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## **Final Report**

### **Evaluation of the Effect of "CHINCHEX" against Highly Resistant Bed bugs**

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**Location of Test Site (physical address):** New Mexico State University, Las Cruces, NM

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A handwritten signature in black ink, appearing to read 'Alvaro Romero', with a horizontal line underneath the name.

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June 21, 2022

Alvaro Romero, PhD

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## Objectives of the study

- Evaluate the effectiveness of a silica-gel based product ‘CHINCHEX’ against the bed bug *Cimex lectularius*
- Test the material under different treatment scenarios, including force exposure, non-force exposure.
- Evaluate the transfer of “CHINCHEX’ from treated bugs to untreated bugs

## Methodology

- Insects

Adult bed bugs were obtained from a colony maintained at 25° C, 50% ±5% relative humidity, and a photoperiod of 12 h:12 h (light:dark). This colony was originally established from bed bugs collected in 2016 from an apartment in Albuquerque, NM, USA. This population was determined to be resistant to deltamethrin, following a method proposed by Romero et al. (discriminating doses of 0.13 mg/cm<sup>2</sup> technical grade deltamethrin; 0% mortality in 20 third to fifth instar nymphs). In the laboratory, the insects were fed defibrinated rabbit blood (Quad Five, Ryegate, MT, USA), heated to 37 °C with a circulating water bath, through a Parafilm-membrane feeder. Evaluations began 8 to 10 days after adult emergence; the insects had been fed as adults three days before the initiation of the experiment.

### **Experiment 1: Forced exposure of bed bugs to various surfaces treated with “CHINCHEX”**

This experiment aims to evaluate the lethality of bed bugs when exposed to concrete, stucco, painted stucco, or popcorn type of ceiling, treated with “CHINCHEX” (Fig. 1). 30 x 30 cm concrete slabs were used to prepare the following surfaces:

1. Concrete only (no “CHINCHEX”) (Control)
2. Concrete
3. Stucco (ready-to-use stucco Patch, Pro-Select)
4. Painted Stucco (color sample, Sherwin Williams)
5. Popcorn Ceiling Patch (Homax)



**Figure 1.** Surfaces that were treated with “CHINCHEX” for the forced exposure experiment with bed bugs. Squares of 12 x 12 cm were covered with one of the following coats stucco, painted stucco, or popcorn ceiling.

The coated surfaces (2, 3,4 and 5, see above) were dried overnight before treatment with “CHINCHEX”. Circles (diameter 9 cm) were marked and their interior treated with “CHINCHEX” using a brush (Les Pinceaux de Chanel) (Fig. 2). The application rate used was 1 pound (453 g) per 1000 square feet (rate regularly used for “Cimexa”). The total amount applied to each circle (area: 63 cm<sup>2</sup>) was 30 mg.



**Figure 2.** Makeup brush used for the application of “CHINCHEX” to concrete, stucco, painted stucco or popcorn ceiling.

Twenty (20) bed bugs were exposed to the treated circles and restrained a ring made of a Petri dish (See Figure 1). The bugs remained forcibly exposed until their dead.

**Results:**

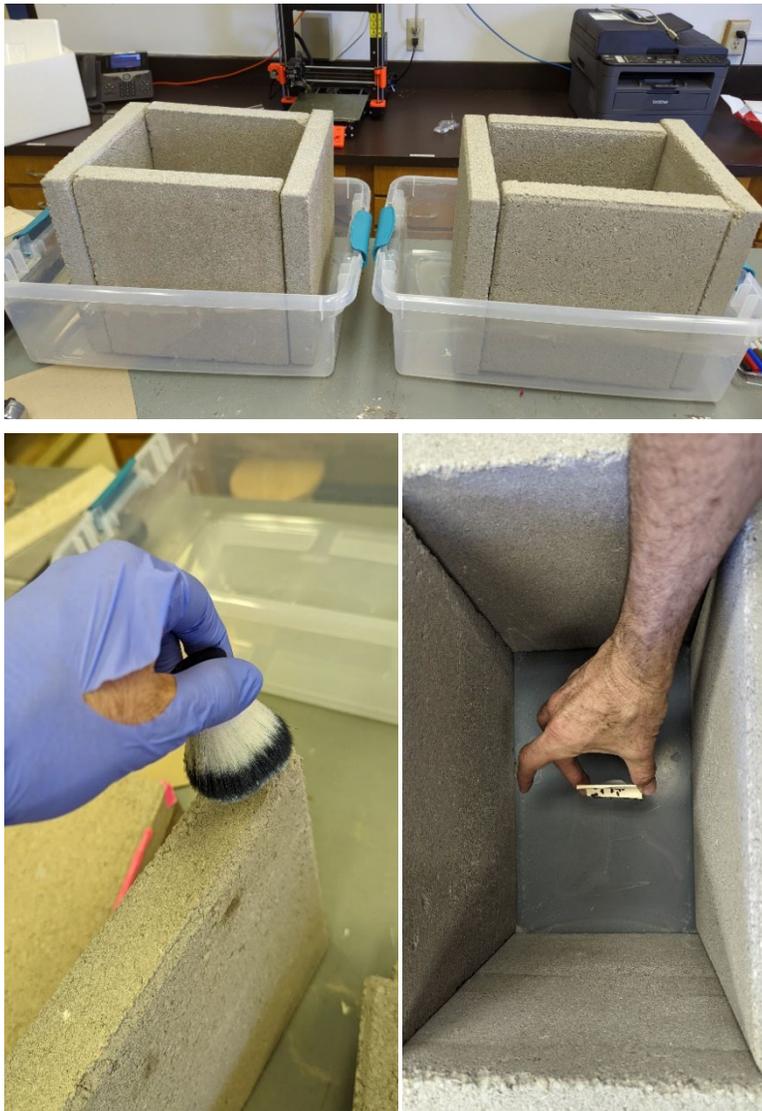
- At the application rate used, bugs died faster when exposed to “CHINCHEX” on stucco than on other surfaces. At 24 h post-exposure, 75% of bugs have died on stucco, while no mortality was recorded at this point on bugs exposed on other surfaces. At 48 h, 100% of bugs exposed on stucco have died, while the onset of mortality was recorded in other treatment (Table 1). Bugs from all treatments have died by day 3 (72h).

**Table 1.** Mortality of bed bugs continuously exposed to four surfaces treated with “CHINCHEX”: concrete, stucco, painted stucco and popcorn ceiling.

	<b>Treatment</b>				
	<b>Control</b>	<b>Concrete</b>	<b>Stucco</b>	<b>Painted Stucco</b>	<b>Popcorn ceiling</b>
<b>24 h</b>	0%	0%	75%	0%	0%
<b>48 h</b>	0%	30%	100%	27%	15%
<b>72 h</b>	0%	100%	100%	100%	100%

## Experiment 2: Effect of Product A on assembled concrete blocks

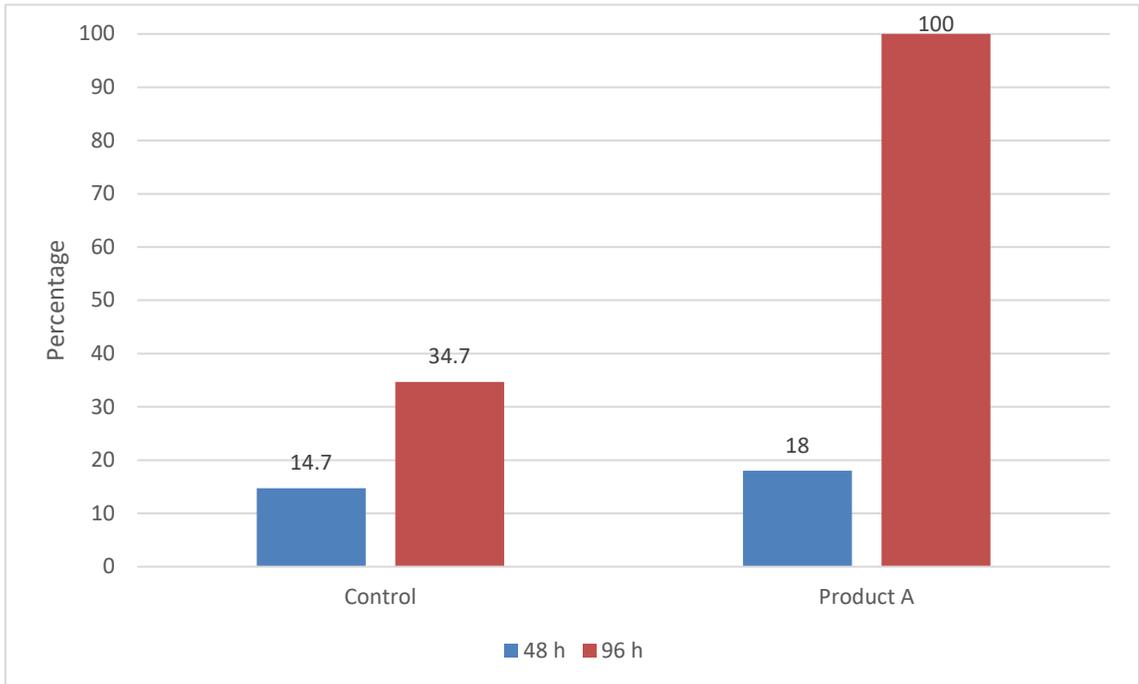
This experiment aims to evaluate the efficacy of “CHINCHEX” in common harborage sites for bed bugs such as crevices. To create the crevices, sets of four blocks were assembled in a square as shown on Fig. 3. After treatment of block edges with “CHINCHEX” (following manufacturer’s recommendations), 75 bugs (25 females, 25 males and 25 nymphs) harboring in a tent were released in the center of the arena and allowed to hide in the blocks for 24 hours.



**Figure 3.** Above, Concrete blocks assembled to form crevices, common bed bug refuges; **left**, application of “CHINCHEX” to external edges of blocks with a makeup brush. After assembling, these edges will conform the bed bug harborages; **right**, bed bugs harboring in a paper tent which was placed in the center of the blocks.

## Results

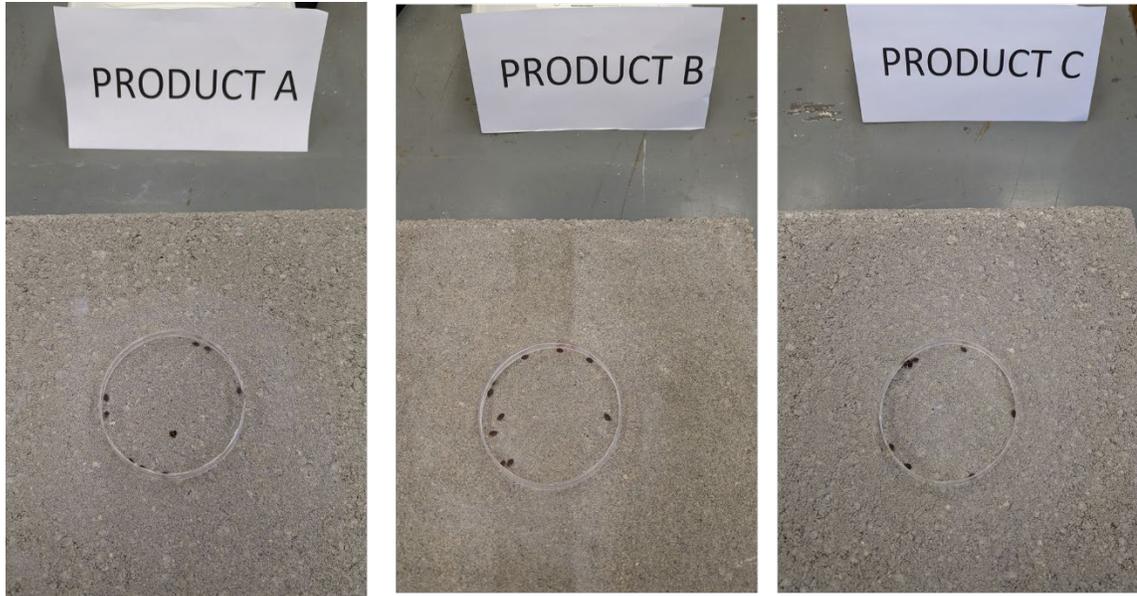
Mortality was evaluated after 48 h post-exposure by disassembling the blocks. While 90% of the bugs were found hidden in the crevices, 14.7% of control were dead and only 34% of “CHINCHEX”-exposed bugs were dead (44% females, 48%, and 12% nymphs). Surviving bugs were removed from the arena and monitored for mortality. 96 h later all bugs were found dead (Fig. 4).



**Figure 4.** Mortality of bed bugs (25 females, 25 males and 25 nymphs), 48 h and 96 h post-exposure.

### **Experiment 3: Forced Exposure to various Formulations of “CHINCHEX”**

“CHINCHEX” has three different formulations and the purpose of this experiment was comparatively evaluate their effectiveness when the bed bugs are continuously exposed to each of them. The test materials were applied to concrete with a brush following manufacturer’s recommendations (Fig. 5). Ten bed bugs were then exposed to the treated surfaces and monitored for mortality since 2 h post-exposure.



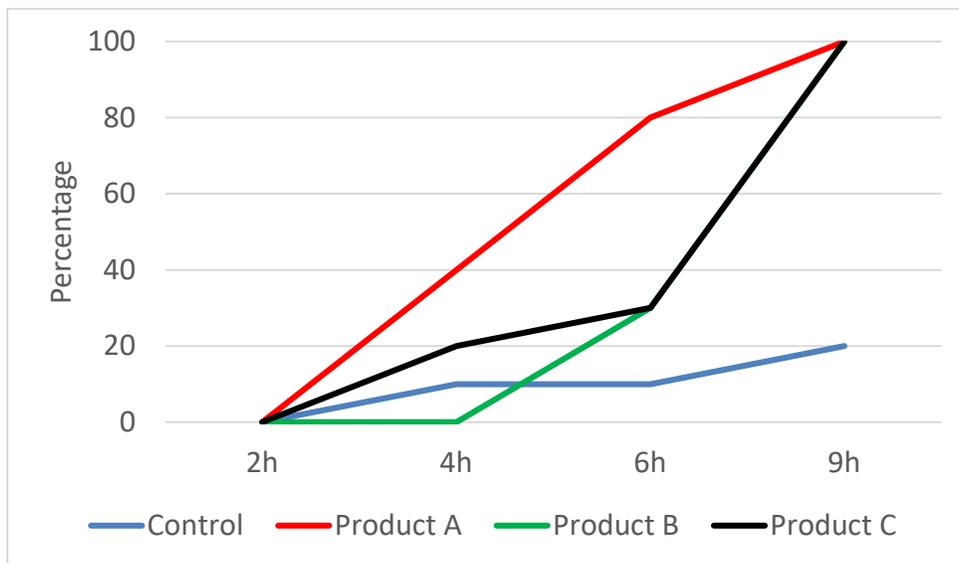
**Figure 5.** Exposure of bed bugs to “CHINCHEX”-treated blocks. Three versions of the products were tested.

## Results

Mortality of bed bugs was detected 4 h post-exposure in Product A and C, and rapidly increased in all versions of the products (Fig. 6). All bed bugs were found dead by hour 9 post-exposure (Fig. 6 and 7).



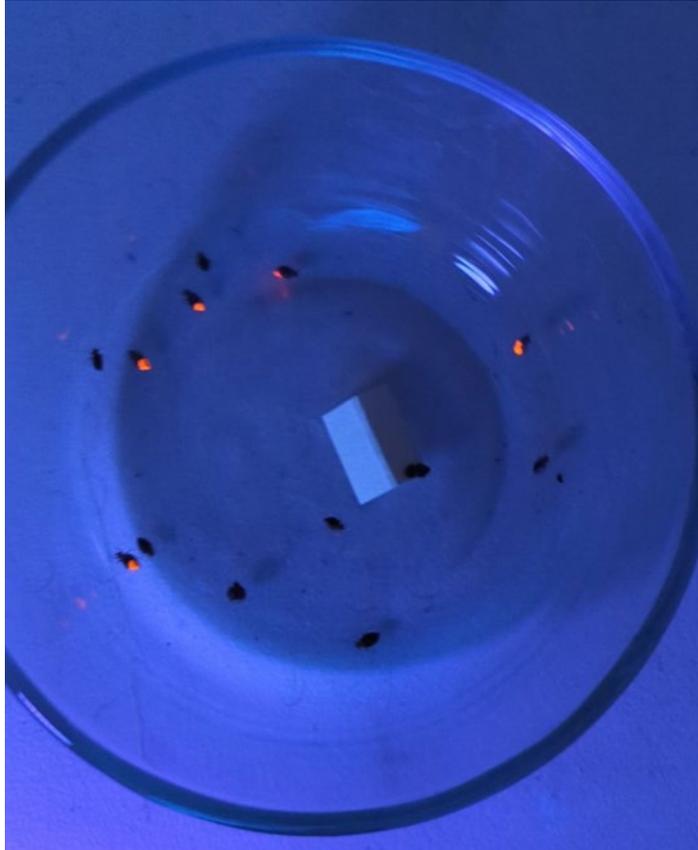
**Figure 6.** Bed bugs found dead after 9 h post-exposure. Notice that bugs are completely covered with the dust.



**Figure 7.** Cumulative mortality of bed bugs exposed continuously to areas treated to three versions of “CHINCHEX”.

## **Experiment 4: Transfer of “CHINCHEX” among Bed Bugs**

The purpose of this experiment was to determine whether “CHINCHEX” is transferred among bed bugs. For this, five bed bugs (donors) were exposed for 5 min to “CHINCHEX” and then transferred to arena where 10 un-exposed bed bugs (recipients) were harboring in a paper tent (Fig. 8). The exposed bugs were marked with a fluorescent dust to differentiate them from the un-exposed one. (Fig. 8).



**Figure 8.** “CHINCHEX”-exposed bugs (orange) interacting with 10 un-exposed bugs in an arena. Mortality of bugs were evaluated daily until all bugs were dead.

### **Results**

24 h post-exposure, while all donor bugs were dead, 55% of recipient were non-viable. By day 3, 95% of recipient bugs were found dead, and 100% mortality was recorded by day 4.

## Conclusions

- All versions of “CHINCHEX” were effective against highly resistant bed bugs
- The rate or speed of mortality caused by “CHINCHEX” depend on the surface or substrate where the material is applied, the type of formulation (Product A, B or C), or the type of assays used
- Although all bed bugs died after 72 h, those exposed to “CHINCHEX” on stucco died faster than on painted stucco, concrete or popcorn ceiling. This clearly indicate that substrate is a crucial factor in the way “CHINCHEX” kill bed bugs. Therefore, the expectations of effectiveness of the product in infested areas highly depend on the presence and dominance of one of these types of surfaces, and perhaps the application rate.
- Rate or speed of mortality of bed bugs seems to depend on the application rates. In assays where the amount of “CHINCHEX” was applied according to manufacturer’s recommendations, 100 mortality was observed 9 h post-exposure.
- Evaluations where bugs were offered crevices (concrete block experiment) demonstrated that most of insects rapidly seek for harborages even thought these areas are treated with “CHINCHEX”. This is evidence that bed bugs do not avoid “CHINCHEX”. All bed bugs died within 96 h (4 days).
- This study shows that bed bugs can transfer “CHINCHEX” to other members of the aggregation. This is an important finding as this is added value of the application of “CHINCHEX” in field conditions. In essence this also suggests that it is not necessary that all individuals of an infestations have direct contact with “CHINCHEX” to be killed.
- ‘CHINCHEX” can be recommended for bed bug management because, 1) kill insecticide resistant bed bugs that are difficult to eliminate with liquid insecticide spray 2) can kill 100% bed bugs within 9 h due to rapid and enhanced adhesion properties of “CHINCHEX” particles to the bed bug body 3) bed bugs do not avoid treated surfaces.

