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TROPICAL BED BUG INFESTATION DYNAMICS IN MALAYSIA: EVALUATION OF INSECTICIDE RESISTANCE LEVEL AND SUSCEPTIBILITY ON PYRETHROID AND CARBAMATE INSECTICIDES (HEMIPTERA: CIMICIDAE)

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Abstract Currently pest control industries are having difficulties in controlling bed bugs, specifically in workers' dormitories and residential houses. Survey of bed bug infestations across Peninsular Malaysia by collecting samples from residential houses, dormitories, hotels and motels and flats and apartments. The survey was carried out in Pulau Pinang, Perlis, Perak, Kedah, Terengganu, Kelantan, Pahang, Selangor, Negeri Sembilan, Melaka, Johor and federal territory of Kuala Lumpur. Highest number of collected species, *Cimex hemipterus*, resulted in Perak (24.8%) in workers' dormitories. No bed bugs were found in Kelantan. They are known to infest mattresses, cracks and crevices of walls and floors as well as cushion seats. Collected bed bugs were then colonized in the laboratory for resistance study and mortality testing on bed bugs using natural enemies of insect, fungi as an introduction to non-chemical method in managing the insect pest. In insecticide resistance bioassay, deltamethrin and pyrethroid were used against live following protocol of World Health Organization (WHO) insecticide-impregnated papers. Triplicates of ten adult bed bugs, each replicate contained impregnated papers and the insect were left exposed for 14 days. Resistance in bed bugs were detected in AR strain (LT_{50} = 466.119h) after exposed to deltamethrin insecticides. Propoxur was highly effective against IP strain due to the low lethal value, LT_{50} = 260.793h. There was a significant difference between the insecticide groups and bed bugs strains. Interaction among the strains exposed to deltamethrin also showed significant difference but not in propoxur**.**

Key words tropical bed bug, Malaysia, infestation dynamics, pyrethroid, carbamate, insecticide resistance.

INTRODUCTION

Recent issues regarding bed bugs control in major infested houses or buildings like dorms, schools, offices including public areas were yet to be solved due to their resurgence due to rapidly develop against pesticides applied (Adelman et al., 2011; Bai et al., 2011). After DDT was banned for its extensive usage, other classes of insecticides showed reduced susceptibility in carbamate, phenylpyrazole, neonicotinoid, organothiophosphate and pyrrole which were also tested for their toxicity in controlling *C. lectularius* colonies in poultry facilities (Steelman et al., 2008). In Australia, bed bugs populations showed lower mortality rate in most insecticide groups except imidacloprid and pirimiphos-methyl (Lilly et al., 2015). However, resistance issue against the pyrethroid group evolved in the insects, including bed bug populations caused difficulties and possessed more challenges to suppress the infestation level. A report by Zhu et al., (2010), proved that common bed bugs in USA involved with kdr-type resistance showed no response towards deltamethrin. Field-collected strain in infested homes in Denmark also reported low efficacy in permethrin and deltamethrin while alternative insecticide, chlorpyrifos resulted in high mortality percentage (Kilpinen et al. 2011). Meanwhile, a survey conducted among pest control companies in Malaysia reported that 25% of them found that the insect became resistant against

pyrethroid class, such as alpha-cypermethrin, deltamethrin, cyphenothrin and lambda-cyhalothrin (Abdul Hafiz and Zulaikha, 2015). Lower efficacy of chlorpyrifos also causes reinfestation to likely occur in residential houses and hotels in Kuala Lumpur. Collected tropical bed bugs in Bangkok, Phuket and Krabi of Thailand were resistant against several insecticide classes like organochlorines, carbamates and pyrethroids compared to imidacloprid and chlorfenapyr which scored higher mortality percentage of the pest (Tawatsin et al. 2011).

This study is to investigate infestation levels of bed bugs across Peninsular Malaysia within the target premises, and to evaluate the resistance level in tropical bed bugs by using impregnated papers of two major classes of insecticide group; pyrethroid and carbamate while determining the status of insecticide resistance among ten populations of bed bugs in Malaysia.

MATERIALS AND METHODS

Bed Bug Survey

A total of 185 surveyed sites in 11 out of 13 states in Malaysia were visually inspected. The sites were categorized based on their state divisions and federal territories on the map. The 11 states were as follows: Pulau Pinang, Perlis, Kedah, Perak, Kelantan, Terengganu, Pahang, Selangor, Negeri Sembilan, Melaka, Johor including a federal territory of Kuala Lumpur**.** The surveys were targeted mainly in the urban areas. Each visit had a limited period of 30-45 minutes per site to collect all bed bugs found in the premises. Infestation level was determined based on number of bed bugs collected in each surveyed sites. For instance, level 1 indicates 1-10 bed bugs found, followed by 2 (11-20 bed bugs); 3 (21-30 bed bugs); 4 (31-40 bed bugs); and 5 (>41 bed bugs). The collected bugs were then marked based on different population obtained at the respective sites.

Table 1. Populations of bed bugs in the resistance bioassay.

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

*Cluster: 1 (Northern region); 2 (East Coast region); 3 (Central region); 4 (Southern region) **Status on field population were in accordance with answered questionnaires by professionals and local residents.

Strain Selection, Insecticide Preparation and Resistance Bioassay

Ten populations were selected to evaluate resistance in them against deltamethrin and propoxur insecticides. Strains were chosen based on the resistance status from recent bed bugs control reports and questionnaires answered by the professionals in Malaysia aside from do-it-yourself treatment of the locals (Abdul Hafiz and Zulaikha 2015). They were represented by each cluster obtained from the previous sampling. The list of selected population (strains) were tabulated in Table 1.

Each treatment required 40 individuals that were chosen from each population group. Insects were separated and maintained according to field population (strains). Selected bed bug populations were tested and observed for their resistance using the insecticide impregnated papers from World Health Organization (WHO) test kit. In this study, deltamethrin and propoxur were evaluated for resistance against tropical bed bugs. 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. Once the bugs were exposed to the treated papers for the first 24 hours, the tubes were covered with fine net cloth to prevent them from escaping. Only adult bed bugs of laboratory strain were used in this study. All tubes were placed in a container at room condition (temperature, 25±2ºC, relative humidity, 50-60%).

The resistance test was conducted following methods by Tawatsin et al. (2011). Ten adults from each population were tested by introducing them first into the test tube containing impregnated papers, deltamethrin (0.05%) or propoxur (0.1%). Each treatment was triplicated with one control. Control bugs were exposed on papers impregnated in silicone oil for deltamethrin treatment. While in propoxur treatment, control papers were treated with risella oil. Prior to the experiment, bed bugs were fed one day on expired human blood. The bed bugs were exposed for 24 hours according to the exposure period for preliminary testing and their mortality were continued and recorded for fourteen 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 at all after 10 seconds of observation. Dead bed bugs were kept for further studies of resistance presence in the bugs in 70% alcohol preservation. Control groups that exceeded 10% of mortality were corrected using Abbott's formula.

Percentage of collected bugs based on locations (strains) were used for statistical analysis. Fisher's exact test was used to calculate bed bugs infestation status according to the number of surveyed premises in each state. Chi-square test for association relating two factors, harborage sites and types of premises were analyzed statistically using SPSS Version 22.0 (IBM Corp., Armonk, NY, USA). Mortality percentage of bed bugs in each treatment was calculated and analyzed daily at 24 hours' interval. Probit analysis was used in determining lethal time (hours) of the bugs at LT_{50}, LT_{90} and LT_{99} with confidence limit of 90-95%. Evaluation on lethal time were made by using software SPSS Version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Results of Fisher's exact test indicated that there was no significant difference between status of bed bugs infestation and number of premises in each state since, $p > 0.05$ (Figure 1).

Tropical bed bugs were less susceptible against deltamethrin compared to propoxur. From the first until the third day of application, all populations showed low mortality within the range of 3-20% in deltamethrin treatment. After a week, both KT and HM populations reached another 20% higher than the third day of exposure. The PC population recorded the highest mortality percentage with more than 70% of the bugs died on day 10 compared to the remaining populations. On day 14, the lowest mortality percentage was observed in AR population with 40% than the others which were ranged between 60-80%. Populations of PC and SW had high susceptibility towards deltamethrin since 90% of them were

completely dead. However, they were still considered to be resistant against this insecticide. Survived bed bugs might be due to the factor of resistance which had developed in them from earlier chemical treatment made by the pest control operators. Meanwhile, propoxur showed high efficacy in killing bed bugs as it caused overall mortality of 90% throughout the treatment period. Only one population, TI had many dead bugs on the first day of exposure with 20% of mortality and day 3 increased to 37%. Nine other populations displayed slow mortality rate until third day of exposure, but increasingly to nearly 50% after day 7. On the 10th day, all populations had reached 80-90 mortality percentages except for the two populations, KT and HM populations. Each of them had 60% and 67% of mortality in bed bugs, respectively. There were three populations including IP, PY and PC, which had all bed bugs die on 14th day of treatment resulting in 100% of mortality. The lowest mortality percentage was found in KT population with 87% while about 90% were recorded for the other seven populations. Lethal time in hours were performed in values of LT_{50}, LT_{90} and LT_{99} within confidence intervals of 95% to determine the efficacy of insecticides against bed bugs (Table 2).

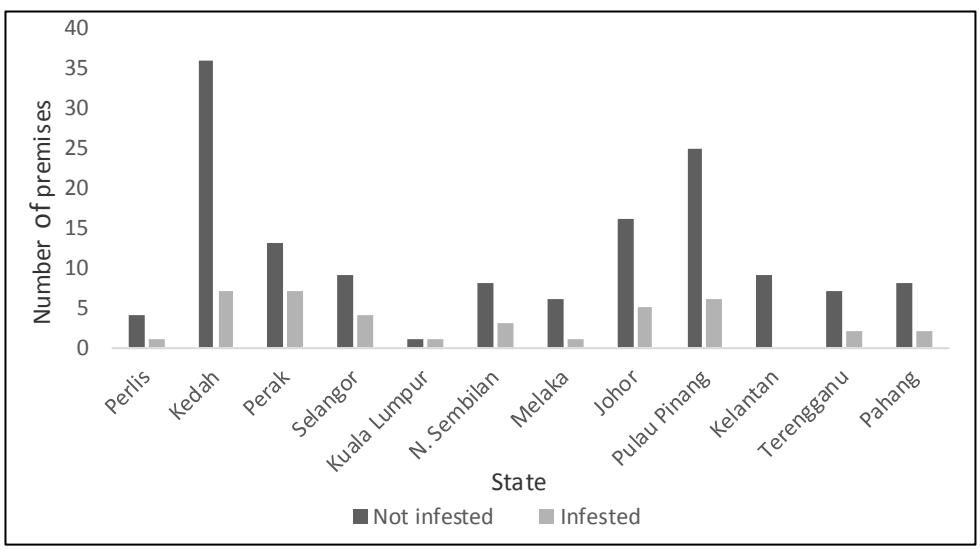

Figure 1. Number of active infestation in all surveyed premises in Peninsular Malaysia.

The results showed that the efficacy of each insecticide class were different in each population of bed bugs. A few populations had high LT_{50} values which were linked to resistance factor. In deltamethrin treatment, the highest LT_{50} values was detected in AR population with 466.119h since there were 60% of survivors on day 14. Increasing LT_{50} values were also exhibited in the following populations; TI, PD, IP, KT and SW. Their lethal time values were ranged between 216.162-328.166h, to kill 50% of bed bug populations using deltamethrin. However, three populations such as KG, HM and PY had decreased LT_{50} values with 207.201h, 203.391h and 200.593h, respectively. The lowest lethal value was found in PC population where LT_{50} = 164.068h, resulting in the most susceptible population to deltamethrin. Exposure on impregnated papers of propoxur demonstrated that half of the population were most susceptible after eight days of treatment for all tested populations. About 153.032h was required to kill 50% of IP population which indicated lowest LT_{50} value compared to the others. Another population exhibited low resistance among bed bugs was TI population with 158.754h. The remaining eight populations had increasing 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 period. Populations of PY, PD, HM, PC, AR, KG and SW had LT_{50} values of 168.238h, 177.739h, 178.756h, 190.135h, 191.67h, 195.85h and 199.483h. KT population however, had the lowest percentage of mortality and thus, had highest LT_{50} value in the population with 203.334 h (Table 2).

Table 2. Comparison of lethal hours of 2 insecticides on 10 populations of bed bugs.

Insecticide group	Population	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* (no time interval)	933.355* (no time interval)	1643.948* (no time interval)
	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)

DISCUSSION

Pyrethroid-based insecticide currently was widely used in Malaysia for treating bed bug infestation (Abdul Hafiz and Zulaikha, 2015). Therefore, via resistance bioassay on strains of bed bugs accumulated across Peninsular Malaysia, we observed that deltamethrin was less effective in controlling the pest. Populations of tropical bed bugs in Sri Lanka also had similar results after exposing them on papers impregnated with permethrin and deltamethrin for 24 hours, each KT_{50} recorded ranges within 0.5-24 hours and 2.5-47 hours respectively (Karunaratne et al., 2007). Effective doses (ED_{50}) values in treated bugs in Thailand although higher compared to other urban pest, triggered their defensive mechanism against pyrethroid-based insectide which were bifenthrin and alpha-cypermethrin (Suwannayod et al. 2010). While *C. hemipterus* showed resistance towards pyrethroid insecticide, *C. lectularius* have already established theirs in many European countries (Lilly et al., 2015; Goddard, 2013; Romero et al., 2007). Rarely reports on tropical bed bugs were published especially on their susceptibility towards chemical pesticides. Sydney strain, Australia showed common bed bugs were highly resistant against 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 based on the study conducted by Lilly et al.

(2015). Upon their act of resistance on pyrethroid insecticides, bed bugs seemed to thicken their cuticle as a defensive mechanism which was positively correlated to knockdown time as well as in 24 hours' survivor bugs (Lilly et al., 2016). In contrast, observation on mortality rate among strains in Malaysia was much higher when exposed to propoxur, carbamate-based insecticide. Significant interaction between percentages of mortality to the lethal time of the insect was detected regardless of strains, showing the insecticide was also susceptible to resistant strains. Minimum number of days required for 50% of the population to be lethal was 6.4 days, whereas 58.8 days indicated as maximum estimation of time needed for lethal values of 99% (LT_{50} = 153.302h, LT_{99} = 1413.259h). Reports from Thailand and Sri Lanka claimed that there was resistance issue in the field strains tested with propoxur (Tawatsin et al., 2011; Karunaratne et al., 2007). Observation on 24 hours' exposure at a concentration of 0.8% displayed 45.5% of the insect were survived in a population regardless of their growth stages. It can be concluded via this study that tropical bed bugs, *C. hemipterus* were highly susceptible against propoxur insecticide. Low lethal values indicated the insecticide was effective in killing these bugs. Pyrethroid-based active ingredient, deltamethrin however, was expected for their low efficacy towards resistant bugs. Malaysian Pest Control Professional can refer the results as their general guideline in controlling the pest population, while considering main active ingredients in their new products.

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