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Susceptibility of Bagworm *Metisa plana* (Lepidoptera: Psychidae) to Chlorantraniliprole

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ABSTRACTS

Bagworm (*Metisa plana*) is an ubiquitous pest in oil palm plantations. Seven insecticides were evaluated for their effectiveness in controlling *M. plana* using a leaf dip bioassay. The evaluation assessed the speeds of action, susceptibility of different instars, ovicide and ovi-larvicide activity of chlorantraniliprole. The lowest LC_{50} (0.25ppm) was found with chlorantraniliprole and trichlorfon, followed by thiamethoxam with 0.70 ppm, indoxacarb with 0.72 ppm, cypermethrin with 0.90 ppm, and for monocrotophos with 15.03 ppm. The highest LC_{50} (18.58 ppm) was found for Bacillus thuringiensis, which was approximately 74 times larger than trichlorfon and chlorantraniliprole. Meanwhile, the speed of action of these insecticides on *M. plana* larvae was also found to differ. Trichlorfon (1900.0 ppm), chlorantraniliprole (50.0 ppm) and cypermethrin (75.0 ppm) were among the three fastest acting insecticides evaluated, with respective LT_{50} values of 12.66, 17.04 and 28.63 minutes and larval mortality of 19.91, 47.27 and 53.06 minutes after exposure to the chemicals. *Bacillus thuringiensis* (324.0ppm) was the slowest acting insecticide, requiring more than 2000 minutes to kill 50% of *M. plana* larvae. The first three instars of *M. plana* larvae were very susceptible to chlorantraniliprole, with LC_{50} below 1 ppm, as compared to LC_{50} of 1.91ppm and 9.62ppm for the 4th and 5th instar larvae. Chlorantraniliprole had low to moderate ovicidal effects on *M. plana*, which caused egg mortality to range from 27.50% to 72.50%, but it was shown to be highly toxic on the neonates emerging from the eggs.

Keywords: Rynaxypyr®, chlorantraniliprole, *Metisa plana*, oil palm.

INTRODUCTION

Bagworm (*Metisa plana*) is one of the most destructive pests in oil palm plantations. Outbreaks of bagworm occur frequently (Ho, 1998). Bagworm has a short life cycle and can have several generations within the narrow span of time (Yap, 2005). Hence, appropriate pest management strategies must be taken to control bagworms and to maintain a healthy and productive oil palm plantation. Foliar application of insecticide is still the best option for bagworm control among oil palm growers.

The concept of integrated pest management (IPM) includes the use of selective insecticides

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(when necessary) to keep the pest population below the economical threshold, and the choice of insecticides that is the least toxic to humans and the environment (Wood, 1971). Currently, organophosphate and pyrethroid are still widely used for bagworm control. Newer alternative effective insecticides, with favourable toxicological and environmental profiles, could be valuable additional tools for oil palm growers.

Chlorantraniliprole (Rynaxypyr®) is a novel insecticide in the anthranilic diamides class. It is a potent and selective activator of insect ryanodine receptors, which are critical for muscle contraction. When the ryanodine receptor in insects is activated, calcium homeostasis in the cell is affected, and this leads to feeding cessation, lethargy, muscle paralysis and ultimately death of the insects (Lahm *et al*., 2007). Chlorantraniliprole is a highly selective insecticide with low mammalian toxicity. The acute oral dosage on rat (LD_{50}) is > 5000mg kg^{-1} (DuPont Crop Protection, 2007). In addition, chlorantraniliprole has demonstrated an excellent efficacy and long lasting control on a broad spectrum of Lepidopteran species in several crops.

In order to manage insecticide resistance, monitoring of pest populations for their susceptibility to various insecticides should be carried out at regular intervals. For comparison of susceptibility, the baseline data for various insecticides must be generated prior to widespread use and they should also be easily available for comparative studies. However, there has been no baseline study of bagworm susceptibility to insecticides in Malaysia to date. *Metisa plana*, just like other Lepidopteran species (like diamondback moth and beet armyworm), has a high potential for the development of resistance to the commonly used insecticides.

The objectives of this study were to: (1) determine the baseline susceptibility of *M. plana* to chlorantraniliprole as compared to other commercial insecticide currently being used in oil palm plantations, (2) evaluate the speed of action of chlorantraniliprole in comparison with current standards on *M. plana* control, (3) understand the level of susceptibility of different larval instars of *M. plana* to chlorantraniliprole, and (4) evaluate the ovicidal and ovi-larvicidal activities of chlorantraniliprole against *M. plana.*

MATERIALS AND METHODS

Insect Preparation

Cocoons of *M. plana* were collected in an oil palm estate and maintained in a growth chamber at 25 – 28 °C. All the insects were reared on oil palm leaves, i.e. their natural food source, until they were ready for bioassays. The larvae of the F1 generation were used for the bioassay studies.

Experiment 1: Susceptibility of M. plana to chlorantraniliprole and other insecticides

The insecticides used in this study are listed in Table 1. The rate (g ai ha^{-1}) of commercially available insecticides in Malaysia tested was based on the recommendations for each product on oil palm, except for chlorantraniliprole, indoxacarb and thiamethoxam. Each insecticide was diluted with distilled water to obtain seven different concentrations (namely, 0, 0.3, 1, 3, 10, 30 and 100 ppm). Consequently, thirty-two healthy 2nd instar larvae were used for each insecticide treatment in the current study.

Leaf-dip bioassay method was used for assaying bagworm susceptibility in this study. An oil palm leaf was collected from healthy oil palm seedling planted in a greenhouse at the DuPont Malaysia Field Research Station (MFRS) under insecticide free conditions. The midrib was removed and the leaf lamina was retained. Rectangular leaf pieces, measuring 40 x 50 mm, were cut from the leaf lamina. The rectangular pieces were individually dipped for 10 seconds in the insecticide solutions, air-dried in the laboratory for 1 hour, and placed in polystyrene trays consisting of 32 cells. One larva was gently released into each cell unit. Meanwhile, distilled water was used to treat the control leaf cut. The cells were sealed with transparent plastic lids. Observed under the stereoscope, the larvae were scored dead if they were unable to move after gentle probing

TABLE 1
Insecticides used in the bioassay

IRAC Insecticide Group	Active Ingredient	Formulation
Group 28: Ryanodione receptor modulator	Chlorantraniliprole	Water-Soluble Concentrate (SC)
Group 22: Voltage dependent sodium channel blockers	Indoxacarb	Water-Soluble Concentrate (SC)
Group 3: Sodium channel modulators	Cypermethrin	Emulsifiable Concentrate (EC)
Group 1: Acetylcholinesterase inhibitors	Trichlorfon	Soluble Powder (SP)
Group 4: Nicotinic acetylcholine receptor agonists	Thiamethoxam	Water-Dispersible Granule (WG)
Group 1: Acetylcholinesterase inhibitors	Monocrotophos	Emulsifiable Concentrate (EC)
Group 11: Microbial disruptors of insect midgut membranes	Bacillus thuringiensis	Water-Dispersible Granule (WDG)

with a small paint brush. The mortality of these larvae was recorded 96 hours after they had been released into the cells.

Experiment 2: The speed of action of chlorantraniliprole on Metisa plana

The speed of action of chlorantraniliprole on *M. plana* was evaluated based on time to stop feeding and time to kill, using five insecticides belonging to distinct chemical classes (Table 2).

Once again, the leaf-dip bioassay method (as described Experiment 1) was used in for this experiment. Thirty-two healthy 2nd instar larvae were used to evaluate each insecticide. While preparing for the bioassay, larvae were starved for 24 hours prior to exposure to the leaf cuts. This procedure standardized the nutritional status of the groups of insects to be tested and ensured immediate initiation of feeding as soon as the larvae were transferred to the cell units with leaf cuttings.

To evaluate the speed of action of the insecticides, each individual larva was observed under the stereoscope from the time it was placed on the leaf cut. Time zero (T_0) was defined as the time when each individual larva initiated feeding on the leaf cut. Time 1 (T_1) was defined as the time when each individual larva permanently stopped feeding. Time to complete feeding cessation and kill (T_{total}) was calculated as (T_{total}) = (T_1) - (T_0). The observations were recorded at 5, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 300, 360, 720, 1440, 2880, 4320 and 5760 minutes. Larvae from each treatment at 0, 1, 2, 3, 4 days were fixed in FAA solution and processed for SEM observation.

TABLE 2
Treatment list for the speed of action study

No.	Treatment	Formulation	Rate (ppm)
1	Chlorantraniliprole	5SC	12.5
2	Chlorantraniliprole	5SC	25.0
3	Chlorantraniliprole	5SC	50.0
4	Indoxacarb	15SC	50.0
5	Cypermethrin	5EC	75.0
6	Trichlorfon	95SP	1900.0
7	Bacillus thuringiensis	54WG	324.0

Speed of Action Categorization

In this study, LT_{50} obtained by probit analysis was categorized into five categories: (I) very fast speed of action = insecticides that stopped feeding or killed *M. plana* within 30 minutes of exposure; (II) fast speed of action = insecticides that stopped feeding or killed *M. plana* in less

than 3 hours (180 minutes) of exposure; (III) moderate speed of action = insecticides that allowed feeding or survived for more than 3 hours (180 minutes) but less than 6 hours (360 minutes) of exposure; (IV) slow speed of action = insecticides that allowed feeding or survived for more than 6 hours (360 minutes) but less than 24 hours (1440 minutes) of exposure; and (V) very slow speed of action = insecticides that allowed feeding or survived for more than 24 hours (≥ 1440 minutes) of exposure.

Preparation for Scanning Electron Microscopy (SEM)

The internal organs of larvae of different instars were viewed under the scanning electron microscope (JOEL 6310). The cuticle of the abdomen of the instars was sliced longitudinally using a sharp razor blade under a stereo-binocular microscope. The larvae with the sliced abdomen were fixed in FAA for 24 hours, washed in 1% cacodylate buffer and post fixed in 1% cacodylate buffered osmium tetroxide for two hours. The larvae were dehydrated in a graded series of alcohol concentrations (i.e. 30, 50, 70, 90, 95 and 100% in Belzers D30, respectively), and Critical Point Drier Balzers using carbon dioxide liquid as an intermediate fluid. Critical point dried samples were mounted on cylinder stubs and sputter coated with gold, and then viewed with scanning electron microscope JEOL 6310 at an acceleration voltage of 10 or 15kV.

Experiment 3: Susceptibility of different instars of M. plana larvae to chlorantraniliprole

Five different instars (1st, 2nd, 3rd, 4th and 5th) of *M. plana* were used in this study. The leaf-dip bioassay method was employed in the study, and the details were as previously described under Experiment 1. Seven different concentrations of chlorantraniliprole (i.e. at 0, 0.3, 1, 3, 10, 30 and 100 ppm) were obtained by diluting the insecticide with distilled water. Each treatment was evaluated using thirty-two healthy larvae.

Experiment 4: Ovicidal and ovi-larvicidal effect of chlorantraniliprole on Metisa plana

Eggs of *M. plana* used in this experiment came from F1 population which was obtained as described above. One day-old eggs were used for the ovicidal and ovi-larvicidal study. To determine the toxicity of chlorantraniliprole and other insecticides to *M. plana* eggs, the eggs were exposed to 12.5 ppm, 25.0 ppm and 50.0 ppm of chlorantraniliprole, 75.0 ppm of cypermethrin and 1900.0 ppm of trichlorfon (Table 3). A total of ten eggs were used per replicate, with four replications per treatment. To ensure uniformity, the eggs collected from the same egg mass were used in each replication. These eggs were dipped in the insecticide solutions for five seconds and air dried for one hour under laboratory conditions. The undipped eggs were used as control. Each replication of ten treated eggs was placed in a 20 ml transparent screw-capped vial. The total number of hatched and unhatched eggs, as well as dead and alive larvae was counted under the stereomicroscope on day 12.

TABLE 3
A treatment list for the ovicidal and ovi-larvicidal study

No.	Treatment	Formulation	Rate (ppm)
1	Chlorantraniliprole	5SC	12.5
2	Chlorantraniliprole	5SC	25.0
3	Chlorantraniliprole	5SC	50.0
4	Cypermethrin	5EC	75.0
5	Trichlorfon	95SP	1900.0
6	Untreated check	-	-

Statistical Analysis

For Experiments 1, 2 and 3, the mortality data were subjected to probit analysis to calculate median lethal concentration (LC_{50}), LC_{90} and Fiducial limits. The probit analysis was carried out using the DuPont Dose Response probit analysis software version 2.0.

For Experiment 4, however, all the treatment data were subjected to ANOVA using FieldPro Biodata Management software, in which the means for each treatment were separated (P≤0.05) using Duncan's Multiple Range Test.

RESULTS

Experiment 1: The Susceptibility of M. plana to Chlorantraniliprole and Other Insecticides

The probit analyses of the susceptibility of *M. plana* 2nd instar larvae to the treated insecticides are presented in Table 4. The lethal concentrations (LC_{50} and LC_{90} values) of *M. plana* varied greatly between the insecticides tested, suggesting that the level of *M. plana* susceptibility to each insecticide differed greatly. The LC_{50} values of trichlorfon and chlorantraniliprole were 0.25 ppm, followed by 0.70 ppm for thiamethoxam, 0.72 ppm for indoxacarb, 0.90 ppm for cypermethrin, and 15.03 ppm for monocrotophos. The highest LC_{50} was observed for *B. thuringiensis,* i.e. at 18.58ppm, and approximately 74 times more than trichlorfon and chlorantraniliprole (0.25 ppm). It is important to note that the mortality in the untreated control for all the treatments was less than 5% after 96 hours.

The LC_{90} value for chlorantraniliprole was the lowest among the seven groups of insecticides, i.e. at 0.64 ppm, followed by cypermethrin, indoxacarb, trichlorfon and monocrotophos at 4.69 ppm, 6.20 ppm, 46.53 ppm, 198.41 ppm, respectively. Meanwhile, Thiamethoxam and *B. thuringiensis* have the highest LC_{90} values at 371.58 ppm 302.65 ppm, respectively, the value which are more than 500 times higher than that of chlorantraniliprole.

Experiment 2: The Speed of Action of Chlorantraniliprole on Metisa plana

Time to Stop Feeding

The highest percentage of larvae that stopped feeding within the first 5 minutes of exposure to the insecticides was found with 50.0 ppm chlorantraniliprole (15.63%), followed by 1900.0 ppm trichlorfon (9.38%) and 25 ppm chlorantraniliprole (3.13%), as shown in Table 5. After 30 minutes of exposure to 50.0 ppm chlorantraniliprole and 1900.0 ppm trichlorfon, 78.13% of *M. plana* stopped feeding. The percentage of the larvae that stopped feeding increased with the time of exposure to pesticides. After 60 minutes, 93.75% of *M. plana* stopped feeding after being exposed to 1900.0 ppm trichlorfon and 75.0 ppm cypermethrin. The descending order of the feeding cessation of *M. plana* larvae to the remaining insecticides after 60 minutes of exposure are 87.50% for

TABLE 4
Dosage-mortality response of the susceptibility bagworm, *Metisa plana* to chlorantraniliprole, indoxacarb, cypermethrin, trichlorfon, thiamethoxam, monocrotophos and *Bacillus thuringiensis,* after 96 hours of insecticide exposure

					LC_{50} (ppm) Fiducial Limit			LC_{90} (ppm) Fiducial Limit	
Insecticides	Slope	Intercept	Chi-Square	LC_{50} (ppm)	Lower 95% CL	Upper 95% CL	LC_{90} (ppm)	Lower 95% CL	Upper 95% CL
Chlorantraniliprole	3.142	1.878	0.013	0.25	0.12	0.33	0.64	0.47	1.40
Indoxacarb	1.374	0.192	12.830	0.72	0.03	2.10	6.20	2.13	804.87
Cypermethrin	1.791	0.078	2.648	0.90	0.60	1.26	4.69	3.07	9.06
Trichlorfon	0.565	0.251	4.999	0.25	0.02	0.72	46.53	15.85	492.53
Thiamethoxam	0.470	0.072	0.291	0.70	0.08	1.90	371.58	72.54	26929.22
Mococrotophos	1.143	-1.346	7.512	15.03	9.80	24.58	198.41	95.56	633.28
Bacillus thuringiensis	1.057	-1.342	9.772	18.58	6.55	105.84	302.65	66.18	62467.42

TABLE 5
Time to stop feeding response of *Metisa plana* to chlorantraniliprole, indoxacarb, cypermethrin, trichlorfon, and *Bacillus thuringiensis,* after 96 hours of insecticide exposure

Time (Minutes)	Treatment (Percentage of larvae stop feeding)						
	12.5ppm Chlorantraniliprole	25.0ppm Chlorantraniliprole	50.0ppm Chlorantraniliprole	50.0ppm Indoxacarb	75.0ppm Cypermethrin	1900.0ppm Trichlorfon	324.0ppm *B. thuringensis*
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	3.13	15.63	0.00	0.00	9.38	0.00
15	9.38	18.75	34.38	3.13	6.25	71.88	0.00
30	34.38	43.75	78.13	3.13	62.50	78.13	0.00
45	43.78	50.00	84.38	3.13	81.25	90.63	0.00
60	53.13	65.63	87.50	3.13	93.75	93.75	0.00
75	56.25	71.88	90.63	6.25	93.75	93.75	3.13
90	56.25	71.88	93.75	9.38	96.88	93.75	6.25
105	68.75	71.88	93.75	9.38	100.00	93.75	6.25
120	68.75	75.00	100.00	15.63	100.00	93.75	6.25
180	81.25	81.25	100.00	40.63	100.00	96.88	6.25
240	96.88	96.88	100.00	53.13	100.00	100.00	9.38
300	100.00	96.88	100.00	56.25	100.00	100.00	9.38
360	100.00	100.00	100.00	65.63	100.00	100.00	9.38
720	100.00	100.00	100.00	71.88	100.00	100.00	9.38
1440	100.00	100.00	100.00	75.00	100.00	100.00	28.13
2880	100.00	100.00	100.00	78.13	100.00	100.00	84.38
4320	100.00	100.00	100.00	87.50	100.00	100.00	87.50
5760	100.00	100.00	100.00	100.00	100.00	100.00	90.63

50.0 ppm chlorantraniliprole, 65.63% for 25.0 ppm chlorantraniliprole, 53.13% for 12.5 ppm chlorantraniliprole, 3.13% for 50.0 ppm indoxacarb, and the feeding of larva continued on to 324.0 ppm *B. thuringiensis* treatment after 60 minutes. Nonetheless, the feeding completely stopped after 105 minutes of exposure to 75.0 ppm cypermethrin, 120 minutes and 240 minutes to 50.0 ppm chlorantraniliprole and 1900.0 ppm trichlorfon, respectively. The first cessation of feeding of the larvae on *B. thuringiensis* treated leaf cut was observed 75 minutes after exposure and 28.13% of the larvae stop feeding after 1440 minutes (1 day) of exposure. All larvae ended feeding within 96 hours exposure to the insecticides, with the exception of larvae which were exposed to 324.0 ppm *B. thuringiensis* and only 90.63% ended feeding.

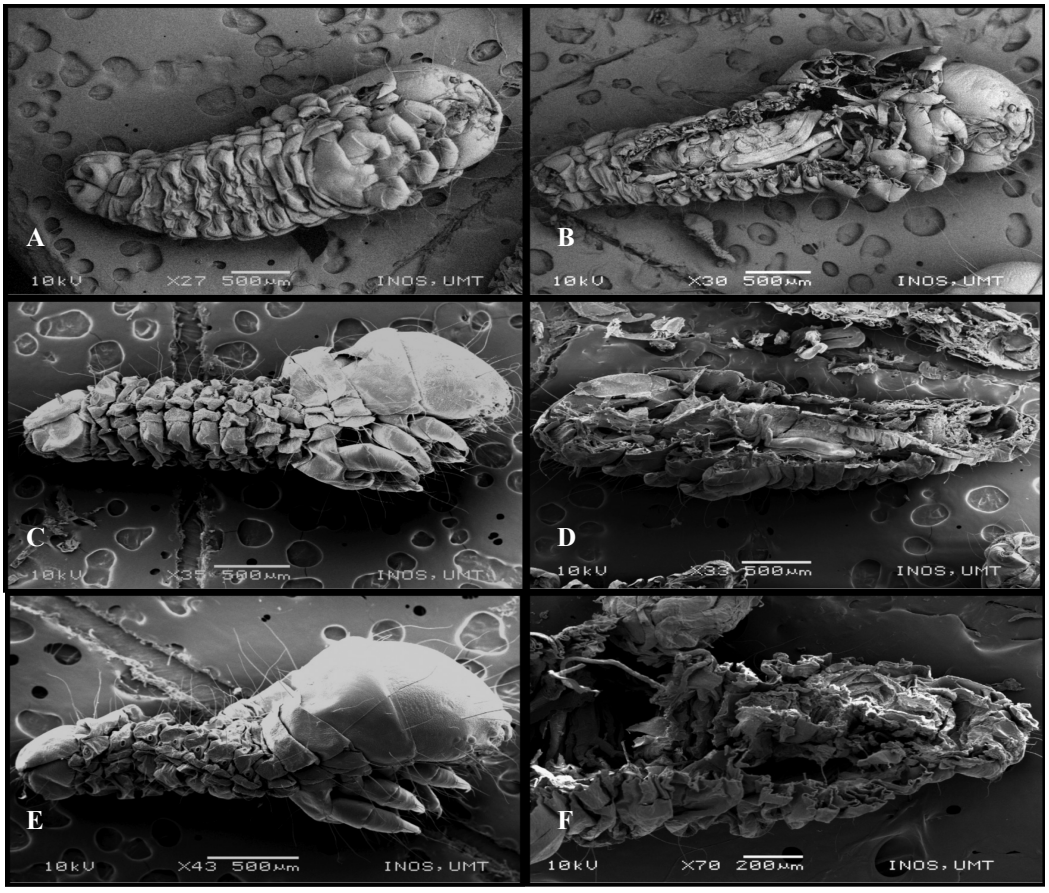

Fig. 1: SEM micrographs of, (A); fresh larva (ventral view; X 27), (B); showing the internal organs of fresh larva (ventral view; X 30), (C); larva exposed to chlorantraniliprole after 1 day of bioassay (side view; X 35), (D); showing the internal organs of day 1 after being exposed to chlorantraniliprole larva (ventral view; X 33), (E): a larva exposed to chlorantraniliprole after 3 days of bioassay (side view; X 43), and (F); showing the internal organs of larva after day 3 of exposure to chlorantraniliprole larva (ventral view; X 70).

TABLE 6
Time to kill response of *Metisa plana* to chlorantraniliprole, indoxacarb, cypermethrin, trichlorfon, and *Bacillus thuringiensis,* after 96 hours of insecticide exposure.

Time (Minutes)	Treatment (Percentage of larvae mortality)						
	12.5ppm Chlorantraniliprole	25.0ppm Chlorantraniliprole	50.0ppm Chlorantraniliprole	50.0ppm Indoxacarb	75.0ppm Cypermethrin	1900.0ppm Trichlorfon	324.0ppm *B. thuringensis*
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	6.25	0.00
15	0.00	0.00	15.63	0.00	0.00	31.25	0.00
30	0.00	15.63	21.88	3.13	15.63	78.13	0.00
45	9.38	37.50	37.50	3.13	31.25	78.13	0.00
60	21.88	37.50	56.25	3.13	50.00	90.63	0.00
75	31.25	53.13	78.13	3.13	81.25	93.75	3.13
90	34.38	56.25	84.38	6.25	87.50	93.75	3.13
105	34.38	56.25	84.38	9.38	96.88	93.75	3.13
120	40.63	56.25	84.38	9.38	100.00	93.75	3.13
180	62.50	71.88	96.88	12.50	100.00	93.75	3.13
240	68.75	78.13	100.00	31.25	100.00	96.88	9.38
300	96.88	81.25	100.00	53.13	100.00	100.00	9.38
360	96.88	96.88	100.00	56.25	100.00	100.00	9.38
720	96.88	100.00	100.00	65.63	100.00	100.00	9.38
1440	96.88	100.00	100.00	68.75	100.00	100.00	9.38
2880	100.00	100.00	100.00	71.88	100.00	100.00	59.38
4320	100.00	100.00	100.00	75.00	100.00	100.00	84.38
5760	100.00	100.00	100.00	75.00	100.00	100.00	87.50

The time to stop feeding response of *M. plana* 2nd instars larvae is presented in Table 7. The larvae exposed to 1900.0 ppm trichlorfon produced the lowest LT_{50} value of 12.66 minutes and this was followed by 17.04 minutes for 50.0 ppm chlorantraniliprole, and 28.63 minutes for 75.0 ppm cypermethrin. The difference of the LT_{50} value between the lowest LT_{50} value of 1900.0 ppm trichlorfon and the second lowest of 50.0 ppm chlorantraniliprole was extremely close, i.e. less than 5 minutes. The difference between the second and third lowest LT_{50} value of 50.0 ppm chlorantraniliprole and 75.0 ppm cypermethrin was more than 10 minutes. The LT_{50} values for 25.0 ppm and 12.5 ppm chlorantraniliprole were at 42.76 and 58.48 minutes, respectively. Meanwhile, the LT_{50} value of 50.0 ppm indoxacarb was slightly higher at 402.91 minutes. At 324.0 ppm, *B. thuringiensis* produced the highest LT_{50} value on *M. plana* larvae at 1451.36 minutes, with 114.6 and 85.1 times higher than 1900.0 ppm trichlorfon and 50.0 ppm chlorantraniliprole, respectively.

When looking at LT_{90} values, 75.0 ppm cypermethrin had the lowest LT_{90} value with 55.56 minutes, followed by 56.13 minutes for 1900.0 ppm trichlorfon, 62.36 minutes for 50.0 ppm chlorantraniliprole, 189.40 minutes for 25.0 ppm chlorantraniliprole, 205.18 minutes for 12.5 ppm chlorantraniliprole and 2714.97 minutes for 50.0 ppm indoxcarb. Meanwhile, 324.0 ppm *B. thuringiensis* still ranked the highest, with the LT_{90} value at 7756.25 and/ or about 5.3 days.

Time to Kill

The mortality time of *M. plana* after exposure to the insecticides is presented in Table 6. The findings revealed that the exposure to trichlorfon (1900.0 ppm) killed 6.25% of the larvae within the first 5 minutes and increased to 31.25% within the first 15 minutes. The killing time of the larvae exposed to the insecticides was observed to vary. After 60 minutes of chemical exposure, 1900.0ppm trichlorfon still ranked the highest, with the percentage of mortality at 90.63%, and this was followed by 50.0 ppm chlorantraniliprole and 75.0 ppm cypermethrin with about 50% mortality. The larvae were killed within 120 minutes of exposure to 75.0 ppm cypermethrin as compared to 240 minutes with 50.0 ppm chlorantraniliprole and 300 minutes with 1900.0 ppm trichlorfon. A longer exposure time was required for 100% kill, with a lower concentration of chlorantraniliprole, 720 minutes and 2880 minutes at 25.0 ppm and 12.5 ppm, respectively. Both 50.0 ppm indoxacarb and 324.0 ppm *B. thuringiensis* achieved only 75.00% and 87.50% kill when the experiment was terminated after 5760 minutes (4 days) of exposure to the tested insecticides.

Table 8 shows the LT_{50} and LT_{90} values of all the treatments tested in terms of the time to kill. The probit analysis result showed that the LT_{50} and LT_{90} values of larvae exposed to 1900.0 ppm trichlorfon remained at the lowest, with 19.91 and 75.80 minutes, respectively. The LT_{50} and LT_{90} values of 50.0 ppm chlorantraniliprole and 75.0 ppm cypermethrin were between 47 to 53 minutes and 91 to 126 minutes, respectively. At 25.0ppm and 12.5ppm, Chlorantraniliprole was ranked the fourth and fifth lowest LT_{50} and LT_{90} values, with the LT_{50} values at 85.59 and 130.31 minutes and LT_{90} values at 326.06 and 372.69 minutes, respectively. The LT_{50} and LT_{90} values of 50.0ppm indoxacarb were 764.01 and 7367.64 minutes, respectively. At 324.0 ppm, *B. thuringiensis* remained the highest with the LT_{50} and LT_{90} values of 2093.82 and 11941.65 minutes.

Speed of Action Categorization

Base on LT_{50} time to stop feeding and probit kill (Tables 7 and 8), the speed of action index of the 5 groups of pesticides was categorized as follows; for feeding cessation, Category I had 1900 ppm trichlorfon, 50 ppm chlorantraniliprole and 75 ppm cypermethrin; Category II had 25 ppm and 12.5 ppm chlorantraniliprole; Category IV had 50 ppm indoxacarb; and Category V had 324 ppm *B. thuringiensis*. As for time to kill, Category I had 1900ppm trichlorfon, Category II had 50 ppm chlorantraniliprole and 75 ppm cypermethrin, Category III had 25 ppm and 12.5 ppm chlorantraniliprole, Category IV had 50 ppm indoxacarb, and Category V had 324ppm *B. thuringiensis*.

TABLE 7
Time to stop feeding response of *Metisa plana* to chlorantraniliprole, indoxacarb, cypermethrin, trichlorfon, and *Bacillus thuringiensis,* after 96 hours of insecticide exposure.

Insecticide	Rate (ppm)	Slope	Intercept	Chi-Square	LT_{50}	LT_{50} Fiducial Limit		LT_{90}	LT_{90} Fiducial Limit	
						Lower 95% CL	Upper 95% CL		Lower 95% CL	Upper 95% CL
Chlorantraniliprole	12.5	2.351	-4.154	10.229	58.48	49.54	67.72	205.18	168.82	264.72
Chlorantraniliprole	25.0	1.983	-3.234	6.866	42.76	34.69	51.07	189.40	152.56	250.42
Chlorantraniliprole	50.0	2.274	-2.801	5.396	17.04	12.97	21.15	62.36	50.88	79.80
Indoxacarb	50.0	1.546	-4.029	32.030	402.91	298.57	568.02	2714.97	1644.85	5669.24
Cypermethrin	75.0	4.451	-6.485	3.836	28.63	24.48	32.55	55.56	48.55	66.06
Trichlorfon	1900.0	1.981	2.185	10.980	12.66	8.98	16.44	56.13	44.66	73.87
Bacillus thuringensis	324.0	1.760	-.5.566	27.409	1451.36	1069.53	2088.80	7756.25	4758.21	15820.94

TABLE 8
Time to kill response of *Metisa plana* to chlorantraniliprole, indoxacarb, cypermethrin, trichlorfon, and *Bacillus thuringiensis,* after 96 hours of insecticide exposure.

Insecticide	Rate (ppm)	Slope	Intercept	Chi-Square	LT50	LT50 Fiducial Limit		LT90	LT90 Fiducial Limit	
						Lower 95% CL	Upper 95% CL		Lower 95% CL	Upper 95% CL
Chlorantraniliprole	12.5	2.808	-5.939	27.170	130.31	109.49	156.07	372.69	286.54	552.56
Chlorantraniliprole	25.0	2.206	-4.263	9.970	85.59	73.34	99.01	326.06	261.76	435.86
Chlorantraniliprole	50.0	3.000	-5.024	8.906	47.27	40.48	53.96	126.40	107.92	155.15
Indoxacarb	50.0	1.302	-3.754	33.396	764.01	528.33	1203.79	7367.64	3821.47	20122.63
Cypermethrin	75.0	5.450	-9.400	5.259	53.06	47.86	57.97	91.18	82.07	104.97
Trichlorfon	1900.0	2.207	-2.867	11.187	19.91	15.31	24.54	75.80	61.99	96.94
Bacillus thuringensis	324.0	1.694	-5.628	26.080	2093.82	1646.67	2773.40	11941.65	7923.48	20735.16

TABLE 9
Probit analysis of dosage-mortality response of the susceptibility bagworm, *Metisa plana* to different instars after 96 hours insecticide exposure.

Instar	Slope	Intercept	Chi-Square	LC50	LC50 Fiducial Limit		LC90	LC90 Fiducial Limit	
					Lower 95% CL	Upper 95% CL		Lower 95% CL	Upper 95% CL
1st	1.418	1.834	4.812	0.05	0.02	0.08	0.40	0.23	0.95
2nd	3.142	1.878	0.013	0.25	0.12	0.33	0.64	0.47	1.40
3rd	2.761	1.073	0.351	0.40	0.22	0.58	1.18	0.81	2.61
4th	1.257	-0.355	3.242	1.91	1.17	3.39	20.02	8.51	143.22
5th	2.154	-2.118	3.869	9.62	6.38	12.57	37.85	27.25	68.77

Structural Changes in Metisa plana Larvae after Exposure to Chlorantraniliprole

The surface of the folded structure of fresh larval abdomen was formed by columnar cells (*Fig. 1A*). Organs of the larvae, such as spiracles, setae and prolegs were clearly defined. The longitudinal section of *M. plana* larva showed that the larval midgut was the longest portion of the alimentary canal, lying convoluted within the larval body cavity (*Fig. 1B*).

When the larva was exposed to chlorantraniliprole after 1 day of bioassay, 96.88 % was obtained (Table 6). The SEM micrograph in *Fig. 1C* showed that the surface morphology of the larva was preserved, but with a less uniformed shape. The spiracles, setae and prolegs were still visible although they were not clearly discernible. The alimentary canal remained intact and the larval intestine shrunk from approximately 100 µm to 35 µm in diameter (*Fig. 1D*). The compact helical shaped intestine was loosening into a straight empty space in the internal body cavity.

After 3 days of bioassay, no survival was obtained and the structure was severely disintegrated. The abdominal body wall fractured and the normal columnar cell disappeared (*Fig. 1E*). Internally, the integrity of the organs started to lose, and the intestine was completely destroyed leaving an empty abdomen (*Fig. 1F*).

Experiment 3: The Susceptibility of Different Instars of M. plana Larvae to Chlorantraniliprole

The results from the probit analysis of dosage-mortality response of all *M. plana* instars larvae are presented in Table 9. No mortality was recorded in the untreated larvae after 96 hours of feeding. In the chlorantraniliprole treated leaves, the LC_{50} values of the 1st to 5th instars larvae were 0.05ppm, 0.25ppm, 0.40ppm, 1.91ppm and 9.62ppm, respectively; whereas the respective LC_{90} values were 0.40ppm, 0.64ppm, 1.18ppm, 20.02ppm and 37.85ppm. These results show that the LC_{50} and LC_{90} values of the first three instars larvae of *M. plana* were generally low (i.e. below 1 ppm), except for the LC_{90} value of 3rd instar larvae which was at 1.18 ppm. Meanwhile, the LC_{50} and LC_{90} values of the 4th and 5th instar larvae increased drastically from 1.91 ppm to 9.62 ppm and from 20.02 ppm to 37.85 ppm, respectively. These results also showed that the 1st, 2nd and 3rd instars larvae were very susceptible to chlorantraniliprole, with relatively 100% mortality and 96 hours exposure to the 3ppm chemical dipped leaf cut; however, the 4th and 5th instars larvae needed a higher concentration of 30 ppm to obtain 100% mortality. Therefore, the results clearly confirmed the greater LC_{50} and LC_{90} values in the older instars than the younger instars. The LC_{50} and LC_{90} values increased drastically beyond the 4th instar larvae stage.

TABLE 10
Percentage of ovicidal and ovi-larvicidal responses of *Metisa plana*.

Treatment	Rate (ppm)	Ovicidal (%)	Ovi-larvicidal (%)
Chlorantraniliprole	12.5	35.00[b]	100.00[a]
Chlorantraniliprole	25.0	72.50[ab]	100.00[a]
Chlorantraniliprole	50.0	27.50[b]	100.00[a]
Cypermethrin	75.0	100.00[a]	100.00[a]
Trichlorfon	1900.0	100.00[a]	100.00[a]
Untreated check	-	27.50[b]	32.50[b]

Note:
1. Ovicidal effect was calculated according to the number of unhatched eggs. The number of effective larval penetration was used to determine the total effect (egg survival).
2. Values within the column having the same superscripts are not significantly different at $P \leq 0.05$.

Experiment 4: The Effect of Chlorantraniliprole on Ovicidal and Ovi-larvicidal of Metisa plana

The results for the ovicidal and ovi-larvicidal effects of the tested pesticides are presented in Table 10. There was a significant difference for the ovicidal response between the treatments. 75.0 ppm cypermethrin and 1900.0 ppm trichlorfon have very strong ovicidal effects resulting in 100.00% unhatched eggs. On the contrary, *M. plana* had low to moderate ovicidal responses to chlorantraniliprole at 12.5 ppm to 50.0 ppm with 27.50% to 72.50%, respectively.

Chlorantraniliprole showed strong ovi-larvicidal property. The total mortality of unhatched eggs (ovicidal) plus immediate death of larvae during and after hatching reached 100.00%. Therefore, the ovi-larvicidal effect of all the treatments was significantly higher than the untreated control.

DISCUSSION

This study has showed that among all the current insecticides in the market, chlorantraniliprole is the most potent insecticide against *M. plana* in oil palm plantations. Based on the LC_{50} and LC_{90} values of 0.25ppm and 0.64ppm, the relative susceptibility of the *M. plana* larvae to chlorantraniliprole was 7.3 times higher than cypermethrin (ranked the second best). Likewise, chlorantraniliprole was found to be 9.6, 72.7, 310.0, 472.8 and 580.5 times more effective to indoxacarb, triclorfon, monocrotophos, *B. thuringiensis* and thiamethoxam, respectively. A low LC_{50} value of chlorantraniliprole was also reported with other pests, such as *Scirphophaga incertulas* (0.03ppm), *Plutella xylostella* (0.11ppm) and *Spodoptera exigua* (0.20ppm) in Malaysia (Kamar *et al*., 2008). Cypermethrin was reported to be the most effective insecticide against the 4th instar bagworm (*Auchmophila kordofensis*) larvae on *Acacia* with the LC_{50} and LC_{90} values of 7.04 ppm and 34.76 ppm, respectively, and these are better than chlorpyrifos and spinosad (Kowkab *et al.*, 2008). However, the current study further revealed that chlorantraniliprole performed even more potent than cypermethrin on *M. plana*. In addition, several synthetic pythrethroid products have shown field failure in the official spraying program against Lepidoptera species, such as *Spodoptera littoralis* (Temerak, 2002). *Spodoptera* and *Helicoverpa* species have been found to develop resistance to synthetic pyrethroids such as cypermethrin (El-Dahan *et al.*, 1985, Tikar *et al.*, 2004). Incidences of *H. armigera* resistance to cypermethrin have frequently been reported in India (Tikar *et al.*, 2004).

Resistance monitoring is an important component for developing IRM strategies. The strategy of insecticide resistance management (IRM) emphasizes on the judicial use of

insecticides to minimize selection for insect resistance and to increase the life-span of the insecticidal molecules. In the recent years, increasing efforts have been made towards incorporating IRM strategies into the larger realm in most of the agrochemical industry. However, the IRM programme for *M. plana* on oil palm is still lacking.

Chlorantraniliprole is a new insecticide with a novel mode of action and it is also effective on lepidopterous pests that have developed resistant to other insecticides. Apparently, chlorantraniliprole has constantly provided a good control on leaf folders and stem borers in rice, as well as beet army worm and diamondback moth in vegetables after several years and numerous crops trials (Kamar *et al*., 2008). Efforts must be made to preserve this high activity; for example, by not applying insecticides in the same chemical class to consecutive generations of the same pest, or by not using those insecticides over more than 50% of the crop time (Insecticide Resistance Action Committee, 2008).

The ability and speed to cease feeding have become one of the critical indicators of insecticidal potency for discovering and developing novel chemical classes of insecticides. Chlorantraniliprole was among the fastest-acting insecticides for feeding cessation (Category I), but not the fastest to kill *M. plana* (Category II). The SEM micrograph showed that chlorantraniliprole had destroyed the internal organs of the larvae. The stomach poisoning effect of chlorantraniliprole was manifested in the deformation of the surface, and disintegration of the intestine. When the cells and the internal organs are damaged, the larvae became lethargic and ultimately die (Lam *et al*., 2007). Chlorantraniliprole was also reported to have caused the fastest feeding cessation on *Plutella xylostella*, *Trichoplusia ni*, *Spodoptera exigua* and *Helicoverpa zea* as compared to emamectin benzoate, indoxacarb, methoxyfenozide and metaflumizone (Hannig *et al*., 2009).

The susceptibility level of chlorantraniliprole gradually decreased with the succeeding instars, as reflected in the values of LC_{50} and LC_{90} in Table 9. The values of LC_{50} and LC_{90} drastically increased with the 4th instar larvae. The larger the larvae, the harder they are to kill with chemicals. Apparently, the best time to apply chlorantraniliprole for *M. plana* control is during the vulnerable stage of the neonate 1st to 3rd instars when the larvae are small and actively feeding on leaves (LC_{50} ranging from 0.05 ppm to 0.40 ppm). The control becomes difficult and less effective with later instars (close to pupation stage), such as 5th instar and above. Moreover, *M. plana* larvae are active feeders from the 1st to 3rd instars, but they slow down near to pupation at the 4th and 5th instar. The LC_{50} of 0.40ppm was sufficient to kill up to the 3rd instar. A higher concentration was required to give a similar result at the 4th and 5th instars. The result of this study concurred with the report of Kumar and Kumar (2008) on *B. thuringiensis* against *Helicoverpa armigera*. The neonates (i.e. the 1st instar) were the most susceptible to *B. thuringiensis* than other later instars. Therefore, the timing of application in the field is very critical to obtain the optimum control. The first 28.0 days of the 1st to 3rd instars larval period are the best time to apply chlorantraniliprole against *M. plana* on oil palm.

The mode of insecticidal action in insect eggs is not well understood and at least two types of mortality have been associated with the death of the developing insect. The embryo in the egg may be killed (true ovicidal effect) and further development (embryogenesis) is halted or the larva dies as it feeds on the chorion during eclosion, an "ovi-larvicidal" effect (Temarak, 2003). Chlorantraniliprole has low to moderate ovicidal effect on *M. plana*, ranging 27.50% to 72.50%, but is potent against emerging neonates. The combined effects against the eggs and larvae of *M. plana* can contribute to its efficacy in the field. Similar finding was also reported on the low ovicidal effect but high ovi-larvicidal effect of chlorantraniliprole on *Lobesia botrana*

(Claudio *et al.*, 2009). The ovi-larvicidal activity of chlorantraniliprole against *M. plana* may increase its benefits in situations where the *M. plana* outbreak occurs over time.

CONCLUSION

Based on the findings of the current study, it can be concluded that Chlorantraniliprole provides a new standard for good residual control of leaf feeders in the tropical climate, and is a good addition to the insecticide resistance management (IRM) strategies and integrated pest management (IPM) of *M. plana* in oil palm. This is based on its low LC_{50} against the larvae of *M. plana,* and also its times of stop feeding and kill (Category I and Category II). Moreover it has demonstrated good ovi-larvicidal and residual properties, and can be regarded as a novel insecticide.

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