

## Toxicity and repellence of aqueous and ethanolic extracts from *Schinus molle* on elm leaf beetle *Xanthogaleruca luteola*

Amanda Huerta<sup>a,\*</sup>, Italo Chiffelle<sup>b</sup>, Karla Puga<sup>a</sup>, Fernando Azúa<sup>a</sup>, Jaime E. Araya<sup>c</sup>

<sup>a</sup> Depto. de Silvicultura, Facultad de Ciencias Forestales y Conservación de la Naturaleza, Universidad de Chile, Av. Santa Rosa 11315 La Pintana, Casilla 9206, Santiago, Chile

<sup>b</sup> Depto. de Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile

<sup>c</sup> Depto. de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile

### ARTICLE INFO

#### Article history:

Received 23 December 2009

Received in revised form

5 April 2010

Accepted 13 April 2010

#### Keywords:

Botanical insecticides

Antifeeding effect

Leaf extracts

### ABSTRACT

Extracts of leaves of *Schinus molle* Rev L. (Anacardiaceae) obtained with water or ethanol as solvents were evaluated in the laboratory for their insecticidal effect on adults of the elm leaf beetle, *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), at 2.5, 3.0, 4.3, and 5.6% w/v for the aqueous extracts and, 2.0, 2.5, 3.5, 4.3, and 4.7% w/v for the ethanol extracts. The extracts were applied onto leaves of elm trees (*Ulmus* sp., Ulmaceae) to observe the feeding of adults, and later their effectiveness and to obtain the LC<sub>50</sub>. Both extracts were effective and caused mortalities greater than 97% with the ethanol extract at the two highest concentrations (4.3 and 4.7% w/v), and near 27% with water at 4.3 and 5.6% w/v. The LC<sub>50</sub> of the ethanol extract, calculated through Probit analysis, was 1.88% w/v on the 2nd day, lower than the LC<sub>50</sub> of 8.52% w/v on the 4<sup>th</sup> day achieved by the aqueous extract. Additionally, the antifeeding effect of both extracts on adults was determined. The aqueous extract inhibited feeding completely (100%), whereas the ethanol extract did not cause any antifeeding effect.

© 2010 Elsevier Ltd. All rights reserved.

### 1. Introduction

The use of chemical insecticides has been a fundamental tool for pest control, but it has had serious consequences such as intoxication of people and animals, contamination of water, air, and soil, residues on food, high persistence in the environment, resistance in pests, and impact on beneficial insects, among other effects (Rodríguez et al., 2003; Regnault-Roger et al., 2004). This has motivated the search for alternative pest controls without the negative effects of synthetic insecticides. Thus, botanical insecticides have become a more ecological and natural alternative for insect control (Rodríguez et al., 2003).

Secondary metabolites in plants function as a means for defense against possible damages by insects and are extracted directly from plants (Carrero, 1996). Many plants synthesize secondary metabolites, such as alkaloids, polyphenols, terpenoids, steroids, essential oils, lignans, sugars and fatty acids, which have important biological properties against insect pests (Silva et al., 2002; Regnault-Roger et al., 2004). The effect of most plants that are used in pest control, more than being insecticidal are insectistatic (growth regulators) (Silva et al., 2002), because they inhibit normal

development of insects. Some plants inhibit feeding in several ways: repelling feeding, and by suppressing or deterring it; others inhibit growth, egg production and development (Metcalfe and Metcalf, 1992; Rodríguez et al., 2003).

Plant extracts have advantages and disadvantages as insecticides. Among the first are their rapid action and degradation, low mammalian toxicity, selectivity, and minimal impact on plants (Cloyd, 2004). As these botanical insecticides possess molecules derived from the secondary metabolism of plants, they exhibit a limited effect on beneficial insects; besides they are rarely toxic to mammals and man, and as they have different action mechanisms, development of resistance in insects is limited (Regnault-Roger et al., 2004). Some disadvantages are high costs, low availability, as well as scarce data of effective results (Cloyd, 2004).

*Schinus molle* L. (Anacardiaceae) is a tree from the Andean region of America, mainly in Peru, its center of origin. This species would have been introduced into Chile from Peru during the Inca period (Silva et al., 2005). It grows naturally in Chile in the Tarapacá province, but it has extended south reaching Santiago (Donoso, 2006).

All plant parts are being used in traditional medicine, as antispasmodic, antiviral, antiseptic, astringent, digestive, purgative, diuretic, healing, coagulant, for fractures, rheumatism, menstrual disorders, pneumonia (Joker et al., 2002; Murray et al., 2005; Donoso, 2006; Ferrero et al., 2006), and anti swelling (Yueqin et al., 2003).

\* Corresponding author.

E-mail addresses: [ahuerta@uchile.cl](mailto:ahuerta@uchile.cl), [ahuertaf@gmail.com](mailto:ahuertaf@gmail.com) (A. Huerta).

*S. molle* has active substances, like terpenes (mainly mono- and sesquiterpenes), tannins, alkaloids, flavonoids, saponins, gums, lipoic acid, oleoresins, mainly in leaves and fruits (Wimalaratne et al., 1996; Steinbauer and Wanjura, 2002; Ferrero et al., 2007; Hayouni et al., 2008), which have diverse properties, such as those mentioned. Some studies of insecticidal and repellent effects of extracts from *S. molle* in different insects have been published recently (Ferrero et al., 2006, 2007; Abdel-Sattar et al., 2009).

In recent years diverse insect pest species have entered Chile, due to trade opening and the greater commercial and travel exchange (Baldini et al., 2005). Thus, one of the world most severe defoliators of elm trees (*Ulmus* spp., Ulmaceae), the elm tree leaf beetle *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae) has been detected in urban trees in the cities of Los Andes and some communes of Santiago city and other regions in central Chile. Elm trees are used in this country mainly as ornamental, cold-resistant trees, adaptable to diverse soils and their importance is given mainly from its high presence in green areas and avenues of Santiago communes. According to Romanyk and Cadahia (2002), this mainly single host pest affects all class of elm trees, at any age. In addition, Muñoz et al. (2003) indicate some infestations in the genus *Aesculus* (e.g. *A. hippocastanum* L., or Indian chestnut tree).

*X. luteola* is distributed in all Europe, northern Africa, the Caucasus, and the near East (Romanyk and Cadahia, 2002). This beetle was introduced into the US from Europe approximately around 1830; was first reported in California in 1920 (Romanyk and Cadahia, 2002; Maistrello et al., 2005), and since then it has become one of the most important urban tree pests in several US states (Dreistadt et al., 2001), as well as in Canada (Romanyk and Cadahia, 2002) and Australia (Leofe, 2002). It is present also in Germany (Meiners et al., 2005), Iran (Sendi et al., 2005; Shekari et al., 2008), Spain (Romanyk and Cadahia, 2002), Portugal (Escada et al., 1979), and Argentina (Defagó et al., 2006). In Chile, *X. luteola* was first detected in 1994 in the city of Los Andes (Valparaíso region), and then in the O'Higgins, Maule, Bío-Bío, Araucanía and Metropolitan regions (SAG, 2010), although Askevold (1991) mentions that the first registry of an adult in Ritoque (a coastal town north of Valparaíso) dates back to April 1982.

Both adults and larvae feed on the parenchyma of leaves, without consuming the veins, and sometimes the damage may affect all the foliage and trees become brown in color. If damages are severe and occur several years in a row, the trees develop deformed canopies, and suffer vigor losses, physiological disorders, and reduced photosynthesis, which predisposes them to the action of other pests, plant disease and stress factors. They become particularly susceptible to scolytid beetles carrying spores of the fungus *Ceratocystis novo ulmi* Brasier, which causes the elm tree disease, a serious threat to survival of these trees (Romanyk and Cadahia, 2002; Muñoz et al., 2003). Defoliation reduces also tree shade in summer and the aesthetical values of elms (Dreistadt et al., 2001).

Given the studies of the insecticide properties of *S. molle* on some insects, and its presence in Santiago and other cities in Chile, the objective of this study was to evaluate in the laboratory the insecticidal effect of leaf extracts on *X. luteola*, to offer new alternatives of selective control of this pest of elm trees in urban areas.

## 2. Materials and methods

The plant material were leaves from *S. molle* obtained from adult trees in the Antumapu Campus of the University of Chile (33°34'S; 70°38'W) in Santiago, Chile, during the summer of 2008. The individuals of *X. luteola* were collected from adult *Ulmus* sp. trees in the Municipality of Maipú, Santiago, during the same period.

Distilled water and 96% p.a. ethanol (Merck) were used to prepare the extracts from *S. molle* leaves.

### 2.1. Collecting and rearing *X. luteola*

Last stage *X. luteola* larvae were collected and taken inside cotton bags to the Forestry Entomology Laboratory at the College of Forestry and Nature Conservation Sciences of the University of Chile. The insects were set in Petri dishes lined with filter paper in the base, wet with distilled water, and were fed with fresh elm tree leaves until they developed into pupae. At this stage they were given only humidity and were covered with more leaves. When the adults emerged, they were fed with fresh leaves and were later used in the bioassays.

### 2.2. Collecting leaves

Approximately around 1 kg leaves from *S. molle* were obtained randomly from different trees, to avoid effects from any individual tree. Mature leaves (hard and dark green) of diverse size were chosen.

### 2.3. Elaboration of extracts

The leaf extracts were prepared at the Chemistry Laboratory of the Department of Agriculture Industry and Enology of the College of Agronomic Sciences, University of Chile. The leaves of *S. molle* obtained were dried in a Memmert® 854 forced air stove at 60 °C, until constant weight in a Shimadzu ELBL 3000 analytical scale, 0.1 g sensibility, max. 3000 g; initial water content was 59.36%. Later, the dried leaves were grinded with an Ufesa® MC 0360 grain mill until obtaining a dust, which was stored in sealed and dated glass vials. To prepare the extracts, this *S. molle* leaf dust was mixed with distilled water or ethanol, in a solution at the highest possible concentration.

The solutions were shaken 18 h in a Heidolph® MR 3001 K magnetic stirrer, heating the first hour to 37 °C. They were then filtered through a Whatman N°1 filter paper and centrifuged in a HN-S centrifuge for 15 min, after which the solutions were filtered again to obtain the stock solution.

To determine the concentration of the base extracts, a fraction of the solutions were dried at 100 °C for 1 h in a forced air stove, and soluble solids were weighed on a Boeco Equilab analytical scale, 0.1 mg sensibility, max. 120 g. Once the concentrations of the solutions were determined, the extracts were prepared at the concentrations to be used in the treatments by dilution. Concentrations of 2.0, 2.5, 3.5, 4.3, and 4.7% w/v were used for the ethanol extracts, and 2.5, 3.0, 4.3, and 5.6 % w/v for the water extracts.

### 2.4. Evaluation of insecticide efficacy in bioassays

The bioassays were set on experiment units with three adults of *X. luteola* with fresh elm tree leaves on wet filter paper in a 10 cm diameter clean Petri dish. The efficacy of *S. molle* leaf extracts were evaluated at the concentrations indicated above for both solvents, plus the controls without any extract, with three replicates, all conducted simultaneously. The extracts were applied by immersing elm leaves in the corresponding solution for around 1 min. The beetles were observed and the survivors were counted periodically during the bioassay. Percentage daily and total mortality  $\pm$  standard errors were obtained. Data were adjusted mathematically to obtain the function most fit, and with it to obtain the LC<sub>50</sub> (lethal concentration to kill 50% of individuals) by Probit analyses (Robertson et al., 1984). Chi<sup>2</sup> tests were used to test data fit to the Probit model.

Once the experimental part ended, data from both ethanol and water extracts were studied separately using a completely random anova with six treatments for ethanol (five concentrations plus a control), and five for water extracts (four concentrations plus a control, with three replicates each). Data were normalized by Bliss [ $\arcsin \sqrt{(\text{percentage adult mortality}/100)}$ ] prior to analyses, to stabilize variance error. Significant differences between treatments were identified with Tukey tests ( $p \leq 0.05$ ), using the InfoStat (2009) statistical software.

### 2.5. Evaluation of the antifeeding action of *S. molle*

This experiment to evaluate the antifeeding effect of extract on *X. luteola* adults was done following the method in Defagó et al. (2006). Five concentrations (2.0; 2.5; 3.5; 4.3; 4.7% w/v) were evaluated for the ethanol extract, and four 2.5; 3.0; 4.3; 5.6% w/v) for the water extract. Each treatment was replicated six times in experiment units with six adults of *X. luteola*. Two elm tree leaves of the same size were selected, and were immersed in each concentration for approx. 1 min and were allowed to dry at room temperatures for 10 min. Control leaves were immersed in distilled water or ethanol and let to dry at room temperature. Each Petri dish was set with a treated leaf, another untreated, and six adult beetles. The percentage of leaf area consumed was measured after 24 h using the Leaf Area Measurement Version 1.3 software (University of Sheffield, 2003). The repellence percentage was calculated as  $(1 - T/C) \times 100$ , where  $T$  and  $C$  are the levels ingested of treated and control leaves, respectively (Schreck et al., 1977). The results obtained were compared with those obtained using other plant species and insects.

## 3. Results

### 3.1. Insecticide effect of leaf aqueous extract of *S. molle* on adults of *X. luteola*

With the water extract, the greatest percentages of mortality of *X. luteola*, near 28%, were obtained with the two greatest concentrations (4.3 and 5.6% w/v). The statistical analyses indicated significant differences between the mortality in each treatment and their control, for water extracts ( $F_{4,35} > 2.64$ ;  $p < 0.05$ ), which reveals that results of mortality were due to the insecticide extract (Table 1).

With the aqueous extract, the greatest percentages of mortality of *X. luteola*, a concentration of 4.3% w/v obtained only 26% mortality, and the maximum 5.6% w/v concentration evaluated increased mortality to almost 28%. The lesser effects occurred with the treatments by lower concentrations (Table 1).

**Table 1**  
Average mortality (%  $\pm$  standard error) of *X. luteola* with water and ethanol extracts of *Schinus molle* at various concentrations.

Concentrations (% w/v)	Extracts	
	Water	Ethanol
5.6	27.78 $\pm$ 5.56 b	–
4.7	–	100.00 $\pm$ 0.00 c
4.3	26.39 $\pm$ 5.92 b	97.22 $\pm$ 2.78 c
3.5	–	87.50 $\pm$ 4.42 bc
3.0	16.67 $\pm$ 5.56 b	–
2.5	15.28 $\pm$ 5.53 b	80.56 $\pm$ 4.58 b
2.0	–	73.61 $\pm$ 5.92 b
0.0	0.00 $\pm$ 0.00 a	8.33 $\pm$ 6.88 a

Different small letters between treatments in a column indicate significant differences between concentrations, according to Tukey tests ( $p \leq 0.05$ ).

Water extracts from leaves of *S. molle* affected daily survival of adults. The water extract, mortality over 20% was obtained from day 4th since the beginning of the bioassay (Fig. 1A).

The results of the Probit analyses indicated for the water extract a mortality response on day 4, with maximum differences between treatments, in a linear response until the end of the experiment (Table 2).

From the Probit analyses, the  $LC_{50}$  was of 8.52% w/v ( $R^2 = 0.87$ ) for the water extract on day 4th (Table 2). However, Probit analysis provides in general good  $LC_{50}$  estimates when mortality obtained varies between 10 and 90%. Beyond these “limits” errors increases and affect calculations.

The effectiveness of the aqueous extract from leaves of *S. molle* was verified in Fig. 2 for all concentrations, and also the direct relationship between concentration and mortality of *X. luteola*.

### 3.2. Insecticide effect of leaf ethanolic extract of *S. molle* on adults of *X. luteola*

With the ethanolic extract, the greatest percentages of mortality of *X. luteola*, over 97%, were obtained with the two greatest concentrations (4.3 and 4.7% w/v). The statistical analyses indicated significant differences between the mortality in each treatment and their control, for ethanol ( $F_{5,42} > 2.44$ ;  $p < 0.05$ ), which reveals that results of mortality were due to the insecticide extract. The lesser effects occurred with the treatments by lower concentrations (Table 1).

The ethanol extracts from leaves of *S. molle* affected daily survival of adults. However, the ethanol extract obtained mortality over 50% in most treatments since the beginning of consumption of treated leaves, while the water extract, mortality over 20% was obtained from day 4th since the beginning of the bioassay (Fig. 1).

The results of the Probit analyses indicated for the ethanol extract a mortality response on day 2, with the greatest differences between treatments, and that this response maintained a linear extract/mortality until the end of the bioassay (Table 2).

From the Probit analyses, the  $LC_{50}$  of 1.88% w/v ( $R^2 = 0.95$ ) for the ethanol extract on day 2 was smaller than that of 8.52% w/v ( $R^2 = 0.87$ ) for the water extract on day 4<sup>th</sup>. Thus, the ethanol extract from leaves of *S. molle* were more effective than the water extract to reach the same mortality (Table 2).

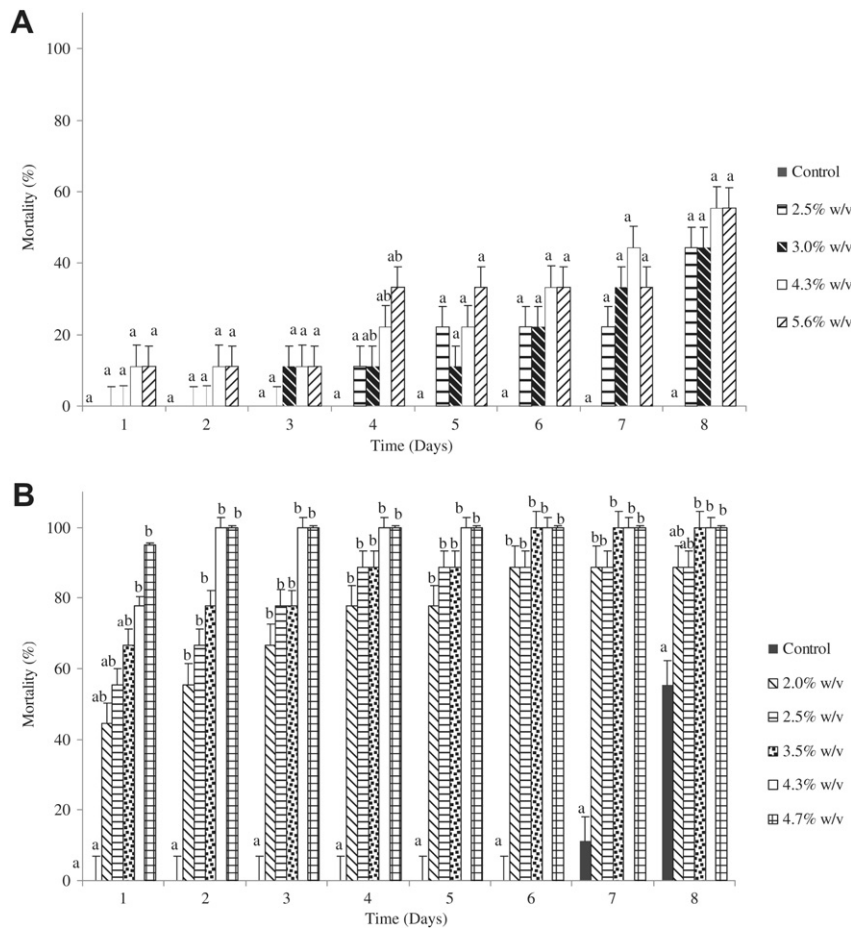
The effectiveness of the ethanol extract from leaves of *S. molle* was verified in Fig. 2 for all concentrations, above that of the water extract, and also the direct relationship between concentration and mortality of *X. luteola* with both solvents. Besides, the  $LC_{50}$  was smaller for the ethanol than the water extracts.

### 3.3. Antifeeding effect of leaf extracts from *S. molle* on adults of *X. luteola*

On the other side, the water extract had a marked antifeeding effect, inhibiting adult feeding completely in all treatments. This means that the insect discriminated between the treated and untreated leaves, and fed only on the latter (Table 3).

On one side, the ethanol extract from the leaves did not cause an antifeeding effect on adults of *X. luteola*, as most of them perished, and those which remained alive did not feed on the treated and also the untreated leaves. However, some toxicity was observed on leaves tested with the ethanol solvent.

Thus, the water extract had a clear and strong antifeeding effect on the adults of *X. luteola*, while the ethanol extracts were toxic, at all concentrations.



**Fig. 1.** Average mortality ( $\pm$  standard error) of adults of *X. luteola* with leaf extract from *S. molle* with several concentrations at several days of evaluation. (A) water; and (B) ethanol. Different letters horizontally indicate significant differences between concentrations, according to a Tukey test ( $p \leq 0.05$ ).

**4. Discussion**

Our results indicate that on adults of *X. luteola*, ethanol extracts at concentrations from 2.5 to 4.7% cause mortality over 80%. Then, our results of the evaluation of efficacy of leaf extracts from *S. molle* were superior than those of Ferrero et al. (2007), of only 53% mortality of *Blattella germanica* L. with ethanol extracts at 15% w/v, a concentration almost three times higher than the greatest evaluated here (5.6%).

Effective results have been obtained with fruit hexane extracts of *S. molle* on neonate larvae of *Cydia pomonella* L. (Lepidoptera: Tortricidae). At concentrations of 0.62, 1.25, 2.5, and 5.0 g/kg diet (equivalent to 0.062, 0.125, 0.25, and 0.5% w/v), these larvae had mortality levels of 9, 21, 39, and 60%, respectively. They also had problems when replacing their exoskeleton, and had malformed

pupae and adults (Chirino et al., 2001). Ethanol extracts at 2, 5, and 10% from young and mature leaves of *Melia azedarach* L. via Soxhlet (extraction of the lipid portion) by Defagó et al. (2006) caused high mortality rates of *X. luteola* adults, near to 100% 14 d after application, results similar to ours with ethanol extracts from *S. molle*.

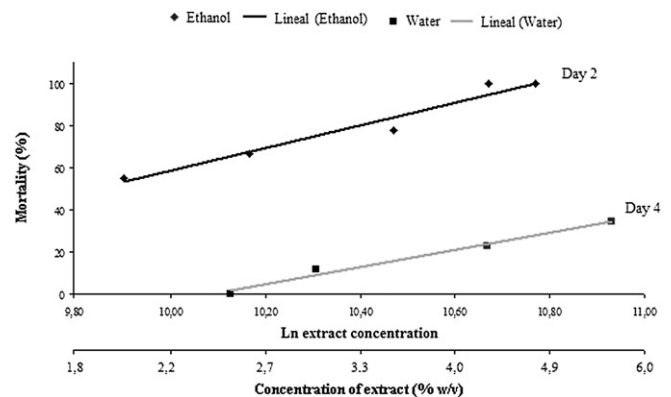
Mortality of larvae of *X. luteola* with ethanol extracts from leaves of *Daphne gnidium*, obtained by Maistrello et al. (2005) using the Soxhlet extraction, with concentrations of 1, 2, and 3 g/L (equivalent to 0.1, 0.2, and 0.3% w/v) were 27, 70, and 73%, respectively.

**Table 2**

Mortality of *X. luteola* adults after treating elm leaves with water and ethanol extracts from leaves of *S. molle*.

Extracts	Time (d)	Slope (mean $\pm$ S.E.)	LC <sub>50</sub> (% w/v)	Chi <sup>2,a</sup>
Water	4	39.5 $\pm$ 2.22	8.52	3.33
	8	27.7 $\pm$ 6.19	4.06	0.65
Ethanol	2	55.2 $\pm$ 2.94	1.88	2.39
	8	15.9 $\pm$ 1.92	0.19	0.14

<sup>a</sup> Means below the Chi<sup>2</sup> tabulated for water (df = 3;  $p \leq 0.05$ ) = 7.81, and Chi<sup>2</sup> tabulated for ethanol (df = 4;  $p \leq 0.05$ ) = 9.49, from which the Probit model fits the experiment data.



**Fig. 2.** Graph of the Probit test for the mortality of *X. luteola* for the ethanol and water extracts from leaves of *S. molle* at several concentrations on days 2 and 4, respectively.

**Table 3**

Effect of water extracts from leaves of *S. molle* on feeding of adults of *X. luteola* in a random choice test.

Extracts (% w/v)	Leaf area consumed (%)		Antifeeding effect (%)
	Treated leaves	Untreated control	
2.5	0.04 a	2.34 a	98.32
3.0	0.00 a	6.67 b	100.00
4.3	0.00 a	3.50 b	100.00
5.6	0.00 a	2.87 b	100.00

Different small letters between treatments in a row indicate significant differences (ANOVA,  $p \leq 0.05$ ) between consumption of treated and untreated leaves.

Concentrations of ethanol extracts of *D. gnidium* smaller than those in our study obtained similar results of mortality when applied to larvae of *X. luteola*, which are comparatively more susceptible than adults of the beetle.

Shekari et al. (2008) evaluated methanol extracts from leaves of *A. annua* on adults of *X. luteola* at 5, 10, 20, 40, and 80%, and at 24 and 48 h obtained LC<sub>50</sub> of 19.14 and 15.43%, respectively. These results indicate that the ethanol extract from *S. molle* in our study would be much more effective on adults of *X. luteola*, requiring much lower concentrations than with *A. annua* to reach the LC<sub>50</sub>.

Our results indicate that water extracts at concentrations from 5.6% w/v cause mortality near 28% on adults of *X. luteola*. As no other studies have been carried out on *X. luteola* × *S. molle*, results with other insects are provided. For example, Iannacone and Lamas (2003) studied the effect of leaf extracts from *S. molle* on *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae), *Trichogramma pinto* Voegelé (Hymenoptera: Trichogrammatidae), and *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae), and had mortality of *T. pinto* of 55, 95, and 90% after 12 h exposure with water, hexane, and acetone extracts at 10%, respectively. In a bioassay of toxicity with 4th stage larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae) with water extracts from leaves and fruits of *S. molle* at 5 and 15%, Pérez-Pacheco et al. (2004) obtained mortality of only 0 and 1.7%, respectively. On larvae, pupae, and adults of *X. luteola*, Sendi et al. (2005) applied water extracts from *Sambucus ebulus* and *Artemisia annua* at 1, 5, and 10%, and obtained average mortality of 77.1 and 91.31%, respectively.

These results of mortality could be due to diverse active substances present mainly in the leaves of *S. molle*, in which Guardiola et al. (1990) have described essential oils such as timol, citronelil acetate, and β-caryophyllene. Wimalaratne et al. (1996) have mentioned *cis*-menth-2-en-1-ol y *trans*-piperitol. Also, Dikshit et al. (1986), and Steinbauer and Wanjura (2002) have identified monoterpenes like α-pinene, α- and β-phellandrene, limonen, cymene, myrcene, β-caryophyllene, cryptone, and α-terpineol. The monoterpenes in the leaves occur also in the fruits (Bernhard et al., 1983; Hayouni et al., 2008), and also triterpenoid acids are extracted from the acid fraction of oleoresins (Pozzo-Balbi et al., 1978).

Respect of toxicity of ethanol solvent observed in our bioassay of antifeeding activity, the action of ethanol on the activity of membrane-bound and soluble acetylcholinesterase (AChE) in sarcoplasmic reticulum of skeletal muscle has been studied. Treatment of membranes with 2.5–12.5% v/v ethanol produced a slight stimulation of the AChE activity and inhibition at higher concentration (Cabezas-Herrera et al., 1992).

The antifeeding effect of the water extracts of *S. molle* on adults of *X. luteola* agrees with the 100% inhibition results obtained by Defagó et al. (2006) on the same insect with extracts from leaves of *M. azedarach* at 2, 5, and 10%. Shekari et al. (2008) obtained also similar results, with an antifeeding effect of the methanol extracts from leaves of *A. annua* superior to 75% for concentrations of 2.5, 5, and 10%.

The insecticide efficacy of the extracts from leaves of *S. molle* has been observed also in their repelling and antifeeding activity on the house fly *Musca domestica* L. by Wimalaratne et al. (1996), who determined that the bioactivity of the volatiles extracted from the leaves is associated to two compounds: *cis*-menth-2-en-1-ol, and *trans*-piperitol, and that this last compound has a greater repellence action against this fly.

## 5. Conclusions

Leaf extracts from *S. molle* were efficacious as bioinsecticides on adults of *X. luteola*, reaching mortality near 100% when obtained with ethanol, and at concentrations of 4.3 and 4.7% w/v. The ethanol extracts caused greater mortality than the water extracts at similar concentrations. On adults of *X. luteola*, the ethanol extracts were mainly toxic, and the water extracts presented antifeeding effect. The least LC<sub>50</sub> on adults of *X. luteola* was obtained with the ethanol extract (1.88%). The results obtained suggest an interesting opportunity to develop bioinsecticides based on extracts from *S. molle* for use in integrated pest management of the elm leaf beetle and other pests that may affect urban trees.

## References

- Abdel-Sattar, E., Zaitoun, A., Farag, M., El-Gayed, S., Harraz, F., 2009. Chemical composition, insecticidal and insect repellent activity of *Schinus molle* L. leaf and fruit essential oils against *Trogoderma granarium* and *Tribolium castaneum*. Nat. Prod. Res. 25, 1–10.
- Askevold, I., 1991. On some poorly known, misidentified and mislabelled Chilean Chrysomelidae (Coleoptera). Rev. Chil. Entomol. 19, 11–15.
- Baldini, A., Cogollor, G., Sartori, A., Aguayo, J., 2005. Control biológico de plagas forestales de importancia económica en Chile. Corporación Nacional Forestal, Gobierno de Chile, Santiago, Chile.
- Bernhard, R., Shibamoto, T., Yamaguchi, K., White, E., 1983. The volatile constituents of *Schinus molle* L. J. Agric. Food Chem. 31, 463–466.
- Cabezas-Herrera, J., Campoy, F.J., Vidal, C., 1992. Differential effects of ethanol on membrane-bound and soluble acetylcholinesterase from sarcoplasm reticulum membranes. Neurochem. Res. 17, 717–722.
- Carrero, J., 1996. Lucha integrada contra las plagas agrícolas y forestales. Mundi Prensa, Madrid.
- Chirino, M., Carriac, M., Ferrero, A., 2001. Actividad insecticida de extractos crudos de drupas de *Schinus molle* L. (Anacardiaceae) sobre larvas neonatas de *Cydia pomonella* L. (Lepidoptera: Tortricidae). Bol. San. Veg., Plagas 27, 305–314.
- Cloyd, R., 2004. Natural instincts: Are natural insecticides safer and better than conventional insecticides? Am. Nurseryman 200 (38), 41.
- Defagó, M., Valladares, G., Banchio, E., Carpinella, C., Palacios, S., 2006. Insecticide and antifeedant activity of different plant parts of *Melia azedarach* on *Xanthogaleruca luteola*. Fitoterapia 77, 500–505.
- Dikshit, A., Naqvi, A., Husain, A., 1986. *Schinus molle*: a new source of natural fungitoxicant. Appl. Environ. Microbiol. 51, 1085–1088.
- Donoso, C., 2006. Las especies arbóreas de los bosques templados de Chile y Argentina. Autoecología. Editorial Masisa Cuneo, Valdivia, Chile.
- Dreistadt, S., Dahlsten, D., Lawson, A., 2001. Elm Leaf Beetle. Publication 7403. UC Statwide IPM Project, University of California, Davis, CA.
- Escada, M., Da Silva, F., Souto, C., 1979. Una plaga de los olmos en Portugal, *Pyrrhalta luteola* Müll. (Coleoptera: Chrysomelidae). Bol. Serv. Plagas 5, 73–78.
- Ferrero, A., Sánchez, C., Werdin, J., Alzogaray, R., 2007. Repellence and toxicity of *Schinus molle* extracts on *Blattella germanica*. Fitoterapia 78, 311–314.
- Ferrero, A., Werdin, J., Sánchez Chopa, C., 2006. Actividad biológica de *Schinus molle* en *Triatoma infestans*. Fitoterapia 77, 381–383.
- Guardiola, V., De Miguel, P., Primo, E., 1990. Repelencia frente a *Blattella germanica* de componentes de *Schinus molle* L. Rev. Agroquím. Tecnol. Alimentos 30, 341–346.
- Hayouni, E., Chraief, I., Abedrabba, M., Bouix, M., Leveau, J., Mohammed, H., Hamdi, M., 2008. Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat. Int. J. Food Microbiol. 125, 242–251.
- Iannacone, J., Lamas, G., 2003. Efectos toxicológicos de extractos de molle (*Schinus molle*) y lantana (*Lantana camara*) sobre *Chrysoperla externa* (Neuroptera: Chrysopidae), *Trichogramma pinto* (Hymenoptera: Trichogrammatidae) (Hymenoptera: Encyrtidae) en el Perú *Copidosoma koehleri*. Agric. Técnica (Chile) 63, 347–360.
- InfoStat, 2009. InfoStat version 2009. Grupo InfoStat, FCA. Universidad Nacional de Córdoba, Argentina.
- Joker, D., Cruz, N., Morales, M., Rojas, E., 2002. *Schinus molle* L. Danida forest seed centre. Seed Leaflets 57, 1–2.

- Leofe, G., 2002. First release of the elm leaf beetle parasitic fly. NRE Frankston: Keith Turnbull Res. Inst, 19, 1–3.
- Maistrello, L., López, M., Soria, F., Ocete, R., 2005. Growth inhibitory activity of *Daphne gnidium* L. (Thymelaeaceae) extracts on the elm leaf beetle (Col., Chrysomelidae). *J. Appl. Entomol.* 129, 418–424.
- Meiners, T., Hacker, N., Anderson, P., Hilker, M., 2005. Response of the elm leaf beetle to host plants induced by oviposition and feeding: the infestation rate matters. *Entomol. Exp. Appl.* 115, 171–177.
- Metcalfe, R., Metcalfe, E., 1992. Plant kairomones in insect ecology and control. Chapman and Hall, New York.
- Muñoz, C., Pérez, V., Cobos, P., Hernández, R., Sánchez, G., 2003. Sanidad Forestal. Ediciones Mundi-Prensa, Madrid.
- Murray, A., Frontera, M., Tomas, M., Mulet, M., 2005. Gas chromatography-mass spectrometry study of the essential oils of *Schinus longifolia* (Lindl.) Speng., *Schinus fasciculata* (Griseb) I.M. Johnston, and *Schinus areira* L. *Z. Naturfor.* 60, 25–29.
- Pérez-Pacheco, R., Rodríguez, C., Lara-Reyna, J., Montes, R., Ramírez, G., 2004. Toxicidad de aceites esenciales y extractos vegetales en larvas de mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae). *Acta Zool. Mex.* 20, 141–152.
- Pozzo-Balbi, T., Nobile, L., Scapini, G., Cini, M., 1978. The triterpenoid acids of *Schinus molle*. *Phytochemistry* 17, 2107–2110.
- Regnault-Roger, C., Staff, V., Philogène, B., Terrón, P., Vincent, C., 2004. Biopesticidas de origen vegetal. Ediciones Mundi-Prensa, Madrid.
- Robertson, J., Smith, K., Savin, N., Lavigne, R., 1984. Effects of dose selection and sample size on the precision of lethal dose estimates in dose-mortality regression. *J. Econ. Entomol.* 77, 833–837.
- Rodríguez, C., Silva, G., Djair, V., 2003. Bases para el manejo racional de insecticidas: Insecticidas de origen vegetal. Facultad de Agronomía, Universidad de Concepción, y Fundación para la Innovación Agraria, Concepción, Chile.
- Romanyk, M., Cadahia, D., 2002. Plagas de insectos en las masas forestales. Ediciones Mundi-Prensa, Madrid.
- SAG (Servicio Agrícola y Ganadero), 2010. Informativo Fitosanitario Forestal N°4. Informativo del Subdepartamento de Vigilancia y Control Oficial Forestal, División Protección Agrícola y Forestal, Santiago, Chile.
- Schreck, C.E., Posey, K., Smith, D., 1977. Repellent activity of compounds submitted by Walter Reed Army Institute of Research. 1. Protection time and minimum effective dosage against *Aedes aegypti* mosquitoes. *Tech. Bull. U.S. Dept. Agric.* 1549, 1–215.
- Sendi, J., Arbab, A., Aliakbar, A., 2005. The efficacy of aqueous plant extracts of wormwood and dwarf elder against elm leaf beetle *Xanthogaleruca luteola* Mull. (Col.; Chrysomellidae). *Agric. Sci. (Tabriz)* 15, 115–120.
- Shekari, M., Sendi, J., Etebari, K., Zibae, A., Shadparvar, A., 2008. Effects of *Artemisia annua* L. (Asteraceae) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). *Pestic. Biochem. Physiol.* 91, 66–74.
- Silva, G., Lagunes, A., Rodríguez, J., Rodríguez, D., 2002. Insecticidas vegetales: una vieja y nueva alternativa para el manejo de plagas. *Manejo Integrado de Plagas y Agroecología (Costa Rica)* 66, 4–12.
- Silva, S., Bozinovic, F., Jaksic, F., 2005. Frugivory and seed dispersal by foxes in relation to mammalian prey abundance in a semiarid thornscrub. *Austral Ecol.* 30, 739–746.
- Steinbauer, M., Wanjura, W., 2002. Christmas beetles (*Anoplognathus* spp., Coleoptera: Scarabaeidae) mistake peppercorn trees for eucalypts. *J. Nat. Hist.* 36, 119–125.
- University of Sheffield, 2003. Program Leaf Area Measurement URL: <http://www.shef.ac.uk/nuocpcpe/ucpe/leafarea.html> (accessed 16.10.08).
- Wimalaratne, P., Slessor, K., Borden, J., Chong, L., Abate, T., 1996. Isolation and identification of house fly, *Musca domestica* L., repellents from pepper tree, *Schinus molle* L. *J. Chem. Ecol.* 22, 49–59.
- Yueqin, R., Recio, M., Manes, S., Giner, R., Cerda, N., Rios, J., 2003. Isolation of two triterpenoides and a biflavanone with anti-inflammatory activity from *Schinus molle* fruits. *Planta Med.* 69, 893–898.