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Surface contact toxicity and synergism of several insecticides against different stages of the tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae)

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Abstract

BACKGROUND: Five formulated insecticides (lambda-cyhalothrin at 10 mg m^{-2}, bifenthrin at 50 mg m^{-2}, fipronil at 10 mg m^{-2}, fenitrothion at 50 mg m^{-2}, imidacloprid at 5 mg m^{-2}) and one active ingredient (DDT at 500 mg m^{-2}) were evaluated using a surface contact method against early and late instars and adults of two strains of the tropical bed bug, *Cimex hemipterus* (F.). Synergism of lambda-cyhalothrin and fipronil using piperonyl butoxide (PBO) was also assessed.

RESULTS: The order of susceptibility of different stages of bed bugs was as follows: early stage – lambda-cyhalothrin > bifenthrin = imidacloprid > fipronil > fenitrothion > DDT; late stage – lambda-cyhalothrin > bifenthrin > fenitrothion > imidacloprid > fipronil > DDT; adult – lambda-cyhalothrin > imidacloprid > bifenthrin > fenitrothion > fipronil > DDT. The late instars exhibited significantly higher LT_{50} among the life stages. The addition of PBO to fipronil increased the susceptibility of the insects.

CONCLUSIONS: Lambda-cyhalothrin, bifenthrin, fenitrothion and fipronil at the recommended application rates were effective against *C. hemipterus*. Although imidacloprid demonstrated good initial response against *C. hemipterus*, the insects showed substantial recovery 72 h post-treatment. The late instars (fourth and fifth instars) should be used as the model for toxicological evaluation.

Keywords: *Cimex hemipterus*; tropical bed bug; surface contact toxicity; synergism; piperonyl butoxide

1 INTRODUCTION

The global resurgence of bed bug infestations has been a major issue in many countries,[1] covering the United States,[2–4] the United Kingdom,[1,5,6] Denmark,[7] Europe,[7,8] Canada,[9,10] Italy,[11] Australia,[12–14] Korea,[15] Malaysia and Singapore.[16] Bed bugs are commonly classified as a nuisance pest. Its bite on humans not only causes haemorrhage anaphylactic-like reactions, pruritic macupopular, erythematous lesions, itchiness, urticaria, inflammation and bullous rashes[17–19] but may also indirectly contribute to delusory parasitosis.[20] Besides that, bed bug infestation is also one of the factors for major economic losses in the hospitality and tourism industry.[14,19,21]

Bed bug infestations typically involve two species of bed bug: the common bed bug, *Cimex lectularius* L., and the tropical bed bug, *Cimex hemipterus* (F.). These two species prefer human hosts and are distributed within temperate and tropical regions respectively.[22] In some regions, however, the two species have an overlapping geographical distribution.[12,13,23–26] Bed bugs are known for their cryptic nature, as they hide in cracks and crevices and in furniture within rooms or buildings. Thus, it can be difficult to detect the presence of live bed bugs. Generally, pest control operators (PCOs) only inspect for signs of bed bug infestation such as blood spots, faecal stains and the presence of exuvia or egg cases.[6,21,22] Owing to the above factors, residual insecticide treatment is usually an efficient method to control bed bug infestations.

To date, only a few insecticide products are labelled for bed bug control, and limited studies have been conducted to assess their toxicological effects on bed bugs,[27–32] particularly on *C. hemipterus*.[29] This study was conducted to evaluate the surface contact toxicity of five insecticide formulations and one active ingredient against *C. hemipterus*. In this study, the effects of the insecticides on three different life stages were assessed – early (first and second instars), late (fourth and fifth instars) and adult (male and female). The effects of piperonyl butoxide (PBO) on the toxicity of lambda-cyhalothrin and fipronil were also evaluated.

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2 MATERIALS AND METHODS

2.1 Insects and experimental conditions

Two field-collected populations of *C. hemipterus* that had been reared in the laboratory since 2006 were used: KMelayu14 from Malaysia and Serangoon from Singapore. Both strains had been reared without any insecticide exposure for the last 4 years. They were kept in glass jars (7 cm diameter × 9 cm height) containing folded brown paper as harbourage sites under the environmental conditions of 27 ± 2 °C, 70 ± 5% RH and a 12 : 12 h light : dark photoperiod. All bed bugs used in this study were direct fed with fresh human blood. All tests were conducted under laboratory conditions of 26 ± 2 °C and 60 ± 5% RH.

2.2 Insecticides

A total of six insecticides from five classes were tested: (1) pyrethroids: bifenthrin 800 g L^{-1} SC (Vic® 80SC) and lambda-cyhalothrin 25 g L^{-1} MC (Quest® MC); (2) organophosphate: fenitrothion 200 g L^{-1} MC (Sumithion® 20MC); (3) phenylpyrazole: fipronil 250 g L^{-1} EC (Termidor® 25EC); (4) chloronicotinyl: imidacloprid 200 g L^{-1} SC (Premise® 200SC); (5) chlorinated hydrocarbon: DDT (technical-grade AI). All insecticide formulations were diluted with water, and DDT was dissolved in acetone. The application rates of the insecticides ranged from 5 to 500 mg AI m^{-2}. These followed the manufacturers' recommendation, or those reported earlier for the common bed bug.[31]

2.3 Contact toxicity of formulated insecticides

Three stages were tested – early instars (first and second instars), late instars (fourth and fifth instars) and adults (males and females at a 1 : 1 ratio) – with four replicates of ten insects each. Insecticide dilutions were spread on ceramic tiles and allowed to dry completely by placing overnight inside a fume hood before use. Test insects were introduced onto treated tiles and exposed for 48 h (bifenthrin, lambda-cyhalothrin, fenitrothion and imidacloprid), or for 72 h if there was limited response at 48 h (fipronil and DDT). Control tiles were treated with distilled water or acetone only. The introduced insects were continously confined to the treated surface with a polyethylene ring. The inner walls of the ring were coated with fluon to prevent the insects from escaping. Initially, data were taken at intervals of 30 min for the first 2 h, and subsequently at intervals of 8 h. A test insect was considered dead if it was unable to move and could not right itself when gently probed. After insecticide exposure, all individuals were transferred into a clean plastic container with folded brown paper (as harbourage) and kept under laboratory conditions to observe the mortality of the treated bugs at 72 h post-treatment. Any recovery of tested bugs after 72 h was also recorded.

2.4 Synergism studies

The effects of piperonyl butoxide (PBO) on the toxicity of lambda-cyhalothrin and fipronil were examined. Technical-grade PBO (98%; FMC Co., Agricultural Chemical Division, Middleport, NY) was diluted in acetone. Individuals were temporarily immobilised by chilling them for 3 min at −17 °C before topically treating them with 20 g of PBO on the dorsal surface of the abdomen. Control insects were topically treated with acetone only. Two hours after the treatment, they were transferred onto treated tiles and exposed for up to 48 h. The replicates and sample sizes of the tested insects were the same as those for the residual test with formulated insecticides. After treatment, all insects were transferred to a clean plastic container with a folded brown paper harbourage and kept under laboratory conditions. Post-treatment mortality was recorded after 72 h. Any recovery of tested bugs at post-treatment mortality after 72 h was also recorded.

2.5 Statistical analysis

Data were pooled and subjected to probit analysis[33] using POLO-PC.[34] The LT_{50} values were later used to obtain the relative toxicological ratio (RTR) of various life stages by dividing the highest LT_{50} value by the corresponding LT_{50} of the specific life stage. The synergism ratio (SR) was calculated by dividing the LT_{50} without PBO treatment by the LT_{50} with PBO treatment. All the observed mortalities were corrected by the Schneider–Orelli formula.[35]

3 RESULTS AND DISCUSSION

3.1 Contact toxicity of insecticides to different stages of *Cimex hemipterus*

Based on the LT_{50} values obtained (Table 1), the order of relative contact toxicity for the three stages of both strains was as follows: early stage − lambda-cyhalothrin > bifenthrin = imidacloprid > fipronil > fenitrothion > DDT; late stage − lambda-cyhalothrin > bifenthrin > fenitrothion > imidacloprid > fipronil > DDT; adults − lambda-cyhalothrin > imidacloprid > bifenthrin > fenitrothion > fipronil> DDT.

The present study indicated that the LT_{50} of adult *C. hemipterus* was achieved within 1.63–1.81 h for both strains (Table 1). Steelman *et al.*[31] also conducted a toxicological study of imidacloprid on bed bugs using adult *C. lectularius*, and they reported a low LC_{50} (0.15–6.17 ppm) and LC_{90} (5.8–9.9 ppm) after 24 h of exposure to a treated surface. However, imidacloprid only caused 69.4–72.2% mortality of late-stage and adult bed bugs in the present study (Fig. 1). Besides that, the late-stage and adult bed bugs treated with imidacloprid showed relatively high recovery percentages: 30–40% of late stage and 28–31% of adult bed bugs of both strains recovered during the 72 h post-treatment mortality observation. The other insecticides did not show such recovery. As a comparison, high recovery results of 54% (after 2 weeks) and 72% (after 5 weeks) have also been reported for blood-sucking fleas, *Oropsylla montana* (Baker), after exposure to imidacloprid.[36] This finding indicates that imidacloprid is an effective fast-acting active ingredient, but it has a low killing impact on *C. hemipterus*.

Among the tested insecticide products, the pyrethroids (lambda-cyhalothrin and bifenthrin) and fenitrothion at the application rates used were the most efficient and fastest-acting active ingredients against all tested strains and stages (Table 1, Fig. 1). Although the application rate of bifenthrin used in these residual tests was higher than that of lambda-cyhalothrin, the latter insecticide showed better efficacy for all stages of all strains, except for the late stage of the Serangoon strain, for which bifenthrin and lambda-cyhalothrin had similar results. This result agreed well with those reported by Steelman *et al.*[31] on *C. lectularius*. Figure 1 shows that neither strain of bed bug showed 100% mortality for adult and late stages when tested against pyrethroids. Only 77.5 and 72.5% mortality were recorded at 72 h post-treatment when exposed to bifenthrin at 50 mg m^{-2}. Some earlier studies had reported pyrethroid resistance on bed bugs,[7,28–32,37,38] which was due to *kdr*-type resistance which contributed to insect nerve insensitivity rather than monooxygenase-type resistance.[32,38] Based on a series of biochemical and molecular analyses of *C. lectularius*, Yoon *et al.*[38] concluded that this resistance is due to mutations

Table 1. Susceptibility of two strains of *Cimex hemipterus* to six insecticides

Insecticide (application rate, mg AI m^{-2})	Strain	Stage	*n*	LT_{50} (h) (95% FL)	LT_{95} (h) (95% FL)	Slope (±SE)	χ^2 (df)	RTR[a]
Lambda-cyhalothrin (10)	KMelayu14	Early	40	0.72 (0.67–0.77)	1.69 (1.46–2.05)	4.41 (±0.36)	3.59 (12)	1.40
		Late	40	1.01 (0.98–1.05)	1.64 (1.53–1.80)	7.90 (±0.63)	6.20 (13)	1
		Male	20	0.54 (0.51–0.57)	0.94 (0.84–1.11)	6.79 (±0.71)	8.04 (11)	1.87
		Female	20	0.52 (0.48–0.55)	0.93 (0.80–1.23)	6.48 (±1.07)	0.70 (8)	1.94
		Adult	40	0.55 (0.52–0.59)	1.17 (1.03–1.41)	5.03 (±0.42)	11.68 (11)	1.84
	Serangoon	Early	40	0.69 (0.61–0.76)	3.48 (2.77–4.69)	2.34 (±0.18)	14.77 (16)	8.03
		Late	40	5.54 (4.11–7.50)	847.76 (374.9–2674.2)	0.75 (±0.07)	13.87 (16)	1
		Male	20	0.52 (0.41–0.63)	1.49 (1.03–4.07)	3.60 (±0.68)	6.88 (6)	10.65
		Female	20	1.25 (1.01–1.66)	9.74 (5.09–40.44)	1.84 (±0.36)	3.50 (9)	4.43
		Adult	40	0.74 (0.57–0.92)	10.42 (6.45–22.40)	1.43 (±0.92)	26.37 (17)	7.49
Bifenthrin (50)	KMelayu14	Early	40	1.63 (1.57–1.69)	2.87 (2.66–3.18)	6.68 (±0.53)	4.41 (15)	1.83
		Late	40	2.98 (2.80–3.19)	7.18 (6.18–8.76)	4.30 (±0.34)	13.27 (14)	1
		Male	20	1.41 (1.31–1.52)	3.12 (2.66–3.91)	4.76 (±0.50)	4.17 (15)	2.11
		Female	20	1.69 (1.55–1.85)	4.12 (3.38–5.73)	4.24 (±0.56)	8.05 (13)	1.76
		Adult	40	1.60 (1.50–1.70)	4.82 (4.09–6.03)	3.43 (±0.29)	14.72 (18)	1.86
	Serangoon	Early	40	2.03 (1.82–2.27)	15.31 (11.75–21.47)	1.88 (±0.13)	13.20 (21)	2.11
		Late	40	4.29 (3.99–4.69)	11.13 (9.15–14.76)	3.98 (±0.39)	1.64 (12)	1
		Male	20	3.39 (2.83–4.66)	11.02 (6.89–35.76)	3.22 (±0.73)	2.01 (6)	1.27
		Female	20	3.06 (2.82–3.33)	7.28 (6.10–9.50)	4.38 (±0.50)	3.65 (14)	1.40
		Adult	40	3.58 (3.32–3.89)	11.72 (9.49–15.71)	3.19 (±0.28)	12.35 (16)	1.20
Fenitrothion (50)	KMelayu14	Early	40	9.56 (9.12–10.00)	20.52 (18.68–23.10)	4.96 (±0.32)	4.43 (18)	1.91
		Late	40	18.23 (17.41–19.17)	38.11 (32.76–48.41)	5.14 (±0.61)	12.85 (14)	1
		Male	20	14.76 (13.77–16.92)	29.81 (25.13–39.48)	5.39 (±0.75)	10.37 (12)	1.24
		Female	20	12.82 (11.86–13.75)	25.66 (22.45–31.46)	5.45 (±0.64)	4.87 (12)	1.42
		Adult	40	13.56 (12.63–14.55)	27.39 (24.06–32.90)	5.39 (±0.41)	16.81 (12)	1.34
	Serangoon	Early	40	5.39 (5.00–5.80)	19.58 (16.87–23.53)	2.94 (±0.18)	9.47 (22)	2.79
		Late	40	15.05 (14.10–16.10)	44.73 (37.65–56.67)	3.48 (±0.30)	15.49 (16)	1
		Male	20	12.27 (11.25–13.36)	28.51 (23.63–38.92)	8.04 (±1.08)	3.20 (11)	1.23
		Female	20	13.40 (12.35–14.50)	27.50 (23.50–35.41)	5.27 (±0.68)	0.97 (10)	1.12
		Adult	40	13.09 (12.48–13.75)	28.65 (25.80–32.76)	4.84 (±0.33)	7.71 (18)	1.15
Fipronil (10)	KMelayu14	Early	40	4.98 (4.71–5.24)	9.71 (8.83–11.01)	5.67 (±0.47)	10.77 (11)	10.28
		Late	40	51.21 (46.81–56.36)	157.57 (127.67–213.20)	3.37 (±0.33)	3.68 (9)	1
		Male	20	22.56 (19.94–25.99)	69.03 (52.81–105.07)	3.39 (±0.41)	3.69 (10)	2.27
		Female	20	29.75 (26.47–33.55)	84.11 (67.17–118.44)	3.64 (±0.42)	3.57 (10)	1.72
		Adult	40	25.73 (23.98–27.66)	77.17 (66.50–93.15)	3.45 (±0.23)	3.63 (16)	1.99
	Serangoon	Early	40	6.11 (5.79–6.47)	13.72 (11.99–16.51)	4.68 (±0.40)	11.83 (12)	22.38
		Late	40	136.80 (104.29–235.75)	856.88 (408.89–4164.34)	2.06 (±0.39)	1.59 (9)	1
		Male	20	42.48 (34.99–52.67)	272.64 (167.50–660.72)	2.04 (±0.32)	2.11 (10)	3.22
		Female	20	40.71 (35.05–47.83)	151.46 (110.79–250.91)	2.88 (±0.37)	1.90 (9)	3.36
		Adult	40	42.90 (39.04–47.34)	193.31 (152.19–267.77)	2.52 (±0.21)	3.54 (15)	3.18
Imidacloprid (5)	KMelayu14	Early	40	1.65 (1.59–1.71)	2.84 (2.64–3.12)	6.96 (±0.48)	9.36 (15)	13.72
		Late	40	22.64 (21.68–23.68)	47.53 (42.85–54.36)	5.11 (±0.36)	15.61 (19)	1
		Male	20	1.81 (1.70–1.93)	3.04 (2.68–3.77)	7.32 (±1.05)	4.22 (8)	12.51
		Female	20	1.86 (1.75–1.99)	3.18 (2.79–4.03)	7.09 (±1.09)	4.22 (8)	12.17
		Adult	40	1.84 (1.73–1.94)	3.23 (2.89–3.86)	6.70 (±0.70)	9.63 (9)	12.30
	Serangoon	Early	40	1.39 (1.35–1.43)	1.97 (1.88–2.11)	10.85 (±0.84)	1.64 (10)	17.24
		Late	40	23.97 (22.48–25.62)	66.92 (57.03–83.13)	3.69 (±0.30)	6.75 (15)	1
		Male	20	1.63 (1.55–1.72)	2.34 (2.13–2.72)	10.52 (±1.46)	1.59 (6)	14.70
		Female	20	1.77 (1.66–1.87)	2.89 (2.57–3.54)	7.69 (±1.13)	3.93 (8)	13.54
		Adult	40	1.71 (1.65–1.77)	2.63 (2.46–2.89)	8.80 (±0.77)	8.81 (10)	14.01
DDT[b] (500)	KMelayu14	Early	40	NA	NA	NA	NA	NA
		Late	40	NA	NA	NA	NA	NA
		Male	20	NA	NA	NA	NA	NA
		Female	20	NA	NA	NA	NA	NA
		Adult	40	NA	NA	NA	NA	NA

Table 1. (*Continued*)

Insecticide (application rate, mg AI m^{-2})	Strain	Stage	*n*	LT_{50} (h) (95% FL)	LT_{95} (h) (95% FL)	Slope (±SE)	χ^2 (df)	RTR[a]
	Serangoon	Early	40	NA	NA	NA	NA	NA
		Late	40	NA	NA	NA	NA	NA
		Male	20	NA	NA	NA	NA	NA
		Female	20	NA	NA	NA	NA	NA
		Adult	40	NA	NA	NA	NA	NA

[a] RTR is the relative toxicological ratio of LT_{50} of the late stage to LT_{50} of the specific insect stage.
[b] NA means that data are unavailable; no mortality was detected or data could not be generated by probit analysis owing to a larger (>0.4) *g* value.[33]

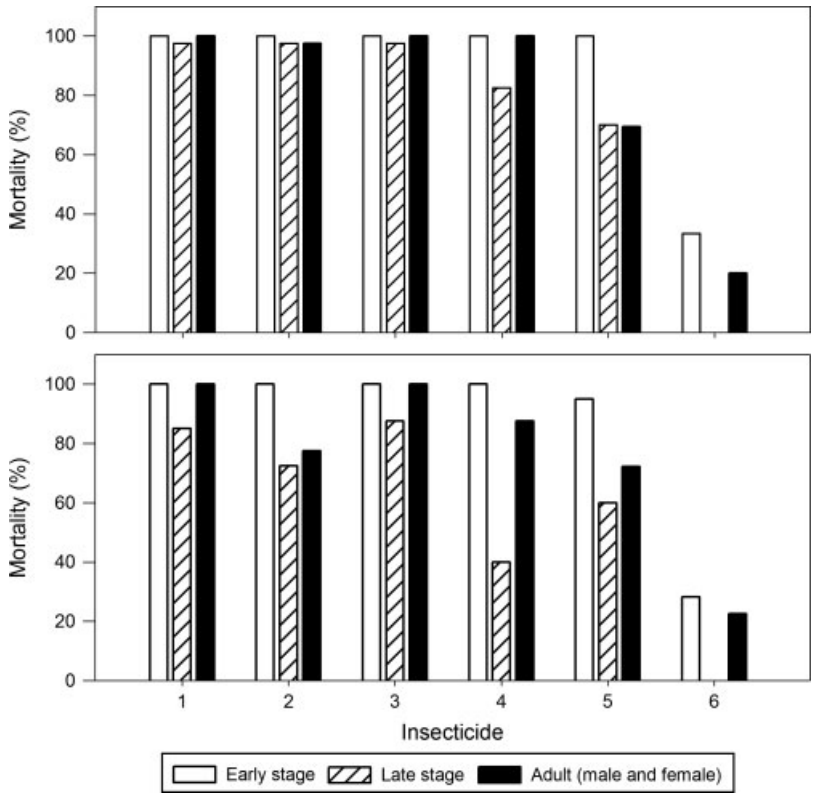

Figure 1. Percentage mortality of the two field strains (A) KMelayu14 and (B) Serangoon of *Cimex hemipterus* at 72 h post-treatment with (1) lambda-cyhalothrin, (2) bifenthrin, (3) fenitrothion, (4) fipronil, (5) imidacloprid and (6) DDT.

within the sodium channel α-subunit gene. It is not possible to establish whether insecticide resistance was present in the strains studied owing to the lack of a laboratory susceptible strain of *C. hemipterus* for comparison.

Cimex hemipterus of the KMelayu14 strain showed high mortality after exposure to fipronil: both early-stage and adult bed bugs showed 100% mortality, and 82.5% of late-stage bed bugs died. However, fipronil did not cause high mortality in adult and late-stage bed bugs of the Serangoon strain (they exhibited 87.5 and 40% mortality respectively) (Fig. 1). The Serangoon strain adults were 2 times less susceptible to the fipronil treatment compared with the KMelayu14 strain adults, based on LT_{50} values, with as long as 42.9 h for adults and 136.8 h for late-stage instars (Table 1).

The RTR explained the observed differences in the lethal time among the various stages of bed bugs. The late stage was the most tolerant stage; it exhibited the longest LT_{50} value (Table 1) and the lowest mortality percentage for all of the tested insecticides (Fig. 1). In general, the late stage showed a higher tolerance coefficient compared with the early stage and adults (1–2-fold RTR) when exposed to surfaces treated with lambda-cyhalothrin, bifenthrin or fenitrothion. This means that the late-stage insects could withstand a one- or twofold greater exposure time compared with early-stage and adult insects. The exception to this was the Serangoon strain exposed to lambda-cyhalothrin, where early-stage and adult bed bugs exhibited much higher than the late stage, 8.03- and 10.65-fold respectively. The RTR difference of the two strains was significantly higher when other life stages were compared with late stage in the residual test of imidacloprid and fipronil. For example, the time required for imidacloprid to kill late-stage individuals of both strains was 12–17-fold higher than the time required to kill other stages. Some results showed that the LT_{50} of females was significantly higher than that of males (KMelayu strains with bifenthrin and fipronil; Serangoon strains with lambda-cyhalothrin and imidacloprid); this finding

Table 2. Effects of PBO on the susceptibility of *Cimex hemipterus* to lambda-cyhalothrin and fipronil

Insecticide	Strain	Stage	*n*	LT_{50} (h) (95% CL)	LT_{95} (h) (95% CL)	Slope (±SE)	χ^2 (df)	SR[a]	Post-treatment mortality 72 h (%)
Lambda-cyhalothrin + PBO	KMelayu14	Late	40	0.80 (0.72–0.88)	3.16 (2.5–4.15)	2.76 (±0.23)	1.27 (12)	1.26	100
		Male	20	0.37 (0.32–0.42)	1.22 (0.93–1.93)	3.15 (±0.44)	4.79 (9)	1.46	100
		Female	20	0.42 (0.36–0.48)	1.46 (1.12–2.21)	3.05 (±0.39)	3.00 (10)	1.24	100
		Adult	40	0.40 (0.37–0.44)	1.33 (1.11–1.70)	3.16 (±0.27)	7.11 (12)	1.38	100
	Serangoon	Late	40	1.19 (1.04–1.37)	9.78 (7.17–14.77)	1.80 (±0.14)	2.69 (15)	4.66	95
		Male	20	0.42 (0.33–0.55)	2.23 (1.41–5.64)	2.28 (±0.35)	9.99 (9)	1.24	100
		Female	20	0.86 (0.69–1.08)	6.05 (3.78–12.95)	1.94 (±0.25)	6.27 (10)	1.45	100
		Adult	40	0.59 (0.50–0.69)	3.49 (2.43–6.10)	2.13 (±0.19)	18.49 (13)	1.25	100
Fipronil + PBO	KMelayu14	Late	40	7.57 (6.92–8.25)	24.53 (20.36–31.73)	3.22 (±0.29)	7.69 (11)	5.08	100
		Male	20	3.83 (3.33–4.43)	14.06 (10.42–23.02)	2.91 (±0.39)	2.15 (10)	5.23	100
		Female	20	4.95 (4.37–5.64)	14.69 (11.40–22.11)	3.48 (±0.45)	2.06 (9)	4.88	100
		Adult	40	4.36 (3.99–4.77)	14.66 (12.23–18.63)	3.13 (±0.24)	3.28 (12)	5.08	100
	Serangoon	Late	40	6.54 (5.78–7.36)	38.77 (28.46–61.20)	2.13 (±0.22)	3.32 (13)	14.53	100
		Male	20	3.47 (2.85–4.24)	21.84 (14.35–43.53)	2.06 (±0.27)	3.17 (11)	10.05	100
		Female	20	3.81 (3.18–4.50)	18.11 (13.10–30.33)	2.43 (±0.31)	1.25 (11)	11.04	100
		Adult	40	3.72 (3.31–4.17)	21.00 (16.43–29.08)	2.19 (±0.17)	6.52 (15)	10.61	100

[a] SR refers to the relative synergism ratio of LT_{50} without PBO to LT_{50} with PBO.

may suggest that the susceptibility of the adult bed bug could be influenced by the sex of the bug.

3.2 Synergism of PBO

The synergistic effect of PBO + lambda-cyhalothrin on two strains of *C. hemipterus* (Table 2) was similar to that reported by Romero *et al*.,[32] who found that synergism between PBO and deltamethrin increased mortality of all three tested strains of *C. lectularius*. They also showed that the effect of PBO with pyrethroid varied among different strains. In the present study, not only were different results between the strains found, but the synergist effects of PBO on *C. hemipterus* also varied with different life stages and sexes. This was particularly obvious for the Serangoon strain, for which the lethal time against lambda-cyhalothrin was reduced by as much as 4.66-fold after the addition of PBO, while only 1.25-fold reductions were observed for adults (Tables 1 and 2).

PBO showed a higher synergistic effect when used together with fipronil. The SR of fipronil was 4.8–5.2-fold and 10.1–14.5-fold for the LT_{50} (of the late stage and adult) of KMelayu14 and Serangoon strains respectively (Table 2). All bed bugs exposed to PBO and surface contact with fipronil died after 48 h, and no recovery was detected after the 72 h mortality observation (Table 1, Fig. 1).

Other studies have also shown synergism between PBO and fipronil for controlling insects, including the housefly *Musca domestica* (L.),[39–41] the hymenopteran *Diaeretiella rapae* (McIntoch),[42] the homopteran *Bemisia tabaci* (Gennadius),[43] the rice stem borer *Chilo suppressalis* (Walker)[44] and many other important agricultural pests.[45] On the other hand, PBO may show an antagonistic effect or no effect with fipronil, for example against the German cockroach *Blattella germanica* (L.)[39,46] and the western corn rootworm *Diabrotica virgifera virgifera* (LeConte).[47] Fipronil is biotransformed by microsomal monooxygenases to a number of metabolites, the most important of which is fipronil-sulfone, but most insects are susceptible to both the fipronil parent and the metabolite. Durham *et al*.[48] showed that, in the European corn borer *Ostrinia nubilalis* (Hübner), the synergist PBO inhibited the activity of the microsomal monooxygenases and so suppressed the biotransformation process. This had no overall effect on the PBO synergism of fipronil against *O. nubilalis*, as both the parent and metabolite molecules were highly toxic to that insect, but the authors pointed out that small differences between the activity of the two compounds against other insects could lead to the observed variations in PBO synergism mentioned above. Durham *et al*.[48] also suggested that the synergistic effect of PBO on fipronil toxicity may be influenced by penetration enhancement or pharmacokinetic differences among various insects.

Moore and Miller[49] reported a field evaluation using both traditional (pyrethroid product) and novel (non-pyrethroid product) treatment regimes against bed bug (*C. lectularius*) infestation. They found that bed bug infestations within premises could not be totally eliminated, even after multiple applications. The results of the present study suggest that a combination of an insecticide and a synergist (e.g. lambda-cyhalothrin + PBO and fipronil + PBO) may effectively manage bed bug infestations. In addition, insecticides of different modes of action would probably be another option that could be applied against pyrethroid-resistant bed bugs, for example chlorfenapyr in either dry residues or aerosol formulation.[50]

Perti *et al*.[51] suggested that late-stage (fourth- and fifth-instar) bed bugs should be tested in addition to adults in toxicological tests, and the results of the present study support this premise. As the late-stage individuals showed significantly higher tolerance to all six tested insecticides compared with adults or early-stage individuals, they should be used for any insecticide evaluation against bed bugs in future. It is also easier and faster to rear bed bug cultures to the late-instar stage, and using this stage for toxicological tests can reduce the issue of variation in fitness between males and females.

4 SUMMARY AND CONCLUSION

Among the five classes of insecticides, pyrethroid (lambda-cyhalothrin and bifenthrin) and organophosphate (fenitrothion) at the recommended application rates were most effective against

the various stages of *C. hemipterus* tested in the laboratory evaluation. Bed bugs treated with imidacloprid showed a greater recovery rate 72 h post-treatment compared with treatment with other insecticides. Synergism with PBO increased the susceptibility of the test insects to fipronil. The last instar was the most tolerant stage among all the stages tested.

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