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LARVICIDAL ACTIVITY OF β-EXOTOXIN AND BEAUVERICIN AGAINST TWO DIPTEROUS SPECIES

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The toxicity of crude β-exotoxin, extracted from *Bacillus thuringiensis* bacterial culture, was tested against larvae of *Musca domestica.* Its LD_{50} was 0.04 % when tested against 3rd instar larvae for 5 hrs. Commercial pure β-exotoxin and beauvericin were treated against larvae and pupae of *Aedes aegypti* and *M. domestica.* β-exotoxin was toxic to 1st and 4th instar larvae and pupae of *A. aegypti,* after exposure of 3, 7, 12 days respectively at doses from 0.3125 μg/ml. water (ppm). Its LC_{50} was 0.52 μg, 2.1 μg and 12 μg respectively. Treatment with *M. domestica,* its LD_{50} was 38 μg/g diet when tested against 3rd instar larvae. Beauvericin was less toxic as compared to β-exotoxin, its relative toxicity was 5-10 fold.

Key words: β-exotoxin, Beauvericin, *Aedes aegypti, Musca domestica.*

INTRODUCTION

Bacillus thurigiensis Berliner, produce an extracellular toxin during their vegetative growth called β-exotoxin. This substance is heat stable and dialysable [1]. The β-exotoxin is reported to have insecticidal activity against various species of dipterous insects [2-7]. Hall and Arakawa [8] showed that 0.025 g of laboratory grown culture of *B. thuringiensis* var. *thuringiensis* (On Difco nutrient agar) caused 90 % to 100 % mortality of larvae of the *M. domestica* in non-resistant, and in D.D.T. and malathion resistant fly strain. Briggs [9] carried extensive experiments with larvae of *M. domestica* using commercial preparations of *B. thuringiensis* var. *thuringiensis* and *B. thuringiensis* var. *sotto.* He also tested 5 strains of *B. thuringiensis* grown in 1 litre flask with Difco nutrient broth aerated with sterile compressed air for 72 hrs. at 30°. Briggs agreed with McConnel and Richard when he suggested that the toxin acting on fly larvae was a soluble fraction.

Burgerjon and de Barjac [10] fed insects representing several orders the supernatant liquid of *B. thuringiensis* var. *thuringiensis.* They have reported that this species produced a measurable amount of exotoxin and that the supernatant liquid had to be fed in large quantities (50 times the volume corresponding to an aqueous suspension of spores and crystals.) to kill the insects.

The commercial preparation of β-exotoxin is currently being used in many countries. The Abbott Laboratories is producing β-exotoxin as Thuringiensis ABG-6162 for control of crop insects.

Another commercial biopesticide Beauvericin [11] is produced from entomopathogenic fungi – *Beauveria bassiana,* has recently attracted the attention of various scientists [12-14] for its use as insecticide.

As the activity of biopesticides is known to vary under different experimental conditions, methods of preperation and its applications, stages of development of insects, treatment time, concentrations and strain specificity, the present work was carried out to determine the extraction of exotoxin from bacteria and its toxicity against larvae and pupae of *M. domestica.* Also standard commercially produced β-exotoxin (IMC 10,0001.2, International Minerals and Chemical Corporation) and Beauvercin (B-7150 Sigma Chemical Co.) were tested against laboratory reared strains of *Aedes aegypti* and *M. domestica.*

MATERIALS AND METHODS

The larvae of *M. domestica* were reared aseptically to the third instar on a diet described by Ashrafi *et. al.* [15].

Extraction of exotoxin from crude supernatant liquid. A crude exotoxin was extracted from a bacterial spore product "Dipel" (Abbott Laboratories, USA), in following manner:

125gm of Dipel was added slowly into 75ml. of distilled water and stirred until properly mixed. The mixture was allowed to settle for 4 hrs. After settling, the supernatant liquid was removed and placed into a centrifuge at speed 3000 rpm for 20 minutes. This supernatant liquid was then removed and freeze dried. Before use, a weighed amount of freeze dried supernatant material was dissolved in distilled water and autoclaved at 15 psi for 15 minutes [2,16].

Preparation of the formulation of standard β-exotoxin. The stock solution of β-exotoxin was prepared by suspending 100 mg of the material in 100 ml distilled water. This suspension was constantly stirred by magnetic stirrer at high speed for 3 minutes. 10 ml of this suspension was immediately diluted in 90 ml tap water to obtain a dilution of 10mg/100ml (w/v). Further dilutions were also prepared by adding appropriate quantity of water. Care was taken to keep the material uniformly distributed in suspensions. Fresh suspensions were prepared for each set of experiments.

Preparation of the formulation of Beauvericin B-7510: 5 mg beauvericin was dissolved in 10 ml acetone with traces of emulsifier (triton x-180) to obtain a stock solution of 0.05 % concentration. Further dilutions were prepared in tap water.

First, 4th instar larvae and 0-4 hrs. old pupae of *A. aegypti* (twenty per beaker) were exposed to biopesticides at doses ranging from 0.3125-20 μg/ml (ppm) in 250 ml water for 3,7, and 12 days respectively. Third instar larvae of *M. domestica* were exposed to β-exotoxin at 6.25 μg to 100 μg/g diet until pupation. Controls were run separately and mortality of larvae and pupae was adjusted for natural mortality in the controls using Abbott's formula [17]. The LC_{50} values were calculated from log dose probit regression analysis.

RESULTS AND DISCUSSION

Feeding tests were carried out with the sterile crude exotoxin from bacterial preparation against 3rd instar larvae of *M. domestica,* including controls (Table 1). Five doses ranging from 0.0156 % to 0.25 % of test medium were used. A probit analysis of the data obtained from these tests, placed the LD_{50} at 0.04 %.

The gross pathological effects of the crude autoclaved exotoxin on larvae pupae of *M. domestica* are illustrated in figures 5 to 7. At levels of 25 mg to 50 mg of supernatant per 10 ml of larval feed, pupation did not begin (Fig. 5). The addition of 6.25 mg to 25 mg resulted in malformation from normal appearing pupae (Fig. 6) showing the anterior end remained in the larval state. Concentration below 6.25 mg resulted normal appearing pupae but the adults were unable to emerge out (Fig. 7). At a level of 1.56 mg many of the adults that emerged were both male and female had vestigial wings and narrow pointed abdomens.

Results of commercial β-exotoxin effectivity showed a direct correlation between doses of *b-exotoxin* and mortality of larvae and pupae of *M. domestica* (Table 2). Most of the larvae reached the pupal stage but died in their pupal cases. There was no emergence at 100 μg/g dose of β-exotoxin in treated diet. The LD_{50} value was 38 μg/g diet.

Ignoffo and Gard [18] reported LD_{50} of this biopesticide against *M. domestica* larvae at 85.5 ppm, a dose considerably higher than our house fly strain. Wasti *et. al.* [19] reported the LD_{50} value of β-exotoxin against two dipterous larvae (*Orthellia* and *Phormia*) in artificial diet at 8.65 ppm and 22.9 ppm respectively. This variation of toxicities towards different dipterous species indicates that toxin in various isolates of *B. thuringiensis* may not be identical.

In comparison, the commercial β-exotoxin is 105.26 times more toxic than the crude exotoxin from the bacterial culture of β. thuringiensis.

In case of *A. aegypti,* after long term exposure to β-exotoxin, (Fig. 1) shows the dose response regression lines plotted against mortality of larvae and pupae. The LC_{50} values against 1st 4th instar larvae and pupae were 0.52 μg/ml, 2.1 μg/ml and 12 μg/ml respectively. 1st instar larvae showed 100 % kill at 20 μg/ml after 24 hrs. Thirty percent 1st instar larvae survived upto 2nd stage after exposure to 10 μg dose. subsequently they died

Table 1. Percentage mortality of 3rd instar larvae of *Musca domestica* (L) after 5 hours feeding on crude exotoxin.

Dose of exotoxin	Mortality percentage
0.0156 %	21.66 ± 1.033
0.0312 %	46.66 ± 2.280
0.0625 %	65.40 ± 2.480
0.125 %	85.00 ± 4.000
0.250 %	86.60 ± 2.874
Control	4.58 ± 1.602

LD_{50} = 0.04 %.

Table 2. Exposure of 3rd instar larvae of *Musca domestica* (L) to β-exotoxin till pupation.

Conc. μg/g diet	Mean % mortality		Total corrected % mortality
	3rd instar larvae	pupae	
100.00	27.67 ± 1.43	72.33 ± 1.43	100.00
75.00	15.20 ± 0.73	70.40 ± 1.87	84.60
50.00	6.66 ± 0.76	67.03 ± 1.37	71.86
25.00	3.75 ± 0.25	27.45 ± 1.31	26.42
12.50	0.00	12.50 ± 0.29	6.42
6.25	0.00	7.50 ± 0.5	1.07

LD_{50} = 38 μg/g diet.

before reaching 3rd stage (Fig. 2). Out of the treated 4th instar larvae none could emerge as adult at 20 μg/ml dose, however, 12.5 % reached the pupal stage at this dose (Fig. 3).

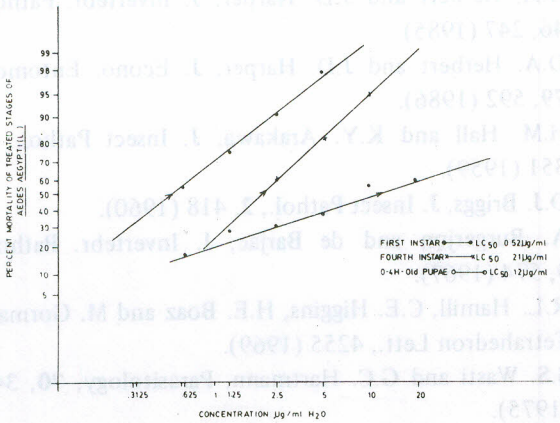

Fig. 1. Dose mortality curves obtained with three stages of *Aedes aegypti* exposed to β-exotoxin of *Bacillus thuringiensis* var. *Thuringiensis* till emergence.

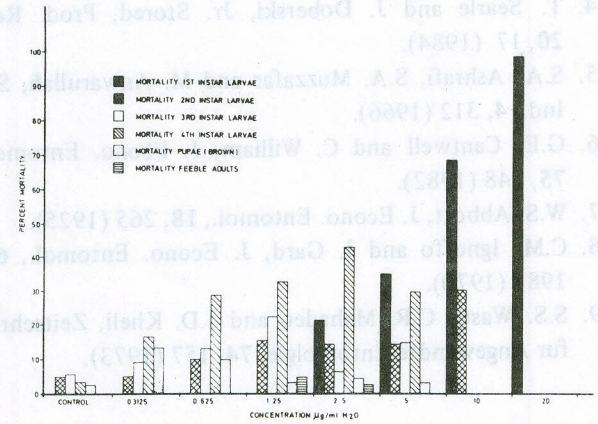

Fig. 2. Susceptibility of ist Instar larvae of *Aedes aegypti* to β-exotoxin following continuous exposure till emergence.

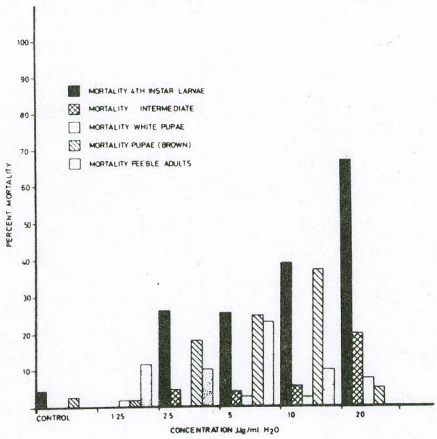

Fig. 3. Susceptibility of 4th instar larvae of *Aedes aegypti* to β-exotoxin following continuous exposure till emergence.

β-exotoxin showed no inhibitory effects on the development of treated stages of larvae of *A. aegypti*. No difference in time period of emergence of adults in treated and control experiments was observed.

Hall and Arakawa [3] reported the toxic values of pathogenic formulations of various strains of β.thurigiensis against 2nd or 3rd instar larvae of different mosquitoes at doses ranging from 0.04 to 10 μg/ml water after an exposure of 7 days. They reported LC_{50} value of *B. thurigiensis* var. *thuringiensis* at 2 μg dose. This level of LD_{50} is very close to our findings against 4th instar larvae of laboratory-reared *A. aegypti*.

Beauvericin was observed to be somewhat toxic to larvae and pupae of *A. aegypti* (Fig. 4) at 10 and 20 ppm doses. It is proved to be less toxic as compared to β-exotoxin, the relative toxicity was observed to be 5 to 10 fold. The data on the survival rate of 1st, 4th instar larvae and

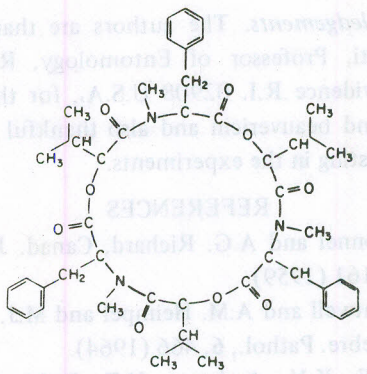

Fig. 4. Beauvericin.

Fig. 5. At 25 mg to 50 mg dose of exotoxin. Popation did not begin.

Fig. 6. At 6.25 mg to 25 mg dose of exotoxin. Normal to malformation of pupae.

Fig. 7. At 6.25 mg/10 ml dose of exotoxin. Normal appearing of pupae but adults were unable to emerge out.

pupae of *A. aegypti* are given in Table 3. This observation is in line with that of Hamill *et al.* [11] who treated larvae of other species of mosquitoes. The efficacy of beauvericin indicates that its potency is low and as such connot be used in the fields for pest control. As no fungal toxins are under development at present for pest control, there is great need and scope to develop effective and cheaper compounds from entomopathogenic fungi for actual use.

Table 3. Response of beauvericin against larvae and pupae of *Aedes aegypti* (L).

Conc.	Mean % survival of adults from treated stage		
ppm.	1st instar	4th instar	pupae
20	29 ± 0.43	6 ± 0.43	–
10	52 ± 0.43	27 ± 0.71	48 ± 0.41
Control	90 ± 0.25	85 ± 1.73	90 ± 0.58

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REFERENCES

1. E. McConnel and A.G. Richard, Canad. Jr. Microbiology, **5**, 161 (1959).
2. G.E. Cantwell and A.M. Heimpel and M.J. Thompson, J. Invertebre. Pathol., **6**, 466 (1964).
3. I.M. Hall, K.Y. Arakawa, H.T. Dulmage and J.A. Correa, Mosquito News, **37**, 246 (1977).
4. M.S. Goettel, M.K. Toohey and J.S. Pillai, Mosquito News, **42**, 163 (1982).
5. L.A. Lacey, M.J. Urbina and C.M. Heitzman, Mosquito News, **44**, 26 (1984).
6. D.A. Herbert and J.D. Harper, J. Invertebr. Pathol., **46**, 247 (1985).
7. D.A. Herbert and J.D. Harper, J. Econo. Entomol., **79**, 592 (1986).
8. H.M. Hall and K.Y. Arakawa, J. Insect Pathol., **1**, 351 (1959).
9. D.J. Briggs, J. Insect Pathol., **2**, 418 (1960).
10. A. Burgerjon and de Barjac, J. Invertebr. Pathol., **9**, 574 (1967).
11. R.L. Hamill, C.E. Higgins, H.E. Boaz and M. Gorman, Tetrahedron Lett., 4255 (1969).
12. S.S. Wasti and G.C. Hartmann, Parasitology, **70**, 341 (1975).
13. M. Kanaoka, A. Isogai, M. Murakoshi, M. Ichinoe, A. Suzuki and S. Tamura, Agric. Chem., **42**, 629 (1978).
14. T. Searle and J. Doberski, Jr. Stored, Prod. Res., **20**, 17 (1984).
15. S.A. Ashrafi, S.A. Muzzafar and M. Anwarullah, Sci. Ind., **4**, 312 (1966).
16. G.E. Cantwell and C. William, J. Econo. Entomol., **75**, 348 (1982).
17. W.S. Abbott, J. Econo. Entomol., **18**, 265 (1925).
18. C.M. Ignoffo and I. Gard, J. Econo. Entomol., **63**, 1987 (1970).
19. S.S. Wasti, C.R. Mahadeo and J.D. Khell, Zeitschrift fur Angewandte Entomolgie, **74**, 157 (1973).