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

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- 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 ($\alpha$-esterase and $\beta$-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 $\alpha$-bisabolol oxide A (35.25%), and trans–$\beta$–farersene (7.75%), while the main components of *Marjorana hortensis* are terpinene–4-ol (23.86%), p-cymene (23.40%) and sabine (10.99%). **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...
**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<sub>90</sub> of the tested oils were evaluated. In this respect, some detoxifying enzymes such as glutathione–S–transferase, nonspecific 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) °C 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) °C 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<sub>50</sub>, LC<sub>90</sub> 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 °C and the supernatants were used directly to determine the activity of alpha and beta esterase, glutathione–S–transferases 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–chlooro–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 LC50 values after 24 h for adults were 0.65%, 1.17% and 1.84%, 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. LC90 values were 2.27%, 5.91% and 7.13% for adults and 4.34%, 9.81% and 28.95% for eggs, respectively.

Table 3 Effect of 24 h treatment by LC50 of essential oils on non specific esterases, phosphatase (alkaline, acid), glutathione-S-transferase and protease activities of adult T. urticae (mean±SD).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>α–Esterase (A) Fold</th>
<th>β–Esterase (B) Fold</th>
<th>Alkaline phosphatase (C) Fold</th>
<th>Acid phosphatase (D) Fold</th>
<th>Glutathione–S–transferase (E) Fold</th>
<th>Protease (F) Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>2.70±0.21a</td>
<td>0.90±0.01a</td>
<td>7.15±0.17a</td>
<td>2.26±0.06a</td>
<td>771.00±28.30b</td>
<td>129.00±4.00a</td>
</tr>
<tr>
<td>Positive control (Triton X–100)</td>
<td>8.62±0.13b</td>
<td>2.72±0.05b</td>
<td>5.70±0.11b</td>
<td>2.22±0.11b</td>
<td>771.70±10.10b</td>
<td>112.20±1.52b</td>
</tr>
<tr>
<td>M. hortensis</td>
<td>19.90±1.34c</td>
<td>7.85±0.14c</td>
<td>4.03±0.15c</td>
<td>2.21±0.04c</td>
<td>881.30±8.50c</td>
<td>80.33±2.52c</td>
</tr>
<tr>
<td>C. recutita</td>
<td>8.99±0.12d</td>
<td>2.65±0.05d</td>
<td>4.66±0.06d</td>
<td>2.50±0.10d</td>
<td>1003.00±15.30c</td>
<td>65.33±1.15c</td>
</tr>
</tbody>
</table>

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 × 10$^-3$/min/gb wt.
3.1. Enzyme activities

Table 3 showed that the activity of glutathione-S-transferase significantly increased after treatment with LC50 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 LC50 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 LC50 of marjoram and chamomile compared with positive control (5.70 ± 0.11) U/gb wt.

Protease enzymes were significantly decreased at LC50 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Percentage (%)</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamomile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Ocimene</td>
<td>13.597</td>
<td>1.435</td>
<td>C10H16</td>
<td>136.23</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>13.963</td>
<td>0.678</td>
<td>C10H16</td>
<td>136.23</td>
</tr>
<tr>
<td>Artemisia ketone</td>
<td>14.255</td>
<td>1.305</td>
<td>C10H16O</td>
<td>152.23</td>
</tr>
<tr>
<td>Bicyclolemene</td>
<td>26.603</td>
<td>0.739</td>
<td>C10H24</td>
<td>204.00</td>
</tr>
<tr>
<td>Trans-β-farnesene</td>
<td>34.379</td>
<td>7.758</td>
<td>C10H18</td>
<td>196.24</td>
</tr>
<tr>
<td>Germacrene–D</td>
<td>34.819</td>
<td>0.122</td>
<td>C10H24</td>
<td>204.19</td>
</tr>
<tr>
<td>α-Farnesene</td>
<td>36.319</td>
<td>1.399</td>
<td>C10H18</td>
<td>196.24</td>
</tr>
<tr>
<td>α-Calacorene</td>
<td>36.702</td>
<td>1.534</td>
<td>C10H18</td>
<td>196.24</td>
</tr>
<tr>
<td>α-Cadin–4,9-diene</td>
<td>43.843</td>
<td>0.893</td>
<td>C10H24</td>
<td>204.35</td>
</tr>
<tr>
<td>Bisabolol oxide A</td>
<td>52.128</td>
<td>35.251</td>
<td>C10H26O</td>
<td>238.54</td>
</tr>
<tr>
<td>Hexahydrofarnesyl acetone</td>
<td>53.690</td>
<td>1.249</td>
<td>C10H18</td>
<td>208.06</td>
</tr>
<tr>
<td>Tricosane</td>
<td>67.160</td>
<td>0.839</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>Heptacosane</td>
<td>70.856</td>
<td>1.636</td>
<td>C10H24</td>
<td>204.35</td>
</tr>
<tr>
<td>Marjoram</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Pinene</td>
<td>8.476</td>
<td>1.757</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>Sabinene</td>
<td>10.473</td>
<td>10.904</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>11.125</td>
<td>1.386</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>13.093</td>
<td>23.400</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>14.512</td>
<td>9.034</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>cis-β-Terpinol-4-ol</td>
<td>15.039</td>
<td>1.152</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>α-Terpinolene</td>
<td>15.645</td>
<td>2.678</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>cis-Sabinol hydrate</td>
<td>16.927</td>
<td>1.685</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>Trans-4-thujanol</td>
<td>16.990</td>
<td>0.164</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>Terpinene–4-ol</td>
<td>21.287</td>
<td>23.860</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>α-Terpinol</td>
<td>21.768</td>
<td>6.421</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>Linalyl acetate</td>
<td>23.707</td>
<td>3.693</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
<tr>
<td>β-Caryophyllone</td>
<td>30.974</td>
<td>4.820</td>
<td>C10H18</td>
<td>196.29</td>
</tr>
</tbody>
</table>

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

Table 5

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>Mass to charge ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamomile</td>
<td>Bisabolol oxide A</td>
<td>228.54</td>
</tr>
<tr>
<td></td>
<td>Trans-β-farnesene</td>
<td>204.19</td>
</tr>
<tr>
<td>Marjoram</td>
<td>Terpinene–4-ol</td>
<td>154.25</td>
</tr>
<tr>
<td></td>
<td>p-Cymene</td>
<td>134.22</td>
</tr>
<tr>
<td></td>
<td>Sabinene</td>
<td>136.24</td>
</tr>
<tr>
<td></td>
<td>γ-Terpinene</td>
<td>136.23</td>
</tr>
<tr>
<td></td>
<td>α-Terpinol</td>
<td>196.29</td>
</tr>
<tr>
<td></td>
<td>β-Caryophyllene</td>
<td>204.35</td>
</tr>
</tbody>
</table>

Chamomile: Non identified peaks=45.872%; Identified peaks=54.238%.
Marjoram: Non identified peaks=10.218%; Identified peaks=89.892%.
analysis of C. recutita (Table 4, Table 5 and Figure 1) proved the presence of thirteen components. The major essential oil contents of C. recutitae 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 oxida A in chamomile.](image)

![Figure 2. Chromatogram of terpinene-4-ol in marjoram.](image)

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 LC₅₀ 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 LC₅₀ 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 Kawakal[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 LD₅₀ 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 ±0.009 1 μmol/20 min). Glutathione–S–transferase, monoxygenase (P450) and esterases activity were detected in resistance in *T. urticae*[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%), tricyclic (11.4%) and β–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 acaricidal 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, further 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.

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