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Evaluation of Cimi-Shield™ as a residual against bed bugs *Cimex lectularius* L.

Ameya D. Gondhalekar; PhD

Research Assistant Professor

Center for Urban and Industrial Pest Management

Department of Entomology

Purdue University

West Lafayette, IN 47907

e-mail:ameyag@purdue.edu

Phone: (765) 494-3839

Study objective:

1. To evaluate the efficacy of dried residues (1-day-old) of Cimi-Shield [an EPA exempt 25 (b) insect control formulation], against bed bugs, *Cimex lectularius* L. at manufacturer recommended application volume

2. To evaluate the residual efficacy of Cimi-Shield, against bed bugs, *Cimex lectularius* at intervals of thirty (30) days, sixty (60) days, ninety (90) days.

Methods:

Description of bioassay arenas

Bioassay arenas were prepared using 10 gallon glass fish tanks (22.00 x 12.00 x 13.50 inches; Walmart, Bentonville, AR), tan colored medium cut pile carpet (Menards, Eau Claire, WI) and electric reptile heating pads attached to a 14 inch long wooden stick with rubber bands (Fig. 1). First the, carpet roll was cut to match the dimensions of the bottom of the fish tank. Each carpet piece (~20 x 10 inches) was then affixed to the bottom of the fish tank using either white or transparent caulk (Menards, Eau Claire, WI). The caulk was allowed to cure for at least 2 to 3 days before using the bioassay arenas for treatment with Cimi-Shield. Use of caulk created a tight seal between the glass and the carpet that prevented bed bugs from moving under the carpet (Fig. 1A).

A single reptile-heating pad attached to a wooden stick with rubber bands was placed hanging over the fish tank ~ 5 cm above from the carpet (Figs. 1B and 1C). The wooden stick allowed researchers to adjust the distance of the heating pad from the carpet as well as to move it across the length of the tank. To prevent bed bugs from climbing onto the heating pad it was never allowed to touch the carpet or the sidewalls of the fish tank. The heating pad was turned on only when required.

Treatment of carpet with Cimi-Shield and residue aging

The product (Cimi-Shield) that was never frozen or exposed to excessive heat was diluted in distilled water as per manufacturer's instructions. An Airofog^{TM} sprayer that had filters removed from the nozzle was used to apply the product over the carpet. The wand or the nozzle was set

to “fan” spray mode during treatment. Manufacturer recommended spray volume was used for treating the carpet. After treatment, the sprayer and the nozzle were rinsed with distilled water. The Cimi-Shield treated carpet was allowed to dry completely (~24 h) before use in bioassays. Carpets in control bioassay arenas were left untreated. During spray treatment, untreated control chambers were placed in another room to avoid contamination caused by spray drift. Additionally, during treatment, bioassay arenas were placed in a rectangular plastic container to avoid contamination of non-target areas of the room (Videos 1 and 2).

Exposing bed bugs to bioassay arenas

Bed bugs of the Harlan laboratory susceptible strain were used for all bioassays. Ten adult male bed bugs that were fed defibrinated rabbit blood 4-5 days before the initiation of bioassays were used per replicate. Three replicates were performed in separate bioassay arenas. Similarly, three independent control replicates were performed for each treatment listed below. Before releasing bed bugs on treated or control tanks, carpet in all arenas was gently rubbed for ~ 1 min (Note: New pair of Nitrile gloves was used for each arena). However, before performing the bioassay test with 3-month old residues, carpet in all bioassay arenas was vacuumed with a portable vacuum cleaner. After releasing 10 adult male bed bugs on completely dry treated (~1 day old or aged) or control (untreated) carpets, the heated reptile pad (temperature of ~ 37 to 40° C or 98 to 104°F) hanging over the carper induced their movement across the length of the bioassay arena. Additionally, fluorescent lights directly above the heating pad were turned off to create a dark area that was attractive to bed bugs. Bed bug movement across the length of the carpet was induced for ~2 hours after releasing the bed bugs. During this 2 hour movement-induction period the position of the heating pad and dark area (lighting control) was rotated every 15 mins. Stimulus-induced bed bug movement was also observed for 1 hour on days 1 and 3 after bed bug release. Bioassays were continued for 14 days and mortality observations were recorded at days 1, 3, 7, 10 and 14 after bed bug release. Bed bugs that were unresponsive, unable to walk or right themselves upon prodding were scored as dead. The temperature of the room where bioassays were conducted was maintained between 22 to 24°C and relative humidity varied between 30 to 45%.

***Treatments*:**

I. Carpets treated with Cimi-Shield using manufacturer recommended dilution rate and application volume x 3 replicates
   a. One-day old residues
   b. One-month old residues
   c. Two-month old residues
   d. Three-month old residues

II. Untreated carpets (control treatment) x 3 replicates
   a. Performed with one-day old residue test
   b. Performed with one-month old residue test
   c. Performed with two-month old residue test
   d. Performed with three-month old residue test

Data analysis:

To determine the differences in movement of bed bugs on treated and untreated (control) carpets, non-parametric Kruskal-Wallis test was performed at the $P<0.05$ significance level. Repeated measures analysis of variance (ANOVA) was used to determine the differences in bed bug mortality between treated (1-day old residues) and untreated carpets over the entire bioassay duration (0 to 14 d). Similarly, repeated measures ANOVA was used to compare mortality differences due to Cimi-Shield residue aging for 1-, 2- or 3-months, followed by a means separation t-test ($P<0.05$).

Results & Discussion:

Bed bug movement in the arena

When bed bugs were released at one end of the bioassay arena, they readily travelled to the other end of the arena due to the presence of the warm heating pad and dark area (Video 3). This movement occurred irrespective of the carpet treatment status. The inner length of the fish tank was ~20 inch and 80 to 100% bed bugs travelled this entire area at least 6 times or more in the first two hours of the bioassay. This indicates that bed bugs travelled ~10 linear

feet or 120 inches right after their release into the bioassay arena. Induction of bed bug movement was done to allow transfer of Cimi-Shield active ingredients from the carpet to the insect cuticle.

Within the first 2 hours of bed bug release into the treated and untreated bioassay arenas, their activity was very high and 70 to 100% of the insects oriented toward the heat and light stimulus (Fig. 2A). At day 1, the number of bed bugs responding to heat and light stimulus decreased to 40–60%, but no differences in movement were found between treated and untreated carpets (Fig. 2B). The decrease in the movement toward the stimulus likely occurred because most bed bugs had found their ideal harborage spot on the carpet within the first 24 h of the bioassay. At day 3, the number of bed bug moving toward the heat pad and dark area was even lower than day 1 (Fig. 2C). However, on day 3 the proportion of bed bugs actively moving was significantly lower ($P<0.05$) in treated arenas in comparison to untreated arenas (Figs. 2A to 2C). The decrease in activity of bed bugs observed on treated carpets at day 3 is indicative of intoxication symptoms caused by exposure to dry residues of Cimi-Shield.

Mortality caused by exposure to Cimi-Shield residues

One-day old dry residues of Cimi-Shield provided complete (100%) mortality of adult bed bugs by day 10 (Fig. 3). Bioassays were also conducted with Cimi-Shield residues for 1-, 2- and 3-months. In comparison to 1-day-old residues, mortality response in aged residue treatments was slightly slower, especially at observation intervals of days 7, 10 and 14 (t-test; $P<0.05$) (Fig. 4). Nonetheless, high mortality (83 to 87%) was observed in aged residue treatments by day 14. The treated carpets were vacuumed before starting the bioassays on 3-month old Cimi-Shield residues. In spite of vacuuming, ~83% bed bug mortality was still achieved by day 14. Mortality in untreated or control bioassay arenas was always under 20% and was also significantly lower than bed bug mortality in treated arenas at all aging intervals ($P<0.05$).

Summary and Conclusions:

1. Cimi-Shield residues affect the ability of bed bugs to actively walk or move after about 72 h of exposure.

2. Aging of Cimi-Shield residues for 1- to 3-months slightly lowers its speed of kill, however, 83–87% bed bug mortality (by day 14) is still achievable.
3. Although Cimi-Shield is a 25 (b) category natural product that is exempt from EPA registration, it displays very high residual efficacy against bed bugs. This is in contrast with many other natural compound products that do not possess residual activity against bed bug or other insects.

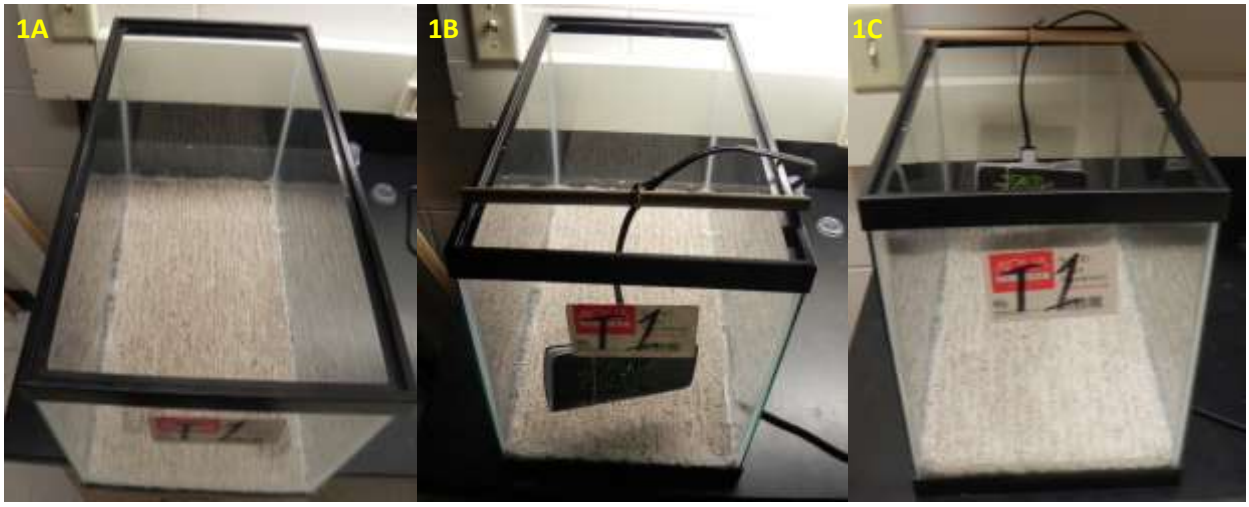

Figure 1. Bioassay arenas prepared using 10-gallon fish tanks, medium cut pile carpet and reptile heating pads. (1A) Shows Cimi-Shield treated bioassay arena. (1B) Shows treated bioassay arena with reptile heating pad suspended/ hanging over the carpet (~5 cm from the bottom). Also, note that fluorescent lighting on the side of the heating pad was turned off to create a relatively dark area. (1C) In this bioassay arena heating pad is moved toward the back of the fish tank and the lights over the heating pad are turned off.

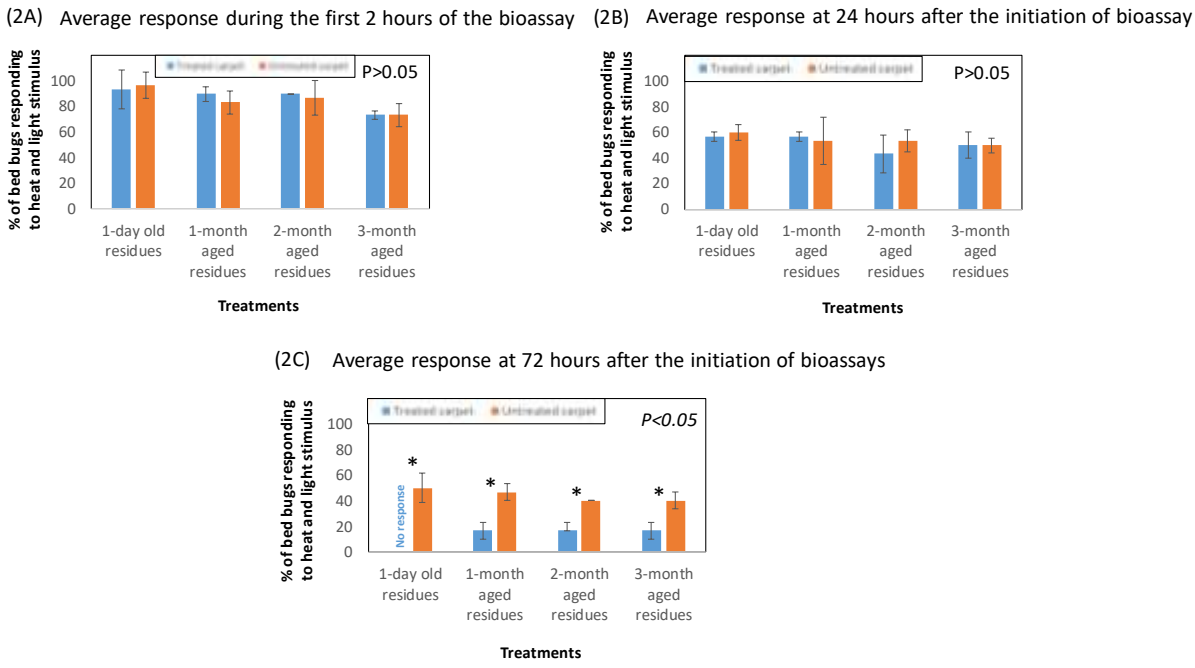

Figure 2. These bar graphs show the average percentage of bed bugs that were attracted to the heat and light stimuli at various time intervals in different treatments. (2A) Represents average percentage of bed bugs attracted to warm and dark areas of the fish tank during the first 2 hours of the bioassay. The position of the stimulus (heat pad and dark area) was changed every 15 mins. (2B) and (2C) Shows the average percentage of bed bugs responding to stimuli at 24 and 72 hours, respectively after the initiation of bioassays. Asterisks (*) next to the bars indicate statistically significant differences in percentage of bed bugs responding to stimuli between treated (blue) and untreated carpets (orange) (Kruskal-Wallis test; *P<0.05*). Differences in insect response between treated and untreated or control carpets can only be seen at the 72 h interval. Error bars indicated (± standard error) values.

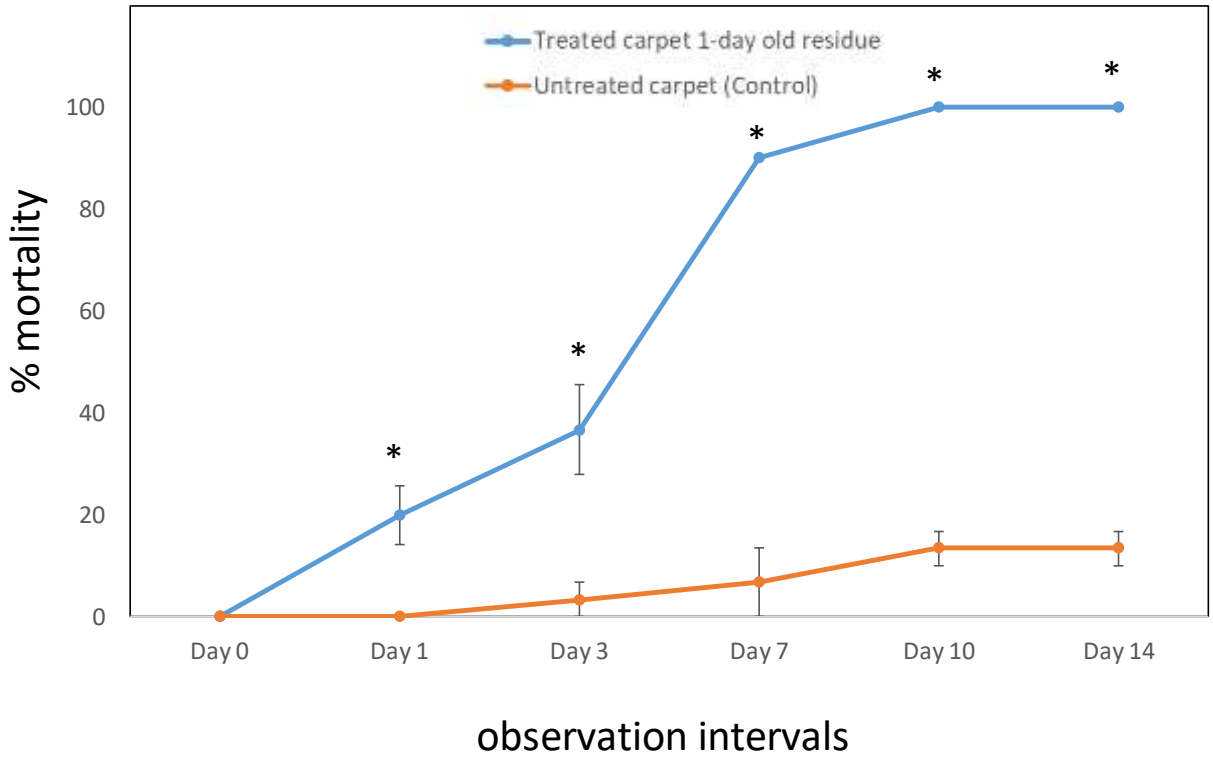

Figure 3. Line graphs depicting bed bug mortality (in %) trends from 0 to 14 days on treated carpets and controls (untreated carpets). The P-value of *<0.05* indicates statistically significant mortality differences between treated and control carpets at different observation intervals, shown by asterisks (*) (Repeated measures ANOVA). Error bars indicate (± standard error) values. Complete 100% mortality was achieved on treated carpet replicates by day 10. Mortality on control carpets was <15%.

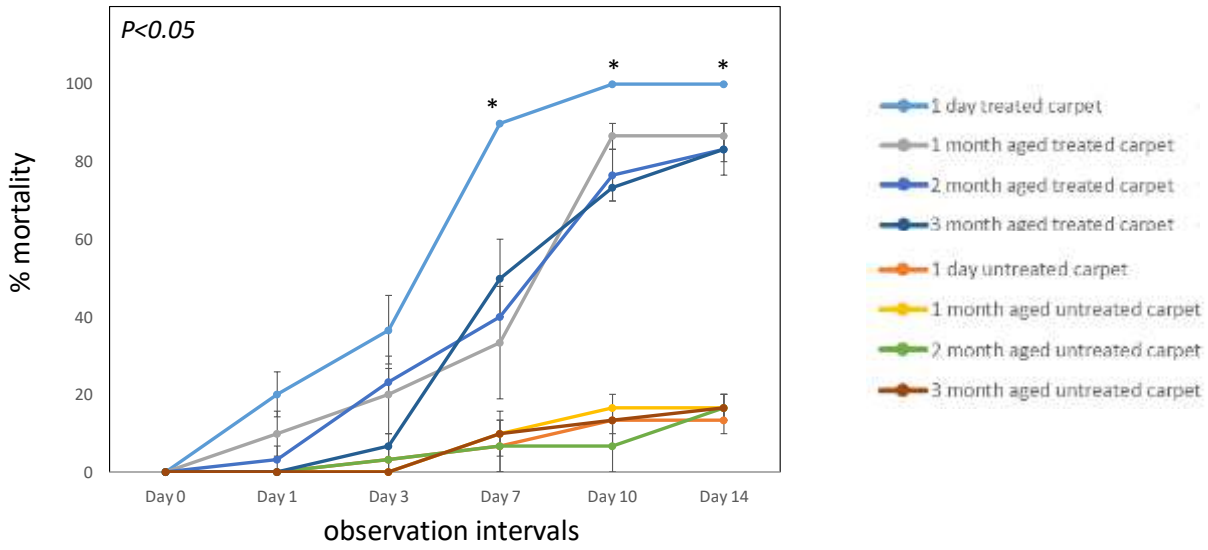

Figure 4. Line graphs depicting bed bug mortality trends (in %) from 0 to 14 days on treated carpet and controls (untreated carpets) aged for various time intervals (1 day to 3 months). P-value of *P<0.05* indicates statistically significant mortality differences between treated and control carpets (Repeated measures ANOVA). A subsequent means separation t-test at the *P<0.05* significance level revealed that mortality in 1-day old residues was significantly higher at observation intervals of day 7, 10 and 14 (shown by asterisks *). Error bars indicated (± standard error) values. Complete 100% mortality was achieved in 1-day old residues by day 10. In 1-, 2- or 3-month residue aging tests 83 to 88% mortality was observed by day 14.