FUMIGANT TOXICITY OF SOME ESSENTIAL OILS ON ADULTS OF SOME STORED-PRODUCT PESTS

Mohammad Mahmoudvand1,2*, Habib Abbasipour1, Moslem Basij1, Mohammad Hossein Hosseinpour1, Fahimeh Rastegar1, and Mohammad Bagher Nasiri1

ABSTRACT

Plant materials with insecticidal properties have been traditionally used for generations in some parts of the world. In this study, fumigant toxicity of some essential oils extracted from Rosmarinus officinalis L., Mentha pulegium L., Zataria multiflora, and Citrus sinensis (L.) Osbeck var. hamlin on adults of stored-product pests, including Tribolium castaneum, Sitophilus granarius, Callosobruchus maculatus, and Plodia interpunctella were investigated. Pure essential oils were used in glass vials for the bioassay. LC50 values of Citrus sinensis var. hamlin against T. castaneum, S. granarius, and C. maculatus were 391.28, 367.75, and 223.48 µL L-1 air after 24 h, 362.40, 20.45, and 207.17 µL L-1 air after 48 h, respectively. Furthermore, LC50 values of the fumigant test of C. sinensis and M. pulegium essential oils against S. granarius were 0.038 and 367.75 µL L-1 air after 24 h, 0.025 and 320.45 µL L-1 air after 48 h, respectively. On the other hand, LC50 values of R. officinalis and Z. multiflora on P. interpunctella moths were 0.93 and 1.75 µL L-1 after 24 h. Results showed that among tested essential oils, C. sinensis var. hamlin had good fumigant toxicity on T. castaneum, S. granarius, and C. maculatus. In addition, M. pulegium essential oil was stronger than C. sinensis var. hamlin on S. granarius. Results also indicated that both Z. multiflora and R. officinalis had fumigant toxicity on P. interpunctella adults. In summary, results indicated that these essential oils have good fumigant toxicity on stored-product pests.

Key words: Fumigant toxicity, Citrus sinensis var. hamlin, Zataria multiflora, Plodia interpunctella.

INTRODUCTION

Protecting crops against agricultural pests is known to depend on the use of synthetic chemical pesticides (Isman, 2000). Broad-spectrum insecticides have been reported to cause development of resistance in insect populations (Bughoio and Wilkins, 2004). In this regard, natural products are generally preferred because of their innate biodegradability and less harmful compounds affecting non-target organisms (Prabakar and Jebanesan, 2004). Plants have acquired effective defense mechanisms that ensure their survival under adverse environmental factors. In addition to morphological mechanisms, plants have also developed chemical defense mechanisms towards organisms such as insects that affect biochemical and physiological functions (Prakash and Rao, 1997). Essential oils are usually extracted from various parts of the plant (Daferera et al., 2000; Isman, 2000). In some Asian and African countries, essential oils are traditionally used through fumigant or contact action to protect grains against storage pests, a suitable method to preserve products stored in warehouses and on small farms (Shaaya et al., 1997). Zataria multiflora Boiss. (Lamiaceae), which is locally known as Avishan-e-Shirazi, is an Iranian native plant that grows only in Iran, Pakistan, and Afghanistan (Mozaffarian, 1998; Ali et al., 2000). Rosemary, Rosmarinus officinalis L. (Lamiaceae), an evergreen shrub with linear leathery leaves grows to a maximum height of 2 m in the Mediterranean region. Rosemary flowers are pale to mid-blue and 10-12 mm long (Blamey and Grey-Wilson, 1998). Mentha pulegium L. (Lamiaceae) is used as insect repellent in Iran because of its antispasmodic, diaphoretic, and anti-inflammatory properties (Marderosian, 2001). Sweet orange, Citrus sinensis L. (Rotaceae), is one of the medicinal plants prescribed as traditional medicine to treat diverse illnesses (Intekhab and Aslam, 2009). It has been used as insect repellent, antibacterial, and larvicide (Han, 1998). The essential oil of C. sinensis also has

1Shahed University, Faculty of Agricultural Sciences, Tehran, Iran.
2Corresponding author (msco_1381@yahoo.com).
3Islamic Azad University, Qom branch, Young Researchers Club, Qom, Iran.
Received: 4 September 2010.
Accepted: 7 November 2010.
The insect colonies were reared at 27 ± 1 °C, 65 ± 5% RH, and dark conditions. Flour, wheat (Triticum aestivum L.) grains, mung bean (Vigna radiata L.) grains, and pistachio (Pistacia vera L.) were employed to rear T. castaneum, S. granarius, C. maculatus, and P. interpunctella, respectively.

Fumigant bioassay of coleopteran pests

The fumigant toxicity of essential oils on T. castaneum, S. granarius, and C. maculatus were tested in glass vials (70 mL) in which 10 adult insects (1- to 7-d-old) were released. Filter paper disks (Whatman N° 1) were cut into 2-cm diameter pieces and fixed under the glass vial screw caps. Filter papers were impregnated with a series of concentrations of each essential oil. Treated insects were transferred to untreated vials after 24 h. Four replicates were run for each concentration and for the control groups. Numbers of dead and live insects were also counted after 3, 6, 9, 12, 24, and 48 h from the start of exposure. The control did not show any mortality.

Fumigant bioassay of P. interpunctella moths

These experiments were similar to those of coleopteran pests although in these tests vials were 600 mL and moths were 1-d-old. In these experiments, mortality was also recorded after 3, 6, 9, 12, and 24 h from the start of exposure.

Statistical analysis

The 50 and 90% lethal concentration (LC50 and LC90) values were assessed by Probit analysis (Finney, 1971) with SAS software (SAS Institute, 1997).

RESULTS

Fumigant toxicity on coleopteran pests

Tables 1 and 2 show LC50 and LC90 values of C. sinensis var. hamlin essential oils on T. castaneum, S. granarius, and C. maculatus after 24 and 48 h. Results indicated that C. maculatus is more susceptible than T. castaneum and S. granarius. LC50 values of C. sinensis var. hamlin on T. castaneum, S. granarius, and C. maculatus after 24 h were 391.28, 367.75, and 223.48 µL L-1 air, respectively. After 48 h, LC50 values were lower than those measured after 24 h, but this decrease was not significant. Values of LC50 after 48 h were 362.40, 320.45, and 207.17 µL L-1 air. Tables 3 and 4 compare C. sinensis var. hamlin and M. pulegium for their fumigant toxicity on S. granarius after 24 and 48 h. Data indicate that LC50 values of M. pulegium were much higher than C. sinensis var. hamlin. Values of LC50 for C. sinensis var. hamlin and M. pulegium were 0.038 and 367.75 µL L-1 air after 24 h, 0.025 and 320.45 µL L-1 air after 48 h, respectively.

In addition, Figures 1 to 3 show mortality trends for T. castaneum, S. granarius, and C. maculatus at different time periods (3, 6, 9, 12, and 48 h).

MATERIALS AND METHODS

Essential oil extraction

Aerial parts of R. officinalis and M. pulegium were collected from the garden of the Medicinal Plants Research Center of Shahed University, Tehran, in July 2009. Leaves of Z. multiflora were collected in the Jiroft region (Kerman Province) in December 2008, their cortex powder was used to extract essential oils. All the collected plant materials were shade-dried at room temperature. Dried materials were then subjected to hydrodistillation with a Clevenger-type distiller (Cavalcanti et al., 2004). To extract essential oils by distillation, 50 g of air-dried plant material was put into water (1:12 w/v) for 4 h. Extracted essential oils were dried via anhydrous sodium sulfate and stored at 4 °C in the dark. Pure essential oils were employed in all the tests.

Insect rearing

The insect colonies were reared at 27 ± 1 °C, 65 ± 5%
concentrations and times after exposure to *C. sinensis* var. *Hamlin*. In Figure 4, the mortality trend of *S. granarius* is depicted at all concentrations after treatment with *M. pulegium* in different time periods. According to Figures 1 to 4, the highest mortality in coleopteran pests occurred after 12 h.

Table 1. Fumigant toxicity of *Citrus sinensis* var. *hamlin* essential oil against three coleopteran stored-products pests after 24 h.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Df</th>
<th>LC50</th>
<th>LC90</th>
<th>Slope ± SE</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tribolium castaneum</td>
<td>200</td>
<td>3</td>
<td>391.28 (375.77-403.55)</td>
<td>482.70 (458.92-529.71)</td>
<td>14.05 ± 2.38</td>
<td>4.37</td>
</tr>
<tr>
<td>Sitophilus granarius</td>
<td>240</td>
<td>4</td>
<td>367.75 (351.20-384.44)</td>
<td>508.91 (470.60-579.52)</td>
<td>9.08 ± 1.27</td>
<td>2.52</td>
</tr>
<tr>
<td>Callosobruchus maculatus</td>
<td>200</td>
<td>3</td>
<td>223.48 (209.49-237.29)</td>
<td>332.80 (301.94-391.07)</td>
<td>7.41 ± 1.06</td>
<td>1.70</td>
</tr>
</tbody>
</table>

1Number of individuals.  
2Degrees of freedom.  
3Chemical concentration that kills 50% of a sample population.  
4Chemical concentration that kills 90% of a sample population.  
5Standard error.

Table 2. Fumigant toxicity of *Citrus sinensis* var. *hamlin* essential oil against three coleopteran stored-products pests after 48 h.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Df</th>
<th>LC50</th>
<th>LC90</th>
<th>Slope ± SE</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tribolium castaneum</td>
<td>200</td>
<td>3</td>
<td>362.40 (339.43-376.45)</td>
<td>442.69 (425.56-474.49)</td>
<td>14.74 ± 2.66</td>
<td>2.62</td>
</tr>
<tr>
<td>Sitophilus granarius</td>
<td>240</td>
<td>4</td>
<td>320.45 (297.86-337.37)</td>
<td>456.48 (424.91-515.28)</td>
<td>8.33 ± 1.28</td>
<td>2.34</td>
</tr>
<tr>
<td>Callosobruchus maculatus</td>
<td>200</td>
<td>3</td>
<td>207.17 (196.57-217.12)</td>
<td>275.83 (258.34-304.68)</td>
<td>10.30 ± 1.33</td>
<td>7.76</td>
</tr>
</tbody>
</table>

1Number of individuals.  
2Degrees of freedom.  
3Chemical concentration that kills 50% of a sample population.  
4Chemical concentration that kills 90% of a sample population.  
5Standard error.

Table 3. Comparing the fumigant toxicity of *Citrus sinensis* var. *hamlin* and *Mentha pulegium* essential oils against *Sitophilus granarius* after 24 h.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Df</th>
<th>LC50</th>
<th>LC90</th>
<th>Slope ± SE</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentha pulegium</td>
<td>240</td>
<td>4</td>
<td>0.038 (0.0311-0.046)</td>
<td>0.137 (0.105-0.209)</td>
<td>2.34 ± 0.32</td>
<td>1.86</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>240</td>
<td>4</td>
<td>367.75 (351.20-384.44)</td>
<td>508.91 (470.60-579.52)</td>
<td>9.08 ± 1.27</td>
<td>2.52</td>
</tr>
</tbody>
</table>

1Number of individuals.  
2Degrees of freedom.  
3Chemical concentration that kills 50% of a sample population.  
4Chemical concentration that kills 90% of a sample population.  
5Standard error.

Table 4. Comparing the fumigant toxicity of *Citrus sinensis* var. *hamlin* and *Mentha pulegium* essential oils against *Sitophilus granarius* after 48 h.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Df</th>
<th>LC50</th>
<th>LC90</th>
<th>Slope ± SE</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentha pulegium</td>
<td>240</td>
<td>4</td>
<td>0.025 (0.018-0.031)</td>
<td>0.091 (0.0722-0.136)</td>
<td>2.29 ± 0.36</td>
<td>4.01</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>240</td>
<td>4</td>
<td>320.45 (297.86-337.37)</td>
<td>456.48 (424.91-515.28)</td>
<td>8.33 ± 1.28</td>
<td>2.34</td>
</tr>
</tbody>
</table>

1Number of individuals.  
2Degrees of freedom.  
3Chemical concentration that kills 50% of a sample population.  
4Chemical concentration that kills 90% of a sample population.  
5Standard error.
Table 5 shows the effects of fumigating *R. officinalis* and *Z. multiflora* essential oils on *P. interpunctella* moths after 24 h. Comparing LC₅₀ values, it was observed that both *R. officinalis* and *Z. multiflora* had fumigant toxicity on *P. interpunctella* moths, but *R. officinalis* was more toxic than *Z. multiflora*. LC₅₀ values of *R. officinalis* and *Z. multiflora* were 0.93 and 1.75 µL L⁻¹ air. Figures 5 and 6 show the mortality rates of *P. interpunctella* in different concentrations and time periods after exposure to *R. officinalis* and *Z. multiflora*. The mortality trend of *P. interpunctella* increased with both essential oils after 12 h.

**Fumigant toxicity on *P. interpunctella* moths**

Figure 1. Mortality of *Tribolium castaneum* adults exposed to *Citrus sinensis* var. *hamlin* essential oil at different concentrations and times.

Figure 2. Mortality of *Sitophilus granarius* adults exposed to *Citrus sinensis* var. *hamlin* essential oil at different concentrations and times.

Figure 3. Mortality of *Callosobruchus maculatus* adults exposed to *Citrus sinensis* var. *hamlin* essential oil at different concentrations and times.

Figure 4. Mortality of *Sitophilus granarius* adults exposed to *Mentha pulegium* essential oil at different concentrations and times.
DISCUSSION

Natural compounds from plants could be efficient alternatives to conventional fumigants because of their low toxicity to mammals, fast degradability properties, and regional availability (Rajendran and Sriranjini, 2008). Our results on fumigant toxicity of *C. sinensis* var. *Hamlin* on *T. castaneum*, *S. granarius*, and *C. maculatus* indicated that this essential oil had good toxicity on these pests by fumigation. Among the examined pests, *C. maculatus* was the most sensitive. On the other hand, comparing LC50 values of *C. sinensis* var. *hamlin* and *M. pulegium* on *S. granarius*, it was found that *C. sinensis* var. *hamlin* was more toxic than *C. sinensis*. In accordance with this study, Mahmoudvand et al. (2011) reported that *M. pulegium* essential oil was more toxic than *Lippia citrodora* Kunth (Verbenaceae), *R. officinalis*, and *Juniperus sabina* (Pinaceae) on *C. maculatus*. Our results also showed that *R. officinalis* and *Z. multiflora* had fumigant toxicity against *P. interpunctella* moths after 24 h. LC50 value of *Z. multiflora* on *P. interpunctella* was 1.75 µL L−1 air. Our study is the first to report the insecticidal activity and fumigant toxicity of *Z. multiflora*, which is native to Iran (Mozaffarian, 1998), on a moth. Rastegar et al. (2010) indicated that by treating *C. maculatus* with *Z. multiflora* essential oil LC50 values were 8.81 µL L−1 after 24 h. In another study, Mahmoudvand et al. (2011) showed that the LC50 value of fumigant toxicity of *R. officinalis* on *C. maculatus* was 46.81 µL L−1 air. These results indicated that moths are more susceptible than coleoptera and low essential oil concentrations are more effective on moths than on beetles. Similar to this study, Shojaaddini et al.
(2008) assessed the effect of *Carum copticum* C.B. Clarke (Apiaceae) essential oil on eggs, larvae, pupae, and adults of *P. interpunctella*. They reported that adult stage was more susceptible than other growth stages. In this study, LC₅₀ values at 48 h were lower than those at 24 h. On the other hand, results indicated that the high rate of mortality occurred between 12 and 24 h, although mortality increased after 24 h.

This showed that the effects of current essential oils were not reversible. To these authors’ knowledge, there are no studies about fumigant effects of essential oil of this variety (*hamlin*) of *C. sinensis* on insects. Zewde and Jember (2010) studied the effect of *C. sinensis* essential oil on Zabrotes subfasciatus L. and showed that *C. sinensis* had contact toxicity on this insect pest.

Results of this study clearly illustrated that insects varied in their susceptibility to different essential oils, which probably refers to the insecticidal ability of their active constituents; however, there is no difference between the time of high mortality for all essential oils and insects.

**CONCLUSIONS**

In conclusion, essential oil of *C. sinensis* var. *hamlin* cortex powder had fumigant toxicity on *T. castaneum*, *S. granarius*, and *C. maculatus*, and susceptibility of *C. maculatus* was the highest. Fumigant toxicity effect of *M. pulegium* was also higher than *C. sinensis* var. *hamlin* on *S. granarius*. Bioassay tests also indicated that the Iranian native plant, *Z. multiflora*, is a good choice for a fumigant toxicity test on *P. interpunctella*, and its toxicity was lower than *R. officinalis*. All essential oils examined in this study exerted good toxicity on stored-product pests and can be used to manage these pests.

**ACKNOWLEDGEMENT**

We thank the members of the Laboratory of Entomology and the Department of Plant Protection of Shahed University, Tehran, Iran, for their assistance.

**RESUMEN**

Toxicidad fumigante de algunos aceites esenciales sobre adultos de algunas plagas de productos almacenados. Materiales vegetales con propiedades insecticidas han sido usados tradicionalmente por generaciones en algunas partes del mundo. En este estudio se estudió la toxicidad fumigante de algunos aceites esenciales extraídos desde *Rosmarinus officinalis* L., *Mentha pulegium* L., *Zataria multiflora* y *Citrus sinensis* (L.) Osbeck var. *hamlin* sobre adultos de plagas de productos almacenados, incluyendo *Tribolium castaneum*, *Sitophilus granarius*, *Callosobruchus maculatus* y *Plodia interpunctella*. Para los bioensayos se usaron aceites esenciales puros en frascos de vidrio. Los valores de LC₅₀ de *C. sinensis* var. *hamlin* contra *T. castaneum*, *S. granarius* y *C. maculatus* fueron 391,28; 367,75 y 223,48 después de 24 h y 362,40; 20,45 y 207,17 µL L⁻¹ aire después de 48 h, respectivamente. Además, los valores de LC₅₀ de la prueba fumigante del aceite esencial de *C. sinensis* y *M. pulegium* contra *S. granarius* después de 24 h fueron 0,038 y 367,75 µL L⁻¹ aire y fueron 0,025 y 320,45 después de 48 h, respectivamente. Por otra parte, los valores de LC₅₀ de *R. officinalis* y *Z. multiflora* en polillas de *P. interpunctella* después de 24 h fueron 0,93 y 1,75 µL L⁻¹. Los resultados mostraron que entre los aceites esenciales, *C. sinensis* var. *hamlin* tuvo una buena toxicidad fumigante sobre *T. castaneum*, *S. granarius*, *C. maculatus*. Además, el aceite esencial de *M. pulegium* fue más fuerte que *C. sinensis* var. *hamlin* sobre *S. granarius*. Los resultados además indicaron que *Z. multiflora* y *R. officinalis* tuvieron toxicidad fumigante sobre adultos de *P. interpunctella*. En conclusión, los resultados indicaron que estos aceites esenciales tienen buena toxicidad fumigante sobre plagas de productos almacenados.

**Palabras clave:** toxicidad fumigante, *Citrus sinensis* var. *hamlin*, *Zataria multiflora*, *Plodia interpunctella*.

**LITERATURE CITED**


