Research

Effectiveness of the botanical insecticide azadirachtin against *Tirathaba rufivena* (Lepidoptera: Pyralidae)

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Abstract

Tirathaba rufivena Walker (Lepidoptera: Pyralidae) is an important pest of areca palm, *Areca catechu* L. (Arecaceae), in China. The effects of azadirachtin on the development and mortality of *T. rufivena* were determined. All larval instars were susceptible to azadirachtin, but the stomach and contact toxicities diminished as the larvae matured. The LC_{25} and LC_{50} dosages had no effect on larval hatch when applied directly to the eggs on different days after deposition, but the LC_{90} treatment retarded hatch from eggs treated 1 to 3 d after deposition. The tested concentrations significantly affected the survival of neonate larvae from treated eggs, especially larvae that emerged from eggs treated 3 d after deposition. Azadirachtin also prolonged larval development and duration of the pupal stage. The percentage of adult emergence decreased, and longevity of the emerged adults was shortened, following treatment. Also, egg production and viability from females treated as larvae with azadirachtin were significantly affected.

Key Words: areca palm; neem; activity; toxicity; development

Resumen

Tirathaba rufivena Walker (Lepidoptera: Pyralidae) es una plaga importante de la palma areca, *Areca catechu* L. (Arecaceae), en China. Se determinaron los efectos de la azadiractina sobre el desarrollo y la mortalidad de *T. rufivena*. Todos los instares de las larvas fueron susceptibles a la azadiractina, pero la toxicidad estómacal y del contacto disminuyeron a medida que las larvas maduraron. Las dosis de CL_{25} y CL_{50} no tuvieron efecto en la eclosión de las larvas cuando se aplicaron directamente a los huevos en diferentes días después de la deposición, pero el tratamiento con CL_{90} retardó la eclosión de los huevos tratados 1 a 3 días después de la deposición. Las concentraciones probadas afectaron significativamente la sobrevivencia de larvas neonatas que nacieron de huevos tratados, especialmente larvas que emergieron de huevos tratados 3 días después de la deposición. Azadirachtin también prolongó el desarrollo larval y la duración del estadio de pupa. El porcentaje de emergencia de adultos disminuyó, y la longevidad de los adultos emergidos fue mas corta, después del tratamiento. Además, la producción de huevos y la viabilidad de las hembras tratadas cuando eran larvas con azadiractina fueron significativamente afectadas.

Palabras Clave: palma areca neem; actividad; toxicidad; desarrollo

Areca palm, *Areca catechu* L. (Arecaceae), has become the second largest economic crop of Hainan Province, China, and the area planted with this crop is almost 50,000 ha (Gan & Li 2004). Areca is important in traditional Chinese medicine, and its fruit and flowers are often used as health-promoting foods. However, the damage to areca caused by *Tirathaba rufivena* Walker (Lepidoptera: Pyralidae) has been severe, significantly affecting production. The damage frequency is 10 to 67% of areca plants and 10 to 40% of areca blossoms and fruit (Fan et al. 1986, 1991).

Currently, the control of *T. rufivena* is still focused on chemical pesticides, which not only causes environmental pollution but also affects human health. The tetranortriterpenoid azadirachtin is the most active insecticide component found in neem seeds and leaves (Butterworth & Morgan 1968), and has generated a great deal of interest because of its bioefficacy and biodegradability. Azadirachtin has a number of biological properties, including repellency, feeding and oviposition deterrence, growth-disrupting activity, and low mammalian toxicity (Schmutterer 1990). Most research has examined the effects of azadirachtin on insect development, including weight reduction (Schlüter 1985; Isman 1993) and mortality (Rembold et al. 1982; Meisner et al. 1986; Zehnder & Warthen 1988; Wilps et al. 1992; Padmanaban et al. 1997; Raguraman & Singh 1999; Raman et al. 2000). However, there have been few studies on the effects of azadirachtin on *T. rufivena*. Here, we present research on the effects of azadirachtin on the development and mortality of *T. rufivena*.

Materials and Methods

INSECTICIDE

A stock solution of 95% azadirachtin (Sigma-Aldrich.Corp, St. Louis, Missouri) was used for the bioassays. The insecticides were diluted with acetone (Guangzhou Chemical Reagent Factory, Guangzhou, Chi-

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na) to the desired concentrations of active ingredient (AI) (120, 60, 30, 15, and 7.5 mg AI/L).

INSECTS

Tirathaba rufivena larvae were collected from an areca field without any history of pesticide spraying, and were fed with areca leaves under controlled conditions (25 ± 1 °C, 70 ± 5% RH, and a 11:13 h L:D photoperiod) so that all development stages were available when necessary.

STOMACH TOXICITY OF AZADIRACHTIN TO LARVAE

Fresh areca leaves were immersed for 10 s in azadirachtin solution at the desired concentration, and the leaves were removed and placed under a chemical hood to dry for 2 h. Different instars of *T. rufivena* were selected and distributed to rearing containers (clean transparent plastic boxes covered with gauze, 10 × 5 × 8 cm). There were 20 larvae per box, and 3 boxes were used for each concentration (120, 60, 30, 15, and 7.5 mg AI/L). To ensure that larval feeding was consistent, the larvae were starved for 24 h and then allowed to feed on the treated leaves for 24 h. Thereafter, they were removed and placed in new rearing boxes containing fresh untreated areca leaves. Leaves immersed in acetone were used as controls. The percentage of mortality was calculated after 48 h and corrected according to Abbott (1925). The slope, LC_{50}, and 95% confidence limits were calculated according to the methods used by Finney (1964).

CONTACT TOXICITY OF AZADIRACHTIN TO LARVAE

The inner walls of the rearing containers were coated with a solution of azadirachtin (60, 30, 15, 7.5, and 3.75 mg AI/L), and boxes coated with acetone were used as controls. After the evaporation of the solvent, 20 larvae of *T. rufivena* were introduced into the rearing box for 12 h, followed by the addition of areca leaves. Each treatment was repeated 3 times. After 48 h, the survival rate was monitored, and the LC_{50} was calculated.

EFFECTS OF AZADIRACHTIN ON HATCHING AND NEONATE LARVAE

Tirathaba rufivena eggs were treated 1, 2, or 3 d after deposition with an LC_{25} (11.35 mg AI/L), LC_{50} (28.79 mg AI/L), or LC_{90} (169.00 mg AI/L) of azadirachtin solution based on stomach toxicity to 1st instars. Areca leaves with 20 eggs were dipped into the solution for 10 s and then removed and placed under a chemical hood to dry for 2 h. For each treatment, 3 replicates were conducted, and all replications were performed at the same time. The mortality was recorded until no additional hatch occurred. To detect the residual effect of azadirachtin on newly hatched larvae, the survival of neonate larvae was observed until the 1st stadium was completed.

EFFECTS ON DEVELOPMENT AND ADULT EMERGENCE

To assess the effects of azadirachtin on the development of *T. rufivena*, areca leaves were immersed in azadirachtin solution of LC_{25}, LC_{50}, and LC_{90} (based on stomach toxicity, as noted for egg treatment) for 10 s, removed, and then placed under a chemical hood to dry for 2 h. Thirty 3-d-old larvae (2nd instar) were fed treated leaves for 24 h and were then kept individually in a separate rearing box and reared on untreated leaves. The development time of 20 surviving larvae that were treated with LC_{25}, LC_{50}, or LC_{90} solution was recorded and averaged. Twenty larvae reared on leaves treated with acetone were used as controls.

The percentage of adult emergence in each treatment also was determined. The longevity and egg production of moths were determined with 10 pairs of moths used for each concentration, and then the hatching rate of eggs was recorded. The adults were fed honey water as supplemental nutrition.

STATISTICAL ANALYSES

For the stomach and contact toxicity of azadirachtin among *T. rufivena* larvae, Abbott's formula (Abbott 1925) was applied to correct the percentage of mortality if the control mortality was between 5 and 20%. Probit regression in the Statistical Package for Social Science (SPSS) Version 12 (http://www.stathome.cn/spss/) was used to determine the LC-P line (log concentration–probability regression line), the lethal concentration values, and the corresponding 95% fiducial limits of the upper and lower confidence limits. A significant difference was determined by the non-overlapping of the 95% confidence limit.

The effects of azadirachtin on egg hatch and development of *T. rufivena* were recorded as the mean ± SE. The results were analyzed with ANOVA and significant differences determined by the Duncan new multiple range test in the Statistical Analysis System (SAS®) software version 8.1 (Hu 2010).

Results

STOMACH TOXICITY OF AZADIRACHTIN TO LARVAE

Based on the LC_{50} values, the 1st instars were 1.53, 2.01, 3.01, and 3.22 times more susceptible than the 2nd, 3rd, 4th, and 5th instars, respectively. A statistically significant difference between the LC_{50} values of 1st instars and the other instars was obtained as a result of the non-overlapping of the 95% confidence limits. Similar differences were found between some other instars, as shown in Table 1, although the 95% confidence limits overlapped among some instars, indicating no significant differences.

CONTACT TOXICITY OF AZADIRACHTIN TO LARVAE

The contact toxicity of azadirachtin to *T. rufivena* larvae is shown in Table 2. No significant difference was evident in the toxicity of azadirachtin to 1st, 2nd, and 3rd instars based on the 95% confidence limits of LC_{50}. However, some significant differences occurred (Table 2) when comparing early instars to late instars.

EFFECTS OF AZADIRACHTIN ON HATCHING AND NEONATE LARVAE

As shown in Table 3, LC_{25} and LC_{50} dosages of azadirachtin had no effect on the percentage of hatch from eggs, whereas significant differences were obtained when using the LC_{90} dosage at 1 d (F = 32.39; df = 3; P < 0.001), 2 d (F = 15.16; df = 3; P < 0.001), or 3 d (F = 31.85; df =

Table 1. Stomach toxicity of azadirachtin to *Tirathaba rufivena* larvae.

Instar	LC-P line[a]	LC_{50} (mg/L)	95% confidence limits (lower–upper)
First	$y = 2.57 + 1.67x$	28.79	24.37–34.00
Second	$y = 2.24 + 1.68x$	44.19	36.89–52.94
Third	$y = 2.27 + 1.55x$	57.75	46.31–72.03
Fourth	$y = 2.01 + 1.54x$	86.73	65.13–115.49
Fifth	$y = 1.61 + 1.72x$	92.70	70.34–122.16

[a]Log concentration–probability regression line.

Table 2. Contact toxicity of azadirachtin to *Tirathaba rufivena* larvae.

Instar	LC-P line[a]	LC_{50} (mg/L)	95% confidence limits (lower–upper)
First	$y = 3.40 + 1.44x$	12.85	10.62–15.55
Second	$y = 3.51 + 1.35x$	12.82	10.47–15.71
Third	$y = 3.58 + 1.15x$	17.07	13.54–21.51
Fourth	$y = 3.54 + 1.09x$	21.97	16.91–28.55
Fifth	$y = 3.17 + 1.21x$	32.34	24.19–43.24

[a]Log concentration–probability regression line.

3; $P < 0.001$) after oviposition. The greatest reduction in hatch (52.6 ± 3.91%) was obtained with treated 3-d-old eggs.

The percentage of survival of neonate larvae was inversely correlated with the concentration of azadirachtin and the age of the eggs (Table 3). Statistical analysis indicated that all tested concentrations affected the hatch of neonate larvae (except the LC_{25} and LC_{50} on 1-d-old eggs), particularly larvae that emerged from the treated 3-d-old eggs ($F = 34.30$; df = 3; $P < 0.001$). Additionally, the proportion of larvae surviving from treated 3-d-old eggs was only 29.3% compared with 92.6% in controls.

EFFECTS OF AZADIRACHTIN ON DEVELOPMENT AND ADULT EMERGENCE

Azadirachtin may significantly prolong larval development ($F = 91.45$; df = 3; $P < 0.001$) and pupal duration ($F = 30.57$; df = 3; $P < 0.001$) (Table 4). The duration of 2nd instars was 2.23 d in the control group. The development of 2nd instars fed leaves treated with an LC_{25}, LC_{50}, or LC_{90} of azadirachtin was prolonged by 8.5, 11.2, and 18.4%, respectively. Similar results were obtained for 3rd, 4th, and 5th instars. Total larval development time was prolonged by 8.2, 10.2, and 13.9% after treatment with LC_{25}, LC_{50}, or LC_{90} of azadirachtin, respectively.

The percentage of emerging moths decreased from 97.8% in the control to 75.6, 50.2, and 26.7% after 2nd instars were exposed for 1 d to LC_{25}, LC_{50}, and LC_{90} azadirachtin treatments, and the percentage of decrease in emergence was 22.7, 48.7, and 72.7%, respectively (Table 5). Statistical analysis showed differences among the controls and different treatment concentrations ($F = 69.57$; df = 3; $P < 0.001$).

Table 3. Effects of azadirachtin on hatch from eggs treated at different ages and on survival of hatched larvae through instar 1 of *Tirathaba rufivena*.

Egg age (d)	Treatment	Hatch (%)	Larval survival (%)
1	LC_{25}	88.5 ± 1.75a	85.3 ± 1.51a
	LC_{50}	88.3 ± 1.67a	83.0 ± 3.22a
	LC_{90}	62.6 ± 3.73b	54.3 ± 4.06c
	Control	91.7 ± 1.67a	92.8 ± 3.61a
2	LC_{25}	85.3 ± 2.62a	77.0 ± 2.94b
	LC_{50}	85.0 ± 2.89a	70.8 ± 2.41b
	LC_{90}	61.7 ± 4.41b	52.2 ± 8.06c
	Control	90.0 ± 2.89a	94.4 ± 0.18a
3	LC_{25}	82.0 ± 1.53a	64.2 ± 6.37b
	LC_{50}	88.3 ± 4.41a	47.4 ± 2.63c
	LC_{90}	52.6 ± 3.91b	29.3 ± 5.62d
	Control	88.3 ± 1.67a	92.6 ± 3.70a

Means (± SE) in the same column followed by the same letter are not significantly different at the probability level of 0.05 determined by the Duncan new multiple range test.

The longevity (Table 5) of the emerged adults was shortened ($F = 21.98$; df = 3; $P < 0.001$) by azadirachtin as compared with the mean longevity of the controls (11.2 d), but the azadirachtin dosages produced equivalent effects.

Egg production by *T. rufivena* was also reduced ($F = 6.80$; df = 3; $P < 0.001$) after treatment with azadirachtin, although there were no detectable differences among the azadirachtin treatments (Table 5). Hatch from eggs of the emerged adults was similarly affected ($F = 48.71$; df = 3; $P < 0.001$)

Discussion

The use of plant-based insecticides has been recommended as an alternative for plant protection with minimal negative risks (Isman 2006; Pavela 2007). Botanical insecticides have long been a subject of research in an effort to develop alternatives to conventional insecticides. Currently, several insecticides based on various plant extracts are used around the world. Azadirachtin is the insecticidal ingredient found in the neem tree and is a naturally occurring substance that belongs to an organic molecule class called tetranortriterpenoids. Azadirachtin is used to control whiteflies, aphids, thrips, fungus gnats, lepidopteran larvae, beetles, mushroom flies, mealybugs, leafminers, gypsy moths, and other insects in food, greenhouse crops, ornamental plants, and turf (Thomson 1992). Our results indicated that azadirachtin had a strong stomach and contact toxicity to *T. rufivena* larvae, and that the contact toxicity was greater than the stomach toxicity.

In this study, azadirachtin affected larval hatch, larval development, pupal duration, adult longevity, and egg production in *T. rufivena*. Azadirachtin produced a significant reduction in the percentage of hatch when it was applied directly to the eggs 1, 2, or 3 d after they had been deposited. Survival of neonate larvae that had hatched from treated eggs diminished, especially when eggs had been treated with a high concentration just before hatch. The ovicidal activity of some plant extracts on other insects such as *Spilosoma obliqua* (Walker) (Lepidoptera: Arctiidae), *Spodoptera litura* F. (Lepidoptera: Noctuidae), and *Dysdercus koenigii* (F.) (Hemiptera: Pyrrhocoridae) was reported by Ghatak & Bhusan (1995) and Suryakala et al. (1995). They suggested that high concentration levels of many plant extracts may inhibit the hatching from insect eggs. Our results confirmed that azadirachtin was toxic to eggs and also affected the neonate larvae from treated eggs.

Azadirachtin is structurally similar to the insect ecdysone hormones, which control the process of metamorphosis as the insects pass from larva to pupa to adult. Metamorphosis requires the careful synchrony of many hormones and other physiological changes to be successful, and azadirachtin seems to be an ecdysone blocker. It blocks the production and release of these vital hormones in insects, and when they are exposed to azadirachtin, insects will not molt, which breaks their life cycle (National Research Council 1992; AgriDyne Technologies, Inc. 1994). The results of this study showed that there was a significant reduction in the development of *T. rufivena* among 2nd instar larvae that survived azadirachtin treatment. The longevity of moths that grew from treated larvae was significantly shorter compared with untreated moths. Additionally, there was a reduction in egg production among females, and hatch from deposited eggs decreased. These findings suggest that toxicity may persist through all life stages from larva to adult, although only 2nd instar larvae were treated with azadirachtin. Therefore, it appears that azadirachtin could effectively suppress *T. rufivena* populations either directly through acute toxic effects on the larvae or indirectly through delayed effects on development.

Table 4. Effects of azadirachtin on *Tirathaba rufivena* larval and pupal development.

Treatment	Development time (d; mean ± SE)							
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Total larval	Pupal	Total larval–pupal
LC_{25}	2.09 ± 0.03a	2.42 ± 0.02b	3.00 ± 0.04b	3.41 ± 0.04b	4.52 ± 0.05c	15.44 ± 0.07b	10.70 ± 0.02b	26.14 ± 0.07c
LC_{50}	2.11 ± 0.03a	2.48 ± 0.02b	3.12 ± 0.03a	3.51 ± 0.03ab	4.61 ± 0.03b	15.81 ± 0.06b	10.71 ± 0.02b	26.53 ± 0.06b
LC_{90}	2.12 ± 0.02a	2.64 ± 0.02a	3.15 ± 0.02a	3.61 ± 0.02a	4.72 ± 0.03a	16.25 ± 0.05a	10.80 ± 0.01a	27.05 ± 0.05a
Control	2.05 ± 0.04a	2.23 ± 0.04c	2.75 ± 0.04c	3.08 ± 0.10c	4.15 ± 0.06d	14.27 ± 0.15c	10.39 ± 0.06c	24.66 ± 0.16d

Means (± SE) in the same column followed by the same letter are not significantly different at the probability level of 0.05 determined by the Duncan new multiple range test.

Table 5. Effects of azadirachtin on *Tirathaba rufivena* adult biology.

Treatment	Emergence (%)	Longevity (d)	No. of eggs per female	Hatch (%)
LC_{25}	75.6 ± 2.22b	8.65 ± 0.30b	71.90 ± 2.18b	70.0 ± 1.38b
LC_{50}	50.2 ± 5.20c	9.00 ± 0.24b	66.80 ± 2.98b	71.8 ± 1.40b
LC_{90}	26.7 ± 3.85d	8.30 ± 0.25b	67.60 ± 3.28b	72.4 ± 2.13b
Control	97.8 ± 2.22a	11.25 ± 0.33a	83.90 ± 3.50a	92.4 ± 0.86a

Means (± SE) in the same column followed by the same letter are not significantly different at the probability level of 0.05 determined by the Duncan new multiple range test.

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