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Toxicity of Three Insecticides on the Predator of Oil Palm Leaf-Eater Pests *Sycanus dichotomus* Stål. (Hemiptera: Reduviidae)

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Abstract: Using dipping and contact methods, three insecticides, namely, cypermethrin, deltamethrin and trichlorfon, were tested for their toxicity effect on *Sycanus dichotomus* Stål. (Hemiptera: Reduviidae), an important biological control agent of oil palm leaf-eater pests. Results in dipping method showed that the mortality percentage of adult *S. dichotomus* were significantly higher when treated with trichlorfon (66 and 76%) than cypermethrin (36 and 57%) and deltamethrin (33 and 52%) at 0.05 and 0.10% concentrations. As for third nymph instar *S. dichotomus*, the mortality percentage of the predator was significantly higher when treated with trichlorfon (31 and 73 %) than cypermethrin (8 and 36%) and deltamethrin (4 and 31%) at 0.002 and 0.02 % concentrations. Similarly, in contact method where trichlorfon caused significantly higher mortality percentage of adult *S. dichotomus* compared to pyrethroids at 0.25-1.5% concentrations. Probit analysis showed that, irrespective of insect sex and developmental stage, the LC_{50} values for *S. dichotomus* treated with trichlorfon were significantly lower than pyrethroids. This indicates that trichlorfon is more toxic to the predators compared to pyrethroids insecticide. Furthermore, female *S. dichotomus* were found to be significantly more tolerant to all the insecticides tested than male. In addition, *S. dichotomus* adults were found to be significantly more tolerant to all insecticides tested than third instar nymphs. To conclude, this study showed the importance of using suitable insecticide to control pest population without harming natural enemies. Therefore, the usage of pyrethroids and beneficial insects in integrated pest management (IPM) programme should be better evaluated.

Key words: Reduviid predator · Bagworms · *Metisa plana* · Trichlorfon · Pyrethroids

INTRODUCTION

Sycanus dichotomus Stål. (Hemiptera: Reduviidae) is one of the natural enemies of oil palm leaf-eater pests (bagworms and nettle caterpillars) and a common insect predator found in oil palm plantations [1,2]. Desmier de Chenon *et al*. [3] has reported that one *S. dichotomus* is able to kill about 430 young larvae of *Metisa plana* (Lepidoptera: Psychidae) (a bagworm species) in its life time. Besides bagworms, *S. dichotomus* has also been reported preying on oil palm nettle caterpillars (Lepidoptera: Limacodidae) such as *Setothosea asigna* van Eecke and *Darna trima* Moore [1,4]. A study carried out by Syari *et al*. [5] on the predatory efficiency of *S. dichotomus* shows that the adult predator killed twice the number of bagworms killed by *Platynopus melacanthus* Biosdural (Hemiptera: Pentatomidae). In addition, having a longer rostrum compared to other bagworm predators mainly from family pentatomidae, gives *S. dichotomus* the ability to attack bigger late instar bagworms and presumably other larger oil palm caterpillars too [6]. It was reported that most pentatomidae predators have trouble controlling late instar bagworms due to the difficulty in penetrating the bags with their short rostrums [7]. This shows that *S. dichotomus* is a good biological control agent in controlling oil palm leaf-eater pests.

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These leaf-eater pests represent a major problem for the oil palm industry in Malaysia. The widespread attack of the pests was first detected in 1966 during the introduction and rapid extension of oil palm cultivation in Malaysia [8]. Wood *et al.* [9] have reported that 50% of crop damage was caused by bagworm from *Metisa plana* Walker species, resulting in a decline in oil palm yield of 43% over the next two years. In fact, even damage as low as 10-13% can cause a similar yield loss [10]. According to a survey conducted by Norman and Basri [11], *Metisa plana* is still the most threatening and dominant oil palm pest in Malaysia.

Until now, the usage of insecticides to control pests is being the main choice among planters. The insecticides that are commonly used to control the bagworms are monocrotophos, methamidaphos, cypermethrin, deltamethrin, trichlorfon and acephate [12]. Although the usage of insecticides have been reported to be successful in controlling pests [13-18] but the extensive usage will also disturb natural enemy population and in turn encourage pests resurgent that can lead to pest outbreak [19].

Realizing the importance of natural enemies in controlling the oil palm leaf-eater pests, a new approach which is integrated pest management (IPM) practices have been taken by the oil palm planters in Malaysia to deal with the problem. The successful in integrating natural enemies and chemicals to suppress pest population can only be achieved if natural enemies were safe against insecticide used [20]. Normally, using natural enemy alone to control the insect pest cannot provide economic control especially during pest outbreak where pest infestation has reached economic threshold level [21,22]. At this level, it can ruin the crop to the point that it is uneconomical to the planter. However with supplemental use of an insecticide, maybe could provide adequate control of the pest [23]. Nevertheless, insecticides should only be used when pest population threaten has exceeded the economic threshold level.

Although IPM practices have started being used in oil palm plantations in Malaysia to control leaf-eater pests, the information of the toxicity effect on *S. dichotomus* is still unknown. Therefore, the laboratory studies were carried out to investigate the effects of the commonly used insecticides in oil palm plantations on the pests' natural enemy, *Sycanus dichotomus*.

MATERIALS AND METHODS

***Sycanus Dichotomus* Rearing:** Adult *Sycanus dichotomus* were collected at the Southern Perak Plantation (SPP) Company, Teluk Intan, Perak. A total of one hundred adult insects, fifty male and fifty female, were released into cages (40.5 x25.5 x30.5cm), each containing 10 adults for mating at room temperature (26 ± 2°C), 60-80% relative humidity and a photoperiod of 11:13 (L:D) hours. *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) were put in a petri dish (9cm diameter x 2cm height) to serve as food to the *S. dichotomus* and a 25ml plastic cup filled with water were placed inside each cage. Three oil palm leaves with their basal parts covered with wet cotton, serving as oviposition site, were put inside each cage. Cages were checked alternate days to replace water and, prey (food) and to collect the eggs. Portions of leaf with eggs attached were cut and put into small plastic cups (7.5cm diameter x 8cm height) for hatching of the eggs. Only one cluster of eggs was put into each plastic cup so that a uniform cohort of nymphs could be reared. Leaves in cage serving as oviposition site were removed and replaced after 2 days. The cage was cleaned twice a week to prevent disease infection. Upon hatching, the nymphs in each cup were provided with moist cotton balls to maintain the humidity and also as drinking source. Two days after hatching the nymphs were gently transferred using a soft paint brush to one plastic cup (12cm diameter x 11cm height) for rearing. Each rearing cup contained only 10 nymphs to prevent crowding. The *S. dichotomus* nymphs were fed as above, but a moist cotton ball was put on top of the muslin cloth covering the rearing cup as drinking source and to maintain the humidity.

Insecticides: Insecticides used in this study were trichlorfon (Dipetex 95.0% SP, Bayer Crop Science) [0,0-dimethyl-(2,2,2 trichloro-1-hydroxyethyl)-phosphonate], cypermethrin (Cypermethrin 5.5% EC, Hextar Chemicals Sdn. Bhd.) [[RS]-α-cyano-3-phenoxybenzyl (1RS)-Cis-trans-3-(2, 2 dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate] and deltamethrin (Decis 2.8% EC, Bayer CropScience) [(S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylate. A pilot study was carried out to determine the maximum and minimum insecticides doses to be used in the experiments. All the insecticides were diluted in distilled water in respective to the concentrations used.

Dipping Method: Direct toxicity by dipping method was conducted following Rahman and Talukder [24] with some modifications. Twelve 3-4 old-day adults of *S. dichotomus* were randomly selected, separated into six males and six females, placed into a plastic cup (62mm height x 90mm diameter) and then immersed in respective prepared insecticide solutions (0.05, 0.1, 0.25, 0.50 and 1.00 % a.i) for 3 seconds. The insects were then removed, air-dried and released separately (base on the sexes) into a plastic cage (220mm height x 120mm diameter). Control insects were treated similarly with distilled water. Ten *T. molitor* larvae were put in a small cup (35mm height x 45mm diameter) and placed inside the plastic cage as a source of food for *S. dichotomus*. Each treatment was replicated eight times. Mortality data was observed at intervals of 24, 48 and 72 h post treatment. Insects were considered dead if they did not react when their legs were touched with the paint brush. A similar test was performed on third nymphal instar of S. *dichotomus* but with different solution of insecticide concentrations (0.002, 0.02 and 0.2 % a.i).

Contact Method: A Contact toxicity test was conducted according to the method of Azimi *et al*. [25] with some modifications. Six randomly selected 3-4 old-day adult males of *S. dichotomus* were placed into each plastic cage (220mm height x 120mm diameter) which previously had been coated with insecticide solution (0.25, 0.50, 1.00, 1.50, 2.00 and 2.50 % a.i) and air drying for 3 h. Tap water was used as a control. The insects were fed with *T. molito*r larvae which had been previously put into a small cup (35mm height x 45mm diameter) and placed inside the plastic cup (as above). Each treatment was replicated eight times. Mortality data was observed at intervals of 24, 48 and 72 h post treatment. Insects were considered dead if they did not react when their legs were touched with a paint brush. A similar test was performed on females of *S. dichotomus* with the same solutions of insecticide concentrations.

Statistical Analysis: LC_{50} at 95% confidence limits was estimated using PROBIT analysis [26] run on SPSS software. Significant differences between the LC_{50} were obtained when there was no overlapping on 95% confidence limits between treatments [27]. Mortality data was first corrected using Abbott's formula before further analysis was carried out [28]. Analysis of variance (ANOVA) was done to determine the differences in mean mortality (%) of *S. dichotomus* among the type of insecticides and/or concentrations and/or sexes but the data was first normalized using square-root of arcsine before analysis. If ANOVA results were significant, Fisher Protected Least Significant Different (LSD) ($P<0.05$) were used to separate the means [29]. ANOVA and LSD analysis were run using MINITAB Statistical Package, Version 15.

RESULTS

Dipping Method: The result shows that there was a significant ($P<0.05$) interaction between effect of insecticides and concentrations of insecticide on adult *S. dichotomus* mortality percentage (Fig. 1; Table 1). However, there were no significant interactions between sexes and concentrations or between insecticides in causing mortality on the predator (Table 1). There was a significant difference in mortality percentage of adult *S. dichotomus* affected by the concentrations ($P< 0.05$) or type of insecticides ($P<0.05$) alone. The mortality percentage was significantly higher when treated with trichlorfon (66 and 76%) than cypermethrin (36 and 57%) and deltamethrin (33 and 52%) at 0.05 and 0.10% concentrations (Fig. 1).

The probit statistic estimates of LC_{50} and their 95% confidence limits (CL) for dipping method are presented in Table 2. The LC_{50} values of cypermethrin and trichlorfon for males were significantly lower than females at 24 h post treatment. In contrast, direct dipping in deltamethrin resulted in significant differences in LC_{50} values between male and female of *S. dichotomus* at 72 h post treatment. Generally, the LC_{50} values of insecticides for male were lower than females. In this study, the LC_{50} value of trichlorfon (LC_{50}= 0.017) for male *S. dichotomus* was significantly lower than LC_{50} values of *S. dichotomus* treated with cypermethrin (LC_{50}= 0.057) and with deltamethrin (LC_{50}= 0.066) at 72 h post treatment. Similarly, the LC_{50} value of females exposed to trichlorfon by dipping method was significantly different from those of other insecticides at 72 h post treatment.

For third nymphal instar, there was a significant interaction between effect of concentrations and insecticides on the *S. dichotomus* mortality ($P<0.05$) (Fig. 2; Table 1). Besides that, different concentrations ($P<0.05$) or type of insecticides ($P<0.05$) alone also gave significantly different effect on mortality percentage on the third instar predator (Table 1). Trichlorfon caused a significantly higher (31 and 73 %) mortality on third instar *S. dichotomus* than when they were treated with cypermethrin (8 and 36%) and deltamethrin (4 and 31%) at concentrations of 0.002 and 0.02 % (Fig. 2). Similar to adults, the LC_{50} values of third instar directly dipped in trichlorfon were significantly lower than cypermethrin and deltametrin at 48 and 72h after treatment (Table 2).

Comparison of LC_{50} values between the third nymphal instar and adult of *S. dichotomus* show that, the LC_{50} values of third nymphal instar treated with cypermethrin (LC_{50}= 0.058) and deltamethrin (LC_{50}= 0.065) were significantly lower than male (LC_{50}= 0.157, cypermethrin;

Table 1: ANOVA statistics for the effect of insecticides on *Sycanus dichotomus* mortality percentage.

Bioassay	Sources	df	Sum of square	F-value	P-value
Dipping Adult	Sex	1	1653.90	1.65	>0.05
	Concentrations	5	229741.00	269.82	<0.05
	Insecticides	2	6285.30	18.45	<0.05
	Sexes x Concentrations	5	1802.70	2.12	>0.05
	Sexes x Insecticides	2	209.00	0.61	>0.05
	Concentrations x Insecticides	10	4309.80	2.53	<0.05
	Sexes x Concentrations x Insecticides	10	745.80	0.44	>0.05
	Error	252	42914.10		
Third nymphal Instar	Concentrations	3	80300.7	187.87	<0.05
	Insecticides	2	4145.3	14.55	<0.05
	Concentrations x Insecticides	6	3884.4	4.54	<0.05
	Error	84	11968.0		
Contact Adult	Sex	1	118.20	1.19	>0.05
	Concentrations	6	330141.10	555.20	<0.05
	Insecticides	2	13314.30	67.17	<0.05
	Sexes x Concentrations	6	1439.00	1.24	>0.05
	Sexes x Insecticides	2	575.60	2.90	>0.05
	Concentrations x Insecticides	12	16707.80	14.05	<0.05
	Sexes x Concentrations x Insecticides	12	2661.90	2.24	<0.05
	Error	294	29137.20		

Table 2: LC$_{50}$ of the male, female and third nymphal instar of *Sycanus dichotomus* exposed to insecticides in dipping method.

		Hour after treatment		
Insecticides		24	48	72
Cypermethrin Female	LC$_{50}$(n)	0.302(48)	0.127(48)	0.088(48)
	95% CL	0.249-0.369	0.068-0.199	0.066-0.111
	Slope ± SE	2.316±0.253	2.015±0.237	2.052±0.261
	X^2(df)	4.711(3)	4.782(3)	3.313(3)
Male	LC$_{50}$(n)	0.157(48)	0.073(48)	0.057(48)
	95% CL	0.116-0.205	0.022-0.124	0.039-0.075
	Slope ± SE	1.500±0.203	1.911±0.267	2.100±0.318
	X^2(df)	3.471(3)	5.587(3)	1.821(3)
Third nymphal instar	LC$_{50}$(n)	0.058	0.047	0.033
	95% CL	0.037-0.097	0.028-0.088	0.021-0.055
	Slope ± SE	1.310±0.193	1.038±0.162	1.186±0.169
	X^2(df)	0.198(1)	0.095(1)	0.197(1)
Deltamethrin Female	LC$_{50}$(n)	0.305(48)	0.141(48)	0.108(48)
	95% CL	0.179-0.568	0.110-0.176	0.088-0.131
	Slope ± SE	2.017±0.233	1.952±0.227	2.553±0.295
	X^2(df)	6.050(3)	3.251(3)	1.578(3)
Male	LC$_{50}$(n)	0.220(48)	0.112(48)	0.066(48)
	95% CL	0.117-0.398	0.080-0.146	0.045-0.084
	Slope ± SE	1.885±0.221	1.590±0.216	1.929±0.276
	X^2(df)	6.204(3)	3.566(3)	1.728(3)
Third nymphal instar	LC$_{50}$(n)	0.065	0.060	0.042
	95% CL	0.041-0.110	0.037-0.106	0.027-0.067
	Slope ± SE	1.288±0.194	1.168±0.178	1.372±0.190
	X^2(df)	0.118(1)	0.002(1)	0.156(1)
Trichlorfon Female	LC$_{50}$(n)	0.244(48)	0.075(48)	0.035(48)
	95% CL	0.185-0.324	0.014-0.142	0.015-0.055
	Slope ± SE	1.459±0.200	1.547±0.228	1.449±0.265
	X^2(df)	3.745(3)	5.901(3)	1.535(3)

Table 2: Continue

		Hour after treatment		
Insecticides		24	48	72
Male	LC_{50}(n)	0.141(48)	0.069(48)	0.017(48)
	95% CL	0.109-0.178	0.045-0.094	0.002-0.034
	Slope ± SE	1.827±0.222	1.615±0.237	1.020±0.242
	X^2(df)	3.463(3)	2.873(3)	1.715(3)
Third nymphal instar	LC_{50}(n)	0.040	0.015	0.006
	95% CL	0.022-0.078	0.008-0.027	0.003-0.010
	Slope ± SE	0.924±0.153	0.892±0.149	0.958±0.160
	X^2(df)	0.224(1)	0.115(1)	0.421(1)

Table 3: LC_{50} of male and female of *Sycanus dichotomus* exposed to insecticides in contact method.

		Hour after treatment		
Insecticides		24	48	72
Cypermethrin	LC_{50}(n)	2.094(48)	1.026(48)	0.593(48)
Female	95% CL	1.844-2.484	0.913-1.139	0.522-0.668
	Slope ± SE	3.506±0.512	4.377±0.438	4.453±0.432
	X^2(df)	4.824(4)	2.749(4)	2.770(4)
Male	LC_{50}(n)	1.386(48)	0.672(48)	0.580(48)
	95% CL	1.076-1.777	0.473-0.884	0.388-0.778
	Slope ± SE	3.690±0.422	3.158±0.303	3.194±0.314
	X^2(df)	7.967(4)	8.219(4)	9.033(4)
Trichlorfon	LC_{50}(n)	1.425(48)	0.448(48)	0.330(48)
Female	95% CL	1.168-1.801	0.365-0.529	0.253-0.402
	Slope ± SE	1.810±0.259	2.830±0.303	2.694±0.331
	X^2(df)	2.199(4)	5.206(4)	2.578(4)
Male	LC_{50}(n)	0.831(48)	0.399(48)	0.308(48)
	95% CL	0.655-1.025	0.342-0.457	0.245-0.365
	Slope ± SE	1.698±0.236	4.022±0.470	3.333±0.443
	X^2(df)	3.504(4)	1.480(4)	2.812(4)
Deltamethrin	LC_{50}(n)	2.438(48)	1.129(48)	0.686(48)
Female	95% CL	2.167-2.951	0.917-1.400	0.570-0.917
	Slope ± SE	4.485±0.785	1.753±0.245	3.187±0.307
	X^2(df)	3.162(4)	5.296(4)	9.365(4)
Male	LC_{50}(n)	1.704(48)	1.006(48)	0.620(48)
	95% CL	1.394-2.110	0.764-1.256	0.426-0.826
	Slope ± SE	5.280±0.660	4.166±0.417	3.274±0.314
	X^2(df)	8.594(4)	8.424(4)	9.117(4)

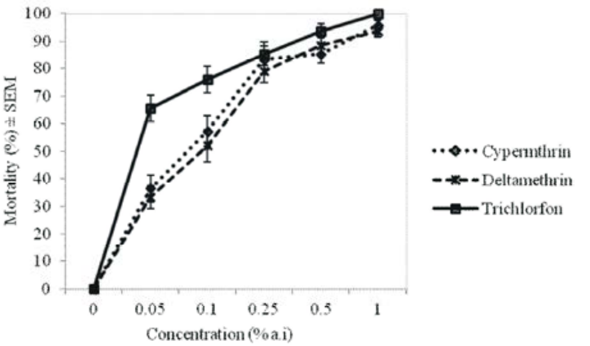

Fig 1: Effect of insecticides and its concentrations on Sycanus dichotomus adult mortality in dipping method.

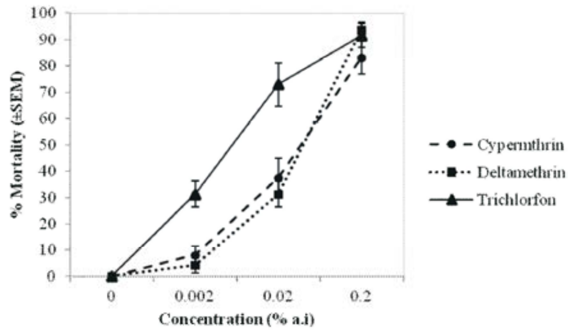

Fig 2: Effect of insecticides and its concentrations on Sycanus dichotomus third nymphal instar mortality in dipping method.

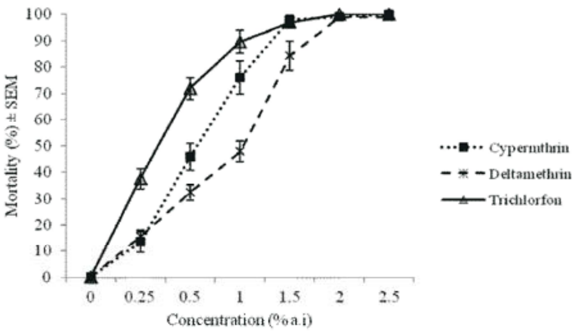

Fig 3: Effect of insecticides and its concentrations on Sycanus dichotomus adult mortality in contact method.

LC_{50}=0.220; deltamethrin) and female (LC_{50}=0.302, cypermethrin; LC_{50}=0.305, deltamethrin) at 24 h post treatment. Whereas in trichlorfon treatment, the LC_{50} values of third nymphal instar were significantly lower than LC_{50} values of both male and female at 24 and 72 h post treatment (Table 2).

Contact Method: There was a significant interaction between concentrations and insecticides ($P<0.05$) and between sexes, concentrations and insecticides ($P < 0.05$) in causing mortality of adult (Fig. 3; Table 1). However, mortality percentage of adult was not significantly affected by the interaction between sexes and insecticides or between concentrations. Insecticides ($P<0.05$) or concentrations of each insecticide ($P<0.05$) alone was found to have significant effect to the mortality percentage of adult (Table 1). As for the direct dipping result, *S. dichotomus* treated with trichlorfon in the contact method also showed significantly higher mortality percentage than pyrethroids at 0.25-1.5% concentrations (Fig. 3).

The LC_{50} values for females seem to be more tolerant to all insecticides than male. Over all, the LC_{50} values for all insecticides for females were significantly different from those of male at 24 h post treatment. Like the dipping method, the contact method also showed a significant difference in LC_{50} values across insecticides. Irrespective of sexes and times, trichlorfon was still more toxic to both male and female insects, with LC_{50} values were significantly different from those of cypermethrin and deltamethrin (Table 3).

DISCUSSION

In this study, the females of *S. dichotomus* were more tolerant to insecticides than males (Tables 1-3). This could be due to *S. dichotomus* female being bigger than male insects. Tillman [30] reported that the different level of susceptibility between the sexes could be due to different sizes and weight of the insect. The female of America cockroach (*Periplaneta americana* L. Blattaria: Blattidae) has been reported to be bigger than the male due to a higher lipids body contain in female, thus reducing the toxicity effect of insecticide [31,32]. Terriere [33] stated that, lipids body is one of the major organs involved in the production of detoxification enzymes that lead to an increase tolerance to insecticide. However, male insects have not always been found to be more susceptible to insecticides than females [34]. Xu *et al.* [35] have reported no significant differences in susceptibility to permethrin of male and female wasps (*Diadegma insulare* Cresson, Hymenoptera: Ichneumonidae) although males were smaller than females similar that of *S. dichotomus*.

Pyrethroids are considered to be the most toxic insecticides to many beneficial arthropods [36]. However, our results clearly demonstrated that *S. dichotomus* adults were less susceptible to the pyrethroid insecticides (cypermethrin and deltamethrin) than organophosphate (trichlorfon) (Figs. 1-3; Table 1-3). Our results are in agreement with the report by Grundy *et al.* [37] that the carbamates (C) (carbaryl and endosulfan) and the pyrethroids (P) (deltamethrin and esfenvalerate) were significantly less toxic than the organophosphates (OPs) (chlorpyrifos and monocrotophos) and left a lower harmful residual to *Pristhesancus plagipennis* Walker (Hemiptera: Reduviidae), a species closely related to *S. dichotomus*. The exact reason for this lower susceptibility of natural enemies, especially insect predators, to pyrethroid are not well understood [38], but it is possibly due to the insects having built up preadapting detoxicifying enzyme systems that eventually lead to development of insecticide resistance [39,40].

Furthermore, the study also shows that nymphs of the predator were highly susceptible to all of the insecticides (cypermethrin, deltamethrin and dipterex) compared to adults (Table 2). This result corroborates the findings of Pereira *et al.* [38], who reported that gamma-cyhalothrin (P) is more toxic against *Podisus nigripinus* Dallas (Hemiptera: Pentatomidae) in the nymph than in the adult stage, perhaps due to thicker cuticle of adults compared to that of nymphs. Brown [41] and Curkovic *et al.* [42] reported that some insects, particularly adults, are protected against the entry of insecticides by thick and sclerotized cuticles and that larvae and nymphs become progressively less permeable as their cuticle thickens. Pereira *et al.* [38] stated that a thicker and more rigid cuticle will slow the rate of penetration of lipophilic insecticides into the cuticle.

In this study, third nymphal instar was chosen as a subject of the investigation because it is the middle instar of *S. dichotomus* nymphal stage and their response to insecticide treatment was expected to be different from the final instar (fifth instar) of *S. dichotomus*, which the final instar may tend to have the same or only slightly different susceptibility when compared to adult *S. dichotomus*. In contrast, the first nymphal instar may have high mortality rate when exposed to similar insecticides. However, further studies on the remaining instars are still needed to investigate the effects of insecticide use in an integrated pest management program of oil palm that integrates *S. dichotomus*.

As a conclusion, our study showed that trichlorfon is highly toxic to *S. dichotomus* compared to pyrethroid insecticides regardless of the method used. However, more laboratory studies are required as the toxicity of insecticides may also be affected by age of the insect, pre-treatment starvation and temperature. Field study also needs to be carried out in order to see the effect of insecticides under the actual environment. Based on the results of this study, it is crucial to have suitable insecticides that are not only effective in controlling the pest population but also safe for the nature enemies.

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