Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(11)60184-6

Control of *Tetranychus urticae* Koch by extracts of three essential oils of chamomile, marjoram and *Eucalyptus*

Abd El-Moneim MR Afify[1*], Fatma S Ali[2], Turky AF[1]

[1]*Department of Biochemistry, Faculty of Agriculture, Cairo University, P. Box 12613, Gamma St, Giza, Cairo, Egypt*
[2]*Departmetn of Zoology and Nematology, Faculty of Agriculture, Cairo University, P. Box 12613, Gamma St, Giza, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 10 June 2011
Accepted 21 June 2011
Available online 28 January 2012

Keywords:
Tetranychidae
Plant essential oils
Enzymes
Glutathione-S-transferase
Non specific esterase
Alkaline phosphatase
Protease
Chamomile
Marjoram
Eucalyptus

ABSTRACT

Objective: To evaluate the acaricidal activity of extracts of three essential oils of chamomile, marjoram and *Eucalyptus* against *Tetranychus urticae* (*T. urticae*) Koch. **Methods:** Extracts of three essential oils of chamomile, marjoram and *Eucalyptus* with different concentrations (0.5%, 1.0%, 2.0%, 3.0% and 4.0%) were used to control *T. urticae* Koch. **Results:** The results showed that chamomile (*Chamomilla recutita*) represented the most potent efficient acaricidal agent against *Tetranychus* followed by marjoram (*Marjorana hortensis*) and *Eucalyptus*. The LC_{50} values of chamomile, marjoram and *Eucalyptus* for adults were 0.65, 1.84 and 2.18, respectively and for eggs 1.17, 6.26 and 7.33, respectively. Activities of enzymes including glutathione-S-transferase, esterase (α-esterase and β-esterase) and alkaline phosphatase in susceptible mites were determined and activities of enzymes involved in the resistance of acaricides were proved. Protease enzyme was significantly decreased at LC_{50} of both chamomile and marjoram compared with positive control. Gas chromatography-mass spectrometer (GC-MS) proved that the major compositions of *Chamomilla recutita* are α-bisabolol oxide A (35.251%), and trans-β-farersene (7.758%), while the main components of *Marjorana hortensis* are terpinene-4-ol (23.860%), *p*-cymene (23.404%) and sabinene (10.904%). **Conclusions:** It can be concluded that extracts of three essential oils of chamomile, marjoram and *Eucalyptus* possess acaricidal activity against *T. urticae*.

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* (*T. urticae*) Koch is one of the most important pests responsible for yielding 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 due to an approximate 5-fold increase in the mixed function of oxidase activity[1]. For several years, chemical control of mites has been extensively practiced[2-4]. In Egypt, different extracts from *Syzygium cumini* (*S. cumini*) L. Skeels (Pomposia) against *T. urticae* Koch were used to control mite population and ethanol extract showed the most potent acaricidal activity[5]. Resistance problems and high residual levels of botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or human health[6]. Therefore, methods used to detect and determine these multiresidue in food products by LC-MS-MS tandem spectroscopy[7] may hinder its marketing. *T. urticae* females were repelled by spinosad and largely oviposited and fed on nonspinosad treated areas. Spinosad did not affect the behavior of *Panonychus ulmi* (*P. ulmi*) females. When *T. urticae* females were released on potted bean plants (two-leaf stage) in which leaves received spinosad sprays on the adaxial or abaxial leaf surfaces, or complete spinosad coverage on one or two of the leaves, mite population increase lagged significantly behind those released on control plants. These results indicate that *S. cumini* L. Skeels (Pomposia) and spinosad have significant acaricidal effects against *T. urticae* but not

*Corresponding author: Abd El-Moneim MR Afify, Department of Biochemistry, Faculty of Agriculture, Cairo University, P. Box 12613, Gamma St, Giza, Cario, Egypt.
E-mail: moneimafify@yahoo.com
Foundation Project: Supported by Cairo University, Faculty of Agriculture, Deptarment of Biochemistry, Cairo, Egypt.

P. ulmi. 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[6,8]. However, the selectivity of these products has to be strictly evaluated for different species of natural enemies as deleterious or sometimes positive effects were recorded among the natural enemies complex[9,10].

In insects, glutathione-S-transferase represents a very interesting enzyme carrying out detoxification mechanism due to their involvement in tolerance to acaricides[5,11]. It is reported that most xenobiotics are subjected to enzymatic modification after penetration through protein binding and transportation in biological system like insects and acaricide. It had been clearly demonstrated that several enzymatic system such as esterase (α and β), and phosphatase (alkaline and acid) can play a vital role in the detoxification of xenobiotics to nontoxic materials[12].

Herein, this study was aimed to evaluate the acaricidal activity of extracts of essential oils of chamomile, marjoram and *Eucalyptus* 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 (α and β), phosphatase (alkaline and acid) and protease enzymes were manipulated. The chemical compositions of the essential oils were studied with gas chromatography-mass spectrometer (GC-MS).

2. Materials and methods

2.1. 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 in diameter) at a rate of 4-5 seeds per pot, and seedlings were infested with *T. urticae* adults. From this culture, adult mites were transferred to aluminum pans (30 cm × 20 cm × 70 cm) on fresh leaves of beef steak (*Acalypha wilkesiana* L.) placed upside down on wet cotton pads. Water was added when needed. Mites were maintained at suitable moisture and kept in incubator at (25±2) ℃ and (70±5)% relative humidity.

2.2. Source of sample

Marjorana hortensis (*M. hortensis*) (Family: Lamiaceae) and *Chamomilla recutita* (*C. 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.

2.3. 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 the method of Calmasur *et al*[13]. Essential oils were extracted by hydro distillation (deionized water for 4 h) under vacuum according to the method of Aroiee *et al*[14]. Essential 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%.

2.4. Treatment of eggs

Leaf discs (3 cm in diameter) of beef steak leaves were used as substrate to ovipositor. Four leaf discs were used for each treatment and five mite females were transferred to each disc and left 24 h to lay eggs, then females were removed. Thereafter, forty eggs, on four discs, were treated with one of the five concentrations (0.5%, 1.0%, 2.0%, 3.0% and 4.0%). Eggs were sprayed by a glass atomizer, with a serial of concentrations for each essential oil. 1 mL/200 cm of the solution was used. Eggs were incubated at (25±2) ℃ for seven days till hatching. The numbers of hatching and non hatching eggs were recorded.

2.5. Treatment of adult females

T. urticae females, 3 days old, were obtained by placing 100 nymphs onto the culture, and wet cotton pads in Petri dishes were placed on excised beef steak leaves. The emerged females and males were transferred to new beef steak leaves for 2-3 days and allowed to mate. Afterwards, forty females were transferred equally to four discs (3 cm in 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[15].

2.6. Biochemical assay

Blood of adult females (10 mg) was homogenized in 1 mL distilled water in ice for 3 min using Teflon Homogenizer. The homogenates were centrifuged at 3 500 rpm for 10 min at 4 ℃ and the supernatants were used directly to determine the activity of alpha and beta esterase, glutathione-S-transferaes and alkaline phosphatase and protease.

Nonspecific esterase alpha esterases (α-esterases) and beta esterases (β-esterase) were determined according to the method of Asperen[16] using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively.

Glutathione-S-transferase was measured according to the method described by Villanueva *et al*[17] using 1-chloro-2, 4 dinitrobenzene (CDNB).

Acid phosphatase and alkaline phosphatase were

determined according to the method described by Manns *et al*[18].

Protease activity was determined according to El-Sharabasy[19] by casein digestion methods.

2.7. 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[20] at $P<0.01$.

3. Results

Essential oils extracts of plants are promising alternative natural products for the control of *T. urticae* Koch. These extracts facilitate the handling and its application, besides they can be an option of lower cost in relation to the studies of chemical control.

Data presented in Table 1 demonstrated that chamomile essential oil extract was the most potent effective acaricidal agent against *T. urticae*, which enhanced the highest adult female mortality and lowest egg hatchability. Adult mortality percentages after 24 h were 42.50%, 75.00%, 90.00%, 95.00% and 100.00% for chamomile by spraying the different concentrations of 0.5%, 1.0%, 2.0%, 3.0% and 4.0%, respectively. The percentages of corresponding mortalities for marjoram were 20.00%, 30.00%, 42.50%, 72.50%, and 85.00%, while 17.50%, 27.50%, 40.00%, 70.00%, and 80.00% for *Eucalyptus*, respectively. Hatchability percentages after six days were 75.00%, 55.00%, 30.00%, 16.00% and 10.00% for chamomile; 95.00%, 87.50%, 80.00%, 72.50%, 57.50% for marjoram and 95.00%, 92.50%, 82.50%, 77.50% and 67.50% for *Eucalyptus*, respectively, for control treatment (Triton X-100 at a rate of 0.1%), adult mortality was 10.00% and egg hatchability was 95.00%.

Table 2 proved that chamomile essential oil extract represented the most potent acaricidal activities 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%, 9.81% and 28.95% for eggs, respectively.

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

Concentration (%)	*C. recutita*		*M. hortensis*		*Eucalyptus* sp.	
	Adult mortality	Egg hatchability	Adult mortality	Egg hatchability	Adult mortality	Egg hatchability
Control	10.00±1.29	95.00±0.58	10.00±1.14	95.00±0.58	10.00±1.29	95.00±0.58
0.5	42.50±1.71	75.00±1.29	20.00±1.29	95.00±0.58	17.50±0.96	95.00±0.58
1.0	75.00±1.70	55.00±2.38	30.00±0.82	87.50±0.50	27.50±1.71	92.50±0.96
2.0	90.00±0.82	30.00±0.58	42.50±2.06	80.00±1.15	40.00±1.41	82.50±1.26
3.0	95.00±0.58	16.00±0.10	72.50±1.50	72.50±1.50	70.00±2.24	77.50±1.71
4.0	100.00±0.00	10.00±1.41	85.00±0.82	57.50±2.36	80.00±0.82	67.50±0.58

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

Toxicity parameters	*C. recutita*		*M. 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 non specific esterases, phosphatase (alkaline, acid), glutathione-S-transferase and protease activities of adult *T. urticae* (mean ±SD).

Treatment	α -Esterase (A)	Fold	β -Esterase (B)	Fold	Alkaline phosphatase (C)	Fold	Acid phosphatase (C)	Fold	Glutathione-S-transferase (D)	Fold	Protease (E)	Fold
Negative control	2.79±0.21^{c}		0.90±0.01^{c}		7.15±0.17^{a}		2.26±0.06^{b}		771.00±38.50^{c}		129.00±4.00^{a}	
Positive control (Triton X-100)	8.62±0.13^{b}		2.72±0.05^{b}		5.70±0.11^{b}		2.22±0.11^{c}		771.70±10.10^{c}		112.20±1.52^{b}	
M. hortensis	19.90±1.34^{a}	2.3	7.85±0.14^{a}	2.8	4.03±0.15^{d}	0.7	2.21±0.04^{b}	0.9	881.30±8.50^{b}	1.1	80.33±2.52^{d}	0.7
C. recutita	8.99±0.12^{b}		2.65±0.05^{b}		4.66±0.06^{c}	0.8	2.50±0.10^{a}	1.1	1 003.00±15.30^{a}	1.2	65.33±1.15^{c}	0.5

Mean bearing different superscript are significantly different at $P<0.01$; A: mg- α -naphthol released/min/gb wt; B: mg- β -naphthol released/min/gb wt; C: U/gb wt; D: nmole/min/mg; E: OD unit$\times 10^{3}$/min/gb wt.

3.1. Enzyme activities

Table 3 showed that the activity of glutathione-S-transferase significantly increased after treatment with LC_{50} of the essential oils and arranged as (1 003.00±15.30), (881.30 ±8.50), and (771.70±10.10) nmole/min/mg for chamomile, marjoram and positive control (Triton X-100), respectively. Regarding α, β esterase, they were significantly increased and reached (19.90±1.34) mg-α-naphthol released/min/gb wt and (7.85±0.14) mg-β-naphthol released/min/gb wt with LC_{50} of marjoram compared with other treatments. While alkaline phosphatase was significantly decreased to (4.03±0.15) and (4.66±0.06) U/gb wt treated with LC_{50} of marjoram and chamomile compared with positive control (5.70±0.11) U/gb wt.

Protease enzymes were significantly decreased at LC_{50} of both chamomile and marjoram compared with positive control, 65.33, 80.33, 112.20, respectively which help mite for recovery and survival and defense against degradation of protein (Table 3).

3.2. GC-MS analysis of essential oil of chamomile

The two essential oil extracts of chamomile and marjoram had the most potent acaricidal activities against *T. urticae*. The detailed chemical compositions of the two essential oils were analyzed by GC/MS as shown in Table 4. GC-MS

Table 4
Composition of chamomile (*C. recutita* L) and marjoram (*M. hortensis* L) essential oil.

	Compound	Retention time (min)	Percentage (%)	Molecular formula	Molecular weight
Chamomile	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
	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
Marjoram	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	23.860	$C_{16}H_{18}O$	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-Caryphollene	30.974	4.820	$C_{15}H_{24}$	204.35
	Spathulenol	39.197	2.876	$C_{15}H_{24}O$	220.00

Chamomile: Non identified peaks=45.872%; Identified peaks=54.238%.
Marjoram: Non identified peaks=10.218%; Identified peaks=89.892%.

Table 5
Major compounds of chamomile and marjoram essential oil and degradation products.

	Compound	Molecular weight	Mass to charge ratio
Chamomile	Bisabolol oxide A	238.54	143-132-125-119-107-99-93-85-79-71-65-59-55-53
	Trans-β-farnesene	204.19	228-216-202-185-173-159-143-129-107-93-71-55
Marjoram	Terpinene-4-ol	154.25	148-140-136-132-125-121-117-111-105-101-97-93-86-81-77-71-67-63-59-55-51
	p-cymene	134.22	134-119-115-107-103-91-87-81-77-65-51
	Sabinene	136.24	136-121-105-93-77-69-63-53
	γ-Terpinene	136.23	136-121-115-105-102-98-80-77-68-65-62-55-51
	α-Terpineol	196.29	136-121-107-97-93-85-81-77-71-67-59-55-51
	β-Caryphllene	204.35	204-189-175-161-147-133-125-125-120-115-110-105-98-93-84-79-69-55-50

analysis of *C. recutita* (Table 4, Table 5 and Figure 1) proved the presence of thirteen components. The major essential oil contents of *C. recutita*e are α-bisabolol oxide A (35.251%), and trans β-farersene (7.758%).

3.3. GC-MS analysis of essential oil of marjoram

The major essential oil of marjoram was terpinen-4-ol (23.860%) (Figure 2 and Table 5) and in chamomile the major component was α-bisabolol oxide A (35.251%) (Figure 1 and Table 5) which may be responsible for controlling *T. urticae*.

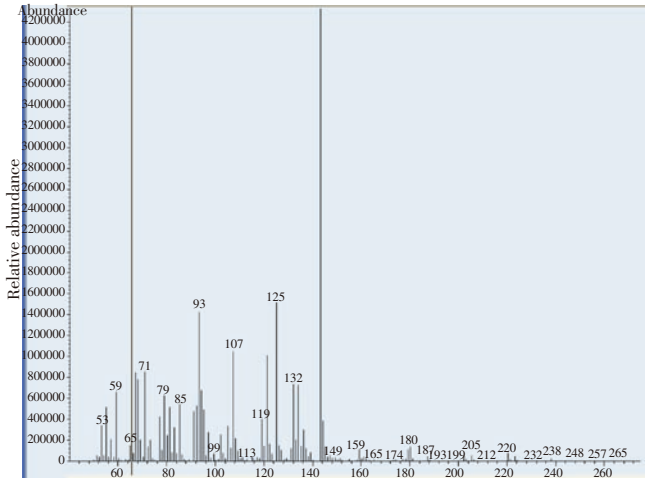

Figure 1. Chromatogram of bisabolol oxid A in chamomile.

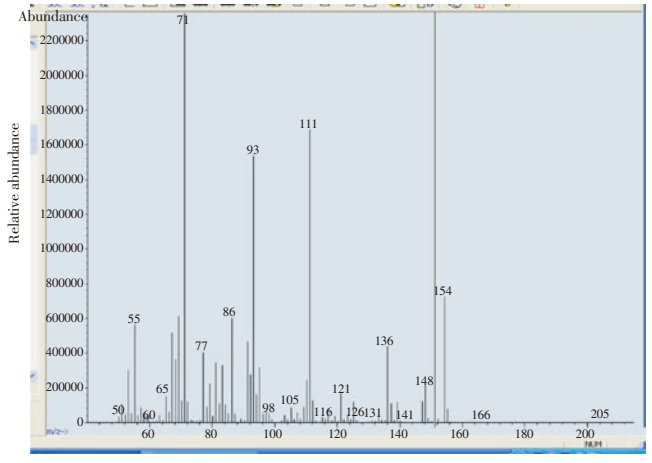

Figure 2. Chromatogram of terpinene-4-ol in marjoram.

4. Discussion

No resistance was noted to essential oils in mites while the difference in the potency of the type of essential oils was reported. The highly effective essential oils were used at an early stage to control mite populations at isolated loci, conserve natural enemies and maximize their role in natural pest control. The rotation of different highly effective extracts for controlling acaricides was an effective method.

The present results of chamomile are in agreement with those documented by Ma *et al*[21] who found that the highest effect of terpinene-4-ol on esterase activity was noted during recover stage of housefly adult (*Musca domestica*). The activities of both acid phosphatase and alkaline phosphatase in insects were induced by terpinen-4-ol. The activities of glutathione-S-transferase were inhibited at exciting, convulsing and paralysis stages, but gradually recovered at recovering stage. The activities of glutathione-S-transferase probably had relations with toxicity of terpinen-4-ol against larvae of the *Mythimna separata*[21]. This point will be taken in our consideration in the near future to clarify inhibition of both phosphatase or its individual one. The activity of glutathione-S-transferase was inhibited in exciting, convulsing and paralysis stages of the 5th star larvae of *Mythimna separata*, but it gradually recovered in the recovery stage. This affected the metabolism and activity of phosphatase and esterase enzymes. On the other hand, the inhibited insect glutathione-S-transferase will inhibit normal metabolism. The activity of glutathione-S-transferase at LC_{50} of the essential oil indicates that the activities of this enzyme were recovered and could defend against free radical and it showed more activity when it could be detected at specific LC_{50} of essential oils extract in recovered mite.

Acaricidal activities of three essential oil extracts (chamomile, marjoram and *Eucalyptus*) against *T. urticae* Koch have been approved that chamomile is the most efficient one[22]. Chamomile and marjoram essential oils showed relationship between essential oil contents and activity of enzyme glutathione-S-transferase, non specific esterase and alkaline phosphatase as well as inhibition of protease enzyme in *T. urticae*. The major essential oil contents of chamomile are α-bisabolol oxide A (35.251%), and trans-α-farersene (7.758%), while the main components of marjoram are terpinen-4-ol (23.860%), *p*-cymene (23.404%) and sabinene (10.904%). The major components of both plant extracts may be responsible for the changes in enzyme activities of *T. urticae*. The present results are in agreement with the data cited by Kawka[23] who studied the effect of chamomile extracts from fresh and dry flowers on *T. urticae*. Leaves extract showed greater mortality. It has been claimed that increased activities of detoxifying and antioxidant enzyme systems in acaricides had been responsible for the resistance[5].

The decrease in proteinase enzyme which is involved in the biological system of defense proves the presence of proteinase inhibitor in the extracts as cited by Manns, Merijn and Azzouz *et al*[18,24,25] who suggest that the extracts can induce defense gene expression of proteinase inhibitor activity. Proteinase inhibitors are proteins that inhibit digestive enzymes in the gut of arthropod herbivores, which can reduce their growth, reproduction. Glutathione-S-transferases are major enzymes involved in metabolic resistance to insecticides, as well as in the detoxification mechanisms of many molecules and, probably, in the transport of physiologically important lipophilic compounds.

Glutathione-S-transferases play an important role in protecting tissues from oxidative damage and stress[11,26].

The changes in the activity of α, β esterase, glutathione-S-transferase and alkaline phosphatase and protease enzymes in target site susceptibility are key biochemical mechanisms of development of active component of essential oils which show more potency against Tetranychidae. These studies laid a solid foundation for further studies on the biochemical mechanisms of resistance in *Tetranychus cinnabarinus* and other spider mites. These points need further investigations in the future to prove our suggestions by using individual component and its effect on the enzyme activities of *T. urticae*. Even this suggestion was approved by Ma *et al*[21] who determined the bioactivity and effect of terpinen-4-ol on activities of some enzymes in adult housefly (*Musca domestica*). The results showed that the LD_{50} of terpinen-4-ol was 23.91 μg/insect. The poisoning symptom of terpinen-4-ol could be divided into four stages *i.e.* excitation, convulsion, paralysis and recover stages. The highest effect of terpinen-4-ol on esterase activity was measured during recover stage (0.216 8±0.009 1 μmol/20 min). Glutathione-S-transferase, monooxygenase (P450) and esterases activity were detected in resistance in *T. urtica*[1]. In contrast, no sesquiterpenes were detected in the fresh resin oil and it was constituted basically by monoterpenes hydrocarbons (42.4%) and oxygen-containing monoterpenes (27.7%), of which α-phellandrene (13.9%) and terpinen-4-ol (7.4%) were the major components, respectively[27]. Conceivably, such challenge has forced the development of mechanisms for survival and adaptation throughout evolution and insecticides activity of these essential oils against *Anopheles stephensi*[28]. Furthermore, and in the above context, induction of detoxifying enzymes by a large number of toxicants has been observed in arthropods[29]. The present results are in agreement with that of Wendel *et al*[27] who studied the evaluation of the acaricidal activity of some essential oils against *T. urticae,* such as fresh and aged resin (*Protium bahianum*) showed higher oil yield 4.6% and 3.2%, respectively. About 22 and 13 components were identified in the oils from the fresh and aged resins, comprising 95.8% and 98.6%, respectively. In the fresh resin oil, monoterpenes (70%) were the major group of constituents, mainly *p*-cymene (18.3%) followed by hydrocarbons, such as α-phellandrene (14.0%), tricyclene (11.4%) and b-phellandrene (9.1%), while the aged resin oil contained sesquiterpenes as the major group with santalol acetate (83.1%) as the principal component.

Treatment with chloroform extract from *Kochia scoparia* enhanced SOD, POD and CAT activities during the 24 hour after treatment[30,31] and traditional Chinese plant can cause toxicity to *Tetranychus cinnabarinus*[32-34] and even glucoside had acaricial effect[35]. Acaricidal activities of *Wikstroemia chamaedaphne* extracts against *Tetranychus* were also reported. Twenty-nine compounds were identified with potential acaricidal activity against *Tetranychus cinnabarinus*[36] and had effect on the activity of *Tetranychus* enzymes[37]. The essential oils from accessions of *Lippia sidoides* Cham. (Verbenaceae) were characterized by GC and GC/MS and investigated for their acaricidal activity against the two-spotted spider mite *T. urticae* Koch[38].

In conclusion, three essential oils of chamomile, marjoram and *Eucalyptus* possess the acaricidal activity against *T. urticae*. However, futher investigation is needed to study the components of theses plants which are responsible for inhibiting the activities of enzymes in *T. urticae*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors would like to thank the Management of the Faculty of Agriculture, Cairo University, Department Biochemistry (Protein Lab) for ongoing cooperation to support research and providing funds and facilities necessary to achieve the desired goals of research.

References

[1] Puinean AM, Denholm I, Millar NS, Nauen R, Williamson MS. Characterisation of imidacloprid resistance mechanisms in the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *Pestic Biochem Physiol* 2010; **97**(2): 129-132.

[2] Soliman MMM. Phytochemical and toxicological studies of *Artemisia* L. (Compositae) essential oil against some insect pests. *Arch Phytopathol Plant Prot* 2007; **40**(2): 128-138.

[3] Tsagkarakou A, Van Leeuwen T, Khajehali J, Ilias A, Grispou M, Williamson MS, et al. Identification of pyrethroid resistance associated mutations in the para sodium channel of the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). *Insect Mol Biol* 2009; **18**(5): 583-593.

[4] Sanil D, Shetty NJ. Genetic study of propoxur resistance-a carbamate insecticide in the malaria mosquito, *Anopheles stephensi* Liston. *Malar Res Treat* 2010; **2010**: 1-6.

[5] Afify AMR, El-Beltagi HS, Fayed SAS, Shalaby EA. Acaricidal activity of different extracts from *Syzygium cumini* L. Skeels (Pomposia) against *Tetranychus urticae* Koch. *Asian Pac J Trop Biomed* 2011: **1**(5): 359-364.

[6] Murray MB. Botanical insecticides detrrents and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol* 2006; **51**: 45-66.

[7] Afify AMR, Mahmoud AM, El-Gammal HA, Attallah ER. Multiresidue method of analysis for determination of 150 pesticides in grapes using quick and easy method (QuEChERS) and LC-MS/MS determination. *Int J Food Agric Environ* 2010; **8**(2): 602-606.

[8] Raina R, Pawan K, Verma NK, Shahid P, Prawez PS.

Induction of oxidative stress and lipid peroxidation in rats chronically exposed to cypermethrin through dermal application. *J Vet Sci* 2009; **10**(3): 257-259.

[9] Khan M, Hossain A, Islam MS. Effect of neem leaf dust and a commercial formulation of neem compound on the longevity, fecundity and ovarian development of melon fly, *Bactrocera cucurbitae* (Coquillet) and oriental fruit by *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Pak J Biol Sci* 2007; **10**(20): 3656-3661.

[10] Jourdie V, Alvarez N, Molina-ochoa J, Wiliams T, Bergvinson D, Benrey B, et al. Population genetic structure of two primary parasitoids of *Spodoptera frugiperda* (Lepidoptera), *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera): to what extent is the host plant important? *Mol Ecol* 2010; **19**(10): 2168-2179.

[11] Gui Z, Hou C, Liu T, Qin G, Li M, Jin B. Effects of insect viruses and pesticides on glutathione-S-transferase activity and gene expression in *Bombyx mori*. *J Econ Entomol* 2009; **102**(4): 1591-1598.

[12] Afify AMR. Biological function of xenobiotics through protein binding and transportation in living cells. *Int J Agric Res* 2010; **5**: 562-575.

[13] Çalmaşur Ö, Aslanand I, Şahin F. Insecticidal and acaricidal effect of three Lamiaceae plant essential oils against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Ind Crops Prod* 2006; **23**(2): 140-146.

[14] Aroiee H, Mosapoor S, Karimzadeh H. Control of greenhouse whitefly (*Trialeurodes vaporariorum*) by thyme and peppermint. *KMITL Sci J* 2005; **5**(2): 511-514.

[15] Finney DJ. *Probit analysis*. 3rd ed. Cambridge: Cambridge University Press; 1971.

[16] Asperen KV. A study of housefly esterase by means of sensitive colourimetric method. *J Insect Physiol* 1962; **8**: 401-416.

[17] Villanueva RT, Walgenbach JF. Acaricidal properties of spinosad against *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). *J Econ Entomol* 2006; **99**(3): 843-849.

[18] Born K, Manns A, Dzeyk K, Lutz-Wahl S, Gau D, Fischer L. Evaluation of ultrasound velocity measurements for estimating protease activities using casein as substrate. *Biotechnol Lett* 2009; **32**(2): 249-253.

[19] El-Sharabasy HM. Acaricidal activities of *Artemisia judaica* L. extracts against *Tetranychus urticae* Koch and its predator *Phytoseiulus persimilis* Athias Henriot (Tetranychidae: Phytoseiidae). *J Biopestic* 2010; **3**(2): 514-519.

[20] Duncan DB. Multiple range and multiple *F* tests. *Biometrics* 1955; **11**: 1-42.

[21] Ma ZQ, Feng J, Guo ZB, Zhang X. Effects of terpinen-4-ol on four kinds of metabolizing enzymes and polyphenol oxidase in *Musca domestica*. *J Zhejiang Univ Agric Life Sci* 2008; **34**(5): 509-515.

[22] Sertkaya E, Kamuran KK, Soylu S. Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.) (Acarina: Tetranychidae). *Ind Crops Prod* 2010; **31**(1): 107-112.

[23] Kawka B. Effect of chamomile extracts on biology of *Tetranychus urticae* Koch feeding on Algerian ivy (*Hedera canariensis* L.). *Ann Warsaw Agric Univ Hortic Landsc Archit* 2004; **25**: 75-79.

[24] Kant MR, Sabelis MW, Haring MA, Schuurink RC. Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defenses. *Proc R Soc Biol Sci* 2008; **275**: 443-452.

[25] Azzouz H, Campan ED, Cherqui A, Saguez J, Couty A, Jouanin L, et al. Potential effects of plant protease inhibitors, oryzacystatin I and soybean Bowman-Birk inhibitor, on the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera, Braconidae). *J Insect Physiol* 2005; **51**(8): 941-951.

[26] Ugurlu S, Konus MB, Iscan M. Pyrethroid resistance and possible involvement of glutathione-S-transferases in *Helicoverpa armigera* from Turkey. *Phytoparasitica* 2007; **35**(1): 23-26.

[27] Wendel JTP, Jose CSD, Claudio AG, Adelmo CHR. Composition and acaricidal activity of the Resin′s essential oil of *Protium bahianum* daly against two spotted spider mite (*Tetranychus urticae*). *J Essent Oil Res* 2007; **19**: 379-383.

[28] Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SPS. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresour Technol* 2005; **96**: 1749-1757.

[29] Cao H, Wang YN, Liu SQ, Li XH, Shi GL. Effects of *Kochia scoparia* extracts to activities of several enzymes of *Tetranychus viennensis*. *Sci Silvae Sinicae* 2008; **42**(2): 68-72.

[30] Wang YN, Cheng J, Jin YS, Ren JJ, Guo HL, Zhao L, et al. *Effects of chloroform extracts from Kochia scoparia on protect enzyme activity of Tetranychus viennensis*. Bioinformatics and Biomedical Engineering (iCBBE), 4th International Conference; 2010, p. 1-4.

[31] Cao H, Wang YN, Liu SQ, Zhao LL, Ping L, Yu TQ, et al. Effects of the chloroform extracts of *Kochia scoparia* to several enzyme systems in *Tetranychus viennensis*. *Sci Silvae Sinicae* 2007; **43**: 68-72.

[32] Ren JJ, Shi GL, Gu JC, Wang JW, Zheng Y, Wang YN. Contact toxicity of crude extracts from thirty-one acaricidal plants in Northeastern China against *Tetranychus cinnabarinus*. *J Beijing Univ Agric* 2009; **24**: 17-21.

[33] Shen ZJ, Wang HX, Shi GL, Wang YN. Biological activities of extracts from 3 species of plants against *Tetranychus cinnabarinus*. *J Beijing Univ Agric* 2008; **23**: 22-24.

[34] Xiao DS, Yang YM, Yu GY. The relationship among the organelles and the implication of yin-yang and wuxing in Chinese traditional medicine. *J Zhejiang Univ Tradit Chin Med* 2008; **32**(3).

[35] Ren XH, Du G, Zhou J, Bing-Feng Zhou BF, Zhang XH, et al. Study on the spectroscopy of two andrographolide glucoside. *Chin J Anal Chem* 2007; **35**(2).

[36] Wang YN, Bu CY, Jin YS, Ren JJ, Guo HL, Zhao L, et al. *Acaricidal activities of Wikstroemia chamaedaphne extracts against Tetranychus urticae and Tetranychus cinnabarinus (Acari: Tetranychidae)*. 4th International Conference on Bioinformatics and Biomedical Engineering; 2010, p. 1-5.

[37] Wang YN, Jin YS, Shi GL, Bu CY, Zhao L, Du J, et al. *Effects of the root extracts of Stellera chamaejasmel L. on the activity of two enzymies of Tetranychus cinnabarinus*. Symposium on Photonics and Optoelectronics; 2009, p. 1-5.

[38] Cavalcanti SC, Niculau Edos S, Blank AF, Câmara CA, Araújo IN, Alves PB. Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). *Bioresour Technol* 2010; **101**(2): 829-832.