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Laboratory bioassay on efficacy of dual mode of action insecticides (beta-cyfluthrin and imidacloprid) towards tropical bed bugs, *Cimex hemipterus* (Hemiptera: Cimicidae)

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Abstract
In testing the efficacy of insecticides, bed bugs were exposed to the dual-action mode insecticides (beta-cyfluthrin and imidacloprid) on a plywood surface. The mortality rate was recorded at 1, 3, 6, 12, 24 hours of each day for seven days until a duration of six weeks post-treatment which showed a decrease in total survival of insect especially the nymphs. The percentage of mortality of bed bugs within the first day of application showed that nymphs were more susceptible than adults at high concentration of insecticide (630 ppm). Varying the concentrations of insecticide and age of bed bugs statistically showed no significant difference. However, both nymphs and adult bed bugs were killed by insecticides of high concentration (0.0.63%). Results showed that there were no statistically significant interactions between the effect of concentration of insecticide and stages of bed bugs, $F(1,108) = 0.051$, $p = 0.822$. However, there were significant difference within concentrations ($p = 0.005$) against different stages of bed bugs ($p = 0.0005$). Survival of bed bugs after the application of insecticide proved that the pest cannot be eliminated easily probably due to their modified mechanisms or development of resistance. As such, more studies should be implemented to investigate the real reasons for their resilience.

Keywords: tropical bed bug, *Cimex hemipterus*, dual-action mode insecticide, mortality

1. Introduction

Bed bugs are nocturnal insects which haunt their victims, mostly after midnight hours as they are attracted to carbon dioxide, heat and fatty acid. During the day, they hide in cracks and crevices or seams of mattresses that makes it hard to detect their infestation [1]. In the early 1900s, steaming and heating were some of the methods used to reduce the bed bug population. Steam disinfectors sanitized clothing and bedding areas for the families in England, whereas in Sweden, fumigation was implemented in the premises and hotel buildings of the citizens [1]. During World War II, soldiers were bitten voraciously by the bugs in their barracks. Therefore, the barracks were filled with hydrogen cyanide fumigation to resolve the problem. The usage of dichlorodiphenyltrichloroethane (DDT) was introduced later in the military quarters and was presumed to be effective and safer to apply around the bunk area.

Discovered in 1948 by Swiss scientist, Paul Muller, the existence of dichlorodiphenyltrichloroethane (DDT) made all the difference in insecticides history as it potentially eliminated pests such as mosquitoes, lice, flies including bed bugs [1]. The effects after application of DDT on a treated surface may last several months to years, effectively boosting the usage of this pesticide at that time. With this, bedding areas only needed to be treated once and when the bugs crawl onto the one-time treated surface, they will collapse and die. Another advantage in DDT usage is its availability in hardware stores and cheaper price [2], causing widespread use, and eventually a great decrease in bed bug populations. However, a few years after its introduction, the insects have developed resistance against formulations of insecticides [3], causing them to once again rise in number.

Over the past 20 years, the infestation of bed bugs has been increasing for unknown reason [4]. Most reports regarding high resistance in bed bugs are of *C. lectularius* species from London and Israel and *C. hemipterus* species from Tanganyika, Hong Kong, Somalia and Gambia [5]. Several factors have been linked to the resurgence of bed bugs such as globalization, socio-economic condition due to the disposal and reuse of furniture, and insufficient knowledgeamong professionals and pest control operators in controlling bed bugs population.

Yet, the greatest factor is the bed bug's resistance to insecticides [3]. Sanitation level becomes crucial in temperate countries, and residents often take action immediately after seeing bed bugs in their premises.

The insecticide resistance causes reestablished infestation to occur in most well-developed countries which affect others as well. For instance, pest control operators often used formulations containing pyrethroid and organophosphate, which are active ingredients to eliminate bed bug infestation in hotels or affected houses. However, to date bed bugs have also developed resistance against certain types of pyrethroids [4, 6]. Recent studies found that pyrethroid has penetrate through several genes in the integument of bed bugs, resisting the insecticide from reaching its nervous system [7]. In species comparison, *C. hemipterus* is more susceptible than *C. lectularius*. Females are more susceptible to insecticides such as DDT and dieldrin compared to males when tested in the laboratory [4]. Perhaps, the females have lower efficiency of mechanisms as they do not have much time to recover after mating occurs.

Due to the high resistance level to pyrethroid and organophosphate insecticides, carbamate pesticide such as Propoxur (2-Isopropoxyphenyl *N*-methylcarbamate) has been proposed for controlling bed bug populations in the 1980s 8]. Propoxur has been used in the agriculture industry mostly to control pests like ants, flies, snails and mosquitoes that may destroy flowering plants and trees. This chemical inhibits production of the enzyme cholinesterase in the nervous system. It gives a rapid-knockdown effect to the insects as the pesticide paralyzes the nervous system, forcing them to die immediately [2]. Studies have shown that the time interval for the system-producing and lethal dose is longer which proves the efficacy of the use of Propoxur in eliminating bed bugs [9]. Nevertheless, the usage of Propoxur has been prohibited by the authorities as a safety measurement as it is highly toxic to both insects and humans [4, 8]. This is due to the effect of the chemical in the air after the first application to the treated surface lasting up to several weeks. Excessive exposure to the pesticide may affect humans especially children and pregnant mothers through dermal and oral means or inhalation that can ultimately lead to death [10].

Active ingredients such as imidacloprid and beta-cyfluthrin (β-cyfluthrin) have broadened the spectrum of insecticides used to control indoor and outdoor pests [1]. Insecticides now possess dual mode of actions caused when different types of active ingredients are combined to reduce number of bed bugs [4]. Imidacloprid contains neurotoxin of nicotine which is found in tobacco, which causes blockage in the transmission of stimuli may paralyze the insects and eventually kill them. β-cyfluthrin is a synthetic pyrethroid type II that has higher acute toxicity than cyfluthrin. It causes rapid knock-down effect to the insects as it is a contact and stomach poison [11]. It has been used widely in agriculture, but subsequently upgraded to become insecticides for public health purposes (cross-resistance). The combination of these two materials produces Temprid, another suspension concentrated (SC) formulation which is proventoeliminate bedbugseffectively [12].

There are other combinations involving neonicotinoid insecticides and pyrethroid that show positive results in handling bed bugs. For instance, imidacloprid works against pierce-sucking insects while bifenthrin stimulate the neurons, sending out unstoppable impulses. The mode of action displayed by both insecticides of different classifications has been used widely in controlling the population in domestic area. The main objective of this research is to evaluate the efficacy of insecticide residual treatment on a wooden surface against tropical bed bugs of different stages (nymph and adult) and the relationship between them.

2. Materials and Methods

The present study was conducted at Household and Structural Urban Entomology Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia from June 2014 until September 2014**.**

Insecticide Preparation

An insecticide used in this bioassay comprises of two different active ingredients on different mode of action which are pyrethroid and neonicotinoid. The ingredients are formulated into suspension concentrate (SC), Temprid® SC consisting of beta-cyfluthrin and imidacloprid. Preparation of residual treatment was made by mixing the required amount of insecticide with water according to the label with high concentration at 0.063% (630ppm) and low concentration at 0.0063% (63ppm).

Bed Bugs and Treatment Preparation

Mass-reared bed bugs were used throughout the experiment comprising of nymph and adult stages. Nymphs of 3rd to 5th instar of *C. hemipterus* from the six strains were selected and split up in the plastic containers (8 cm diameter x 8 cm height) 48 hours earlier. Adult bed bugs were segregated into male and female with a sex ratio of 1:1 for each treatment. Two days prior to the experiment, they were blood fed on human hosts and were later separated into different containers (each replicate 8 bed bugs, 32 bed bugs per treatment). The experiment was conducted to observe the mortality rate of the insects based on their maturity stages by exposing them to the treated surface of plywood (Figure 1). The plywood was cleaned and dried three days before the bioassay started.

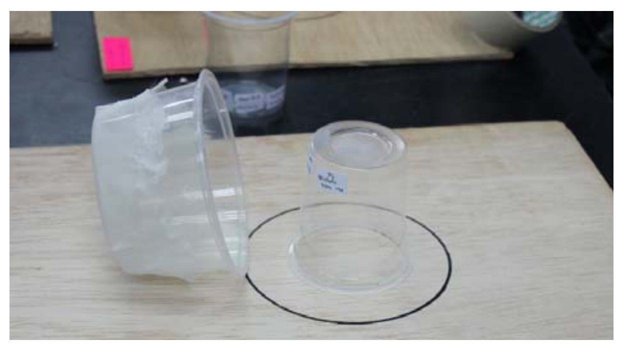

Fig 1: Bed bugs are inserted first before plastic containers with net cloth cover up the treated surface.

Bioassay Treatment

Three circles, each with 11 cm diameter, representing treated surfaces were drawn on a plywood with a size of (36 cm x 18 cm). The insecticide was diluted in distilled water to the concentrations of 0.0063% and 0.063% as they were close to the recommended level of application. The insecticide was then evenly applied to the surface of plywood using a fine mist sprayer. The plywood was left to air dry overnight. On the other hand, circles of the untreated surface were drawn on separated plywood to avoid any chemical residue that could affect the result of the experiment. The untreated surface was sprayed with distilled water.

Nymphs and adult bed bugs were exposed to the treated plywood simultaneously in the inverted containers of (11 cm diameter x 8 cm height). Net cloth covered the containers to avoid the insect from escaping (Figure 2). The observation of mortality rate was recorded at 1, 3, 6, 12, and 24 hours of each day for seven days. The plywood was covered with black plastic to prevent overexposure to light. Temperature and humidity in the laboratory were maintained at 26 ± 2 °C and 60 ± 10% respectively. Visual observation of the number of eggs that hatches into a first-instar nymph were assessed and recorded for six weeks to test the survival of the progeny. Percentage of mortality was used to determine which effect of concentrations works best on stages of bed bugs; (Percentage mortality= (total number of dead bed bug/ total number of bed bug in bioassay) x 100).

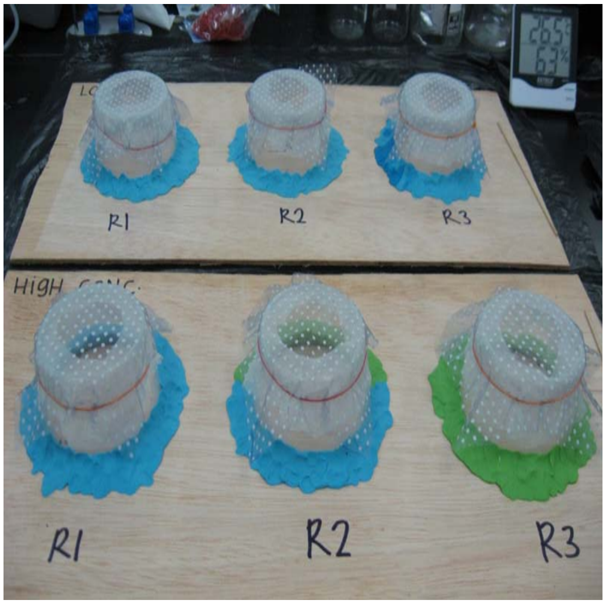

Fig 2: Residual bioassay of bed bugs on plywood surface. Net cloth with rubber band is used to cover containers

Statistical Analysis

The total survival rate of bed bugs for both stages after post-treatment was calculated in percentages for six weeks. Interactions between stages of bed bugs (nymph and adult) on the effect of insecticides of different concentrations have been tested using two-way ANOVA.

3. Results and Discussion

To evaluate the effectiveness of insecticides made with a combination of beta-cyfluthrin and imidacloprid as its main active ingredients, bed bugs were exposed to a plywood surface treated with said solution. The mortality rate at each time interval (by the hour and day) is taken in the form of percentage (Figure 3 and 4) to observe the effectiveness of insecticide on bed bugs according to stages of nymph and adult. Based on the results, it can be observed that the nymph stage is more susceptible against the residual treatment since high concentration (630 ppm) kills more than 50% (87.5%) of bed bugs on a daily basis for a week. Recording at each interval also proves that approximately 50% of the nymphs are killed using a similar concentration of insecticide.

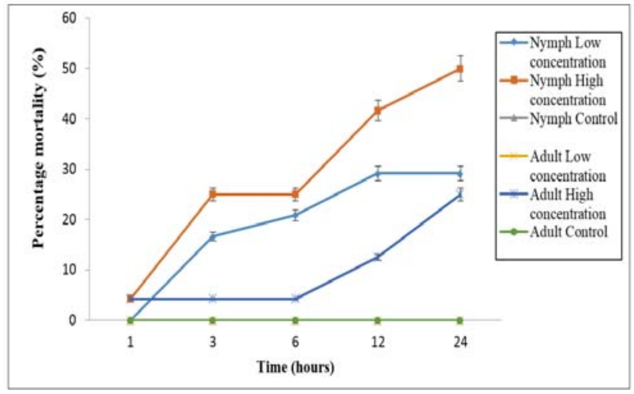

Fig 3: Percentage of mortality within hours on the first day application

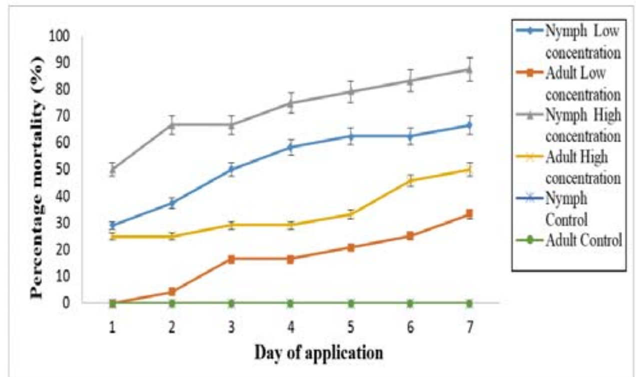

Fig 4: Bioassay of bed bugs using low and high concentration on both stages nymph and adult bed bugs

High concentration of insecticide shows highest efficacy compared to that of low concentration. As for low concentration (63 ppm), 66.7% of nymphs are harmed, whereas only 37.5% of adult insects managed to be killed. Concentration of residual treatment however, was applied below the recommended application which causes survival on tested bed bugs.

Results of two-way ANOVA shows that there is no statistically significant interaction between the effect of concentration of insecticide and stages of bed bugs, $F (1,108) = 0.051$, $p = 0.822$. However, there is a significant difference within concentrations ($p = <0.005$) and stages of bed bugs ($p = <0.0005$).

The survival rate of bed bugs after post-treatment is taken in percentage for six weeks (Table 1). Residual bioassay at nymph stage reports that the first week of exposure to low concentration insecticides (63 ppm) resulted in survival rate of 16.67%. Week 2 and 3 both show that 12.5% nymphs survive but during week 4 until week 6, only 8.33% nymphs survive. High concentrations (630 ppm) reports that the survival rate of the nymphs from the first week was 12.5% while 8.33% do not die from the second to the sixth week.

Table 1: Percentage on bed bugs survival for both stages after six weeks post-treatment

Bed bug stage	Concentration	Percentage of survival bed bugs (%)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Nymph	Low 63 ppm	16.67 (4)	12.5 (3)	12.5 (3)	8.33 (2)	8.33 (2)	8.33 (2)
	High 630 ppm	12.5 (3)	8.33 (2)	8.33 (2)	8.33 (2)	8.33 (2)	8.33 (2)
Adult	Low 63 ppm	29.17 (7)	25 (6)	25 (6)	25 (6)	25 (6)	25 (6)
	High 630 ppm	41.67(10)	25 (6)	25 (6)	25 (6)	20.83 (5)	20.83 (5)

* Values in brackets refer to number of live bed bugs per total replicates

Post-treatment results for adult bed bugs after six weeks are also reported in percentages. At a concentration of 63 ppm, the first week many adults survive with 29.17% and the following weeks 25% of the adults are still alive. In 630 ppm concentration, week 1 shows almost 50% adult bed bugs are still alive with new hatch nymphs produced on the treated surface. In week 2 until week 4, 25% of the population survives but in week 5 and 6, the population is reduced to 20.83%. Based on the results obtained, a mixture between the two active ingredients such as beta-cyfluthrin and imidacloprid works effectively on bed bugs especially at nymph stage but are much slower acting than pyrethroid insecticides [13]. The percentage of mortality of bed bugs on the first day of application shows that nymphs are more susceptible than adults towards high concentration of insecticide (630 ppm). The adults have likely developed metabolic activities in their bodies which enable them to survive even after being exposed to high concentration [9].

Similar patterns are displayed after seven days of insecticide exposure. Nymph stage is susceptible to high concentration of insecticide. However, studies regarding efficacy testing using pyrethroid insecticides has shorter lethal time values (LT_{50} = 20 minutes) [10]. One hundred percent of mortality is not achieved in this experiment since only 87.5% of nymph population is susceptible to high concentration. This is probably due to the late instar nymphs that have higher tolerance level, causing the remaining of population to stay alive. Varying the concentrations of insecticide and stages of bed bugs, however, has no significant difference statistically even though both stages are killed at high concentration (0.0.63%). The reason for the stages not overlapping with each other is because the nymphs are highly susceptible compared to the adults when exposed to both concentrations (0.0063% and 0.063%). In this study, adults show high tolerance towards the tested insecticide to the extent that they are capable of producing eggs on the treated surface. As the applied insecticide is not up to the recommended levels, the test is unable to tell whether or not the metabolic resistance developed in the integuments of the insect. Resistance in bed bugs is positively correlated to different generations of pyrethroid insecticides which causes high survival chances of this insect pest [12].

There are reports on resistance against pyrethroid insecticides in field strained bed bugs which is also displayed in reared bed bugs used in the study [12]. Observation on the survival of the bed bugs has been examined after six weeks post-treatment and the insects that remained are found to be still alive but the exact reason for this cannot be well-explained. Assumptions which can be made for the survival is that resistance may have developed in the bed bugs or that modification in the insects' bodies occurred such as higher metabolic rates or toxicity. However, further studies must be performed to support the above assumptions. For instance, molecular studies or post genomics tests on bed bugs can prove whether resistance did arise in the collected bed bugs or likewise [13].

4. Conclusion

It can be concluded that the efficacy of dual action mode insecticide was less than the pyrethroid insecticide for both nymph and adult stages. The adults showed high tolerance towards the insecticide. In comparison of concentrations, both stages were susceptible towards high concentration but the nymph stage insects showed higher percentage of mortality. Survival of bed bugs after application of insecticide proved that the pest cannot be eliminated easily probably due to their modified mechanisms or development of resistance. As such, more studies should be carried out to investigate the real reasons for their resilience.

5. Acknowledgements

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