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EFFICACY OF *BACILLUS THURINGIENSIS* BERLINER SUBSPECIES *KURSTAKI* AND *AIZAWAI* AGAINST THE BAGWORM, *METISA PLANA* WALKER ON OIL PALM

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Abstrak: Efikasi *Bacillus thuringiensis* subspesies *kurstaki (Btk)* dan *aizawai (Bta*) terhadap larva instar ketiga dan kelima *Metisa plana* pada kelapa sawit telah dikaji. Tujuan kajian ialah untuk menentukan tahap kematian yang boleh dicapai oleh enam produk *Bt* terpilih terhadap *M. plana*. Bioasai di makmal telah dijalankan ke atas formulasi yang mengandungi *Btk*: Dipel® ES, Dipel® DF, Dipel® WP dan ABG-6429 FC® (formulasi untuk penyelidikan), dan *Bta*: Florbac® SC dan Xentari® WG pada suhu 24°C–29°C dan 55%–80% kelembapan bandingan (RH) dengan menggunakan kaedah celupan daun. Kedua-dua subspecies telah menunjukkan keberkesanannya terhadap ulat bungkus itu. Dalam kebanyakan keadaan, larva instar ketiga memerlukan sedikit *Bt* serta masa yang singkat untuk mengakibatkan kematian berbanding larva instar kelima.

Kata Kunci: Bioasai, Ulat Bungkus, *Metisa plana*, *Bacillus thuringiensis* subspecies *kurstaki* dan *aizawai*

Abstract: The efficacy of *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*) and *aizawai* (*Bta*) against the third and fifth instar larvae of *Metisa plana* on oil palm was studied. The objective of the study was to determine the level of kill achievable with six selected *Bt* products on *M. plana*. Laboratory bioassays were performed on formulations containing *Btk*: Dipel® ES, Dipel® DF, Dipel® WP and ABG-6429 FC® (research formulation), and *Bta*: Florbac® SC and Xentari® WG at temperatures of 24°C–29°C and 55%–80% relative humidity (RH) by using leaf dipping method. Both subspecies were shown to be effective on the bagworms. In most cases, the third instar larvae required lesser amount of *Bt* and shorter time for a satisfactory kill compared to the fifth instar larvae.

Keywords: Bioassay, Bagworms, *Metisa plana*, *Bacillus thuringiensis* subspecies *kurstaki* and *aizawai*

INTRODUCTION

The bagworms *Metisa plana* and *Pteroma pendula* were first recorded on oil palm in Malaysia in 1947 and they attained pest status in 1954 when they infested about 1,000 acres of oil palm in an estate in the state of Perak (Yunus 1966). During those years, broad spectrum and long residual contact insecticides like DDT, dieldrin and endrin were applied on oil palms to control the bagworms (Wood 1968; 1971). The usage of these broad spectrum insecticides which were generally detrimental to the natural enemies caused population explosion of the

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bagworms. After these dramatic results, Wood (1971) introduced the idea of integrated pest management (IPM) on oil palm. One of the ideas was the choice for selective insecticides, permitting the survival of natural enemies and substantially reducing bagworm numbers. The most likely candidates were stomach poisons, contact poisons with a degree of selectivity against Lepidoptera or those with short-lived residues. In line with these ideas, *Bacillus thuringiensis* was first suggested by Wood (1968) for the IPM of leaf-eating caterpillars on oil palm. The biocide is advantageous because it is toxic on target insects but harmless to natural enemies, man, fish and livestock (Tryon 1986). There are two common subspecies that act specifically against many species of Lepidoptera, namely *kurstaki* (*Btk*) and *aizawai* (*Bta*). Both *Bt* subspecies were tested against *M. plana* in the laboratory by Basri *et al.* (1994; 1996). They found that *Bta* was more efficacious than *Btk* and the overall larval control by *Btk* was poor. In these studies, the mass rearing of *M. plana* was not described and the the original percentage of larval mortality in the control was not mentioned.

With the establishment of a rearing protocol for *M. plana* (Tan *et al.* 2006), relatively healthy and standardized insects were produced for laboratory bioassay of *Bt* against the bagworms. The use of the insects fulfilled the principle of bioassay that in its widest sense covers all experiments where the potency of an insecticide is measured by reference to standardized insect colonies (Busvine 1971; Reichelderfer 1985). Therefore, the screening of *Bt* on *M. plana* was repeated. The objective of the study was to determine the level of kill achievable with six selected *Bt* products on *M. plana*.

MATERIALS AND METHODS

Metisa plana was obtained from colonies reared on young oil palm seedlings maintained in the screenhouse of Universiti Putra Malaysia; the rearing protocol was as described by Tan *et al.* (2006).

The larvae were starved overnight before exposure to *Bt*. Six formulations were obtained from Abbott Laboratories, U.S.A. in 1999 and tested at concentrations of 0, 2, 4, 6, 8 and 10 gl^{-1} against the third (L3) and fifth instar (L5) larvae (Table 1). The efficacy of each *Bt* formulation on *M. plana* was determined using the leaf-dip method. Oil palm leaflets used were collected from the middle stratum of palm canopy. These leaflets were prepared by briefly soaking and then rinsing three times with running tap water and cut to 25.0 cm from the leaf tip. *Bt* aliquots were prepared in distilled water with the addition of 0.05% (v/v) of Triton-X 100. The excised leaflets were completely immersed for five seconds in a 100–150 ml aliquot. For control, excised leaflets were immersed in the aqueous solution of Triton-X-100 only. The treated leaflets were then allowed to air-dry for about 20 min. Per test unit, 10 larvae of *M. plana* were caged with two treated excised leaflets in a modified cage system consisting of a 1.5 l polyethylene terephthalate bottle (PET) overlaid with clear polythene sheet. The PET bottle was then placed upright in a clear plastic container of 2 cm distilled water. The treated excised leaflets were replaced with fresh leaflets four days after treatment (DAT). The bioassay was conducted in an ambient

environment of 24°C–29°C and 55%–80% RH. Each treatment was replicated six times.

Table 1: Formulations of *Bt* tested on the specific larval instars of *M. plana*.

Formulations	Instar stages
Dipel® DF (*Bt* subspecies *kurstaki*; 32 000 I.U. mg^{-1})	3rd and 5th
Dipel® ES (*Bt* subspecies *kurstaki*; 17 600 I.U. mg^{-1})	3rd and 5th
Florbac® SC (*Bt* subspecies *aizawai*; 8 500 I.U. mg^{-1})	3rd and 5th
Xentari® WG (*Bt* subspecies *aizawai*; 15 000 I.U. mg^{-1})	3rd and 5th
Dipel® WP (*Bt* subspecies *kurstaki*; 16 000 I.U. mg^{-1})	3rd
ABG-6429 FC (research formulation) (*Bt* subspecies *kurstaki*; 10 600 I.U. mg^{-1})	3rd

Dead larvae were recorded daily up to seven DAT. The larvae were considered dead when they did not respond to prodding. The results were subjected to probit analysis using the POLO-PC Package (LeOra Software, 1987). The percentage of larval mortality was calculated and subjected to arc sine transformation. Transformed values were analyzed using analysis of variance (ANOVA) and the mean larval mortalities between concentrations were separated using least significant difference (LSD) tests at α = 0.05. Statistical analyses were carried out using SAS version 8.12 (SAS System, 2001).

RESULTS AND DISCUSSION

Figures 1 to 6 show the mean percentage mortality of L3 and L5 obtained from six *Bt* formulations at three and seven DAT. Controls in the experiment exhibited less than 10% mortality up to seven DAT. Results of larval mortalities showed that formulations containing either *Btk* or *Bta* were intrinsically effective on *M. plana* and high larval mortality (70%–100%) could be achieved at 2 to 10 gl^{-1} concentrations by seven DAT.

Probit analyses were performed on results generated from 2–4 DAT depending on whether the dose-response curve could be fitted under the probit requirements. The overall account of the results summarized in Tables 2 and 3 show the dose responses of six *Bt* formulations against the 3rd and 5th instar larvae of *M. plana*.

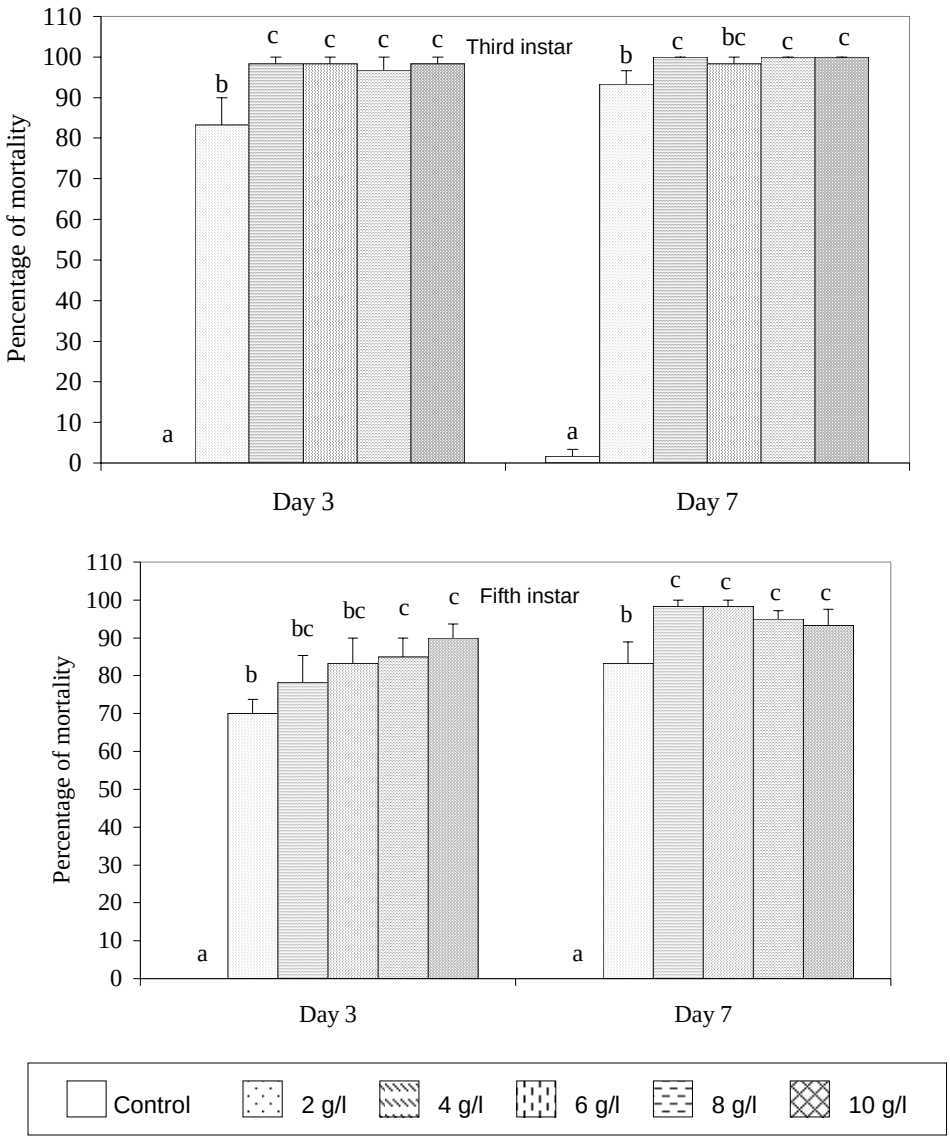

Figure 1: The levels of kill achievable with the L3 and L5 of *M. plana* at various concentrations of Dipel® DF on 3 and 7 DAT. The vertical lines are standard errors of means.

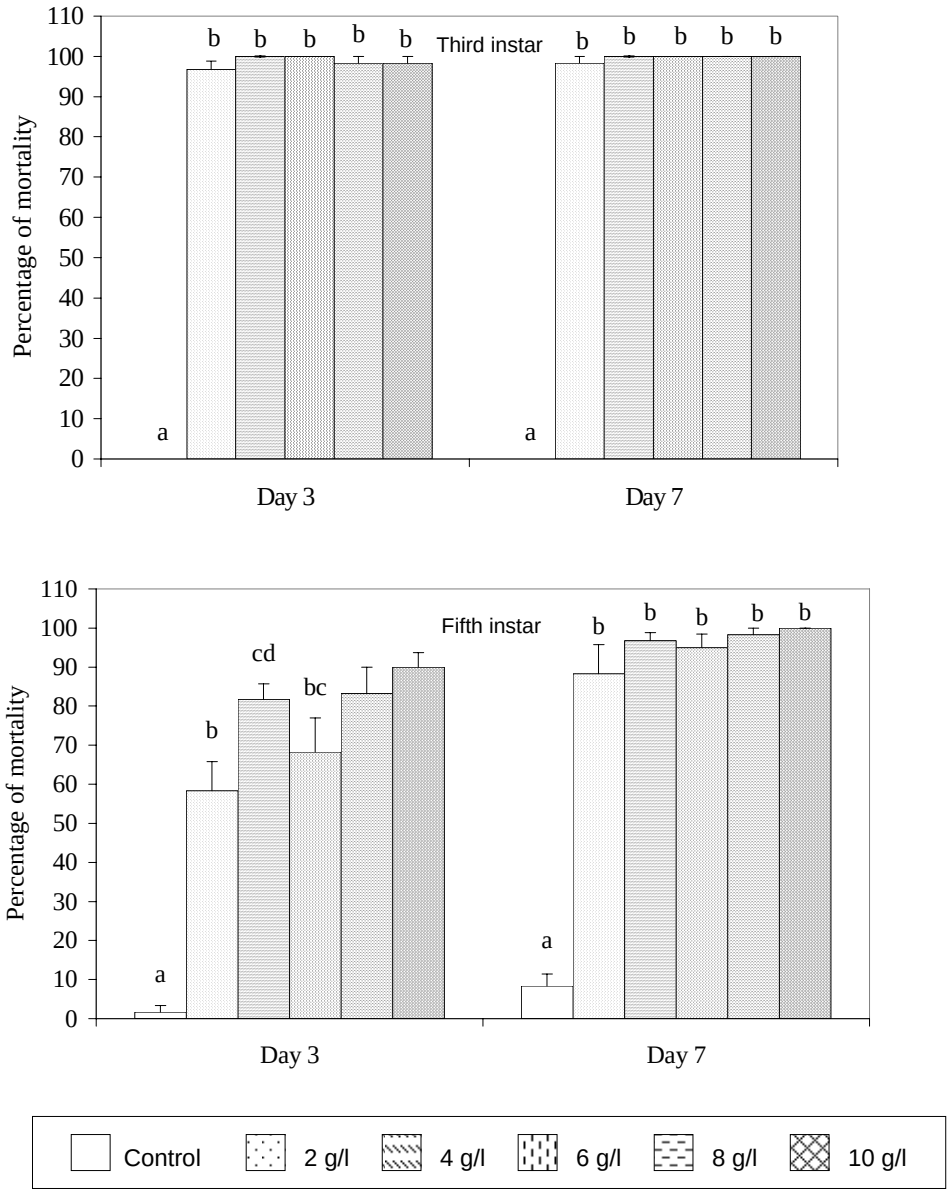

Figure 2: The levels of kill achievable with the L3 and L5 of *M. plana* at various concentrations of Dipel® ES on 3 and 7 DAT. Vertical lines are standard errors of means.

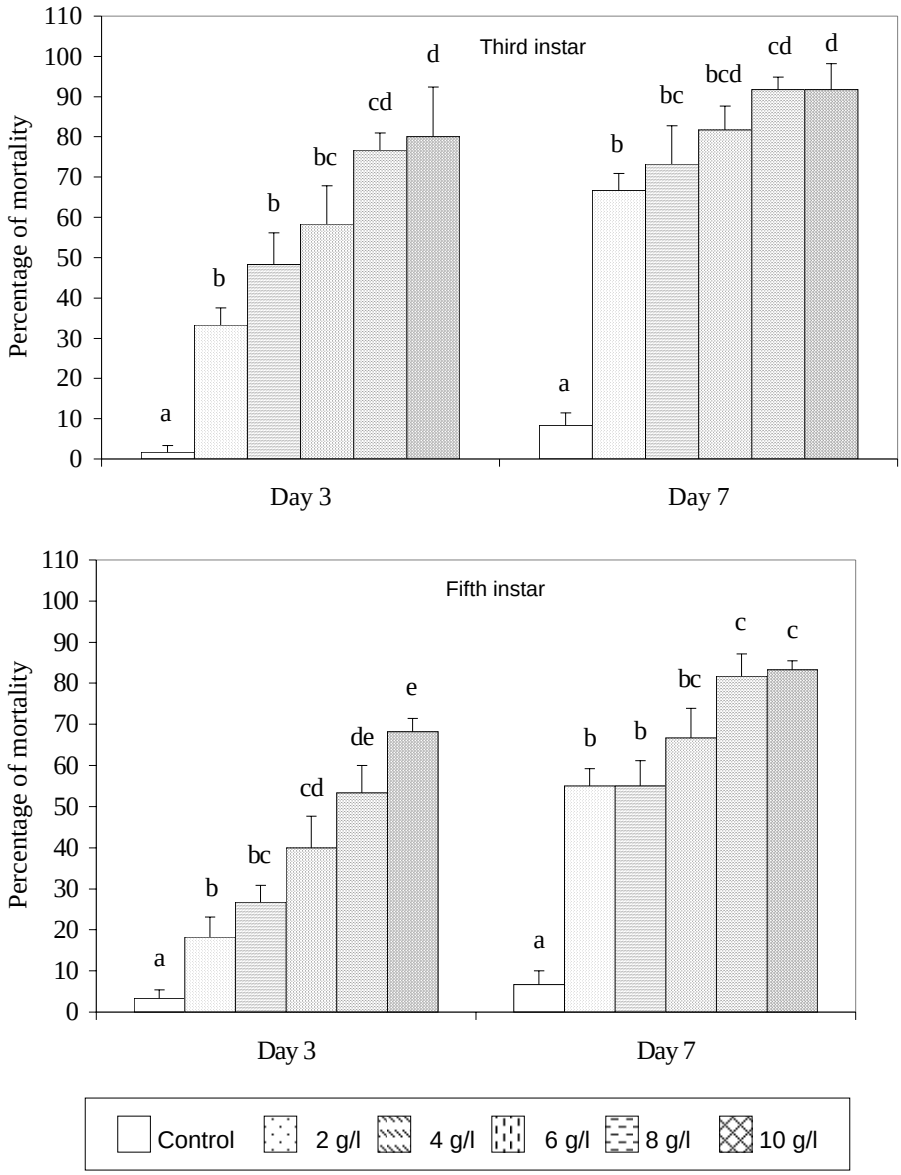

Figure 3: The levels of kill achievable with the L3 and L5 of *M. plana* at various concentrations of Florbac® SC on 3 and 7 DAT. The vertical lines are standard errors of means.

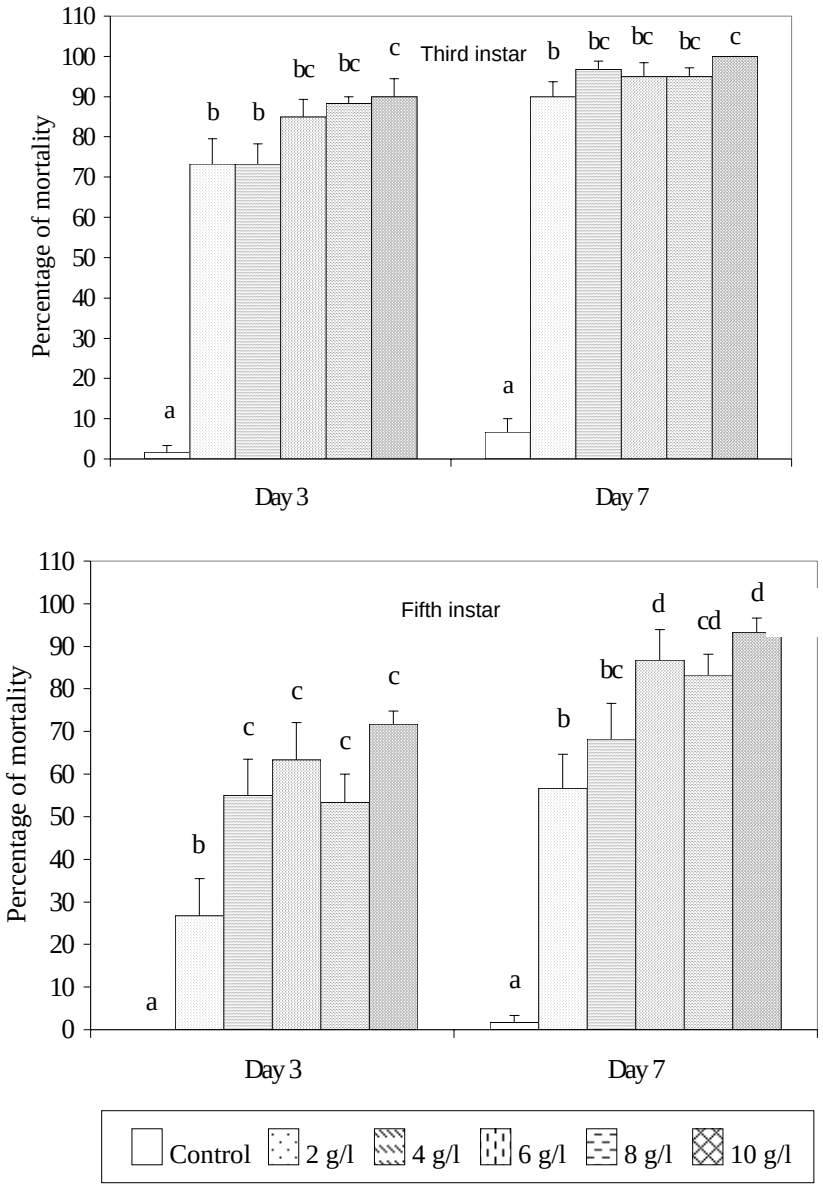

Figure 4: The levels of kill achievable with the L3 and L5 of *M. plana* at various concentrations of Xentari® WG on 3 and 7 DAT. The vertical lines are standard errors of means.

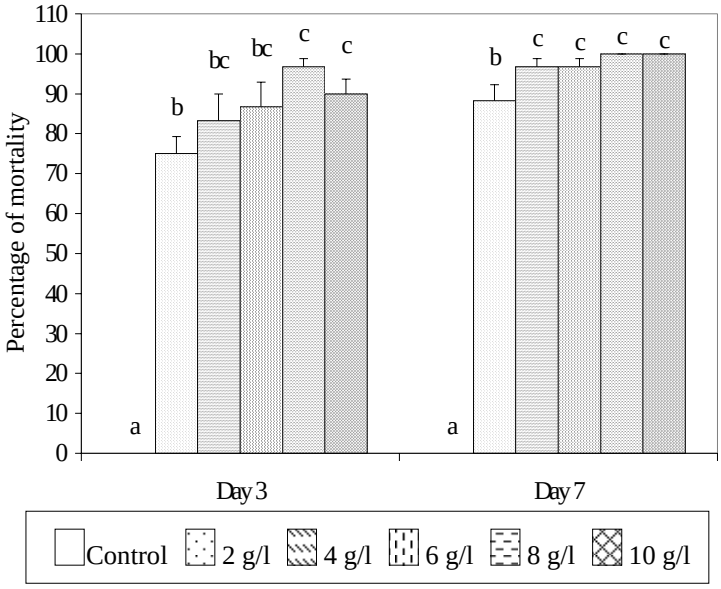

Figure 5: The levels of kill achievable with the L3 of *M. plana* at various concentrations of Dipel® WP on 3 and 7 DAT. The vertical lines are standard errors of means.

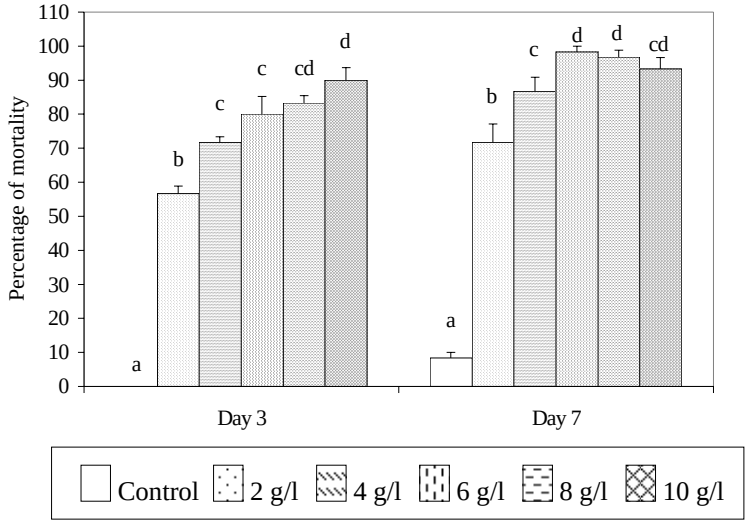

Figure 6: The levels of kill achievable with the L3 of *M. plana* at various concentrations of ABG-6429 FC on 3 and 7 DAT. The vertical lines are standard errors of means.

Table 2: Dose response of six *Bt* formulations against the 3rd instar larvae of *M. plana*.

Formulations	DAT[a]	LC_{50} (95% FL)[b]	LC_{95} (95% FL)[b]	χ^2 (P<0.05)	Slope ± SE
Dipel® DF	2	1.49 (0.32-2.33)	25.77 (11.74-595.92)	0.001	1.33 ± 0.42
Dipel® ES	2	1.46 (0.77-1.95)	6.62 (4.94-12.57)	0.008	2.50 ± 0.55
Florbac® SC	3	3.74 (2.84-4.79)	26.11 (14.52-103.72)	0.850	1.95 ± 0.42
Xentari® WG	3	0.60 (0.01-1.44)	25.86 (12.33-754.33)	1.937	1.01 ± 0.34
Dipel® WP	2	2.88 (1.36-4.09)	63.14 (20.87-7030.34)	0.147	1.23 ± 0.39
ABG-6429 FC	2	3.30 (1.78-4.80)	75.29 (23.28-12075.0)	0.253	1.21 ± 0.39

[a] Results of the day fitted for Probit analysis.
[b] g *Bt* formulation l^{-1} water.
n = 360; 10 larvae/replicate; 6 replicates and 6 treatments per test.

Table 3: Dose response of six *Bt* formulations against the 5th instar larvae of *M. plana*.

Formulations	DAT[a]	LC_{50} (95% FL)[b]	LC_{95} (95% FL)[b]	χ^2 (P<0.05)	Slope ± SE
Dipel® DF	3	0.62 (0.01-1.47)	28.49 (13.09-1075.16)	0.28	0.99 ± 0.34
Dipel® ES	4	0.67 (0.02-1.39)	8.96 (5.44-85.47)	0.52	1.46 ± 0.52
Florbac® SC	3	8.15 (5.95-16.06)	64.077 (25.88-1023.6)	0.86	1.84 ± 0.48
Xentari® WG	4	2.973 (1.36-4.15)	25.856 (13.69-200.27)	4.90	1.75 ± 0.32

[a] Results of the day fitted for Probit analysis.
[b] g *Bt* formulation l^{-1} water.
n = 240; 10 larvae/replicate; 6 replicates and 4 treatments per test.

The current laboratory results support the findings of insecticide screening by Chung and Narendran (1996) conducted in a polybag trial. The authors reported that both *Bta* and *Btk* gave satisfactory kill (70%–100%) of the early instar larvae of *M. plana* after one week. The results in this study were, however, partly contradictory to the findings of laboratory assays by Basri *et al*. (1994; 1996); the authors reported that *Bta* rather than *Btk* were potent on *M. plana* and *Mahasena corbetti*. Overall, in view that the present laboratory bioassays of the *Bt* against *M. plana* were performed under the principle of bioassay, the findings clarified that both *Btk* or *Bta* were intrinsically effective against the bagworm *M. plana*.

The larval control by *Bt* was generally slow and it normally takes two to three days for the larvae to die (Knowles 1994) and sometimes it even extended beyond one week for larger larvae (Glare & Callaghan 2000). After ingestion, the endotoxins bind specifically to receptor sites on the mid-gut cell walls, causing perforations in the membranes. This causes loss of control in the ion exchange between epithelial cells and the gut lumen, resulting in rapid death at high doses. However, lower doses would result in the reduction of pH in the gut lumen before allowing spore germination, rapid vegetative multiplication, penetration into the haemocoel, gross septicemia and eventual death (Glare & Callaghan 2000). In this experiment, L3 seemed to be more susceptible to *Bt* than L5, and in fact the speed of killed was faster on the younger larvae (Figures 1 to 6). Sandoz (1974) also reported that small and young larvae were easier to killed compared to late instar larvae as lesser amount of insecticide was needed based on weight factor.

Figures 1 and 2 show the dose response of Dipel® DF and Dipel® ES to be almost identical; they consistently gave high kill of the L3 and L5. Dipel® DF is a balanced formulation containing five bacterial protein toxins which give effective kill on the caterpillars that infest vegetables, while Dipel® ES is formulated in petroleum solvent and contains emulsifiers that provide uniform mixture between the *Btk* and oil. The petroleum oil in the formulation might have acted as a contact insecticide (Johnson 1980) which physically interfered with the respiration systems of the insect. During the bioassay, it was observed that the bags of *M. plana* were lined with oil absorbed from the treated leaf surface. The *Btk* and oil could have acted synergistically to cause the high larval mortality.

In this study, the dose response curve observed with Florbac® SP showed that the formulation seemed to perform well between 8–10 gl^{-1} on both the early and late instar larvae. Even though the field recommended rate is 5 gl^{-1}, it was shown by both Chung and Narendran (1996) and Basri *et al*. (1994; 1996) that this rate was able to provide excellent control of *M. plana* in polybag test system and laboratory bioassays, respectively. Xentari® WG and Dipel® WP at 2 gl^{-1} were effective in the early instars and this concentration is within the recommended rates of both formulations in oil palm fields. ABG-6429 FC is not commercially recommended for bagworm spray application in Malaysia and therefore, no label recommendation was available during the study.

Interpretation of the results in this study was not based on comparisons between formulations due to certain unavoidable shortcomings during the preparation of the bioassays. First, there might be natural variations in the insect's susceptibility level since test insects were collected from different locations and rearing was done separately for each batch of bagworms collected. Other than that, the use of oil palm foliage as test substrate may not be as standardized compared to artificial diet which is commonly used in bioassays. Thirdly, the various types of *Bt* formulations could have different effects on target species resulting from varying feeding behavior (Glare & Callaghan 2000). In view of the inadequacy of running a standardized bioassay as recommended by Dulmage *et al.* (1971) and, Burges and Thomson (1971), this experiment was conducted to examine the level of kill achievable with various concentrations of the *Bt* formulations against *M. plana* while no comparison was made on the potency of the different formulations. In fact, Watkinson (1994) mentioned that

there was no easy way to compare potency among products and probably the only way to compare the performance of different *Bt* products is by assessing their cost-effectiveness in the field.

Bacillus thuringiensis subspecies *kurstaki* or *aizawai* are shown to be effective on the bagworm *M. plana* under the condition of the experiment. In most cases, the 3rd instar larvae required lesser amount of *Bt* and shorter time for a satisfactory kill compared to the 5th instar larvae.

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