Industrial Crops and Products 43 (2013) 523–528

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Proximal analysis and insecticidal effects of extracts from pepper tree (*Schinus molle*) leaves on elm leaf beetle (*Xanthogaleruca luteola*) larvae

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ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 13 July 2012
Accepted 31 July 2012

Keywords:
Elm leaf beetle
Botanical extract
Pepper tree

ABSTRACT

Among a series of exotic pests that have been reported in Chile, *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), a defoliating insect of elms (*Ulmus* spp.), is causing important damage in urban trees in central Chile. Leaves from *Schinus molle* L. (Anacardiaceae), an interesting South American plant, were characterized and compared physically and chemically through proximal analysis. Also, the insecticidal effects of ethanol and water extracts from young and mature leaves of *S. molle* were evaluated on third instar larvae of *X. luteola* at concentrations of 0.5–4.3% w/v. Water and lipid contents presented the greatest differences in both leaf maturity stages. At 12 d, the maximum concentrations obtained with ethanol and water from young and mature leaves caused mean mortalities of 89 and 67, and 78 and 63%, respectively. The lowest 50% lethal concentration was 1.28% w/v, obtained at the 7th day of evaluation with the ethanol extract from young leaves. Hence, extracts from *S. molle* leaves may have a potential use as a bioinsecticide in Integrated Pest Management plans against *X. luteola* and other similar pests defoliating urban trees, to decrease the risk of using conventional pesticides in public areas.

1. Introduction

A series of exotic forest pests have been reported in Chile, one of them the elm leaf beetle *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), a defoliating insect associated to the *Ulmus* genus (Ulmaceae), which has caused important damage in elm wind barriers in farms and urban trees in central Chile, and for which there is still not and efficacious treatment (Huerta et al., 2011).

The elm leaf beetle is distributed mainly in central and southern Europe, North Africa, west and central Asia, southern Australia, and temperate areas in North and South America (Romanyk and Cadahía, 2002; Borowiec and Sekerka, 2010). In Chile, it has been detected in the central zone, from the Valparaiso through the Araucanía regions (Servicio Agrícola y Ganadero, 2010).

Larvae and adults of *X. luteola* feed on the parenchyma of the leaves. Successive infestations render the tree susceptible to varied pests and diseases, mainly scolytid beetles (*Scolytus* spp.) that affect the bark and transmit the fungus causing the elm disease, *Ophiostoma* (= *Ceratocystis*) spp. in Europe (not present in Chile) (Pérez, 2003).

In general, synthetic insecticides have been important tools in pest and disease management in crops, and have determined a significant increase in production. However, the history also shows that overzealous use of synthetic insecticides led to numerous problems unforeseen at the time of their introduction: acute and chronic poisoning of applicators, farm workers, and even consumers; destruction of fauna; disruption of natural biological control and pollination; extensive groundwater contamination, potentially threatening human and environmental health, and the evolution of resistance to pesticides in pest populations (Perry et al., 1998; National Research Council, 2000).

In this context, it is necessary to search for new alternative pesticides with a low risk, easily accessible and renewable, such as plant extracts, which support the role of synthetic pesticides but diminish their negative effects, and offer environmental security and an efficient option for agriculture (Isman, 2000, 2006). The capacity of plants to synthesize secondary metabolites with biological properties on insects, such as repellence, feeding dissuasion, toxicity, reproductive effects, and regulation activity of growth and development, have been documented (Koul and Dhaliwal, 2001; Rodríguez et al., 2003; Regnault-Roger et al., 2005). The toxic and biocide effects of several genera in the Anacardiaceae family, widely distributed in South America has been reported, like *Mangifera* (mango), *Toxicodendron* (*Rhus*) (poison ivy, poison oak, poison sumac, lacquer tree), *Anacardium* (cashew), *Schinus* (pepper tree, molle, huingán), *Pistacia* (pistachio), and *Lithraea* (litre), among the most studied. These plants produce secondary metabolites like tannins, alkaloids, saponines, steroids, xanthones, terpenes, flavonoids, lacquers, latex and caustic oleoresins, and hot

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http://dx.doi.org/10.1016/j.indcrop.2012.07.062

spices, that may cause strong dermal and mucous membrane irritation (Ghalem and Mohamed, 2009; Bendaoud et al., 2010; Zahed et al., 2010).

Schinus molle L., or pepper tree, is an interesting plant that has been used worldwide as an insecticide and in ethnic medicine (Machado et al., 2007; Iannacone and Alvariño, 2010). This perennial dioic tree is originary from Andean South America and is distributed from Ecuador through Chile and Bolivia (Orozco and Lentz, 2005; Vidaurre, 2006). It is also naturalized in southern US, Central America (Díaz et al., 2008), Colombia, southern Brazil, Paraguay, Uruguay and Argentina (Guala et al., 2009). It is found as a wild, invasive, or urban tree in the Mediterranean region, the near East, Canary Islands (Danin, 2000; Sanz et al., 2004), north, south and east Africa (Hayouni et al., 2008; Iponga et al., 2009), Pakistan (Nasir, 1983), India (Dikshit et al., 1986), south and west Australia (Hosking et al., 2007), New Zealand (Burckhardt and Basset, 2000), and Cook Islands (Meyer, 2000).

The essential oil from *S. molle* leaves and fruits has demonstrated to be a strong repellant and insecticide, which effect is associated mainly to *cis*-menth-2-en-1-ol and *trans*-piperitol (Wimalaratne et al., 1996).

Different authors have evaluated the effect of *S. molle* extracts on varied pests, both important on crops and forests (Abdel-Sattar et al., 2009; Benzi et al., 2009; Huerta et al., 2010). Given this information, a research was conducted to evaluate the insecticidal effects of extracts young and mature leaves of *S. molle* on *X. luteola* larvae in laboratory bioassays to contribute to the development of integrated pest management (IPM). Also, for characterize of plant material a proximal analysis and color determination were conduced.

2. Materials and methods

2.1. Leaf sampling and water content and dry weight determination

Young (light green and tender) and mature (darker green and harder) leaves (1 kg each) from *S. molle* were collected randomly from the canopy at 1.5 m from the soil, of trees with the trunk at least 10 cm diameter, in the Antumapu Campus (33°24′27″S, 70°37′59″W), Universidad de Chile, Santiago, Chile, during the summer of 2010. The leaves were washed with distiller water by reduce pollution and air-dried in Chemistry Laboratory, Department of Agroindustry and Enology, College of Agronomic Sciences, University of Chile, Santiago, Chile. Then those leaves were separated from the rachis (which were discarded) and were dried in a forced air oven (model 18, Thelco) at 37 °C during 60 h. Next, water content and dry weight were determined by weight differences.

2.2. Preparing of powders

Then dried leaves were grinded in a grain mill with a 60 mesh (0.25 mm) in Chemistry Laboratory. The resulting powder was used to obtain by proximal analysis, color determination and the extract preparing.

2.3. Proximal analysis and color determination

Young and mature leaf powders (10 g each) were characterized and compared physically and chemically after drying in a forced air oven at 105 °C; lipids were extracted via Soxhlet; raw fiber by acidic and basic hydrolysis; proteins through the micro-Kjeldahl method; ashes from muffle incineration at 550 °C; and the non-nitrogen extract as the difference between the previous analyses and the weight until it became constant (Association of Official Analytical Chemists, 1984). Color was determined for both maturity stages with a CR 300 Minolta (New York, USA) colorimeter through the CIELab method using three values: L^*, the difference between light (where $L^* = 100$) and dark (where $L^* = 0$); a^*, the difference between green ($-a^*$) and red ($+a^*$); and b^*, the difference between yellow ($+b^*$) and blue ($-b^*$) (Ngo et al., 2007). All of the analyses were done three times in the Chemistry Laboratory. For leaf maturity stages was an ANOVA for each determination of proximal analysis followed by Tukey tests ($p \leq 0.05$), when significant differences occurred between leaf maturity stages (Canavos, 1988).

2.4. Insect sampling and rearing

First instar *X. luteola* larvae were hand collected from urban elm trees in the San Miguel Municipality, Metropolitan Region, Santiago, Chile, during the summer of 2010. These larvae were placed on Petri dished lines with filter paper slightly wet with distilled water and given fresh clean elm leaves as food, until they reached the third instar, when they were used for the bioassays, at the Forest Entomology Laboratory, Department of Forestry and Nature Conservation, College of Forestry and Nature Conservation Sciences, Universidad de Chile, Santiago, Chile. All insects were maintained at 22 ± 3 °C and 61 ± 4% RH.

2.5. Elaboration of extracts

The extracts were prepared at the Chemistry Laboratory. The powders obtained (150 g each) from the young and mature leaves were used to prepare extracts using water (1:10 powder:water) or ethanol (1:6 power:ethanol). The first phase was to prepare a solution with the highest possible concentration, which was agitated during 24 h, the first hour at 37 °C and at room temperature the remaining hours. This procedure was done to prevent evaporation of volatile active principles. To determine their concentration, fractions of the solutions were dried at 100 °C for 1 h in a forced air oven, and soluble solids were weighed on a Boeco Equilab analytical scale, 0.1 mg sensitivity, maximum 120 g. Then, several dilution factors were used to prepare extract solutions with different concentrations for use in the bioassay. Water and ethanol as solvents were used, because they are less toxic. The ethanol and water extracts from young and mature leaves were obtained at 0.5, 1.1, 2.2, and 4.3% w/v. These concentrations were used to obtain mortality values from 25 to 75%, an adequate range for Probit analysis (Robertson et al., 1984).

2.6. Bioassays

The bioassays were set in the Laboratory of Forest Entomology under natural light at 28 ± 3 °C and 30 ± 7% RH. The four concentrations determined for each solvent (water and ethanol) were evaluated using five replicates, plus the controls (elm leaves only with the solvent, water or ethanol) from young and mature *S. molle* leaves, determining insect mortality. The experiment units had 10 third instar larvae obtained in the laboratory, on Petri dishes lined with Whatman No. 1 filter paper slightly wet with distilled water, and two fresh and clean *Ulmus* sp. leaves each. The extracts were applied by immersion of the leaves during 60 s in the prepared extract solutions, and the leaves were let to dry before adding the larvae. The larvae were carefully observed until the adult if they contained parasitoid or fungi; however they have not been reported in the country yet.

2.7. Evaluation of the insecticidal effects

Mortality (% ± SD) was determined daily. Dead larvae and those reaching pupation were counted. Insects without movement and with necrosis were considered dead. The percent mortality was

Table 1
Initial humidity content and dry weight (means ± SD) from leaves (1 kg) from *S. molle* at two maturity stages.

Leaf maturity stages	Initial humidity (%)[a]	Mean dry weight (g)
Young	66.5 ± 0.9a	335 ± 9a
Mature	61.7 ± 0.8b	383 ± 8b

[a] Different letters between means in a column indicate significant differences between leaf maturity stages, according to Tukey tests ($P < 0.05$).

normalized by Bliss (arcsen$\sqrt{}$porcentage larval mortality/100), corrected with Abbott's (1925) formula to eliminate natural mortality in the controls. After the evaluation, data were submitted a factorial design (5×4) ANOVA where the factor A was the concentration and the factor B extract effect. When significant differences occurred between treatments, they were separated using Tukey tests ($P \leq 0.05$) (Canavos, 1988). The effectiveness of the extracts with both solvents was used to determine lethal concentration (LC_{50}) to kill 50% of *X. luteola* larvae exposed by Probit analyses (Throne et al., 1995). Data fit to the Probit model was verified with χ^2 tests.

3. Results

3.1. Proximal analysis of S. molle leaves

The average humidity of young and mature leaves was significantly different ($F_{1,4} = 50.24$, $P < 0.05$), being greatest (slightly over 66%) in young leaves (Table 1). In the chemical characterization of the leaf powders, all parameters were significantly different, with humidity ($F_{1,4} = 112.9$, $P < 0.05$) and lipid ($F_{1,4} = 176.4$, $P < 0.05$) contents with the greatest differences (Table 2). The humidity and lipid contents were 90% and over 120% greater in young than older leaves. The powder from young leaves had a significantly lighter (L^*) ($F_{1,4} = 58.57$, $P < 0.05$) green color (Table 3).

3.2. Mortality of X. luteola larvae

The treatments with the ethanol extract from young leaves caused a greater mortality than the water extract. The statistical analysis detected significant differences in mortality between larvae exposed and the controls, which indicates that mortality was caused by the biocide action of the extracts (Table 4). Concentration ($F_{4,12} = 196.0$; $P < 0.05$) and extract effect ($F_{3,12} = 43.1$; $P < 0.05$) factors were significant.

The mortality of *X. luteola* larvae was gradual, and reached 89% [corrected with Abbott's (1925) formula] at 12 d with the highest concentration (4.3% w/v) of the ethanol leaf young extract. The mortality with the water extract from young leaves occurred faster than with the ethanol extract treatments. This response at a lower concentration demonstrates a greater insecticide of the *S. molle* young leaf powder when using ethanol as solvent than when using water (Table 4).

The average mortality obtained with the ethanol extract from mature leaves of *S. molle* was smaller than that of the same extract from young leaves at the same concentration, although both were slightly over 60% (Table 4).

Table 3
Color (means ± SD) of powder obtained from *S. molle* young and mature leaves.

Leaf maturity stages	Color parameters[a,b]		
	L^*	a^*	b^*
Young	56.50 ± 0.5a	−8.00 ± 0.03a	23.90 ± 0.12a
Mature	47.63 ± 2.0b	−8.54 ± 0.34a	27.30 ± 0.85b

[a] L^*: clarity; a^*: spectrum from green to red; b^*: spectrum between yellow and blue.
[b] Different letters between means in a column indicate significant differences between leaf maturity stages, according to Tukey tests ($P < 0.05$).

Mortality reached at least 35% in the bioassay with the water extract from mature leaves at the lowest concentration evaluated (0.5% w/v). There occurred significant differences between exposure treatments and the controls. Lower mortalities (56 and 63%) were obtained with water extracts from mature leaves at 2.2 and 4.3% w/v, as the greatest mortality levels were reached (81 and 89%) with the ethanol extracts from young leaves at equal concentrations (Table 4).

Mortality with both the ethanol and water extracts from young leaves was greater than with those from mature leaves. However, the mortality with the highest concentrations evaluated were >55%, which demonstrates their insecticide potential.

3.3. Determination of insecticidal effects

Herein, the ethanol extract presented a stronger insecticide effect than the water one. Thus, the least LC_{50} (1.28% w/v) with the ethanol extract from young leaves occurred at the 7th day evaluation. The order of decreasing effectiveness of insecticide and solvent extracts of leaves was: young-ethanol ($LC_{50} = 1.28\%$ w/v), mature-ethanol ($LC_{50} = 2.51\%$ w/v), young-water ($LC_{50} = 2.56\%$ w/v) and mature-water ($LC_{50} = 13.6\%$ w/v) at the 7th day after application of the extracts (Table 5).

4. Discussion

Each plant species protects itself in multiple ways against insect damage through the expression of a series of secondary metabolites, and not just from any one in particular, and they may accomplish an insecticide action depending on their concentration (Silva et al., 2002; Regnault-Roger et al., 2005). In this case, the secondary metabolites from *S. molle* could increase the insecticidal effect on *X. luteola*, without isolating an active compound in particular. As this botanical insecticide posses molecules derived from the secondary metabolism of plant, 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., 2005). Also, this bioinsecticide has biodegradability, a renewable character and to reduce the natural resistance against pests, could contribution to the sustained management of this pest in urban tree.

Table 2
Proximal analysis (means ± SD) of the powders obtained from *S. molle* young and mature leaves.

Leaf maturity stages	Proximal analysis (%)[a]					
	Humidity	Lipids	Raw fiber	Proteins	Ashes	Non-nitrogenated extract[b]
Young	5.10 ± 0.30a	4.88 ± 0.10a	5.95 ± 0.45a	2.47 ± 0.15a	7.40 ± 0.20a	74.20 ± 0.80a
Mature	2.67 ± 0.25b	10.67 ± 0.24b	7.00 ± 0.42b	3.00 ± 0.10b	9.40 ± 0.30b	67.30 ± 0.70b

[a] Different letters between means in a column indicate significant differences between leaf maturity stages, according to Tukey tests ($P < 0.05$).
[b] Obtained by weight difference.

Table 4
Mean mortality (% ± SD) of *X. luteola* larvae after 12 d by effect of the ethanol and water extracts from young and mature leaves from *S. molle*.

Concentrations (% w/v)	Young leaves[a]		Mature leaves	
	Water	Ethanol	Water	Ethanol
0.0	14 (0) ± 6a	26 (0) ± 6a	14 (0) ± 6a	26 (0) ± 6a
0.5	50 (42) ± 7b	64 (51) ± 6b	44 (35) ± 6b	54 (38) ± 6b
1.1	56 (49) ± 6b	70 (60) ± 7b	46 (37) ± 6b	60 (46) ± 7b
2.2	68 (63) ± 8c	86 (81) ± 9c	62 (56) ± 5b	76 (68) ± 6c
4.3	72 (67) ± 5c	92 (89) ± 5c	68 (63) ± 8c	84 (78) ± 6d

[a] Means with different letters original values between parentheses corrected with Abbott's formula in a column are significantly different, according to Tukey tests ($P < 0.05$).

In *S. molle* they are mainly caustic oleoresins. Using a Clevenger distillator, Dikshit et al. (1986) determined that *S. molle* leaves contain 2.1–2.3% oil. Barrachina et al. (1997) indicated a 9.7% of lipids in leaves collected in Valencia, Spain. Also, Viturro et al. (2000) obtained 2.8–3.2% essential oil from leaves and tender twigs. Guenther (1952) obtained 0.5, 5.5, and 7.7% essentials oil from *S. molle* leaves and fruits using water vapor extraction, in samples from South Africa, Mexico, and Spain, respectively. Using the same method, Bendaoud et al. (2010) extracted near 2.7% essential oil from *S. molle* fruits obtained in Tunisia. These differences may be due to the extraction techniques used, the origin and plant structure processed, and the phenology of the trees at collection.

Huerta et al. (2010) evaluated the toxicity of ethanol and water extracts from *S. molle* leaves on *X. luteola* adults, at 2.0–4.7% w/v for the ethanol extract and 2.5–5.6% w/v for the water one, and obtained mortality from 73.6 to 100% and 15.3 to 27.8%, respectively. These authors obtained also greater mortality with the ethanol extract, at concentrations similar to those in the study herein, but they not separated between maturity states of leaves. The water extract caused in that study a smaller mortality on adults with concentrations comparable that those evaluated here with larvae. Also, they obtained LC_{50} levels for *X. luteola* adults with the ethanol extract from *S. molle* of 1.88 and 0.19% w/v at the two and eight day evaluation, while with the water extract they were 8.52 and 4.06% w/v at the 4th and 8th days, respectively. This demonstrates that *X. luteola* adults, which also cause damage to elm leaves (Huerta et al., 2011), are more affected than third instar larvae to the extracts from *S. molle* leaves.

Moreover, these extracts of *S. molle* produce mortality in at least two developmental stages of *X. luteola*, in larvae (tested in this study) and adults (Huerta et al., 2010). These same authors found in adults of this insect antifeedant effect (100%) of aqueous extracts tested at 24 h. Therefore, although the LC_{50} in this study occurred in larvae on the seventh day, there would be a protective effect on the leaves from the time consumed by the antifeedant effect of biopesticide.

Werdin et al. (2008) evaluated the insecticidal effects of essential oils by hydrodistillation of *S. molle* leaves in nymphs II of *Nezara viridula* L. (Hemiptera: Pentatomidae) at a concentration of 0.176% w/v. The difference observed when comparing our results was due to the further purification of the oil and the insect itself.

Other researchers have studied, using different extraction and application techniques, the toxic action of extracts obtained from other components of *S. molle* onto other insect pests. For example, Iannacone and Lamas (2003a) evaluated the insecticidal effects of water, ethanol and acetone extracts from *S. molle* leaves at 10% w/v onto the potato moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), and obtained 90.4, 49.2, and 88.1% larval mortality after mixing them with the diet, and 11.8, 8.8, and 32.4% adult mortality through contact with residues, respectively. There, larval mortality of *P. operculella* larvae treated with the water extract was greater than the results obtained herein with the same water extract onto *X. luteola* larvae, which was 60% lower at 4.3% w/v for extracts from young and mature *S. molle* leaves. Further, Iannacone and Lamas (2003b) evaluated the effect of the same extracts through contact exposure to residues of some natural enemies of *P. operculella*. At the 12 h evaluation they observed 28.9, 92.0, and 84.1% mortality on adults of *Trichogramma pintoi* Voegelé (Hymenoptera: Trichogrammatidae). At 48 h, the lethal effect was 11.1, 0.0, and 27.8% for larvae I of *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae), and 86.7, 33.3, and 26.7% for adults of *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae), respectively.

The studies cited above allow stating that the insecticidal effects of the extracts from *S. molle* vary among plant parts, maturity state of the plant, extraction techniques (For example: hydrodestillation of essential oils, extraction with solvents, or other), application method, and target insect, anyhow with promising results worth investigating. According to the maturity state of the plant used, the higher mortality of larvae of *X. luteola* occurred with extracts from young leaves of *S. molle*, which could be explained by the higher concentration of active compounds in this state of maturity of the leaves (Gross et al., 1985).

Other studies have reported on the toxic effects of extracts from different plants on *X. luteola*. For example, Chiffelle et al. (2011) evaluated the effect of extracts from *Peumus boldus* Molina (Monimiaceae) leaves, being the most effective from young-ethanol leaf extract (LC_{50} 1.2% w/v) as obtained in this study but with the difference that was achieved at 2 d in adult insects, whereas in our to 7 d but in larvae. Maistrello et al. (2005) evaluated the effect of the ethanol extract from *Daphne gnidium* L. (Thymelaceae) leaves obtained with a Soxhlet extractor, on mortality of second instar *X. luteola* larvae. At 0.1, 0.2, and 0.3% w/v applied at 6.4, 12.7, and 19.1 μg/cm^2, respectively, onto elm (*U. minor* Mill.) leaves, they obtained 27.3, 70.4, and 73.4% mortality, respectively. Those results are similar to our values obtained here with the water extract, but they obtained them with lesser concentrations. The differences may be due to the application of variable amounts of the extract to the

Table 5
Insecticidal capacity of leaf extracts from *S. molle*, as indicated by the LC_{50} on *X. luteola* larvae.

Leaf maturity stages	Solvents	Days	Slopes (mean ± SD)	LC_{50} (% w/v)	χ^{2}[a]
Young	Ethanol	7	16.4 ± 0.48	1.28	5.92
	Water	7	15.8 ± 0.38	2.56	6.95
Mature	Ethanol	7	16.2 ± 1.87	2.51	6.85
	Water	7	10.7 ± 0.50	13.62	5.19

[a] χ^2 values obtained below those tabulated for young and mature leaves for both water and ethanol ($\chi^2 = 7.81$, df = 3; $P \leq 0.05$), from which the Probit model fits the experiment data.

leaves offered to the insects, and to a greater efficacy of the extract from *D. gnidium*.

Also, Defagó et al. (2006) evaluated the insecticidal effects of ethanol extracts from *M. azedarach* leaves at 2, 5, and 10%, and obtained 80–100% mortality on *X. luteola* adults with the extract from young leaves. For the extract from mature leaves they obtained 25, 65, and 100%. Those results are similar to ours with the ethanol extract herein.

Shekari et al. (2008) evaluated the toxic effects of the topical application of the methanol extract in acetone from *Artemisia annua* L. (Asteraceae) leaves onto *X. luteola* adults and larvae, at 5, 10, 20, 40, and 80%. At the 2 d evaluation they obtained a LC_{50} on adults of 15.43% and on third instar larvae of 43.77%. From this, it is clear than the adults are also more susceptible than third instar larvae to the leaf extract from *A. annua*, similarly to the results in Huerta et al. (2010). These authors mention also that the insects return to feed on the leaves treated at 2 d, which indicates that the organic compounds effective on the leaves are probably volatile. That observation agrees with the results of the bioassay with the ethanol extract from *S. molle* leaves evaluated herein, where at the 2 d evaluation began to occur larval mortality, as most of them remained on the container walls during the first 1–1.5 d.

5. Conclusions

The ethanol extract from young leaves caused a greater *X. luteola* larval mortality that the water one at similar concentrations, directly proportional to the concentration in both cases. The LC_{50} at the 7th day of evaluation was smaller with the ethanol than the water extract, for both leaf development stages. Given the risk of applying conventional insecticides on elms in streets, this species represent a bioinsecticide source of use in urban tree IPM. This requires field trials to verify the results obtained in the laboratory.

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