

- 4 Warren, J. R. and Winstead, N. N., *Phytopathology*, 1965, 55, 244
- 5 Uma, G., 1993, A survey on the incidence of aflatoxins in food and feed in Gulbarga city, MPhil Dissertation, Gulbarga University, Gulbarga, p. 28.
6. Seitz, L. M. and Mohr, M. E., *Cereal Chem.*, 1974, 51, 487.
7. Seitz, L. M. and Mohr, M. E., *Cereal Chem.*, 1977, 54, 179.

ACKNOWLEDGEMENTS. We are grateful to Dr A. H. Rajasab, Chairman, Department of Botany, Gulbarga University, Gulbarga, for facilities, Dr S. B. Angadi of our Department for sparing *Chlorella* culture and Dr Ir H. P. van Egmond, Bilthoven, The Netherlands, for kindly sending aflatoxin standards.

Received 7 March 1994, accepted 15 June 1994

Synergistic action of different strains of *Bacillus thuringiensis* against cotton leaf worm *Spodoptera littoralis* (Boisduval)

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The insecticidal activity of the crystal toxin of different strains of bacteria, *Bacillus thuringiensis* was tested against the cotton leaf worm, *Spodoptera littoralis* (Boisduval). According to the LC_{50} values against the neonate larvae, the order of toxicity was *B. thuringiensis* var. *entomocidus* > *aizawai* > *berliner* > *kurstaki*. The strains *aizawai* and *entomocidus* when combined showed only additive action whereas *entomocidus*-*berliner* and *aizawai*-*kurstaki* combinations showed potentiation suggesting a synergistic action.

LITTLE work is conducted to study the biological activity of mixtures of different strains of *Bacillus thuringiensis*. Hence an attempt is made in this study to see the joint action of different strains of *B. thuringiensis*. The test insect used was cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae).

Culture of *S. littoralis*, maintained for several generations in the laboratory at La Minere (INRA Research Station), France, was used for conducting the experiment. The insect was reared in 20–25°C and 75% RH on an artificial diet. Adult moths laid the eggs on filter paper folds and they were sterilized in the fumes of formalin for 20 min and kept at 25°C for eclosion. In about four days the larvae hatched out and the first instar unfed larvae were used. Different strains of *B. thuringiensis* in the crystal (endotoxin) form were

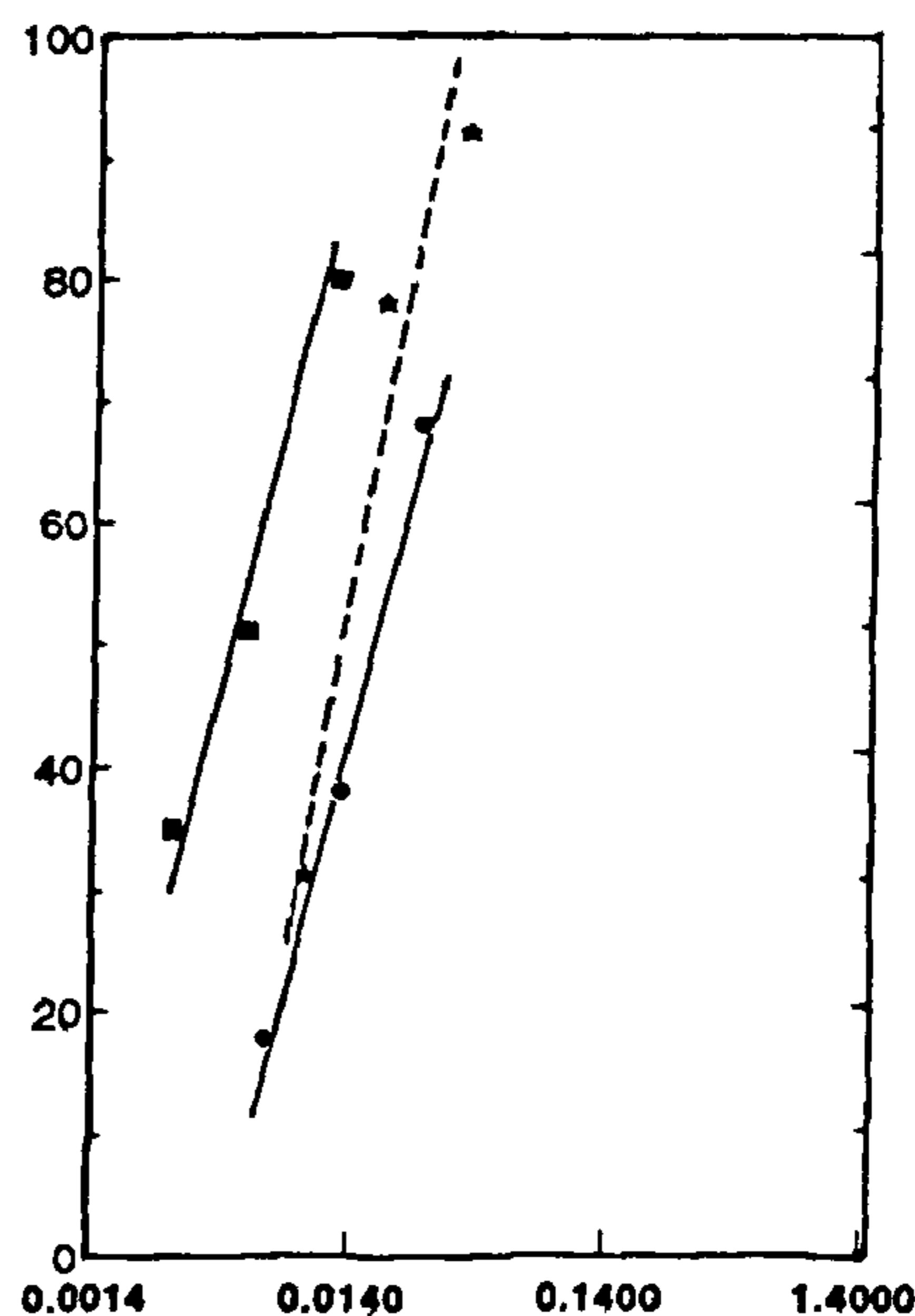


Figure 1. Toxicity of *entomocidus*, *aizawai* and their combination.

Table 1. Toxicity of different strains of *B. thuringiensis* to cotton leafworm *S. littoralis* (Boisduval)

Strains	Code no	Stock soln	LC ₅₀ (72 h)	No of assays*
		(% concentration)		
<i>B. thuringiensis</i> var. <i>entomocidus</i>	605	0.33	0.0055 ± 0.001	6
<i>aizawai</i>	635	0.44	0.011 ± 0.002	9
<i>berliner</i>	663	0.34	0.124 ± 0.074	8
<i>kurstaki</i>	672/564	0.35	0.218 ± 0.121	5

*each assay is with four replications of 20 observations each

supplied by Pasteur Institute, Paris, France (courtesy Miss La Cadet).

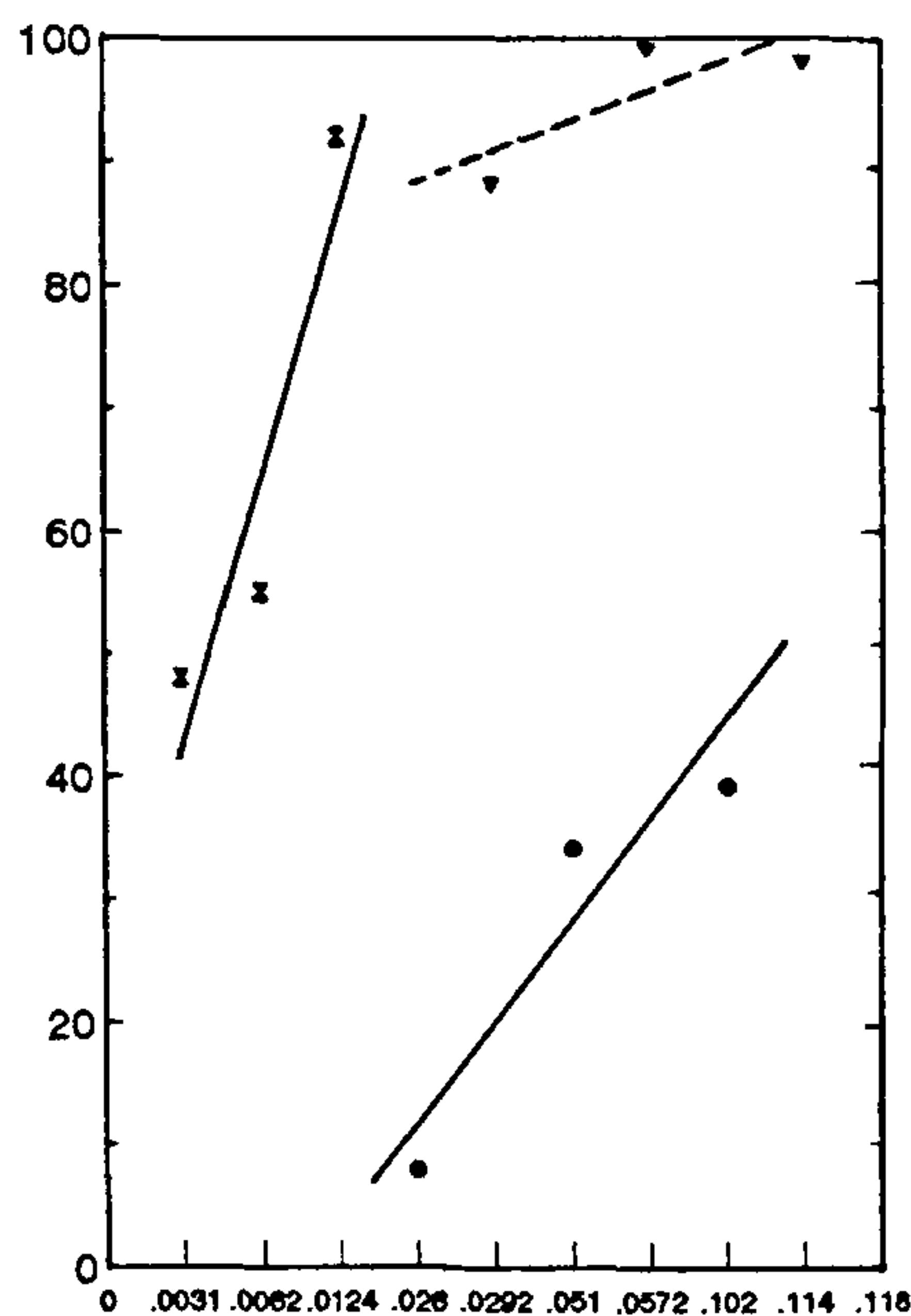
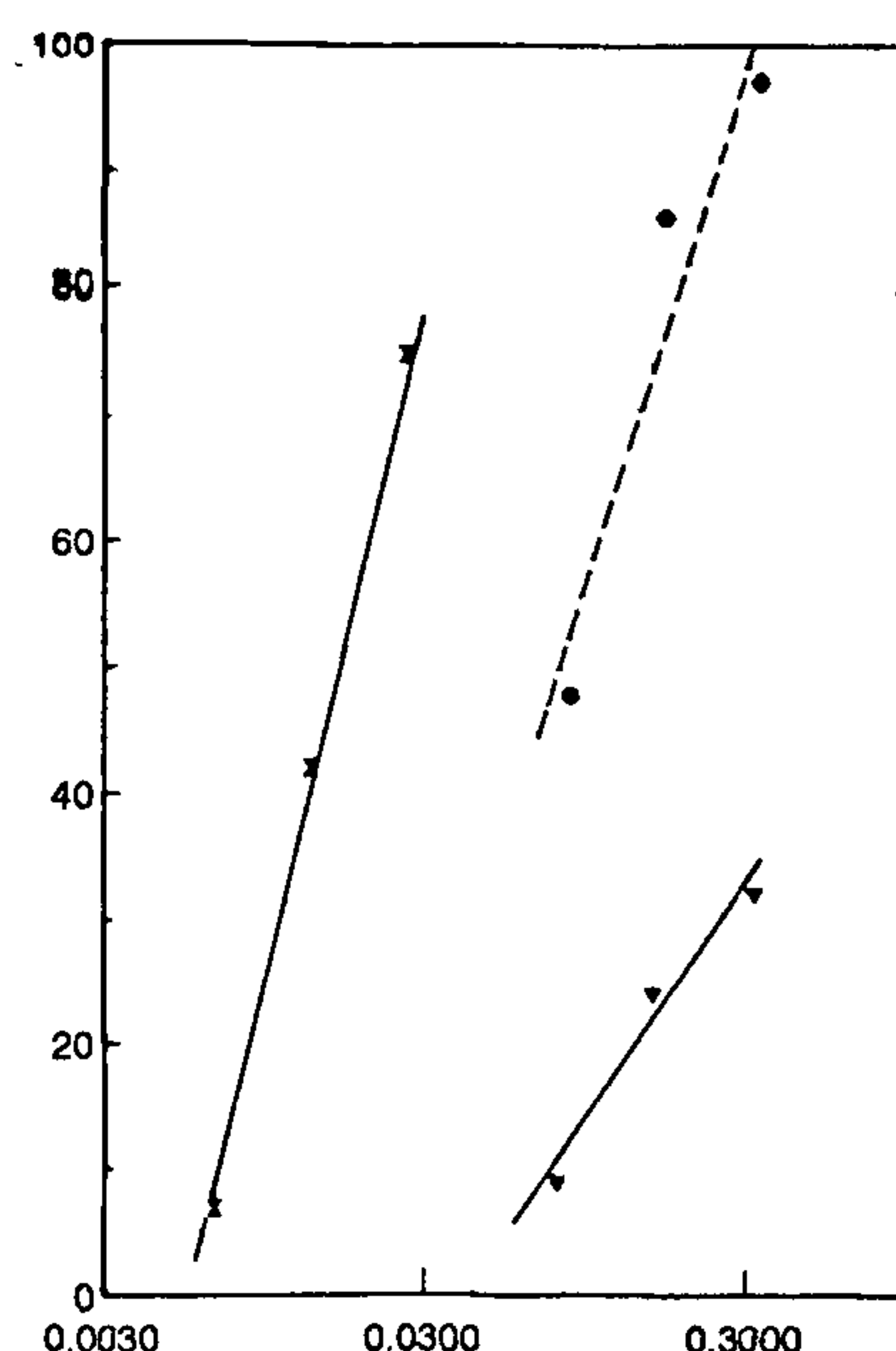
The stock solutions for different strains were prepared as follows. Vials having crystal suspension were agitated for 10–15 min in a vertical shaker and diluted with sterile water. A serial dilution procedure was followed. The concentrations of stock solutions are shown in Table 1. Control was only sterile water with 0.5 ml of wetting agent. Spraying was done using Burgerjon tower¹ on 1 cm diameter circular bits of cabbage leaves, which were airdried and used to feed 20 neonate larvae. The containers were kept at 25°C and 75% RH.

All the treatments were sprayed at a time and the mortality rate at LC_{25} , LC_{50} and LC_{90} was plotted. The

Table 2.

Combinations	Cototoxicity coefficient			Cototoxicity factor		
	48 h	72 h	Overall mean	48 h	72 h	Overall mean
<i>B. thuringiensis</i> strains						
<i>aizawai</i> + <i>entomocidus</i>	114.95 ± 12.82 (2)	87.24 ± 11.28 (2)	101.10	-8.22 (4)	-19.52 (4)	-13.97
<i>entomocidus</i> + <i>berliner</i>	162.4 ± 38.7 (3)	703.29 ± 262.79 (3)	432.85	+43.45 (5)	+32.76 (3)	+38.11
<i>aizawai</i> + <i>kurstaki</i>	682.2 ± 567.6 (3)	151.81 ± 32.89 (3)	417.01	110.8 (4)	67.08 (3)	+88.94

Values in parentheses indicate the number of bioassay studies conducted. Each assay is with 4 replications of 20 observations each.

Figure 2. Toxicity of *entomocidus*, *berliner* and their combination.Figure 3. Toxicity of *aizawai*, *kurstaki* and their combination.

nature of joint action, i.e. synergism, antagonism or additive effect was worked out. Methods of Sun and Johnson² and Mansour *et al.*³ were used to find out the cototoxicity factor (by dosage mortality curve) and cototoxicity coefficient (by expected and observed mortality formula) respectively.

Based on the LC_{50} values at 72 h after treatment, the order of toxicity of the strains was *entomocidus* > *aizawai* > *berliner* > *kurstaki* (Table 1). Among them *B. thuringiensis* var. *entomocidus* with the lowest LC_{50} value of 0.0005 ± 0.001 was considered highly toxic. This was similar to the earlier studies with *S. littoralis* where *entomocidus* [Inst. Past. 17 (ref. 4) and Isolate 24 serotyped as H6 (ref. 5)] was found more lethal.

The maximum mortality percentage in *entomocidus* when used alone was around 50 and 80 and in *aizawai* it was 42 and 70% at 48 and 72 h. The same was 77.5 and 91.5% respectively in the combination (Figure 1). The dosage mortality curve for the combination was not parallel to the curves of individual strains. This was due to increased mortality in higher dose combination. The cototoxicity coefficient was 87.24 and the cototoxicity factor was -19.52 at 72 h, suggesting there was no synergistic action but only additive effect (Table 2).

The LC_{50} for the *entomocidus* and *berliner* combination was much lower than those for *entomocidus* and *berliner* individually (Figure 2). The mean cototoxicity coefficient 162.40 ± 38.7 and 703.29 ± 269.69

and cotoxicity factor +43.45 and +32.76 at 48 and 72 h were high. This indicated potentiation or synergism in *entomocidus* and *berliner* combination (Table 2).

Kurstaki strain was less toxic since the mortality was only 43% in 72 h, even at a higher dose (Figure 3). When it is jointly used with *aizawai*, the mortality was 48% in the lowest dose, which was higher than in both the strains applied individually. The cotoxicity coefficient was 682.2 ± 567.6 and 151.81 ± 32.89 and the cotoxicity factor was +110.8 and +67.08 in 48 and 72 h respectively (Table 2). This suggested a synergistic action when these strains were combined. Similarly high toxicity was noticed when *aizawai* HD133 and *kurstaki* HD1 were jointly administered to *Heliothis armigera* (Hubner)⁶ and *entomocidus* and *kurstaki* HD73 to *S. littoralis*⁶.

The outcome of this study may be used practically for activation of mild strains or controlling the pests occupying similar ecological niche but susceptible to different strains. Joint application of strains showing synergism may be useful.

- 1 Burgerjon, A., *Ann Epiphyt.*, 1956, 4, 677-686
- 2 Sun, Y. P. and Johnson, E. R., *J Econ Entomol*, 1960, 53, 887-892
- 3 Mansour, N. A., Elfoffrawi, M. E., Topozada, A. and Zeid, M., *J Econ. Entomol*, 1966, 59, 307-311
- 4 Salama, H. S., Foda, M. S. and El-Sharaby, A. M., *Z. Angew Entomol*, 1981, 92, 388-398
- 5 Sneh, B., Schuster, S. and Broz, M., *Entomophagha*, 1981, 26, 179-190
- 6 Salama, H. S., Foda, M. S. and El-Sharaby, A. M., *Z Angew Entomol*, 1983, 95, 69-74

ACKNOWLEDGEMENTS. L. N. thanks the Indian and French Governments for offering post-doctorate fellowship, IRHO, Paris, INRA, LaMinere, for the facilities provided and Kerala Agricultural University for granting leave.

Received 25 August 1993, revised accepted 8 July 1994

Prospects and problems in cage culture of giant tiger shrimp in Vellar estuary

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The culture of the giant tiger shrimp *Penaeus monodon* was planned to be carried out for 90 days covering premonsoon and monsoon seasons in a cage (5 × 4 × 1 m) in Vellar estuary, feeding with a supplementary feed. Seeds (65 mm in size and weight

1.5 g) were stocked at the rate of 120,000 seeds/ha (240 seeds/20 m²). The juvenile shrimps were fed twice a day with the pelletized feed to 10% of their body weight. The average length of the shrimps after 60 days was 127 mm and the average weight was 17.8 g having a growth rate of 1.03 mm and 0.27 g per day. The autoentrants were also collected and recorded. The culture experiment could be carried out only for 60 days after which there was an unprecedented flood lasting for 10 days in Vellar estuary due to maximum rainfall in the season, which ultimately damaged the cage, resulting in total loss of shrimps. The environmental parameters like salinity, temperature, hydrogen ion concentration and dissolved oxygen were monitored fortnightly and were found to be ranging from 0.3 to 30.0‰, 24 to 34°C, 7.5 to 8.4 and 3.6 to 5.7 ml/l respectively.

ALONG with the traditional culture practised in a few states of India, the intensive culture practices are also slowly coming up. The cage culture method is being practised in countries like Indonesia, Thailand, Japan, Malaysia, Singapore, etc. Since it is a three-dimensional culture, it gives enough room for optimum utilization of its potential.

Some pilot scale cage and pen culture studies were done in areas like Tuticorin^{1,2}, Kovalam^{3,4}, Ennore estuary⁵, Kundakkal channel⁶ and Killai backwaters^{7,8}. Vellar estuary is one of the few estuaries in India thoroughly studied but its potential for culture practices has not been exploited so far. We used the culture of *Penaeus monodon* in a cage to study the suitability of cage culture operations of giant tiger shrimp *P. monodon* in Vellar estuary during the premonsoon and monsoon seasons and the prospects and problems involved.

A cage of the size of 5 × 4 × 1 m made of synthetic velon screen with 16 p mesh size was erected in Vellar estuary in such a position that two-thirds of the cage was kept inside the water column and the bottom of the cage about 30 cm above the bottom sediments⁴. The *P. monodon* seeds were collected from Vellar estuary using a push net having a mesh size of 1.5 mm. The seeds of almost uniform size (from tip of the rostrum to telson 65 mm length and 1.5 g in weight) were selected and acclimatized in a hapa in the estuary itself and then released into the cage after 2 or 3 days at a stocking density of 240 seeds/20 m² (120,000 seeds/ha).

The animals were fed at the rate of 10% of the body weight assuming a 100% survival in the cage in two instalments at dawn and dusk. The feed was formulated using the locally available ingredients such as cuttle fish (24%), fish meal (24%), prawn head waste (12%), rice bran (5%), ground nut oil cake (10%), maida (10%), tapioca flour (14%) and fish oil (10%). The amount of feed in the first 15 days of culture was about 36 g/day and after every 15 days, the amount was raised to 105 g, 218 g, 333 g and 427 g respectively depending upon the