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# Food consumption, utilization, and detoxification enzyme activity of the rice leaffolder larvae after treatment with *Dysoxylum* triterpenes

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**Abstract**

The toxicity and physiological (enzyme and nutritional indices) effect of *Dysoxylum* triterpenes 3β,24,25-trihydroxycycloartane and beddomei lactone were evaluated on the rice leaffolder *Cnaphalocrocis medinalis* (Guenée). The $LC_{50}$ [6.66 ppm (SD = 0.31), 5.79 ppm (SD = 0.33) for 3β,24,25-DHCL and BL, respectively] and $LC_{90}$ [14.63 ppm (SD = 0.36), 13.49 ppm (SD = 0.27) for 3β,24,25-DHCL and BL, respectively] were identified by probit analysis. Fourth instars were exposed to various concentrations (1.5, 3, 6, and 12 ppm) of *Dysoxylum* triterpenes. Results showed that treated larvae exhibited reduced food consumption and enzyme activity. Food consumption, digestion, relative consumption rate, efficiency of conversion of ingested food, efficiency of conversion of digested food, and relative growth rate values declined significantly but the approximate digestibility of treated larvae was significantly higher as a result of treatment (in particular 6 and 12 ppm). Likewise, the gut enzymes acid phosphatases, alkaline phosphatases, and adenosine triphosphatases were significantly inhibited by the *Dysoxylum* triterpenes. The high biological activity of these triterpenes from *Dysoxylum* sp. could be used as an active principle during the preparation of botanical insecticides for insect pest like rice leaffolder.


*Keywords:* Rice leaffolder; *Dysoxylum*; Triterpenes; Nutrition; Enzyme ACP; ALP; ATPase; Toxicity

## 1. Introduction

Botanical insecticides may provide alternatives to currently used synthetic insecticides because many of them are often active against a limited number of species, are often biodegradable to non-toxic products, and are potentially suitable for use in IPM [1–3]. Much effort has, therefore, been focused on plant-derived materials for potentially useful products as commercial insect-control agents [4,5].

The family Meliaceae includes many plant species that are sources of valuable secondary metabolites called limonoids (tetranortriterpenes) [5,6]. *Dysoxylum malabaricum* Bedd. and *Dysoxylum beddomei* Hiern (Meliaceae) were critically endangered and economically important trees of Western Ghats, Southern India. The leaves of *Dysoxylum* sp. contain several triterpenes [7]. Leaf extracts of *D. malabaricum* affect insects in a variety of ways, acting as an antifeedant, retarding development having direct toxicity to larvae. [7–9]. Many other investigators have isolated triterpenes from *Dysoxylum* species [10–13].

Secondary metabolites from plants are deleterious to insect and other herbivores in diverse ways, such as through acute toxicity, enzyme inhibition, and interference with the consumption and/or utilization of food [14–18]. In many cases, however, the modes of action for these metabolites are unknown. In an effort to determine the effects of, and herbivore responses to, dietary allelochemicals, their consumption and utilization of food is often quantified and various food utilization efficiencies are calculated [19,20]. Various methods have been employed in an effort to

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determine whether post-ingestive changes in food utilization and growth can be accounted for by the altered consumption that frequently occurs when an insect feeds on an allelochemical-laced diet, or whether such changes result from true post-ingestive action of the ingested allelochemical [17].

In insects, acid phosphatase (ACP, E.C.3.1.3.2),[1] known as a lysosomal marker enzyme [21], is active in the guts [22–25]. Alkaline phosphatase (ALP, E.C.3.1.3.1) is a brush border membrane marker enzyme [26] and is especially active in tissues with active membrane transport, such as intestinal epithelial cells [24,27] and malpighian tubules [24,28,29]. The larval midgut of the silkworm, *Bombyx mori*, is the most studied insect tissue for ALP [30].

In *B. mori*, gut ALP is believed to participate in the transport of glucose and fatty acids across intestinal wall membranes [22]. Furthermore, m-ALP is thought to be involved with digestion and absorption of nutrients by the columnar epithelial cells, but s-ALP, with ATPase activity, is present in goblet cells and is involved in the regulation of ionic balance [27,31]. Also Yi and Adams [30] identified that the patterns of s- or m-ALP-specific activity in the beetle midgut showed correlation with feeding activity of Colorado potato beetle, *Leptinotarsa disseminate* Say.

Attempts have been made in insects to localize these enzymes in particular tissues [32], and there is strong evidence that alkaline phosphatase is located in cells that are most active in the synthesis of fibrous proteins. Likewise, the presence of high alkaline phosphatase activity in the malphigian tubules [33] and digestive tract [34], it is suggestive that its role is in the resorption of metabolites in general and of sugars in particular. Attempts have also been made to correlate the changes in the activity of these metabolites to growth in two insects, viz., the stable fly, *Stomoxys calcitrans*, and the housefly, *Musca domestica* [25,35,36]. Although these authors could not draw any definite conclusions because of the variations observed. In this paper are presented results which show a definite relationship between the changes in the alkaline and acid phosphatases on the one hand and reduced food utilization on the other in the rice leaffolder *Cnaphalocrocis medinalis*.

Rice leaffolder (RLF) *C. medinalis* (Guenée) (Lepidoptera: Pyralidae) is considered major pests in many Asian countries [18,37–39]. It is widespread in the tropics and occurs in most of the rice-growing regions of Asia including India, China, Korea, and Japan. The RLF folds the leaves longitudinally and fastens leaf margins. The larvae feed by scraping the green mesophyll from within the folded leaves. This results in a linear pale white stripe damage as a result of stunted growth of rice plants. Insecticide use against leaffolder is widespread, but may not be justified due to tolerance of the leaffolder, also synthetic pesticides produced resistance and resurgence to leaffolder population [38–40].

Objective of the present research work is to find out the effect of *Dysoxylum* triterpenes on food consumption, absorption, and utilization in relation with detoxification enzyme activity of the rice leaffolder.

## 2. Materials and methods

### 2.1. Laboratory mass culture of Cnaphalocrocis medinalis

*Cnaphalocrocis medinalis* larvae were reared in a greenhouse on potted rice plants covered with mesh sleeves at $27 \pm 2$ °C in a 14:10 light–dark photoperiod and 85% relative humidity. Rice plants were grown in earthenware pots, 18 cm tall with a 20-cm diameter top, each pot held 15 plants and possessed about 62 tillers. The pots were placed in about 10 cm of water in a metal tray in the greenhouse [18]. The culture was initiated with partly grown larvae from the field. Thereafter, newly hatched larvae were placed on ca. 50-day-old plants of the rice variety 'IR20'.

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% honey solution to enhance oviposition. After 2 days the potted plants were removed from the oviposition cage. Leaf portions containing eggs were clipped and placed on moist filter paper in Petri dishes. These eggs were used to establish the culture of *C. medinalis*.

### 2.2. Preparation of Dysoxylum pure compounds

The triterpenes, 3β,24,25-trihydroxycycloartane (3β,24,25-DHCL) and beddomei lactone (BL) (Fig. 1), were isolated from *D. malabaricum* and *D. beddomei*, respectively, and were received from Dr. G. Jayakumar, M.G. College, Trivandrum, Kerala, India [12]. They were dissolved in isopropanol and different concentrations were prepared by dilution with isopropanol.

### 2.3. Bioassay

The rice leaves were treated with 1.5, 3, 6, and 12 ppm of 3β,24,25-DHCL and BL. Control leaves were treated with 1% isopropanol and air-dried. The leaves were allowed to dry at room temperature for 10 min and were then placed in 15 cm diameter Petri dishes. The experiments were carried out with newly moulted 4 h starved fourth instars (10 larvae per concentration, five replicates). After 24 h, the larvae were transferred to fresh untreated rice leaves and maintained until they closed or died. Total number of normal adults emerging was noted. The larvae were observed for mortality. The percent mortality data after correction [41] were subjected to probit analysis [42] to calculate mean lethal concentrations

[1] *Abbreviations used:* RLF, rice leaffolder; 3β,24,25-DHCL, 3β,24,25-trihydroxycycloartane; BL, beddomei lactone; RGR, relative growth rate; RCR, relative consumption rate; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; ACP, acid phosphatases; ALP, alkaline phosphatases; ATPase, adenosine triphosphatases; LC, lethal concentration; SD, standard deviation; SE, standard error.

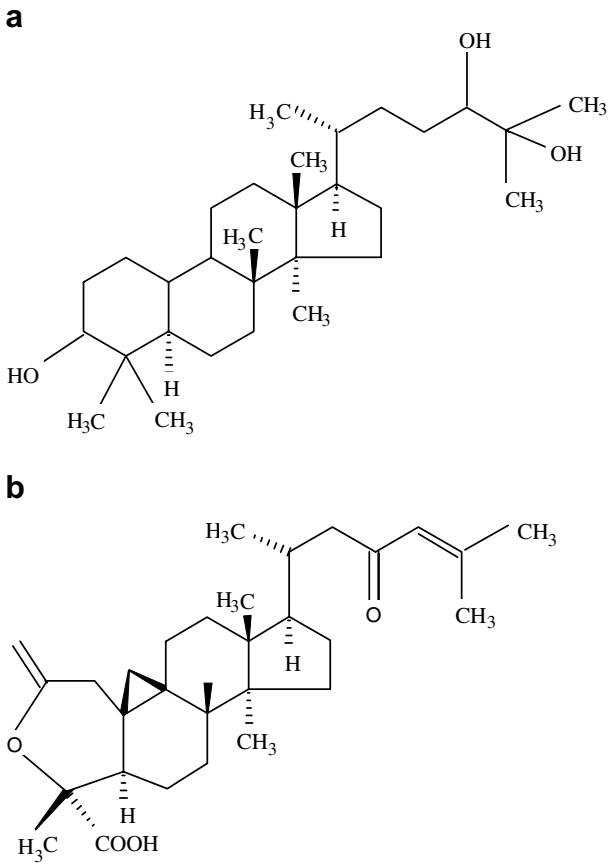


Fig. 1. Structure of *Dysoxylum* triterpenes tested against *C. medinalis*. (a) 3β,24,25-DHCL; (b) BL.

($LC_{50}$ and $LC_{90}$). From the mean lethal concentration, the treatment concentrations were selected for enzyme studies.

### 2.4. Quantitative food utilization efficiency measures

A gravimetric technique was used to determine weight gain, food consumption, and faeces produced. All weights were measured using a monopan balance accurate to 0.1 mg. The fresh rice leaves (*Oryza sativa* L) were sprayed with 1.5, 3, 6, and 12 ppm concentrations of 3β,24,25-DHCL and BL. The formulations were applied to leaves (five leaves) with a regulator-controlled spray applicator (5 ml). Control leaves were treated with isopropanol and air-dried. The newly moulted fourth instars were starved for 4 h. After measuring the initial weight of the larvae, they were individually introduced into separate containers. The larvae (10 larvae per concentration, five replicates) were allowed to feed on five leaves of weighed quantities of triterpenes treated and untreated IR20 rice leaves, for a period of 24 h. The uneaten leaves were removed after 24 h and replaced with fresh untreated leaves. Larvae were again weighed and the difference in weight of the larvae was used as fresh weight gained during the period of study. Sample larvae were weighed, oven dried (48 h at 60 °C), and re-weighed to establish a percentage dry weight of the experimental larvae. 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 for the estimation of diet dry weight. 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. Faeces were collected 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 a traditional manner [18–20,43], such as: relative consumption rate (RCR) $= E/TA$, 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 $A$ is the mean dry weight of animal during $T$, $E$ is the dry weight of food eaten, $F$ is the dry weight of faeces produced, $P$ is the dry weight gain of insect, and $T$ is the duration of experimental period.

### 2.5. Preparation of enzyme extract

Two-day-old fourth instars of treated *C. medinalis* were used to quantify the enzyme activities. The method used to prepare the enzyme extract was that of Applebaum [44] and Applebaum et al. [45]. Individuals were anaesthetized with cotton pads soaked in ether and the entire digestive tract was dissected out in ice-cold insect Ringer's solution (distilled water containing 8.6 g sodium chloride, 0.3 g potassium chloride, and 0.33 g calcium chloride per litre). 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 (E.C.3.1.3.2) and alkaline phosphatases (E.C.3.1.3.1)

The enzyme assays were carried out as described by Bessey et al. [46]. 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.

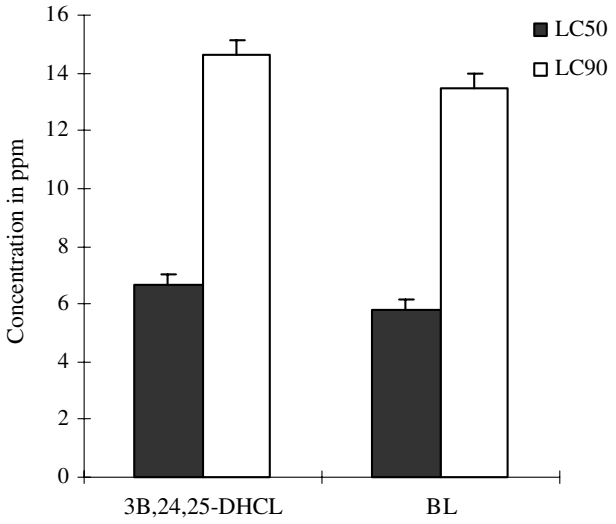


Fig. 2. Lethal concentrations ($LC_{50}$ and $LC_{90}$) of 3β,24,25-HDCL and BL against fourth-instar larvae of *C. medinalis* (values are means of five replicates with SD).

### 2.7. Estimation of adenosine triphosphatases (ATPases)

The specific activity of sodium- and potassium-dependent ATPase in the gut was assayed according to the method described by Shiosaka et al. [47].

The quantity of inorganic phosphorous liberated was assayed according to the method of Fiske and Subbarow [48]. 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-napthol-4-sulphonic acid (ANSA) reagent to produce blue colour. The intensity of the colour is proportional to the amount of phosphorous present.

### 2.8. Statistical analysis

The lethal concentrations (both $LC_{50}$ and $LC_{90}$) were calculated using probit analysis [42] and values were expressed as means ± standard error (SEM) of five replicates. For enzyme activity, linear regression technique of Microcal Software (Origin 7.5) was used. Data from nutritional indices are subjected to analysis of variance (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test ($P \leqslant 0.05$) [49,50].

## 3. Result

The $LC_{50}$ (6.21 and 5.96 ppm for 3β,24,25-DHCL and BL, respectively) and $LC_{90}$ (14.65 and 13.93 ppm for 3β,24,25-DHCL and BL, respectively) value of *Dysoxylum* triterpenes against rice leaffolder is shown in Fig. 2. Concentration-dependent mortality was evident after treatment with 3β,24,25-DHCL and BL. The *Dysoxylum* triterpenes killed the fourth instars within 48 h, with about 40% with peak host mortality (daily rates) occurring 48 h after treatment.

The treatment of *Dysoxylum* triterpenes into the rice leaves significantly reduced larval growth of rice leaffolder compared to controls (Tables 1 and 2). There was a concentration-dependent reduction in growth from 1.5 to 12 ppm. Efficiency of conversion of ingested and digested food (ECI and ECD) into biomass of rice leaffolder larvae was reduced except the control. The reduction in these parameters was irrespective of any significant change in relative

Table 1
Nutritional indices of fourth-instar larvae of *C. medinalis* after treatment with 3β,4,25-DHCL

| Treatment | RGR (mg/mg/day) | RCR (mg/mg/day) | AD (%) | ECI (%) | ECD (%) |
|---|---|---|---|---|---|
| Control | $5.130 \pm 1.02^{a}$ | $1.185 \pm 0.237^{a}$ | $43.15 \pm 8.63^{c}$ | $23.0 \pm 4.60^{a}$ | $54.58 \pm 10.91^{a}$ |
| 1.5 | $4.848 \pm 0.969^{a}$ | $0.718 \pm 0.151^{b}$ | $44.56 \pm 8.91^{bc}$ | $22.5 \pm 4.51^{a}$ | $51.46 \pm 10.29^{a}$ |
| 3 | $4.353 \pm 0.870^{b}$ | $0.603 \pm 0.120^{b}$ | $45.70 \pm 9.14^{b}$ | $20.53 \pm 4.10^{ab}$ | $45.66 \pm 9.13^{ab}$ |
| 6 | $3.748 \pm 0.749^{b}$ | $0.378 \pm 0.076^{bc}$ | $47.21 \pm 9.44^{b}$ | $19.61 \pm 3.92^{b}$ | $41.61 \pm 8.32^{b}$ |
| 12 | $2.353 \pm 0.470^{c}$ | $0.240 \pm 0.048^{c}$ | $49.73 \pm 9.94^{a}$ | $16.78 \pm 3.35^{c}$ | $36.13 \pm 7.22^{c}$ |

Means standard error (±SEM) followed by the same letter within columns indicate no significant difference ($P \leqslant 0.05$) in a Tukey test.
*Abbreviations:* RGR, relative growth rate; RCR, relative consumption rate; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food.

Table 2
Nutritional indices of fourth-instar larvae of *C. medinalis* after treatment with BL

| Treatment | RGR (mg/mg/day) | RCR (mg/mg/day) | AD (%) | ECI (%) | ECD (%) |
|---|---|---|---|---|---|
| Control | $5.146 \pm 1.029^{a}$ | $1.055 \pm 0.517^{a}$ | $42.31 \pm 8.46^{c}$ | $23.71 \pm 4.74^{a}$ | $54.61 \pm 10.92^{a}$ |
| 1.5 | $4.891 \pm 0.978^{a}$ | $0.678 \pm 0.334^{b}$ | $43.73 \pm 8.74^{c}$ | $22.43 \pm 4.48^{a}$ | $51.48 \pm 10.29^{a}$ |
| 3 | $4.443 \pm 0.888^{a}$ | $0.586 \pm 0.287^{bc}$ | $44.86 \pm 8.97^{bc}$ | $20.78 \pm 4.15^{ab}$ | $47.31 \pm 9.46^{ab}$ |
| 6 | $3.751 \pm 0.750^{ab}$ | $0.448 \pm 0.220^{c}$ | $46.36 \pm 9.27^{b}$ | $19.30 \pm 3.86^{b}$ | $41.60 \pm 8.32^{b}$ |
| 12 | $2.436 \pm 0.487^{b}$ | $0.380 \pm 0.187^{c}$ | $49.66 \pm 9.93^{a}$ | $17.58 \pm 3.51^{c}$ | $37.81 \pm 7.56^{b}$ |

Means standard error (±SEM) followed by the same letter within columns indicate no significant difference ($P \leqslant 0.05$) in a Tukey test.
*Abbreviations:* RGR, relative growth rate; RCR, relative consumption rate; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food.

consumption rates and the only significant reduction in consumption relative to controls was observed at the highest treatment dose of 12 ppm. (Table 1). For example, the ECD in the control was 54.5% and decreased to 41.6% with 6 ppm treatment of 3β,24,25-DHCL and was further reduced to 36.1% in 12 ppm concentration of the same treatment ($F = 24.9772$; df = 24; $P < 0.0001$). The results also showed that relative growth and consumption (RGR, RCR) rates were significantly lower among fourth instars confined to a diet containing *Dysoxylum* triterpenes.

Daily consumption and digestion revealed a continuous decrease in food consumption and digestion in 6 ppm triterpenes treated larvae relative to untreated larvae. Food consumption in the *Dysoxylum* triterpenes treated groups remained at a significantly lower level throughout the experimental period. Approximate digestibility (AD) of 6 ppm triterpenes treated larvae was significantly higher than the control in the 6 and 12 ppm treatments during the experimental periods ($F = 24.4053$, df = 24; $P < 0.0001$ for 3β,24,25-DHCL; $F = 24.4871$, df = 24; $P < 0.0001$ for BL, respectively).

The RCR generally declined over the course of larval development for the treatment groups. The highest AD was recorded for BL-treated RLF larvae. The letters give the groups of significance according to the one-way ANOVA. Error numbers denote the standard error of the means. ECI values for the 6 ppm triterpenes treated RLF larvae declined until pupation and were significantly different according to ANOVA (Tables 1 and 2). The efficiency of food conversion of 6 ppm triterpenes treated larvae was significantly lower than that of control larvae during the treatment period.

Exposure of *Dysoxylum* triterpenes in larval diet reduced enzyme activities in fourth instars. Figs. 2–5 demonstrate the effect of 3β,24,25-DHCL and BL against ACP, ALP, and ATPase activity of rice leaffolder. The effect on gut enzyme activities was concentration-dependent (Fig. 3–5). Both 3β,24,25-DHCL and BL were potent enzyme inhibitors. ACP activity of fourth instars was markedly reduced (about 52%) in 3β,24,25-DHCL treatment ($R^2 = 0.886$, $P < 0.0001$) (Fig. 3a). Significant reduction in activity of ATPase (55%) was observed in BL treatment ($R2 = 0.863$, $P < 0.0001$) (Fig. 4b). As shown in Figs. 6a and b, ACP, ALP, and ATPase activities showed significant reduction after treatment with *Dysoxylum* triterpenes than control counterparts.

## 4. Discussion

The triterpenes used in these experiments affect the food consumption and utilization of rice leaffolder and decreased the enzyme activities. Obtained results also show that *Dysoxylum* triterpenes affected the gut physiology of RLF and produced visible external symptom like malformed pupae and premature pupae. Decreases in enzyme activity and reduced food consumption were further indication of disturbance of general metabolism in 6 ppm

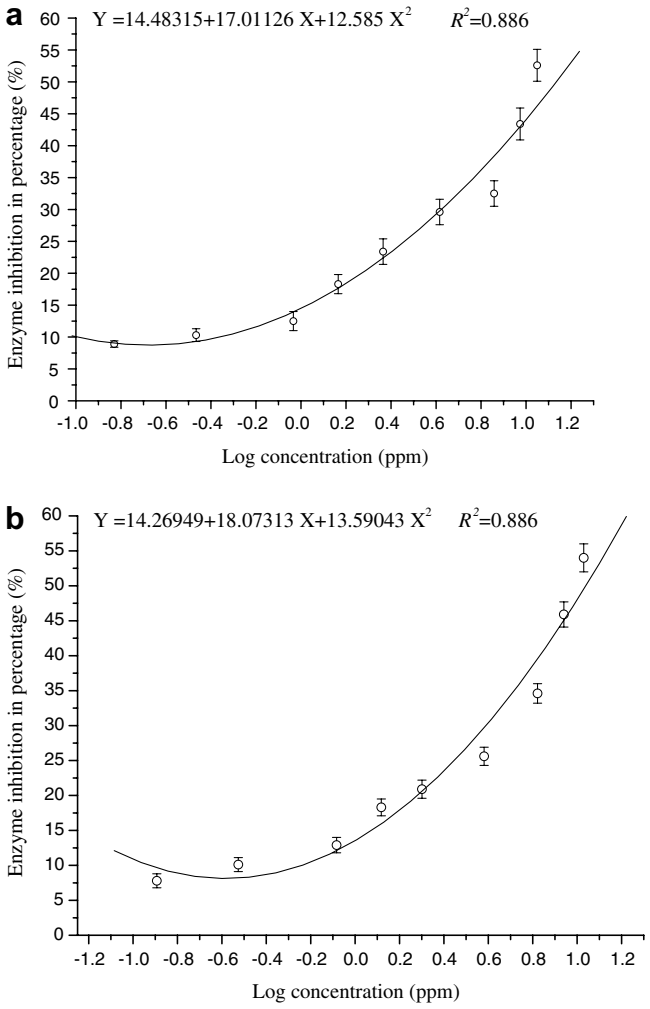


Fig. 3. Percentage reduction of ACP activities of fourth-instar larvae of *C. medinalis* after treatment with 3β,24,25-HDCL (a) and BL (b). The data were fitted on polynomial (regression) model, where as vertical bars indicate standard error (±SEM).

*Dysoxylum* triterpenes treated leaffolder larvae. Similar studies on the effects of pure compounds and extracts from Meliaceae plants on enzyme activity [18,51–53] and food consumption [16,18,54] have been previously conducted. It may be inferred from the previous studies that the decreased larval growth coupled with lower RGR, which is more likely due to longer retention of food in the gut for maximization of AD to meet the increased demand of nutrients, [54,55]. The results of the current study revealed that although the treated larvae were capable of maintaining the AD (increased during treatment), they failed to maintain the RGR during larval development (Tables 1 and 2). AD could not be maintained due to a continuous decline in RGR. The RGR reached it's lowest level in the 12 ppm treatment (Tables 1 and 2).

The consumption and conversion efficiency were highly correlated with the gut enzyme activity of *C. medinalis*. Tetranortriterpenoid contains enzyme-inhibiting components, which reduce the conversion rate of food [56–58]. The percentage reduction in ECI and ECD results from a food

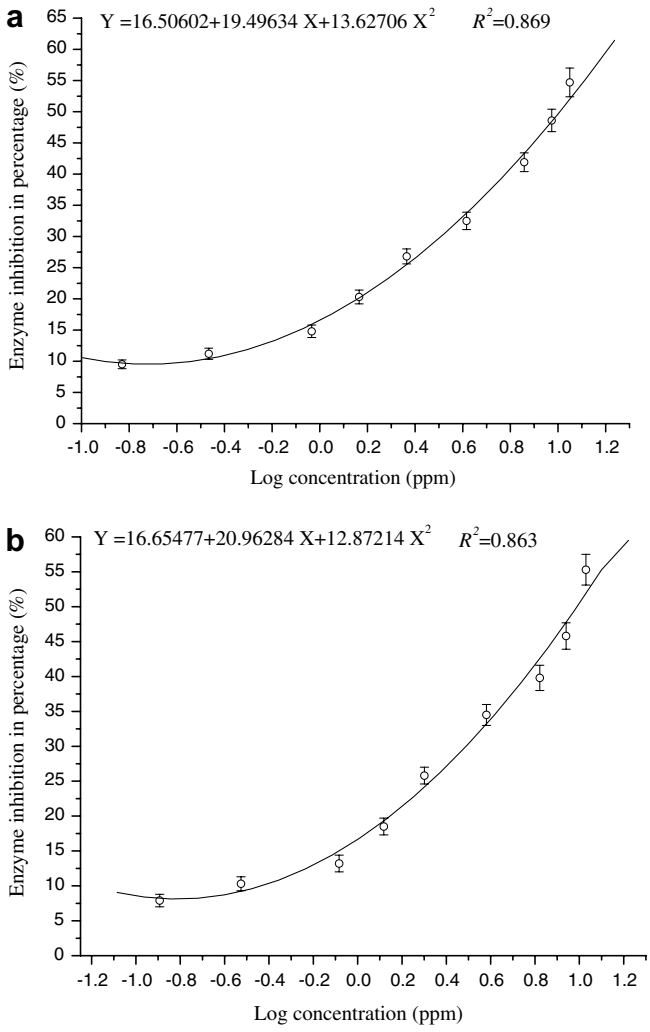


Fig. 4. Percentage reduction of ALP activities of fourth-instar larvae of *C. medinalis* after treatment with 3β,24,25-HDCL (a) and BL (b). The data were fitted on polynomial (regression) model, where as vertical bars indicate standard error (±SEM.)

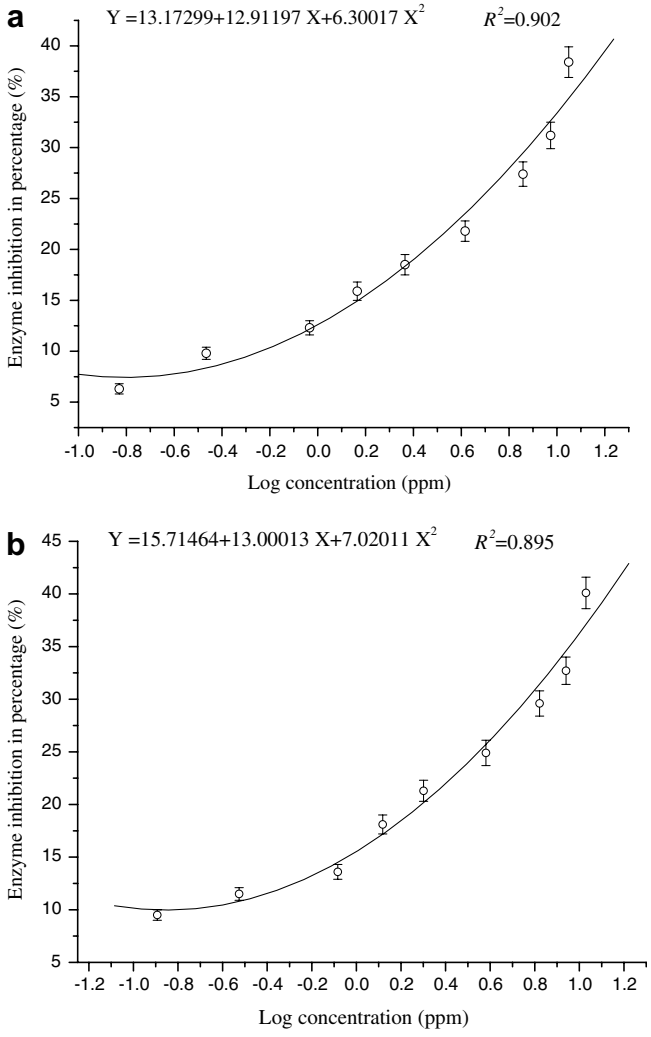


Fig. 5. Percentage reduction of ATPase activities of fourth-instar larvae of *C. medinalis* after treatment with 3β,24,25-HDCL (a) and BL (b). The data were fitted on polynomial (regression) model, where as vertical bars indicate standard error (±SEM).

conversion deficiency, which reduces growth perhaps through a diversion of energy from biomass production into detoxification [17].

Previous reviews [5,6] of triterpenes bioactivity revealed that the majority of triterpenes tested show some level of antifeedant activity. When fourth-instar rice leaffolder were fed *Dysoxylum* triterpenes, growth rate fell as triterpenes concentration increased. This corresponded to a decrease in consumption rate. It is likely that this decrease in consumption rate is due to the antifeedant nature of the triterpenes and this accounts for the majority of the decrease in growth rate. However, both ECI and ECD also decrease as extract concentration increases. ECI is an overall measure of an insect's ability to utilize the food that it ingests for growth. ECD also decreases as the proportion of digested food metabolized for energy increases. Decreasing ECI and ECD values indicate that ingested *Dysoxylum* triterpenes also exhibit some chronic toxicity. Similar results were also seen with *Trichilia americana* extract [16], rotenone (isoflavonoid) [17], chinaberry extract (*Melia azedarach* L) [18], and neem limonoids [54], when tested against lepidopteran pests. It was proved that triterpenes have different modes of action depending on the test insect species and that they can exhibit both antifeedant and toxic modes of action, e.g., Azadirachtin [52,54]. Azadirachtin acts as both an antifeedant and a chronic toxin and work with rice leaffolder larvae suggests that these activities are independent. Treatment with azadirachtin and other triterpenes from neem reduces RGR, RCR, ECI, and ECD [54]. Further work has investigated the effect of neem limonoids on midgut enzymes, in particular midgut ACP and ALP. This is the primary hydrolytic enzymes, which hydrolyse phosphomonoesters under alkaline or acid conditions, found in the gut of many lepidopteran insects [59]. Treatment with neem limonoids and *M. azedarach* L extract causes a drastic reduction in the activity of ALP and ACP [18,49]. Koul et al. [60] also found that azadirachtin interferes with growth via digestive impairment by inhibiting the secretion

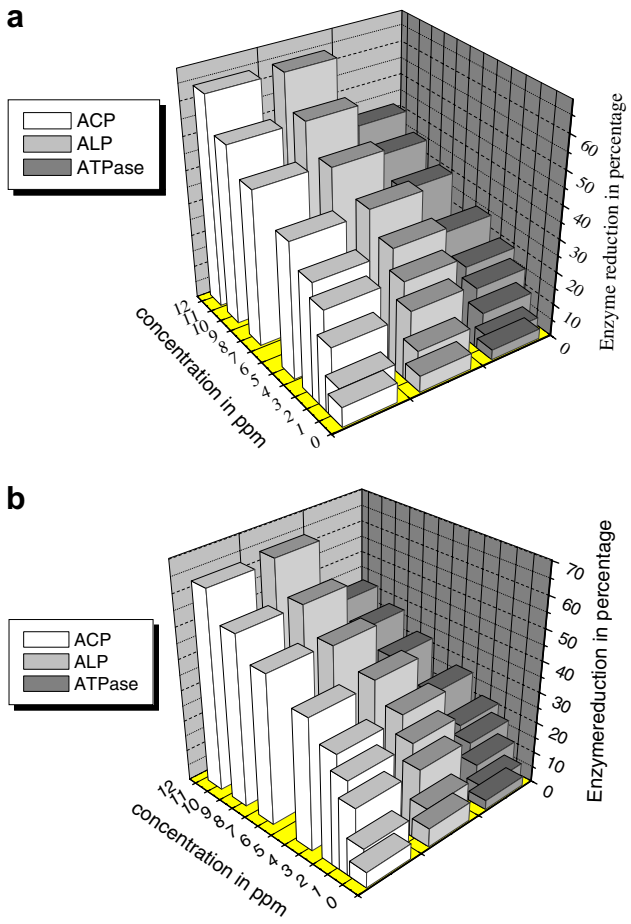


Fig. 6. Comparison of enzyme reduction percentages of ACP, ALP, and ATPase of fourth-instar larvae of *C. medinalis* after treatment with 3β,24,25-HDCL (a) and BL (b).

of trypsin-type proteinases from gut epithelial cells. Timmins and Reynolds [61] also found the similar result on *Manduca sexta* L.

In conclusion, our results indicate that *Dysoxylum* triterpenes exert various effects on growth and physiology of RLF. However, especially at the higher concentrations (6 and 12 ppm) tested, larval growth declined, associated with reduced food consumption and enzyme activity. The triterpenes interfered with the digestion and/or absorption of ingested food and with the conversion of absorbed food to biomass.

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