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Insecticide effect of leaf extracts from *Schinus molle* on larvae of *Gonipterus platensis*

Efecto insecticida de extractos de hojas de *Schinus molle* en larvas de *Gonipterus platensis*

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ABSTRACT

Keywords:

Schinus molle
Gonipterus platensis
Botanical insecticides
Integrated pest
management

The insecticide effect of new and mature leaf extracts obtained with ethanol and water from *Schinus molle* L. (Anacardiaceae) were evaluated at 0.5 - 3.4% w/v and 0.5 - 4.8% w/v, respectively, onto larvae third instar of *Gonipterus platensis* Marelli (Coleoptera: Curculionidae), an important defoliating pest on eucalypt (*Eucalyptus* spp., Myrtaceae) plantations. The extracts were applied to eucalypt leaves that were given as food to the larvae, and their effects were determined by determining mortality and the LC_{50} and LT_{50} by Probit analysis on the larvae. Both extracts were effective, especially those from new leaves; the ethanol extract from them caused the greatest mortality. The highest concentrations of the water and ethanol extracts (3.4 and 4.8% w/v, respectively) caused average mortality of 100 and 88.9% with new leaf extracts, and 94.7 and 86.4% with mature ones, respectively. The insecticide effectiveness 6 d after treatment in decreasing order were: new leaves-ethanol (LC_{50} = 0.79% w/v), new leaves-water (LC_{50} = 2.08% w/v), mature leaves-ethanol (LC_{50} = 0.63% w/v) mature leaves-water (LC_{50} = 12.01% w/v). The LT_{50} at 1.7% w/v was 5.2 d in new leaves-ethanol. These results of the insecticide effect on *G. platensis* larvae allow to consider the leaf extracts from *S. molle* an interesting alternative as a bioinsecticide source for an integrated pest management system of this pest.

RESUMEN

Palabras clave:

Schinus molle
Gonipterus platensis
Insecticidas botánicos
Manejo integrado de
plagas

Se evaluaron extractos insecticidas etanólicos y acuosos obtenidos desde hojas jóvenes y maduras de *Schinus molle* L. (Anacardiaceae), en concentraciones de 0,5 a 3,4% p/v y 0,5 a 4,8% p/v, respectivamente, sobre larvas de tercer estadio de *Gonipterus platensis* (Coleoptera: Curculionidae), importante plaga defoliadora de eucaliptos (*Eucalyptus* spp., Myrtaceae). Los extractos se aplicaron sobre hojas de eucalipto como alimento para las larvas, y su efectividad se determinó a través de la mortalidad y la determinación mediante análisis Probit de la concentración y tiempo letal (CL_{50} y TL_{50} , respectivamente). Ambos extractos fueron eficaces contra las larvas, especialmente aquellos obtenidos de hojas jóvenes; el extracto etanólico de estas mismas hojas causó la mayor mortalidad. Con las mayores concentraciones de los extractos en etanol (3,4% p/v) y agua (4,8% p/v) se obtuvieron mortalidades promedio de 100 y 88,9% con hojas jóvenes, y 94,7 y 86,4% con hojas maduras, respectivamente. El orden decreciente de la efectividad insecticida de los extractos de hojas y solvente fue: hojas joven-etanol (CL_{50} = 0,79% p/v), hojas joven-agua (CL_{50} = 2,08% p/v), hojas maduras-etanol (CL_{50} = 2,63% p/v) y hojas maduras-agua (CL_{50} = 12,01% p/v) a seis días después de la aplicación de los extractos. El TL_{50} fue 5,2 días a la concentración de extractos de hojas nuevas en etanol de 1,7% p/v. Las propiedades insecticidas de los extractos de hojas de *S. molle* sobre las larvas de *G. scutellatus* permiten considerar a esta planta una alternativa interesante como fuente bioinsecticida para el sistema de manejo integrado de esta plaga.

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G*onipterus platensis* Marelli (formerly *G. scutellatus* Gyllenhal) (Col.: Curculionidae) is distributed worldwide, feeding both as larvae and adults on eucalypt leaves, causing defoliation and stopping growth (Mapondera *et al.*, 2012). The damage affects mainly the upper third of the tree, particularly on the new leaves of the upper part of the canopy. This pest may present 2-4 cycles a year (Muñoz *et al.*, 2003; Alvarado and Sartori, 2006; Elgueta, 2010). In Chile, it has become an important forest pest because of the large eucalypt plantations, where it has caused economic losses to producers (Alvarado and Sartori, 2006).

The first and second stage larvae damage the leaf epidermis at both sides, and at the third and fourth stages they consume all the lamina. The adults feed at the leaf borders, where they leave a characteristic serrate border (Muñoz *et al.*, 2003; Alzugaray *et al.*, 2004; Sartori, 2006).

The use of chemical insecticides is an important tool on pest control, but they have also negative effect, like the development of pest and disease resistance, the appearance of new pests, the reduction of beneficial fauna, and contamination of the environment and feeding crops (Silva *et al.*, 2002; Montesino, 2009). The use of plant extracts appears as an alternative to chemical treatments, as they are healthier and ecologically acceptable (Silva *et al.*, 2002; Millán, 2008).

The plants are important sources of chemical molecules, and they have evolved for million years developing defense mechanisms to reduce insect damage (Regnault-Roger *et al.*, 2004; Montesino, 2009). The plants synthesize secondary metabolites, natural products that do not participate directly in processes essential for growth, development and reproduction, but are determinant in plant resistance against insect damage or adverse factors like herbivore consumption, infection by microorganisms or competition for light, water or soil, among others (Sepúlveda *et al.*, 2003; Ávalos and Pérez-Urria, 2009; Montesino, 2009).

The Bolivian pepper tree *Schinus molle* L. (Anacardiaceae), a Peruvian tree common in the Andes region of South America, has been efficacious as bioinsecticide (Ferrero *et al.*, 2007; Werdin *et al.*, 2008; Fuentes *et al.*, 2010). This ever green tree with leaves rich in essential oils may

reach up to 15 m high (Donoso, 2006). Some of its major secondary metabolites are tannins, alkaloids, flavonoids, saponins, sterols, terpenes, gums, resins, and essential oils, present mainly in its fruit and foliage (Ferrero *et al.*, 2006, 2007; Alba *et al.*, 2009).

Other studies demonstrate the insecticide effect of the essential oils in *S. molle* leaves and fruit. For example, Deveci *et al.* (2010) determined the antimicrobial activity and repellence effect of the essential oil and the hexane extracts from the leaves and fruit of *S. molle* on *Blattella orientalis*, and on 9 bacteria strains. Also, Werdin *et al.* (2008) evaluated the insecticide effect of essential oils on leaves and fruit of *S. molle* on nymphs II of *Nezara viridula* L. (Hem.: Pentatomidae), a soybean pest. At the greatest concentrations (88 and 176 µg mL⁻¹) they obtained at 48 h a mortality >95%, while the least concentrations (11 and 22 µg mL⁻¹) caused >70% mortality.

Benzi *et al.* (2009) evaluated the repellence and fumigant action of essential oils from *S. molle* leaves and fruit on the rice weevil, *Sitophilus oryzae* L. (Col.: Curculionidae). The essential oil from the leaves caused repellence at 24 h at 0.04 and 0.4% w/v, but those from the fruit had no effect.

The insecticide effect of new and mature leaf ethanol and water extracts of *S. molle* were evaluated on larvae of *G. platensis* in laboratory bioassay tests, to contribute to integrated management of this pest.

MATERIALS AND METHODS

The larvae of *G. platensis* were collected in October 2011 at a five years old *E. globulus* plantation in the Metropolitan Region, Chile (33°47'41"S; 71°30'12"W), that were taken in cloth bags to the Forest Entomology Laboratory, where they were set in Petri dishes underlined with slightly wet filter paper, and provided daily with fresh eucalypt leaves.

In the bioassays, one kg of new and mature *S. molle* leaves were used, collected at random from adult trees at the College of Forestry and Nature Conservation Sciences, University of Chile, Santiago, during the fall and winter of 2011.

To prepare the extracts the leaflets were taken from the central leaf vein, weighed fresh and then dried at 37 °C in a Memmert® 854 (Schwabach, Germany) forced air

oven until constant weight at 60 h on a Shimadzu ELBL 3000 balance. Then the dry leaflets were grinded with an Ufesa® MC 0360 knife mill. The dust obtained was mixed with water or 96 % w/v ethanol, shaking 18 h on a Heidolph® MR 3001K magnetic stirrer (Schwabach, Germany), and was heated at 37 °C during the first hour. Then, the mixes were filtered with paper, and set 15 min in a centrifuge (HN-S, USA), to obtain the base extracts. To determine the concentrations of those base extracts, a small volume of them were dried and the concentrations of total solids in % w/v were obtained by weight difference. The solutions used were prepared by serial dilution of the leaf extracts (Huerta *et al.*, 2010).

The bioassays had experiment units with five third instar larvae of *G. platensis* in plastic containers lined with slightly wet filter paper and fresh and clean eucalypt leaves, at room temperature (19±2 °C). The treatment was standardized at 60 s immersion of eucalypt leaves in the extracts. These leaves were maintained for two d on exposure to the third stage larvae then every two d were changed to fresh leaves without extract until completing 10 d (Huerta *et al.*, 2010).

The efficacy of the leaf extracts were evaluated by determining daily mortality with the extracts and

concentrations. The results with the extracts from new and mature leaves were analyzed separately, using bifactorial anovas with two solvents x five concentrations, including controls with only the solvents, with five replicates. The results were normalized by Bliss [$\arcsen \sqrt{X}$ (% adult mortality/100)] prior to the analyses, to stabilize the error of the variance. Significant differences between treatments were separate with Tukey ($P \leq 0.05$) tests. Mortality percentages were corrected by Abbott's (1925) formula to eliminate natural mortality in the controls, particularly from the toxic effect of ethanol. The LC_{50} and the LT_{50-95} were obtained in Probit analyses of separate bioassays (Thrones *et al.*, 1995).

RESULTS AND DISCUSSION

Evaluation of mortality

The larval mortality of *G. platensis* obtained with the ethanol extract from new *S. molle* leaves was greater than with the water extract. The statistical analysis indicated significant differences between the treatments and their respective controls. The least mortality obtained was >33%, with the least concentration (0.5% w/v). The greatest final mortality (100%) occurred with the ethanol extract from new leaves at the greatest concentration (3.4% w/v), at the 10 d evaluation (Table 1, Figure 1).

Table 1. Mortality (%) of *G. platensis* larvae by effect of the ethanol and water extracts from new and mature leaves of *S. molle* at 10 d.

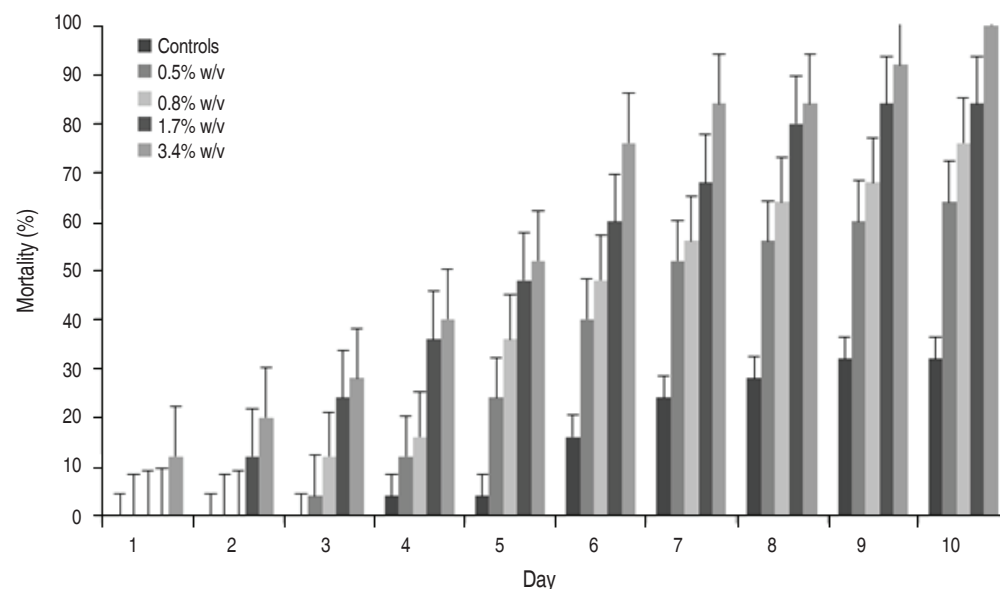
Concentrations of the extracts (%w/v) from new leaves					
Solvents	0.0 (controls)	0.5	0.8	1.7	3.4
Ethanol	32.0 ± 4.9 a (0.0)	64.0 ± 7.5 b (47.1)	76.0 ± 7.5 b (64.7)	84.0 ± 4.0 bc (76.5)	100.0 ± 0.0 c (100)
Water	28.0 ± 4.9 a (0.0)	52.0 ± 4.9 ab (33.3)	72.0 ± 10.2 bc (61.1)	80.0 ± 6.3 bc (72.2)	92.0 ± 8.0 c (88.9)
Concentrations of the extracts (%w/v) from mature leaves					
	0.0 (controls)	0.5	1.2	1.7	4.8
Ethanol	24.0 ± 4.0 a (0.0)	60.0 ± 11.0 b (47.4)	72.0 ± 12.0 bc (63.2)	84.0 ± 4.5 bc (78.9)	96.0 ± 4.0 c (94.7)
Water	12.0 ± 4.9 a (0.0)	44.0 ± 4.0 b (36.4)	60.0 ± 6.3 bc (54.5)	76.0 ± 7.5 cd (72.7)	88.0 ± 8.0 d (86.4)

Mortality values in parenthesis corrected by Schneider-Orelli's (1947) formula. Means in a line with different letters are significantly different, according to Tukey ($P \leq 0.05$) tests.

The bioassay with the ethanol extract from mature *S. molle* leaves yielded a minimum larval mean mortality of 47.4% at the least concentration (0.5% w/v). The maximum mortality at 10 d for both bioassays occurred with the greatest concentrations, 1.7 and 4.8% w/v (Table 1, Figure 1).

The mortality levels obtained with the ethanol and water extracts from new leaves were greater than those from mature foliage. The larvae that consumed leaves treated with the ethanol extract from new leaves at the maximum concentration (3.4% w/v) presented 100% mortality.

New-ethanol



New-water

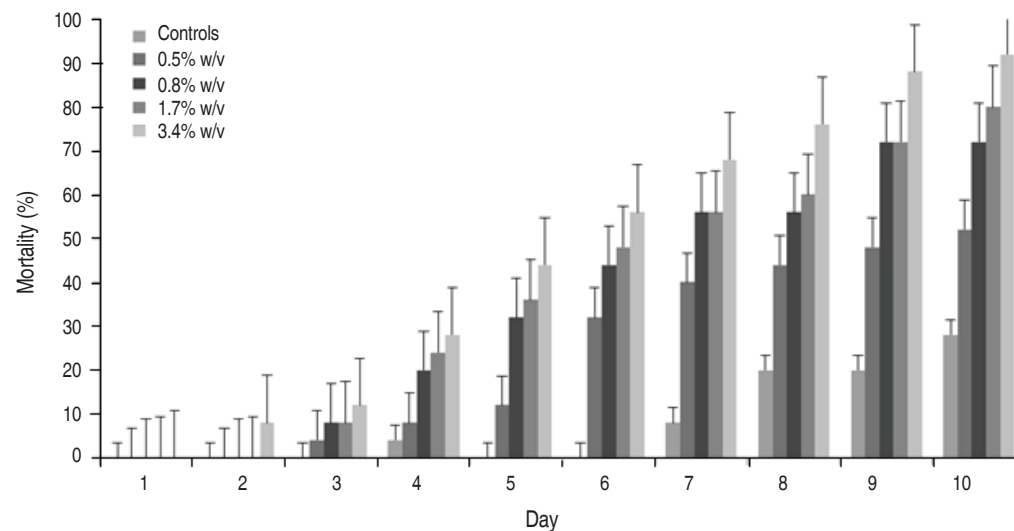


Figure 1. Mortality means (%) of larvae of *G. platensis* exposed to ethanol and water extracts of new *S. molle* leaves.

It is noteworthy to see that mortality at the two greatest concentrations evaluated in each bioassay was >72%, which demonstrates the insecticide value of both extracts (Table 1). The results obtained with the extract from mature foliage are presented in Table 1 and Figure 2.

Insecticide effectiveness

The results of the Probit analyses indicated a greater insecticide effect of the ethanol than the water extracts

on the larvae of *G. platensis*. The least LC_{50} was 0.79% w/v with the ethanol extract from new leaves at day six (Table 2). The extracts from new *S. molle* foliage caused greater effects than those of mature leaves on mortality of *G. platensis* larvae. The least lethal time (TL_{50}) at 1.7% w/v was 5.2 d with the ethanol extract from new leaves, while the greatest lethal time was 8.4 d with the water extract from mature foliage (Table 2).

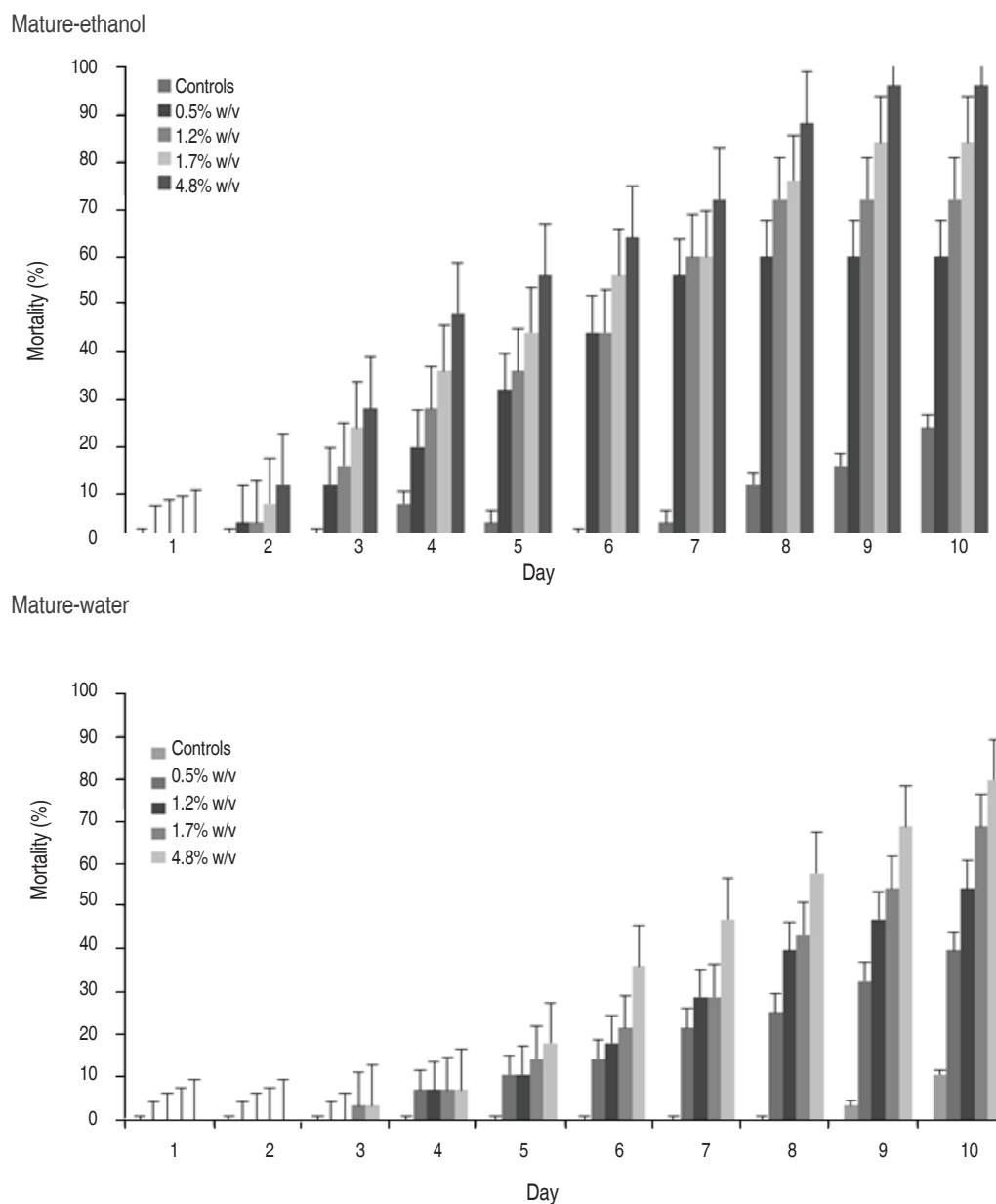


Figure 2. Mortality means (%) of larvae of *G. platensis* exposed to ethanol and water extracts of mature *S. molle* leaves.

The effectiveness of *S. molle* as botanical insecticide has been demonstrated in various studies on several pests. For example, Huerta *et al.* (2010) evaluated the toxicity of ethanol and water extracts of mature *S. molle* leaves onto adults of *Xantogaleruca luteola* Müller (Col.: Chrysomelidae), a defoliating pest of elm trees (*Ulmus* spp., Ulmaceae), at 2.0 to 4.7% w/v with ethanol and 2.5 to 5.6% w/v with water, and achieved mortality from 73.6 to 100% and 15.3 to 27.8 %, respectively. Chiffelle

et al. (2013) analyzed the toxicity of ethanol and water extracts from new and mature *S. molle* leaves on third stadium *X. luteola* larvae, at 0.5 to 4.3% w/v for the ethanol and water extracts from new leaves, and 0.7 to 5.9% w/v and 0.5 to 4.3% w/v for mature leaves, respectively. Average mortality occurred with the greatest concentrations in ethanol and water: 89.2 and 67.4% from new leaves, and 78.4 and 62.8% from mature ones, respectively. In both studies the greatest

Table 2. Insecticide effectiveness (LC_{50} and LT_{50-95} at 1.9% w/v) on larvae of *G. platensis* of the leaf extracts from *S. molle*.

Leaf stages	Solvents	Days	Slopes (mean± SE)	LC ₅₀ (% w/v)	X ²
New	Ethanol	4	15.23 ± 2.86	5.42	5.69
		5	12.30 ± 4.47	2.33	2.22
		6	17.52 ± 3.47	0.79	4.49
	Water	5	9.41 ± 4.41	7.29	1.53
		6	11.13 ± 2.20	2.08	1.67
Mature	Ethanol	5	10.97 ± 7.53	4.46	2.00
		6	10.03 ± 7.21	2.63	1.50
	Water	6	10.65 ± 1.99	12.11	3.32
		7	11.73 ± 3.11	5.82	1.81
		8	15.72 ± 1.76	1.49	3.90
LT ₅₀ (days)		LT ₉₅ (days)	Slopes (mean± SE)		X ²
New	Ethanol	5.2	9.6	0.2 ± 0.7	5.20
	Water	6.8	11.4	9.8 ± 0.8	6.50
Mature	Ethanol	6.7	11.7	9.0 ± 1.0	10.33
	Water	8.4	13.4	9.0 ± 1.3	9.22

The X^2 values calculated for the LC_{50} and LT_{50-95} were smaller than those in the table (df 3; $P \leq 0.05=7.8147$, and df 4; $P \leq 0.05=9.4877$, thus the Probit model was adjusted to the bioassay results. \pm standard deviation.

mortality occurred with the ethanol extract from new leaves, similarly to our results herein.

Iannacone and Lamas (2003) evaluated the insecticide effect of water, hexane y acetone extracts at 10% w/v from *S. molle* leaves on larvae and adults of the potato moth *Phthorimaea operculella* Zeller (Lep.: Gelechiidae). For the larvae the solutions were added to the diet, and caused 90.4, 49.2 and 88.1% mortality, respectively. Those results are similar to *G. scutellatus* larval mortality obtained with the water extracts from new and mature leaves were 92% at 3.7% w/v, and 88% at 3.3% w/v, respectively.

Chiffelle *et al.* (2013) obtained a LC_{50} for *X. luteola* larvae with the ethanol extract from new *S. molle* leaves, of 1.28% w/v at seven d. This result is different to ours, where the LC_{50} was 0.79% and occurred at six d. Thus, the larvae of *G. platensis* are more susceptible than those of *X. luteola* to the extracts from *S. molle* leaves.

Also, Huerta *et al.* (2010) obtained with the ethanol extract

from *S. molle* leaves a LC_{50} for *X. luteola* adults of 1.88 and 0.19% w/v at two and eight d, while that with the water extract was 8.52 and 4.06% w/v at four and eight d, respectively. This indicates that the adults of *X. luteola* are more sensible to the larvae of *G. platensis* to the extracts from the leaves of *S. molle*.

Descamps *et al.* (2008) evaluated the contact toxicity of ethanol and hexane extracts from *S. molle* leaves and fruit on larvae and adult *Tribolium castaneum* Herbst. (Col.: Tenebrionidae), at 6.8 and 4.8 % w/v of the extracts, respectively. A LC_{50} at 72 h for the foliage and fruit ethanol extracts were 13 and 29 $\mu\text{g larva}^{-1}$, and 11 y 17 $\mu\text{g adult}^{-1}$, respectively. The ethanol extract from the leaves was more effective than that from the fruit.

Iannacone and Alvarino (2010) evaluated the ecotoxicological effects of water extracts from *S. molle* leaves onto four controllers of crop pest in Perú. Concentrations of 1.5, 2.5, 5.0, 10.0, and 20.0% w/v obtained at 48 h a LC_{50} of 3.7% for larvae of the

neuropterans (Chrysopidae) *Ceraeochrysa cincta* (Schneider), 32.2% for larvae of *Chrysoperla asoralis* (Bank), 40.9% for adults of *Telenomus remus* Nixon (Hym.: Scelionidae), and 14.2% for adults of *Orius insidiosus* Say (Hem.: Anthocoridae). The third instar larvae of *G. platensis*, both the aqueous extracts of new and mature leaves, are more sensitive than the biological controllers evaluated, given that the LC_{50} 7.29% w/v to five d in new leaves and LC_{50} 12 % w/v to six d in mature leaves.

CONCLUSIONS

The extracts from new and mature *S. molle* leaves were efficacious as bioinsecticides on *G. platensis* larvae, which were more susceptible to the extracts from new over mature leaves. The ethanol extract from *S. molle* new leaves caused at 3.4% w/v the greatest larval mortality (100%). The least LC_{50} (0.79% w/v) of *G. platensis* larvae occurred with the ethanol extract from new leaves, at day six, and also the least LT_{50} (5.2 d) at 1.7% w/v with the same extract. Our results of the insecticide properties of *S. molle* leaf extracts on *G. platensis* larvae allow to consider this tree an interesting alternative for a bioinsecticide to be used for integrated management of this pest.

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