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Glasshouse Evaluation on the Efficacy of *Bacillus thuringiensis* (*Bt*) Against Bagworm, *Metisa plana* and Non-target Effect on Oil Palm Pollinator, *Elaeidobius kamerunicus* (Faust)

YUSDAYATI RASHID, CIK MOHD RIZUAN ZAINAL ABIDIN, THEN KEK HO AND NOOR HISHAM HAMID

Pest Management Department, FGV R&D Sdn Bhd, PPP Tun Razak, 26400 Jengka, Pahang Darul Makmur, Malaysia

Evaluations on the performance of selected bioinsecticides and recommended chemical insecticides to suppress the bagworm, Metisa plana *(Walker) infesting oil palm, were carried out in a glasshouse environment. The results showed that a series of biological products using* Bacillus thuringiensis (Bt) *is effective in killing 80 per cent of bagworm larvae within 7 to 15 days after application (DAA). Among the biological products, the Bt1 product showed the highest efficacy, killing 99.72 per cent of the bagworm population at 7 DAA. Although slow in reacting,* Bt *products were highly toxic to the adults, and less effective on the emergence of pollinator weevil,* Elaeidobius kamerunicus *(Faust). The chemical insecticide, cypermethrin was the most effective compared to all other treatments ($p<0.05$), killing 100 per cent of the bagworm larvae in only 3 DAA. All non-*Bt *based insecticides, including cypermethrin, were toxic to both adults and emergence of the weevils. There was no significant difference in the number of adult emergence between the non-treated (control) and* Bt*-insecticides, but significantly was lower when treated with other insecticides ($F= 93.75$ $p<0.05$). Based on its high performance, the biopesticide Bt1 product has a higher potential to be tested in the field for integrated leaf-eating caterpillar management programme. To ensure consistency in their efficacy, these bio-insecticides need further evaluation in large-scale trials, especially in oil palm fields infested with bagworms in epidemic proportions.*

Keywords*:* Bagworms, *Bacillus thuringiensis*, non-target effect, pollinator, oil palm.

Chemical control is popularly practiced to control oil palm pests in Malaysia mainly due to its high efficacy, fast-acting, relative cheapness in the short-term and easily handled by operators (Pedigo, 2009). Despite their advantages, chemical insecticides have some well-known downside effects. The extensive use of chemical pesticides against insect pests may have contributed to the loss of pollinators (Blacquiere *et al.*, 2012) and its natural enemies (Norman *et al.*, 2011). It was reported that the application of cypermethrin could have a direct effect on the larvae and adults of *Elaeidobius kamerunicus* (Yusdayati & Noor Hisham, 2015). As a consequence of routine use in pest management, many insect pests have developed resistance towards various insecticides (Sarwar & Salman, 2015). Malaria vector (*Anopheles funestus*) was reported to have developed resistance to pyrethroid insecticide in South Africa, enabling the population of the species to increase their

email: yusdayati.r@fgvholdings.com

ranges into areas where pyrethroids were being exclusively used for malaria control resulting in unprecedented malaria epidemic (Wood *et al.*, 2010; Hargreaves *et al.*, 2000).

Furthermore, the increase in outbreak areas has led to increased consumption of pesticide. Since the cost of insecticide production has doubled in recent years, the price of chemical pesticides has increased compared to 10 years ago. The Malaysian Agricultural Digest reported that cost of consumption of agrichemicals in Malaysia has increased from RM328 million in 2005 to RM563 million in 2012 (Noorhazwani *et al.*, 2017). All these factors sufficiently prove that crop management using chemical control is costly and less desirable.

The effectiveness of insecticides is very much related to the properties of the insecticides, application method and the behaviour of the pests (Chung, 1998). Some pests such as bagworms are more difficult to control because they thrive in protective bags hung on the underside of the leaves (Rhainds *et al.*, 2009). Additionally, the timing of application is also very crucial to match the stage of their life cycle that are more susceptible to the insecticides (Yap, 2005), and have to be thoroughly sprayed too (Hasber, 2010). To reduce the shortcomings of chemical insecticides, particularly their detrimental effects on the pollinators, bio-insecticides such as *Bacillus thuringiensis* (*Bt*), which are more effective and with favourable toxicological and environmental profiles were introduced in the market (Mohd Najib *et. al.* 2009).

Bacillus thuringiensis (*Bt*) is a gram-positive spore-forming bacterium that produces crystalline proteins called delta-endotoxins during its stationary phase of growth (Saiyad, 2017). This bacterium is found naturally in soil, water, insect remains and grain dust (Valicente *et al.*, 2010). Various strains of *Bt* produce different parasporal inclusion proteins, which exhibit specific activity against the larvae of Lepidoptera, Diptera, Coleoptera, Hemiptera, Hymenoptera and Mallophaga (Morris *et al.*, 1997; Schnepf *et al.*, 1998). The mode of action of *Bt* involves a series of critical steps which are ingestion by the insect, specific binding to brush border membrane receptors and then membrane insertion which leads to spore formation (Federici, 1999). The protein crystal of *Bt* is highly lethal to certain Lepidopteran larvae and it is relatively harmless to other organisms. *Bt* was also claimed to be safe to beneficial insects such as predators, parasitoids and pollinator weevils, *Elaeidobius kamerunicus* (Coleoptera:Curculionidae) (Mohd Najib *et al.*, 2009).

At present, various *Bt* products with different efficacies and affinities to various pests are available in the market. Their favourable properties and effectiveness are highly desirable as alternative candidates for controlling oil palm pests in Malaysia. To determine the most suitable product against bagworms, a study to evaluate their effectiveness was undertaken. Their effects on the oil palm weevil pollinator, *E. kamerunicus* was also evaluated. The performances of the *Bt* products were compared with several chemical insecticides that had been widely used to control insect pests in ornamental plants.

MATERIALS AND METHODS

The effectiveness of *Bt* against bagworm larvae

Layout of treatments

In this study, four products of *Bt* were selected based on their commercially widespread use in controlling bagworms in oil palm. Two commonly used insecticides were used for comparison of the performances of the *Bt* products. Their active ingredients and concentrations used in this study are listed in *Table 1*. The treatments were also compared with two types of chemical insecticides that have been used as standard practice in oil palm plantation for controlling bagworm infestations.

Stock culture preparation of Metisa plana

The pupal cases of *M. plana* which contain eggs were collected from FELDA Besout region. The pupal cases were taken to the glasshouse and attached to the leaves of oil palm seedlings. The newly hatched larvae were reared on the seedlings for two weeks, following which healthy third instar larvae were collected and transferred to the seedlings for treatment one day prior to the application of treatments.

Experimental design of insecticides trials

The trial was arranged based on a completely randomised design (CRD). Twelve-month-old DxP oil palm seedlings were prepared to serve as a growth medium for *M. plana*. Fifty healthy third instar larvae of *M. plana* were placed on the oil palm seedlings one day prior to the treatments.

The insecticide was applied using a manual hand sprayer calibrated to 200 ml per seedling to ensure the seedling was completely covered with the insecticide. The untreated oil palm seedlings were sprayed with tap water in the control treatment. The number of bagworms that survived after the insecticide treatment was recorded at 1, 3, 7 and 15 days after application (DAA). Each treatment consisting of five replications with a total of 35 seedlings and 1 750 larvae was used in this trial. The mortality percentages of the bagworm larvae for each treatment was calculated for each sampling occasion (DAA).

Effect of various insecticides on mortality of Elaiedobius kamerunicus

This study was carried out in the Entomology Glasshouse, Felda Global Venture (FGV), Pusat Penyelidikan Pertanian Tun Razak (PPPTR), Jengka, Pahang. *E. kamerunicus* adults were collected from male inflorescences of 7-year- old DxP oil palms in PPPTR. The weevils were treated with corresponding insecticides for bagworm as listed in *Table 1*.

TABLE 1
LIST OF TREATMENTS, THEIR ACTIVE INGREDIENTS (A.I) AND RECOMMENDED RATES USED FOR CONTROLLING BAGWORMS IN A GLASSHOUSE TRIAL

Treatments	*Active ingredient (a.i.)*	*Recommended rate (ml/litre of water)*
Bt1product	*Bacillus thuringiensis* subsp. *kurstaki* (17 600 I.U./mg)	3.4
Bt2 product	*Bacillus thuringiensis* subsp *kurstaki* 8.12% w/w	2.5
Bt3 product	*Bacillus thuringiensis* subsp *kurstaki* 25% w/w	50.0
Bt4 product	*Bacillus thuringiensis* subsp. *kurstaki* (16 000 I.U./mg)	1.0
Chlorantraniliprole	Chlorantraniliprole 5%w/w	1.0
Cypermethrin	Cypermethrin 5.5% w/w	3.27
Tap water (Control)	-	-

In this study, fresh oil palm full anthesis spikelets (average 20 cm long) were used as refuges for the weevils. For each treatment, three fresh oil palm spikelets free from weevils were placed into a small container and sprayed with approximately 0.7 ml of insecticide solution per spikelet at the recommended rate using a manual hand sprayer. After 30 seconds of spraying, 20 healthy weevils were introduced into the container. Each treatment has four replications and the number of live and dead weevils were counted and recorded on a daily basis for five consecutive days. The mortality of weevils was calculated as follows:

% cumulative mortality =
100 - [(a / b) x 100]

Where,
a = total live weevil after treatment
b = total live weevil before treatment

Meanwhile, corrected cumulative mortality was calculated using Abott's Corrected Mortality formula as below:

% corrected mortality =

$$\frac{\%T - \%C}{100 - \%C} \times 100$$

Where,
% T = percentage of dead weevil in particular treatment
% C = percentage of dead weevil in control treatment

The differences of the mean weevil mortalities among the treatments were analysed using ANOVA and significant means were subsequently tested using Tukey *post-hoc* test.

Effect of various insecticides on the emergence of E. kamerunicus

The insecticide treatments in this study were similar in experiments 1 and 2 (*Table 1).* Oil palm male inflorescence (DxP) was collected from a 7-year-old DxP oil palm and brought to the Entomology Lab, PPPTR. The spikelets in each full anthesis inflorescence that contained weevil eggs were identified and three or four spikelets in the middle of the inflorescence were cut and sprayed with the respective treatments. The spikelets of each treatment were placed in a small container and observed for the development of the adult emergence. Each treatment was replicated four times. The numbers of emerged adults from each treatment were counted daily and recorded until the last adult emerged. The differences in the mean number of adult emergences among treatments were analysed using one-way ANOVA.

RESULTS

Effectiveness of *Bt* against bagworm larvae

This study shows that chemical insecticides were fast-acting, as expected and more effective in controlling the bagworm population. The mortality rate of bagworms treated with cypermethrin and chlorantraniliprole was almost 100 per cent within a span of 3 days. In fact, cypermethrin killed more than 98 per cent and chlorantraniliprole eliminated more than 72 per cent of the bagworms at 1 DAA (*Table 2*). All *Bt* products did not show much effectiveness at 1 DAA, but Bt1 caused relatively high mortality (69.25%) at 3 DAA. It was recorded after a week up to 99.75 per cent of the bagworms were killed and 100 per cent mortality was achieved after 2 weeks by Bt1 application, which was comparable to the toxic effect of the chemical insecticides. Generally, the *Bt* products were slow in action and a noticeable amount of mortality was only recorded at 7 DAA.

TABLE 2
CUMULATIVE MORTALITY PERCENTAGE OF BAGWORM (*METISA PLANA*) AFTER THE APPLICATION OF VARIOUS INSECTICIDES

Treatments	*Cumulative mortality (% ± SE)*			
	Days after application (DAA)			
	1	*3*	*7*	*15*
Bt1 product	1.25±0.75 c	69.25±1.75 b	99.75±0.25 a	100±0 a
Bt2 product	0.75±0.48 c	16.5±3.48 d	80±6.28 bc	96.5±1.85 a
Bt3 product	1.25±0.75 c	39.25±3.38 c	89.25±3.66 ab	95±3.72 a
Bt4 product	0.25±0.25 c	23.75±3.28 d	73.25±2.32 c	93.75±1.6 a
Chlorantraniliprole	72.75±6.06 b	99.5±0.5 a	100±0 a	100±0 a
Cypermethrin	98.25±0.85 a	100±0 a	100±0 a	100±0 a
Untreated Control	0.5±0.5 c	1±1 e	9.75±1.31 d	50.25±11.66 b

Note: Means within rows followed by the same letter are not significantly different (P>0.05) by Tukey test

TABLE 3
CORRECTED MORTALITY PERCENTAGE OF BAGWORMS (*METISA PLANA*) AFTER THE APPLICATION OF VARIOUS INSECTICIDES

Treatments	*Corrected mortality (% ± SE)*			
	Days after application (DAA)			
	1	*3*	*7*	*15*
Bt1 product	0.75±0.75 c	68.94±1.75 b	99.72 ±0.25 a	100±0.00 a
Bt2 product	0.25± 0.86 c	15.66±3.63 d	77.84 ±6.95 bc	92.96±2.89 a
Bt3 product	0.75±0.48 c	38.64±3.09 c	88.089±3.81 ab	89.95±4.78 a
Bt4 product	0±0.26 c	22.98±2.71 d	70.36±2.25 c	87.44±3.11 a
Chlorantraniliprole	72.61±6.10 b	99.49±0.52 a	100±0.00 a	100±0.00 a
Cypermethrin	98.24±0.85 a	100±0.00 a	100±0.00 a	100±0.00 a

Note: Means within rows followed by the same letter are not significantly different (P>0.05) by Tukey test

Toxicity effect of *Bt* applications against oil palm pollinator, *Elaeidobius kamerunicus*

The cumulative and corrected mortality of *E. kamerunicus* after the respective insecticide treatments at 1-5 DAA are shown in *Table 4* and *Figure 1*. Similar to its effect on bagworms, cypermethrin was the most toxic insecticide killing all weevils (100% mortality) as early as 1 DAA. Meanwhile, chlorantraniliprole had significantly reduced the number of *E. kamerunicus* by 65.0 per cent on the following day after treatment. Among all *Bt* products, Bt1 caused the highest mortality of 66.25 per cent 24 hours after application of treatment.

This study shows that Bt1 product was highly toxic to the pollinator weevil which is equivalent to the toxicity of chemical insecticides, cypermethrin and chlorantraniliprole from 2 DAA until 5 DAA. Although it did not kill all the weevils, however, their mortality was very high

TABLE 4
CUMULATIVE MORTALITY OF POLLINATING WEEVIL, *ELAEIDOBIUS KAMERUNICUS*, AFTER THE APPLICATION OF VARIOUS INSECTICIDES

Treatment	*Cumulative mortality (%±SE)*				
	Days after application (DAA)				
	1	*2*	*3*	*4*	*5*
Bt1 product	66.25±11.97 ab	91.25 ±5.9 a	91.25±5.9 ab	93.75±4.73 ab	97.5±1.44 a
Bt2 product	38.75±8.0 bc	46.25 ±9.87 bc	48.75±8.75 bc	51.25±8.26 ab	63.75±10.07 ab
Bt3 product	61.25±17.96 ab	66.25 ±19.83 ab	66.25±19.83 ab	71.25±16.38 ab	81.25±9.66 ab
Bt4 product	35.0±11.73 bc	60 ±12.25 ab	66.25±12.14 ab	66.25±12.14 ab	77.5±7.5 ab
Chlorantraniliprole	65.0±3.54 ab	93.75 ±2.39 a	100±0 a	100±0 a	100±0 a
Cypermethrin	100±100 a	100 ±0 a	100±0 a	100±0 a	100± a
Untreated Control	6.25±4.73 c	6.25 ±4.73 c	6.25±4.73 c	20±13.07 b	40.75±21.05 b

Note: Means within rows followed by the same letter are not significantly different (P>0.05) by Tukey test

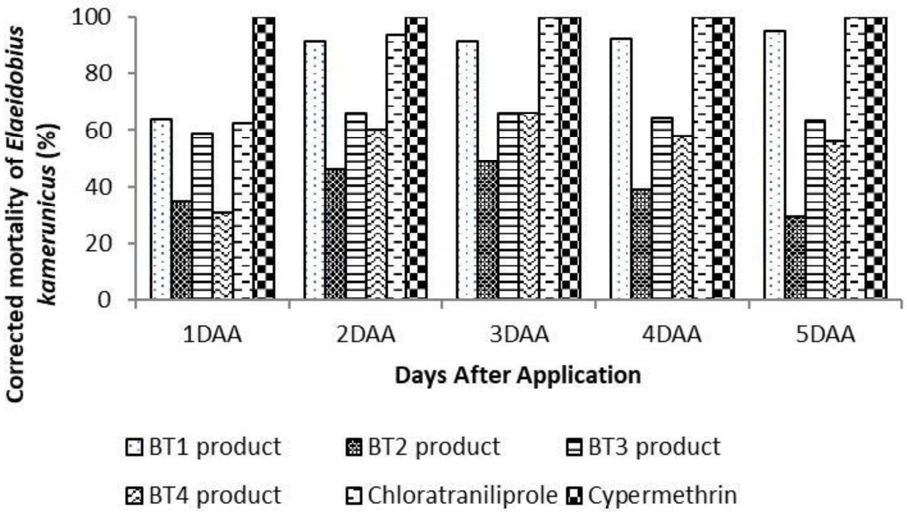

Figure 1 The corrected mortality of Elaeidobius kamerunicus *after 5 days of application of treatments*

at 91.25 to 97.5 per cent within 4 days after treatment application and showed similar mortality as cypermethrin. Among the *Bt* products, Bt2 and Bt4 were relatively safer to *E. kamerunicus*, whereby their weevil mortalities were lower compared with the other insecticides and the daily mortality increment rates were also slower. Generally, all Bt products and chemical insecticides were toxic to *E. kamerunicus* and their mortalities were not statistically different at P=0.05.

The emergence of weevils from treated spikelets was not very much affected by the toxicity of the *Bt* products (*Figure 2*). In all four *Bt* products, the number of adults that emerged was not significantly different from

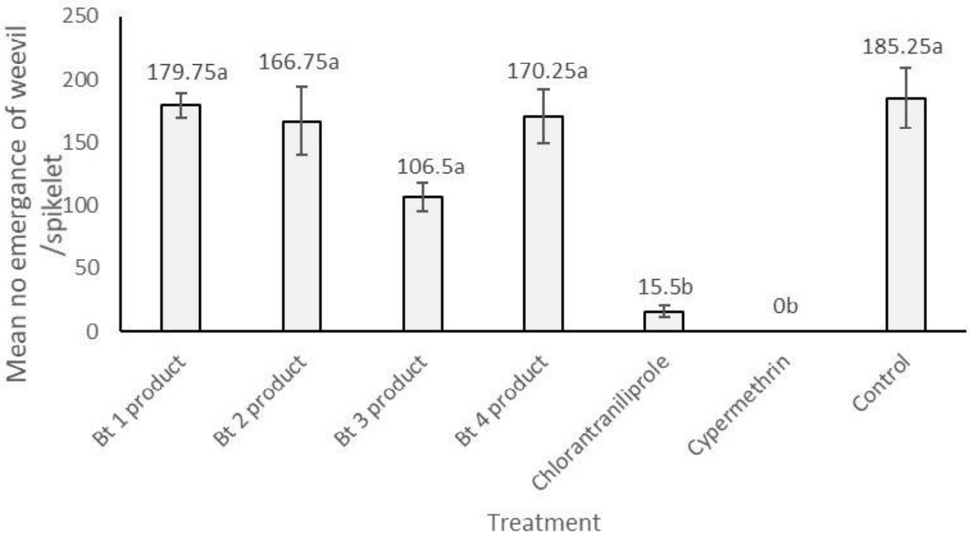

Figure 2 The number of adult E. kamerunicus *emergence per spikelet after 7 days of exposure to application of insecticides*

that of the untreated spikelets (P=0.05). The highest emergence was observed in Bt1 product treated spikelets (97% closer to control), followed by Bt2 and Bt4 products. However, slightly lower emergence was recorded for Bt3 product although it was not significantly different from the untreated spikelets. All non-*Bt* insecticides were very toxic to the development of the weevil as reflected by very low adult emergence (0% to 15.75%) from each treatment, especially cypermethrin. Somehow, chlorantraniliprole was not significantly different as compared with cypermethrin ($p>0.05$).

DISCUSSION

The *Bt* products in this study were derived from a bacterium *Bacillus thuringiensis*, a biological organism that attacked the digestive system of the insect pests and subsequently kill the hosts. The caterpillars that eat *Bt* on the leaves die when *Bt* spores are activated in the insects' stomach (Reardon *et al.,* 1994; Siti Ramlah & Basri, 1997). Therefore, the poisoning process requires a little time to produce the detrimental effect and the mortality of the pests takes place slightly later than what is normally observed in chemical insecticides. Extremely low mortality of bagworm larvae was detected in *Bt* products at 24 hours after application, but a drastic increment of the effect was observed from 3 DAA until 15 DAA. Satisfactory control of the bagworms by all products was recorded at 7 DAA. According to Knowles (1994), insect larvae normally take 2 to 3 days to die after exposure to *Bt*. This period may extend beyond one week for larger larvae (Glare & Callaghan, 2000). Chung and Narendran (1996) also reported the slow action of *Bt* insecticides on *M. plana.* A satisfactory kill (70% to 100%) of the early instar larvae was observed one week after the application of the bioinsecticides. Meanwhile, Noorhazwani *et. al.* (2017) reported the effectiveness of *Bt* in a field trial by aerial spraying but multiple sprayings are required.

The application of *Bt* insecticides to control bagworm in this study did not show any toxic effect on the emergence of *E. kamerunicus*. Due to the mode of action (pest need to ingest), *Bt* was a very selective insecticide and more environmentally friendly (Basri *et al.*, 1996; Siti Ramlah *et al.*, 2003). Since all *Bt* proteins have a very restricted spectrum of activity, each usually only exhibits toxicity to insects of a single order (Lepidoptera, Diptera or Coleoptera). Thus, the use of *Bt* proteins is much more environmentally compatible than chemical insecticides. But, since certain *Bt* products can adversely affect Coleopteran, this explains why *Bt* can affect the population of *E. kamerunicus* in this study.

The results of this study suggest that *Bt* products do not affect the development of *E. kamerunicus* larvae. When the insecticides were applied onto oil palm male inflorescences, there was no detrimental effect on the development of the weevil from larva to the adult stages. Insect eggs are usually safe from the harmful effect of pesticides because of their protective eggshell covering. In oil palm plantations, many insecticides applied to control bagworms showed low effectiveness due to continuous hatching of the eggs. In this case, some plantations struggled to follow the standard practice in controlling certain pests when it involves targetting a specific stage of pest development.

Bt insecticides have minimal effect on the emergence *of E. kamerunicus* and open an opportunity to the oil palm planters to optimise the control of oil palm pests especially the bagworms. Application of highly effective *Bt* products such as the emulsion solution (ES) during the anthesis of male inflorescence would kill the targetted pest but protect the pollinators from the harmful effects of the insecticide.

Statistically, there was no significant difference in the efficacies of all treatments tested and the results of this study clearly indicate that the insecticides, cypermethrin, chlorantraniliprole and *Bt* products were suitable for use against bagworms through ground spraying with high volume of water. But due to the high toxicity of cypermethrin and 100 per cent effect on the adults and emergence of pollinator weevils, it is not recommended to be used in the field. *Bt* product with ES formulation had displayed high toxicity to larvae of bagworms and was the safest insecticide to *E. kamerunicus* emergence. Proper timing of *Bt* application would satisfactorily control the bagworms and protect the pollinator population. This bio-insecticide makes a good candidate for testing in bagworm control in oil palm plantations, especially during sporadic infestation.

CONCLUSION

As outlined previously, *Bt* products were effective against the bagworm population in glasshouse conditions but relatively toxic to oil palm pollinator, *E. kamerunicus* in the adult stage. However, they are safer towards *E. kamerunicus* emergence compared to other treatments. Bt1 product was the most effective and the safest product to the emergence of *E. kamerunicus* and therefore suitable for use in the management programme of bagworms in oil palm plantations. Proper timing of application of *Bt* products to control bagworm infestation would provide optimum benefit to the oil palm planters.

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