

# STATUS REPORT ON THE USE OF *Bacillus* *thuringiensis* IN THE CONTROL OF SOME OF OIL PALM PESTS

**Keywords:** *Bacillus thuringiensis*; nettle caterpillars; bunch moth; bagworms; *Metisa plana*; *Tirathaba rufivena*.

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**P**rogress in the use of *B. thuringiensis* in the control of bunch moth (*Pyralidae*), nettle caterpillars (*Limacodidae*) and bagworms (*Psychidae*) is reviewed. *Bacillus thuringiensis* Berliner (*Thuricide*) was more effective than diflubenzuron, cyfluthrin and endosulfan in the control of the bunch moth. Few field trials on the use of *B. thuringiensis* against nettle caterpillars have been reported to date. In all reported instances except one, it was found to be ineffective.

The performance of *B. thuringiensis* against bagworms is similar to that against nettle caterpillars: there has been no field success. Laboratory investigations with the bagworm, *Metisa plana*, revealed that of the eight products tested (*Bactospeine*, *Thuricide*, *BCBT II*, *Florbac*, *Foray*, *Dipel*, *Biobit FC* and *CGA-BT-237218*), only *Florbac* showed some potential in control, causing 80% mortality of the fourth instars after seven days. The mortality values with the other products, as well as with *Florbac* against second instars, were generally less than 60 per cent.

Investigations on concentration-mortality responses revealed that a high concentration would be required to effect a 75% kill, the level regarded as acceptable for biocontrol agents. Subsequently, various suggestions are made on how to overcome the lack of potency of *B. thuringiensis* against the larvae of *M. plana*.

## INTRODUCTION

The main types of insect pests of oil palm in Malaysia are the bagworms (Lepidoptera : Psychidae), nettle caterpillars (Lepidoptera : Limacodidae), rhinoceros beetles (Coleoptera : Dynastidae) and bunch moths (Lepidoptera : Pyralidae) (Wood, 1968). Bagworms and nettle caterpillars are regarded as occasional pests, capable of producing outbreaks, when there is a breakdown in natural control. Incidences of bagworm outbreaks have been reported in Malaysia by Wood (1982) for the period 1975–1980 and by Wahid *et al.* (1988) for the period 1981–1985. More outbreaks were reported in the later period, suggesting the increased importance of bagworms as pests of oil palm. Norman and Wahid (1992) noted that for the period 1980 to 1990 only 31 estates reported outbreaks of nettle caterpillars.

Rhinoceros beetle is not generally a problem except at replanting, when rotting oil palm trunks provide ideal breeding sites. The bunch moth seldom attains pest status: there was one isolated case in Perak (Wahid *et al.*, 1991).

Sufficient 'management tools' are available for the control of these pests. Most of them concentrate on the use of selective insecticides such as Trichlorfon and Methamidophos for bagworms (Wood and Nesbit, 1969; Conway, 1966; Wood *et al.*, 1974), and Endosulfan and Cypermethrin for nettle caterpillars (Wood *et al.*, 1977; Chung, 1989). A granulosis virus has also been used successfully in the field for the control of nettle caterpillar outbreaks in Sarawak (Tiong and Munroe, 1977). The use of selective chemicals permits the conservation of natural enemies thus integrating chemical and natural biological control.

The use of *Bacillus thuringiensis* as a biocontrol agent also needs to be considered because of the many associated advantages as compared with the use of a chemical insecticide (Tryon, 1986); it is toxic only to the target insect and is generally harmless to natural enemies, man, fish and livestock. This paper reports the status of the use of *B. thuringiensis* in the control of nettle caterpillars and bunch moths in the field. It also reports laboratory results on

the use of *B. thuringiensis* for bagworm control.

## NETTLE CATERPILLARS

Field trials on the use of commercial formulations of *B. thuringiensis* against nettle caterpillars have been reported by Chung (1989), Ho (1988) and Ho and Sidhu (1986). A summary of the details of the trials is shown in Table 1. Of the seven reported trials, only two demonstrated that *B. thuringiensis* was effective against *Setora nitens* in Sabah, applied at rates of 0.6, 0.9 and 1.2 kg of formulated product (16 000 IU/mg) per hectare (Ho and Sidhu, 1986). Below 0.6 kg product/ha, the *B. thuringiensis* product was reported as not effective.

*Bacillus thuringiensis* has also been field-tested against *Ploneta diducta*, *Darna trima* and *S. nitens*, but it gave poor results with all of them (Chung, 1989 and Ho, 1988).

## BUNCH MOTH

A field trial was done on the effect of *B. thuringiensis* on the bunch moth, *Tirathaba rufivena* in 1988 (Wahid *et al.*, 1991). The trial was carried out in a mature oil palm plantation, planted in 1974, with a plot size of 4 × 6 palms and 6 replicates. Besides *B. thuringiensis*, which was applied at a rate of 0.5 kg (16 000 IU/mg) formulated product per hectare, several chemical insecticides, namely Cyfluthrin, Diflubenzuron and Endosulfan were also included as treatments. The insecticides were applied three times at two-week intervals using a knapsack power sprayer with a 25 to 30 foot telescopic aluminium lance, meant to raise the spray nozzle to the level of the palm crowns. The results of the trials showed that *B. thuringiensis*, Cyfluthrin and Diflubenzuron were more effective than Endosulfan in the control of the bunch moth. This was assessed on the basis of larval densities and bunch damage after treatment. Overall however, *B. thuringiensis* gave the best performance, followed by cyfluthrin and diflubenzuron. Further, the cost of application of *B. thuringiensis* was much cheaper than that of diflubenzuron.

The field effectiveness of *B. thuringiensis* has been confirmed by Tan and Mukesh (per-

TABLE 1. A SUMMARY OF PAST FIELD TRIALS ON THE EFFECTS OF *B. thuringiensis* AGAINST VARIOUS SPECIES OF NETTLE CATERPILLARS

Trial No.	Species	Palm age (yrs)	Plot size/ number of replications	Sprayer	Rate of applications	Remarks	References
1.	<i>Setora nitens</i>	< 5	4x4 palm/3	Portable mist-blower	1.2 kg f/ha *	Effective, 95% protection at 7 DAT	Ho and Sidhu (1986)
2.	<i>S. nitens</i>	< 5	4x4 palm/3	Portable mist-blower	1.2 kg f/ha 0.9 kg f/ha 0.6 kg f/ha *	Effective) 99% protection Effective) at 7 DAT	Ho and Sidhu (1986)
3.	<i>S. nitens</i>	< 5	4x4 palm/3	Portable mist-blower	0.4 kg f/ha 0.3 kg f/ha *	Not effective 73% to 75% protection at 7 DAT	Ho and Sidhu (1986)
4.	<i>S. nitens</i>	7	6x7 palm/3	Portable mist-blower	19 g a.i./ha *	Not effective, poor results	Chung (1989)
5.	<i>Ploneta diducta</i>	7	5 ha/2	Centrifugal fan mist-sprayer	0.6 kg f/ha *	Not effective, poor results Poor spray coverage	Ho (1988)
6.	<i>P.diducta</i>	7	6x7 palm/3	Turbo mist-blower	19 g a.i./ha	Not effective, poor results	Chung (1989)
7.	<i>Darna trima</i>	7	6x7 palm/3	Turbo mist-blower	19 g.a.i./ha *	Not effective, poor results	Chung (1989)

Note: DAT = days after treatment  
f/ha = formulated product per hectare  
\* = single application

sonal communication) who found that 90% control of *Tirathaba* could be obtained. As a result, this biological insecticide has completely replaced chemical insecticides for bunch moth control over the last four years.

#### BAGWORMS

A summary of past field trials testing the effects of *B. thuringiensis* against the bagworm is shown in Table 2. No effective results have yet been reported (Ho, 1987, 1988; Ho and

Sidhu, 1986). A PORIM estate survey on bagworm also reported that Thuricide and Dipel had not been found effective against *M. plana* and *Pteroma pendula*, when used in outbreak situations (Wahid *et al.*, 1988). Liau (personal communication) also reported that Dipel had been applied aerially to control *Mahasena corbetti*. However, the treatment was not considered effective because after one week the percentage kill was only 70 per cent.

Because of the poor performance of *B. thuringiensis* under field conditions, PORIM

initiated laboratory investigations of this bioinsecticide against the bagworm *M. plana*. The objectives were to determine the effects of the various commercial formulations of *B. thuringiensis* against the bagworm and to determine a suitable dosage for control. The details are presented in this paper.

## MATERIALS AND METHODS

### Screening of commercial formulations

Eight commercial formulations of *B. thuringiensis* were evaluated in this study. Details of them, as well as their rates of application are shown in *Table 3*. All these products have been registered for field application in Malaysia except<sup>1</sup> for BCBT II and CGA-BT-237218. The controls were Methamidophos (7.5 ml formulated product/4.5 litre water; 50% active ingredient) and a blank solution of 0.05% Triton X-100. Suspensions of *B. thuringiensis* at the commercially recommended rates of application were prepared in 0.05% Triton X-100. The suspensions were stirred continuously by using two magnetic stirring plates prior to dipping of sterilized oil palm leaflets. The continuous stirring ensured a homogeneous suspension.

Oil palm leaflets, sampled from frond number 17, were cut into segments each with an approximate exposed area of 32 cm<sup>2</sup>. These leaflet segments were washed in 1% Teepol solution, surface sterilized with 75% ethanol and rinsed repeatedly in distilled water. The sterilized leaflets were air-dried and inoculated with *B. thuringiensis* by dipping into the suspensions prepared earlier. The dipping technique (Morris, 1988) is an alternative to feeding known concentrations of products on leaf-disks or in an artificial diet. Excess liquid was shaken off gently before allowing each inoculated leaflet segment to stand in a vial of distilled water for surface dryness, at a room temperature of 25 ± 2°C.

The test insect was the bagworm, *M. plana*, originally collected from the field. The larvae used were newly moulted first generation second and fourth instars, which had been reared in a

controlled environment room. Their selected weight ranges were 0.2–2.0 mg and 5.0–10.0 mg respectively.

The experiments were carried out in three replicates. For each replicate, five active larvae were allowed to feed on each of the inoculated leaflet segments prepared earlier. Each leaflet segment was inserted into a vial of distilled water which was placed in an enclosed transparent cylinder. These cylinders were incubated in the controlled environment room at a day temperature of 27 ± 1°C, a night temperature of 24 ± 1°C, 35%–50% relative humidity and a photoperiod of 12:12 (light:darkness) for one week.

Larval feeding, mortality and leaflet damage were recorded daily for seven days. After one week, the leaf area damaged was then measured with a Delta-T leaf area meter.

The data on percentage mortality were first transformed by arc-sine, while the data on leaf area damaged were analysed without any transformation. The effects of various treatments were then compared by analysis of variance.

### Lethal concentration of *B. thuringiensis*

This investigation was undertaken for Thuricide, Florbac and CGA-BT against second and fourth instars of *M. plana*. The procedure was similar to the method described above, except that five different concentrations were used for each formulation. A series of suspensions were prepared for dipping, containing 100, 20, 2, 1 and 0.1 ml or g of powdered product or soluble concentrate per litre of distilled water, to which 0.05% Triton X-100 had been added, (Morris, 1988). The manner of recording data was similar to that described above.

The LC<sub>50</sub> and LC<sub>75</sub> values were estimated by Probit analysis (Finney, 1971).

### Confirmation of pathogenicity

#### *Phase-contrast microscopy*

The tested larvae were removed by cutting open the bags. They were placed separately in vials containing a freshly prepared mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3. The gut was

TABLE 2. A SUMMARY OF PAST FIELD TRIALS ON THE EFFECTS OF *B. thuringiensis* AGAINST VARIOUS SPECIES OF BAGWORMS

Trial No.	Species	Palm age (yrs)	Plot Size/ Number of replications	Sprayer	Rate of applications	Remarks	References
1.	Mixture of <i>Pteroma pendula</i> <i>plana</i>	< 5	4x4 palm/2	Portable mist-blower	0.3 and 0.5 kg f/ha 2 applications	3 brands at 2 rates tested; none effective 30%–71% protection only	Ho and Sidhu (1986)
2.	<i>P. pendula</i>	7	5 ha/2	Centrifugal fan sprayer	0.6 kg f/ha *	Poor control	Ho (1988)
3.	<i>P. pendula</i>	9 and 15	6 ha/0	Pulsfog K4 super Bio	0.6 kg and 1.2 kg f/ha	Test fogging of <i>B. thuringiensis</i> No control; technique unsuitable for bagworm control	Ho (1987)
4.	<i>Clania tertia</i>	< 5	4x4 palm/3	Portable mist-blower	1.2 kg f/ha	Not effective 13% protection at 7 DAT	Ho and Sidhu (1986)

Note: DAT = days after treatment  
f/ha = formulated product per hectare  
\* = single application

TABLE 3. DETAILS OF *B. thuringiensis* PRODUCTS TESTED AND THEIR RESPECTIVE RATES OF APPLICATION AS RECOMMENDED BY THE MANUFACTURERS

No.	Brand names/details	Concentration of <i>B. thuringiensis</i>
1	Bactospeine (Pasteur Institute, Paris); 16 000 I.U./mg	1.10 g/litre
2	Thuricide (Sandoz Ltd., Basle Switzerland); 16 000 I.U./mg	0.89 g/litre
3	BCBT II (Bactec Corporation, Houston, USA); 16 000 I.U./mg	0.37 g/litre
4	Florbac (Duphur B.V. Weesp, Holland); 8500 I.U./mg	3.00 ml ( = 2.56 g) litre <sup>a</sup>
5	Foray (Novo-Nordisk a/s, Denmark) 12 700 I.U./mg	3.72 ml ( = 1.74 g) litre <sup>a</sup>
6	Dipel (Abbot Laboratories North Chicago, USA); 16 000 I.U./mg	0.83 g/litre
7	Biobit FC (Novo-Nordisk a/s, Denmark); 8400 I.U./mg	3.33 ml ( = 3.08g)/litre <sup>a</sup>
8	CGA-BT-237218 (Ciba-Geigy Ltd., Basle, Switzerland); 16 000 I.U./mg	1.50 g/litre

<sup>a</sup>Product is a soluble concentrate

squashed opened, stained with Gram's stain and observed directly under a Jenaval phase contrast light microscope.

#### **Electron microscopy**

The dead larvae were fixed briefly *in situ* with a fresh mixture of 2% para formaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer. They were rinsed with cacodylate buffer and postfixed in osmium tetroxide for 2 hours at 2°C before rinsing twice with double distilled water. Dehydration was done with acetone, followed by pre-infiltration, post-infiltration and embedding in 100% agar araldite. Three days later, semi-thin and ultra-thin sections were prepared using a Reichert Jung ultracut microtome; these were stained and scanned in a Philips 400 transverse electron microscope.

## **RESULTS**

### **Screening of commercial formulations at recommended rates**

Although in most cases, significant effects of various *B. thuringiensis* products on larval mortalities of *M. plana* were obtained (in comparison with the control) at both the second and fourth instars (Tables 4 and 5), none of the products inflicted high mortalities even by the seventh day after treatment (Figures 1 to 5).

At the second instar stage, the highest mortality recorded was 60%, found for CGA-BT, Dipel and Florbac. The mortalities caused by the rest of the *B. thuringiensis* formulations (Bactospeine, Thuricide, Foray, BCBT II and Biobit) were all below 40 per cent. It was also

found that generally the *B. thuringiensis* formulations were slow-acting and mortality among the larvae became noticeable only by about the fourth day after treatment.

At the fourth instar stage, a relatively high mortality was obtained with Florbac, reaching 80% at 7 days after treatment (7 DAT) (Figure 3). The next highest mortality was recorded for CGA-BT and Bactospeine, at 60% (Figures 4 and 5). The rest of the *B. thuringiensis* products (Foray, Thuricide, BCBT II, Biobit and Dipel) performed poorly in respect of control. As with the second instars, mortality among the fourth instars generally became apparent by the fourth day after treatment, except in the case of Florbac, where it was noticeable by the second day after treatment.

Methamidophos, which is currently a recommended insecticide for bagworm control, performed well, causing 100% mortality to both second and fourth instars of *M. plana* at 7 DAT. None of the *B. thuringiensis* formulations tested could match this performance.

The results on the effects of various treatments on the area of leaf damaged by second and fourth instars of *M. plana* (Tables 6 and 7) showed that none of the *B. thuringiensis* products performed as well as Methamidophos, in spite of significant reductions in leaf area damaged in comparison with the control.

Among the various formulations of *B. thuringiensis*, the largest reduction in percentage leaf area damaged (LAD) was 76.5%, for Thuricide (against the fourth instar). The formulations of

TABLE 4. ANALYSIS OF VARIANCE ON THE EFFECT OF *B. thuringiensis* ON THE MORTALITY OF SECOND INSTARS OF *M. plana*

No.	Treatments	Transformed means	Difference from control
1	Bactospeine	38.36	38.36**
2	Thuricide	30.00	30.00*
3	BCBT II	30.79	30.79*
4	Florbac	51.15	51.15**
5	Foray	35.01	35.01*
6	Methamidophos	90.00	90.00**
7	Dipel	51.15	51.15**
8	Biobit FC	25.78	25.78 ns
9	CGA BT	50.77	50.77**
10	Control	0	

Note:  $LSD_{0.05} = 27.75$   
 $LSD_{0.01} = 37.20$

TABLE 5. ANALYSIS OF VARIANCE ON THE EFFECTS OF *B. thuringiensis* ON THE MORTALITY OF FOURTH INSTARS OF *M. plana*

No.	Treatments	Transformed means	Difference from control
1	Bactospeine	55.78	45.52**
2	Thuricide	46.92	36.66**
3	BCBT II	42.70	32.44*
4	Florbac	68.07	57.81**
5	Foray	42.70	32.44*
6	Methamidophos	90.00	79.74**
7	Dipel	38.86	28.60*
8	Biobit FC	43.08	32.82**
9	CGA BT	56.15	45.89**
10	Control	10.26	

Note:  $LSD_{0.05} = 24.16$   
 $LSD_{0.01} = 32.74$

*B. thuringiensis* showing reductions in LAD above 70% were Thuricide (against second and fourth instars), CGA-BT (against second), Foray (against fourth) and Bactospeine (against fourth).

However, with Thuricide, the relatively high reduction in LAD was not accompanied by a high larval mortality. The recorded mortalities were low, at 33.3% and 46.1% for the second and fourth instars respectively. This means that although most of the larvae were alive, they were no longer feeding or they were feeding at a much reduced rate, accounting for the relatively high percentage reduction in LAD for Thuricide. A similar phenomenon was recorded for Foray against fourth instars.

#### Lethal concentration of *B. thuringiensis*

A high mortality up to 100%, can be obtained by applying high concentrations of Thuricide, Florbac and CGA-BT against both second and fourth instars of *M. plana* (Figures 6

to 11). With the exception of Florbac, concentrations of 2.0% and 10.0% active ingredient (a.i.) gave 100% mortality against fourth instars.

For the purpose of determining the  $LC_{50}$  and  $LC_{75}$  values of Thuricide and Florbac, data with 100% mortality were excluded because the regression analysis could not include points with this level of mortality. Suitable sets of data in which the higher levels of mortality were slightly above 90% were selected. As a result, the  $LC_{50}$  and  $LC_{75}$  were examined at different days after treatments. Significant concentration-mortality response relationships were obtained for both Thuricide and Florbac, and their respective  $LC_{50}$  and  $LC_{75}$  values could be determined from the regression equations (Table 8).

#### Confirmation of pathogenicity

Phase-contrast microscopical examination of dead bagworm larvae subjected to various treatments revealed that they were infected



TABLE 6. THE ANALYSIS OF VARIANCE ON THE EFFECT OF *B. thuringiensis* ON THE LEAF AREA DAMAGED BY SECOND INSTARS OF *M. plana*

No.	Treatment	Mean leaf area damaged (cm <sup>2</sup> )	Difference from control	% Reduction in damage
1	Bactospeine	1.15	1.58***	57.9
2	Thuricide	0.72	2.01***	73.6
3	BCBT II	1.91	0.82*	30.0
4	Florbac	1.14	1.59***	58.2
5	Foray	1.12	1.61***	59.0
6	Methamidophos	0	2.73***	100.00
7	Dipel	0.97	1.76***	64.5
8	Biobit FC	1.02	1.71***	62.0
9	CGA BT	0.74	1.99***	72.9
10	Control	2.37		

Note: LSD<sub>0.05</sub> = 0.77  
 LSD<sub>0.01</sub> = 1.04  
 LSD<sub>0.001</sub> = 1.40

by numerous spore-bearing crystalliferous bacilli (Figure 12). The rods varied in form and size. In the case of BCBT II, the rods were short with a clearly visible central spore. Bagworms treated with Florbac, Thuricide and Bactospeine revealed considerable numbers of short rods and abundant extracellular proteinaceous toxic crystals. Treatments with Foray and CGA-BT-237218 gave rise to equal multiplication of both long and short rods within the larval midgut, while Dipel resulted in the proliferation of numerous very long and a few short spore-bearing bacilli. The bacilli were similar to those in cultures from the respective commercial products. The ability of these bacilli to produce crystals was confirmed by plate cultures. Histopathological examination in a transverse electron microscope showed that these bacilli ultimately caused intensive destruction of the midgut. Total destruction of intestinal columnar cells, goblet cells,

microvilli, transitional cells and underlying muscle fibres was observed. As a consequence, the whole gut content was intoxicating the hemolymph and forming semi-solid material comprising numerous bacilli and toxic proteinaceous crystals (Figure 13). A normal gut structure is as shown in the control (Figure 14).

## DISCUSSION

Efforts have been made to exploit *B. thuringiensis* in the control of oil palm pests such as nettle caterpillars and bagworms (Ho and Sidhu, 1986; Chung 1989). However the level of field success is extremely low. Such a low rate of success may be associated to the lack of laboratory studies involving screening and the establishment of dosage-response curves for each target pest, from which suitable products and appropriate field application rates could be

recommended. These studies ought to be regarded as pre-requisites for field trials and the importance of such studies has been emphasized by Dulmage (1986) who commented that two species of the same genus may respond differently to the same product containing *B. thuringiensis*.

The use of *B. thuringiensis* for the effective control of bunch moth (Wahid *et al.*, 1991; Tan and Mukesh, personal communication) without any laboratory studies is an exception to the above remarks. It was by chance that the *B. thuringiensis* product and the rate tested were found effective in the field.

The field work by Ho and Sidhu (1986), against *S. nitens* illustrated the importance of

the right dosage for effecting control. From their field trials, it was seen that a dosage of more than 0.6 kg of formulated product per hectare was required for satisfactory results.

For the purpose of biological control, a mortality of 75% would appear acceptable, sufficient to bring about a reduction of the pest population, as advocated by Ooi (1978) after his investigation on the coconut leaf moth, *Artona catoxantha*. Among the commercial formulations of *B. thuringiensis* examined on bagworms, all, with a partial exception in the case of Florbac, showed poor performances (< 75% mortality) even at seven days after treatments. With Florbac, the effectiveness was marginal because high

TABLE 7. ANALYSIS OF VARIANCE OF THE EFFECTS OF *B. thuringiensis* ON THE LEAF AREA DAMAGED BY FOURTH INSTARS OF *M. plana*

No.	Treatment	Mean leaf area damaged (cm <sup>2</sup> )	Difference from control	% Reduction in damage
1	Bactospeine	1.48	3.75***	71.7
2	Thuricide	1.23	4.00***	76.5
3	BCBT II	3.13	2.10*	40.2
4	Florbac	2.11	3.12**	59.7
5	Foray	1.34	3.89***	74.4
6	Methamidophos	0.03	5.20***	99.4
7	Dipel	5.46	-0.23ns	0
8	Biobit FC	4.24	0.99ns	18.9
9	CGA BT	2.85	2.38	45.5
10	Control	5.23		

Note: LSD<sub>0.05</sub> = 1.86  
LSD<sub>0.01</sub> = 2.53  
LSD<sub>0.001</sub> = 3.38

TABLE 8. CONCENTRATION-MORTALITY RESPONSE EQUATIONS OF THURICIDE AND FLORBAC AGAINST LARVAE OF *M. plana*

Product	Larval instar	DAT	Regression equations	R <sup>2</sup>	F.Value	LC <sub>50</sub> (% a.i.)	LC <sub>75</sub> (% a.i.)
Thuricide	2	2	$Y = 5.272 + 0.433 \log_e X$	0.969	92.83***	0.53	2.54
	4	3	$Y = 5.392 + 0.366 \log_e X$	0.946	35.24*	0.34	2.17
Florbac	2	4	$Y = 5.244 + 0.397 \log_e X$	0.946	52.25**	0.54	2.96
	4	5	$Y = 5.671 + 0.403 \log_e X$	0.954	62.55***	0.19	1.01

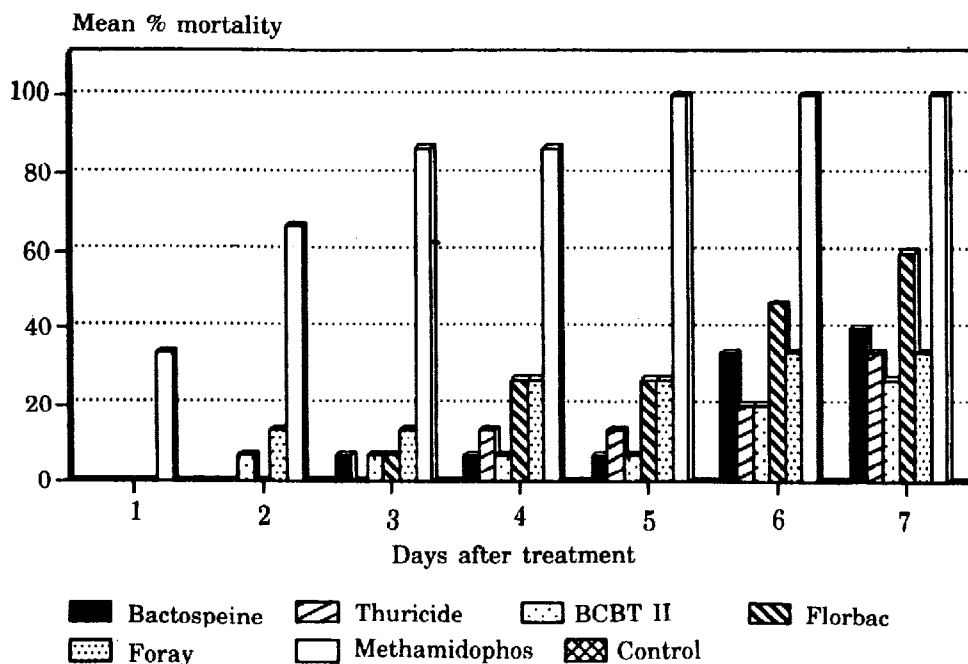
Note: Y = probit of per cent corrected mortality  
 X = per cent concentration  
 \*, \*\*, \*\*\* = significance at 5%, 1%, 0.5% respectively  
 % a.i. = percentage active ingredient

mortality (80%) was obtained only at the fourth instar stage and not at the second. This could be associated with the higher feeding rate of the fourth instars than of the second (Wahid 1993). This laboratory finding was consistent with the poor field performance of *B. thuringiensis* as reported by other workers (e.g. Ho and Sidhu, 1986).

The concentrations of *B. thuringiensis* recommended for control by the various manufacturers may not be suitable for *M. plana*. Accordingly, the concentration-mortality response investigation was carried out for Thuricide and Florbac. The extrapolated results indicated that in order to achieve a 75% kill of bagworms within a palm, 20 litres of water containing 502 g product of Thuricide would be required. The corresponding value for Florbac is 592 ml. These values were 28 and 9 times the respective concentrations recommended by the manufacturers (Table 3). This suggests that a high concentration of *B. thuringiensis* would be required for bagworm control and partly explains the poor performance of *B. thuringiensis* in the laboratory and in the field.

However, increasing the concentration of *B. thuringiensis* would not be desirable because it would entail a substantial increase in cost, and to levels far above those incurred with a chemical insecticide such as Monocrotophos. For Thuricide and Florbac, it would cost RM20.00 and RM40.25 respectively to spray an entire palm, while with Monocrotophos, the cost is only RM0.65 per palm (Chung, 1989). It is therefore imperative that the causes for the lack of effectiveness of various commercially available *B. thuringiensis* be examined. These causes may be associated with gut pH, proteases, the presence and type of receptor (toxin-binding) protein, and membrane interactions with the cytolytic domains of the toxin, (Milne *et al.*, 1990).

Besides examining commercially available formulations of *B. thuringiensis*, the task of collecting and identifying local strains of *B. thuringiensis* for bagworm control should be initiated. Such an approach was advocated by Tryon and Litsinger (1988) who reported that several among 312 strains of *B. thuringiensis* in the Philippines were more toxic to rice, vegetable and corn insect pests than the commer-



Note: The value for control was zero.

Figure 1. Mortality of second instars of *Metisa plana* subjected to *Methamidophos* and five formulations of *Bacillus thuringiensis*.

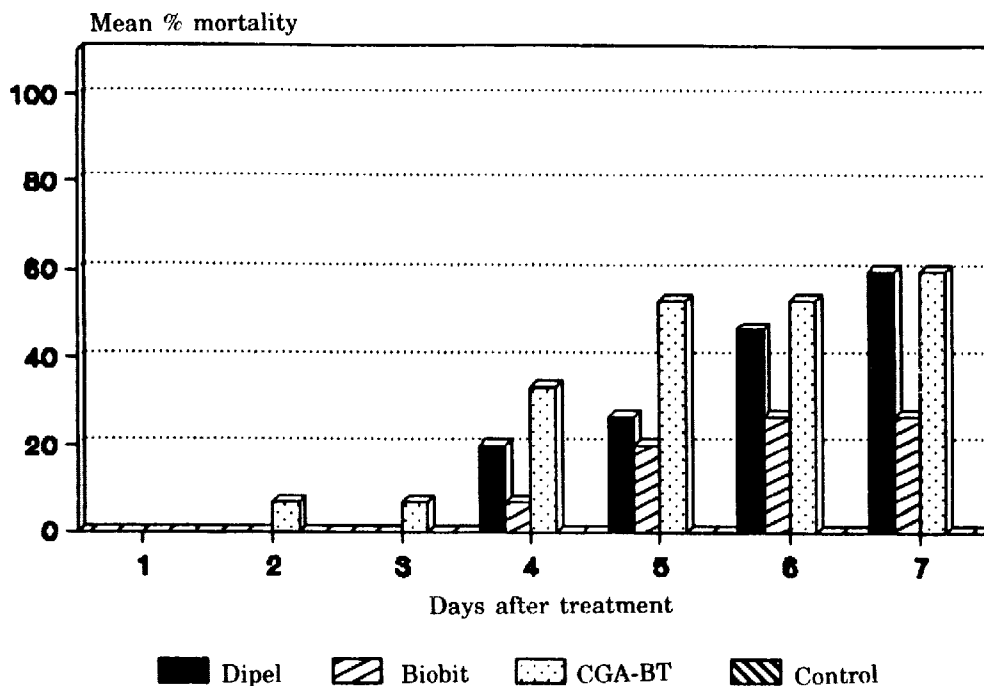


Figure 2. Mortality of second instars of *Metisa plana* subjected to three formulations of *Bacillus thuringiensis*.

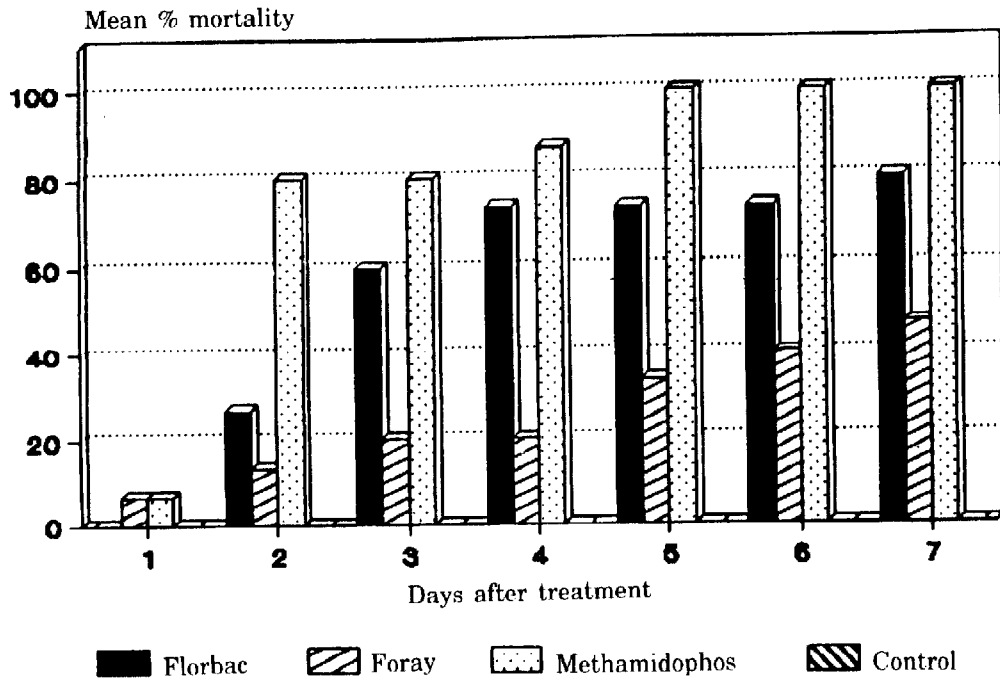


Figure 3. Mortality of fourth instars of *Metisa plana* subjected to two formulations of *Bacillus thuringiensis*.

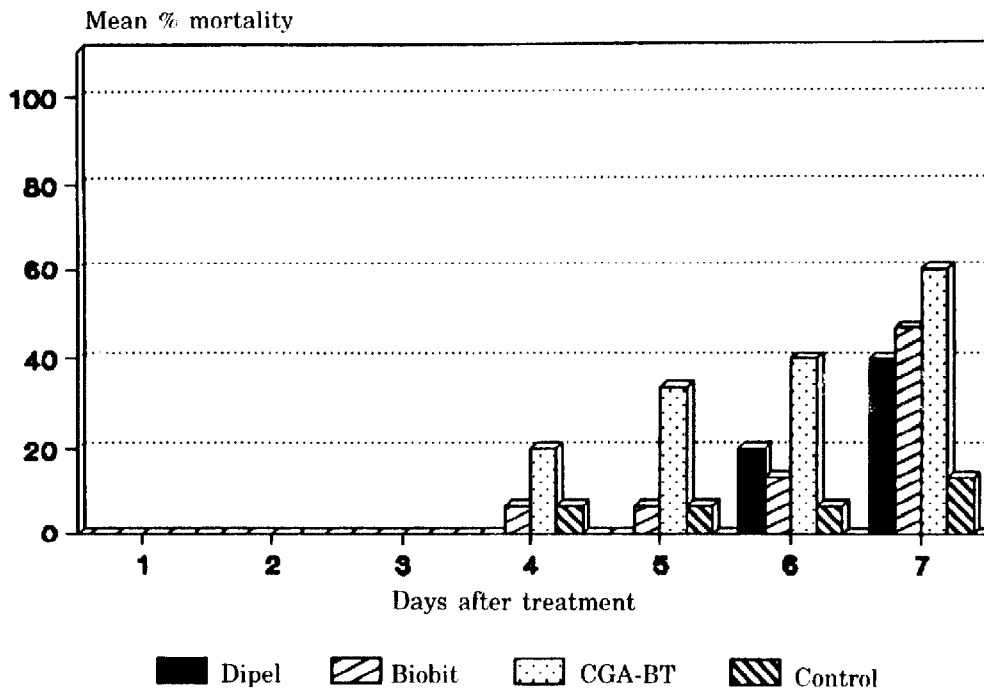


Figure 4. Mortality of fourth instars of *Metisa plana* subjected to three formulations of *Bacillus thuringiensis*.

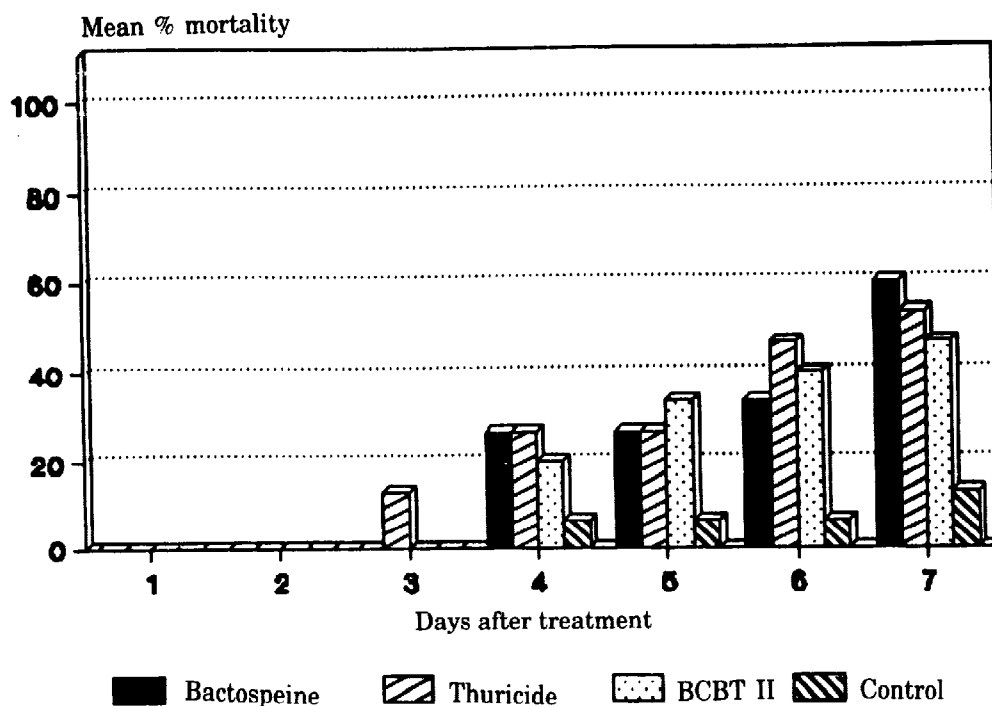


Figure 5. Mortality of fourth instars of *Metisa plana* subjected to three formulations of *Bacillus thuringiensis*.

cial strains of *B. thuringiensis*. Other advocates of such an approach include Maddox (1982) and Jangi and Mahadi (1990).

There is a prospect of finding suitable *B. thuringiensis* isolates for bagworm control, because in our present investigations we have been able to confirm the pathogenicity of *B. thuringiensis* products for *M. plana*. Histopathological examination of dead larvae of *M. plana* under a transverse electron microscope revealed total destruction and disintegration of the mid-gut epithelia. Similar results have been reported by Mathavan *et al.* (1989) and by Percy and East (1983) on the silkworm, *Bombyx mori*.

### CONCLUSIONS

Success in the use of commercial formulations of *B. thuringiensis* in the control of the bunch moth and a species of nettle caterpillar suggests that there may be a prospect for the exploitation of *B. thuringiensis* to control pests of oil palm. Past field trials were based on commercial recommendations

which were not supported by basic laboratory studies. Such studies are essential to establish the rate of field application so as to ensure a reasonable kill, as demonstrated by the laboratory study on *M. plana*.

Various commercial products containing *B. thuringiensis* were generally not potent against *M. plana*. Factors governing such a lack of potency need to be elucidated if the benefits of *B. thuringiensis* as a biocontrol agent are to be realized. In addition, a search for more virulent local strains of *B. thuringiensis* ought to be made.

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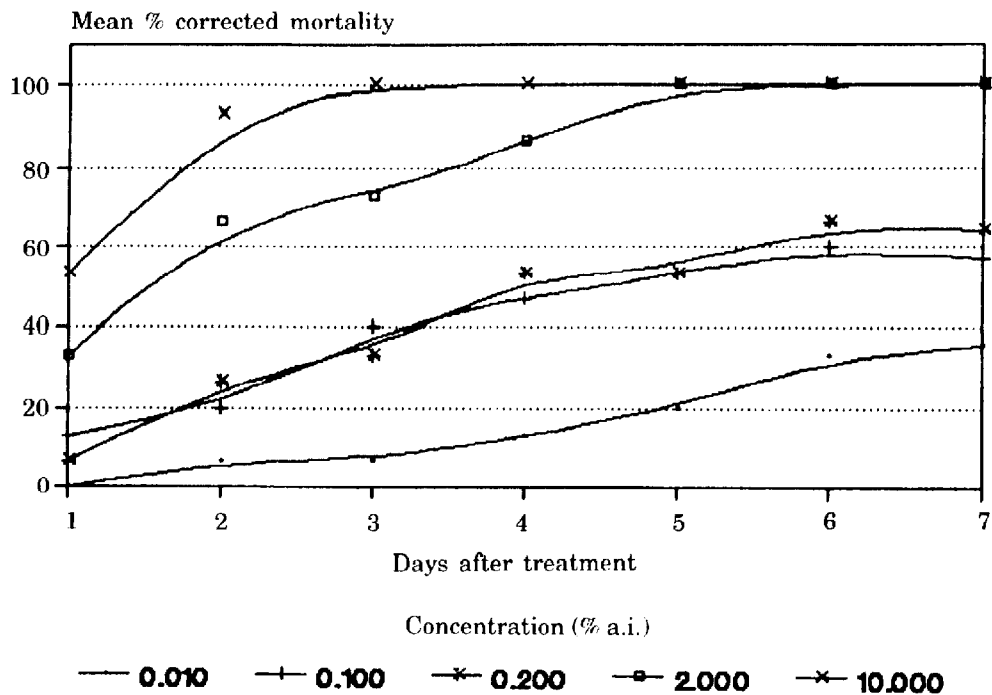


Figure 6. Concentration/response curves of second instars of *Metisa plana* treated with Thuricide.

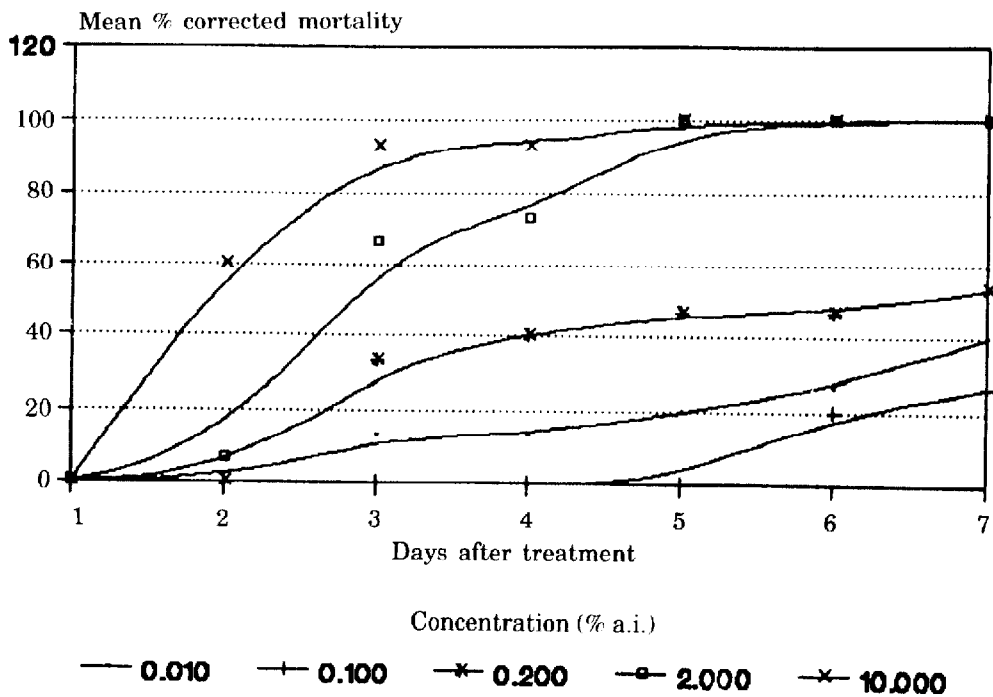


Figure 7. Concentration/response curves of fourth instars of *Metisa plana* treated with Thuricide.

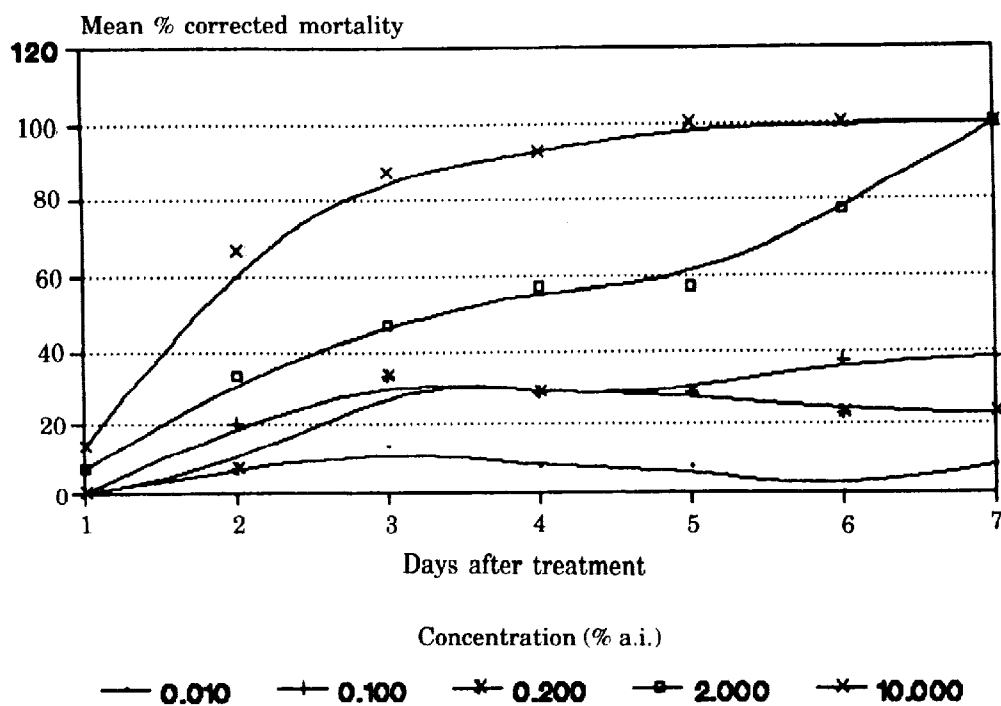


Figure 8. Concentration/response curves of second instars of *Metisa plana* treated with Florbac.

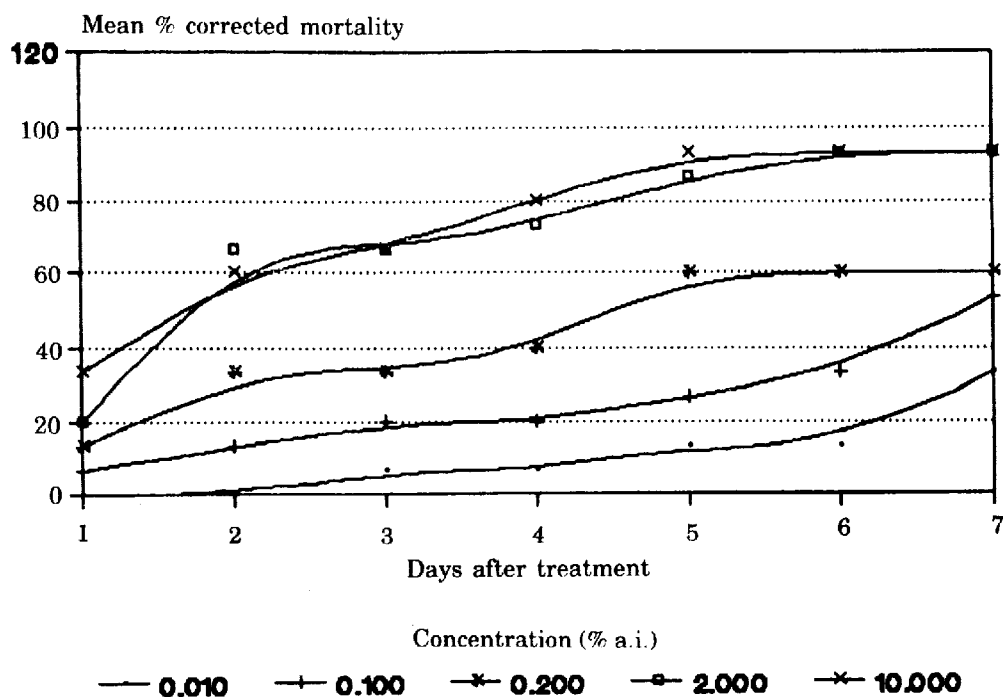


Figure 9. Concentration/response curves of fourth instars of *Metisa plana* treated with Florbac.



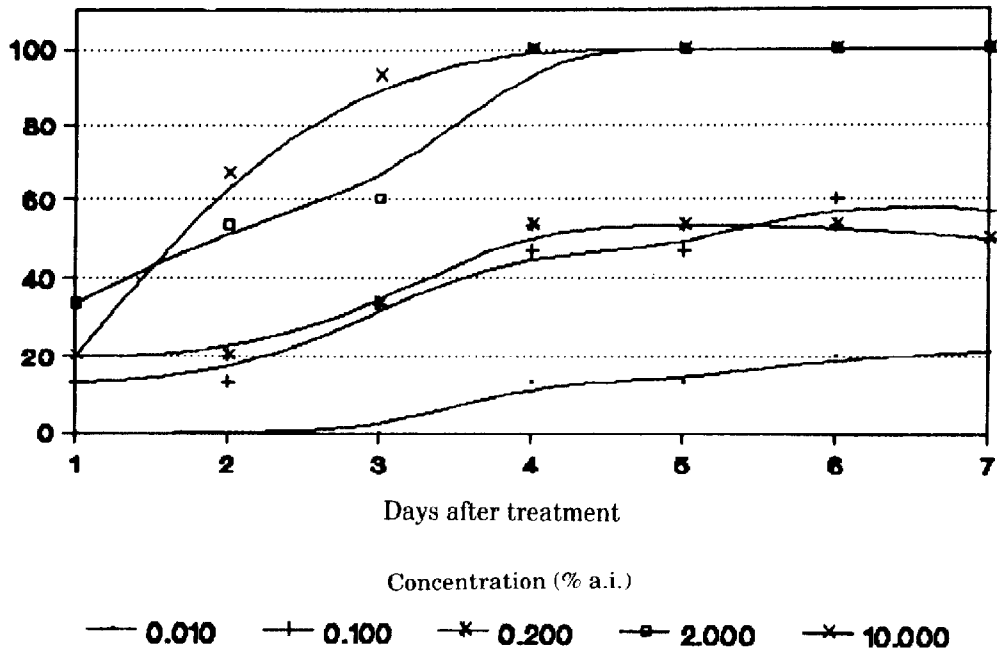


Figure 10. Concentration/response curves of second instars of *Metisa plana* treated with CGA-BT.

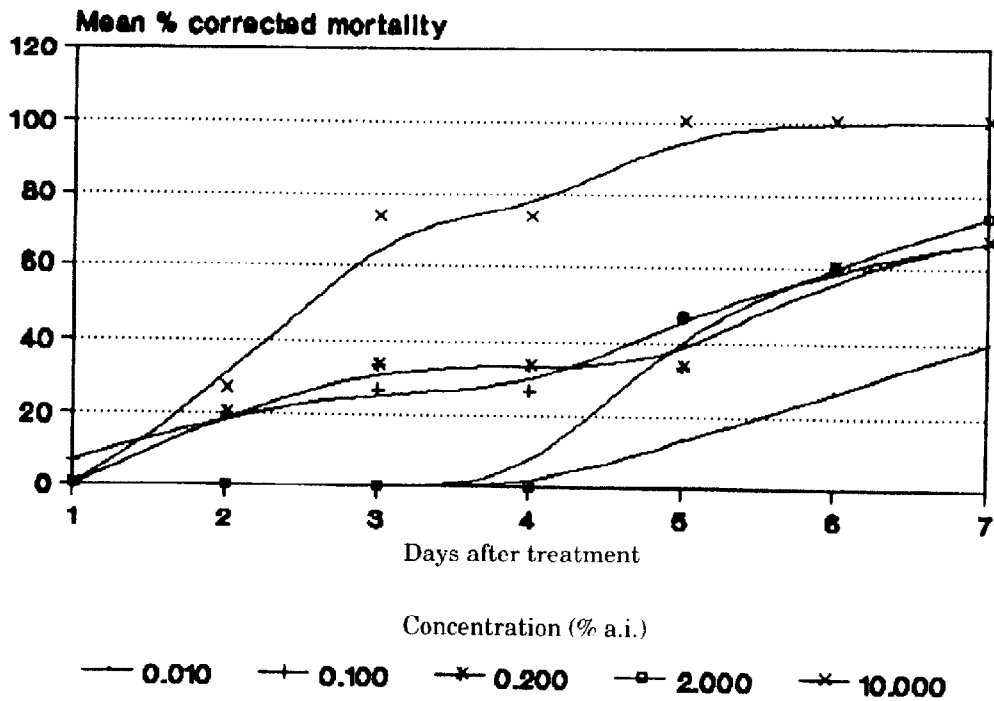


Figure 11. Concentration/response curves of fourth instars of *Metisa plana* treated with CGA-BT.



Figure 12. Phase-contrast micrograph showing the squashed portion of dead *Metisa plana* larva releasing numerous pathogenic bacilli (b) and the proteinaceous toxic crystals (c).x 650.

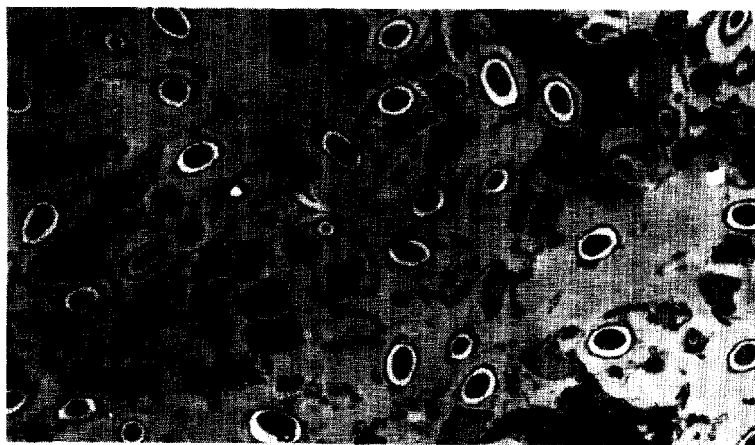


Figure 13. Transmission electron micrograph of severely degenerated gut of *Metisa plana* larva with *Bacillus thuringiensis* spores (b) and crystals (c). x 20 000.



Figure 14. Ultrastructure of normal *Metisa plana* gut, showing the microvilli (mv) and part of the columnar epithelium cell (ce). x 20 000.

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