LABORATORY EFFICACY OF INSECT GROWTH REGULATORS ON THE MORTALITY RATE OF TROPICAL BED BUGS, *Cimex hemipterus* (F.)

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ABSTRACT

Cimicidae are blood feed ectoparasites of mammals and birds. The *Cimex hemipterus* has been pestering humans since before globalisation and urbanisation. Till today, this pest has shown resilience and resurgence in infestation cases as documented in North and South America, Africa, Asia, and Europe. Hence, research on the efficacy of insect growth regulators was conducted using surface contact bioassay on a plastic surface using six insecticides at four concentrations (100ppm, 500ppm, 1000ppm, and 10 000ppm). Bioassay was performed in triplicates comprising of five-bed bugs (an adult male, two adult females, and two nymphs) per replicate. Six exposure times were designed; 1hr, 2hr, 3hr, 24hr, 48hr, 72hr and continuous exposure until mortality was achieved. The mean mortality of bed bugs within the first week of exposure demonstrated significant difference (P=0.004) while when evaluated for continuous exposure of 13 weeks tropical bed bugs showed no significant interaction (P=0.126) with zero knockdown rate. Treated bed bugs indicate significant difference between the mean egg deposited and hatched (P<0.001). During the exposure, chlorfluazuron resulted in the fastest mortality rate, followed by beta-cyfluthrin+imidacloprid, tebufenozide, methoprene, chlorfluazuron, and pyriproxyfen. Better killing efficacy was observed in insecticide at high concentrations, followed by lower concentrations with a slightly slower mortality assessment interval. The mortality of bed bugs proves that insect growth regulators do work on bed bugs. Further studies should be done before better control measures could be proposed to curb the infestation of bed bug populations.

Keywords: *Cimex hemipterus*, insect growth regulators, surface contact bioassay, knockdown rate

ABSTRAK

Cimicidae merupakan sejenis ektoparasit yang menghisap darah mamalia dan burung. *Cimex hemipterus* telah mengganggu hidup manusia sejak sebelum bermulanya globalisasi dan urbanisasi. Sehingga kini, makhluk perosak ini masih berjaya memperlihatkan kerintangan dan peningkatan dalam kes infestasi seperti yang dilaporkan di Amerika Utara dan Selatan, Afrika, Asia dan Eropah. Maka kajian terhadap keberkesanan pengatur pertumbuhan serangga dijalankan menggunakan enam racun serangga pada empat kepekatan (100ppm, 500ppm, 1000ppm dan 10 000ppm) di atas permukaan plastik melalui kaedah bioasai sentuh permukaan. Kaedah bioasai dilaksanakan dalam tiga replikasi yang menggunakan lima ekor pepijat terdiri daripada seekor jantan dewasa, dua betina dewasa dan dua nimfa bagi setiap replikasi. Kajian ini merangkumi enam masa pendedahan iaitu 1jam, 2jam, 3jam, 24jam, 48jam, 72jam serta pendedahan berterusan sehingga mortaliti dicapai. Purata kematian pepijat dalam minggu pertama pendedahan racun serangga menunjukkan terdapat perbezaan signifikan (P=0.004) manakala bagi penilaian pendedahan berterusan selama 13 minggu, tiada interaksi signifikan (P=0.996) pada purata mortaliti dikesan dan kadar ketumbangan sifar direkodkan. Pepijat yang dirawat juga menunjukkankan terdapat perbezaan signifikan di antara bilangan telur dan bilangan telur menetas (P<0.001). Sepanjang tempoh pendedahan berterusan pada racun serangga, racun jenis chlorfluazuron yang telah dikilangkan menunjukkan kadar mortaliti terpantas diikuti oleh beta-cyfluthrin+imidacloprid, tebufenozide, methoprene, chlorfluazuron dan pyriproxyfen. Kadar kematian yang positif telah dikesan pada racun serangga berkepekatan tinggi diikuti oleh racun berkepekatan rendah dengan jangka kematian yang lebih perlahan. Mortaliti pepijat membuktikan pengatur pertumbuhan serangga berkesan ke atas pepijat. Kajian lanjut perlu dilaksanakan bagi mencadangkan langkah kawalan yang lebih baik untuk membendung infestasi populasi pepijat.

Kata kunci: *Cimex hemipterus*, pengatur pertumbuhan serangga, bioasai sentuh permukaan, kadar ketumbangan

INTRODUCTION

Bed bugs are members of the Cimicidae family, which includes the tropical bed bug *Cimex hemipterus* and the common bed bug *C. lectularius* (Doggett et al. 2012). Bed bugs are hematophagous nocturnal ectoparasites that prey on mammals and birds. They are capable of finding blood meal from the carbon dioxide, heat, and fatty acid released by their host (Ab Majid & Zahran 2015; Zahran et al. 2017), which is similar to mosquito host-seeking behavior (Dieng et al. 2015; Dieng et al. 2017) Bed bugs have been associated with humans since ancient times, but they were not commonly known, particularly by the advanced economic nation. Thus, bed bugs are reclaiming their status as a major household pest as they are found across the globe, from North and South America to Africa, Asia, and Europe (Doggett et al. 2012). Although bed bugs have traditionally been seen as a problem in developing countries, they have recently spread rapidly in parts of the United States, Canada, the United Kingdom, and other parts of Asia (CDC 2020). Since 1990s until today the number of reported infestation cases has shown an aggressive global inclination andsurprisingly bed bugs are found almost anywhere human beings can venture (Ashcroft et al. 2015; Potter 2011). In those days, bed bugs were commonly found in houses, hospitals, dorms, and modes of public transportation, but in this era, bed bugs could be found in malls, theme parks, restaurants, and other public hotspot areas filled with people. This proves the resurging problem that had become a setback for eradication.

The fundamental factor for this failure is bed bugs themself, where they managed to counter every control measure by evolving their resistance mechanisms against insecticide applied throughout the years. Bed bugs are capable of four types of resistance that includes penetration, target site, metabolic and behavioral resistance, which prevents them from being controlled to the fullest (Dang et al. 2017a; Romero 2018).Earlier, highly effective organochlorine insecticide likeDichlorodiphenyltrichloroethane (DDT) was considered the key control measure that was expected to put an end to bed bug infestation since it provides a long residual effect, inexpensive and easily accessible. Yet, it began to show resistance within five years of introduction as it failed to control the bed bugs population and was first noted at the Pearl Harbour barracks of the Naval Receiving Station in 1947 (Davies et al. 2012). Excessive and continuous use inclined the pace of resistance (Dang et al. 2017b). Furthermore, the United States Environmental Protection Agency (EPA) issued a cancellation order for DDT due to its negative environmental impact and probable health risk to humans, causing it to be no longer used (United States Enviromental Protection Agency 2021). Thus, the National Pest Control Association recommends malathion as an alternative to other products such as carbamate and organophosphate (Potter 2011). Despite the advancement of numerous chemicals, such as pyrethroid, neonicotinoids, and pyrrole, bed bugs can rapidly develop resistance to insecticides, making it difficult to implement effective control measures up to this point, resulting in insect growth regulators (IGR) as the most recent method of influencing insect growth and development and frequently employed by pest management experts (Gordon et al. 2014). IGRs have two modes of action which are a juvenile hormone analogue and a chitin synthesis inhibitor. They are not designed to kill immediately, but they may cause abnormalities that make it difficult for the insect to thrive, reproduce, and eventually dies (Das 2013). Generally, IGRs have a reasonable margin of safety for human and domestic animals as its species and stage-specific is higher than other conventional insecticides that suit the existing IPM programs system (Singh & Mandal 2013). Undoubtedly, bed bugs have been a nuisance to humans battling with this pest for centuries since insecticide resistance have been a crucial predictor of bed bug resistance. Thus, this study evaluates the efficacy of manufactured chlorfluazuron, chlorfluazuron, tebufenozide, pyriproxyfen, methoprene, and β-cyfluthrin+imidacloprid that serves as insect growth regulators at different concentrations (100ppm, 500ppm, 1000ppm and 10 000ppm) on a plastic surface against tropical bed bugs and their relationship.

MATERIALS AND METHODS

Bed Bugs Preparation

The study was carried out at the Household and Structural Urban Entomology Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia for 91 days. *Cimex hemipterus used* for the study were collected from residential areas around Penang Island. The tropical bed bugs were mass-reared in the laboratory at a temperature of 26(±1)^{o}C, relative humidity of 65(±5)%, and photoperiods of 12:12 (L:D). Bed bugs were fed fresh with human blood two days prior to the exposure (Figure 1). Nymphs of 5th instar and adult bed bugs were selected to be exposed to the insecticide. 5th instar nymphs were in the last nymphal stage before emerging to adulthood and their emergence capability was noted during the study. This study was conducted in triplicates to observe the mean mortality of 450-bed bugs exposed to insecticides at various concentrations.

Figure 1. Bed bugs fed with fresh human blood using a urine container covered with a mesh net bug/ number of replicates

Insecticides Preparation

Six insecticides comprised two modes of action which are chitin synthesis inhibitor (CSI) and juvenile hormone analogue (JHA), including a positive control with pyrethroid and neonicotinoid active ingredients, were used in this study. The positive control are effective against all strains of bed bugs, even killing eggs and newly hatched nymphs that could be used to compare with other tested insecticides. Positive controls are used to demonstrate the anticipated outcome of the treatment, allowing for comparison with the effectiveness of the tested insecticides. The surface contact bioassay was prepared by mixing the required amount of insecticide with distilled water to achieve the design concentration, which were 100ppm, 500ppm, 1000ppm, and 10 000ppm, respectively calculated using the formula mg/L (Figure 2).

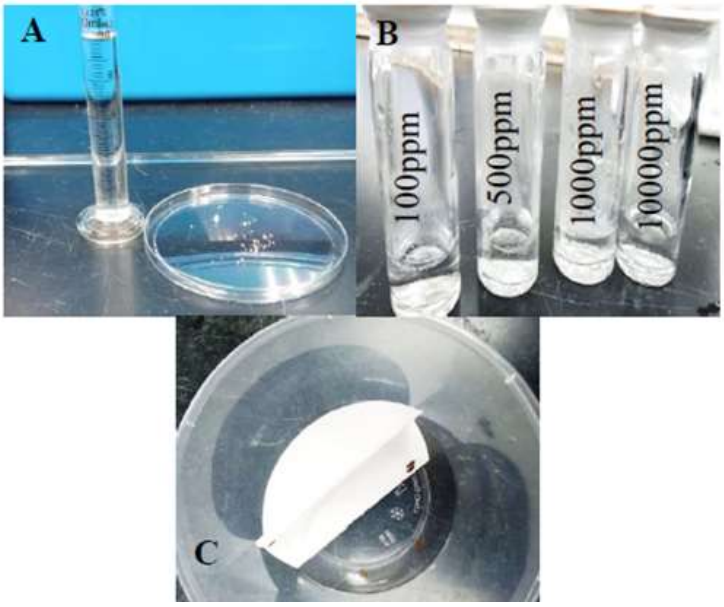

Figure 2. A. Preparation of insecticide (white powder) in 0.01L distilled water. B. Insecticides of four concentrations stored in vial bottle. C. Nymph and adult bed bugs added into the treated plastic container.

Surface Contact Bioassay Treatment

Plastic containers were treated with designated insecticides with varying concentrations. The insecticide was evenly applied on the plastic container surface using a fine mist sprayer and allowed to air dry for 24 hours. Three sets of replicates and control were prepared for each concentration. Two 5th instar nymphs, two female adults and a male adult, were exposed to the insecticide simultaneously. Harbourage paper was included in the container to allow movement and egg deposition. The mortality rate was recorded at 1-hour, 2-hour, 3-hour, 12-hour, 24-hour, 48—hour, 72-hour and continuous exposure until bed bugs achieved mortality. The plastic container was covered with black plastic to maintain the photoperiods of 12:12 (L:D). Temperature and humidity were maintained at 26(±1)^{o}C and 65(±5)%, respectively. Mean mortality was used to identify the insecticides at which concentration indicates the best effect on bed bugs; Mean mortality = (total dead bed bug/number of replicates).

Knockdown Assessment

Knockdown is defined as treated insects completely cease to move as all of their body parts remain immobile for an extended period. A knockdown bed bug may regain movement in a matter of seconds (Phillips et al. 2019). As for this study, bed bugs were required to remain stationary for 30 seconds to be considered knockdown. A wooden skewer was used to probe the bed bugs to verify any movements. If the bed bugs continue to move around throughout the exposure, it indicates they lack in response.

Nymphal and Egg Development Assesment

Bed bug nymph takes at least five weeks to mature to adult upon receiving blood meal at every stage. In this study, 5th stage nymph was used and usually developed into adults between 3-7 days (Delaunay et al. 2011). Bed bug development is classified as fast if it begins within 3 days, moderate if it takes between 4-9 days, and slow if it takes more than 9 days (Evison et al. 2018). The growth phase and physical alterations were observed in treated and control nymphs, such as cuticle formation and exuviae attachment. Egg deposition and egg hatch into the first instar, together with the survival of bed bugs progeny, were observed and recorded. Eggs deposited onto the harbourage paper were calculated and compared with the number of first-instar progeny that emerged to indicate the number of eggs hatched. Bed bugs progeny's survival and mortality duration were recorded based on the number of exposure days.

Statistical Analysis

The total mean mortality of both nymph and adult bed bugs was calculated at 13 weeks intervals. The mean mortality of bed bugs within first week of exposure and the effect of insect growth regulators on mortality of bed bugs at 13 weeks interval were analysed using Kruskal Wallis H test. The deposition of the eggs and the number of eggs hatched were evaluated using Wilcoxon Signed-Rank Test (Ab Majid & Zahran 2015).

RESULTS

The efficacy of two modes of action, by varying insecticide concentration and a positive control exposed to plastic container surfaces, were evaluated. The mean mortality of all insecticides at the designed time interval was calculated based on an average of three replicates. In the first week, higher mortality was observed in manufactured chlorfluazuron and β-cyfluthrin+imidacloprid, followed by pyriproxyfen and methoprene, while chlorfluazuron and tebufenozide had the lowest mortality rate based on exposure hours (Table 2).

Table 2. Mean mortality of tropical bed bugs upon exposure to insecticides at first week interval

Insecticides	PPM	Mortality	Std Error	Sig Diff
Manufactured Chlorfluazuron	100	2.33	0.33970	0.002
	500	1.00	0.14286	0.000
	1000	1.00	0.14326	0.257
	10000	1.67	0.23867	0.120
	Control	1.00	0.14008	0.006
Chlorfluazuron	100	0.00	-	-
	500	0.00	-	-
	1000	0.00	-	-
	10000	0.33	0.04714	0.000
	Control	0.00	-	-
Tebufenozide	100	0.00	-	-
	500	0.00	-	-
	1000	0.33	0.04714	0.000
	10000	0.00	-	-
	Control	0.00	-	-
Pyriproxyfen	100	0.00	-	-
	500	0.33	0.04714	0.000
	1000	0.33	0.04714	0.000
	10000	1.67	0.22730	0.019
	Control	0.00	-	-
Methoprene	100	0.33	0.06086	0.000
	500	1.33	0.40924	0.000
	1000	0.67	0.39673	0.000
	10000	1.00	0.14286	0.000
	Control	1.00	0.14278	0.000
Beta Cyfluthrin + Imidacloprid	100	0.67	0.09571	0.000
	500	1.00	0.14286	0.000
	1000	0.67	0.09571	0.000
	10000	2.67	0.35679	0.006
	Control	0.33	0.06086	0.000[1]

[1]Zero mortality recorded

Based on Table 3, the total mortality of insecticides at each concentration shows that manufactured brand chlorfluazuron, β-cyfluthrin+imidacloprid and methoprene had the fastest mortality rate at 10 000ppm while pyriproxyfen at 1000ppm and chlorfluazuron and tebufenozide at 100ppm, respectively. Overall, tropical bed bugs achieved mortality between 1000ppm and 10 000ppm first, followed by 100ppm and 500ppm, apart from chlorfluazuron, which had mortality beginning from the lowest to the highest concentration. Although the mortality duration varies among the bed bugs tested with different insecticides at varying concentrations, bed bugs possess a similar effect of the insecticide (Table 4).

Table 3. Mean mortality of tropical bed bugs upon exposure to insecticides at four concentrations

Insecticides	Ppm	Mean Mortality/ Time Interval																		Std. error	Sig. diff (*p*)
		1hr	2hr	3hr	24hr	48hr	72hr	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	
Manufactured Chlorfluazuron	**100**	0.00	0.00	1.33	1.33	1.33	2.00	2.33	3.00	3.00	4.33	4.33	4.67	4.67	4.67	4.67	4.67	5.00	5.00	5.00	0.40577	0.005
	500	0.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00	3.00	4.00	4.00	4.67	4.67	5.00	5.00	5.00	5.00	5.00	5.00	0.51263	0.000
	1000	0.00	0.00	0.33	0.67	0.67	0.67	1.00	2.00	2.67	4.33	4.67	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	0.49288	0.000
	10000	0.00	0.00	0.00	0.00	0.67	0.67	1.67	2.67	3.33	4.33	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	0.49661	0.000
	Control	0.00	0.00	0.00	0.00	0.33	0.33	1.00	1.33	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	0.19960	0.000
Chlorfluazuron	**100**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	2.00	3.00	3.00	3.33	4.67	5.00	5.00	5.00	5.00	5.00	5.00	0.49272	0.002
	500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00	2.67	3.67	4.00	4.33	4.33	4.67	5.00	5.00	5.00	5.00	0.50224	0.001
	1000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	1.33	1.67	2.33	3.00	4.33	4.33	4.33	5.00	5.00	5.00	5.00	0.47756	0.002
	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.33	2.00	2.67	3.33	3.67	4.33	4.33	4.67	5.00	5.00	5.00	5.00	0.49051	0.002
	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.00	1.33	1.67	2.00	2.00	2.00	2.00	2.00	2.00	0.21379	0.000
Tebufenozide	**100**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.33	3.33	4.00	4.67	4.67	4.67	5.00	5.00	5.00	5.00	5.00	0.53605	0.000
	500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.67	2.67	3.67	4.00	4.00	4.00	5.00	5.00	5.00	5.00	5.00	0.51492	0.000
	1000	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.67	2.33	2.67	4.33	4.33	4.33	5.00	5.00	5.00	5.00	5.00	5.00	0.51349	0.001
	10000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00	2.33	3.67	3.67	4.33	5.00	5.00	5.00	5.00	5.00	5.00	0.51257	0.001
	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.67	1.33	1.33	1.33	1.33	1.67	2.00	2.33	2.67	2.67	0.22746	0.003
Pyriproxyfen	**100**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.33	2.33	2.67	2.67	3.33	4.67	5.00	5.00	5.00	5.00	5.00	0.49275	0.002
	500	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	1.00	1.00	2.33	3.00	3.00	3.33	3.33	3.33	5.00	5.00	5.00	0.43856	0.004
	1000	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.00	1.67	1.67	3.00	3.67	4.00	4.33	4.33	4.67	4.67	4.67	5.00	0.47053	0.002
	10000	0.00	0.00	0.00	0.33	0.33	0.67	1.67	2.67	3.00	3.33	3.67	4.00	4.33	5.00	5.00	5.00	5.00	5.00	5.00	0.46959	0.004
	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	1.00	1.00	1.33	1.33	1.33	2.00	2.00	2.67	2.67	0.21848	0.002
Methoprene	**100**	0.00	0.00	0.33	0.33	0.33	0.33	0.33	2.00	2.33	2.67	3.33	4.00	4.67	4.67	4.67	4.67	4.67	5.00	5.00	0.46879	0.002
	500	0.00	0.00	0.67	0.67	0.67	0.67	1.33	1.67	3.33	3.33	4.00	4.00	4.33	4.67	5.00	5.00	5.00	5.00	5.00	0.45460	0004
	1000	0.00	0.00	0.00	0.00	0.00	0.33	0.67	1.00	1.67	2.33	2.67	3.00	3.67	4.00	5.00	5.00	5.00	5.00	5.00	0.47418	0.005
	10000	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.33	2.33	2.67	3.00	3.33	4.00	4.33	5.00	5.00	5.00	5.00	5.00	0.48047	0.004
	Control	0.00	0.00	0.00	0.00	0.00	0.33	1.00	1.33	1.33	1.67	1.67	1.67	1.67	2.33	2.33	2.33	2.33	2.33	2.33	0.21895	0.003
Beta Cyfluthrin + Imidacloprid	**100**	0.00	0.00	0.00	0.00	0.00	0.00	0.67	2.33	2.67	300	3.00	3.00	3.33	3.67	4.33	5.00	5.00	5.00	5.00	0.45866	0.006
	500	0.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00	2.33	3.00	3.67	4.67	5.00	5.00	5.00	5.00	5.00	5.00	5.00	0.51068	0.001
	1000	0.00	0.00	0.00	0.00	0.00	0.00	0.67	1.67	2.33	3.33	3.67	3.67	4.00	4.67	5.00	5.00	5.00	5.00	5.00	0.49288	0.002
	10000	0.00	0.00	0.00	0.67	0.67	0.67	2.67	4.33	4.67	4.67	4.67	4.67	4.67	5.00	5.00	5.00	5.00	5.00	5.00	0.48845	0.000
	Control	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33	0.67	0.67	0.67	1.00	1.67	1.67	2.00	2.33	2.67	3.00	3.00	0.24800	0.008[1]

NOTE: W=Week; [1]Zero mortality recorded

Table 4. Effect of Insecticides on Tropical Bed bugs upon continuous exposure

Effect on Bed Bug/ Insecticides	Manufactured Chlorfluazuron	Chlorfluazuron	Tebufenozide	Pyriproxyfen	Methoprene	B-Cyfluthrin + Imidacloprid
Knockdown	-	-	-	-	-	-
Nymphal Development	Fast	Moderate	Moderate	Moderate	Moderate	Moderate
Exuviae	X	/	/	/	/	X
Egg Laid	/	/	/	/	/	/
Egg Hatched	/	/	/	/	/	/
1st Instar Survival	±1 Week	±1 Week	±1Week	±1Week	±1 Week	±1 Week*

Note: *No knockdown; X-No exuviae attached to bed bug; /-Exuviae remain attached to bed bug

The mean mortality of tropical bed bugs upon exposure to insecticides at different concentrations was recorded to observe the efficacy of the insect growth regulator. Figures 3 and 4 shows the mean mortality of bed bugs within one week of exposure and 13 weeks of exposure where a total of 450 bed bugs were used. The result shows higher concentrations resulted in the highest efficacy from week one of exposure (Figure 3) As for the lower concentration, the effectiveness was gradually noted throughout the exposure until bed bugs showed total mortality (Figure 4).

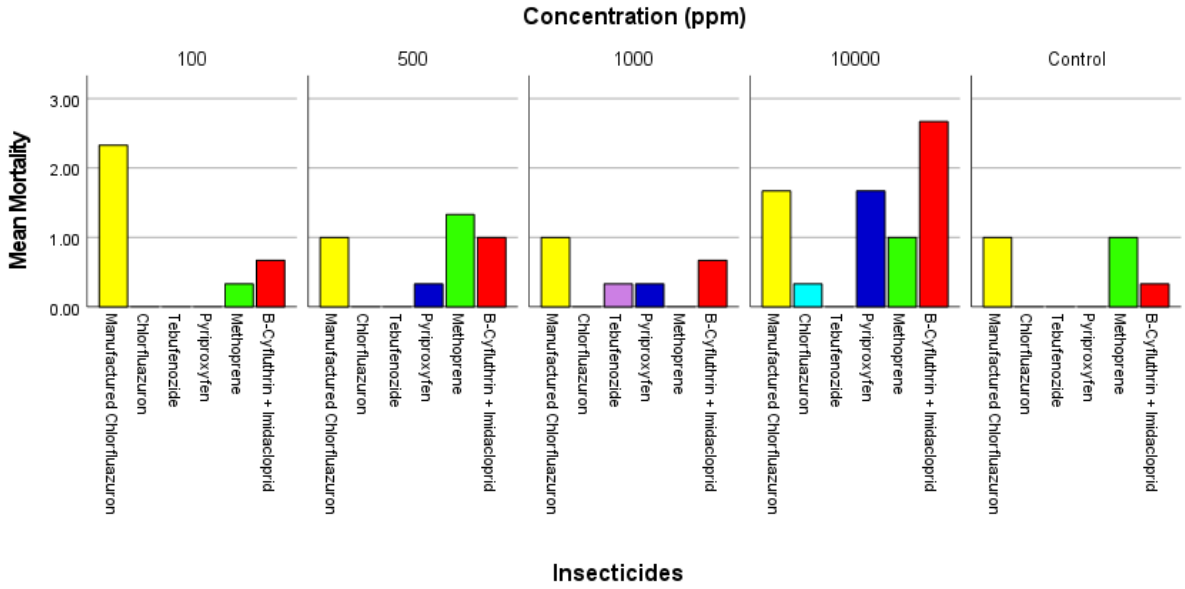

Figure 3. Mean mortality of bed bugs at one week of exposure against insecticides at different concentrations (P=0.004)

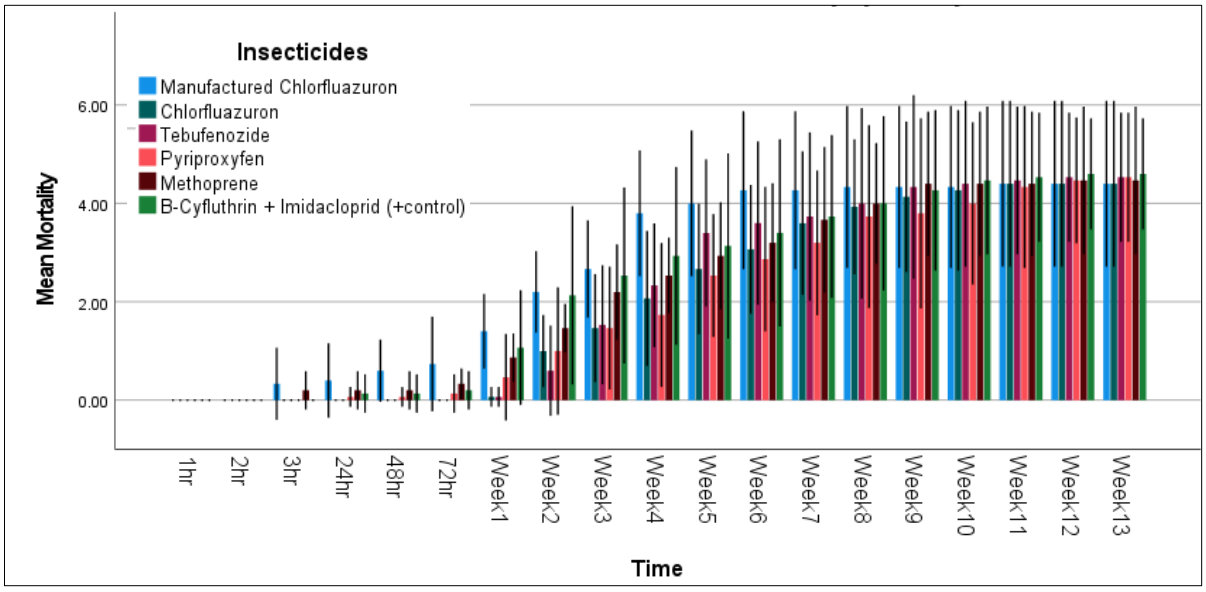

Figure 4. Mean mortality of bed bugs at 13-weeks of exposure against insecticides at four different concentrations (P=0.126)

This study demonstrates that bed bugs imply mortality at high and low concentrations. The Kruskal Wallis indicates there is significant difference between mean mratlity of bed bugs at the first week of insecticides exposure H= 17.066, df = 5, P=0.004. For the mean mortality of *C. hemipterus* upon exposure to different insect growth regulators resulted in no statistically significant interaction with the value of H = 8.596, df =5, P=0.126. Figure 5 shows the mean difference between eggs deposited and hatched per insecticide. Chlorfluazuron and pyriproxyfen had the highest egg hatched, followed by methoprene, tebufenozide and manufactured chlorfluazuron, while β-cyfluthrin+imidacloprid had the lowest number of mean eggs hatched. Wilcoxon Signed Rank test indicated insect growth regulators at varying concentration had a significant influence in the number of eggs deposited and hatched Z=-4.789, *P<0.001*.

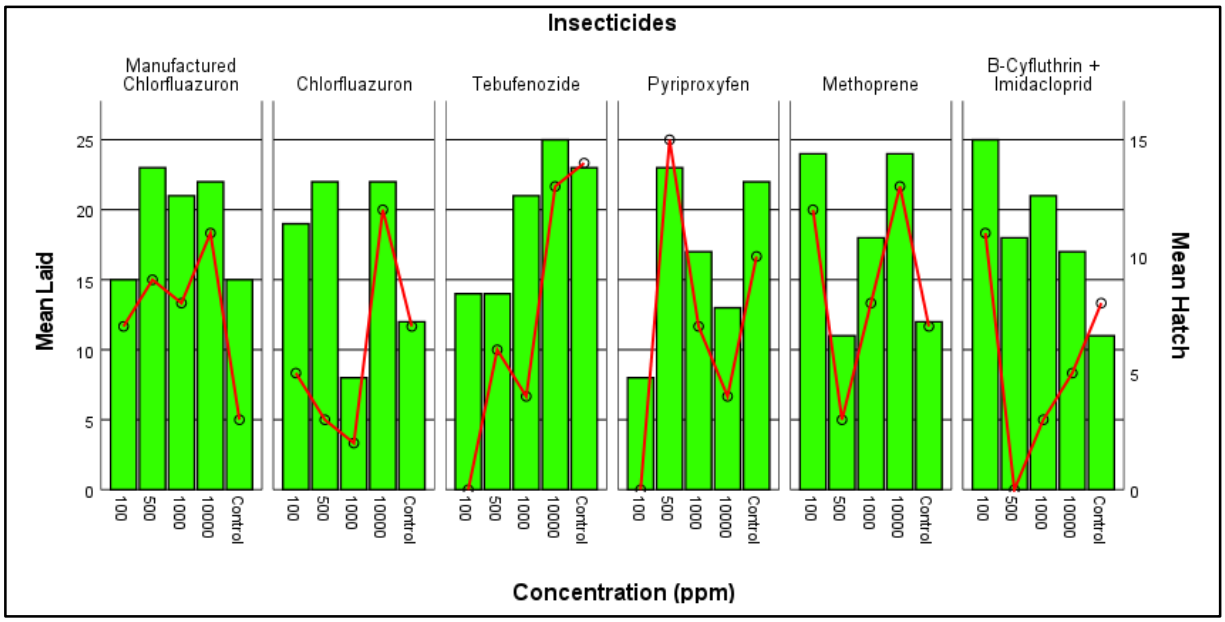

Figure 5. Mean egg laid and hatched upon exposure to insecticides at different concentrations (*P*<0.00). Green Bar – Mean egg laid; Red Line - Mean egg hatched

DISCUSSION

This study demonstrates the knockdown responses of tropical bed bugs when tested against insect growth regulator at different concentrations on a plastic surface was similar. No knockdown response was recorded throughout the study, as bed bugs regained their strength when the plastic container was moved or slightly shaken. This was due to the study being performed via surface contact instead of ingestion, which resulted in the slow acting of the insecticides(Sierras & Schal 2020). During observation, bed bugs were seen hiding between the folding of the harborage paper, and there was less contact with the treated surface. Thus, bed bugs that have escaped continuous contact with the treated surface or insecticide require a longer time to work on their exoskeleton and reach their system (Leong et al. 2020). Suppose the bed bugs hide on the harborage paper provided; the potency of insecticide transmission will be lower since it has been evaporated, which may lead to irregular delivery of insecticides to the tropical bed bugs (Dang et al. 2017b) .Paper was placed in the container to allow bed bugs to live, thrive, reproduce, and move, as they prefer to congregate on a rough surface rather than

a smooth surface (Mathison & Pritt 2021). The study was carried out after bed bugs received blood meals. Each replicate prepared with two nymphs at the 5th instar stage took at least two weeks to develop into an adult. However, the adults have a nymphal exoskeleton called the exuviae that remain attached as they fail to shed. Juvenile hormone analogue (JHA) delayed the imaginal molt and increased cuticular melanization, causing the 5th nymphal stage of bed bugs takes more than two weeks to develop to adult after receiving blood meal and insecticide exposure (Sierras & Schal 2020). Similar to the effect of JHA, Chitin synthesis inhibitors (CSI) obstruct chitin biosynthesis, preventing tropical bed bugs from developing a new exoskeleton and shedding the old one that leads to the remaining of exuviae attached to bed bugs (Campbell et al. 2017). Not all nymphs developed to adulthood from the recorded data, as nymphal mortality was also recorded. The developed nymph and adult bed bugs also succeed in laying eggs; not all deposited eggs hatched to first instar nymphs. Those eggs that hatched remain in the first nymphal stage for a week and eventually die. This proves insect growth regulators were proficient in oviposition and egg retainment. The hatched first instar nymph receives prolonged exposure from embryogenesis, which slows its development and results in mortality (Goodman et al. 2013). JHA and CSI were competent as ovicidal since JHA could encourage sterilization, behavior, and diapause disruption. At the same time, CSI disrupts embryo development, resulting in the development of non-viableeggs and eggs failing to hatch (Sarwar 2020). Bed bug eggs, nymphs, and adults were susceptible to the insect growth regulator employed in the study. However, the action of insecticides takes time to work because the study was a surface contact bioassay that allows the transmission of the insecticides to bed bugs only when there is contact on the treated surface. (Singh et al. 2013) JHA and CSI play their role where development, reproduction, embryogenesis, and chitin formation in the bed bugs were disrupted, eventually causing them to reach mortality without any progeny. Thus, further studies are required to prove any occurrence of resistance that leads to prolonged mortality intervals.

CONCLUSION

It can be concluded that the efficacy of insect growth regulators at different concentrations works in all stages of tropical bed bugs. Higher concentrations had higher bed bug mortality, followed by lower concentrations at the beginning of the study. In comparing insect growth regulators, both juvenile hormones analogue (JHA) and chitin synthesis inhibitors (CSI) had similar effects on bed bugs of all stages. The survival of bed bugs up to almost 13 weeks of intervals proved that pests have the capability of survival and adaptation of resistance against insecticides. Hence, further studies are required to seek advanced bed bug management technique employing insect growth regulators to have a broader understanding on how to completely eradicate this pest infestation.

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AUTHORS DECLARATIONS

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Universiti Sains Malaysia Research Ethics Committee (Human) JEPeM, Code: USM/JEPeM/19120868.

Data Availability Statement
The manuscript has no associated data.

Authors' Contributions
NurHidayah Taibukahn: Methodology, Investigation, Data Curation, Formal analysis, writing – original draft, Writing – review & editing. Abdul Hafiz Ab Majid: Conceptualization, Supervision, Project administration, Resources, Funding acquisition, writing – review & editing.

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