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Potential of Fipronil as a Feeding Toxicant Against the Subterranean Termite *Heterotermes indicola* (Rhinotermitidae: Isoptera)

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Studies were conducted to find the dose-response relationship, feeding deterrence and transfer of fipronil to unexposed nest mates when offered to the subterranean termite *Heterotermes indicola* in a substrate. Dose response studies revealed that values of the effective lethal time to kill 90% of the treated termites (ELT 90) ranged from 2 to 6 d for 5 to 50 ppm. Projected ELT 90 value was protracted for 1 ppm, showing a value of 11 d. Choice feeding and exposure studies indicated that no significant difference was observed between the consumption of treated and untreated substrates at a concentration range of 1– 20 ppm, while at 30 and 50 ppm, the termites preferred to feed on untreated blotting paper. When termite workers (donors) force-fed on fipronil were confined with untreated workers (recipients) for a period of 10 d, all concentrations greater than 1 ppm were able to inflict more than 50% mortality on the untreated workers. Relevance of the results is discussed with a possibility to use fipronil as a spot treatment bait in agricultural fields.

Key Words: fipronil, *Heterotermes indicola*, subterranean termites, slow acting toxicant, termite control

INTRODUCTION

Termites are an important agricultural pest in different parts of the world especially in the tropics where they damage agronomic crops such as sugarcane, cotton, rice, maize, wheat and peanuts (Harris 1969; Rajagopal 1987) and orchards (Stansly et al. 2001; Salihah et al. 2012). Termites can physically damage the structural support of plants, interfere with their food and water supply, and eventually cause their death. They can defoliate plants close to the ground and tunnel into, or eat stems and roots (Pearce 1997). Attack to the root system can also lead to increased lodging susceptibility of mature plants followed by the invasion of grains by soil fungi (Anonymous 2000). In some arid areas, losses of 90% to 100% have been recorded in sugarcane at the germination stage (Avasthy 1967). Salihah et al. (1988) reported that termite infestation caused 40% destruction of buds in sugarcane, resulting in yield loss of 33%.

Damages to crop plants and orchards are largely caused by subterranean termites belonging to the families Rhinotermitidae and Termitidae which build diffuse subterranean nests and below-ground galleries. In agroecosystems, conventional termite control uses pesticides as seed dressing to reduce access of termites to the plants. Pesticides are also used as general soil application (in irrigation water) and suppress the populations of subterranean termites to prevent plant loss. The commonly used pesticides are organophosphates and pyrethroids as well as non-repellent insecticides such as imidacloprid and fipronil (Alam et al. 2001; Anonymous 2000). Soil application of insecticides uses several hundred grams to kilograms of active ingredient which can lead to undesirable environmental effects. A safer alternative would be spot application of non-repellent termiticides to suppress the populations of subterranean termites.

Fipronil is a widely used non-repellent chemical against subterranean termites. It is highly toxic to termites even in very small quantities, i. e., a lethal dose or toxicity (LD_{50}) of 0.16 ng per termite for *Reticulitermes heperus* (Saran and Rust 2007) and 1.33 ng per termite for *Coptotermes formosanus* (Ibrahim et al. 2003). Along with its delayed mode of action, fipronil has the capability of horizontal transfer within nest mates (Shelton and Grace 2003), mainly through body contact (Saran and Rust 2007).

Most of the studies conducted on fipronil have focused more on its contact toxicity when applied as a barrier treatment than on its use as a feeding toxicant against subterranean termites. Huang et al. (2006) tested fipronil in a bait matrix in the field and reported that approximately 3–5 mg of fipronil could suppress foraging populations of *Odontotermes formosanus* containing 0.4–0.7 million foragers per colony.

Our study was conducted to explore the potential of fipronil use as a feeding toxicant to be dispensed in a bait matrix against *Heterotermes indicola* (Wasmann), a major subterranean termite causing economic damage to crops, buildings and orchards in Pakistan (Salihah et al. 2012). The parameters studied included dose-response relationship of fipronil when used in a cellulose-based substrate along with its acceptability/deterrence and the potential of its transfer to unexposed nestmates. Dose-response relationship refers to the relationship between the quantities of a chemical and its overall effect on the mortality rate of a given insect population.

MATERIALS AND METHODS

Termite Collection

A stake-survey and trapping method was used to capture the termites. Wooden stakes (30 cm high × 3.5 cm wide × 2 cm thick) of *Poplus* sp. were driven 20 cm deep into the soil of lawns and sidewalks surrounding the building where there was either observed or suspected termite infestation. The stakes were placed 5 m apart and examined fortnightly. The infested stakes were replaced by underground monitoring stations by digging a cavity in the soil to fit the station so that the upper margin of the station touched the ground surface. The monitoring station was comprised of a slice bundle surrounded by a 2-mm thick plastic collar (17 cm diameter, 22 cm high). The slice bundle consisted of five rectangular wooden slices (*Poplus* sp., 15 cm high × 8 cm wide × 1 cm thick) wrapped in a blotting paper and held together by a rubber band. The space between the slices and the plastic collar was filled with soil. The station was covered with polyethylene bag to prevent entry of rain or irrigation water. The monitoring stations were checked on alternate weeks and the infested slice bundles were removed for termite collection and replaced with new ones.

Termites present in the slices of wood bundle were collected and weighed to estimate the number of captured termites. One gram of reference sample was used to count the number of workers and soldiers and multiplied by the weight of the termite to get an estimate of termite numbers. Seven grams of the cleaned termites were kept in glass Petri dishes (14 cm diameter, 3 cm high) that had two moist blotting papers (diameter 14.0 cm) at the bottom as food and placed in round glass chambers (as mentioned above), having water at the bottom. The acclimatized termite specimens were used after 2–3 d.

Dose-Response Relationship

Agenda (Jaffer Brothers, Pakistan), a commercial formulation containing 25 g L^{-1} of fipronil, was used in the studies. One hundred termite workers (plus five soldiers) were placed in petri dishes (9.0 cm diameter, 1.5 cm high) provisioned with a pair of 9-cm treated circular blotting papers (Millat paper art, Karachi, Pakistan). The blotting papers (0.21 g each) were dipped in aqueous solution of the termiticide for 5 s to yield the desired concentrations of 1, 5, 10, 20, 30 and 50 ppm (wt/wt) of the toxicant in the blotting paper. For example, 5 ppm was prepared by dipping a piece of 0.21 g of blotting paper in 0.1 g L^{-1} dilution of fipronil. Five-second dipping adsorbed about 0.3 mL of the solution, resulting in a concentration of 0.00105 mg of active ingredient per 0.21 g of paper, which is the equivalent of 5 ppm wt of toxicant/wt of paper. The treated blotting paper was dried at room temperature and moistened with 2 mL of deionized water before it was used in the experiment. All treatments were replicated four times.

Termites were force-fed on the treated paper for 24 h and the survivors were transferred to similar Petri dishes containing untreated blotting paper. Dead or moribund workers were recorded and removed from each Petri dish daily until all the termites were dead. Probit analysis was conducted to estimate effective lethal time (ELT 50 and ELT 90), the time required for a fixed dosage to kill 50% or 90% of the test insects, respectively, by using days as independent variable for each dose (Su 1987).

Feeding Deterrence Studies

Plastic Petri dishes (9 cm diameter, 1.5 cm high), having their bottoms brushed with sandpaper to provide traction for termites, were used as experimental units. Two rectangular pieces (3 cm × 2 cm) of blotting papers were placed 3 cm apart horizontally, and covered with 25 g of sterilized sand (60 mesh size) moistened with 20% (w/v) of deionized water. One piece of blotting paper was treated by dipping in an insecticide solution to get the required concentration (wt of a. i. /wt of blotting paper) while the second piece was dipped in deionized water only. The pieces of blotting paper were kept in an oven at 120 °C for 6 h before use, after which their dry weights were determined. Each concentration was considered as a treatment and was replicated four times. Two hundred workers and 10 soldiers of *H. indicola* were introduced into each unit. All the units were kept in round glass chambers (30 cm diameter, 25 cm high) with an air tight lid, having water at the bottom to maintain 90 ± 5% RH, and kept in the laboratory at 25 ± 2 °C. The units were disassembled after 2 wk and data were recorded on the number of termites still alive. The blotting papers were cleaned to remove any sand particles and their dry weights were determined. Consumption rates of both treated and untreated blotting papers in each concentration were determined by subtracting the final dry weights from the initial ones and were compared using a paired t-test (Su and Scheffrahn 1993).

Transfer Studies

To distinguish donors from recipients, the former were stained using Nile blue (The British Drug Limited, UK). The workers collected from the traps were force-fed for 72 h on blotting paper (Whatman No. 42) soaked in aqueous solution of Nile blue to yield a 0.2% concentration (w/w) of the dye in the paper. Only well-stained and active workers were used in the study. The donors were confined on blotting paper treated with fipronil to yield concentrations of 1, 5, 10, 20, 30 and 50 ppm (wt/wt) in Petri dishes (10 × 15 cm) for 24 h as earlier mentioned. The treated donors (50 workers) were then allowed to interact with the same number of untreated recipients in similar glass Petri dishes, their bottoms lined with a circular blotting paper moistened with 2 mL of distilled water as food source. The Petri dishes were kept in round glass chambers (as earlier mentioned) with water at the bottom to maintain RH of 90 ± 5% and kept in the dark at room temperature (25 ± 3 °C). Mortalities of donor and recipient termites were recorded daily for 10 d but the dead termites were not removed. At the end of experiment, the total number of dead and live termites was counted to account for missing individuals that may have been cannibalized by other workers. Mortality of both donors and recipients and percentage of missing workers were subjected to analysis of variance (ANOVA) and means were separated using Tukey's HSD mean separation test.

RESULTS

Dose-Response Relationship

A very high mortality rate was observed in termites for dose applications of more than 1 ppm of fipronil. Obvious steep slopes indicated a very rapid rate of kill at doses of 10, 20, 30 and 50 ppm. A dose of 1 ppm, however, showed a shallow slope, ultimately reaching 100% mortality in 12 d. The 5 ppm dose showed an intermediate response where an initial fast rate of kill was observed, which slowed down later, culminating in total mortality at day 8.

ELT 50 was estimated to be 7.5, 1.84, 1.41, 1.2, 1.8 and 1.01 d at doses of 1, 5, 10, 20, 30 and 50, ppm respectively (Table 1). ELT 90 values ranged from 2 to 6 d for 5–50 ppm. Projected ELT 90 value was protracted for 1 ppm, showing a value of 11 d.

Feeding Deterrence Studies

At a concentration range of 1–20 ppm, no significant difference (p values for paired t-test = 0.08–0.53) was observed between consumption of treated and untreated substrates while at 30 and 50 ppm, termites preferred to feed on untreated blotting paper (Table 2). Population count of termite workers 2 wk after the start of choice test indicated an overall significant difference ($F=189$, $p<0.001$). Except for 1 ppm (showing 25% mortality), there was complete mortality of termites after 2 wk in all the concentrations used.

Transfer Studies

Table 3 shows the results of studies aimed at finding the possibility of horizontal transfer of fipronil from the workers exposed to different concentrations (donors) and mixed with untreated workers (recipients) for a period of 10 d. Recipient mortality ranged from 40% to 71%, indicating a strong potential of horizontal transfer of fipronil ($F_{5,21} = 4.80$, $p= 0.007$).

All concentrations greater than 1 ppm were able to inflict more than 50% mortality in the untreated workers. An overall variability was observed in the number of missing dead donors for the concentrations of fipronil used ($F_{5,\ 21} = 21.4$, $p<0.001$). The missing individuals were assumed to have been consumed by the other workers. A higher number of dead donors was missing in 1 ppm (45%) compared with the rest of the doses used. However, there was no significant difference among the other doses for missing dead donors.

DISCUSSION

Dose-Response Relationship

Fipronil was very effective in killing *H. indicola* even at a concentration of 1 ppm. Fipronil is known to cause termite mortality at very low concentrations. In *C. formosanus*, the LD_{50}s of fipronil at 72 h after treatment were <2.0 ng per insect, with no significant differences in the tested workers/soldiers or colonies (Ibrahim et al. 2003) while LD_{50} was approximately 0.2 ng per termite

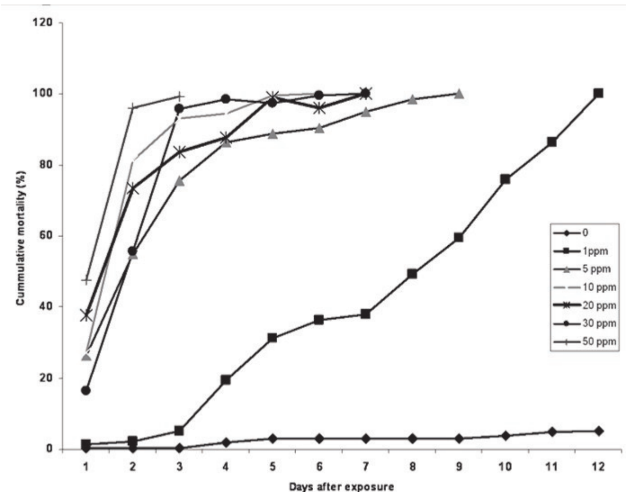

Fig. 1. Cumulative mortality (not adjusted against control) of *Heterotermes indicola* after exposure to different concentrations of fipronil.

expressed between day 4 and 7 against *R. hesperus* (Saran and Rust 2007). Remmen and Su (2005a) also found that 24-h exposure of *C. formosanus* on filter paper treated with 1 ppm fipronil resulted in 31% mortality at 24 h and 84% mortality at 7 d, indicating its delayed toxicity at this concentration. We noted that at 5 ppm, mortality started from day 1. Half of the termites were dead after 2 d, however, the rate of kill slowed down later, culminating in total mortality at day 8. A strong dose-dependent rate of kill for fipronil was observed where a dose of 10–50 ppm, when offered in no choice feeding, resulted in total mortality of treated termites within 3–4 d.

Feeding Deterrence Studies

Studies to determine deterrence of different concentrations of fipronil showed that although termites consumed at least some part of the blotting papers treated with all the concentrations, a concentration-dependent response was obvious where increasing concentration showed a decline in consumption of the treated substrate. Fipronil was not deterrent to feeding by *H. indicola* at a concentration range of 1–20 ppm. It is reportedly a non-repellent termiticide. Remmen and Su (2005b) reported that sand treated with fipronil did not repel *C. formosanus* and *R. flavipes* at concentrations as high as 64 ppm. Our studies further indicated that the termites would even feed on substrate treated at a concentration range of 1–20 ppm so that fipronil impregnated in a bait matrix can be used as a slow-acting toxicant.

Based on mortality data in the deterrence test, there was an evident concentration-dependent mortality effect of fipronil, indicating that despite the deterrence effect of fipronil at concentration >20 ppm, termites did not completely avoid feeding on the medium treated with higher concentrations of fipronil, thus leading to a higher mortality rate. Mortality data recorded after 2 wk showed almost complete mortality in all the concentrations, except at 1 ppm, in which 25% mortality was observed.

Table 1. Estimated lethal time (d) required for 50% and 90% mortality (ELT 50, ELT 90 ± 95% FL) of *Heterotermes indicola* after exposure to varying concentrations of fipronil.

Dose (ppm)	ELT 50 Estimate (d)	95% CI	ELT 90 Estimate (d)	95% CI	Regression Equation
1	7.5	7.02, 7.9	11.6	11.04, 12.3	ELT 50 = −2.3+28 × dose
5	1.84	0.021, 3.06	5.2	4.2, 6.1	ELT 50 = −0.6+18 × dose
10	1.41	0.42, 2.24	2.8	1.9, 3.7	ELT 50 = −1.2+0.89 × dose
20	1.2	ND, 3.02	3.7	ND, 5.2	ELT 50 = −0.28 × dose
30	1.8	ND, ND	3.0	ND, ND	ELT 50 = −1.8+1.03 ×dose
50	1.01	ND, ND	1.8	ND, ND	ELT 50 = −1.5+1.5 ×dose

CI – Confidence interval
ELT 50 – effective lethal time to kill 50% of treated termites
ELT 90 – effective lethal time to kill 90% of treated termites
ND – not determined

Table 2. *Heterotermes indicola* consumption of untreated blotting paper and paper treated with different concentrations of fipronil after 2 wk.

Dose (ppm)		Consumption (mg) Mean ± SE	t statistics (p value)	Living Termites after 2 wk (out of 200)*
1	Control	5.75± 1.83	0.692 (0.539)	51±5.1 a
	Treated	4.27± 1.46		
5	Control	9.93±2.80	2.707 (0.073)	0.5 ± 0.25 b
	Treated	6.83±1.67		
10	Control	7.20± 1.06	0.698 (0.535)	0.5 ± 0.25 b
	Treated	5.07±2.1		
20	Control	10.30±1.85	2.578 (0.082)	0 b
	Treated	4.35±1.21		
30	Control	9.76±0.14	4.842 (0.017)	0 b
	Treated	6.50±0.81		
50	Control	6.88±0.30	5.178 (0.014)	0 b
	Treated	3.84±0.88		

*Means followed by the same letter are not significantly different at p≤0.05 using Tukey's HSD test.

Table 3. Mean cumulative mortality of donors and recipients of *Heterotermes indicola* in groups of 50 workers (donor: recipient ratio of 1:1) 8 d after mixing the donors treated with different concentrations of fipronil.

Dose (ppm)	Donor Mortality ± SE (%)	Recipient Mortality ± SE (%)	Dead Donor Missing* (%)
1	52 ± 4.0 a	30.9 ± 10.0 a	45.3 ± 4.6 b
5	81.0 ± 5.0 b	69 ± 9.9 b	6.08 ± 0.9 a
10	74.6 ± 13.3 ab	61.3 ± 71.3 ab	20.71± 0 a
20	89.3 ± 4.8 b	69.3 ±1.3 b	19.9 ± 5.1 a
30	87.0 ± 4.1 b	71.0 ±4.4 b	6.04 ± 3.1 a
50	100 b	58 ± 6.2 ab	1.54 ± 1.9 a

*Means followed by the same letter are not significantly different at p≤0.05 using Tukey's HSD test.

At 1 ppm, the force feeding test indicated complete mortality of termite workers after 12 d while a low mortality rate was observed in the choice test. A possible explanation may be the learnt behavior of avoidance after getting a sublethal exposure to the chemical (Su et al. 1995).

Transfer Studies

Studies on the transfer of fipronil in the confined arena showed that concentrations greater than 1 ppm were able to inflict more than 50% mortality in untreated workers interacting with their treated counterparts. Shelton and Grace (2003) also found lethal transfer of fipronil to be dose-dependent. They found that *C. formosanus* suffered a significant increase in recipient mortality over control mortality when donor workers were treated with 100 ppm fipronil, while exposure of donors to 1 ppm insecticide did not consistently lead to lethal transfer of the insecticides. Bagneres et al. (2009) noted that transfer from exposed donors to unexposed recipients occurred within 24 h. Donors transferred approximately 46% of the toxicant to recipients. Transfer of toxicants was attributed to social behaviors such as contact and grooming.

During the course of recording data, we observed head capsules without bodies, an indication of the process of necrophagy (or cannibalism) in the termites in which only the head (a much sclerotized part of the body) was left behind. In order to estimate the number of dead bodies that were eaten out, we recorded data on both living and dead donor termites in each treatment unit. When the sum of both was less than 25 (original number of donors), the remainder was assumed to have been consumed by their fellow workers and was reported as percentage of dead donors. We compared the number of missing dead bodies for various doses and found a higher number of dead donors missing in 1 ppm (45%) compared with a very low proportion of dead donors missing in 50 ppm (1.5%). This result may have been due to avoidance of recipients of the donors treated with high concentrations of fipronil.

Ideal slow-acting bait is assumed to be the one that has delayed mode of action, has horizontal transfer capability at effective concentrations and is dose-independent. Our studies showed that fipronil is not a typical slow-acting toxicant because at effective concentrations (>1 ppm), the potential of its transfer via trophallaxis is limited due to rapid mortality of the exposed termites. But since the living termite workers did not avoid contact with the dead workers intoxicated with fipronil and would rather consume their dead fellows, another opportunity for toxicant transfer was created via contact and cannibalism. This condition may have contributed to the spread of the chemical within the colony (even at shorter distances). Hu et al. (2006) also noted that dying termites (intoxicated with fipronil) seemed to attract active colony mates to provide them with intensive grooming and care and were more likely to be cannibalized by active workers.

Huge quantities of imidacloprid or fipronil are applied to protect agricultural fields and orchards against termites through contact toxicity. However, spot application of fipronil has the potential to replace general soil application of termiticides.

Termites can be attracted in large numbers to a trap (similar to the one we used for termite collection) to establish multiple foraging points. We were able to collect as many as 50,000 termite workers within 2 wk (in an agricultural field, presence of poplar slices would be more attractive for termites than living plant material). If such multiple foraging points are established 10–15 m apart and then replaced with a substrate treated with 5–10 ppm of fipronil, termites would readily feed on this bait, leading to the mortality of the ones coming in contact with the chemical and those feeding on this substrate. This mortality will be supplemented by that of the unexposed termites coming in contact with intoxicated workers while providing grooming care to the dying termites or feeding on the dead nest mates. This situation could lead to suppression, if not elimination, of termite populations and thus reduce crop damage. This technique would need far lesser amounts of the chemical and reduce non-target effects of the chemical, making it a more economical and environmentally safer approach for suppressing termite populations than flooding the soil with the termiticide. Apart from very low amounts of fipronil needed to kill termite populations, another desirable feature of this toxicant is its stability in the soil. It is relatively immobile in soil and has low potential to leach into groundwater. Fipronil degrades slowly on vegetation and relatively slowly in soil and in water, with a half-life ranging between 36 h and 7.3 mo depending on substrate and conditions (Tingle et al. 2003), making it a good candidate for use in agriculture.

Our field observations support this approach where 1–5 ppm of fipronil-treated paper, when used as bait in all the foraging points, led to consumption of the bait followed by suppression of the population of *Odontotermes* sp. in a lawn. Huang et al. (2006) also showed that approximately 3–5 mg of fipronil could suppress foraging populations of *Odontotermes formosanus* containing 0.4–0.7 million foragers per colony. Field studies are needed to compare the efficacy of general soil application with the proposed spot treatment of fipronil to reduce the damage of subterranean termites in agricultural fields.

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CONCLUSION

Fipronil was able to inflict significant mortality in *H. indicola* (though with a relatively faster rate of kill) at a concentration of 5–20 ppm, along with its capability of horizontal transfer. Within a concentration range of 1–20 ppm, fipronil did not deter feeding by *H. indicola* when incorporated in a blotting paper matrix. Fipronil can be tested for spot application in agricultural fields for suppressing populations of subterranean termites.

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