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Fumigant toxicity of essential oils of leaves and fruits of *Platycladus orientalis* to *Lasioderma serricorne* (F.)

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Abstract. Fumigant toxicity of essential oils of leaf and fruit from oriental arborvitae, *Platycladus orientalis* (Cupressaceae), was investigated against adults of cigarette beetle, *Lasioderma serricorne*. Fresh leaves and fruits were subjected to hydrodistillation using a Clevenger-type apparatus. The chemical composition of the volatile oils was analyzed by GC-MS. Twenty-six (92.9%) and twenty-three constituents (97.8%) were identified in leaves and fruit oils, respectively. The major components of both leaves and fruit oils were α-Pinene (35.2%, 50.7%), α-Cedrol (14.6%, 6.9%) and Δ-3-Carene (6.3%, 13.8%), respectively. Both oils in the same concentration were tested for their fumigant toxicity on adult insects. Results showed that leaf oil was more toxic than the fruit oil. LC_{50} values of leaf and fruit oils at 24 h were estimated 43.86 µl/l air and 59.69 µl/l air, respectively. These results suggested that *P. orientalis* oils may have potential as control agents against *L. serricorne*.

Key words: Cigarette beetle, fruit oil, fumigant toxicity, *Lasioderma serricorne*, leaf oil, *Platycladus orientalis*.

Introduction

The cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), is known to successfully breed and develop on a variety of grain-based products, spices, and tobacco, and infest these commodities during storage, manufacturing and at the retail level (Howe 1957, Bry et al. 1974, Jacob 1992). Control of *L. serricorne* populations around the world is primarily dependent upon continued applications of phosphine (PH_3) (White & Leesch 1995, Kim et al. 2003). The use of phosphine is increasing due to the convenience of formulations, the relatively short-term hazard, and low retention of residues (Lee et al. 2002). However, PH_3 fumigation may become increasingly limited in use because resistance of stored grain insects to phosphine has been discovered in more than 45 countries. (Bell & Wilson 1995, Chaudhry 1995). Naturally occurring substances are an alternative to conventional pesticides and plant essential oils have traditionally been used to kill insects (Isman 2000). Essential oils are potential sources of alternative compounds to currently used fumigants. Essential oils have low toxicity to warm-blooded animals, high volatility and strong toxicity to insect pests (Lee et al. 2002). Some works have been done to manage *L. serricorne* by using aromatic medicinal plants despite their excellent pharmacological actions (kim et al. 2003, Ebadollahi et al. 2010). Essential oils derived from plant species of *Platycladus* have been evaluated for insecticidal properties (Keita et al. 2000, Pavela 2005, Jeon et al. 2005). However, no report on insecticidal activity of essential oils of *P. orientalis* against *L. serricorne* is available.

Platycladus orientalis (L.) Franco [*Thuja orientalis* L.] locally named Sarv-e Khomreii or Nosh is an evergreen species, which grows naturally in Iran. In addition, this species is widely cultivated as a common ornamental plant in Iran and other countries (Assadi 1998). *Platycladus* is a genus from Family Cupressaceae, with five species, two native to North America and three native to eastern Asia, they are commonly known as arborvitae (Vines 1987). *Platycladus* was an old remedy for delayed menstruation, being also a stimulant to smooth muscles such as those of uterus and bronchial passages so it is used for treatment of bronchitis (Mabey et al. 1948) and also as cough suppressant in traditional Chinese medicine (Takao et al. 1985). The present study was conducted to determine the efficiency of the essential oils from *P. orientalis* as a fumigant in the management of *L. serricorne*.

Materials and methods

Insect culture

Lasioderma serricorne was reared in glass containers (1 liter) containing wheat flour that was covered by a fine mesh cloth for ventilation. The culture was maintained in the dark in an incubator set at 27±2°C and 60±5% RH. Parent adults were obtained from laboratory stock cultures maintained at the Department of Plant Protection, Urmia University, Iran. Adult insects, 7-14 days old, were used for fumigant toxicity tests. 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 period 2010 from the shrubs cultivated in the Department of Horticultural University of Urmia, the region of Nazlo, 12 km in the west of Urmia (latitude: 37° 32', longitude: 45° 05'; altitude: 1313 m), 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 were transferred to the glass flasks that were filled to the top and were 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 × 0.25 mm; 0.25 µm film thickness). The carrier gas was helium with flow 1 ml/min. The oven temperature was held at 60°C for 3 min, programmed at 6°C /min 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* 35-350 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).

Bioassay

To determine the fumigant toxicity of the *P. orientalis* oils, same concentrations of the leaf and the fruit oils including 8, 10.4, 13.5, 17.6 and 22.8 µl with 280 ml capacity jars, corresponding to 22.4, 29.1, 37.8, 49.2, and 63.8 µl/l air were tested on *L. serricorne.* They were applied to filter papers (Whatman No. 1, cut into 4 × 5 cm paper strip) and put into 280 ml glass jars. Doses were applied based on calculated concentrations. Twenty adults of *L. serricorne* (7-14 days 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 airtight lids. Mortality was determined after 24, 48 and 96 h from commencement of the exposure. Each concentration and control were replicated four times. In the control jars, no essential oils were used.

Data analysis

Mortality percentages were calculated by the Abbott correction formula for natural mortality in the untreated control (Abbott, 1925). Tests were arranged as completely randomized design and data were analyzed by one-way ANOVA using the SAS software version 9.1. Lethal concentrations (LC_{50} and LC_{95}) and lethal time values (LT_{50} and LT_{95}) were calculated with SPSS software (version 16.0). Comparison of means were done through Tukey's test (α=0.01).

Results

Chemical constituents of essential oils

The main components of both leaves and fruits essential oils from *P. orientalis* were *a*-Pinene (35.2%, 50.7%), *a*-Cedrol (14.6%, 6.9%), Δ-3-Carene (6.3%, 13.8%), and Limonene (6.1%, 1.5%), respectively (Table 1).

Bioassay

The insecticidal activity varied with plant-derived material, different concentrations of the oils and exposure time. The effects of fumigation of essential oils of leaves and fruits of *P. orientalis* were very toxic on adult of *L. serricorne*. Probit analysis showed that at exposure time of 24 h, *L. serricorne* was more susceptible to leaf oil with LC_{50} = 43.86 µl/l air than fruit oil LC_{50} = 59.69 µl/l air (Tables 2 and 3). Furthermore, with the increase of exposure time to 96 h, mortality increased and LC_{50} values decreased to 24.84 µl/l air and 28.6 µl/l air for the leaf and fruits oils, respectively (Tables 2, 3 and Fig. 1). LT_{50} values and their corresponding information were calculated at the different concentrations of oils (Tables 2 and 3).

According to the results of ANOVA, the mortality percents significantly increased depending on the increase in the essential oils concentration. Adults of *L. serricorne* were significantly influenced in a different manner being exposed to essential oils.

Discussion

Essential oil from the leaf oil was more toxic than the fruit oil on the insect (Fig. 1). Papachristos & Stamopoulos (2002) tested 13 essential oils against *Acanthoscelides obtectus* (Say). They reported that the fruit oil of *P. orientalis* had lowest repellent activity in comparison with other oils. Evaluation of larvicidal activities of *P. orientalis* oils against 4th-instar larvae of *Aedes aegypti* and *Culex pipiens pallens* was revealed by Jeon et al. (2005). Larvicidal activities of leaf oils were significantly higher than stem, fruit, and seed oils. The essential oils from leaves and fruits at 400 ppm exhibited 100 and 71.6% mortalities against *A. aegypti*, and 100 and 53.1% against *C. pipiens*, respectively. However, the essential oils of stems and seeds showed no mortality at 400 ppm. The essential oils of leaves and fruits exhibited 100 and 18.7% mortalities against *A. aegypti* and 92.1 and 18.7% against *C. pipiens* at 200 ppm, respectively. 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).

Papachristos & Stamopoulos (2002) tested the essential oil of the fruit of *P. orientalis* against *Acanthoscelides obtectus*. The tests revealed that the oil reduced fecundity, decreased egg hatchability, increased neonate larval mortality and adversely influences offspring emergence. The oils of *P. orientalis* revealed larvicidal activity against two mosquito species (Jeon et al. 2005). Extracts of *P. orientalis* have shown insecticidal action against larvae of malaria and Japanese encephalitis vector (Sharma et al. 2005). Molluscicidal activity of extracts and leaf powder of plant was studied against the snail *Lymnaea acuminata* (Lamarck) (Singh & Singh 2009).

Based on fresh weights, the essential oils of leaves and fruits gave yellowish oils with a yield of 0.3% and 0.95%, respectively. GC-MS analyses of the oils identified twenty-six compounds (92.9%) and twenty-three constituents (97.8%) in the leaf and the fruit oils, respectively (Table 1). Leaf and fruit oils had major compositions similar to those of other *P. orientalis* essential oils analyzed in Iran (Hassanzadeh et al. 2001, Nickavar et al. 2002, Afsharypuor & Nayebzadeh 2009, Emami et al. 2011a, b). There are few previous reports on the phytochemical studies of the oil of *P. orientalis* in other regions of the world (Emami et al. 2011b). Zhu et al. (1995) reported that the yield of leaf oil of *P. orientalis* from China was 0.3% and had 38 constituents. The major components were α-pinene (4.3%), Δ-carene (6.1%) and cedrol (22.3%). Tanker et al. (1977) showed that the amount and main components of the leaf oil of two different samples of *P. orientalis* collected from Turkey in two different regions of Anatolia (Mersin and Ankara) in different times (August and January) were different. The main components of the Mersin oil were a mixture of α-pinene and α-thujene (33.5%), β-Pinene (16.6%), a mixture of Myrcene and α-phellandrene (12.5%), limonene (12.0%), and Δ3-carene (8.7%). The main components of the Ankara January oil sample were a mixture of α-pinene and α-thujene (32.0%), β-Pinene (23.1%), sabinene (14.3%), limonene (8.2%), and a mixture of myrcene and α-phellandrene (7.11%). While the major constituents of Ankara August sample oil were a mixture of α-pinene and α-thujene (51.8%), limonene (11.3%), β-Pinene (10.8%) and a mixture of myrcene and α-phellandrene (8.5%). The yield oils were 1.66% for the sample collected from Mersin and 0.79% from Ankara (in January) and 0.84% (in August), respectively.

In comparison with the published data, it can be clearly shown that the ingredients of the essential oils of leaves and the fruit of *P. orientalis* are similar, but with differences in

Table 1. Main chemical components (%) of the leaf and the fruit oils of *Platycladus orientalis.*

Compound	Retention Index	% Leaf oil	% Fruit oil
α-Pinene	936	35.2	50.7
Sabinene	971	1.5	2.1
Myrcene	993	3.3	3.8
α-Phellandrene	1005	1.6	2.1
Δ3-Carene	1013	6.3	13.8
ρ-Cymene	1021	1.4	2.0
Limonene	1032	6.1	1.5
Terpinolene	1063	2.1	1.7
β-Caryophyllene	1423	5.8	4.1
a-cedrol	1614	14.6	6.9
Total		92.9	97.8

Table 2. Result of the leaf oil probit analysis to calculate LC_{50}, LC_{95}, LT_{50}, and LT_{95} values.

Insect species	Time	LC_{50}	LC_{95}	χ2 [df=3]	*p*	Intercept	Slope
	24	55.00	117.13	0.27[a]	0.96	-3.72	10.01
L. serricorne	48	43.86	102.48	0.36[a]	0.94	-2.31	9.45
	96	24.84	38.73	5.60[b]	0.13	-6.90	13.53
Concentrations (µl/l air)		LT_{50} [h]	LT_{95} [h]	χ2 [df = 1]	*P*	Intercept	Slope
22.4		104.69	295.10	0.03[a]	0.86	-2.38	8.65
29.1		89.63	421.66	0.04[a]	0.83	0.23	7.44
37.8		43.92	109.49	6.27[b]	0.10	-1.81	9.14
49.2		32.12	105.01	4.51[b]	0.03	0.19	8.19
63.8		21.19	73.54	1.16[a]	0.28	0.87	8.04

[a] Since goodness-of-fit Chi square is not significant (P > 0.15), no heterogeneity factor is used.
[b] Since goodness-of-fit Chi square is significant (P < 0.15), a heterogeneity factor is used.

Table 3. Result of the fruit oil probit analysis to calculate LC_{50}, LC_{95}, LT_{50}, and LT_{95} values.

Insect species	Time	LC_{50}	LC_{95}	χ2 [df=3]	*p*	Intercept	Slope
	24	59.69	116.33	0.67[a]	0.87	-5.08	10.67
L. serricorne	48	50.75	127.29	0.41[a]	0.93	-2.02	9.11
	96	28.56	49.87	0.24[a]	0.97	-4.89	11.79
Concentrations (µl/l air)		LT_{50} [h]	LT_{95} [h]	χ2 [df = 1]	*P*	Intercept	Slope
22.4		157.97	577.56	0.35[a]	0.55	-1.42	7.92
29.1		94.16	293.81	0.00[a]	0.96	-1.57	8.32
37.8		59.72	208.51	1.79[a]	0.18	-0.38	8.02
49.2		39.13	147.24	3.67[b]	0.05	0.45	7.85
63.8		24.89	88.55	2.42[b]	0.12	0.84	7.98

[a] Since goodness-of-fit Chi square is not significant (P > 0.15), no heterogeneity factor is used.
[b] Since goodness-of-fit Chi square is significant (P < 0.15), a heterogeneity factor is used.

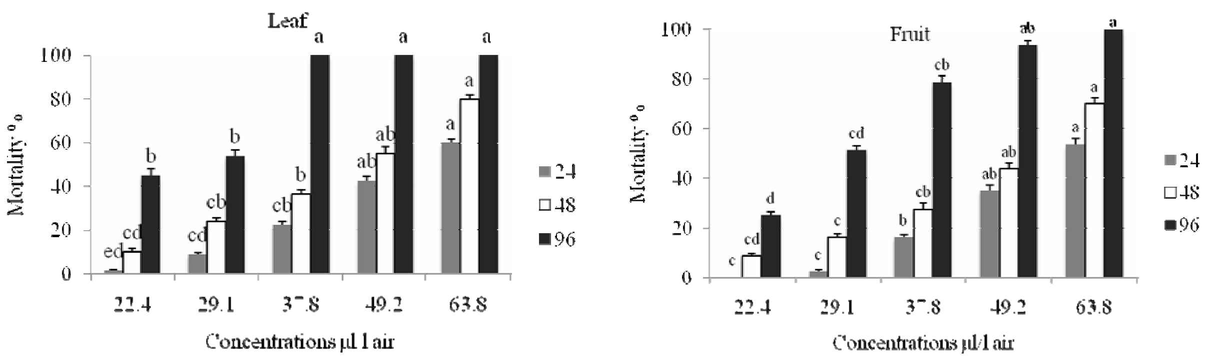

Figure 1. Mean mortality (%) of *Lasioderma serricorne* exposed to different concentrations of the leaf and the fruit oils of *Platycladus orientalis*. (Different letters over similar columns indicate significant differences according to Tukey test, *P<0.001*).

their percentage depending distinctly on the region in which they are grown. Most notable differences observed in the composition of *P. orientalis* grown in Iran included the low percentage of α-thujene, β-Pinene, γ-terpinene, and α-Phellandrene, the absence of Δ-carene, and the high percentage of α-pinene (Tanker et al. 1977, Zhu et al. 1995).

The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Coats et al. 1991, Konstantopoulou et al. 1992, Regnault-Roger & Hamraoui 1995). Monoterpenoids 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).

The toxic effects of *P. orientalis* can be attributed to major constituents such as α-Pinene. The monoterpene α-pinene might have broad insecticidal activity against stored-product insects and act as the fumigant in *P. orientalis* oil. There are numerous reports on biological activity of α-pinene. Ojimelukwe & Adler (1999) found α-pinene to be toxic to *Tribolium confusum* (du Val). Huang et al. (1998) reported antifeedant and growth inhibitory effects of this monoterpene toward *Tribolium castaneum* (Herbst). α-pinene possesses important repellent effects toward *Tribolium confusum* (Tapondjou et al. 2005), and has shown strong fumigant toxicity against *Acanthoscelides obtectus* (Regnault-Roger & Hamaroui 1995).

Limonene (6.1, 1.5%), β-caryophyllene (5.8, 4.1%), myrcene (3.3, 3.8%), ρ-cymene (1.4, 2.0%), and terpinolene (2.1, 1.7%) are the other components of leaf and the fruit of *P. orientalis* oils, respectively, that have insecticidal activity. For example, Limonene had insecticidal and repellent activities to *Tribolium castaneum* (Lee et al. 2002; Garcia et al. 2005). Albuquerque et al. (2004) reported that β-caryophyllene from *Eupatorium betonicaeforme* (D.C.) Baker (Asteraceae) had larvicidal effect toward *Aedes egyptii* (L.). Toxic effect of myrcene has been reported on *Sitophilus oryzae* (L.) (Coats et al. 1991), ρ-cymene had fumigant toxicity on *Acanthoscelides obtectus* (Regnault-Roger & Hamraoui, 1995) and terpinolene showed contact and fumigant toxicity against *Sitophilus oryzae* (Park et al. 2003).

The insecticidal activity of an essential oil could be attributed either to the major compound of the oil, or to the synergic and/or antagonistic effects of all the components of the oil. Krishnarjah et al. (1985) demonstrated that the association of p-cymene and β-pinene resulted in a higher toxicity in *Sitotroga cerealella* (Olivier) than that of the components used separately.

We observed that, the insecticidal activity of the essential oils of *P. orientalis* varied according to the exposure time rather than the dose. Rahman & Schmidt (1999) studied the effect of *Acorus calamus* (L.) (Araceae) essential oil vapours from various origins on *Callosobruchus phaseoli* (Gyllenhal). In that study, the period of exposure appeared to be the most important factor affecting the efficiency of the vapours rather than dosage. Su (1991) reported similar results for the contact toxicity of *A. calamus* oil to adults of *Callosobruchus maculatus* (F.), *Sitophilus oryzae* and *L. serricorne*.

According to the results obtained from the current study, it is suggested that *P. orientalis* essential oils can be used to control stored-product insect pest. However, further studies also need to evaluate the cost, efficacy, negative effects (residues and resistance) and safety of these essential oils on wide range of pests (e.g. *Rhyzopertha dominica* (Fabr.)) in commercial store.

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