

DETRIMENTAL EFFECTS OF COMMONLY USED INSECTICIDES IN OIL PALM TO POLLINATING WEEVIL, *Elaeidobius kamerunicus* FAUST. (Coleoptera: Curculionidae)

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ABSTRACT

Elaeidobius kamerunicus is the main insect pollinator of oil palm in Malaysia. It has improved oil palm pollination, thus leading to a better yield. The lethal concentrations (LC_{50}) of four currently used oil palm commercial insecticides, namely cypermethrin, trichlorfon, fipronil and *Bacillus thuringiensis* on *E. kamerunicus* were determined using residual film, topical spray and oral bioassays. The weevil mortality was recorded at 24, 48 and 72 hr after treatment. Probit analysis was used to determine LC_{50} . The result showed that male weevils were more susceptible to trichlorfon and fipronil than female weevils. However, there was no difference between sexes in terms of susceptibility towards cypermethrin. Among the tested insecticides, fipronil had higher lethality on weevil whereas *B. thuringiensis* was less harmful to weevil mortality by <20% even when treated at the highest concentration (0.26% product). Regardless of the type of insecticides and exposure time, the weevil was less sensitive to the insecticide applied via spray method as compared to oral and residual film. The study findings provide useful information in determining the less harmful insecticides to this pollinating weevils with various mode of actions.

Keywords: *Elaeidobius kamerunicus*, oil palm, insect pollinator, insecticides.

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INTRODUCTION

Elaeidobius kamerunicus (Faust.) is the main pollinator in the oil palm plantations in Malaysia. It is an exotic insect, which was imported from Cameroon, West Africa to improve the oil palm pollination efficiency in Malaysia (Syed *et al.*, 1982). It was first released

to the oil palm plantation in Pamol and Mamor, Kluang, Johor, Malaysia in 1981 and the following year its population had spread over to most of oil palm plantations in Peninsular Malaysia (Basri *et al.*, 1983). Prior to that, *Thrips hawaiiensis* (Morgan) was the main pollinating agent in oil palm plantations but it was less efficient than the weevil (Syed, 1979). Besides, hand pollination was also used by most growers, especially for the young palms (Hussein and Rahman, 1991).

Elaeidobius kamerunicus has left huge impact on the Malaysian oil palm industry and economy (Chiu, 1984). The yield increased from 48%-52% to 62.3%-89.6% after the weevil was introduced (Syed *et al.*, 1982; Basri *et al.*, 1987). Besides, a million dollar was saved through cessation of hand-assisted

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pollination (Chiu, 1984; Kevan *et al.*, 1986). These contribute to the increase in Malaysian revenue, making Malaysia as the second highest global oil palm producer and exporter (Chiu, 1984; Ferdous Alam *et al.*, 2015; Kushairi, 2019). Therefore, the weevil is known as the multimillion dollar weevil (Greathead, 1983).

However, the reduction in oil extraction rate (OER) over the last four years become a major concern among the oil palm growers in Malaysia (BEPI, 2018). One of the possible factors resulting in the decline in OER is the reduction in the yield and quality of fruit (Donough *et al.*, 1996; Rao and Law, 1998). According to Lawton (1982), the reduction in the number of fruit set is probably caused by poorer pollination, which is associated with *E. kamerunicus* effectiveness as the oil palm pollinator.

The *E. kamerunicus* activities and population is affected by weather and its natural enemies (Syed and Saleh, 1988; Sugih *et al.*, 1996; Poinar *et al.*, 2002; Ponnamma *et al.*, 2006). In addition, the extensive use of pesticides to control oil palm pest can leave negative impact on weevil population and pollination activity (Rosma Hasibuan *et al.*, 2002; Kalidas *et al.*, 2008; Kok *et al.*, 2010; Yudayati and Hamid, 2015; Prasetyo *et al.*, 2018; Wahyu Syahputra *et al.*, 2018). Although several studies on the lethality effect of insecticides used in oil palm against the pollinating weevil have been reported, there is still lack of information about the susceptibility of this weevil to insecticides especially in determining the insecticides median lethal concentration (LC_{50}) of the weevil. In fact, the determination of the LC_{50} value will help to examine the relationship between dose/concentration response and animal mortality (Chandra *et al.*, 2014). The smaller the LC_{50} value, the more toxic is the insecticide to the animal. Besides, the LC_{50} value is also used to evaluate the lethality of a particular insect species in response to diverse insecticides mode of actions under similar test condition (Paramasivam and Selvi, 2017). The value serves as a basic data to label insecticides and can be used in sub-chronic and chronic studies. Therefore, the aims of this study were to determine the LC_{50} of insecticides commonly used in oil palm plantations and to evaluate their lethality and efficacy on *E. kamerunicus*.

MATERIAL AND METHODS

Insects

All *E. kamerunicus* used in bioassays came from the post-anthesis stage of oil palm male inflorescences. The samples were collected from the Malaysian Palm Oil Board (MPOB) plantation in Keratong, Pahang, Malaysia. This site was chosen due to its insecticide-free status as there was no

incident of insect pest outbreak in this area besides the rodent issue. Thus, the *E. kamerunicus* samples collected in this experiment were more credible and assumed to not have any resistance developed towards the insecticides that would be tested on them. The collected spikelets were kept in rearing cage (16 cm in height and 10 cm in diameter) and maintained in the Centre for Insect Systematic (CIS), Universiti Kebangsaan Malaysia (UKM), Selangor under room temperature and relative humidity of 60%-80% with 12L:12D photoperiod. The emerging adult weevils were sexed by examining according to the length of their snout, body size and the presence of setae on the edge of elytra. The male weevils have shorter snout and larger body size than females. The presence of setae on the edge of elytra indicates male weevil. Male and female weevils were then transferred into new rearing cage separately. The freshly anthesised oil palm male inflorescences free from *E. kamerunicus* were used as the food source for the weevils. All *E. kamerunicus* adults used in the bioassays emerged from the inflorescences were at three days of age.

Host Plant Material

The spikelets of oil palm male inflorescence at the anthesis stage were cut with pruning scissors and stored in refrigerator at $1.93 \pm 0.27^\circ\text{C}$ with $66.09\% \pm 2.96$ relative humidity. At this stage, the wild *E. kamerunicus* can be observed on the spikelet. The wild *E. kamerunicus* will be dead once the spikelets were stored in the refrigerator. All the dead wild *E. kamerunicus* attached to the spikelets were removed once they were taken out from the refrigerator prior to the experiment. This was to avoid confusion regarding the *E. kamerunicus* mortality data.

Insecticides

The insecticides (treatments) used in the bioassays were 5.5% cypermethrin EC, 95% trichlorfon SC, 80% fipronil WG and *Bacillus thuringiensis* subsp. *Kurstaki* ES. Distilled water was used as control.

Bioassay Methods

The residual film, topical spray and oral (feeding) were used as the bioassay methods. Bioassays were conducted in the UKM insect laboratory at room temperature and relative humidity of 60%-80% with 12L:12D photoperiod. To determine the LC, a preliminary study was performed to find the concentration range of the insecticides against the weevils. Each insecticide was tested using a serial dilution where distilled water was used as the solvent. Half of the recommended label rate for field application was used as a starting solution in the serial

dilution except for *B. thuringiensis* (Bt) where the full field rate (0.26% product) was used. In the test, each of the tested insecticide has different concentration range due to different insecticide has different recommended label rate for field application.

Residual film. Insecticide-diluted solutions were applied inside the glass jar surface (9 cm in height by 5 cm diameter). Distilled water was used as control. To get a uniform coverage throughout the jar surface, 250 µl diluted solution was inserted using micropipette and the jar was then manually rotated until all the surface was covered with the solution. The jar was left to dry in fume hood for 12 hr before the weevils were released into it. Ten randomly selected three-day old male weevils were placed in each jar. The glass jar was then covered with organza to allow ventilation, and secured with a rubber band at the mouth ring to prevent the weevils from escaping. The weevils were fed with untreated male inflorescence at the anthesis stage (2 cm in length) which was held at the centre of the cloth covering the glass jar. The weevils were deemed dead if they did not react when their legs were touched with the paint brush. Similar procedure was conducted on female weevils. Each treatment including control was replicated four times. The number of dead *E. kamerunicus* was recorded at 24, 48 and 72 hr after treatment. The experiment was arranged following complete randomised design (CRD). Residual test was conducted according to the method of Desneux *et al.* (2006) with modification.

Topical spray. Ten randomly selected three-day old male weevils were put into plastic cup (200 ml) before being sprayed with 3 ml insecticides using a handheld sprayer. The weevils were removed from treated area right after spraying and transferred into new, insecticide-free plastic cup. The amount of spray applied on each adult weevil was not determined. An insecticide-free spikelet of oil palm anthesising male was put into the cup as the weevil food source. The weevils were deemed dead if they did not react when their legs touched the paint brush. Similar procedure was repeated on female weevils. Each treatment was replicated four times following CRD experimental design. The number of dead *E. kamerunicus* was recorded at 24, 48 and 72 hr after treatment. Topical spraying test was conducted according to the method of Kaakeh (2006) with modification.

Oral (feeding). Ten randomly selected three-day old male weevils were starved for 24 hr before being introduced with treated spikelet into the testing area (using plastic cup as mentioned above). An anthesising male spikelet was used as the weevil food source. The spikelet was dipped for 60 s in the insecticide solutions or distilled water (control) and allowed to dry up for 4 hr in the fume chamber. Later,

the treated spikelet was transferred inside a plastic cup (200 ml) containing starved male weevils. The weevils were deemed dead if they did not react when their legs touched the paint brush. Similar procedures was conducted on female weevils. Each treatment was replicated four times following CRD design. The number of dead *E. kamerunicus* was recorded at 24, 48 and 72 hr after treatment. Oral method was conducted following bolt-dip method by Carrillo *et al.* (2013) with modification.

Data Analysis

The number of dead *E. kamerunicus* was corrected according to Abbot (1925) prior to analysis. The LC₅₀ was determined using Probit analysis in the PoloPlus software. The significant difference between the LC₅₀ values is demonstrated when the 95% confidence limits (CL) between treatments do not overlap with each other. Meanwhile, the data of weevil mortality in percentage was based on concentration-dependent fashion that was analysed using two-way analysis of variance (ANOVA) (sex and concentration as the variables). When ANOVA result was significant ($p < 0.05$), Tukey test was used to separate the treatment means. However, the data needed to be normalised using $\arcsin\sqrt{\chi}$ transformation prior to two-way ANOVA. The mortality data of *E. kamerunicus* treated with various Bt concentrations were analysed with Kruskal-Wallis test due to non-normal data distribution. All ANOVA and Kruskal-Wallis tests were done using the Minitab Software version 17 (Minitab Inc.).

RESULTS

From the Probit analysis, the estimated LC₅₀ values with 95% CL and the slope of regression lines are presented in Table 1. Regardless of the methods used and exposure time, the LC₅₀ values of male and female weevils were not significantly different when treated with cypermethrin, which is denoted by the overlapping of 95% CL of the LC₅₀ values. In contrast, the susceptibility of male and female weevils towards trichlorfon at different exposure times showed no overlapping of the LC₅₀ 95% CL. The LC₅₀ value of trichlorfon tested on female weevil (LC₅₀ = 27.56 ppm) in residual method was significantly higher than male (LC₅₀ = 13.57 ppm) after 24 hr treatment whereas in the spray and oral methods, the LC₅₀ values of trichlorfon for male weevils (spray: LC₅₀ = 192.11 ppm; oral: LC₅₀ = ppm 9.80) were significantly lower than females (spray: LC₅₀ = 356.21 ppm; oral: LC₅₀ = 11.66 ppm) after 72 hr treatment. Irrespective of exposure time, there was no difference in LC₅₀ values of fipronil for male and female weevils in residual method. However, the LC₅₀ values of fipronil for males were significantly lower than for females

in spray and oral methods. There were no LC₅₀ values for orally Bt-treated weevils because even at the highest concentration of Bt (recommended field concentration) their mortality was <20%.

In terms of insecticide toxicity effect in all bioassays on male *E. kamerunicus*, it was shown that fipronil had the lowest LC₅₀ values followed by

cypermethrin and trichlorfon across the exposure times. Similar results were obtained for female weevils, where fipronil showed the lowest LC₅₀ values as compared to cypermethrin and trichlorfon. Therefore, the results suggested that fipronil and trichlorfon had the highest and lowest acute toxicity on *E. kamerunicus*, respectively.

TABLE 1. LETHAL CONCENTRATION (LC₅₀) OF THREE INSECTICIDES ON *E. kamerunicus* AFTER EXPOSED TO INSECTIDES AT 24, 48 AND 72 hr BY THREE DIFFERENT BIOASSAY METHODS

Insecticide	Sex	Method		Hour after treatment (HAT)		
				24	48	72
Cypermethrin	♂	Residual	LC ₅₀	0.85	0.59	0.36
			95% CL	0.70-1.03	0.49-0.72	0.29-0.44
			Slope ± SE	2.39±0.24	2.28±0.22	2.22±0.24
			χ ² (dk,χ _t)	1.84(5,11.07)	4.08(5, 11.07)	3.46(4,9.49)
	♀	Residual	LC ₅₀	1.14	0.67	0.44
			95% CL	0.88-1.52	0.50-0.87	0.34-0.56
			Slope ± SE	1.68±0.20	1.80±0.20	2.17±0.27
			χ ² (dk,χ _t)	3.87(5, 11.07)	4.66(5, 11.07)	3.98(4,9.49)
	♂	Spray	LC ₅₀	1.40	1.11	1.00
			95% CL	1.19-1.64	0.91-1.33	0.79-1.22
			Slope ± SE	3.49±0.42	2.84±0.36	3.05±0.44
			χ ² (dk,χ _t)	2.32(3,7.82)	2.93(3,7.82)	2.82(3,7.82)
	♀	Spray	LC ₅₀	1.49	1.31	1.19
			95% CL	1.15-1.87	0.97-1.69	0.84-1.57
			Slope ± SE	1.96±0.26	1.76±0.25	1.62±0.25
			χ ² (dk,χ _t)	2.74(3,7.82)	1.52(3,7.82)	1.47(3,7.82)
	♂	Oral	LC ₅₀	0.24	0.07	0.05
			95% CL	0.16-0.33	0.04-0.10	0.03-0.07
			Slope ± SE	1.73±0.28	2.19±0.34	2.11±0.36
			χ ² (dk,χ _t)	3.98 (5, 11.07)	4.87(5, 11.07)	1.31(4,9.49)
	♀	Oral	LC ₅₀	0.21	0.06	0.03
			95% CL	0.17-0.26	0.05-0.08	0.02-0.04
			Slope ± SE	2.17±0.22	1.92±0.20	1.86±0.26
			χ ² (dk,χ _t)	4.58(5, 11.07)	4.24(5, 11.07)	2.57(4, 9.49)
Trichlorfon	♂	Residual	LC ₅₀	13.57	13.90	10.61
			95% CL	10.87-16.81	9.27-17.60	6.88-14.08
			Slope ± SE	2.08±0.23	3.48±0.73	2.84±0.54
			χ ² (dk,χ _t)	2.39(4, 9.49)	2.39(4, 9.49)	2.59(3,7.82)
	♀	Residual	LC ₅₀	27.56	14.04	11.78
			95% CL	22.53-34.20	11.04-17.42	8.75-15.04
			Slope ± SE	2.27±0.25	2.48±0.30	2.59±0.38
			χ ² (dk,χ _t)	3.68(4, 9.49)	3.88(4, 9.49)	2.93(3,7.82)
	♂	Spray	LC ₅₀	269.72	243.26	192.11
			95% CL	230.47-316.43	206.60-287.03	157.42-241.76
			Slope ± SE	3.39±0.41	3.19±0.37	2.60±0.38
			χ ² (dk,χ _t)	2.43(3,7.82)	1.61(3,7.82)	1.79(2, 5.99)

TABLE 1. LETHAL CONCENTRATION (LC₅₀) OF THREE INSECTICIDES ON *E. kamerunicus* AFTER EXPOSED TO INSECTIDES AT 24, 48 AND 72 hr BY THREE DIFFERENT BIOASSAY METHODS (continued)

Insecticide	Sex	Method		Hour after treatment (HAT)		
				24	48	72
Trichlorfon	♀	Spray	LC ₅₀	359.01	352.18	356.21
			95% CL	289.56-469.97	280.36-470.57	261.66-612.42
			Slope ± SE	2.22±0.31	2.05±0.30	1.91±0.37
			χ ² (dk,χ _t)	1.617(3,7.815)	2.325(3,7.815)	1.11(2,5.99)
	♂	Oral	LC ₅₀	20.77	11.91	9.80
			95% CL	17.84-23.88	9.83-14.00	7.90-11.56
			Slope ± SE	3.20±0.30	3.56±0.42	4.04±0.63
			χ ² (dk,χ _t)	5.57(6,12.59)	3.84(6, 12.59)	3.87(5, 11.07)
	♀	Oral	LC ₅₀	21.87	16.59	11.66
			95% CL	18.99-24.80	14.17-18.70	9.15-13.93
			Slope ± SE	3.93±0.39	5.03±0.64	4.16±0.66
			χ ² (dk,χ _t)	5.42(6, 12.59)	4.33(6, 12.59)	1.00(5, 11.07)
Fipronil	♂	Residual	LC ₅₀	0.52	0.14	0.11
			95% CL	0.40-0.68	0.11-0.16	0.09-0.13
			Slope ± SE	1.76±0.21	2.39±0.28	2.59±0.33
			χ ² (dk,χ _t)	1.59(4, 9.49)	3.99(4, 9.49)	2.90(3,7.82)
	♀	Residual	LC ₅₀	0.47	0.174	0.10
			95% CL	0.38-0.58	0.12-0.22	0.07-0.14
			Slope ± SE	2.41±0.29	3.04±0.51	2.90±0.49
			χ ² (dk,χ _t)	3.27(4, 9.49)	3.98(4,9.49)	2.98(3,7.82)
	♂	Spray	LC ₅₀	0.49	0.30	0.29
			95% CL	0.46-0.53	0.26-0.34	0.24-0.32
			Slope ± SE	7.51±0.90	5.07±0.80	5.02±0.89
			χ ² (dk,χ _t)	2.90(3,7.82)	2.89(3,7.82)	1.86(2, 5.99)
	♀	Spray	LC ₅₀	0.90	0.55	0.47
			95% CL	0.81-1.06	0.50-0.61	0.41-0.56
			Slope ± SE	4.93±0.77	4.96±0.60	3.26±0.63
			χ ² (dk,χ _t)	1.82(3,7.82)	1.97(3,7.82)	1.38(2, 5.99)
	♂	Oral	LC ₅₀	0.026	0.007	0.004
			95% CL	0.020-0.031	0.002-0.010	0.000-0.008
			Slope ± SE	2.70±0.37	2.55±0.74	1.94±0.69
			χ ² (dk,χ _t)	3.23(5, 11.07)	0.80(5, 11.07)	0.41(4, 9.49)
	♀	Oral	LC ₅₀	0.08	0.03	0.01
			95% CL	0.07-0.10	0.02-0.03	0.01-0.02
			Slope ± SE	2.74±0.28	7.59±1.54	3.45±0.71
			χ ² (dk,χ _t)	4.55(5, 11.07)	0.01(5, 11.07)	3.25(4, 9.49)
<i>Bacillus thuringiensis</i> subsp. <i>Kurstaki</i>	♀♂	Oral	LC ₅₀	*-	-	-
			95% CL	-	-	-
			Slope ± SE	-	-	-
			χ ² (dk,χ _t)	-	-	-

Note: *Analysis is not conducted due to the mortality of weevil <50% even though treated with the higher concentration (recommended rate in field).

CL - confidence limits.

SE - standard error.

In general, males *E. kamerunicus* that were exposed to insecticides by spraying method had significantly higher LC_{50} than those exposed to residual and oral insecticides over time. Similar results were obtained for females *E. kamerunicus*, where those exposed to insecticides by spray method had significantly higher LC_{50} value than those exposed to the respective insecticides tested by other methods.

All the calculated chi-squared (χ^2) values were lesser than the tabulated chi-squared (χ_c) values, indicating the χ^2 values are not significantly different (Table 1). In general, the slopes for male weevils were steeper than females for all methods and treatments. Regardless of the methods and sex, the LC_{50} slopes for weevils treated with fipronil were steeper than trichlorfon and cypermethrin. For trichlorfon, the LC_{50} slopes for male weevils in oral method (4.04 ± 0.63) were steeper than residual (2.84 ± 0.54) and spray (2.59 ± 0.38) methods after 72 hr exposure while for the female weevils, the oral method slopes were steeper than those of other methods after 48 hr and 72 hr exposure. For fipronil, the LC_{50} slopes for male weevils in spray method were steeper than residual and oral methods at all exposure times. This is similar to the female weevils, where the LC_{50} slopes in spray method (4.93 ± 0.77) were steeper than those of residual (2.41 ± 0.29) and oral methods (2.74 ± 0.28) after 24 hr treatment. However, after 48 hr exposure, the LC_{50} slopes of female weevils in oral method (7.59 ± 1.54) were steeper than residual (3.04 ± 0.51) and spray methods (4.96 ± 0.60).

The percentage mortality of *E. kamerunicus* caused by insecticides is based on concentration-dependent relationship as shown in Table 2 and Figures 1 to 6. For the residual film method, there was no significant interaction between sex and concentration as well as between the sexes alone for all the tested insecticides that cause the death of *E. kamerunicus*. However, each insecticide concentration was found to have a significant effect to the weevil mortality percentage (Table 2 and Figure 1). The exposure to the two highest concentrations of cypermethrin and trichlorfon caused significantly higher weevil mortality than other concentrations. Almost 90% weevils were dead when they were in contact with the fipronil residue at the highest concentration (1.56 ppm) (Figure 1). However, the weevil mortality percentage decreases significantly after being exposed to the lower insecticide concentrations. For spray method, the interaction between sex and concentration as well as between the sexes alone had no significant effect on weevil death when they were treated with cypermethrin and trichlorfon (Table 2). Cypermethrin caused significantly higher weevil mortality at the two highest concentrations

(4.10 ppm and 8.19 ppm) in comparison to other concentrations. Meanwhile for trichlorfon, almost 80% weevils were dead when being exposed to the highest concentration (600 ppm) but the mortality was <3% at the lowest concentration (50 ppm) (Figure 2). In contrast, there was significant interaction between sex and concentration on the weevil mortality when they were exposed to fipronil. Besides, different concentrations and sex alone significantly affected weevil mortality (Table 2). At the concentration of 0.38 ppm to the highest, the mortality of male weevils was significantly higher than females (Figure 3). This shows that male weevils are more susceptible to fipronil than the females. Female weevils treated with the two highest fipronil concentrations (0.77 ppm and 1.02 ppm) had significantly higher mortality than those exposed to the lowest concentration (0.26 ppm), with mortality of only <3%. Meanwhile, the male weevils exposed to the two highest fipronil concentrations (0.77 ppm and 1.02 ppm) had highest mortality (>95% death) as compared to other concentrations (Figure 3). For oral method, the interaction between sex and concentration as well as between the sexes had no significant effect on weevil death when they were treated with cypermethrin and trichlorfon (Table 2). The contact with cypermethrin and trichlorfon via oral method at the two highest concentrations had resulted in significantly higher weevil mortality than the remaining concentrations (Figure 4). However, there was no difference in weevil mortality at the three lowest cypermethrin concentrations, in which the percentage of weevil death was similar to the control. There was significant interaction between sex and fipronil concentration that contributed to the weevil death (Table 2). Sex and fipronil concentration alone also significantly affected the percentage of weevil mortality. As the concentration increased, the percentage of male weevil mortality had increased significantly higher than female percentage mortality, where only 62.50% females were killed but all male weevils (100%) were killed when exposed to the highest fipronil concentration (0.10 ppm) (Figure 5). The male *E. kamerunicus* orally treated with different Bt concentrations showed no difference in weevil mortality throughout the exposure times when analysed using Kurskal-Wallis tests [24 and 48 hr after treatment (HAT), $H=5.07$; $df=4$; $P=0.28$; 72 HAT, $H=8.04$; $df=4$; $P=0.09$]. Similar results were observed for female weevils, where there was no significant difference in mortality between different Bt concentrations at all exposure times (48 and 72 HAT, $H=5.07$; $df=4$; $P=0.28$). Except at 24 hr after treatment, the data cannot be generated due to the absence of weevil mortality when exposed to different Bt concentrations (Figure 6).

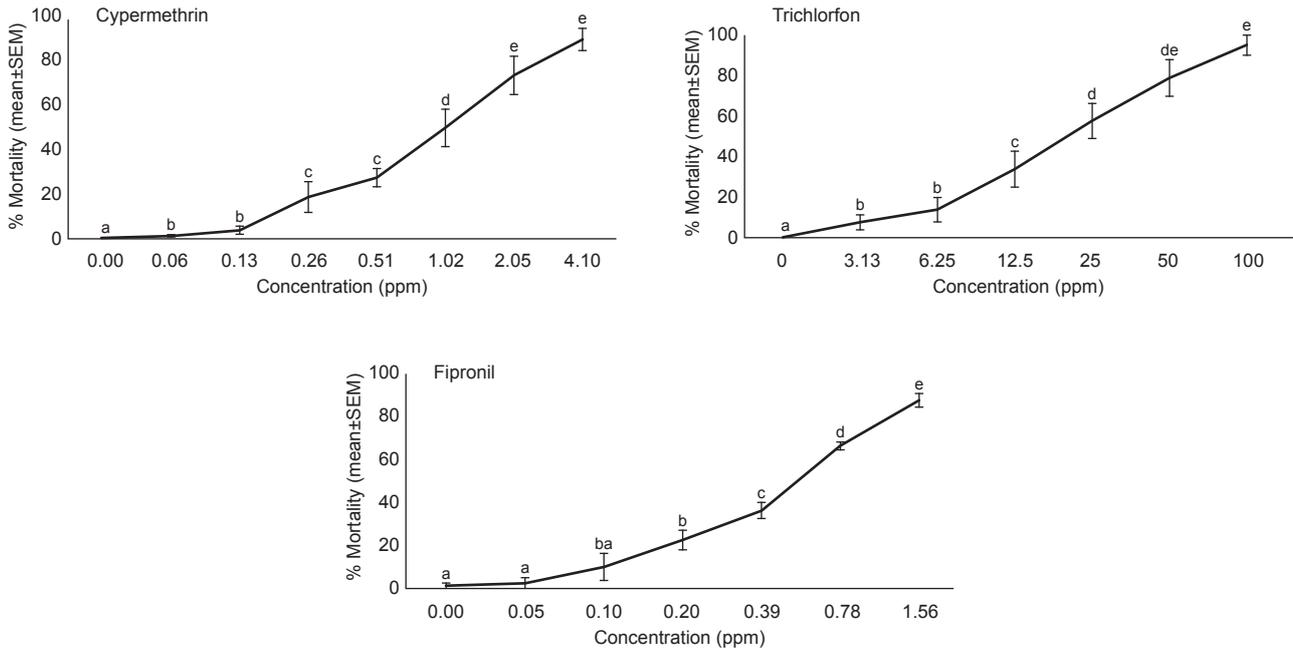


Figure 1. Percentage mortality of *E. kamerunicus* 24 hr after exposure to different concentrations of selected insecticides by residual film method. The insecticide concentration '0' represents the no-insecticide control treatment. Error bars represent the scanning electron microscope (SEM).

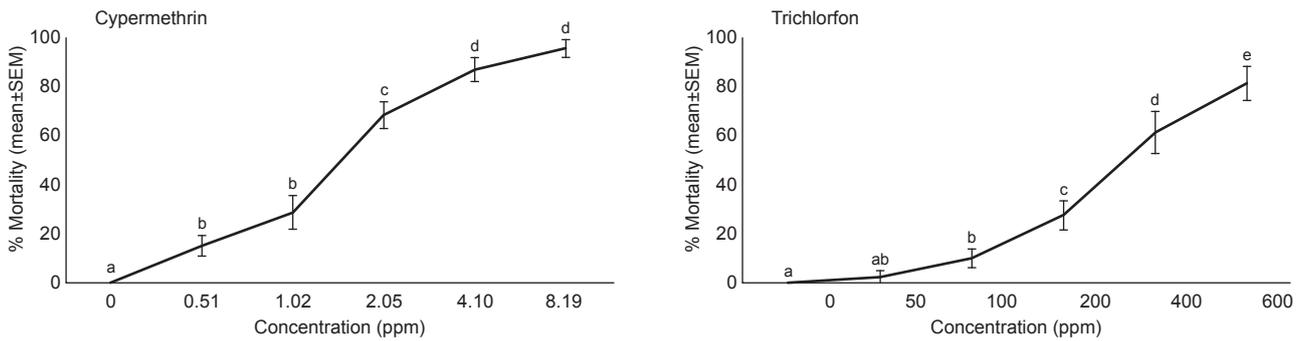


Figure 2. Percentage mortality of *E. kamerunicus* 24 hr after exposure to different concentrations of cypermethrin and trichlorfon by spraying method. The insecticide concentration '0' represents the no-insecticide control treatment. Error bars represent the scanning electron microscope (SEM).

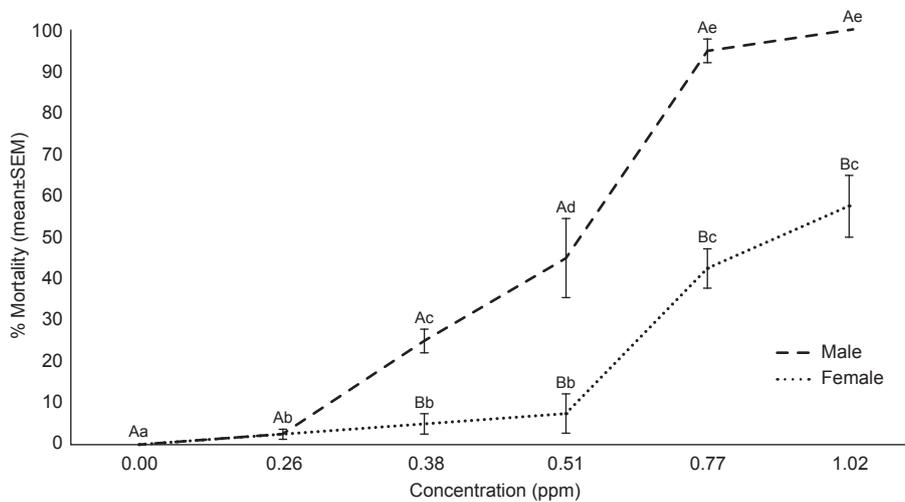


Figure 3. Percentage mortality of *E. kamerunicus* male and female 24 hr after exposure to different concentrations of fipronil by spraying method. The insecticide concentration '0' represents the no-insecticide control treatment. Error bars represent the scanning electron microscope (SEM).

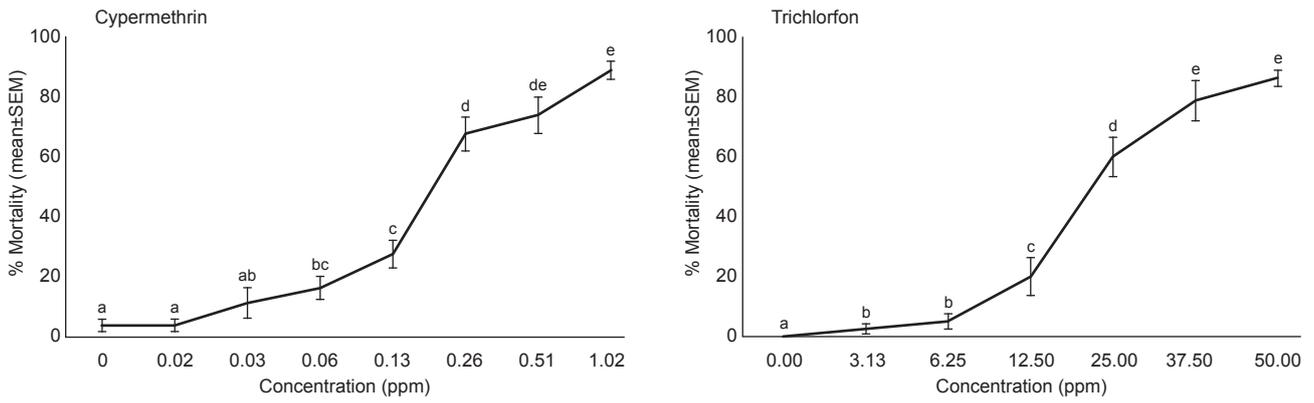


Figure 4. Percentage mortality of *E. kamerunicus* 24 hr after exposure to different concentrations of cypermethrin and trichlorfon by oral method. The insecticide concentration '0' represents the no-insecticide control treatment. Error bars represent the scanning electron microscope (SEM).

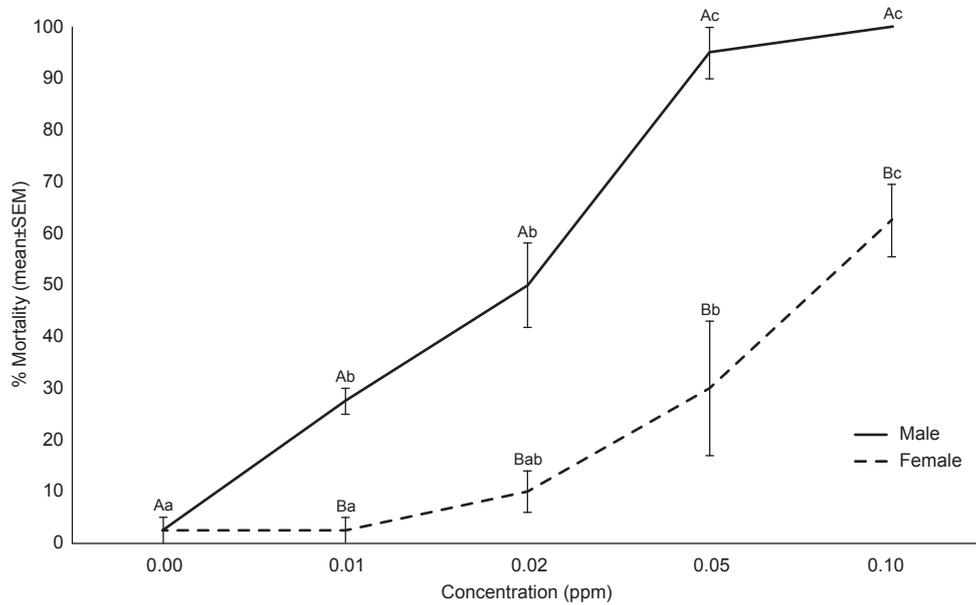


Figure 5. Percentage mortality of *E. kamerunicus* 24 hr after exposure to different concentrations of fipronil by oral method. The insecticide concentration '0' represents the no-insecticide control treatment. Error bars represent the scanning electron microscope (SEM).

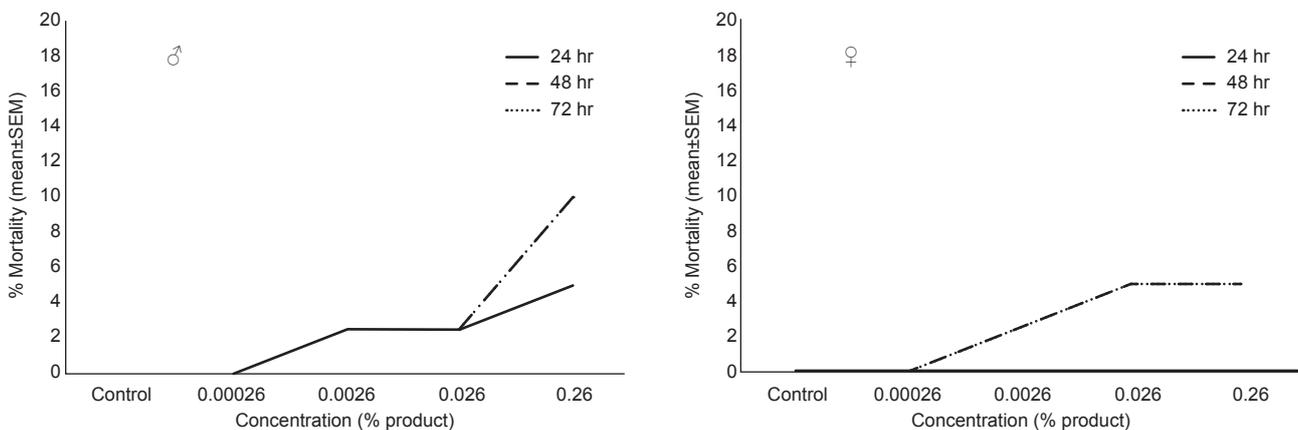


Figure 6. Percentage mortality of *E. kamerunicus* when treated with *Bacillus thuringiensis subsp. Kurstaki* by oral bioassay.

TABLE 2. ANALYSIS OF VARIANCE (ANOVA) STATISTICS FOR THE EFFECT OF SELECTED INSECTICIDES ON *E. kamerunicus* MORTALITY PERCENTAGE AT 24 hr AFTER EXPOSURE

Method	Chemicals	Sources	DF	SS	MS	F-value	P-value
Residual	Cypermethirn	Sex	1	279.90	279.90	1.72	0.20
		Concentration	7	47 047.80	6 721.10	41.34	0.00
		Interaction	7	774.40	110.70	0.68	0.69
		Error	48	7 804.70	162.60	-	-
	Trichlorfon	Sex	1	2 357	2 357	2.28	0.14
		Concentration	6	45 619.00	7 603.20	34.81	0.00
		Interaction	6	1 140.70	190.10	0.87	0.53
		Error	42	9 173.40	218.40	-	-
	Fipronil	Sex	1	44.6	44.60	0.38	0.54
		Concentration	6	53 285.70	8 881.00	75.74	0.00
		Interaction	6	342.90	342.90	0.49	0.81
		Error	42	4 925.00	117.30	-	-
Spray	Cypermethirn	Sex	1	52.10	52.10	0.29	0.59
		Concentration	5	64 568.80	12 913.80	72.92	0.00
		Interaction	5	1 085	217.10	1.23	0.32
		Error	36	6 375	177.10	-	-
	Trichlorfon	Sex	1	118.70	118.70	0.73	0.40
		Concentration	5	29 588.10	5 917.60	36.58	0.00
		Interaction	5	1 015.40	203.10	1.26	0.30
		Error	36	5 823.70	161.80	-	-
	Fipronil	Sex	1	7 752.10	7 752.10	93.81	0.00
		Concentration	5	46 018.80	9 203.80	111.37	0.00
		Interaction	5	4 985.40	997.10	12.07	0.00
		Error	36	2 975.00	82.60	-	-
Oral	Cypermethirn	Sex	1	394.90	394.94	0.57	0.45
		Concentration	7	37 129.00	5 304.14	55.76	0.00
		Interaction	7	1 244.00	177.72	1.87	0.10
		Error	48	4 565.70	95.12	-	-
	Trichlorfon	Sex	1	0.60	0.60	0.01	0.94
		Concentration	6	41 399.10	6 899.80	72.52	0.00
		Interaction	6	702.00	117.00	1.23	0.31
		Error	42	3 995.90	95.10	-	-
	Fipronil	Sex	1	23 715.20	5 928.81	40.72	0.00
		Concentration	4	8 935.80	8 935.80	61.37	0.00
		Interaction	4	3 075.30	768.81	5.28	0.00
		Error	30	4 368.20	145.61	-	-

Note: DF - degree of freedom; SS - sum of squares; MS - mean square.

DISCUSSION

Oil palm is a monoculture crop that provides habitat for a number of plant-eating insect pests such as lepidopteran pest (*i.e.* bagworms, nettle caterpillars and bunch moth), termite and beetle (*i.e.* *Oryctes rhinoceros*) due to the abundant and sustainable food sources. Hence, to prevent from the loss of yield, oil palm plantation has implemented an integrated pest management (IPM) practise to address the issue (Kuntom *et al.*, 2007). Implementation of IPM

in agroecosystem helps in reducing the usage of insecticides through the increase of natural enemies. Although there are natural enemies to control the oil palm insect pests, their population is insufficient for effective control especially during the insect pest outbreak (Young, 1971). Thus, insecticides become the main tool in reducing the insect pest population during the outbreak. Most of the planters use broad spectrum insecticides (*e.g.* cypermethrin, trichlorofon and fipronil) due to the presence of different insect herbivoures species in oil palm

plantation. Furthermore, cheaper in price, easily accessible, low cost to operate and fast result make these insecticides the main choice among planters (Norman, 1994; Kuntom *et al.*, 2007). However, overdosing and frequent use of insecticides as preventive applications can lead to adverse effects on human health and also can lead to environmental pollution (Basiron, 2007; RSPO, 2013). Besides, some insects have developed physiological and behaviour resistance to insecticide (Martínez *et al.*, 2009). In addition, excessive use of insecticide also can give harmful effect to the beneficial organisms such as natural enemies and the oil palm pollinators. Therefore, the impact of insecticides that are commonly used in oil palm plantation either directly or indirectly on *E. kamerunicus* need to be evaluated as this weevil plays a major role in oil palm pollination.

The study results showed that female weevils are less susceptible to insecticides than males (Table 1, Figures 3 and 5). This is consistent with the previous studies on several insect species (Rathman *et al.*, 1992; Weaver *et al.*, 1994; Shearer and Usmani, 2001; Noor Farehan *et al.*, 2013). There are factors that lead to differences in terms of susceptibility towards insecticides between sexes, size and metabolism (Rathman *et al.*, 1992; Weaver *et al.*, 1994; Tillman, 1995). The bigger the size, the higher is the tolerance to insecticide (Abdelrahmen, 1973; Scott and Rutz, 1988; Rathman *et al.*, 1992; Weaver *et al.*, 1994; Shearer and Usmani, 2001; Noor Farehan *et al.*, 2013). In fact, the insects with larger body size have higher amount of lipid that helps them to be more tolerant to insecticides (Munson and Gottfried, 1953; Roy and Prasad, 2018). Lipid is one of the molecules stored in body fat that is involved in synthesising detoxification enzymes; it can reduce the effect of insecticides on the insects (Terriere, 1982; Roy and Prasad, 2018). Interestingly, female *E. kamerunicus* has smaller body size than males. One of the reasons the female *E. kamerunicus* are more tolerant to insecticides is due to vitellogenesis. Vitellogenesis is the process of yolk formation, which occurs during egg development (Telfer, 2009). This process is important as it provides nutrients for embryo development in the insects (Valle, 1993). According to Telfer (2009), during egg production, the female body fat secretes proteins that will be used during vitellogenesis. Despite having smaller body size than males, the female weevils still have higher amount of lipid as they need fat to produce eggs. Another reason for the different susceptibility towards insecticides is that the detoxification activity of the female weevils is higher than males. Based on the study done by De Lame *et al.* (2001) on the Oriental fruit moth, *Grapholita molesta*, even though the females have larger body size, it was found that the male moths are more tolerant to organophosphate (OP) than females due to differences in the level

of degrading acetylcholinesterase (AChE) enzyme and insensitivity enzyme AChE to OP, making the female moths to be more susceptible. However, further study needs to be conducted to prove this assumption on *E. kamerunicus*.

Both male and female *E. kamerunicus* treated with fipronil had lower LC₅₀ values as compared to the weevils treated with cypermethrin and trichlorfon (Table 1). The lower susceptibility of *E. kamerunicus* towards cypermethrin and trichlorfon could be due to the weevils having already developed resistance to both insecticides, since both are being widely used to control oil palm leaf defoliator insect pests such as bagworms and nettle caterpillars (Wood, 1968; Chung, 1989; Norman and Basri, 1992; Kuntom *et al.*, 2007, Kok *et al.*, 2012). Besides that, although all these insecticides are classified under neurotoxic group but the differences in mode of actions or targeted site of action in the nervous system could contribute to difference degree of toxicity effect on *E. kamerunicus*. The mode of action of fipronil involves the blocking of γ -aminobutyric acid (GABA)-gated chloride channel, thus leading to hyperexcitation at lower dose, as well as paralysis and death at higher dose (Cole *et al.*, 1993; Brooks, 2010; Gupta and Milatovic, 2014). Meanwhile, cypermethrin causes knockdown effect in invertebrates by disrupting the voltage-gated sodium channels (Bloomquist and Miller, 1986; Baumler and Potter, 2007; Macoris *et al.*, 2018). Trichlorfon interferes with the normal nerve function by inhibiting AChE enzyme located at the postsynaptic membrane (Costa *et al.*, 2008) and insects normally show behavioural changes such as uncoordinated movement, hyperactivity and ovipositor extrusion (Alix *et al.*, 2001; Stürmer *et al.*, 2014). Throughout the observation, *E. kamerunicus* that were exposed to lower concentration of these neurotoxic insecticides displayed temporary altered mobility and muscle contraction. However, as the concentration increased, there were some *E. kamerunicus* showing constant paralysis without recovery signs, which eventually led to death (Figures 1 to 5). Thus, the application of these insecticides in oil palm plantations, especially fipronil, should be carefully managed because it may not only affect *E. kamerunicus* but other useful insects too.

Unlike neurotoxic insecticides, Bt is a pest-specific microbial insecticide used in oil palm to kill lepidopteran larvae. This bio pesticide contains crystal toxin proteins (δ -endotoxins) that binds to the gut receptor of the targeted insect and causes osmotic lysis, resulting in death of the insect (Ramlah, 2000; Ghribi *et al.*, 2006; Norman *et al.*, 2017). In this experiment, less than 20% weevils were killed upon treatment with Bt at the concentration recommended in the field (0.26% product) (Figure 6). Our results are in line with previous studies on weevil response to Bt (Mohd Najib *et al.*, 2009; 2012; Yusdayati and Hamid, 2015). Based on the International Organisation

Biological Control (IOBC) 2005, if the mortality of the tested insect against pesticide is less than 30%, the pesticide is harmless to the insect (Hassan *et al.*, 1983; Boller *et al.*, 2005). Thus, the use of Bt to control the population of oil palm insect pests is highly recommended.

The comparison between the type of bioassays suggests that the effect of using spray method insecticide give less detrimental impact on *E. kamerunicus* than oral and residual film methods. This maybe because the residual film and oral methods provide more time for *E. kamerunicus* to absorb/come in contact with more insecticide than the one-time contact with the spray method. This means higher amount of insecticides can enter into the insect body. Similarly, a study by Immaraju *et al.* (1990) showed that the citrus thrips were most sensitive to fluvalinate and dimethoate via leaf spray (residual bioassay) than using microapplicator as the thrips were repeatedly in contact with the toxicant in leaf spray method compared to receiving only one-time acute dosing in topical method. However, in the field, the combination of contact via spray and residues on plants/oil palm flowers may occur. Thus, the study results provide information regarding safe-to-use pesticides for *E. kamerunicus*.

There were non-significant difference of the chi-squared values in Probit analysis indicating no variation between individual weevils within a treatment, weevils are genetically the same. Besides, chi-squared values are also used to test whether the data used to estimate the median LC (LC_{50}) fit to the Probit model or not (Robertson *et al.*, 2007). If the chi-squared values are significantly different at $p=0.05$, then the data does not fit with the model. Thus, other models that fit to the data should be used.

The regression slope indicates the degree of homogeneity of a population on its response to the toxicant. According to Schouest and Miller (1988), steeper slope indicates a population is more homogenous (*i.e.* have similar age) or has higher consistency in terms of exposure to chemical substance. The slope also shows the degree of sensitivity of insect to a toxicant. Steeper slope indicates that insect is more sensitive to the toxicant.

In this study, the regression slope of male weevils is steeper than female, this demonstrates male weevils are more sensitive to the insecticides than the females. Besides, the steeper slope from the fipronil treatment shows that weevils are more sensitive to fipronil than cypermethrin and trichlorfon. However, when the slopes between methods for each insecticide were compared, it was found that weevils were more sensitive to trichlorfon treated orally but they were more sensitive to fipronil in spray application. Weevil has similar sensitivity towards cypermethrin regardless of bioassay methods.

CONCLUSION

The use of insecticide is one of the effective ways to control insect pests in oil palm plantation especially during pest outbreak but it may affect oil palm pollinator as well. Our findings demonstrated that fipronil exhibits the strongest insecticidal effect on *E. kamerunicus* under laboratory condition. Although the lethality of cypermethrin, trichlorfon and Bt is lower than fipronil, the side effect of these chemicals should not be neglected. Hence, the sublethal tests (*i.e.* the effect of insecticide on insect biology, physiology and behaviour) on insect should be carried out to find out the impact of insecticide on other insects especially the insect natural enemies. The bioassay data in the laboratory should be compared with the actual field data so that it could tell us which bioassay techniques could reflect the real effect of insecticides in the actual environment where the insect come in contact with direct spray, plant residues or food contaminated with insecticides. Therefore, semi-field (field cage) and field tests should also be conducted to better determine the toxicity of insecticides on weevils under the actual environment.

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