EFFECT OF Bt PRODUCTS, LEPCON-1, BAFOG-1 (S) AND ECOBAC-1 (EC), AGAINST THE OIL PALM POLLINATING WEEVIL, *Elaeidobius kamerunicus*, AND BENEFICIAL INSECTS ASSOCIATED WITH *Cassia cobanensis*

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ABSTRACT

The effects of Bacillus thuringiensis *products, Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC), on the oil palm pollinating weevil,* Elaeidobius kamerunicus, *and beneficial insects, especially parasitoids, were studied. For pollinating weevils, oil palm male spikelets with weevils were treated by spraying with Lepcon-1 and Ecobac-1 (EC) at five different concentrations, ranging from* 5.2×10^{7} *to* 5.2×10^{11} *cfu* ml^{-1} *for Lepcon-1, and* 7.34×10^{7} *to* 7.34×10^{11} *cfu* ml^{-1} *for Ecobac-1 (EC). For Bafog-1 (S), male spikelets with weevils were fogged at a dose of* 7.7×10^{11} *cfu* ml^{-1}*. The Bt products Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) were safe* for E. kamerunicus. *Direct spraying of Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) at the highest concentrations caused 13%, 10% and 11% corrected mortality at nine days after treatment (DAT), respectively. Cypermethrin was toxic to the pollinating weevils as it killed 100% of the weevils at as early as five DAT. Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) were also harmless to beneficial insects. Application of these products by spraying at the highest concentrations resulted in low mortality of the beneficial insects, ranging from 8% to 13%. Cypermethrin was toxic to the parasitoids as spraying of the chemical at 7.5% w/w killed 100% of the beneficial insects at as early as five DAT. The laboratory study showed that Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) were safe for the oil palm pollinating weevils and beneficial insects and, therefore, they are recommended for use in the integrated control of bagworms in oil palm.*

Keywords: *Bacillus thuringiensis*, cypermethrin, toxicity, *Elaeidobius kamerunicus*, beneficial insects.

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INTRODUCTION

Bacillus thuringiensis (Bt) proteins have insecticidal properties and they have been used commercially as biopesticides for more than 40 years. Bt-based biopesticides have contributed up to 90% of the global biopesticide market, and have been considered valuable alternative products to chemical insecticides (NPTN, 2004). Bt products are known to be safe to non-target organisms; they are used when insects develop resistance to chemical insecticides. Their residues do not persist in the environment (NPTN, 2004). Bt is a gram-positive bacterium that upon sporulation produces protein crystals toxic to certain insect pests. Numerous research studies have been conducted on different strains of Bt that affect the larvae of insects in the Orders of Lepidoptera, Diptera and Coleoptera (NPTN, 2004). The active ingredients in Bt products are a mixture of spores and crystal toxin proteins that are produced aerobically during the lag

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phase of the growth cycle of the bacteria (NPTN, 2004). These ingredients are easily harvested and formulated. When ingested by susceptible insect larvae, the δ-endotoxins are activated by the gut protease enzymes (Ghribi *et al*., 2006). Several Cry proteins or δ-endotoxins have receptor proteins in the gut lining of insects, such as the oil palm bagworm, *Metisa plana* (Ramlah Ali, 2000). The activated toxins bind to the gut receptors and cause osmotic lysis, resulting in the death of the larvae.

Prior to the registration of a biopesticide, it is mandatory to conduct appropriate safety tests on the toxicity of the biopesticide (DeBach and Rosen, 1991). According to the Pesticide Board Malaysia, toxicological data are required for pesticide registration with the aim of defining possible hazards to man, non-target organisms and the environment. The tests include toxicology studies against a wide range of natural enemies and also non-target organisms. In oil palm plantations, there are numerous natural enemies such as parasitoids and predators that are responsible for reducing the population levels of bagworms and other insect pests (Basri *et al.*, 1995; Howard *et al*., 2001). The Malaysian Palm Oil Board (MPOB) is currently developing three formulations of Bt, namely, Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC), for bagworm control. Although it is known that Bt products do not affect natural enemies (Howard *et al.*, 2001), it is still essential to evaluate the effects of these three Bt products against the natural enemies such as predators or parasitoids which are commonly associated with a beneficial plant, *Cassia cobanensis*. This study also evaluates the effects of the Bt products against the oil palm pollinating weevil, *Elaeidobius kamerunicus*, and the natural enemies of oil palm pests.

MATERIALS AND METHODS

Bt Products and their Application

Three different preparations of Bt products were tested. All three products contained an indigenous strain of *B. thuringiensis* isolated in Malaysia. The products are Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) which contain the same δ-endotoxins. The products were formulated after 48 hr of liquid fermentation of MPOB Bt1 at 30°C using AgroNat medium (patent No. PI2011000307). Fermentation was conducted in a 300-litre bioreactor at the Microbial Technology and Engineering Centre (MICROTEC), MPOB. Lepcon-1 is a flowable concentrate applied by spraying from the bottom up using a turbo mist blower. Bafog-1(S) is a formulated solution, applied via fogging suitable for smallholdings with a small area of infestation, particularly in peat areas where the use of a turbo mist blower is not possible due to the soft ground conditions. Bafog-1 (S) is produced with inert ingredients added into the concentrated culture. Ecobac-1 (EC) is an emulsified concentrate, produced via the sedimentation process of the concentrated culture. The amount of Bt in the concentrate was standardised at 16 000 IU mg^{-1}, and the product is to be applied via aerial spraying in extensive areas of bagworm infestation.

Effects of Bt Products against Oil Palm Pollinating Weevil, *E. kamerunicus*

***Source of* E. kamerunicus.** The adults of the pollinating weevil, *E. kamerunicus*, were obtained from anthesising male inflorescences of *tenera* palms collected from the MPOB Research Station in Bangi, Selangor. The adult weevils were transferred into a Petri dish that was lined with Whatman filter paper No. 1 at the bottom of the dish. Each Petri dish contained 15 weevils. Two spikelets from a male inflorescence, approximately 10-12 cm long, were placed in the Petri dish as a food source for the weevils. The spikelets were pre-treated by drying them overnight in an oven at 37°C to prevent the growth of saprophytic fungi.

Bioassay method. A total of 15 adult weevils were transferred from the Petri dish onto each dried spikelet. Fifteen spikelets were treated with five concentrations each of Lepcon-1 and Ecobac-1 (EC). The spikelets were sprayed in a fume-cupboard with 2 ml of each product using a hand-held sprayer. The various concentrations of the Bt products were prepared by serial dilution. The concentrations of Lepcon-1 were: C1 = 5.2 x 10^7 cfu ml^{-1}, C2 = 5.2 x 10^8 cfu ml^{-1}, C3 = 5.2 x 10^9 cfu ml^{-1}, C4 = 5.2 x 10^{10} cfu ml^{-1} and C5 = 5.2 x 10^{11} cfu ml^{-1}, while the concentrations of Ecobac-1 (EC) were: C1 = 7.34 x 10^7 cfu ml^{-1}, C2 = 7.34 x 10^8 cfu ml^{-1}, C3 = 7.34 x 10^9 cfu ml^{-1}, C4 = 7.34 x 10^{10} cfu ml^{-1} and C5 = 7.34 x 10^{11} cfu ml^{-1}.

As for Bafog-1 (S), only one concentration was tested, because a lower dilution of Bafog-1 (S) contained more water than the concentrated culture and mixed poorly with diesel in the 7-litre solution tank of the fogging machine. The concentration of Bafog-1 (S) used was 7.43 x 10^{11} cfu ml^{-1}. The spikelets were fogged inside a 500-ml customised and sterilised cylinder.

For each product, the experiment was replicated three times with 15 weevils used in each replicate. Each treated spikelet was transferred into the 500-ml sterilised cylinder, and placed in an indoor insectory maintained at a temperature of 24°C to 28°C and 50% RH. The mortality of *E. kamerunicus* was recorded at 5, 9 and 13 days after treatment (DAT).

In the chemical control, the weevils were treated by spraying with 2 ml of 5% cypermethrin (Hextar Cyper 5.5EC) per spikelet. For the blank or untreated control, the weevils were sprayed with distilled water at 2 ml/spikelet. Spraying of the weevils was done in the fume cupboard using a 1-litre hand-held sprayer.

Effect of Bt Products on Beneficial Insects

Source of beneficial insects. In general, there are two types of beneficial insects in the oil palm ecosystem, namely, the predators and the parasitoids. In this study, bioassays were conducted against three species of parasitoids, namely, *Dolichogenidea metesae*, *Goryphus bunoh* and *Brachymeria carinata.* These species were collected by the sweep net technique from the planted beneficial plants, *Cassia cobanensis*, at the MPOB Research Station, Bangi, Selangor. Collections were done around 10.00 am, as the parasitoids were still active at this time. Prior to collection, the sweep net was sterilised by spraying with 70% alcohol and left to dry. The collected insects were placed in a customised cage with dimensions of 47 cm x 47 cm x 105 cm height, and immediately sent to the laboratory. In the laboratory, the insects were placed in a sterilised Petri dish lined with sterilised filter paper. Sorting was then conducted by separating the insects according to species. A sum of 20 mixed species of beneficial insects was placed inside each cage.

Bioassay method. In this study, bioassays were conducted using parts of the branches from one-year-old beneficial plants, *C. cobanensis*, planted in the nursery of MPOB Research Station, Bangi, Selangor. The beneficial plants were sprayed with 5 ml of Lepcon-1 and Ecobac-1 (EC) using a 1-litre hand-held sprayer. The various concentrations of the Bt products were prepared by serial dilution. Only three concentrations of two of the products were tested. The concentrations of Lepcon-1 were: C3 = 6.4 x 10^9 cfu ml^{-1}, C4 = 6.4 x 10^{10} cfu ml^{-1} and C5 = 6.4 x 10^{11} cfu ml^{-1}, while the concentrations of Ecobac-1 (EC) were: C3 = 8.4 x 10^9 cfu ml^{-1}, C4 = 8.4 x 10^{10} cfu ml^{-1} and C5 = 8.4 x 10^{11} cfu ml^{-1}.

In the case of Bafog-1 (S), only one concentration was tested, which was 7.43 x 10^{11} cfu ml^{-1}. The beneficial plants were fogged with a mixture of Bafog-1 (S): diesel at 1:1 (v/v) using a fogging machine, model Z-FOG. Each treated plant was then transferred into an individual cage. A sum of 20 mixed species of beneficial insects was introduced into each cage.

The treatment design used was completely randomised. A chemical control and blank were prepared to compare with each tested product. For each tested product, the experiment was replicated three times. The cages were placed in an outdoor insectory at 24°C-28°C. As an additional nutrient source for the parasitoids, 80% diluted honey solution was placed inside each cage. Mortality of the parasitoids was recorded at five, nine and 13 DAT.

In the case of the chemical control, the beneficial plants were treated by spraying with cypermethrin (Hextar Cyper 5.5EC) with 5% active ingredient at 5 ml/plant. For the blank, the plants were sprayed only with distilled water at 5 ml/plant. Spraying of plants was done in the fume cupboard using a 1-litre hand-held sprayer.

Data Analysis

Data on mortality were converted to corrected mortality using the Abbot Formula, then analysed using a one-way ANOVA by the SPSS software, version 11.5. The means were compared by the Least Significant Difference (LSD) test using the same software.

RESULTS AND DISCUSSION

Effect of Lepcon-1 on Beneficial Insects

This study showed that Lepcon-1 was harmless to the beneficial insects as compared with cypermethrin. At five DAT, treatment with Lepcon-1 at the highest rate of C5 (6.4 x 10^{11} cfu ml^{-1}), or 100 times more concentrated than the recommended dose, caused only 16% corrected mortality, which was significantly lower ($P<0.05$) as compared with caused by treatment with cypermethrin. Spraying with cypermethrin at 7.5% w/w was highly toxic, causing 100% corrected mortality of the beneficial insects.

Spraying of the Bt product, Lepcon-1 at concentrations C3 (6.4 x 10^9 cfu ml^{-1}) to C5 (6.4 x 10^{11} cfu ml^{-1}) did not affect the survival of the beneficial insects. Spraying with Lepcon-1 at the concentration of 6.4 x 10^{11} cfu ml^{-1}, which was 100-fold higher than the recommended dose (6.4 x 10^9 cfu ml^{-1}), caused only 8% corrected mortality in the beneficial insects at nine DAT (*Table 1*). According to Mohd Najib *et al.* (2009), the surroundings and feeding conditions can affect the mortality of beneficial insects. In a

TABLE 1. CORRECTED MORTALITY OF BENEFICIAL INSECTS AFTER TREATMENT WITH FIVE DIFFERENT CONCENTRATIONS OF LEPCON-1

Days after treatment	Treatment			
	Lepcon-1, cfu ml^{-1}			Cypermethrin
	C3	C4	C5	
5	0a	0a	16±7b	100c
9	0a	8±6a	8±8a	100b
13	0a	0a	38±8b	100c

Note: Doses: C3 = 6.4 x 10^9 cfu ml^{-1}, C4 = 6.4 x 10^{10} cfu ml^{-1} and C5 = 6.4 x 10^{11} cfu ml^{-1}.

previous study, the Bt product Teracon-1 led to 38% mortality of the beneficial insects at a dose of C5 at seven DAT. Therefore, this suggests that Lepcon-1 was safe and harmless to beneficial insects, especially the parasitoids.

Effect of Bafog-1 (S) on Beneficial Insects

Application of Bafog-1 (S) caused low mortality to the parasitoids. At five DAT, fogging with Bafog-1 (S) at the highest dose of C5 (7.43×10^{11} cfu ml^{-1}) caused only 11% corrected mortality, significantly lower ($P<0.05$) as compared with mortality for the treatment with cypermethrin. Application of cypermethrin was hazardous to the beneficial insects, as the insecticide caused 100% mortality at five, nine and 11 DAT. This study showed that the application of Bafog-1 (S) at the highest concentration of C5 = 7.43×10^{11} cfu ml^{-1} was harmless to the beneficial insects, as it killed only 9% of the insects at 9 DAT (*Table 2*).

Effect of Ecobac-1 (EC) on Beneficial Insects

Application of Ecobac-1 (EC) at a rate which was 100-fold more concentrated than the recommended dose, C5 (8.4×10^{11} cfu ml^{-1}) caused 0% corrected mortality to the beneficial insects at five DAT. Application of cypermethrin at the recommended dose of 7.5% w/w was highly toxic to the beneficial insects as it caused 100% mortality at five DAT. These results indicate that Ecobac-1 (EC) was safe and harmless to the beneficial insects. Application of the product at the concentration 8.4×10^{11} cfu ml^{-1}, which was 100-fold higher than the recommended dose of 8.4×10^{9} cfu ml^{-1}, caused only 13% corrected mortality at 9 DAT (*Table 3*). Meanwhile, application of Ecobac-1 (EC) at dose of C5 led to higher mortality of the insects, 29% at 13 DAT. According to Basri *et al*. (1999), insufficient nectar disrupts the life cycle of the parasitoids and can also cause high mortality to the parasitoids.

Effect of Lepcon-1 on Oil Palm Pollinating Weevil, *E. kamerunicus*

This study showed that the pollinating weevil, *E. kamerunicus*, was not affected after treatment with Lepcon-1, suggesting that the Bt product was safe for the weevils. Treatment with this Bt product at concentration C5 (5.2×10^{11} cfu ml^{-1}), which was 100-fold higher than the recommended concentration C3 (5.2×10^{9} cfu ml^{-1}) caused only 11% corrected mortality to *E. kamerunicus* at nine DAT (*Table 4*). Cypermethrin was highly toxic to *E. kamerunicus*, causing 100% mortality even at as early as one DAT (Mohd Najib *et al*., 2009). The ANOVA analysis showed that treatments with Lepcon-1 at concentrations ranging from 5.2×10^{7} to 5.2×10^{11} cfu ml^{-1} did not cause any difference ($P>0.05$) in the mortality of *E. kamerunicus*.

TABLE 2. CORRECTED MORTALITY OF BENEFICIAL INSECTS TREATED WITH BAFOG-1 (S)

Days after treatment	Treatment	
	Bafog-1 (S) (C5), cfu ml^{-1}	Cypermethrin
5	7±7a	100b
9	9±5a	100b
11	11±9a	100b

Note: Dose: C5 = 7.43×10^{11} cfu ml^{-1}.

TABLE 3. CORRECTED MORTALITY OF BENEFICIAL INSECTS TREATED WITH DIFFERENT CONCENTRATIONS OF ECOBAC-1 (EC)

Days after treatment	Treatment			
	Ecobac-1 (EC), cfu ml^{-1}			Cypermethrin
	C3	C4	C5	
5	0a	0a	0a	100b
9	0a	0a	13±7b	100c
13	0a	0a	29±8b	100c

Note: Doses: C3 = 8.4×10^{9} cfu ml^{-1}, C4=8.4×10^{10} cfu ml^{-1} and C5=8.4×10^{11} cfu ml^{-1}.

TABLE 4. CORRECTED MORTALITY OF OIL PALM POLLINATOR, *E. kamerunicus*, AFTER TREATMENT WITH FIVE DIFFERENT CONCENTRATIONS OF LEPCON-1

Days after treatment	Treatment					
	Lepcon-1, cfu ml^{-1}					Cypermethrin
	C1	C2	C3	C4	C5	
5	0a	4±4ab	4±4ab	8±5ab	17±4b	100c
9	0a	5±9a	0a	11±9a	11±9a	100b
13	7±7a	7±7a	13±8a	13±8a	13±8a	100b

Note: Doses: C1 = 5.2×10^{7} cfu ml^{-1}, C2 = 5.2×10^{8} cfu ml^{-1}, C3 = 5.2×10^{9} cfu ml^{-1}, C4 = 5.2×10^{10} cfu ml^{-1} and C5 = 5.2×10^{11} cfu ml^{-1}.

Effect of Bafog-1 (S) on Oil Palm Pollinating Weevil, *E. kamerunicus*

This study showed that the Bt product, Bafog-1 (S), was not toxic to *E. kamerunicus.* At five DAT, spraying of the product at the highest concentration C5 (7.7×10^{11} cfu ml^{-1}) killed only 4% of *E. kamerunicus*. Cypermethrin was toxic to *E. kamerunicus*, because it killed 100% *E. kamerunicus* at five, nine and 13 DAT. The Bt product, Bafog-1 (S), was safe for *E. kamerunicus* as the application of the product at the highest concentration C5 (7.7×10^{11} cfu ml^{-1}) caused only 10% corrected mortality at nine DAT (*Table 5*).

Effect of Ecobac-1 (EC) on Oil Palm Pollinating Weevil, *E. kamerunicus*

Results of this experiment show that Ecobac-1 (EC) was safe and not toxic to *E. kamerunicus*. Treatment with Ecobac-1 (EC) at two different concentrations ranging from C3 (7.34×10^{9} cfu ml^{-1}) to C4 (7.34×10^{10} cfu ml^{-1}) did not cause any significant difference ($P>0.05$) in the mortality of *E. kamerunicus* at nine DAT. Ecobac-1 (EC) was harmless to the oil palm pollinating weevil, as it caused only 13% corrected mortality of *E. kamerunicus* when applied at C5 (7.34×10^{11} cfu ml^{-1}) at nine DAT, which was 100-fold higher than the recommended dose of C3 (7.34×10^{9} cfu ml^{-1}) (*Table 6*).

TABLE 5. CORRECTED MORTALITY OF OIL PALM POLLINATOR, *E. kamerunicus*, AFTER TREATMENT WITH BAFOG-1 (S)

Days after treatment	Treatment	
	Bafog-1 (S), cfu ml^{-1}	Cypermethrin
	C5	
5	4±4a	100b
9	10±6a	100b
13	7±9a	100b

Note: Dose: C5 =7.7×10^{11} cfu ml^{-1}.

CONCLUSION

This study showed that the Bt-based biopesticides, Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC), are not toxic to the oil palm pollinating weevil, *E. kamerunicus*, and to beneficial insects as compared with cypermethrin. Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) are target-specific biopesticides to oil palm insect pests such as bagworms, nettle caterpillars and other lepidopterous pests of other crops. The chemical insecticide, cypermethrin, is toxic to non-target organisms such as *E. kamerunicus* as well as beneficial insects. Application of this chemical in the oil palm plantation should be avoided whenever possible, and preferably be replaced with Bt products. Bt product, Bafog-1 (S), can be mixed with diesel and applied using a fogging machine to control bagworm outbreaks in oil palm plantations. Furthermore, Ecobac-1 (EC) can be applied via aerial spraying to control an extensive bagworm infested area. The use of Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) is recommended for the management of bagworms and other Lepidopteran insect pests. Thus, this will reduce the current reliance on toxic chemical insecticides.

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TABLE 6. CORRECTED MORTALITY OF OIL PALM POLLINATOR WEEVIL, *E. kamerunicus*, TREATED WITH DIFFERENT CONCENTRATIONS OF ECOBAC-1 (EC)

Days after treatment	Treatment					
	Ecobac-1 (EC), cfu ml^{-1}					Cypermethrin
	C1	C2	C3	C4	C5	
5	0a	4±4ab	4±8ab	8±5ab	17±4b	100c
9	0a	5±8a	5±8a	11±8a	13±9a	100b
13	7±7a	7±7a	13±8a	13±8a	13±8a	100b

Note: Doses: C1 = 7.34×10^{7} cfu ml^{-1}, C2 = 7.34×10^{8} cfu ml^{-1}, C3 = 7.34×10^{9} cfu ml^{-1}, C4 = 7.34×10^{10} cfu ml^{-1} and C5 = 7.34×10^{11} cfu ml^{-1}.

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