

# TOXICITY OF NEEM-DERIVED INSECTICIDES TO VARIOUS LIFE STAGES OF THE ELM LEAF BEETLE

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**Abstract.** A series of laboratory and field experiments to determine the suitability of neem oil derived insecticides for elm leaf beetle control was performed. Laboratory experiments determined mortality and antifeedant effects to elm leaf beetle larvae. Ovicidal activity was also evaluated in the laboratory. Field trials were conducted on mature elm trees to evaluate the efficacy of neem on elm leaf beetle larvae. Results of laboratory studies indicated significant mortality to elm leaf beetle larvae as a result of neem applications. In addition, greater mortality was observed to eggs treated with neem. Also, anti-feedant effects of neem-treated leaves was observed in all three elm leaf beetle larval instars. Neem-treated trees showed significantly less elm leaf beetle feeding damage than control trees for approximately one month after application. This study demonstrates the potential of neem oil formulations for elm leaf beetle control.

The elm leaf beetle, *Xanthogaleruca luteola*, is a major defoliator of elm trees in many urban areas of the United States (4). As a result of the extensive aesthetic damage, elm trees are one of the most widely insecticide-treated trees in the urban environment.

A potential control for elm leaf beetle has become available with the development of insecticides extracted from the seeds of the neem tree, *Azadirachta indica*. Azadirachtin has been shown to have a wide range of effects on many insect pests, with the primary effects being antifeedant activity and disruption of hormonal processes (1). Depending on the species of insect, azadirachtin has been observed to cause incomplete molting and metamorphosis (7), disruption of mating (9) and starvation by influencing swallowing and gut motility (11).

Azadirachtin has demonstrated high efficacy rates against lepidopteran ornamental pests such as the gypsy moth, *Lymantria dispar* (5) and the spruce budworm, *Chorsitopneura fumiferana* (12).

Efficacy against leaf beetles on shade trees has not been evaluated. However, azadirachtin has been found to be effective against the Colorado potato beetle, *Leptinotarsa decemlineata*, a foliage feeder on potatoes (3).

Neem-derived insecticides have several advantages for use in urban areas. These advantages include low mammalian toxicity (1,6), minimal impact on beneficial insects and short field persistence (10). The purpose of this study was to determine if neem-derived insecticides can be an effective control agent for the control of elm leaf beetle.

## Methods and Materials

**Larval bioassays.** Elm leaf beetle eggs were field collected from the Ft. Collins, Colorado area in June of 1991. The eggs were placed in Petri dishes, sealed with Parafilm and maintained in a growth chamber (photoperiod 16 L:8 D) at 25 ±1°C. Sufficient number of eggs were collected to allow a uniform age of 48 to 72 h post-eclosion for each of the three instars.

Serial dilutions ranging from 1:80 to 1:640 were prepared with Margosan-O<sup>®</sup> (0.3% azadirachtin, Grace-Sierra Crop Protection Company, Milpitas, CA). Freshly collected Siberian elm (*Ulmus pumila*) leaves were dipped for 5 seconds and allowed to air dry (approximately 30 minutes). The larvae were then placed onto the foliage in petri dishes and sealed with Parafilm. Fifteen 1st and 2nd instar larvae and twelve 3rd instar larvae were placed in each petri dish, respectively. There were six replications per dilution for each instar. Mortality was recorded at 48, 96 and 216 hr. In addition, mortality of 3rd instar was monitored through 13

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days (312 hr) to determine if the treated larvae would develop into adults. Fresh untreated Siberian elm leaves were placed in the Petri dishes after 48 hr. The larvae were maintained in an incubator (photoperiod 16 L:8 D) at a constant  $25 \pm 1^\circ\text{C}$  for the duration of the study. Differences in larval mortality between concentrations were analyzed by ANOVA and means were separated by the Student-Newman-Keuls multiple range test ( $P < 0.05$ ) (SAS 1988).

Antifeedant activity was assessed by painting both surfaces of one-half of a freshly collected Siberian elm leaf with an azadirachtin treatment. Four concentrations of Margosan-O<sup>®</sup> (1:80, 1:160, 1:320 and 1:640) were evaluated for all three instars. The remaining half of each treated leaf was then treated with water. Larvae were placed on the midrib of the leaf which separated the treated and untreated halves of the leaf. The larvae were maintained in a Parafilm-sealed petri dish (photoperiod 16 L:8 D) at a constant  $25 \pm 1^\circ\text{C}$ . After 48 hr, larval location was recorded as off leaf, on treated or untreated portion of leaf.

**Ovicide testing.** Eleven elm leaf beetle egg masses (24-48 hr old) were flagged on field grown Siberian elm trees. Six of the egg masses were treated with Bio-Neem<sup>®</sup> (0.3% azadirachtin, 1:100 dilution, Ringer Corporation, 9959 Valley View Road, Eden Prairie, MN 55344) and five egg masses were treated with water. All treatments were sprayed to the point of run-off on the evening of 10 May, 1992. Eggs were allowed to remain on the trees until hatch was completed. On 11 June, they were returned to the laboratory and percentage of egg hatch assessed. A total of 107 untreated eggs and 140 treated eggs were counted in the trial.

**Field trials.** Field trials were conducted during 1993 on mature elm trees (*Ulmus* sp.) located in a municipal park in Denver, Colorado. The field trial was conducted on 15 June 1993, when elm leaf beetle eggs had just begun to hatch and minimal injury had occurred to the leaves. A single treatment of Azatin EC<sup>®</sup> (3% azadirachtin, 16 fl. oz/100 gal, Agri Dyne Technologies, Inc., Salt Lake City, UT 84108) was applied to run-off. The treatment was applied with a commercial hydraulic sprayer (Meyers Corp. #7360, maximum output

60 gpm). Single trees were considered individual plots, with a buffer tree separating each treatment tree to avoid effects from drift. There were six replications arranged in a randomized complete block design. Plots were evaluated 26 June, 14 and 26 July 1993 by determining the percent of leaves with feeding injury from terminals collected from the four cardinal points of each tree. This involved use of injury rating of 0-10 (0 = no damage, 1 = 1-10% of leaves with injury, 2 = 11-20% of leaves with injury, etc.) similar to that used by Brewer (2). Differences in leaf injury between treatments were determined using the Student-Newman-Keuls Test ( $P < 0.05$ ) (SAS 1988).

## Results and Discussion

**Larval bioassays.** *1st Instar.* After 216 hr (9 days), mortality among treated larvae was significantly greater than the control, at all concentrations (Table 1). After 216 hr, mortality ranged from 98.9% to 100% among the four different concentrations. Background mortality among the control larvae was comparable to previously observed mortality of greater than 50% in field observations of 1st instar elm leaf beetle larvae (13).

*2nd Instar.* Mortality was significantly higher among the four azadirachtin concentrations than the control at all three time intervals (Table 1). After 48 hr, mortality at the concentrations of 1:80 and 1:160 was significantly higher than the lower concentrations of 1:320 and 1:640. After 96 hr, there was no significant difference in mortality rates between all four concentrations (mortality ranged from 87.8% to 93.3%). This may indicate that a minimum amount of azadirachtin is required to initiate the physiological activity required to induce initial high levels of mortality.

*3rd Instar.* After 96 hr, mortality among all 4 concentrations was not significantly different than the control (Table 1). After 312 hr, mortality (97.2% to 100%) for all four concentrations was significantly higher than the control.

Developmental disorders were observed in all three instars 48 hr after feeding on the azadirachtin treated foliage. The first and second instar larvae that survived showed precocious development. In the process of ecdysis, the larvae were observed to have difficulty in extracting themselves from

**Table 1. Percent mortality of Instar I elm leaf beetle (*Xanthogaleruca luteola*) after consuming Siberian elm leaves (*Ulmus pumila*) treated with various concentrations of Margosan-O® (AI = 0.3% azadirachtin) after 48, 96 and 216 hours.**

Margosan-O® dilution	N	Post-treatment interval		
		48 hr	96 hr	216 hr
<b>Instar I</b>				
Control	90 <sup>a</sup>	21.1±20.4 B <sup>b</sup>	33.3±26.7 D	52.2±30.2 B
1:80	90	51.1±18.7 A	94.4±7.8 A	100.0±0.0 A
1:160	90	18.9±15.4 B	64.4±17.7 BC	98.9±2.7 A
1:320	90	42.2±18.2 AB	76.7±15.1 AB	100.0±0.0 A
1:640	90	27.8±14.9 AB	48.9±20.5 CD	100.0±0.0 A
<b>Instar II</b>				
Control	90 <sup>c</sup>	0.0±0.00 C <sup>d</sup>	2.2±4.4 B	1.1±3.3 B
1:80	90	83.3±11.2 A	93.3±8.7 A	97.8±4.4 A
1:160	90	75.6±28.8 A	87.8±13.9 A	91.1±13.6 A
1:320	90	43.3±27.8 B	91.1±10.5 A	96.7±7.1 A
1:640	90	25.6±25.1 B	88.9±11.7 A	98.9±3.3 A
<b>Instar III</b>				
Control	72 <sup>e</sup>	15.3±27.0 A <sup>f</sup>	25.0±29.7 B	38.9±26.9 B
1:80	72	16.7±22.5 A	62.5±23.7 A	98.6±4.8 A
1:160	72	16.7±17.4 A	45.8±27.6 AB	95.8±10.4 A
1:320	72	27.8±20.5 A	45.8±28.5 AB	100.0±0.0 A
1:640	72	23.6±32.6 A	45.8±33.4 AB	97.2±9.6 A

<sup>a</sup> 15 larvae per Petri dish. Total of 6 replications.

<sup>b</sup> Means within columns followed by the same upper case letter are not significantly different ( $P < 0.05$ ; Student-Newman-Keuls test, 48 h;  $F = 3.76$ ,  $df = 25$ , 96 h;  $F = 9.76$ ,  $df = 25$ , 216 h;  $F = 14.7$ ,  $df = 25$ ).

<sup>c</sup> 10 larvae per petri dish. Total of 9 replications.

<sup>d</sup> Means within columns followed by the same upper letter are not significantly different ( $P < 0.05$ ; Student-Newman-Keuls test, 48 h;  $F = 22.9$ ,  $df = 40$ , 96 h;  $F = 130.5$ ,  $df = 40$ , 216 h;  $F = 293.8$ ,  $df = 40$ ).

<sup>e</sup> 6 larvae per petri dish. Total of 12 replications.

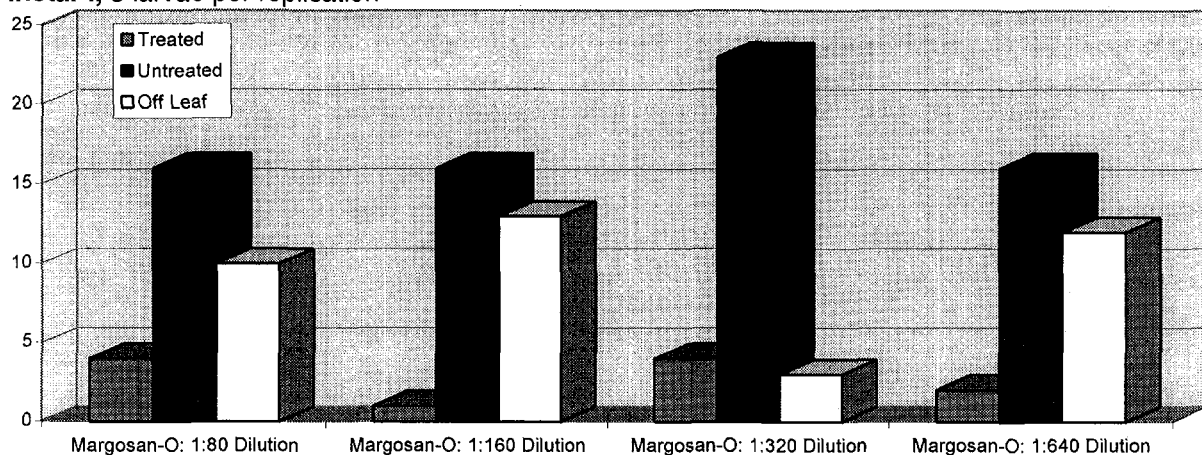
<sup>f</sup> Means within columns followed by the same upper case letter are not significantly different ( $P < 0.05$ ; Student-Newman-Keuls test, 48 h;  $F = 0.59$ ,  $df = 55$ , 96 h;  $F = 2.56$ ,  $df = 55$ , 216;  $F = 4.8$ ,  $df = 55$ ). All larvae were 48 - 72 hr old. Margosan-O® contains 0.3 % azadirachtin as the only active ingredient. Larvae were maintained at  $25 \pm 1^\circ \text{C}$  for duration of experiment.

their exoskeleton. Many 1st and 2nd instar larvae were found with head and thorax protruding from the old exoskeleton with the abdomen still attached. The azadirachtin also appeared to accelerate the maturation of an instar. The early stages of an instar, which are typically dark in color, would begin to prematurely take on the extensive yellowing of the latter stage of the same instar.

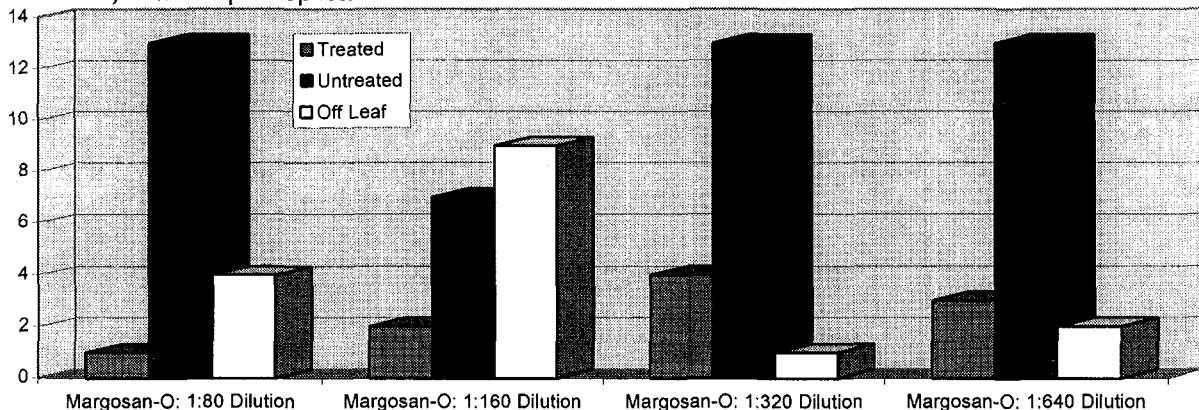
Several growth abnormalities were observed in all azadirachtin treated third instars. Third instar larvae were noticeably smaller than those larvae from the control group. The azadirachtin also appeared to accelerate the maturation of the third

instar. Pre-pupal third instar larvae normally curl into a C-shape prior to pupating. This behavior appeared very early in the third instar stage for those larvae which fed on the treated foliage at all concentrations. Third instar larvae also developed a noticeable bulging of the thorax. After 13 days, only 7 larvae (all were still in the pre-pupal stage) from the treated group survived. Only 1 larva actually completed pupation and emerged as an adult. This adult was pink in color as opposed to olive green and black. In the control group, 50 third instar larvae out of the original 72 larvae pupated to adults.

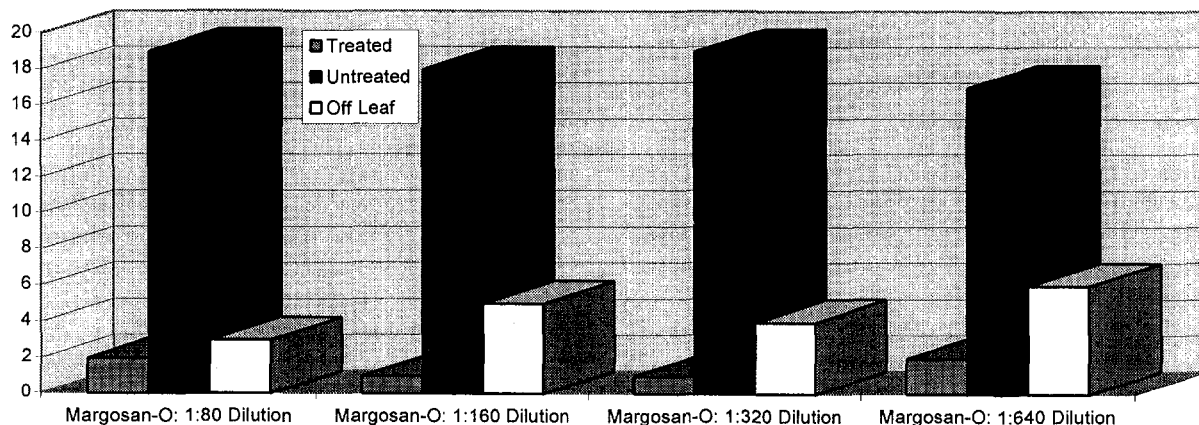
**Instar I, 5 larvae per replication**



**Instar II, 4 larvae per replication**



**Instar III, 3 larvae per replication**



**Figure 1. Location of Instar I, II, and III elm leaf beetle larvae 48 hours after being given a choice of leaf position on Margosan-O® (AI 0.3% azadirachtin) treated or untreated foliage. Total of six replications.**

In the antifeedant study, larvae of all instars showed evidence of avoidance of azadirachtin-treated foliage after 48 hr (Figure 1). Few larvae remained on the treated leaf surfaces, and most which did were found along the mid-rib. A minimum amount of treated foliage was consumed.

**Ovicide testing.** Treatment of elm leaf beetle egg masses with Bio-Neem<sup>®</sup> indicated ovicidal activity (Table 2). Treated masses contained large numbers of eggs that darkened within days of treatment and failed to hatch. Overall, 25.0 percent of the treated eggs did hatch, in comparison to 76.6 percent of those in the water check treatment. However, the majority of egg hatch occurred in only one of the six treated egg masses (33 of 35 eggs hatched). Furthermore, larvae observed to emerge from neem-treated eggs usually died shortly after hatch.

**Field trials, neem insecticides.** Field applications of the neem-derived insecticide, Azatin<sup>®</sup>, achieved excellent control of elm leaf beetle larvae (Table 3). Nearly complete control of feeding was observed throughout the season on treated trees. A small second generation of beetles prevented assessment of the persistence of these treatments. However a slight increase in foliage damage did occur during midsummer on treated trees, suggesting that persistence on foliage was limited to control of first generation larvae.

Commercial neem formulations that contain azadirachtin have been found to be effective against all three larval instars of the elm leaf beetle. This allows for an expanded application window for the arborist. The ovicide study indicates that neem formulations which contain azadirachtin also may have potential as an ovicide.

**Table 2. Effect of Bio-neem<sup>®</sup> insecticide (AI 0.3% azadirachtin) application on hatching of elm leaf beetle eggs.**

Treatment and concentration	No. egg masses	% egg hatch <sup>a</sup>
Bio-Neem <sup>®</sup> 1:100 dilution	6	25.0
Water Check	4	76.6

<sup>a</sup> Totals of 107 untreated and 140 treated egg masses, respectively.

**Table 3. Control of elm leaf beetle defoliation from an application of Azatin<sup>®</sup> (AI 3.0% azadirachtin) insecticide, Denver, CO 1993. Applications made 15 June 1993.**

Treatment and rate	Avg. foliar damage rating <sup>a</sup>		
	26Jun	14July	26July
Azatin <sup>®</sup> EC, 16 fl oz/100 gal	2.0 b	1.8 b	3.4 a
Untreated Check	5.2 a	5.2 a	5.1 a

<sup>a</sup> Foliar damage rating system based on percent of leaves showing feeding injuries. An 11-point system was used: 0 = 0% of leaves with injury; 1 = 1-10% of leaves with injuries, 2 = 11-20% of leaves with injuries, etc. Numbers followed by the same letter are not significantly different ( $P = 0.05$ ) by SNK test.

Azadirachtin's low mammalian toxicity and short persistence give the arborist a safe alternative to currently used broad-spectrum insecticides.

**Acknowledgment.** Funding for this research was supported, in part, from Colorado Agricultural Experiment Station project 319.

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**Zusammenfassung.** Um festzustellen, inwieweit sich aus dem Öl des Neem-Baum gewonnene Insektizide zur Kontrolle von Ulmenblattkäfern eignen, wurden eine Reihe von Labor- und Feldstudien durchgeführt. Bei Laborexperimenten wurden Sterblichkeit und verminderte Fraßfähigkeit bei den Käferlarven festgestellt. Ebenso wurde im Labor die Eiablage-aktivität bewertet. Die Felstudien wurden an ausgewachsenen Ulmen durchgeführt, um die Effizienz zu bewerten. Die Ergebnisse der Laboruntersuchungen zeigten eine deutliche Larvensterblichkeit. Eigelege, die mit Neem-Öl behandelt wurden, zeigten eine noch größere Sterblichkeitsrate. Die Auswirkungen der mit Neem-Öl behandelten Blätter auf das Fraßverhalten wurde in allen drei Larvenstadien untersucht. Behandelte Bäume zeigten nach schätzungsweise einem Monat deutlich weniger Fraßschäden als unbehandelte Kontrollbäume.