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Bioassay of *Bacillus thuringiensis* parasporal crystal and its toxic components using the maize borer, *Chilo partellus* (Swinhoe)

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Abstract

A method for determining the toxicity of *B. thuringiensis* parasporal crystal and its toxic components to the maize borer, *Chilo partellus*, is described. *Chilo partellus* is highly sensitive to the σ-endotoxin and provides a highly sensitive and reproducible bioassay that can be used to compare relative toxicities of crystals from other subspecies as well as the toxic components of the crystals.

Key words: *Bacillus thuringiensis*, *Chilo partellus*, parasporal crystal, δ-endotoxin, insecticide, bioassay, artificial diet, probit-mortality, LC_{50}, toxic component.

1. Introduction

Bacillus thuringiensis produces intracellular proteinaceous crystal that is toxic to insects, primarily lepidopteran larvae[1–3]. In our laboratory, we are studying the biochemistry of the crystal and the insecticidal properties of the toxic components obtained by various methods from the crystals. To evaluate the toxicity of the various components of the crystal, a highly sensitive and reproducible bioassay is a prerequisite and the available test systems include *Phylosamia ricini*[4], *Pieris brassicae*[5], *Bombyx mori*[6] and the best syxtem in terms of quantitative analysis of toxicity being that of *Manduca sexta* described by Schesser *et al*[7]. In our laboratory conditions, we have found *Chilo partellus* Swinhoe, which could be reared easily on the artificial diet, as highly susceptible to the δ-endotoxin among the insects tested. Most bioassays for the crystal have employed formulations containing spores[8,9] that are also toxic.

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Chilo partellus, commonly known as maize or jowar stem borer[12,13], has become a serious menace to rice[13] in the eastern paddy tracts of India and constituted about 35–80 per cent of the rice borers depending on the locality. It has also been shown to attack sugarcane[14,15], bajra (*Pennisetum typhoides*)[12], manduwa (*Eleusine caracana*)[12], sudan grass (*Sorghum vulgare* var. *sudadese*)[12], Job's tears (*Coix lachryma-jobi*)[12], Johnson grass (*Sorghum halepense*)[13], burgur (*Polytoca barbata*)[13], *Eragrostis* species[13], *Eleusine verticilata*[11] and *Trinanthema monogyna*[16].

2. Materials and methods

2.1. *Test organism*

The eggs of *Chilo partellus* were obtained from Dr. H. Nagaraja, Commonwealth Institute for Biological Control, Indian Station, Bangalore. The neonate larvae were grown on artificial diet until 5 days in big bottles. The diet used was that of Nagaraja[17] (unpublished data), which is based on the modified formulae of Chatterji *et al*[18]. and Dange *et al*[19]. It consisted of 100 gm of Kabuligram (*Cicer arietinum*) flour, 10 gm maize leaf powder, 30 gm casein, 16 gm Brewers yeast, 3 gm ascorbic acid, 1 gm sorbic aicd, 2 gm methyl parahydroxy benzoate, 1·5 gm salt mixture No. 2 (a BDH preparation), 2 ml of 10 per cent formaldehyde, 2 capsules of multivitaplex (Dumex), 10 gm agar and 350 ml of distilled water and 325 ml of tap water. Five-day old larvae were used for toxicity tests.

The serovar used in these studies was *B. thuringiensis* var. *thuringiensis* procured from Dr. H. de Barjac, Institute Pasteur, Paris. The bacterium was grown in a nutrient broth containing 0·3 per cent sodium chloride at 30° C on a rotary shaker (250 rpm) for 72 hr. Spores and crystals were collected from the culture medium by centrifugation at 7,000 rpm for 10 min in a Sorvall GSA3 rotor. The crystals were subsequently separated from spores and cellular debris by the method developed earlier[20]. The crystals were lyophilized to constant weight and the purity was checked by viable spore count method, carbol fuchsin staining method and protein estimation method. The purity of the crystal preparation was about 99 per cent.

The method of introducing the crystal or the toxic component to the test insect larvae involved its incorporation into the agar-based diet while still in the liquid state. Freshly prepared artificial diet was poured up to 10 gm level in 50 ml glass vials. Dilutions to give 2 mg to 0·01 μg of crystals per ml of solution were prepared and 1 ml of each was mixed with the diet before solidification and allowed to air dry. The applications resulted in treatments ranging from 2 mg/10 gm to 0·01 μg/10 gm of the diet. Three larvae were introduced into each container and the vials were closed with sterile cotton plugs and incubated at room temperature (20–28° C). Controls received distilled water instead of the crystal suspension. A mortality count was made after 10 days of exposure to the treated diet. The same procedure was adopted for the evaluation of the toxicity of the toxic component of M.Wt. of 2,75,000 daltons isolated from the purified crystals[21]. Dilutions of the toxin ranging from 15 μg/ml to 10 ng/ml

Table I

Effect of parasporal crystals and toxic component on the larvae of *C. partellus* after 10 days

Crystals μg/100 gm diet	No. of larvae (n)	Mortality (r)	Mortality %	Log conc. (x)	Probit of mortality	Expected probit	Expected mortality	Expected np	Descrepancy $(r - np)$	$(r - np)^2 / np(1 - p)$
Crystal										
150	20	20	100	2·18	..	..	..	..	..	..
125	20	19	95	2·10	6·64	6·64	95·0	19·0	0·0	0·00
100	20	18	90	2·00	6·28	6·38	91·6	18·3	0·3	0·06
80	20	17	85	1·90	6·04	6·13	87·1	17·4	0·4	0·07
50	20	15	75	1·70	5·68	5·62	73·2	14·6	0·4	0·04
40	20	14	70	1·60	5·52	5·37	64·4	12·9	1·1	0·26
25	20	9	45	1·40	4·87	4·87	45·0	9·0	0·0	0·00
20	20	7	35	1·30	4·62	4·61	34·8	7·0	0·0	0·00
10	20	3	15	1·00	3·96	3·85	12·5	2·5	0·5	0·11
5	20	0	0	0·70	..	..	..	..	..	..
										$K^2_{(6)} = 0·54$
Toxic component										
120	25	25	100	2·08	..	..	..	..	..	..
100	25	24	96	2·00	6·75	6·87	96·9	24·2	−0·2	0·05
80	25	23	92	1·90	6·41	6·59	94·4	23·6	−0·6	0·27
50	25	22	88	1·70	6·18	6·01	84·4	21·1	1·9	1·10
40	25	19	76	1·60	5·71	5·73	76·7	19·2	−0·2	0·01
20	25	12	48	1·30	4·95	4·87	44·8	11·2	0·8	0·10
15	25	9	36	1·18	4·64	4·53	31·9	8·0	1·0	0·18
10	25	4	16	1·00	4·01	4·01	16·1	4·0	0·0	0·00
5	25	0	0	0·70	..	..	..	..	..	..
										$K^2_{(6)} = 1·71$

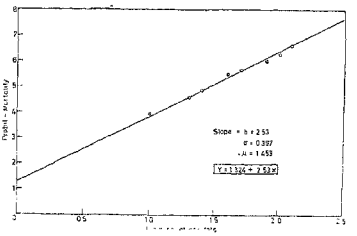

FIG. 1. Dosage mortality curve for the larvae of *C. partellus* fed on crystal preparation.

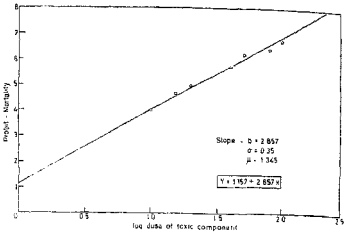

FIG. 2. Dosage mortality curve for the larvae of *C. partellus* fed on the toxic component.

were prepared. The applications resulted in 15 μg/10 gm to 10 ng/10 gm of the diet The bioassay was repeated three times to check reproducibility.

3. Results and discussion

The effect of the crystal preparation of *B. thuringiensis* on the maize borer, *C. partellus* is shown in Table I. After 10 days, about 55 per cent of the larvae survived at a crystal concentration of 2·5 μg/10 gm diet and none at 150 μg/10 gm diet. When the probit mortality is plotted against the logarithm of the dose of the crystal used[22], the lethal concentration values and 95 per cent confidence limits shown in Table II and Fig. 1 are obtained. For convenience, all the concentrations in Tables I and II and Figs. 1 and 2, are expressed as μg/100 gm diet. The parasporal crystal is an extremely effective insecticide with a mean lethal concentration value (LC_{50}), for the larvae of *C. partellus*, of only 284 μg/kg diet.

Table II

Lethal doses and confidence limits for crystal and the toxic component

End point	Concentration μg/100 gm		Lower limit μg/100 gm		Upper limit μg/100 gm	
	Cr	tc	Cr	tc	Cr	tc
50	28·38	22·13	22·75	18·32	35·40	26·73
90	91·20	61·66	65·46	46·24	127·40	82·22
95	125·90	81·28	88·51	57·81	179·10	114·30
99	234·40	141·30	132·70	88·31	414·00	225·90
99·9	..	269·20	..	143·70	..	503·50

Cr = crystals, tc = toxic component.

With the toxic component, purified from crystal, the lethal concentration of the toxin (LC_{50}) has been found to be about 221 μg/kg diet, which is less than that of crystals (Tables I and II and Fig. 2).

The microbial insecticide severely affects the normal growth of the surviving larvae. As expected, all control larvae have pupated. The larvae surviving after the crystal or toxic component treatment, however, hardly grew and showed severe reduction in size and weight and eventually died. A concentration of 100 μg/kg diet brought about significant reduction in size after 10-day incubation period. The results indicate that the crystal causes either a drastic reduction in the amount of diet consumed or a severe interference with the movement of food through the gut wall. From known physical effects of the δ-endotoxin, it seems likely that the toxin inhibits the feeding mechanism at lower doses.

The bioassay described in this report presents a highly susceptible experimental insect, *C. partellus*, that responds to a crystal dose (LC_{50}) of 284 μg/kg diet and a dose of the toxic component of 221 μg/kg diet. Now with a reliable bioassay available, the evaluation of the toxic properties of the various components obtained from crystals by various methods would be facilitated. The toxic component, when modified by acetylation, maleylation, succinylation and reversible citraconylation and alkylation with iodoacetate, showed no toxicity towards *C. partellus* as well as to the larvae of *Bombyx mori*, when tested at a concentration 200 times the LC_{50} value for the toxic component (unpublished data). Apparently, any modification of the toxin involving acidic or sulfhydryl groups, results in the loss of toxic property. When the citraconyl groups are removed even under mild conditions, the toxicity is not restored.

B. thuringiensis can be used as an effective insecticide to control the serious pest of rice and other economic crops.

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References

1. BURGES, H. D. — In *Insect pathology and microbial control*, P. A. Van der Laan (ed.), *Proceedings of the international colloquium on insect pathology, microbial control*, Wageningen, The Netherlands, 1966. North Holland Publishing Co., Amsterdam, 1967, 306–338.

2. COOKSEY, K. E. — In *Microbial control of insects and mites*, H. D. Burges and N. W. Hussey (eds.), Academic Press Inc., New York, 1971, 247–274.

3. LECADET, M. M. — In *Microbial toxins*, Vol. III, T. C. Montie, S. Kadis, S. J. Ajl (eds.), Academic Press Inc., New York, 1970, 437–471.

4. PENDELTON, I. R. — Characterization of crystal protein and an activated toxin from *Bacillus thuringiensis* var. *entomocidus*, *J. Invertebr. Pathol.*, 1973, **21**, 46–52.

5. LECADET, M. M. AND MARTOURET, D. — II. Toxicity of the different fractions of the hydrolysate for larvae of *Pieris brassicae*, *J. Invertebr. Pathol.*, 1967, **9**, 322.

6. FAUST, R. M., HALLAM, G. M. AND TRAVERS, R. S. — Degradation of the parasporal crystal produced by *B. thuringiensis* var. *kurstaki*, *J. Invertebr. Pathol.*, 1974, **23**, 365.

7. SCHESSER, J. H., KRAMER, K. J. AND BULLA, L .A. Jr. — Bioassay for homogeneous parasporal crystal of *B. thuringiensis* using the tobacco hornworm, *Manduca sexta*, *Appl. Environmental Microbiol.*, 1977, **33**, 878–880.

8. BEGG, J. A. — The effectiveness of a microbial insecticide against the tomato and tobacco hornworms (protoparce) under laboratory conditions, *J. Invertebr. Pathol.*, 1963, **4**, 492–494.

9. IGNOFFO, C. M., HOSTETTER, D. L., PINNELL, R. E. AND GARCIA, C. — Relative susceptibility of six soybean caterpillars to a standard preparation of *B. thuringiensis* var. *kurstaki*, *J. Econ. Entomol.* 1977, **70**, 60–63.

10. SOMERVILLE, H. J. AND POCKETT, H. V. — An insect toxin from spores of *B. thuringiensis* and *B. cereus*, *J. Gen. Microbiol.*, 1975, **87**, 359–369.

11. BANERJEE, S. N. AND PRAMANIK, L. M. — In *The proceedings of a symposium at the International Rice Research Institute (IRRI)*, Sept. 1964, Published for the IRRI by the Johns Hopkins Press, Baltimore, Maryland, 1967, 119–121.

12. FLETCHER, T. B. AND GHOSH, C. C. — Borers in sugarcane, rice, etc., 1920, *Rep. Proc. 3rd Entom. Meet. Pusa*, 1919, **1**, 354–417.

13. TREHAN, K. N. AND BUTANI, D. K. — Notes on the life history, bionomics and control of *Chilo zonellus* Swinhoe in Bombay Province, *Indian J. Entom.*, 1949, **11**, 47–59.

14. DESHPANDE, V. G. — Important pests of sugarcane in the Bombay Presidency, *Curr. Sci.*, 1937, **6** (1), 33–35.

15. PRUTHI, H. S. AND NARAYANAN, E. S. — A study of the behaviour of some common varieties of sugarcane in reference to the attack of borers at Pusa (Bihar) during 1935–1936, *Proc. Nat. Inst. Sci. India*, 1938, **4**, 87–107.

16. SINGH, J., DHAWAN, N. C. AND JOSHI, A. B. — New host plants of the top shoot borer of maize (*Chilo zonellus* Swinhoe), *Curr. Sci.*, 1961, **30**, 109–110.

17. Nagaraja, H. — Unpublished data.

18. CHATTERJI, S. M., SIDDIQUI, K. H., PAWAR, V. P. S., SHARMA, G. C. AND YOUNG, W. R. — Rearing of the maize stem borer, *Chilo zonellus* Swinhoe on artificial diet, *Indian J. Entom.*, 1968, **30**, 8–12.

19. DANG, K., ANAND, M. AND JOTWANI, M. G. — A simple improved diet for mass rearing of sorghum stem borer, *Chilo zonellus* Swinhoe, *Indian J. Entom.*, 1970, **32**, 130–133.

20. RAO, C. D. AND SHETHNA, Y. I. — A simple technique for purification of the parasporal crystal (δ-endotoxin) of *B. thuringiensis* var. *thuringiensis*, *J. Indian Inst. Sci.*, 1980, **62** (C), 1–11.

21. RAO, C. D. AND SHETHNA, Y. I. — Unpublished data.

22. FINNEY, D. J. — *Probit Analysis*, Cambridge University Press, London, 1971.