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Susceptibility of Malaysian Tropical Bed Bug *Cimex hemipterus* F. (Hemiptera: Cimicidae) Populations to Deltamethrin and Propoxur Insecticides

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Highlights

- Several field-collected tropical bed bug populations shown to be resistance to pyrethroid-based insecticides.
- Avoid any pyrethroid-based insecticides for controlling tropical bed bug infestation.
- Carbamate-based insecticides can be an alternative insecticide for controlling tropical bed bug population that shown resistance to insecticide.

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Susceptibility of Malaysian Tropical Bed Bug *Cimex hemipterus* (F.) (Hemiptera: Cimicidae) Populations to Deltamethrin and Propoxur Insecticides

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Abstrak: Pepijat telah dikumpul dari seluruh Semenanjung Malaysia untuk melihat tahap kerintangan terhadap dua kelas racun serangga, piretroid dan karbamat. Dalam kajian ini, deltamethrin dan propoxur yang tersedia secara komersial digunakan untuk menguji status kerintangan pepijat tropika*, Cimex hemipterus* di Malaysia. Status kerintangan mereka dinilai dengan menggunakan kertas yang diresapi racun serangga, suatu kaedah bioesei yang disyorkan oleh Pertubuhan Kesihatan Sedunia (WHO). Sepuluh pepijat dewasa dalam setiap populasi diletakkan di dalam setiap tiub dengan kertas yang diresapi 0.05% deltamethrin dan 0.1% propoxur. Pada mulanya, pepijat terdedah selama 24 jam, kemudian tempoh pendedahan dilanjutkan hingga 14 hari. Bioesei dilakukan dalam triplikat. Tahap kerintangan tertinggi dipamerkan oleh populasi AR dengan nilai masa maut (LT_{50}) sebanyak 466.119 j di dalam tiub yang mengandungi deltametrin. Sebaliknya, propoxur sangat berkesan terhadap populasi IP (Ipoh) kerana nilai masa maut yang paling rendah, (LT_{50} = 153.032 j). Perbezaan yang signifikan ($p < 0.005$) didapati antara kelas racun serangga dan populasi pepijat. Purata kematian di kalangan populasi AR, SW, HM, PY, dan PC menunjukkan perbezaan yang signifikan apabila terdedah kepada deltametrin. Sebagai kesimpulan, propoxur menunjukkan keberkesanan yang tinggi dalam mengawal populasi pepijat. Walau bagaimanapun, keberkesanannya hanya di tempat tertentu. Sementara itu, rawatan menggunakan deltamethrin harus dielakkan oleh profesional kawalan perosak kerana keberkesanannya yang rendah.

Kata kunci: *Cimex hemipterus*, Kerintangan Racun Serangga, Masa Maut, Kerentanan, Malaysia

Abstract: Bed bugs were collected across Peninsular Malaysia to investigate their resistance to two types of insecticide classes, pyrethroid and carbamate. In this study, commercially-available deltamethrin and propoxur were used to test the resistance status

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of tropical bed bug, *Cimex hemipterus* in Malaysia. Their resistance status was evaluated using insecticide-impregnated papers, a bioassay method recommended by the World Health Organization (WHO). Ten adult bed bugs in each population were placed inside the tubes with papers impregnated with 0.05% deltamethrin and 0.1% propoxur. At first, the bugs were exposed for 24 h, then, the exposure period was extended to 14 days. The bioassay was performed in triplicates. The highest resistance level was exhibited by the AR population with a lethal time value (LT_{50}) of 466.119 h in the deltamethrin-containing tube. In contrast, propoxur was highly effective against the IP population due to its lethal time value (LT_{50} = 153.032 h) was the lowest. A significant difference ($p < 0.005$) was found between insecticide classes and bed bug populations. Mean mortality among AR, SW, HM, PY and PC populations showed a significant difference when exposed to deltamethrin. In conclusion, propoxur showed high efficacy in controlling bed bug populations. However, its efficacy was only at particular places. Meanwhile, a treatment using deltamethrin should be avoided by pest control professionals due to its low efficacy.

Keywords: *Cimex hemipterus*, insecticide resistance, lethal time, susceptibility, Malaysia

INTRODUCTION

Over the last two decades, the resurgence of bed bugs has become one of the primary problems in bed bug control strategy worldwide. Generally, the elimination of the bed bug infestations involves the use of chemical insecticides. However, a continual application of chemical insecticides has resulted in bed bug resistance (Dang *et al.* 2017) and have created widespread worries among pest control professionals. To make matter worse, a cryptic and nocturnal lifestyle of the bed bugs further complicate the elimination strategy (Reinhardt & Siva-Jothy 2007). Numerous studies have demonstrated that the evolution of insecticide resistance likely plays a major role in the resurgence of these blood-sucking insects (Reinhardt & Siva-Jothy 2007; Dang *et al.* 2017).

The main reason linked to this matter is that the evolution of their body systems, causing multiple resistance mechanisms to rapidly develop against pesticides applied (Adelman *et al.* 2011; Bai *et al.* 2011). After DDT was banned for its persistence and negative environmental impact (Carson 1963), other classes of insecticides with reduced susceptibilities such as carbamate, phenylpyrazole, neonicotinoid, organothiophosphate and pyrrole were tested for their toxicity in controlling *Cimex lectularius* colonies in poultry facilities (Steelman *et al.* 2008). In Australia, bed bug populations showed lower mortality rate for most insecticide classes tested, except imidacloprid and pirimiphos-methyl (Lilly *et al.* 2015).

To date, a majority of pest control companies prefer to use synthetic pyrethroids as they are known to effectively kill insects such as mosquitoes, flies, head louse and bed bugs (Zhu *et al*. 2016). However, evolved resistance to the pyrethroids by insects including bed bugs has led to difficulties and posed more challenges in suppressing their infestation. A report by Zhu *et al.* (2010) proved that common bed bugs in the USA showed no response towards deltamethrin are associated with kdr-type resistance triggered by two major amino acids i.e. V419L

and L925I. Field-collected strains in infested homes in Denmark also reported the low efficacy of permethrin and deltamethrin, while the alternative insecticide, chlorpyrifos resulted in higher mortality percentage (Kilpinen *et al.* 2011).

Two knockdown resistance (kdr) mutations associated with resistance to pyrethroids have been recently detected in *C. hemipterus* (Dang *et al*. 2015). These kdr mutations affect the efficacy of pyrethroid, a class of insecticide that attack a voltage-gated sodium channel (VGSC) in the nervous system. An alteration of sequences of binding sites in the sodium channel counteracts the mode of action of pyrethroid and DDT, resulting in the insensitivity to both classes of insecticides (Soderlund & Knipple 2003). The kdr mutations have also been detected in several hematophagous insects such as mosquitoes, *Anopheles gambiae, Culex pipiens,* cat flea, *Ctenocephalides felis* and *C. hemipterus* (Bass *et al*. 2004; Dang *et al*. 2015).

In general, *C. hemipterus* is more susceptible than *C. lectularius*. However, some of them possess a higher tolerance level towards insecticides which can be passed on to their progeny (Busvine 1958). Pest control companies in Southeast Asia rely mostly on chemical and spray methods as treatments (Wang *et al*. 2011; Ab Majid & Zahran 2015a; 2015b). However, due to resistant factors, the companies hardly reduce pest populations despite various insecticides are readily available in the market. Pyrethroid, a class of insecticide which has been widely used as a control agent was reported to demonstrate a little or no effect on bed bugs and other insect vectors (Wood *et al.* 2010; Miller 2014). Bed bugs may also develop their resistance to other classes of insecticides with similar active ingredients if no alternative control measure is immediately sought to avoid the circumstance (Moore & Miller 2006).

Numerous findings related to chemical and non-chemical methods that resulted in effective control of bed bugs have been reported by many. These include the application of diatomaceous earth and spraying of insecticides such as chlorfenapyr, propoxur, and other classes (Wang *et al.* 2009, Manuel 2010). Unlike termites, bed bugs lack grooming activity to affect other members in a population. Nevertheless, horizontal transfer through contact and ingestion of insecticides might occur among bed bugs (Akhtar & Isman 2013). A few studies found that simultaneous practices of two control methods such as a combination of heating and chemical treatment can effectively eliminate resistant populations compared to a single method; either by spraying or dust application (Wang *et al*. 2009, Akhtar & Isman 2013). Tropical bed bugs sampled in Bangkok, Phuket and Krabi of Thailand have developed resistance to several insecticide classes i.e. organochlorines, carbamates, and pyrethroids but noted a higher mortality when treated with imidacloprid and chlorfenapyr (Tawatsin *et al.* 2011).

Multiple resistance mechanisms have been identified in bed bugs including penetration resistance through cuticle thickening or remodeling, metabolic resistance by increasing the activities of detoxification enzymes (e.g. cytochrome P450 monooxygenases and esterases), and knockdown resistance by kdr mutations (Dang *et al.* 2017). Other potential resistance mechanisms such as behavioural and physiological changes (e.g. increasing esterase activities

by point mutations, glutathione S-transferase, target site insensitivity including altered AChEs, GABA receptor insensitivity, and altered nAChRs), and symbiont-mediated mechanisms are yet to be discovered. It is also worthwhile to mention that research addressing the mechanisms of insecticide resistance in the tropical bed bug, *C. hemipterus* is generally lacking although the mechanisms are likely similar to the temperate species, *C. lectularius*.

In Malaysia, the tropical bed bugs have been reported exhibiting a resistance to chlorinated hydrocarbons, particularly DDT, HCH, d-allethrin, and dieldrin group (Dang *et al*. 2017). However, the resistance status of the Malaysian bed bugs to other classes of insecticides such as pyrethroid and carbamate is currently unknown. Therefore, this study was conducted to evaluate the resistance status of *C. hemipterus* collected across Peninsular Malaysia to two major classes of insecticides i.e. pyrethroid (deltamethrin) and carbamate (propoxur). The efficacy of both insecticides on the bed bugs was also determined.

MATERIALS AND METHODS

Bed Bugs Rearing and Population Selection

Bed bugs were acquired from the sample collection across Peninsular Malaysia. They were maintained, reared under room conditions (25 ± 2C, 50%–60% of relative humidity) and were fed weekly on expired human blood supplied by Penang General Hospital. Modifications of artificial feeding system were set up by stretching parafilm membrane on a glass jar filled with the expired blood based on the feeding maintenance of bed bugs by Montes *et al.* (2002) and Abd Rahim *et al.* (2015). Ten populations were selected based on the resistance status of recent bed bugs control reports and feedback from questionnaires given to the pest control companies in Malaysia (Ab Majid & Zahran 2015a). They were represented by each cluster obtained from the previous sampling. Bed bugs were separated and maintained according to field and laboratory strains. F1, F2 and F3 generations were labeled and kept in different containers.

Insecticide Preparation

Two insecticides i.e. deltamethrin and propoxur were used to evaluate the resistance of bed bugs using insecticide-impregnated papers from the World Health Organization (WHO) test kit (Busvine 1958). The papers (12 cm x 15 cm) were impregnated with 0.1% propoxur and 0.05% deltamethrin. Impregnated papers then were cut into smaller pieces (3 x 5 cm), rolled and inserted into glass test tubes of 12.5 cm x 1.5 cm. When the bugs were exposed to the treated papers, the tubes were covered with fine net cloth to prevent them from escaping. All tubes were placed in a container at room condition (temperature, 25 ± 2ºC, relative humidity, 50%–60%).

Resistance Bioassay

The resistance bioassay was conducted following methods by Busvine (1958) and Tawatsin *et al.* (2011). Only adult bed bugs from the F2 generation were used in this bioassay. Each bioassay required 40 bed bugs from each population. The selected populations are tabulated in Table 1. Ten adults from each population were tested by introducing them into the test tube containing 0.05% deltamethrin-impregnated papers - or 0.1% propoxur-impregnated papers. The concentrations of insecticides and exposure times were as recommended by WHO (Busvine & Nash 1953; Tawatsin *et al*. 2011; DeVries *et al*. 2015) and based on the previously established baseline data (unpublished data). Each treatment was done in triplicates with one control. Filter papers were impregnated with standard oil solutions of insecticides incorporated with the insecticide resistance kit provided by the WHO (Busvine & Nash 1953; Tawatsin *et al.* 2011). Bed bugs exposed to papers impregnated with silicone oil (deltamethrin treatment), and risella oil (propoxur treatment) were used as controls. Bed bugs were fed on expired human blood for 24 h prior to experimental procedures. The bed bugs were exposed for 24 h for preliminary testing. Observations on their mortality were continued and recorded for 14 days. They were starved during the continuous bioassay. Mortality was scored based on their posture and movement in the tubes. Dead bugs were in an upside-down position with no signs of movement after 10 sec of observation. Survived bed bugs were kept in 70% alcohol for further observation of resistance in the bugs. Control groups exceeding 10% of mortality were corrected using Abbott's formula (Abbott 1925).

Table 1: Strains of bed bugs in the resistance bioassay.

Location and strain/ population	Cluster	Date collected	Total bed bugs in both treatments	Resistance status
Arau, Perlis (AR)	1	24 September 2014	80	Unknown
Ipoh, Perak (IP)	1	17 October 2014	80	Highly resistant
Teluk Intan, Perak (TI)	1	18 October 2014	80	Resistant
Hutan Melintang, Perak (HM)	1	18 October 2014	80	Resistant
Sg. Petani, Kedah (PY)	1	26 September 2014	80	Resistant
Langkawi, Kedah (PC)	1	27 October 2014	80	Resistant
Kuala Terengganu, Terengganu (KT)	2	20 August 2014	80	Unknown
Klang, Selangor (KG)	3	25 November 2014	80	Resistant
Port Dickson, Negeri Sembilan (PD)	4	29 November 2014	80	Unknown
Senawang, Negeri Sembilan (SW)	4	29 November 2014	80	Unknown

*Status on field strain/population were in accordance via questionnaires by professionals and local residents.

Statistical Analysis

Percentage of mortality in each treatment was calculated and analysed daily at 24 h interval. Probit analysis was used in determining lethal times (hours) of the bugs at LT_{50}, LT_{90}, and LT_{99} with a confidence limit of 10%–90%. Evaluations of lethal times were made by using software SPSS Version 22.0 (IBM Corp., Armonk, NY, USA). Mean comparison was performed using one-way and two-way analysis of variance (ANOVA) to determine the significant difference between the populations and the effect of insecticide on bed bug populations.

RESULTS

Based on the results, tropical bed bugs were less susceptible against deltamethrin compared to propoxur (Fig. 1 and Fig. 2). From the first until the third day of application, all populations showed low mortality within the range of 3%–20% in the deltamethrin treatment. Among the tested populations, the KT and HM exhibited 20% higher in mortality after one week of exposure. The PC population recorded the highest mortality (>70%) on day 10 compared to the other populations. On day 14, the AR population exhibited the lowest mortality (40%). The other populations exhibited a moderate to high mortality; ranging from 60% to 80%. Two populations, PC and SW had the highest mortality rate (90%), further indicating that both populations were highly susceptible towards deltamethrin. They were, however, still considered as resistant to this insecticide. Survived bed bugs might have developed resistance in them due to the previous treatment of different insecticides by pest control operators.

Meanwhile, propoxur showed high efficacy in killing bed bugs as it caused an overall mortality of 90% throughout the treatment period (Fig. 1). Only one population i.e. TI noted high mortality on the first day of exposure i.e. 20% and increased to 37% after day 3. Nine other populations displayed a low mortality rate until the third day of exposure, but the mortality increased to nearly 50% after day 7. On the 10th day, all populations reached 80%–90% mortality except for two populations (KT and HM), where the mortality rates were 60% and 67%, respectively. Three populations namely IP, PY, and PC noted 100% mortality after the 14th day of the treatment. The lowest mortality was observed in the KT population with 87%, while ~ 90% were recorded for the other seven populations.

Lethal time (in hours) was performed to determine the efficacy of insecticides against bed bugs (Table 2). The results showed that the efficacy of each insecticide class was different in each population of bed bugs. A few populations had high LT_{50} values which can be linked to the resistance factor. For deltamethrin, the highest LT_{50} value was detected in the AR population with 466.119 h. However, three populations i.e. KG, HM, and PY had low LT_{50} values with 207.201 h, 203.391 h, and 200.593 h, respectively. The PC population exhibited the lowest lethal value (164.068 h), indicating it as the most susceptible population to deltamethrin.

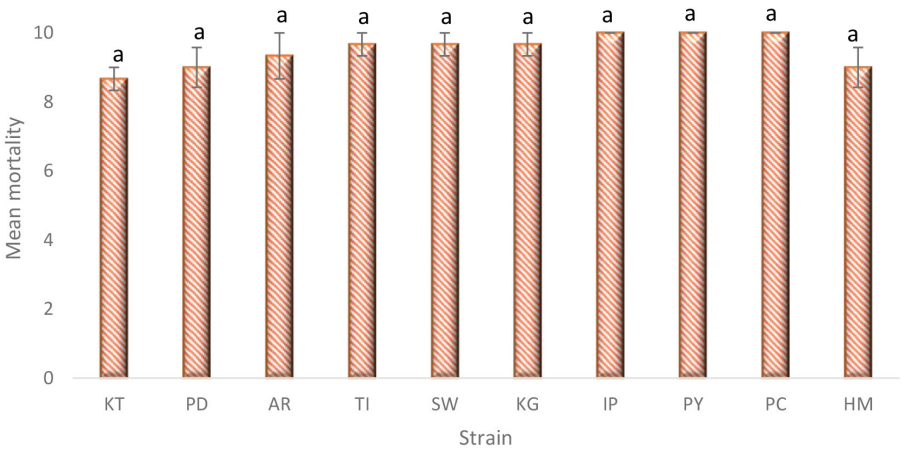

Figure 1: Effect of propoxur on strains of bed bugs. Bars of same letters had no significant difference.

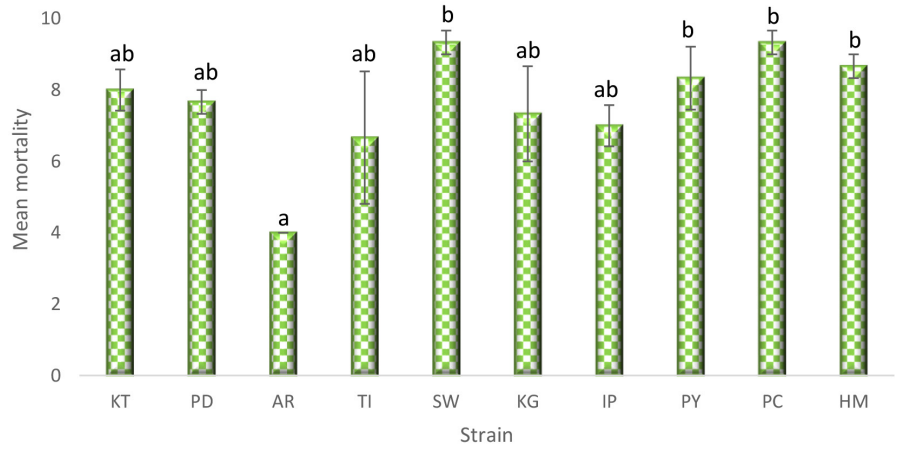

Figure 2: Effect of deltamethrin on strains of bed bugs. Bars of same letters had no significant difference.

Trends of LT_{50} (in hours) over time for all populations are presented in Table 2. Most of the tested populations showed high susceptibility to propoxur after eight days of exposure. Approximately 153.032 h was required to kill 50% of the IP population, exhibiting the lowest resistance compared to others. Another population exhibited low resistance was the TI with 158.754 h. The remaining eight populations showed increased lethal time values but were still considered to have low resistance since up to 30%–53% of bed bugs were killed within 7–8 days of exposure periods. The KT population, on the other hand, had the highest LT_{50} with 203.334 h, exhibiting the lowest percentage of mortality.

Table 2: Comparison of lethal hours of two insecticides on ten populations of bed bugs.

Insecticide group	Strain	Slope ± SE	LT_{50} (hours)	LT_{90} (hours)	LT_{99} (hours)
Deltamethrin (Pyrethroid)	TI	3.530 ± 1.431	284.804 (240.644–328.166)	463.274 (379.422–947.871)	688.802 (492.113–2515.216)
	IP	2.320 ± 0.954	260.793 (232.406–289.819)	468.215 (393.439–664.890)	754.473 (563.208–1403.948)
	SW	3.354 ± 1.383	237.864 (216.162–254.595)	343.034 (314.216–399.939)	462.348 (397.345–620.013)
	AR	5.924 ± 2.377	466.119*	933.355*	1643.948*
	PD	4.114 ± 1.671	264.429 (239.107–283.148)	376.335 (342.284–455.285)	501.796 (425.178–723.230)
	PY	3.248 ± 1.293	200.593 (95.664–249.387)	400.629 (342.782–589.981)	704.157 (514.451–2245.255)
	KG	2.136 ± 0.882	207.201 (170.238–233.596)	393.510 (339.133–522.291)	663.832 (505.724–1183.800)
	KT	2.108 ± 0.860	247.848 (173.481–307.909)	549.310 (404.198–1650.044)	1050.986 (619.948–8425.492)
	HM	1.862 ± 0.777	203.391 (173.900–227.031)	385.057 (332.895–496.535)	647.902 (501.074–1060.090)
	PC	1.901 ± 0.798	164.068 (120.478–193.927)	301.371 (259.628–382.042)	494.759 (388.286–830.287)
Propoxur (Carbamate)	TI	2.492 ± 1.048	158.754 (116.636–186.392)	280.187 (245.834–340.010)	445.237 (360.467–695.171)
	IP	1.644 ± 0.735	153.032 (143.223–162.228)	224.445 (209.445–245.345)	306.698 (275.686–356.010)
	SW	2.684 ± 1.119	199.483 (172.598–219.028)	307.926 (280.695–354.980)	438.687 (375.006–585.523)
	AR	2.557 ± 1.075	191.670 (159.364–213.146)	290.600 (261.759–347.293)	407.987 (342.834–591.645)
	PD	3.697 ± 1.485	177.739 (69.581–226.661)	334.460 (284.997–438.197)	559.993 (431.029–1565.418)
	PY	3.266 ± 1.408	168.238 (142.984–184.959)	231.404 (212.569–260.921)	300.083 (265.020-382.786)
	KG	3.597 ± 1.505	195.850 (171.461–212.711)	269.887 (251.511–296.361)	350.520 (314.812–424.036)
	KT	1.677 ± 0.681	203.334 (133.444–251.892)	591.646 (441.068–1236.819)	1413.259 (817.121–6474.890)
	HM	1.672 ± 0.672	178.756 (139.948–207.008)	376.324 (324.764–481.492)	690.455 (525.655–1175.958)
	PC	4.465 ± 1.895	190.135 (168.293–204.621)	250.897 (234.988–275.576)	314.544 (284.094–381.439)

*No time interval shown

A two-way analysis of variance (ANOVA) was performed to determine the effect of insecticide groups on different populations of bed bugs. Based on the results, there was a significant interaction ($F (9, 40) = 2.741$ at $p < 0.005$) between insecticide groups and different populations of bed bugs. Comparison between two main active ingredients revealed that propoxur was highly effective ($p < 0.0001$) than deltamethrin in controlling bed bugs. Mean values of mortality in bed bugs for propoxur and deltamethrin were 9.5 and 7.6, respectively. Interactions between bed bug populations, however, had different results depending on which insecticides they were treated.

Therefore, one-way ANOVA was used to investigate the differences in bed bugs populations treated with propoxur and deltamethrin. As shown in Fig. 1 and Table 2, exposure to propoxur exhibited no significant difference among the populations ($F (9, 20) = 1.468$, $p > 0.05$) while, populations treated with deltamethrin showed a significant difference among the populations ($F (9, 20) = 3.453$, $p < 0.05$). A significant interaction of mean mortality was found between populations of AR, SW, HM, PY, and PC (Fig. 2 and Table 2).

DISCUSSION

To date, pyrethroid-based insecticides are widely used in Malaysia to control bed bug infestations in various premises (Ab Majid & Zahran 2015a). Failure in chemical control is the major factor of resistance development following repetitive exposure of similar insecticides to bed bugs. As such, a bioassay was used to assess the effect of two insecticides against populations of bed bugs in Peninsular Malaysia. The method is relatively simple to perform and provides standardized data in monitoring insecticide resistance (Dang *et al*. 2017). Based on the results, deltamethrin was less effective in controlling the populations. Similar results were obtained in the tropical bed bug populations in Sri Lanka where high KT_{50} values were observed, ranging from 0.5 h to 24 h and from 2.5 h to 47 h after being exposed to permethrin and deltamethrin for 24 h (Karunaratne *et al.* 2007). Low doses of bifenthrin and alpha-cypermethrin were used to treat bed bugs in Thailand had caused high effective dose (ED_{50}) values compared to other urban pests (Suwannayod *et al.* 2010). Bed bugs might have their systems triggered by these pyrethroid-based insecticides, leading to developed resistance in them (Karunaratne *et al.* 2007).

While *C. hemipterus* showed resistance towards pyrethroid-based insecticides, *C. lectularius* have already developed theirs in many European countries (Romero *et al.* 2007; Goddard 2013; Lilly *et al.* 2015). Rarely reports on tropical bed bugs are published especially on their susceptibility to chemical pesticides. On the other hand, topical bioassays on common bed bugs in Sydney, Australia showed that the pests were highly resistant to permethrin (1,235,000 times), deltamethrin (370,000 times) and bendiocarb (250 times) due to their high LD_{50} values compared to other insecticide groups (Lilly *et al.* 2015). They seemed

to thicken the cuticle as a defense mechanism, which was positively correlated with a knockdown time of survived bugs after 24 h forced-exposure (Lilly *et al.* 2016). Furthermore, the effect of feeding and starvation status can influence the insecticide tolerance in bed bugs. In this study, bed bugs were fed on expired human blood for 24 h prior to experimental procedures to prevent mortality due to starvation. Based on the study by Busvine (1958), the tolerance level might be compromised due to starvation which could lead to biased results. DeVries *et al*. (2015) also suggested that the blood meal had a little impact on tolerance of 9-day and 2-day starved *C. lectularious* to deltamethrin. Thus, we decided to feed the bed bugs prior to bioassay to prevent mortality due to starvation which could give biased results.

A study on the resistance levels in several populations of bed bugs in the United States has been recently reported by Romero and Anderson (2016). They detected a low lethal dose (LD_{50}) in four types of nicotinoids (imidacloprid, acetamiprid, dinotefuran, and thiamethoxam). Esterase activities have been associated with the increased resistant populations, which probably acquired from the previous exposure to other insecticide groups (Mamidala *et al.* 2011). Although many formulations are marketed extensively, newer products, however, do not entirely solve the resistant issues among bed bug populations across the world. A mixture of imidacloprid and beta-cyfluthrin in a form of concentrated suspension, Temprid SC also exhibited less efficacy against several selected strains due to the presence of pyrethroid components (Wang *et al.* 2015; Wang *et al.* 2016; Gordon *et al.* 2014). In a study by Ab Majid and Zahran (2015b), adult bed bugs were not 100% killed, although they applied Temprid at a high concentration (630 ppm). In another study, the exposure of the product on bed bugs exhibited high resistance ratio in the first nymphal stage with a value of 9.4 for Epic Center strain and 10.0 for Harlan strain (Campbell & Miller 2015).

Ashbrook *et al.* (2017) assessed susceptibility levels of 10 bed bugs populations from six different states in the USA within seven days of exposure to two types of insecticides i.e. chlorfenapyr and bifenthrin. Among these, they detected a significantly reduced susceptibility in three and five populations to chlorfenapyr and bifenthrin. They found that populations with more than 25% survivors were deemed to have reduced susceptibility to the insecticides. They also found an unexpected correlation between chlorfenapyr and bifenthrin susceptibility of the different bed bug populations although the modes of action of both insecticides are different (Ashbrook *et al*. 2017).

Based on the results, a high percentage of mortality were observed when the populations were exposed to propoxur. There was a significant interaction between mortality percentage and lethal time. It was shown that propoxur was also susceptible to resistant populations. Minimum days required for 50% of the population to die was 6.4 days, whereas 58.8 days was the maximum days required for 99% of the population to die (LT_{50} = 153.302 h, LT_{99} = 1413.259 h). In the USA, a residual bioassay on bed bugs collected in a poultry house exhibited 100% mortality when 1% propoxur was used (Goddard 2013). The bugs did not rapidly die with less than 80% of mortality was noted when exposed to other non-

propoxur products within 24 hours. In general, propoxur acts as a neurotoxin that inhibited the binding process of acetylcholine to the receptor which is also similar to the role of organophosphate insecticide in the nervous systems of insects.

However, the application of propoxur is risky as it remains intact in the atmosphere in the infested places for several weeks (Berg 2010). Apart from the pungent smell of the active ingredient, propoxur is harmful to children and pregnant mothers (Doggett *et al.* 2012). Reports from Thailand and Sri Lanka claimed that there were resistance issues in the field populations tested with propoxur (Tawatsin *et al.* 2011; Karunaratne *et al.* 2007). Observation on 24 h exposure at a concentration of 0.8% displayed that 45.5% of bed bugs survived regardless of their growth stages. This could be related to the previous enzyme detoxification on other insecticide groups which resulted in multiple cross-resistance in bed bugs. Thus, the United States Environmental Protection Agency (EPA 2016) has prohibited the use of propoxur, particularly around the children. The insecticide is, however, allowed to be used in urban areas including commercial and industrial buildings like office properties, warehouses, hotels, malls and medical centers (EPA 2016).

Diatomaceous earth dust is another option in bed bug control measures for it has similar characteristics to insecticides (Doggett *et al.* 2012; Berg 2010). The effectiveness of this non-chemical treatment in eliminating the infestation is higher since it is able to kill bed bugs at all stages whether or not they have been treated with insecticides (Berg 2010). A study by Wang *et al.* (2012), suggested that the non-chemical treatment was able to eliminate bed bugs in the apartment building rather than spraying residual insecticides which can reintroduce the colony within a short period of time. Furthermore, the suppression of bed bug populations will be more effective if the implementation of integrated control management is included in the treatment method. Heating and steaming in between 50°C-60°C along with spreading the powdered diatomaceous earth to tiny gaps could definitely reduce the number of bugs in the infested house (Puckett *et al.* 2013; Akhtar & Isman 2013). It is critical to continue a periodic monitoring and to implement a preventive control measure after successful elimination of bed bugs has been achieved to minimise the re-infestation rate and cost associated with bed bug controls (Bennet *et al*. 2016). It also requires community-wide and proactive, bed bug management programs (Romero *et al.* 2017), in order to provide a more effective, sustainable, economically viable, and affordable solutions to the housing residents and premise owners.

CONCLUSION

It can be concluded that the tropical bed bug, *C. hemipterus* was highly susceptible to propoxur. Low lethal values indicated that the insecticide was effective in killing this bug. The pyrethroid-based active ingredient, deltamethrin, however, was expected for its low efficacy towards resistant bugs. The only limitation of this study was no susceptible populations of *C. hemipterus* can be used for comparison.

A little information is available regarding tropical bed bug, *C. hemipterus* and its resistance towards insecticides (Dang *et al.* 2017). However, this study provided a preliminary reference for local pest control professionals to avoid the use of any potential resistant products to control tropical bed bug infestations in Malaysia.

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