

## Laboratory Evaluation of Insecticide Treated Soil against *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae)

by

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### Abstract

Four termiticide formulations (biflex 2.5% EC, chlorfenapyr 36% SC, fipronil 25 EC and imidacloprid 20% SC) were evaluated against *Coptotermes heimi* (Wasmann) in the laboratory by using modified glass tube and petri dish methods. Four concentrations (12.5, 25, 50 and 100 ppm w/w) of all termiticides were evaluated in the glass tube method, while seven concentrations (1.57, 3.125, 6.25, 12.5, 25, 50, 100 ppm) were evaluated for each termiticide in the petri dish method. It was revealed from results that with the exception of biflex, all termiticides confirmed non-repellent properties and biflex showed its maximum repellent and toxic properties against *C. heimi* (Wasmann). According to LC<sub>50</sub> values the insecticides were arranged for toxicity in the following ascending order of preferences, Biflex > Chlorfenapyr > Fipronil > Imidacloprid.

**Key words:** *Coptotermes heimi*, Tunneling, Soil Toxicity, LC<sub>50</sub>, Termiticides, Repellency

### Introduction

*C. heimi* (Wasmann) are urban as well as agricultural pests and cause severe damage to wooden structures and cash crops (sugarcane, cotton and wheat) in Pakistan. It is distributed in different parts of Pakistan and accounts for significant damage to agricultural crops, forest plantations and wood works in buildings (Manzoor, 2010). Soil termiticide treatments are easy to use and have been in practice since 1900's. Long term protection and maintenance of structures is achieved by applying termiticides after five or more years. (Su *et al.*, 1999; Richman *et al.*, 2006). Both repellent and non repellent termiticides have been proved effective barriers, when applied properly against termites.

Present study was carried out to examine the laboratory performance of biflex, fipronil, imidacloprid and chlorfenapyr, repellent and non-repellent termiticides at different concentrations against the Subterranean termite *Coptotermes heimi* (Wasmann) using evaluation methods; (modified glass tube, and the petri-dish methods).

### Materials and methods

*Coptotermes heimi* (Wasmann) were collected from underground monitoring stations established earlier at the University campus Lahore College for women university, Lahore, Pakistan. Termites were brought back to the laboratory and separated from soil debris using the method described by Tamashir *et al.*, (1987). Only termites from the same colony were used in this study and were kept in a plastic container having moistened filter paper as their food source. Before

exposure to insecticides, termites were kept in laboratory at  $26 \pm 2$  °C and 80% relative humidity to eliminate injured and inactive termite. Only healthy and active termites were used for the experiment. Termites were kept in petri plates (90mm x 15mm) containing moistened filter papers until the experiments were conducted.

#### **Modified glass tube method**

This method was used to assess the tunneling activity of termite species in soil treated with different concentrations of different termiticides. The glass tube method is a modified version of the one as adopted by Su & Scheffrahn (1990), and then conducted by Yeoh, B. H. and Lee, C. Y. (2007) in which a 16-cm glass tube (1.4 cm diam.) contained a 8 cm long moisten soil layer and a 2 cm termiticides treated soil layer sandwiched between 2 cm sawdust and 2 cm 5% agar. Two cut pieces of moisten filter paper were placed into the 3 cm void adjacent to the agar layer, serving as a temporary food source. Both ends of the glass tube were sealed with several layers of aluminium foil. 40 workers and 10 soldier termites were introduced into the void and allowed to tunnel freely. The tube was held horizontally in a dark chamber ( $25.2 \pm 0.2$  °C,  $56.3 \pm 0.7$  % RH). The cumulative tunneling distance was measured daily up to 9 days post-treatment. After this period, the setup was disassembled and the numbers of surviving insects were counted. Four different concentrations (12.5, 25, 50 and 100 ppm) were tested and each concentration was replicated 3 times.

#### **Petri dish method**

This method was used a polyethylene petri dish separated into 2 sections by a piece of glass as adopted by Yeoh, B. H. and Lee, C. Y in which one section contained 10 g of untreated soil, while the other contained an equal amount of soil that was treated with the termiticide. Recommended concentrations were obtained by mixing the equivalent amount of diluted termiticide formulation and soil at the ratio of 1:20 (v/w). Four pieces of oven-dried, pre weighted filter paper, measuring 2 x 1 x 1 cm were placed in each section to serve as food. Fifty workers and 10 soldiers were introduced into the untreated section and allowed to acclimatize for 48 hr. After that period, the glass pieces were removed and termites were allowed to forage freely for 2-weeks. Each treatment was replicated 3 times. All bioassays were held in a dark chamber ( $25.2 \pm 0.2$  °C,  $56.3 \pm 0.7$  % RH). Tunneling activities in both treated and untreated sections were qualitatively scored.

Data in percentages were subjected to Mean, Standard error and analysis of variance (ANOVA). Means were separated with Tukey's HSD. All analysis were performed using Graph pad prism version 4.00 for windows, Graph pad software, San Diego California USA, ([www.graphpad.com](http://www.graphpad.com)). The  $LC_{50}$  values of all the termiticides were determined by using EPA probit analysis program, (Version 1.5). Results with  $P < 0.05$  were considered statistically significant.

## **Results and discussion**

In petri dish method, Mean Percentage mortality of *C.heimi* (Wasmann) after 15 days of test arena was 17.22% with lowest concentration (1.562 ppm) and 60.00% with highest concentration (100 ppm) of imidacloprid. Analysis of variance revealed that the tunneling distance and percentage mortality of *C.heimi* (Wasmann) were significantly different in treated and untreated sections of imidacloprid ( $F = 4.647$  ;  $df = 2$  ;  $P < 0.0213$ ).(table 1). Table 1 also reveals that Mean percentage mortality of *C.heimi* (Wasmann) when treated with fipronil was 19.44%, with lowest concentration (1.562 ppm) and 62.22% with highest concentration (100 ppm) .The analysis of variance revealed

**Table 1:** Mean tunneling activity and mean percentage mortality (Mean  $\pm$  S.E) of *C.heimi* (Wasmann) in response to different concentrations of imidacloprid, fipronil, chlorfenapyr and biflex by petri dish method ( after 15 days).

Termiticides	Termiticide concentration in ppm	Untreated section	Treated section	Mean percentage mortality
		Mean tunneling activity (cm)	Mean tunneling activity (cm)	
Imidacloprid	Control	22.0 $\pm$ 0.289 <sup>a</sup>	20.8 $\pm$ 0.536 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
	1.562	20.1 $\pm$ 0.100 <sup>b</sup>	17.83 $\pm$ 0.167 <sup>b</sup>	17.22 $\pm$ 0.333 <sup>b</sup>
	3.125	16.4 $\pm$ 0.291 <sup>c</sup>	12.83 $\pm$ 0.167 <sup>c</sup>	28.88 $\pm$ 0.333 <sup>c</sup>
	6.25	10.5 $\pm$ 0.289 <sup>d</sup>	6.66 $\pm$ 0.441 <sup>d</sup>	36.66 $\pm$ 0.000 <sup>d</sup>
	12.5	9.16 $\pm$ 0.167 <sup>e</sup>	5.43 $\pm$ 0.296 <sup>e</sup>	45.55 $\pm$ 0.333 <sup>e</sup>
	25	8.16 $\pm$ 0.167 <sup>f</sup>	3.5 $\pm$ 0.289 <sup>f</sup>	53.33 $\pm$ 0.000 <sup>f</sup>
	50	6.3 $\pm$ 0.167 <sup>g</sup>	3.0 $\pm$ 0.289 <sup>g</sup>	56.66 $\pm$ 0.577 <sup>g</sup>
Fipronil	100	2.8 $\pm$ 0.167 <sup>h</sup>	0.35 $\pm$ 0.145 <sup>h</sup>	60.00 $\pm$ 0.577 <sup>h</sup>
	1.57	11.83 $\pm$ 0.167 <sup>b</sup>	8.5 $\pm$ 0.289 <sup>b</sup>	19.44 $\pm$ 0.333 <sup>b</sup>
	3.125	8.83 $\pm$ 0.167 <sup>c</sup>	5.16 $\pm$ 0.601 <sup>c</sup>	30.55 $\pm$ 0.333 <sup>c</sup>
	6.25	8.4 $\pm$ 0.208 <sup>d</sup>	4.1 $\pm$ 0.100 <sup>d</sup>	38.33 $\pm$ 0.577 <sup>d</sup>
	12.5	5.33 $\pm$ 0.167 <sup>e</sup>	2.36 $\pm$ 0.318 <sup>e</sup>	46.66 $\pm$ 0.577 <sup>e</sup>
	25	4.26 $\pm$ 0.267 <sup>f</sup>	1.33 $\pm$ 0.203 <sup>f</sup>	51.66 $\pm$ 0.155 <sup>f</sup>
	50	3.33 $\pm$ 0.167 <sup>g</sup>	0.46 $\pm$ 0.033 <sup>g</sup>	58.33 $\pm$ 0.577 <sup>g</sup>
Chlorfenapyr	100	2.0 $\pm$ 0.00 <sup>h</sup>	0.2 $\pm$ 0.067 <sup>h</sup>	62.22 $\pm$ 0.333 <sup>h</sup>
	1.57	10.6 $\pm$ 0.208 <sup>b</sup>	6.16 $\pm$ 0.306 <sup>b</sup>	27.77 $\pm$ 0.333 <sup>b</sup>
	3.125	8.37 $\pm$ 0.079 <sup>c</sup>	5.21 $\pm$ 0.103 <sup>c</sup>	33.88 $\pm$ 0.333 <sup>c</sup>
	6.25	6.17 $\pm$ 0.093 <sup>d</sup>	3.16 $\pm$ 0.048 <sup>d</sup>	43.88 $\pm$ 0.333 <sup>d</sup>
	12.5	5.51 $\pm$ 0.217 <sup>e</sup>	2.40 $\pm$ 0.166 <sup>e</sup>	50.55 $\pm$ 0.333 <sup>e</sup>
	25	4.45 $\pm$ 0.247 <sup>f</sup>	1.27 $\pm$ 0.134 <sup>f</sup>	60.55 $\pm$ 0.333 <sup>f</sup>
	50	2.7 $\pm$ 0.355 <sup>g</sup>	0.53 $\pm$ 0.172 <sup>g</sup>	67.22 $\pm$ 0.333 <sup>g</sup>
Biflex	100	1.1 $\pm$ 0.104 <sup>h</sup>	0.5 $\pm$ 0.167 <sup>h</sup>	77.22 $\pm$ 0.333 <sup>h</sup>
	1.57	8.83 $\pm$ 0.441 <sup>b</sup>	5.65 $\pm$ 0.191 <sup>b</sup>	36.11 $\pm$ 0.333 <sup>b</sup>
	3.125	7.67 $\pm$ 0.096 <sup>c</sup>	3.53 $\pm$ 0.177 <sup>c</sup>	42.22 $\pm$ 0.333 <sup>c</sup>
	6.25	6.38 $\pm$ 0.087 <sup>d</sup>	2.42 $\pm$ 0.223 <sup>d</sup>	48.88 $\pm$ 0.000 <sup>d</sup>
	12.5	5.15 $\pm$ 0.040 <sup>e</sup>	1.45 $\pm$ 0.114 <sup>e</sup>	56.11 $\pm$ 0.333 <sup>e</sup>
	25	3.20 $\pm$ 0.151 <sup>f</sup>	0.46 $\pm$ 0.163 <sup>f</sup>	62.77 $\pm$ 0.333 <sup>f</sup>
	50	2.03 $\pm$ 0.060 <sup>g</sup>	0.2 $\pm$ 0.067 <sup>g</sup>	70.55 $\pm$ 0.333 <sup>g</sup>
	100	1.25 $\pm$ 0.124 <sup>h</sup>	0.00 $\pm$ 0.000 <sup>h</sup>	85.90 $\pm$ 0.333 <sup>h</sup>

that there were significant differences between tunneling activity and percentage mortality against *C .heimi* (Wasmann) with different concentrations of fipronil (F = 10.17; d.f = 2; P <

0.0008). It is also evident from table 1 that Mean percentage mortality of *C.heimi* (Wasmann) was 27.77% with lowest concentration (1.562ppm) and 77.22% with highest concentration (100 ppm) of chlorfenapyr. The analysis of variance revealed that there were significant differences between the tunneling activity and percentage mortality of *C.heimi* (Wasmann) with different concentrations of chlorfenapyr (F = 9.112 ; d.f = 2 ; P < 0.0014).

Regarding treatment with Biflex, Mean percentage mortality of *C.heimi* (Wasmann) after 15 days was 36.11% with lowest concentration (1.562 ppm) and 85.90% with highest concentration (100 ppm) of biflex. In control set the percentage mortality was 0.00%. There were significant difference between the tunneling activity and percentage mortality of *C.heimi* (Wasmann) with different concentrations of biflex (F = 16.67; d.f = 2; P < 0.0001). Table 2 shows the mean tunneling activity and percentage mortality of *C.heimi* (Wasmann) against different termiticides. The mean tunneling activity with 12.5, 25, 50 and 100 ppm concentrations of imidacloprid was 2.05, 1.73, 1.66 and 1.27 cm after 9 days with percentage mortality of 16.0%, 20.66%, 25.33% and 29.66%.

**Table 2:** Tunneling activity and mean percentage mortality (Mean ±S.E.) of *C.heimi* (Wasmann) in response to different concentrations of Imidacloprid , fipronil, Chlorfenapyr and, Biflex after 9 days of treatment

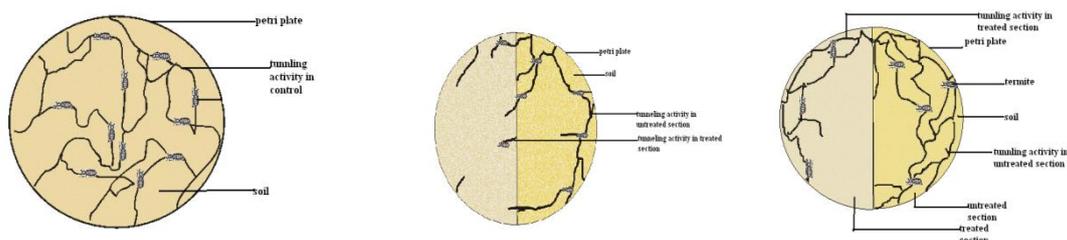
Termiticide	Conc. in ppm	Mean tunneling distance	Percentage survivorship
	Control		7.09 ± 0.012 <sup>a</sup>
Imidacloprid	12.5	2.05 ± 0.009 <sup>b</sup>	16.0 ± 0.577 <sup>b</sup>
	25	1.73 ± 0.012 <sup>c</sup>	20.66 ± 0.333 <sup>c</sup>
	50	1.66 ± 0.003 <sup>d</sup>	25.33 ± 0.333 <sup>d</sup>
	100	1.27 ± 0.012 <sup>e</sup>	29.66 ± 0.333 <sup>e</sup>
Fipronil	12.5	2.03 ± 0.058 <sup>a</sup>	17.33 ± 0.333 <sup>a</sup>
	25	1.81 ± 0.012 <sup>b</sup>	21.66 ± 0.333 <sup>b</sup>
	50	1.46 ± 0.029 <sup>c</sup>	26.0 ± 0.577 <sup>c</sup>
	100	1.09 ± 0.018 <sup>d</sup>	31.33 ± 0.333 <sup>d</sup>
Chlorfenapyr	12.5	1.67 ± 0.007 <sup>a</sup>	18.66 ± 0.333 <sup>a</sup>
	25	1.54 ± 0.012 <sup>b</sup>	22.33 ± 0.333 <sup>b</sup>
	50	1.31 ± 0.012 <sup>c</sup>	25.33 ± 0.333 <sup>c</sup>
	100	1.03 ± 0.007 <sup>d</sup>	29.0 ± 0.577 <sup>d</sup>
Biflex	12.5	1.79 ± 0.024 <sup>a</sup>	32.66 ± 0.333 <sup>a</sup>
	25	1.54 ± 0.017 <sup>b</sup>	48.33 ± 0.333 <sup>b</sup>
	50	0.78 ± 0.020 <sup>c</sup>	54.0 ± 0.577 <sup>c</sup>
	100	0.34 ± 0.018 <sup>d</sup>	69.33 ± 0.343 <sup>d</sup>

Tunneling activity with 12.5, 25, 50 and 100ppm of fipronil was 2.03, 1.81, 1.46 and 1.09 cm while the percentage mortality was 17.33%, 21.66%, 26.0% and 31.33%. Mean tunnel distance covered by *C.heimi* (Wasmann) termite workers was 1.67, 1.54, 1.31 and 1.03 cm with the 12.5, 25, 50 and 100 ppm concentration of chlorfenapyr while percentage mortality was 18.66%, 22.33%,

25.33% and 29.0%. The mean tunneling activity with 12.5, 25, 50 and 100 ppm of biflex formulation was 1.79, 1.54, 0.78 and 0.34 cm with percentage mortality of 32.66%, 48.33%, 54.0% and 69.33%. The control set showed the maximum tunnel distance and least termite mortality which was 7.09 cm and 0.00% respectively (Table 2). Results indicated that all the termiticide formulations (with the exception of biflex) showed non repellent characteristics during the modified glass tube method. Termites readily tunneled through the treated layers of these evaluated termiticide formulations and showed the delayed mode of action due to their non repellent characteristics. Similarly , LC<sub>50</sub> values of imidacloprid, fipronil, chlorfenapyr and biflex were 25.91, 23.13, 10.88 and 6.63 for petri dish method while for the modified glass tube method the LC<sub>50</sub> values were 48.68, 44.62, 40.92 and 16.69 respectively (Table 3). According to LC<sub>50</sub> values the insecticides were arranged for toxicity in the following ascending order of preferences, Biflex > Chlorfenapyr > Fipronil > Imidacloprid. Due to least LC<sub>50</sub> Value of biflex, it is also revealed that its efficacy is more than other termiticides by applying in lower concentration.

**Table 3:** Relative toxicity of four insecticides Imidacloprid, Fipronil, Chlorfenapyr and, Biflex against workers of *C.heimi* (Wasmann) by petri dish and modified glass tube method.

Termiticide	Worker termites LC <sub>50</sub> (ppm) in petri dish method			Worker termites LC <sub>50</sub> (ppm) in modified glass tube method		
	Lc <sub>50</sub>	Fit of probit line		Lc <sub>50</sub>	Fit of probit line	
		slop± S. E.	X <sup>2</sup>		Slop ± S. E.	X <sup>2</sup>
Imidacloprid	25.912	0.636260±0.061964	11.070	48.689	0.779343 ± 0.155523	5.991
Fipronil	23.132	0.620063±0.61664	11.070	44.625	0.787297 ± 0.155311	5.991
Chlorfenapyr	10.886	0.728967±0.62411	11.070	40.925	0.573042 ± 0.153823	5.991
Biflex	6.637	0.632217±0.061831	11.070	16.696	1.006360 ± 0.160612	5.991



**Fig. Tunneling activity of *C .heimi* (Wasmann) in petri dishes containing untreated and treated soil (A) imidacloprid treated (B) biflex treated (c) Control.**

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