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Acaricidal Activity of Essential Oils of Chamomile, Marjoram and Eucalyptus against the Two-Spotted Spider Mite, *Tetranychus urticae* Koch: Biology and Enzymes

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ABSTRACT

Different concentrations of three essential oil extracts (0.5, 1, 2, 3 and 4%) were used against *Tetranychus urticae.* (Koch) Chamomile; *Chamomilla recutita* (L.), proved to be the most efficient agent against *T. urticae* followed by Marjoram; *Marjorana hortensis* (L.), and Eucalyptus (*Eucalyptus* sp.) The LC_{50} values of these oils were 0.651 &1.17; 1, 84 & 6.26 and 2.18 & 7.33 for adults and eggs, respectively. Herein, the bioassay experiments of the two essential oils, Chamomile and Marjoram showed relationship between essential oil contents and activity of enzyme glutathione S-transferase, non specific esterase and alkaline phosphatase in *T.urticae*. GC-MS analysis of *C. recutita* and *M. hortensis* proved the presence of 13 and 14 essential oil components, respectively. The major essential oil contents of *C. recutita* are α-Bisabolol oxide A (35.25%), and Trans-β-farersene (7.98%), while the main components of *M. hortensis* are Terpinen-4-ol (23.86%), P-cymene (23.40%) and Sabinene (10.90%). The major components of both plant extracts (Terpinen-4-ol 23.86% and α-Bisabolol oxide A 35.25% essential oils) may be responsible for the activity of enzymes of *T. urticae* (glutathione S-transferase, non specific esterase and alkaline phosphatase).

Key Words: Tetranychidae, Plant essential oils, Enzymes, Glutathione S-transferase, Non specific esterase and Alkaline phosphatase.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch is one of the most important pests responsible for yield losses to many horticultural ornamental and agronomic crops. A major problem in the control of *T. urticae* is the response to develop resistance to many acaricides (Stumpf and Nauen, 2002).

For several years, chemical control of mites has been extensively practiced in Egypt to check mite population increase. Resistance problems and high residual levels in food products may hinder its marketing. Such undesirable consequences have caused alienating effects on the irrational use of chemical agents (Ali, 2004).

Therefore, the use of essential oils of plant extracts in pest management programs has recently attracted the attention of many scientists. Pesticides of plant origin seem to be recommended as they generally have a very short persistence in the plant (Isman, 1997 and Carbaras *et al*., 2002).

However, the selectivity of these products has to be strictly evaluated for different species of natural enemies as deleterious or some times positive effects were recorded among the natural enemies complex (Stark *et al*., 1990, 1992, Tsolakis and Ragusa, 1999).

Glutathione S-transferases (GSTs) catalyse the conjugation of a large variety of compounds bearing an electrophilic site, with reduced glutathione (Mannervik and Danielson, 1988). In insects they represent a very interesting detoxification mechanism due to their involvement in tolerance to insecticides (Motoyama and Dauterman, 1980 and Kostaropoulos *et al*., 2001).

It is reported that most xenobiotics are subjected to enzymatic modification after penetration of the insect body. It had been clearly demonstrated that several enzymatic system in insect such as esterase and phosphatase can play a vital role in the detoxification of xenobiotics to nontoxic materials (Busvine, 1971, and Croft, 1977).

Herein, this study aimed to evaluate the ability of plant extracts essential oils against *T. urticae.* The biochemical changes due to treatment of *T. urticae* by LC_{50} of the tested oils were evaluated. In this respect, some detoxifying enzymes such as glutathione S-transferase, nonspectific esterase and alkaline phosphatase were manipulated in *T.urticae.*

MATERIALS AND METHODS

Test mite

T. urticae was collected from infested cucumber plants (*Cucumis sativus* L.). Bean (*Phaseolus vulgaris* L.) seeds were planted in plastic pots (14 cm diameter) at a rate of 4-5 seeds per pot, and seedlings were infested with *T. urticae* adults from this culture, adult mite were transferred to aluminum.

Pans (30 X 20 X 70cm) on fresh leaves of beef steak (*Acalyph wilkesiana* L.) placed upside down on wet cotton pads. Water was added when needed

maintaining suitable moisture and kept in incubator at 25±2°C and 70±5%R.H.

Source of sample

Three essential oils extracted from plants belong to three different families of which Marjoram (*Marjorana hortensis*) Family Lamiaceae and Chamomile (*Chamomilla recutita*) family Asteraceae were collected from Sekam farm in Belbase (Sharqia Governorate). Leaves of Eucalyptus (*Eucalyptus sp*.) Family Myrtaceae, were collected from the Faculty of Agriculture farm of Cairo University.

Preparation of essential oils

The whole plants (herbs) of Marjoram and Chamomile and leaves of Eucalyptus were dried for a week at room temperature, and then crushed according to Aslan *et al.* 2004. Essential oils were extracted from by hydro distillation (deionized water for 4 hr) under vacuum according to (Hoelzl and demuth, 1975). All oils and components were kept under freezing until used. Series of aqueous concentrations of each essential oil were prepared with Triton X-100 as surfactant at a rate of 0.1%.

Treatment of eggs

Leaf discs, 3 cm diameter, of Beef steak leaves were used as substrate to oviposition. Four leaf discs were used for each treatment and five mite females were transferred for each disc and left 24 h to lay eggs, then females were removed. Thereafter, fourty eggs, on four discs, were treated with one of the five concentrations (0.5, 1, 2, 3 and 4%). Eggs were sprayed by a glass atomizer, with a serial of concentrations for each essential oil; 1ml/200cm of the solution was used. (Tsolakis & Ragusa, 2008). Eggs were incubated at 25±2°C for seven days till hatching. The numbers of hatching and non hatching eggs were recorded.

Treatment of adult females

T. urticae females, 3 days old, were obtained by placing 100 dueto- nymphs from the culture, and on excised Beef steak leaves placed on wet cotton pads in Petri dishes. The emerged females and males were transferred to new Beef steak leaves for 2-3 days and allowed to mate. Afterwards, fourty females were transferred equally to four discs, 3cm diameter, and then treated with one of the previous treatments. Control treatment was operated by Triton X-100 at a rate of 0.1%. Mortality was estimated for the adult females after 24 h of spraying and estimated by Abbot's formula (1925) and LC_{50}, LC_{90} and slope values were estimated according to Finney (1971).

Biochemical assay

Preparation of samples for enzymes determination

Adult females (10 mg) were homogenized in 1ml distilled water in ice for three minutes using Teflon Homogenizer. The homogenates were centrifuged at 3500 r.p.m for 10 minutes at 4ºC and the supernatants were used directly to determine the activity of alpha and beta esterase, glutathione S-transferaes and alkaline phosphatase according to Sakunwarin *et al*., (2004).

Determination of Non specific esterase

α-esterase and β-esterase were determined according to the method of Van Asperen (1962).

Determination of Glutathione-S-transferase

Glutathione-S-transferase (GST) was measured according to the method described by Habig *et al*. (1974) who used 1-chloro -2, 4 Dinitro benzene (CDNB).

Determination of Alkaline phosphatase

Alkaline phosphatase was determined according to the method described by Powell and Smith (1954).

Statistical Analysis

Experimental data were statistically analyzed by using Costa software (cohort software, Berkeley). Significance of results was obtained by randomized one way ANOVA, and the means were separated using the Duncan's multiple range test (1955) at $p<0.01$.

RESULTS AND DISCUSSION

Data presented in table (1) demonstrate that Chamomile essential oil extract was the most effective against *T. urticae*, which enhanced the highest adult female mortality and lowest egg hatchability. Adult mortality percentages after 24 h were 42.5, 75, 90, 95 and 100% for Chamomile by spraying the different concentrations 0.5, 1, 2, 3 and 4%, respectively. The percentage corresponding mortalities for Marjoram were 20, 30, 42.5, 72.5, and 85%, while 17.5, 27.5, 40, 70, and 80% for Eucalyptus, respectively. Hatchability percentages after six days were 75, 55, 30, 16 and 10% for Chamomile; 95, 87.5, 80, 72.5, 57.5% for Marjoram and 95, 92.5, 82.5, 77.5 and 67.5% for Eucalyptus respectively, for control treatment (Triton X-100 0.1%), adult mortality recorded 10% and egg hatchability 95%.

The present results are in agreement with that of Baker (2003) who studied the evaluation of the

acaricidal activity of some essential oils against *T. urticae* as the percentage mortality of adults at both 2 and 4% concentrations recorded 38 and 78%, respectively for Eucalyptus and recorded 70% at the 3% concentration for Marjoram . It is also in agreement with that of El-Halawany and Sawires (1988) who tested six essential volatile oils against *T. urticae*, whereas repellency percentage ranged between 70% and 85% for Marjoram treatment. Mansour *et al*., (1986) reported that 0.5, 1and 2% concentrations of *Ocimum basilicum* oil reduced *T. cinnabarinus* female's fecundity by 15, 38 and 92%, respectively. On the other hand, the same concentration of Chamomile oil reduced female's fecundity by 42.5, 75 and 90%, respectively.

Table 2 proved that Chamomile essential oil extract was the most potent followed by Marjoram and Eucalyptus. The LC_{50} values after 24 h for adults were 0.65, 1.84 and 2.18 %, respectively, while for eggs 1.17, 6.26 and 7.33 % were recorded after seven days. The slope values of the regression line were 2.41, 2.53 and 2.49 for adults and 2.28, 1.89 and 2.15 for eggs, respectively. LC_{90} values were 2.27, 5.91 and 7.13% for adults and 4.34, 9.81and 28.95 % for eggs, respectively.

The present results were in agreement with the data cited by Kawka (2004) who studied the effect of Chamomile (*Matricaria recutita* [*recutita*]) extracts from fresh and dry flowers on *T. urticae*; mortality of immatures was the highest on leaves treated with extract from dried flowers.

Table 3 showed that the activity of GST significantly increased after treatment with LC_{50} and the activity was 881.3, 1003, 771.7 and 771.3 n mole/min/mg for Marjoram, Chamomile, positive control (Triton.X-100) and negative control, respectively. Regarding α, β esterase, it significantly increased in 19.9 and 7.85 mg α, β-naphthol released/min/g.b.wt treated with LC_{50} of Marjoram compared with other treatments. Alkaline phosphatase significantly decreased in 4.03 and 4.66 U/g.b.wt treated with LC_{50} of Marjoram and Chamomile compared with other treatments.

Similar data were obtained by Cao *et al.* (2003) who studied the efficiency of four essential oil extracts from *Stellera chamaejasme* L. [*Wikstroemia chamaejasme* (L.)] against. *Tetranychus viennensis* Zacher [*Amphitetranychus viennensis* (Zacher)]. The effect of the essential oil extracts on the three most important enzyme systems of *T. viennensis* was also studied. The extracts of *S. chamaejasme* exhibited strong pesticidal activities against *T. viennensis*.

Table (1): Effect of three essential oil plant extracts against *T.urticae* egg hatchability and adult mortality.

Conc. %	*Chamomilla recutita*		*Marjorana hortensis*		*Eucalyptus* sp.	
	Adult mort.	Egg hatch.	Adult mort.	Egg hatch.	Adult mort.	Egg hatch.
0.5	42.5	75.0	20.0	95.0	17.5	95.0
1	75.0	55.0	30.0	87.5	27.5	92.5
2	90.0	30.0	42.5	80.0	40.0	82.5
3	95.0	16.0	72.5	72.5	70.0	77.5
4	100.0	10.0	85.0	57.5	80.0	67.5
Control	10.0	95.0	10.0	95.0	10.0	95.0

Conc. = Concentration mort. = mortality hatch. = hatchability

Table (2): Toxicity of three essential oil plant extracts against *T. urticae* adult females and eggs.

Toxicity parameter	*Chamomilla recutita*		*Marjorana hortensis*		*Eucalyptus* sp.	
	Adults	Eggs	Adults	Eggs	Adults	Eggs
LC_{50}	0.65	1.17	1.84	6.26	2.18	7.33
Lower limit	0.46	0.94	1.53	4.18	1.82	4.74
Upper limit	0.82	1.45	2.21	25.40	2.67	39.05
Index	100.00	100.00	35.44	19.11	29.82	16.31
Slope	2.41	2.28	2.53	1.89	2.49	2.15
LC_{90}	2.27	4.34	5.91	9.81	7.13	28.95

Table (3): Effect of 24 h treatment by LC_{50} of essential oils on enzyme activities of *T. urtice* adult.

Enzymes	Treatment			
	Negative Control	Positive Control (Triton X-100)	*Marjorana hortensis*	*Chamomilla recutita*
Glutathione s-transferase (n mole/min/mg)	771 ± 38.5^{c}	771.7 ± 10.1^{c}	881.3 ± 8.5^{b}	1003 ± 15.3^{a}
α- Esterase (mg– α- naphthol released/min/g.b.wt)	2.79 ± 0.21^{c}	8.62 ± 0.13^{b}	19.9 ± 1.34^{a}	8.99 ± 0.115^{b}
β-Esterase (mg β-naphthol released/min/g.b.wt)	0.904 ± 0.007^{c}	2.72 ± 0.05^{b}	7.85 ± 0.138^{a}	2.65 ± 0.05^{b}
Alkaline phosphatase (U/g.b.wt)	7.15 ± 0.17^{a}	$5.7\pm o.11^{b}$	4.03 ± 0.15^{d}	4.66 ± 0.06^{c}

Mean bearing different subscript are significantly different at $P<0.01$
Data are presented as mean ±SD.

The activities of protease, Glutathione S-transferase and esterase isoenzymes were induced with chloroform + extract direct treatment, while ingestion of leaves with chloroform inhibited esterase isoenzyme activity only. Also, Wang *et al.,* (2007) tested several important enzyme systems in *T. cinnabarinus* treated with petroleum ether extract of *Jungians raga* leaves. The extracts had strong acaricidal activities against *T. cinnabarinus* as it induced strong activities of glutathione-S-transferase in the mite. Because the two essential oil extracts of Chamomile and Marjoram had most potent activities against *T.urticae,* the detailed chemical composition of the two essential oils were analyzed by GC/MS as shown in tables (4) and (5). GC-MS analysis of *C.recutita* (table, 4): proved the presence of thirteen components. The major essential oil contents of *C. recutita*e α-bisabolol oxide A (35.25%), and trans β-farersene (7.98%). The present result is in agreement with that of Pino *et al.* (2002) who studied the chemical composition of *C. recutita* essential oil by GC/MS which contained α-bisabolol oxide A (43.8%) as the major one. GC-MS analysis of *M. hortensis* (Table, 5) proved the presence of fourteen components, respectively. The major essential oil contents are Terpinen-4-ol (23.86%), ρ-cymene (23.40%), Sabinene (10.90%), γ-Terpinene (9.034%), α-Terpinene (6.421). The present results are in agreement with those documented by .Ma *et al.,* (2004) who found that the highest effect of terpinene -4-ol on esterase activity was noted during recover stage of housefly adult (*Musca domesticae*). Ma *et al*., (2008) reported that activities of both acid phosphatase (ACP) and alkaline phosphates (AKP) in insects were induced by terpinen-4-ol , but ACP was inhibited at paralysis stage .The activity of Glutathione S-transferase (GSTs) were inhibited in exciting, convulsing and paralysis stages of 5*th* star larvae of *M.separata,* but it gradually recovered in the recovery stage. This affected the metabolism and activity of phophatase and esterase enzymes. On the other hand the inhibited insect GST will inhibit normal metabolism. The major essential oil of marjoram was Terpinen-4-ol (23.86%), (Fig.1&Table 6) and in chamomile was α-Bisabolol oxide A (35.25%), (Fig.2&Table 6) may be responsible for controlling T. *urticae.* These points need further investigations in the future to prove our suggestions by using individual component and its effect on the enzymes of T. *urticae*. Even this suggestion was approved by Ma *et al.*, (2004) and Ma *et al.*, (2008) who cited that Terpinen-4-ol hydrolyzed metabolism by activitying phophatase .in addition that Terpinen-4-ol inhibited protection and immunity system and speeded up death of insects.

Table (4): Composition of chamomile (*Chamomilla recutita* (L.) essential oil

compound	R_t (min)	%	M. formula	M.W.
β-Ocimene	13.597	1.435	$C_{10}H_{16}$	136.23
γ-terpinene	13.963	0.678	$C_{10}H_{16}$	136.23
Artemisia ketone	14.255	1.305	$C_{10}H_{16}O$	152.23
Bicycloe lemene	26.603	0.739	$C_{15}H_{24}$	204.00
Trans β-farnesene	34.379	7.758	$C_{15}H_{24}$	204.19
Germacrene-D	34.819	0.122	$C_{15}H_{24}$	204.19
α-farnesene	36.319	1.399	$C_{15}H_{24}$	204.19
α-calacorene	36.702	1.534	$C_{15}H_{24}$	204.35
6 α-Cadina-4,9-diene	43.843	0.893	$C_{15}H_{24}$	204.35
α-bisabolol oxide A	52.128	35.251	$C_{15}H_{26}O$	238.54
Hexahydrofarnesyl acetone	53.690	1.249	$C_{18}H_{36}O$	268.00
Tricosane	67.160	0.839	$C_{23}H_{48}$	324.63
Heptacosane	70.856	1.636	$C_{27}H_{56}$	380.00

R_t = Retention time M. formula = Molecular formula
M.W. = Molecular weight

Table (5): Composition of Marjoram (*Marjorana hortensis* (L.)) essential oil

Compound	R_t (min.)	%	M.formule	M.W.
α-pinene	8.476	1.757	$C_{10}H_{16}$	136.23
Sabinene	10.473	10.904	$C_{10}H_{16}$	136.24
β-Myrcene	11.125	1.386	$C_{10}H_{16}$	136.24
p-cymene	13.093	23.404	$C_{10}H_{14}$	134.22
γ-terpinene	14.512	9.034	$C_{10}H_{16}$	136.23
Cis β-Terpineol	15.039	1.152	$C_{10}H_{18}O$	154.24
α-terpinolene	15.645	2.678	$C_{10}H_{16}$	136.23
Cis-Sabinehydrate	16.927	1.685	$C_{16}H_{18}O$	154.24
Trans-4-Thujanol	16.990	0.164	$C_{16}H_{18}O$	154.25
Terpinene-4-ol	21.287	23.86	$C_{16}H_{18}O$	154.25
α-Terpineol	21..768	6.421	$C_{12}H_{20}O$	196.29
Linalyl acetate	23.707	3.693	$C_{16}H_{18}O$	154.28
β-Caryophllene	30.974	4.820	$C_{15}H_{24}$	204.35
Spathulenol	39.197	2.876	$C_{15}H_{24}O$	220.00

R_t = Retention time M. formula = Molecular formula
M.W. = Molecular weight

Table (6): Major compounds of Marjoram and Chamomile essential oil

Compound	M.W.	M/Z
Terpinene -4-ol	154	154-148-140-136-132-125-121-117-111-105-101-97-93-86-81-77-71-67-63-59-55-51
Bisabolol oxide A	238	143-132-125-119-107-99-93-85-79-71-65-59-55-53

M.W = Molecular weight M/Z = Mass- To- charge ratio

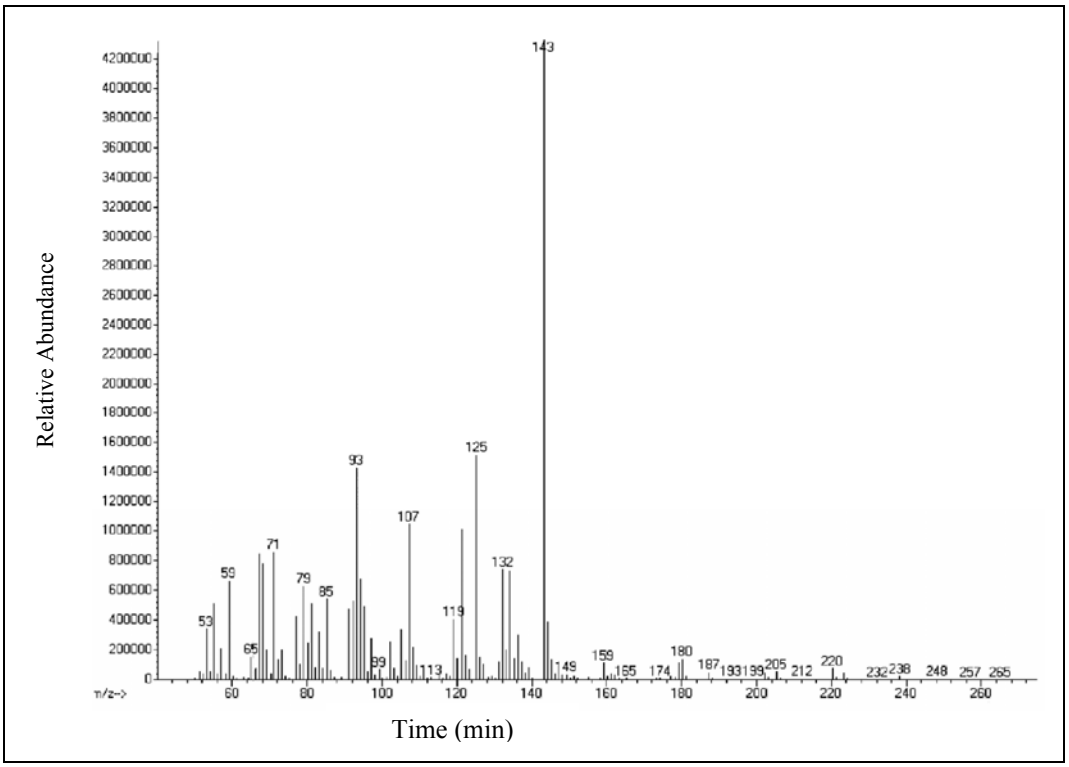

Fig. (1): Chromatogram of Bisabolol oxid A in Chamomile

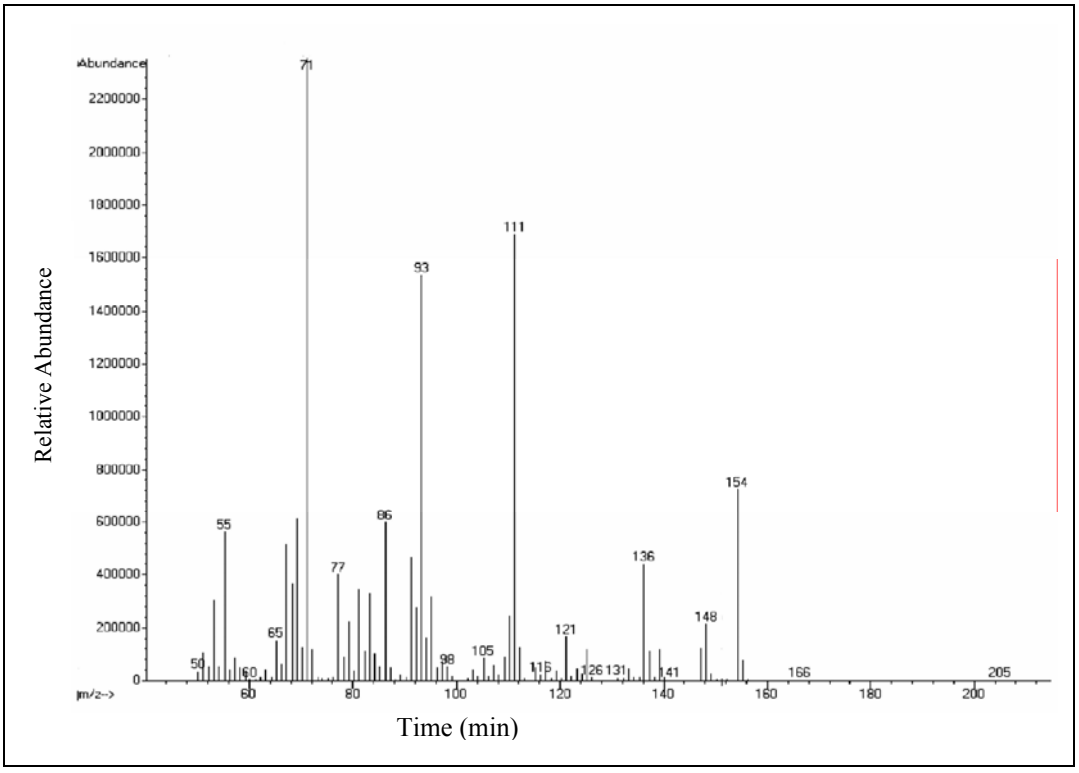

Fig. (2): Chromatogram of Terpinene–4-Ol in Marjoram

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