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TOXICITY AND REPELLENCY OF DIFFERENT INSECTICIDES AGAINST *Heterotermes indicola* (ISOPTERA: RHINOTERMITIDAE)

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ABSTRACT

In Laboratory bioassays four Insecticides Imidacloprid, Chlorfenapyr, Bifenthrin, and Fipronil at concentrations 1.56, 3.125, 6.25, 12.50, 25, 50, 100 ppm andCadusafos at concentrations 0.098, 0.195, 0.391, 0.781, 1.562, 3.125 and 6.25 ppm were used to explore the feasibility of controlling the *H. indicola,* the toxicities and repellencies of these chemicals to the workers in the present study. Toxicity was expressed as F-1(0.97) for 8 hrs and one month old treated soil. For *H. indicola* when treated with Imidacloprid, Chlorfenapyr, Bifenthrin, and Fipronil it took more than 8 hours to obtain 97% mortality in 8 hrs and one month old treatments. LC_{50} values for *H. indicola* were 346.75, 75.86, 14.45, 1.05, 0.46 for Imidacloprid, Chlorfenapyr, fipronil, Bifenthrin, and Cadusafos, respectively after 8 hrs exposure and corresponding values after one month exposure the values were 1230.27, 190.5, 28.18, 10.47 and 2.51, respectively. Present study also reveals that Chlorfenapyr was non-repellent and Fipronil was repellent only above 25ppm and Bifenthrin was repellent at all tested concentrations.

Key words: Toxicity, Mortality, *H. indicola,* Repellency, LC_{50}.

INTRODUCTION

Heterotermes indicola (Wasmann) are considered to be one of the most destructive termites in urban and Agricultural areas (Manzoor *et al.,* 2010). This subterranean termite species construct diffused type of nests in the soil and reaches timber and other materials by means of galleries. However it is capable of establishing satellite nests in rafters and other wood material of large dimensions. At times, hanging satellite like tubes re built in search of contact with food and moisture (Sarma-Sen, 1975). It is estimated that billions of dollars are spent annually to control termites worldwide (Tsunoda, 2003).

Various kinds of termite management systems such as chemical and physical control methods have been developed to combat destructive termites. Chemical control with liquid termiticides treated soil, treating foraging areas of termites with dusts and baiting systems have been used as long lasting and cost-effective methods. However, termite control appears to be very difficult to achieve in the post chlordane era even with the newest chemicals and technologies (Potter, 2002). Numerous studies have examined the efficacy of new insecticides for the control of formosan termites (Delgarde and Rouland-Lefevre 2002; Shelton and Grace, 2003). However little informations are available on toxicity of insecticides to *H. indicola* (Wasmann).

Effectiveness of insecticides against termites depends mainly on the toxicity and the mode of activity of the compound and factors such as susceptibility of termite to insecticides, soil properties (e.g. pH, soil group, particle size, organic matters, and compactness), application protocol, and formulation applied (Osbrink *et al*., 2001).The penetration of termites in a soil treated with termiticides compounds is essential in evaluating the efficacy of the termiticides (Su *et al.,* 1997). Recently, repellent compounds for soil treatment are going to be replaced by non-repellents. For example non-repellent termiticides fipronil (Termidor), imidacloprid (Premise), and Chlorfenapyr (Phantom) are preferred over Bifenthrin and Cadusafos. A non-repellent termiticide allow termites to enter into treated soil (Thorne and Breisch, 2001; Kubota *et al.,* 2007) however with sufficient toxicity it could kill any termite come in contact with the soil before it can cause structural damage. In case of repellent termiticides, they prevent termites from acquiring lethal doses, reducing termite mortality in spite of any toxicity (Smith and Rust, 1990). The main aim of the studies was to design preventive and remedial control of *H. indicola* and find alternatives to the broadly used insecticides. The aim was achieved by determining the repellency of Fipronil, Imidacloprid, Chlorfenapyr (non repellent) and compared with Bifenthrin and Cadusafos (repellent). The most common method of determining termiticide repellency is by measuring how far termites will tunnel into soil treated at a concentration however in our studies we measured repellency by counting the number of termites on treated and untreated soil (Smith *et al* 1979). Furthermore, we were also interested in determining the termite mortality across increasing concentrations of termiticides against *H. indicola*.

MATERIALS AND METHODS

Termite Collection: Third instar workers and soldiers of *Heterotermes indicola* were collected using underground open-bottom bucket traps (Hu and Appel, 2004) from different locations of Lahore college for women university, Lahore. The collected soldiers and workers were maintained on moist filter papers in a plastic container at 26±2°C and 80% relative humidity to eliminate injured and inactive termites. All termites were tested within a week of collection and only active and healthy termites were used for the experiments.

Chemicals. Commercial formulation of five insecticides used for the experiments were, Bifenthrin (Biflex® 100EC, FMC Pakistan), Fipronil (Agenda® 2.5 EC Bayer France), Imidacloprid (Mirage® 45 EC Makhteshim-Agan SA), Chlorfenapyr (Phantom® 21.45% w/w, BSF NJ, US) and Cadusafos (Rugby® 100G FMC Pakistan).

Susceptibility of soldiers and workers to insecticides. The soil used for the laboratory experiments was sandy loam and free from any insecticidal residues as insecticides had never been used on the land where the soil was collected from. To make the soil clean from debris, it was sieved through a (10×18 mm) mesh screen and was oven-dried at 100°C for 24 hours. For this test, procedure was as adopted by Smith (1979) with few changes. Five grams of untreated soil was spread evenly at the bottom of each Petri dish (9 cm) and soil samples were treated with seven different concentrations of each insecticide and each concentration including controls was replicated three times with 10 termites (workers and soldiers) per replication and thus 30 insects were exposed per concentration. The termites were examined after every half an hour for up to 8 hours and the number of active, sluggish, ataxic, moribund and dead termites were recorded. The termite toxicity was expressed as F-1(0.97) according to Smith (1979). After 8 hours, the termites were removed and the insecticides treated soil was kept for one month at room temperature and the termites were again exposed to soil to determine residual activity of the insecticides. Data from the three replicates were pooled and analyzed by probit analysis (Finney, 1971). Lethal time to 50% mortality (LT_{50}) and lethal concentration to 50% mortality (LC_{50}) was estimated for each insecticide tested. Comparison of the slopes was made using a likelihood ratio test of parallelism where the slopes of the probit lines are constrained to the same line.

Repellency bioassays. For repellency test, insecticides concentrations for the soil treatment were same as those for toxicity test however in this case one of half of the soil was untreated. One half of bottom of a 9 cm Petri dish was covered with 2.5 grams of untreated soil and the second half with treated soil. Then 10 termites (mixture of soldiers and workers) were placed in the center of each dish. The soil samples were treated with seven different concentrations of each insecticide and each concentration including controls was replicated three times with 10 termites per replication and thus 30 insects were exposed per concentration. The experiment was carried at a temperature 26°C with 80% relative humidity. The number of termites on either treated or untreated soil was recorded at 15 minutes intervals for each Petri dish for upto 10 observations. A concentration of an insecticide was considered repellent when 21 or more of the 30 termites tested, were observed on untreated soil after 10 observations.

Calculation of LC_{50} and statistical analysis: Insect mortality data were corrected by Abbott's formula (1925), LC_{50} values (the concentration at which 50% of the larvae were immobilized) and LT_{50} were calculated by probit analysis using the PROBIT.

RESULTS AND DISCUSSION

Susceptibility of insecticides against *H. indicola*: Cadusafos was the most toxic product compared with all the compounds tested (Table 2) with LC_{50} significantly lower (non-overlapping 95% FL; $P < 0.05$) followed by Bifenthrin, Fipronil and Chlorfenapyr. The Imidacloprid was the least toxic with LC_{50} of 463 ppm which could reflect its heavy usage in the field to control termites. However previously reported studies for Imidacloprid had shown that the product was also least toxic than other neonictoids (Rust and Saran 2008). Though we have not tested other nictoids but present and previous studies suggest that the termite may have inheritant tolerance to Imidacloprid. The insecticidal activity of pyrethroid (Bifenthrin) and organophosphate (Cadusafos) was the highest than any other compound tested. Similarly the steeper slopes of the probit lines of the pyrethroids and organophosphates also reflect the fact that the lower doses of the Bifenthrin and Cadusafos caused sublethal caused significantly higher toxicity than either Imidacloprid or fipronil (Table 1) possibly contributing to steeper slope for Bifenthrin and Cadusafos.

When the insecticides were used to determine the time to kill, the Chlorfenapyr and Bifenthrin had similar time ($P > 0.05$) to kill 50% population but significantly less ($P < 0.05$) than other compounds tested. Similarly, the Fipronil and Cadusafos had same time to kill 50% population but it was significantly less than Imidacloprid which took 56 hours to kill 50% population (Table 3) which was 8-fold slower than Chlorfenapyr and Bifenthrin and 7-fold compared with Fipronil and Cadusafos (Table 3).

To determine if the LC_{50} of compounds tested was a correlated with LT_{50}, the correlation analysis suggested that there was strong and significant correlation between the LC_{50}s of the products and LT_{50} after 8 hour treatments (Fig 1A; $R^2 = 0.98$; d.f. 3 $P < 0.01$). The data further suggested that higher the LC_{50} of an insecticide greater time it took to kill a population. It was also possible that if the population previously had an exposure to a particular insecticide then due to inheriting tolerance the termites would be able to withstand the harmful affect of insecticides for longer period of time. This was also in agreement with the fact that at higher LC_{50} greater amount insecticidal toxin was required to be ingested before it could have an impact on target site. Similar results were obtained one month after the treatment (Fig 1B) suggesting that due to high residual activities time to kill 50% population was similar as it was at 8 hours treatment.

Repellency of Insecticides: The repellency of termiticide may be indicated by the height of the intersection of a percentage of termites recorded on treated curve and a percentage of termite mortality curve. An intersection of the two curves at a low percentage indicates a high level of repellency, whereas an intersection of the two curves at a high percentage indicates a low level of repellency (Remmen and Su, 2005). Based upon this definition, Imidacloprid, Chlorfenapyr and Fipronil treatments are clearly the least repellent termiticide after 8 hours and one month old treatment, because the mortality and repellency curves intersected at high % level (Fig. 2A-C; Fig 3A-C). This lack of repellency is consistent with that observed for Chlorfenapyr (Rust and Saran, 2006) and Fipronil (Ibhrahim *et al.,* 2003). In contrast Bifenthrin and Cadusafos were repellent at all concentration after 8 hours and one month old treatment (Fig 2D,E ; Fig 3D,E)

Results obtained in our studies were with specific formulations of the tested active ingredients. Other formulations with unique properties may have different effects on termites under similar test conditions. Furthermore, field conditions not adequately replicated in laboratory bioassays may affect the products not observed in our experiments. Imidacloprid, Chlorfenpyra and Fipronil may potentially be more effective in eliminating termites after they come into directly contact with treated soil. A variety of termiticides with repellent and nonrepellent properties are available to adequately protect structures. However, understanding the different active ingredients, their effect on termites, mobility in soil, compatibility with other active ingredients, and other properties may be important in designing more effective termiticides.

Table 1. Lethal time response at lethal concentration of insecticides against *Heterotermes indicola*

Insecticides	Immediate exposure					Exposure after one month				
	LT_{50} (95% FL) (Hours)	Fit of Probit line				LT_{50} (95% FL) (Hours)	Fit of Probit line			
		Slope ± SE	χ^2	d.f	P		Slope ± SE	χ^2	d.f	P
Imidacloprid	56.3 (40.9-88.6)	0.96± 0.09	10.6	15	0.78	36.5(30.3-47.1)	2.05±0.17	7.67	15	0.94
Chlorfenapyr	6.48(6.18-6.98)	7.47±0.60	2.10	15	0.99	6.72(6.59-6.86)	8.07±9.35	2.39	15	0.99
Fipronil	7.90(7.71-8.14)	9.34± 0.55	3.23	15	0.99	9.85(8.10-17.8)	4.94±1.3	18.3	15	0.25
Bifenthrin	6.72(6.59-6.85)	8.07 ±0.35	2.39	15	0.99	7.91 (7.70-8.16)	8.61±0.50	0.54	15	0.99
Cadusafos	7.46(7.26-7.70)	6.70(±0.34)	2.62	15	0.99	9.17(9.74-9.75)	6.99±0.51	3.07	15	0.99

Table 2. Relative toxicity of different insecticides to *Heterotermes indicola* after 8 hours and one month old treatments

Insecticides	Immediate exposure					Exposure after one month				
	LC_{50} (95% FL) (µg a.i. ml^{-1})	Fit of Probit line				LC_{50} (95% FL) (µg a.i. ml^{-1})	Fit of Probit line			
		Slope ± SE	χ^2	d.f	P		Slope ± SE	χ^2	d.f	P
Imidacloprid	462.8(321-287)	1.16± 0.29	1.6	6	0.95	1231(1123-1289)	1.05±0.43	1.53	6	0.96
Chlorfenapyr	75.9(40.6-149.3)	0.83±0.17	0.47	6	0.99	189.5(83.9-220)	0.87±0.19	0.28	6	0.99
Fipronil	13.8(6.56-30.8)	0.59± 0.15	0.93	6	0.99	24.3(14.0-52.9)	0.74±0.15	1.29	6	0.97
Bifenthrin	1.10(0.89-1.78)	1.15 ±0.15	1.39	6	0.97	10.3(5.85-17.2)	0.82±0.15	0.79	6	0.99
Cadusafos	0.43(0.29-0.61)	1.28(±0.18)	1.02	6	0.98	2.18(1.48-3.67)	1.17±0.18	2.08	6	0.91

For the last many years, researchers have been testing the repellent and non-repellent termiticides but due to environmental safety non-repellent termiticides are more frequently used now a day because they have delayed mode of action (Hu, *et. al.,* 2005; Su, 2005). Various studies have been carried out to test the efficacy of different insecticides on different termite species in different parts of the world. Mauldin (1986) in his ground board method evaluated six insecticides to study termite control. Similar views have been published by Beal in 1986 regarding chlorpyrifos. Su, *et al.,* (1982) reported that both species *Coptotermes heimi* and *Micromeres obesi* do not penetrate Permethrin treated soil. Rummen and Su (2005) also proved the efficacy of Fipronil and found that 2 ppm of Fipronil that can fully stop the penetration of *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* Kollar in treated barrier layer.

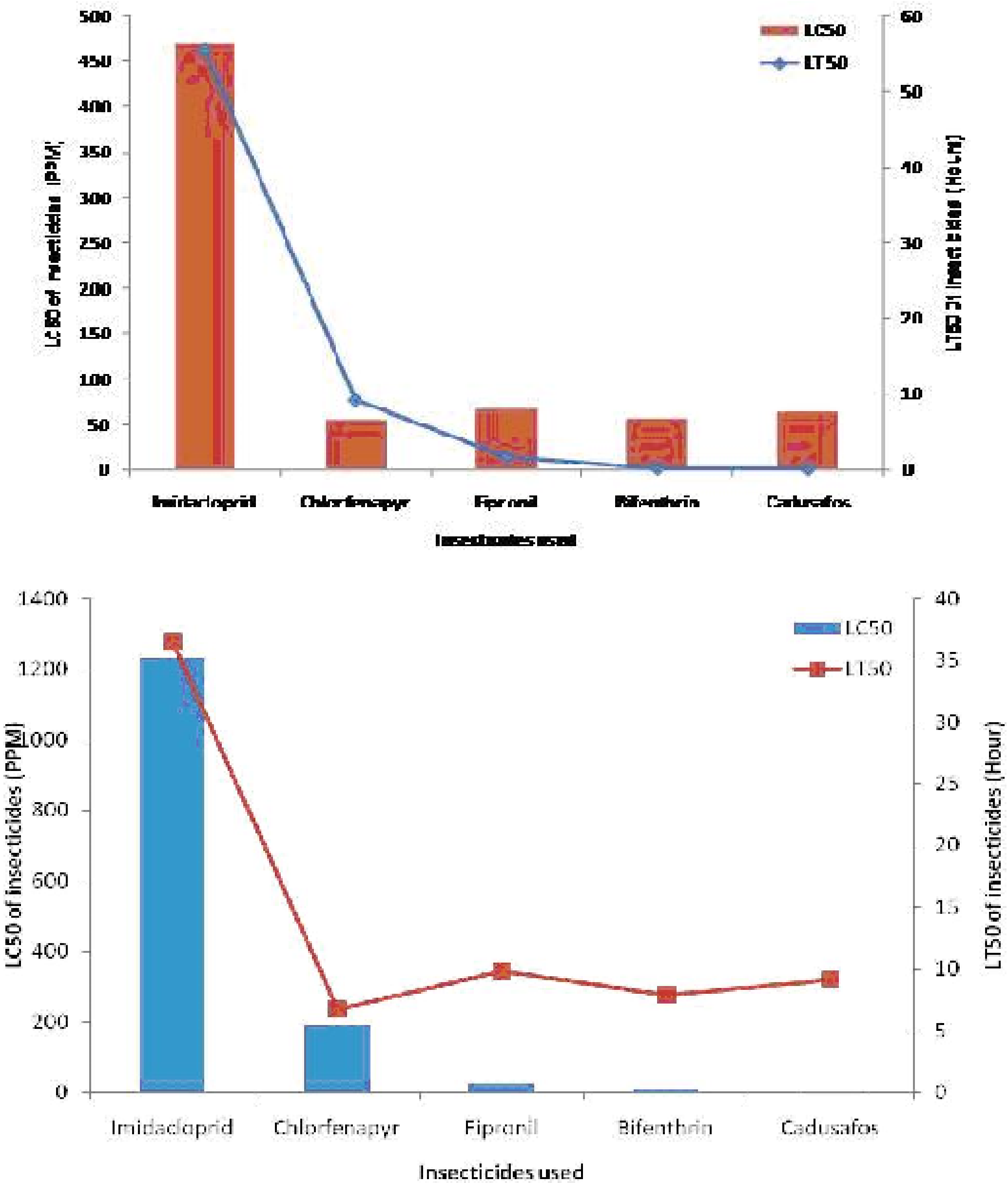


Figure 1 Relationship between LT_{50} and LC_{50} of insecticides tested against *Heterotermes indicola* for (a) 8 hours treatment (b) one month treatment.

Our outcome showed that cadusafos is highly toxic against termites after 8 hours treatment than Bifenthrin, Fipronil, Chlorfenapyr and Imidacloprid even at lowest concentrations. Bifenthrin was more toxic than Imidacloprid, Chlorfenapyr and Fipronil but less than cadusafos. Imidacloprid, Chlorfenapyr and Fipronil are slow acting insecticides and showed less mortality when compared to Bifenthrin. Our results also agree with Yoeh and Lee (2007). The results showed that Bifenthrin is repellent termiticide at 30 ppm with high termite mortality and high tunneling at untreated zone and no tunneling behaviour at treated zone. Osbrink *et al.,* (2001) also proved the relatively slow speed of Fipronil against soldiers. Even though Fipronil is slow acting termiticide but it acts faster on workers as compared to soldiers. Osbrink (2001) also tested its slow speed on soldiers allowing them more time to interact with workers before getting toxifying and dying. He also found that its early poisoning activity on workers causes the soldiers to identify the sick and dead workers and soldiers to avoid infected (infested) workers.

From this study we also proposed that even though cadusafos is nematicide but when we compare its toxicity against termites with other termiticides, it can be used for termite control in the fields .

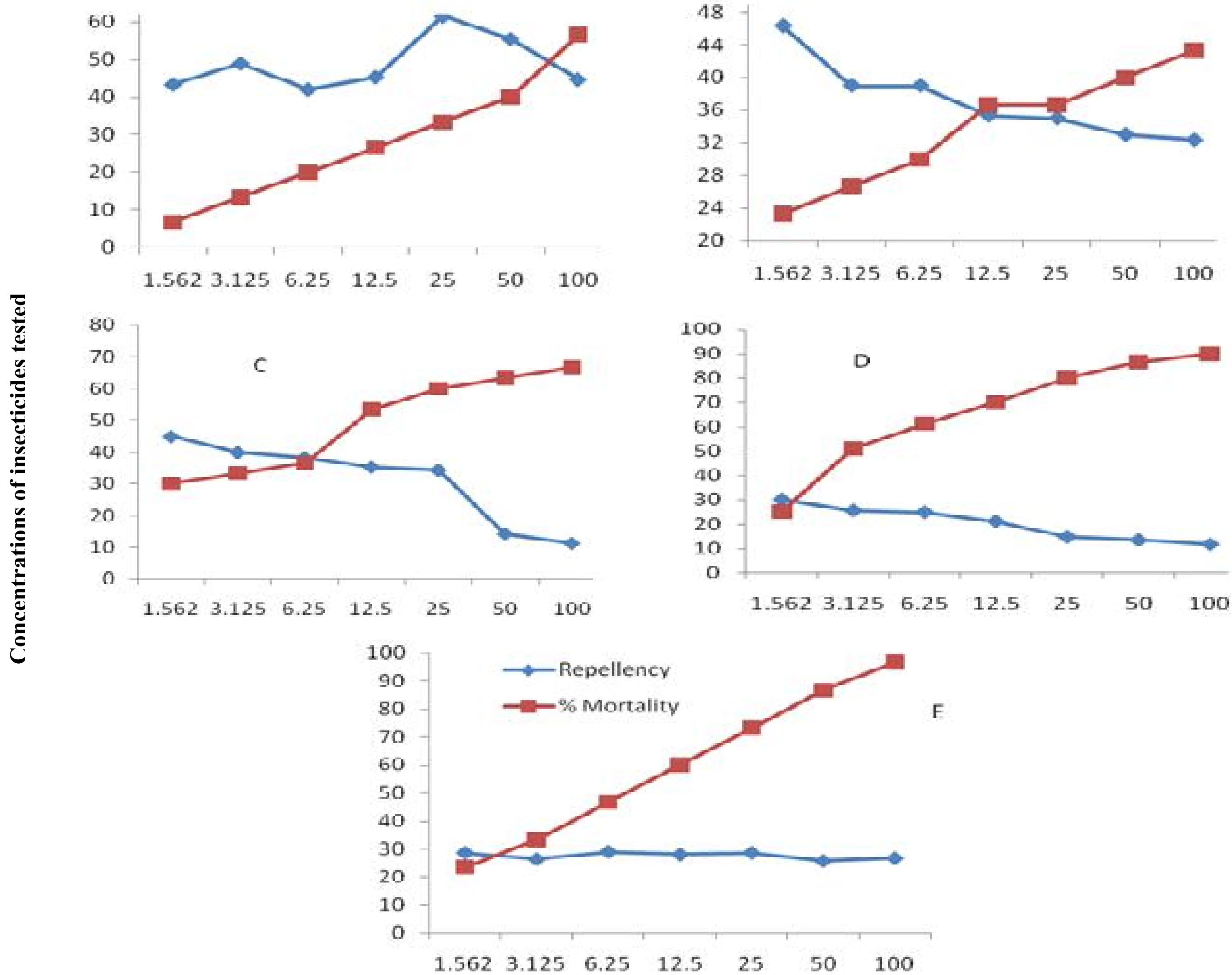
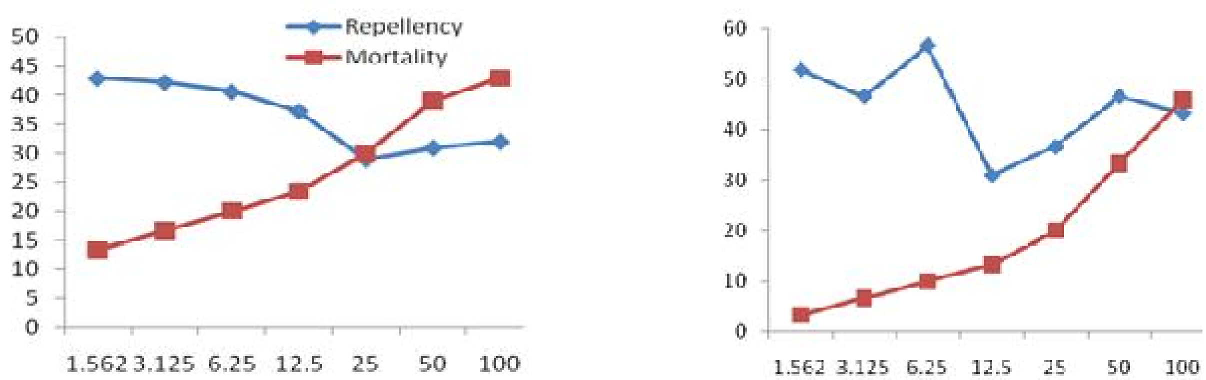


Figure 2. Percentage of termites distribution on treated soil and percent mortality of (A) imidacloprid (B) chlorfenapyr (C) fipronil (D) bifentherin and (D) cadusafos after 8 hour exposure to insecticides



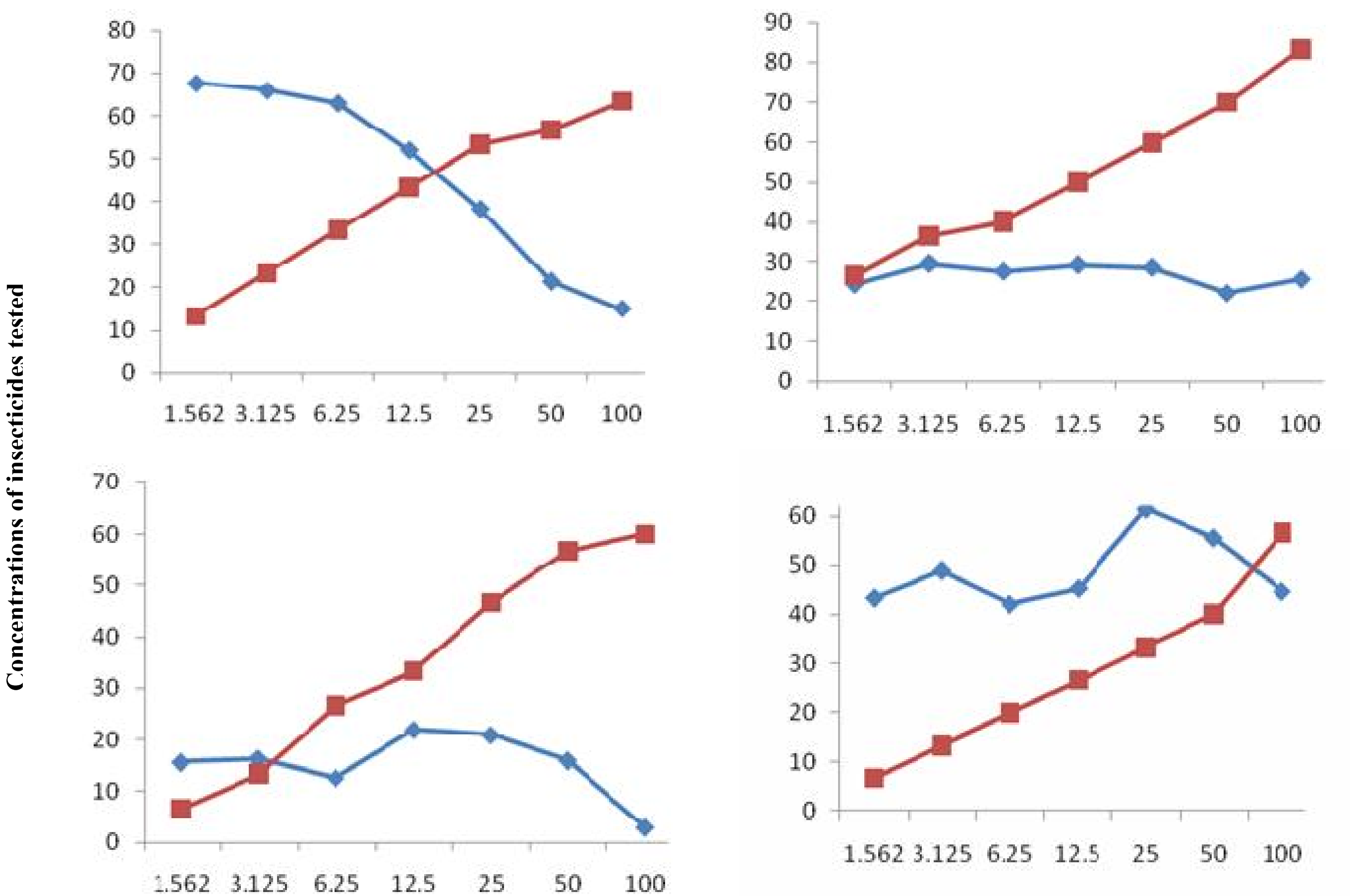


Figure 3. Percentage of termites distribution on treated soil and percent mortality of (A) imidacloprid (B) chlorfenapyr (C) fipronil (D) bifentherin and (D) cadusafos one month after treatment to insecticides

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