EFFECT OF *Bacillus thuringiensis*, *TERAKIL-1®* AND *TERACON-1®* AGAINST OIL PALM POLLINATOR, *Elaeidobius kamerunicus* AND BENEFICIAL INSECTS ASSOCIATED WITH *Cassia cobanensis*

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ABSTRACT

A laboratory study on the effect of Bacillus thuringiensis *(Bt) product,* Terakil-1® *and* Teracon-1® *on oil palm pollinating weevil,* Elaeidobius kamerunicus *and beneficial insects such as predators and parasitoids was studied to evaluate the safety and specificity of Bt product against non-target organisms in particular the beneficial insects as compared to chemical. The male spikelets were sprayed with* Terakil-1® *and* Teracon-1® *at different doses, ranging from* $3.7x10^{7}$ *cfu* ml^{-1} *-* $3.7x10^{11}$ *cfu* ml^{-1} *and 0.1124 µg* ml^{-1} *– 1124 µg* ml^{-1}*, respectively. At five days after treatment (DAT),* Terakil-1® *and* Teracon-1® *being target specific at dose of C5,* $3.7x10^{11}$ *cfu* ml^{-1} *and 11.24 µg* ml^{-1}*, resulted in 21% and 25% mortality of beneficial insects, respectively. Whereas, at seven DAT,* Terakil-1® *and* Teracon-1® *being a safe Bt products at dose of C5,* $3.7x10^{11}$ *cfu* ml^{-1} *and 11.24 µg* ml^{-1}*, resulted in 22% and 23% corrected mortality of beneficial insects, respectively. Cypermethrin at 5.5% w/w as a chemical control led to 100% corrected mortality of beneficial insects at five DAT. As for oil palm pollinating weevil,* E. kamerunicus, Terakil-1® *and* Teracon-1® *being a safe and environmental-friendly products, each resulted in 26% and 33% mortality of* E. kamerunicus *at seven DAT at 100-fold higher dose than the recommended dose of C3,* $3.7x10^{9}$ *cfu* ml^{-1} *and 11.24 µg* ml^{-1}*. Whereas, at seven DAT,* Terakil-1® *and* Teracon-1® *being a safe Bt products at dose of C5,* $3.7x10^{11}$ *cfu* ml^{-1} *and 1124 µg* ml^{-1}*, resulted in 14% and 16% corrected mortality of* E. kamerunicus*, respectively. At five to seven DAT, the mean mortality of the adult* E. kamerunicus *subjected to the five different doses,* $3.7x10^{7}$*cfu* ml^{-1} *to* $3.7x10^{11}$ *cfu* ml^{-1} *and 0.1124 µg* ml^{-1} *– 1124 µg* ml^{-1} *of* Terakil-1® *and* Teracon-1®*, respectively was not significantly different at P=0.05 when tested using Duncan's Multiple Range Test, implying that even at very high doses, Bt products were equally safe to the weevils. Cypermethrin was significantly toxic to pollinating weevils as compared to high doses of* Terakil-1® *and* Teracon-1®*. Unlike chemicals,* B. thuringiensis, Terakil-1® *and* Teracon-1® *were found to be safe for beneficial insects under the oil palm and is therefore recommended for integrated control of bagworm under oil palm.*

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

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INTRODUCTION

Bacillus thuringiensis (Bt) proteins have insecticidal properties and have been used commercially for more than 30 years. Bt-based biopesticides, which

make up 90% of the global biopesticide market, are widely used and are considered a valuable alternative to chemical insecticides in terms of safety to non-target organisms and when resistance to chemical insecticides has developed. Bt mode of action can be divided into a series of critical steps. First, ingestion by the insect. Second, specific binding to brush border membrane receptors. Third, membrane insertion which leads to pore formation (EPA, 1999). The protein crystal of Bt is very toxic to certain lepidopterous larvae and it is relatively harmless to other organisms. Being gut poison, the spores and crystals must be ingested. It is most effective against leaf-eating larvae which have a strongly alkaline gut, pH 8.9 and gut enzyme to dissolve the spore crystal and release the toxin (Matthews, 1984).

Health and safety legislation makes it mandatory in some countries for the appropriate safety tests on toxicity of biopesticides prior to registration (Paul, 1991). In many areas, especially in the hot and humid tropical climate, conditions are not conducive to wearing protective clothing, even if users can afford it, so it is not surprising that deaths have occurred following the use of certain highly toxic chemical pesticides (Matthews, 1984). Consideration on the safety of non-target organisms is vital. With integrated control using Bt, beneficial organisms, the predators and parasitoids are not affected (Howard *et al*., 2001).

Oil palm pollinating weevil, *Elaeidobius kamerunicus* was introduced into Malaysia from Cameroon, Africa in 1981. The introduction of *E. kamerunicus* has increased number of bunches per hectare and oil extract rate (OER) (Basri *et al*., 1985). In addition, the weevil has greatly saved the pollinating costs between RM 100-RM 150 ha^{-1} yr^{-1} and saved up to RM 2 million per year for a big plantation (Syed, 1982).

This article reports the effect of MPOB local isolate of Bt, MPOB Bt1 products, *Terakil-1®* and *Teracon-1®* on the oil palm pollinator, *E. kamerunicus* and beneficial insects associated with *Cassia cobanensis* as compared to cypermethrin, a common insecticide used in the control of oil palm insect pests. It is essential to study the safety of Bt product towards non-target organisms in oil palm plantation to ensure that the ecological systems and environment are not affected when these are used for the control of oil palm insect pests. Besides, the documented data in this article will be used to fulfill the requirement for registration of MPOB Bt1 products as suggested by Department of Agriculture (DOA).

MATERIALS AND METHOD

MPOB Bt1 Products

Terakil-1® and *Teracon-1®* is a wettable powder and protein concentrate of indigenous *Bacillus thuringiensis*, Bt. Both Bt products were generated via 48 hr fermentation at the Microbial Technology and Engineering Centre (MICROTEC), Malaysian Palm Oil Board (MPOB). In the downstream process, the cell was subjected to high centrifugation forces. Fermented liquid culture was spun and the concentrate was stored in storage tank. The concentrate was then spray-dried using fluidized spray dryer to form wettable powder or *Terakil-1®*. The effluent was filtered through 0.45 μm cartridge to get rid off debris and remaining cells. The filtrate passed through two different cartridges and the retentate produced was then collected aseptically. The light liquid or retrievable soluble proteins concentrate called *Teracon-1®*. Cypermethrin was used as a chemical control with another untreated control.

The experiment was conducted using five different concentrations of *Terakil-1®* and *Teracon-1®* in replicates of three. For each replicate of the treatment, 15 pollinators were used. Treatments include control such as cypermethrin, at 5.5% w/w and water (as blank), each in replicates of three. Concentrations of *Terakil-1®* used were 3.7 x 10^7 cfu ml^{-1} (C1), 3.7 x 10^8 cfu ml^{-1} (C2), 3.7 x 10^9 cfu ml^{-1} (C3), 3.7 x 10^{10} cfu ml^{-1} (C4) and 3.7 x 10^{11} cfu ml^{-1} (C5) and 0.1124 μg ml^{-1} (C1), 1.124 μg ml^{-1} (C2), 11.24 μg ml^{-1} (C3), 112.4 μg ml^{-1} (C4) and 1124 μg ml^{-1} (C5) for *Teracon-1®*. Controls used were cypermethrin and water (untreated).

E. kamerunicus Adult

E. kamerunicus adult were obtained from one-week-old male inflorescences of DxP palms collected from MPOB/UKM Station, Bangi, Selangor (*Figure 1*). To avoid fast growing saprophytic fungi, moisture content of the spikelets (without pollinators) were reduced by drying in an oven at 37°C overnight. Prior to drying of the spikelets, all pollinators were sorted and isolated from male spikelet in a petri dish layered with Whatman filter paper (*Figure 2*). Each petri dish contained 15 pollinators. Forty-eight spikelets were placed in a petri dish layered with Whatman filter paper and kept in the oven overnight.

Figure 1. One-week-old male inflorescences were cut for the experiment.

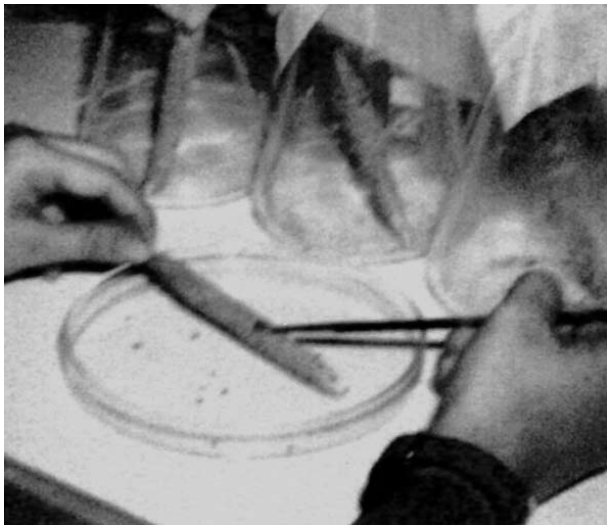

Figure 2. Sorting and isolating adult of E. kamerunicus *from male spikelet.*

Bioassay Treatments against *E. kamerunicus*

Forty-eight spikelets were transferred from the oven into the fume cupboard. Four types of treatments were prepared including *Terakil-1®*, *Teracon-1®*, cypermethrin and control/untreated. Ten-fold of *Terakil-1®* and *Teracon-1®* dilution, $3.7x10^{7}$ cfu ml^{-1} – $3.7x10^{11}$ cfu ml^{-1} and 0.1124 μg ml^{-1} – 1124 μg ml^{-1}, respectively were prepared prior to the experiment. The spikelets were sprayed with *Terakil-1®* and *Teracon-1®* at the various dosages, each with 5 ml of *Terakil-1®* and *Teracon-1®* per dosage inside the fume cupboard (*Figure 3*). The treated spikelets were placed into a sterilized cylinder in indoor insectory at 24°C- 28°C with relative humidity of 39 g m^{-3} (*Figure 4*). Fifteen *E. kamerunicus* were transferred from the petri dish onto each treated spikelets. Mortality of *E. kamerunicus* was recorded for 13 days.

Source of Beneficial Insects

Beneficial insects consisted of two different group of insect for the control of oil palm pest, namely predator and parasitoids. Predators such as *Sycanus dichotomus* and *Cosmolestes picticeps*, directly attack and kill bagworms by sucking the bagworms tissue through their proboscis. Whereas, parasitoids namely, *Dolichogenidea metesae*, *Goryphus bunoh* and *Brachymeria carinata*, attack the bagworm by injecting its egg into the bagworm body via ovipositor. Nectar located on a stipule of *Cassia cobanensis* is provided as feed for the beneficial insects.

The beneficial insects were swept and collected at MPOB/UKM (*Figure 5*) when they were active at 10 am. The sweep net was sterilized using 70% alcohol and left to dry to avoid any contamination to the insects. Then, the collection of insects was transported in cages to the laboratory. In the laboratory, the insects were placed into sterilized petri dishes layered with sterilized filter paper for sorting (*Figure 6*).

Bioassay Treatments Against Beneficial Insect

The beneficial insects were collected and kept inside a sterilized cylinder. Ten-folds of *Terakil-1®* and *Teracon-1®* dilution, $3.7x10^{7}$ cfu ml^{-1} – $3.7x10^{11}$ cfu

Figure 3. Spraying of 5 ml of Terakil-1® *onto oil palm spikelet.*

Figure 4. Oil palm pollinating weevil, Elaeidobius kamerunicus *was incubated in indoor insectory.*

Figure 5. Beneficial insects were caught at MPOB/ UKM using sweep net.

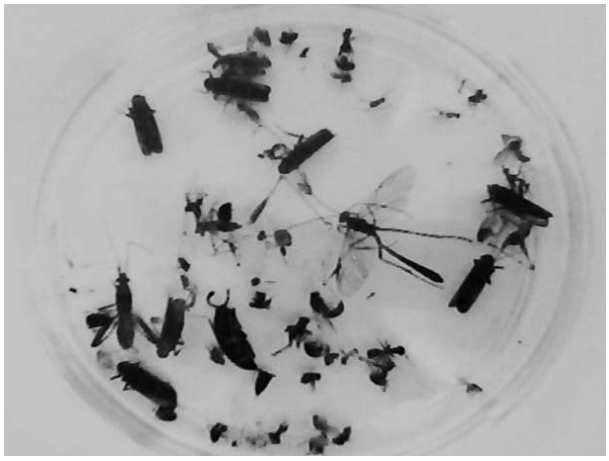

Figure 6. Collection of insects was placed onto sterilized petri dish.

ml^{-1} and 0.1124 $\mu g\ ml^{-1}$ – 1124 $\mu g\ ml^{-1}$, respectively was prepared prior to the experiment. The beneficial plant, *C. cobanensis* were sprayed with 5 ml of *Terakil-1®* (*Figure 7*) and *Teracon-1®*, using all the five different dosages. Eighty percent honey dilution was used as feed for the beneficial insect. Treatments include cypermethrin and water (as blank) as control. The plants and feed were placed inside individual cages and incubated in outdoor insectory, at 24ºC – 28ºC (*Figure 8*). Twenty beneficial insects were introduced into each cage for every treatment. Mortality of the beneficial insects was recorded for 13 days.

Data Analysis

The data was angular-transformed before analysed using ANOVA according to the general linear model procedure, PROC GLM. Mean for all parameter was compared by Duncan's Multiple Range Test (DMRT) at P=0.05 (Ramle *et al.*, 1999).

RESULTS AND DISCUSSION

Effect of *Terakil-1®* on Beneficial Insects

In this study, *Terakil-1®* is confirmed to be significantly harmless and no adverse effect was observed on beneficial insects as compared to cypermethrin at 5 DAT at P=0.001 when tested using DMRT analysis. *Terakil-1®* led to 21% mortality of beneficial insects at five DAT at dose of C5, $3.7x10^{11}$ cfu ml^{-1} as compared to cypermethrin (*Table 1*). At seven DAT, due to surrounding and feeding condition during the experiment, high mortality of beneficial insects, 34% was recorded at dose of C5. The same result was observed for control, which resulted in 25% mortality of the insects. Cypermethrin at 5% w/w is highly toxic and its adverse effect on the insecticide led to 100% mortality of beneficial insects at five DAT. While, *Terakil-1®* is significantly did not affect the beneficial insects at P>0.05 as compared

Figure 7. Spraying of 5 ml of Terakil-1® *onto* Cassia cobanensis.

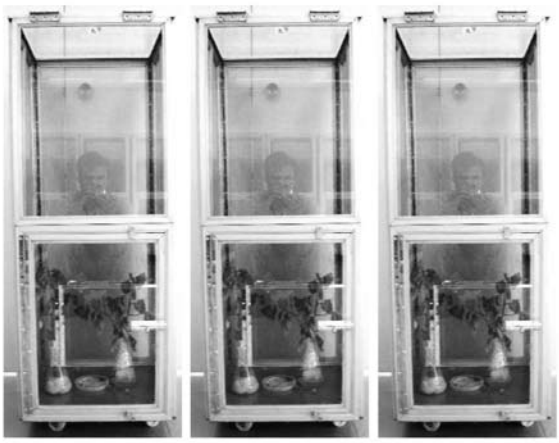

Figure 8. Beneficial insects associated with Cassia cobanensis *were incubated in individual cages in the indoor insectory.*

to control. It is implying that *Terakil-1®* only gave 22% corrected mortality of beneficial insects at concentration 100-fold higher than the recommended dose, 3.7 x 10^{11} cfu ml^{-1} (*Figure 9*).

at one to seven DAT, led to high mortality, 100% of beneficial insect at five DAT.

Result of this study indicated that *Teracon-1®* has no adverse effect and is significantly harmless to the beneficial insect. It resulted in only 23% corrected

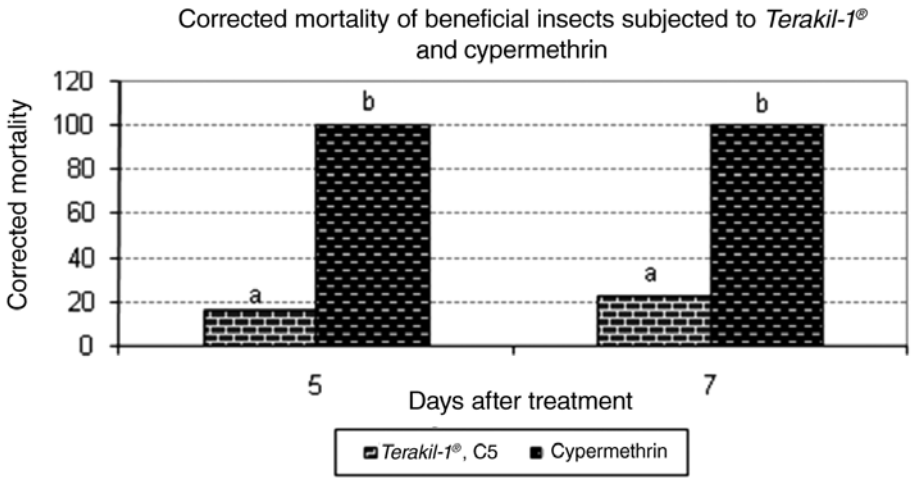

Figure 9. Corrected mortality of beneficial insects attributed by Terakil-1® *as compared to cypermethrin. Bars in groups with the same letters are not significantly different (P>0.05) after Duncan's Multiple Range Test. Corrected mortality* $= \left(\frac{\%Treatment - \%Control}{100 - \%Control} \right) x\ 100\%$.

Effect of *Teracon-1®* on Beneficial Insects

No adverse effect of *Teracon-1®* was observed on the beneficial insects. At five DAT, *Teracon-1®* gave low mortality of the beneficial insects, 25% at the highest dose of C5, 1124 μg ml^{-1} as compared to cypermethrin (*Table 2*). Due to surrounding and feeding condition during the experiment, a slightly high mortality of beneficial insects, 38% was recorded at dose of C5 at seven DAT. The same result was observed for control, which resulted in 26% mortality of the insects. Cypermethrin with the adverse effect mortality of the beneficial insect at seven DAT at 100 folds higher than it recommended dose, 1124 μg ml^{-1} show in *Figure 10*.

Effect of *Terakil-1®* on Oil Palm Pollinating Weevil, *Elaeidobius kamerunicus*

As for oil palm pollinating weevil, *E. kamerunicus*, *Terakil-1®* resulted in 26% mortality of *E. kamerunicus* at seven DAT at 100-fold higher dose, 3.7 x 10^{11} cfu ml^{-1} than the recommended dose of C3, 3.7x10^{9} cfu ml^{-1} (*Table 3*). With regard to *Terakil-1®*, at the

TABLE 1. PERCENTAGE MORTALITY OF BENEFICIAL INSECTS SUBJECTED TO DIFFERENT TREATMENTS

	Treatments		
Day after treatment	***Terakil-1®* (C5), cfu ml^{-1}**	**Cypermethrin**	**Control**
5	21	100	6
7	34	100	25

Note: Dose: C5 = 3.7x10^{11} cfu ml^{-1}.

TABLE 2. PERCENTAGE MORTALITY OF BENEFICIAL INSECTS SUBJECTED TO DIFFERENT TREATMENTS

	Treatments		
Day after treatment	***Teracon-1®* (C5), μg ml^{-1}**	**Cypermethrin**	**Control**
5	25	100	6
7	38	100	26

Note: Dose: C5 =1124 μg ml^{-1}.

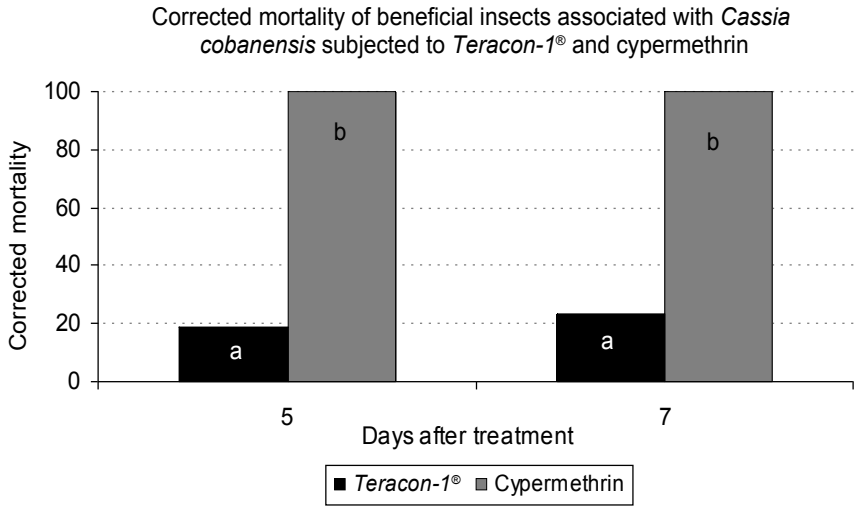

Figure 10. Corrected mortality of beneficial insects attributed by Teracon-1® *as compared to cypermethrin. Bars in groups with the same letters are not significantly different (P>0.05) after Duncan's Multiple Range Test. Corrected mortality* $= \left(\frac{\%Treatment - \%Control}{100 - \%Control} \right) x\ 100\%$.

recommended dose of $3.7x10^9$ cfu ml^{-1} and at 100-fold higher dose of C5, $3.7x10^{11}$ cfu ml^{-1}, it was significantly non-toxic at P=0.001 as compared to cypermethrin. Cypermethrin has an adverse effect and being highly toxic led to 100% mortality of *E. kamerunicus* at one to seven DAT. The corrected mortality of *E. kamerunicus* was not significantly different for three series of concentrations, $3.7x10^9$ - $3.7x10^{11}$ cfu ml^{-1} at P>0.05 when tested using Duncan's Multiple Range analysis.

Terakil-1® being harmless to oil palm pollinator, gave only 14% corrected mortality of *E. kamerunicus* at seven DAT at 100-fold higher (C5) than the recommended dose of C3, 3.7 x 10^9 cfu ml^{-1}as compared to cypermethrin (*Figure 11*).

Effect of *Teracon-1*® on Oil Palm Pollinating Weevil, *Elaeidobius kamerunicus*

Teracon-1® at the economical dose, C3, 11.24 µg ml^{-1} and 100-fold higher dose of C5, 1124 µg ml^{-1} was significantly non-toxic at P=0.015 as compared to cypermethrin. *Teracon-1*® resulted in only 33% mortality of *E. kamerunicus* at the highest dose of C5, 1124 µg ml^{-1}, each at five and seven DAT. Cypermethrin at the original concentration, led to 100% mortality of *E. kamerunicus* at five and seven DAT (*Table 4*).

Study on the effect of *Teracon-1*® against oil palm pollinating weevil, *E. kamerunicus* indicated that corrected mortality of *E. kamerunicus* was

TABLE 3. PERCENTAGE MORTALITY OF OIL PALM POLLINATOR, *E. kamerunicus* SUBJECTED TO DIFFERENT TREATMENTS

	Treatments				
	Terakil-1®, cfu ml^{-1}				
Day after treatment	C3	C4	C5	Cypermethrin	Control
5	20	26	26	100	13
7	20	26	26	100	13

Note: Dose: C3 = $3.7x10^9$ cfu ml^{-1}, C4 = $3.7x10^{10}$ cfu ml^{-1} and C5 = $3.7x10^{11}$ cfu ml^{-1}.

TABLE 4. PERCENTAGE MORTALITY OF OIL PALM POLLINATOR, *E. kamerunicus* SUBJECTED TO DIFFERENT TREATMENTS

	Treatments						
	Teracon-1®, µg ml^{-1}						
Day after treatment	0.1124	1.124	11.24	112.4	1124	Cypermethrin	Control
5	20	27	27	27	33	100	20
7	20	27	27	33	33	100	20

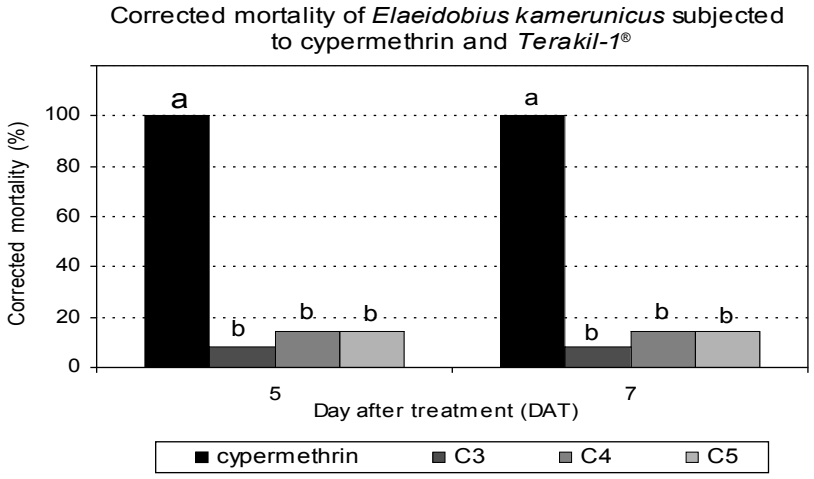

Figure 11. Corrected mortality of Elaeidobius kamerunicus *attributed by* Terakil-1® *as compared to cypermethrin. Dose: C3 = 3.7x10⁹ cfu ml⁻¹, C4 = 3.7x10¹⁰ cfu ml⁻¹ and C5 = 3.7x10¹¹ cfu ml⁻¹. Bars in groups with the same letters are not significantly different (P>0.05) after Duncan's Multiple Range Test. Corrected mortality* $= \left(\frac{\%Treatment - \%Control}{100 - \%Control} \right) x\ 100\%$.

significantly different (P<0.05) between *Teracon-1®* and cypermethrin at five and seven DAT. The corrected mortality of *E. kamerunicus* was not significantly different for five series of concentrations, 0.1124 μg ml⁻¹ – 1124 μg ml⁻¹ at P=0.05 when tested using Duncan's Multiple Range analysis. *Teracon-1®* caused only 16% corrected mortality of *E. kamerunicus* at seven DAT at the highest dose of C5, 1124 μg ml⁻¹ as compared to cypermethrin (*Figure 12*).

CONCLUSION

Terakil-1® and *Teracon-1®* as biopesticides were significantly harmless and have no adverse effect on the *E. kamerunicus* and beneficial insects as compared to cypermethrin. *Terakil-1®* and *Teracon-1®* were target specific to palm insect pests such as bagworms and nettle caterpillars and also effective against lepidopterous pests of other crops. Chemical pesticide, cypermethrin has an adverse effect and being toxic toward non-target organisms such as *E. kamerunicus* and beneficial insects should be avoided when possible. *Terakil-1®* and *Teracon-1®*

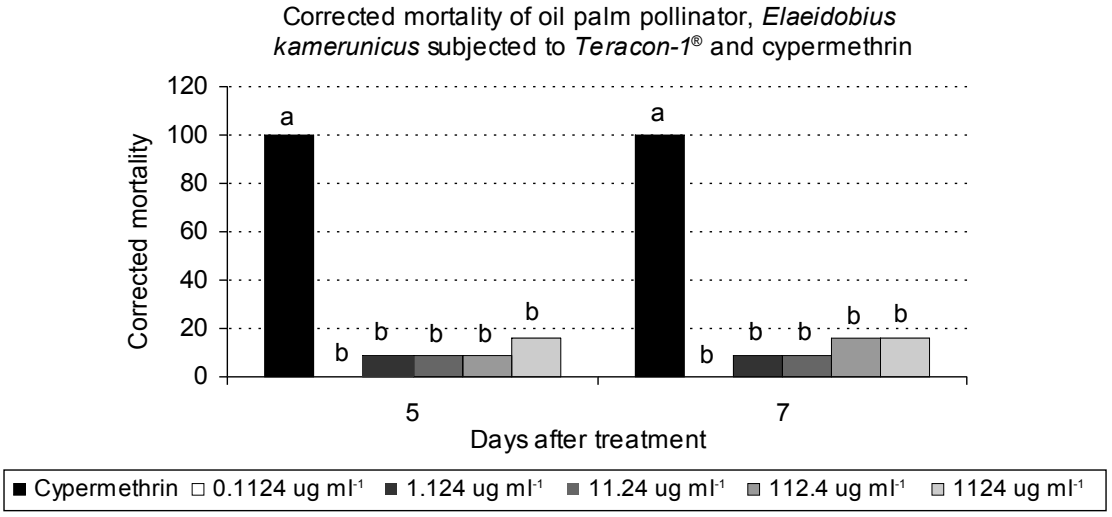

Figure 12. Corrected mortality of Elaeidobius kamerunicus *attributed by* Teracon-1® *as compared to cypermethrin. Dose: C1 = 0.1124 μg ml⁻¹, C2 = 1.124 μg ml⁻¹, C3 =11.24 μg ml⁻¹, C4 = 112.4 μg ml⁻¹ and C5 =1124 μg ml⁻¹. Bars in groups with the same letters are not significantly different (P>0.05) after Duncan's Multiple Range Test. Corrected mortality* $= \left(\frac{\%Treatment - \%Control}{100 - \%Control} \right) x\ 100\%$.

are recommended to decrease reliance on such toxic chemical pesticide.

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