Laboratory Bioassay for the Efficacy of Coriander and Rosemary Extracted Essential Oils on the Citrus Brown Mite, *Eutetranychus orientalis* (Actinidida: Tetranychidae)

Ashraf S. Elhalawany, Aziza, M. Abou-Zaid and Ahmad I. Amer
Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt, drashrafsaid2@gmail.com.

ABSTRACT

The objective of this study was to evaluate the toxicity and repellency effect of Rosemary, *Rosmarinus officinalis* L. (herb) and Coriander, *Coriandrum sativum* L. (fruits) essential oils against *Eutetranychus orientalis* (Klein). Essential oils were extracted using aqueous extract. The chemical composition of the extracted essential oils was characterized by GC. The main components were Linalool and camphor, respectively. Concentrations of 0.5, 1, 2, 3 and 4% were tested. Results indicated significant differences in efficiency of the tested essential oils on the developmental stages of *E. orientalis*. Coriander essential oil was more toxic for controlling different stages of *E. orientalis* than Rosemary ones. For eggs, LC_{50} and LC_{90} were 4.82%, while for Rosemary; it was 1.49 and 7.94%, respectively after 7 days. Relative values after 3 days for larval stage were: 0.160, 1.340, 0.280 and 20.080 respectively. For nymphal stage relative values were: 0.21, 1.52, 0.20 and 4.37. For adult stage relative values were: 0.77, 4.09, 0.37 and 6.10, respectively. It was concluded that Coriander essential oil was more potent to *E. orientalis* than Rosemary ones. Repellency of different tested concentrations for adult females indicated significant effects of the two oils, concentration and time. Coriander was significantly more repellant than Rosemary. Repellency significantly increased with concentration increased and decreased by time increase. It was concluded that Coriander and Rosemary essential oils could potentially used for the management of *E. orientalis.* More efforts are suggested to evaluate these oils and their components as natural ones for controlling this pest.

Key words: Essential oils, *Eutetranychus orientalis*, Rosemary, Coriander, Toxicity**,** Repellency.

INTRODUCTION

The citrus brown mite, *Eutetranychus orientalis* (Klein) is an important pest of citrus being a persistent pest in Upper Egypt. It also infests a several crops including apple, peach, grape, guava, papaya, date palm, grapevines, quince, cotton, eggplant, castor bean, cucurbits and a variety of ornamentals being reported on 228 host plants of 58 families worldwide. The mites colonize the upper side of leaves where it feed, and their damage develops as yellow-grey stippled spots which cause leaf wilting and drop (Zaher, 1984; Elhalawany, 2001).

As results of the continuous application of pesticides, spider mites became resistant to miticides. Food contamination, mammalian toxicity and pollution of the environment are other problems to be uncounted. Thus approach in pest control such as natural plant products must receive considerable attention (El-Halawany *et al.* 1989).

Studies on plant extracts having acaricidal influences on the citrus brown mite *E. orientalis* are few. Among these are the essential oils of *Majorana hortensis* Moench, *Rosmarinus officinalis* L., *Ocimum basilicum* L., *Lavandula officinalis* Chaix, *Francoeria crispa* (Forssk), Orange Peel, Lemon grass (El-Safty, 1993; Amer, *et al.*, 1993; Amer *et al.*, 2001and Abdel-Khalek *et al.*, 2010). Elhalawany and Dewidar (2017) studied the effect of seven oils of the plants Lemon grass, Spearmint, Rosemary, Marjoram, fennel, Coriander and Chamomile on *Tetranychus urticae* Koch, suggesting that the essential oils of the seven plants have potential to be used for management of *T. urticae*. The aim of this work was to study the toxicity and repellency of Coriander and Rosemary oils extracts on *E. orientalis* and the potential of using them as an alternative to pesticides.

MATERIALS AND METHODS

Plant material, extraction and analysis of volatile oils:

Two essential oils extracted from Rosemary herb and Coriander fruits were tested. Plant materials were obtained from the medicinal and aromatic plants Department Farm, Horticulture Research Institute, Agriculture Research Center, El-Qanater El-Khayreya, Qalyubia, Egypt.

The air dried plants were hydro distilled in a Clevenger-type apparatus for 4 h, according to the procedure described in the Egyptian Pharmacopeia (2005) to determine the volatile oil percentage (volume/weight). The obtained oils were dehydrated by filtration through anhydrous sodium sulfate and kept in a refrigerator in dark bottles for GC analysis. The Extraction of volatile oils and its components were carried out at Medicinal and Aromatic Plants Research Department Laboratory, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

Gas chromatography analysis (GC):

The GC analysis of the volatile oil samples was carried out using gas chromatography instrument at

the Medicinal and Aromatic plants Dept. Laboratory, Horticulture Research Institute. DsChrom 6200 Gas Chromatograph was equipped with a flame ionization detector, Column: BPX-5, 5% phenyl (equiv.) polysillphenylene-siloxane 30 m x 0.25 mm ID x 0.25 µm film. Sample size: 1 µl, Temperature program ramp increased with a rate of 10 ºC/min from 70 to 200 ºC, Detector temperatures (FID): 280°C. Carrier gas: nitrogen. Flow rate: N_2 30 ml/min; H2 30 ml/min; air 300 ml/min. Main compounds of the volatile oils were identified by matching their retention times with those of the authentic samples injected under the same conditions. The relative percentage of each compound was calculated from the area of the peak corresponding to each compound.

Preparation of the emulsions:

Emulsions of the two essential oils were prepared for different concentrations by mixing of Triton-x 100 with oils and completed with distilled water in exact volume.

Stock culture of *E. orientalis*

Culture of *E. orientalis* was collected from infested castor bean plants in Qaha Agriculture Research Station (ARC), Qualyubia governorate, Egypt in May 2018. Seeds of castor bean plants were planted in pots containing soil and leaf compost. After suitable growth, plants were infected with *E. orientalis*. The stock culture was maintained in small greenhouse 5X5m^2. After several generations, mites from the stock colony were used for the tests.

Experimental design

An experimental foam dish (15x20 cm) with a castor bean leaf disc (3 cm in diameter) kept upside down on moistened cotton pads resting on sponge. Water was replaced, as required to prevent the mites from escaping and to keep the culture healthy. A total of 30 experimental foam dishes were divided into two treatments and a control, with ten replicates in each treatment.

Treatment of *E. orientalis* eggs

Leaf discs of castor bean leaves were used as substrate to oviposition. Ten leaf discs were used for each treatment and ten mite females were transferred to each disc and left 24 h to lay eggs, then females were removed. Each test contained 5 concentrations and each concentration had 10 replicate (10 eggs/ replicate). Eggs were sprayed by a glass atomizer with each concentration for every essential oil and other with distilled water (control). Eggs were maintained at temperature 28°C conditions for 7 days till hatching. The numbers of hatched and non hatched eggs were recorded.

Treatment of *E. orientalis* larvae, nymphs and adult females

Ten individuals of larvae, nymphs and adult females of *E. orientalis* were transferred to the lower surface of each castor bean leaf disc. Each test contained 5 concentrations and each concentration had 10 replicates (10 individuals/replicate) treated previously, using a fine camel hairbrush. Leaf discs were treated with one of previous treatments. In each test, a control was included using distilled water and two drops of Triton X-100. Each treatment was replicated six times. Mortality was recorded after 24, 48 and 72 h post treatments under a binocular microscope. Mites were considered to be dead if their bodies or appendages did not move when prodded with fine camel hairbrush (Elhalawany and Dewidar, 2017).

Repellency test procedures for *E. orientalis* females:

The repellency tests were performed according to method described by Amer *et al.* 2011. Castor bean leaf discs (5 cm diam.) were prepared with surfaces upside-down in Petri- dish, lined with moist cotton wool. Half of each disc was dipped with five of aqueous concentrations of each essential oil for 5 seconds and left to dry, while the other half left untreated as control. Ten females were put on the middle of the leaf disk using a fine tipped paint brush. The number of mites on treated or control half was recorded after 24 and 48-hr. Ten replicate leaf discs were used per concentration of each essential oil. Each treatment was repeated for three times. The repellency index was calculated by RI = (C–T/ C+T) × 100 (Pascual-Villalobos and Robledo, 1998). In this formula RI stands for repellency index, T is the number of mites in treatment and C is the number of mites in the control.

Statistical analysis

Data obtained from each dose-response bioassay were subjected to probit analysis (Finney, 1971) to estimate LC50 and LC_{90} values using Ldp line software http://www.ehabsoft.com/ldpline/. Repellency data were analyzed using analysis of variance (ANOVA) and Least Significant Difference Test (LSD) in SAS Program version 9.1.3 (SAS Institute, 2003).

RESULTS AND DISCUSSION

Chemical compositions of extracted essential oils:

The obtained results are illustrated in Figs (1&2). They present the gas chromatography analysis GC-MS for two essential oils. Gas chromatography analysis for volatile oils showed that, the Coriander seed oil contains 12 compounds of which the major

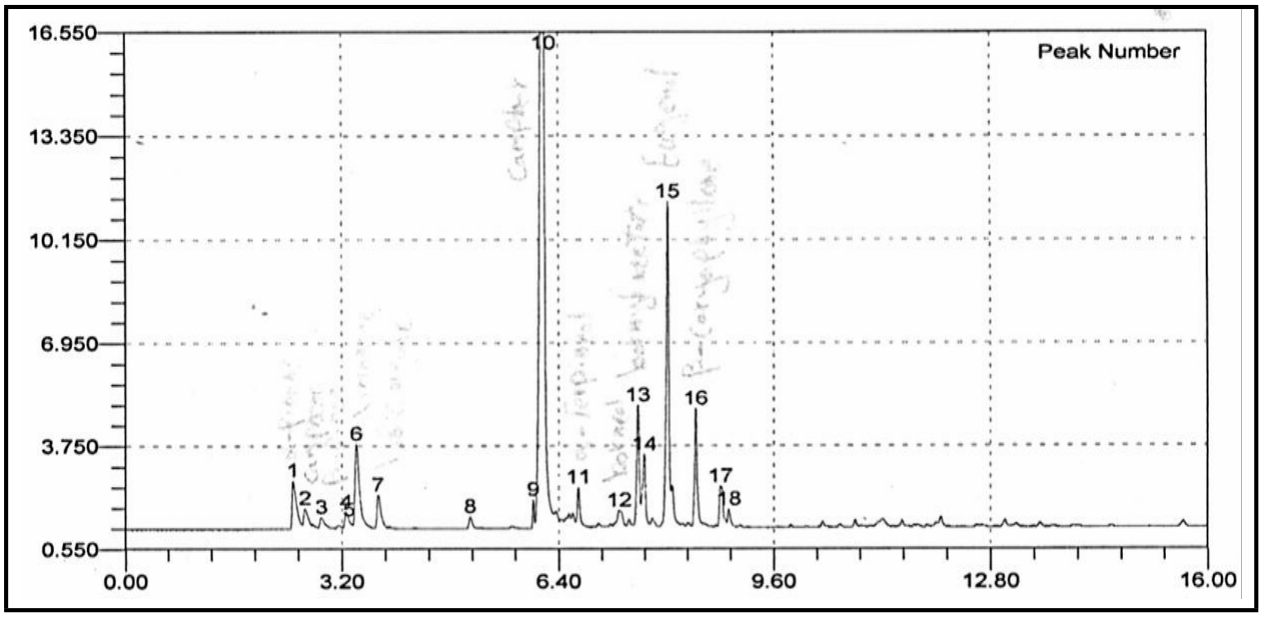

Fig. (1): GC analysis chromatogram for Rosemary volatile oil.

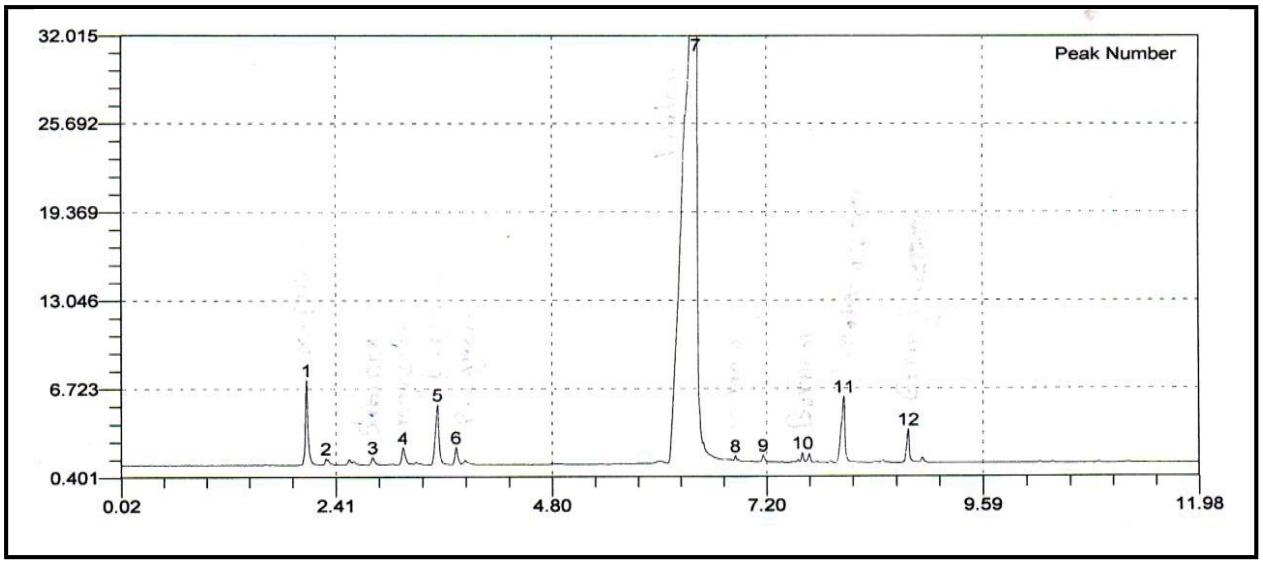

Fig. (2): GC analysis chromatogram for Coriander volatile oil.

compounds were linalool (85.60%), α-Pinene (2.44%), β–Pinene (2.40%) and Linalyl acetate (3.23%). Whereas Rosemary volatile oils contained 18 compounds mostly of camphor (54.36%), Eugenol (14.17%), α-Pinene (3.05%), Limonene (5.10%), Bornyl acetate (4.66%) and β-caryophyllene (4.30%). Comparable results were obtained by Bhuiyan *et al.* (2009) who indicated that the Coriander seed oil contained 53 compounds where the major compounds were linalool (37.7%) and geranyl acetate (17.6%). Linalool was reported as major constituents of Coriander seeds (Coleman and Lawrence, 1992). Other constituents were 1,8-cineol (33.08-37.75%), camphor (13.55-18.13%), a-pinene (8.58-9.32%), a-terpineole (6.79-8.17%), camphene (5.07-5.58 %), borneol (4.08-5.48 %), limonene (3.19-3.04 %) and p-cymene (2.42-3.11%). Yıldırım (2018) indicated that the Rosemary containd 27 components, the major components were 1.8 cineole (41.25%-45.96%), isoborneol (11.96%-14.89%), α-pinene (9.28%-11.22%) and α-terpineol (4.65%-8.41%). Camphene (3.29%-3.51%), Limonene (2.89%-3.61%), p-cymene (3.64%-4.26%) and Bornyl acetate (2.02%-3.13%) found as moderately high compounds.

Toxicity of the two essential oils for *E. orientalis* eggs

Obtained results are presented in Table (1). The two tested essential oils had toxic effects on *E. orientalis* eggs hatchability. Coriander oil was the more potent (LC_{50}= 1.26 and LC_{90}= 4.82), while Rosemary oil was the less toxic (LC_{50}=1.49 and LC_{90}=7.94). The slopes for Coriander and Rosemary were 2.20 and 1.76, respectively. Elhalawany and Dewidar (2017) indicated that LC_{50} values for eggs of *T. urticae* were 1.54, 6.44, 0.96, 1.72, 1.30, 14.67 and

Table (1). Toxicity effect of two essential oils against *E. orientalis* stages after 24, 48 and 72h

Stage	Essential oils	Time (h)	LC_{50}	LC_{90}	Lower limit %	Upper limit %	Slope	Toxicity index
Egg	Coriander	7 days	1.26	4.82	3.93	6.37	2.2	100.00
	Rosemary	7 days	1.49	7.94	5.86	12.4	1.76	60.71
Larva	Coriander	24	0.20	1.51	1.88	2.04	1.47	88.68
		48	0.18	2.58	1.88	4.40	1.21	51.87
		72	0.16	1.34	1.03	1.80	1.40	100.00
	Rosemary	24	1.07	39.7	14.91	409.76	0.81	3.37
		48	0.50	41.02	13.03	1311.8	0.67	3.26
		72	0.28	20.08	8.01	294.13	0.69	6.67
Nymph	Coriander	24	0.36	2.97	2.28	4.47	1.4o	51.26
		48	0.25	2.17	1.71	2.99	1.38	70.27
		72	0.21	1.52	1.20	2.05	1.50	100.00
	Rosemary	24	0.48	9.64	5.6	28.72	0.99	15.81
		48	0.27	5.91	3.59	17.97	0.95	25.76
		72	0.20	4.37	2.88	10.24	0.95	34.84
Adult	Coriander	24	1.27	6.13	4.73	8.88	1.87	66.69
		48	0.91	4.52	3.57	6.31	1.85	90.49
		72	0.77	4.09	3.22	5.75	1.70	100.00
	Rosemary	24	0.94	16.11	8.67	53.37	1.04	25.40
		48	0.56	13.4	7.07	51.43	0.93	30.54
		72	0.37	6.1	3.94	13.98	1.05	67.08

Toxicity index was calculated with respect to the most effective compound LC_{90}

Table (2) Repellency effects of two plants essential oils on *E. orientalis* females

Concentration %	24 h		48 h	
	Coriander	Rosemary	Coriander	Rosemary
0.5	44.0±12.6	38.0±17.5	32.0±19.3	26.0±13.5
1	54.0±13.5	42.0±14.8	42.0±11.4	30.0±19.4
2	72.0±14.0	52.0±16.9	52.0±21.5	44.0±22.7
3	84.0±12.6	64.3±18.4	70.3±23.4	54.0±19.0
4	92.0±10.3	70.0±19.4	82.2±14.2	60.0±23.1

Table (3) Factorial analysis for repellency effects on *E. orientalis* females

Factor	Level	Mean
Extracted oil	Coriander	62.45^{a}
	Rosemary	48.03^{b}
F value		68.95
P value		0.0001
LSD		3.752
Time (hours)	24	61.23^{a}
	48	49.25^{b}
F value		47.59
P VALUE		0.0001
LSD		3.752
Concentration (%)	0.5	35^{e}
	1	42^{d}
	2	55^{c}
	3	68.15^{b}
	4	76.05^{a}
F value		78.57
P value		0.0001
LSD		5.932

Different letters on the same column indicate significant difference ($P \leq 0.05$).

0.95% for lemon grass, spearmint, Rosemary, marjoram, fennel, Coriander and chamomile essential oils, respectively.

Toxicity effect of two essential oils against *E. orientalis* different stages

Larval stage:

The for mentioned results in (Table 1) clarified that, the corresponding LC_{50} values of Coriander and Rosemary against *E. orientalis* larvae after 24h of treatment were 0.20 and 1.07%, and the corresponding LC_{90} values were 1.51 and 39.70%, respectively. While the corresponding LC_{50} values after 48h of treatment against the larvae of *E. orientalis* were 0.18 and 0.50%, and the consequent LC_{90} values were 2.58 and 41.02%, respectively. Coriander recorded the highest slope value after 24 and 48 h 1.47& 1.21, respectively; whereas the lowest slope values were 0.81 & 0.67 for Rosemary oil after 24 and 48 h, respectively. On the other hand, the corresponding LC_{50} values after 72 h for larvae were 0.16 and 0.28% and the consequent LC_{90} values were 1.34 and 20.08%, respectively. The slope values of regression line were 1.4 and 0.69 for Coriander and Rosemary after 72 h for larvae, respectively. At the level LC_{50} the relative potency levels expressed as number of folds indicated that Rosemary 20.08 times as toxic as Coriander oil after 72h. It was statistically determined that the effect of the two essential oils toxicity to *E. orientalis* larvae increased with an increase in concentration.

Nymphal stage:

Applying the same tests (Table 1) indicated that significant differences occurred between the two essential oils. Coriander extracted oil was more toxic than Rosemary ones on nymphal stages of *E. orientalis.* The corresponding LC_{50} values of Coriander and Rosemary against the nymphal stages of *E. orientalis* were 0.36& 0.48, 0.25& 0.27, and 0.21& 0.2% after 24, 48 and 72h, respectively; whereas the LC_{90} values were 2.97& 9.64, 2.17&5.91 and 1.52& 4.37% at the same times, respectively.

Adult female:

As presented in Table (1) LC_{50} values increased as time increased after application, the Ldp-lines of toxicity effects of two essential oils on adult females of *E. orientalis*. When compare between the effects of essential oils on mortality percentage of *E. orientalis* females after 72h from treatment it can be conducted that Rosemary was more toxic with LC_{50} value 0.37%; LC_{90} value 6.1% and the slope values gave 1.05, whereas Coriander was less toxic to adult of females of *E. orientalis* with LC_{50} values 0.77%; LC_{90} values 4.09% and the slope values gave 1.7.

The obtained results are in harmony with that detected by Iskander (1993) who determined that LC_{50} were 250.95 and 406.44 ppm, while LC_{90} values were 564.93 and 696.31 ppm for Duranta and Lantana for *Eutetranychus anneckei* Meyer (*E. orientalis*). Elhalawany and Dewidar (2017) found that LC_{50} values for the *T. urticae* adult females after 72h were 1.28, 0.85, 0.53, 1.61, 0.44, 3.11 and 0.46% for Lemon grass, Spearmint, Rosemary, Marjoram, Fennel, Coriander and Chamomile, respectively.

Repellency effects of two plants essential oils on *E. orientalis* females.

The repellent effects of different concentrations of Coriander and Rosemary oils on *E. orientalis* adults are presented in Table (2). Factorial analyses of these results are presented in Table (3). Repellency of different tested concentrations for adult females indicated significant effects of the two oils, concentration and time. Coriander was repellant than Rosemary. Repellency increased with concentration increased and decreased by time increase.

The obtained results are in agreement with that recorded by Schauer and Schmutterer (1981) who indicated that aqueous extracts and high concentrations (10, 5 and 2.5%) of methanolic extracts of Neem seed were strongly repellent to *T. urticae* adult mites. The application of the 10% methanolic extract to eggs resulted in high mortality of larvae and nymphs. Mansour *et al.* (1986) found that bean leaf discs sprayed with concentrations of the acetonic solutions of the Rosemary oil from 0.1 to 2% caused mortality and induced repellency to *T. cinnabarinus* (Boisd) within 48 h of placing adult females on the discs and consequently egg-laying was reduced. El-Halawany and Sawires (1988) tested six essential volatile oils against *T. urticae*, whereas repellency percentage ranged between 70% and 85% for Marjoram treatment. Both Rosemary and Sweet Marjoram oils gave 100% repellency to *T. urticae* at 10% level of concentration (Sawires *et al.,* 1988). Amer *et al.*, (1993), indicated that the Orange Peel oil showed a remarkable deterrent effect with *E. orientalis* than *T. urticae*. El-Safty (1993) showed that at concentration 10% the repellency percentages were 32.35, 30.56 and 52.77% after 72hr. of adult *E. orientalis*. Hori and Komatsu (1997) found that Rosemary volatile oil and its principle component 1,8-cineole was repellent against *Neotoxoptera formosana*. Amer *et al.* (2001) indicated that Rosemary oil proved to be completely deterrent for *E. orientalis* even after 72h.

It was concluded that Coriander and Rosemary essential oils and their two major constituents

Linalool and camphor could potentially use for the management of *E. orientalis*. More efforts are suggested to evaluate these components as natural ones for controlling this pest.

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