.*, 2008). Therefore, there is an urgent need to find safer alternatives that have the potential to replace synthetic pesticides and are appropriate for control of *T. urticae* (El-Zemity *et al.*, 2009).

Essential oils derived from plants can be potential alternative for mite control, because some of them are selective, biodegradable, and have few effects on non-target organisms and the environment (Isman, 2000).

According to our previous studies, essential oils derived from different plant species such as *Thymus vulgaris*, *Lippia sidoides*, *Ocimum*

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basilicum, *Carum carvi*, *Eucalyptus citriodora* and *Rosmarinus officinalis* have acaricidal activity against *T. urticae* (Chiasson *et al.*, 2001; Miresmailli *et al.*, 2006; El-Zemity *et al.*, 2009; Cavalcanti *et al.*, 2010; Han *et al.*, 2010). So far, several reports have dealt with the use of essential oils extracted from *Cuminum cyminum*, *Syzygium aromaticum* and *Mentha spicata* plants to control insect pests and some phytophagous and ectoparasite mites such as *Sitophilus oryzae* , *Acanthoscelides obtectus* , *Tetranychus cinnabarinus, and Rhipicephalus microplus* (Kim *et al.*, 2013; Tunç *et al.*, 2000 ; Jumbo *et al.*, 2014; Ho *et al.*, 1994; George *et al.*, 2009; Sertkaya *et al.*, 2010; Martinez-Velazquez *et al.*, 2011). But, there are no reports about acaricidal and ovicidal properties of these oils against *T. urticae*.

Therefore, in this study, our specific objectives were: to determine the fumigant toxicities of cumin (*Cuminum cyminum* L. var. Kerman) (Apiaceae), clove (*Syzygium aromaticum* Thun. var. Nelson) (Myrtaceae), and spearmint (*Mentha spicata* L. var. Crispa) (Lamiaceae) essential oils against *T. urticae*; and to evaluate repellent effect of different concentrations of the essential oils on this pest.

MATERIALS AND METHODS

Mite Rearing

Spider mites, *T. urticae*, were collected from infested greenhouses of Pakdasht in Tehran Province, during 2011-2012. Mites were reared on 3-week-old kidney bean (*Phaseolus vulgaris* L.) plants in the growth chamber at 25±1°C, 60±5% RH and a photoperiod of 16:8 L: D hour.

Essential Oil Extraction and Analysis

The seeds of cumin, flower buds of clove and leaves of spearmint were collected from Kohan Abad Village, Semnan, Iran. The essential oils were extracted by hydro distillation using Clevenger-type apparatus. A total of 50 g of dried plant materials and 500 mL of distilled water were used, and the distillation was carried out for 4 hours. The oils were collected in plastic tubes and stored in the refrigerator at 4°C until used.

GC analysis was performed by a GC (9-A-Shimadzu) gas chromatograph equipped with a flame ionization detector in Tehran University. Quantitation was carried out on Euro Chrom 2000 from KNAUER by the area normalization method. The analysis was carried out using a DB-5 fused-silica column (30 m×0.25 mm, film thickness 0.25 μm) using a temperature program of 40-250°C at a rate of 4°C min^{-1}, injector temperature 250°C, detector temperature 265°C, carrier gas was helium (99.99%). The GC/MS unit consisted of Varian-3400 gas chromatograph coupled to a Saturn II ion trap detector. The column was the same as that of the GC under the same conditions as stated above. The constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with the literature data (Abdelwahab *et al*., 2014).

Fumigant Toxicity Bioassay

To determine fumigant toxicity of the tested essential oils on *T. urticae* adults, we followed the bioassay method described by Choi *et al.* (2004). Twenty adult female mites were transferred onto excised bean leaves (2 cm diameter) placed with its dorsal side on wet cotton pad in glass container (27 ml volume) using a fine brush. Different concentrations of the tested essential oils including 2.22, 2.81, 3.4, 4.07, 4.88 and 5.92 μL L^{-1} air for cumin, 3.33, 3.7, 5.55, 7.4, 9.62 and 11.11 μL L^{-1} air for clove, and 5.18, 5.92, 6.88, 7.92, 9.03 and 10.37 μL L^{-1} air for spearmint oils, were prepared by dissolving in ethanol. A 10 μL aliquot of each concentration was applied on filter paper pieces attached to the inner surface of

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the container lid. Control filter papers received 10 μL of ethanol. The lids of containers were sealed tightly with Parafilm. Five replications were made for each concentration. The experimental units were incubated in a chamber at 25±1°C, 65±5% RH and a photoperiod of 16:8 (L:D) hour. Mortality was determined 24 hours after treatment. Mites were considered to be dead if appendages did not move when they were prodded with fine brush.

For treatment of eggs, five *T. urticae* females were allowed to oviposit for 24 h on 2 cm diameter of bean leaf discs placed with its dorsal side on wet cotton pad in glass container. Then, adults were removed and twenty eggs were kept on per disc. Previous treatments were applied on filter paper pieces attached to the inner surface of the container lid. The concentration ranges used for the mite egg, were 4-10 μL L^{-1} air for cumin, 4-11 μL L^{-1} air for clove and 5-13 μL L^{-1} air for spearmint oils. The lids were sealed with Parafilm. The experiment was incubated in a chamber at 25±1°C, 65±5% RH and a photoperiod of 16:8 (L:D) hour. Egg mortality was recorded when the hatched mites in the control treatment had reached the larval stage. For each concentration five replications were used.

Repellency Tests

The repellency tests were performed according to method described by Kogan and Goeden (1970). Leaf discs of kidney bean of 3-cm diameter were used. Half of the disk was infected with an ethanolic solution of the oils in four concentrations (equal to LC_{10}, LC_{15}, LC_{20} and LC_{25} per oils) and the other half of the disk was immersed in pure ethanol that was used as control. Both treated and untreated leaf disks were placed in 9 cm diameter Petri dish, with 5 cm diameter hole in lid as ventilation. There were five replications for each treatment. Ten adults of *T. urticae* were transferred in the middle of treated and untreated leaf discs. After 24 hours, the number of mites present on treated or control leaf discs was counted (Pontes *et al.*, 2007). The Repellence Index (RI) of the oils were obtained according to the equation: $RI = 2G/(G+P)$

Where, G= Mite number in the treatment and P= Number of mites in the control.

The security interval used to consider oil as repellent or not was obtained based on the mean value of *RI* and the respective standard deviations (SD). If the mean value of the *RI* was less than 1-*SD*, the oil was repellent, while for a mean value higher than 1+*SD*, the oil was attractant, and for mean values between 1–*SD* and 1+*SD*, the oil was indifferent (Pontes *et al.*, 2007).

Data Analysis

If mortality in the control group was found, the corrected mortality was used according to Abbott (1925). Data obtained from each dose-response bioassay were subjected to probit analysis (Finney, 1971) to estimate LC_{50} values using SAS software version 9.1 (SAS Institute, 2002). For repellency experiments, one-way ANOVA ($P < 0.05$) was used. Means were compared by Duncan's test.

RESULTS

Chemical Compositions of Essential Oils

The results of the oils analyses are presented in Table 1. Based on GC–MS investigations, α-pinene (29.1%), limonene (22%) and 1,8-cineole (17.9%) were recorded as the most abundant components in *C. cyminum* essential oil. The oil of *S. aromaticum* was particularly rich in eugenol (78.5%) and β-caryophyllene (13.8%). The main components in *M. spicata* oil were carvone (59.4%), limonene (9.8%) and 1,8-cineole (7.4%).

Table 1. Relative composition of major chemical components of three essential oils.

Component	Mean composition (%)		
	C. cyminum	*S.aromaticum*	*M. spicata*
Isobutyl isobutyrate	0.8	-	-
α-Thujene	0.3	-	-
α-Pinene	29.1	-	1.5
β-Pinene	-	-	2.3
Sabinene	0.6	-	0.8
Myrcene	0.2	-	0.6
p-Cymene	0.3	-	-
Limonene	22	-	-
1,8-Cineole	17.9	-	7.4
γ-Terpinene	0.6	-	1.5
Limonene	-	-	9.8
Terpinolene	0.4	-	-
Linalool	10.4	-	-
Terpinene-4-ol	0.6	-	2.6
α-Terpineole	3.2	-	-
trans-Carveole	0.4	-	2.6
Geraniol	1.1	-	-
Carvone	-	-	59.4
Linalyl acetate	4.8	-	-
Methyl geranate	0.2	-	-
α-Terpinyl acetate	1.3	-	-
Dihydrocarvyl acetate	-	-	1.6
Eugenol	-	78.5	-
Methyl eugenol	1.6	-	-
α-Compaene	-	0.2	-
β-Caryophyllene	0.4	13.8	4.8
α-Humulene	0.2	2.8	0.4
Eugenyl acetate	-	4.4	-
Caryophyllen oxide	0.1	0.2	-
Acetocyclohexane dione	0.4	-	-

Fumigant Toxicity Bioassay

The acaricidal effects of the three plant essential oils obtained from *C. cyminum*, *S. aromaticum* and *M. spicata* against *T. urticae* are summarized in Table 2. The results showed that all three essential oils had low LC_{50} values. Thus, the plant extractions were toxic against *T. urticae*. For female adults, the lowest LC_{50} value was recorded for cumin oil (3.74 μL L^{-1} air) followed by clove (6.13 μL L^{-1} air) and spearmint (7.53 μL L^{-1} air). There were significant differences between LC_{50} values of all essential oils (based on non-overlap in 95% confidence limits of LC_{50} values). Mortality in the control treatment (ethanol only) was 9%. The highest ovicidal activity was recorded for cumin oil (LC_{50}= 7.65 μL L^{-1} air) followed by clove (LC_{50}= 8.73 μL L^{-1} air) and spearmint (LC_{50}= 9.01 μL L^{-1} air) (Table 3). Based on 95% CL (Confidence Limit), the difference between LC_{50} values were not significant (Figure 1).

Repellency Tests

According to Table 4, mean value of Repellence Index (RI) for each applied concentration was determined. Means with

Table 2. The toxicity analysis of three essential oils, applied as fumigants, against *T. urticae* adult females.

Essential oil	n[a]	LC_{50} value and its 95% CL[b] (μL L^{-1} air)	Slope±SE	*Chi*-square value	*P* value
C. cyminum	600	3.74 (3.47 - 4.02)	3.79 ± 0.45	2.39	0.66
S.aromaticum	600	6.13 (5.56 - 6.74)	2.89 ± 0.32	1.97	0.74
M. spicata	600	7.53 (7.09 - 8.00)	4.53 ± 0.59	0.78	0.94

[a] Number of individuals used, [b] Confidence Limit.

Table 3. The toxicity analysis of three essential oils, applied as fumigants, against *T. urticae* eggs.

Essential oil	n[a]	LC_{50} value and its 95% CL[b] (μL L^{-1} air)	Slope±SE	*Chi*-square value	*P* value
C. cyminum	600	7.65 (7.29-8.44)	3.79 ± 0.45	2.69	0.61
S.aromaticum	600	8.73 (8.25-9.22)	5.03 ± 0.56	1.56	0.81
M. spicata	600	9.01 (8.55-9.51)	5.21 ± 0.67	1.50	0.83

[a] Number of individuals used, [b] Confidence Limit.

Table 4. Repellent effect for four different concentrations of each of oils on *T. urticae* adults.

Essential oil	n[a]	Corresponding *LC* value	Concentration (μL L^{-1} air)	Mean value of Repellence Index (RI)[b]	*SD*	Effect
C. cyminum	50	10	1.92	1.36 [a]	0.16	attractant
	50	15	2.2	1.08 [b]	0.22	indifferent
	50	20	2.46	0.84 [c]	0.08	repellent
	50	25	2.7	0.56 [efg]	0.16	repellent
S.aromaticum	50	10	2.21	1.08 [b]	0.22	indifferent
	50	15	2.69	0.80 [cd]	0.14	repellent
	50	20	3.14	0.60 [def]	0.14	repellent
	50	25	3.58	0.36 [g]	0.16	repellent
M. spicata	50	10	3.68	1.36 [a]	0.16	attractant
	50	15	4.13	1.16 [ab]	0.16	indifferent
	50	20	4.52	0.76 [cde]	0.16	repellent
	50	25	4.89	0.40 [fg]	0.14	repellent

[a] Number of individuals used, [b] *RI* calculated according to the equation described by Kogan and Goeden (1970).

different letters are significantly different (df = 11, F= 21.17, P< 0.0001). *RI* values ranged from 0.36 to 1.36. The lowest value of *RI* (0.36) was recorded for clove oil, followed by spearmint (0.40) and cumin (0.56) oils.

DISCUSSION

The acaricidal activity of the essential oils may be due to those known major components. This theory is in accordance with Badawy *et al.* (2010) who reported that monoterpenes such as 1,8-cineole, limonene and carvone have a strong fumigant activity against *T. urticae*. Moreover, Lee *et al.* (2001b) showed that monoterpenes, such as 1,8-cineole and eugenol are toxic to the rice weevil, *Sitophilus oryzae*.

Vapors of all of the tested oils showed a varying degree of toxicity to the adults of *T.*

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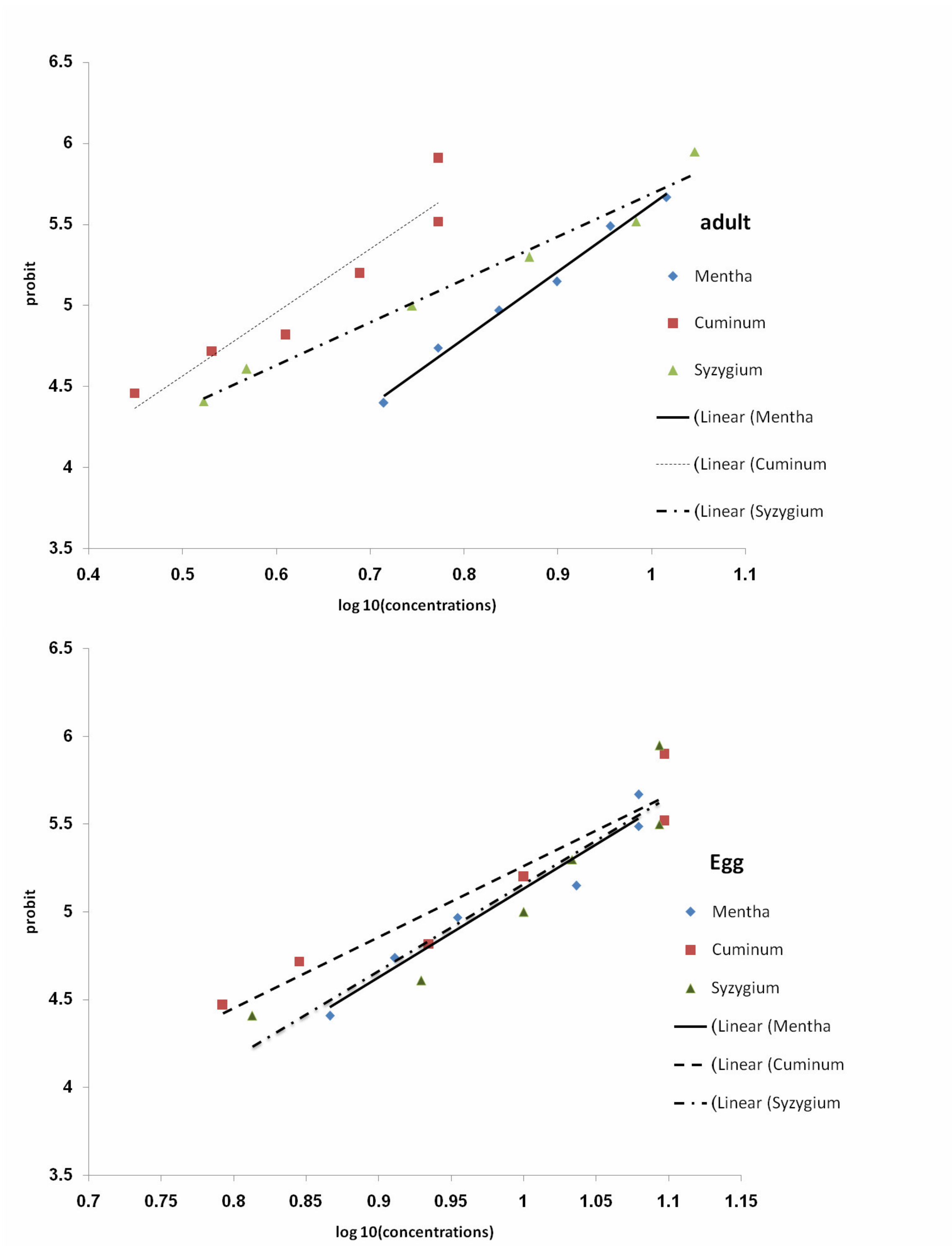

Figure 1. Concentration-mortality lines of three plant essential oils applied as fumigants at the egg and adult stages of *T. urticae*

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urticae. The observed low values of LC_{50} could be due to the fact that essential oils have fumigant action (Kim *et al.*, 2003; Koul, 2004) and volatile oil could penetrate organism via the respiratory system (Choi *et al.*, 2004) resulting in enhanced efficacy. Toxicity was different due to the oil type and applied concentration. Similar results were reported by Motazedian *et al.* (2012), Pontes *et al.* (2007), Aslan *et al.* (2004) and Çalmaşur *et al.* (2006) with oils obtained from other aromatic plants against *T. urticae.* Our experiment demonstrated that cumin oil had high toxicity in the adult and egg stages of two-spotted spider mite. This finding was confirmed by Martinez-Velazquez *et al.* (2011), Tunç and Sahinkaya (1998), and Tunç *et al.* (2000) who reported high toxicological effect for cumin oil when tested on *Rhipicephalus microplus* tick , *T. cinnabarinus,* and *Aphis gossypii,,*and eggs of *Tribolium confusum* and *Ephestia kuehniella*, respectively. For all these pests, the mortality percentage was 100%, which was somehow higher than our finding. This incoherence may be due to differences in the concentrations used or differences in relative amounts of chemical components of the oil. The high acaricidal activity of the cumin essential oil is perhaps attributable to the high level of α-pinene, limonene and 1,8-cineole compounds which the other tested plant oils lack or contain in lower amounts. Our results showed that the LC_{50} values of all tested oils for adults were lower than *T. urticae* eggs. This is similarly reported by Choi *et al.* (2004) and Afify *et al.* (2012).

Results demonstrated that only the two highest concentrations of the oils indicated repellency effects on *T. urticae* (RI were lower than 1–SD). In fact, by increasing concentration of oils repellency effect was increased. This result is in agreement with the data cited by Pontes *et al.* (2007) who reported that only the resin oil in concentrations higher than 0.5% were repellent against *T urticae*. Clove oil represented the most repellent property. This kind of activity may be due to the high content of eugenol compound. This is in agreement with Araújo *et al.* (2012) who reported that eugenol component had a strong repellency property on *T. urticae*. Moreover, Del Fabro and Nazzi (2008) evaluated the repellency activity of eugenol compound against *Ixodes ricinus* ticks. Jantan and Zaki (1998) found that the formulations made of *Cinnamonum camphora* Linnaeus (Lauraceae), *Mentha pulegium* Linnaeus (Labiatae) essential oils and the camphor component were effective to remove pests for a long time and some monoterpenoids such as α-pinene, limonene, terpinolene, citronellol, citronellal and camphor have repellency effects as well. Hori and Komatsu (1997) found that *Rosmarinus officinalis* L. essential oil and the main component 1,8-cineole have repellency effect on *Neotoxoptera formosana* (Takahashi) (Homoptera: Aphididae). The 1,8-cineole and camphor components of sage and rosemary were found to be high in this study. It is seen that both of these essential oils have repellency effect on *T. urticae* adults and nymphs. Araújo *et al*. (2012) found that *Piper aduncum* essential oil and its main components, nerolidol, α-humulene and β-caryophyllene, do not have any repellency effect on *T. urticae*.

In conclusion, essential oils extracted from aromatic plants have considerable potential for pest control. Experimental oils indicated toxicity and repellency effects as fumigant on *T. urticae*. Certain plant essential oils, or their chemical constituents, are toxic to a broad spectrum of economic insect pests, with some selectivity favoring biocontrol agents. Moreover, essential oils consist of the terpenoid component mixture and, thus, rapid resistance development in spider mites will be slower compared to insecticides consisting of one active substance. However, more research is needed on the tested oils, such as acaricidal, effects of each major compound, and their modes of action and efficacies in greenhouse and field conditions.

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سمیت و دورکنندگی سه اسانس روغنی گیاهی علیه کنه تارتن دولکه ای *Tetranychus urticae* (Acari: Tetranychidae)

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چکیده

بمنظور معرفی کنه کش اکولوژیکی بی خطر، اثرات اسانس های روغنی مشتق شده از گیاهان زیره سبز، میخک و نعناع سبز علیه کنه تارتن دولکه ای در شرایط دمایی ۱ ±۲۵ درجه سلسیوس، رطوبت ۵ ±۶۵ درصد و دوره نوری ۸:۱۶ (روشنایی: تاریکی) ساعت مورد آزمایش قرار گرفت. اسانس های روغنی استخراج شده توسط تقطیر با آب با دستگاه GC و GC–MS مورد تجزیه قرار گرفتند. زیست سنجی بوسیله انتشار برروی کاغذ صافی و بدون تماس مستقیم صورت گرفت. اسانس های زیره سبز، میخک و نعناع سبز بترتیب دارای آلفاپینن (۲۹/۱٪)، اوژنول (۷۸/۵٪) و کارون (۵۹/۴٪) بعنوان ترکیبات اصلی تشکیل دهنده بودند. کمترین مقدار LC_{50} علیه بالغین برای اسانس زیره (۳/۷۴ میکرولیتر بر لیتر هوا) و پس از آن میخک (۶/۱۳ میکرولیتر بر لیتر هوا) و نعناع (۷/۵۳ میکرولیتر بر لیتر هوا) ثبت شد. بیشترین خاصیت تخم کشی برای اسانس زیره (LC_{50}= ۷/۶۵ میکرولیتر بر لیتر هوا) و پس از آن میخک (LC_{50}= ۸/۷۳ میکرولیتر بر لیتر هوا) و نعناع (LC_{50}= ۹/۰۱ میکرولیتر بر لیتر هوا) ثبت گردید. مطابق آزمایشات دورکنندگی، با افزایش غلظت اسانس ها، اثر دورکنندگی افزایش یافت. بیشترین خاصیت دورکنندگی برای اسانس میخک و پس از آن نعناع و زیره به ثبت رسید. هر سه اسانس گیاهی استخراج شده بنظر منابع مناسبی از ترکیبات تدخینی فعال می باشند که قادرند جایگزین آفت کش های شیمیایی برای کنترل آفت مذکور گردند.

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