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Research Article

Laboratory Evaluation of Imidacloprid against *Microtermes obesi* (Holmgren) (Isoptera: Macrotermitinae)

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Abstract: A laboratory bioassay was developed to test the toxicity, repellency and tunneling behavior of imidacloprid against *Microtermes obesi* (Holmgren). In this study >90% of termites died after 96 h exposure to 100 µg mL^{-1} of imidacloprid and after 168 h all the termites died at all the tested concentrations. When tested for persistence (exposure to soil aged one month after treatment), >90% mortality was observed in soil treated with 100 µg mL^{-1}, 50 µg mL^{-1} and 25 µg mL^{-1} after 120 h exposure. Repellency test results proved imidacloprid to be a non-repellent insecticide at all tested concentrations (i.e., 100 µg mL^{-1}, 50 µg mL^{-1}, 25 µg mL^{-1}, 12.5 µg mL^{-1}, 6.25 µg mL^{-1}, 3.125 µg mL^{-1} and 1.562 µg mL^{-1}). When tested for F-1 (0.97) toxicity, it was also evident that it took more than 8 h to give 97% mortality. Tunneling behavior was also studied for cumulative tunnel distance, maximum tunnel height and number of tunnels. At higher concentrations, not only was there more mortality, but also less cumulative tunnel distance and a reduced number of tunnels.

Keywords: Toxicity, repellency, tunneling, imidacloprid, *Microtermes obesi*

1. INTRODUCTION

Microtermes obesi (Holmgren) is one of the most common termite species of Pakistan. It has been found attacking forest plants as well as timbers which are used as building material; doors and windows and ventilators are commonly destroyed. It builds underground nests in the soil. Despite many other measures available to control termites, including treated woods, baits, physical barriers, biological control agents, etc., the most effective and widely used method of control is the use of insecticides. Chemicals like silafluofen, imidacloprid, didecyl dimethyl ammonium chloride (DDAC), chlorpyrifos, fenvalerate, cypermethrin, permethrin, fenvalerate, spinosad, chlorfenapyr, thiamethoxam, bifenthrin, flufenoxuron, fipronil and acetamiprid have been found effective for the control of different termite species [3–11, 14, 15, 17, 18, 20–23].

The present study aims to determine the efficacy of a slow acting insecticide, imidacloprid against *M. obesi* in fresh and one month post-treated laboratory soil.

2. MATERIALS AND METHODS

Workers of *M. obesi* used in this study were collected by traps placed in the lawns of the Lahore College for Women University, Lahore, Pakistan. The traps were brought to the laboratory, all the debris was removed and termites (workers and soldiers) were kept in plastic boxes with moist filter papers in constant darkness at 25-28°C and 80% r.h. for more than 14 days prior to assay in order to eliminate inactive termites and get active and healthy termite workers.

The soil (5–10 cm depth) used in this study was a sandy loam, free of contamination. It was

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collected from Botanical Garden of the University. The soil was ground and passed through a 2 mm sieve, spread on a tray and oven-dried at 100°C for 24 h.

Imidacloprid (20 S.L.) was serially diluted to seven different concentrations, i.e., 100 µg mL^{-1}, 50 µg mL^{-1}, 25 µg mL^{-1}, 12.5 µg mL^{-1}, 6.25 µg mL^{-1}, 3.125 µg mL^{-1} and 1.562 µg mL^{-1}. Contact toxicity testing of imidacloprid (20%) was done in a Petri dish (14 cm diameter). Each sample of 8.0 g soil was treated with 2 mL of a specific concentration of the termiticide with the help of a micropipette, then air dried for a few minutes. There were three replicates for each concentration. In the control sample, the soil was treated with distilled water only. Fourteen worker termites were released in each Petri dish and the dishes were sealed with parafilm. The dishes were kept in an incubator at 25-26°C and 80% r.h. The termites were examined at half hour intervals for 8 hours and subsequently after every 24 h. The number of dead termites was counted and tabulated at each examination. The contact toxicity bioassay of imidacloprid (20%) was terminated till 100% mortality was achieved. In order to determine the persistence of the insecticide, termite mortality was also checked in the one month old treated soil.

For the contact repellency test, the procedure was the same as for the contact toxicity test except that the soil was divided into two halves. One half of the soil was treated with 1.0 mL of the respective termiticide concentration and the other half with 1mL of the solvent. Repellency readings were taken after every 15 minutes for 2.5 h; the number of termites were counted on the treated and untreated sides and tabulated. The contact repellency test was conducted for freshly treated soil and for the one month post-treated soil. A treatment concentration was considered as repellent when 30 or more of the termites (sum of three replicates) were observed on the untreated soil [17].

Tunneling bioassays were performed by the procedure suggested by Grace [10]. The apparatus (Fig. 1) consisted of the following three compartments:

Component 1: A plastic vial containing untreated soil and filter paper of size of vial. An untreated filter paper weighing about 0.07 grams was placed in one of the two vials, which also contained the moistened soil.

Fig. 1. Tunneling test apparatus.

Component 2: A plastic "sandwich" or tunneling arena, containing treated soil.

Component 3: A second vial containing untreated sand and an additional food source like moist filter paper the size of the vial.

The vials were connected serially by 1.5 cm long Tygon tube. Each of these vials contained 10.0 g untreated soil, 2.0 mL distilled water and filter paper as food. The treated soil was poured into the tunneling arena, and 2.0 mL distilled water added by pipette along the open top edge. This water moistened the soil of the arena. The top edge of the each tunneling arena was sealed with transparent plastic tape in order to reduce evaporation. One hundred termites were released in the one of the two vials. The vials were capped and the caps were pierced with air holes by using a heated insect pin. Each concentration of insecticide and the control were replicated thrice. All experimental units were kept in an incubator at 26°C. Test setups were examined at 24 h intervals for 7 days. At each examination, the number of tunnels, the length of the tunnels, and the number of surviving and affected individual termites were measured and counted.

The data were analyzed statistically. Means were separated by Tukeys Honestly Significant Difference (HSD) test using GraphPad Prism Version 4.00 for Windows, GraphPad Software, San Diego California USA, (www.graphpad.com).

3. RESULTS AND DISCUSSION

The mean percent mortality of *M. obesi* (Holmgren) treated with different concentrations of imidacloprid: 100% mortality was observed at 100 µg mL^{-1}, after 6 days of exposure to insecticide (Table 1). After

8 days of treatment, 100% mortality was observed at all the tested concentrations. The mortality was 0% with the control treatment. For testing the persistence of imidacloprid, one month old treated soil stored in the dark at 26°C was also tested for toxicity. The mortality was 100% with 100 µg mL^{-1} after 7 th day of the testing period, and all the termites exposed to other experimental dosages were observed dead after 9th day of the test period. There were significant differences between all treatments ($P<0.001$). For example, there existed a significant relationship between the testing time

Table 1. Mortality (mean ± SD) of *Microtermes obesi* (Holmgren) in soil treated with different concentrations of imidacloprid after 8 days exposure.

Hours	Mean and Standard Deviation of Mortality at								P[1]
	100 µg mL^{-1}	**50** µg mL^{-1}	**25** µg mL^{-1}	**12.5** µg mL^{-1}	**6.25 µg mL^{-1}**	**3.125** µg mL^{-1}	**1.562** µg mL^{-1}	**Control**	
8 (day 1)	A* 21.43±1.00	a 19.04±1.00	a 19.04±1.00	a 19.04±1.00	a 14.29±1.00	a 14.29±1.00	a 14.29±1.00	a 0.00±0.00	P<0.0001
24 (day 2)	b 35.71±1.00	b 33.33±1.00	b 28.57±1.00	B 26.19±1.00	b 23.81±1.00	b 21.43±1.00	b 21.43±1.00	a 0.00±0.00	P<0.0001
48 (day 3)	c 80.95±1.00	c 76.19±1.00	c 73.81±1.00	C 71.43±1.00	c 64.29±1.00	c 61.90±1.00	c 52.38±1.00	a 0.00±0.00	P<0.0001
72 (day 4)	c 83.33±1.00	c 78.57±1.00	c 78.57±1.00	C 78.57±1.00	d 76.19±1.00	d 71.43±1.00	d 66.67±1.00	a 0.00±0.00	P<0.000
96 (day 5)	d 92.86±1.00	d 88.10±1.00	d 88.10±1.00	D 85.71±1.00	e 85.71±1.00	e 85.71±1.00	e 80.95±1.00	a 0.00±0.00	P<0.0001 ***
120 (day 6)	d 100.0±1.00	d 100.0±1.00	e 100.0±1.00	F 97.62±1.00	f 97.62±1.00	f 90.48±1.00	e 85.71±1.00	a 0.00±0.00	P<0.00
144 (day 7)	d 100.0±1.00	d 100.0±1.00	e 100.0±1.00	F 100.0±1.00	f 100.0±1.00	f 100.0±1.00	f 90.48±1.00	a 0.00±0.00	P<0.0001 ***
168 (day 8)	d 100.0±1.00	d 100.0±1.00	e 100.0±1.00	f 100.0±1.00	f 100.0±1.00	f 100.0±1.00	g 100.0±1.00	a 0.00±0.00	P<0.0001 ***

* Means followed by similar letters within the column indicate non-significant differences (Tukey's HSD test).

Table 2. Mortality (mean ± SD) of *Microtermes obesi* (Holmgren) in soil treated with different concentrations of imidacloprid (1 month old treatment).

Hours	Mean and Standard Deviation of Termite Mortality at								P[1]
	100 µg mL^{-1}	**50 µg mL^{-1}**	**25 µg mL^{-1}**	**12.5 µg mL^{-1}**	**6.25 µg mL^{-1}**	**3.125 µg mL^{-1}**	**1.562 µg mL^{-1}**	**Control**	
8 (day 1)	A* 14.28±1.00	a 11.90±1.00	a 11.90±1.00	A 7.140±1.00	a 0.00±0.00	A 0.00±0.00	a 0.00±0.00	a 0.00±0.00	P<0.0001 ***
24 (day 2)	b 19.04±1.00	b 16.66±1.00	a 14.28±1.00	A 9.520±1.00	b 7.140±1.00	B 4.760±1.00	b 4.760±1.00	a 0.00±0.00	P<0.0001 ***
48 (day 3)	c 40.47±1.00	c 38.09±1.00	b 33.33±1.00	B 21.42±1.00	c 19.04±1.00	C 16.66±1.00	c 16.66±1.00	a 0.00±0.00	P<0.0001 ***
72 (day 4)	d 61.90±1.00	d 59.52±1.00	c 57.14±1.00	C 54.76±1.00	d 52.38±1.00	D 52.38±1.00	d 50.00±1.00	a 0.00±0.00	P<0.0001 ***
96 (day 5)	e 78.57±1.00	e 76.19±1.00	d 73.8±1.00	D 71.42±1.00	e 71.42±1.00	E 69.04±1.00	e 64.28±1.00	a 0.00±0.00	P<0.0001 ***
120 (day 6)	f 97.61±1.00	f 92.85±1.00	e 90.47±1.00	E 88.09±1.00	f 88.09±1.00	F 88.09±1.00	f 85.71±1.00	a 0.00±0.00	P<0.0001 ***
144 (day 7)	g 100.0±1.00	g 100.0±1.00	e 97.61±1.00	F 97.61±1.00	g 95.23±1.00	G 95.23±1.00	g 92.85±1.00	a 0.00±0.00	P<0.0001 ***
168 (day 8)	g 100.0±1.00	g 100.0±1.00	f 100.0±1.00	F 100.0±1.00	h 100.0±1.00	Gh 97.61±1.00	gh 97.61±1.00	a 0.00±0.00	P<0.0001 ***
192 (day 9)	g 100.0±1.00	g 100.0±1.00	f 100.0±1.00	F 100.0±1.00	h 100.0±1.00	Gh 100.0±1.00	gh 100.0±1.00	a 0.00±0.00	P<0.0001 ***

* Means followed by similar letters within the column indicate non-significant differences (Tukey's HSD test).

and the dose applied. So the results showed, not unexpectedly, that the highest termite mortality was observed at 100 µg mL^{-1} and 50 µg mL^{-1} and lowest mortality was observed at 1.562 µg mL^{-1}. As discussed in Materials and Methods, it is evident that imidacloprid was non-repellent at all the tested concentrations against the termites even after one month exposure**.**

Laboratory experiments were also conducted to test the tunneling behavior of *M. obesi* (Holmgren) against imidacloprid at all the seven tested concentrations of insecticides. The objective of this study was to evaluate cumulative tunnel distance, maximum tunnel height and number of tunnels after the first and seventh days of the treatment.

The maximum tunnel height after the first day of treatment, for the soil treated with different concentrations of imidacloprid, was 61.53, 60.27, 58.83, 55.60, 47.47, 44.57, 36.80 and 33.63 mm for the control, and 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 µg mL^{-1} treatments, respectively. Different insecticide concentrations highly significantly affected maximum tunnel height after day-one ($P<0.0001$). The mean tunnel height recorded after one day was 10.0, 9.0, 7.0, 6.0, 4.3, 3.3 and 2.3 mm for 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 µg mL^{-1}, respectively. For control treatment the mean number of tunnels was 11.0. The ANOVA test revealed that different concentrations of the insecticide significantly affected the number of tunnels after day 1 ($P<0.0001$; Table 3).

After 7^{th} day of treatment application, the cumulative tunnel distance was 282.5 mm in control and 374.6, 369.3, 338.7, 302.3, 257.6, 239.4 and 208.3 mm in soil treated with 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 µg mL^{-1}, respectively. Highly significant differences were observed in tunnel distance with different concentrations of imidacloprid ($P<0.0001$). Mean maximum tunnel distance after 7^{th} day of soil treatment application with different concentrations of imidacloprid was 80.3, 76.7, 75.5, 70.8, 65.5, 61.6 mm with 53.6 for 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 µg mL^{-1}, respectively. In control treatment, maximum tunnel length after 7^{th} day was 82.6 mm. Different insecticide concentrations affected maximum tunnel distance highly significantly ($P<0.0001$; Table 3).

Mean number of tunnels after 7^{th} day was 17.0, 15.7, 15.0, 13.0, 12.0, 8.0 and 5.0 with insecticide concentrations of 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 µg mL^{-1}, respectively. With control treatment, the number of tunnels was 17.7. Analysis of variance revealed significant differences for this parameter at different concentrations of imidacloprid ($P<0.0001$; Table 3).

Fipronil acts at the same target site as the organochlorinecyclodines, with similar effects on insects [2]. Fipronil has been shown to have much greater affinity for insect GABA receptors as

Table 3. Tunneling behaviour of *Microtermes obesi* (Holmgren) (mean ± SE) on 7^{th} day after treatment with imidacloprid.

Concentration (µg mL^{-1})	Cumulative tunnel distance (mm)	Maximum tunnel height (mm)	No. of tunnels
Control	482.5±0.491	92.6±0.491	27.7±0.333
1.562	374.6±0.406	80.3±0.208	17.0±0.577
3.125	369.3±0.569	76.7±0.491	15.7±0.333
6.25	338.7±0.463	75.5±0.433	15.0±0.577
12.50	302.3±0.754	70.8±0.467	13.0±0.577
25	257.6±0.463	65.5±0.406	12.0±0.577
50	239.4±0.404	61.6±0.436	8.0±0.577
100	208.3±0.462	53.6±0.462	5.0±0.577

compared to those in mammals [12].

For many years, researchers have been testing both repellent and non-repellent termiticides, but due to environmental safety considerations, non-repellent termiticides are now more frequently used because they have a delayed mode of action [13, 25]. Various studies have been carried out to test the efficacy of different insecticides on different termite species in different parts of the world. Su *et al* [26] reported that neither species *Coptotermes heimi* nor *M. obesi* would penetrate permethrin treated soil. Remmen and Su [25] also proved the efficacy of fipronil and found that 2 µg mL^{-1} of fipronil that can fully stop the penetration of *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* Kollar in a treated barrier layer.

In Pakistan, various researchers have also tested the efficacy of different commercially available insecticides on different termite species. Akhtar and Shazia [1] reported that nimbokil and disodium tetraborate decahydrate were not toxic to *M. championi* even at higher doses. Imidacloprid was not tested for any termite species in Pakistan pior to the present study. Sheikh et al [24] evaluated the toxicity of Tenekil® (polychlorinated petroleum hydrocarbon), Termidor® (fipronil) and Terminus ® (chlorpyrifos) against *Heterotermes indicola* (Wasmann) in soil. Similarly, Manzoor *et al* [16] studied the repellence, toxicity and tunneling ability of *Coptotermes heimi* in response to bifenthrin (Biflex®), phenylpyrazole, fipronil (Termidor) and polychlorinated petroleum hydrocarbon (Tenekil), the results of the study were that Biflex is repellent, while Termidor and Tenekil did not show repellent effect at any concentration. Our data cannot be directly extrapolated to field situations, so investigations for performance in the field must still be carried out. Toxicity, repellency and tunneling response were tested in this study only in the sandy loamy soil, and previous studies revealed that soil type also influences the bioavailability of imidacloprid. Ramakrishnan et al [19] studied feeding inhibition and mortality in various soils and concluded that there was significant interaction between imidacloprid and the soils. So different soil types should be a part of any future efficacy testing.

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