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 (PR50 = 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
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. x 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/m², while that of Tandem was 204 mg/m².

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

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 used for comparison as there is no known susceptible strain of C. hemipterus worldwide (Dang et al. 2015, 2021; Leong et al. 2018). An insecticide-susceptible strain (Monheim) was determined 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.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Location</th>
<th>Year</th>
<th>Deltamethrin</th>
<th>Imidacloprid</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. lectularius</td>
<td>Monheim</td>
<td>Monheim, Germany, laboratory colony</td>
<td>Late 1960s</td>
<td>Susceptible</td>
<td>Susceptible</td>
</tr>
<tr>
<td>C. hemipterus</td>
<td>QLD-AU</td>
<td>North Queensland, AUSTRALIA</td>
<td>2007</td>
<td>Resistant1</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>KL-MY</td>
<td>Kuala Lumpur, MALAYSIA</td>
<td>2005</td>
<td>Resistant1</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>BM-MY</td>
<td>Bukit Mertajam, Penang, MALAYSIA</td>
<td>2015</td>
<td>Resistant1</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>BP-MY</td>
<td>Bayan Point, Penang, MALAYSIA</td>
<td>2015</td>
<td>Resistant1</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>SAJ-MY</td>
<td>Saujana, Penang, MALAYSIA</td>
<td>2015</td>
<td>Resistant1</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>TT-MY</td>
<td>Tanjung Tokong, Penang, MALAYSIA</td>
<td>2015</td>
<td>Resistant1</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>CH-MY</td>
<td>Christian, Penang, MALAYSIA</td>
<td>2015</td>
<td>Resistant1</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>GL-MY</td>
<td>Green Lane, Penang, MALAYSIA</td>
<td>2015</td>
<td>Resistant1</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

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 CO₂ 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 (Fioroni, Ingre, France) at room temperature (23 ± 2°C). 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 24 h. Tandem also registered low mortality with the C. hemipterus strains, with PR50s ranging from 1.8 to 8.3-fold (Table 3). Tandem resulted in varying mortality of C. hemipterus strains 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 (PR50 = 6.5-fold), moderate performance on the BM-MY, GL-MY, SAJ-MY, and QLD-AU strains (PR50 = 12.8 to 21.4-fold), poor performance on the BP-MY (PR50 = 48.2-fold) and KL-MY (PR50 = 49-fold) strains, and very poor performance on the CH-MY strain (PR50 = 128.2-fold).

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 determine the KT50 value of the resistant strain by the corresponding KT50 value of the susceptible Monheim strain. The classification of performance was as follows: excellent performance (PR50 ≤ 1-fold), high performance (1-fold<PR50≤10-fold), moderate performance (10-fold<PR50≤25-fold), poor performance (25-fold<PR50≤50-fold), and very poor performance (PR50>50-fold). The higher the PR50, 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). KT50 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 (PR50 = 6.5-fold), moderate performance on the BM-MY, GL-MY, SAJ-MY, and QLD-AU strains (PR50 = 12.8 to 21.4-fold), poor performance on the BP-MY (PR50 = 48.2-fold) and KL-MY (PR50 = 49-fold) strains, and very poor performance on the CH-MY strain (PR50 = 128.2-fold).

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 KT50 values (Table 4). Compared with fresh residues, the QLD-AU strain survived longer on ≥1 wk old residues (including 1 wk [χ² = 25.41, df = 1, P < 0.0001], 2 wk [χ² = 23.09, df = 1, P < 0.0001], and 4 wk [χ² = 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
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 KT50 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, $P = 0.0062$])
The performance of the fresh and 1 wk old residues was similar, of which, the bed bug survival time on the 1 wk old residues (Fig. 5A). The performance of the fresh and 1 wk old residues are > 0.05) was registered for all residual times (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 strain (Figs. 3 and 5). Although the residual efficacy of both products declined with aging,

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 (P < 0.05) 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).

### 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

<table>
<thead>
<tr>
<th>Formulation</th>
<th>N</th>
<th>Strain</th>
<th>KT$_{50}$ (95% CI) min</th>
<th>KT$_{90}$ (95% CI) min</th>
<th>Slope ± SE</th>
<th>χ²(df)</th>
<th>Mortality % (24 h)</th>
<th>Mortality % (120 h)</th>
<th>PR$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temprid SC</td>
<td>30</td>
<td>Monheim</td>
<td>≥7,200</td>
<td>≥7,200</td>
<td>-</td>
<td>1.7(8)</td>
<td>100 ± 1.1</td>
<td>100 ± 1.4</td>
<td>a 1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>TT-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>36.7 ± 8.8</td>
<td>10 ± 5.8</td>
<td>b &gt;233</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>SAJ-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>6.7 ± 3</td>
<td>26.7 ± 6.8</td>
<td>10 ± 5.8</td>
<td>b &gt;233</td>
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<tr>
<td></td>
<td>30</td>
<td>QLD-AU</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>13.3 ± 3.3</td>
<td>10 ± 5.8</td>
<td>b &gt;233</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>BM-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>13.3 ± 3.3</td>
<td>10 ± 5.8</td>
<td>b &gt;233</td>
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<tr>
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<td>GL-MY</td>
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<td>-</td>
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<td>10 ± 5.8</td>
<td>b &gt;233</td>
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<tr>
<td></td>
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<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>13.3 ± 3.3</td>
<td>10 ± 5.8</td>
<td>b &gt;233</td>
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<tr>
<td></td>
<td>30</td>
<td>KL-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>13.3 ± 3.3</td>
<td>10 ± 5.8</td>
<td>b &gt;233</td>
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<tr>
<td></td>
<td>30</td>
<td>CH-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>13.3 ± 3.3</td>
<td>10 ± 5.8</td>
<td>b &gt;233</td>
</tr>
</tbody>
</table>

**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**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>N</th>
<th>Strain</th>
<th>KT$_{50}$ (95% CI) min</th>
<th>KT$_{90}$ (95% CI) min</th>
<th>Slope ± SE</th>
<th>χ²(df)</th>
<th>Mortality % (24 h)</th>
<th>Mortality % (120 h)</th>
<th>PR$_{50}$</th>
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<tr>
<td>Temprid SC</td>
<td>30</td>
<td>Monheim</td>
<td>≥7,200</td>
<td>≥7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
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<tr>
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<td>TT-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
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<tr>
<td></td>
<td>30</td>
<td>SAJ-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
<td>a 1</td>
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<td></td>
<td>30</td>
<td>QLD-AU</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
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<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
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<td>GL-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
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<td>BP-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
<td>a 1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>KL-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
<td>a 1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>CH-MY</td>
<td>&gt;7,200</td>
<td>&gt;7,200</td>
<td>-</td>
<td>0</td>
<td>10 ± 5.8</td>
<td>100 ± 1.4</td>
<td>a 1</td>
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</tbody>
</table>
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*
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. The surface type also affects the residual efficacy of insecticides. Generally, an insecticide applied on a nonporous surface provides 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 more excellent performance than a more porous substrate (Chadwick 2018, 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 on the KT 50 values, producing high mortality (100%) after 120 h exposure. All PR 50s of *C. hemipterus* strains to Tandem ranged higher than 7,200 - - 6.7 ± 6.7 26.7 ± 12 a 7,200 0.7 ± 0.2 0.3(3) 13.3 ± 3.3 40 ± 11.5 a 7,200 1.0 ± 0.3 0.7(3) 26.7 ± 8.8 63.3 ± 3.3 a 7,200 0.7 ± 0.2 0.3(3) 13.3 ± 3.3 40 ± 11.5 a 7,200 1.0 ± 0.2 1.4(4) 13.3 ± 3.3 50 ± 10 a 7,200 1.4 ± 0.3 2.5(4) 30 ± 5.8 70 ± 10 ab 7,200 1.2 ± 0.2 2.3(4) 33.3 ± 3.3 53.3 ± 8.8 b 7,200 1.0 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab 7,200 1.4 ± 0.2 4.7(4) 100 ± 5.8 a 7,200 1.2 ± 0.2 2.3(4) 30 ± 5.8 70 ± 10 ab.
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/m², 30.5 mg/m²) 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 \( \chi^2 = 2.05, \text{df} = 1, P = 0.1518 \)); (ii). 1 d < 2 wk (\( \chi^2 = 5.42, \text{df} = 1, P = 0.0199 \)); (iii). 1 d < 4 wk (\( \chi^2 = 7.50, \text{df} = 1, P = 0.0062 \)). B. KL-MY strain: (i). 1 d = 1 wk (\( \chi^2 = 0.14, \text{df} = 1, P = 0.7054 \)); (ii). 1 d < 2 wk (\( \chi^2 = 11.89, \text{df} = 1, P = 0.0006 \)); (iii). 1 d < 4 wk (\( \chi^2 = 14.97, \text{df} = 1, P = 0.0001 \)).

**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.

*B. 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 run-off’. 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. Pre-existing 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 *B. 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 (Crossthaite 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 to 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.

**Acknowledgments**

We thank Universiti Sains Malaysia for a post-doctoral fellowship to K.D.; Dr. C. Toi (Westmead Hospital) for assistance with statistical analyses; Dr. D.-Y. Kim (presently Kasetsart University, Thailand) and Dr. X.-Y. Leong (presently Ecolab, Malaysia) for help with bed bug rearing.

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