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Repellent and Oviposition-Deterring Activity of Rosemary and Sweet Marjoram on the Spider Mites *Tetranychus urticae* and *Eutetranychus orientalis* (Acari: Tetranychidae)

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The deterrent and toxic effect of two essential oils, *Majorana hortensis.* Moench and *Rosmarinus officinalis* L. on the two tetranychid mites *Tetranychus urticae* Koch and *Eutetranychus orientalis* (Klein) were studied under laboratory conditions. Both materials used were more potent for *E. orientalis* than against *T. urticae* with a significant increase in repellency.

Leaf discs treated with increasing concentrations of the two oils showed increased mortality of both spider mites and reduction in the total numbers of eggs laid. This result could be due to the oil of the higher oxygenated compounds content that was more effective in this respect.

Keywords: *Tetranychus urticae, Eutetranychus orientalis, Majorana hortensis, Rosmarinus officinalis,* essential oil.

The use of chemical pesticides offers problems such as atmospheric pollution and residual toxicity in food. For these reasons, research on non-contaminating pesticides is needed. Research in recent years has been turning more towards selective biorational pesticides, such as plant-derived compounds, because they are perceived to be safer than the synthetics (Arnason et al., 1989). Few studies have been done on such plants having acaricidal influences are assumed to be acceptable because they are alterable in nature (Schauer and Schmutterer, 1981; Amer 1984; Rasmy 1985; Amer et al., 1989; Dimetry et al., 1990). Among these botanicals, the essential oils exhibit antifeedant activity and toxic to some important insects (Sy et al., 1972; Purohit et al., 1983; Don-Pedro, 1985; Tare and Sharma, 1988).

In Egypt, very few research has been reported on the effect of the essential oils on mites (Amer et al., 1993; Ibrahim et al., 1993; El-Gengaihi et al., 1996).

Both *Majorana hortensis* Moench and *Rosmarinus officinalis* L. (Fam. Labiatae) are considered as two of the most valuable aromatic and medicinal plants. Rosemary oil has been reported by Guenther (1961) as an insect repellent, and as an ingredient in rubefacient liniments. He stated also that its oil contains: α-pinene, camphene, cineole, dipentene, camphor, borneol and sesquiterpene compounds. Sweet marjoram is used medicinally as carminative and stimulant due to its strong, highly aromatic and pleasing odor (Guenther, 1961). Marjoram oil contains terpinene 4-01, α-terpinene, D-limonene, linalool, linalyl acetate, P-cymene, β-pinene and α-terpineol.

This is the first report, dealing with the acaricidal properties and biological activity of both *M. hortensis* and *R. officinalis* on two of the most serious pests in Egypt, namely *Tetranychus urticae* Koch and *Eutetranychus orientalis* (Klein) under laboratory condition.

Materials and Methods

Plant materials

Both plants essential oils (rosemary and sweet marjoram) were obtained in June and November 1998, respectively from the air-dried herbs about seven to nine months old, originally grown as plants in the Experimental Farm of National Research Center at Giza.

Preparation of the oil

The essential oil was extracted by submitting the air-dried herb of each to stem distillation for 3 hours in a Clevenger apparatus. The oil collected from each was dehydrated over anhydrous sodium sulfate and subjected to GC-MS analysis.

Chromatographic investigation of the volatile oil

The obtained essential oils were subjected for GC-MS analysis under the following conditions:

GC-MS Finnigan mat SSO 7000 Digital DEC 3000. Work station: Digital DEC 3000. Ionization mode Eleven 70. Column: Capillary column of Fused silica, DB-5 (5% phenyl), 30 m length, 0.32 mm i.d and 0.25 µm thickness. Carrier gas: Helium at 13 psi. Temperature-programming: initial temp. 60 ºC for 15 min, 60–220 ºC, at a rate of 5 ºC/min., final temp. 250 ºC for 15 min., ionization voltage: 70 eV. The identification of the compounds was based on comparison of the retention times and mass spectra with corresponding data components of references oils and pure compounds whenever possible. Mass spectra were compared with those of the published data by Adams (1989). Qualitative determination was based on peak area measurements.

Preparation of the emulsions

Emulsions of rosemary (*R. officinalis* L.) and sweet marjoram (*M. hortensis* Moench) were prepared respectively by mixing five drops of Triton-X 100 emulsifier with 2 ml of oil plus an amount of water to obtain 2% concentration of the oil emulsion. A series of dilution i.e. 1, 0.5, 0.25 and 0.125% were prepared from the stock 2% solution using water.

Maintenance of mite stock cultures

The stock cultures of two spotted spider mite *T. urticae* were collected from infested lima bean (*Phaseolus vulgaris* L.) in the laboratory at N.R.C. Cairo. The citrus brown mite, *E. orientalis,* was obtained from cultures maintained on sweet potato. The mites were reared in a controlled climate room at 25–27 ºC and 60± 5% R.H.

Pots of lima bean seedlings cultivated in soil were placed in center of the cage (measured 70 x 40 x 60 cm). A continuous light source of (60 watt) flourcent was provided. Large numbers of *T. urticae* were placed on leaf of the lima bean.

Treatment

REPELLENCY TEST PROCEDURE FOR ADULT FEMALES OF *T. URTICAE* AND *E. ORIENTALIS*

Raspberry leaf discs were placed with the lower surface upwards in petri-dish lined with moist cotton wool. Half of each disc was painted with the proper concentration, while the other half treated with water only. Adult females were placed on the midrib and observations on repellency and oviposition were taken after 24, 48 and 72 h, respectively. Each treatment comprised 5 replicates, each replicate contained 10 females. The repellency (mites which had left the discs were considered as repelled) was calculated according to Lwande et al. (1985).

TOXICITY AND BIOLOGICAL EFFECTS OF ROSEMARY AND SWEET MARJORAM ON ADULT FEMALES OF *T. URTICAE* AND *E. ORIENTALIS*

Adult females of the same age of both species were placed individually on raspberry leaf discs treated with different concentrations of both oils. Twenty replicate leaf discs were used and a similar number of discs treated with water only as control. The total number of eggs laid on the treated discs were recorded over a period of 10 days. The mortality of both tetranychid mites was also recorded.

All the experiments reported herein were carried out in the laboratory at 27±2 ºC and 70–75 % R.H.

Statistical analyses were carried out by using “F” test .

Results and Discussion

Chemical constituents of rosemary and sweet marjoram oils

GC-MS and GLC analysis of *R. officinalis* and *M. hortesis* essential oils, detected the presence of the following compounds:

1. Hydrocarbon terpenes.
2. Oxygenated terpenes.
3. Hydrocarbon sesquiterpenes.
4. Oxygenated sesquiterpenes.

One can see in *Tables 1, 2, 3* and *Figs. 1, 2* the identification of forty-four components which represent about 90.5% of the total rosemary oil. The oil is characterized by the dominant occurrence of oxygenated hydrocarbon terpenes which contributed with 79.922% of oil content. As assessed from the total peak area, cineole represented the major compound (ca 22%), followed by camphor (9.77%) and linalool (5.01%). The same tables indicated also that the oxygenated sesquiterpenes represented the least content value (2.94%) only.

Table 1

The content of the terpene hydrocarbon compounds in sweet marjoram and rosemary oils

Sweet marjoram oil			Rosemary oil		
Peak No.	Compounds	%	Peak No.	Compounds	%
1	Tricyclene	0.003	1	α-Pinene	5.006
2	α-Thujene	2.054	2	Camphene	1.886
3	α-Pinene	1.126	3	β-Pinene	0.526
4	Camphene	0.046	4	Myrecene	1.762
5	Sabinene + β-Pinene	13.999	5	2-Carene	1.500
6	Myrecene	1.251	6	β-Phellandrene	0.168
7	α-Phyllendrene	0.574	8	γ-Terpinene	0.516
8	3-Carene	0.499	9	Terpinolene	0.700
9	α-Terpinene	2.260		Unknown	1.128
10	p-Cymene	7.802		Total	13.192
11	Limonene	1.778			
12	γ-Terpinene	21.456			
	Unknown	0.035			
	Total	52.883			

Table 2

The content of the oxygenated terpene compounds in sweet marjoram and rosemary oils

Sweet marjoram oil			Rosemary oil		
Peak No.	Compounds	%	Peak No.	Compounds	%
13	cis-p-Menth-2-en-1-ol	1.040	7	Cineole	21.957
14	Terpinolene	0.692	10	Fenchone	0.260
15	trans-p-Menth-2-en-1-ol	3.054	11	Linalool	7.433
16	Linalool	0.566	12	Camphor	9.771
17	cis-Sabinene hydrate	0.241	13	Isoborneol	6.009
18	Citronellal	4.624	14	Borneol	6.915
19	Borneol	0.711	15	Terpinene-4-ol	0.120
20	Terpinene-4-ol	8.246	16	α-Terpineol	6.402
21	α-Terpineol	6.196	17	Verbenone	7.133
22	Nerol	0.293	18	new-iso-Dihydro carveol	1.220
23	Linalyl acetate	1.836	19	cis-Myrtanol	1.901
24	Bornyl acetate	0.212	20	Carvone oxide	0.266
25	Isomenthyl acetate	2.869	21	Bornyl acetate	2.600
26	trans-Carvyl acetate	0.170	22	Carvacrol	0.038
27	Terpinenyl acetate	0.014	24	Eugenol	0.215
28	Neryl acetate	0.308	25	cis-Carvyl acetate	0.189
30	Geranyl acetate	0.413	28	Methyl eugenol	0.271
	Unknown	4.996	30	Geranyl acetate	0.617
	Total	36.48		Unknown	6.618
				Total	79.922

Table 3

The content of sesquiterpene compounds in sweet marjoram and rosemary oils

Sweet marjoram oil			Rosemary oil		
Peak No.	Compounds	%	Peak No.	Compounds	%
		I–Sesquiterpene hydrocarbon compounds			
29	α-Copaene	0.005	23	D-Elemene	0.113
31	β-Bourbonene	0.016	26	α-Copaene	0.132
32	β-Cubebene	0.025	27	β-Elemene	0.034
33	β-Caryophyllene	3.981	29	β-Caryophyllene	2.170
34	Aromadendrene	0.104	31	α-Humulene	0.453
35	α-Humulene	0.279	32	γ-Muurolene	0.179
36	Germacrene-D	0.021	33	Germacrene-B	0.065
37	β-Bisabolene	2.914	34	α-Longipinene	0.117
38	γ-Cadinene	0.019	35	β-Bisabolene	0.090
39	trans-γ-Bisabolene	0.021	36	γ-Cadinene	0.334
	Unknown	0.642	37	Calaminene	0.031
	Total	8.027	38	Trans-γ-Bisabolene	0.053
				Unknown	0.680
				Total	4.451
		II–Oxygenated sesquiterpene compounds			
40	Germacrene-4-ol	0.028	39	Nerolidol	0.013
41	Spathulenol+Caryophyllene oxide	1.048	40	Spathulenol	0.597
42	Globulol	0.150	41	Caryophyllene oxide	0.036
43	Viridiflorol	0.140	42	Tau-Cadinol	0.194
44	Tau-Cadinol	0.138	43	α-Cadinol	0.271
45	α-Cadinol	0.043	44	Epi-α-Bisabolol	0.215
46	Epi-α-Bisabolol	0.003		Unknown	1.109
	Unknown	1.061		Total	2.435
	Total	2.610			

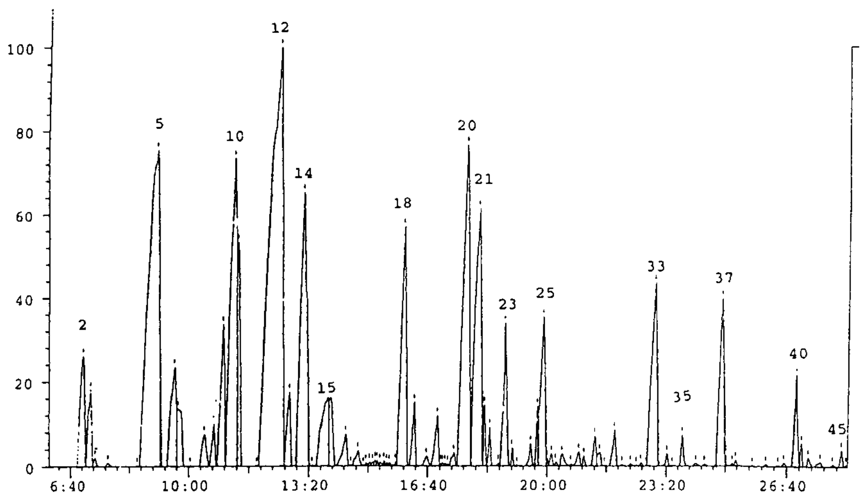

Fig. 1. Chromatogram of marjoram oil

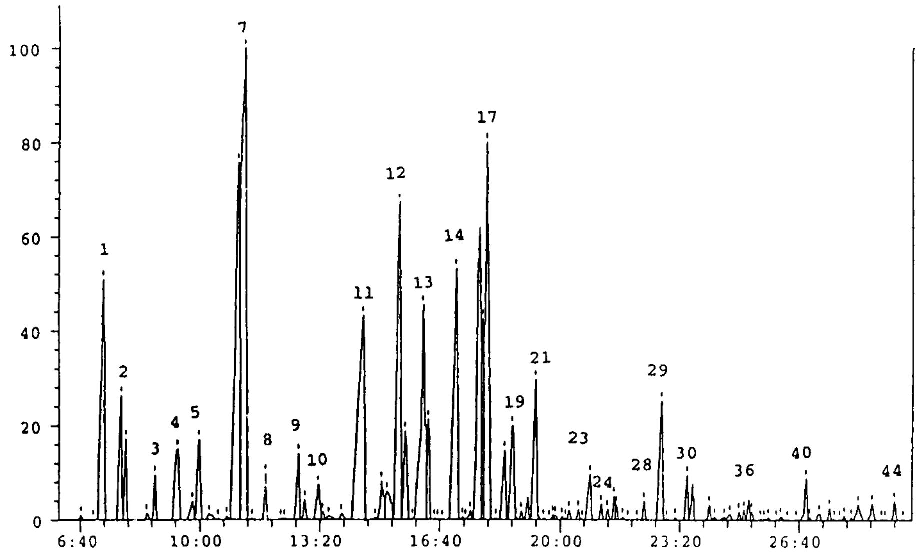

Fig. 2. Chromatogram of rosemary oil

Data in *Tables 1, 2, 3* show that forty-six compounds of sweet marjoram could be identified representing about 93.3% of the total content. It is evident that about 53% of the total oil consisted of terpene hydrocarbons with γ-terpinene accounting about 21.5% of total oil, sabinene + β-pinene and p-cymene represented about 14% and 7.8% of total oil content, respectively. The oxygenated terpenes contributed with 36.48% of the oil content, terpineol-4 and α-terpineol represented about 8.2% and 6.2% of the total oil content, respectively. On the other hand sesquiterpenes and oxygenated sesquiterpenes represented about 8% and 2.6% of the oil content respectively.

Repellency and oviposition deterrence

In *T. urticae,* percent repellency gradually increased with both essential oil concentrations *(Table 4).* On the contrary, rosemary oil proved to be completely deterrent for *E. orientalis* even after 72 h, of treatment and at the lowest concentrations *(Table 5).* Repellency of sweet marjoram oil decreased at 0.125% conc. in comparison with other concentrations *(Table 5).* As for oviposition behavior [Oviposition deterrent indices (ODI) as defined by Lundgren 1975] in case of *E. orientalis,* both oils hindered adults to lay eggs except at 0.125% conc. of sweet marjoram *(Table 5).* The average number of eggs laid by females of *T. urticae* after 72 h of treatment varied according to concentrations *(Table 4).* 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. Mazeed (1987) demonstrated that marjoram oil form

(Origanum majorana) was effective for controlling the spread of *Acaropis woodi* (Rennie) to young workers introduced to infested colonies. Both rosemary and sweet marjoram oils gave 100% repellency to *T. urticae* at 10% level of concentration (Sawires et al., 1988).

The varying effect of the different oils may be due to the varying constituents of oxidative substances within the solid part of the volatile oil.

Table 4

Relative distribution and oviposition of *T. urticae* on treated leaf discs with rosemary and sweet marjoram oils

% concentrations	% distribution on treated part after			% repellency	Avg. No. of eggs/female after 72 h		ODI
	24 h	48 h	72 h		T	C	
Rosemary							
2	15	20	30	91	0.15	1.65	83.33
1	15	25	30	89.5	0.2	1.9	80.95
0.5	25	30	35	82.1	0.35	1.95	69.57
0.25	35	35	50	67.3	0.9	2.75	50.68
0.125	40	50	80	46.6	1.55	2.9	30.34
Sweet marjoram							
2	5	5	5	100	0	0.7	100
1	10	10	15	100	0	0.7	100
0.5	15	20	25	92.5	0.2	2.65	85.96
0.25	15	25	30	85.5	0.4	2.75	74.60
0.125	20	25	40	69.6	0.85	2.8	53.42

C = control; T = treated

Table 5

Relative distribution and oviposition of *E. orientalis* on treated leaf discs with rosemary and sweet marjoram oils

% concentrations	% distribution on treated part after			% repellency	Avg. No. of eggs/female after 72 h		ODI
	24 h	48 h	72 h		T	C	
Rosemary							
2	0	0	0	100	0	0	0
1	0	0	0	100	0	0.25	100
0.5	0	0	0	100	0	0.35	100
0.25	0	0	0	100	0	0.45	100
0.125	0	0	0	100	0	0.45	100
Sweet marjoram							
2	0	0	0	100	0	0.65	100
1	0	0	0	100	0	1.25	100
0.5	5	0	5	100	0	1.45	100
0.25	5	5	5	100	0	2.7	100
0.125	25	25	25	90.8	0.25	2.7	83.05

C = control; T = treated

Concentration effects on oviposition and mortality

Significant reduction in the total number of eggs laid by both tetranychid mites were recorded during 10 days period for all concentration tested for both essential oils *(Table 6).* Fecundity was severely reduced as the concentration of both oils increased. Previous studies have shown that the essential oils of *Callistemon lanceolatus* DC. and

Table 6

Effect of rosemary and sweet marjoram oils on reproduction and mortality of *T. urticae* and *E. orientalis*

Conc.	Rosemary		Sweet marjoram oil	
%	Total No. of eggs/female/10 days**	% corrected mortality after 10 days*	Total No. of eggs/female/10 days**	% corrected mortality after 10 days*
		T. urticae		
2	1.75	53.85	0.25	90.91
1	2.10	15.38	3.25	27.27
0.5	6.50	8.33	4.80	25.00
0.25	6.90	7.69	10.20	23.10
0.125	13.10	7.14	17.15	21.43
Control	58.5	–	58.5	–
		E. orientalis		
2	0	100	0	100
1	0	100	0.20	100
0.5	1.10	71.43	0.50	92.86
0.25	1.50	60.00	1.90	86.67
0.125	2.35	60.00	2.30	80.00
Control	15.4	–	15.4	–

*Counts according to Abbott's Formula (1925).
**Highly significantly at $P < 0.01$
[statistical analysis based on Least Significant Difference (L.S.D. test)]

Cotula cinerea L. had deterrent effects on females of *T. urticae* and spraying females with LC_{50} significantly reduced its fecundity (Ibrahim and Amer, 1992; Ibrahim et al., 1993). However, percentage mortality was significantly concentration dependent. In case of *E. orientalis* at 2% and 1% conc. it was high with both oils and reached 100% with *T. urticae* the trend was similar albeit at a slightly lower rate increasing from 7.14 and 21.43% to 53.85 and 90.91% with rosemary and sweet marjoram, respectively. The results clearly depended on the mite used for experiments, as marked differences in mortality were recorded. Amer et al. (1993) reported that the orange peel oil showed a remarkable deterrent effect with *E. orientalis,* whereas the essential oil of lemon grass in addition to the orange peel oil was toxic to females and eggs of *T. urticae* and *E. orientalis.* Regnault and Hamraoui (1995) suggested that lipid as well as non-lipid allelochemicals, such as phenolics, or non-protein amino acids or flavonoids, may be involved in the toxicity of extracts of aromatic plant *R. officinalis* and *Eucalyptus globulus* to *Acanthoscelides obtectus.* El Gengaihi et al. (1992) confirmed the role of volatile oil as having antifeedant and growth inhibiting effects of *Vitex agnus costus* on *Spodoptera littoralis* larva. Rosemary and sweet marjoram oils were promising for possible use against *T. urticae* and *E. orientalis.* It is worth mentioning that oils with such properties will not disturb ecosystems and consequently, will not cause outbreaks of new pests, as persistent insecticides are apt to do.

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