RESEARCH

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

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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. LC_{50} values of *Citrus sinensis* var. *hamlin* against *T. castaneum*, *S. granarius*, and *C. maculatus* were 391.28, 367.75, and 223.48 $\mu L\ L^{-1}$ air after 24 h, 362.40, 20.45, and 207.17 $\mu L\ L^{-1}$ air after 48 h, respectively. Furthermore, LC_{50} values of the fumigant test of *C. sinensis* and *M. pulegium* essential oils against *S. granarius* were 0.038 and 367.75 $\mu L\ L^{-1}$ air after 24 h, 0.025 and 320.45 $\mu L\ L^{-1}$ air after 48 h, respectively. On the other hand, LC_{50} *values of R. officinalis* and *Z. multiflora* on *P. interpunctella* moths were 0.93 and 1.75 $\mu L\ L^{-1}$ 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 (Bughio 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 (Mozzaffarian, 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

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fumigant toxicity against *Aedes aegypti* L. mosquitoes (Omomouwajo *et al*., 2005).

One of the major storage pests of cowpea (*Vigna sinensis* L.) is *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) (Epidi *et al*., 2008), which is an important insect pest of many grains, such as chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), and soybean (*Glycine max* L.) (Mahfuz and Khalequzzaman, 2007). In addition, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is also a major storage pest that prefers grain products such as flour (Lecato, 1976; Campbell and Runnion, 2003). Granary weevil, *Sitophilus granarius* (L.), is ranked among the important stored grain pests. It was a primary pest in the past (Kucerova *et al*., 2003). Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is a worldwide and major stored-product pest that infests a wide range of stored products, including nuts, beans, processed foods, and dried fruits (Simmons and Nelson, 1975; Lecato, 1976).

In this study, the fumigant toxicity of cortex powder of a variety of *C. sinensis, C. sinensis* var. *hamlin* on adults of three important pests, *T. castaneum*, *Sitophilus granarius*, and *C. maculatus* after 24 and 48 h was investigated. The toxicity of *C. sinensis* var. *hamlin* and *M. pulegium* against adults of *Sitophilus granarius* after 24 and 48 h was also compared. In addition, toxicity of *R. officinalis* and *Z. multiflora* essential oils against *P. interpunctella* moths after 24 h was explored, and LC_{50} values of these essential oils were calculated. Insect mortality was also recorded for the examined insects at various 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 Nour-Abad region (Fars Province) in May 2009. After collecting fruits of *C. sinensis* var. *hamlin* 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% RH, and dark conditions. Flour, wheat (*Triticum aestivum* L.) grains, mung bean (*Vigna radiata* (L.) R. Wilczek) 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 (LC_{50} and LC_{90}) 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 LC_{50} and LC_{90} 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*. LC_{50} 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 $\mu L\ L^{-1}$ air, respectively. After 48 h, LC_{50} values were lower than those measured after 24 h, but this decrease was not significant. Values of LC_{50} after 48 h were 362.40, 320.45, and 207.17 $\mu L\ L^{-1}$ 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 LC_{50} values of *M. pulegium* were much higher than *C. sinensis* var. *hamlin*. Values of LC_{50} for *C. sinensis* var. *hamlin* and *M. pulegium* were 0.038 and 367.75 $\mu L\ L^{-1}$ air after 24 h, 0.025 and 320.45 $\mu L\ L^{-1}$ air after 48 h, respectively.

In addition, Figures 1 to 3 show mortality trends for *T. castaneum*, *S. granarius*, and *C. maculatus* at different 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.

	n[1]	Df[2]	LC_{50}[3]	LC_{90}[4]	Slope ± SE[5]	χ^2
			μL L^{-1} air			
Tribolium castaneum	200	3	391.28 (375.77-403.55)	482.70 (458.92-529.71)	14.05 ± 2.38	4.37
Sitophilus granarius	240	4	367.75 (351.20-384.44)	508.91 (470.60-579.52)	9.08 ± 1.27	2.52
Callosobruchus maculatus	200	3	223.48 (209.49-237.29)	332.80 (301.94-391.07)	7.41 ± 1.06	1.70

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

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

	n[1]	Df[2]	LC_{50}[3]	LC_{90}[4]	Slope ± SE[5]	χ^2
			μL L^{-1} air			
Tribolium castaneum	200	3	362.40 (339.43-376.45)	442.69 (425.56-474.49)	14.74 ± 2.66	2.62
Sitophilus granarius	240	4	320.45 (297.86-337.37)	456.48 (424.91-515.28)	8.33 ± 1.28	2.34
Callosobruchus maculatus	200	3	207.17 (196.57-217.12)	275.83 (258.34-304.68)	10.30 ± 1.33	7.76

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

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

	n[1]	Df[2]	LC_{50}[3]	LC_{90}[4]	Slope ± SE[5]	χ^2
			μL L^{-1} air			
Mentha pulegium	240	4	0.038 (0.0311-0.046)	0.137 (0.105-0.209)	2.34 ± 0.32	1.86
Citrus sinensis	240	4	367.75 (351.20-384.44)	508.91 (470.60-579.52)	9.08 ± 1.27	2.52

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

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

	n[1]	Df[2]	LC_{50}[3]	LC_{90}[4]	Slope ± SE[5]	χ^2
			μL L^{-1} air			
Mentha pulegium	240	4	0.025 (0.018-0.031)	0.091 (0.0722-0.136)	2.29 ± 0.36	4.01
Citrus sinensis	240	4	320.45 (297.86-337.37)	456.48 (424.91-515.28)	8.33 ± 1.28	2.34

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

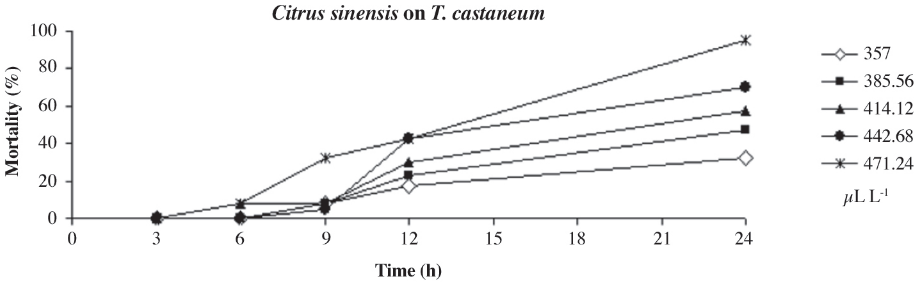

Figure 1. Mortality of *Tribolium castaneum* adults exposed to *Citrus sinensis* var. *hamlin* essential oil at different concentrations and times.

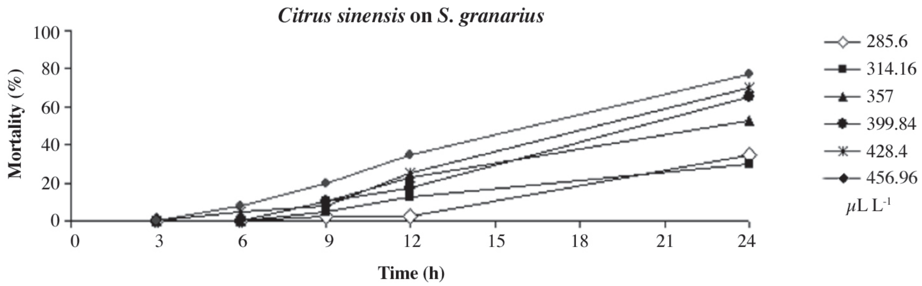

Figure 2. Mortality of *Sitophilus granarius* adults exposed to *Citrus sinensis* var. *hamlin* essential oil at different concentrations and times.

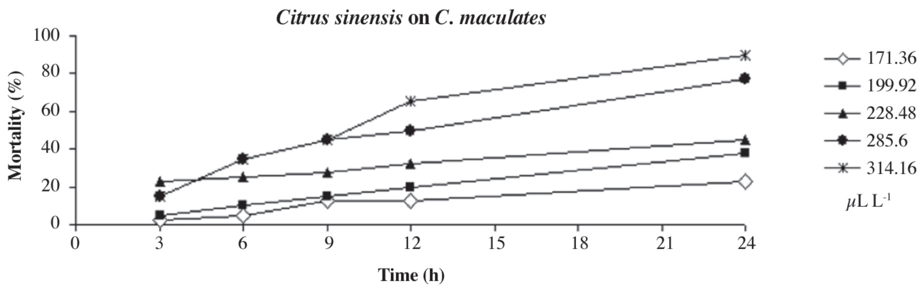

Figure 3. Mortality of *Callosobruchus maculatus* adults exposed to *Citrus sinensis* var. *hamlin* essential oil at different concentrations and times.

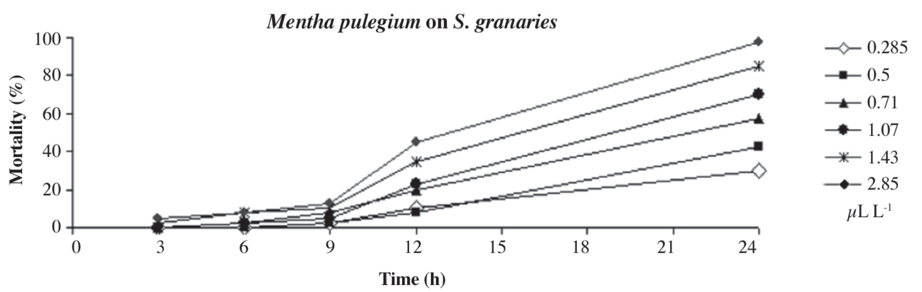

Figure 4. Mortality of *Sitophilus granarius* adults exposed to *Mentha pulegium* essential oil at different concentrations and times.

Fumigant toxicity on *P. interpunctella* moths

Table 5 shows the effects of fumigating *R. officinalis* and *Z. multiflora* essential oils on *P. interpunctella* moths after 24 h. Comparing LC_{50} 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*. LC_{50} values of *R. officinalis* and *Z. multiflora* were 0.93 and 1.75 $\mu L\ L^{-1}$ 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.

Table 5. Fumigant toxicity of *Rosmarinus officinalis* and *Zataria multiflora* essential oils against *Plodia interpunctella* moths after 24 h.

	n[1]	Df[2]	LC_{50}[3]	LC_{90}[4]	Slope ± SE[5]	χ^2
			μL L^{-1} air			
Rosmarinus officinalis	200	3	0.93 (0.71-1.21)	6.33 (3.47-27.19)	1.54 ± 0.33	4.90
Zataria multiflora	240	4	1.75 (1.28 -2.45)	24.26 (11.40-114.13)	1.12 ± 0.20	3.40

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

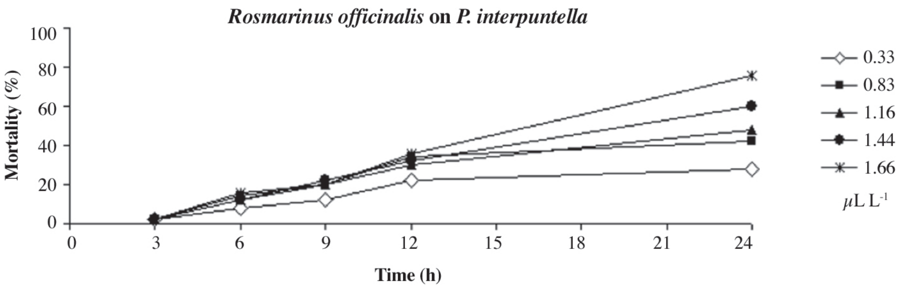

Figure 5. Mortality of *Plodia interpunctella* moths exposed to *Rosmarinus officinalis* essential oil in different concentrations and times.

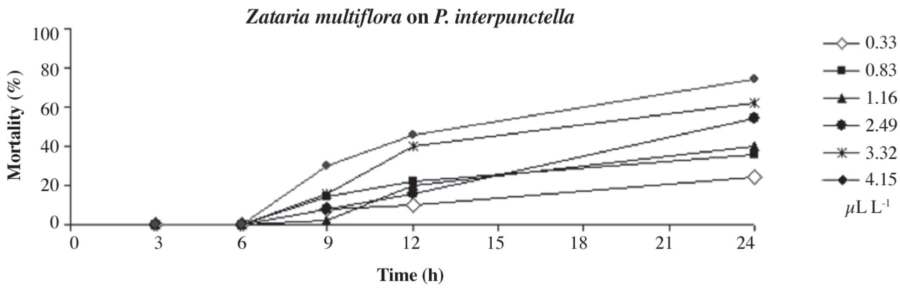

Figure 6. Mortality of *Plodia interpunctella* moths exposed to *Zataria multiflora* essential oil in different concentrations and times.

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 LC_{50} 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. LC_{50} 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 (Mozzaffarian, 1998), on a moth. Rastegar *et al.* (2010) indicated that by treating *C. maculatus* with *Z. multiflora* essential oil LC_{50} values were 8.81 μL L^{-1} after 24 h. In another study, Mahmoudvand *et al.* (2011) showed that the LC_{50} 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, LC_{50} 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 Jembere (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.

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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 LC_{50} 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 $\mu L\ L^{-1}$ aire después de 48 h, respectivamente. Además, los valores de LC_{50} 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 $\mu L\ L^{-1}$ aire y fueron 0,025 y 320,45 después de 48 h, respectivamente. Por otra parte, los valores de LC_{50} de *R. officinalis* y *Z. multiflora* en polillas de *P. interpunctella* después de 24 h fueron 0,93 y 1,75 $\mu L\ L^{-1}$. 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* and *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*.

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