Full Length Research Paper

# Bioactivity of essential oil from *Satureja hortensis* (Laminaceae) against three stored-product insect species

Mollaei Maedeh<sup>1</sup>, Izadi Hamzeh<sup>1</sup>\*, Dashti Hossein<sup>2</sup>, Azizi Majid<sup>3</sup> and Ranjbar Karimi Reza<sup>4</sup>

<sup>1</sup>Department of Plant Protection, Vali-e-Asr University, Rafsanjan, Iran.
 <sup>2</sup>Department of Agronomy and Plant Breeding, Vali-e-Asr University, Rafsanjan, Iran.
 <sup>3</sup>Department of Horticulture, Ferdowsi University of Mashhad, Iran.
 <sup>4</sup>Department of Chemistry, Vali-e-Asr University, Rafsanjan, Iran.

Accepted 20 May, 2011

Human health problems and environmental hazards caused by the indiscriminate use of chemical pesticides during the past three decades, led scientists to look for less persistent and biodegradable alternatives. Essential oils from aromatic plants are recognized as proper alternatives. In this experiment, toxicity of *Satureja hortensis* essential oil that was isolated via hydrodistillation was investigated against 1 to 7 day-old adults of the red flour beetle, *Tribolium castaneum* (Herbst), 12 to 14 day-old larvae of the Mediterranean flour moth, *Ephestia kuehniella* (Zell.) and Indianmeal moth, *Plodia interpunctella* (Hübner). Repellency of this oil on all the three pest species adults was also studied. After 48 h of exposure, the LC<sub>50</sub> value for *T. castaneum* was 192.35  $\mu$ /L. LC<sub>50</sub> values were calculated as 80.9  $\mu$ /L and 139.8  $\mu$ /L after 9 h for *E. kuhniella* and *P. interpunctella*, respectively. *S. hortensis* oil showed more contact toxicity against *P. interpunctella* (LC<sub>50</sub> = 0.19  $\mu$ /cm<sup>2</sup>) than *E. kuehniella* (LC<sub>50</sub> = 0.27  $\mu$ /cm<sup>2</sup>). Repellency of this oil on all the insect species was high. Relationship between exposure time and oil concentration on mortality of all species indicated that mortality was increased by increasing the oil concentration and exposure time.

Key words: Tribolium castaneum, Ephestia kuehniell, Plodia interpunctella, repellency, fumigant toxicity, contact toxicity.

# INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst), Mediterranean flour moth, *Ephestia kuehniella* (Zell.) and Indianmeal moth, *Plodia interpunctella* (Hübner) are the three primary and most destructive pests of stored nuts and grains which cause severe quantitative and qualitative losses throughout the world. Alleviation of these pest's problems in storage, relies mostly on synthetic pesticides and specially methyl bromide and phosphine. These may have drawbacks, including toxicity to nontargets or grains, development of pest resistance and resurgence, and environmental contamination

(Talukder and Howse, 1993; Sahaf et al., 2008; Subramanyam and Hagstrom, 1995). Hence, there is a worldwide interest in the development of alternative strategies; therefore, repellents, fumigants, feeding deterrents and insecticides of natural origin are all rational alternatives to synthetic pesticides. Among these potential alternatives, plant derived insecticides play an important role in traditional methods of pest control. The plant kingdom can be a rich source of a variety of chemicals with the potential for development as successful pest control agents (Atta-ur-Rahman et al., 1999). Researchers in pest control have recently concentrated their efforts on the search for active natural products derived from plants as alternatives to conventional insectcides. Many plant secondary metabolites are known to have various activities against insects and their

<sup>\*</sup>Corresponding author. E-mail: izadi@vru.ac.ir. Tel: +983913202156. Fax: +983913202042

secondary metabolites have been formulated as botanical pesticides for plant protection. These compounds do not leave toxic residue to the environment, have lower toxicity to mammals, and medicinal properties for human (Duke, 1985; Coats et al., 1991; Chiasson et al., 2004; Garcia et al., 2005; Sahaf et al., 2008; Negahban et al., 2007).

Essential oils are volatile, natural complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites, and play an important role in the protection of the plants as antibacterial, antiviral, antifungal and insecticide. Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries (Coats et al., 1991). The Satureja is a genus of aromatic plants of the family Laminaceae, related to rosemary and thyme. There are about 30 species called savories, of which Summer savory and Winter savory are the most important in cultivation. Satureia species are native to warm temperate regions and may be annual or perennial. S. hortensis (Summer savory) is the better known of the savory species. It is an annual, but otherwise, is similar in use and flavor to the perennial Winter savory. Summer savory has a strong aromatic flavor, which could be compared to thyme (particularly, thyme harvested in summer). Essential oils, obtained by the distillation of plant foliage and even the foliage itself of certain aromatic plants have traditionally been utilized to protect stored grains and legumes against different pests (Isman and Passreiter, 2001). The medicinal and antibacterial properties of S. hortensis have long been recognized. Essential oils of this species are formed in special pits around the leaves, and contain carvacrol, cymol and other phenolic compounds (Omidbaigi, 2004). However, its insecticidal bioactivities have not yet been investigated. This study was therefore undertaken in order to investigate the bioactivities of the essential oils of S. hortensis against three major stored-product pests: T. castaneum, E. kuehniella and P. interpunctella.

#### MATERIALS AND METHODS

#### The insects

Adults of red flour beetle, *T. castaneum*, were taken from the Division of Plant Protection, Ferdowsi University of Mashhad. Different developmental stages of Indianmeal moth, *P. interpunctell* were from Pistachio Research Center of Rafsanjan and eggs of Mediterranean flour moth, *E. kuehniella*, were from the Division of Plant Protection, Agricultural Research Center of Mashhad. *T. castaneum*, *E. kuehniella* and *P. interpunctella* were reared on wheat flour mixed with yeast (2:1, w/w), wheat flour and pistachio nuts, respectively. Colonies were maintained in a climate chamber set at  $25\pm2^{\circ}$ C,  $65\pm5^{\circ}$  RH and with a photoperiod of 16:8 (L:D). Mediterranean flour moth and Indianmeal moth were reared in rectangular plastic jars (25 cm length, 18 cm width and 10 cm height). Red flour beetle was reared in columnar plastic containers. Plastic funnel covered with net cloth secured with adhesive glue was used for egg laying. 1 to 7 day-old adults of red flour beetle

and 12 to 14 day-old larvae of Indianmeal moth and Mediterranean flour moth were used for fumigant toxicity tests.

### Plant materials

Leaves of *S. hortensis* were collected from Mashhad, Iran, and dried naturally on laboratory benches at room temperature (23 to  $24 \,^{\circ}$ C) for 5 days until crisp. The dried materials were stored in a deep freezer (-24  $^{\circ}$ C) until used and then hydro distilled to extract their essential oil (Negahban et al., 2007).

#### Extraction of essential oil

Negahban et al. (2007) method with some modification was used for the extraction of the essential oil. Essential oil was extracted from the leaves using a Clevenger-type apparatus where the plant material was subjected to hydrodistillation. Conditions of extraction were: 200 g dried leaf, 500 ml distilled water and 3 h distillation. Anhydrous sodium sulphate was used for removing water after extraction. The extracted oil was kept in a refrigerator at 4 °C.

#### Analysis of essential oil

The elemental analyses for C, H, and N were performed using Heraeus CHN-O-Rapid analyzer. The <sup>13</sup>C NMR and <sup>1</sup>H NMR spectra were recorded at 125 MHz for <sup>13</sup>C and 500 MHz for <sup>1</sup>H. GC-mass spectra were taken by a Micromass Platform II: EI mode (70 eV). Column chromatography was performed using silica (Merck #60). Silica plates (Merck) were used for TLC analysis.

#### Fumigant toxicity assay

Keita et al. (2000) method with some modification was used for fumigant toxicity bioassay. Glass vials (10 ml) were used for the bioassay. 1 to 7 day-old adults of red flour beetle, 14 day-old larvae of Mediterranean flour moth and 12 to 14 day-old larvae of Indianmeal moth were separately transferred to the vials in the group of 10 insects. The vials were covered with fine steel gauze secured with adhesive tape. Different volumes (10, 14.45, 21.38, 31.62, 46.77 and 70 µl for T. castaneum, 0.5, 0.87, 1.51, 2.36, 4.57 and 8 µl for E. kuehniella and 1, 1.51, 2.29, 3.47, 5.25 and 8 µl for P. interpunctella) of oil were placed in a vial and then the vial containing the insects was turned upside down over the vial containing the oil. As such, the oil vapor saturated the atmosphere of the container containing the insects. The control consisted of a similar setup but without essential oil. This procedure was replicated 6 times for each volume. The vials were placed at 25±2℃ and 65±5% RH with a photoperiod of 16:8 (L: D). Mortality was scored at 24, 36 and 48 h after treatment for red flour beetle and 6. 9 and 12 h after treatment for Mediterranean flour moth and Indian meal moth.

#### Contact toxicity assay

To evaluate contact toxicity of *S. hortensis* essential oil, Kim et al. (2003) method with some modification was used. In this method, the insecticidal activity of essential oil was determined by direct contact application. Different volumes (2.5, 3.58, 4.51, 5.67, 7.14 and 9  $\mu$ l for *E. kuehniella* and 1.5, 2, 2.82, 3.98, 5.62 and 8  $\mu$ l for *P. interpunctella*) of oil were dissolved in 400  $\mu$ l acetone and applied to filter papers (Rundfilter MN, cut into 5.5 cm diameter pieces) (0.11, 0.16, 0.26, 0.32 and 0.41  $\mu$ /cm<sup>2</sup> for *E. kuehniella* and 0.07, 0.09, 0.13, 0.18, 0.25 and 0.36  $\mu$ L/cm<sup>2</sup> for *P. interpunctella*). The filter

Table 1. Averages	assigned to
different classes.	

Class	Percent repulsion
0	>0.01 to <0.1
I	0.1-20
II	20.1-40
Ш	40.1-60
IV	60.1-80
V	80.1-100

paper was dried under room temperature for 5 min and put in Petri dish (5.5 cm diameter and 1 cm height). In each set of experiments, ten 14 day-old larvae of Mediterranean flour moth or ten 12 to 14 day-old larvae of Indianmeal moth were put in Petri dishes. The control consisted of a similar setup but without essential oil. Each volume was replicated six times. Mortality was counted after 18 h.

#### **Repellent activity**

To evaluate repellency of S. hortensis essential oil, Asawalam et al. (2006) method with some modification was used. Two 125 ml glass iars were connected together at their rims by means of a 2×10 cm hyaline nylon tube. A circular hole was cut at the middle of the tube for the introduction of test insects. In this devise, it was possible to test large quantities of materials. Different concentrations of essential oil (0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4 and 0.8 µl) were applied to the filter papers (Rundfilter MN, cut into 2 cm diameter pieces) by a programmable microsyringe (Stoleting, USA) and put in the bottom of one jar. Another jar was untreated and acted as control. In the case of T. castaneum, 1 g wheat grain was applied in both jars. Twenty (20) 1 to 7 day-old adults of T. castaneum or ten 1 day-old adults of each moth species were introduced into the nylon tube trough circular hole and the hole was closed by tape. The number of insects present in the control (Nc) and treated (Nr) jars were recorded after 1 h exposure. There were four replications in the bioassay. Percent of repellency (P<sub>R</sub>) were computed as:  $P_{R} = [(N_{C}-N_{T})/(N_{C}+N_{T})] \times 100$ . Positive values express repellency and negative values attractancy. The averages were then assigned to different classes using the scales in Table 1 (Talukder and Howse, 1993). Each experiment was replicated four times.

#### Data analyses

Data were analyzed with Minitab 14 software followed by MSTATC and Duncan's multiple range tests to compare effects among treatments. In order to meet normality and stability of the variances within treatments, before analysis of variance, the data for fumigant effect of the oil on *T. castaneum* and *E. kuehniella* were transformed to arcsine square-root values. The results were expressed as means ( $\pm$ SE) of untransformed data and considered significantly different at P < 0.05. Probit analysis was used for estimation of LC<sub>50</sub> by POLO-PC 2002 software.

# RESULTS

# **Fumigant toxicity**

In all cases, considerable differences in mortality of

insects to essential oil were observed with different concentration and times. The results of two-way analysis of variances showed that effect of oil concentration (F= 6.14; df = 5, 51; P < 0.001) and time exposure (F= 86.94; df = 1, 51; P < 0.001) on beetle adults mortality were significant. As it is evidence from Table 2, there was no adult's mortality of T. castaneum after 24 h in all the concentrations and control. The highest mortality of T. castaneum (50%) after 48 h was observed with 3500 µl/L air. The data in Table 3 illustrate that the effect of oil concentration (F= 112.71; df = 6, 103; P < 0.001) and time exposure (F= 54.17; df = 2, 103; P < 0.001) on larval mortality of E. kuehniella were significant. Effect of oil concentration (F= 99.68; df = 6, 105; P < 0.001) and time exposure (F= 26.25; df = 2, 105; P < 0.001) on larval mortality of P. interpunctella were also significant (Table 4). At 400 µl/L air, maximum mortality was obtained as 100 and 98.33% for E. kuehniella and P. interpunctella larvae, respectively. Although data probit analysis showed that the LC<sub>50</sub> value after 9 h for *P. interpunctella*  $(LC_{50} = 139.8 \mu I/I air)$  was more than that for *E. kuehniella*  $(LC_{50} = 80.9 \ \mu l/l \text{ air})$ , the  $LC_{50}$  ratios with their lower and upper 95% confidence limits (0.78-3.81) showed that there was no significant difference between these values.

# **Contact toxicity**

As shown in Tables 5 and 6, effect of oil concentration on larval mortality of *E. kuehniella* and *P. interpunctella* was significant (F= 41.54 and 49.92; df = 6, 25; P < 0.001), whereas, effect of higher rate of oil (4.08  $\mu$ l/cm<sup>2</sup>) on *T. castaneum* after 96 h, was too low in the primary experiments, therefore, more continuation of this experiment was avoided. After 18 h, the highest mortality of *E. kuehniella* and *P. interpunctella* (85 and 80%) were observed with 0.41 and 0.36  $\mu$ l/ cm<sup>2</sup>, respectively. LC<sub>50</sub> value comparison using the LC<sub>50</sub>s ratio (1.5) and their lower and upper 95% confidence limits (1.25 and 1.53) showed that LC<sub>50</sub> value for *E. kuehniella* (0.27  $\mu$ l/cm<sup>2</sup>).

# **Repellent activity**

In this study, effect of oil concentration on insects repellency was significant (F= 10.82 to 14.67; df= 8, 27; P < 0.001). The results in Table 7 demonstrated that *S. hortensis* essential oil was highly repellent to the adults of *T. castaneum*, *E. kuehniella* and *P. interpunctella*. It repelled the insects significantly even at very low concentration. Higher repellency of *T. castaneum* (92.5%) and *E. kuehniella* (85%) adults was obtained at 6.4  $\mu$ l/L air, whereas, there was no significant difference between repellency of *P. interpunctella* were repelled significantly by

		Mortality (%) maan+85	-	
Concentration (µl/L air)	<u>Mortality (%) mean±SE</u> 24 h after treatment 36 h after treatment 48 h after treatment			
500.0	0.00±0.00	11.67±4.01 <sup>b</sup>	30.00±4.72 <sup>b</sup>	
722.5	0.00±0.00	6.67±3.33 <sup>b</sup>	31.67±5.43 <sup>b</sup>	
1069.0	0.00±0.00	12.00±3.74 <sup>b</sup>	41.67±3.07 <sup>ab</sup>	
1581.0	0.00±0.00	20.00±3.65 <sup>ab</sup>	40.00±3.65 <sup>ab</sup>	
2338.5	0.00±0.00	8.00±4.90 <sup>b</sup>	45.00±2.89 <sup>ab</sup>	
3500.0	0.00±0.00	36.00±2.45 <sup>a</sup>	50.00±0.00 <sup>a</sup>	

 Table 2. Fumigant activity of Satureja hortensis essential oil against Tribolium castaneum.

Means within a column followed by different letters are significantly different (P < 0.001; by Duncan's multiple range test).

Table 3. Fumigant activity of Satureja hortensis essential oil against Ephestia kuehniella larvae.

Concentration (ul/l air)	Mortality (%) mean±SE		
Concentration (µl/l air)	6 h after treatment	9 h after treatment	12 h after treatment
00.00	3.33±2.11 <sup>°</sup>	8.33±3.07 <sup>d</sup>	13.33±3.33°
25.00	11.67±4.77 <sup>°</sup>	26.00±6.78 <sup>c</sup>	31.67±8.33b <sup>c</sup>
43.50	15.00±4.28 <sup>c</sup>	26.67±5.58 <sup>c</sup>	43.33±4.22 <sup>b</sup>
75.50	12.00±5.83 <sup>c</sup>	48.33±7.92b <sup>c</sup>	58.33±9.46 <sup>b</sup>
118.0	43.33±4.94 <sup>b</sup>	73.33±2.11 <sup>b</sup>	91.67±3.07 <sup>a</sup>
228.50	66.67±9.19 <sup>b</sup>	93.33±3.33 <sup>a</sup>	100.00±0.00 <sup>a</sup>
400.00	90.00±4.47 <sup>a</sup>	98.33±1.67 <sup>a</sup>	100.00±0.00 <sup>a</sup>

Means within a column followed by different letters are significantly different (P < 0.001; by Duncan's multiple range test).

Table 4. Fumigant activity of Satureja hortensis essential oil against Plodia interpunctella larvae.

Concentration (ul/l air)	Mortality (%) mean±SE		
Concentration (µl/l air)	6 h after treatment	9 h after treatment	12 h after treatment
00.00	1.67±1.67 <sup>e</sup>	3.33±2.11 <sup>e</sup>	5.00±2.24 <sup>d</sup>
50.00	18.33±4.01 <sup>de</sup>	21.67±5.43 <sup>de</sup>	26.67±6.67 <sup>c</sup>
75.50	16.67±4.94 <sup>de</sup>	26.67±5.58 <sup>d</sup>	35.00±7.19 <sup>c</sup>
114.5	30.00±5.16 <sup>cd</sup>	50.00±6.83 <sup>c</sup>	58.33±6.01 <sup>b</sup>
173.5	43.33±3.33 <sup>bc</sup>	55.00±6.71 <sup>bc</sup>	61.67±5.43 <sup>b</sup>
262.5	50.00±5.77 <sup>b</sup>	70.00±4.47 <sup>ab</sup>	85.00±4.28 <sup>a</sup>
400.0	75.00±5.00 <sup>ª</sup>	88.33±4.77 <sup>a</sup>	98.33±1.67 <sup>a</sup>

Means within a column followed by different letters are significantly different (P < 0.001; by Duncan's multiple range test).

*S. hortensis* at 0.48  $\mu$ /l air and above the concentrations. Higher repellency of *P. interpunctella* adults (80%) was obtained at 6.4  $\mu$ /L air. graphy on silica gel (ethyl acetate/n-hexane, 1:3); gave 5isopropyl-2-methylphenol (carvacrol) and 1-isopropyl-4methylcyclohexa-1,4-diene (γ-Terpinene).

#### Analysis of essential oil

Carvacrol and  $\gamma$ -Terpinene were extracted with dichloromethane. The solvent was evaporated to yield the crude product, which was then purified by column chromate-

## DISCUSSION

In this study, the essential oil of *S. hortensis* demonstrated fumigant and contact toxicity as well as repellent activity against *T. castaneum*, *E. kuehniella* and

Table5.Contact toxicity ofSaturejahortensisessentialagainstEphestia kuehniellalarvae.

Concentration (µl/cm <sup>2</sup> )	Mortality (%) mean±SE
0.00	10.00±3.65 <sup>d</sup>
0.11	18.33±3.07 <sup>cd</sup>
0.16	23.33±6.15 <sup>cd</sup>
0.21	31.67±4.77 <sup>bc</sup>
0.26	43.33±4.22 <sup>b</sup>
0.32	83.33±5.58 <sup>ª</sup>
0.41	85.00±5.00 <sup>a</sup>

Means within a column followed by different letters are significantly different (P < 0.01; by Duncan's multiple range test).

P. interpunctella. It is well obvious from our results that insecticidal activity of this oil varied with insect species. concentration of the oil, method of application, and exposure time. The insecticidal constituents of many plant extracts and essential oils are monoterpenoids (Coats et al., 1991; Konstantopoulou et al., 1992; Regnault-Roger and Hamraoui, 1995; Ahn et al., 1998). Monoterpenoids are naturally occurring compounds that are found in higher-order plants. These compounds are secondary metabolites that seem to play no major role in the metabolic functioning of the plants. One role of monoterpenoids in the plants is to defend against plantdirected pathogens, herbivores, or competing plant species. These compounds are good leads for synthesis or isolation of more effective insecticides. Due to their high volatility, these oils have fumigant activity.

In this study, two monoterpenoids, carvacrol and yterpinene were extracted from the oil as major constituents. The toxic effects of S. hortensis essential oil could be attributed to these two major constituents. This finding is in agreement with the results of Sampson et al. (2005), Pavela et al. (2008), Pavela (2009), Darbour et al. (1990), and Sefidkon et al. (2006). Carvacrol or cymophenol -  $C_6H_3CH_3(OH)(C_3H_7)$  - is a monoterpenoid phenol. It has a characteristic pungent, warm odor of oregano and is highly volatile with broad insecticidal and acaricidal activity against agricultural, stored-product and medical pests (Budavari et al., 1989). Gamma-terpinene -C<sub>10</sub>H<sub>16</sub> - is a monoterpene and a major component of essential oils of different plants. This essential oil has a characteristic lemon odor of rather low tenacity. In addition to S. hortensis, analysis of essential oil of S. thymbra (Gören et al., 2004; Ayvaz et al., 2010; Cetin et al., 2010) and phytochemical analysis of S. montana and S. cuneifolia essential oils (Bezić et al., 2005), showed carvacrol and y-terpinene as the major constituents of the oils. So, the insecticidal action of S. hortensis oil is likely due to its major constituents, carvacrol and y-terpinene.

Table 6	. Contact	toxicity	of
Satureja	hortensis	essential	oil
against	Plodia	interpunct	ella
larvae.			

Concentration (µl/cm <sup>2</sup> )	Mortality (%) mean±SE
0.00	3.33±2.11 <sup>d</sup>
0.07	15.00±3.41 <sup>d</sup>
0.09	16.67±2.11 <sup>cd</sup>
0.13	31.67±4.01 <sup>°</sup>
0.18	48.33±6.01 <sup>b</sup>
0.25	63.33±4.94 <sup>b</sup>
0.36	80.00±3.65 <sup>ª</sup>

Means within a column followed by different letters are significantly different (P < 0.01; by Duncan's multiple range test).

Moreover, in carvacrol combination with  $\gamma$ -terpinene or other monoterpenoid substances, very good synergistic effect has been documented by Hummelbrunner and Isman (2001).

Insecticidal mode of action of carvacrol may be attributable to fumigant action, although there is also significant contact toxicity (Ahn et al., 1998). In this investigation, insect mortality was increased significantly with increasing oil concentration and time exposure. This finding is in agreement with the results of Tunc et al. (2000), Aslan et al. (2006), Wang et al. (2006), Negahban et al. (2006), Sahaf et al. (2008), Ogendo et al. (2008) and Shojaaddini et al. (2008). Our result revealed that percent of repellency was increased significantly with increasing oil concentration, which is relevant to the results of Asawalam et al. (2006), Ogendo et al. (2008) and Tripathi and Upadhyay (2009). It has been well established that certain plant -derived extracts and phytochemicals may provide potential alternatives to the currently used insecticides. Regarding this, there are numerous reports on the insecticidal and repellent activity of the essential oils from Satureja species (Park et al., 2005; Isman and Passreiter, 2001; Pavela et al., 2008; Pavela, 2008, 2009; Sampson et al., 2005; Aslan et al., 2004; Regnault-Roger et al., 2004; Nottingham et al., 1991; Laurent et al., 1997; Cetin et al., 2010; Karpouhtsis et al., 1998; Mezzoug et al., 2007; Ayvaz et al., 2010; Michaelakis et al., 2007).

Upon comparing our determinations of  $LC_{50}$  of *S.* hortensis essential oil with other authors, it was found that essential oils from Artemisia sieberi (Negahban et al., 2006, 2007), Vitex pseudo-negundo (Sahaf et al., 2008), Rosmarius officinalis, Citrus limonum, C. auratifolia, Pimenta racemosa and Mentha piperata (Lee et al., 2002) were more toxic than *S. hortensis* oil against *T. castaneum* adults. No report exists for the comparison of susceptibility of *E. kuehniella* and *P. interpunctella* to

Test insect	Concentration (µl/l air)	Average repellency (%)	Repellency class*	Mean number of insect repelled
T. castaneum	0.08	-2.5	_	9.75±1.18 <sup>c</sup>
	0.16	32.5	Ш	13.25±0.75 <sup>bc</sup>
	0.32	57.5	111	15.75±0.75 <sup>ab</sup>
	0.48	60.0	111	16.00±1.22 <sup>ab</sup>
	0.64	77.5	IV	17.75±1.44 <sup>a</sup>
	0.80	80.0	IV	18.00±0.71 <sup>ª</sup>
	1.60	90.0	V	19.00±0.41 <sup>a</sup>
	3.20	82.5	V	18.25±1.03 <sup>a</sup>
	6.40	92.5	V	19.25±0.48 <sup>ª</sup>
E. kuehniella	0.08	-10.0	_	9.00±0.58 <sup>b</sup>
	0.16	5.00	I	10.50±1.26 <sup>b</sup>
	0.32	70.0	IV	17.00±1.00 <sup>a</sup>
	0.48	75.0	IV	17.50±0.96 <sup>a</sup>
	0.64	65.0	IV	16.50±0.96 <sup>a</sup>
	0.80	80.0	IV	18.00±0.82 <sup>a</sup>
	1.60	85.0	V	18.50±0.96 <sup>a</sup>
	3.20	80.0	IV	18.00±0.82 <sup>a</sup>
	6.40	85.0	V	18.50±0.96 <sup>a</sup>
P. interpunctella	0.08	-15.0	_	8.50±0.96 <sup>c</sup>
	0.16	-5.00	_	9.50±1.50 <sup>c</sup>
	0.32	0.00	0	10.00±1.41 <sup>bc</sup>
	0.48	40.0	II	14.00±1.41 <sup>ab</sup>
	0.64	60.0	111	16.00±0.82 <sup>a</sup>
	0.80	60.0	111	16.00±1.15 <sup>a</sup>
	1.60	75.0	IV	17.50±0.50 <sup>a</sup>
	3.20	55.0	111	15.50±0.50 <sup>a</sup>
	6.40	80.0	IV	18.00±0.82 <sup>a</sup>

**Table 7.** Repellency of Satureja hortensis oil to Tribolium castaneum, Ephestia kuehniella and Plodia interpunctella.

\*See materials and methods for details. For each insect species, means within a column followed by different letters are significantly different (P < 0.01; by Duncan's multiple range test).

## S. hortensis and other plant essential oils.

The development of natural insecticides would help to decrease the negative impact of synthetic insecticides, such as persistence of residues, resistance and damage to the environment and human health. In this respect, naturally occurring compounds may be effective, selective, biodegradable, and less harmful to the environment. In conclusion, the results presented in this study suggest that the essential oil of *S. hortensis* had moderate repellency, fumigant and contact toxicity against three major stored pests; *T. castaneum, E. kuehniella* and *P. interpunctella* and could be integrated with other pest management procedures.

## ACKNOWLEDGEMENT

This work was supported by grant to Dr. H. Izadi. We are grateful to the research vice presidency of Vali- e-Asr University of Rafsanjan for this research grant.

#### REFERENCES

- Ahn YJ, Lee SB, Lee HS, Kim GH (1998). Insecticidal and acaricidal activity of caravacrol and β-thujaplicine derived from *Thujopsis dolabrata* var. *hondai* sawdust. J. Chem. Ecol. 24: 1-90.
- Asawalam EF, Emosairue SO, Hassanali A (2006). Bioactivity of Xylopia aetiopica (Dunal) A. rich essential oil constituents on maize weevil Sitophilus zeamais Mostchulsky (Coleoptera: Curculionidae).

Elec. J. Environ. Agric. Food. Chem. 5: 1195-1204.

- Aslan İ, Özbec H, Çalmaşur Ö, Şahİn F (2004). Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. Ind. Crop Prod. 19: 167-173.
- Aslan I, Kılıc T, Goren AC, Topcu G (2006). Toxicity of acetone extract of *Sideritis trojana* and 7-epicandicandiol, 7-epicandicandiol diacetate and 18-acetylsideroxol against stored pests *Acanthoscelides obtectus* (Say), *Sitophilus granarius* (L.) and *Ephestia kuehniella* (Zell.). Ind. Crop. Prod. 23: 171-176.
- Atta-ur-Rahman, Choudhary MI, Thomsen JW (1999). Manual of bioassay techniques for natural product research. Harward Academic Press, Amsterdam.
- Ayvaz A, Sagdic O, Karaborklu S, Ozturk I (2010). Insecticidal activity of the essential oils from different plants against three stored-product insects. J. Insect. Sci. 10: p. 21.
- Bezić N, Skočibušič M, Dunkić V (2005). Phytochemical composition and antimicrobial activity of *Satureja montana* L. and *Satureja cuneifolia* Ten. essential oils. Acta. Bot. Croat. 64: 313-322.
- Budavari SB, Neil OMJ, Smtih A, Heckelman PE (1989). The Merck Index. Merck & Co., Inc. Rahway, New Jersey.
- Cetin H, Cilek JE, Oz E, Aydin L, Deveci O, Yanikoglu A (2010). Acaricidal activity of *Satureja thymbra* L. essential oil and its major components, carvacrol and γ-terpinene against adult *Hyalomma marginatum* (Acari: Ixodidae). Vet. Parasitol. 170: 287-290.
- Chiasson H, Vincent C, Bostanian NJ (2004). Insecticidal properties of a Chenopodiumbased botanical. J. Econ. Entomol. 97: 1378-1383.
- Coats JR, Karr LL, Drewes CD (1991). Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. In: Hedin PA (ed). Naturally Occurring Pest Bioregulators. ACS symposium Series No. 449. American Chemical Society, Washington DC, pp. 305-3165.
- Darbour N, Baltassat F, Raynaud J, Pellecuer J (1990). Flavonoid glycosides in leaves of *Satureja hortensis* L. (Labiateae). Pharma. Acta. Helv. 65: 239-240.
- Duke JA (1985). Handbook of Medicinal Herbs. CRC Press.
- Garcia M, Donadel OJ, Ardanaz CE, Tonn CE, Sosa ME (2005). Toxic and repellent effects of *Baccharis salicifolia* essential oil on *Tribolium castaneum*. Pest Manage. Sci. 61: 612-618.
- Gören AC, Topçu G, Bılsel G, Bılsel M, Wilkinson JM, Cavanagh HMA (2004). Analysis of essential oil of *Satureja thymbra* by hydrodistillation, thermal desorber, and headspace GC/MS techniques and its antimicrobial activity. Nat. Prod. Res. 18: 189-195.
- Hummelbrunner LA, Isman MB (2001). Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on tobacco cutworm, *Spodoptera litura* (Lep: Noctuidae). J. Agric. Food Chem. 49: 715-720.
- Isman MB, Wan AJ, Passreiter C (2001). Insecticidal activities of essential oils to the tobacco cutworm, *Spodoptera litura*. Fitoterapia, 72: 65-68.
- Karpouhtsis I, Pardali E, Feggou E, Kokkini S, Scouras ZG, Mavragani-Tsipidou P (1998). Insecticidal and genotoxic activities of oregano essential oils. J. Agric. Food Chem. 46: 1111-1115.
- Keita SM, Vincent C, Schmit JP, Belanger A (2000): Essential oil composition of *Ocimum basilicum* L., *O. gratissimum* L. and *O. suave* L. in the Republic of Guinea. Flavour Fragr. J. 15: 339-341.
- Kim SI, Park C, Ohh MH, Cho HC, Ahn YJ (2003). Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). J. Stored Prod. Res. 39: 11-19.
- Konstantopoulou LL, Vassilopoulou L, Mavragani-Tsipidou P, Scouras ZG (1992). Insecticidal effects of essential oils, A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. Experimentia, 48: 616-619.
- Laurent D, Vilaseca LA, Chantraine JM, Ballivian C, Saavedra G, Ibanez R (1997). Insecticidal activity of essential oils on *Triatoma infestans*. Phytother. Res. 11: 285-290.
- Lee BH, Lee SE, Annis PC, Pratt SJ, Park BS, Tumaalii F (2002). Fumigant toxicity of essential oils and monoterpenes against the red flour beetle, *Tribolium castaneum* Herbst. J. Asia-Pacific Entomol. 5: 237-240.
- Mezzoug N, Elhadri A, Dallouh A, Amkiss S, Skali NS, Abrini J, Zhiri A, Baudoux D, Diallo B, El Jaziri M, Idaomar M (2007). Investigation of

the mutagenic and anti mutagenic effects of *Origanum compactum* essential oil and some of its constituents. Mutat. Res. 629: 100-110.

- Michaelakis A, Theotokatos SA, Koliopoulos G, Chorianopoulos NG (2007). Essential Oils of Satureja Species: Insecticidal Effect on *Culex pipiens* Larvae (Diptera: Culicidae). Molecules, 12: 2567-2578.
- Negahban M, Moharramipour S, Sefidkon F (2006). Insecticidal Activity and Chemical Composition of *Artemisia sieberi* Besser Essential Oil from Karaj, Iran. J. Asia-Pacific Entomol. 9: 61-66.
- Negahban M, Moharramipour S, Sefidkon F (2007). Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. J. Stored. Prod. Res. 43: 123-128.
- Nottingham SF, Hardie J, Dawson GW, Hick AJ, Pickett JA, Wadhams LJ, Woodcock, CM (1991). Behavioral and electrophysiological responses of aphids to host and non-host plant volatiles. J. Chem. Ecol. 17: 1231-1242.
- Ogendo JO, Kostyukovsky M, Ravid U, Matasyoh JC, Deng AL, Omolo EO, Kariuki ST, Shaaya E (2008). Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. J. Stored. Prod. Res. 44: 328-334.
- Omidbaigi R (2004). Essential oil content and compositions of *Satureja hortensis* of two different regions. J. Essent. Oil Bear. Plant, 7: 175-178.
- Park BS, Choi WS, Kim JH, Kim KH, Lee SE (2005). Monoterpenes from thyme(*Thymus vulgaris*) as potential mosquito repellents. J. Am. Mosq. Control Assoc. 21: 80-83.
- Pavela R (2008). Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say Iarvae (Diptera: Culicidae). Parasitol. Res. 102: 555-559.
- Pavela R (2009). Larvicidal property of essential oils against *Culex quinquefasciatus* Say (Diptera: Culicidae). Ind. Crop Prod. 30: 311-315.
- Pavela R, Sajfrtova M, Sovova H, Bárnet M (2008). The insecticidal activity of *Satureja hortensis* L. extracts obtained by supercritical fluid extraction and traditional extraction techniques. Appl. Entomol. Zool. 43: 377-382.
- Regnault-Roger C, Hamraoui A (1995). Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). J. Stored Prod. Res. 31: 291-299.
- Regnault- Roger C, Ribodeau M, Hamraoui A, Bareau I, Blanchard P, Gil-Munoz MI, Barberan FT (2004). Polyphenolic compounds of Mediterranean Lamiaceae and investigation of orientational effects on *Acanthoscelides obtectus* (Say). J. Stored Prod. Res. 40: 395-408.
- Sahaf BZ, Moharramipour S, Meshkatalsadat MH (2008). Fumigant toxicity of essential oil from *Vitex pseudo-negundo* against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L). J. Asia-Pacific Entomol. 11: 175-179.
- Sampson BJ, Tabanca N, Kirimer N, Demirci B, Can Baser KH, Khan IA, Spiers JM, Wedge DE (2005). Insecticidal activity of 23 essential oils and their major compounds against adult *Lipaphis pseudobrassicae* (Davis) (Aphididae: Homoptera). Pest Manage. Sci. 61: 1122-1128.
- Sefidkon F, Abbasi K, Khaniki GB (2006). Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*. Food Chem. 99: 19-23.
- Shojaaddini M, Moharramipour S, Sahaf BZ (2008). Fumigant toxicity of essential oil from *Carum copticum* against Indian meal moth, *Plodia interpunctella*. J. Plant Prot. Res. 48: 411-419.
- Subramanyam B, Hagstrum D (1995). Resistance measurement and management. In: Subramanyam B, Hagstrum D (Eds). Integrated management of insects in stored products. Marcel Dekker Inc. New York, pp. 331-398.
- Talukder FA, Howse PE (1993). Deterrent and insecticidal effects of extracts of pithraj, *Aphanamixis polystachya* (Meliaceae) against *Tribolium castaneum*, in storage. J. Chemical Ecol. 19: 2463-2471.
- Tripathi AK, Upadhyay Sh (2009). Repellent and insecticidal activities of *Hyptis suaveolens* (Lamiaceae) leaf essential oil against four storedgrain coleopteran pests. Int. J. Trop. Insect Sci. 29: 219-228.
- Tunc I, Berger BM, Erler F, Dagli F (2000). Ovicidal activity of essential

oils from five plants against two stored-product insects. J. Stored Prod. Res. 36: 161-168.

Wang J, Zhu F, Zhou XM, Niu CY, Lei CL (2006). Repellent and fumigant activity of essential oil from *Artemisia vulgaris* to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 42: 339-347.