

ANTIFEEDING AND INSECTICIDE PROPERTIES OF AQUEOUS AND ETHANOLIC FRUIT EXTRACTS FROM *Melia azedarach* L. ON THE ELM LEAF BEETLE *Xanthogaleruca luteola* Müller

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ABSTRACT

Xanthogaleruca luteola Müller (Coleoptera: Chrysomelidae), a defoliator of *Ulmus* species currently present in several regions of central Chile, causes severe damage to trees, mainly in park areas, street tree-lines and gardens. The antifeeding and insecticidal activities of extracts from immature fruit of *Melia azedarach* L. (Meliaceae) were determined on adults of *X. luteola* in laboratory bioassays. Several concentrations of the extracts obtained with water and ethanol were used and their effectiveness and LC₅₀ were determined. The antifeeding action of the water extracts caused 100% deterrence over concentrations of 3.6% w/v. Both extracts were effective insecticides against adults, causing 86% mortality (2.4% w/v), with a better performance of the ethanol extracts, with a LC₅₀ of 0.9% w/v on the 3rd day after exposure, and 6.6% w/v on the 5th day with the water extract.

Key words: Botanical insecticides, ethanol extract, water extract, *Ulmus*.

Elms (*Ulmus* spp.), ornamental trees originally from Europe, Asia, and America, grow in Chile mainly in parks and avenues in the central and south-central zones (Fu *et al.*, 2003; Loewe and González, 2005). Elm trees in diverse communes of the Metropolitan (including Santiago and surrounding areas) and other regions are being attacked by the elm leaf beetle, *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), a monophagous pest native to Europe that defoliates *Ulmus* spp., with preference for European species (Romanyk and Cadahia, 2002). It is considered the greatest defoliator of elms in plantations and ornamental trees in Europe, and has become the most important urban forestry pest in the USA, Argentina, Canada, and Australia (Lawson and Dahlsten, 2003). Its first recorded appearance in Chile occurred in April 1982, in Ritoque, a coastal resort North of Valparaíso (Askevold, 1991). Recently, this pest has been found in the Metropolitan area and the Libertador General Bernardo O'Higgins, Bío Bío, and La Araucanía Regions (SAG, 2010).

Damage by *X. luteola* begins with first-stage larvae,

which consume the leaves starting at their underside epidermis, leaving the upper side untouched, which skeletonizes the laminae gradually during larval development. Later, when the adults emerge, a second period of foliar destruction ensues, when the adults feed on the leaves, leaving them with circular perforations. The two kinds of damage are often found together, indicating certain overlap between generations (Romanyk and Cadahia, 2002). The result may vary from partial to total defoliation, which reduces the aesthetic value of the tree, weakens it and makes it more susceptible to other pests, such as scolytid beetles transmitting Dutch elm disease (De Liñán, 1998). The level of damage is related to the number of generations per year. In regions where weather conditions are mild, the insect may complete up to three generations a season, as happens in Spain (Romanyk and Cadahia, 2002), and south-central California (Dreistadt *et al.*, 2004).

Botanical insecticides have been used in agriculture for at least 2000 yr in Asia and the Near East (Thacker, 2002). The interest in botanical compounds for pest control is based on their efficacy, degradability, and physiological activity (Rodríguez, 1998; Isman, 1999). The neem tree, *Azadirachta indica* L., and the Chinese neem, *Melia azedarach* L. (Meliaceae) are native to Asia and Australia and possess important insecticidal properties. Both species are used as ornamentals and have been naturalized in tropical and subtropical countries (Villalobos, 1996).

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Melia azedarach is native to Iran, India, and China (Hong and Ellis, 1998). In Chile it is commonly found on streets and avenues. The plant has become the object of studies to evaluate properties from different plant structures, in particular insecticide, antiviral, antioxidant, bactericide, and antiparasitic activities (Carpinella *et al.*, 2003; Ahmed *et al.*, 2008; Gende *et al.*, 2008).

The insecticidal activity of *M. azedarach* is found on leaves, fruits, and seeds, and is due to a group of biologically active triterpenoids that have antifeeding effects (Valladares *et al.*, 1997; Isman, 2006). Generally, extracts from green fruits and leaves have been those most efficacious because of their antifeeding effect, mainly on beetles and lepidopterans (Carpinella *et al.*, 2003; Nathan and Kim, 2005; Defagó *et al.*, 2006). However, *M. azedarach* has had a modest development as a commercial insecticide compared *A. indica*, due mainly to the content of meliatoxin in the fruit, a triterpenoid compound that is toxic to mammals (Schmutterer, 2002). In any event, the chemical composition of *M. azedarach* varies enormously from its wild form to the cultivated one. Trees in Argentina, for example, have fruit without meliatoxins, although they contain other triterpenoids, especially meliartenin, a strong insect antifeedant (Carpinella *et al.*, 2003; 2005).

Considering the results of diverse studies of the insecticidal effectiveness of diverse parts of *M. azedarach*, and that in Chile this plant is grown as an urban tree may be used to prepare natural extracts for use against diverse pests, particularly *X. luteola* on elms.

The hypothesis of this study is that there are differences in the antifeeding and insecticidal properties of immature (green) fruit of *M. azedarach* when different solvents are used for extraction.

This research presents results of a study on the preparation of extracts using two solvents from immature fruit of *M. azedarach*, and the evaluation of their antifeeding effects on adult *X. luteola* in laboratory bioassays, to contribute to the integrated management of this insect pest or others defoliator insects.

MATERIALS AND METHODS

Collecting *X. luteola*

Last stage *X. luteola* larvae were manually collected from adult *Ulmus minor* Mill. trees in the Municipality of Maipú, Santiago, during the same period, and transported in cotton bags to the Forestry Entomology Laboratory, College of Forestry and Nature Conservation Sciences, Universidad de Chile, Santiago. They were set in Petri dishes lined with Whatman N° 1 filter paper and moistened with distilled water. The larvae were fed fresh elm tree leaves until developing into pupae. At this stage they were provided only with humidity and covered with

more leaves. When the adults emerged, they were fed with fresh leaves and subsequently used in the bioassays.

Collecting and preparation of fruit extracts

Approximately 1 kg of bright green fruit from *M. azedarach* (20 fruits per tree) were obtained randomly from 32 adult trees growing as ornamentals, to avoid effects of any individual tree. The trees were located at the Antumapu Campus, Universidad de Chile (33°34' S; 70°38' W) in Santiago, Chile and the fruit were collected during the summer of 2008. Green fruit were selected because of their greater insecticide effectiveness (Chiffelle *et al.*, 2009). The collected fruit were washed superficially with distilled water. Fruit extracts were prepared in the Chemistry Laboratory of the Departamento de Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago. First they were dried in a forced air stove (Memmert, Schwabach, Germany) at 45 °C until a constant weight 0.01 g sensitivity analytical scale (ELB3000, Shimadzu Scientific Instruments, Columbia, Maryland, USA). Fruit extracts were then ground with a grain mill (MC 0360, Ufesa, Spain) until obtaining a dust that was stored in sealed and dated glass vials. To prepare the extracts, the *M. azedarach* fruit dust was mixed with distilled water or 96% pro-analysis ethanol (Merck, Darmstadt, Germany) in a solution at the highest possible concentration. The solutions were shaken 18 h in a magnetic stirrer (MR 3001K, Heidolph, Kelheim, Germany), heating the first hour to 37 °C. They were then filtered through a Whatman N° 1 filter paper and centrifuged in a centrifuge (HN-S Centrifuge, International Equipment Co., Philadelphia, USA) 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 0.1 mg sensitivity analytical scale (Boeco Equilab, Hamburg, Germany). Once the concentrations of these solutions were determined, the concentrations to be used in the treatments were prepared by dilution.

Evaluation of the antifeeding action of *M. azedarach*

The antifeeding effect of extracts on *X. luteola* adults was determined following the feeding choice test method in Defagó *et al.* (2006), with modifications. Six concentrations were evaluated for the ethanol extract (1.0, 1.8, 2.4, 3.0, 3.6 and 4.2% w/v), and for the water extract (2.4, 3.0, 3.6, 4.2, 6.0, and 7.5% w/v), with six replicates per treatment, using experimental units with three adults of *X. luteola*. Two elm tree leaves of the same size were selected per unit, and one leaf was coated on both sides with 500 µL of the reported concentrations, and the other leaf was coated with 500 µL of solvent

(water or ethanol). The leaves were left to dry at room temperature. Each Petri dish (10 cm diameter) was set randomly with a treated leaf, another untreated, and three adult beetles. All the experiments were carried under a 18:6 photoperiod at 20 °C (Meiners and Hilker, 1997). The leaf area was measured initially and after 24 h using the Leaf Area Measurement Version 1.3 software (University of Sheffield, 2003). The ingested area was determined by the difference. The repellence percentage was calculated as $(1-T/C) \times 100$, where T and C are the areas ingested of treated and control leaves, respectively (Schreck *et al.*, 1977). Data were normalized by Bliss ($\arcsin\sqrt{\text{percentage leaf area consumed}}$) prior to analysis, to stabilize variance error. Once the experimental part was completed, data from water extracts were studied with the Wilcoxon Signed-Rank Test using InfoStat statistical software (InfoStat, 2009).

Evaluation of insecticide efficacy

The bioassays were established with experiment units of three *X. luteola* adults with fresh elm tree leaves on moist filter paper in a clean 10-cm-diameter Petri dish. Concentrations of 3.9, 6.8, 7.3, 8.6 and 10.0% w/v were used for the water extracts, and 0.4, 0.6, 1.0, 1.8 and 2.4% w/v for the ethanol extracts. These concentrations were used to obtain mortality values from 25 through 75%, adequate for Probit analysis (Robertson *et al.*, 1984). The efficacy of fruit extracts was evaluated at the concentrations indicated plus the controls without any extract, with three replicates, all conducted simultaneously. The extracts were applied by immersing elm leaves for nearly 1 min in the corresponding solution. The beetles exposed afterwards were observed and the survivors were counted daily during the bioassay. All the experiments were carried out under the same environmental conditions described above. The percentages of daily and total mortality \pm standard error were obtained. Mortality values were corrected using Abbott's formula (Abbott, 1925) to eliminate natural mortality in the control (Silva *et al.*, 2003). Data were adjusted to obtain the function with the best mathematical fit, as well as to obtain the LC₅₀ by Probit analyses (Robertson *et al.*, 1984) using Stadplus 5.4.0 software (AnalystSoft, 2008). Chi² tests were used to test the data fit to the Probit model.

Following the experimental component, data from the ethanol and water extracts were studied separately using a completely random ANOVA with five treatments for ethanol and five for water (five concentrations plus a control), with three replicates each. Data were normalized by Bliss ($\arcsin\sqrt{\text{percentage adult mortality}}$) prior to analysis, to stabilize variance error. Significant differences among treatments were identified with Tukey tests ($p \leq 0.05$), using InfoStat statistical software (InfoStat, 2009).

RESULTS

Antifeeding effect

The extracts obtained with the solvents had different antifeeding effects. The ethanol extract was not evaluated as an antifeedant because most of the insects died and those that survived did not feed on either the control or treated leaves. The water extract had a strong antifeeding effect at all evaluated concentrations, particularly at 3.6% w/v or higher, where inhibition reached 100% (Table 1).

Insecticide efficacy of the aqueous extracts onto adults of *X. luteola*

The survival of the insects fed with leaves treated with concentrations of the water extract were numerically lower than the control, starting day one, and these differences became statistically significant from day five (Figure 1a).

The LC₅₀ of the water extract was 6.6% w/v ($R^2 = 0.87$) on day 5 (Table 2). In general, the probit analysis provides good LC₅₀ estimates when mortality obtained varies between 10 and 90%. Beyond these limits, errors increase and affect calculations.

The maximum mortality (76%) with the water extract was obtained when applying the highest concentration (10.0% w/v) (Figure 1a). The levels of mortality were lower with lower concentrations of the extract. However, the mortality levels were always significantly different from those with the control, indicating that mortality rates were due to the insecticidal extract.

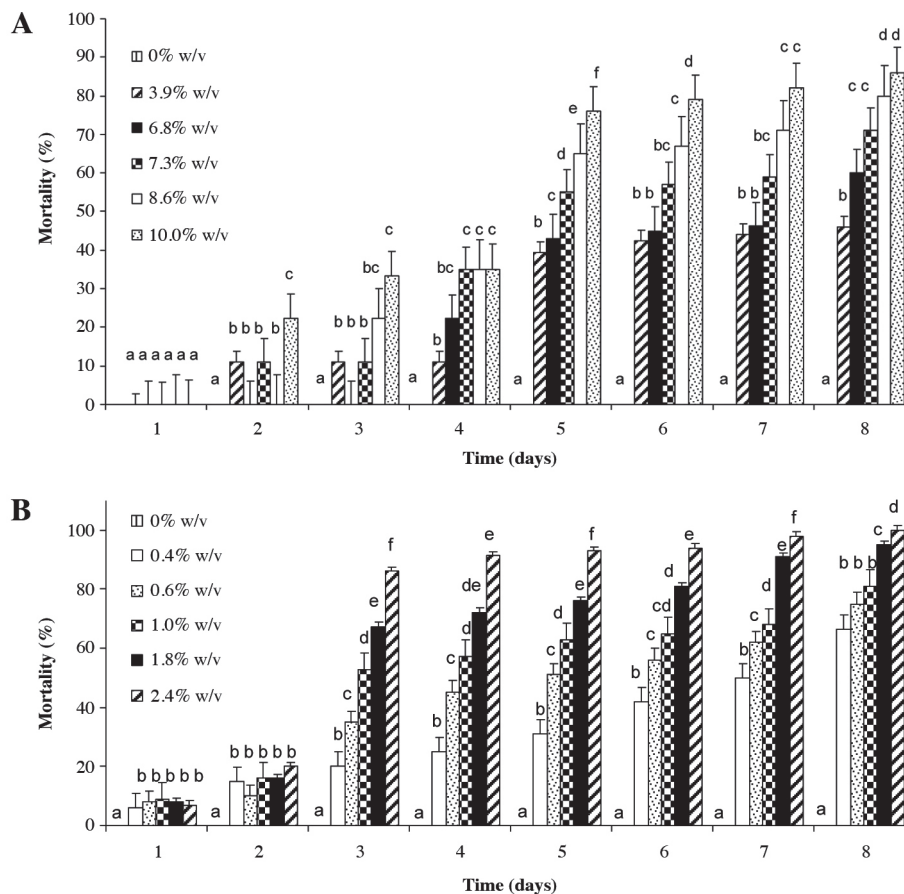
Insecticide efficacy of the ethanol extracts on *X. luteola* adults

The survival rate of adults fed with leaves treated with the ethanol extract was lower than that of the control from day one, with significant differences between days 3 and 6 (Figure 1b). The most significant differences in

Table 1. Effect of water extracts from fruit of *Melia azedarach* on the feeding of *Xanthogaleruca luteola* adults on elm leaves in a random choice test.

Extracts	Leaf area consumed		Antifeeding effect
	Untreated control	Treated leaves	
% w/v	%		
2.4	1.1	0.1*	87.1
3.0	3.1	0.1*	97.7
3.6	3.3	0.0*	100.0
4.2	2.9	0.0*	100.0
6.0	2.5	0.0*	100.0
7.5	2.2	0.0*	100.0

*Significant differences between consumption on control and treated leaves (Wilcoxon signed-rank test, $P < 0.05$).



Different letters horizontally indicate significant differences among concentrations, according to Tukey tests ($p \leq 0.05$). SE: standard error.

Figure 1. Average mortality (% \pm SE) of *Xanthogaleruca luteola* adults with leaf extracts from *Melia azedarach* at several concentrations at 1-8 d of evaluation. A) Water; B) ethanol.

mortality rates among concentrations occurred on day 3. The values for the Probit fit for the calculation of the LC_{50} are presented in Table 2. The LC_{50} of the ethanol extract was 0.9% ($R^2 = 0.91$) on day 3.

The highest mortality of *X. luteola* adults (86%) with the ethanol extract (2.4% w/v) and the water extract

(10.0% w/v) were obtained with the highest concentrations (Figure 1). Smaller concentrations caused lower levels of mortality. All concentrations were statistically different from the control, with a clear trend toward increased mortality in response to higher concentrations of the extract.

Table 2. Mortality of *Xanthogaleruca luteola* adults after treating elm leaves with water and ethanol extracts from fruit of *Melia azedarach*.

Extracts	Time	Slope	LC			Chi ^{2*}
			LC ₁₀	LC ₅₀	LC ₉₀	
	d	mean \pm SE	%			
Water	5	33.3 \pm 6.5	2.0	6.6	21.7	2.79
	8	31.9 \pm 9.8	0.9	3.1	11.1	1.38
Ethanol	3	34.9 \pm 8.1	0.3	0.9	3.0	2.07
	8	9.4 \pm 0.4	0.0	0.1	1.8	0.15

*Means below the Chi² tabulated (df = 5; $p \leq 0.05$) = 11.05 indicate that the Probit model fits the experiment data for both extracts. SE: standard error.

DISCUSSION

The results in this study verified the antifeeding effect of the water extract from fruit of *M. azedarach* on *X. luteola* adults, with 100% inhibition at 3.6% w/v, while the ethanol extracts were not evaluated as antifeedants because most of the insects died and the surviving ones did not feed on either the control or the treated leaves. Defagó *et al.* found antifeeding effects of 98% on *X. luteola* with 0.25% concentrations of an ethanol fraction and 100% effect with 1, 2, and 5% concentrations. The latter results are comparable to ours, since those authors used the same insect and fruit maturity stage, although we evaluated crude extracts, while they used Soxhlet extracted with EtOH, which could explain the differences in results (Defagó *et al.*, 2006). Other studies on the antifeeding effects of ethanol extracts of *M. azedarach* with other insects found close to 100% antifeeding effects with ethanol extracts from mature fruit at similar concentrations, 2, 5, and 10%, applied to *Spodoptera eridania* Cramer (Lepidoptera: Noctuidae) (Rossetti *et al.*, 2008), and other studies found that the ethanol extract at 10% from senescing leaves of *M. azedarach* had a total antifeeding effect (100%) when applied to the coleopterans *Diabrotica speciosa* (Germar) (Chrysomelidae), *Epilachna paenulata* Germar (Coccinellidae), and *Sitophilus oryzae* (L.) (Curculionidae) (Valladares *et al.*, 2003).

With respect to the toxicity of the ethanol solvent observed in our antifeeding activity bioassay, treatment of membranes with 2.5-12.5% w/v ethanol produced a slight stimulation of acetylcholinesterase activity in skeletal muscles and inhibition at higher concentrations (Cabezas-Herrera *et al.*, 1992).

Other authors have studied the insecticidal effect of fruit extracts from *M. azedarach*. Applying water extracts from green fruits at 5% to the spider mite *Tetranychus urticae* Koch (Tetranychidae), Castiglioni *et al.* found 63% mortality (2002). Other authors evaluated the insecticidal effect of these extracts on *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) nymphs and found that at a 20% concentration, mortality was slightly over 15%, which increased to nearly 72% by adding a coadjuvant (Tween-20 detergent at 0.5%) (Jazzar and Hammad, 2003).

Mortality levels > 70% were registered with *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) adults by applying water extracts at a 1.07% w/v concentration (Huerta *et al.*, 2008).

Levels of mortality in the literature indicate an insecticidal effect of water extract of fruits of *M. azedarach* on diverse insects, and coincide in part with

the results obtained in our study, although at lower mortality rates than those registered by Huerta *et al.* (2008) with adults of *D. melanogaster*.

As per the Soxhlet extracted with ethanol from green fruits of *M. azedarach*, concentrations of 10 and 20% applied to *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae) third instar larvae caused 55 and 65% mortality of pupae, respectively (Banchio *et al.*, 2002). In the study by Defagó *et al.* (2006), the insecticide activity of mixed solvents (water, acetone, ethanol, and methanol) from green fruits was evaluated on adults of *X. luteola*, with average cumulative mortality levels of 91, 100, and 100% obtained for concentrations of 2, 5, and 10%, respectively. Similar levels of mortality were obtained in our study at the given concentrations. The results of Defagó *et al.* (2006) are important as they were obtained on the same insect, although with differences in the preparation of the extract, which they obtained via Soxhlet, while crude extracts were used in our work.

Other studies have evaluated the insecticidal effect of extracts obtained with other solvents, such as methanol, hexane, petroleum ether, and acetone, among others (Nunes *et al.*, 2004). Ethanol extracts caused mortality effects in a shorter period of time, probably because of the greater solubility of the organic compounds in this solvent, which has a 25 absolute dielectric constant at 25 °C and lower polarity compared to the 79 level for water at the same temperature (Weast and Selby, 1966).

The LC₅₀ obtained in our study differs from that in Huerta *et al.* (2008) for extracts from green fruit of *M. azedarach*, where concentrations of 2071 and 4382 ppm were obtained for ethanol and water, respectively. However, the application method in that research was different, where the extracts were mixed directly with the rearing substrate of *D. melanogaster* adults. In any event, in both studies, the effectiveness of the ethanol extracts was greater than that obtained with water.

Others authors have found a similar LC₅₀ for the methanol bark extract from *M. azedarach* applied to larvae of two species of nocturnal lepidopterans, *Trichoplusia ni* Hübner (Noctuidae) and *Pseudaletia unipuncta* Haworth (Noctuidae). When the extract was applied on a foliar disc (feeding test), the LC₅₀ for *T. ni* and *P. unipuncta* were 6 and 3.1% w/v. However, when the extract was sprayed directly on the body of *T. ni* larvae, an LC₅₀ of 12.6% w/v was found at day (Akhtar *et al.*, 2008).

In *X. luteola*, Shekari *et al.* (2008) found that at 48 h, the methanol extract from *Artemisia annua* (L.) (Asterales: Compositae) presented an LC₅₀ of 43.8 and 15.4% when applied on third stage larvae and adults,

respectively. This latter result is close to that found for the ethanol extract in our study.

CONCLUSIONS

The water extracts from green fruits of *M. azedarach* evaluated in laboratory bioassays had pronounced antifeeding effects on adults of *X. luteola* at all tested concentrations. The water ethanol extracts were more efficacious as bioinsecticides, causing 86% mortality at the highest concentration (2.4% w/v). The levels of mortality were lower with lower concentrations of the extract, although they were always significantly different from the control, indicating that mortality rates were due to the insecticidal extract. The lowest LC₅₀ (0.9% w/v) on adults of *X. luteola* was obtained with the ethanol extracts. The results obtained suggest an interesting opportunity for development of this bioinsecticide from green fruits of *M. azedarach*, for use in integrated pest management of *X. luteola* and possibly other pests.

RESUMEN

Propiedades antialimentaria e insecticida de extractos acuosos y etanólicos del fruto de *Melia azedarach* L. en el escarabajo de la hoja del olmo *Xanthogaleruca luteola* Müller. *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), un defoliador de especies de *Ulmus* presente en varias regiones de Chile central, causa daño severo en árboles principalmente en áreas de parques, árboles de calles, y jardines. Se determinó el efecto antialimentario e insecticida de extractos de frutos inmaduros de *Melia azedarach* L. (Meliaceae) sobre adultos de *X. luteola* en bioensayos de laboratorio. Se usaron varias concentraciones con agua y etanol de los extractos y se determinó su efectividad y su CL₅₀. La acción antialimentaria de los extractos de agua causó un 100% de deterrencia sobre concentraciones de 3,6% p/v. Ambos extractos fueron efectivos como insecticidas contra los adultos, causando un 86% de mortalidad (2,4% p/v), con un mejor comportamiento de los extractos con etanol con una CL₅₀ de 0,9% p/v al tercer día después de la exposición, y 6,6% p/v al quinto día con los extractos acuosos.

Palabras clave: Insecticidas botánicos, extracto de etanol, extracto de agua, *Ulmus*.

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