Bioefficacy and Mode-of-Action of Aglaroxin B and Aglaroxin C from *Aglaia elaeagnoidea* (syn. *A. roxburghiana*) against *Helicoverpa armigera* and *Spodoptera litura*

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ABSTRACT The bioefficacy of aglaroxin B and aglaroxin C from *Aglaia elaeagnoidea* (syn. *A. roxburghiana*) was assessed using the gram pod borer, *Helicoverpa armigera* (Hübner) and Asian armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). The compounds exhibited strong growth inhibition in diet bioassay, with 0.63 and 0.68 ppm of aglaroxin B and 0.61 and 0.69 ppm of aglaroxin C reducing growth by 50 per cent in *H. armigera* and *S. litura* neonate larvae, respectively, whereas a growth inhibition of 95 per cent was achieved in the range of 2.0 to 2.18 ppm; this was comparable to azadirachtin treatments used as standard. Rocaglamide, a sister compound from the same plant, also used for comparison, was comparatively less active than aglaroxins. Both aglaroxin B and aglaroxin C were toxic to various stadia. Nutritional analysis revealed the antifeedant properties of the compounds; however, nutritional indices indicated that the reduction in growth of the larvae was not entirely due to starvation but partly due to toxic effects of the ingested compounds. This was further confirmed in topical treatments. When relative growth rate was plotted against relative consumption rate, the growth efficiency of larvae fed on diet containing aglaroxin B and C was significantly less than that of control larvae. These results further indicate that aglaroxin B and C act as both antifeedant and chronic toxins. Structure/ activity relationships drawn from the present findings show that methylenedioxy phenyl moiety plays a significant role in the activity of these compounds. However, it is obvious from the present findings that aglaroxins do not absolutely follow the activity pattern of azadirachtin or more related compound rocaglamide known in lepidopterans. Moult inhibition is also a characteristic action of these compounds.

KEY WORDS : Aglaroxin B, aglaroxin C, *Aglaia elaeagnoidea*, Meliaceae, *Helicoverpa armigera*, *Spodoptra litura*, toxicity, antifeedant

INTRODUCTION

Plants provide the source of secondary metabolites possessing biological activities against pests. Plants of Meliaceae and Rutaceae have shown substantial promise in insect control, at least partly owing to the presence of limonoids or triterpenes characteristic of the order Rutales (Champagne *et al*., 1992). *Azadirachta indica* A. Juss. metabolites are well known for their antifeedant and growth inhibitory and growth regulatory activities against various insect pests (Koul, 1992, 1996) and have already been developed and marketed (Koul and Wahab, 2004).

Screening for feeding deterrence and growth inhibitory effects of extracts from other members of the Meliaceae against insect pests has also been

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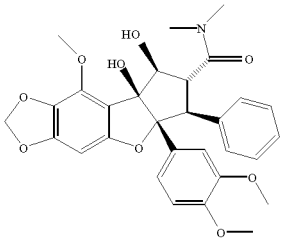
carried out, and in the last decade the genus *Aglaia* has attracted considerable attention as a possible new source for unique natural products. The studies have revealed that extracts from nearly 30 different *Aglaia* species possessed promising bioactivity against cotton leaf worm, *Spodoptera littoralis* (Boisduval), gypsy moth, *Lymantria dispar* (Linnaeus), variegated cutworm, *Peridroma saucia* (Hübner), etc. (Greger *et al*., 2001). From the Meliaceae plant, *Aglaia odorata* Lour., rocaglamide was isolated in 1992 and found to be potent insecticide against variegated cutworm, *P saucia* (Satasook *et al*., 1992; Ishibashi *et al*., 1993; Janprasert *et al*., 1993). Recently several novel rocaglamide derivatives, isolated from different *Aglaia* species have shown to have similar insecticidal activity mostly against neonate larvae of *Spodoptera* and *Ostrinia* species (Ewete *et al*., 1996; Güssregen *et al*. 1997; Nugroho *et al*., 1997a,b, 1999), cytotoxic (Ohse *et al*., 1996; Heebyung *et al*., 1997) or fungicidal properties (Fuzzati *et al*., 1996; Engelmeier *et al*., 2000). These reports clearly emphasize the potential of *Aglaia* as a source for biopesticides and provide the impetus for a systematic investigation of the components against variety of insect pests.

Out of about 130 species of *Aglaia* found in Indo-Malaysia, South China and the Pacific Islands, 23 species are reported to occur in India (Wealth of India, 1985). Our research programme of Meliaceae plants to find anti-insect allelochemicals recently revealed that extracts of *Aglaia elaeagnoidea* (A. Juss.) Benth (syn. *A. roxburghiana*, found in the tropical forest of the hills of South India), has promising bioactivity (Koul *et al*., 1997) with rocaglamide as potential toxic compound against *Helicoverpa armigera* (Hubner) larvae (Koul *et al*., 2004). Aglaroxin A from the same plant was also studied in detail recently (Koul *et al*., 2005) and found more effective than rocaglamides. The present investigation, therefore, was undertaken to study the insecticidal activity of other active compounds, aglaroxin B and aglaroxin C from this plant, to establish their bioefficacy and the physiological mode of action.

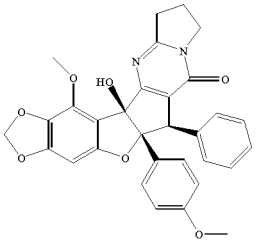
MATERIALS AND METHODS

Chemistry

A. elaeagnoidea (Fuzzati *et al*., 1996) plant material was collected from Southern India. The twigs with bark were shade dried and powdered (4.0 kg). The powder was extracted with methanol (3x5 l), the extract evaporated in vacuum to dryness to give semi-solid extract (250 g). The crude extract was purified on silica gel with dichloromethane + methanol gradient (atmospheric pressure), then with medium



AGLAROXIN-B



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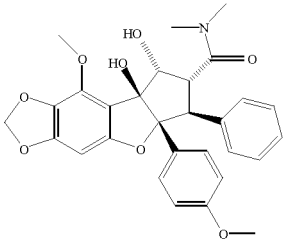

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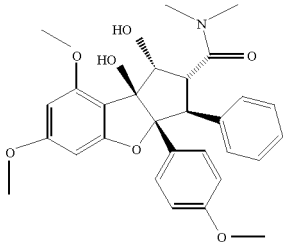

ROCAGLAMIDE

Fig. 1. Aglaroxins and rocaglamide from *A. elaeagnoidea*

pressure column chromatography (ethyl acetate + dichloromethane + methanol) and finally with flash chromatography (hexane + ethyl acetate + methanol). Further separation and confirmation was done by high-pressure liquid chromatography to yield rocaglamide and aglaroxins (Fig. 1) using the known procedures (Janprasert *et al*., 1993; Molleyres *et al*., 1999). Aglaroxins isolated were reconfirmed by HPLC analysis with authentic sample received through the courtesy of Louis-Pierre Molleyres (Novratis Crop Protection AG). For comparison of the activity azadirachtin (> 95% purity), based on fast bombardment mass spectroscopy, was isolated from Indian neem tree (*Azadirachta indica*) seeds by modified Nakanishi method (Schroeder and Nakanishi 1987) and evaluated in a similar fashion as the aglaroxins

Insects

The gram pod borer, *H. armigera* and Asian armyworm, *Spodoptera litura* (Fabricius) were taken from laboratory cultures reared on artificial diet prepared in the laboratory (Koul *et al*., 1997). The cultures were kept at 27 + 1^{O}C at L16:8D photoperiod. Generally neonate, 2nd, 3rd and 5th stage larvae were used in various experiments.

Growth Evaluation

The compounds were dissolved in acetone and individually mixed with the dry portion of the artificial diet at concentrations ranging from 0.1 to 5.0 ppm in acetone. Control diet was treated with carrier alone and the carrier solvent in the treated and control diet was evaporated before the final preparation of the diet.

Following hatching, two 24-h-old neonate larvae were placed on 1g fresh weight diet in an individual solo cup (30 g) as described earlier (Koul *et al.,* 1990). The cups were kept in a plastic tray lined with moisten filter paper to maintain humidity. The experiments were carried out in a growth chamber at 27 + 2^{O}C at L16:8D photoperiod. Larval growth was assessed as a percentage of the controls after 7 days, based on larval weight. Larval mortality, if any, was also recorded. Forty larvae were used for each concentration. The concentration inhibiting 50 per cent growth relative to controls (EC_{50}) was determined by regression analysis. This procedure was also followed for the evaluation of azadirachtin and EC_{50} values were similarly determined. LC_{50} and LC_{95} values (concentrations inducing 50 and 95 % mortality, respectively) were also calculated using probit analysis.

Early second instar larvae (av. weight 8 + 2 mg) were also used to determine effects on growth in artificial diet for this stadium as mentioned above. However, treatment range of various compounds was 1 to 5 ppm.

Fifth instar larvae (0-day-old) of both species were also fed treated diets at 5 and 10 ppm concentrations. The procedure followed for diet preparation and treatment was similar to those used for younger larvae, as mentioned above. However, these larvae were allowed to grow till pupation was completed and all the morphological and developmental abnormalities were recorded. As significant abnormalities were observed in the development and during the moulting process at the time of pupation, the larvae were injected with exogenous doses of 20-hydroxy ecdysone (5 µg/ larva) (Sigma) after 24, 48 and 72 hours of treatment, respectively in order to see if the effect was not due to the depletion of the moulting hormone in these larvae.

Nutritional Analysis

In order to segregate the behavioural effects from toxicity, aglaroxin B and aglaroxin C were subjected to nutritional analysis. The experiments were carried out using both *H. armigera* and *S. litura* early 3rd instar larvae. In these experiments 30 larvae were provided with each compound at a dietary concentration of 1, 3 and 5 ppm. Relative growth rate per unit weight of the insect at the outset of experiment (RGR_i) and relative consumption rate at the outset of experiment (RCR_i) were calculated on dry weight basis after 3 days of feeding. Index of efficiency of conversion of ingested food (ECI) was calculated as described earlier (Koul *et al*., 1997). Dietary utilization experiment was also carried out in similar fashion using azadirachtin at EC_{50} level. In another set of experiments aglaroxin B or aglaroxin C was topically applied at 0.5 and 1.0 µg/larva, and azadirachtin at 0.05 µg/larva, respectively. Larvae were treated on the dorsal surface with a single 0.5

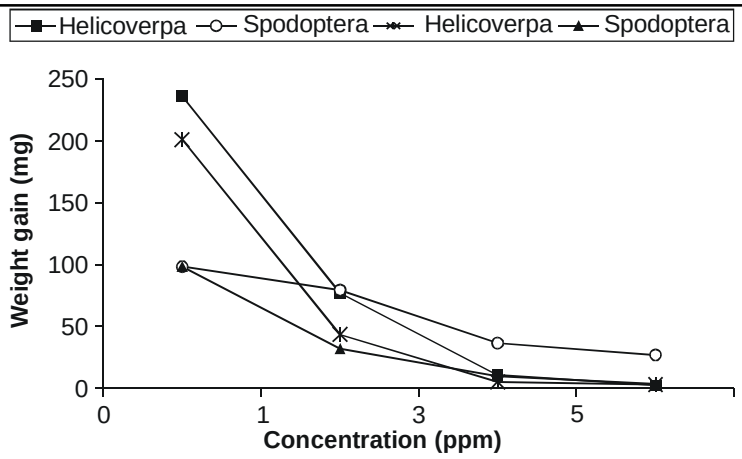

Fig. 2. Effect of aglaroxin B (■–■ ; o–o) and aglaroxin C (∗–∗; ▲–▲) on growth of 2nd instar *H. armigera* and *S. litura* larvae.

µl drop of each compound in acetone using a fine 25 µl syringe (7105 series syringe, Hamilton Co., Reno, Nevada, USA) attached to a repeating dispenser (PB-600, Hamilton Co.). Controls were treated with acetone alone. Care was taken to avoid any contact with the mouthparts of the larvae during topical application. The larvae were then allowed to feed on untreated diet.

Comparison of RGR_is and RCR_is in each case was made using the procedure followed by Wheeler and Isman (2001). This experiment tested the toxic effect over and above any antifeedant effect of aglaroxins. A standard curve, relating RGR_is and RCR_is was constructed by feeding larvae different amounts of food (0, 50, 100, 200 mg and unlimited, n = 20 for each weight). Larvae were weighed and placed on the diet, one per cup. The assay trays were stored as mentioned above. After 3 days, larvae, frass and diet were separated, dried and weighed. For the determination of effect of aglaroxin B and aglaroxin C, diets were incorporated with different amounts of the compounds to get the following concentrations (0.1, 0.5, 1.0, 3.0 and 5.0 ppm, n = 20 in each case). The diet was weighed (ca. 1 g per larva) and the experiment conducted as above. Both experiments were carried out simultaneously at the same time and for same duration. RGR_is and RCR_is were calculated and linear regression analysis performed, correlating control RGR_is and RCR_is and test RGR_is and RCR_is. Difference between regression coefficients was used to test for differences between the two regressions.

Table 1. Effective concentrations (ppm) of aglaroxin B and aglaroxin C inhibiting growth (neonates) of *H. armigera* and *S. litura* in a dietary assay (n = 120). Rocaglamide and azadirachtin used for comparisons.

	H. armigera			*S. litura*		
Compound	EC_{50} (95% CI)	EC_{95} (95% CI)	Slope + SE	EC_{50} (95% CI)	EC_{95} (95% CI)	Slope + SE
Aglaroxin B	0.63 (0.51-0.77)	2.00 (1.23-3.24)	3.44 + 0.77	0.68 (0.50-0.78)	2.11 (1.24-3.48)	3.48 + 0.68
Aglaroxin C	0.61 (0.48-0.72)	2.05 (1.27-3.42)	3.46 + 0.68	0.69 (0.50-0.76)	2.18 (1.36-3.56)	3.51 + 0.73
Rocaglamide	0.88 (0.78-1.08)	3.8 (2.32- 4.88)	2.47 + 0.43	0.87 (0.66-1.10)	3.62 (2.35-4.90)	2.51 + 0.51
Azadirachtin	0.25 (0.19-0.32)	1.35 (0.44-4.26)	2.95 + 0.45	0.24 (0.16-0.35)	1.38 (0.46-3.95)	2.28 + 0.68

Table 2. Toxicity (ppm) of aglaroxin B and aglaroxin C to neonates of *H. armigera* and *S. litura* in a dietary assay (n = 120). Rocaglamide and azadirachtin used for comparisons.

	H. armigera			*S. litura*		
Compound	LC_{50} (95% CI)	LC_{95} (95% CI)	Slope + SE	LC_{50} (95% CI)	LC_{95} (95% CI)	Slope + SE
Aglaroxin B	1.30 (1.21-1.58)	3.56 (2.83-4.27)	3.87 + 0.58	1.38 (1.27-1.81)	3.68 (2.15-4.64)	3.97 + 0.54
Aglaroxin C	1.33 (1.24-1.60)	3.54 (2.78-4.30)	3.81 + 0.60	1.40 (1.19-2.00)	3.66 (2.08-4.73)	3.98 + 0.59
Rocaglamide	1.65 (1.41-1.82)	4.02 (2.89-4.34)	4.12 + 0.66	1.70 (1.48-1.95)	4.08 (2.88-4.47)	4.11 + 0.70
Azadirachtin	1.23 (1.08-1.54)	3.05 (2.54-3.66)	4.02 + 0.55	1.26 (1.10-1.57)	3.08 (2.32-3.84)	4.14 + 0.53

Table 3. Feeding, growth and efficiency of conversion of ingested food by 3rd instar *H. armigera* and *S. litura* larvae (n = 30 in each treatment) after dietary feeding of aglaroxin B and aglaroxin C. Azadirachtin was used as a standard.

Compound	Dose (ppm)	Nutritional index (mean ± SE)* RGRi (mg/mg/d)	RCRi (mg/mg/d)	ECI (%)
H. armigera				
Aglaroxin B	1	1.41 + 0.05b	2.87 + 0.5b	49.1 + 4.8a
	3	0.79 + 0.03c	1.98 + 0.1c	40.3 + 5.3b
	5	0.35 + 0.02d	1.05 + 0.1d	33.5 + 2.5c
Aglaroxin C	1	1.37 + 0.04b	2.90 + 0.6b	47.3 + 5.0ab
	3	0.80 + 0.02c	1.95 + 0.3c	41.0 + 5.4b
	5	0.29 + 0.02d	1.00 + 0.1d	29.0 + 2.7c
Azadirachtin	0.4	1.42 + 0.06b	2.73 + 0.3b	52.1 + 2.7a
Control	–	1.85 + 0.07a	3.45 + 0.8a	51.0 + 5.3a
S. litura				
Aglaroxin B	1	1.48 + 0.09b	2.87 + 0.5b	51.4 + 6.8a
	3	0.88 + 0.07c	2.20 + 0.4c	40.2 + 4.8b
	5	0.43 + 0.04d	1.84 + 0.4d	26.9 + 2.7c
Aglaroxin C	1	1.50 + 0.09b	2.91 + 0.7b	51.5 + 7.2a
	3	0.91 + 0.06c	2.17 + 0.3c	40.9 + 6.6b
	5	0.44 + 0.02d	1.76 + 0.3d	25.0 + 3.4c
Azadirachtin	0.4	1.43 + 0.04b	2.86 + 0.3b	50.9 + 5.5a
Control	–	2.69 + 0.3a	5.00 + 0.7a	53.8 + 4.8a

*Means within a column followed by the same letter are not significantly different ($P > 0.05$) based on Tukey's test.

Table 4. Feeding, growth and efficiency of conversion of ingested food by 3rd instar *H. armigera* and *S. litura* larvae (n = 30 in each treatment) after topical application of aglaroxin B and aglaroxin C. Azadirachtin was used as a standard

Compound	Dose (ppm)	Nutritional index (mean ± SE)* RGRi (mg/mg/d)	RCRi (mg/mg/d)	ECI (%)
H. armigera				
Aglaroxin B	0.5	1.48 + 0.13b	3.44 + 0.6ab	42.9 + 3.3ab
	1.0	1.04 + 0.03c	3.14 + 0.6b	33.2 + 2.4b
Aglaroxin C	0.5	1.51 + 0.11b	3.36 + 0.7ab	44.0 + 3.8ab
	1.0	1.00 + 0.04c	3.11 + 0.7b	32.3 + 1.8b
Azadirachtin	0.05	1.44 + 0.06b	3.75 + 0.3a	38.4 + 2.8b
Control	-	1.76 + 0.07a	3.48 + 0.8a	50.8 + 5.3a
S. litura				
Aglaroxin B	0.5	2.17 + 0.3b	5.06 + 0.7a	42.6 + 6.7b
	1.0	1.52 + 0.1c	4.72 + 0.9b	32.2 + 3.4c
Aglaroxin C	0.5	2.21 + 0.4b	5.00 + 0.8a	44.2 + 7.1b
	1.0	1.48 + 0.1c	4.82 + 0.7b	30.9 + 2.8c
Azadirachtin	0.05	1.36 + 0.04c	5.11 + 0.3a	26.6 + 5.8c
Control	-	2.59 + 0.3a	5.13 + 0.7a	51.0 + 4.9a

*Means within a column followed by the same letter are not significantly different (P > 0.05) based on Tukey's test.

Results

The addition of aglaroxin B and aglaroxin C to the artificial diet retarded growth of neonate larvae of *H. armigera* and *S. litura* in a dose dependent manner, with EC_{50} values of 0.63 and 0.68 ppm (aglaroxin B) and 0.61 and 0.69 ppm (aglaroxin C), respectively (Table 1). These values compared favourably with azadirachtin (EC_{50} = 0.24 and 0.25 ppm) and rocaglamide (EC_{50} = 0.88 and 0.87 ppm). However, azadirachtin was substantially more potent and rocaglamide less effective than the candidate compounds in inducing growth inhibition.

Aglaroxin B was found to have LC_{50} and LC_{95} values of 1.30 and 3.54 ppm against neonate larvae of *H. armigera* and 1.38 and 3.68 ppm against *S. litura* larvae after 7 days of treatment (Table 2). This was quite similar to azadirachtin treatment for similar duration (Table 2). Rocaglamide was again less effective in comparison to other compounds evaluated. Treatment of the second instar larvae also showed a similar pattern of efficacy (Fig. 2). The effect on the growth by aglaroxin B and aglaroxin C was almost similar for both species (Fig. 2).

Nutritional analysis revealed that incorporation of aglaroxin B and aglaroxin C into artificial diet reduced RGR_i and RCR_i with a significant change in the ECI values. Reduction in growth was not only correlated with dietary concentrations ($P<0.05$). When the compounds were applied topically to the 3rd instar larvae, significant effects were also seen in the growth and ECI parameters, though the consumption was not reduced (Tables 3 and 4).

Another approach used to confirm this was comparison of RGR_i and RCR_i values, with one set of insects being fed varying amounts of control diet (0 to excess) and one set of treated diets (0.1 to 5 ppm) (Wheeler and Isman, 2001). Plotting relative growth rates against consumption rates was used to determine the differentiation of deterrent and toxic effects of the compounds. Two lines were generated for each, one calibration curve, where a range of RCR_is were generated and correlated to RGR_is and one test line, where larvae were fed diets containing a range of compound concentrations. RGR_i and RCR_i for each set of larvae were subjected to linear regression analysis (Figs. 3 and 4). The slope (regression coefficient) of the regression line represents the growth efficiency of the larvae. The two regression coefficients were compared by calculating the vari-

ance of the difference between the two estimates of the regression coefficients (Searle, 1977). This test showed that the growth efficiency of the *H. armigera* fed on treated diet was significantly less than that of the control larvae ($P < 0.01$), with the insects fed in two different cohorts growing differently for a given RCR_i. This again indicates that the reduced growth of these larvae under the influence of aglaroxin A is not entirely due to starvation; some of the growth reduction is due to toxic effect of the ingested compound.

Treatment to 5th instar *H. armigera* larvae revealed very interesting results as there was substantial effect on the morphology of the larvae, moulting process and ecdysis. The larval period was extended and pupal weight was reduced by 43 per cent in comparison to the controls. In higher treatments of 10 ppm aglaroxin B or aglaroxin C in diet, all the pupae formed were abnormal and subsequently 100 per cent mortality was induced in larval and prepupal stages. At 5 ppm level also 80 per cent mortality was recorded with the development of partially pupated larvae in *H. armigera*. As the foregoing effects were clearly noticed during the moulting process, 20 hydroxyecdysone was injected to record recovery, if any. The larvae receiving 20-hydroxyecdysone did not, however, recover from the induced effects of aglaroxin A. Similar results were obtained in *S. litura* treatments.

DISCUSSION

Naturally occurring allelochemicals from Meliaceae are well known to possess insecticidal, antifeedant or growth inhibitory activity against insects (Koul and Dhaliwal, 2001). Most detailed studies of the effects of a natural product on insect behaviour and physiology have been those carried out on the limonoid, azadirachtin, from the neem tree, *A. indica*. However, in recent past a lot of emphasis has been on the isolation and characterization of the rocaglamide type of compounds and different furan analogues from *Aglaia* species, a genus of potential importance after *Azadirachta* or *Melia* species in the family Meliaceae. Our studies against *H. armigera* and *S. litura* treated with aglaroxin B and aglaroxin C from *A. elaeagnoidea* showed that larvae gained less weight when fed on treated diets. The activity was concentration dependent. Interestingly, the activity with a much higher dosage was comparable with azadirachtin, the most active compound in *A. indica*. Though activity was related to low consumption but at the same time mortality induced in different stadia did not correspond strictly to starvation. The efficacy of these compounds, therefore, was exactly similar to the ac-

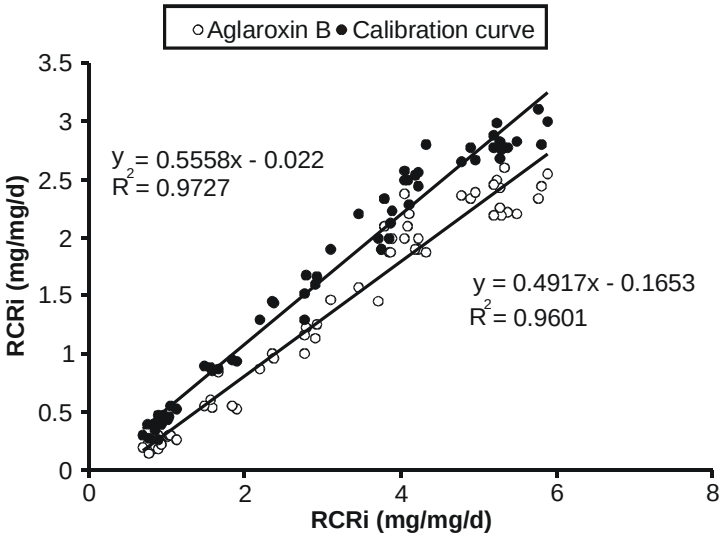

Fig. 3. Relationship between relative consumption rates and relative growth rates of *H. armigera* larvae fed on different quantities of artificial diet (calibration curve), and larvae fed on a diet containing varying amounts of aglaroxin B.

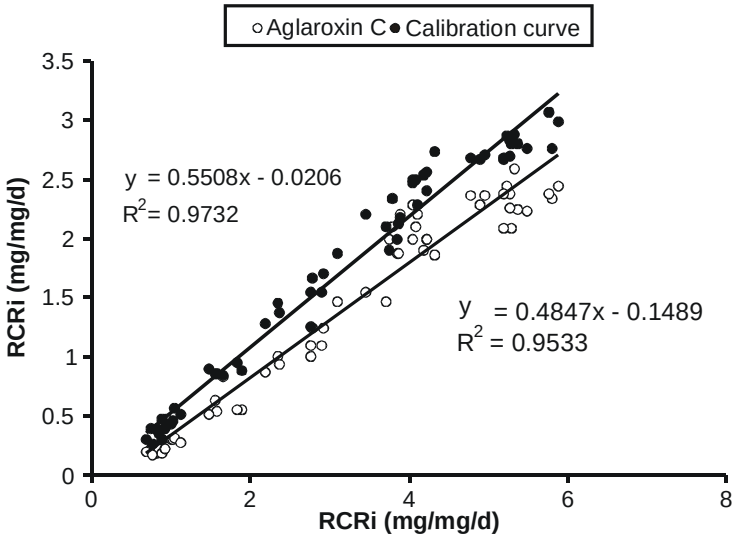

Fig. 4. Relationship between relative consumption rates and relative growth rates of *H. armigera* larvae fed on different quantities of artificial diet (calibration curve), and larvae fed on a diet containing varying amounts of aglaroxin C.

tivity of aglaroxin A against same insect species reported in our recent studies (Koul *et al*., 2005).

Experiments were carried out to investigate whether the efficacy was purely a feeding deterrence or toxicity mediated physiological inhibition. Using nutritional indices, it was established that there was a fall in growth rate concomitant with the reduction in consumption and accounted partially for the decrease in growth rate as there was reduction in ECI values. ECI is an overall measure of an insect's ability to utilize the food that it ingests for growth. Therefore, change in ECI values indicate that ingested allelochemicals do exhibit chronic toxicity and is not merely an antifeedant affect. This gets further support from topical treatment experiments where values for both growth and ECI parameters showed a reduced trend, though consumption was not affected. Therefore, it is obvious that both aglaroxins act as a physiological toxin against insects in addition to having antifeedant action as was observed for aglaroxin A earlier (Koul *et al.*, 2005).

Comparison of RGR_is and RCR_is using variance of the difference between the two estimates of the regression coefficients (Figs. 3 and 4) also showed that reduced growth of these larvae under the influence of aglaroxin B and aglaroxin C is not entirely due to starvation; some of the growth reduction is due to toxic effect of the ingested compound. Previous reports, for instance on citrus limonoids, demonstrate that limonoids do act as antifeedants and not toxins against *Spodoptera frugiperda* (J.E. Smith) larvae (Mendel *et al*., 1993) or *Leptinotarsa decemlineata* (Say) beetles (Liu *et al*., 1990). This, however, cannot be a generalization as same limonoids could be post-ingestive toxins for other insect species (Mendel *et al*., 1991; Chen *et al*., 1995). Thus present study demonstrates that aglaroxins act as antifeedants as well as post-ingestive toxins, and could have different primary modes-of-action depending on the test insect species by exhibiting both antifeedant and toxic modes of action (Koul *et al.*, 2002). However, there is a possibility, as is obvious from the present study, that antifeedant effect could be an outcome of the chronic toxicity induced by these allelochemicals. At this stage it is difficult to give a generalized conclusion, as no other detailed study of mode-of-action of aglaroxin series of compounds is known so far and the nutritional indices do not give any indication that two activities of aglaroxins, are independent of each other. Azadirachtin is a potential example for this as its oral administration reduces RGR and RCR, but not ECI and efficiency of conversion of digested food (ECD), i.e. antifeedant activity. Topical application of azadirachtin, on the other hand, results in reduction of RGR, ECI and ECD, but not RCR, indicating

toxicity, where energy is diverted from biomass production to detoxification, i.e. increase in costs (Koul and Isman, 1991; Koul *et al*., 1996).

However, the activity of aglaroxin B and aglaroxin C has exact similarity with a related compound aglaroxin A (Koul *et al*., 2005) and some similarity with rocaglamide from the same plant species, which acts as cytotoxic agent at non-specific cellular levels (Koul *et al*., 2004). Rocaglamide derivatives do act as protein synthesis inhibitors (Ohse *et al*., 1996) and induce cellular death (Heebyung *et al*., 1997). Some derivatives of rocaglamide have also been demonstrated to act as antiplatelet aggregation principles (Wu *et al*., 1997). Obviously, from biological activity point of view, rocaglamide seems to be different than the aglaroxin type of compounds. This difference could be explained on the basis of structural activity relationship. The major difference is the presence of a methylenedioxyphenyl moiety in aglaroxins A, B and C in addition to the cyclopentatetrahydrobenzofuran ring that is present in both aglaroxins as well as the rocaglamide. Cyclopentatetrahydrobenzofuran ring has been reported as responsible for insecticidal activity (Molleyres *et al*., 1999) and obviously making both aglaroxins and rocaglamide biologically active against insects. The presence of methylenedioxyphenyl moiety (known to inhibit mixed function oxidases) in aglaroxins A, B and C, however, makes them more potent in terms of chronic toxicity and as moult inhibitors at least against *H. armigera* and *S. litura*. This explanation, however, does not hold true for aglaroxins D, G, H and I, which do possess the methylenedioxyphenyl moiety but are less active (Molleyres *et al*., 1999) in comparison to aglaroxin A, B or C. The reduced activity in these compounds could be due to the presence of planar pyrimidinone ring, which display a completely different steric configuration.

If dietary utilization data is compared, the cytotoxicity of aglaroxin B and aglaroxin C differs from rocaglamide in reducing the growth and consumption significantly in both oral and topical applications. On the contrary, rocaglamide reduces growth rate by about 50 per cent without having very significant effect on the relative consumption rate in dietary treatments (7 to 16 % reduction only) (Koul *et al*., 2004). This shows that the consumption rate of larvae was not reduced concomitant with a reduction in relative growth rate. Obviously these results do not implicate any absolute antifeedant effect but the toxicity effect due to reduced ECI. This also shows that feeding deterrence is not primary mode of action of rocaglamide but whatever, little reduction in consumption is a centrally mediated toxic anorexic effect, which could be due to the induced cytotoxicity. There is already the evidence for cytotoxic nature of rocaglamide as it does have the antileukemic activity against P388 lymphocytic leukemia (King *et al*., 1982) cells. However, in view of the above findings and comparative analysis of data with that of agalroxin B and aglaroxin C, it may be concluded that these compounds act as both antifeedant and chronic toxin and does not seem to absolutely follow the pattern of activity of rocaglamide.

Interesting observation in the present study was the effect of aglaroxin B and aglaroxin C on 5th instar larvae of *H. armigera* and *S. litura* where abnormal pupation occurred or larvae died prematurely in half cast moults. Dominant ecdysteroid in moulting process is 20-hydroxy ecdysone that exists in double peak prior to pupation in lepidopterous larvae and circulates as free hormone unit that interacts with target tissues. We presumed that somehow these aglaroxins may be depleting this free ecdysteroid in the circulatory system and, therefore, the subsequent abnormal pupation and ecdysis. Thus exogenous 20-hydroxyecdysone was injected into the treated insects every 24 hours to record any possible recovery. However, the effect was not reversible. This shows that effect is not mediated through the inactivation of moulting hormone, an observation made in our earlier study with agalroxin A as well (Koul *et al*., 2005). Such morphological abnormalities induced seem to correlate with azadirachtin treatments. When feeding does occur in antifeedant trials with azadirachtin, significant insect mortality is often recorded, particularly in no-choice situations, due to the predominance of secondary toxic effects of azadirachtin. Various characteristic effects like abnormal larval ecdysis, incomplete sclerotization and pigmentation of new cuticle, incomplete metamorphosis, etc. are common effects of azadirachtin. In fact,

it has been proved in various studies that the insect growth inhibitory and antifeedant effects of azadirachtin are independent of each other against this insect species as well as in other lepidopteran species (Koul and Isman, 1991; Koul *et al*., 1996). Aglaroxin B and C treated 5th instar larvae, therefore, do show similar effects as that of azadirachtin induced morphological aberrations, but do not seem to follow the pattern of azadirachtin action as there is significant variation in food utilization pattern under treatment conditions, as demonstrated in our previous studies with aglaroxin A (Koul *et al*., 2005).

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