Tunneling Responses of the Asian Subterranean Termite, *Coptotermes gestroi* in Termiticide-Treated Sand (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Six termiticide formulations (bifenthrin 24% EC, chlorfenapyr 24% SC, chlorantraniliprole 18.5% SC, fipronil 2.5% EC, imidacloprid 20% SC and indoxacarb 14.5% SC) were evaluated against the Asian subterranean termite *Coptotermes gestroi* (Wasmann) in the laboratory using glass tube and petri-dish methods. Four concentrations (1, 10, 50 and 100 ppm w/w) were evaluated in the glass tube method, while only the manufacturer's recommended concentration for each termiticide was tested in the petri dish method. Results showed that with the exception of bifenthrin, all termiticides demonstrated non-repellent properties. All termiticide formulations (at concentrations > 10 ppm) were able to stop termite soil penetration, either by killing or repelling them. Termite mortalities caused by the evaluated termiticides were concentration-dependent. Termiticides at 1 ppm were unable to prevent termite tunneling activity, regardless of repellent or non-repellent properties, while termiticides at higher concentrations (eg. 100 ppm) negated or minimized tunneling activity. We suggest that termiticide properties (repellent or non-repellent) may be dependent on the concentrations of the termiticides used.

Key Words: *Coptotermes gestroi*, termiticides, non-repellent, repellent, tunneling, concentration-dependent.

INTRODUCTION

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann) is an economically important subterranean termite species in South East Asia, where it contributes between 63 - >90% of the total termite damage in buildings

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and structures in Peninsular Malaysia, Singapore and Thailand (Lee 2002; Lee *et al.* 2003; Kirton & Azmi 2005; Lee *et al.* 2007). This species has also spread from its origin in the Orient to the Turks and Caicos Island in the Caribbean (Scheffrahn *et al.* 1990), Florida, USA (Su *et al.* 1997a) and Brazil (Milano & Fontes 2002).

Management of subterranean termites has relied heavily on chemical methods such as soil treatment, baiting and dusting (Lee *et al.* 2003). Soil termiticide treatment creates a chemical barrier for exclusion of subterranean termites from buildings and structures (Bläske *et al.* 2003), and has been a popular mode of termite control over the past 50 years (Su *et al.* 1997b; Miller 2001; Ibrahim *et al.* 2003; Jones 2003). In the past, repellent termiticides have been the principal approach to exclude termites from structures. Control of subterranean termites was dominated by the usage of chlordane in the past (Appel 2003) until it was banned in many countries between 1980 and 2000 due to environmental and health-related concerns. Pyrethroids then became key candidates for repellent termiticides.

However, over the last several years, there has been increasing popularity in the use of non-repellent termiticides. This characteristic is crucial because termites will not be able to detect the presence of the treated termiticide in soil, and thus will continue to forage through the soil (Shelton & Grace 2003). These compounds have a delayed mode of action (Hu *et al.* 2005; Su 2005). At suitable concentrations, termites can forage without realizing the presence of the chemical, contactng ample amounts of toxicant on their bodies, enabling transfer of lethal doses to happen (Shelton & Grace 2003). In this study, we examine the laboratory performance of several repellent and non-repellent termiticides at different concentrations against the Asian Subterranean termite *C. gestroi* using two evaluation methods, namely the glass tube, and the petri-dish methods.

MATERIALS AND METHODS

Insects

The Asian subterranean termites (*C. gestroi)* were collected from underground monitoring stations established earlier at the Universiti Sains Malaysia, Minden campus. Termites were brought back to the laboratory and separated from soil debris using the method described by Tamashiro *et al.* (1973). Only termites from the same colony were used in this study.

Insecticides

Six termiticide formulations, i.e. bifenthrin 24% SC [FMC, USA], chlorfenapyr 24% SC [WellTech Healthcare Co. Ltd, Thailand], chlorantraniliprole 18.5% SC [DuPont Professional Products, USA], fipronil 2.5% EC [Bayer Environmental Science, Malaysia], imidacloprid 20% SC [Bayer Environmental Science, Malaysia] and indoxacarb 14.5% SC [DuPont Professional Products, USA] were evaluated in this study.

Modified glass tube method

The glass tube method is a modified version of the one described by Su & Scheffrahn (1990) (Fig. 1). It consists of a 30-cm glass tube (1.4 cm diam.) that contains a 21-cm long moistened sand layer and a 2-cm termiticide-treated sand layer sandwiched between 2-cm sawdust and 2-cm 10% agar. Two cut pieces of moistened 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. Two hundred 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 ± 0.2 °C, 56.3 ± 0.7 % RH). The cumulative tunneling distance was measured daily up to 7-d post-treatment. After this period, the setup was disassembled and the number of surviving insects was counted. Four different concentrations [1, 10, 50 and 100 ppm (w/w)] were tested and each concentration was replicated 3 times.

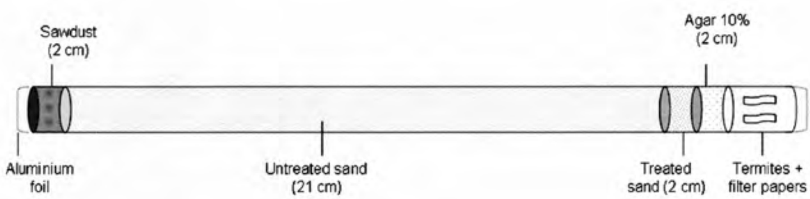

Fig. 1. Glass tube method used in this study

Petri dish method

This method used a polyethylene petri dish (16 cm diam. x 6 cm height) separated into 2 sections by a piece of glass (Fig. 2). One section contained 140 g of untreated sand, while the other contained an equal amount of sand that was treated with the termiticide. Recommended concentrations were obtained by mixing the equivalent amount of diluted termiticide formulation and sand at the ratio of 1 : 20 (v/w). Two pieces of oven-dried, pre-weighted rubber wood, *Hevea brasiliensis* (Wild. ex A. Juss.) Muell. Arg. measuring 2 x 1 x 1 cm were placed in each section to serve as food. Four hundred workers and 20 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-wk. Each treatment was replicated 5 times. All bioassays were held in a dark chamber (25.2 ± 0.2 °C, 56.3 ± 0.7 % RH). Tunnel formations were scanned using a flat scanner (Canon CanoScan LiDE20, Canon Inc., China) and tunneling activities in both treated and untreated sections were qualitatively scored with 0 for no tunneling activity, 1 for tunneling activities covering ≤ 25 % of the total arena, 2 for tunneling activities covering 26 – 50 % of the total arena, 3 for tunneling

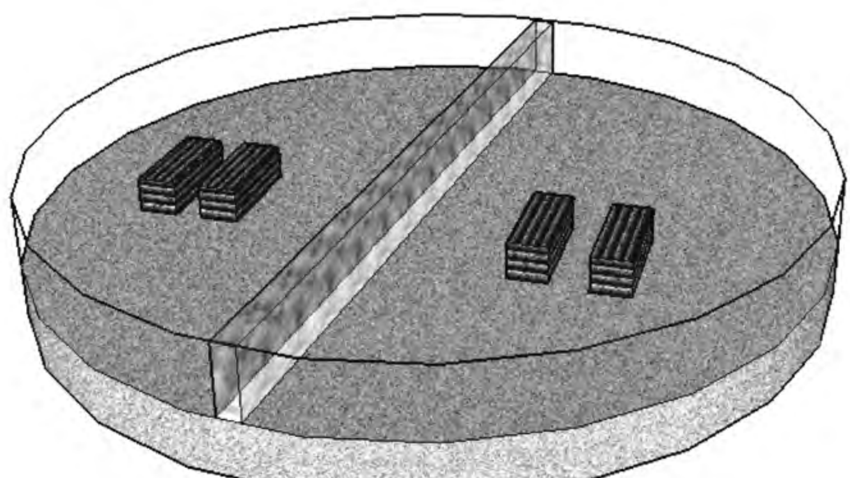

Fig. 2. Petri dish method used in this study

activities covering 51 – 75 % of the total arena, and 4 for tunneling activities covering ≥ 75 % of the total arena.

Data analysis

Data in percentages were subjected to arcsine transformation before analysis of variance (ANOVA). Means were separated with Tukey HSD. Non-parametric data were analyzed with Krustal-Wallis (KW) analysis of variance and means were separated with KW multiple range test (Siegel & Castellan 1988). All analyses were performed using Statistix® Version 7.0 (Analytical Software, Tallahassee, Florida).

RESULTS AND DISCUSSION

Modified glass tube method

Results indicated that all termiticide formulations evaluated (with the exception of bifenthrin) showed non-repellent characteristics (Table 1). Termites readily tunneled through chlorfenapyr, chlorantraniliprole, fipronil or indoxacarb-treated layers at all concentrations tested resulting in considerable termite mortality (~ 70 – 100 %) after 7 days. In addition, chlorfenapyr, chlorantraniliprole and indoxacarb showed a delayed mode of action, as evidenced by long tunneling distances (50 -100 % of total distance tunneled).

Chlorantraniliprole caused only approximately 40 % mortality at 10 ppm even though the termites had reached the maximum tunneling distance, indicating the slow-acting properties of this compound. If the experiment would have been held for longer than one week, it is believed that the delayed effects of chlorantraniliprole (or other non-repellent termiticides) may have increased the mortality of the test insects. Newer technologies and many non-repellent termiticides are slow acting and require longer amounts of time to express their lethal effects (Su *et al.* 1987).

At higher concentrations, most termites were killed even with slight contact (100% mortality was recorded at 100 ppm with very minimal penetration). Both fipronil and chlorfenapyr showed similar efficacy against the tested insects. However, at lower concentrations (eg. 10 and 50 ppm), termites tunneled twice as long in the chlorfenapyr treatment than in the fipronil treatment, suggesting that fipronil was a faster killing agent than chlorfenapyr. For most fipronil concentrations evaluated (with the exception of 1 ppm), termites

Table 1. Performance of termiticide formulations evaluated using the glass tube method.

Termiticide	Conc. (ppm) (w/w)	Evaluated parameters[1] % cumulative tunneling distance	Termite survivorship (%)
Control	(water)	100.0 ± 0.0a	86.5 ± 2.3abc
bifenthrin	1	100.0 ± 0.0a	51.7 ± 22.9a-e
	10	1.6 ± 0.5d	62.7 ± 17.6a-d
	50	5.6 ± 2.2d	14.5 ± 14.5def
	100	0.5 ± 0.3d	0.0 ± 0.0f
chlorfenapyr	1	100.0 ± 0.0a	51.5 ± 22.8a-e
	10	51.9 ± 12.5a-d	0.0 ± 0.0f
	50	48.7 ± 22.1a-d	0.0 ± 0.0f
	100	29.8 ± 0.93bcd	0.0 ± 0.0f
chlorantraniliprole	1	100.0 ± 0.0a	73.7 ± 0.6abc
	10	100.0 ± 0.0a	59.5 ± 2.3a-e
	50	20.9 ± 9.1cd	9.2 ± 9.2ef
	100	11.9 ± 1.0cd	0.0 ± 0.0f
fipronil	1	87.7 ± 1.1ab	0.0 ± 0.0f
	10	20.4 ± 8.5cd	0.0 ± 0.0f
	50	20.5 ± 1.9cd	0.0 ± 0.0f
	100	19.2 ± 0.8cd	0.0 ± 0.0f
imidacloprid	1	69.3 ± 25.7abc	87.8 ± 1.9ab
	10	35.9 ± 30.2bcd	81.0 ± 2.0abc
	50	0.3 ± 0.3d	50.7 ± 13.0a-e
	100	0.0 ± 0.0d	23.0 ± 11.6c-f
indoxacarb	1	100.0 ± 0.0a	93.7 ± 0.7a
	10	68.8 ± 20.0abc	31.2 ± 27.4b-f
	50	38.3 ± 14.7bcd	0.0 ± 0.0f
	100	2.0 ± 1.06d	0.0 ± 0.0f

[1] Means followed by the different letters within the same column and insecticide are significantly different (Tukey HSD; $P < 0.05$).

were killed before they could tunnel longer than several cm. It was relatively fast-acting especially at higher concentrations. Remmen & Su (2005a) had found that 2 ppm fipronil was sufficient to fully stop *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* Kollar from penetrating a treated barrier layer. Generally, at higher concentrations, most formulations showed a faster killing action, as evident from the shorter tunneling distance of termites and the higher termite mortality. There was minimal mortality of termites in the control replicates and the maximum tunneling distance was achieved within 48 hours post-treatment.

In this study, it was observed that repellent or non-repellent properties were dependent on concentrations used. As observed with the repellent termiticide bifenthrin at the lowest concentration (1 ppm), the test insects completed the maximum tunneling distance with approximately 50 % mortality. This implied that the termites were not repelled by the termiticide at this concentration. Su & Scheffrahn (1990) reported that the threshold concentration, the minimum concentration to totally prevent termite tunneling for bifenthrin, was 6 ppm for *C. formosanus* and *R. flavipes*. Conversely, imidacloprid, a non-repellent termiticide, completely negated tunneling activity at its highest concentration (100 ppm). Su (2005) reported that a non-repellent insecticide at higher concentrations was similar to fast-acting insecticides, in which the barrier efficacy was caused by secondary repellency (Su *et al.* 1982). Su & Scheffrahn (1990) also found the same phenomenon for non-pyrethroids. At lower concentrations (1 and 10 ppm), termites in imidacloprid did not fully penetrate the treatment, with relatively high numbers of survivors remaining at the end of the experiment. This was possibly caused by the latent effect of the compound at low concentrations. Remmen & Su (2005b) also observed similar phenomena with *R. flavipes* in thiamethoxam at 8 ppm.

Petri dish method

Evaluation using the petri dish method confirmed the characteristics of the compounds evaluated in the earlier experiment. Termites showed extensive tunneling activity in the control sets in both treated and untreated sections (Fig. 3a). They also showed significantly ($P < 0.05$) higher wood consumption in both treated and untreated sections (0.2016 ± 0.0323 g and 0.2400 ± 0.0325 g, respectively; Table 2). In chlorfenapyr replicates, termites were found to forage freely between the two sections (treated and untreated; Fig. 3b) and complete mortality of termites was recorded at the end of the test period (Table 2). Despite the foraging activity of termites in the treated zone, the amount of wood consumption was still relatively low (0.0458 ± 0.0072 g). This compund was found to be an excellent non-repellent termiticide candidate at the recommended concentration. Termites in chlorantraniliprole, imidacloprid and indoxacarb treatments showed moderate tunneling activities in treated zones with slight wood damages. These compounds had also demonstrated good non-repellent insecticide properties. Fipronil, however,

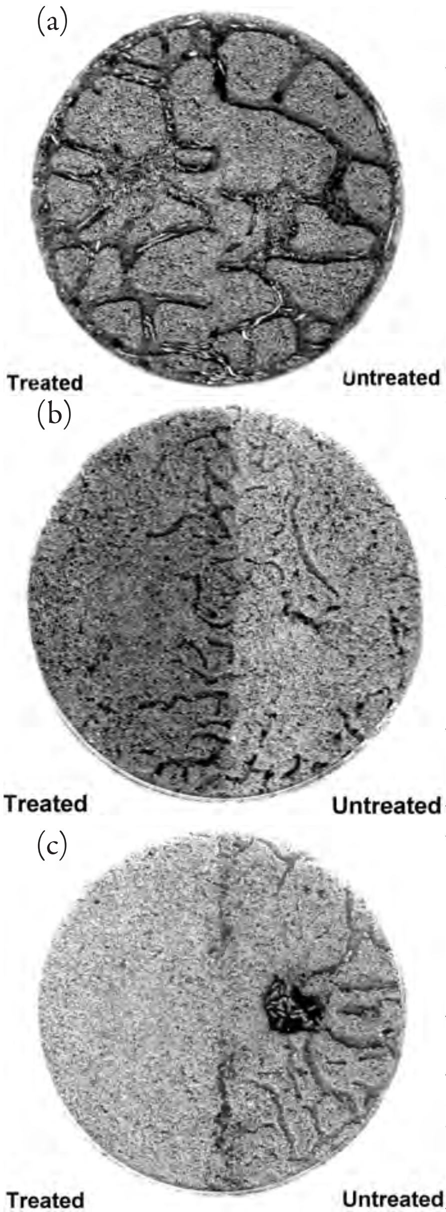

Fig. 3. Tunneling activity of *C. gestroi* in petri dishes containing untreated and treated sands: (a) control (water), (b) chlorfenapyr treated, and (c) bifenthrin treated.

showed a quicker killing action with limited tunneling activities of termites in treated zones.

Bifenthrin showed repellent activity at 30 ppm (w/w) with high termite tunneling activity in untreated section and no activity in the treated zone (Fig. 3c). Termites were killed in bifenthrin treatments, and this suggested the movement of the chemical from treated into untreated sections. Su & Scheffrahn (1990) reported the movement of a pyrethroid (tralomethrin) from treated sand to the agar layer in their experiment against *R. flavipes,* causing high mortalities even though the termites did not reach the treated area. Many other researchers also reported the role of vapor phase in their experiments. Ebeling & Pence (1958) noticed that the vapor phase of chlorinated hydrocarbons had penetrated into areas beyond the treated soil and was killing the termites. Isoborneol has also shown a high rate of evaporation in sand (Bläske *et al.* 2003). Although bifenthrin has low volatility when applied on dry soil, it possesses a higher migration potential in wetter conditions (Fecko 1999). The movement of the termiticide (and emulsifier) was probably facilitated by water solubility (Smith & Rust 1990; 1991) and vapor pressure (Su *et al.* 1982; Smith & Rust 1990; 1991). When the vapor pressure is high enough, some of

Table 2. Performance of termiticide formulations evaluated using petri dish method.

Termiticide	Conc. (ppm w/w)	Untreated section: Termite activities[1] (mean ± SE)	Untreated section: Wood consumption[2] (mean ± SE)	Treated section: Termite activities[1] (mean ± SE)	Treated section: Wood consumption[2] (mean ± SE)	Termite survivorship[2] (%)
Control (water)	-	4.0 ± 0.0a	0.2400 ± 0.0325a	4.0 ± 0.0a	0.2016 ± 0.0323a	90.0 ± 0.5a
bifenthrin	30	3.2 ± 0.6ab	0.0838 ± 0.0147b	0.0 ± 0.0b	0.0458 ± 0.0072b	3.6 ± 1.4b
chlorfenapyr	30	2.6 ± 0.4ab	0.0380 ± 0.0073b	1.8 ± 0.4ab	0.0608 ± 0.0075b	0.0 ± 0.0b
chlorantraniliprole	100	2.2 ± 0.6ab	0.0710 ± 0.0173b	1.6 ± 0.2ab	0.0435 ± 0.0063b	0.6 ± 0.3b
fipronil	11	4.0 ± 0.0a	0.0565 ± 0.0036b	1.0 ± 0.0ab	0.0410 ± 0.0078b	0.0 ± 0.0b
imidacloprid	25	1.4 ± 0.2b	0.0960 ± 0.0104b	1.0 ± 0.0ab	0.0278 ± 0.0112b	3.9 ± 1.1b
indoxacarb	100	1.6 ± 0.2a	0.0772 ± 0.0092b	1.4 ± 0.2ab	0.0476 ± 0.0125b	0.0 ± 0.0b

[1] Intensity of tunnels formed: 0 = no tunneling activity; 1 = tunneling activities covering ≤ 25 % of total arena; 2 = tunneling activities covering 26 – 50 % of total arena; 3 = tunneling activities covering 51 – 75 % of total arena; 4 = tunneling activities covering ≥ 75 % of total arena. Means followed by the different letters within the same column are significantly different (Kruskal-Wallis multiple Range Test; $P < 0.05$).
[2] Means followed by different letters within the same column are significantly different (Tukey HSD; $P < 0.05$).

the termites might have satisfactory amounts of toxicant deposited on the cuticle. Penetration of the toxicant through the cuticle will slowly affect the termites without direct termiticide contact (Su *et al.* 1982; Smith & Rust 1991). This may explain the death of the termites in the untreated section.

To achieve greater colony suppression or even elimination, the termiticide used is required to be slow-acting and non-repellent. This is because termites demonstrate necrophobic behavior, where quick killing of the poisoned termites may give rise to abandonment or sealing of tunnels that lead to the treated zone by healthy colony members (Su *et al.* 1982; Su 2005). Once decomposed carcasses accumulate near and in treated zones, the healthy termites will associate them with the treated zone and avoid entering it, thus surviving the treatment. Termiticides also need to be applied at suitable concentrations (Su 2005) so that termites are able to return to their nest for transmission of lethal doses before it causes any effects to the contaminated individuals.

In this study, two methods were used to evaluate the termiticide performance. The modified glass tube method provides a reliable quantitative method that permits measurement of tunneling distance of test insects in the presence of a chemical barrier (layer). The longer untreated section in this method provided a longer tunneling distance from the initial treatment section, and we believe that this will enable a better demonstration of termite response after termiticide exposure. Alternatively, the petri dish method provides a simple qualitative approach to study the termites' tunneling response towards chemically treated areas while creating the vapor phase that may occur in field applications (especially underslab soil termiticide application). This method also enables accurate determination of the wood damage in both treated and untreated zones. It is believed that the combination of all parameters from the two methods will provide a relatively reliable interpretation of the actual performance of termiticide formulations. Finally, we also found that repellent or non-repellent characteristics of termiticides were dependent on the concentrations of termiticides used. However, more extensive studies are required before the current findings can be further substantiated.

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