Indian Journal of Experimental Biology
Vol. 41, December 2003, pp. 1463-1465

Toxicity assessment of wild bean seed protein - Arcelin on Asian armyworm, *Spodoptera litura* (Fabricius)

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Received 25 April 2003; revised 1 August 2003

Arcelin, an anti-metabolic protein was purified from the seeds of wild bean, *Lablab purpureus*. The feeding assay containing arcelin at 5, 10 and 15 μg concentrations revealed no antifeedant effect against fifth instar larvae of *S. litura*. However, the enhanced activity of α- and β-naphthyl esterases in the mid-gut samples of *S. litura* treated with arcelin suggests countermeasure against the toxic effect of arcelin.

Keywords: Bean seed protein – Arcelin, *Spodoptera litura*, Toxicity

The use of plant derived compounds and transgenic crop plants possessing characteristics such as antixenosis and antibiosis is in the increasing trend in Integrated Pest Management programmes. Incorporation and exploitation of natural resistance, mainly of secondary compounds in wild varieties of crop plants is a non-chemical method and is one of the most acceptable components of pest control strategies. Arcelin, the seed proteins of common bean, *Phaseolus vulgaris* are very effective in imparting resistance to stored product pests[1].

The polyphagous pest, *Spodoptera litura* is a serious pest of many crops[2]. Various synthetic insecticides are available to control this pest, but often, they are environmentally offensive and also ineffective due to the development of resistance by the pest. Current literature supports the general hypothesis that plant secondary compounds have the potential to protect plants against herbivorous insects[3]. In this communication purification of arcelin from wild bean, *Lablab purpureus* and antimetabolic or insecticidal property of arcelin against *S. litura* are reported.

Seeds of wild *Lablab purpureus* were collected from Palney Hills, Western Ghats (Latitude 10°, Longitude 78°, and Altitude 2010m from MSL), South India. The dried seeds were decorticated and seed flour was prepared. The flour was extracted with 30 ml of 25 m*M* sodium acetate buffer, *p*H 4.6 at 4°C for 12 hr. The extract was centrifuged at 9000 *g* for 30 min at 4°C and the supernatant collected. The pre-swollen CM-cellulose was packed in a glass column (1.5 mm width and 25 cm length) and calibrated with sodium acetate buffer, *p*H 4.6. The supernatant was then loaded on this column and the unbound material was eluted using sodium acetate buffer, *p*H 4.6. Later, it was washed with the same acetate buffer. The bound material was eluted with a sodium chloride gradient (0.00 to 0.5 *M*) in sodium acetate buffer, *p*H 4.6 (ref.4). Column fractions were analyzed by non-denatured and denatured gel electrophoresis[5]. Arcelin eluting as a single fraction was pooled, dialyzed against distilled water and freeze-dried.

Sets of four experimental larvae (first day-fifth instar) were provided with a known weight of untreated (distilled water) and arcelin treated (5,10 and15 μg of arcelin) fresh castor leaves. After arcelin application, the leaves were air dried under moist conditions. Larvae were allowed to feed for 24 hr *ad libitum*. After feeding, the antifeedant index was calculated as per Blaney *et al*[6]. The larvae were then sacrificed for collection of mid-gut samples (for esterase analysis). The method of Van Asperen[7] was followed for quantitative analysis of esterases. The method followed for the identification of isozymes of esterases was Georghiou and Pasteur[8].

Arcelin obtained consisted of polypeptides of 36,000 to 40,000 molecular weight (Fig. 1). The molecular weight of native form of arcelin was estimated as 1,14,000 (Fig. 1). The antifeedant activity of arcelin against the fifth instar larvae of

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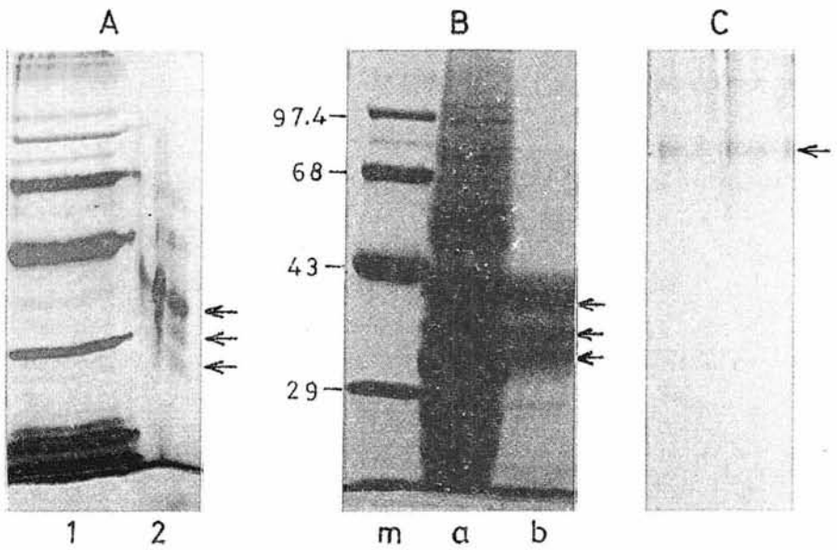

Fig. 1 — A: Lane1-Protein molecular weight markers; Lane 2-Purified arcelin fraction showing polypeptides stained with silver nitrate. B: Lane M-Protein molecular weight markers; Lane a-SDS-PAGE (10%) analysis of total soluble wild seed proteins of *L. purpureus*; Lane b-Purified arcelin showing various polypeptides (indicated by arrowheads) of 40,000 to 36,000 molecular weight. C: Native PAGE (12%) analysis showing arcelin protein at MW 1,14,000

S. litura indicated insignificant effect on their feeding at concentrations of 5, 10 and 15 μg which recorded ineffective antifeedant indices of 3.32, 6.08 and 8.28 respectively (Table 1). Detoxification of arcelin in terms of esterase activity was also assessed. The results recorded significant increase in the activity of α- and β-napthyl esterases in all the treatments when compared to untreated larvae (Table 2). The esterase isozyme electrophoretic patterns of mid-gut samples revealed higher staining intensities of isozyme fractions in treated larvae (Fig. 2).

Polyphagous insects are likely to adapt more readily and become resistant to allelochemicals than insects having a restricted host range. The expression of insect control proteins of multiple gene inheritance in crop varieties from wild plants would be an essential component for insect pest management programmes[9]. Evaluation of antifeedant activity of arcelin on *S.litura* revealed insignificant antifeedant property at all the concentrations tested, though this protein is responsible for imparting resistance against several bruchids[10]. Paes *et al*[3]. have also reported that such anti-metabolic effects on gut enzymes limited the digestive physiology of herbivorous insects. Polyphagous insects with a polysubstrate carboxylenase system can detoxify many compounds, particularly secondary plant products[11]. Ingestion of toxic proteins induce isozymes of carboxylesterases in insects[12]. In this investigation, the larvae treated with higher concentrations of arcelin (10 μg) recorded enhanced activity of esterases and this

Table 1 — Antifeedant activity of arcelin against fifth instar larvae of *S. litura*

[Values are mean ± SD of 5 replicates]

Treatment of arcelin (μg)	Food consumption (mg/individual/day)	Antifeedant index
Control	2118.50 ±106.7	—
5	1982.16 ± 92.5	3.32
10	1875.70 ± 86.8	6.08
15	1784.50 ± 74.9	8.28

Table 2 — *In vitro* assay of α- and β-napthyl esterases from the mid-gut samples of the fifth instar larvae of *S. litura*

[Values are mean ± 5 replicates]

Treatment duration (hr)	Doses of arcelin (μg)	α-NA esterase activity (nmol/min/mg protein)	β-NA esterase activity (nmol/min/mg protein)
4	C	795.00 ± 3.16	4594.00 ± 4.08
	5	1194.50 ± 4.65	5093.75 ± 4.99*
	10	1792.00 ± 2.16*	6495.25 ± 2.22*
	15	1993.75 ± 2.75*	8592.50 ± 2.65*
12	C	794.6 ± 2.61	4595.25 ± 2.75
	5	1794.50 ± 4.65*	6495.50 ± 2.65*
	10	2291.50± 1.29*	7596.00 ± 2.94*
	15	2493.75 ± 2.5*	9495.75 ± 2.63*
24	C	792.75 ± 2.22	4595.25 ± 2.75
	5	1793.25 ± 5.56*	6595.50 ± 3.11*
	10	2494.00 ± 5.23*	8395.25 ± 2.75*
	15	2693.25 ± 2.99*	10196.00 ± 3.61*

C = Control; *$P < 0.05$ [Data analysed using Student's *t* test]

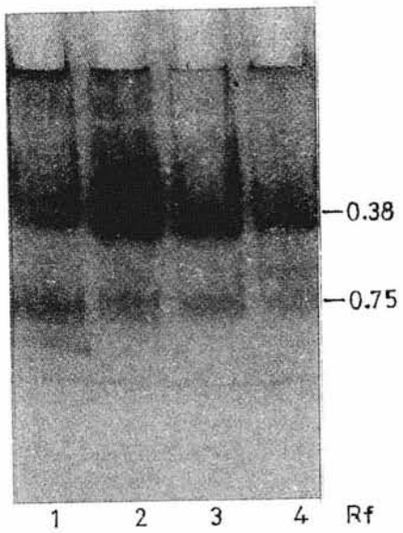

Fig. 2—Electrophorogram showing esterase pattern of the mid-guts of the larvae treated with various doses of arcelin (lane 1 = control; lanes 2-4 = arcelin treatment; 2: 15 μg; 3: 10 μg and 4: 5 μg)

suggests that the detoxification of ingested arcelin in *S. litura*. This bioassay confirmed that arcelin effectively induced higher rate of esterase activity by exhibiting its toxic nature to *S. litura* system.

S J thanks Department of Science and Technology for financial assistance.

References

1 Osborn T C, Alexander D C, Sun S S M, Cardona C & Bliss FA, Insecticidal activity and lectin homology of arcelin seed protein, *Science*, 240 (1988) 207.

2 Sajap A S, A preliminary observation on microsporidian infection in the armyworm, *Spodoptera litura*, *Malay Appl Bio*, 24 (1996) 47.

3 Paes N S, Gerhardt I R, Coutinho M V, Yokoyama M, Santana E, Harris N, Chrispeels M J & Fatima Grossi de Sa M, The effect of arcelin-1 on the structure of the midgut of bruchid larvae and immunolocalisation of arcelin protein, *J. Insect Physiol*, 46 (2000) 393.

4 Minney B H P, Gatehouse A M R, Dobie P, Dendy J, Cardona C & Gatehouse J A, Biochemical basis of seed resistance to *Zabrotes subfasciatus* (bean weevil) in *Phaseolus vulgaris* (common bean): a mechanism of arcelin toxicity, *J Insect Physiol*, 36 (1990) 757.

5 Laemmli U K, Cleavage of structural proteins during assembly of the head of bacteriophage T4, *Nature*, 227 (1970) 680.

6 Blaney W M, Simmonds M S J, Ley S V, Anderson J C, Smith S C & Wood A, Effect of azadirachtin derived Decalin (perhydronapthalene) and Dihydrofuracetal (furo (2,3,6) pyran) fragments on the feeding behaviour of *Spodoptera litura*, *Pestic Sci*, 40 (1994)169.

7 Van Asperen K J, A study of housefly esterases by means of a sensitive colorimetric method, *J Insect Physiol*, 8 (1962) 401.

8 Georghiou G P, & Pasteur N, Electrophoretic esterase patterns in insecticide resistant and susceptible mosquitoes, *J Econ Entomol*, 71 (1978) 201.

9 Hilder V A, Gatehouse A M R, Sheerman S E, Barker R F & Boulter D, A novel mechanism of insect resistance engineered into tobacco, *Nature*, 300 (1987) 160.

10 Hartweck L M, Cardona C & Osborn T C, Bruchid resistance of common bean lines having an altered seed protein composition, *Theor Appl Genet*, 95 (1997) 1018.

11 Salama M S, Schouest L P & Miller T A, Effect of diet on the esterase patterns in the haemolymph of the corn earworm and the tobacco budworm (Lepidoptera: Noctuidae), *J Econ Entomol*, 85 (1992) 1079.

12 Shiotsuki T, & Kato Y, Induction of carboxylesterase isozymes in *Bombyx mori* by *E. coli* infection, *Insect Biochem Mol Biol*, 29 (1999) 731.