COMPARATIVE TOXICITY OF FIVE INSECTICIDES AGAINST SUBTERRANEAN TERMITE, *AMITERMES VILIS* (ISOPTERA: TERMITIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT: Subterranean termite, *Amitermes vilis* (Hagen) is one of the most important insect pests in Iran, which cause a serious economic damage to agricultural and urban areas, natural resources, and historic monuments. Toxicity of five insecticides including Agenda EC 25 (fipronil), Consult EC 100 (hexaflumuron), Admiral EC 100 (pyriproxyfen), Confidor SC 350 (imidacloprid), Reldan EC 400 (chlorpyrifos methyl) were evaluated against *A. vilis*. To determine LC_{50} of the insecticides, bioassay was carried out on worker termites of *A. vilis*. The LC_{50} values for fipronil, pyriproxyfen, hexaflumuron, imidacloprid and chlorpyrifos methyl were 3.48, 9.56, 32.02, 2.02 and 0.09 ppm, respectively. The LT_{50} value of pyriproxyfen was shorter than hexaflumuron. The results showed that fipronil and imidacloprid were respectively the most effective insecticides for a short term control of the termite. It could be concluded that the toxicity of pyriproxyfen was higher than hexaflumuron.

KEY WORDS: *Amitermes vilis*, fipronil, pyriproxyfen, hexaflumuron, imidacloprid, chlorpyrifos methyl, bioassay.

Termites cause a serious economic damage to different crops, natural resources, forestry and historic monuments, as well as electrical cables and other building materials (Pearce, 1997; Sheets et al., 2000). Subterranean termites are difficult insects to control because they have complex social patterns and form great extent colonies generally made of several thousands individuals with an outstanding ability to develop from one caste to another (Sheets et al., 2000). Even though the queen dies, secondary reproductives can get that function. Thus, the removal of foragers is usually not enough to eliminate the colony (Nunes & Nobre, 2003).

In the United States the public cost of subterranean termites are approximately $1.2 billion each year. Historic buildings and structures that have been used wood as a building material are susceptible to damage by subterranean termites. Termites' damage to historic buildings is both costly and impossible to reverse and can diminish the quality of a structure (Su et al., 1998). Furthermore, termites cause crop loss in agriculture, damaging forest and destroying stored products and other essential or interested materials of human (Kramm et al., 1982).

There are usually three major strategies in termites control (Nunes & Nobre, 2003): (1) inserting chemical and/or physical barriers to prevent termites from entering to buildings or attacking timbers in contact with the ground, (2) using naturally durable timbers or impregnation of susceptible ones with a wood preservatives that act as a termiticide (3) eliminating the termites colonies using chemicals directly to the nests or indirectly via toxic baits that are eaten and distributed through the colony, leading to their destruction.

Despite many secure and simple practices to manage termites including cultural and biological control, queen removal, plant resistance, physical barriers based on natural products and baiting systems, insecticides are still playing a key role for the control of the termites (Kumawat, 2001; Rana et al., 2001). Imidacloprid, hexaflumuron, thiamethoxam, fipronil and bifenthrin in different formulations have been found effective for the control of different species of termites (Sheets et al., 2000; Anonymous, 2001; Delgarde & Rouland-Lefevre, 2002a,b; Ahmed et al., 2005a,b; Remmen & Su, 2005).

Soil insecticide treatment makes a chemical barrier for exclusion of subterranean termites from considered area (Blaske et al., 2003), and has been used a general method of termite control over the past 55 years (Su et al., 1997; Ibrahim et al., 2003; Jones, 2003).

Over the last several years, there has been increasing popularity in the use of non-repellent termiticides because forage termites could not detect the presence of the treated soil with this kind of termiticide, and thus they would continue to forage through the treated soil and would receive amounts of insecticides on their bodies, and then would be died (Shelton & Grace, 2003).

Baits containing the insect growth regulators such as hexaflumuron, have been effectively used to eliminate subterranean termite colonies in around structures (Clement et al., 1996; Forschler & Ryder, 1996). Hexaflumuron inhibits the synthesis of chitin (Nakagawa et al., 1992), which is essential for the formation of insect exoskeleton but it is virtually harmless to vertebrates. Because of its slow lethal effect on mammalian (LD50 > 5,000 mg/kg) as well as on nontarget organisms, it is registered in the least toxic category, "caution." Using baiting procedure, hexaflumuron is delivered by foraging termites to eliminate the entire colony populations of several million individuals (Su, 1994).

In spite of the economic importance of subterranean termite, *Amitermes vilis* (Hagen) (Isoptera: Termitidae) as an insect pest of some agricultural crops, buildings, natural resources, and historic monuments in Iran especially in southern regions of Iran, only limited information is available on the efficiency of termiticides used to its control in Iran. Therefore, the aim of our research was to determine the toxicity effect of five termiticides on mortality of workers *A. vilis* exposed to the termiticides tested under laboratory conditions.

MATERIALS AND METHODS

Insecticides. The insecticides tested for the bioassay were fipronil, pyriproxyfen, hexaflumuron imidacloprid and chlorpyrifos methyl against *A. vilis*. The formulations and active ingredient percentage of insecticides are given in Table 1.

Collection of termite. Subterranean termite, *A. vilis* was collected from termite-infested monument (Jame mosque of Saveh by using a trapping technique in Iran. The traps consisted of five thin wood slices (25 × 6 × 0.5 cm) that were buried in ground station by a PVC pipe (20 cm in diameter) with a lid to protect from rain and sunlight. They had a great potential for attraction and harboring thousands of the termites inside the crevices of the slices. Trapped termites were transferred to the laboratory of Iranian Research Institute of Plant Protection (IRIPP) located in Tehran. They were maintained in plastic boxes, with fresh cardboard, in a growth chamber 25 ± 2 °C, 80 ± 5% RH in dark conditions to further use. Distilled water was sprayed on the inside walls of the container to keep the relative humidity above 80%. Mature worker and soldier termites were separated from logs or nest debris by breaking and sharply tapping materials into plastic trays containing moist paper towels. The worker termites were used for bioassay within one hour of segregation.

Bioassay. Filter papers were deepened in the different concentrations of the insecticides and allowed to dry for hree hours. After drying, they were placed in Petri dishes (70 mm diameter, 20 mm height), and left in an incubator at 25 ± 2°C in the dark conditions. The mortality of termites exposed to fipronil, imidacloprid and chlorpyrifos methyl were recorded after 24 hours of the exposure. The record of mortality due to the insect growth regulator (IGR) insecticides was continued up to 10 days after treatment. The experiments were carried out in triplicate using 10 workers per a dish. Mortality data were submitted to Probit analyses. The corrected mortality was estimated by the difference between total death in the treatment and the control.

RESULTS

Table 2 shows the LC_{50} values of five insecticides tested against *A. vilis*. Among different insecticides used, chlorpyrifos methyl and hexaflumuron had respectively lowest (0.09 ppm) and highest (32.02) LC_{50} values as compared to the other insecticides tested.

LT_{50} values of *A. vilis* after treatment with hexaflumuron and pyriproxyfen are presented (Table 3). Our findings demonstrated that LT_{50} value of pyriproxyfen at highest concentration (1000 ppm) was shorter (6.61 ± 1.73 days) than that of hexaflumuron (8.29 ± 1.32 days). LT_{50} values of hexaflumuron (50 ppm) and pyriproxyfen (5 ppm) were 15.52 ± 0.172 and 12.74 ± 1.73 days, respectively.

The mortality percentage of *A. vilis* after exposure to two IGR insecticides for 10 days is presented (Figs. 1 and 2). The highest mortality of *A. vilis* exposed to hexaflumuron and pyriproxyfen was observed in 10^{th} days after treatment. Thus effective time of these insecticides occurred in 10^{th} days of treatment. Our results showed that pyriproxyfen was found to be higher lethal effect than hexaflumuron.

DISCUSSION

The subterranean termite, *A. vilis* is an important urban insect pest causing a tremendous amount of damage to different natural resources and structures in southern regions of Iran. The complex behavioral patterns of social insects such as termites in conjunction with the cryptic nature of their foraging make them challenging to manage with conventional insecticides (Sheets et al., 2000). In the current research, we investigated the mortality of workers *A. vilis* after exposure to different insecticides in order to determine the toxicity effect of the tested insecticides.

Comparing the LC_{50} of different insecticides used in this research indicated that chlorpyrifos methyl had the lowest LC_{50} in compared with the other insecticides, suggesting that the termiticidal efficacy of chlorpyrifos methyl against *A. vilis* was the highest. This result is in agreement with the findings of Ahmed & Farhan (2006), who reported that the chlorpyrifos 40 EC gave the best results in controlling the population of subterranean termites in sugarcane compared with imidacloprid. Moreover, Mishra (1999) reported the highest efficacy of chlorpyrifos 10 G as a soil treatment and chlorpyrifos 20 EC as seed dressing in ground nuts against termites.

Regarding the LC_{50} values of imidacloprid, fipronil, hexaflumuron and pyriproxyfen, it would be suggested that imidacloprid was more toxic than the above-mentioned insecticides. This result is in confirmation with Ahmed & Farhan (2006), who stated that imidacloprid 50 SL had the highest toxicity effect compared with thiamethoxam 70 WP and flufenoxuron 10 DC. According to the reports by Ramakrishnan et al. (2000), imidacloprid was active to control termite *Reticulitermes flavipes* (Kollar) in a variety of soil types. Additionally, the above-mentioned researchers demonstrated that imidacloprid enhances the susceptibility of *R. flavipes* to a fungal entomopathogen, thus reducing the chemical concentrations required.

As can be seen in Table 3, the mean time required to kill half of the workers *A. vilis* was affected by the concentration of hexaflumuron to which they were exposed. The 1000 ppm concentration resulted in an about 7 days shorter LT_{50} than the 50 ppm one, which is in consistency with the results of Sheets et al. (2000), who reported the 0.5% concentration diet exposed hexaflumuron had an about 8 days sooner LT_{50} than the 0.1% diet for Eastern subterranean termites. This faster time to kill workers exposed to the higher concentrated diet is probably due to the insects obtaining and maintaining the threshold concentration required for lethality in a shorter period, because of the more rapid uptake kinetics at the higher diet dosage (Sheets et al., 2000). Baits containing slow-acting toxicants, such as hexaflumuron, have been used as alternatives to conventional soil insecticides because of its certifiable effects at very low concentrations in bait stations to manage termites (Su et al., 1997). In addition, hexaflumuron can transfer from treated to untreated termites in a highly efficient manner, predominately by trophallaxis (Sheets et al., 2000). Such distribution of a slow-acting toxicant may be ideal for bait control of social insects such as termites, where the active ingredient can be spread throughout the colony, even to those insects not directly exposed to the insecticide (Su et al., 1987). The LT_{50} value of pyriproxyfen was shorter than that of hexaflumuron. Additionally there were great differences in LC_{50} value between these two IGR insecticides, indicating that the concentration of pyriproxyfen required to kill 50% of the workers was lower than that of hexaflumuron. Therefore, the toxic effect of pyriproxyfen was higher than hexaflumuron.

According to the laboratory results from the study carried out to find out the effective insecticides against *A. vilis*, it would be concluded that chlorpyrifos methyl and imidacloprid were effective insecticides for a temporary period of treatment. However, pyriproxyfen and hexaflumuron as IGR's can be more effective on *A. vilis* for a long time of treatment. Further studies are required to investigate the termiticidal potential of the other insecticides registered in Iran against dominant species of termites in the field and laboratory conditions.

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Table 1. Different formulations of insecticides, chemical group and their concentrations used against *Amitermes vilis*.

Trade name	Entry name	Formulation	Chemical group	Concentration (ppm)
Consult	hexaflumuron	EC[1] 10%	IGR[2]	5, 50, 100, 500, 1000
Admiral	pyriproxyfen	EC 10%	IGR	5, 50, 100, 500, 1000
Agenda	fipronil	EC 2.5%	Phenylpyrazole	50, 100, 500, 1000
Confidor	imidacloprid	SC[3] 35%	Neonicotinoid	0.5, 1.5, 10, 100
Reldan	chlorpyrifos methyl	EC 40%	Organophosphate	0.05, 0.1, 1

[1] Emulsifiable concentration [2] Insect growth regulator [3] Suspension concentrate

Table 2. LC_{50} values of hexaflumuron, pyriproxyfen, fipronil, imidacloprid and chlorpyrifos methyl against *Amitermes vilis*.

Insecticide	No. insects tested	No. concent rations	Slope (±SE)	Inter cept (±SE)	LC_{50} (95% CI) (ppm)	X^2	Probability
hexaflumuron	220	5	0.17 0.77	0.36 - 1.94	32.02 (17.64-94.75)	1.74	0.41
pyriproxyfen	150	5	0.11 0.57	0.24 - 1.14	9.56 (3.89-24.36)	1.53	0.67
fipronil	400	4	0.15 1.75	0.11 - 0.95	3.48 (2.85-4.20)	5.49	0.64
imidacloprid	350	5	0.11 0.82	0.08 - 0.25	2.02 (1.27-3.18)	2.48	0.47
chlorpyrifos methyl	400	3	0.13 1.45	0.16 - 1.52	0.09 (0.06-0.11)	3.34	0.18

Table 3. LT_{50} values of *Amitermes vilis* after treatment with hexaflumuron and pyriproxyfen.

Insecticide	**Concentration (ppm)**	**Correlation coefficient**	**Regression model**	**(LT_{50}±SE) in days**
hexaflumuron				
	1000	0.99	Logistics	8.29 ±1.32
	500	0.99	Logistics	12.60 ±1.31
	100	0.97	Logistics	12.11 ±1.88
	50	0.99	Logistics	15.52 ±0.172
pyriproxyfen				
	1000	0.99	Logistics	6.61 ±1.73
	5	0.99	Logistics	12.74 ±1.73

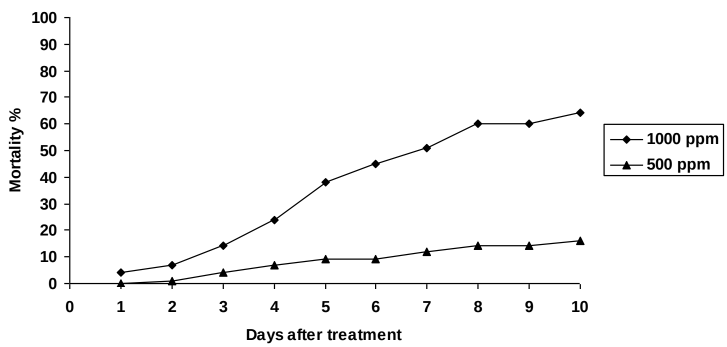

Figure 1. Mortality percentage of *Amitermes vilis* after treatment with pyriproxyfen for 10 days.

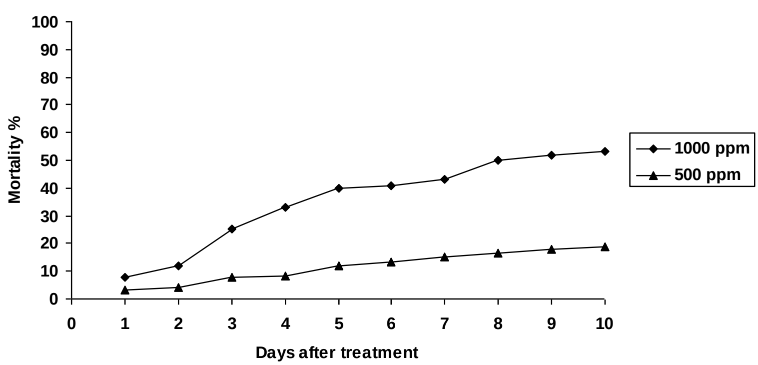

Figure 2. Mortality percentage of *Amitermes vilis* after treatment with hexaflumuron or 10 days.