ISSN 0719-3882 print
ISSN 0719-3890 online

TOXICITY AND REPELLENCY OF BOTANICAL INSECTICIDES TO THE ELM LEAF BEETLE, *Xanthogaleruca luteola*, IN THE LABORATORY

Jaime E. Araya[1*], Gianella Vergottini[1], Amanda Huerta[2]

[1] Departamento de Silvicultura y Conservación de la Naturaleza, Facultad de Ciencias Agrarias, Universidad de Chile, Casilla 1004, Santiago, Chile.
[2] Departamento de Silvicultura y Conservación de la Naturaleza, Universidad de Chile, Casilla 9206, Santiago, Chile.
Corresponding author E-mail: jearaya@uchile.cl

RESUMEN

Durante 2008-2009 se evaluó en laboratorio la toxicidad y repelencia de los insecticidas botánicos Biomilbe, Biobug, Garlic Barrier y Bugitol, sobre *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), obtenido desde olmos (*Ulmus* sp., Ulmaceae) en el Parque O'Higgins, Santiago, Chile. Los productos se aplicaron en una torre Potter en cuatro concentraciones, más un control con agua. Su toxicidad por ingestión se evaluó después de asperjar las soluciones sobre hojas, las que a los 20 min se proporcionaron a larvas y adultos; el efecto de contacto se determinó después de la aspersión sobre individuos de *X. luteola*. La repelencia de hojas tratadas se comparó en bandejas con hojas tratadas sólo con agua. Las pruebas de toxicidad se hicieron con un diseño estadístico completamente al azar, con cuatro insecticidas más el control, y cuatro repeticiones. La unidad experimental fue una placa Petri con 20 individuos. La mortalidad se evaluó a las 24 h de la aspersión sobre hojas, y también a 1 y 24 h de la inmersión de hojas en las soluciones. Los porcentajes de mortalidad obtenida se sometieron a ANDEVA y pruebas para separar promedios. En el ensayo de repelencia se compararon los porcentajes de larvas en las hojas tratadas y sin tratar. Los niveles de mortalidad fueron muy bajos. La mortalidad mayor se obtuvo con Bugitol al 4%, con 37,5% de mortalidad larvaria. Se observó una clara repelencia larvaria en las hojas tratadas con Biomilbe y Biobug, y las larvas se concentraron en las hojas tratadas sólo con agua.

Palabras clave: Biobug, Biomilbe, Bugitol, Garlic Barrier, insecticidas botánicos, vaquita del olmo.

ABSTRACT

The toxicity and repellency of the botanical insecticides Biomilbe, Biobug, Garlic Barrier, and Bugitol to the elm leaf beetle, *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), were evaluated in the laboratory during 2008-2009. Elm leaf beetles were collected from elm trees (*Ulmus* sp., Ulmaceae) in Parque O`Higgins, Santiago, Chile. The products were applied at four concentrations each in a Potter tower; a control treatment only with water was also included. Toxicity by ingestion was evaluated after spraying the insecticides on leaves, which were given as food to larvae and adults after 20 min. Contact effects were determined after direct application of these products onto *X. luteola* specimens. In addition, their repellency on treated leaves was compared on trays with other leaves treated only with water. The toxicity tests were set in a completely randomized statistical design, with four insecticides plus the control treatment and four replicates. The experiment unit was a Petri dish with 20 individuals. Mortality was evaluated after 24 h of direct spray, and after 1 and 24 h when applied by leaf immersion. The mortality obtained was subjected to ANOVA and mean separation tests. For the repellency test, the percentages of larvae, on both treated and untreated leaves,

Received: 08 January 2015. Accepted: 16 March 2015.

were compared. Mortality levels were very low. The highest mortality was obtained with Bugitol at 4%, recording 37.5% larval mortality. The repellency bioassay revealed marked effects on the leaves treated with Biomilbe and Biobug, while larvae concentrated on the leaves treated only with water.

Key words: Biobug, Biomilbe, Bugitol, Garlic Barrier, plant insecticides, elm leaf beetle.

INTRODUCTION

Urban trees can make a city a more pleasant place to live in, to work, and for recreation. Therefore, to protect plant health by taking care of pests and diseases is a key issue in urban tree management. This should include cultivation and biological and chemical techniques, making urban trees as environmentally and economically friendly as possible in order to reduce their damage to tolerable levels (Sánchez, 2003).

The elm (*Ulmus* spp.) is a beautiful native ornamental tree from eastern and central U.S. and Europe, which is common in streets and parks in Santiago, Chile. There are several cultivated species, like the American elm *Ulmus americana* L., the Siberian ulm *U. pumila* L., the English elm *U. campestris* L., and the European elm *U. procera* Salisbury.

The elm leaf beetle, *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), is a monofagous pest that lives on all kinds of elms (Romanyk and Cadahía 2002). The eggs, larvae, pupae, and adults (Fig. 1) are described in De Liñán (1998), and larvae and adults in Romanyk and Cadahía (2002). The adults and larvae feed on the leaves, while larvae devour especially the parenchyma and respect only the veins (Cobos et al., 2003). Such damages may defoliate elms totally. If the infestations occur on several consecutive years, elm trees weaken and are likely to be affected by other insects and diseases (De Liñán, 1998).

The species was first detected in Chile in 1994 in Los Andes, Valparaíso Region, and later in the Bernardo O´Higgins, Bíobío, and Metropolitan regions. It was found on elms in the Maule region (SAG, 2005) in March of 2005. Currently, it has spread down south to the La Araucanía region (SAG, 2010).

According to De Liñán (1998), the use of insecticides is very frequent among the control alternatives, but pest management in urban areas must also consider interactions with people and domestic animals, as well as the risk for contamination of drinking water, and the distance between the treated area and housing. Therefore, the toxic effects of pesticides need to be determined before their application in order to select the least harmful to the environment (Vargas and Ubillo, 2001).

As pests emerge from hibernation and before eggs are laid, insecticides target adults in order to decrease their number and avoid the first damage. This treatment must be completed with a second application when most larvae of the first cycle have appeared. If sprays are preferred, it is advisable to apply organochlorine or pyrethroid insecticides (Romanyk and Cadahía, 2002). In addition, abamectin, imidacloprid or acefate can be injected to the trunks. However, the cost of this technique limits its use to valuable individual trees, and the wood also has limitations in its capacity to restore sap movement.

Fig. 1. Life stages and damage by larvae and adult *X. luteola* on elm trees. From left to right: eggs mass, larvae on leaves, pupas on the soil, adult.

Plants have evolved during millions of years, and to decrease insect damage they have developed protection mechanisms that include insecticidal and repellent effects (Silva, 2007). Plant oils have been used since ancient times and they can act as contact insecticides. Different toxicity types are due to their physical properties, by inhalation of volatile compounds, by contact, forming an impermeable film that isolates the insect from the air and asphyxiates it, and by deep penetration (Casida and Quistad, 1998). Most of the plants that are used in plant protection exhibit an insectistatic effect rather than an insecticidal effect. In practice, they inhibit the normal development of insects through different types of action mechanisms, which affect their growth, inhibit their feeding, and act confounding them (Silva, 2007).

Plant extracts with repelling properties are chemicals that protect plants and other commodities from insect damage, making them less attractive for feeding or affecting their environment (Isman, 2006).

Garlic (*Allium sativum* L.) is known for its properties against insect pests due to its content of allicin, which is a sulphur compound, and the flavonoid rutin. Chili pepper (*Capsicum annuum* L.) contains capsaicin, an irritant and repelling alkaloid (Gómez and Soto, 2002).

The objective of this research was to determine in the laboratory the direct and residual toxicity of four formulations of plant extracts, Biomilbe, Bugitol, Biobug, and Garlic Barrier, onto larvae and adults of the elm leaf beetle *X. luteola*, and their repellent effects on larval feeding.

MATERIALS AND METHODS

This study was carried out in the Toxicology Laboratory, Dept. of Crop Protection, Coll. of Agronomic Sciences, Universidad de Chile, from October 2008 through January 2009.

Collecting and insect rearing. Larvae were collected from Nov. 2008 through Jan. 2009, on elm trees in Parque O´Higgins, Santiago, Chile (33°27′51″ S, 70°39′36″ O). They were taken to the Toxicology laboratory, kept separate according to their development, and fed daily with elm leaves. To ensure a sufficient number of adults, the pupae obtained by rearing were added to others collected at the end of the cycle on infested elms. Finally, 2nd instar larvae were used for the experiment. Periodic observations were made of the *X. luteola* individuals on the trees, to determine the number of cycles in the season. The criterion used to estimate the end of a generation, along with the reduction of individuals on the foliage, was the presence of a great number of pupae on the ground at the base of the trees.

Application of insecticides. The insecticides applied and evaluated in the laboratory at the concentrations indicated in Table 1 were:

Biomilbe: active ingredient, 50% plant fatty acids (Bioland S.A., Santiago, Chile). It corresponds to an organic, oily insecticide and miticide. It is innocuous for people, animals and biological control agents. It becomes biodegraded in hours. It asphyxiates fixed individuals, and causes cell plasmolysis, dehydration, and loosening of dead or dehydrated individuals.

Bugitol (capsaicin and related capsaicinoids, allyl isothiocyanate): 100% natural and biodegradable insecticide and repellant, formulated with chili pepper (*Capsicum*) extracts and essential oil from mustard (*Sinapsis* spp., Brassicaceae). It is a double action product: it kills on contact and repels over a long period of time (Gómez and Soto, 2002).

Biobug: 1:1 mix of Biomilbe and Bugitol.

Garlic Barrier: wide action spectrum organic repellant, formulated from garlic (*Allium sativum* L., Amaryllidaceae) varieties, which contain *allicin*, an organic sulfur compound that causes a systemic repellency. It masks pheromones and decreases mating, and it also has an antifeeding effect as well as an over stimulation effect of the nervous system (Garlic Research Labs, Inc., Glendale, California, USA)

Five concentrations were evaluated of each product. These were applied to the leaves and directly on the insects by spraying 0.5 mL of the corresponding solution in an ST4 Potter tower, on Petri dishes, a volume equivalent to 700 L ha^{-1}. The experiment unit was a Petri dish with 20 larvae, and with four replicates. When treating the leaves, once their surfaces were dry, after ~30 min, the insects (2nd stage larvae or adult *X. luteola*) were placed on top and were left there 2 d. Absorbent paper was set under the leaves to diminish foliage dehydration. Dead individuals were counted after 24 h. In the direct application on the 20 larvae, these were placed on absorbent paper in the Petri dish and sprayed in the Potter tower with the corresponding solutions. Once dried, the larvae were placed on clean dishes with elm leaves for feeding. Dead larvae were counted after 30 and 60 min. In both experiments, a Petri dish with just distilled water was used as an untreated control, with four replicates per treatment.

The upper part of the dishes had cloth-covered windows for ventilation to avoid a lethal

Table 1. Botanical insecticides evaluated on larvae and adults of *X. luteola*.

Commercial products	Recommended doses	Concentrations evaluated
	(%)	
Biomilbe	2.0	0.0, 0.5, 1.0, 2.0, 4.0
Bugitol	0.8	0.0, 0.5, 0.8, 2.0, 4.0
Biobug	2.5	0.0, 0.5, 1.0, 2.5, 4.0
Garlic Barrier	1.0	0.0, 0.8, 1.0, 2.0, 4.0

chamber. Repellency to larval feeding was also determined for concentrations recommended by the manufacturer. By using aluminum trays, two groups of three leaves were set, one with leaves sprayed with the recommended concentration and the other with leaves treated only with distilled water. On each tray with leaves, 20 larvae were set free. Their distribution was observed after 1 and 24 h.

Experimental design and statistical analysis. A completely randomized statistical design was used for the experiments with leaves sprayed and immersed with 4 concentrations of each insecticide and four replicates. Mortality was determined in those experiments, and the number of larvae was determined for the treated and untreated leaves in the trays. The main analysis used the Kruskal-Wallis test. Dunns test was used later, applying the Graphpad Prism 5.0 (Motulsky, 2007) and Statgraphic (2009). The repellency results were analyzed independently one on one, comparing them with the control treatment based on a Mann Whitney test with Welch's correction. A two-way ANOVA was used to determine an interaction between factors.

RESULTS AND DISCUSSION

Monitoring the life stages of *X. luteola*. The pest was monitored since early September 2009. The appearance of the first adults started on October 1, together with the first signs of leaf damage. The maximum number of larvae of the 3 stadia occurred around November 10. The second maximum number was recorded around the following January 12 but their number decreased considerably, due to the great increase in cumulative damage on the leaves. Finally, 3 cycles were observed during the season, and the last adults appeared at the end of March. Huerta et al. (2011) described the development of four generations a year in the city of Santiago, Chile.

Insecticide evaluation after foliar spray. The results of larvae and adults' mortality after 24 and 48 h from foliar application of the treatments are presented in Tables 2 and 3, respectively.

Table 2. Mean values (% ± SD) for mortality levels of larvae and adult *X. luteola* after 24 h from foliar spray of Biomilbe, Bugitol, Biobug, and Garlic Barrier.

Concentrations*	Larval mortality means**	Adult mortality means**	P value for larvae	P value for adults
		Biomilbe		
0.0	1.25 (± 1.2) a	1.25 (± 0.8) a		
0.5	12.50 (± 1.4) a	1.25 (± 1.2) a		
1.0	10.00 (± 5.8) a	5.00 (± 2.0) a	0.137	0.036
2.0	7.50 (± 4.3) a	7.50 (± 1.4) b		
4.0	10.00 (± 4.6) a	7.50 (± 3.3) b		
		Bugitol		
0.0	1.25 (± 0.8) a	1.25 (± 0.7) a		
0.5	5.00 (± 2.0) a	1.25 (± 1.3) a		
0.8	16.25 (± 6.3) a	6.25 (± 2.4) a	0.0003	0.0002
2.0	32.50 (± 4.8) b	23.75 (± 3.8) b		
4.0	33.75 (± 5.5) b	27.50 (± 3.2) b		
		Biobug		
0.0	1.25 (± 0.8) a	1.25 (± 0.7) a		
0.5	1.25 (± 1.3) a	3.75 (± 2.4) a		
1.0	3.75 (± 3.8) a	1.25 (± 1.3) a	0.616	0.031
2.5	7.50 (± 4.3) a	10.00 (± 2.0) a		
4.0	6.25 (± 3.8) a	13.75 (± 4.3) b		
		Garlic Barrier		
0.0	1.25 (± 1.3) a	1.25 (± 1.3) a		
0.8	7.50 (± 1.4) a	8.75 (± 2.4) a		
1.0	12.50 (± 3.8) a	12.50 (± 3.2) a	0.0041	0.0021
2.0	12.50 (± 2.5) a	17.50 (± 3.2) b		
4.0	18.75 (± 3.8) b	22.50 (± 4.3) b		

*mL commercial product 100 mL solution^{-1}. **Means in a column for each insecticide with different letters are significantly different (P ≤ 0.05) from the untreated control, according to non-parametric ANOVA.

Mean values of larval and adult mortality obtained after 24 h of insecticide spraying increased with the concentration applied (Table 2).

When spraying Biomilbe, larval mortality after 24 h did not show significant differences between the concentrations applied. However, results with a 5% concentration show a slight increasing trend, which was not significant with the control. This may happen because the definition of the test decreases when using a non-parametric test, especially if the number of data is small. This suggests that an increased number of observations could detect significant differences. The adults presented significant differences with concentrations at 2% and 4%. When spraying Biobug at 4.0 or 2.5% onto adults, significant differences were observed with respect to the control. The greatest mortality levels of adults occurred with Garlic Barrier at 4% (Table 2).

The Bugitol sprayings at 2.0 or 4.5% onto larvae presented significant differences with the control. With the highest concentration, mortality reached 33.75 and 46.3% after 24 and 48 h, respectively. Significant differences were observed at 2.0 and 4.0% concentrations on adults with respect to the control, this did not occur at 0.8 or 0.5% (Tables 2 and 3).

After 48 h from spraying on the leaves (Table 3), Biomilbe presented differences with respect to the 24 h reading, showing a significant although small increase in larval mortality with the 4% concentration. Biobug was more efficacious than Biomilbe, and caused significant mortality at 2.0 and 4.0%.

Evaluation of the insecticide applied directly. The results on larval and adult mortality after 30 min and 1 h from direct application are shown in Tables 4 and 5, respectively.

Garlic Barrier presented significant differences in larval mortality at 4% concentration only after 1 h from direct application. Bugitol was the only product that showed significant differences at 2.0 and 4.0% with respect to the control in terms of mortality of both larvae and adults; the highest larval mortality level reached 37.5%. Biomilbe and Biobug produced only a slight mortality after direct application, which suggests that the main

Table 3. Mean values (% ± SD) for mortality levels of larvae and adult *X. luteola* after 48 h from foliar spray of Biomilbe, Bugitol, Biobug, and Garlic Barrier.

Concentrations*	Adult mortality means**	P value means**	P value for larvae	Larval mortality for adults
		Biomilbe		
0.0	1.87 (± 1.3) a	1.87 (± 0.7) a		
0.5	12.50 (± 1.4) a	2.50 (± 1.4) a		
1.0	12.50 (± 4.8) a	5.00 (± 2.0) a	0.021	0.0031
2.0	12.50 (± 3.2) a	10.00 (± 2.0) b		
4.0	15.00 (± 4.6) b	15.00 (± 4.6) b		
		Bugitol		
0.0	1.90 (± 0.8) a	1.90 (± 0.8) a		
0.5	7.50 (± 1.4) a	2.50 (± 1.4) a		
0.8	20.00 (± 5.4) a	10.00 (± 2.0) a	< 0.0001	< 0.0001
2.0	41.30 (± 4.3) b	31.25 (± 3.8) b		
4.0	46.30 (± 6.9) b	37.50 (± 8.8) b		
		Biobug		
0.0	1.90 (± 0.8) a	1.90 (± 0.7) a		
0.5	3.75 (± 2.4) a	6.25 (± 2.4) a		
1.0	6.25 (± 3.1) a	12.50 (± 3.2) a	0.039	0.0001
2.5	21.25 (± 8.5) b	20.00 (± 4.1) b		
4.0	20.00 (± 7.9) b	28.75 (± 1.3) b		
		Garlic Barrier		
0.0	1.90 (± 1.3) a	1.90 (± 1.3) a		
0.8	12.50 (± 3.2) a	10.00 (± 2.9) a		
1.0	20.00 (± 3.5) a	13.75 (± 3.1) a	0.0013	0.0009
2.0	25.00 (± 5.4) b	26.25 (± 5.5) b		
4.0	26.30 (± 4.7) b	30.00 (± 5.4) b		

*mL commercial product 100 mL solution^{-1}. ** Means in a column for each insecticide with different letters are significantly different ($P \leq 0.05$) from the untreated control, according to non-parametric ANOVA.

Table 4. Mean values of larval and adult mortality (% ± SD) of *X. luteola* after 30 min from direct application of Biomilbe, Bugitol, Biobug, and Garlic Barrier.

Concentrations*	Larval mortality means**	Adult mortality means**	P value for larvae	P value for adults
	Biomilbe			
0.0	0.00(± 0,00) a	1.25(± 1.3) a		
0.5	0.00(± 0,00) a	1.25(± 1.3) a		
1.0	1.25(± 1.3) a	1.25(± 1.3) a	0.241	0.417
2.0	3.75(± 2.4) a	2.25(± 1.4) a		
4.0	13.75(± 9.4) a	5.00(± 2.0) a		
	Bugitol			
0.0	0.00(± 0.0) a	1.25(± 1.3) a		
0.5	1.25(± 1.3) a	2.50(± 1.4) a		
0.8	7.50(± 3.2) a	5.00(± 2.0) a	0.009	0.0215
2.0	12.50(± 1.4) a	10.00(± 2.0) b		
4.0	13.75(± 3.1) b	12.50(± 3.2) b		
	Biobug			
0.0	0.00(± 0.0) a	1.25(± 1.3) a		
0.5	0.00(± 0.0) a	0.00(± 0.0) a		
1.0	0.00(± 0.0) a	0.00(± 0.0) a	0.402	0.385
2.5	1.25(± 1. 3) a	1.25(± 1.3) a		
4.0	0.00(± 0.0) a	2.25(± 1.4) a		
	Garlic Barrier			
0.0	0.00(± 0.0) a	1.25(± 1.3) a		
0.8	3.75(± 2.4) a	0.00(± 0.0) a		
1.0	3.75(± 2.4) a	0.00(± 0.0) a	0.149	0.247
2.0	3.75(± 2.4) a	5.00(± 2.9) a		
4.0	7.50(± 1.4) a	3.75(± 2.4) a		

* mL commercial product 100 mL solution$^{-1.}$ ** Means in a column for each insecticide with different letters are significantly different (P ≤ 0.05) from the untreated control, according to non-parametric ANOVA.

effect of these products is changes in metabolism.

Two-way ANOVA were done to determine if life stage, either larva or adult, had a significant importance on mortality. Biomilbe presented differences between life stages, but only after 48 h, with a greater effect on larvae rather than on adults (P = 0.0104). In contrast, Biobug and Garlic Barrier produced an equivalent larval and adult mortality after 24 and 48 h, with no differences between both life stages. The effect of Bugitol was greater on larvae rather than on adults (P = 0.0179 and P = 0.0227 after 24 and 48 h, respectively).

In summary, differences were observed in terms of mortality depending on the life stages (larva o adult) with Bugitol after 24 and 48 h and with Biomilbe after 48 h on elm leaves, while larvae were more affected than the adults. These results differ from those obtained by Shekari et al. (2008) with extracts from *Artemisia annua* L. (Asteraceae) onto larvae and adult *X. luteola*, in tests where the adults were 2.4 times more susceptible than the larvae.

Evaluation of the repellent effect on leaves in trays. The results of repellency to larval feeding on elm leaves treated with the botanical insecticides, evaluated after 1 and 24 h, are presented in Figure 2.

Both Biomilbe and Biobug caused repellency to *X. luteola* larvae after 1 h, as a greater percentage of them preferred to feed on the untreated leaves. Bugitol and Garlic Barrier did not show this effect after 1 h or 24 h (Fig. 2).

Direct application of the insecticides onto *X. luteola* larvae and adults presented low mortality levels and represent an inefficient control method compared to foliar sprays. In another study, Defagó et al. (2006) evaluated extracts from fruits, green leaves and senescing foliage of *Melia azedarach* L. (Meliaceae) onto *X. luteola*. Their results showed that the three extracts had a strong antifeeding effect, and inhibited completely feeding at 1, 2, 5, and 10% concentrations. Only concentrations ≤ 0.5% did not cause differences in the feeding election. In that research, , the survival of the insects fed with elm leaves treated with the

Table 5. Mean values of larval and adult mortality (% ± SD) of *X. luteola* after 1 h from direct application of Biomilbe, Bugitol, Biobug, and Garlic Barrier.

Concentrations*	Larval mortality means**	Adult mortality means**	P value for larvae	P value for adults
		Biomilbe		
0.0	0.00(± 0.0) a	1.25(± 1.3) a		
0.5	1.25(± 1.3) a	1.25(± 1.3) a		
1.0	2.50(± 1.4) a	2.50(± 1.4) a	0.4280	0.067
2.0	3.75(± 2.4) a	3.75(± 1.3) a		
4.0	13.75(± 9.4) a	7.50(± 1.4) a		
		Bugitol		
0.0	0.00(± 0.0) a	1.25(± 1.3) a		
0.5	2.50(± 1.4) a	2.50(± 1.4) a		
0.8	11.25(± 5.2) a	6.25(± 1.3) a	0.0043	0.0031
2.0	30.00(± 5.5) b	17.50(± 1.3) b		
4.0	37.50(± 6.0) b	27.50(± 1.3) b		
		Biobug		
0.0	0.00(± 0.0) a	1.25(± 1.3) a		
0.5	0.00(± 0.0) a	0.00(± 0.0) a		
1.0	0.00(± 0.0) a	0.00(± 0.0) a	0.2021	0.385
2.5	1.25(± 1.3) a	1.25(± 1.3) a		
4.0	2.50(± 1.4) a	2.50(± 1.4) a		
		Garlic Barrier		
0.0	0.00 (± 0.0) a	1.25 (± 1.3) a		
0.8	3.75 (± 2.4) a	0.00 (± 0.0) a		
1.0	3.75 (± 2.4) a	1.25 (± 1.3) a	0.052	0.073
2.0	6.25 (± 3.8) a	3.75 (± 1.3) a		
4.0	12.50 (± 1.4) b	10.00(± 3.5) a		

*mL commercial product 100 mL solution^{-1}. **Means in a column for each insecticide with different letters are significantly different (P ≤ 0.05) from the untreated control, according to non-parametric ANOVA.

extracts was very small after 6-10 d from application, compared to the control treatment (leaves treated with distilled water). Mortality reached 50% after 3 d and went up to 100% after 8 d.

Huerta et al. (2010) studied the insecticidal effectiveness of water and ethanol extracts from *Schinus molle* L. leaves at several concentrations to control *X. luteola*. Both extracts were effective, as the ethanol caused > 97% mortality at the highest concentrations (4.3 and 4.7% w/v), and close to 27% with the aqueous extract at 4.3 and 5.6% w/v). Onto the adults, the ethanol extract mainly had a toxic effect, while the aqueous one had an antifeeding effect.

Furthermore, the insecticidal effects of ethanol and water extracts from new and matures leaves of *S. molle* were evaluated by Chiffelle et al. (2013) on third instar larvae of *X. luteola* at concentrations of 0.5-4.3% w/v. After 12 h, the maximum concentrations obtained with ethanol and water from new and mature leaves caused mortality levels that reached mean values of 89 and 67, and 78 and 63%, respectively. The lowest LC_{50} was 1.28% w/v, obtained on the 7th day of evaluation with the ethanol extract from new leaves.

Benzi et al. (2009) evaluated the insecticidal effects of foliar and fruit extracts from *S. molle* against the rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae), and Upadhyay and Ahmad (2011) recommend them as management strategies against stored grain insect pests in farmer stores and public warehouses.

In a laboratory study, Girmay et al. (2014) found that extracts from new and mature *S. molle* fruit are also toxic to larvae and pupae of *Culex quinquefasciatus* Say (Diptera: Culicidae), causing up to 93.3 and 83.3% larval mortality, respectively.

In the research of Shekari et al. (2008) with extracts from *Artemisia annua* L., the highest repellent effect after 24 h occurred at concentrations of 10 and 5%, while the lowest effect was observed at a concentration of 0.625%. The effect decreased after 48 h, as the adults of *X. luteola* went back to feed on the treated leaves, which indicates that the components of the extract are probably volatile.

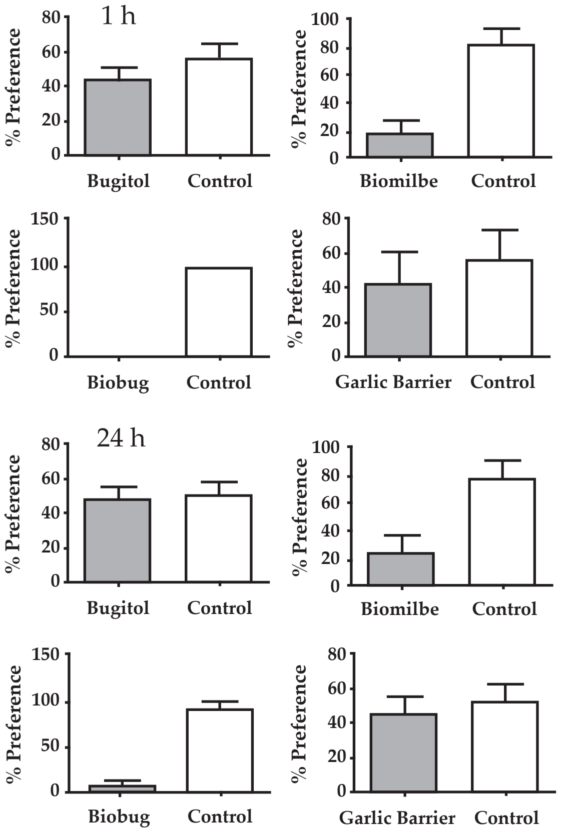

Fig. 2. Preference (%) of larvae for leaves treated with the botanical insecticides (grey columns) or water (white columns), after 1 and 24 h from application.

Herein, Bugitol and Garlic Barrier were the products with the greatest lethal effect onto *X. luteola* larvae and adults, at the highest concentrations evaluated of 2 and 4%, after 48 h from application on the leaves. Biomilbe and Biobug presented repellent effects. Therefore, these insecticides would be an excellent alternative to chemical insecticides in integrated management of *X. luteola*. Being good repellents, Biomilbe and Biobug could be applied at the emergence of the pest, to reduce its damage. The effect of Bugitol is more effective on larvae, as they are more sensitive than adults to this product. The results would improve with more than one spray, considering that moth deaths occurred after 48 h from the treatment. In addition, applying Bugitol sprays along with Garlic Barrier sprays are likely to increase mortality.

Direct sprays onto both stages of *X. luteola* caused very low mortality, which complement the results on the leaves. Biomilbe and Biobug demonstrated repellency onto *X. luteola* larvae that were more susceptible particularly to Bugitol. Mortality obtained with all the organic pesticides evaluated herein was < 50%. Further studies should evaluate higher concentrations or mixed products, including repellency to adults and the effects on the eggs and pupae.

For years, organic vegetable producers have combined the repellent effects of garlic, onion, and hot pepper to make all-purpose sprays against insect pests. Nowadays, there are commercial organic sprays that list garlic oil and/or hot pepper as active ingredients.

CONCLUSIONS

Bugitol and Garlic Barrier were the products with the highest lethal effect onto *X. luteola* larvae and adults, at the maximum concentrations evaluated, which correspond to 2% and 4%, after 48 h from application on the leaves. Biomilbe and Biobug also presented repellence effects.

LITERATURE CITED

Anwar, A., M. Groom, and D. Sadler-Bridge. 2009. Garlic: from nature's ancient food to nematicide. Pesticide News 84:18-20.

Casida, J.E. and G.B.Quistad. 1998. Golden age of insecticide research: past, present or future? Annu. Rev. Entomol. 43:1-16.

Benzi, V., N. Stefannazzi, and A.A. Ferrero. 2009. Biological activity of essential oils from leaves and fruits of pepper tree (*Schinus molle* L.) to control rice weevil (*Sitophilus oryzae* L.). Chilean J. Agric. Res. 69:154-159.

Chiffelle, I., A. Huerta, M. Célis, and J.E. Araya. 2013. Proximal analysis and insecticidal effects of extracts from pepper tree (*Schinus molle*) leaves on elm leaf beetle (*Xanthogaleruca luteola*) larvae. Industrial Crops and Products 43:523-528.

Cobos, P., A. Hernández, C. Muñoz, V. Pérez, y G. Sánchez. 2003 Sanidad forestal. Guía en imágenes de plagas, enfermedades y otros agentes presentes en los bosques. 575 p. Mundi Prensa, Madrid, España.

Defagó, M., G. Valladares, E. Banchio, C. Carpinella, and S. Palacios. 2006. Insecticide and antifeedant activity of different plant parts of *Melia azedarach* on *Xanthogaleruca luteola*. Fitoterapia 77:500-505.

De Liñán, V. 1998 Entomología agroforestal. Insectos y ácaros que dañan montes, cultivos y jardines. 1309 p. Ediciones Agrotécnicas, Madrid, España.

Ferrada, I. y A. Farías. 2004. Uso de extractos de ajo como repelente de áfidos (Hemiptera: Aphididae) en un cultivo orgánico de papas. Disponible en http://es.scribd.com/doc/119766732/publicacionAJOCOMOREPELENTESDEAFIDOS-pdf#scribd (Consulta 13 mayo 2015).

Girmay, K., B. Fikre, A. Asmelash, B. Getachew, E. Tekle, and N. Raja. 2014. Evaluation of water and ethanol extracts of *Schinus molle* Linn. against immature *Culex quinquefasciatus* Say (Diptera: Culicidae). J. Coastal Life Med. 2(6):471-477.

Gómez, C., y A. Soto. 2002. Evaluación de extractos vegetales para el manejo del picudo negro del plátano (*Cosmopolites sordidus* Germar). Boletín 65. Comité de Investigación Aplicada para la Gestión Ambiental, Universidad de Caldas, Manizales, Colombia.

Huerta, A., I. Chiffelle, K. Puga, F. Azúa, and J.E. Araya. 2010. Toxicity and repellence of aqueous and ethanolic extracts from *Schinus molle* on elm leaf beetle *Xanthogaleruca luteola.* Crop Protection 29:1118-1123.

Huerta, A., I. Chiffelle, K. Puga, R. Jiménez, and J.E. Araya. 2011. Life cycle of *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae) in Santiago, Chile, and sex fenotype differentiation of adults. Bol. San. Veg. Plagas 37:57-64.

Isman, M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Ann. Rev. Entomol. 51:45-66.

Motulsky, H. 2007. Prism 2.5 Statistics Guide. GraphPad Software Inc., San Diego, California, USA. Available at www.graphpad.com (Accessed 22 May 2013).

Romanyk, N., y D. Cadahía. 2002. Plagas de insectos en la masa forestal. 334 p. Sociedad Española de Ciencias Forestales, Madrid, España.

SAG. 2005. Informativo Fitosanitario Forestal. Año 1 Nº 2. Ministerio de Agricultura, Servicio Agrícola y Ganadero (SAG), Santiago, Chile.

SAG. 2010. Informativo Fitosanitario Forestal Nº 4. Servicio Agrícola Ganadero (SAG), Subdepartamento de Vigilancia y Control Oficial Forestal, División Protección Agrícola y Forestal, Santiago, Chile.

Sánchez, H. 2003. Manejo de insecticidas en ambientes urbanos. In Silva, G. y R. Hepp (eds.). Bases para el manejo racional de insecticidas. 236 p. Fundación para la Innovación Agraria (FIA). Facultad de Agronomía, Universidad de Concepción, Chillán, Chile.

Shekari, M., J.J. Sendi, K. Etebari, A. Zibaee, and A. Shadparvar. 2008 Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). Pesticide Biochemistry and Physiology 91:66-74.

Silva, G. 2007. Insecticidas vegetales. Boletín técnico del Área Pest Control. Edición Nº 17. ANASAC. Santiago, Chile.

Statgraphics. 2009. Statgraphics Centurion XVI User Manual. Available at http://www.statgraphics.com/main.pdf (Accessed 22 May 2013).

Upadhyay, R.K., and S. Ahmad. 2011. Management strategies for control of stored grain insect pests in farmer stores and public warehouses. World J. Agric. Sci. 7:527-549.

Vargas, R., y A. Ubillo. 2001. Toxicidad de pesticidas sobre enemigos naturales de plagas agrícolas. Agricultura Técnica (Chile) 61(1):35-41.

Vergottini, G. 2011. Toxicidad de algunos insecticidas de origen vegetal sobre *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae). Disponible en http://www.tesis.uchile.cl/handle/2250/112804 (Consulta 10 abril 2012)