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Acaricidal and molluscicidal potential of three essential oils isolated from Egyptian plants

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ABSTRACT

The chemical composition of essential oil isolated from *Eucalyptus camaldulensis* Dehnh was analyzed by gas chromatography/mass spectrometry (GC / MS). Acaricidal and molluscicidal activities of this oil and the essential oils of *Mentha microphylla* C. Koch and *Lantana camara* L. were evaluated against adults and eggs of the two-spotted spider mite, *Tetranychus urticae* Koch, and terrestrial snails, *Theba pisana* and *Eobania vermiculata*. To determine the effects of the essential oil vapors against the adults of *T. urticae*, concentrations of 1, 2.5, 5 and 10 µl / l air were tested. Responses varied with essential oil, dose and exposure time. *Mentha microphylla* oil exhibited the strongest acaricidal activity at all of the tested concentrations followed by *E. camaldulensis* oil, while *L. camara* oil showed the weakest one. At concentrations of 1 and 2.5µl / l, *M. microphylla* oil caused 76.67 and 100 % mortalities after 48h of treatment, respectively. In a leaf disc dipping assay with eggs of *T. urticae*, the essential oil of *M. microphylla* revealed also the highest activity with LC_{50} value of 137.51µg / ml, whereas the essential oil of *L. camara* showed the lowest activity with LC_{50} value of 272.27 µg / ml. This study may suggest that these essential oils have potential to be used for management of *T. urticae* under greenhouse conditions. In addition, the essential oils of *L. camara* and *M. microphylla* exhibited pronounced molluscicidal activity against *T. pisana* snails. The oil of *L. camara* showed molluscicidal activity greater than methomyl as a reference at all of the tested concentrations.

Keywords: Acaricidal activity, molluscicidal activity, plant essential oils, *Tetranychus urticae*, *Theba pisana*, *Eobania vermiculata*

INTRODUCTION

Essential oils of higher plants consist of complex mixtures of monoterpenes, sesquiterpenes, and others. However, their biological activities are attributed to smaller group of constituents acting additively and synergistically (Singh and Agarwal, 1988). They are part of the chemical defense system against different plant pests (Rice and Coats, 1994). Besides remarkable medicinal properties, plant essential oils have been recognized as an important natural resource of pesticides. They exhibited interesting insecticidal, antibacterial and antifungal activities (Curtis *et al.,* 1991; Isman *et al.,* 2001; El-Zemity and Ahmed, 2005 and El-Zemity *et al.,* 2006).

The two-spotted spider mite, *Tetranychus urticae* is a worldwide economic pest both in the field and in the greenhouse. On the other hand, *Theba pisana* (Muller) and *Eobania vermiculata* (Muller), cause great damage to ornamental plants, vegetables and citrus trees (Godan, 1983). Intensive use of pesticides on various agricultural crops led these pests to develop a resistance to most classes of chemical pesticides (Choi *et al.,* 2004). Therefore, there is an urgent need to develop safer and efficient alternatives that have potential to replace synthetic pesticides and are convenient to use for control *T. urticae*, *T. pisana* and *E. vermiculata*. One of the alternatives which have been widely investigated is essential oils. Their toxicities, arresting and repellent effects to stored-product insects and greenhouse pests have been of interest during the last several years (Huang *et al.,* 1997; Aslan *et al.,* 2004; Choi *et al.,* 2004; Tedonkeng Pamo *et al.,* 2005 and Çalmaşur *et al.,* 2006).

The present study was aimed to investigate the acaricidal and molluscicidal activities of three essential oils isolated from three plant species, *Eucalyptus camaldulensis*, *Mentha microphylla* and *Lantana camara* against adults and eggs of the two spotted spider mite, *T. urticae*, and terrestrial snails, *T. pisana* and *E. vermiculata*.

MATERIALS AND METHODS

1. Plant materials: Leaves of *Eucalyptus camaldulensis* Dehnh (Myrtaceae), *Mentha microphylla* Koch (Labiatae) and *Lantana camara* L. (Verbenaceae)

were collected during the flowering stage in August, 2005 from the Faculty of Agriculture Farm, Alexandria, Egypt. The taxonomic identification of plant species were preformed by Dr. Ahmed Mohareb, Department of Timber Trees and Wood Technology, Faculty of Agriculture, Alexandria University. Voucher specimens have been deposited in Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University.

2. Isolation of *E. camaldulensis*, *M. microphylla* and *L. camara* essential oils: The leaves were dried at room temperature (27 ± 1 ºC) for five days. Essential oils were extracted from the partially dried leaves by hydrodistillation in a Clevenger-type apparatus for 2 h. The essential oils, which were pale yellow, were obtained with yields 0.6, 0.36 and 0.4 % (v / w) for *M. microphylla*, *L. camara* and *E. camaldulensis*, respectively. The obtained essential oils were dried over anhydrous sodium sulfate, and stored at 4 °C until used for analysis and biological activity.

3. Analysis of *E. camaldulensis* essential oil: Essential oil of *E. camaldulensis* was diluted in diethyl ether and 1 µl was injected into a gas chromatography (TRACE GC 2000, THERMO) /mass spectrometry (SSQ 7000, FINNIGAN) (GC/MS) set-up. The GC column was a 60m (0.25mm i.d.) DB-5 (5%-Phenyle) Methylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 220 ºC; column temperature, isothermal at 40 ºC for 2 min, then programmed to 250 ºC at 5 ºC/2 min and held at this temperature for 2 min; ion source temperature, 200 ºC. Helium was used as the carried gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 400 amu for 5 s.

4. Test organisms: The two-spotted spider mite, *Tetranychus urticae* (Koch), was obtained from culture maintained in the Pesticide Chemistry Department, Faculty of Agriculture, Alexandria University and was reared on caster-oil plant, *Ricinus communis* L. The colony was kept under laboratory conditions at 25 ± 1 °C and 65 ± 5 % R.H., with a 12:12 light : dark photoperiod. *Theba pisana* (Muller) and *Eobania vermiculata* (Muller) terrestrial snails were collected from Faculty of Agriculture Garden, Alexandria, Egypt in August,

2005. The snails were acclimatized to laboratory conditions for one week and were fed on fresh lettuce leaves.

5. Fumigant toxicity assay: A piece of a double faced scotch tape was pressed tightly to the surface of a glass slide. With the aid of a stereomicroscope, 30 adult females were fixed to the tape on the dorsal part of the hysterosoma by using a moistened Chinese writing brush (Dittrich, 1962). The slides infested with *T. urticae* were brought to plastic jars with a capacity of 1L as test chambers. The essential oils were applied on filter paper pieces (2 cm × 3 cm) inside the jars with amounts of 1, 2.5, 5, 10 µl per jar. Three replicates of each concentration and control (without essential oil) were carried out. The treatments were kept in a holding chamber of 25 ± 1 °C and 65 ± 5 % R. H., with a 12:12 light: dark photoperiod. Number of dead mites was recorded after 24 and 48 h of treatment. Test mites were considered dead if appendages did not respond after being touched with a camel hair brush under a stereoscopic microscope (10x to 40x, Optika microscope, SZM-2). The activity of the essential oils against mites was expressed as mortality percentages.

6. Ovicidal activity assay: Acaricidal activity of the essential oils against *T. urticae* eggs were determined by a leaf-dipping method (Siegler, 1947). Disks (2 cm diameter) of castor-oil leave *Ricinus communis* L. were placed on cotton pads in Petri dishes. Five adult females were put on each disc and left for 24 hours and then the adult females were removed and the eggs were counted. The essential oils were dissolved in acetone and diluted with distilled water to obtain a series of concentrations (0, 25, 50, 100, 200 and 400 µg / ml). The leaf disks infested with eggs were dipped in a test solution for 5 seconds with gentle agitation. Three replicates with three disks were maintained for each concentration and control. The experimental units were kept in a holding chamber of about 25 ± 1 °C and 95 % R. H., with a 12:12 light : dark photoperiod. The eggs mortality was calculated when the hatched mites in the control treatment have reached to the deutonymphal stage, i.e., all the present eggs were hatched or nonviable.

7. Bioassay against terrestrial snails: The efficiency of essential oils was evaluated on adult snails (Hussein *et al.,* 1994 and El-Zemity and Radwan, 1999) of *T. pisana* (17mm shell diam.) and *E. vermiculata* (26 mm shell diam.).

Essential oils were prepared in acetone and tested at doses of 0.1, 0.25, 0.5 mg / snail and 0.25, 0.5, 1 mg / snail against *T pisana* and *E vermiculata*, respectively. These doses contained in 5µl of acetone solution in the case of *T pisana* and 25 µl in the case of *E vermiculata* were gently applied on the surface of the snail body inside the shell using a micropipette. Three replicates (five snails each) of each concentration were used. Control snails were treated with the same volumes of acetone. The snails were fed on fresh lettuce leaves during the course of experiment. Methomyl (98 %, Wako, Japan) was used at same concentrations as a reference. The mortality percentages were recorded after 24 hours of treatment.

8. Data analysis: Mortality percentages were corrected by Abbott's formula (1925). LC_{50} values were calculated by using the probit-analysis method of Finney (1971).

RESULTS AND DISCUSSION

1. Isolation of essential oils and chemical composition of *E. camaldulensis* oil: The essential oils, with pale yellow colors, were obtained by hydrodistillation in a Clevenger-type apparatus from leaves of *E. camaldulensis, M. microphylla* and *L. camara* with the yields of 0.4, 0.6 and 0.36 % (v / w) on dry weight basis, respectively. The essential oil of *E. camaldulensis* was analyzed by GC–MS and the chemical constituents with their percentage and retention times are summarized in Table 1. A total of 47 components were identified and accounted for 78.9 % of the essential oil. Chemical constitutes of *M. microphylla* and *L. camara* essential oils analyzed by the same method were reported in a previous study (Abdelgaleil, 2006). The chemical classes of the identified compounds are monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenes, triterpenes and others. Some of identified compounds of the isolated essential oils were matched with those reported in the literatures (Traboulsi *et al.,* 2002; Hussein, 2005 and Randrianalijaona *et al.,* 2005). However, these components were present with different concentrations. The reason of this variation may be attributed to that the chemical composition of essential oils depends on climatic, seasonal, and geographic conditions, harvest period, and isolation technique.

Table (1): Chemical composition of the essential oil isolated from *Eucalyptus camaldulensis*

Compound	RT (min)	%
1. 2,2-Dimethyl-1,3-dioxane-4,6-dione	4.26	0.80
2. 4-Hydroxy-4-methyl-2-pentanone	5.82	5.77
3. α-Pinene	8.25	0.08
4. α-Humulene	10.72	0.07
5. 1,8-Cineole	11.17	0.54
6. γ-Terpinene	11.95	0.36
7. 1-Octanol	12.37	1.32
8. Linalool	13.23	0.91
9. endo-Borneol	13.96	0.12
10. Camphor	14.62	0.08
11. L-Menthone	14.87	0.10
12. l-4-Terpineol	15.60	0.71
13. 1-Methanol 4-trimethyl-3-cyclohexene	16.03	0.45
14. 2-Ethylhexyl acetate	16.41	19.36
15. n-Octyl acetate	17.23	0.04
16. 2-Methyl-5-(1-methylethenyl)- 2-Cyclohexen-1-one	17.40	0.45
17. Ingenol triacetate	19.73	0.04
18. ε-Elemene	19.87	0.36
19. α-Copaene	20.96	0.19
20. α-Elemene	21.31	3.33
21. 24,25-Dihydroxycholecalciferol	21.56	0.02
22. trans-Caryophyllene	22.10	0.18
23. γ-ElemeneL	22.33	0.25
24. Curzerene	23.95	2.67
25. 2,3-dicyano-7,7-dimethyl-5,6-benzonorbornadiene	24.13	3.40
26. γ-Selinene	25.12	0.14
27. Torreyol	25.27	0.13
28. Spathulenol	25.97	0.11
29. (-)-Caryophyllene oxide	26.10	0.37
30. 3-Methyl-28-nor-3,4-seco-5à-lanosta-4,8-dien-3-one	27.08	3.46
31. 3-Phenyl-4,5,6,7-tetrahydro-(3H)-isobenzofuran-1-one	27.28	1.21
32. Elemol	27.99	2.06
33. Heptadecane	28.74	1.96
34. Bisabolol oxide A	29.80	0.40
35. Hexadecamethyl heptasiloxane	30.42	1.06
36. Octadecane	30.88	3.76
37. Isochiapin B	31.73	0.30
38. Nonadecane	32.94	2.64
39. bis(2-methylpropyl) ester 1,2-Benzenedicarboxylic acid	33.98	2.00
40. Eicosane	34.89	3.34
41. (+-)-cis-3,4,6,9-tetrahydro-10-hydroxy-1,3,8-trimethyl-1H-naphtho[2,3-c]pyran-6,9-dione	35.15	2.32
42. Heneicosane	36.76	3.79
43. Nerolidol-epoxyacetate	37.94	2.09
44. Docosane	38.57	1.77
45. Tricosane	40.29	1.05
46. Di-(2-ethylhexyl)phthalate	44.04	2.46
47. Ethyl iso-allocholate	44.73	0.86

2. Acaricidal activity of essential oils against the adult females of *T. urticae*: The acaricidal activity of the three essential oil vapors of *E. camaldulensis, M. microphylla and L. camara* against the adult females of *T. urticae* is shown in Table 2. Two of the three tested essential oils exhibited strong acaricidal activity. Reponses varied depended on oil, concentration and exposures time. Essential oil of *M. microphylla* revealed the highest acaricidal activity at all of the tested concentrations and exposure times followed by *E. camaldulensis* oil while *L. camara* oil was the less active one. At concentrations of 1 and 2.5µl / l, *M. microphylla* essential caused 76.67 and 100 % mortality after 48h of treatment, respectively. This strong activity of *M. microphylla* essential oil compared with *L. camara* oil was also observed when these two oils were tested for their insecticidal and antifungal activities against stored product insects and plant pathogenic fungi (Abdelgaleil, 2006). Essential oils of *M. microphylla* and *E. camaldulensis* were more toxic to adults of *T. urticae* than the essential oils of *Satureja hortensis* L., *Ocimum basilicum* L., *Micromeria fruticosa* L., *Nepeta racemosa* L. and *Origanum vulgare* L. However, they showed comparable activity with *Thymus vulgaris* L. essential oil tested by the same assay (Aslan *et al.,* 2004 and Calmasur *et al*. 2006).

Table (2): Acaricidal activity of the essential oils of *E. camaldulensis M. microphylla and L. camara* against adult female of *T. urticae*

Essential oil	Conc. (µl / l air)	Mortality (%) (Mean ± SE)	
		24h	48h
Control	0	0 ± 0.0	6.67 ± 0.33
E. camaldulensis	1	26.67 ± 0.33	56.67 ± 0.33
	2.5	53.33 ± 1.20	83.33 ± 1.20
	5	100 ± 0.0	100 ± 0.0
	10	100 ± 0.0	100 ± 0.0
M. microphylla	1	56.67 ± 0.33	76.67±0.67
	2.5	83.33 ± 0.33	100±0.0
	5	100 ± 0.0	100±0.0
	10	100 ± 0.0	100±0.0
L. camara	1	6.67 ± 0.33	23.33 ± 0.33
	2.5	6.67 ± 0.33	26.67 ± 0.33
	5	13.33 ± 0.33	53.33 ± 0.67
	10	26.67 ± 0.67	66.67 ± 0.67

3. Ovicidal activity of essential oils against the eggs of *T. urticae*: The acaricidal activity of the three isolated essential oils against the eggs of *T. urticae* is presented in Table 3. Essential oil of *M. microphylla* showed the highest toxicity against the eggs with LC_{50} value of 137.51 µg / ml. Both *E. camaldulensis* and *L. camara* essential oils exhibited lower toxicity to the eggs than *M. microphylla* oil with LC_{50} values of 261.58 and 272.27 µg / ml, respectively.

Table (3): Acaricidal activity of the essential oils of *E. camaldulensis, M. microphylla and L. camara* against eggs of *T. urticae*

Essential oil	LC_{50} (µg/ml)	95% Confidence limits of LC_{50}		Slope ± SE
		Lower	Upper	
E. camaldulensis	261.58	206.81	357.51	1.37 ± 0.16
M. microphylla	137.51	71.48	365.22	1.56 ± 0.15
L. camara	272.27	203.22	415.33	1.08 ± 0.15

Comparing the results of the two experiments of this study, indicating that the essential oils were more toxic to the adults than the eggs. It seems that the acaricidal mode of action of these oils may be largely attributable to fumigant toxic action through respiratory system. The previous studies have been shown that toxicity of essential oils from aromatic plants against insects and mites is related to the fumigant action of their monterpenoids components (Isman *et al.,* 2001).

4. Molluscicidal activity of essential oils against the terrestrial snails *T. pisana* and *E. vermiculata*: Table 4 presents mortality percentages of the two snails *T. pisana* and *E. vermiculata* treated topically by *M. microphylla* and *L. camara* oils. The results showed that *T. pisana* snail was more sensitive to both essential oils at all of the tested doses. *Lantana camara* oil exhibited higher mortality than a reference insecticide, methomyl, against *T. pisana* after 24 hours at the tested concentrations while *M. microphylla* oil showed

comparable toxicity with methomyl against the same snail. Molluscicidal activities of the isolated essential oils against *T. pisana* were stronger than those of *Eucalyptus camaldulensis* oil, monoterpenoids (α-terpineol, pulegone and anisole) and dichloromethane extracts of *Magnolia grandiflora* but close to those of *Lavandula ﷽ entate* and *Ruta chalepensis* oils and monoterpenoids thymol and eugenol (El-Zemity, 2001; Abdelgaleil, 2005 and Hussein, 2005).

Table (4): Mortality percentages of *T. pisana* and *E. vermiculata* after 24 hours of treatment with essential oils of *M. microphylla* and *L. camara*

Plant oil	Dose mg/snail	*T. pisana*	*E. vermiculata*
M. microphylla	0.1	40.0	-[a]
	0.25	40.0	6.7
	0.5	53.3	13.3
	1	-	26.7
L. camara	0.1	26.7	-
	0.25	53.3	0
	0.5	60.0	13.3
	1	-	20.0
Methomyl	0.1	20.0	-
	0.25	40.0	53.3
	0.5	53.3	60.0
	1	-	86.7
Control	0	0	0

[a]- = not tested.

The results of the present study demonstrated that the isolated essential oils possess interesting acaricidal and molluscicidal activities against the adults of *T. urticae* and *T. pisana* snail. They may be used as useful agents for control of *T. urticae* and *T. pisana* snail in the closed areas and greenhouses. However, more detail studies need to be carried out on chemistry, formulation, and large scale biological activity of these oils before taking a decision of commercial applications.

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