

# **Laboratory Assays to Evaluate Insecticide Product Efficacy (Knockdown and Residual Activity) for Control of Bed Bugs (*Cimex lectularius* L.)**

Report for

**Tom Alsup**

Project Director:

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## Objectives:

1. To quantify and compare the mortality of bed bugs exposed to direct spray applications of enzymatic products.

## Materials and Methods

1. **Start:** December 11th, 2006  
**Completion:** December 19th, 2006

### 2. Test Facilities:

Laboratory assays were conducted at the Dodson Urban Pest Management Laboratory (DUPML), Virginia Tech campus in Blacksburg, Virginia.

### 3. Bed Bug Rearing:

The "South Carolina" (field strain) bed bugs was used in the laboratory assays. The South Carolina strain bed bugs have been in colony at the DUPML since November 2005. The bed bugs are currently maintained on a diet of blood obtained from human volunteers (VT IRB #06-165). The bed bugs are fed every 7 d for 30 minutes. Bed bugs are housed in glass mason jars containing a folded cardboard for harborage. Jars are closed with a mesh top to allow feeding through the mesh without opening the jar. Rearing jars are kept in an environmental chamber at 27° C, 55% RH, and on a 12:12 L:D photoperiod.

### 4. Bioassay Designs

*Direct spray assay:* Groups of 10 adult (5 male, 5 female) South Carolina strain bed bugs were removed from rearing containers without anesthesia and confined under an inverted Petri dish (35mm X 5mm) on the surface of a hardboard panel (6 in. X 6 in.). The bed bugs were then sprayed with 2.3 ml of one of the 3 following treatments: tap water (control); Bed bug Terminator; Enzatech (mixed 1: 3 with water). Each product treatment was replicated 5 times; 2 control assays were conducted. Applications were made to bed bugs using 500-ml capacity polyethylene hand sprayers that used a finger-action trigger to spray. A cone type spray was adjusted to a diameter of 7–8 cm at 10–15 cm from the nozzle. Two pumps from the sprayer delivered the 2.3 ml of product. After application, bed bug knockdown was recorded at regular intervals (seconds) until 100% knockdown or mortality was observed.

Bed bug knockdown was analyzed using Probit analysis (PoloPlus 2001) to determine the  $KT_{50}$  and  $KT_{90}$  values (the time necessary to knock down 50% and 90% of the bed bugs tested) for all treatments. The percentage of bed bugs knocked down was compared using Students t-test (SAS Institute 2006).

### 5. Results

Both Bed bug Terminator and the Enzatech solution caused very rapid knock down (complete immobilization) of bed bugs in these tests. The time it took for each product to knock down the bed bugs was not significantly different from each other, as compared by individual t-tests at specific times, and by comparison of  $KT_{50}$  values.

In the Bed bug Terminator assays 58% of the bed bugs were immobilized within 10 seconds of being sprayed (Table 1). The percentage of bed bugs knocked down rose to 88% by 30 seconds, and 94% by 60 seconds. The percentage of bed bugs knocked down led to a calculated  $KT_{50}$  value of 6.43 seconds and a  $KT_{90}$  value of 46.3 seconds (table 2).

The Enzatech spray caused 62% of the bed bugs were immobilized within 10 seconds of being sprayed (Table 1). The percentage of bed bugs knocked down rose to 92% by 30 seconds, and 94% by 60 seconds. The percentage of bed bugs knocked down led to a calculated  $KT_{50}$  value of 6.36 seconds and a  $KT_{90}$  value of 32.7 seconds (Table 2).

Table 1. Percent of bed bugs knocked down after being sprayed with enzymatic products.

Treatment	n	Percent knocked down after spray application (time in seconds)					
		0	10	20	30	45	60
Bed Bug Terminator	5	0	58 + 2.0	76 + 8.1	88 + 5.8	92 + 3.7	94 + 2.5
Enzatech (1:3)	5	0	62 + 2.0	82 + 4.9	92 + 3.7	94 + 4.0	94 + 4.0
Control	2	0	0	0	0	0	0

Table 2. Comparison of  $KT_{50}$  values calculated for field strain bed bugs (South Carolina strain) directly sprayed with enzymatic products (n = 5 replicates).

Treatment	N	$KT_{50}$ (seconds)	95% CIs	Slope $\pm$ SE
Bed Bug Terminator	50	6.43	2.62 – 10.09	1.50 $\pm$ 0.28
Enzatech (1:3)	50	6.36	2.57 – 9.66	1.80 $\pm$ 0.37

Bed bug mortality was recorded at 12 and 24 hours after spray exposure. Bed bug mortality in the Bed Bug Terminator tests was 80% at 12 hours and 82% at 24 hours. Bed bug mortality in the Enzatech spray test was 70% at 12 hours and remained unchanged at 24 hours. The mortality recorded for both spray solutions were not significantly different from each other.

## 6. Discussion

Both solutions (Bed Bug Terminator and Enzatech) caused rapid knockdown when sprayed directly on bed bugs. When sprayed, the bed bugs ran approximately 1-2 cm and then froze in place with a “raised abdomen” posture (Figure 1 and figure 2). We observed that once a bed bug took on this “raised abdomen” posture, it did not recover from exposure to the sprays.

A small percentage of the bed bugs recovered from the initial knockdown caused by the spray solutions as can be seen by comparing the percentage of bugs knocked down at 60 seconds with the mortality recorded at 12 hours. The bugs that recovered

were individuals that became immobilized less rapidly than the bugs displaying the “raised abdomen” posture. The bed bugs appeared to recover because they received a “less than lethal” dose of the spray. Subsequent spraying of these individuals would likely cause mortality.

Both the Bed Bug Terminator and Enzatech solutions appear to act as insecticidal soaps which disrupt the structure and permeability of the insects' cell membranes. The cell contents are able to leak from the damaged cells, and the insect quickly dies. Both solutions are capable of causing rapid knock down and mortality when sprayed directly on bed bugs, but do not have any residual insecticidal activity.



Figure 1. Bed bugs dying in "abdomen up" posture after being sprayed with Enzatech solution.



Figure 2. Close up dead bed bug which died from exposure to spray of Bed Bug Terminator.

**7. Invoice:****Bioassays:**

<b>Adult Bed Bugs Bioassays</b>	<b>Treatments</b>	<b>Reps</b>	<b>Price/ Replicate</b>
<b>Direct Spray Assay</b>	Bed bug Terminator	5	\$150.00 / \$750.00 total
<b>South Carolina strain</b>	Alternative product	5	\$150.00 / \$750.00 total
	Controls	2	\$150.00 / \$300.00 total
			<b>Total \$1800.00</b>

Payment information:

**Please make check payable to:** Treasurer of Virginia Tech

**Please include payment with the following letter:** EXACTLY AS WRITTEN

*Dear Dr. Miller:*

*Enclosed please find a check made out to the **Treasurer of Virginia Tech** in the amount of \_\_\_\_\_. These funds are provided in support of laboratory trials evaluating the effects of insecticides on bed bugs.*

*Sincerely,*

If you have any questions about this proposal, please call Dr. Dini Miller at (540) 231-4918 or Tim McCoy at 231-4045.

Thank you so much for your support of our bed bug research program.