

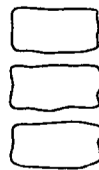
Strips stained with AgNO ₃	Corresponding strips for bioassay		
	Shoot length	Section number	Root length
	+ 24.41%	7	+ 32.62%
	+ 13.05%	6	+ 38.49%
	+ 18.57%	5	+ 39.93%
	- 25.21%	4	- 29.77%
	+ 17.53%	3	+ 17.67%
	+ 33.67%	2	+ 24.79%
	+ 46.76%	1	+ 28.41%
×			×

Figure 2. Inhibitory and stimulatory influences of different sections of the chromatogram developed with 0.5 ml of *Sterculia foetida* pulp extract. Solvent— butanol : acetic acid : water :: 4 : 1 : 2.2. Width of the sections 1 and 2 is 2.3 cm and the remaining sections is 2 cm. (Mean of 25 seeds recorded in mm.)

blank run in identical conditions). Three such experiments were performed. Shoot and root length were measured after 4 days.

For bioassay, two sets of measurements were performed, the treated set with aqueous pulp extract of *S. foetida* and the control with distilled water. Length of shoot and root in the two sets was measured after 4 days. Table 1 shows inhibition in shoot and root length in a treated set.

Paper chromatography showed three spots (one brown, one violet and one black or slate colour) which were visible after staining with AgNO₃ reagent.

A. erioloba reveals 6 phenolic spots¹ but the upper three are missing in *S. foetida*.

Bioassay on different sections of preparative chromatogram shows inhibition of both shoot and root length only at section 4 (Figure 2). Stimulation of both shoot and root length was observed in sections 1–3 and 5–7. Three phenolic spots were observed in sections 2–4.

Thus, two stimulators and one inhibitor were revealed. The inhibitory effect on the shoot length is more pronounced than on the root length.

Thus the situation is very close to that

of *A. erioloba*, a zoochoric fruit¹. This is not what should be theoretically expected. But in the case of *S. foetida* the pulp of the fruit was not fully mature. Perhaps in view of the thick wooden rind, water is not easily accessible to the seeds. Thus we have to address an interesting question— whether the stimulators and inhibitors have evolved without any zoochoric selection pressure or, in other words, whether these are metabolic by-products of the basic physiology and biochemistry of the plants? Generation of a large set of data on the chemical aspects of zoochoric and non-zoochoric fruits including the presence of laxatives in pulp² is likely to help us to address the question.

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ACKNOWLEDGEMENT. We thank Mr N. Banik of Embryology Unit, ISI for valuable assistance in the laboratory.

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Synergistic effect of Cry1Ac and Cry1F δ -endotoxins of *Bacillus thuringiensis* on cotton bollworm, *Helicoverpa armigera*

Insecticidal δ -endotoxins of *Bacillus thuringiensis* (Bt) have acquired great significance in recent years because of their specificity to target insects, toxicity at very low concentration and environment friendly nature¹. Genes coding for Bt δ -endotoxins have been deployed in a wide range of transgenic crop plants with considerable success². One of the major

concerns in field level application of Bt transgenic plants is development of resistance in insects towards δ -endotoxins upon continuous selection pressure³. Various strategies have been suggested to prevent or delay the resistance development among which gene pyramiding/mixture is an important measure⁴. A combination of Bt genes coding for δ -endo-

toxins which differ in their mode of action, receptor binding and sequence homology needs to be worked out in relation to each target insect. Recently we have reported the toxicity of eleven lepidoptera specific δ -endotoxins of Bt towards *Helicoverpa armigera*, an important polyphagous pest on cotton, chickpea, pigeonpea, tomato, sunflower,

Table 1. Toxicity of different combinations of CryIAC/CryIF toxin mixtures to *H. armigera*

Ratio (CryIAC : CryIF)	Observed EC ₅₀ ^a	Slope ± SE	Expected EC ₅₀ ^{**}	Expected/observed
1 : 0	8.0	0.86 ± 0.01	—	—
0 : 1	870.0	0.49 ± 0.03	—	—
2 : 1	0.5	1.15 ± 0.04	11.9	23.8
1 : 1	0.6	0.89 ± 0.02	15.8	26.3
1 : 2	1.9	1.15 ± 0.04	23.6	12.3

EC₅₀^a: Concentration of Cry toxins causing 50% larval growth reduction.

EC₅₀^{**}: Calculated by the formula used by Tabashnik⁴.

sorghum, etc.⁵. Here we report synergism between two Bt δ-endotoxins in relation to their toxicity to *H. armigera*.

The genes (*cryIAC*, *cryIAB*, *cryIF* and *cry2Aa*) coding for Bt δ-endotoxins were overexpressed in *E. coli* strain JM103 using the expression vectors (from Donald Dean, Ohio State University, USA). The protoxins were purified and solubilized as described by Lee *et al.*⁶. The solubilized protoxin was digested with trypsin in a trypsin : protoxin ratio of 1 : 25 (by mass) for 2 h at 37°C. Activated toxins were dialysed against 50 mM sodium carbonate buffer, pH 9.5. The purity of the protoxin and activated toxin was examined on 10% SDS-PAGE⁷. The toxins at different concentrations were spread on semi-artificial diet in 24-well Costar plates and one larva (1 instar) per well was released⁵. Larval mortality and growth inhibition were recorded after 6 days. The data were subjected to probit analysis⁸. Synergism between the toxins was calculated according to the equation of Tabashnik⁹.

$$LD_{50(m)} = \left[\frac{r_a}{LD_{50(a)}} + \frac{r_b}{LD_{50(b)}} \right]^{-1}$$

where LD_{50(m)} represents the expected LD₅₀ of the mixture, LD_{50(a)} and LD_{50(b)} represent the LD₅₀ for toxin a and b respectively, and r_a and r_b represent the

relative proportion of toxins a and b in the mixture respectively.

We have previously observed that four toxins, viz. CryIAC, CryIAa, CryIAB and Cry2Aa were highly effective against *H. armigera* larvae and toxins such as CryIF caused only growth inhibition⁵. Three combinations of toxins (CryIAC + CryIAB, CryIAC + Cry2Aa and CryIAC + CryIF) were tested for their efficacy in the present study. No significant alteration in the toxicity was observed when the combinations of CryIAC + CryIAB and CryIAC + Cry2Aa were used (data not shown). On the other hand, CryIAC and CryIF exhibited an interesting interactive effect (Table 1). CryIAC toxin is about 100 times more toxic than CryIF toxin towards *H. armigera*. Mixture of CryIAC and CryIF toxin (1 : 1) showed a synergistic effect in that the EC₅₀ of CryIAC toxin was lowered 13 times due to the presence of CryIF. The observed toxicity of the CryIAC + CryIF mixture was about 26 times higher than the expected toxicity, strongly suggesting a synergism. Although we have no definitive explanation for the synergistic effect of the CryIAC and CryIF mixture, two possible mechanisms can be speculated. The mechanism of Bt δ-endotoxin action involves binding of the toxins to the receptors, insertion into the membrane and pore formation¹. It has been suggested that the toxin might oligomerize before or after binding to the

receptors¹⁰. It is possible that a hetero-oligomer comprising CryIAC and CryIF has better insertional ability than the CryIAC homo-oligomer complex, either during or subsequent to receptor binding. Another possibility is that the toxins CryIAC and CryIF bind to different receptors in the midgut epithelium of the larvae and each individual pore made by different toxins act together and show higher toxicity than the individual pores. Receptor binding analysis and voltage clamp experiments can resolve the mode of synergism.

In conclusion, we suggest that the toxins CryIAC and CryIF can be expressed together in transgenic crop plants for effective control of *H. armigera* and also as a durable resistance management strategy.

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Plant regeneration from mature leaves and roots of *Eryngium foetidum* L., a food flavouring agent

Eryngium is a South American genus of family Apiaceae, with approximately 230 species, of which *Eryngium foetidum*, one of the rare species, is restricted to certain

parts of India. It is an aromatic plant, commonly called spiny coriander, and as important as *Coriandrum sativum*. It is strong smelling and contains aromatic

essential oil comprised of about forty compounds^{1,2}. The leaves are used as a condiment for culinary purposes in north-east India³. Its aromatic oil is indispen-