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Special Collection: Perspective on Biology and Management of Bed Bugs

Performance of Pyrethroid-Neonicotinoid Mixture Formulations Against Field-Collected Strains of the Tropical Bed Bug (Hemiptera: Cimicidae) on Different Substrates

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Abstract

The residual performance of two pyrethroid-neonicotinoid mixture formulations: Temprid SC (10.5% beta-cyfluthrin and 21% imidacloprid) and Tandem (3.5% lambda-cyhalothrin and 11.6% thiamethoxam) on two substrates (glass and filter paper) against eight pyrethroid-resistant strains (BM-MY, BP-MY, CH-MY, GL-MY, KL-MY, SAJ-MY, TT-MY, and QLD-AU) of the tropical bed bug, *Cimex hemipterus* (F.) (Hemiptera: Cimicidae) collected from Malaysia, and Australia were evaluated. The aging effect of treatment residues on glass was also investigated. A susceptible *C. lectularius* L. strain (Monheim) was used for comparison. Temprid SC showed varying levels of performance against all *C. hemipterus* strains: TT-MY (PR_{50} = 6.5-fold, high performance), BM-MY, GL-MY, SAJ-MY, and QLD-AU (12.8–21.6-fold, moderate performance), BP-MY, and KL-MY (48.2–49-fold, poor performance), CH-MY (128.2-fold, very poor performance). On the other hand, Tandem displayed high performance against all *C. hemipterus* strains (1.8–8.3-fold). Tandem caused faster mortality than Temprid SC for all strains. Temprid SC and Tandem residues killed *C. hemipterus* significantly faster on glass than filter paper. Compared with fresh residues, the efficacy of Temprid SC residues significantly declined after one week of aging, while the effectiveness of Tandem residues declined after two weeks of aging. Further investigations using the topical assay method with a diagnostic dose of imidacloprid found two strains (CH-MY and GL-MY) resistant to imidacloprid. The six other strains (BM-MY, BP-MY, KL-MY, SAJ-MY, TT-MY, and QLD-AU) were susceptible.

Key words: imidacloprid, beta-cyfluthrin, thiamethoxam, lambda-cyhalothrin, insecticide formulation performance

The common bed bug, *Cimex lectularius* L., and the tropical bed bug, *Cimex hemipterus* (F.), are cryptic, nocturnal ectoparasites adapted to blood-feeding on humans (Usinger 1966). Both bed bug species have undergone a major worldwide resurgence over the last two decades (Dang et al. 2017a, 2021; Doggett et al. 2018). Similar to *C. lectularius*, high levels of pyrethroid resistance have been reported in *C. hemipterus* (Dang et al. 2015, 2021; Zulaikha and Majid 2019, Leong et al. 2020a, Soh and Veera Singham 2021). Due to the resistance to multiple insecticide classes, bed bug management is becoming increasingly challenging. Although numerous control methodologies are available including nonchemical options, many pest control operators still rely on insecticides to manage both bed bug species (Potter et al. 2015, Lee et al. 2018).

Several pyrethroid-neonicotinoid spray formulations have been introduced to the market in recent years and registered for bed bug management. These include, Temprid SC (10.5% beta-cyfluthrin and 21% imidacloprid), Transport GHP (27.27% bifenthrin and 22.73% acetamiprid), Transport Mikron (6% bifenthrin and 5% acetamiprid), CrossFire Bed Bug Concentrate (0.1% metofluthrin, 4% clothianidin, and 10% piperonyl butoxide), and Tandem (3.5%

lambda-cyhalothrin and 11.6% thiamethoxam). Several studies reported on the excellent performance of mixture formulations in managing *C. lectularius* populations (Reid et al. 2010, Potter et al. 2012, Wang et al. 2015, 2016) due to the mixture of two different modes of action (Reid et al. 2010, Potter et al. 2012). However, a laboratory study demonstrated that the performance of Temprid SC could be decreased against *C. lectularius* populations over one generation through the deliberate selection and breeding of the more resistant individuals (Gordon et al. 2014). Furthermore, these authors reported varying levels of performance of Temprid SC against field *C. lectularius* populations. Subsequently, high levels of neonicotinoid resistance were found in pyrethroid-resistant *C. lectularius* strains in the U.S. (Romero and Anderson 2016). Similarly, neonicotinoid resistance was also detected in Australian *C. lectularius* strains (Lilly et al. 2018).

Information on neonicotinoid resistance and the efficacy of pyrethroid-neonicotinoid insecticides is limited in *C. hemipterus* (Tawatsin et al. 2011, Majid and Zulaikha 2015, Lee et al. 2018, Lilly et al. 2018, Leong et al. 2020a, b). This study evaluated the residual performance of two pyrethroid-neonicotinoid mixture products (Temprid SC and Tandem) using the surface-contact method, on glass and filter paper, against eight strains of *C. hemipterus* collected in Australia and Malaysia. We also tested the effects of aging on the performance of both products on glass. Lastly, we determined the imidacloprid susceptibility status of all strains using a topical assay with a diagnostic insecticide dose.

Materials and Methods

Bed Bug Strains

Bed bug populations of *C. hemipterus* were collected from the field and maintained in the Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia, at 27 ± 2°C, 75 ± 10% relative humidity, and a 12-h photoperiod (Table 1). They were blood-fed using an artificial blood-feeding system (Hemotek membrane feeding system, Discovery Workshops, Accrington, UK), with freshly drawn rabbit blood in a lithium heparin tube (Vacutest Kima SRL, Arzergrande [PD], Italy) [Animal ethics approval: USM/Animal Ethics Approval/2016/(104)(819)]. An insecticide-susceptible *C. lectularius* strain (Monheim) was used for comparison as there is no known susceptible strain of *C. hemipterus* in colony worldwide (Dang et al. 2015, 2021; Leong et al. 2020a, b). None of the strains underwent insecticide selection. The species of all the bed bug strains were confirmed using Usinger (1966). All bed bugs were fed to repletion 5 d before testing.

Insecticides

Two pyrethroid-neonicotinoid mixture products, Temprid SC (SC: suspension concentrate, Bayer Environmental Science, Singapore) and Tandem (ZC: capsule suspension and suspension concentrate, Syngenta Asia Pacific, Singapore) were evaluated in this study. All formulations were diluted with deionized water and tested at the prescribed label rate: Temprid SC (label application rate = 0.075%, this equates to 0.05% imidacloprid and 0.025% beta-cyfluthrin) and Tandem (label application rate = 0.13%, equating to 0.1% thiamethoxam and 0.03% lambda-cyhalothrin). For the topical assay to determine imidacloprid susceptibility, technical grade imidacloprid (95%, Bayer Australia Ltd., Sydney, Australia) diluted in acetone (R & M Marketing, Essex, UK) was used.

Preparation of Insecticide on Different Substrate Surfaces

Two surface types were selected for surface-contact exposure: filter paper (90 mm diam. Whatman filter paper grade 1) and glass (90 mm diam. × 15 mm height glass Petri dish). The filter paper was evenly treated with 1 ml of insecticide solution and then left to air dry for 24 h in a fume hood. The glass was rotated by hand to make sure that 1 ml of the water-soluble insecticide evenly coated the inner bottom surface of the glass Petri dish. To keep the insecticide coated evenly at the inner bottom surface, the treated glass was left on a horizontal laboratory table to air dry for 24 h. The filter paper and glass controls were treated with 1 ml of deionized water. The final application rate of Temprid SC was 118 mg/m^2, while that of Tandem was 204 mg/m^2.

Residual Insecticide Assays

Ten randomly selected adult bed bugs of mixed sex and age were placed onto the filter paper treated with insecticide for the continuous exposure experiment. Another ten insects were transferred onto filter paper treated with deionized water as controls. For the Monheim strain, the cumulative number of knocked-down bed bugs was recorded at intervals of 2.5 min until all insects were knocked down. Mortality was recorded at 24 h. For the field strains, the cumulative number of knocked-down bed bugs was recorded at intervals of 1 h for the first 12 h and subsequently at intervals of 24 h up to 120 h. Mortality was recorded at intervals of 24 h for up to 120 h. An insect was considered knocked down if it could not right itself when gently touched. An insect was deemed dead if it failed to move when probed. The experiments above were repeated on glass treated with the insecticide formulations. For Temprid SC

Table 1. The *Cimex hemipterus* strains and the insecticide-susceptible Monheim *Cimex lectularius* strain used in this study

Species	Strain	Location	Year	Deltamethrin	Imidacloprid[2]
C. lectularius	Monheim	Monheim, Germany, laboratory colony	Late 1960s	Susceptible	Susceptible
C. hemipterus	QLD-AU	North Queensland, AUSTRALIA	2007	Resistant[1]	Susceptible
	KL-MY	Kuala Lumpur, MALAYSIA	2005	Resistant[1]	Susceptible
	BM-MY	Bukit Mertajam, Penang, MALAYSIA	2015	Resistant[3]	Susceptible
	BP-MY	Bayan Point, Penang, MALAYSIA	2015	Resistant[3]	Susceptible
	SAJ-MY	Saujana, Penang, MALAYSIA	2015	Resistant[3]	Susceptible
	TT-MY	Tanjung Tokong, Penang, MALAYSIA	2015	Resistant[1]	Susceptible
	CH-MY	Christian, Penang, MALAYSIA	2015	Resistant[1]	Resistant
	GL-MY	Green Lane, Penang, MALAYSIA	2015	Resistant[1]	Resistant

[1] Dang et al. (2021).
[2] Susceptibility to imidacloprid was identified in this study.
[3] Unpublished data.

residues on glass, the cumulative number of knocked-down bed bugs was recorded simultaneously as for filter paper. For Tandem residues on the glass against *C. hemipterus* strains, the cumulative number of knocked down bed bugs was recorded at intervals of 30 min for the first 3 h and subsequently at intervals of 1 h until all bed bugs were knocked down. Mortality was recorded at intervals of 24 h for up to 120 h. Three replicates were carried out for each insecticide formulation on each substrate.

Aged Residual Assays

Insecticide residues and the controls on glass were aged indoors with a photoperiod of 16:8 h (L:D) for 1 wk, 2 wk, and 4 wk at room temperature (23 ± 2°C). The residual bioassay experiments described above were conducted using the QLD-AU and KL-MY strains on the treated aged residual glass. The susceptible Monheim strain was used as a control. Aged residual assays on filter papers were not carried out, as the mortality of the eight *C. hemipterus* strains after Temprid SC and Tandem assays on filter papers did not exceed 50% (Table 3).

Topical Assays

Adults (mixed sex and age) were immobilized using CO_2 for 5 s (Dang et al. 2021). The diagnostic dose of 0.1 g/l of imidacloprid was employed (Lilly et al. 2018). Ten adult insects were topically treated with 1 µl of the acetone-diluted imidacloprid (= 0.1 µg imidacloprid per insect) on the ventral surface of the abdomen using a microapplicator (Burkard Scientific PAX 100 Automatic Micro-Dispensing System, Burkard Manufacturing Co. Ltd, Hertfordshire, UK) equipped with a 250-µl glass syringe (Eterna Matic, Sanitex, Switzerland). After treatment, each group of ten insects was held in a plastic Petri dish (90 mm diam. × 15 mm height, Favorit, Malaysia) at room temperature (23 ± 2°C) for 30 min before being transferred into a plastic Petri dish (60 mm diam. × 15 mm height, Citotest Labware Manufacturing Co. Ltd., Jiangsu, China) lined with a filter paper (diam. 55 mm, Filtres Fioroni, Ingre, France) at room temperature (23 ± 2°C) for 24 h. Another ten insects were treated with 1 µl acetone for the control set. Mortality was recorded after 24 h postexposure. Three replicates were carried out for each strain.

Statistical Analysis

Knockdown and mortality of insects in the tests were corrected using Abbott's (1925) formula. Data were pooled and subjected to probit analysis (Finney 1971) using Polo Plus (Robertson et al. 2003) to generate KT_{50}s and KT_{95}s. The KT_{50}s were considered significantly different ($P < 0.05$) when their 95% confidence intervals (CIs) did not overlap (Payton et al. 2003, Wheeler et al. 2006). Mean cumulative mortality of insects after 24 h and 120 h exposure was examined for statistical significance using one-way ANOVA. The means were separated using Tukey HSD test in GraphPad Prism software 5.00 (GraphPad Software Inc., San Diego, CA). The survival time data from different bioassays were analyzed using Survival Analysis in the GraphPad Prism. Kaplan-Meier survival curves from different bioassays were generated, and the statistical significance of differences in survival curves was determined using log-rank test at $P < 0.05$. Performance ratios (PR_{50}s) were calculated by dividing the KT_{50} value of the resistant strain by the corresponding KT_{50} value of the susceptible Monheim strain. The classification of performance was as follows: excellent performance ($PR_{50} \leq 1$-fold), high performance (1-fold$< PR_{50} \leq$10-fold), moderate performance (10-fold$< PR_{50} \leq$25-fold), poor performance (25-fold$< PR_{50} \leq$50-fold), and very poor performance (PR_{50}>50-fold). The higher the PR_{50}, the poorer the performance.

Results

Performance of Temprid SC and Tandem on Filter Paper and Glass

Filter Paper

Temprid SC produced low mortality in all *C. hemipterus* strains after 24 h and 120 h exposure (24 h: 0 to 16.7 ± 6.7%, 120 h: 3.3 ± 3.3% to 36.7 ± 8.8% [Mean ± S.E.: 17.1 ± 4.1%]) (Fig. 1A–B). However, all test insects of the Monheim strain were killed by 24 h (Table 2, Fig. 1A). Similarly, Tandem also recorded low mortality in the *C. hemipterus* strains after 24 h (Fig. 1C). Excluding SAJ-MY (6.7 ± 3.3%) and BM-MY (13.3 ± 3.3%) strains, there was no mortality in the remaining strains (Fig. 1C). After 120 h exposure, Tandem also registered low mortality with the *C. hemipterus* strains tested, ranging from 0 to 36.7 ± 12% (Mean ± S.E.: 12.8 ± 4.1%) (Fig. 1D). KT_{50}s of eight *C. hemipterus* strains to Temprid SC and Tandem could not be generated, as the mortality did not exceed 50% (Table 2, Fig. 1). There was no mortality in the control groups on filter paper.

Glass

Temprid SC tested with all *C. hemipterus* strains resulted in variable mortality after 24 h, ranging from 10 ± 10% to 100% (Fig. 2A). After 120 h exposure, the mortality ranged from 43.3 ± 3.3% to 100% (Mean ± S.E.: 83.6 ± 7.8%) (Fig. 2B). The mortality of the Monheim strain was 100% at 24 h. Compared with the Monheim strain, Temprid SC displayed significantly poorer ($P < 0.05$) performance against all *C. hemipterus* strains (Table 3). The residues of Temprid SC showed high performance on the TT-MY strain (PR_{50} = 6.5-fold), moderate performance on the BM-MY, GL-MY, SAJ-MY, and QLD-AU strains (PR_{50}s = 12.8 to 21.4-fold), poor performance on the BP-MY (PR_{50} = 48.2-fold) and KL-MY (PR_{50} = 49-fold) strains, and very poor performance on the CH-MY strain (PR_{50} = 128.2-fold).

Tandem resulted in varying mortality of *C. hemipterus* strains after 24 h, ranging from 36.7 ± 3.3% to 100% (Fig. 2C). After 120 h exposure, Tandem caused 100% mortality in all *C. hemipterus* strains (Fig. 2D). The mortality of the Monheim strain was 100% at 24 h. Tandem displayed high performance on all the *C. hemipterus* strains, with PR_{50}s ranging from 1.8 to 8.3-fold (Table 3). Tandem also killed all strains significantly ($P < 0.05$) faster than Temprid SC (Table 3). All control mortality was less than 10% after 120 h.

Compared with both Temprid SC and Tandem deposits on filter paper, the deposits on glass caused significantly ($P < 0.05$) faster (Table 2 and 3) and higher mortality (Figs. 1 and 2) in all the *C. hemipterus* strains.

Aged Residual Assays

Temprid SC

The performance of the fresh (1 d) Temprid SC residues on glass was higher ($P < 0.05$) than that of the ≥1 wk old residues against the QLD-AU strain based on KT_{50} values (Table 4). Compared with fresh residues, the QLD-AU strain survived longer on ≥ 1wk old residues (including 1 wk [$\chi^2 = 25.41$, df = 1, $P < 0.0001$], 2 wk [$\chi^2 = 23.09$, df = 1, $P < 0.0001$], and 4 wk [$\chi^2 = 26.78$, df = 1, $P < 0.0001$]) (Fig. 3A). However, the mortality after 120 h exposure was significantly lower (Tukey HSD test, $P < 0.05$) only on the 2 wk old residues (53.3 ± 8.8%), than that on the fresh residues (100%). The

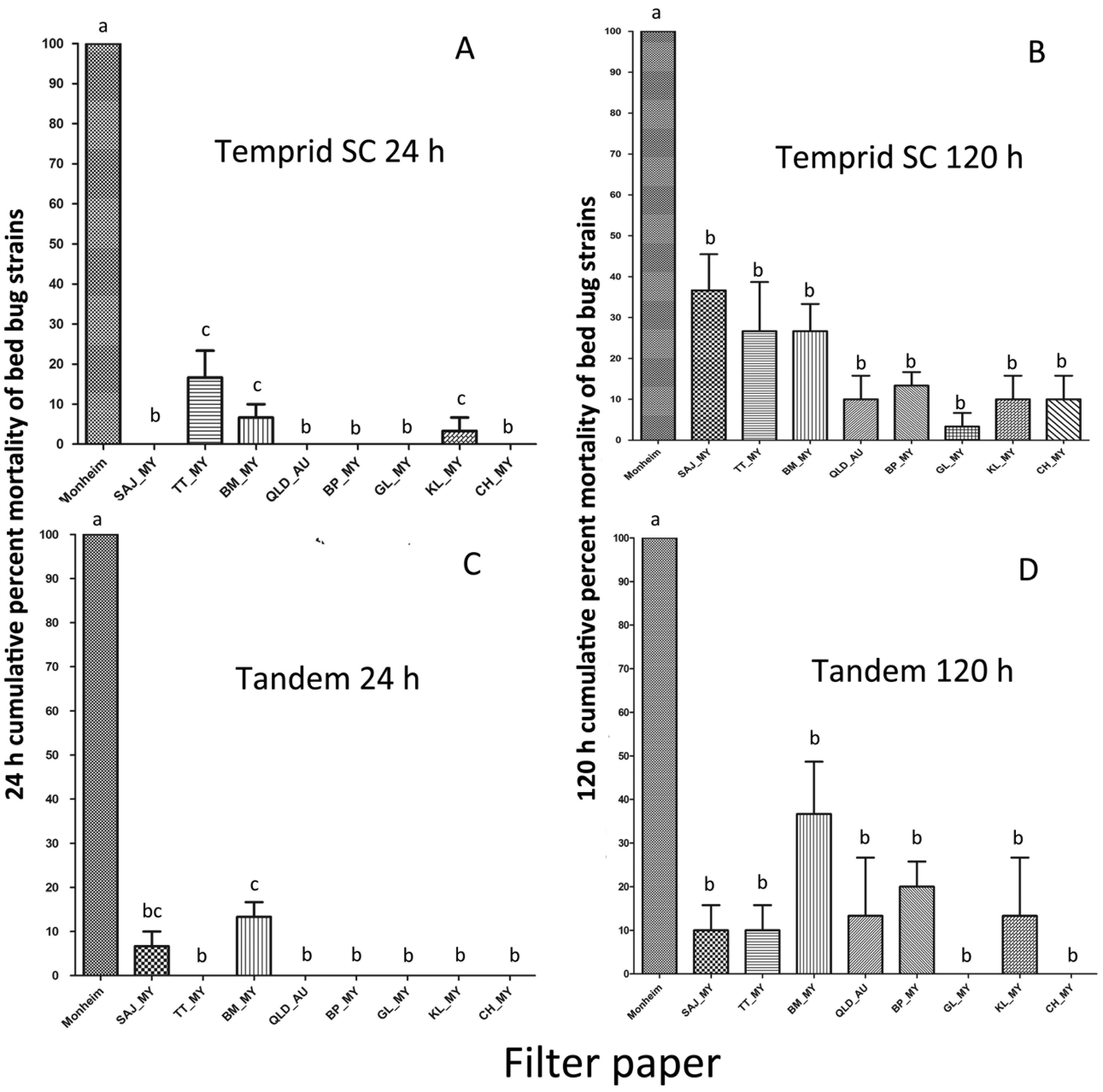

Fig. 1. Cumulative percent mortality of bed bug strains (Mean ± S.E.%) exposed to Temprid SC (A–B), and Tandem (C–D) on filter paper, for 24 h and 120 h exposure. Significant differences are indicated by different letters above each bar.

mortality on the 1 wk and 4 wk old residues was 76.7 ± 3.3% and 70 ± 10%, respectively, which were similar (Tukey HSD test, $P > 0.05$) to the mortality (100%) on the fresh residues.

For the KL-MY strain, the performance of the fresh residues was similar to that of the 1 wk and 2 wk old residues based on KT_{50} values. However, the performance was ($P<0.05$) higher than that of 4 wk old residues(Table 4). Compared with the fresh residues, the KL-MY strain survived longer ($\chi^2 = 5.73$, df = 1, $P = 0.0167$) only on 4 wk old residues (Fig. 3B). The survival time on 1 wk ($\chi^2 = 0.55$, df = 1, $P = 0.46$) and 2 wk ($\chi^2 = 2.28$, df = 1, $P = 0.1311$) old residues was similar to that on the fresh residues (Fig. 3B). After 120 h exposure, there were no statistical differences (Tukey HSD test, $P > 0.05$) in mortality on different residual ages (Table 4). However, mortality gradually declined with residual aging (Table 4). Furthermore, residues aged for ≥2 wk did not kill 50% of the KL-MY strain after 120 h.

In the Monheim strain, different aged residues of Temprid SC displayed slightly different performances (Fig 4A). The performance of ≥1 wk old residues higher than that of the fresh residues of Temprid SC, of which bed bugs survived longer ($\chi^2 = 17.45$, df = 3, $P = 0.0006$) on the fresh residues than that on ≥1 wk old residues (Fig. 4A). While there was a statistical difference, all bed bugs in both treatments were knocked down in under 40 min (Fig. 4A). After 24 h exposure, all residues irrespective of age produced 100% mortality (Table 4).

Tandem

The performance of the fresh Tandem residues on glass was higher ($P < 0.05$) than that of ≥2 wk old residues against the QLD strain, of which, bed bugs survived longer on ≥2 wk old residues (2 wk [$\chi^2 = 5.42$, df = 1, $P = 0.0199$], and 4 wk [$\chi^2 = 7.50$, df = 1, $P = 0.0062$])

Table 2. Performance of Temprid SC (118 mg/m²) and Tandem (204 mg/m²) on filter paper tested on the Monheim *Cimex lectularius* strain and the eight *Cimex hemipterus* strains

Formulation	N	Strain	KT_{50}(95% CI) min	KT_{95}(95% CI) min	Slope±SE	χ^2(df)	Mortality % (24 h)	Mortality % (120 h)[1]		PR_{50}
Temprid SC	30	Monheim	30.8(29.6–31.9)	43.0(40.6–46.6)	11.3 ± 1.1	1.7(8)	100	100	a	1
	30	TT-MY	>7,200	>7,200	-	-	16.7 ± 6.7	26.7 ± 12	b	>233
	30	SAJ-MY	>7,200	>7,200	-	-	0	36.7 ± 8.8	b	>233
	30	QLD-AU	>7,200	>7,200	-	-	0	10 ± 5.8	b	>233
	30	BM-MY	>7,200	>7,200	-	-	6.7 ± 3.3	26.7 ± 6.8	b	>233
	30	GL-MY	>7,200	>7,200	-	-	0	3.3 ± 3.3	b	>233
	30	BP-MY	>7,200	>7,200	-	-	0	13.3 ± 3.3	b	>233
	30	KL-MY	>7,200	>7,200	-	-	3.3 ± 3.3	10 ± 5.8	b	>233
	30	CH-MY	>7,200	>7,200	-	-	0	10 ± 5.8	b	>233
Tandem	30	Monheim	34.3(33.3–35.2)	41.8(40.2–44.4)	19.1 ± 2.3	1.0(5)	100	100	a	1
	30	TT-MY	>7,200	>7,200	-	-	0	10 ± 5.8	b	>210
	30	KL-MY	>7,200	>7,200	-	-	0	13.3 ± 13.3	b	>210
	30	QLD-AU	>7,200	>7,200	-	-	0	13.3 ± 13.3	b	>210
	30	BP-MY	>7,200	>7,200	-	-	0	20 ± 5.8	b	>210
	30	GL-MY	>7,200	>7,200	-	-	0	0	b	>210
	30	SAJ-MY	>7,200	>7,200	-	-	6.67 ± 3.3	10 ± 5.8	b	>210
	30	BM-MY	>7,200	>7,200	-	-	13.3 ± 3.3	36.7 ± 12	b	>210
	30	CH-MY	>7,200	>7,200	-	-	0	0	b	>210

[1]Different letters within the same column of the same formulation indicate significant differences ($P < 0.05$).

Table 3. Performance of Temprid SC (118 mg/m²) and Tandem (204 mg/m²) on glass tested on the Monheim *Cimex lectularius* strain and the eight *Cimex hemipterus* strains

Formulation	N	Strain	KT_{50} (95% CI)[1] min		KT_{95} (95% CI) min	Slope ± SE	χ^2(df)	Mortality% (24 h)	Mortality % (120 h)[1]		PR_{50}
Temprid SC	30	Monheim	27.0(26.0–27.9)	a	35.0(33.2–37.7)	14.7 ± 1.8	0.9(5)	100	100	a	1
	30	TT-MY	175.5(131.9–225.4)	b	1,295.7(844.5–2576.0)	1.9 ± 0.253	1.8(5)	80 ± 11.6	100	a	6.5
	30	SAJ-MY	346.9(315.0–380.7)	c	709.7(605.5–907.8)	5.3 ± 0.7	4.3(5)	100	100	a	12.8
	30	QLD-AU	356.1(333.5–381.7)	c	549.1(488.6–669.8)	8.7 ± 1.3	1.6(3)	70 ± 10	100	a	13.2
	30	BM-MY	396.5(329.7–484.2)	cd	1,986.5(1,347.8–3,731.3)	2.4 ± 0.3	2.3(6)	76.7 ± 6.7	100	a	14.5
	30	GL-MY	583.1(456.2–818.1)	d	4,582.2(2,344.1–18,954.0)	1.8 ± 0.3	2.9(4)	43.3 ± 14.5	70 ± 11.6	b	21.6
	30	BP-MY	1,301.2(890.8–1,939.2)	e	5,249.4(3,034.8–22,413.0)	2.6 ± 0.3	6.8(4)	60 ± 15.3	93.3 ± 3.3	a	48.2
	30	KL-MY	1,324.6(913.2 –2,073.0)	e	>7,200	1.1 ± 0.2	0.7(6)	26.7 ± 8.8	63.3 ± 3.3	b	49.0
	30	CH-MY	3,464.1(2383.0–5,293.2)	f	>7,200	1.9 ± 0.3	4.0(4)	10 ± 10	43.3 ± 3.3	c	128.2
Tandem*	30	Monheim	23.8(22.7–24.8)	a	32.9(31.0–35.9)	11.8 ± 1.4	1.9(6)	100	100	a	1
	30	TT-MY	42.8(31.9–51.3)	b	88.0(74.3–116.7)	5.2 ± 0.8	3.3(3)	96.7 ± 3.3	100	a	1.8
	30	KL-MY	73.9(61.2–83.8)	c	160.9(136.3–215.8)	4.9 ± 0.8	1.7(3)	36.7 ± 3.3	100	a	3.1
	30	QLD-AU	95.6(68.4–114.4)	cd	294.0(227.1–518.7)	3.4 ± 0.7	2.7(3)	80 ± 5.8	100	a	4.0
	30	BP-MY	115.8(105.2–125.0)	d	189.8(169.2–230.3)	7.7 ± 1.2	0.7(3)	73.3 ± 3.3	100	a	4.9
	30	GL-MY	128.5(114.4–141.2)	d	260.0(223.2–334.5)	5.4 ± 0.8	1.2(4)	70	100	a	5.4
	30	SAJ-MY	148.9 (130.5–164.3)	d	302.3(259.8–392.2)	5.3 ± 0.8	2.2(4)	93.3 ± 6.7	100	a	6.2
	30	BM-MY	152.6(132.4–169.4)	d	333.3(281.7–447.4)	4.8 ± 0.8	2.7(4)	90 ± 5.8	100	a	6.4
	30	CH-MY	196.6 (173.7–219.3)	e	499.3(407.6–696.4)	4.1 ± 0.5	1.9(5)	46.7 ± 8.8	100	a	8.3

[1]Different letters within the same column of the same formulation indicate significant differences ($P < 0.05$).
*Tandem provides significant faster mortality of *C. hemipterus* than Temprid SC.

(Fig. 5A). The performance of the fresh and 1 wk old residues are similar, of which, the bed bug survival time on the 1 wk old residues was similar (χ^2 = 2.05, df = 1, P = 0.1518) to that on the fresh residues (Fig. 5A). After 120 h exposure, all aged residues caused high mortality (≥96.7 ± 3.3%) with the QLD-AU strain (Tukey HSD test, $P > 0.05$) (Table 4).

When tested on the KL-MY strain, higher (P<0.05) mortality was registered with ≤1 wk old residues than that of ≥ 2 wk old residues. Compared with fresh residues, the KL-MY strain survived longer on ≥2 wk old residues (2 wk [χ^2 = 11.89, df = 1, P = 0.0006], and 4 wk [χ^2 = 14.97, df = 1, P = 0.0001]) (Fig. 5B). After the 120 h exposure, high mortality (≥86.7 ± 13.3%) of KL-MY strain (Tukey HSD test, $P > 0.05$) was registered for all residual times (Table 4).

Similar to what was observed with Temprid SC, different aged residues of Tandem displayed a slightly variable performance against the Monheim strain (Fig. 4B), with the performance of the 1 wk and 2 wk old residues higher than that of the fresh residues. The performance of 4 wk old residues was similar ($P > 0.05$) to that of the fresh residues. Bed bugs survived longer (χ^2 = 10.70, df = 3, P = 0.0135) on the fresh residues than that on ≥1 wk old residues (Fig. 4B). After 24 h exposure, all residues irrespective of age produced 100% mortality (Table 4).

Compared with the susceptible Monheim strain (Fig. 4), treatment aging on glass significantly reduced the efficacy of Temprid SC and Tandem against the *C. hemipterus* strains (Figs. 3 and 5). Although the residual efficacy of both products declined with aging,

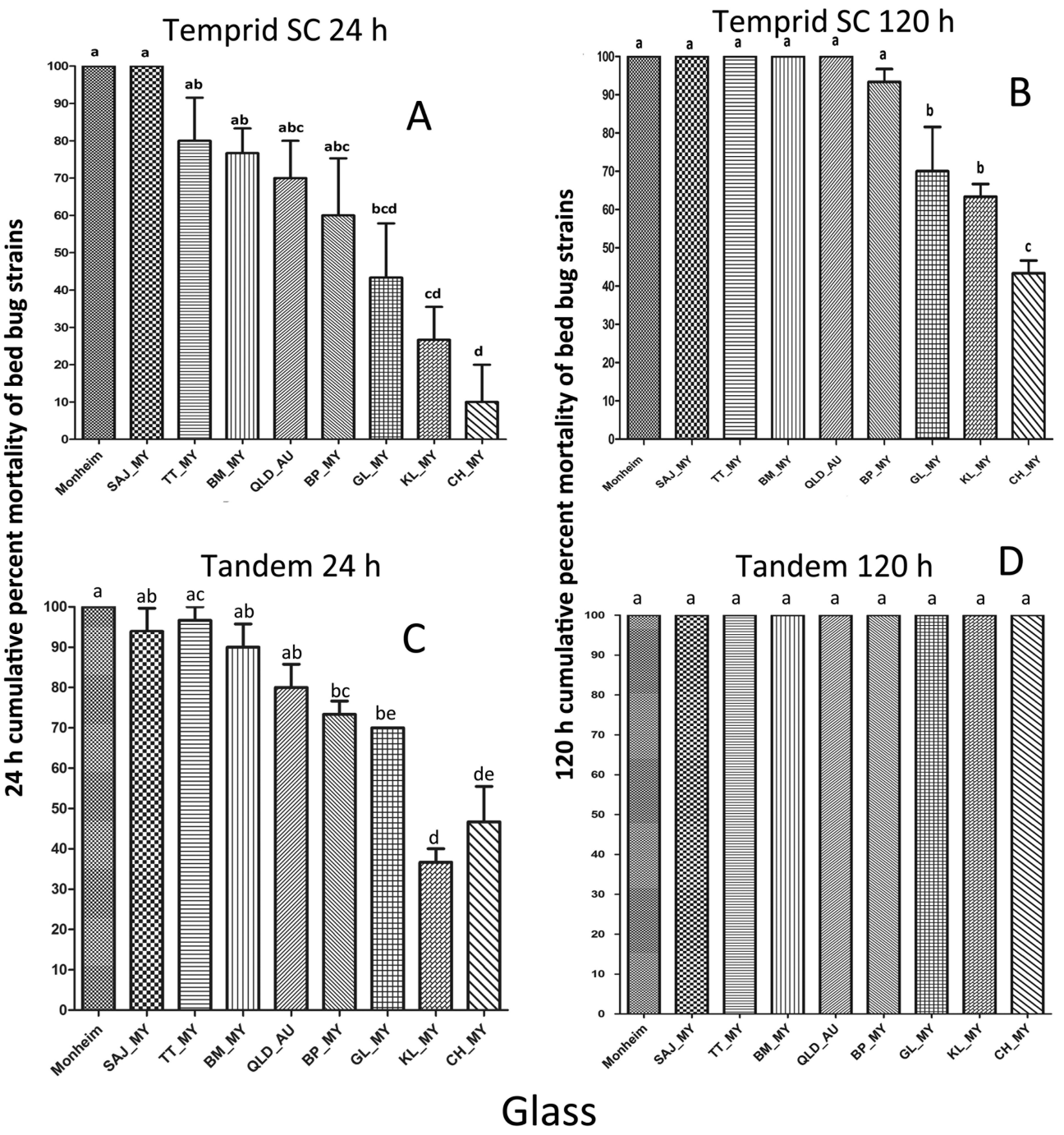

Fig. 2. Cumulative percent mortality of bed bug strains (Mean ± S.E.%) exposed to Temprid SC (A–B), and Tandem (C–D) on glass, for 24 h and 120 h exposure. Significant differences are indicated by different letters above each bar.

Tandem residues aged ≥2 wk still exerted significantly higher mortality (Tukey HSD test, $P < 0.05$) after 120 h exposure than that of Temprid SC, especially with the KL-MY strain (Table 4). All control mortality was less than 10% after 120 h.

Imidacloprid Susceptibility

Diagnostic dose assays against all strains resulted in varying mortality at 24 h, ranging from 23.3 ± 3.3% to 100% (Fig. 6). Statistical analysis indicated that mortality of the CH-MY (23.3 ± 3.3%) and GL-MY (30 ± 5.8%) strains was significantly (Tukey HSD test, $P < 0.05$) lower than that of the other six *C. hemipterus* strains (BM-MY [90 ± 5.8%], BP-MY [76.7 ± 8.8%]), KL-MY [76.7 ± 8.8%], SAJ-MY [100%], TT-MY [100%], and QLD-MY [80 ± 5.8%], as well as the Monheim strain (100%). There were no significant differences (Tukey HSD test, $P > 0.05$) between the latter six strains and the Monheim strain (Fig. 6).

There was no mortality in control group.

Discussion

The study tested the performance of Temprid SC (a pyrethroid-neonicotinoid insecticide formulation) against eight *C. hemipterus*

Table 4. Performance of aged residues of Temprid SC (118 mg/m²) and Tandem (204 mg/m²) on glass tested on the Monheim *Cimex lectularius* and the two *Cimex hemipterus* strains (QLD-AU and KL-MY)

Strain	N	Aged period	KT_{50} (95% CI)[1] min		KT_{95}(95% CI) min	Slope ± SE	χ^2(df)	Mortality % (24 h)	Mortality % (120 h)[1]	
Monheim	30	1 d Temprid	27.0(26.0–27.9)	a	35.0(33.2–37.7)	14.7 ± 1.8	0.9(5)	100	100	a
	30	1 wk Temprid	19.6(18.6–20.7)	b	30.1(27.7–33.9)	8.9 ± 1.0	2.9(6)	100	100	a
	30	2 wk Temprid	22.1(21.2–23.1)	b	31.4(29.3–34.6)	10.8 ± 1.1	4.7(6)	100	100	a
	30	4 wk Temprid	23.6(22.4–24.7)	b	36.1(33.3–40.5)	8.9 ± 0.9	3.9(7)	100	100	a
	30	1 d Tandem	23.8(22.7–24.8)	a	32.9(31.0–35.9)	11.8 ± 1.4	1.9(6)	100	100	a
	30	1 wk Tandem	19.1(18.2–20.1)	b	28.4(26.3–31.7)	9.6 ± 1.0	3.2(5)	100	100	a
	30	2 wk Tandem	19.8(18.9–20.7)	b	28.1(26.2–30.9)	10.9 ± 1.1	3.4(6)	100	100	a
	30	4 wk Tandem	22.6(21.5-23.6)	a	33.4(31.2–38.5)	9.6 ± 1.0	3.1(7)	100	100	a
QLD-AU	30	1 d Temprid	356.1(333.5–381.7)	a	549.1(488.6–669.8)	8.7 ± 1.3	1.6(3)	70 ± 10	100	a
	30	1 wk Temprid	901.7(710.1–1,156.9)	b	6,601.5(4,155.2–13,758.0)	1.9 ± 0.2	1.0(5)	40 ± 5.8	76.7 ± 3.3	ab
	30	2 wk Temprid	1,388.4(940.1–2,552.3)	bc	>7,200	1.2 ± 0.2	2.3(4)	33.3 ± 3.3	53.3 ± 8.8	b
	30	4 wk Temprid	1,841.4(1,341.4–2,807.2)	c	>7,200	1.4 ± 0.3	2.5(4)	30 ± 5.8	70 ± 10	ab
	30	1 d Tandem	102.6(79.9–123.9)	a	272.6(204.5–504.7)	3.9 ± 0.6	4.3(4)	80 ± 5.8	100	a
	30	1 wk Tandem	129.0(112.8–145.5)	ab	303.132(294.4–504.3)	3.7 ± 0.5	1.4(6)	76.7 ± 8.8	100	a
	30	2 wk Tandem	161.7(145.5–179.3)	b	355.346(295.6–479.2)	4.8 ± 0.6	1.7(5)	90 ± 5.8	100*	a
	30	4 wk Tandem	170.0(134.8–212.0)	b	927.274(599.8–2,051.0)	2.2 ± 0.4	3.8(4)	90 ± 10	96.7 ± 3.3	a
KL-MY	30	1 d Temprid	1,372.5(858.6– 3,244.2)	a	>7,200	1.0 ± 0.3	0.7(3)	26.7 ± 8.8	63.3 ± 3.3	a
	30	1 wk Temprid	2,226.9(1,439.3–4,210.5)	a	>7,200	1.0 ± 0.2	1.4(4)	13.3 ± 3.3	50 ± 10	a
	30	2 wk Temprid SC	4,711.6(2,190.3–23,506.0)	ab	>7,200	0.7 ± 0.2	0.3(3)	13.3 ± 3.3	40 ± 11.5	a
	30	4 wk Temprid	>7,200	b	>7,200	-	-	6.7 ± 6.7	26.7 ± 12	a
	30	1 d Tandem	71.4(61.7–80.6)	a	167.3(140.7–217.0)	4.4 ± 0.6	2.1(4)	36.7 ± 3.3	100	a
	30	1 wk Tandem	54.5(41.9–66.1)	a	221.4(167.4–349.6)	2.7 ± 0.4	1.2(5)	60 ± 10	86.7 ± 13.3	a
	30	2 wk Tandem	136.4(110.0–167.6)	b	991.1(656.1–1,890.3)	1.9 ± 0.2	5.0(7)	40	90 ± 5.8*	a
	30	4 wk Tandem	111.9(97.9–127.0)	b	270.4(217.5–390.7)	4.3 ± 0.6	2.3(3)	80	100*	a

[1]Different letters within the same column of the same strain and same formulation indicate significant differences ($P < 0.05$).
* Indicates significant differences ($P < 0.05$) in mortality between Temprid SC and Tandem at the same residual age.

strains. The results demonstrated that all *C. hemipterus* strains exposed to deposits of Temprid SC on glass showed high mortality (mean: 83.6%) after 120 h exposure, even though the CH-MY strain demonstrated low mortality (43.3 %). Similar findings were reported previously in both *C. lectularius* (Reid et al. 2010, Potter et al. 2012, Wang et al. 2015, 2016) and *C. hemipterus* (Leong et al. 2020a). However, Temprid SC displayed varying degrees of performance against our *C. hemipterus* strains (PR_{50}s = 6.5–128.2-fold): TT-MY (high performance), BM-MY, GL-MY, SAJ-MY, and QLD-AU (moderate performance), BP-MY, and KL-MY (poor performance), and CH-MY (very poor performance). In a previous investigation, the five CH-MY, GL-MY KL-MY, TT-MY, and QLD-AU strains, displayed high resistance to a range of pyrethroids (Dang et al. 2021), while BM-MY, BP-MY, SAJ-MY strains also showed high resistance to deltamethrin (unpublished data). In contrast, all *C. hemipterus* strains were collected when none of the neonicotinoid products were registered for use against bed bugs in Australia and Malaysia. Hence, none of the strains could have been exposed to a neonicotinoid before collection. Furthermore, the present results also showed that most of these strains (6/8) were susceptible to imidacloprid (Fig. 6). Therefore, pyrethroid resistance plays a significant role responsible for affecting the performance of Temprid SC in these *C. hemipterus* strains, which was also found in *C. lectularius* (Gordon et al. 2014). Gordon et al. (2014) also found that through selection with Temprid SC, the performance of Temprid SC and other pyrethroid-neonicotinoid formulations (e.g., Transport GHP) against *C. lectularius* was rapidly reduced. Additionally, in our investigations, the tested cohort of the CH-MY strain was not completely killed with Temprid SC, with mortality less than 50 % even after 120 h exposure. Thus, the performance of pyrethroid-neonicotinoid insecticide products against *C. hemipterus* in the field should be continually monitored.

This study also examined the performance of Tandem against the eight *C. hemipterus* strains, which is another pyrethroid-neonicotinoid formulation that contains different actives to Temprid SC. In comparison, Tandem displayed higher performance based on the KT_{50} values, producing high mortality (100%) after 120 h exposure. All PR_{50}s of *C. hemipterus* strains to Tandem ranged from 1.8 to 8.3-fold. Tandem also killed the bed bugs significantly faster ($P < 0.05$) than Temprid SC. The possible reason for the more rapid knockdown could be related to the concentration of the neonicotinoid in Tandem being almost twice that of Temprid SC at the prescribed label rate (Wang et al. 2015). Thus, to avoid failure in the management of *C. hemipterus* by using a pyrethroid-neonicotinoid formulation, the formulation with the higher concentration of active at the label application rate (namely Tandem) should preferentially be used.

Furthermore, P450s are the major detoxification enzymes responsible for metabolic resistance to pyrethroids and neonicotinoids (Scott 1999, Bass et al. 2015, Nauen et al. 2021). These enzymes can be inhibited by pretreatment of piperonyl butoxide (PBO) (Romero et al. 2009, Lilly et al. 2016, Dang et al. 2021). The result is that a greater amount of insecticide can reach the target sites. This means that a mixture formulation incorporating PBO (e.g., CrossFire Bed Bug Concentrate (MGK Co. Minneapolis, Minnesota) containing 4% clothianidin, 0.1% metofluthrin, and 10% PBO) should have higher efficacy against field strains of *C. hemipterus*. However, this has yet to be tested and ascertained.

The surface type also affects the residual efficacy of insecticides. Generally, an insecticide applied on a nonporous surface provides more excellent performance than a more porous substrate (Chadwick 1985, Rust et al. 1995, Dang et al. 2017b). This study showed that the efficacy of both Temprid SC and Tandem deposits was significantly impacted by the surface type (glass vs. filter paper). Both Temprid SC and Tandem residues on glass killed bed bugs faster than those

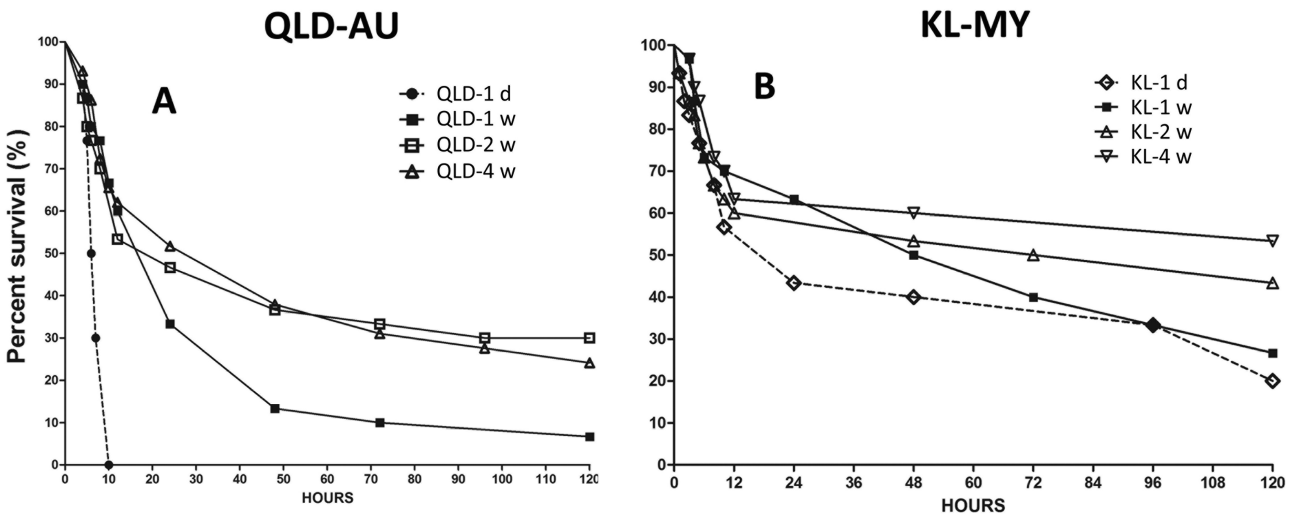

Fig. 3. Kaplan-Meier survival analyses for the QLD-AU (A) and KL-MY (B) strains when exposed to Temprid SC deposits on glass, aged for 1 d, 1 wk, 2 wk, and 4 wk. A. QLD-AU strain: (i). Comparison of percent survival between the fresh (1 d) and 1 wk old residues (1 d < 1 wk [$\chi^2 = 25.41$, df = 1, $P < 0.0001$]); (ii). 1 d < 2 wk ($\chi^2 = 23.09$, df = 1, $P < 0.0001$); (iii). 1 d < 4 wk ($\chi^2 = 26.78$, df = 1, $P < 0.0001$). B. KL-MY strain: (i). 1 d = 1 wk ($\chi^2 = 0.55$, df = 1, $P = 0.46$); (ii). 1 d = 2 wk ($\chi^2 = 2.28$, df = 1, $P = 0.1311$); (iii). 1 d < 4 wk ($\chi^2 = 5.73$, df = 1, $P = 0.0167$).

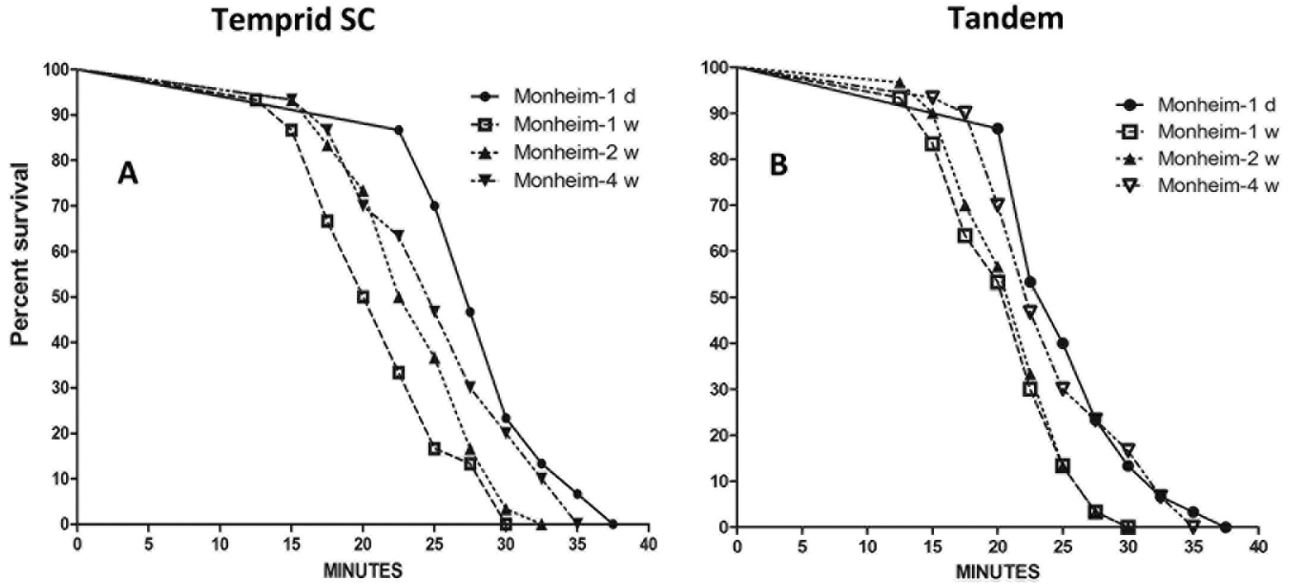

Fig. 4. Kaplan-Meier survival analyses for the Monheim strain when exposed to Temprid SC (A), and Tandem deposits (B) on glass, aged 1 d, 1 wk, 2 wk, and 4 wk. A. Comparison of percent survival on Temprid SC between fresh (1 d) and other aged (≥ 1 w) residues ($\chi^2 = 17.45$, df = 3, $P = 0.0006$). B. Comparison of percent survival on Tandem between fresh (1 d) and other aged (≥ 1 w) residues ($\chi^2 = 10.70$, df = 3, $P = 0.0135$). $P < 0.05$ indicates significant differences of percent survival between the fresh (1 d) and other aged (≥ 1 w) residues.

on the filter paper. This observation has been reported in multiple studies (Fletcher and Axtell 1993, Rojas de Arias et al. 2003, Arthur et al. 2008, Bennett et al. 2016, Wang et al. 2016, Dang et al. 2017b, Gaire and Romero 2020). For example, Wang et al. (2016) reported that the residual efficacy of Tandem on three different substrates was fabric (porous) < unpainted wood (semiporous) < vinyl (nonporous), against *C. lectularius*. Dang et al. (2017b) reported that the residual efficacy of malathion and imidacloprid on glass provided significantly faster knockdown of *C. hemipterus* than on filter paper. Gaire and Romero (2020) reported that the residual effects of Temprid SC and Transport GHP on tiles produced a more rapid mortality with the Turkestan cockroach, *Blatta lateralis* (Walker), than on wood. With a more porous surface, the absorption of a freshly applied insecticide will be higher, resulting in less insecticide available on the surface to contact the insect (Rozendaal and WHO 1997, Rojas de Arias et al. 2003, Bennett et al. 2016, Wang et al. 2016, Gaire and Romero 2020). As a result, the bed bugs presumably picked up less insecticide deposits on the filter paper than on glass. Interestingly, the efficacy of insecticides (e.g., Temprid SC) appears to be reduced more by the filter paper against *C. hemipterus*, compared with *C. lectularius* in other studies. However, such trials with both species have yet to be undertaken in parallel within the same laboratory. For example, despite the different laboratory environments, Temprid SC residues, even with a lower application rate (e.g., 16.5 mg/m^2, 30.5 mg/m^2) on various porous substrates, including wood, fabric, and filter paper, provided excellent mortality with several *C.*

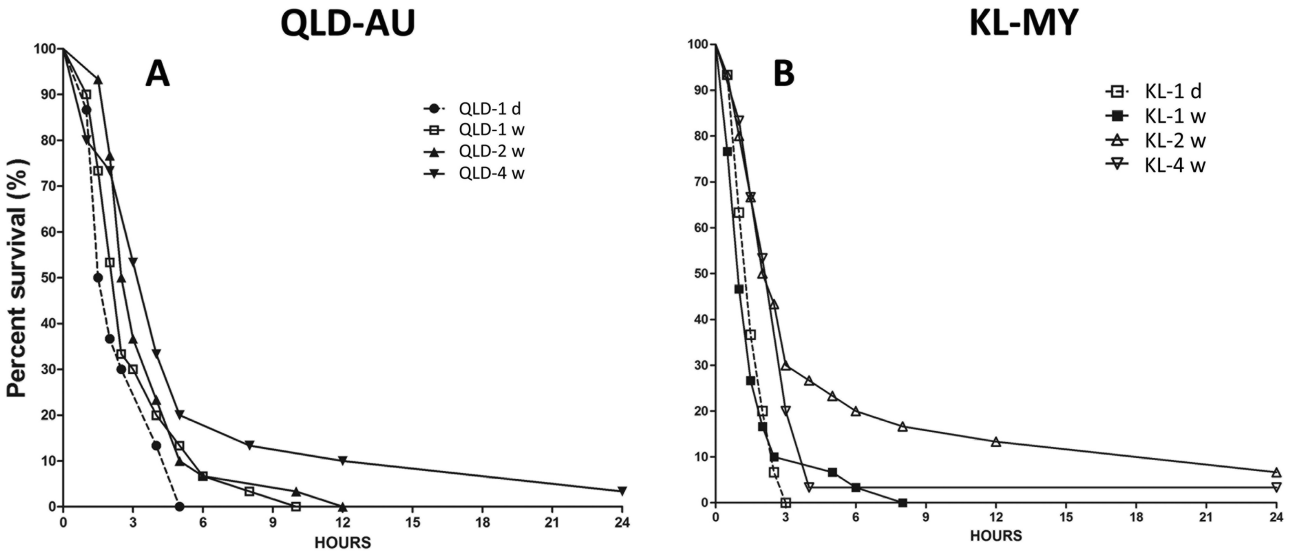

Fig. 5. Kaplan-Meier survival analyses for the QLD-AU (A) and KL-MY (B) strains when exposed to Tandem deposits on glass, aged for 1 d, 1 wk, 2 wk, and 4 wk. AQLD-AU strain: (i). Comparison of percent survival between the fresh (1 d) and 1 wk old residues (1 d = 1 wk [χ^2 = 2.05, df = 1, P = 0.1518]); (ii). 1 d < 2 wk (χ^2 = 5.42, df = 1, P = 0.0199); (iii). 1 d < 4 wk (χ^2=7.50, df = 1, P = 0.0062). B. KL-MY strain: (i). 1 d = 1 wk (χ^2 = 0.14, df = 1, P = 0.7054); (ii). 1 d < 2 wk (χ^2 = 11.89, df = 1, P = 0.0006); (iii). 1 d < 4 wk (χ^2 = 14.97, df = 1, P = 0.0001).

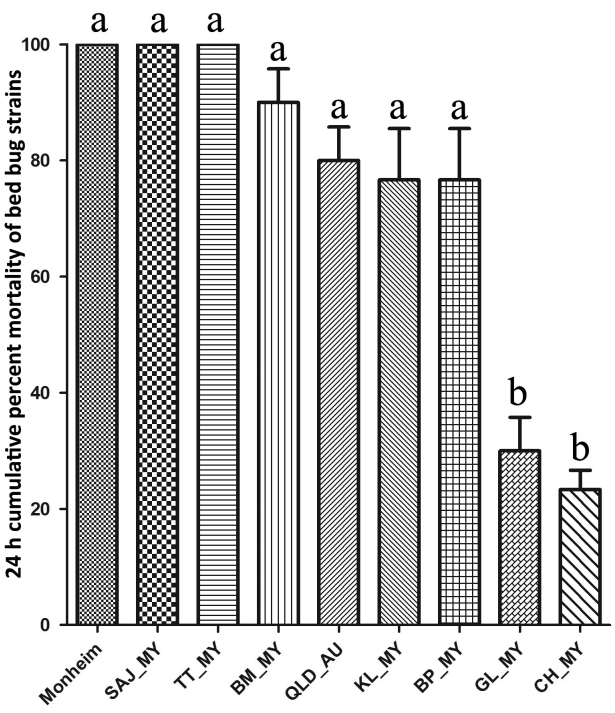

Fig. 6. Cumulative percent mortality of bed bug strains (Mean ± S.E. %) treated with 0.1 µg/insect of imidacloprid via a topical assay after 24 h postexposure (three replicates of 10 bed bugs). Significant differences (P < 0.05) are indicated by different letters above each bar.

lectularius strains (Reid et al. 2010, Potter et al. 2012, Gordon et al. 2014, Wang et al. 2016). Bed bugs typically avoid smooth surfaces and preferentially prefer rough, porous substrates, such as furniture, in wall cracks, carpet, unpainted wood, fabric, wallpaper, concrete, paper, and plaster (Wang et al. 2016) and thus insecticide pressure on the insect will be reduced. Hence, rotation of insecticides with different modes of action (e.g., essential oil-based products, diatomaceous earth products) (Wang et al. 2014, Akhtar and Isman 2016) and the use of noninsecticidal control methods (e.g., heat, vacuuming, steam) (Doggett 2013) should be incorporated in best management practices.

A confounding factor is the label instructions that could influence efficacy on different surfaces. Some formulations recommend applying the product at a standard rate (as we have in our study), while others recommend applying the product to the 'point of runoff'. A porous surface will take much more product, which may overcome the inherent differences in surface efficacy.

Insecticide aging also impacts residual efficacy against bed bugs (Fletcher and Axtell 1993). In our study, the residual age significantly impacted the effectiveness of insecticides against the *C. hemipterus* strains. In our trials, Tandem performed substantially better than Temprid with aging. As mentioned above, Tandem has a higher concentration of the neonicotinoid active ingredients at the label application rate, which may have prolonged the residual efficacy.

Although imidacloprid is one of the most widely used neonicotinoids in agricultural and urban pest management (Sheets 2010), the usage of the insecticide as an indoor residual spray against *C. hemipterus* has been limited. The topical assays found that two strains (CH-MY and GL-MY) were resistant to imidacloprid. Preexisting metabolic resistance mechanisms (e.g., P450s) found in the CH-MY and GL-MY strains may confer resistance to imidacloprid (Scott 1999, Bass et al. 2015, Nauen et al. 2021, Dang et al. 2021). Similar observations have been reported in *C. lectularius* (Romero and Anderson 2016, Lilly et al. 2018). In addition, glutathione S-transferases (GSTs) may also play a role in imidacloprid resistance in our *C. hemipterus* strains, as it has been identified in other insects (Bass et al. 2015, Romero and Anderson 2016, Yang et al. 2020a, b, 2021). Beyond metabolic resistance, neonicotinoid resistance could also be caused by target-site mutations in the nicotinic acetylcholine receptor (nAChR) subunits, such as the mutation R81T (Crossthwaite et al. 2014). Although our strains (without the neonicotinoid exposure) may not possess altered nAChR target-site resistance to imidacloprid, this mechanism could become common in the future, especially now with the widespread use of generic neonicotinoid spray formulations. Cuticle thickening may also contribute to resistance against imidacloprid in the CH-MY and

GL-MY strains (Soh and Veera Singham 2021). Further studies are warranted to investigate the neonicotinoids resistance mechanisms in *C. hemipterus*.

In summary, this study investigated the residual efficacy of pyrethroid-neonicotinoid insecticide products and factors (e.g., insecticide resistance, surface types, and residual age) that could potentially impact product performance against *C. hemipterus*. Further studies are warranted to investigate other factors such as insecticide repellency, humidity, and temperature on the efficacy of the pyrethroid-neonicotinoid mixtures. Due to cost factors, liquid insecticide treatments remain the most common strategy employed to treat bed bug infestations in Asia. However, after repeated applications, the performance of pyrethroid-neonicotinoid insecticides can be quickly reduced, as bed bug populations evolve to develop resistance the actives. Hence, periodic insecticide performance monitoring and an insecticide rotation strategy should be incorporated into a bed bug IPM management program. This would efficiently manage bed bug infestations and slow down the development of insecticide resistance.

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