Chemosphere 72 (2008) 1393–1400

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The toxicity and physiological effect of goniothalamin, a styryl-pyrone, on the generalist herbivore, *Spodoptera exigua* Hübner ☆

Sengottayan Senthil-Nathan [a,b,*], Man-Young Choi [a,c], Chae-Hoon Paik [a], Kandaswamy Kalaivani [a]

[a] *Plant Environment Division, Honam Agricultural Research Institute (HARI), National Institute of Crop Science (NICS), Rural Development Administration (RDA), #381 Songhak-dong, Iksan, Chonbuk 570-080, Republic of Korea*
[b] *Division of Biopesticides and Environmental Toxicology, Sri Paramakalyani Centre for Environmental Sciences (SPKCES), Manonmaniam Sundaranar University, Alwarkurichi 627 412, Tirunelveli, Tamil Nadu, India*
[c] *Agricultural Pest Division, National Institute of Agricultural Sciences and Technology (NIAST), RDA, Seodoon dong, Suwon, Republic of Korea*

ARTICLE INFO

Article history:
Received 4 October 2007
Received in revised form 4 February 2008
Accepted 13 March 2008
Available online 21 May 2008

Keywords:
Goniothalamin
Styryl-pyrone
Beet armyworm
Moulting
Nutrition
Gut histology

ABSTRACT

Efficacy of Goniothalamin a styryl-pyrone isolated from *Goniothalamus wightii* Hook.f. and Thoms, against beet armyworm, *Spodoptera exigua* (Hübner), populations was determined under laboratory condition. The experiments were carried out with concentrations of 5, 10, 15 and 30 ppm in an artificial diet and compared with control insects. Laboratory bioassay showed that the goniothalamin had a strong effect on food utilization, moulting and gut histology. The food consumption and conversion of ingested and digested food to body matter decreased with increasing pyrone concentration. The antifeedant activity was also observed in larvae of *S. exigua*. The treated third instar larvae exhibited mortality in a dose dependent manner. At 5, 10, 15 and 30 ppm/insect, the pyrone gave 23%, 45% 63% and 100% mortality respectively. The larvae of *S. exigua* gained significantly less weight until pupation in the 10 and 15 ppm pyrone concentrations. Duration of larval period was also affected after treatment with pyrone. The metamorphosis was delayed with additional moulting (7th instar) after treatment with 10 and 15 ppm of goniothalamin, the percentage of larvae successfully moulted into progressive instars was significantly decreased with an increase in pyrone concentrations. The effects of goniothalamin on midgut ultrastructure of third instar larvae of *S. exigua* were investigated by using light microscopy. Cross sections of the midgut showed that the epithelial cells were destroyed. Significant damage of the midgut epithelium was observed along with lysis.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

The random and unwise uses of pesticides have resulted in widespread contamination in agricultural products (Bhanti and Taneja, 2007). Several pesticides including organophosphorous and organochlorine pesticides are commonly used. The continuous use of organophosphorous or organochlorine pesticides has been associated with several concerns including alteration of the aquatic environment (Albanis et al., 1986). Therefore, a serious effort has been made to find alternative methods of pest control.

For centuries natural pesticides of plant origin were used for controlling agricultural pests. Plants themselves have proven to be sources of some of the most potent pest control products, and they have their own chemical defenses against insects (Levin, 1976). Effective, sustainable botanical insecticides can be utilized in the management of insects or in the development of environmentally safe materials to control pests. These products are either synthetic analogues of a plant-derived compound (bioresmethrin) or derived from natural sources (Regnault-Roger et al., 2005). The deleterious effects of natural insecticides on insects work in several ways: antifeedant, insect growth regulator, affect the hormone system, affect the nervous system, and have direct effects on organs or tissues such as the stomach and muscle (Schmutterer, 1990; Mordue and Blackwell, 1993; Senthil-Nathan et al., 2006a, 2007). Goniothalamin, a bioactive styryl-pyrone (Fig. 1), is isolated from the leaves of *Goniothalamus* species (Annonaceae), which are widely distributed throughout Asia and are used as medicinal plants. The bioactive principles are comprised of two types of compounds known as styryl-lactones and acetogenins. Styryl-lactones are a group of secondary metabolites ubiquitous in the genus

---

☆ This paper was given in an abstract form at the International Congress of Insect Biotechnology and Industry, Daegu, Korea, 19–24th August 2007, Abstract No. GP02-43.

* Corresponding author. Address: Division of Biopesticides and Environmental Toxicology, Sri Paramakalyani Centre for Environmental Sciences (SPKCES), Manonmaniam Sundaranar University, Alwarkurichi 627 412, Tirunelveli, Tamil Nadu, India. Tel./fax: +91 4634 283270.

E-mail addresses: senthil@ms-university.org, senthilkalaidr@hotmail.com, senthil@glay.org (S. Senthil-Nathan).

0045-6535/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2008.03.037

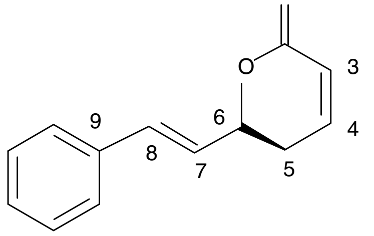

Fig. 1. Chemical structure of goniothalamin tested against *S. exigua*.

Goniothalamus, which have anti-proliferative activity against cancer cells (de Fatima et al., 2005; Wiart, 2007).

The beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) is a cosmopolitan polyphagous, defoliating insect pest, which has migrated all over the world. The pest has a wide host range of plants such as vegetables and field crops (Metcalf et al., 1962; Capinera, 2005). Armyworm larvae are difficult to control with insecticides once an infestation is well established (Hill, 1983; Zhu, 1992; Wang et al., 2006). The abundance of the pest and the serious economic consequences of infestation have resulted in frequent application of insecticides to foliage that have led to ecological and toxicological hazards; a lot of effort has been dedicated to finding alternative control measures for the pest. Protection of crops from serious pests with chemical pesticides has led to increasing resistance to many chemical pesticides as well as pest resurgence and secondary pest infestations (Brewer and Trumble, 1994; Glenn et al., 1994; Perez et al., 2000), there is an increasing interest among entomologists and phytochemists in the use of botanical pesticides such as neem, the pure neem limonoid, azadirachtin, *Melia azederach* L., pyrethrum, *Bacillus thuringiensis*, entomopathogenic fungi, and other allelochemicals, biopesticides and repellants used in agriculture of the past and present (Copping and Menn, 2000; Goettel et al., 2004; Senthil-Nathan et al., 2006b).

Hence an attempt has been made to determine out the effects of goniothalamin, a styryl-pyrone, on the behavior and nutritional physiology of the beet armyworm.

2. Materials and methods

2.1. Laboratory mass culture of S. exigua

The experimental insects were obtained from a culture of *S. exigua* maintained on soybean leaves (*Glycine max* L. Merr.) in the laboratory. The susceptible strain of *S. exigua* has been maintained for more than 2 years in the laboratory of the Honam Agricultural Research Institute, Rural Development Administration (RDA), Iksan, South Korea without any exposure to insecticide. The soybean plants were grown in a green house and were 2–3 weeks old. Prepupae were separated and provided with clay or sand soil as pupation sites. Emerging adult moths were transferred to cages and fed on a 10% sucrose solution. Moths were transferred at a ratio of 1 male:2 females to oviposition cages containing soybean leaves. The leaves containing eggs were moistened and kept in plastic containers (500 × 356 × 200 mm) to allow hatching. All experiments and culture were carried out at 28 ± 2 °C, 65% relative humidity, with a 14:10 light:dark cycle.

2.2. Bioassays and treatments

Goniothalamin was isolated from *G. wightii* and was received from Dr. G. Jeyakumar, M.G. College, Trivandrum, India. Bioassays were performed with first to fourth instars of *S. exigua* larvae using artificial diet (Beet armyworm diet, Bio-Serv®, NJ) amended with concentrations of 5, 10, 15 and 30 ppm of goniothalamin. After preparing the diet in the laboratory, aliquots of 5 ml methanol solution containing of 100, 200, 300 and 600 ppm, were added to 95 g portions of the diet during the normal course of cooling (50 ± 2 °C). Control diets received methanol only.

A group of 10 larvae per concentration were used for all the treatments with three replicates. The uneaten diets were removed after 24 h, and replaced with fresh untreated diets. The duration of larval and pupal stages were recorded. Pupae of all test larvae were weighed on the first day after pupation. Deformities in the larvae, pupae and emerged adults were recorded. Mortality was recorded every 24 h and final mortality was recorded after 12 days. The percentage mortality was calculated by using the formula (1) and corrections for natural mortality when necessary were done by using Abbott's (1925) formula.

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \qquad (1)$$

The percent mortality data after correction were subjected to probit analysis (Finney, 1971) to calculate mean lethal concentrations (LC_{50} and LC_{90}).

2.3. 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 (Sartorius, CP2245). 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 were allowed to feed on weighed quantities of treated and untreated diet, for a period of 24 h. Larvae were again weighed. The differences in weights of the larvae were the fresh weights gained during the period of study. Sample larvae were weighed, oven dried (48 h at 60 °C) and later re-weighed to establish a dry weight of the experimental larvae. The diet remaining at the end of each day was oven dried and re-weighed to establish a 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. Feces were collected daily and weighed, then oven dried and re-weighed to estimate the dry weight of excreta.

Consumption, growth rates and post-ingestive food utilization efficiencies (all based on dry weight) were calculated in the traditional manner (Waldbauer, 1964, 1968; Slansky and Scriber, 1985), relative consumption rate (RCR) = dry weight of food eaten/duration of feeding (days) × mean dry weight of the larva during the feeding period, relative growth rate (RGR) = dry weight gain of larva during the period/duration of feeding (days) × mean dry weight of the larva during the feeding period, efficiency of conversion of ingested food (ECI) = 100 × dry weight gain of larva/dry weight of food eaten, efficiency of conversion of digested food (ECD) = 100 × dry weight gain of larva/(dry weight of food eaten – dry weight of feces produced), approximate digestibility (AD) = 100 × (dry weight of food eaten – dry weight of feces produced)/dry weight of food eaten.

To confirm the toxicity of goniothalamin, we compared the relative growth rate and consumption rate (Blau et al., 1978). For the experiment an aluminum tray was used to provide an artificial diet. The larvae received diet containing 0, 25, 50, 100 mg of goniothalamin and unlimited artificial untreated diet (10 larvae per concentration). A small piece of (2 cm × 2 cm) moistened cotton was placed in the aluminum tray where larvae received zero diet. The experiment was conducted for 72 h. After 72 h the larvae were weighed and the consumption rate was calculated. The calculated relative consumption rate was plotted against relative growth rate by using Minitab® linear regression analysis.

2.4. Histology

Small pieces of gut tissue from treated and control insects were fixed overnight in Bouin's solution. The specimens were embedded in an embedding medium (optimal cutting temperature (OCT), Tissue-Tek, Sakura, USA). The blocks were cooled −27 °C for 3 h and cut into 1.5 μm ribbons with an ultra-cryo-microtome (Cryocut 1800, Leica, Germany). The ribbons were stained with Delafield's haematoxylin and counter-stained with eosin, and mounted after drying. The sections were observed and photographed under light microscope (Leica, DMRE, Germany).

2.5. Statistical analysis

Data from bioassay and food utilization were subjected to analysis of variance (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's family error rate by Minitab® statistical software. Difference between means were considered significant at $P \leqslant 0.05$ (Snedecor and Cochran, 1989). The lethal concentrations (both LC_{50} and LC_{90}) were calculated using probit analysis (Finney, 1971) and values were expressed as means of five replicates. Mortality was corrected using Abbott's (1925) formula, if it was necessary.

3. Result

3.1. Larval duration, moulting, growth and weight gain of *S. exigua* after treatment with styryl-pyrone

When goniothalamin was fed to *S. exigua* at different concentrations (5–30 ppm) several effects were noticed. The effective weight of the growing insects was changed from third instar larvae (1-day-old) to pupation. Control larvae increased weight significantly until 12th day. Due to increased weight, larvae began to stop feeding prior to pupation and larval period duration was reduced and stabilized as larvae approached the pre-pupal stage. The developmental effects on *S. exigua* larvae were significantly different at the highest concentration (15 ppm) of goniothalamin compared with control diet.

As the concentration of goniothalamin was increased (10 ppm and 15 ppm), *S. exigua* larvae consistently lost weight and consumed significantly less than those exposed to the lowest concentration (5 ppm) and controls; and the time taken to reach maximum weight increased. This antifeedant effect was inversely correlated with concentration. On average, control larvae took 13.4 (SE = 0.32) days to reach maximum weight, those larvae fed diet containing 5, 10, and 15 ppm took 16.2 (SE = 0.41), 20.7 (SE = 0.68) and 23.9 (SE = 1.12) days to reach maximum weight, respectively. A dose dependent increase in the time until pupation was noticed at all concentrations (Fig. 2). The same trend was also noticed in pupal stage. The pupae attained maturity with in the time, but, in larvae which received more than 5 ppm goniothalamin, it took significantly more time. After treatment with goniothalamin, the complete life cycle of *S. exigua* was lengthened in a dose dependent manner.

The longest larval period was observed in the 15 ppm treatment, while the shortest larval period was observed among the larvae fed on unamended control diet. The larval period for larvae fed on 30 ppm was not obtained, as no larvae survived after treatment (48 h). Survival, either calculated on 48 h day or over the whole larval period, decreased as the concentration of the pyrone increased. The survival of larvae fed on control diet was about 95% after 48 h, but, in case of goniothalamin treatment, only 70%, 58%, 32% and 0% of larvae fed on diets containing 5, 10, 15 and 30 ppm of the pyrone, respectively, survived to 48 h or more (Fig. 3) ($F = 11088.53$; d.f. = 14; $P < .0001$). The pupal weight was significantly reduced after treatment with goniothalamin ($F = 1520.58$; d.f. = 11; $P < .0001$). The estimated LC_{50} and LC_{90} values of 4th instar larvae of *S. exigua* were 14.2 ppm and 25.5 ppm, respectively. As the dose of goniothalamin increased, the effects on the development of *S. exigua* increased causing greater mortality and more severe deformities in both larval and pupal stage (Fig. 4).

As shown in Fig. 5 when 10 and 15 ppm of goniothalamin was applied to third stadium of *S. exigua* larvae 6 h after moulting, nearly 60% and 85% of the treated larvae were affected during the moulting stage. In control, the larval development was divided into six larval instars. The last larval instar could be divided into two stages; a period of growth and the pre-pupal stage. The duration of the feeding period was 4 days. On the last day of the feeding period, the larvae reached maximal body weight and size. This was followed by the pre-pupal stage and pupal stage. But in the case of 15 ppm treatment, larvae extended their larval stages up to 7 instars. Only 25% of the larvae successfully moulted in to the pupal stage. In the control group, larvae reached the pupal (96%) stage

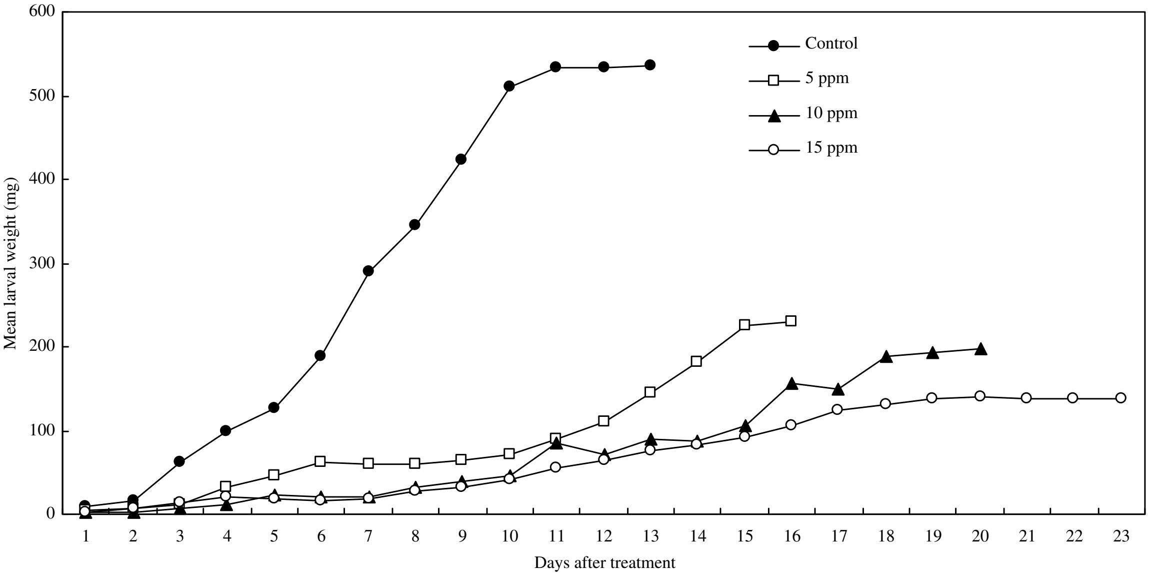

Fig. 2. A comparison between mean larval duration versus mean larval weight of *S. exigua* after treatment with goniothalamin (values are means of three replicates).

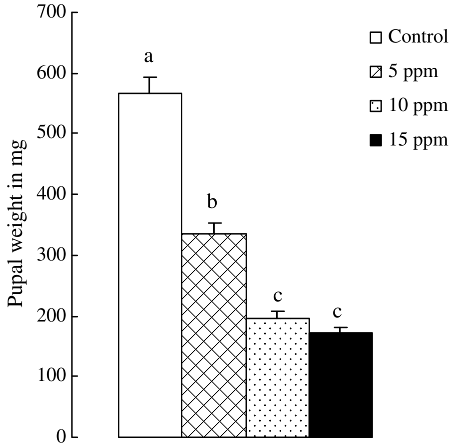

Fig. 3. Percentage mortality of fourth instar larvae of *S. exigua* after treatment with goniothalamin. Means (SEM±) followed by the same letters above bars indicate no significant difference ($P \leqslant 0.05$) according to a Tukey test.

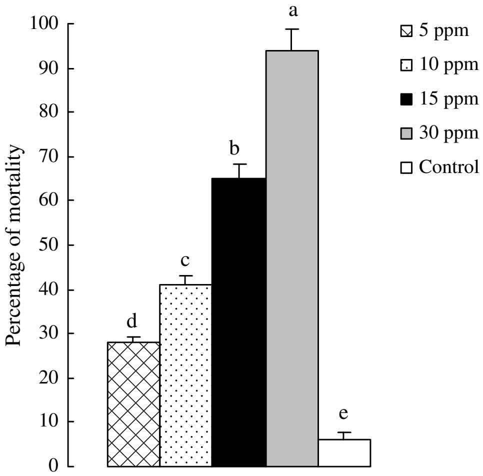

Fig. 4. Mean pupal weight of *S. exigua* after treatment with goniothalamin. Means (SEM±) followed by the same letters above bars indicate no significant difference ($P \leqslant 0.05$) according to a Tukey test.

24–25 days after starting the experiments (Fig. 5). In the group treated with a concentration of 5 ppm goniothalamin only 56% of larvae began metamorphosis in 31 days (Fig. 5). In contrast, concentrations of 10 and 15 ppm goniothalamin inhibited the moult of 60 and 85% (Fig. 5), respectively, from 41 to 42 d after starting treatment. Larvae in both groups were moulted but remained a smaller size when compared with the control group. Treatment with high doses of goniothalamin (10–15 ppm) elicited ecdysis in order to form an additional larval stage (7th stage) in about 20% (Fig. 5). However, these larvae showed severe deformities in the thoracic appendages. On the other hand, control treatment with 1% methanol in the third stadium neither prolonged metamorphosis nor changed the duration of any stadia (Fig. 5). Goniothalamin treatment applied at 5 ppm to third stadium larvae again prolonged the duration of the fourth, fifth and sixth stadia, but did not change the number of moults (Fig. 5).

Only the lowest concentration (5 ppm) allowed the larvae to complete moulting, but, at higher concentrations of the goniothalamin, the moult was incomplete. The moulting period was prolonged with an increasing dose. However in 5 and 10 ppm treatment, several anomalies, probably related to defective moulting, were observed in goniothalamin treatments. Particularly at the 15 ppm goniothalamin treatment, parts of the old cuticle adhered to the larval cuticle. And, also, body setae and structures located at the extremities of the legs and mouth parts were lost. Growth-regulatory effects such as formation of larval–pupal intermediates occurred in the same treatments. Goniothalamin diet resulted in irreversible damage to physiological processes essential to the development of S. *exigua.* At higher concentrations, affected larvae assumed a characteristic cuticle discoloration where they turned to a uniform dark grey to blackish brown color, and the black dot patterns were more pronounced. The treated larvae showed some sort of alteration in the pigmentation of the cuticle with black spots resulting from cuticular melanization. All control larvae in these treatments developed normally to the adult stage. At 15 ppm goniothalamin, the larvae in the last three instars either had their body or at least several thoracic segments dorsally swollen. In addition, 100% of the larvae showed an anomalous pattern of color or spots on the cuticle in the later larval instars. At the pre-pupal stage, insects fed on 10 ppm and 15 ppm goniothalamin-treated diet were mostly dead and those remaining showed some abnormalities such as wrinkled, swollen or stretched bodies, different patterns of cuticle color with spots, a prolonged pre-pupal period, or failed to pupate. At the 10 ppm concentration, 58% of the insects completed the larval period, and pupated. The moulted insects showed some sort of damage during the period and were reduced in size as compared with the control larvae.

3.2. Effect of goniothalamin on food utilization of S. exigua

Nutritional analysis revealed that the compound goniothalamin specifically acts as an antifeedant. Goniothalamin when incorporated into artificial diet reduced relative growth and consumption rates without any significant change in the efficiency of conversion of ingested food values. Reduction in RGR and RCR was correlated with goniothalamin dietary concentrations. The reduction depended on the dose of the treated diet. Larval growth was maximal on the control diet, but the growth rate decreased significantly on diets with higher concentration values (Table 1). Both relative growth rate and final larval mass were significantly lower for larvae fed on diet with 10 and 15 ppm ($F = 1113.83$; d.f. = 11; $P < .0001$) concentrations than for larvae fed control diet. This relative reduction in growth rate arises because of compensatory changes in consumption rate and approximate digestibility (Table 1). In addition, larval consumption was highly dependent upon the goniothalamin concentration in the diets. As the dose of goniothalamin increased, a greater reduction in consumption efficiency was noted. As the concentration of goniothalamin in the diet increased, there were corresponding decreases in the amount of food consumed, weight gained and the approximate digestibility was increased significantly ($F = 36.32$; d.f. = 11; $P < .0001$). A significant reduction in all nutritional indices was observed at, 5, 10 and 15 ppm concentrations. Relative growth rates against consumption rates was plotted to determine the differentiation of the deterrent and toxic effects of the goniothalamin. Calibration curves generated for the RCR and correlated with RGR, revealed that the relatively reduced growth rates of larvae that were fed on treated diets had significantly more effects than that of the control larvae (Fig. 6). The regression coefficients of the RCR–RGR relations for control and treated larvae were significantly different ($R^2 = 0.982$, $R^2 = 0.887$). The regression lines represent the growth efficiency of the larvae, and again indicate that the reduced growth of these larvae fed on goniothalamin-treated diets is not entirely due to starvation; some of the growth reduction is from toxicity.

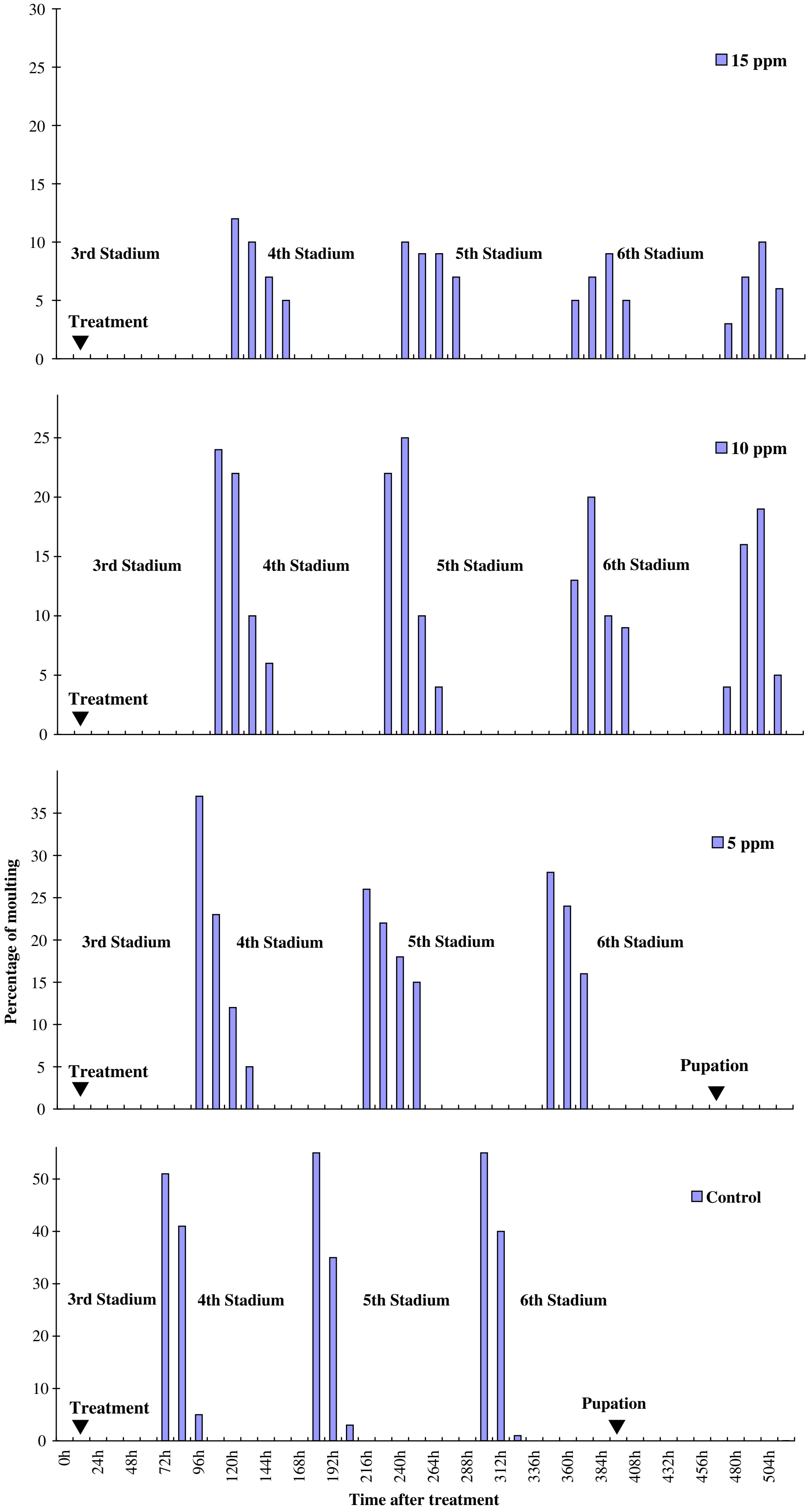

Fig. 5. Moulting time and responses of *S. exigua* to goniothalamin applied at various concentrations after moulting to the third stadium as indicated in the figure (data are means of three replicates).

Table 1
Nutritional indices of fourth instar larvae of *S. exigua* after treatment with goniothalamin

Treatment in ppm	mg/mg/day		ECI (%)	ECD (%)	AD (%)
	RGR[A]	RCR			
5	1.26 ± 0.014^b	2.45 ± 0.011^b	28.23 ± 0.68^b	37.16 ± 0.68^b	43.90 ± 0.17^b
10	0.99 ± 0.015^c	1.84 ± 0.014^c	21.83 ± 0.76^c	30.40 ± 0.41^c	44.66 ± 0.20^b
15	0.50 ± 0.011^d	0.94 ± 0.014^d	16.33 ± 0.29^d	22.33 ± 0.38^d	46.46 ± 0.12^a
Control	1.68 ± 0.017^a	3.45 ± 0.020^a	33.93 ± 0.18^a	45.16 ± 0.38^a	41.46 ± 0.52^c

Means (±SEM) followed by the same letter within columns indicate no significant difference ($P \geqslant 0.05$) in a Tukey test.

[A] RGR, relative growth rate, RCR, relative consumption rate, ECI, efficiency of conversion of ingested food, ECD, efficiency of digested food, and AD, approximate digestibility.

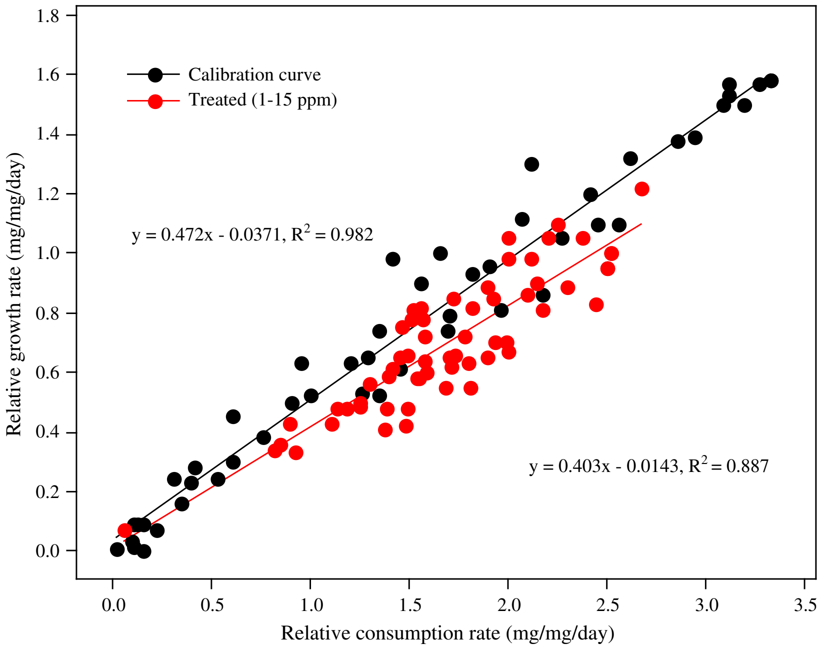

Fig. 6. Correlation between the relative consumption rates and relative growth rates of *S. exigua* fed on different quantities of control diet (calibration curve) and larvae fed on diet containing different concentrations of goniothalamin. Regression equations are displayed. The regression coefficients of the two lines are significantly different (R^2 = 0.982, R^2 = 0.887).

Growth was maximal on the control, but decreased significantly on diets with higher concentration values. Both relative growth rate and final larval mass were significantly lower for larvae fed the 10 and 15 ppm concentration diet than for larvae fed control diet. This relative reduction in growth rate arises because of compensatory changes in consumption rate and approximate digestibility. In addition, larval consumption is highly dependent upon the goniothalamin concentration in the diets; the effect of reduced consumption efficiency is obviously less for larvae fed on control diet than for larvae fed on 5, 10 and 15 ppm goniothalamin-amended diet.

3.3. Mid gut histology of S. exigua after treatment with goniothalamin

A considerable difference was found between midgut sections from 10 to 15 ppm goniothalamin-treated larvae and control larvae of *S. exigua* (Fig. 7A). In controls, the epithelium of midgut appears formed by large uniform cells with nuclei and microvilli. At the concentration of 10 ppm, the peritrophic membrane was damaged and it mixed with epithelial cells (Fig. 7B). The columnar cells of the gut were also damaged by 10 ppm treatment of goniothalamin (Fig. 7B). After treatment with 15 ppm of goniothalamin the destruction of the midgut epithelium was complete, with a disappearance of cellular components like nuclei (Fig. 7C).

4. Discussion

4.1. Effect of goniothalamin on biology and metamorphosis

The styryl-pyrone, goniothalamin, is an effective botanical insecticide for polyphagous pest like *S. exigua*. Most detailed studies of the effects of secondary metabolites on insect physiology have been carried out during last three decades (Kubo, 2006). Also, our studies on goniothalamin-treated *S. exigua* showed that larval growth and metamorphosis were affected and pupae gained less weight after being fed with styryl-pyrone. The activity was dose dependent. The concentration-dependent effects of styryl-pyrone on the growth, development and metamorphosis of *S. exigua* were similar to those reported for other insect species tested with pure allelochemicals like azadirachtin, and other neem limonoids (Sieber and Rembold, 1983; Dorn et al., 1986; Barnby and Klocke, 1987; Govindachari et al., 1996; Senthil-Nathan et al., 2006b,c), terpenoids (Qiu et al., 1998), aglaroxin-A (Koul et al., 2004), β-carbolines (Cavin and Rodriguez, 1998), quassin, cinnamaldehyde (Hertel and Muller, 2006), avocadofurans (Rodriguez-Saona and Trumble, 1999). Although metamorphosis is known to be inhibited by limonoids and terpenes, there is no study on the effect of styryl-pyrone on insect metamorphosis. In the present study on *S. exigua*, 12-h-old third stadium larvae were affected by prolonged/

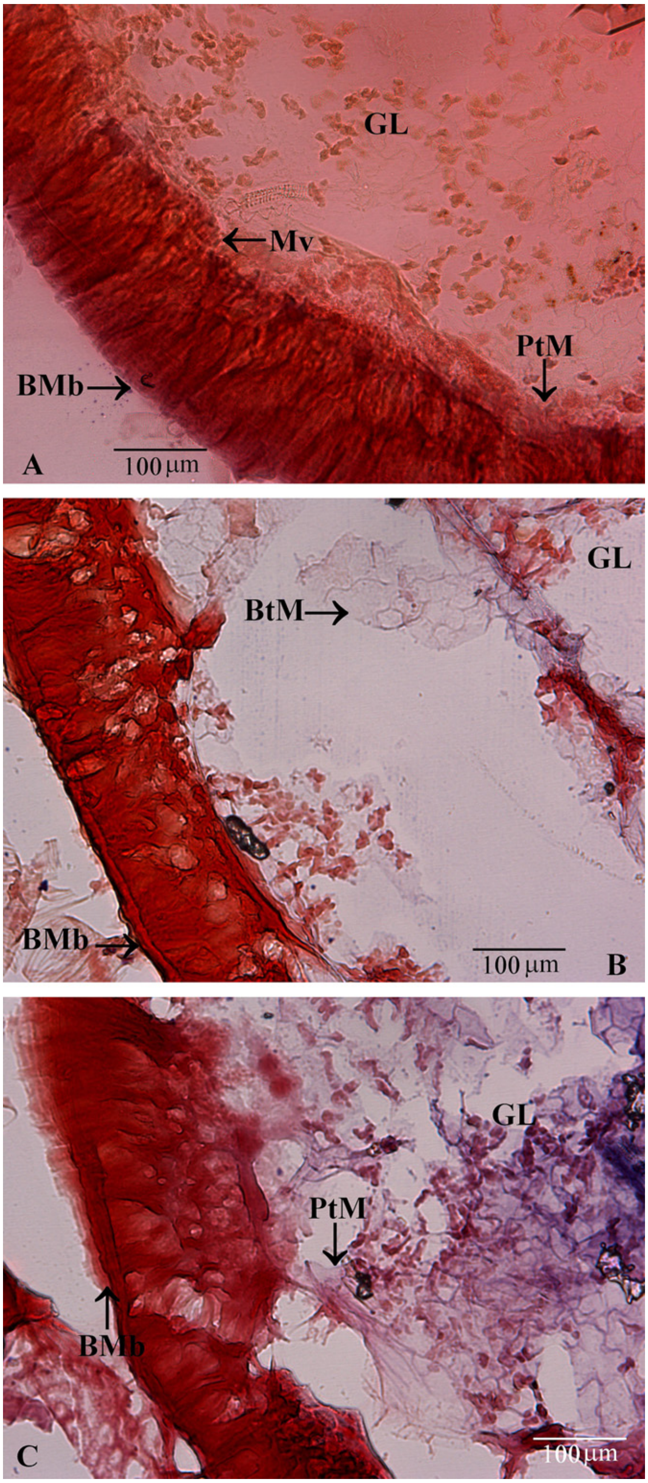

Fig. 7. Mid gut histology of *S. exigua* after treatment with goniothalamin (A, control, B, treated with 10 ppm and C, treated with 15 ppm (BMb, basement membrane, Mv, microvilli, BtM, peritrophic membrane, GL, gut lumen; bar = 100 μm).

deformed moulting after ingesting the styryl-pyrone, goniothalamin. These effects are also related to disruption of other endocrine systems controlling moulting. The moulting effects are due to a disruption in the synthesis and release of ecdysteroids (moulting hormone) and other classes of hormones and this was proved by injections of neem limonoid AZA into the hemolymph of nymphs of *L. migratoria* (Mordue et al., 1985).

Our data also showed that compared to the control group, larvae treated with goniothalamin at concentrations of 15 and 30 ppm in an artificial diet exhibited increased mortality of 85% and 100%, respectively, during development of larvae. The effect was very apparent at the concentration of 30 ppm goniothalamin with 100% mortality of third instar larvae. These results are in agreement with the earlier studies of Kabir et al. (2003) who showed high mortality of *Culex quinquefasciatus* Say treated with goniothalamin.

Significant reduction in weight gain in response to treatment doses at 10 ppm concentration level could indicate that the styryl-pyrone had a strong antifeedant effect on the larvae. Despite the mortalities in response to the higher concentrations of goniothalamin, the extended period of larval duration and slowed larval growth in the 5 ppm treatment indicated that the lower concentration also had physiological effects. At the 15 ppm concentration, growth was almost completely inhibited because 85% of larvae could not develop to pupa and near100% final mortality occurred.

4.2. Effect of goniothalamin on nutritional indices of *S. exigua*

The RGR and RCR for each set of larvae were subjected to a linear regression analysis (Fig. 6). The nutritional indices clearly indicates that the reduced growth of these larvae under the influence of goniothalamin is not entirely due to starvation; some of the growth reduction is due to toxic effects of the ingested compound. The dry weight of feces, weight gain and growth rate were positively affected. The relative growth and consumption rate (RCR and RCR), measuring the growth and consumption of *S. exigua* was decreased significantly at concentrations higher than 5 ppm. This result was similar with other lepidopteran insects tested with pure allelochemicals and extract (Wheeler and Isman, 2001; Sadek, 2003; Koul et al., 2004; Senthil-Nathan et al., 2007b). The efficiency of conversion of ingested food (ECI) measuring the ingested food which converted into the body matter. These results clearly indicated that the styryl-pyrone has strong effects on food absorption and digestion. Plotting the RCR against the RGR, revealed that the extract had significant toxic effects as was shown by the relatively low growth rates of larvae fed on treated diets (Fig. 6). The regression coefficients of the RCR–RGR relations for control and treated animals were significantly different; indicating that the reduction in growth of larvae fed on extract-containing diets was not entirely a result of lower food intake. Nutritional analysis revealed that the compounds specifically act as antifeedants. The compounds, when incorporated into artificial diet reduced RGR and RCR with significant change in the ECI.

4.3. Effect of goniothalamin on gut histology of *S. exigua*

Our study has shown that histology of *S. exigua* larvae was similar to that for other active plant secondary metabolites such as AZA (Nasiruddin and Mordue, 1993), microbial toxins such as δ-endotoxins from *Bacillus thuringiensis* (Gill et al., 1992) and, insecticidal proteins from fungal isolates, *Metarhizum anisopliae*, *Beauveria bassiana*, *B. brongniartii* and *Scopulariopsis brevicaulis* (Yu et al., 1997). The histological changes to the gut varied with the treatment doses. Ingestion of styryl-pyrone in artificial diet of *S. exigua* led to swelling of columnar cells and later the gut epithelium was completely destroyed by 15 ppm. The changes or damage in the structure of epithelial cells and brush border membrane after treatment with pure plant allelochemicals AZA Nasiruddin and Mordue, 1993) and *Melia azedarach* plant extract (Schmidt et al., 1997) was already been reported. The midgut of the digestive system is an important part of an insect, because this is where the digestive enzymes are secreted.

5. Conclusion

In conclusion, the styryl-pyrone, goniothalamin, resulted in increased mortality, reduced food consumption, reduced weight gain of larva and pupa, moulting inhibition via prolonged moulting period, and damage to the gut in *S. exigua*. These effects were dose dependent.

Acknowledgments

This research was completely supported by the Rural Development Administration, NICS, Korea as a part of the organic agriculture research consortium. The authors express gratitude to Prof. J. Powell Smith for his keen interest, advice and comments on an earlier version of the manuscript. We thank Profs. Richard W Mankin and B. Merle Shepard for providing valuable reprints, related articles and advice. We are grateful to Drs. D.K. Lee, H.K. Shim, and T.H. Noh to permit to avail the laboratory facilities especially for microtome assay. We thank (Mrs.) S.S. Kang and S.M. Kang for their technical assistance and insect rearing.

References

Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Albanis, T.A., Pomonis, P.J., Sdoukos, T.A., 1986. Organophosphorous and carbamates pesticide residues in the aquatic system of Ioannina basin and Kalamas River (Greece). Chemosphere 15, 1023–1034.

Barnby, M.A., Klocke, J.A., 1987. Effects of azadirachtin on the nutrition and development of the tobacco budworm, *Heliothis virescens* (Fab.) (Noctuidae). J. Insect Physiol. 33, 69–75.

Bhanti, M., Taneja, A., 2007. Contamination of vegetables of different seasons with organophosphorous pesticides and related health risk assessment in northern India. Chemosphere 69, 63–68.

Blau, P.A., Feeny, P., Contardo, L., 1978. Allylglucosinolate and herbivorous caterpillar: a contrast in toxicity and tolerance. Science 200, 1296–1298.

Brewer, M.J., Trumble, J.T., 1994. Beet-armyworm resistance to fenvalerate and methomyl-resistance variation and insecticide synergism. J. Agri. Entomol. 11, 291–300.

Capinera, J.L., 2005. Beet Armyworm, *Spodoptera exigua* (Hübner) (Insecta: Lepidoptera: Noctuidae). University of Florida Extension report no. EENY-105. <http://edis.ifas.ufl.edu/IN262> (accessed on 5th May, 2007).

Cavin, J.C., Rodriguez, E., 1998. The influence of dietary- carboline alkaloids on growth rate, food consumption, and food utilization of larvae of *Spodoptera exigua* (Hubner). J. Chem. Ecol. 14, 475–484.

Copping, L.G., Menn, J.J., 2000. Biopesticides: a review of their action, applications and efficacy. Pest Manag. Sci. 56, 551–576.

de Fatima, A., Kohn, L.K., Antonio, M.A., de Carvalho, J.E., Pilli, R.A., 2005. (R)-Goniothalamin: total synthesis and cytotoxic activity against cancer cell lines. Bioorg. Med. Chem. 13, 2927–2933.

Dorn, A., Rademacher, J.M., Sehn, E., 1986. Effects of azadirachtin on the moulting cycle, endocrine system, and ovaries in last-instar larvae of the milkweed bug, *Oncopeltus fasciatus*. J. Insect Physiol. 32, 231–238.

Finney, D.J., 1971. Probit Analysis. Cambridge University Press, London, UK. p. 383.

Gill, S.S., Cowles, E.A., Pietrantonio, P.V., 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Ann. Rev. Entomol. 37, 615–636.

Glenn, D.C., Hoffman, A.A., Mc Donald, G., 1994. Resistance to pyrethroids in *Helicoverpa armigera* (Lepidoptera: Noctuidae) from corn: adult resistance, larval resistance and fitness effects. J. Econ. Entomol. 87, 1165–1171.

Goettel, M.S., Eilenberg, J., Glare, T.R., 2004. Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert, L.I., Iatrou, K., Gill, S. (Eds.), Comprehensive Molecular Insect Science. Elsevier, pp. 361–406.

Govindachari, T.R., Narasimhan, N.S., Suresh, G., Partho, P.D., Gopalakrishnan, G., 1996. Insect antifeedant and growth-regulating activities of salannin and other C-seco limonoids from neem oil in relation to azadirachtin. J. Chem. Ecol. 22, 1453–1461.

Hertel, W., Muller, P.J., 2006. Physiological effects of the natural products quassin, cinnamaldehyde and azadirachtin on *Periplaneta americana* (L.). J. Appl. Entomol. 130, 323–328.

Hill, D.S. (Ed.), 1983. Agricultural Insect Pests of the Tropics and Their Control. Cambridge University Press, UK.

Kabir, K.E., Khan, A.R., Mosaddik, M.A., 2003. Goniothalamin-a potent mosquito larvicide from *Bryonopsis laciniosa* L. J. Appl. Entomol. 127, 112–115.

Koul, O., Singh, G., Singh, R., Multani, J.S., 2004. Bioefficacy and mode-of-action of aglaroxin-A from *Aglaia elaeagnoidea* (syn. A. roxburghiana) against *Helicoverpa armigera* and *Spodoptera litura*. Entomol. Exp. Appl. 114, 197–204.

Kubo, I., 2006. New concept to search for alternate insect control agents from plants. In: Rai, M., Carpinella, M. (Eds.), Naturally Occurring Bioactive Compounds 3. Elsevier, Amsterdam, pp. 61–80.

Levin, D.A., 1976. The chemical defenses of plants to pathogens and herbivores. Ann. Rev. Ecol. Syst. 7, 121–159.

Metcalf, C.L., Flint, W.P., Metcalf, R.L., 1962. Destructive and Useful Insects their Habits and Control. McGraw-Hill Book Co., New York. p. 1087.

Mordue, A.J., Cottee, P.K., Evans, K.A., 1985. Azadirachtin: its effect on gut motility, growth and moulting in *Locusta*. Physiol. Entomol. 10, 431–443.

Mordue, A.J., Blackwell, A., 1993. Azadirachtin: an update. J. Insect Physiol. 39, 903–924.

Nasiruddin, M., Mordue, A.J., 1993. The effect of azadirachtin on the midgut histology of the locust *Schistocerca gregaria* and *Locusta migratoria*. Tissue Cell 25, 875–884.

Perez, C.J., Alvarado, P., Narvaez, C., Miranda, F., Hernandez, L., Vanegas, H., Hruska, A., Shelton, A.M., 2000. Assessment of insecticide resistance in five insect pests attacking field and vegetable crops in Nicaragua. J. Econ. Entomol. 93, 1779–1787.

Qiu, Y.T., Joop, J.A., Loon, V., Roessingh, P., 1998. Chemoreception of oviposition inhibiting terpenoids in the diamondback moth *Plutella xylostella*. Entomol. Exp. Appl. 87, 143–155.

Regnault-Roger, C., Philogene, B.J.J., Vincent, C., 2005. Biopesticides of Origin. Lavoisier and Intercept Ltd., Paris. p. 313.

Rodriguez-Saona, C.R., Trumble, J.T., 1999. Effect of avocadofurans on larval survival, growth, and food preference of the generalist herbivore, *Spodoptera exigua*. Entomol. Exp. Appl. 90, 131–140.

Sadek, M.M., 2003. Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). J. Appl. Entomol. 127, 396–404.

Schmidt, G.H., Ahmed, A.A.I., Breuer, M., 1997. Effect of *Melia azedarach* extract on larval development and reproduction parameters of *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.) (Lep., Noctuidae). J. Pest Sci. 70, 4–12.

Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann. Rev. Entomol. 35, 271–297.

Senthil-Nathan, S., Kalaivani, S., Murugan, K., 2006a. Behavioural responses and changes in biology of rice leaffolder following treatment with a combination of bacterial toxin and botanical pesticides. Chemosphere 64, 1650–1658.

Senthil-Nathan, S., Kalaivani, K., Chung, K., Murugan, K., 2006b. Effect of neem limonoids on lactate dehydrogenase (LDH) of the rice leaffolder, *Cnaphalocrocis medinalis* (Guenée) (Insecta: Lepidoptera: Pyralidae). Chemosphere 62, 1388–1393.

Senthil-Nathan, S., Choi, M.Y., Paik, C.H., Seo, H.Y., 2007a. Food consumption, utilization, and detoxification enzyme activity of the rice leaffolder larvae after treatment with *Dysoxylum* triterpenes Pest. Biochem. Physiol. 88, 260–267.

Senthil-Nathan, S., Choi, M.Y., Paik, C.H., Seo, H.Y., Kim, J.D., Kang, S.M., 2007b. The toxic effects of neem extract and azadirachtin on the brown planthopper, *Nilaparvata lugens* (Stål) (BPH) (Homoptera: Delphacidae). Chemosphere 67, 80–88.

Senthil-Nathan, S., Kalaivani, K., Chung, P.G., Murugan, K., 2006c. The toxicity and behavioural effects of neem limonoids on *Cnaphalocrocis* medinalis (Guenée) the rice leaffolder. Chemosphere 62, 1381–1387.

Sieber, K.P., Rembold, H., 1983. The effects of azadirachtin on the endocrine control of moulting in *Locusta migratoria*. J. Insect Physiol. 29, 521–523.

Slansky, F., Scriber, J.M., 1985. Food consumption and utilization. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergaman Press, New York, pp. 63–87.

Snedecor, G.W., Cochran, W.G., 1989. Statistical Methods. Iowa State Univ. Press, Ames, IA.

Waldbauer, G.P., 1964. The consumption, digestion and utilization of solanaceous and non-solanaceous plants by larvae of the tobacco hornworm, *Protoparce sexta* (Johan.) (Lepidoptera: Sphingidae). Entomol. Exp. Appl. 7, 253–269.

Waldbauer, G.P., 1968. The consumption and utilization of food by insects. In: Beament, J.W.L., Treherne, J.E., Wigglesworth, V.B. (Eds.), Advances in Insect Physiology. Academic Press, London and New York, pp. 229–288.

Wang, W., Mo, J., Cheng, J., Zhuang, P., Tang, Z., 2006. Selection and characterization of spinosad resistance in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) Pest. Biochem. Physiol. 80, 180–187.

Wheeler, D.A., Isman, M.B., 2001. Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. Entomol. Exp. Appl. 98, 9–16.

Wiart, C., 2007. Goniothalamus species: a source of drugs for the treatment of cancers and bacterial infections. eCAM, pp. 1–13.

Yu, C.G., Mullins, M.A., Warren, G.W., Koziel, M.G., Estruch, J.J., 1997. The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. Appl. Environ. Microbiol. 63, 532–536.

Zhu, G., 1992. Research Progress and Control Technique of Insect Pests and Disease of Major Vegetables Agricultural Publishing House, Beijing, China, pp. 89–112.