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EFFECTS OF THREE PLANT ESSENTIAL OILS ON TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* (ACARI:ETRANYCHIDAE)

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Abstract: Effects of essential oils derived from *Cuminum cyminum*, *Eugenia caryophyllata* and *Mentha spicata* were determined on *Tetranychus urticae* adults, at 25±1^{0}C, 65±5%RH and a photoperiod of 16:8 (L:D) h. The essential oils extracted by hydrodistillation 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 was recorded for cumin oil (3.74 µl/L air) followed by clove (6.13 µl/L air) and spearmint (7.53 µl/L air). The efficiency of the fumigant activity was increased with concentration for the all studied oil types. 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. Three extracted essential oils seem to be suitable sources of active vapors that can be used as alternatives for chemical pesticides to control of this pest.

Keywords: *Tetranychus urticae*, essential oil, toxicity, repellency.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari:Prostigmata), is known as the most serious pests in ornamental plants and vegetable crops grown in greenhouses worldwide (Kwon et al., 2009; Rauch & Nauen, 2003; Leeuwen et al., 2006). 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 & Snyder, 2006). Nevertheless, chemical control of this pest has created problems (Chueca, 2010). Spider mites have been resistant to most available pesticides and the loss of acaricidal efficacy is the major problem encountered (Ay, 2005). Increasing levels of resistance to the commonly used acaricides leads to the use of multiple treatments and excessive doses, fostering serious environmental and human health concerns (Han et al., 2010). Therefore, there is an urgent need to find safer and more efficient alternatives that have the potential to replace synthetic pesticides and are appropriate for control of *T. urticae* (El-Zemity, 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). *Lippia sidoides* essential oil has been found to be active against *T. urticae* (Cavalcanti, 2010). Fumigant activity of several essential oils against adults of *T. urticae* was assessed by Han et al. (2010).

In this study our objective was to determine the fumigant toxicity of *Cuminum cyminum* L. (Apiaceae), *Eugenia caryophyllata* Thun. (Myrtaceae) and *Mentha spicata* L. (Lamiaceae) essential oils against *T. urticae* and to evaluate repelling effect of different concentrations of the essential oils of this pest.

MATERIALS AND METHODS

Mite rearing

A population of *T. urticae* was obtained from the Acarology Laboratory in the Agriculture Faculty of Tehran University, Iran and reared on 3-week-old kidney bean (*Phaseolus vulgaris*) plants.

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 50g of dried plant materials and 500ml of distilled water were used, and the distillation was carried out for 4h. The oils were collected in plastic tubes and stored at refrigerator at 4°C until used. GC analysis was performed by a GC (9-A-Shimadzu) gas chromatograph equipped with a flame ionization detector. 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 using a temperature programme of 40-250^{0}C at a rate of 4^{0}C/min, injector temperature 250^{0}C, detector temperature 265^{0}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 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.

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. in 2004. Twenty adult female mites were transferred onto excised bean leaves (2cm diameter) placed with its dorsal side on wet cotton pad in glass container (27 ml volume) using a fine brush. Different concentrations of essential 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 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^{0}C, 65±5% RH and 16:8h. Mortality was determined 24 h after treatment. Mites were considered to be dead if appendages did not move when they were prodded with fine brush.

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 ethanol solution of the oils in four concentrations (equal to LC_{10}, LC_{15}, LC_{20} and LC_{25} per oils) and the other half was immersed in pure ethanol that was used as control. Both treated and untreated leaf disks were placed in a Petri dish. 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 h, the number of mites present on treated or control leaf disc was counted. The Repellence Index (RI) of 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 minor than 1 -

SD, the oil is repellent, while for a mean value higher than 1 + SD, the oil is attractant, and for mean values between 1-SD and 1+SD, the oil is indifferent.

Data analysis

If mortality in control group was found, the corrected mortality was used (Abbott, 1925)**.** Data from each dose-response bioassay were subjected to probit analysis (Finney, 1961) to estimate LC_{50} values using SAS software version 9.1. Samples for which the 95% confidence limits did not overlap were considered to be significantly different ($p<0.05$). For repellency experiments, one-way ANOVA ($p<0.05$) was used. Means were compared by Duncan's test.

RESULTS AND DISCUSSIONS

Chemical compositions of essential oils

The results of the oils analysis 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 *E. caryophyllata* was particularly rich in eugenol (78.5%) and β-Caryophyllene (13.8%). The main components in *M. spicata* oil are carvone (59.4%), limonene (9.8%) and 1.8-cineole (7.4%). The acaricid activity of the essential oils is attributed to those known major components and the resulting synergistic or antagonistic action.

Fumigant toxicity bioassay

The acaricid effect of three plant essential oils obtained from *C. cyminum*, *E. caryophyllata* and *M. spicata* against *T. urticae* were summarized in Table 2. The results showed that all three essential oils have low LC_{50} values. So, the plant extractions are toxic against *T. urticae*. The low values LC_{50} observed 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 resulting in enhanced efficacy. Toxicity was different due to the oil type and concentration. Similar results were reported by Pontes et al. (2007), Aslan et al. (2004) and Çalmaşur et al. (2006) with oils from other aromatic plants against *T. urticae*. There were significant differences between LC_{50} values of all essential oils (based on non-overlap in 95% confidence limits). The lowest LC_{50} value was recorded for cumin oil (3.74 µl/L air) followed by clove (6.13 µl/L air) and spearmint (7.53 µl/L air). This finding is in line with Martinez-Velazquez et al. (2011) results. They found that cumin oil has a high toxicological effect producing 100% mortality on *Rhipicephalus microplus* tick. The high acaricid 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 their or contain in lower amounts. Mortality in the control treatment (ethanol only) was 9%. Since the *Chi-square* values are small ($df = 4$, $P > 0.05$), there was good fit between the observed points and bioassay regression lines for all tested oils.

Repellency tests

According to table 3, mean value of Repellence Index for each applied concentration was determined. Means with different letters are significantly different ($df = 11$, $F=21.17$, $P< 0.0001$). Results demonstrated that only the two highest concentrations of oils indicated repellency effects on *T. urticae* (RI were lower than 1-SD). In fact, by increasing concentration of oils repellency effect was increased. RI values were 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. So, clove oil represented the most repellency activity. This kind of activity may due to the high content of eugenol compound. Our result is in agreement with Araújo et al. (2012) who reported that eugenol component has a strong repellency property 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*. However, more research is needed on the tested oils, such as their acaricide modes of action and their cost-efficacy for improving the acaricid potency and stability

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Table 1. Retention index and relative composition of major chemical components of three essential oils

Component	RI	Mean composition% area		
		Cumin	Clove	Spearmint
Isobutyl isobutyrate	892	0.8	-	-
α- Thujene	922	0.3	-	-
α- Pinene	931	29.1	-	1.5
β- Pinene	962	-	-	2.3
Sabinene	971	0.6	-	0.8
Myrcene	981	0.2	-	0.6
δ-3- Carene	998	0.2	-	-
p- Cymene	1013	0.3	-	-
Limonene	1025	22	-	-
1,8-Cineole	1028	17.9	-	7.4
(*E*)-Ocimene	1038	0.1	-	-
γ- Terpinene	1051	0.6	-	1.5
Limonene	1061	-	-	9.8
Terpinolene	1082	0.3	-	-
Linalool	1089	10.4	-	-
δ- Terpineole	1154	0.09	-	-
Terpinene-4-ol	1169	0.5	-	2.6
α- Terpineole	1180	3.17	-	-
trans-Carveole	1213	0.4	-	2.6
Geraniol	1242	1.1	-	-
Carvone	1245	-	-	59.4

Linalyl acetate	1248	4.8	-	-
Methyl geranate	1310	0.2	-	-
α- Terpinyl acetate	1342	1.3	-	-
Dihydrocarvyl acetate	1345	-	-	1.6
Eugenol	1356	-	78.5	-
Methyl eugenol	1369	1.6	-	-
α- Compaene	1376	-	0.2	-
β- Caryophyllene	1430	0.2	13.8	4.8
α- Humulene	1463	0.2	2.8	0.4
Eugenyl acetate	1524	-	4.4	-
Caryophyllen oxide	1586	0.1	0.2	-
Acetocyclohexane dione	1704	0.4	-	-

Table 2. The toxicity of three essential oils, applied as fumigants, against *T. urticae* for 24 h

Essential oil	n[a]	LC_{50} value and its 95% CL[b] (µl/l 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
E. caryophyllata	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]CL: Confidence Limit

Table 3. Repellent effect on *T. urticae* adults, for four different concentrations of each of oils

Essential oil	n[a]	Corresponding LC value	Concentration (µl/l air)	Mean 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
E. caryophyllata	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)