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Evaluation of Selected Insecticides for Managing Larvae of *Erionota thrax* and Effects on its Parasitoid (*Brachymeria albotibialis*)

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ABSTRACT

Banana skipper (*E. thrax*) is an important folivorous insect pest of *Musa* spp. Mortality effects of Dipel® (*Bacillus thuringiensis*), Capture 605® (cypermethrine), and Wesco Malathion 57® (malathion) were evaluated on larvae of *E. thrax* (laboratory and field studies) and on a major pupal parasitoid (*B. albotibialis*) of *E. thrax* (laboratory studies). For young instar larvae, Capture 605® and Wesco Malathion 57® caused 100% mortality after 12 h while Dipel® caused 100% only after 30 h. For mature instar larvae, Capture 605® caused 100% mortality after 12 h while Dipel® and Wesco Malathion 57® caused 100 and 92% mortality, respectively after 30 h. After 6, 18, and 24 h, mortality of mature instar larvae caused by Dipel® and Wesco Malathion 57® were not significantly different. Based on direct exposure of adult parasitoids to the treatments, Capture 605® and Wesco Malathion 57® caused 100% mortality after 24 h while Dipel® caused 45%. Generally, Capture 605® and Wesco Malathion 57® were harmful to the parasitoids while Dipel® was harmless. Athough Capture 605® caused highest mortality of *E. thrax* larvae in the field (90% after 6 h and 85.3% after 24 h), that for Dipel® and Wesco Malathion 57® were not significantly different. This research therefore shows that *B. thuringiensis* (a microbial control agent) could be used as an important component for integrated management of *E. thrax*.

Keywords: banana, *Bacillus thuringiensis*, biocontrol, cypermethrine, malathion, pest

INTRODUCTION

Bananas are important to many people in the world (Lassoudiere 2007) and in South East Asia where they serve as food and their leaves play an important role as biological plates (using plant leaves or parts to keep or serve food) (Waterhouse and Norris 1989; Khoo *et al*. 1991). There are many insect pests on bananas and the most important folivorous one is the banana skipper (Okolle *et al*. 2006a). Although the banana skipper (*Erionota thrax*) is often considered as a minor pest in its native Southeast Asia (Ahmad and Balasubramaniam 1975; Kalshoven 1981; Khoo *et al*. 1991; Hasyim *et al*. 1994), it is a serious pest when its natural enemies are absent especially outside its native area (Sand et al. 1993; Okolle *et al*. 2006b, 2006c, 2009). *Erionota thrax* usually lays its eggs in clusters on leaves of banana plants 3-5 months old (Okolle *et al*. 2006c) and after 5-8 days, the eggs hatch and the young larvae begin to feed and construct leaf rolls on the edges of the leaves. Waterhouse *et al*. (1998) suggested that since the larvae and pupae are concealed within leaf rolls, chemical applications might not be effective. Although defoliation of bananas in its native area is usually low, occasionally it is very serious, especially during outbreaks (Ahmad and Balasubramaniam 1975; Khoo *et al.* 1991) and when it is found outside its native area (Waterhouse and Norris 1989).

With the injudicious use of insecticides in some banana plantations, coupled with the increasing status and invasion of this pest to non-native areas, basic ecological research on *E. thrax* and its natural enemies in its native areas are necessary for implementing any meaningful and effective classical biological control or integrated management. Although various management options have been reported (e.g. use of parasitoids, hand picking, pruning of leaf rolls, and use of numerous chemicals) (Okolle *et al*. 2010), there seems to be no study on the effects of different groups of insecticides on larvae and on parasitoids of *E. thrax*. In most farms and/or plantations in Malaysia, conventional insecticides are sprayed on a calendar basis. Insecticides used in the plantation where this research was carried out are found in **Table 1**. Of all the insecticides, Capture 605® (cypermethrin – a pyrethroid), Wesco Malathion 57® (malathion – an organophosphate), and Dipel® (*Bacillus thuringiensis* – a microbial control agent (MCA)) were the most commonly used and the frequency of spraying was usually once or twice per month. According to Nikpay (2007), due to food and environmental safety concerns as well as development of resistant pest populations, there is increasing need to

Table 1 Application mode and doses of insecticides applied in a banana plantation, Penang, Malaysia.

Insecticides	Active ingredient	Application mode	Application dose
Capture 605®	Cypermethrin	Mist blower (tractor and motorized knapsack)	220 ml/300 L water
Wesco Malathion 57®	Malathion	Mist blower (tractor and motorized knapsack)	135 ml/300 L water
Decis®	Deltamethrin	Mist blower (tractor and motorized knapsack)	Not available
Endotox 555®	Endosulfan	Mist blower (tractor and motorized knapsack)	530 ml/300 L water
Dipel®	*Bacillus thuriengensis*	Mist blower (tractor and motorized knapsack)	Not available

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assess alternative or biorational compounds. The objective of this research was therefore to find out the toxic effects that different groups of insecticides commonly used in the plantations have on *E. thrax* larvae and on a major parasitoid of the pupae (*Brachymeria albotibialis*) as reported by Okolle *et al*. (2006b, 2008).

MATERIALS AND METHODS

Experimental site

The research was carried out in a commercial plantation (Synergy Farm) consisting of about 60% Cavendish bananas and 40% of assorted fruit orchards, fishponds, and wild flowers. The plantation is made up of sandy loam clay soil that also supports a variety of weeds and it is surrounded entirely by oil palm (*Elaeis guineensis* Jacq.) and located in Ara Kuda, Daerah Seberang Prai – Penang State, Malaysia. Ara Kuda is positioned at latitude 5° 30' North and longitude 100° 30' East with an altitude of 52 m above sea level. Laboratory tests were carried out in the laboratory of the plantation.

Bioassay on *E. thrax* larvae

Four treatments were used for this experiment: Dipel® (an entomopathogen with active ingredient as *Bacillus thuringiensis* var Kustaki), Capture 605® (classical pyrethroid insecticide with active ingredient as cypermethrin), Wesco Malathion 57® (classical organophosphate with active ingredient as malathion), and a control (potable tap water). Hextar Chemical Sdn Bhd of Malaysia supplied Dipel® and Capture 605® to the plantation while Wesco Agencies (M) Sdn Bhd supplied Wesco Malathion 57®. Two ml of each insecticide was mixed with 500 ml of potable water inside a beaker and the resulting solution was stirred gently. 25 m^2 pieces of fresh banana leaves (var. Cavendish - AAA) were cut from the field and placed in the insecticide solutions for 5 min. The leaves were then removed, air-dried for 5 min and then placed singly into 40 plastic containers (15 cm in diameter and 12 cm in height) (10 containers for each treatment) whose open ends were covered with perforated polyethylene. These plastic containers together with their contents were kept in the laboratory at mean temperature of 29.9 ± 2°C, mean relative humidity of 66.8 ± 4%, and a 14-h photoperiod. The temperature and relative humidity were measured using a mini hygro-thermometer (Extech Instruments Corp., Model 445702; China). Mature larvae (3^{rd} – 5^{th} instars) collected from the field were then placed singly into the containers. This experiment was replicated 6 times such that there were 10 larvae per replicate. In addition, this same experiment was repeated for young larvae (1^{st} and 2^{nd} instars) collected from the field. Cumulative mortalities of these young and mature larvae were recorded at 6-h intervals for a maximum of 30 h.

Bioassay on a major pupal parasitoid of *E. thrax*

1. Collection of adult parasitoids

Banana leaf rolls with parasitized pupae within were collected from the banana plantation. Generally, pupae of *E. thrax* parasitized by *Brachymeria* spp are dark brown with three distinct dark rings around the abdominal region (Okolle *et al*. 2008, 2010). These parasitized pupae were kept in cages in the laboratory until adult parasitoids emerged.

2. Effects of contaminated banana leaves on adult parasitoids

The experiment was similar to that for *E. thrax* larvae except that instead of larvae, the adult parasitoids (*B. albotibialis*) of *E. thrax* pupae were placed into the plastic containers. The experiment was repeated 4 times. Cumulative mortalities of these parasitoids were recorded after 6 and 24 h.

3. Dipping parasitized E. thrax pupae into insecticide solutions

Parasitized *E. thrax* pupae were collected from the field. Such pupae usually had 3 black or dark rings or bands on the abdominal region, serving as typical sign of parasitism of *Brachymeria* spp., which is a major parasitoid (Okolle *et al*. 2008, 2010). For each of the treatments (*Bacillus thuringiensis*, cypermethrin, malathion and the control), 40 parasitized *E. thrax* pupae were dipped into the solution and left there for 5 sec. Pupae from the different treatments were placed separately into plastic containers (the containers were 10 cm in height and 10 cm in width and each contained 10 parasitized pupae) with perforated lids. The pupae were then observed daily for emergence of adult parasitoids. After 2 weeks, if no emerged adult parasitoids were seen, the pupae were dissected under a light microscope to record any dead parasitoid larvae, pupae or adults.

Effects of insecticides on *E. thrax* larvae in the field

A highly infested block from the plantation that had been left unsprayed for 6 months was chosen and from this, 3 rows were selected for each treatment. The 3 rows were separated from each other by 10 unsprayed rows. Using a motorized backpack sprayer, the different insecticides (treatments) (20 ml per 10 L of water each) were applied on the leaves of the plants. After application of the treatments, leaf rolls were collected after 6 and 12 h. This short period of time was considered because the purpose of this experiment was to determine the acute toxicity of the chemicals to larvae that are usually found inside leaf rolls. These leaf rolls were taken to the laboratory, opened carefully and then cumulative mortalities of larvae (young and old combined) were recorded.

Data analyses

Cumulative numbers of dead larvae and parasitoids were converted to percentage mortalities. The percentages or proportions were arcsine transformed and then subjected to a one-way analysis of variance (ANOVA). These transformations and analyses were done using the STATDISK V 9.1 software (Triola *et al*. 2003). Upon rejection of the null hypothesis (equal means), the means were further subjected to Tukey's means separation test of equal sample sizes (Fowler *et al*. 1998) to find out those that were significantly different at $\alpha = 0.05$. In all cases, only untransformed values are reported. Concerning mortality on the adult parasitoids, harmful impact of the insecticides was interpreted according to the IOBC standards (Sterk *et al*. 1999). These standards included four categories; (1) harmless (mortality ≤ 30%), (2) slightly harmful (31-79% mortality), (3) moderately harmful (80-99% mortality), and (4) harmful (mortality > 99%).

RESULTS

Mortality on young and mature instar larvae

After 6, 12, and 18 h, for mortality of young instar larvae, there was a significant difference between Dipel® and the conventional insecticides (Capture 605® and Wesco Malathion 57®) (Critical F $(CF)_{16,3} = 3.1$, Test Statistics (TS) = 153.7 at $\alpha = 0.05$); $CF_{16,3} = 3.1$, TS = 178 at $\alpha = 0.05$; $CF_{16,3} = 3.1$, TS = 251.9 at $\alpha = 0.05$, respectively; **Table 2**). However, there was no significant difference after 24 and 30 h ($CF_{16,3} = 3.1$; TS = 842.7 at $\alpha = 0.05$ and $CF_{16,3} = 3.1$; TS = 153.7 at $\alpha = 0.05$, respectively). Capture 605® and Wesco Malathion 57® resulted to 100% mortality of young instar larvae after 12 h while Dipel® resulted to 100% mortality only after 30 h.

Generally, for mature instar larvae, after 6, 18, and 24 h, mortality resulting from Dipel® and Wesco Malathion 57® were not significantly different ($CF_{16,3} = 3.2$; TS = 36.4 at $\alpha = 0.05$; $CF_{16,3} = 3.2$; TS = 76.5 at $\alpha = 0.05$; and $CF_{16,3} = 3.2$; TS = 82.1 at $\alpha = 0.05$, respectively) (**Table 2**). Capture 605® caused 100% mortality after 12 h while Wesco Malathion 57® and Dipel® caused 100 and 92%, respectively only after 30 h.

In all the bioassay concerning *E. thrax* larvae, after about 30 min, all the banana leaf pieces that were immersed in Capture 605® solution became decolorized (turning from green to dark brown patches). Furthermore, in the Capture

Table 2 Mean percent mortalities (±SE) of young and mature *E. thrax* instar larvae after exposure to *Bacillus thuringiensis* (Dipel), cypermethrine (Capture) and malathion (Wesco) in the laboratory.

Chemical treatments	Young instar larvae					Mature instar larvae				
	6 h	12 h	18 h	24 h	30 h	6 h	12 h	18 h	24 h	30 h
Dipel®	73.3 ± 6.1 a	91.7 ± 3.9 a	96.7 ± 3.3 a	98.3 ± 1.6 a	100 ± 0.0 a	34 ± 5.9 a	64 ± 10 a	86 ± 7.6 a	88 ± 7.5 a	92 ± 3.8 a
Capture®	98.3 ± 1.7 b	100 ± 0.0 a	100 ± 0.0 b	100 ± 0.0 a	100 ± 0.0 a	86 ± 5.2 b	100 ± 0.0 b	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 b
Wesco®	96.7 ± 1.7 b	100 ± 0.0 a	100 ± 0.0 b	100 ± 0.0 a	100 ± 0.0 a	56 ± 11.9 a	92 ± 3.8 c	98 ± 2.1 a	98 ± 2.1 a	100 ± 0.0 b
Control	0 ± 0.0 c	1.7 ± 1.6 b	1.7 ± 1.6 c	0 ± 0.0 b	0 ± 0.0 b	0 ± 0.0 c	0 ± 0.0 d	2 ± 0.9 b	2 ± 0.9 b	2 ± 0.9 c

Within a column, means with the same letters are not significantly different at P≥0.05 (Tukey's mean separation test).

Table 3 Mean percent mortalities (±SE) of a major parasitoid (*Brachymeria albotibialis*) of *E. thrax* pupae after exposure to *Bacillus thuringiensis* (Dipel), cypermethrine (Capture) and malathion (Wesco) in the laboratory.

Chemical treatments	Time interval			
	Direct contact of adults with chemical treatments		Parasitized *E. thrax* pupae dipped in chemical treatments	
	After 6 h	After 24 h	Parasitoid died within *E. thrax* pupae	Parasitoid emerged but died after 6 h
Dipel®	27.5 ± 9.3 a (1)	45 ± 9.8 a (2)	25 ± 3.3 a (1)	23 ± 2.9 a (1)
Capture®	100 ± 0.0 b (4)	100 ± 0.0 b (4)	52.5 ± 2.6 b (2)	59.8 ± 10.3 b (2)
Wesco®	97.5 ± 1.7 b (3)	100 ± 0.0 b (4)	37.5 ± 6.6 b (2)	44.8 ± 2.1 b (2)
Control	0 ± 0.0 c (1)	2.5 ± 2.5 c (1)	0 ± 0.0 c (1)	0 ± 0.0 c (1)

Within a column, means with the same letters are not significantly different at P≥0.05 (Tukey's mean separation test). Numbers in parenthesis represent harmful impacts according to IOBC standards (1 = harmless, 2 = slightly harmful, 3 = moderately harmful, 4 = harmful)

605® treatments, none of the larvae were able to roll leaves and instead of fecal pellets (frass), the affected larvae sent out watery stool before dying. However, unlike the Capture 605® treatment, all the leaves used in the Wesco Malathion 57® Malathion 57 and Dipel® treatments were rolled by the larvae although all dead larvae were found outside the leaf rolls. In all the treatments, most of the surviving mature larvae were 5th instars (i.e., those transforming from larvae to pupae). For the young instars, those surviving after 6 h were those in the process of moulting (i.e., changing from one instar larva to another).

Table 4 Mean percent mortalities (±SE) of *E. thrax* larvae after spraying of *Bacillus thuringiensis* (Dipel), cypermethrine (Capture) and malathion (Wesco) in the field.

Chemical treatments	After 6 h	After 24 h
Dipel®	23.3 ± 7.5 a	33.5 ± 2.2 a
Capture 605®	90.0 ± 0.0 b	85.3 ± 4.5 b
Wesco Malathion 57®	28.5 ± 9.5 a	41.5 ± 2.0 a
Control	0 ± 0.0 c	0 ± 0.0 c

Within a column, means with the same letters are not significantly different at P≥0.05 (Tukey's mean separation test).

Mortality on adult parasitoids

Direct exposure of the parasitoids to the treatments after 6 and 24 h, resulted to a significant difference between Dipel® and the other insecticides ($CF_{12,3}$ = 3.49; TS = 141.4 at α = 0.05; and $CF_{12,3}$ = 3.49; TS = 125.1 at α = 0.05 respectively) (**Table 3**). Capture 605® and Wesco Malathion 57® caused 100% mortality after 24 h while Dipel® caused 45%. Furthermore, according the IOBC standards, Capture 605® and Wesco Malathion 57® were found to be generally harmful while Dipel® was harmless after 6h and slightly harmful (45% mortality) after 24h.

After dipping parasitized *E. thrax* pupae in the treatment solutions, there was a significant difference between Dipel® and the conventional insecticides but there was no significant difference between Capture 605® and Wesco Malathion 57® for parasitoids dying within *E. thrax* pupae and those dying 6 h after emerging from *E. thrax* pupae ($CF_{12,3}$ = 3.49; TS = 33.1 at α = 0.05; and $CF_{12,3}$ = 3.49; TS = 23.9 at α = 0.05, respectively) (**Table 3**). However, in this situation, the IOBC standards showed that Capture 605® and Wesco Malathion 57® were slightly harmful while Dipel® was harmless. In all cases, Capture 605® caused the highest mortality of adult parasitoids (>50% and reaching a peak of about 60%) (**Table 3**).

Mortality of *E. thrax* larvae in the field

Although highest mortalities were recorded for Capture 605® after 6h (90%) and 24 h (85.3%) (**Table 4**), there was no significant difference between Dipel® and Wesco Malathion 57® Malathion after 6h and 24 h of spraying in the field ($CF_{12,3}$ = 3.49; TS = 37.5 at α = 0.05; and $CF_{12,3}$ = 3.49; TS = 158.8 at α = 0.05, respectively).

DISCUSSION

According to Fitzgerald (2004), although many laboratory bioassays assess only short-term acute toxicity of pesticides, they are quicker and less labour intensive than field experiments and therefore a useful way of evaluating a range of pesticides for managing a given pest. However, such short-term evaluations would hardly show sub-lethal effects (e.g. oviposition and fecundity) of the tested chemicals on the pest. Generally, in our study, mortality of younger *E. thrax* larvae was higher than those of mature larvae especially after 6 h of exposure to the treatments. Studies have also shown that younger larvae of insect pests are more susceptible to insecticides (Mann *et al*. 2009; Sial and Brunner 2010). First and second instar larvae of *E. thrax* are usually very active, searching suitable locations for food, constructing leaf rolls, eating more food and have no protective waxy powder on their bodies (Okolle 2006; Okolle *et al*. 2008, 2010) and all these increases exposure of the insects to insecticides found on banana leaf surfaces. On the contrary, mature instar larvae of *E. thrax* are less active, feed less and have bodies covered with a thick white powder (Khoo *et al.* 1991; Okolle *et al*. 2006b, 2008). This therefore reduces exposure of the mature insects to insecticides and hence lower mortality.

Although the conventional insecticides resulted to 100% mortality of *E. thrax* larvae after 12 h, Dipel® also resulted in 100 and 92% of young and mature larvae, respectively only after 30 h. This shows that the MCA (Dipel®) needs a relatively longer period to cause mortality in the insect pest and to subsequently control the insect pest population. These high mortalities on young and mature larvae of *E. thrax* caused by Dipel® are an indication that the MCA can conveniently and effectively be used alternately with conventional insecticides in an IPM program. With the exception of Capture 605® that caused 85.3% mortality of larvae in the field after 24 h, that for Dipel® and Wesco Malathion 57® was less than 50% and not significantly different (**Table 4**). This shows that the high frequency of spraying Wesco Malathion 57® in the plantation might only increase cost of production as it was not found to be superior to Dipel®. Besides, our laboratory studies showed that

Wesco Malathion 57® is significantly more harmful to pupal parasitoids of *E. thrax* than (Dipel® *B. thuringiensis*) (**Table 3**).

Generally, considering direct exposure of the adult parasitoids (*B. albotibialis*) as well as dipping parasitized *E. thrax* pupae into the treatment solutions, our results showed a significant difference between Dipel® and the conventional insecticides (Wesco Malathion 57® and Capture 605®). Although broad spectrum insecticides such as organophosphates have been reported to provide stable management of leaf rollers and other major pests (Sial and Brunner 2010), use of organophosphates such as malathion has been controversial because of human health concerns and harmful effects on beneficials (Urbaneja *et al.* 2009) as well as their relatively high mammalian toxicity (Scharf 2003). In addition, according the IOBC standards, direct contact of the parasitoid with the insecticides in this research shows that Wesco Malathion 57® and Capture 605® are harmful (100% mortality after 24-h exposure) (**Table 3**). This therefore supports several studies that have shown that most conventional insecticides are harmful to natural enemies of crop pests (Takahashi and Kiritani 1973; Haubruge and Amichot 1998; Elzen 2001; Desneux *et al*. 2007; Asogwa and Dongo 2009; imidacloprid significantly reduced the instantaneous rate of increase of *Galendremus occidentalis* (Stavrinides and Mills 2009); Urbaneja *et al*. 2009 where malathion and spinosad were found to be toxic to two parasitoids (*Lysiphlebus testaceipes* and *Aphytis melinus*) in Spanish citrus orchards; Bayram *et al*. 2010; Dhillon and Sharma 2010). However, organophosphates such as fenithrothion were reported to be almost non-toxic to *Lycosa* spp., a common predator of rice pests in Japan (Takahashi and Kiritani 1973).

Contaminated adult parasitoids in this study usually trembled for a few seconds before dying. Desneux *et al*. (2007) also reported pesticide effects such as trembling and abdomen cleaning on natural enemies of pests. Percent mortalities of adult parasitoids that emerged from their parasitized *E. thrax* pupal hosts were higher than those found within the hosts. *B. albotibialis* adults in pupal cocoons within parasitized *E. thrax* pupae are inactive while emerged adult parasitoids are very active and were usually seen rubbing their proboscis on the treated surfaces found in their confined laboratory habitat. Schneider *et al*. (2004) have also reported a decrease in adult emergence of an endoparasitoid (*Hyposoter didymator*) after exposure to spinosad. The higher mortality/harmful effect of Wesco Malathion 57® and Capture 605® on this important parasitoid of *E. thrax* show that these two insecticides might not be suitable candidates for implementing an IPM program on bananas. In support of this, Elzen (2001) reported malathion as one of the insecticides not recommended or compatible for IPM of cotton pests while Fritzerald (2004) reported use of pyrethroids as incompatible with IPM in strawberry production as they are toxic to predatory mites.

This research has therefore shown that Dipel® (i.e., *Bacillus thuringiensis*) is an important IPM component for the management of *E. thrax* in banana agro-ecosystems. Casida and Quistad (1998) reported that *B. thuringiensis* has been used for decades to control lepidopterous larvae. Although this microbial agent is relatively slow in killing larvae of *E. thrax*, it is less harmful to a major pupal parasitoid. Contrary to Dipel®, the conventional insecticides (Capture 605® and Wesco Malathion 57®) are found to be harmful to the parasitoids, and besides, the action of Dipel® on *E. thrax* larvae in the field is comparable to that of Wesco malathion 57®. To really conclude on the effectiveness of Dipel® in managing *E. thrax* larvae, it would be good if sub-lethal effects of such microbials on parasitoids of *E. thrax* are studied.

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