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Efficacy of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae) the rice leaffolder

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Abstract

In leaf cut choice assay and topical application experiments, the neem (*Azadirachta indica*) limonoids azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione, and deacetylnimbin showed strong antifeedant and growth inhibitor activity against fifth instar larvae of *Cnaphalocrocis medinalis*, the rice leaffolder, a serious pest of rice in Asian countries. The parameters, used to evaluate the activity of the limonoids were larval food utilization, quantity of ingested and digested food; consumption index and feeding deterrence index. Azadirachtin, salannin, deacetylgedunin showed high bioactivity at all doses, while the other neem limonoids proved to be less active, and only at the higher doses did they display antifeedant activity. Azadirachtin was the most potent in all experiments. Gross dietary utilization (efficiency of conversion of ingested food) of *C. medinalis* was also decreased after treatment with limonoids. Our results suggest that neem limonoids may be used in IPM programs for rice leaffolder and should be evaluated for efficacy under field conditions.

Keywords: *Cnaphalocrocis medinalis*; Rice leaffolder; Neem limonoids; Antifeedant; Food utilization

1. Introduction

Many compounds with diverse chemical structures and different modes of action are classified as botanical insecticides. The Indian neem tree, *Azadirachta indica* A. Juss (Meliaceae) is a promising source of botanical insecticides given that several constituents of its leaves and seed show marked insect control potential. (Schmutterer, 1990; Liang et al., 2003; Senthil Nathan et al., 2004; Venzon, et al., 2004). Limonoids are bitter tetranortriterpenes found predominantly in Meliaceae and Rutaceae (Champagne, et al., 1989). It is generally believed that bioactivity of neem is due to their azadirachtin (complex limonoids) content (Butterworth and Morgan, 1971; Mordue and Blackwell, 1993; Nisbet et al., 1996 Mordue and Nisbet, 2000; Huang et al., 2004; Senthil Nathan et al., 2005), but their spectrum and level of efficacy are unknown to the rice leaffolder.

The rice leaffolder (RLF) *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae) is a major insect pest of rice (*Oryza sativa* L). It appears to have become increasingly important with the spread of high-yield rice varieties and the accompanying changes in cultural practices. (Dale, 1994; Senthil Nathan et al., 2004). In this study we investigated the effect of neem limonoids on feeding physiology of *C. medinalis* (Lepidoptera: Pyralidae), the rice leaffolder.

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2. Materials and method

2.1. Laboratory mass culture of Cnaphalocrocis medinalis

Cnaphalocrocis medinalis larvae were collected from the paddy fields in and around Coimbatore district, Tamilnadu, India and Paddy Breeding Station (PBS), Tamilnadu Agricultural University, Coimbatore. Larvae were reared in a greenhouse on potted rice plants covered with mesh sleeves at $27 \pm 2^{\circ}C$; 10:14LD: 85% RH. Rice plants were grown in earthenware pots, 18 cm tall with a 20 cm diameter top each pot held 15 plants and gave 62 tillers. The pots were placed in about 10 cm of water in a metal tray in the greenhouse. The culture was initiated with partly grown larvae from the field. Thereafter, newly hatched larvae were placed on plants of the rice variety TN1, about 60-day old.

After pupation, adults emerged on plants in the sleeves. To maintain the culture, 12 female and 13 male moths were placed in an oviposition cage containing one potted plant. The moths were fed with 10% sucrose solution fortified with a few drops of vitamin mixture (Multidec drops®, Ashok Pharmaceuticals, Chennai-24, India) drops to enhance oviposition. After 2 days the potted plants were removed from the oviposition cage. The leaf portions containing the eggs were clipped and placed on moist filter paper in a petri dish. These eggs were used to maintain the culture.

2.2. Neem limonoids

Six neem limonoids, azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin (Fig. 1) were received from Dr. M. Ishida, Central Research Laboratories, Taiyo Kayaku Co. Ltd., Japan. They were dissolved in isopropanol and different concentrations were prepared by dilution with isopropanol.

2.3. Bioassays and treatment

Bioassays were performed with fifth instars of *C. medinalis* using concentration of 0.5,1,2 and 4 ppm of azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deaceytlnimbin. Control leaves were treated with isopropanol and air dried. A minimum of 30 larvae/concentration were used for all the experiments and the experiments were replicated five times. Larval weight/mortality was recorded after 7 days at 28 °C and 16:8 (L: D) photoperiod and the effective concentration (EC_{50}) was calculated using Probit analysis (Finney, 1971). The fresh rice leaves (*Oryza sativa* L) were coated with 0.25, 0.5, and 1 ppm concentrations of limonoids and air-dried. Control leaves were treated with isopropanol and air tried. Fifth

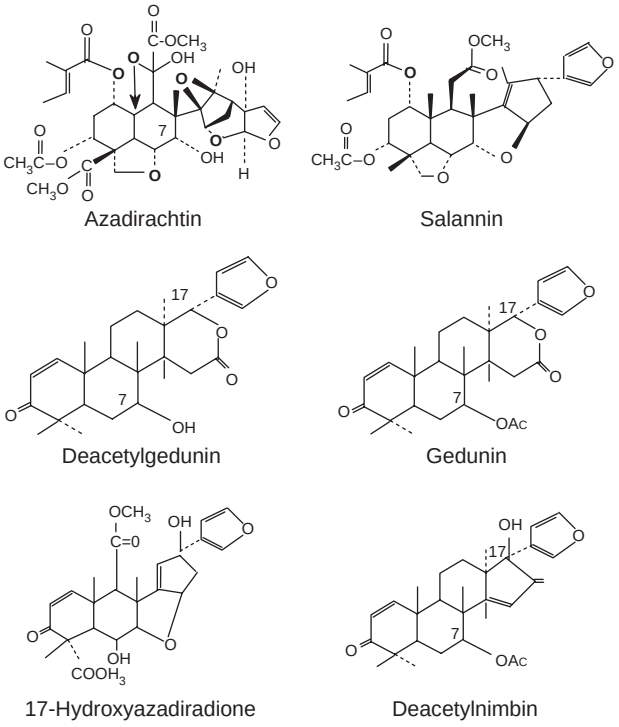

Fig. 1. Structure of neem limonoids tested against *C. medinalis*.

instars were starved for 4 h and were individually fed with different concentrations of limonoids. The uneaten leaves were removed every 24 h, and placed with freshly treated leaves. A minimum of 10 larvae/concentration were used for all the experiments and the experiments were replicated 5 times.

2.4. Feeding deterrence index

Feeding deterrence index was estimated by using a leaf cut-choice test (Isman et al., 1990; Khan et al., 1996). In a 15 cm diameter petridish lined with a moist filter paper disc. 5 cm long leaf cuts from TN 1 rice plants were treated on each surface with 5 µl of aqueous solutions of the neem limonoids emulsified with Triton-X100 (0.1%). Controls were treated with isopropanol alone. The leaf cuts were dried at room temperature and then 4 h starved fifth instars were introduced into each arena containing one treated and untreated leaf discs in alternate position line with moist filter paper disc. Experiments were carried out with 10 larvae per concentration. Each experiment was replicated five times. Consumption was recorded using a digitizing leaf area meter (Model LI-3000, Li-cor, USA) after 12 h. The index of feeding deterrency was calculated as $(C - T)/(C + T) \times 100$, where C is the consumption of control leaf cut and T is the treated leaf cut.

2.5. Quantitative food utilization efficiency measures

A gravimetric technique was used to determine weight gain, food consumption and feces produced. All weights were measured using a monopan balance accurate to 0.1 mg. The newly moulted fourth instar larvae were starved for 4 h. After measuring the initial weight of the larvae, they were individually introduced into separate containers. The larvae (10 larvae/concentration, five replicates) were allowed to feed on weighed quantities of neem limonoids treated and untreated TN 1 rice leaves, for a period of 24 h. Larvae were again weighed. The difference in weight of the larvae gives the fresh weight gained during the period of study. Sample caterpillars were weighed, oven dried (48 h at 60 °C) reweighed to establish a percentage dry weight of the experimental caterpillars. The leaves remaining at the end of each day were oven dried and re-weighed to establish a percentage dry weight conversion value to allow estimation of the dry weight of the diet given to the larvae. The quantity of food ingested was estimated by subtracting the diet (dry weight) remaining at the end of each experiment from the total dry weight of the diet provided. Feces were collected daily and weighed, then oven dried and re-weighed to estimate the dry weight of excreta. The experiment was continued for 4 days and observations were recorded every 24 h.

Consumption, growth rates and post-ingestive food utilization efficiencies (all based on dry weight) were calculated in the traditional manner (Waldbauer, 1968). Consumption index (CI) = E/TA, relative growth rate (RGR) = P/TA, Approximate digestibility, (AD)-$100(E-F)/E$, Efficiency of conversion of ingested food (ECI) = $100\ P/E$, Efficiency of conversion of digested food (ECD) = $100\ P/(E-F)$, where A = mean dry weight of animal during T, E = dry weight of food eaten, F = dry weight of faeces produced, P = dry weight gain of insect and T = duration of experimental period.

2.6. Statistical analysis

Data from feeding deterrence and nutritional indices were analyzed with ANOVA of arcsine transformed percentages followed by Tukey's multiple range test ($P \leqslant 0.05$) (SAS Institute, 2001).

3. Results and discussion

Neem-based insecticides containing azadirachtin that was derived from the extracts of neem tree (*Azadirachta indica*) have played important roles in crop protection. Of six limonoids tested against rice leaffolder, azadirachtin showed the highest antifeedant activity (Table 1) Incorporation of neem in the diet (rice leaves) significantly reduced the growth of *C. medinalis* larvae. Azadirachtin at 2 ppm produced the highest feeding deterrence (93% in fifth instar) than all other limonoids (Table 1). All the limonoids showed a positive dose-dependent antifeedant activity. The reduced consumption of limonoids-treated leaves is likely to be the main cause of growth inhibition (Tables 1 and 2) (Murugan et al., 1998). Neem limonoids treatment had detrimental effect upon *C. medinalis* larval growth and development. Azadirachtin, however, proved to be the most detrimental to the larvae at the concentrations tested. At the concentrations tested (i.e. 2 ppm in azadirachtin), the feeding deterrence (93%) was higher in azadirachtin-treated insects when compared to other treatments. (Murugan et al., 1998).

Table 1
Antifeedant activities of neem limonoids against fifth instar larvae of *C. medinalis*

Concentrations (ppm)	Feeding deterrence index (%) (fifth instar)
Control	0.05 ± 0.003^{a}
Azadirachtin	
2.00	93.2 ± 7.6^{a}
Salannin	
2.00	$49.6 \pm .5.2^{b}$
Deacetylgedunin	
2.00	23.6 ± 1.8^{c}
Gedunin	
2.00	18.8 ± 1.5^{c}
17-Hydroxyazadiradione	
2.00	23.6 ± 2.1^{c}
Deacetylnimbin	
2.00	21.0 ± 1.8^{c}

Within columns, means (±SE) followed by a same letter do not differ significantly (Tukey's test, $P \leqslant 0.05$).

Dietary utilization by *C. medinalis* was severely affected when fed on leaves treated with neem limonoids (Table 2). The adverse effect of neem limonoids on the feeding and growth of *C. medinalis* as evident from the nutritional experiment. In addition to significant decreases in ECI and ECD, treatments with limonoids also produced significant decreases in relative consumption rate (RCR) and relative growth rate (RGR). Azadirachtin at 1 ppm was the most potent growth inhibitor of the tested limonoids reducing RGR from 0.524 mg/mg/day in control insects to 0.027 mg/mg/day.

The reduction in dietary utilization suggests that reduction in growth may result from both behavioral and physiological (post-ingestive) effects (Koul and Isman, 1991;Venzon, et al., 2004). This conclusion is corroborated by the results of direct bioassays for feeding deterrence, and the leaf cut-choice tests (where substantial feeding inhibition on treated leaf cut was observed). The results also showed that relative consumption and growth rates were significantly lower

Table 2
Nutritional indices [consumption index (CI), relative growth rate (RGR), approximate digestibility (AD), efficiency of conversion of ingested food, (ECI) and efficiency of conversion of digested food (ECD)] of fifth instar larvae of *C. medinalis* after treatment with neem limonoids

Treatments (ppm)	CI (mg/mg/day)	RGR (mg/mg/day)	RCR (mg/mg/day)	AD (%)	ECI (%)	ECD (%)
Control	2.52 ± 0.15^{a}	0.524 ± 0.03^{a}	1.10 ± 0.10^{a}	44.81 ± 4.12^{b}	20.81 ± 1.58^{a}	46.45 ± 4.12^{d}
Azadirachtin 1.00	0.42 ± 0.02^{c}	0.027 ± 0.00^{d}	0.18 ± 0.00^{c}	52.71 ± 4.91^{a}	6.50 ± 0.56^{c}	12.35 ± 1.00^{c}
Salannin 1.00	1.55 ± 0.12^{ab}	0.257 ± 0.01^{bc}	0.55 ± 0.02^{b}	49.61 ± 3.91^{a}	16.62 ± 1.50^{ab}	33.52 ± 2.78^{b}
Deacetylgedunin 1.00	2.20 ± 0.21^{a}	0.385 ± 0.02^{b}	0.92 ± 0.10^{a}	48.45 ± 4.21^{a}	17.50 ± 1.63^{a}	36.12 ± 3.21^{ab}
Gedunin 1.00	2.35 ± 0.25^{a}	0.470 ± 0.03^{ab}	1.00 ± 0.12^{a}	46.45 ± 4.45^{a}	20.0 ± 1.85^{a}	43.15 ± 4.11^{a}
17-Hydroxyazadiradione	2.30 ± 0.21^{a}	0.419 ± 0.03^{b}	0.95 ± 0.10^{a}	47.21 ± 4.12^{a}	18.24 ± 1.60^{a}	38.65 ± 3.32^{a}
1.00 Deacetylnimbin 1.00	2.32 ± 0.21^{a}	0.424 ± 0.03^{b}	0.95 ± 0.10^{a}	46.74 ± 4.48^{a}	18.28 ± 1.60^{a}	39.11 ± 3.52^{a}

Within columns, means ($\pm$SE) followed by a same letter do not differ significantly (Tukey's test, $P\leqslant0.05$).

among fourth instars confined to a diet containing neem limonoids. Furthermore, utilization efficiencies for larvae exposed to limonoids were significantly reduced.

In conclusion, we have demonstrated that the neem limonoids exhibit significant inhibition of feeding of *C. medinalis* larvae on rice leaves. In particular, azadirachtin was the most potent. These limonoids will play an important role in the management of agricultural ecosystems because they do not persist in the environment.

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