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EFFICACY OF PYRETHROID-NEONICOTINOID MIXTURE FORMULATIONS AGAINST DIFFERENT DEVELOPMENTAL STAGES OF TROPICAL BED BUG (HEMIPTERA: CIMICIDAE)

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ABSTRACT

Bed bugs are urban pests as they are closely associated with humans to obtain blood meals. Many insecticides have been used against them, rendering them resistant to most of the insecticide formulations. Thus, novel insecticide formulations are in high demand. This study aimed to evaluate the efficacy of pyrethroid-neonicotinoid mixture formulations against *Cimex hemipterus*. The Penang Island-strain *C. hemipterus* was collected and subjected to residual insecticide bioassay and egg immersion treatment. The analysis of the knockdown and lethal time values imposed on 50% and 95% populations (10-d KT_{50}, 10-d KT_{95}, 10-d LT_{50}, and 10-d LT_{95}) revealed the orders of relative contact toxicity for all developmental stages (except eggs) of *C. hemipterus* as follows: porous surface – bifenthrin and imidacloprid > lambda-cyhalothrin > alpha-cypermethrin and dinotefuran; non-porous surface – alpha-cypermethrin and dinotefuran > bifenthrin and imidacloprid > lambda-cyhalothrin. The hatching rates of *C. hemipterus* eggs remained high (84.17 – 100.00%) after being immersed in the tested insecticides, except alpha-cypermethrin and dinotefuran diluted at 50000 ppm. Thus, the overall performance of novel pyrethroid-neonicotinoid insecticide mixture formulations was mediocre.

Keywords: *Cimex hemipterus*, pyrethroid-neonicotinoid mixture formulation, residual insecticide bioassay, tropical bed bug

ABSTRAK

Pepijat dianggap sebagai perosak di penempatan manusia di bandar kerana ia berkait rapat dengan manusia untuk memperoleh darah sebagai sumber makanan. Banyak racun serangga telah digunakan untuk membasmi pepijat dan situasi ini telah mencetuskan masalah rintangan pepijat terhadap kebanyakan formulasi racun serangga. Oleh itu, formulasi racun serangga baharu mendapat permintaan yang tinggi. Kajian ini bertujuan menilai keberkesanan campuran formulasi piretroid-neonikotinoid terhadap *Cimex hemipterus*. *Cimex hemipterus* strain Pulau Pinang dikumpul dan digunakan untuk ujian bioasai sisa racun serangga dan rawatan rendaman

telur. Analisis kajian yang berdasarkan nilai masa diperlukan untuk mencapai mati merata dan kematian dalam kalangan 50% dan 95% populasi (10-d KT_{50}, 10-d KT_{95}, 10-d LT_{50} dan 10-d LT_{95}) menunjukkan susunan prestasi ketoksikan secara relatif yang merangkumi semua peringkat hayat (kecuali telur) *C. hemipterus* adalah seperti berikut: permukaan berliang – bifenthrin dan imidacloprid > lambda-cyhalothrin > alpha-cypermethrin dan dinotefuran; permukaan tidak berliang – alpha-cypermethrin dan dinotefuran > bifenthrin dan imidacloprid > lambda-cyhalothrin. Selain alpha-cypermethrin dan dinotefuran yang dicairkan pada 50000 ppm, kadar penetasan telur *C. hemipterus* kekal tinggi (84.17 – 100.00%) walaupun setelah direndam dalam racun serangga yang diuji. Justeru, prestasi keseluruhan formulasi racun serangga campuran formulasi piretroid dan neonikotinoid ini adalah sederhana.

Kata kunci: *Cimex hemipterus*, campuran formulasi piretroid dan neonikotinoid, ujian bioasai sisa racun serangga, pepijat tropika

INTRODUCTION

From the viewpoint of taxonomic classification, bed bugs are recognized under the order Hemiptera and the family Cimicidae (Usinger 1966). They are hematophagous ectoparasites throughout their lifespans (Araujo et al. 2009). While they can obtain blood meals from other warm-blooded animals such as bats, chickens, and birds, their preferred host is currently humans (Doggett et al. 2012; Lim & Ab Majid).

Although bed bugs are not associated with transmitting diseases to humans (Aigbodion & Megbuwe 2008), their bites are of great medical concern as they may cause symptoms ranging from skin irritation, allergic hypersensitivity, hemoglobin iron deficiency, cutaneous wheal formation, and secondary infection (Goddard & DeShazo 2009; How & Lee 2010; Zulaikha et al. 2016). Therefore, control and management of bed bug are imperative.

The current practical approach to bed bug control still relies heavily on insecticides, which are responsible for the occurrence of insecticide resistance in bed bugs (Doggett et al. 2012; Zulaikha & Ab Majid 2015). Multiple molecular studies have been conducted to comprehend the underlying mechanisms (metabolic, behavioral, and physiological aspects) leading to insecticide resistance, and some results affirmed the statement mentioned earlier (Adelman et al. 2011; Bai et al. 2011; Benoit et al. 2016; Dang et al. 2015; Dang et al. 2017; Ghavami et al. 2021; Hardstone et al. 2015; Hosokawa et al. 2009; Hwang et al. 2014; Koganemaru et al. 2013; Mamidala et al. 2012; Lilly et al 2018; Romero et al. 2009; Romero & Anderson 2016; Rosenfeld et al. 2016; Seong et al. 2012; Yoon et al. 2008; Zhu et al. 2013; Zhao et al. 2020). Moreover, while the assessment of insecticide resistance of bed bugs has been progressively reported, it is mainly restricted on the nymphal and adult stages (Leong et al. 2020). Hence, the status of insecticide resistance on bed bug eggs is not fully known.

In response to the challenge of insecticide resistance, a comprehensive understanding of the biology and toxicology of bed bugs is crucial (How & Lee 2010). Progressive exploration of innovative insecticide formulations is underway to address resistance issues in bed bugs. Additionally, the synergistic effects of various insecticides are being investigated to enhance the efficacy of chemical treatments against bed bugs (How & Lee 2011).

The objective of this study is to determine the knockdown and lethal time values imposed on 50% and 95% population (KT_{50}, KT_{95}, LT_{50}, and LT_{95}) of three developmental stages of *C. hemipterus* (adults, late instars, and early instars) treated with three insecticide

formulations (Bithor SC – bifenthrin and imidacloprid, Ridesco WG – alpha-cypermethrin and dinotefuran, and Icon CS – lambda-cyhalothrin) on two contact surfaces (porous and non-porous) at three concentrations (50000 ppm, 10000 ppm, and 1000 ppm). Additionally, how well the insecticides impact the hatching rates of bed bug eggs at these concentrations is also evaluated in this study.

MATERIALS AND METHODS

Bed Bugs Collection

Bed bugs were collected from different locations on Penang Island, Malaysia, where bed bug problems occurred between December 2021 and March 2022 (Table 1). The inspected places were typically urban areas, crowded spaces with people, messy, and had poor hygiene conditions. Various housing types were visited, including residential houses, flats, and apartments.

Table 1. Field sampling of *C. hemipterus* (data on coordinates, location, collection date and notes)

Coordinates	Location	Collection Date	Note
5° 24' 0'' N 100° 19' 4'' E	Malaysia: Penang, Pinang Court, Lebuh Sungai Pinang	08 Dec 2021	Flat
5° 23' 6'' N 100° 16' 54'' E	Malaysia: Penang, Lorong Semarak Api 2, Bandar Baru Air Itam	20 Dec 2021	Residental house
5° 23' 26'' N 100° 17' 42'' E	Malaysia: Penang, Greenlane Heights, Georgetown	24 Dec 2021	Flat
5° 23' 8'' N 100° 16' 60'' E	Malaysia: Penang, Semarak Api Flat, Bandar Baru Air Itam	14 Jan 2022 25 Jan 2022 18 Mar 2022	Flat
5° 23' 52'' N 100° 19' 43'' E	Malaysia: Penang, Maritime Luxury Suite, Persiaran Karpal Singh	16 Feb 2022	Apartment homestay
5° 22' 56'' N 100° 18' 39'' E	Malaysia: Penang, Rumah Intan Old Folks Home, Georgetown	18 Feb 2022	Old folks' home
5° 23' 0'' N 100° 16' 34'' E	Malaysia: Penang, Lebuh Rambai 8, Paya Terubong	18 Feb 2022	Residential house
5° 23' 3'' N 100° 16' 54'' E	Malaysia: Penang, Lorong Semarak Api 2, Bandar Baru Air Itam	19 Feb 2022	Flat

Only live bed bugs in nymphal or adult stages and eggs were considered target samples. Collected bed bugs were placed in 30.0 mL sterile vial bottles with a folded paper serving as an artificial hiding spot. All these collected bed bugs were referred to as the Penang Island-strain.

Morphological Identification of Bed Bug

The collected bed bugs were taken to the Household and Structural Urban Entomology Laboratory at the School of Biological Sciences, Universiti Sains Malaysia. Ten bed bug samples were randomly chosen from each collection site for identification purposes. Morphological identification followed the key characteristics outlined by Usinger (1966), covering features such as the head, thorax, abdomen, legs, and setae of both late nymphal instars and adults.

Bed Bug Rearing

The collected samples were identified as *C. hemipterus*, commonly referred to as tropical bed bugs. All bed bugs were cultured in 200.0 mL plastic containers covered with a mesh cloth (≤0.1 mm pore sizes). These containers were kept in a laboratory at a temperature of 26±2°C (How & Lee 2011; Zulaikha et al. 2016).

To minimize the physiological impacts on bed bugs, including excretion, digestion, and blood depletion (Benoit et al. 2009), the bed bugs were fed directly on humans following guidelines from the Human Research Ethics Committee USM (HREC) (Code reference: USM/JEPeM/19120868). The feeding was performed on human volunteers' arms or thighs once a week, with volunteers fully informed about the procedure, implications, and associated risks. The blood-feeding duration was approximately ten minutes. Bed bugs were fed until three days before commencing the insecticide bioassays.

Insecticide Formulations

All tested insecticide formulations (Bithor SC – bifenthrin and imidacloprid, Ridesco WG – alpha-cypermethrin and dinotefuran, and Icon CS – lambda-cyhalothrin) were diluted with deionized water to achieve the tested concentrations (50000 ppm, 10000 ppm, and 1000 ppm). The standard application rates ranged from 15.00 g/m^2 to 100.00 g/m^2, which were in accordance with the manufacturers' recommendations (Table 2).

Table 2. Insecticide products and dilution rate used

Trade name			Bithor (from Ensystex (M) Sdn. Bhd., Malaysia)	Ridesco (from BASF Corporation, USA)	Icon (from Syngenta Crop Protection Sdn. Bhd., Malaysia)
Insecticide class			Pyrethroid-neonicotinoid mixture	Pyrethroid-neonicotinoid mixture	Pyrethroid
Active ingredients			Bifenthrin[a] (4.51%), Imidacloprid[b] (5.50%)	Alpha-cypermethrin[a] (10.00%), Dinotefuran[b] (20.00%)	Lambda-cyhalothrin[a] (2.44%)
Formulation type			Suspension concentrate (SC)	Water-dispersible granule (WG)	Capsule suspension (CS)
Application rate for non-porous surface (g/m^2)			50.00	40.74	15.00
Application rate for porous surface (g/m^2)			100.00	81.48[c]	30.00
Dilution rate[d]	**1000 ppm**	**Porous**	22.5 µL: 22500.0 µL	8.8 µL: 17620.0 µL	9.0 µL: 6750.0 µL
		Non-porous	22.5 µL: 11250.0 µL	4.4 µL: 8810.0 µL	9.0 µL: 3375.0 µL
	10000 ppm	**Porous**	22.5 µL: 2250.0 µL	8.8 µL: 1762.0 µL	9.0 µL: 675.0 µL
		Non-porous	22.5 µL: 1125.0 µL	4.4 µL: 881.0 µL	9.0 µL: 337.5 µL
	50000 ppm	**Porous**	22.5 µL: 450.0 µL	8.8 µL: 352.0 µL	9.0 µL: 135.0 µL
		Non-porous	22.5 µL: 225.0 µL	4.4 µL: 176.0 µL	9.0 µL: 67.5 µL

[a]Pyrethroid

[b]Neonicotinoid

[c]The manufacturer does not recommend the application on the porous surface. The value is generated in reference to the trend exhibited on the other two insecticide products (double the application rate from non-porous surfaces).

[d]The dilution ratio is presented in the form of an insecticide-to-water ratio. Dilution calculation is based on the surface area coverage of 0.0225 m^2. The total volume of insecticide dilution applied to the treatment surfaces follows 10000 ppm (excluding 50000 ppm).

Surfaces Treatment

Two types of surfaces were evaluated: (1) porous surface: plywood (15.0 cm × 15.0 cm) and (2) non-porous surface: ceramic tile (15.0 cm × 15.0 cm). The total surface area was 225.00 cm^2 (equivalent to 0.0225 m^2). The diluted insecticides were transferred onto the surfaces using a 100-1000 µL pipette and were spread evenly using an L-shaped cell spreader. All treated surfaces were allowed to air dry thoroughly in a fume hood for 24 hours. The surfaces treated only with distilled water were used as negative controls.

Toxicity Bioassay of Formulated Insecticides

The toxicity bioassay procedure was adapted from Leong et al. (2020) and How & Lee (2011). The experiment commenced by testing three bed bug stages – early instars (first and second instars), late instars (third, fourth, and fifth instars), and adults (males and females in a 1:1 ratio) – with triplicates of five insects each. Five bed bugs were placed onto the treated surface (Figure 1), and a Petri dish (90.0 × 15.0 mm) (Brandon, Malaysia) was inverted over the treated surface to confine the introduced insects consistently.

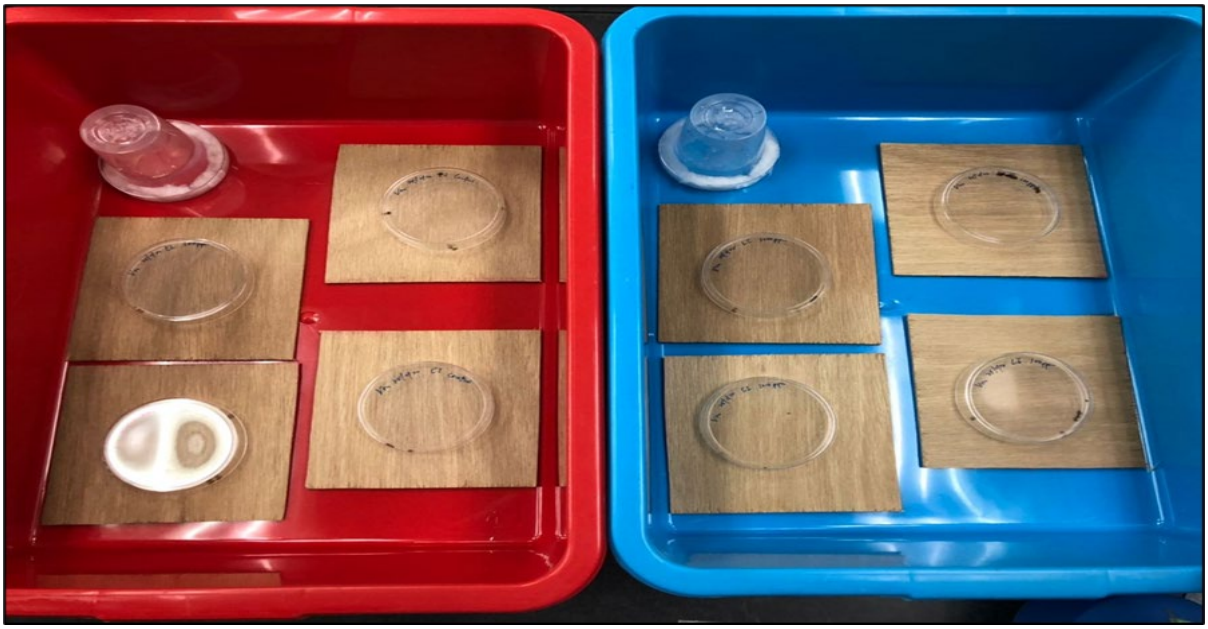

Figure 1. The setting of insecticide bioassay. The treated surfaces were placed in the basins, where the moisture level and temperature were regulated optimally. Bed bugs were confined inside the Petri dishes

Knockdown was identified when the insect either remained immobile with minimal antennal and leg movement or was positioned upside down and unable to right itself within 20 seconds after gentle probing with forceps. Mortality was recorded when the insects remained utterly motionless. Data were recorded at predetermined intervals, precisely ten days for knockdown and mortality.

Egg Immersion Treatment

The immersion method followed the protocols outlined by Leong et al. (2020) and Campbell & Miller (2015). Ten newly fed adult *C. hemipterus* (five males and five females) were introduced into a Petri dish containing a 90.0 mm diameter filter paper (Advantec, Japan). Each week, one female was expected to lay approximately 5-7 eggs on the filter paper. The adults

were given a one-week period to lay eggs. The selected age range for the eggs was 4-5 days, allowing for maximum embryonic development.

For the bioassay, the eggs on the filter paper were immersed in insecticide dilution for 5 seconds, while eggs immersed in deionized water for the same duration were used as control. Afterward, they were air-dried in a fume hood for 4 hours and transferred to a clean Petri dish. Triplicates were employed for each insecticide dilution, and the egg hatching rates (%) were recorded ten days post-treatment. The baseline was that lower efficacy performance of insecticide would lead to higher hatching rates of *C. hemipterus* eggs.

Data Analysis

The collected data was pooled and subjected to Probit analysis. At 10 days posttreatment, the knockdown times (hours) were determined at 10-d KT_{50} and 10-d KT_{95}, and the lethal times (hours) were determined at 10-d LT_{50} and 10-d LT_{95}. A 10% - 90% confidence limit using time-response data was deployed. Chi-squared (χ^2) goodness-of-fit tests were conducted to ensure the homogeneity test was achieved (Dang et al. 2017). The values were considered significantly different when their 95% fiducial limits (FLs) did not overlap (Payton et al. 2003; Wheeler et al. 2006). All statistical analyses were performed using software SPSS Version 26.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to analyze the effect of insecticides on the percentage of hatching rates of *C. hemipterus* eggs at fixed concentrations at $P = 0.05$.

RESULTS

Knockdown and lethal time values of Penang Island-Strain *C. hemipterus*

The ten-day knockdown time (10-d KT_{50} and 10-d KT_{95}) values of *C. hemipterus* adults (Table 3), late instars (Table 4), and early instars (Table 5) were demonstrated. The overview of generated knockdown time values allowed the revelation of the order of contact toxicity for all developmental stages of *C. hemipterus*: porous surface – Icon CS (lambda-cyhalothrin) > Bithor SC (bifenthrin and imidacloprid) > Ridesco WG (alpha-cypermethrin and dinotefuran); non-porous surface – Ridesco WG (alpha-cypermethrin and dinotefuran) > Bithor SC (bifenthrin and imidacloprid) > Icon CS (lambda-cyhalothrin).

In the stance of porous surface, *C. hemipterus* adults were statistically proven most susceptible to Bithor SC (bifenthrin and imidacloprid) diluted at the concentration of 50000 ppm (10-d KT_{50} = 26.7 h [95% FL: 18.3 – 41.5 h], 10-d KT_{95} = 230.1 h [95% FL: 113.6 – 954.0 h], and Icon CS (lambda-cyhalothrin) with a diluted concentration of 10000 ppm (10-d KT_{50} = 123.1 h [95% FL: 60.1 – 1289.2 h], 10-d KT_{95} = 2084.5 h [95% FL: 392.7 – 1.3×10^6 h]) (Table 3). Icon CS (lambda-cyhalothrin) exhibited excellency in knocking down *C. hemipterus* late instars at higher diluted concentrations (50000 ppm, 10-d KT_{50} = 97.2 h [95% FL: 51.2 – 537.0 h], 10-d KT_{95} = 1644.0 h [95% FL: 362.9 – 2.1×10^5 h]; 10000 ppm, 10-d KT_{50} = 244.8 h [95% FL: 83.4 – 24063.7 h], 10-d KT_{95} = 8563.4 h [95% FL: 784.5 – 7.0×10^7 h]) (Table 4). *C. hemipterus* early instars were persistently knocked down at a faster rate treated with Bithor SC (bifenthrin and imidacloprid), Ridesco WG (alpha-cypermethrin and dinotefuran), and Icon CS (lambda-cyhalothrin) diluted at 10000 ppm in contrast to 50000 ppm (Table 5).

Table 3. Knockdown time (KT_{50} and KT_{95}) values of *C. hemipterus* adults treated with three insecticides (pyrethroid-neonicotinoid and pyrethroid) on two contact surfaces (porous and non-porous) at three concentrations (1000 ppm, 10000 ppm, and 50000 ppm)

Insecticide	Con. (ppm)	Porous Surface				Non-Porous Surface			
		LT_{50} (95% FL) (h)	**LT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**	**LT_{50} (95% FL) (h)**	**LT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**
Bithor SC	50000	26.7 (18.3–41.5)a	230.1 (113.6–954.0)b	1.3±2.0	2.6(7)	2.8 (1.5–5.1)c	19.4 (9.5–89.5)a	1.4±0.0	11.3(7)
	10000	235.1 (87.9–8.8×10^9)a	2633.7 (331.2–7.5×10^{20})a	1.1±3.0	0.6(7)	9.2 (6.4–13.0)b	62.5 (38.5–132.4)a	2.0±2.0	1.2(7)
	1000	1587.4 (164.3–1.6×10^{11})a	8.8×10^5 (6171.6–3.7×10^{24})a	0.4±1.6	2.3(7)	94.9 (46.6–456.6)a	2706.0 (528.1–2.0×10^5)a	0.9±1.6	5.5(7)
Ridesco WG	50000	408.5	3044.1	0.0±1.5	0.6(7)	0.2 (0.1–0.3)	0.7 (0.5–1.4)	2.9±2.6	3.1(10)
	10000	408.5	3044.1	0.0±1.5	0.6(7)	0.2 (0.1–0.2)	0.6 (0.4–1.3)	1.7±1.0	5.2(10)
	1000	1301.9 (160.3–6.1×10^{75})a	96828.2 (1530.7–1.1×10^{157})a	0.5±2.1	1.1(7)	0.2 (0.1–0.3)b	0.7 (0.5–1.4)c	2.9±2.6	3.2(10)
Icon CS	50000	322.9 (92.1–44799.4)a	21448.6 (1341.2–3.4×10^9)a	0.5±1.6	2.2(7)	49.0 (24.8–171.4)a	2950.4 (544.6–1.6×10^5)a	0.6±1.2	3.4(7)
	10000	**123.1 (60.1–1289.2)a**	**2084.5 (392.7–1.3×10^6)a**	0.7±1.8	5.7(7)	129.3 (59.8–1306.2)a	2895.6 (487.8–1.5×10^6)a	0.8±2.0	1.4(7)
	1000	ND	ND	ND	ND	6049.2	5.1×10^5	0.0±1.5	3.8(7)

ND: Not determined; no mortality was detected, or data could not be generated by Probit analysis owing to a larger (>0.4) g value.
Knockdown values followed by same latter in each row are not significantly different (overlapping of fiducial values).
Bold letters wordings represent values following dose-independent trend.

Table 4. Knockdown time (KT_{50} and KT_{95}) values of *C. hemipterus* late instars treated with three insecticides (pyrethroid-neonicotinoid and pyrethroid) on two contact surfaces (porous and non-porous) at three concentrations (1000 ppm, 10000 ppm, and 50000 ppm)

Insecticide	Con. (ppm)	Porous Surface				Non-Porous Surface			
		KT_{50} (95% FL) (h)	**KT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**	**KT_{50} (95%FL) (h)**	**KT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**
Bithor SC	50000	175.1 (74.3–8588.9)a	2943.5 (434.8–5.4×10^7)a	1.0±2.6	1.2(7)	19.8 (13.3–30.5)b	211.3 (104.9–760.5)a	0.4±0.4	8.7(7)
	10000	303.8 (93.3–2.0×10^5)a	10259. 3 (787.1–5.7×10^{10})a	0.5±1.8	5.3(7)	39.3 (29.5–58.5)b	**168.7 (95.8–638.0)a**	2.5±4.0	2.4(7)
	1000	ND	ND	ND	ND	110.6 (73.2–2.7×1011)	315.8 (131.4–4.8×10^{26})	4.0±8.2	0.3(7)
Ridesco WG	50000	287.9	1901.2	2.0±4.9	0.5(7)	0.4 (0.3–0.6)	3.2 (1.8–7.9)	1.8±0.6	1.0(10)
	10000	ND	ND	ND	ND	1.2 (0.9–1.8)	9.0 (5.1–22.7)	1.8±0.2	4.5(10)
	1000	1238.4	14487.7	ND	1.1(7)	36.6 (19.1–100.4)	2364.0 (531.9–42423.7)	1.0±1.5	2.7(10)
Icon CS	50000	97.2 (51.2–537.0)a	1644.0 (362.9–2.1×10^5)a	1.2±2.8	2.6(7)	106.7 (20.0–1.3×10^9)a	5.3×10^6 (9718.5–4.4×10^{41}) a	0.5±0.8	1.4(7)
	10000	244.8 (83.4–24063.7)a	8563.4 (784.5–7.0×10^7)a	0.8±2.4	2.6(7)	66.7 (40.0–199.0)a	863.8 (258.0–24846.1)a	1.2±2.4	1.0(7)
	1000	1238.4	14487.7	ND	1.1(7)	603.4 (118.2–2.7×10^6)	84749.6 (2401.4–3.4×10^{13})	0.4±1.6	2.1(7)

ND: Not determined; no mortality was detected, or data could not be generated by Probit analysis owing to a larger (>0.4) g value.
Knockdown values followed by same latter in each row are not significantly different (overlapping of fiducial values).
Bold letters wordings represent values following dose-independent trend.

Table 5. Knockdown time (KT_{50} and KT_{95}) values of *C. hemipterus* early instars treated with three insecticides (pyrethroid-neonicotinoid and pyrethroid) on two contact surfaces (porous and non-porous) at three concentrations (1000 ppm, 10000 ppm, and 50000 ppm)

Insecticide	Con. (ppm)	Porous Surface				Non-Porous Surface			
		KT_{50} (95% FL) (h)	**KT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**	**KT_{50} (95% FL) (h)**	**KT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**
Bithor SC	50000	**796.5 (119.4–1.8×10^7)a**	**4.2×10^5 (5323.1–2.7×10^{16})a**	0.6±1.6	1.6(7)	8.3(3.7–17.9)b	106.9 (39.6–1179.8)a	0.7±0.2	14.3(7)
	10000	**122.3 (62.2–1637.8)a**	**1483.9 (311.2–1.6×10^6) a**	1.4±2.9	4.3(7)	**48.4 (30.7–103.5)a**	**592.6 (213.8–6278.0)a**	1.3±2.0	4.7(7)
	1000	4338.0	8.8 × 10^5	0.0±1.5	1.5(7)	36.3 (26.4–55.5)	196.2 (103.6–912.1)	2.5±4.5	1.3(7)
Ridesco WG	50000	**4338.0**	**8.8 × 10^5**	0.0±1.5	1.5(7)	0.2 (0.2–0.3)	0.9 (0.6–1.6)	2.5±1.5	1.9(10)
	10000	**601.7 (124.2–1.7×10^{13})a**	**22927.2 (922.5–1.4×10^{27}) a**	0.8±2.4	1.4(7)	0.2 (0.1–0.3)b	0.8c (0.5–1.6)	2.5±2.0	1.5(10)
	1000	ND	ND	ND	ND	14.5 (7.5–36.1)	2314.5 (467.4–46881.5)	0.8±1.2	6.1(10)
Icon CS	50000	**441.9 (107.9–1.3×10^6)a**	**25977.9 (1256.6–3.0×10^{12})a**	0.4±1.5	2.4(7)	7.4 (3.8–14.3)b	624.3 (166.2–9857.2)a	0.8±0.9	3.3(7)
	10000	**194.0 (74.8–7935.4)a**	**5477.8 (636.4–6.8×10^7) a**	0.4±1.3	7.2(7)	13.6 (5.4–59.2)b	13438.1 (848.0–1.4×10^8)a	0.8±1.1	4.1(7)
	1000	816.5	16066.4	0.0±1.5	1.5(7)	200.8 (80.3–91634.5)	2997.7 (400.1–9.7×10^9)	1.1±3.0	2.7(7)

ND: Not determined; no mortality was detected, or data could not be generated by Probit analysis owing to a larger (>0.4) g value.
Knockdown values followed by same latter in each row are not significantly different (overlapping of fiducial values).
Bold letters wordings represent values following dose-independent trend.

Table 6. Lethal time (LT_{50} and LT_{95}) values of *C. hemipterus* adults treated with three insecticides (pyrethroid-neonicotinoid and pyrethroid) on two contact surfaces (porous and non-porous) at three concentrations (1000 ppm, 10000 ppm, and 50000 ppm)

Insecticide	Con. (ppm)	Porous Surface				Non-Porous Surface			
		LT_{50} (95% FL) (h)	**LT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**	**LT_{50} (95% FL) (h)**	**LT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**
Bithor SC	50000	148.5 (124.8–182.2)a	524.0 (358.2–1098.7)b	3.8±7.5	7.6(14)	**843.8 (338.9–7868.4)b**	**1.0×10^5 ($9891.3–5.5\times10^7$)c**	0.7±1.9	3.9(14)
	10000	206.9 (166.2–302.1)a	916.4 (516.0–3475.1)a	3.1±7.0	1.6(14)	**414.4 (260.7–1760.3)a**	**3130.8 (1000.8–1.6×10^5)a**	2.5±6.0	3.8(14)
	1000	223.7 (152.0–436.1)a	3839.5 (1333.7–39133.6)a	1.0±2.4	2.9(14)	619.1 (322.6–30974.0)a	4386.8 (1031.9–4.5×10^7)a	1.7±4.6	2.4(14)
Ridesco WG	50000	242.2 (192.8–387.5)a	930.0 (516.0–4223.5)b	2.0±4.8	3.3(14)	30.9 (21.8–41.2)c	272.8 (182.5–488.4)a	1.6±2.0	6.4(14)
	10000	267.7 (206.3–486.6)a	1113.1 (570.4–6990.5)b	2.5±6.0	2.1(14)	52.5 (39.8–66.3)c	290.3 (201.6–516.2)a	8.6±10.6	3.4(13)
	1000	257.2 (176.1–518.6)a	3279.5 (1197.1–32303.8)a	1.3±2.9	5.4(14)	62.3 (46.2–83.0)b	565.9 (338.3–1316.6)a	1.6±2.0	15.7(13)
Icon CS	50000	460.1 (288.5–21360.0)a	1709.5 (613.7–1.5×10^7)a	16.7±40.0	11.9(14)	146.9 (234.0–1066.6)a	1842.2 (733.1–43682.1)a	2.0±5.2	1.3(14)
	10000	904.3 (380.2–23645.3)a	23375.1 (3038.3–8.8 $\times10^7$)a	0.7±2.4	12.6(14)	434.2 (265.8–2134.2)a	3611.3 (1074.7–2.8×10^5)a	1.0±3.0	3.0(14)
	1000	ND	ND	ND	ND	5117.5	52032.8	ND	3.4(14)

ND: Not determined; no mortality was detected, or data could not be generated by Probit analysis owing to a larger (>0.4) g value.
Knockdown values followed by same latter in each row are not significantly different (overlapping of fiducial values).
Bold letters wordings represent values following dose-independent trend.

In the stance of non-porous surface, Ridesco WG (alpha-cypermethrin and dinotefuran) demonstrated a consistently excellent knockdown effect against all developmental stages of *C. hemipterus* regardless of the concentration (10-d KT_{50} = 0.2 – 1.2 h [95% FL: 0.1 – 1.8 h], 10-d KT_{95} = 0.6 – 9.0 h [95% FL: 0.4 – 22.7 h], except for 1000 ppm treated against late instars and early instars) (Tables 3, 4 and 5).

The ten-day lethal time (10-d LT_{50} and 10-d LT_{95}) values of *C. hemipterus* were shown in Tables 6 (adults), 7 (late instars), and 8 (early instars). Based on the LT values generated, the order of relative toxicity against all developmental stages of *C. hemipterus* was as follows: porous surface – Bithor SC (bifenthrin and imidacloprid) > Icon CS (lambda-cyhalothrin) > Ridesco WG (alpha-cypermethrin and dinotefuran); non-porous surface – Ridesco WG (alpha-cypermethrin and dinotefuran) > Bithor SC (bifenthrin and imidacloprid) > Icon CS (lambda-cyhalothrin).

In the stance of porous surface, Bithor SC (bifenthrin and imidacloprid) outperformed the other insecticides in terms of killing *C. hemipterus* adults irrespective of the diluted concentrations (10-d LT_{50} = 148.5 – 223.7 h [95% FL: 124.8 – 436.1 h], 10-d LT_{95} = 524.0 – 3839.5 h [95% FL: 358.2 – 39133.6 h]) (Table 6). *C. hemipterus* late instars were most susceptible to Icon CS (lambda-cyhalothrin) at three diluted concentrations (10-d LT_{50} = 220.9 – 515.8 h [95% FL: 163.3 – 1.2 × 10_{10} h], 10-d LT_{95} = 1800.5 – 1978.2 h [95% FL: 624.6 – 5.9 × 10_{10} h]) (Table 7). The contact toxicity of all insecticide treatment for *C. hemipterus* early instars on porous surfaces revealed that the diluted concentration of 10000 ppm caused higher mortality efficacy in comparison to 50000 ppm, with Bithor SC (bifenthrin and imidacloprid) contributing the lowest 10-d LT_{50} (148.7 h [95% FL: 114.7 – 200.9 h]) and 10-d LT_{95} (1014.9 h [95% FL: 560.0 – 3227.8 h]) values (Table 8).

In the stance of non-porous surface, Ridesco WG (alpha-cypermethrin and dinotefuran) was regarded the most ideal option to cause mortality on *C. hemipterus* over the shortest time (10-d LT_{50} = 15.4 – 249.6 h [95% FL: 9.7 – 464.8 h], 10-d LT_{95} = 272.8 – 2164.7 h [95% FL: 182.5 – 16224.8 h]) (Tables 6, 7 and 8). Dose-independent response was observed occasionally in the diluted concentration of 10000 ppm among all insecticide products applied at non-porous surface (Bithor SC (bifenthrin and imidacloprid) against adults and late instars; Ridesco WG (alpha-cypermethrin and dinotefuran) against late and early instars; Icon CS (lambda-cyhalothrin) against late instars).

Table 7. Lethal time (LT_{50} and LT_{95}) values of *C. hemipterus* late instars treated with three insecticides (pyrethroid-neonicotinoid and pyrethroid) on two contact surfaces (porous and non-porous) at three concentrations (1000 ppm, 10000 ppm, and 50000 ppm)

Insecticide	Con. (ppm)	Porous Surface				Non-Porous Surface			
		LT_{50} (95% FL) (h)	**LT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**	**LT_{50} (95% FL) (h)**	**LT_{95} (95% FL) (h)**	**Slope±SE**	**χ^2 (df)**
Bithor SC	50000	329.3 (217.3–888.0)a	3382.1 (1125.8–71485.3)a	1.0±2.8	2.6(14)	456.1 (273.8–2395.7)a	3904.1 (1127.9–3.3×10^5)a	1.8±4.4	2.4(14)
	10000	411.1 (243.6–1466.9)a	**6715.7 (1741.0–2.8×10^5)a**	1.7±4.0	2.7(14)	585.8 (314.0–2.5×10^{34})a	**2301.9 (639.8–6.2×10^{71})a**	8.3±20.6	5.6(14)
	1000	707.0 (336.4–1.7×10^8)	4153.5 (879.5–2.6×10^{15})	1.0±3.5	1.3(14)	ND	ND	ND	ND
Ridesco WG	50000	**1875.5**	**24790.7**	0.0±1.5	1.8(14)	44.7 (18.1–100.2)c	671.2 (232.6–11501.9)c	1.0±1.5	60.2(14)
	10000	ND	ND	ND	ND	82.3 (66.0–100.2)	**360.8 (259.7–612.2)**	3.3±6.0	6.0(14)
	1000	**1083.0**	**7322.9**	0.0±1.5	1.7(14)	112.4 (85.1–154.7)	1096.8 (585.9–3394.7)	2. ±3.6	10.3(14)
Icon CS	50000	220.9 (163.3–374.9)a	1800.5 (807.3–10853.8)a	1.3±2.7	5.8(14)	771.7 (349.2–9263.0)a	21974.1 (3225.6–1.5×10^7)a	1.0±2.8	2.3(14)
	10000	464.4 (283.1–2962.0)a	**2924.3 (921.7–3.2×10^5)a**	1.5±4.0	3.9(14)	**402.8 (263.1–1662.6)a**	**2244.0 (813.1–93757.5)a**	1.8±4.4	3.9 14)
	1000	515.8 (298.2–1.2×10^6)a	1978.2 (624.6–5.9×10^{10})a	2.0±5.6	1.3(14)	2591.8 (621.3–1.4×10^7)a	1.5×10^5 (6964.0–4.4×10^{13})a	0.4±2.1	2.0(14)

ND: Not determined; no mortality was detected, or data could not be generated by Probit analysis owing to a larger (>0.4) g value.
Knockdown values followed by same latter in each row are not significantly different (overlapping of fiducial values).
Bold letters wordings represent values following dose-independent trend.

Table 8. Lethal time (LT_{50} and LT_{95}) values of *C. hemipterus* early instars treated with three insecticides (pyrethroid-neonicotinoid and pyrethroid) on two contact surfaces (porous and non-porous) at three concentrations (1000 ppm, 10000 ppm, and 50000 ppm)

Insecticide	Con. (ppm)	Porous surface LT_{50} (95% FL) (h)	Porous surface LT_{95} (95% FL) (h)	Slope±SE	χ^2 (df)	Non-porous surface LT_{50} (95% FL) (h)	Non-porous surface LT_{95} (95% FL) (h)	Slope±SE	χ^2 (df)
Bithor SC	50000	205.7 (149.0–348.4)a	2183.1 (931.7–13271.9)b	1.8 ±3.8	5.8(14)	40.2 (26.1–60.9) c	1696.1 (746.6–6336.5)b	0.8±1.0	7.3(14)
	10000	**148.7 (114.7–200.9)a**	**1014.9 (560.0–3227.8)b**	2.1 ±4.5	1.0(14)	161.8 (121.0–245.1)a	1627.8 (762.9–7599.3)b	2.5±5.0	2.6 14)
	1000	3875.3 (784.6–5.4×10^6)a	5.7×10^5 (17069.3–9.7×10^{12})a	0.7 ±2.3	6.1(14)	185.9 (144.5–275.5)b	1199.7 (619.0–4998.2)a	1.6 ±3.3	7.0(14)
Ridesco WG	50000	244.6 (198.4–391.3)a	790.9 (459.6–3240.7)b	5.0 ±11.5	8.1(14)	17.9 (10.3–29.3)c	1843.9 (701.0–8844.9)b	1.3 ±1.3	14.0(14)
	10000	**187.4 (138.4–298.7)a**	**1855.1 (840.2–9521.8)b**	1.8 ±3.8	4.8(14)	**15.4 (9.7–23.4)c**	**611.5 (308.3–1674.2)b**	1.2 ±0.9	14.4(14)
	1000	276.6 (216.8–538.3)a	918.8 (493.6–6934.1)b	5.0 ±12.0	3.3(14)	249.6 (179.2–464.8)a	2164.7 (905.8–16224.8)b	2.5 ±5.0	8.4(14)
Icon CS	50000	**2886.0 (674.0–6.2×10^5)a**	**5.8×10^5 (20193.4–2.9×10^{11})a**	0.3 ±1.5	4.2(14)	274.2 (177.8–631.9)b	5142.5 (1575.7–81937.8)a	1.7 ±4.0	1.6(14)
	10000	**1048.0 (400.8–16566.6)a**	**77225.2 (7358.0–1.1×10^8)a**	0.5 ±1.8	4.1(14)	289.1 (171.7–750.4)a	11521.0 (2784.5–2.6×10^5)a	1.4 ±3.4	3.8(14)
	1000	**965.5 (392.9–3.3×10^5)**	**12604.5 (1774.4–8.2×10^9)**	1.0 ±3.2	1.8(14)	670.8	**2726.2**	0.0 ±1.1	3.2(14)

ND: Not determined; no mortality was detected, or data could not be generated by Probit analysis owing to a larger (>0.4) g value.
Knockdown values followed by same latter in each row are not significantly different (overlapping of fiducial values).
Bold letters wordings represent values following dose-independent trend.

Hatching Rates of Insecticide-Treated *C. hemipterus* Eggs

The hatching rates of *C. hemipterus* eggs immersed in three concentrations three technical-grade insecticide formulations at ten days posttreatment were illustrated (Figure 2). The treatment of Ridesco WG (alpha-cypermethrin and dinotefuran) diluted at 50000 ppm contributed a significantly lower mean hatching rate of *C. hemipterus* eggs (3.13%) as compared to the other dilutions from Ridesco WG (alpha-cypermethrin and dinotefuran), Bithor SC (bifenthrin and imidacloprid), and Icon CS (lambda-cyhalothrin) (between 84.17 to 100.00%) (ANOVA; $F_{11,24} = 9.709$, df = 11, $P<0.001$). It was statistically proven to be the only insecticide with a strong ovicidal effect in this study.

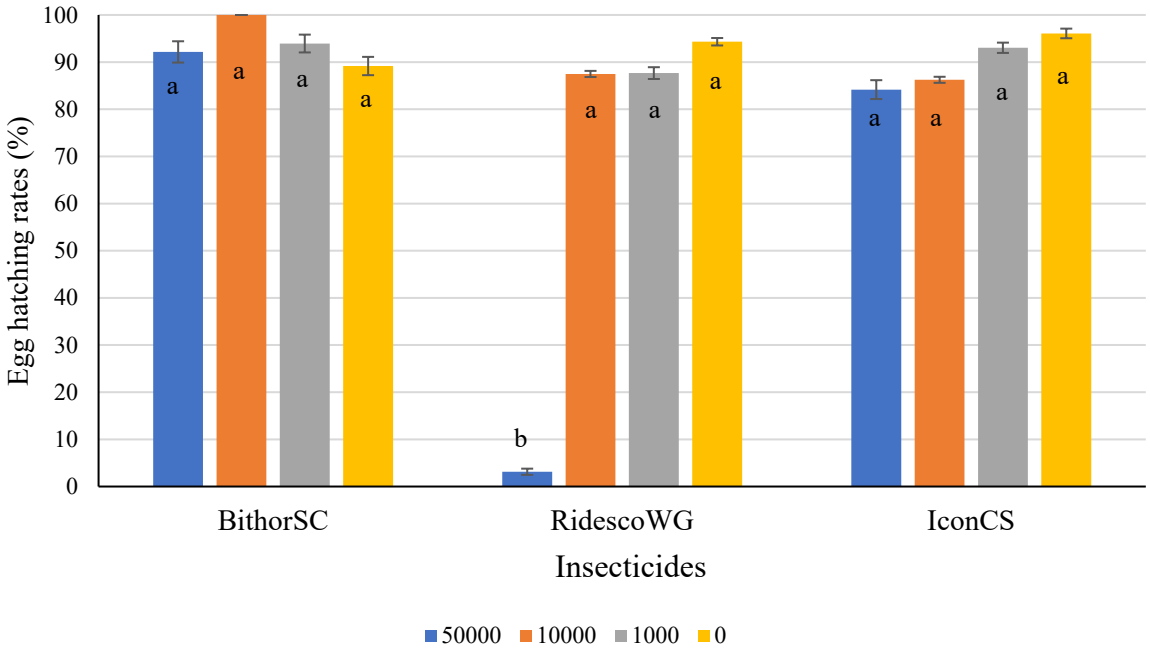

Figure 2. Influence of insecticides (Bithor SC, Ridesco WG, and Icon CS) on hatching rates of *C. hemipterus* eggs at 10 days posttreatment. Bars with different letters are significantly different (one-way ANOVA, $P < 0.05$). Error bars indicate ± standard error (SE) values

DISCUSSION

The overall insecticidal efficacy against tropical bed bugs in this study was summarized as follows: Bithor SC (bifenthrin and imidacloprid) performed the best on porous surfaces, followed by Icon CS (lambda-cyhalothrin) and Ridesco WG (alpha-cypermethrin and dinotefuran), while Ridesco WG (alpha-cypermethrin and dinotefuran) was the most effective on non-porous surfaces, followed by Bithor SC (bifenthrin and imidacloprid) and Icon CS (lambda-cyhalothrin). These results suggested that the Penang Island-strain bed bugs have moderate to high susceptibility to pyrethroid-neonicotinoid insecticides but lower to moderate susceptibility to pyrethroid insecticides.

Typically, insecticides comprised of pyrethroid alone exhibit reduced knockdown and mortality efficacy while the combination of pyrethroid and neonicotinoid, attributed to the

resistance of bed bugs to pyrethroid insecticides (Adelman et al. 2011; Davies et al., 2012; Zhu et al. 2013). This may explain the low susceptibility of bed bugs to pyrethroid (Icon CS) in this study. As for the moderate susceptibility to pyrethroid-neonicotinoid insecticides, this may be due to bed bugs having developed cross-resistance. The mode of action of pyrethroids is to interfere with sodium channels, while neonicotinoids affect nicotinic acetylcholine receptors (Khalid et al. 2019). Despite acting on different parts of the nerve neurons, there is a high probability of developing cross-resistance between these two types of insecticides due to the fact that they collectively act on similar neuronal sites (Gordon et al. 2014). Dang et al. (2017) highlighted similar mechanisms of detoxification observed between pyrethroids and neonicotinoids, and these detoxification mechanisms are pathways through which insects may develop resistance to insecticides.

The literature reviews on the toxicity of dual-action insecticides against bed bugs are limited. Cross-referencing with studies involving other organisms can reveal the efficacy performance profiles of dual-action insecticides. For example, Larson et al. (2014) showed that premix insecticides of chlothianidin (neonicotinoid) and bifenthrin (pyrethroid) had a significantly higher knockdown effect on beneficial insects (ground beetles, scarab grubs, black cutworms, and bumble bees) but did not exhibit synergistic or additive effects while Jones et al. (2018) reported a mixture of bifenthrin and imidacloprid compromised the life table of tarnished plant bugs, showing an antagonistic effect. Nevertheless, the Fluodora Fusion, a novel product combining clothianidin (neonicotinoid) and deltamethrin (pyrethroid), showed high efficacy in pyrethroid-resistant *Anopheles gambiae* populations in multiple African countries (Agossa et al. 2018; Zoh et al. 2021; Dieng et al. 2017). The outcomes of these studies demonstrate the species-independent nature of pyrethroid-neonicotinoid insecticides. The inconsistent and ambiguous results indicate that the effects may vary across different species.

Delayed efficacy in both knockdown and mortality of bed bugs was noted for each tested insecticide, except for Ridesco WG when applied on a non-porous substrate, demonstrating only little delay in efficacy. The delay may be due to the variation in insecticide dilutions and toxicity ratios. Even though insecticide dilutions were adjusted from the recommended label application rates, there was a consistent tendency to apply differing amounts of active ingredients. This leads to an imbalance in toxicity ratios among the tested insecticide products (Ashbrook et al. 2017). Other than this, the delay could also be due to the physicochemical characteristics of the treatment surface. Porous surfaces generally absorb more insecticides compared to non-porous surfaces (Jenson et al. 2009; Rust 1995). The performance of tested insecticide formulations (suspension concentrate, water-dispersible granule, and capsule suspension) was influenced by the type of substrate. Thus, it is essential to understand which insecticide is suitable for which substrate before conducting residual treatment (Wang et al. 2016).

Results of our study also revealed occasional responses independent of concentration for each tested insecticide. Based on knockdown and lethal time values from Probit analysis, the insecticides showed greater efficacy at the recommended concentration (10000 ppm) than at a higher concentration (50000 ppm). These observations imply that the bed bugs from a particular region may be linked to the historical use of certain insecticides in that area (Gordon et al. 2014). This could suggest that bed bugs in different locations may develop unique traits or resistance patterns based on their exposure to specific insecticides over time.

All treatments exhibited high hatching rates from *C. hemipterus* eggs, except for Ridesco SC (diluted at 50000 ppm) (Figure 2), indicating a potential occurrence of insecticide

resistance. This study demonstrated that Ridesco WG insecticide effectively acted against bed bug eggs, provided it was used at five times the label application rate. This may be due to this level of concentration of Ridesco WG being high toxicity to *C. hemipterus* eggs or the eggs having weak chorionic permeability (Hinson et al. 2016). Other factors influencing susceptibility in bed bug eggs encompass eggshell adaptations, structures, and the development of body systems (Campbell & Miller 2015).

CONCLUSION

The efficacy of pyrethroid-neonicotinoid mixture formulations used in this study against tropical bed bugs was mediocre, highlighting the importance of understanding insecticide resistance mechanisms and exploring innovative formulations to manage resistance issues. This study also demonstrated delayed efficacy in knockdown and mortality of bed bugs with varying responses across different insecticide concentrations and surface types, emphasizing the need for tailored insecticide strategies to control bed bug populations effectively.

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AUTHORS DECLARATIONS

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Conflict of Interest
The authors declared no conflict of interest.

Ethics Declarations
The method of bed bug rearing was approved by the Human Research Ethics Committee USM (HREC) (Code reference: USM/JEPeM/19120868).

Data Availability Statement
This is a Final Year Project (FYP) and the data are currently in thesis of Kok Yean Von (2022).

Authors' Contributions
Kok Yean Von (KYV): Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Validation; Visualization; Writing – original draft; Writing – review & editing. Li Lim (LL) : Writing – original draft; Writing – review & editing. Abdul Hafiz Ab Majid (AHAM): Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Validation; Writing – review & editing.

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