

# CHEMICAL CONSTITUENTS AND TOXICITY OF ESSENTIAL OILS OF ORIENTAL ARBORVITAE, *Platycladus orientalis* (L.) FRANCO, AGAINST THREE STORED-PRODUCT BEETLES

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Plant secondary metabolites play an important role in plant-insect interactions and therefore such compounds may have insecticidal or biological activity against insects. Fumigant toxicity of essential oils of leaves and fruits from oriental arborvitae (*Platycladus orientalis* [L.] Franco) (Cupressaceae) was investigated against adults of cowpea weevil (*Callosobruchus maculatus* Fab.), rice weevil (*Sitophilus oryzae* L.), and red flour beetle (*Tribolium castaneum* Herbst). Fresh leaves and fruits were subjected to hydrodistillation using a Clevenger-type apparatus and the chemical composition of the volatile oils was studied by gas chromatography-mass spectrometry (GC-MS). Twenty-six compounds (92.9%) and 23 constituents (97.8%) were identified in the leaf and the fruit oils, respectively. The major components of both leaves and fruits oils were  $\alpha$ -pinene (35.2%, 50.7%),  $\alpha$ -cedrol (14.6%, 6.9%) and  $\Delta$ -3-carene (6.3%, 13.8%), respectively. Both oils in the same concentration were tested for their fumigant toxicity on each species. Results showed that leaf oils were more toxic than fruit oils against three species of insects. *Callosobruchus maculatus* was more susceptible than *S. oryzae* and *T. castaneum*. LC<sub>50</sub> values of the leaf and the fruit oils at 24 h were estimated 6.06 and 9.24  $\mu\text{L L}^{-1}$  air for *C. maculatus*, 18.22 and 21.56  $\mu\text{L L}^{-1}$  air for *S. oryzae*, and 32.07 and 36.58  $\mu\text{L L}^{-1}$  air for *T. castaneum*, respectively. These results suggested that *P. orientalis* oils may have potential as a control agent against *C. maculatus*, *S. oryzae*, and *T. castaneum*.

**Key words:** Botanical insecticides, fumigation, *Platycladus orientalis*, stored-product insects.

Insect pest often cause extensive damage to stored grains and their products and this may amount to 5-10% in the temperate zone and 20-30% in the tropical zone (Haque *et al.*, 2000). In such a situation, protection of stored grain and agricultural products against insect infestation is an urgent need. Synthetic insecticides and fumigation are the main methods in stored product insect pest control. Furthermore, an uncontrolled use of these synthetic insecticides causes a great hazard for environment and consumers due to residues (Isman, 2006). Naturally occurring substances are an alternative to conventional pesticides and plant essential oils have traditionally been used to kill insects (Isman, 2000). Essential oils obtained from plants are under particular investigation for their broad-spectrum pest control properties. They have been shown to possess fumigant activity for stored-product pests (Negahban *et al.*, 2007; Negahban and Moharrampour, 2007; Sahaf *et al.*, 2007; 2008) as contact insecticides (Park *et al.*, 2003). Moreover, their repellent, antifeedants, growth inhibiting, and reproduction-retarding effects have been demonstrated against several storage pests

(Huang *et al.*, 1998; Papachristos and Stamopoulos, 2002; Taponjoui *et al.*, 2005; Benzi *et al.*, 2009). Essential oils derived from plant species of *Platycladus* have been evaluated for insecticidal properties (Keita *et al.*, 2001; Pavela, 2005). However, no report on insecticidal activity of essential oils of *P. orientalis* against cowpea weevil, *Callosobruchus maculatus* (Fab.), rice weevil, *Sitophilus oryzae* (L.), and red flour beetle, *Tribolium castaneum* (Herbst) was available.

All aromatic Iranian conifers belong to Cupressaceae family. In Iran this family consists of one species of *Platycladus*, one species of *Cupressus*, and five species of *Juniperus*. Oriental arborvitae, *Platycladus orientalis* (L.) Franco [*Thuja orientalis* L.], locally named Sarv-e Khomreii or Nosh, is an evergreen species, which grows naturally in Iran. Also, this species is widely cultivated as a common ornamental plant in Iran and other countries (Assadi, 1998).

*Platycladus orientalis* is a medicinal plant whose fresh leaves have been used as an anti-inflammatory (Panthong *et al.*, 1986), while the dried leaves have been used to treat flu and cough (Comerford, 1996), high blood pressure (Panthong *et al.*, 1986), bleeding arthralgia (Mikage *et al.*, 1984), cancer (Sharma *et al.*, 1993), haemostatic (Kosuge *et al.*, 1981), and has also been used in Chinese medicine for the treatment of gout, rheumatism, diarrhea, and chronic tracheitis (Zhu *et al.*, 2004).

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The aim of this study was to analyze chemical constituents of essential oils of leaves and fruits of *P. orientalis* and to identify the active chemical constituents of the essential oils as well as to evaluate its fumigant toxicity in the management of *C. maculatus*, *S. oryzae*, and *T. castaneum*.

## MATERIALS AND METHODS

### Insect culture

*Callosobruchus maculatus*, *S. oryzae*, and *T. castaneum* were reared on bean grains, whole rice, and wheat flour mixed with yeast (10:1, w/w), respectively. Adult insects, 1-7-d old, were used for fumigant toxicity tests. The cultures were maintained in dark in a growth chamber set at  $27 \pm 2$  °C and  $60 \pm 5\%$  RH. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. All experiments were carried out under the same environmental conditions.

### Plant material

The vegetal organs (leaves and fruits) of the *P. orientalis* were collected during the summer 2010 from the shrubs cultivated in the Department of Horticultural, Urmia University, Nazlo area, 12 km in the west of Urmia ( $37^{\circ}32'$  N,  $45^{\circ}05'$  E; 1313 m a.s.l.), Iran. Plant taxonomists in the Department of Biology at Urmia University, confirmed the taxonomic identification of plant species. The voucher specimens have been deposited at the herbarium of the Department of Plant Protection at Urmia University.

### Extraction and analysis of essential oils

Fresh leaves and fruits of the plant were separately hydrodistilled in a Clevenger type apparatus where the plant materials were subjected to hydrodistillation. Conditions of extraction were: 100 g of fresh sample; 1:10 plant material/water volume ratio, 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Extracted oils transferred to glass flasks that were filled to the top and kept at the temperature of 4 °C in a refrigerator.

The constituents of *P. orientalis* essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard 6890/5972 system with a HP-5MS capillary column (30 m  $\times$  0.25 mm; 0.25  $\mu$ m film thickness). The carrier gas was helium with flow 1 mL  $\text{min}^{-1}$ . The oven temperature was held at 60 °C for 3 min, programmed at 6 °C  $\text{min}^{-1}$  to 220 °C and then held at this temperature for 3 min. Mass spectra were taken at 70 eV. Mass range was from  $m/z$  35D350 amu. The injector temperature was 240 °C. Relative percentage amounts were calculated from peaks total area by apparatus software. The compounds were identified by comparing mass spectra and retention indices with those in literatures

(Adams, 1995) and by computer searching followed by matching the mass spectra data with those held in a computer library (Wiley 275.L).

### Fumigant toxicity

To determine the fumigant toxicity of the *P. orientalis* oils, some concentrations of the leaf and the fruit oils including 2, 5, 7, 10, and 13  $\mu\text{L L}^{-1}$  air were tested on *C. maculatus*. Suceptibility of *S. oryzae* was evaluated at 8, 14, 19, 23, and 28  $\mu\text{L L}^{-1}$  air of essential oils. Concentrations were 15, 21, 28, 36, and 47  $\mu\text{L L}^{-1}$  air for *T. castaneum*. They were dissolved in 100  $\mu\text{L}$  acetone and applied to filter papers (Whatman N° 1, cut into 4  $\times$  5 cm paper strip) was dried in air for 2 min. The impregnated filter papers were put into 1 L glass bottles. Twenty adults of *C. maculatus*, *S. oryzae*, and *T. castaneum* (1-7-d old) were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical centre of glass bottles, which were then sealed with air-tight lids. In the control bottles, only acetone was applied on the filter papers. Mortality was determined after 24, 48, and 72 h from commencement of the exposure.

When no leg or antennal movements were observed, insect was considered dead. Insect mortality percentages were calculated using the Abbott correction formula for natural mortality in untreated control (Abbott, 1925).

### Data analysis

Tests arranged as completely randomized design and data were analyzed by one-way ANOVA using the SAS software version 9.1. Lethal concentrations ( $\text{LC}_{50}$  and  $\text{LC}_{95}$ ) and lethal time values ( $\text{LT}_{50}$  and  $\text{LT}_{95}$ ) were calculated with SPSS software (version 16.0). Comparison of means were done through Tukey's test ( $\alpha = 0.01$ ).

## RESULTS AND DISCUSSION

### Chemical constituents of essential oils

The main components of both leaves and fruits essential oils from *P. orientalis* were  $\alpha$ -pinene (35.2%, 50.7%),  $\alpha$ -cedrol (14.6%, 6.9%),  $\Delta$ -3-carene (6.3%, 13.8%), limonene (6.1%, 1.5%),  $\beta$ -caryophyllene (5.8%, 4.1%), and myrcene (3.3%, 3.8%), respectively (Table 1).

### Fumigant toxicity

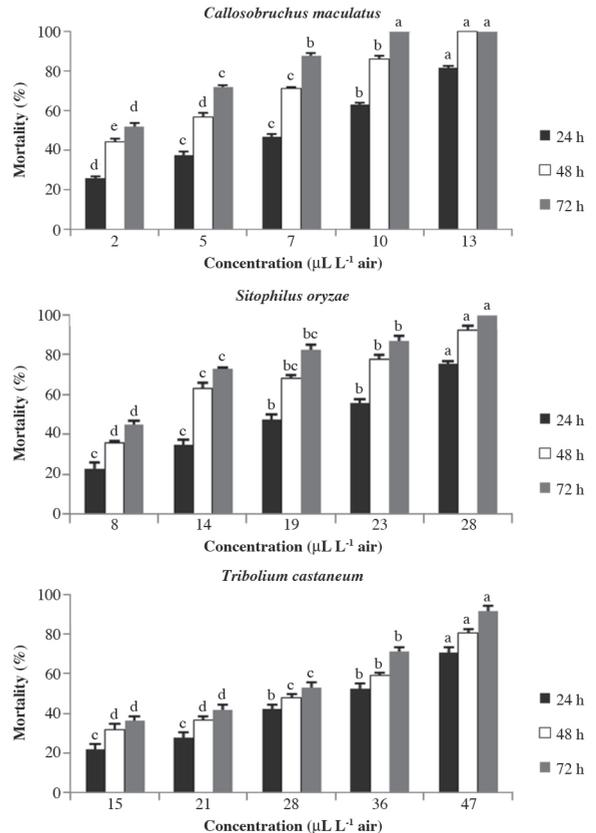
The concentration of 13  $\mu\text{L L}^{-1}$  air of the leaf oil recorded 100% mortality of *C. maculatus* after 48 h, but in the same concentration from the fruit oil 100% mortality of *C. maculatus* was achieved in 72 h exposure time. The concentration of 28  $\mu\text{L L}^{-1}$  air from the leaf oil killed 100% of *S. oryzae* after 72 h exposure. By contrast with the fruit oil about 93% mortality was achieved for *S. oryzae* at the same time exposure. At 47  $\mu\text{L L}^{-1}$  air the leaves oil caused 71% mortality on *T. castaneum* after 24 h exposure and about 91% mortality after 72 h. At this concentration from

**Table 1. Chemical composition (%) of essential oils of oriental arborvitae, *Platycladus orientalis*.**

Compound	Retention index	Leaf oil		Fruit oil	
		%			
$\alpha$ -Thujene	928	0.6	-	-	-
$\alpha$ -Pinene	936	35.2	50.7	-	-
$\alpha$ -Fenchene	944	1.2	1.6	-	-
Sabinene	971	1.5	2.1	-	-
$\beta$ -Pinene	977	0.1	0.9	-	-
Myrcene	993	3.3	3.8	-	-
$\alpha$ -Phellandrene	1005	1.6	2.1	-	-
$\Delta$ 3-Carene	1013	6.3	13.8	-	-
$\rho$ -Cymene	1021	1.4	2.0	-	-
Limonene	1032	6.1	1.5	-	-
$\gamma$ -terpinene	1058	0.4	0.5	-	-
Terpinolene	1063	2.1	1.7	-	-
cis-Sabinene hydrate	1092	-	0.2	-	-
Linalool	1101	1.2	-	-	-
Terpinen-4-ol	1177	0.1	0.3	-	-
$\alpha$ -Terpineol	1241	-	0.1	-	-
Bornyl acetate	1285	0.7	1.3	-	-
$\alpha$ -Terpenyl acetate	1376	0.5	-	-	-
$\beta$ -Elemene	1390	0.7	0.3	-	-
$\beta$ -Cedrene	1417	1.8	0.9	-	-
$\beta$ -Caryophyllene	1423	5.8	4.1	-	-
Thujopsene	1435	2.1	1.7	-	-
$\alpha$ -Humolene	1456	1.0	0.4	-	-
Germacrene-D	1483	2.2	-	-	-
$\Delta$ -Cadinene	1515	0.3	0.3	-	-
Elemol	1541	1.5	0.6	-	-
$\alpha$ -Cedrol	1614	14.6	6.9	-	-
$\alpha$ -Cadinol	1651	0.6	-	-	-
Total		92.9	97.8		

the fruit oil about 66% mortality was achieved after 24 h and 84% mortality after 72 h for *T. castaneum* (Figures 1 and 2).

Probit analysis showed that at exposure time of 24 h, *C. maculatus* was more susceptible than *S. oryzae*, and *T. castaneum* to both leaf and the fruit oils (Tables 2 and 3). Furthermore, with the increase of exposure time to 72 h, mortality increased and LC<sub>50</sub> values decreased to 2.21 and 2.60  $\mu\text{L L}^{-1}$  air for *C. maculatus*, 9.07 and 10.90  $\mu\text{L L}^{-1}$  air for *S. oryzae*, and 22.54 and 25.63  $\mu\text{L L}^{-1}$  air for *T. castaneum* from the leaf and fruits oils, respectively. Moreover, slopes of probit lines estimated that any increase in essential oil concentration, was imposed the least mortality to *C. maculatus* (2.64 and 2.33, respectively, for leaf and fruit essential oils at 72 h) when compared to other tested insects. Furthermore, intercept of probit line for this pest was higher than *S. oryzae* and *T. castaneum*, showing the higher response threshold (Tables 2 and 3, and Figures 1 and 2). Considering the R<sup>2</sup> values, a linear model was fitted for lethal time analysis. LT<sub>50</sub> values were 12.87 and 18.89 h for *C. maculatus*, 15.25 and 14.55 h for *S. oryzae*, and 11.31 and 10.72 h for *T. castaneum* for the leaf and fruits oils, respectively. LT<sub>50</sub> values and their corresponding information were calculated at the highest concentrations (13  $\mu\text{L L}^{-1}$  air for *C. maculatus*, 28  $\mu\text{L L}^{-1}$  air for *S. oryzae*, and 47  $\mu\text{L L}^{-1}$  air for *T. castaneum*). Comparison of slopes of regression lines among three insects showed that *T. confusum* mortality was slowly influenced by time, and conversely, *C. maculatus* mortality was highly affected by time spent when compared with

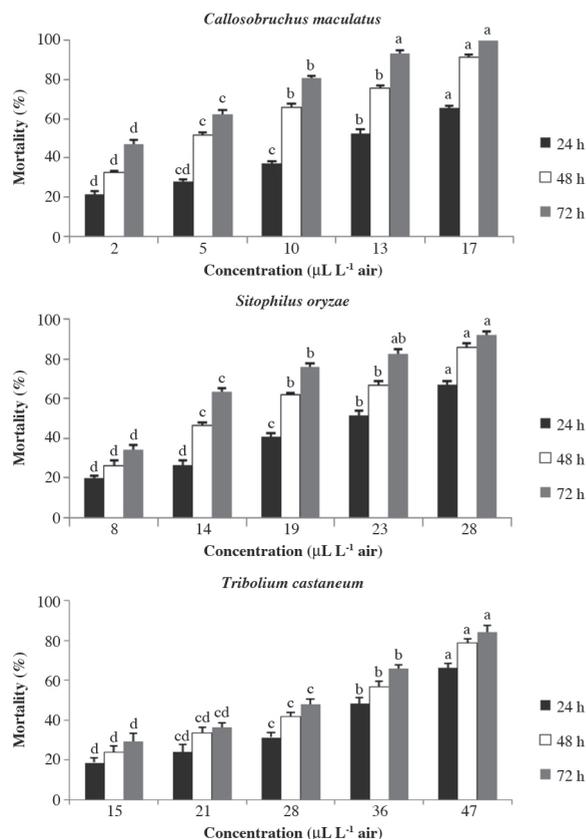


Different letters over columns indicate significant differences according to Tukey test at  $\alpha = 0.01$ . Columns with the same letter are not significantly different. Vertical bars indicate standard error ( $\pm$ ).

**Figure 1. Mean mortality (%) of *Callosobruchus maculatus*, *Sitophilus oryzae*, and *Tribolium castaneum* exposed to different concentrations of *Platycladus orientalis* leaves oil.**

other insects. Intercept of time analysis lines showed that mortality of *C. maculatus* started faster than other tested insects (Tables 2 and 3).

Our study showed that insecticidal activity of *P. orientalis* varied with plant derived material, insect species, different concentrations of the oils and exposure time (Figures 1 and 2). Essential oil of leaves was more toxic than the fruit oil on the three species of insects. In the same way, Jeon *et al.* (2005) revealed that insecticidal activity of *P. orientalis* leaves oils against 4<sup>th</sup>-instar larvae of *Aedes aegypti* and *Culex pipiens pallens* was significantly higher than stem, fruit, and seed oils. The observed differences in the effects produced by the essential oils could be due to the presence of different secondary metabolites in both vegetal organs (Murray *et al.*, 2005). The essential oil of the fruit of *P. orientalis* was tested against *Acanthoscelides obtectus*. The test revealed increased neonate larval mortality (Papachristos and Stamopoulos, 2002). The oils has also shown antimicrobial and fungitoxic properties (Hassanzadeh *et al.*, 2001; Guleria *et al.*, 2008) and anti-tumoral, cytotoxic, and antioxidant effects (Kosuge *et al.*, 1985; Emami *et al.*, 2005; Emami *et al.*, 2011a; 2011b).



Different letters over columns indicate significant differences according to Tukey test at  $\alpha = 0.01$ . Columns with the same letter are not significantly different. Vertical bars indicate standard error ( $\pm$ ).

**Figure 2.** Mean mortality (%) of *Callosobruchus maculatus*, *Sitophilus oryzae*, and *Tribolium castaneum* exposed to different concentrations of *Platycladus orientalis* fruit essential oil.

Extracts of *P. orientalis* have been shown to possess a range of biological activities, including insecticidal action against larvae of malaria and Japanese encephalitis vector (Sharma *et al.*, 2005), control of *Potato leaf roll virus* (PLRV) in potato plants (Al-Ani *et al.*, 2010), antioxidant effects (Nizam and Mushfiq, 2007), and antifungal activity (Alinezhad *et al.*, 2011). The molluscicidal

activity of extracts and leaf powder of *P. orientalis* was studied against the snail *Lymnaea acuminata* (Lamarck) (Singh and Singh, 2009).

In this research the essential oils of leaves and fruits from *P. orientalis* collected from region of Urmia, gave yellowish oils with a yield of 0.3% and 0.95%, respectively, based on fresh weights. The yield of essential oils from leaf is relatively higher than other studies on *P. orientalis* in Iran (Nickavar *et al.*, 2003; Emami *et al.*, 2011a; 2011b). By contrast the yield of fruit oil is lower than other studies in Iran (Hassanzadeh *et al.*, 2001; Nickavar *et al.*, 2003; Emami *et al.*, 2011a; 2011b).

GC-MS analyses of the oils were identified 26 compounds (92.9%) and 23 constituents (97.8%) in the leaf and the fruit oils, respectively (Table 1). The leaf and the fruit oils had compositions similar to those of other *P. orientalis* essential oils analyzed in Iran. Hassanzadeh *et al.* (2001) reported the main components of both the fruit and leaves oils were  $\alpha$ -pinene (23.5%, 15%) sabinene (11.1%, 10%) and  $\alpha$ -cedrol (7.2%, 11.7%). Nickavar *et al.* (2003) also studied the composition of the leaf and fruits oils of *P. orientalis*. The leaf oil contained  $\alpha$ -pinene (21.9%),  $\alpha$ -cedrol (20.3%), and  $\Delta^3$ -carene (10.5%) as main components; while the fruit oil contained  $\alpha$ -pinene (52.4%),  $\Delta^3$ -carene (14.2%), and  $\alpha$ -cedrol (6.5%). Emami *et al.* (2011a) reported the main components of the leaf oil were  $\alpha$ -pinene (15.5%),  $\Delta^3$ -carene (10.8%), and lingofolene (9.8%). In the other hand  $\alpha$ -pinene (37.3%), sabinene (8.0%), and  $\beta$ -phellandrene (7.9%) were the major components of the oil of the fruit of this plant. In the another study the major compounds in both oils with  $\alpha$ -pinene being the major constituent at levels of 14.5% and 39.3% for leaf and fruit oil, respectively (Emami *et al.*, 2011b). Afsharypuor and Nayebzadeh (2009) analyzed oils of young stem, leaf and fruit of *P. orientalis* and reported the main constituents of the fruit oil were  $\alpha$ -pinene (38.7%),  $\Delta^3$ -carene (20.4%), and  $\alpha$ -fenchene (5.0%), while the major components of the leaf oil were  $\alpha$ -pinene (30.0%),  $\Delta^3$ -carene (21.7%), and  $\beta$ -caryophyllene (6.9%). On the other hands, the main constituents of the young stem oil were  $\Delta^3$ -carene

**Table 2.** Results of the leaf oil probit analysis to calculate LC<sub>50</sub>, LC<sub>95</sub>, LT<sub>50</sub>, and LT<sub>95</sub> values.

Insect species	Time	LC <sub>50</sub>	LC <sub>95</sub>	$\chi^2$ [df = 3]	<i>p</i>	Intercept	Slope
<i>Callosobruchus maculatus</i>	24	6.06	52.46	2.23 <sup>a</sup>	0.52	3.63	1.75
	48	2.99	18.06	4.37	0.22	4.00	2.10
	72	2.21	9.27	3.37	0.33	4.09	2.64
<i>Sitophilus oryzae</i>	24	18.22	85.95	1.15	0.76	1.93	2.44
	48	11.08	43.44	1.09	0.77	2.11	2.77
	72	9.07	28.26	1.67	0.64	1.81	3.33
<i>Tribolium castaneum</i>	24	32.07	129.91	0.40	0.94	0.93	2.70
	48	26.31	114.69	1.27	0.73	1.35	2.57
	72	22.54	74.68	2.23	0.52	0.73	3.16
Insect species	LT <sub>50</sub> [h]	LT <sub>95</sub> [h]	$\chi^2$ [df = 1]	<i>p</i>	Intercept	Slope	
<i>C. maculatus</i>	12.87 <sup>b</sup>	37.50	0.55 <sup>a</sup>	0.45	1.07	3.54	
<i>S. oryzae</i>	15.25	48.40	0.56	0.45	1.12	3.27	
<i>T. castaneum</i>	11.31	123.28	0.21	0.64	3.33	1.58	

<sup>a</sup>Since goodness-of-fit Chi square is not significant ( $P > 0.15$ ), no heterogeneity factor is used.

<sup>b</sup>Lethal time values were calculated at the highest concentrations (13 µL L<sup>-1</sup> for *Callosobruchus maculatus*, 28 µL L<sup>-1</sup> for *Sitophilus oryzae* and 47 µL L<sup>-1</sup> for *Tribolium castaneum*).

**Table 3. Results of the fruit oil probit analysis to calculate LC<sub>50</sub>, LC<sub>95</sub>, LT<sub>50</sub>, and LT<sub>95</sub> values.**

Insect species	Time	LC <sub>50</sub>	LC <sub>95</sub>	$\chi^2$ [df = 3]	<i>p</i>	Intercept	Slope
<i>Callosobruchus maculatus</i>	24	9.24	122.33	1.44 <sup>a</sup>	0.69	3.59	1.46
	48	3.98	27.19	1.43	0.69	3.82	1.97
	72	2.60	13.19	3.43	0.32	4.03	2.33
<i>Sitophilus oryzae</i>	24	21.56	107.62	1.18	0.75	1.86	2.35
	48	14.27	54.15	0.90	0.82	1.72	2.84
	72	10.90	36.73	0.16	0.98	1.77	3.11
<i>Tribolium castaneum</i>	24	36.58	148.65	0.81	0.84	0.78	2.70
	48	29.23	107.57	0.96	0.81	0.74	2.90
	72	25.63	89.16	1.09	0.77	0.99	3.03

Insect species	LT <sub>50</sub> [h]	LT <sub>95</sub> [h]	$\chi^2$ [df = 1]	<i>p</i>	Intercept	Slope
<i>C. maculatus</i>	18.89 <sup>b</sup>	50.94	0.51 <sup>a</sup>	0.47	0.13	3.81
<i>S. oryzae</i>	14.55	92.52	0.00	0.94	2.62	2.04
<i>T. castaneum</i>	10.72	245.91	0.00	0.97	3.76	1.20

<sup>a</sup>Since goodness-of-fit Chi square is not significant ( $P > 0.15$ ), no heterogeneity factor is used.

<sup>b</sup>Lethal time values were calculated at the highest concentrations (13  $\mu\text{L L}^{-1}$  for *Callosobruchus maculatus*, 28  $\mu\text{L L}^{-1}$  for *Sitophilus oryzae* and 47  $\mu\text{L L}^{-1}$  for *Tribolium castaneum*).

(24.3%),  $\alpha$ -pinene (15.4%), and cedrol (17.7%).

In comparison with published data, it could be clearly shown that ingredients of the essential oil of fruit and leaves of *P. orientalis* are similar, but with differences in their percentage depending distinctly on the region in which they are grown. Most notable differences observed in the composition of *P. orientalis* grown in Urmia (Nazlo) included the absence of *cis*-thujopsene, camphene,  $\alpha$ -copaene, *O*-cymene, *cis*-*p*-menth-2-en-1-ol, and viridiflorene, and the high percentage of  $\alpha$ -pinene 35.2 and 50.7% for leaves and the fruit, respectively (Hassanzadeh *et al.*, 2001; Nickavar *et al.*, 2003; Afsharypuor and Nayebzadeh, 2009; Emami *et al.*, 2011a; 2011b).

$\alpha$ -Pinene, a monoterpenoid, is the major component in *P. orientalis* essential oil. There are numerous reports on biological activity of  $\alpha$ -pinene. Ojmelukwe and Adler (1999) found  $\alpha$ -pinene was toxic to *Tribolium confusum* du Val. The antifeedant and growth inhibitory effects of this monoterpene toward *T. castaneum* were observed by Huang *et al.* (1998).  $\alpha$ -Pinene possesses important repellent effects toward *T. confusum* (Tapondjou *et al.*, 2005), and has shown strong fumigant toxicity against *Acanthoscelides obtectus* (Regnault-Roger and Hamraoui, 1995).

Limonene,  $\beta$ -caryophyllene, myrcene,  $\rho$ -cymene, terpinolene, and bornyl acetate are the other components of *P. orientalis* oil that have insecticidal activity. For example, limonene had insecticidal and repellent bioactivities to *T. castaneum* (Lee *et al.*, 2002; Garcia *et al.*, 2005).  $\beta$ -Caryophyllene from *Eupatorium betonicaeforme* (D.C.) Baker (Asteraceae) has been reported as larvicidal toward *Aedes aegyptii* (L.) (Albuquerque *et al.*, 2004). Toxic effect of myrcene have been evaluated on *S. oryzae* (Coats *et al.*, 1991),  $\rho$ -cymene had fumigant toxicity on *Acanthoscelides obtectus* (Regnault-Roger and Hamraoui, 1995) and terpinolene and bornyl acetate showed contact and fumigant toxicity against *S. oryzae* (Park *et al.*, 2003).

So the toxic effects of *P. orientalis* oil could be attributed to  $\alpha$ -pinene and other components. As major constituents of *P. orientalis* are monoterpenoids, they are

typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions (Lee *et al.*, 2002). Due to their high volatility they have fumigant activity that might be of importance for controlling stored-product insects (Ahn *et al.*, 1998).

Experiments showed that *T. castaneum* is more tolerant than *S. oryzae* and *C. maculatus* (Tables 2 and 3). Negahban and Moharrampour (2007) reported fumigant toxicity of essential oils from *Eucalyptus intertexta* R.T. Baker, *Eucalyptus sargentii* Maiden and *Eucalyptus camaldulensis* Dehnh (Myrtaceae) against *T. castaneum*, *S. oryzae*, and *C. maculatus*. The LC<sub>50</sub> values to the selected essential oils were between 2.55 and 3.97  $\mu\text{L L}^{-1}$  air for *C. maculatus*, 6.93 and 12.91  $\mu\text{L L}^{-1}$  air for *S. oryzae*, and 11.59 and 33.50  $\mu\text{L L}^{-1}$  air for *T. castaneum*. Sahaf *et al.* (2007) studied fumigant toxicity of *Carum copticum* C.B. Clarke (Apiaceae) essential oil against *S. oryzae* and *T. castaneum*. They reported that *T. castaneum* (LC<sub>50</sub> = 33.14  $\mu\text{L L}^{-1}$  air) was significantly more tolerant than *S. oryzae* (LC<sub>50</sub> = 0.91  $\mu\text{L L}^{-1}$  air). Ogendo *et al.* (2008) demonstrated that *T. castaneum* was more tolerant than other tested species. LC<sub>50</sub> values for *S. oryzae*, *Rhyzopertha dominica* (F.), *Oryzaephilus surinamensis* (L.), and *Callosobruchus chinensis* (L.) adults ranged from 0.20 to 14  $\mu\text{L L}^{-1}$  air, 0.01 to 17  $\mu\text{L L}^{-1}$  air, and 0.80 to 23  $\mu\text{L L}^{-1}$  air 24 h after treatment with *Ocimum gratissimum* L. (Lamiaceae) essential oil, eugenol, and *b*-(*Z*)-ocimene, respectively. Fumigant toxicity of *Artemisia sieberi* Besser (Asteraceae) essential oil against *T. castaneum*, *S. oryzae*, and *C. maculatus* was evaluated by Negahban *et al.* (2007). *Callosobruchus maculatus* was significantly more susceptible than *S. oryzae* and *T. castaneum*; the LC<sub>50</sub> values were 1.45  $\mu\text{L L}^{-1}$  air for *C. maculatus*, 3.86  $\mu\text{L L}^{-1}$  air for *S. oryzae*, and 16.76  $\mu\text{L L}^{-1}$  air for *T. castaneum*. Fumigant toxicity of *Vitex pseudonegundo* (Hausskn.) Hand.-Mazz. (Lamiaceae) essential oil against *T. castaneum* and *S. oryzae* was evaluated by Sahaf *et al.* (2008), demonstrating that *S. oryzae* (LC<sub>50</sub> = 31.96  $\mu\text{L L}^{-1}$  air) was more susceptible than *T. castaneum* (LC<sub>50</sub> = 47.27  $\mu\text{L L}^{-1}$  air). These

findings are consistent with the results of this study as *C. maculatus* was more susceptible to the essential oils, and *T. castaneum* was more tolerant than *S. oryzae* and *C. maculatus*.

## CONCLUSIONS

This study demonstrated that the essential oil from the leaf of *P. orientalis* was more toxic than the fruit oil on *C. maculatus*, *S. oryzae*, and *T. castaneum*. Both oils in the same concentration were tested on each species and *T. castaneum* was more tolerant than other species. GC-MS analysis of the oils revealed that the percentage of monoterpene hydrocarbons was higher than other components. The major hydrocarbon was  $\alpha$ -pinene both in the leaf and the fruit. Therefore, oils of *P. orientalis* possess a potential for use in the management of *S. oryzae*, *T. castaneum*, and especially *C. maculatus*. Further studies need to be conducted on these essential oils against other insects (e.g. *Rhyzopertha dominica*).

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### Componentes químicos y toxicidad de aceites esenciales de tuya oriental, *Platyclusus orientalis* (L.) Franco, contra tres escarabajos de productos almacenados.

Los metabolitos secundarios de las plantas juegan un papel importante en las interacciones planta-insecto, y por lo tanto pueden tener actividad insecticida o biológica en los insectos. La toxicidad fumigante de los aceites esenciales de hojas y frutos del árbol oriental de la vida (*Platyclusus orientalis* (L.) Franco) (Cupressaceae) fue investigada contra adultos de gorgojo del guisante (*Callosobruchus maculatus* Fab.), gorgojo del arroz (*Sitophilus oryzae* L.), y escarabajo rojo de la harina (*Tribolium castaneum* Herbst). Las hojas frescas y las frutas fueron sometidas a hidrodestilación utilizando un aparato tipo Clevenger y la composición química de los aceites volátiles se estudió por cromatografía de gas-espectrometría de masa (GC-MS). Se identificaron 26 (92,9%) y 23 compuestos (97,8%) en los aceites de hoja y de frutos, respectivamente. Los componentes principales de los aceites de hojas y frutos fueron  $\alpha$ -pineno (35,2%, 50,7%),  $\alpha$ -cedrol (14,6%, 6,9%) y  $\Delta$ -3-careno (6,3%, 13,8%), respectivamente. Ambos aceites fueron probados en la misma concentración por su toxicidad fumigante en cada especie. Los aceites de hoja fueron más tóxicos que los de fruto contra tres especies de insectos. *Callosobruchus maculatus* fue más susceptible que *S. oryzae*, y *T. castaneum*. Los valores de CL<sub>50</sub> de aceites de hojas y frutos a las 24 h se estimó 6,06 y 9,24  $\mu$ L L<sup>-1</sup>de aire de *C. maculatus*, 18,22 y 21,56  $\mu$ L L<sup>-1</sup>de aire para *S. oryzae*, y 32,07 y 36,58  $\mu$ L L<sup>-1</sup>de aire para

*T. castaneum*, respectivamente. Estos resultados sugieren que aceites de *P. orientalis* pueden tener potencial como agente de control de *C. maculatus*, *S. oryzae*, y *T. castaneum*.

**Palabras clave:** insecticidas botánicos, fumigación, *Platyclusus orientalis*, insectos de productos almacenados.

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