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The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) the rice leaffolder

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Abstract

The effect of neem (*Azadirachta indica*) limonoids azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione, and deaceytlnimbin on gut enzyme activity of the rice leaffolder larvae was investigated. When fed a diet of rice leaves treated with limonoids in bioassays, gut tissue enzymes—acid phosphatases (ACP), alkaline phosphatases (ALP), and adenosine triphosphatases (ATPase) activities of rice leaffolder (*Cnaphalocrocis medinalis*) larvae are affected. Azadirachtin was most potent in all experiments. Larvae that were chronically exposed to limonoids showed a reduction in weight (59–89%) and exhibited a significant reduction in ACP, ALP, and ATPase activities. These results indicate neem limonoids affects gut enzyme activities. These effects are most pronounced in early instars.

Keywords: *Cnaphalocrocis medinalis*; Neem limonoids; Azadirachtin; Salannin; Deacetylgedunin; Gedunin; 17-Hydroxyazadiradione; Deaceytlnimbin; Gut enzyme; ACP; ALP; ATPase

1. Introduction

The Indian neem tree, *Azadirachta indica* A. Juss (Meliaceae) has been found to be a promising source of natural pesticides. Several constitutions of its leaves and seed show marked insect control potential and due to their relative selectivity, neem products can be recommended for many programs on Integrated Pest Management (IPM) [1]. The neem seed kernel extract is known to suppress the feeding, growth, and reproduction of insects. Neem leaves contain organic compounds that have insecticidal and medicinal properties [2].

During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. Limonoids are bitter tertranortriterpenes found

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predominantly in Meliaceae and Rutaceae [3]. It is generally believed that bioactivity of neem is due to the azadirachtin (complex limonoids) content in them [4]. Azadirachtin in known to have adverse effects on more than 200 insect species [5].

Neem limonoids or derivatives affect insect physiology in many different ways and their modes of action are relatively unstudied. To examine the effects of neem liminoids on the insect midgut, we conducted a study of the activities of alkaline and acid phosphatases and ATPase activities in a susceptible insect pest, the rice leaffolder, *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). Alkaline phosphatase (ALP, EC 3.1.3.1) and acid phosphatase (ACP, EC 3.1.3.2) are hydrolytic enzymes, which hydrolyse phosphomonoesters under acid or alkaline conditions, respectively. ALP is mainly found in the intestinal epithelium of animals and its primary function is to provide phosphate ions from mononucleotide and ribonucleoproteins for a variety of metabolic processes. ALP is involved in the transphosphorylation reaction [6]. ATPases are essential for transport of glucose, amino acids, etc. Any impairment in their activity will affect the physiology of gut. These enzymes are located in the midgut, Malpighian tube, muscles, and nerve fibres of the lepidopertan insects. [7]. The midgut has the highest ALP and ACP activity as compared to other tissues. The ALP and ACP activities are low during the larval moulting stage and increased gradually after moulting [8]. The highest activity appeared before the full appetite gluttonous stage of the fifth instar and the lowest activity was found in the mature larval stage [8].

2. Materials and methods

2.1. Laboratory mass culture of C. medinalis

Cnaphalocrocis medinalis larvae were collected from the paddy fields in and around Coimbatore district, Tamil Nadu, India and Paddy Breeding Station (PBS), Tamil Nadu Agricultural University, Coimbatore. Larvae were reared in a greenhouse on potted rice plants covered with mesh sleeves at 27 ± 2 °C; 10:14 LD:85% Rh. Newly hatched larvae were placed on plants of the rice variety TN1, about 60-day-old. Rice plants were grown in earthenware pots, 18 cm tall with a 20 cm diameter opening. 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.

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) to enhance oviposition. After two 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 (purity >99%) namely azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione, and deaceytlnimbin (Fig. 1) were received as a gift from Dr. M. Ishida, Central Research Laboratories, Taiyo Kayaku, Japan. They were dissolved in isopropanol and different concentrations were prepared by dilution with isopropanol.

2.3. Bioassays

Bioassays were performed with third to fifth instars of *C. medinalis* using concentration from 0.10 to 0.50 (ppm). Control leaves were treated with isopropanol and air dried. A minimum of 30 larvae/concentration were used for all the experiments and this experiments were replicated five times. Larval weight/mortality was recorded after seven days at 28 °C and 16:8 (L:D) photoperiod and he effective concentration (EC_{50}) was calculated using Probit analysis [9].

2.4. Treatments

The fresh rice leaves (*Oryza sativa* L.) were coated with different concentration of limonoids and air-dried. Control leaves were treated with iso-

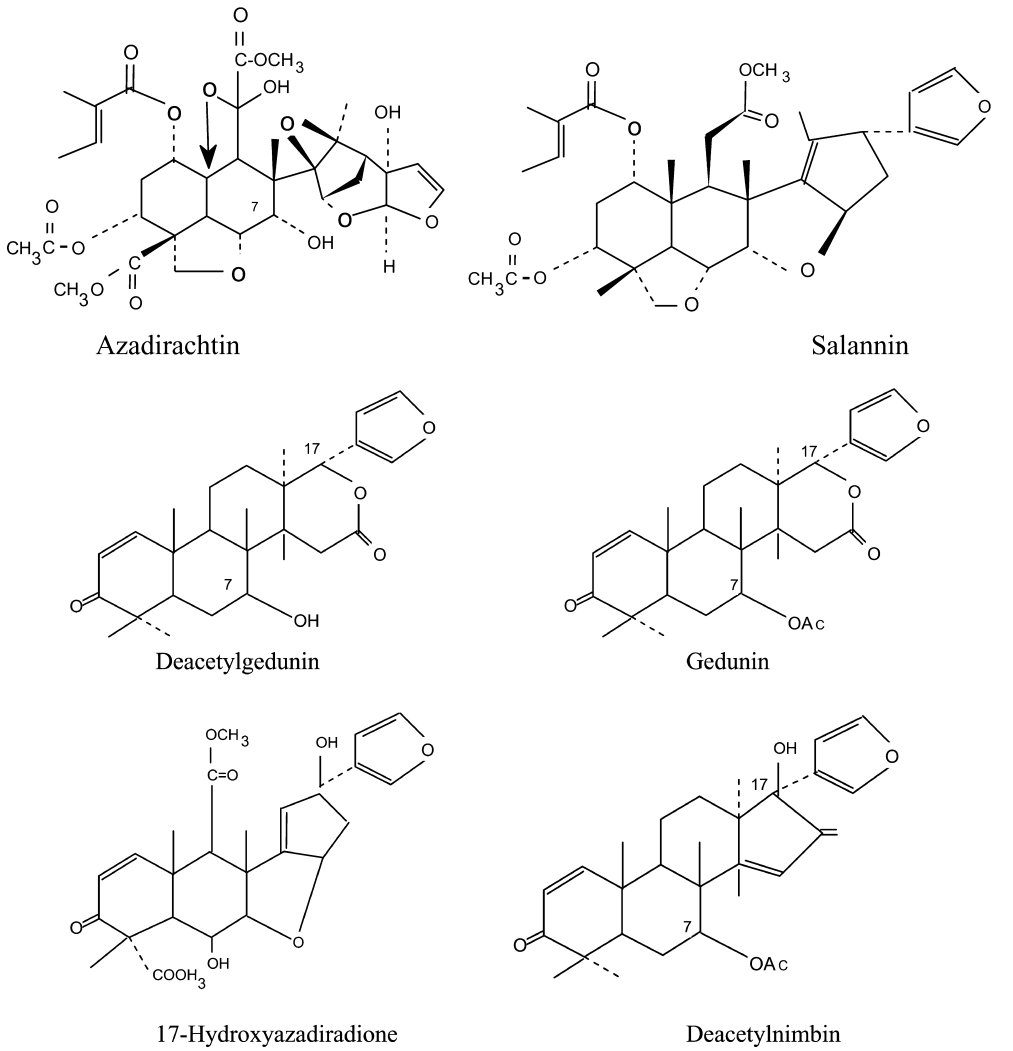

Fig. 1. Structure of neem limonoids tested against *Cnaphalocrocis medinalis*.

propanol and air-dried. Third to fifth larval 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 fresh treated leaves. A minimum of 10 larvae/concentration were used for all the experiments and these experiments were replicated five times.

2.5. Preparation of enzyme extract

Two-day-old fourth and fifth instars of treated *C. medinalis* were used to quantify the enzyme activities. The method used to prepare the enzyme extract was that of Applebaum [10] and Applebaum et al. [11]. Individuals were anaesthetized with cotton pads soaked in ether and the entire digestive tract dissected out in ice cold insect Ringer's solution. The Malpighian tubules, adhering tissues, and gut contents were removed. The gut was split into regions, weighed (accuracy in mg) and homogenized for 3 min at 4 °C in ice-cold citrate-phosphate buffer (pH 6.8) using a tissue grinder. Homogenized gut was suspended in ice-cold buffer and made up

to 1 ml. The homogenate was centrifuged at 500 rpm for 15 min and the supernatant was used as the enzyme source.

2.6. Estimation of acid (EC 3.1.3.2) and alkaline phosphatases (EC 3.1.3.1)

The enzyme assays were carried out as described by Bessey et al. [12]. The buffered substrate was incubated with tissue extract for 30 min. Alkali was added to stop the reaction and to adjust the pH for the determination of the concentration of the product formed. The spectral absorbance of *p*-nitrophenolate was maximal at 310 nm. The molar absorbance of *p*-nitrophenolate at 400 nm is about double that of *p*-nitrophenyl phosphate at 310 nm. On converting the *p*-nitrophenolate into *p*-nitrophenol by acidification, the absorption maximum is shifted to about 320 nm with no detectable absorption at 400 nm.

2.7. Estimation of adenosine triphosphatases

The specific activity of sodium and potassium dependent ATPase in the gut was assayed according to the method described by Shiosaka et al. [13].

The quantity of inorganic phosphorous liberated was assayed according to the method of Fiske and Subbarow [14]. In this method the protein is precipitated with trichloroacetic acid. The protein free filtrate is treated with acid molybdate solution and the phosphoric acid formed is reduced by the addition of 1-amino-2-naphthol-4-sulphonic acid (ANSA) reagent to produce blue color. The intensity of the color is proportional to the amount of phosphorous present.

Table 1
Enzyme activities (in μmol/mg/h) of fourth instar larvae of *C. medinalis* fed on limonoids treated *O. sativa* leaves

Treatments (ppm)	Acid phosphatase (ACP)	Alkaline phosphatase (ALP)	Adenosine triphosphate (ATPase)
Control	$10.38 \pm 0.78^{a,*}$	$13.96 \pm 0.95^{a,*}$	$75.52 \pm 5.62^{a,*}$
Azadirachtin			
0.25	5.78 ± 0.23^{e}	9.25 ± 0.75^{d}	52.61 ± 3.85^{d}
0.50	4.46 ± 0.31^{h}	6.10 ± 0.45^{f}	41.84 ± 2.95^{e}
1.00	2.91 ± 0.15^{i}	4.80 ± 0.29^{h}	31.22 ± 2.09^{f}
Salannin			
0.25	8.35 ± 0.41^{c}	10.63 ± 0.79^{cd}	58.23 ± 4.21^{c}
0.50	7.14 ± 0.32^{d}	9.06 ± 0.75^{g}	47.19 ± 3.26^{d}
1.00	4.50 ± 0.12^{h}	5.76 ± 0.45^{g}	39.47 ± 2.56^{e}
Deacetylgedunin			
0.25	8.92 ± 0.52^{b}	11.25 ± 0.90^{c}	60.14 ± 4.52^{bc}
0.50	7.84 ± 0.41^{cd}	9.92 ± 0.85^{d}	49.25 ± 3.58^{d}
1.00	5.21 ± 0.32^{g}	6.43 ± 0.45^{f}	40.65 ± 3.21^{e}
Gedunin			
0.25	9.20 ± 0.78^{b}	12.75 ± 1.02^{b}	66.71 ± 5.21^{b}
0.50	8.50 ± 0.52^{bc}	11.15 ± 0.98^{c}	58.32 ± 5.01^{c}
1.00	5.98 ± 0.40^{e}	8.25 ± 0.75^{e}	48.62 ± 3.25^{d}
17-Hydroxyazadiradione			
0.25	9.15 ± 0.42^{b}	12.26 ± 0.97^{b}	62.37 ± 5.29^{b}
0.50	8.23 ± 0.61^{c}	11.00 ± 0.95^{c}	56.25 ± 4.63^{cd}
1.00	5.55 ± 0.40^{ef}	8.10 ± 0.75^{e}	45.04 ± 3.29^{e}
Deacetylnimbin			
0.25	9.10 ± 0.75^{b}	12.64 ± 1.21^{b}	64.25 ± 5.42^{b}
0.50	8.45 ± 0.49^{bc}	11.09 ± 1.06^{c}	54.21 ± 3.39^{c}
1.00	5.76 ± 0.26^{e}	7.84 ± 0.65^{e}	46.25 ± 2.76^{de}

* Within columns, means followed by a same letter do not differ significantly (Tukey's test, $P < 0.05$).

2.8. Statistical analysis of enzyme activity

Data from all experiments were subjected to analysis of variance (ANOVA) and means separated using Tukey's test [15].

3. Results

Exposure of neem limonoids in larval diet depressed or reduced enzyme activities in fourth and fifth instars. Tables 1 and 2 demonstrate the efficacy of neem limonoids against gut enzyme activity of rice leaffolder. The effect on ACP, ALP, and ATPase activities was concentration-dependent (Tables 1 and 2). An EC_{50} value of neem limonoids against rice leaffolder was shown in Table 4. Azadirachtin was most potent in all experiments with least EC_{50} (0.042, 0.059, and 0.062 ppm for third, fourth, and fifth instars, respectively).

Differences in acid phosphatase (ACP), alkaline phosphatase (ALP), and adenosine triphosphatase (ATPase) activities in the gut between the control and treated fourth instar larvae are shown in Table 1. The maximal suppression of gut enzyme activity was obtained by azadirachtin at 1 ppm in fourth and fifth instars. The ACP activity in the fourth instar (control insect) was 10.38 μmol/mg/h. ACP activity was reduced to 5.21 μmol/mg/h (50%) by deacetylgedunin treatment (1 ppm) and it was further reduced to 2.91 μmol/mg/h (72%) by azadirachtin at 1 ppm treatment. Similarly, there was significant reduction in the activities of ALP (maximum of 66%) and ATPase (maximum of 59%) in azadirachtin at 1 ppm treatment (Fig. 2). Gut enzyme activity was considerably decreased when

Table 2
Enzyme activities (in μmol/mg/h) of fifth instar larvae of *C. medinalis* fed on limonoids treated *O. sativa* leaves

Treatments (ppm)	Acid phosphatase (ACP)	Alkaline phosphatase (ALP)	Adenosine triphosphate (ATPase)
Control	$13.25 \pm 0.98^{a,*}$	$19.05 \pm 1.29^{a,*}$	$88.45 \pm 4.25^{a,*}$
Azadirachtin			
0.25	9.45 ± 0.75^{c}	14.11 ± 1.11^{d}	59.81 ± 3.21^{d}
0.50	6.21 ± 0.78^{e}	10.49 ± 1.09^{f}	44.37 ± 3.26^{f}
1.00	4.19 ± 0.71^{f}	6.59 ± 0.44^{g}	37.21 ± 2.45^{g}
Salannin			
0.25	10.95 ± 0.81^{bc}	15.50 ± 0.98^{bc}	68.64 ± 5.21^{c}
0.50	9.78 ± 0.78^{c}	13.45 ± 0.91^{d}	60.23 ± 5.01^{d}
1.00	6.60 ± 0.75^{e}	9.42 ± 0.74^{f}	46.65 ± 4.58^{ef}
Deacetylgedunin			
0.25	11.10 ± 0.91^{bc}	15.74 ± 1.32^{b}	70.37 ± 2.78^{bc}
0.50	10.05 ± 0.94^{c}	13.87 ± 1.31^{d}	62.41 ± 5.01^{cd}
1.00	7.21 ± 0.61^{d}	9.92 ± 0.98^{f}	48.29 ± 3.25^{e}
Gedunin			
0.25	11.91 ± 0.94^{b}	17.02 ± 1.42^{b}	77.21 ± 6.21^{b}
0.50	9.96 ± 0.92^{c}	15.21 ± 1.36^{bc}	66.43 ± 5.12^{c}
1.00	7.61 ± 0.52^{d}	12.44 ± 0.96^{de}	53.21 ± 4.52^{e}
17-Hydroxyazadiradione			
0.25	11.24 ± 0.75^{b}	16.58 ± 1.36^{b}	73.91 ± 5.98^{b}
0.50	9.34 ± 0.56^{c}	14.93 ± 1.02^{c}	65.42 ± 5.25^{c}
1.00	7.49 ± 0.51^{d}	12.07 ± 1.09^{e}	49.29 ± 3.57^{e}
Deacetylnimbin			
0.25	11.45 ± 0.75^{b}	16.71 ± 1.23^{b}	75.42 ± 6.32^{b}
0.50	9.51 ± 0.71^{c}	15.10 ± 1.15^{c}	66.93 ± 3.01^{c}
1.00	7.35 ± 0.70^{d}	12.27 ± 1.09^{e}	51.76 ± 4.25^{e}

* Means within columns followed by the same letter are not significantly different (Tukey's test, $P < 0.05$).

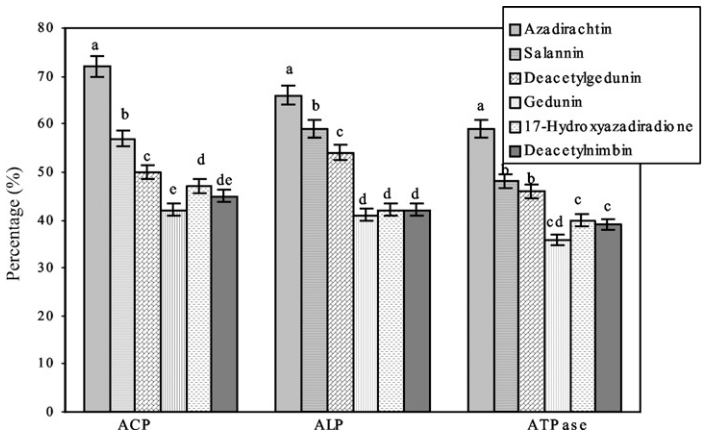

Fig. 2. Percent reduction of enzyme activities in fourth instar larvae of *C. medinalis* after treatment with neem limonoids. Within bar (Individual enzyme), means followed by a same letter do not differ significantly (Tukey's test, $P < 0.05$).

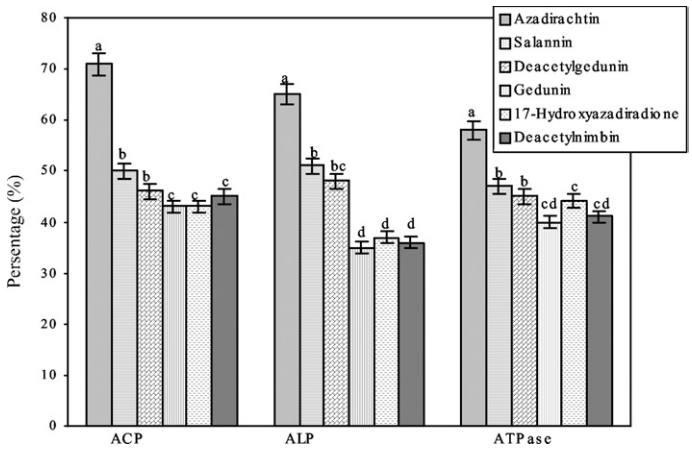

Fig. 3. Percent reduction of enzyme activities in fifth instar larvae of *C. medinalis* after treatment with neem limonoids. Within bar (Individual enzyme), means followed by a same letter do not differ significantly (Tukey's test, $P < 0.05$).

the insects were fed on leaves treated with neem limonoids, compared to control treatment. Gut enzyme activities significantly decreased with increasing concentration of limonoids. The activities of ACP, ALP, and ATPase were maximum decreased in the fourth instar larvae of *C. medinalis* after treatment with azadirachtin at 1 ppm concentration (72, 66, and 59%, respectively) (Fig. 3).

The adverse effect of neem limonoids on the activity of the gut enzymes of *C. medinalis* was evident during the feeding. ALP activity of fifth instar larva was markedly reduced to 6.60 μmol/mg/h (50%) in salannin at 1 ppm and in azadirachtin treatment at 1 ppm the enzyme activity was drastically decreased to 4.19 μmol/mg/h (68%). Significant reduction in activity of ATPase (58%) was observed in azadirachtin treatment at 1 ppm. (Table 2). There were statistically significant differences ($P < 0.05$) in enzyme activities between azadirachtin and other limonoids (Tables 1–3). As shown in Fig. 3 ACP, ALP, and ATPase activities showed significant reduction after treatment with azadirachtin than other limonoids (71, 65, and 58%, respectively).

Table 3
Larval and pupal weight of *C. medinalis* after treatment with neem limonoids

Treatments (ppm)	Larval weight (mg) (10 larvae)			Pupal weight (mg) (10 pupae)
	Larval instars			
	III	IV	V	
Control	$17.2 \pm 1.02^{a,*}$	$24.2 \pm 1.22^{a,*}$	$33.4 \pm 0.75^{a,*}$	$23.2 \pm 1.23^{a,*}$
Azadirachtin				
0.25	11.4 ± 0.98^{d}	18.1 ± 0.98^{c}	25.4 ± 1.21^{c}	15.1 ± 0.56^{d}
0.50	9.3 ± 0.87^{e}	12.0 ± 0.96^{f}	18.8 ± 0.98^{e}	10.4 ± 0.59^{f}
1.00	6.5 ± 0.81^{h}	9.2 ± 0.95^{g}	13.4 ± 0.97^{h}	6.3 ± 0.51^{h}
Salannin				
0.25	12.5 ± 1.12^{cd}	20.0 ± 1.25^{b}	28.5 ± 0.95^{b}	18.7 ± 0.89^{b}
0.50	10.0 ± 0.71^{de}	14.2 ± 0.98^{c}	21.9 ± 0.96^{de}	15.2 ± 0.85^{d}
1.00	7.8 ± 0.75^{g}	12.0 ± 0.98^{f}	15.6 ± 0.95^{g}	9.3 ± 0.81^{g}
Deacetylgedunin				
0.25	12.7 ± 0.78^{c}	20.2 ± 0.93^{b}	29.2 ± 1.23^{b}	19.1 ± 0.96^{b}
0.50	10.7 ± 0.79^{d}	15.8 ± 0.96^{d}	23.2 ± 1.56^{d}	15.4 ± 1.21^{cd}
1.00	8.2 ± 0.75^{f}	13.2 ± 1.22^{ef}	17.2 ± 1.52^{f}	11.1 ± 1.15^{f}
Gedunin				
0.25	15.3 ± 0.91^{b}	21.7 ± 0.23^{b}	31.2 ± 1.52^{ab}	21.7 ± 1.22^{ab}
0.50	13.7 ± 0.96^{c}	17.5 ± 1.25^{cd}	26.7 ± 1.45^{c}	17.2 ± 1.18^{c}
1.00	11.4 ± 0.95^{d}	14.9 ± 163^{e}	20.1 ± 1.50^{e}	12.1 ± 1.15^{e}
17-Hydroxyazadiradione				
0.25	14.9 ± 0.96^{b}	21.0 ± 0.92^{b}	31.0 ± 1.71^{ab}	21.5 ± 1.25^{ab}
0.50	13.2 ± 0.96^{c}	16.9 ± 0.98^{cd}	25.8 ± 1.52^{c}	16.5 ± 1.18^{c}
1.00	11.0 ± 0.86^{d}	13.8 ± 0.85^{e}	19.2 ± 1.56^{e}	12.1 ± 1.11^{e}
Deacetylnimbin				
0.25	15.1 ± 1.03^{b}	21.5 ± 1.22^{b}	31.2 ± 2.21^{ab}	21.5 ± 1.56^{ab}
0.50	13.5 ± 1.00^{c}	17.2 ± 1.36^{cd}	26.3 ± 1.98^{c}	16.7 ± 1.61^{c}
1.00	11.2 ± 0.92^{d}	14.4 ± 1.25^{e}	19.5 ± 1.96^{e}	12.5 ± 0.98^{e}

[*] Means within columns followed by the same letter are not significantly different (Tukey's test, $P < 0.05$).

Table 4
Effective concentrations (EC_{50}) of neem limonoids against third-fifth instar larvae of *C. medinalis*

Concentrations (ppm)	Larval instar		
	III	IV	V
Azadirachtin	$0.042 \pm 0.00^{f,*}$	$0.059 \pm 0.00^{f,*}$	$0.062 \pm 0.00^{e,*}$
Salannin	0.091 ± 0.00^{e}	0.113 ± 0.01^{e}	0.123 ± 0.01^{d}
Deacetylgedunin	0.121 ± 0.03^{d}	0.134 ± 0.04^{d}	0.156 ± 0.04^{c}
Gedunin	0.148 ± 0.04^{c}	0.167 ± 0.05^{c}	0.186 ± 0.06^{b}
17-Hydroxyazadiradione	0.172 ± 0.03^{a}	0.189 ± 0.05^{a}	0.203 ± 0.06^{a}
Deacetylnimbin	0.161 ± 0.04^{b}	0.179 ± 0.05^{b}	0.196 ± 0.06^{a}

[*] Within columns, means followed by a same letter do not differ significantly (Tukey's test, $P < 0.05$).

Larval weight was gradually decreased in third to fifth instar larvae due to treatment with neem limonoids. Larval body weight was 33.4 mg in fifth instar (larvae control). At 0.5 ppm a concentration of 17-hydroxyazadiradione larval weight fall to 25.8 mg (22%) and it was further reduced to 13.4 mg (60%) in azadirachtin treatment at 1 ppm (Table 3). The data show a gradual decrease

in the pupal weight of insects fed on neem limonoids separately.

4. Discussion

Our results show that limonoids affected the gut physiology of *C. medinalis* at several doses. Sub lethal effects on larval may greatly hinder the survival and fitness of leaffolder adults. The doses of neem limonoids also affected the weight of the larvae. Changes in metabolism and decreases in the gut enzyme activity of neem treated individuals may be expected to affect enzyme titers and activities. Our data support this hypothesis (Tables 1–3). There were statistically significant differences in activity among treatment doses. We conclude that neem limonoids treatments in larval diet influences ACP, ALP,and ATPase activities in larvae and pupae.

The findings that increasing the amounts of neem limonoids consumed by larvae results in decreased enzymatic conversion efficiency and decreased larval body weight, suggest that besides antifeedant properties, limonoids contain digestive components inhibiting enzymes activities and reducing conversion rate. Several studies have shown that feeding is necessary for the stimulation of enzymes activities [16,17]. Higher enzyme activity in the midgut of control insects is most probably due to consumption as well as utilization of large quantities of food. Imbalance in enzyme–substrate complex and inhibition of peristaltic movement of the gut [18] might have inhibited the enzyme activity in the treated insects. Chapman [18] reported that enzyme production is clearly related to the feeding behavior (amount of food that passes through the alimentary canal). The activity of these enzymes is related to the physiological situation of RLF and reflects the absorption, digestion, and positive transport of nutrients in the midgut.

Changes in ALP and ACP activities after treatment with neem limonoids toxin indicate that changing the physiological balance of the midgut might affect these enzymes. In the present study, the decrease in the activity of these enzymes after neem limonoids were fed to *C. medinalis* suggests that limonoids are affecting gut physiological events (i.e., ion transport) that might influence these enzymes.

Decreased levels of ACP at higher concentrations of azadirachtin suggests a reduced phosphorous liberation for energy metabolism, decreased rate of metabolism as well as decreased rate of transport of metabolites, and may be due to the direct effect of azadirachtin and other limonoids on enzyme regulation. The significant decrease in the rate of maturation caused by neem treatment appear to be due to poor nutrition in the treated insects and also related to altered feeding physiology.

ATPases are membrane bound enzymes. The role of membrane lipids and their micro-environmental changes at physical and chemical levels may be responsible for the differential response observed at the level of ATPases activity after feeding neem limonoids treatment. Membrane ATPase, especially in the intestinal epithelium, assists transport and reabsorption of metabolites and nutrients, and also secondary transport of ions and non-electrolytes [19,20]. Babu et al. [21] showed that the ATPase activity in the gut of *Helicoverpa armigera* was significantly decreased due to toxic effects of azadirachtin. In the present study, the ATPase activity in the gut was also significantly reduced by neem treatments. ATPase inhibition may affect active ion transport leading to alteration in electrolyte regulation. After neem limonoids treatments decrease in enzymatic activity denotes reduced metabolism in the insect and may be due to the toxic effects of neem limonoids on membrane permeability, especially on the gut epithelium [22–24].

It is evident that exposure to neem limonoids in larval diet has significant effects on several enzymes activities found in the late instars larvae and pupae of *C. medinalis*. This effect appeared severe at the highest treatment dose for larvae (0.5 and 1 ppm). In the fourth instar all limonoids doses significantly reduced enzyme activities compared with controls. This may indicate that neem limonoids activity was more pronounced in early instars than later instars. Our data also suggest that neem limonoids had a stronger effect on enzyme activities in early instars. Azadirachtin has

been shown to be the most active compound in the limonoids in terms of its effects on insect physiology. Botanical insecticides such as neem may interfere with the production of certain types of proteins. This activity is apparently strongest during pupation; pupae were very susceptible after larvae were exposure [23]. The chemical structures of the assayed compounds are shown in Fig. 1. In this study, the potent enzyme inhibitors were azadirachtin, salannin, deacetylgedunin, and gedunin. These four compounds have some common structural features such as furan ring and an α,β-unsaturated ketone in their A-ring. Azadirachtin is by far the most potent enzyme inhibitor among all the limonoids, being more than five times as effective as the least potent enzyme inhibitor, gedunin.

In conclusion, neem limonoids had significant effects on larval and pupal *C. medianlis* and they caused reduction of weight and enzyme activity. The adult physiology is thus impaired after larvae were exposed neem limonoids. These changes were much more pronounced in early instars. These neem limonoids may therefore serve as effective alternatives to conventional pesticides in the control of rice leaffolder.

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