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Research

Performance of Commercial Insecticide Formulations Against Different Developmental Stages of Insecticide-Resistant Tropical Bed Bugs (Hemiptera: Cimicidae)

Xin-Yeng Leong,[1] Dae-Yun Kim,[1,2] Kai Dang,[3] G. Veera Singham,[4] Stephen L. Doggett,[5] and Chow-Yang Lee[1,6,7]

[1]Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11700 Penang, Malaysia, [2]Present address: Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkean, 10900 Bangkok, Thailand, [3]Ecolab Australia, 2 Drake Avenue, Macquarie Park, NSW 2113, Australia, [4]Centre for Chemical Biology, Universiti Sains Malaysia, 11900 Bayan Lepas, Penang, Malaysia, [5]Department of Medical Entomology, Pathology West - ICPMR, Westmead Hospital, Westmead, NSW 2145, Australia, [6]Present address: Department of Entomology, University of California, 900 University Avenue, Riverside, CA 92521, and [7]Corresponding author, e-mail: chowyang.lee@ucr.edu

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Abstract

This study examined the presence of insecticide resistance in different developmental stages (adults, first instars, and eggs) of the tropical bed bug, *Cimex hemipterus* (F.) using several insecticide formulations. Adults and first instars of five strains (Queensland, Kuala Lumpur, Bukit Mertajam, Saujana, and Krystal Point) were evaluated using the surface contact method and compared with a susceptible strain (Monheim) of the common bed bug *Cimex lectularius* L. The insecticide formulations were used at their label rates in this study: Tandem (thiamethoxam [11.6%], lambda-cyhalothrin [3.5%]) at 183.96 mg/m^2; Temprid SC (imidacloprid [21%], beta-cyfluthrin [10.5%]) at 106.13 mg/m^2; Sumithion 20CS (fenitrothion [20%]) at 250 mg/m^2; Pesguard FG161 (d-tetramethrin [4.4%], cyphenothrin [13.2%]) at 110 mg/m^2; and Sumithrin 10SEC (d-phenothrin [10%]) at 100 mg/m^2. Results showed a very high level of resistance to Pesguard FG161 (388.3 to >605.0 times) and Sumithrin (302.9 to >365.5 times) in all adults of the strains tested, whereas low to high levels of resistance were registered for Tandem (1.4–4.7 times), Temprid (7.3–16.7 times), and Sumithion (1.2–14.6 times) for adults of all bed bug strains. For first instars, resistance to the former two formulations were high to very high (31.4–118.1 times). In contrast, they showed lower resistance to Tandem, Temprid, and Sumithion (1.0–10.2 times). An immersion method used to test on bed bug eggs found high to very high resistance toward all tested formulations. Results demonstrate that the resistance level varies between bed bug developmental stages.

Key words: *Cimex hemipterus*, developmental stage, insecticide resistance, insecticide formulation

Bed bugs have become an important urban insect pest worldwide in the past two decades. The two species of bed bugs that commonly infest human dwellings are the common bed bug (*Cimex lectularius* L.) and the tropical bed bug (*Cimex hemipterus* (F.)). The modern resurgence of bed bugs is thought to be largely due to the development of insecticide resistance (Boase 2001; Dang et al. 2015a; Lilly et al. 2015; Dang et al. 2017a, b; Doggett et al. 2018; Romero 2018).

Insecticide resistance in bed bugs has been reported for most classes of insecticides (Dang et al. 2017b), including the pyrethroids (Myamba et al. 2002, Durand et al. 2012, Vander Pan et al. 2014, Dang et al. 2015a, Palenchar et al. 2015), carbamates (Karunaratne et al. 2007, Steelman et al. 2008, Tawatsin et al. 2011), organophosphates (Karunaratne et al. 2007, Kilpinen et al. 2011, Tawatsin et al. 2011), chlorinated hydrocarbons (Karunaratne et al. 2007, Tawatsin et al. 2011, Dang et al. 2017a), neonicotinoids (Romero and Anderson 2016, Lilly et al. 2018), and pyrroles (Ashbrook et al. 2017). Furthermore, tolerance has been reported in a pyrethroid-resistant strain of *C. lectularius* when tested on sublabel rate of a desiccant dust (Lilly et al. 2016a). Tolerance in this context is the natural ability to withstand insecticide action, and is not the result of heritable changes caused by the insecticide selection pressure. On the other hand, resistance is the ability of a pest population to withstand the label recommendation of an insecticide product via a genetic change in its susceptibility (Dang et al. 2017b).

Insecticide resistance studies of bed bugs often focus on evaluation of technical grade insecticides (Steelman et al. 2008, Tawatsin

et al. 2011, Dang et al. 2017a, Lee et al. 2018, Romero 2018) as well as commercial insecticide formulations (Campbell and Miller 2015, Vander Pan et al. 2019). Most previous studies evaluated resistance status solely for adult insects (Romero et al. 2010; Kilpinen et al. 2011; Dang et al. 2015b; Lilly et al. 2016a, b; Romero and Anderson 2016; Berenji et al. 2019), although some tested the first instars (How and Lee 2011, Campbell and Miller 2015, Dang et al. 2015a, Hinson et al. 2016) and eggs (Campbell and Miller 2015, Hinson et al. 2016). Additionally, most of these studies concentrated on insecticide resistance in *C. lectularius* but not *C. hemipterus*.

Zahran and Ab Majid (2019) evaluated insecticide resistance of field-collected *C. hemipterus* from Malaysia by exposing them to the World Health Organization (WHO) insecticide-impregnated filter paper, and they reported deltamethrin resistance. Similarly, Karunaratne et al. (2007) tested insecticide resistance in Sri Lanka strains of *C. hemipterus* using WHO insecticide-impregnated papers and found resistance toward several insecticides (DDT, propoxur, malathion, permethrin, and deltamethrin). In another study, Myamba et al. (2002) reported pyrethroid resistance in the Tanzania strain of *C. hemipterus* when using modified WHO mosquito test kits. Dang et al. (2015c) tested for resistance in *C. hemipterus* using d-allethrin-impregnated mats that normally are used for adult mosquito control, and they found pyrethroid resistance. Further investigation using molecular techniques revealed the presence of *kdr* mutations. In addition to *kdr* mutations, other resistance mechanisms such as metabolic resistance also were reported in *C. hemipterus* (Karunaratne 2007, How and Lee 2011, Punchihewa et al. 2019).

In this study, we assessed the resistance of *C. hemipterus* to five commercial insecticide formulations (Tandem, Temprid SC, Sumithion 20CS, Pesguard FG161, and Sumithrin 10SEC) using an immersion method for the egg stage and the surface contact method for the first instar and adult stages.

Materials and Methods

Insects

Five strains of *C. hemipterus* collected from Malaysia (Kuala Lumpur, Bukit Mertajam, Saujana, and Krystal Point) and Australia (Queensland) were used in this study (Table 1). Due to the unavailability of a susceptible strain of *C. hemipterus*, a *C. lectularius* susceptible strain was used for comparison (Monheim). All bed bugs were reared under conditions of 27 ± 2°C, 70 ± 5% RH, and 12 h photoperiod. The insects were kept in 0.5-liter glass jars containing folded brown paper that served as a harborage. They were fed once a week to repletion with defibrinated rabbit blood using an artificial membrane feeder (Hemotek Ltd., Blackburn, England, United Kingdom).

Insecticide Formulations

Five commercial insecticide formulations were evaluated (Table 2). All formulations were diluted with deionized water and tested at the prescribed label rates.

Surface Contact Method

0.9 ml of diluted insecticide was pipetted onto a 90-mm-diameter glass Petri dish, spread evenly, and allowed to dry in a fume hood for 24 h. The control Petri dish was treated with water. Ten unfed first instars aged 5–7 d from each strain were tested. The first instars were introduced to the treatment surface and knockdown time of the insects was observed at regular time interval up to 72 h. Once all the insects were knocked down, they were then transferred to a clean Petri dish and provided with a small piece of folded paper to serve as a harborage. An insect is considered knocked down when it could not right itself within 20 s after being probed gently with soft forceps. Mortality was scored at 72 h posttreatment. Each experiment was replicated three times.

The experiment was repeated using adult bed bugs (five males and five females in each replicate). The adults were introduced to the treatment surface and knockdown time of the adults were observed at regular time interval up to 120 h. Upon all insects were knocked down, they were transferred into a clean Petri dish and provided with a small piece of folded paper to serve as a harborage. Mortality of the tested adult bed bugs was scored at the end of the experiment (120 h posttreatment).

Immersion Method for Eggs

To collect the eggs for the experiment, 30 each of newly fed adult male and female bed bugs (sex ratio 1:1) were placed into a Petri dish containing filter paper (diameter 90 mm, Filtres Fioroni, Ingre, France, Reference No.: 0601A00006). After 3–4 d, the females laid eggs on the filter paper. The number of eggs laid was recorded daily, and all adult insects were removed when the number of eggs deposited reached 50. The immersion test method used was previously described by Campbell and Miller (2015). Eggs aged 4–6 d were used for the experiment to allow maximum development of the embryo. The eggs on the filter paper were immersed in the insecticide solution (Table 2) for 5 s, whereas control eggs were immersed in deionized water. The treated eggs on the filter papers were dried in a fume hood for 4 h before being transferred to a clean Petri dish. Three replicates were used for each insecticide formulation. The hatching rate (%) of the eggs was assessed after 14 d.

We also determined the LC_{50} of each insecticide formulation on eggs. Using a similar method described above, filter papers containing eggs were immersed into a series of different insecticide concentrations and allowed to dry for 4 h before egg hatchability at 14 d posttreatment was observed. Control eggs were treated with deionized water. Each concentration was replicated three times.

Data Analysis

The control mortalities were corrected using Abbott's formula (Abbott 1925) before being subjected to probit analysis using PoloPlus. The knockdown time (KT_{50} and KT_{95}) values were generated using time-response data, whereas concentration-response data obtained from egg immersion experiments were used to generate

Table 1. The bed bug strains employed in this study

Species	Strain	Year established	Notes
C. hemipterus	Kuala Lumpur	2005	Collected from a hotel
	Queensland	~2004	Collected from multiple sources in Queensland, Australia
	Bukit Mertajam	2015	Collected from migrant worker quarters
	Saujana	2015	Collected from university dormitory
	Krystal Point	2015	Collected from migrant worker quarters
C. lectularius	Monheim	~Late 1960s	Lab colony, Monheim, Germany

lethal concentration (LC_{50} and LC_{95}) values. Resistance ratios (RR_{50}) were calculated by dividing the KT_{50} or LC_{50} value of the resistant strain with the corresponding value of the Monheim strain. The classification of resistance followed that of Lee and Lee (2004): ≤1 time = no resistance; >1 to ≤5 time(s) = low resistance; >5 to ≤10 times = moderate resistance; >10 to ≤50 times = high resistance; >50 times = very high resistance. Knockdown time of both adults and first instars were subjected to Kaplan–Meier analysis at $P = 0.05$ using software SPSS v22 (IBM Corp., Armonk, NY).

Results

Surface Contact Method

Tandem caused 100% mortality in the first instars of all resistant strains and killed >80% of all adults except for in the Kuala Lumpur strain (53.3%) (Table 3). Temprid caused >80% mortality of the first instars (except for the Kuala Lumpur strain [63.3%]) but performed poorly against adults of all resistant strains tested (Table 3). Overall, the adults and first instars from all resistant strains showed low resistance to Tandem and moderate to high resistance to Temprid (Table 3). Bed bugs of all resistant strains exposed to Tandem were knocked down in less than 5 h, and the value for Temprid was less than 14 h. The susceptibility of adult bed bugs to Tandem and Temprid was significantly lower than that of first instar bed bugs for all strains (Figs. 1 and 2).

Pesguard FG161 and Sumithrin showed poor performance against adults of resistant strains of *C. hemipterus*, with 120 h posttreatment mortality of 20.0–56.7% and 13.3–50.0%, respectively (Table 4). However, these pyrethroid formulations had a better knockdown effect on first instars. Knockdown time for adults tested with Pesguard FG161 and Sumithrin were significantly longer than that of first instars

Table 2. Insecticide formulations and their active ingredients

Insecticide formulations	Active ingredients (%)	Formulation type	Concentration at which adults and first instars were tested (mg/m²)	Concentration in which eggs were immersed (mg/liter)
Tandem	Thiamethoxam[a] (11.6%), lambda-cyhalothrin[b] (3.5%)	Microencapsulated (M)	183.96	1,300
Temprid SC	Imidacloprid[a] (21%), beta-cyfluthrin[b] (10.5%)	Suspension concentrate (SC)	106.13	750
Sumithion	Fenitrothion[c] (20%)	Capsule suspension (CS)	250	5,000
Pesguard FG161	d-Tetramethrin[b] (4.4%), cyphenothrin[b] (13.2%)	Emulsifiable concentrate (EC)	110	2,200
Sumithrin	d-Phenothrin[b] (10%)	Microemulsion (ME)	100	2,000

[a]Neonicotinoid.
[b]Pyrethroid.
[c]Organophosphate.

Table 3. Knockdown time (KT_{50} and KT_{95}) values of different life stages of bed bugs treated with insecticides formulated with a mixture of pyrethroids and neonicotinoids that were applied at the label rate

Insecticide formulations	Stage	Strain	KT_{50} (95% FL) (minute)	KT_{95} (95% FL) (minute)	Slope ± SE	χ^2 (df)	Percentage mortality (%)[a]	RR_{50}
Tandem	First instar	Monheim	27.3 (26.1–28.3)	35.8 (33.6–40.2)	13.9 ± 1.7	8.3 (7)	100	–
		Queensland	48.7 (45.8–51.9)	121.2 (105.0–147.1)	4.2 ± 0.4	9.3 (17)	100	1.8
		Kuala Lumpur	27.2 (26.0–28.2)	35.8 (34.0–39.0)	13.9 ± 2.0	3.1 (5)	100	1.0
		Bukit Mertajam	28.7 (27.4–30.1)	49.1 (45.0–55.6)	7.1 ± 0.7	8.4 (11)	100	1.1
		Saujana	31.4 (29.4–33.3)	59.0 (52.8–69.2)	6.0 ± 0.6	1.6 (9)	100	1.2
		Krystal Point	34.5 (32.7–36.1)	52.6 (48.8–58.5)	9.0 ± 0.9	2.5 (7)	100	1.3
	Adult	Monheim	26.6 (25.9–27.4)	37.6 (35.5–40.8)	10.9 ± 1.0	8.3 (13)	100	–
		Queensland	60.3 (56.4–64.1)	130.7 (115.7–154.7)	4.9 ± 0.4	3.3 (13)	93.3 ± 6.7	2.3
		Kuala Lumpur	35.9 (34.1–37.5)	56.9 (52.6–63.7)	8.2 ± 0.9	2.4 (9)	53.3 ± 12.0	1.4
		Bukit Mertajam	66.8 (60.6–74.0)	261.9 (197.2–409.0)	3.9 ± 0.5	1.9 (8)	83.3 ± 3.3	2.5
		Saujana	124.6 (115.1–136.1)	271.5 (231.4–340.2)	4.9 ± 0.5	4.8 (9)	96.7 ± 3.3	4.7
		Krystal Point	71.9 (68.5–75.2)	116.4 (105.6–135.5)	7.8 ± 1.0	0.8 (8)	86.7 ± 3.3	2.7
Temprid	First instar	Monheim	21.7 (20.7–22.6)	29.5 (27.8–32.5)	12.4 ± 1.7	1.1 (5)	100	–
		Queensland	113.8 (112.5–115.0)	125.2 (122.9–128.6)	39.9 ± 4.4	1.6 (6)	86.7 ± 3.3	5.3
		Kuala Lumpur	138.7 (136.1–141.2)	172.6 (166.4–181.9)	17.4 ± 1.7	9.2 (14)	63.3 ± 8.8	6.4
		Bukit Mertajam	27.4 (24.2–31.1)	123.4 (95.2–176.9)	2.5 ± 0.2	3.3 (12)	100	1.3
		Saujana	220.2 (200.5–239.8)	467.6 (403.6–583.6)	5.0 ± 0.6	0.7 (6)	100	10.2
		Krystal Point	185.8 (149.8–242.6)	1,090.6 (690.3–2,208.0)	2.1 ± 0.3	1.3 (6)	90.0 ± 5.8	8.6
	Adult	Monheim	22.9 (20.9–24.6)	38.4 (34.2–46.7)	7.3 ± 0.9	6.1 (6)	100	–
		Queensland	376.1 (341.4–409.5)	801.0 (710.0–942.1)	5.0 ± 0.5	1.5 (8)	56.7 ± 3.3	16.4
		Kuala Lumpur	230.5 (215.9–245.2)	454.9 (398.7–556.9)	5.6 ± 0.7	7.5 (9)	56.7 ± 3.3	10.1
		Bukit Mertajam	166.4 (151.0–180.2)	329.7 (289.0–405.6)	5.5 ± 0.7	1.6 (6)	60.0 ± 0.0	7.3
		Saujana	374.2 (351.4–396.2)	601.0 (540.9–714.3)	8.0 ± 1.1	3.5 (5)	70.0 ± 5.8	16.3
		Krystal Point	382.7 (370.9–392.5)	471.9 (454.1–500.6)	18.1 ± 2.2	1.6 (6)	43.3 ± 3.3	16.7

[a]Percentage mortality of first instars at 72 h, adults at 120 h.

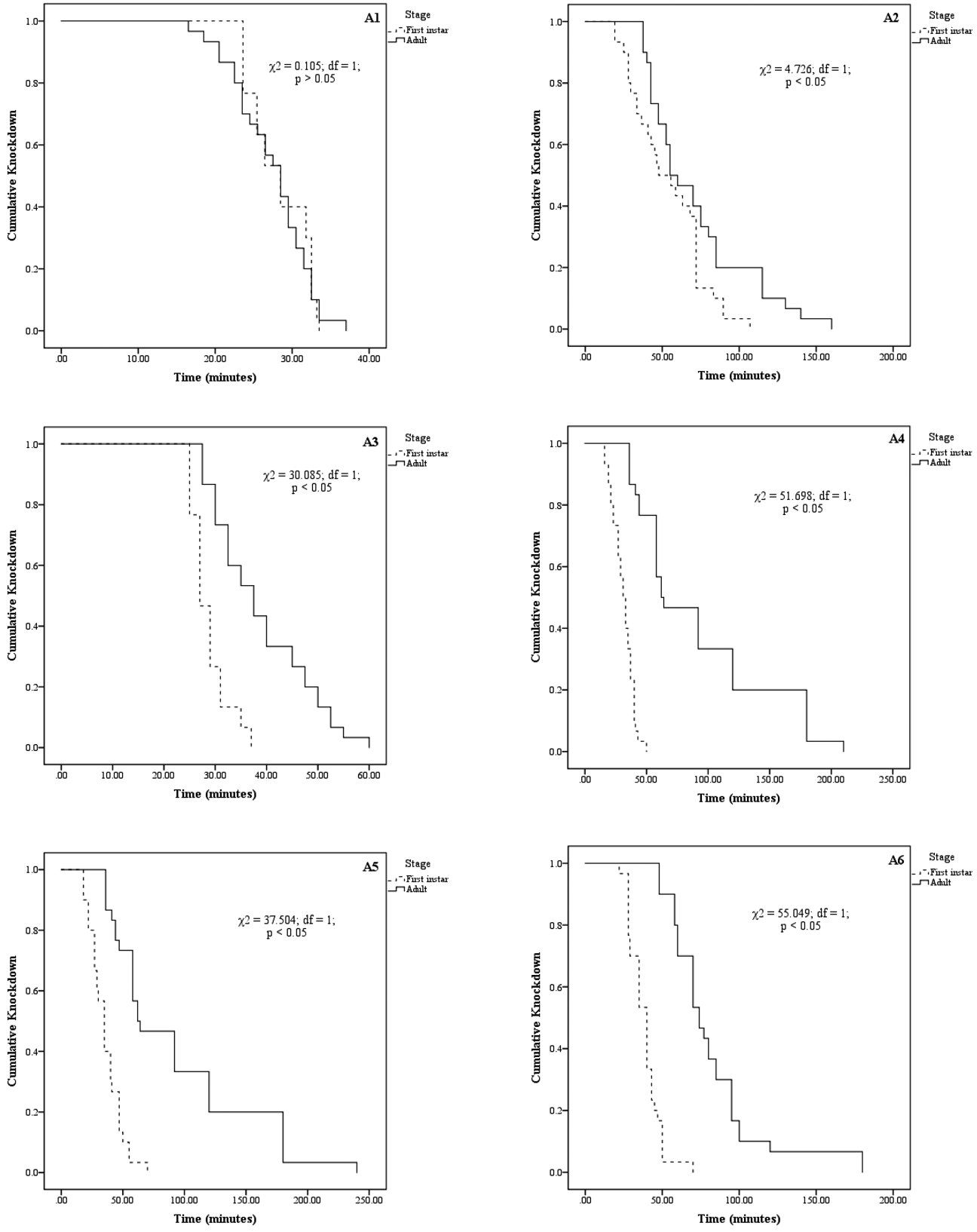

Fig. 1. Kaplan–Meier analysis of knockdown response of first instars and adults of six bed bug populations when exposed to Tandem at the label rate (A1: Monheim, A2: Queensland, A3: Kuala Lumpur, A4: Bukit Mertajam, A5: Saujana, and A6: Krystal Point).

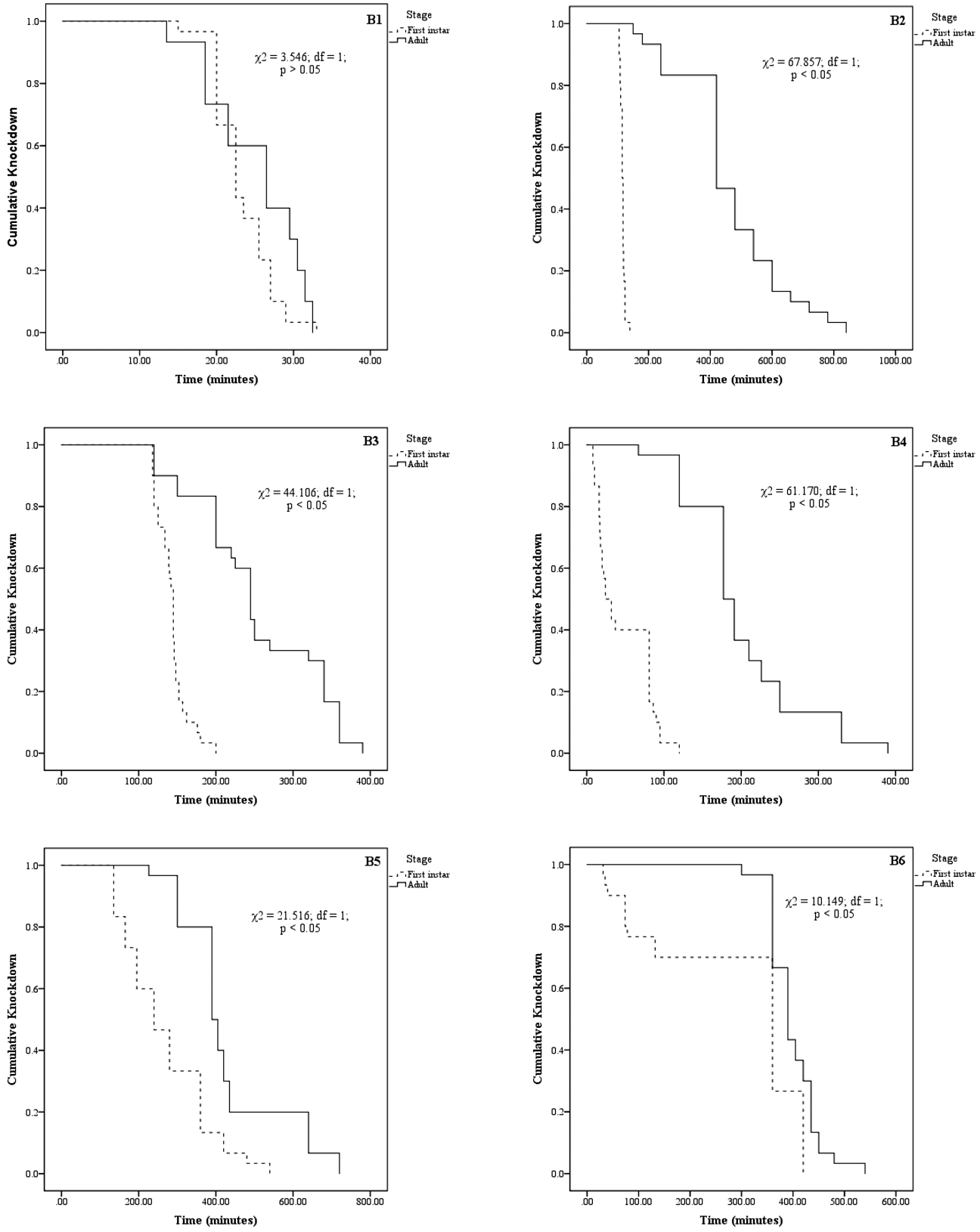

Fig. 2. Kaplan–Meier analysis of knockdown response of first instars and adults of six bed bug populations when exposed to Temprid at the label rate (B1: Monheim, B2: Queensland, B3: Kuala Lumpur, B4: Bukit Mertajam, B5: Saujana, and B6: Krystal Point).

Table 4. Knockdown time (KT_{50} and KT_{95}) values of different life stages of bed bugs treated with insecticides formulated with pyrethroids that were applied at the label rate

Insecticide formulations	Stage	Strain	KT_{50} (95% FL) (minute)	KT_{95} (95% FL) (minute)	Slope ± SE	χ^2 (df)	Percentage mortality (%)[a]	RR_{50}
Pesguard 161	First instar	Monheim	11.8 (11.1–12.3)	16.9 (15.8–18.8)	10.6 ± 1.4	1.9 (6)	100	–
		Queensland	458.6 (409.0–512.5)	1,244.8 (1,003.4–1,750.0)	3.8 ± 0.5	5.5 (6)	100	38.9
		Kuala Lumpur	761.2 (680.0–847.3)	2,968.5 (2,425.0–3,893.8)	2.7 ± 0.2	6.9 (14)	93.3 ± 3.3	64.5
		Bukit Mertajam	370.6 (316.2–435.7)	1,778.9 (1,334.5–2,645.5)	2.4 ± 0.2	1.9 (9)	83.3 ± 3.3	31.4
		Saujana	1,113.7 (1,037.5–1,186.3)	2,312.7 (2,013.8–2,869.1)	5.2 ± 0.6	0.9 (10)	100	94.4
		Krystal Point	1,393.9 (1,276.5–1,515.9)	3,145.3 (2,671.2–4,036.9)	4.7 ± 0.5	2.1 (7)	80.0 ± 0.0	118.1
	Adult	Monheim	11.9 (11.3–12.5)	20.3 (18.6–22.7)	7.1 ± 0.6	5.5 (11)	100	–
		Queensland	>7,200	>7,200	NA	NA	23.3 ± 3.3	>605.0
		Kuala Lumpur	>7,200	>7,200	NA	NA	20.0 ± 5.8	>605.0
		Bukit Mertajam	4,620.5 (3,544.0–8,608.1)	>7,200	1.9 ± 0.5	0.4 (3)	56.7 ± 3.3	388.3
		Saujana	>7,200	>7,200	NA	NA	26.7 ± 8.8	>605.0
		Krystal Point	>7,200	>7,200	NA	NA	23.3 ± 3.3	>605.0
Sumithrin	First instar	Monheim	18.3 (17.3–19.5)	28.2 (25.1–35.3)	8.8 ± 1.1	6.3 (6)	100	–
		Queensland	941.8 (857.1–1,036.2)	2,531.7 (2,136.0–3,183.8)	3.8 ± 0.3	6.1 (10)	96.7 ± 3.3	51.5
		Kuala Lumpur	1,007.2 (899.0–1,116.0)	3,219.8 (2,642.6–4,284.9)	3.3 ± 0.3	4.9 (10)	96.7 ± 3.3	55.0
		Bukit Mertajam	1,405.2 (1,017.8–2,078.6)	7,811.3 (4,283.5–28,206.0)	2.2 ± 0.3	13.9 (7)	80.0 ± 11.5	76.8
		Saujana	2,068.3 (1,883.9–2,303.3)	5,135.4 (4,148.5–7,169.3)	4.1 ± 0.5	2.9 (7)	80.0 ± 15.3	113.0
		Krystal Point	1,688.2 (1,568.0–1,827.5)	3,342.0 (2,885.6–4,155.5)	5.5 ± 0.6	2.5 (7)	93.3 ± 3.3	92.3
	Adult	Monheim	19.7 (18.6–20.7)	30.7 (28.2–35.1)	8.5 ± 1.0	1.2 (6)	100	–
		Queensland	>7,200	>7,200	NA	NA	16.7 ± 3.3	>365.5
		Kuala Lumpur	>7,200	>7,200	NA	NA	16.7 ± 3.3	>365.5
		Bukit Mertajam	5,966.3 (4,632.2–11,365.0)	>7,200	2.7 ± 0.3	2.9 (9)	50.0 ± 11.5	302.9
		Saujana	>7,200	>7,200	NA	NA	33.3 ± 3.3	>365.5
		Krystal Point	>7,200	>7,200	NA	NA	13.3 ± 3.3	>365.5

[a]Percentage mortality of first instars at 72 h, adults at 120 h.

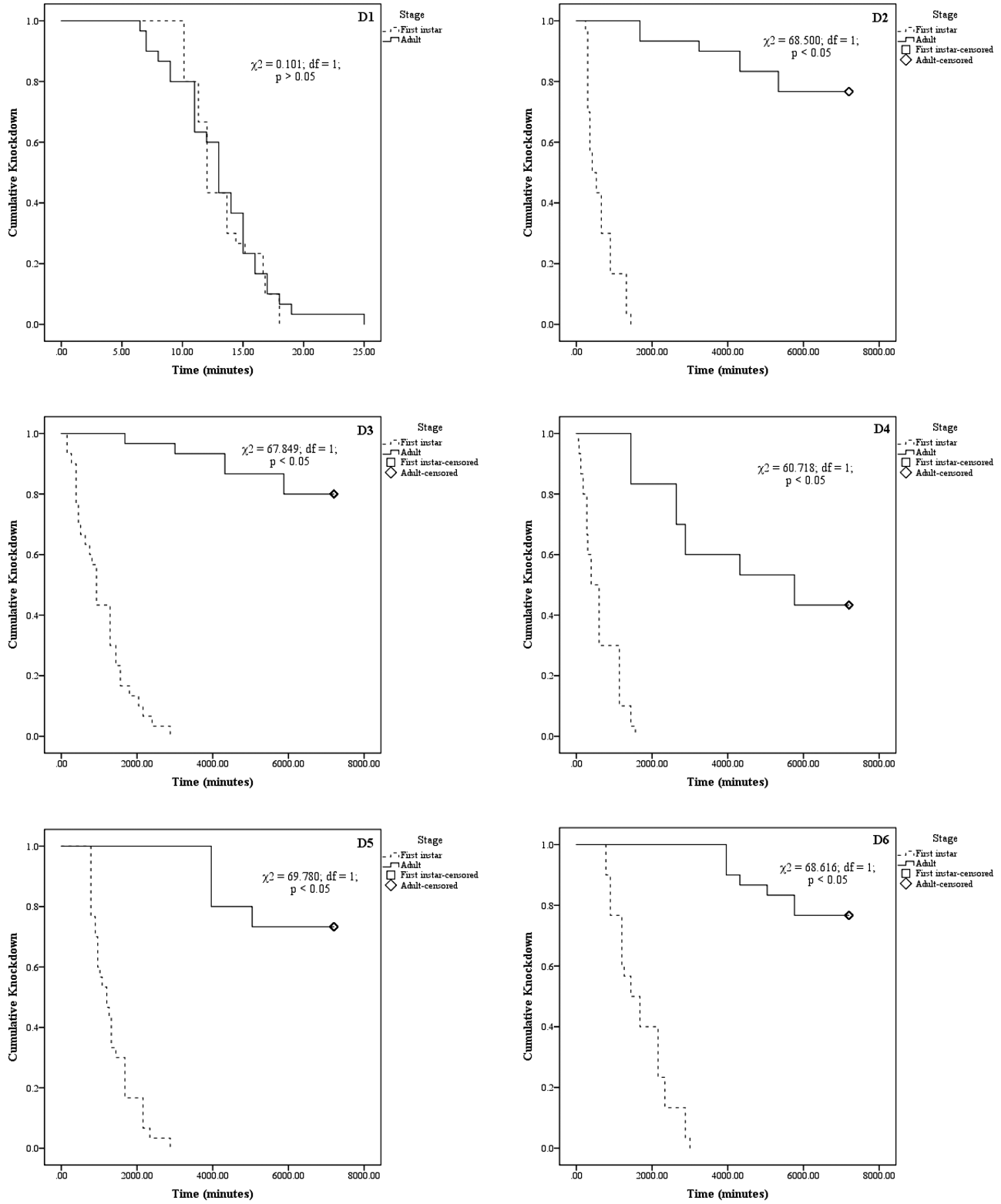

Fig. 3. Kaplan–Meier analysis of knockdown responses of first instars and adults of six bed bug populations when exposed to Pesguard 161 at the label rate (D1: Monheim, D2: Queensland, D3: Kuala Lumpur, D4: Bukit Mertajam, D5: Saujana, and D6: Krystal Point).

for resistant strains (Figs. 3 and 4). Adults of all resistant strains tested with Pesguard FG161 or Sumithrin showed less than 60% knockdown in 120 h, whereas first instars were knocked down completely in 72 h.

Sumithion showed excellent performance against all resistant strains, with resistance level ranging from 1.5 to 7.7 times for first instars and 1.2 to 14.6 times for adults (Table 5). A significant difference in knockdown time between first instars and adults was observed for all strains, with adults having a substantially longer knockdown time compared to that of the first instars (Fig. 5). Mortality reached 100% at 72 h for all first instars and at 120 h for all adults (Table 5).

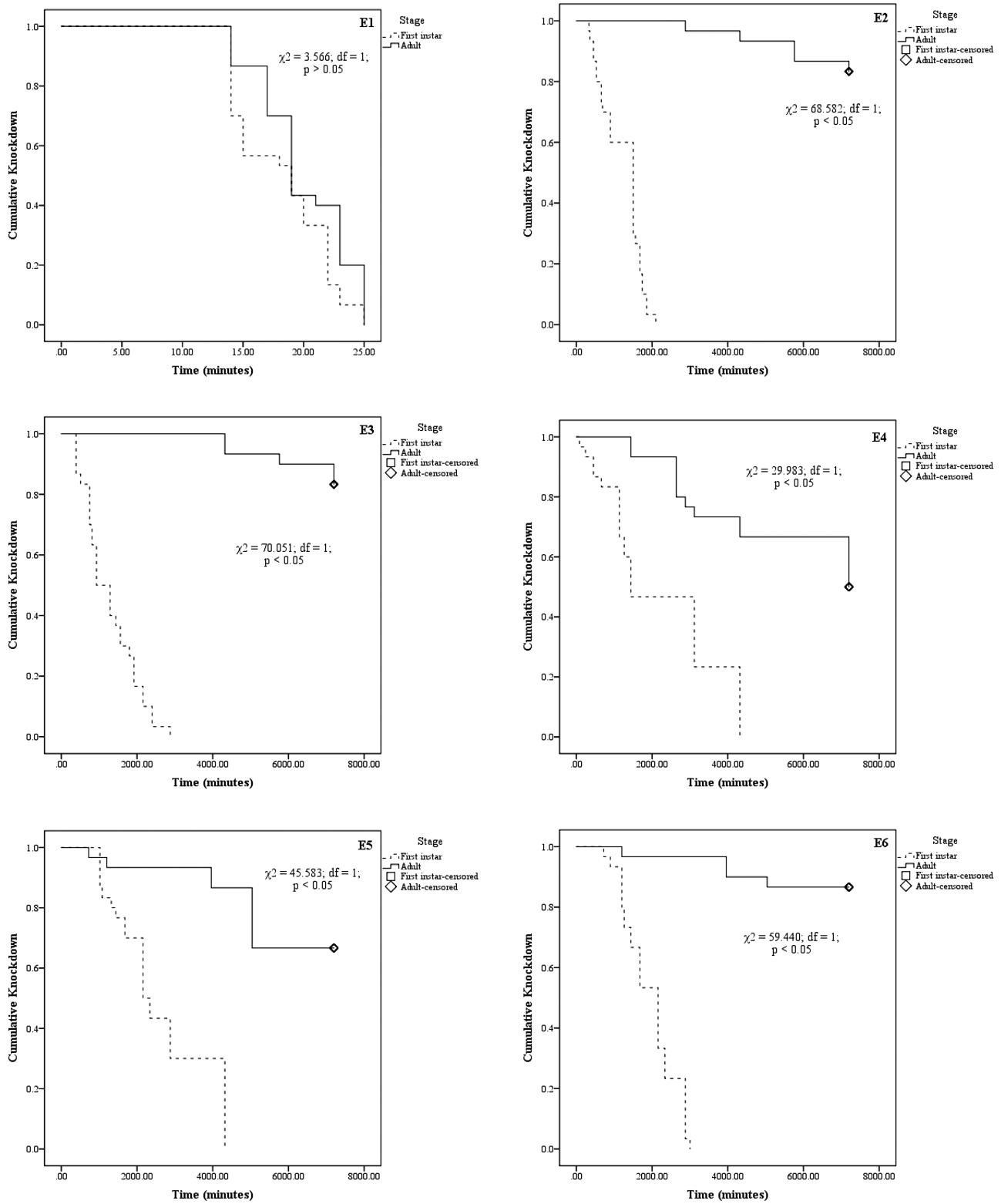

Fig. 4. Kaplan–Meier analysis of knockdown response of first instars and adults of six bed bug populations when exposed to Sumithrin at the label rate (E1: Monheim, E2: Queensland, E3: Kuala Lumpur, E4: Bukit Mertajam, E5: Saujana, and E6: Krystal Point).

Immersion Method for Eggs

Both Queensland and Kuala Lumpur strains showed low egg mortality when tested with each insecticide formulation (Fig. 6). Low egg mortality was recorded for the Bukit Mertajam strain when tested with each insecticide formulation (7.3–29.0%), except for Sumithion (91.3%). Mortality of the eggs of the Krystal Point strain was moderate for Tandem (51.3%), Temprid (54.7%), and Sumithion (68.7%) (Fig. 6). Saujana strain showed high mortality

Table 5. Knockdown time (KT_{50} and KT_{95}) values of different life stages of bed bugs treated with Sumithion that was applied at the label rate

Stage	Strain	KT_{50} (95% FL) (minute)	KT_{95} (95% FL) (minute)	Slope ± SE	χ^2 (df)	Percentage mortality (%)[a]	RR_{50}
First instar	Monheim	58.4 (56.8–60.0)	83.2 (78.9–89.3)	4.5 ± 0.7	2.3 (15)	100	–
	Queensland	359.8 (341.2–373.7)	482.9 (457.8–526.3)	12.9 ± 1.5	10.2 (9)	100	6.2
	Kuala Lumpur	448.8 (421.9–476.2)	794.34 (710.4–938.3)	6.6 ± 0.7	3.1 (7)	100	7.7
	Bukit Mertajam	91.9 (77.6–104.4)	251.1 (205.1–351.0)	3.8 ± 0.5	2.2 (5)	100	1.6
	Saujana	85.0 (79.5–90.4)	142.8 (128.0–169.4)	7.3 ± 0.9	0.7 (5)	100	1.5
	Krystal Point	399.2 (371.8–428.7)	729.3 (643.7–876.8)	6.3 ± 0.7	4.9 (6)	100	6.8
Adult	Monheim	118.5 (107.05–126.74)	198.04 (178.89–237.09)	7.4 ± 1.2	0.4 (5)	100	–
	Queensland	1,224.9 (1,125.5–1,315.0)	2,123.1 (1,921.0–2,452.8)	6.9 ± 0.8	2.8 (5)	100	10.3
	Kuala Lumpur	1,733.1 (1,501.1–1,980.2)	7,145.3 (5,560.1–10,269.0)	2.7 ± 0.3	2.9 (9)	100	14.6
	Bukit Mertajam	304.6 (272.1–346.7)	1,462.3 (1,079.1–2,267.0)	3.4 ± 0.3	2.4 (9)	100	2.6
	Saujana	1,38.5 (122.7–151.0)	247.4 (216.0–318.8)	6.5 ± 1.1	1.3 (3)	100	1.2
	Krystal Point	1,074.3 (991.1–1,150.1)	1,901.8 (1,721.0–2,194.6)	6.6 ± 0.7	1.7 (6)	100	9.1

[a]Percentage mortality of first instars at 72 h, adults at 120 h.

toward Temprid (96%) and Sumithion (100%) but less than 20% mortality were observed toward Tandem (18%), Pesguard FG161 (7.3%), and Sumithrin (6%) (Fig. 6).

The eggs of Queensland and Kuala Lumpur strains showed low mortality to all formulations tested (>82 to >312.5 times) (Table 6). Bukit Mertajam and Saujana strains showed very high resistance to Tandem, Temprid (Bukit Mertajam only), Pesguard FG161, and Sumithrin, but had moderate resistance to Sumithion (Table 6). Krystal Point strain exhibited very high resistance to all formulations, except to Temprid (33 times) (Table 6).

Discussion

This study revealed the presence of resistance to five commercial insecticide formulations in several developmental stages of resistant *C. hemipterus*. Despite the prevalence of reports of pyrethroid resistance in bed bugs, this class of insecticides remains the most used group incorporated into insecticide products used in the management of bed bug infestations. Among the five formulations tested in this study, four contained pyrethroids. Pesguard FG161 and Sumithrin, which contained only pyrethroids, performed poorly against all stages of resistant strains, which suggested the presence of pyrethroid resistance. Pyrethroid resistance in bed bugs is often associated with knockdown resistance (*kdr*), metabolic detoxification, and penetration resistance. Pyrethroid-resistant Kuala Lumpur and Queensland strains were previously reported to possess *kdr* mutations (Dang et al. 2015c). Punchihewa et al. (2019) also detected *kdr* mutation was associated with pyrethroid resistance of *C. hemipterus* in Sri Lanka. Adelman et al. (2011) also detected *kdr* mutations in pyrethroid-resistant *C. lectularius* and found the involvement of detoxification enzymes (cytochrome P450 and carboxylesterase). A synergism study conducted by Gonzalez-Morales and Romero (2018) found increased susceptibility toward pyrethroids in pyrethroid-resistant bed bugs (*C. lectularius*) after pretreatment with synergists (PBO, DEM, and DEF). Similarly, How and Lee (2011) reported that pretreatment with PBO increases pyrethroid susceptibility of *C. hemipterus*. Beside *kdr* and metabolic detoxification, penetration resistance also could possibly be involved. It was described earlier by Koganemaru et al. (2013) and Lilly et al. (2016b) that cuticular modification of bed bugs could reduce cuticular penetration of insecticides.

All *C. hemipterus* strains in this study were cultured in the laboratory and had been free from insecticide exposure (Table 1). According to Gordon et al. (2015), due to trade-off between resistance development and life history parameters, if the insects are not exposed to insecticides, susceptibility of resistant populations to insecticides may return rapidly. In Australia, the Sydney strain of *C. lectularius* exhibited reduced resistance to deltamethrin (LD_{50}) from RR_{50} of 400,000 detected in 2009 (Lilly et al. 2009) to 130 detected in 2014 (Lilly and Doggett, pers. comm. June 21, 2019). In contrast, the Kuala Lumpur and Queensland strains of *C. hemipterus* in our study were still highly resistant to pyrethroids, despite having no exposure to insecticides for approximately 14 yr. A strategy of insecticide rotation, which is often recommended as resistance management strategy, maybe ineffective against this bed bug strain.

We also evaluated the performance of mixture formulations (Tandem and Temprid), which contain a pyrethroid and a neonicotinoid. Both Tandem and Temprid failed to completely kill the resistant strains. Because all strains were pyrethroid-resistant, it is likely that cross-resistance to neonicotinoid also occurred. Previously, cytochrome P450 monooxygenases were shown to detoxify both pyrethroids and neonicotinoids (Scott 1999, Nauen and Denholm 2005, Romero and Anderson 2016). Therefore, it is likely that metabolic detoxification is part of or the contributing resistance mechanism in these strains.

Although both formulations are pyrethroid-neonicotinoid mixtures, the bed bugs showed significantly higher RR_{50} (with the exception of the first instars of the Bukit Mertajam population, which showed no significant difference) to Temprid than to Tandem. Romero and Anderson (2016) also reported that *C. lectularius* from the same population could have varying degrees of resistance to different neonicotinoids. As all the resistant strains in our study may be subjected to varying insecticide selection pressures prior to collection, they could have developed different resistance mechanisms and caused varying levels of resistance to thiamethoxam and imidacloprid.

Varying levels of resistance (RR_{50}) to Sumithion (fenitrothion) were detected in the resistant strains tested despite the occurrence of complete mortality. In the past, organophosphates such as chlorpyrifos, fenitrothion, and pirimiphos-methyl have been used to control bed bugs in Asia (Lee et al. 2018). Tawatsin et al. (2011) and Dang et al. (2017a) reported organophosphates (malathion and fenitrothion) resistance in the *C. hemipterus* strains that they evaluated. In our study, the possible involvement of penetration resistance and metabolic resistance mechanisms (e.g., cytochrome P450 monooxygenase and carboxylesterase) could have contributed to fenitrothion resistance.

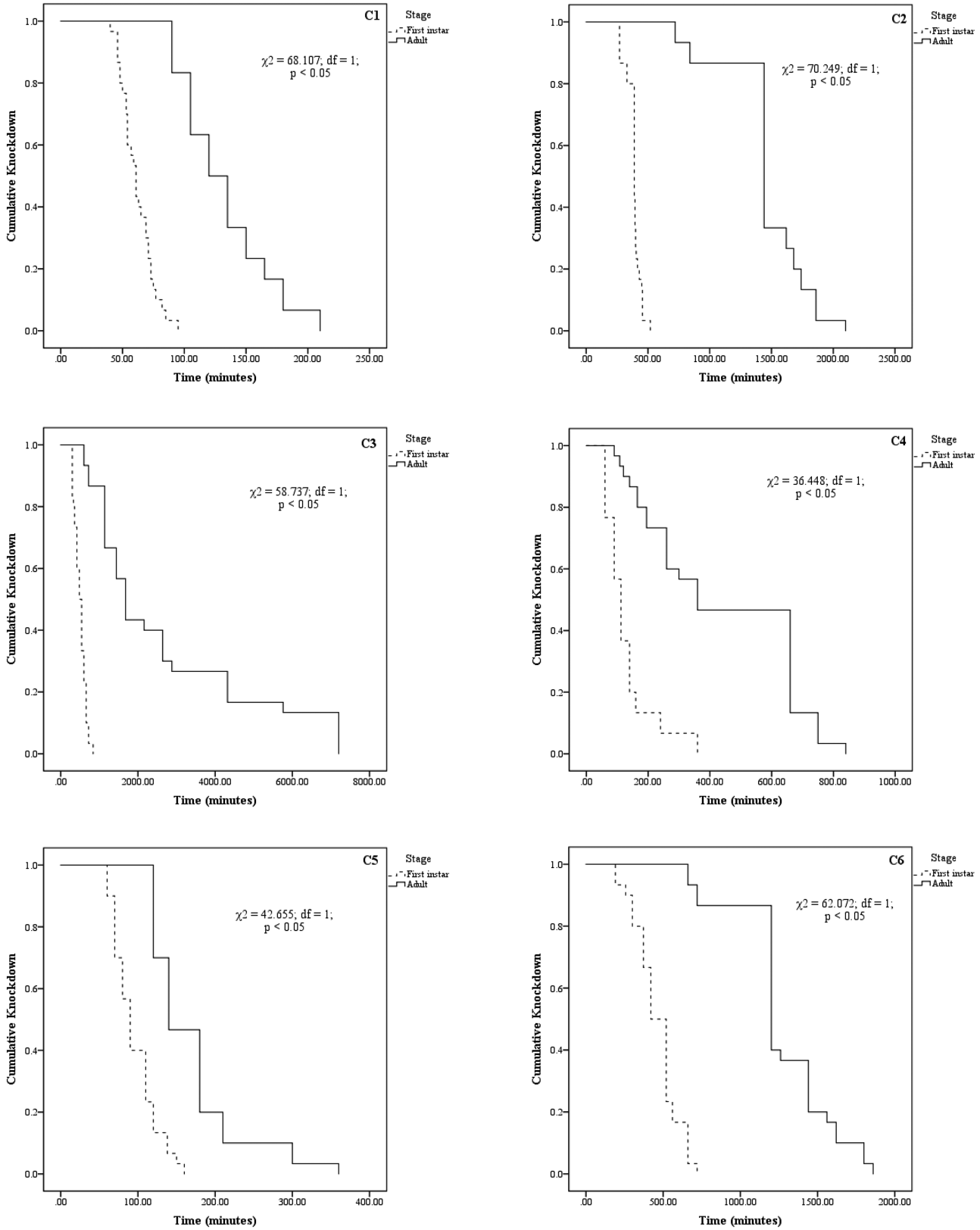

Fig. 5. Kaplan–Meier analysis of knockdown response of first instars and adults of six bed bug populations when exposed to Sumithion at the label rate (C1: Monheim, C2: Queensland, C3: Kuala Lumpur, C4: Bukit Mertajam, C5: Saujana, and C6: Krystal Point).

Ovicidal effects of the insecticides were evaluated in this study. O'Brien (1960) and De Villar et al. (1980) suggested that for an ovicidal action of a neurotoxic insecticide to occur, the embryo needs to be dependent on its nervous system. Neurotoxic insecticides are only lethal to the embryo at a later stage of the developmental process, as insects lack a functional nervous system during the early

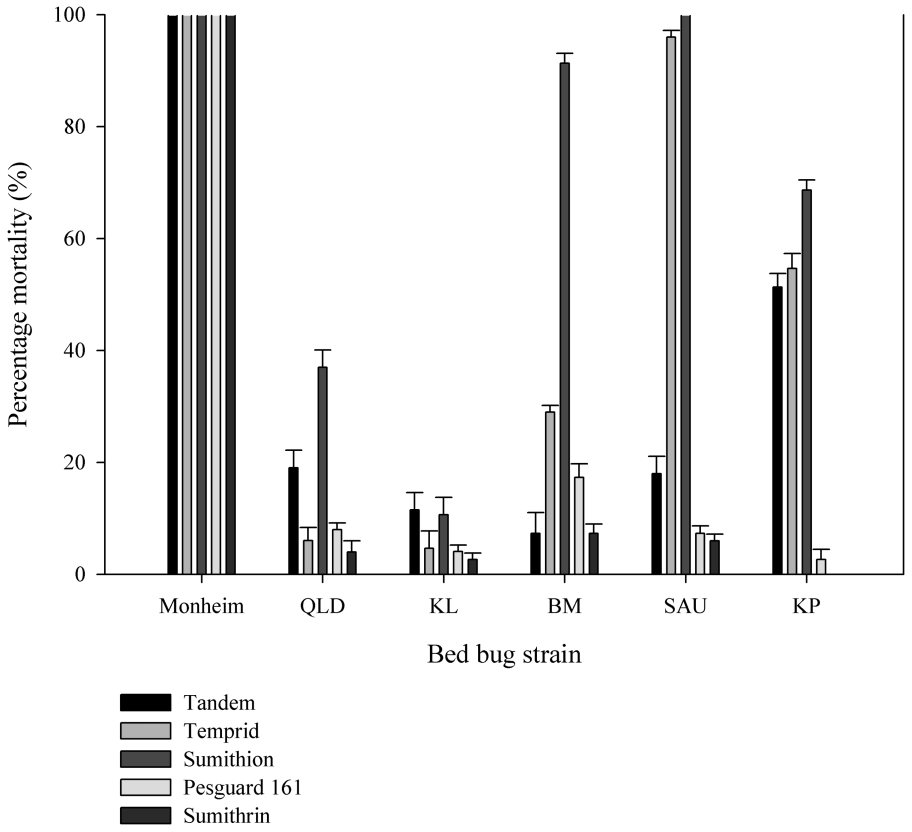

Fig. 6. Percentage mortality of bed bug eggs from six bed bug populations (Monheim, Queensland, Kuala Lumpur, Bukit Mertajam, Saujana, and Krystal Point) treated with Tandem, Temprid, Sumithion, Pesguard FG161, and Sumithrin at the label rate.

embryonic stage (Smith 1955). The pyrethroid-only formulations (Pesguard 161 and Sumithrin) showed the poorest ovicidal effect on the eggs of the resistant strains. The LC_{50} values (except that of the Monheim strain) could not be determined because <40% egg mortality was recorded at the highest concentrations used. It is likely that pyrethroid resistance is present in bed bug eggs, as reported previously by Campbell and Miller (2015) for *C. lectularius*. Sumithion, Tandem, and Temprid performed well against the first instars but had varying degrees of efficacy against the eggs. Campbell and Miller (2015) reported that eggs and first instars exhibited different insecticide resistance levels, with eggs being slightly more resistant than the first instars. When tested on *Triatoma infestans*, variations in resistance levels between first instars and eggs, in several field populations also were demonstrated (Toloza et al. 2008).

Little is known about resistance mechanisms in bed bug eggs. Hinson et al. (2016) suggested that it is not suitable to compare resistance of eggs with that of the free-living stages because these two developmental stages are fundamentally different. Therefore, aside from resistance mechanisms, the combined effects of the egg shell (such as its composition, thickness, and permeability) and the insecticide(s) used in the formulations could have contributed to the poor ovicidal effects of the formulations tested (Hinson et al. 2016).

For the free-living stages, knockdown time for adults and first instars of the resistant strains differed significantly according to the Kaplan–Meier analysis ($P < 0.05$). Lower mortality and substantially longer knockdown time were observed in adults. In this study, to avoid molting during the course of the experiment, first instars were not provided with a blood meal prior to or during the testing. Furthermore, similar to the insecticides that are applied in the field, the first instars are exposed to the insecticide residue when the eggs hatch out. Hence, we chose to test the unfed first instars.

On the other hand, the adult bed bugs were given a blood meal 7 d prior to the experiment. A blood meal could affect knockdown responses as well as the mortality of first instars and adults of the resistant populations. According to Choe and Campbell (2014), survival times of fed bed bugs were significantly longer than those of unfed individuals. Blood intake could be a stimulus that induces a chain of metabolic processes or the production of metabolic enzymes, which eventually could lead to an increase in the resistance level of the insects (Spillings et al. 2008). Studies of other hematophagous insects also showed that resistant insects were more tolerant to insecticide treatments when given a recent blood meal (Sanders et al. 2003, Machani et al. 2019).

Age differences in an insect population also can affect insecticide susceptibility. In this study, first instars had lower resistance than adults. The lower resistance level of first instars likely is a trade-off for the nymphal stage, when resources are needed for developmental processes. Valles et al. (1994) found that microsomal monooxygenase systems based on cytochrome P450 content and enzyme activity were different in the nymphal and adult stages. Koehler et al. (1993) reported a higher level of resistance in older nymphs than in adults of the German cockroach *Blatella germanica*. The resistance level decreased as the cockroach nymphs molted into the adult stage. Park and Kamble (1998) conducted metabolic assays and found higher esterase activity in German cockroach nymphs than in adults.

In summary, we evaluated the performance of several liquid insecticide formulations against resistant populations of *C. hemipterus*. All strains were highly resistant to pyrethroid formulations (Pesguard FG161 and Sumithrin) and had low to high

Table 6. Lethal concentration (LC_{50} and LC_{95}) values for eggs of several bed bug populations treated with five insecticide formulations

Insecticide formulations	Strain	LC_{50} (95% FL) (mg/liter)	LC_{95} (95% FL) (mg/liter)	Slope ± SE	χ^2 (df)	RR_{50}
Tandem	Monheim	18.8 (11.2–29.9)	256.4 (134.6–701.4)	1.5 ± 0.1	8.9 (4)	–
	Queensland	>3,000	>3,000	NA	NA	>160.6
	Kuala Lumpur	>3,000	>3,000	NA	NA	>160.6
	Bukit Mertajam	>3,000	>3,000	NA	NA	>160.6
	Saujana	>3,000	>3,000	NA	NA	>160.6
	Krystal Point	1,106.9 (695.6–1,871.3)	18,645.0 (7,430.7–133,750.0)	1.3 ± 0.1	5.3 (3)	58.9
Temprid	Monheim	15.7 (11.4–21.9)	60.4 (39.1–127.3)	2.8 ± 0.2	4.1 (2)	–
	Queensland	>3,000	>3,000	NA	NA	>191.1
	Kuala Lumpur	>3,000	>3,000	NA	NA	>191.1
	Bukit Mertajam	1,045.0 (787.8–1,319.7)	4,267.0 (2,967.2–8,248.1)	2.7 ± 0.2	4.9 (3)	66.6
	Saujana	104.8 (59.0–187.7)	1,406.5 (593.5–8,333.8)	1.5 ± 0.1	2.2 (2)	6.7
	Krystal Point	517.8 (423.1–617.9)	6,391.8 (4,571.5–10,054.0)	1.5 ± 0.1	3.8 (4)	33.0
Sumithion	Monheim	26.6 (19.1–35.3)	121.5 (75.7–352.3)	2.5 ± 0.2	6.6 (3)	–
	Queensland	>5,000	>5,000	NA	NA	>188.0
	Kuala Lumpur	>5,000	>5,000	NA	NA	>188.0
	Bukit Mertajam	157.1 (46.3–384.3)	13,855.0 (3,718.40–227,560.0)	0.8 ± 0.1	7.5 (3)	5.9
	Saujana	117.5 (88.5–148.1)	1,551.5 (1,048.8–2,725.3)	1.5 ± 0.2	0.7 (2)	4.4
	Krystal Point	3,405.3 (2,067.6–9,266.2)	21,030.0 (8,231.8–826,760.0)	2.1 ± 0.2	4.0 (2)	128.0
Pesguard 161	Monheim	9.6 (7.2–12.5)	55.5 (36.3–110.8)	2.2 ± 0.2	3.7 (3)	–
	Queensland	>3,000	>3,000	NA	NA	>312.5
	Kuala Lumpur	>3,000	>3,000	NA	NA	>312.5
	Bukit Mertajam	>3,000	>3,000	NA	NA	>312.5
	Saujana	>3,000	>3,000	NA	NA	>312.5
	Krystal Point	>3,000	>3,000	NA	NA	>312.5
Sumithrin	Monheim	36.6 (28.6–44.5)	142.8 (104.7–241.6)	2.8 ± 0.2	3.5 (3)	–
	Queensland	>3,000	>3,000	NA	NA	>82.0
	Kuala Lumpur	>3,000	>3,000	NA	NA	>82.0
	Bukit Mertajam	>3,000	>3,000	NA	NA	>82.0
	Saujana	>3,000	>3,000	NA	NA	>82.0
	Krystal Point	>3,000	>3,000	NA	NA	>82.0

resistance to pyrethroid-neonicotinoid mixtures (Tandem and Temprid) and Sumithion. Resistance levels varied with the different stages tested. Due to the varying types of resistance mechanisms at different stages of development, we propose that more research is warranted especially tests involving immature stages using technical grade active ingredients that could provide additional clarity on stage-dependent resistance.

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