# Determination of Lethal Effects of ChinChex Aerosol Spray on Tropical Bed Bug *Cimex hemipterus* (Hemiptera: Cimicidae)

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## Objective

Evaluate the lethal effect of ChinChex aerosol spray on tropical bed bugs under laboratory conditions.

## Materials

ChinChex aerosol spray from NOBEDBUGS-HK.

## Materials and Methods

Tropical bed bugs used in this test were collected from Guangzhou City, Guangdong Province, China in 2018 (Zhang et al., 2021b). They were maintained under laboratory conditions (26 ± 1ºC, 40 ± 10% relative humidity at 12 h:12 h light:dark photoperiod) according to the methods of Zhang et al. (2021a). Only adult males (unknown age) and nymphs (third–fifth instar) were used for testing, and all of them were fed with defibrinated rabbit blood 3–4 d prior to experiment. Adult females were not used to prevent oviposition in the experimental arenas. Two experiments were conducted for evaluating the lethal effect of ChinChex aerosol spray on tropical bed bugs.

*Experiment I. Choice exposure bioassay.* This experiment simulated field application of dusts where bed bugs may come in contact with a narrow band of dust multiple times, or avoid the dust deposit, when they have a choice to stay between treated and untreated substrates (Singh et al., 2016). Clean plastic pallet (18 by 12 by 6 cm; Jinmingfeng Hardware and Plastic Products Factory, Shenzhen, Guangdong Province) with the bottom sticked with a piece of filter paper and the inner wall coated with a thin layer of polytetrafluoroethylene was used as experimental arena (Fig. 1A). ChinChex aerosol spray was applied on the rectangular

band with a width of 2 cm around the inner bottom of the experimental arena, and the spraying amount was 5 mg/cm$^2$. After spraying, the experimental arena was left under laboratory conditions for 30 min to ensure that the solvent in the spray was completely volatilized. A folded paper harborage (5 cm long by 3 cm wide) containing bed bug feces (conditioned harborage) was placed in the center of each arena. At half an hour before entering the light cycle, 10 adult males and 10 nymphs were placed onto the conditioned harborage in the center of a plastic ring (7 cm diameter and 5 cm height) to confine the bed bugs for 30 min. The plastic ring confining the bed bugs was then removed to initiate the experiment. Control arenas received all the materials as test arenas except the ChinChex aerosol spray. This experiment was conducted under laboratory conditions and was replicated four times. Bed bug mortality was recorded daily until 14 d after treatment or until all the bed bugs in the treatment group died. A bed bug was considered dead if there was no movement when it was prodded with forceps.


**Fig. 1.** Experimental arenas of Experiment I (A) and Experiment II (B).

*Experiment II. Lethal harborage bioassay.* This experiment simulated the situation of installing ChinChex treated lethal harborage in the field or directly spraying ChinChex aerosol spray on the hiding places of bed bugs (Kerdsawang et al., 2023). The experimental arena (except the ChinChex aerosol spray treated rectangular band) used in Experiment I was also selected as the experimental arena for this experiment (Fig. 1B). The folded paper harborage containing bed bug feces (conditioned harborage) was treated with ChinChex aerosol spray, and the spraying amount on the harborage was the same as Experiment I (5 mg/cm$^2$), and then was placed in the center of each arena at 0–1 h after entering the light cycle. The treated harborage was left under laboratory conditions for 30 min to ensure that

the solvent in the spray was completely volatilized. Subsequently, 10 adult males and 10 nymphs were immediately introduced into the experimental arena and the experiment began. Control group received all the materials as test arenas except the lethal harborage, and the lethal haborage was replaced by a no-spray treated conditioned harborage. This experiment was conducted under laboratory conditions and was replicated four times. Bed bug mortality was recorded following the same method as that in Experiment I.

**Statistical analysis**

The mortality data of nymphs and adult males was pooled together for statistical analysis. Independent samples *t* test was conducted for analyzing the mortality rate of bed bugs between control and treatment groups under the same time point. The percentage data were arcsine square-root transformed prior to analysis to meet the assumptions of normality and homogeneity of variance, if necessary. All data analyses were conducted using IBM SPSS Statistics version 22 (International Business Machines Corp., Armonk, NY, USA) (IBM Corporation, 2013).

## Results

Both in the choice exposure bioassay (Experiment I) and the lethal harborage bioassay (Experiment II), compared with the negative control (unexposed bed bugs), significant mortality rates of bed bugs began to appear from the 1st day after treatment (all $P < 0.05$) (Fig. 2 and 3). In the choice exposure bioassay and the lethal harborage bioassay, the mortality rates of bed bugs on the 1st day after treatment were 45 ± 13.4% and 93.8 ± 4.7%, respectively. In the following period, the mortality rates of bed bugs in the ChinChex treatment group were significantly higher than that in the negative control group (all $P < 0.05$). In the choice exposure bioassay, all bed bugs were killed after 8 days of exposure to ChinChex. Surprisingly, in the lethal harborage bioassay, all bed bugs died on the 2nd day after treatment.

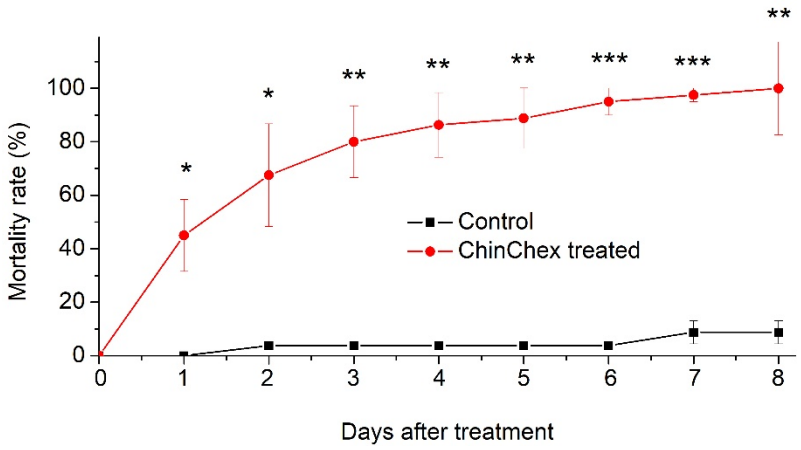


**Fig. 2.** Efficacy of ChinChex aerosol spray in choice exposure bioassay. Within each time point, the stars above the lines indicate significant differences in bed bug mortality rate between different treatments (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; independent samples *t* test).

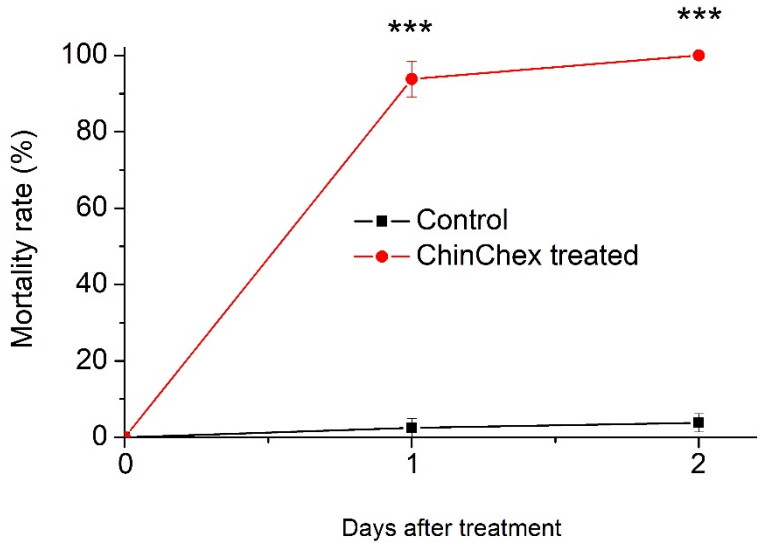


**Fig. 3.** Efficacy of ChinChex aerosol spray in lethal harborage bioassay. Within each time point, the stars above the lines indicate significant differences in bed bug mortality rate between different treatments (*** $P < 0.001$; independent samples *t* test).

**Conclusion:** ChinChex aerosol spray can be used to effectively kill tropical bed bugs.

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## Raw data

*Experiment I. Choice exposure bioassay.*

**Daily number of dead bed bugs**

| Treatment | | 1d | 2d | 3d | 4d | 5d | 6d | 7d | 8d |
|---|---|---|---|---|---|---|---|---|---|
| CK-1 | Adult male | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 |
| | Nymph | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| CK-2 | Adult male | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Nymph | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| CK-3 | Adult male | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Nymph | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CK-4 | Adult male | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Nymph | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Tr-1 | Adult male | 0 | 0 | 4 | 2 | 0 | 3 | 0 | 0 |
| | Nymph | 1 | 1 | 2 | 0 | 1 | 2 | 2 | 2 |
| Tr-2 | Adult male | 2 | 5 | 1 | 2 | 0 | 0 | 0 | 0 |
| | Nymph | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tr-3 | Adult male | 4 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Nymph | 7 | 2 | 0 | 0 | 1 | 0 | 0 | 0 |
| Tr-4 | Adult male | 5 | 3 | 2 | 0 | 0 | 0 | 0 | 0 |
| | Nymph | 7 | 2 | 0 | 1 | 0 | 0 | 0 | 0 |

*Experiment II. Lethal harborage bioassay.*

**Daily number of dead bed bugs**

| Treatment | | 1d | 2d |
|---|---|---|---|
| CK-1 | Adult male | 0 | 0 |
| | Nymph | 0 | 1 |
| CK-2 | Adult male | 0 | 0 |
| | Nymph | 2 | 0 |
| CK-3 | Adult male | 0 | 0 |
| | Nymph | 0 | 0 |
| CK-4 | Adult male | 0 | 0 |
| | Nymph | 0 | 0 |
| Tr-1 | Adult male | 9 | 1 |
| | Nymph | 10 | 0 |
| Tr-2 | Adult male | 7 | 3 |
| | Nymph | 9 | 1 |
| Tr-3 | Adult male | 10 | 0 |
| | Nymph | 10 | 0 |
| Tr-4 | Adult male | 10 | 0 |
| | Nymph | 10 | 0 |