

INSECTICIDE CAPACITY OF AQUEOUS EXTRACT FROM FRUITS OF *Melia azedarach* SPRAYED ON *Ulmus* sp. (ULMACEAE) INFESTED WITH *Xanthogaleruca luteola* (COLEOPTERA: CHRYSOMELIDAE) ADULTS

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Abstract

The elm leaf beetle, *Xanthogaleruca luteola*, defoliates severely *Ulmus* spp. (Ulmaceae) in many countries, including central and southern Chile, and can even kill young trees. It still has no effective control treatment. Herein, the insecticide capacity of the aqueous extract from green fruits of *Melia azedarach* (Meliaceae), a tree for ornamental use, was evaluated at three concentrations on *X. luteola* adults on elm tree in Santiago, Chile. Five 50 cm branches selected at random were sprayed per extract, infested with 15 newly emerged adults, and enclosed with cloth. Mortality obtained with 0.0 (control), 1.8, 3.6, and 7.1% w/v concentrations and three replicates were evaluated periodically from day 3 through 15 after spray. Statistics were analysed through ANOVA and significance differences between treatments and times after spray were estimated with the Tukey test. The LC_{50} was calculated using the Probit method and adjusted for Chi-square. All concentrations had insecticide effect compared to the control. The maximum mean mortality (90%, at 7.1% w/v) was reached at 9 d. The lowest LC_{50} was 10.94% w/v on day 6. Beetles' mortality shown a positive linear response, increasing with extract concentration. Also, the phenology of *M. azedarach* was observed during a year in Santiago, for further use in pest control studies. We suggest the green fruits should be harvested the first half of December. These results could be applied for the integrated management of *X. luteola* on infested elms.

Keywords: Botanical insecticide, elm leaf beetle, integrated pest management, plant extract.

1. INTRODUCTION

The elm leaf beetle, *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae) is a severe defoliator of elms (*Ulmus* spp., Ulmaceae), with a wide distribution (Borowiec & Sekerka 2010, Vatanparast et al. 2012, Lefoe et al. 2014). Severe infestations can cause total defoliation (Soudi & Moharramipour 2011). In Chile, it has a great impact in the central and southern zones (Servicio Agrícola y Ganadero 2010), in farm windbreaks and on urban trees, and it does not yet have an effective control treatment.

Due to their high efficacy and low cost, the synthetic insecticides that appeared in the 1940s have been widely used against pests (Silva et al. 2002). Despite growing evidence of environmental damage and human health concerns, the global use of insecticides has grown continuously the last 50 yr (Isman 2020). To reduce their negative effects, new control methods have been developed using plant insecticides based on extracts of leaves, fruits, or other plant parts, with diverse results. These compounds have biodegradability, reduction of pest resistance, less impact on natural enemies, among others positive properties (Amri et al. 2013).

Among the phytochemical compounds with pesticide action are those obtained from species of the Meliaceae family (Schmutterer 1995), due to azadirachtins, limonoids that inhibit insect feeding

(Carpinella et al. 2003, Isman 2020, Khoshraftar et al. 2020). Among these plants, the widely distributed ornamental paradise or melia tree, *Melia azedarach* L. (Meliaceae), stands out in Chile and Argentina. This deciduous species is native to Persia, India and China and has become naturalized in many countries in Africa, Australia, and America. Fruits are 1 cm diam globe drupes with 4-5 seeds, arranged in highly decorative light-yellow clusters that remain on the trees all winter (Pennington & Sarukán 1998, Fuentes et al. 2001).

Studies with extracts from leaves and fruits of *M. azedarach* have shown strong feeding inhibition, in addition to lethal effects on several pest species (Mazzonetto & Vendramin 2003, Pérez-Pacheco et al. 2004, Defagó et al. 2006).

Xanthogaleruca luteola adults exposed to green fruit extracts obtained from *M. azedarach*, using either aqueous or ethanol as solvents have caused mortality; ethanol extracts being more effective, with a LC_{50} of 0.9% at day 3 in the laboratory (Chiffelle et al. 2011). On larvae, extracts from green fruits obtained a LC_{50} of 1.49% w/v at day 8 (Chiffelle et al. 2019).

Given the relative scarcity of field studies of plant extracts, this study evaluated the effectiveness of aqueous extracts from *M. azedarach* fruits at various concentrations on elms infested with adults of *X. luteola*. The phenology of this tree was also studied in Santiago, Chile, to contribute with further pest control studies with extracts.

2. MATERIAL AND METHODS

A trial was carried out in a 1 ha of juvenile elms forest (*Ulmus* spp.) with furrow irrigation, infested by *X. luteola* (Fig. 1) surrounded by a sclerophyllous forest with boldos (*Peumus boldus* Molina), quillayes (*Quillaja saponaria* Molina) and litres [*Lithraea caustica* (Molina) H. et A.], in the South Plantation Sector A at the summit of the Santiago Metropolitan Park (33°25' S, 70°37' W), with no history of applications of chemicals. The climate is warm temperate with a 7- or 8-months dry season, 312.5 mm annual precipitation mostly between May and August. The average annual temperature is 14.4 °C, being January the warmest month and July the coldest one (Rioseco & Tesser 2007). Green fruits (4 kg) (Chiffelle et al. 2009), were collected in December from adult trees (> 30-yr-old), in the Santiago Metropolitan Park.



Fig. 1. Elm leaves with holes caused by emerged adult *Xanthogaleruca luteola* and skeletonized by larvae (light areas at right).

The green fruits aqueous extract was prepared in the Chemistry Laboratory, Department of Agroindustry and Enology, Faculty of Agronomic Sciences, and in the Forest Entomology Laboratory, Faculty of Forestry and Nature Conservation, University of Chile. The fruits were dried in a forced air oven (Memmert®, model 854 Schwabach, Germany) at 40 °C until constant weight. Then, the

complete nuts, with shells, seeds, and pulp, were grinded in a mechanical mill with a graduated sieve (No 60 mesh) into 0.25 mm particles, which was stored in hermetically sealed and dated jars. Increasing volumes of distilled water was added to the milled material to obtain a two phases liquid-solid mixture. Then, this mixture was stirred with a magnetic stirrer (Heidolph, MR 3001K, Schwabach, Germany), at 37 °C the first hour, and at room temperature to complete 24 h. Subsequently, the homogenized mixture was filtered through a Whatman N° 1 filter paper and centrifuged for 15 min at 1500 rpm, to obtain the supernatant (extract). To calculate maximum concentration (mc) of total solids, 15 mL of each extract were dried until constant weight, at 100 °C in a forced air oven. Then, this mc was next mixed with distilled water, in the following proportions: 1 L of concentrated solution (CS) (7.1% w/v), 0.5 L CS plus 0.5 L of distilled water (3.6% w/v), and 0.25 L CS plus 0.75L of distilled water (1.8% w/v).

The effect on the extract on *X. luteola* was observed directly on newly emerged adults (lighter brown color than those older) (Fig. 1). For the tests, uncut 50 cm elm branches (EU) were randomly selected on different trees, which were marked with the treatment, washed with water, and allowed to air dry for 15 min, after which they were sprayed with the corresponding treatment with a 1.5 L manual sprayer (total 180 branches, 5 per treatment). Afterwards the sprayed branches were infested with 15 young adults, covered with a cloth bag, and tied to avoid escapes. The living and dead adults were counted every three days until day 15 in each EU.

The mean mortality $\pm$ SE for each concentration was calculated. The results were normalized by Bliss. A random factorial design (4x5) Anova was applied for analysis, where factor A was the extract concentration (0.0 [control], 1.8, 3.6, and 7.1% w/v), and factor B, the days from application (3, 6, 9, 12 and 15) and three replicates. Significant differences were established with the Tukey test ($p \leq 0.05$). The results of mortality were adjusted to mathematical functions to identify the most suitable concentration and with it to obtain the LC_{50} using the Probit procedure (Robertson et al. 1984). Chi-square tests were used to ensure the fitness of the results to the model. All statistical analysis using the InfoStat (2009) software.

The phenology of adult trees was studied from September 2009 through October 2010 in the Antumapu Campus, College of Forestry and Nature Conservation Sciences, University of Chile, in Santiago, Chile (33°34'S, 70°37'W).

3. RESULTS

There were statistical differences in mortality on young *X. luteola* adults between the *M. azedarach* fruits concentrations ($F_{3,12}$ (0.05) = 6.06) and the days since its application ($F_{4,12}$ (0.05) = 4.77). All the aqueous extract concentrations had significant insecticide effects compared with the control. Within a particular date, mortality between concentrations was not significantly different at day 3. However, from day 6 through 15 mortality significantly increased at least once, ranging from 28 to 90%. The significantly highest mortality occurred quicker, on day 9, with the maximum concentration (7.1% w/v) (Table 1).

Table 1. Accumulated mean mortality $\pm$ SE of young *X. luteola* adults caused by aqueous extract from fruits of *M. azedarach* at several concentrations sprayed, several days from application on branches infested.

Days from Application	Concentrations (% w/v)			
	0.0	1.8	3.6	7.1
3	0.1 $\pm$ 0 aA	28.1 $\pm$ 2 bA	32.9 $\pm$ 3 bA	37.9 $\pm$ 4 bA
6	2.0 $\pm$ 1 aA	37.9 $\pm$ 2 bA	44.4 $\pm$ 4 cB	68.6 $\pm$ 4 dB
9	5.0 $\pm$ 1 aA	63.4 $\pm$ 1 bB	63.4 $\pm$ 2 bC	90.0 $\pm$ 4 cC
12	5.0 $\pm$ 0 aA	75.1 $\pm$ 2 bC	90.0 $\pm$ 2 cD	90.0 $\pm$ 4 cC
15	5.0 $\pm$ 1 aA	90.0 $\pm$ 2 bD	90.0 $\pm$ 2 cD	90.0 $\pm$ 2 cC

Different minor letters on each line and capital letters on each column indicate significant differences between concentrations and days from application, respectively, using the Tukey test ($p \leq 0.05$).

From the Probit analyses, the lowest LC_{50} was 10.94% w/v on day 6 (Table 2).

Table 2. Mean CL_{50} obtained with aqueous extracts from *Melia azedarach* fruits at several days from treatment on young *Xanthogaleruca. luteola* adults.

Days	Slopes $\pm$ SE *	Crossing	LC_{50} (% w/v)	Chi-square
6	-16.1 $\pm$ 0.8	-236.8	10.94	0.44
15	-1.5 $\pm$ 0.1	-70.1	52.49	3.97

Means were lower than tabled Chi-square ($df = 3$; $p \leq 0.05$) = 5.99, thus the results were adjusted to the Probit model.

The phenology of the trees studied is presented in Table 3.

Table 3. Phenology stages of adult *Melia azedarach* trees in the Antumapu Campus, University of Chile, Santiago, Chile.

Phenology stages	Beginning	Observations
Foliage sprouts	2nd wk of September	Light green foliage sprouts in branches and trunk.
Flower clusters	1st wk of October	First panicles of whitish-violet 5-petal fragrant flowers, that remain present most of the year (Figure 2, left).
Green fruits	3rd wk of October	Light-green globe drupes 12-18 cm diam (Figure 2, center).
Mature fruits	1st and 2nd wk of May	Drupes turn light yellow and later darken to ochre and shrink slightly, to remain present the year (Figure 2, right).
Foliage falls	3rd wk of May	The leaves fall in winter, but mature and dry fruits from previous years remain.



Fig. 2. Phenology of *Melia azedarach*. Flower panicles (left), green fruits (center), mature fruits (right).

As mentioned, Chiffelle et al. (2009), the best insecticide effect extract is obtained from green fruits, which should be harvested the first half of December.

4. DISCUSSION

The results verified a strong insecticide effectiveness of the aqueous extract from green fruits of *M. azedarach* against young *X. luteola* adults on elm branches sprayed in the field. Smaller concentrations caused lower mortality, although all concentrations differed statistically from the control, with a clear trend toward increased mortality in response to higher concentrations and time after continuous exposure.

In the laboratory, the insecticide effects of ethanol and aqueous extracts of green *M. azedarach* fruits were effective at several concentrations on *X. luteola* adults. The highest mortality of adults (86%) with the ethanol extract (2.4% w/v) and the aqueous one (10.0% w/v) were obtained with the highest concentrations at day 8 (Chiffelle et al. 2011). Here, very similar results were achieved, 90% mortality with a 7.1% w/v concentration on day 9.

It must be noted that Defagó et al. (2006) obtained high adult mortality rates of *X. luteola*, close to 100% with concentrations of 2, 5, and 10% w/v with ethanol extracts of *M. azedarach* from leaves but 16 days after exposure. Therefore, our results here on elm branches in the field with green fruits extract are more effective than the leaf extract.

M. azedarach extracts have also been evaluated on other beetles. For example, on adults of *Epilachna paenulata* (German) (Curculionidae), Valladares et al. (2003) obtained with 5 and 10% w/v leaf extract significant results from day 4 compared the control, while with the lowest concentration (1.8% w/v) these differences occurred from day 3, revealing a greater effectiveness on *E. paenulata* than on *X. luteola* adults.

Also, leaf, fruits and woody stems powders from *M. azedarach* applied to *Sitophilus zeamais* Motschulsky (Curculionidae) adults were tested by Espinoza et al. (2012). The highest contact mortality was obtained with the powder of leaves and fruits at 2% w/v, with reached mortality 91% 15 d after exposure, which is close to our results.

In comparison, herein the lowest LC₅₀ was 10.94% w/v on day 6. Also, Chiffelle et al. (2011) found an LC₅₀ of 6.55% w/v with the aqueous extract on day 5, whereas Defagó et al. (2006) found an LC₅₀ of 2% w/v on day 9 after exposure, suggesting the concentrations needed to kill 50% of the population were higher in our study, possibly by different field conditions.

Our phenology study of *M. azedarach* agrees with others in México (Calderón & German 1993; Pennington & Sarukán 1998), where flowers and fruits are present most of the year.

Piccolo & Gregolim (1980) observed in Rio Claro, Brazil, that the leaves fall twice, in July-August, and January-February, while herein it occurred at the end of May. Flowering began in their study in

October and lasted most of the year, while in our study fruits develop from the end of November through mid-May. These differences are surely due mostly to the fall of foliage due to climatic factors.

Cavusoglu and Sulusoglu (2015) observed in Kocaeli, Turkey, the phenology of *M. azedarach*. Sprouting began in March, with first leaves developed in April, with more leaf shots forming along the summer, but stopped in September. Flowers appeared in April and were synchronized with foliage development; full bloom occurred in May, when old fruits from previous years remained on the tree; and in June all flowers disappeared. First dark green fruits appeared in June, together with old yellow and other ones from previous years. The new green fruits turned yellowish-green in November, in January mostly light yellow fruits and a few light green fruits were observed even in snowy days. In February, all fruits were light yellow. The mature fruits remained on the tree 16 months (from May to October). Leaves began to fall in October, with the last fall in November. During the winter the tree kept with no foliage, keeping only mature and over mature fruits. In another study in Thailand, Palakit et al. (2018), leaf flushed in January-July, matured in January-November, and leaf abscission occurred in May-June and October-December in the northern part of the country.

Differences in phenology with our study may be due by the different geographic and edaphic conditions that directly affect the phenology of *M. azedarach*.

5. CONCLUSIONS

All the concentrations evaluated of the aqueous extract from green fruits of *M. azedarach* tested caused mortality to young adults of *X. luteola* in the field compared with the control. The maximum 90% mean mortality was obtained quicker with the greatest concentration (7.1% w/v) at day 9. The least LC₅₀ was 10.94% w/v on day 6. In addition, there was a relatively proportional response, the mortality increased with concentration and time of exposure. These results favor further use of this extract on infested trees using integrated pest management, although further phenology studies are required.

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