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Original Article

Toxicity of Acetamiprid to Workers of *Reticulitermes flaviceps* (Isoptera: Rhinotermitidae), *Coptotermes formosanus* (Isoptera: Rhinotermitidae) and *Odontotermes formosanus* (Isoptera: Termitidae)

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The toxicity of acetamiprid was tested on *Reticulitermes flaviceps* (Oshima), *Coptotermes formosanus* Shiraki and *Odontotermes formosanus* (Shiraki), important pests in buildings, dams and trees in China. The LD_{50} value of acetamiprid among worker termites of the three species was 4.41×10^{-5}, 13.8×10^{-5} and 146×10^{-5} μg/termite, respectively, at 72 hr post-treatment. More than 90% of individuals tested died within 120 hr of coming into contact with sandy soil containing 4.8 ppm of acetamiprid. A 10-cm thick barrier of loam treated with 8.0 ppm of acetamiprid retarded completely the penetration by *C. formosanus* and *O. formosanus*.

Keywords: acetamiprid, toxicity, *Reticulitermes flaviceps*, *Coptotermes formosanus, Odontotermes formosanus*.

INTRODUCTION

Subterranean termites cause considerable economic damage. A common measure of control is to establish an insecticide-treated barrier of soil around the structure to be protected.[1] Insecticides for treating soil include chlordane, chlorpyrifos, permethrin, cypermethrin, bifenthrin, imidacloprid, cyfluthrin, and fipronil, but chlordane and chlorpyrifos have been banned in the United States because of public health concerns.[2–3] In China, due to a lack of feasible economical alternatives, chlordane is still used extensively for subterranean termite control in some areas. To eliminate the use of chlordane in termite control as soon as possible, there is an urgent need to find highly effective and environmentally sound alternatives.

Acetamiprid, invented by Nippon Soda Co., Ltd., affects the acetylcholine receptor of the insect central nervous system and shows excellent efficacy against several agricultural pests.[4–6] It thus has become an important component of integrated pest management since it was registered commercially in 1995. However, little information has been available on the efficacy of acetamiprid against termites except for a few reports from our laboratory.[7–10] To explore the potency of acetamiprid for termite control, its toxicity in three of the most important species of termites in China was evaluated in the laboratory.

MATERIALS AND METHODS

1. Termites and Insecticides

Reticulitermes flaviceps (Oshima) (Isoptera: Rhinotermitidae), *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) and *Odontotermes formosanus* (Shiraki) (Isoptera: Termitidae) are subterranean termites of great economic importance in China. *R. flaviceps* and *C. formosanus* damage mainly buildings, trees and turf in urban ecosystem.[11] *O. formosanus* damages mainly dams, dykes, and trees in rural ecosystem.[11] Adult workers of these three species were collected by trapping from the Jade-Emperor Mountain in Hangzhou City within one week of the tests and were maintained in a rearing room at 26±1°C and 65±5%RH.

Acetamiprid (purity≧99.99%) and its commercial formulation (Mospilan® 200 g kg^{-1} SP), both provided by the Nippon Soda Co., Ltd. (Tokyo, Japan), were used for this study.

2. Susceptibility of Worker Termites to Acetamiprid

Acetamiprid was diluted with acetone into serial concentrations. An aliquot of 0.5 μl of acetamiprid solution or acetone was topically applied to the ventral backside of each termite worker. Sixty healthy adult workers were treated for each concentration. The treated workers were introduced into Petri dishes (ϕ9.0 cm) paved with wet sandy soil, and provided

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with a piece of wet filter paper (2 cm×2 cm) as food. All Petri dishes were moved into a dark incubator maintained at 26±1°C and 65±5%RH. Mortality was recorded every 24 hr. Termites that did not respond to prodding with a pencil tip were considered dead. The data obtained were subjected to a probit analysis,[12] using a personal computer.

3. Response of Worker Termites to Toxic Sandy Soil

Ten grams of sifted sandy soil (ϕ<0.28 mm) dried for 1 day at 40°C before the test was paved on the bottom of a Petri dish (ϕ9.0 cm). The soil was evenly wetted with 3 ml of distilled water or acetamiprid solution diluted from 20% acetamiprid SP. The concentrations of acetamiprid in sandy soil were 0.0384, 0.192, 0.96, 4.8, 24, and 120 ppm (weight of active ingredient/weight of sandy soil), respectively. Ten replicates were made for every treatment. Thirty healthy adult workers were introduced into each Petri dish. Other steps were the same as for the determination of susceptibility.

4. Anti-Tunneling Effect of Acetamiprid on Worker Termites

Because relatively few *O. formosanus* workers survive more than 10 days in a small container, only *R. flaviceps* and *C. formosanus* were used in this test. Loam (ϕ<0.90 mm) with or without acetamiprid was introduced into a glass pipe 3.0 cm in diameter and 15.0 cm in length and a 10.0 cm pillar of soil pillar was formed in the middle of the pipe. Then a wood block (2.0 cm×2.0 cm×1.0 cm) was placed on one side of the soil pillar as food to attract the termites. Five milliliters of 5% agar was put on the other side of the pillar, and 100 healthy adult workers and one piece of wet filter paper (2 cm^2) were introduced after the agar had cooled.[9] Both ends of the glass pipe were connected to one plastic cup (5.0 cm in diameter and 8.0 cm in height) filled 5.0 cm high with wet loam. To prevent the water from evaporating and termites from escaping, the plastic cups were sealed with two layers of plastic membrane with five pinholes. The concentrations of acetamiprid in the loam were 0.50, 1.0, 2.0, 4.0, and 8.0 ppm (weight of active ingredient/weight of soil), respectively. There were 5 replicates for every treatment. The glass pipes with plastic cups were put in the dark incubator (26±1°C and 65±5%RH) during the test. The length from the top of the tunnel to the surface of the soil pillar was determined and the activity of the termites was observed every 24 hr. For the results obtained, an analysis of variance (ANOVA)[13] was used to assess the significance of the main effects. The significance of the F-statistic was tested at the α=0.05 level.

RESULTS

1. Susceptibility of Worker Termites to Acetamiprid

After 2–3 hr exposure to acetamiprid, worker termites showed symptoms of poisoning such as a staggering gait, body trembling, immobility, tumbling, and laying on their backs with occasional twitching of the legs and antennae. Eight hours later, most of the termites treated topically with the 100 ppm acetamiprid acetone solution were knocked down, while those termites treated with the 0.8, 4.0 and 20.0 ppm acetamiprid acetone solutions were mostly immobile and moved just a few steps if touched lightly with the top of flat tip forceps. After 24 hr, the abdomen of some dead termites was shrunken and wizened.

Table 1. Susceptibility of workers of three species of termites to acetamiprid

Species	Period of treatment (hr)	LD_{50} (95% Confidence interval) ($\times 10^{-5}$ μg/termite)
R. flaviceps	24	277.7 (174.1–463.7)
	48	5.42 (2.13–15.37)
	72	4.41 (1.39–8.52)
	96	4.35 (4.12–5.33)
	120	4.03 (3.41–5.27)
C. formosanus	24	119 (78.1–187.8)
	48	31.3 (20.2–51.0)
	72	13.8 (10.1–18.9)
	96	8.09 (5.58–11.34)
	120	5.80 (4.23–7.76)
O. formosanus	24	1157(456–1927)
	48	269(168–399)
	72	146 (101–206)
	96	102 (77.6–138)
	120	112 (85.9–155)

The workers of *R. flaviceps*, *C. formosanus* and *O. formosanus* were highly susceptible to acetamiprid. Of which, the *R. flaviceps* was most susceptible. The LD_{50} value was 4.03×10^{-5} μg/termite for *R. flaviceps*, 5.80×10^{-5} μg/termite for *C. formosanus* and 112×10^{-5} μg/termite for *O. formosanus* at 120 hr post-treatment: the LD_{50} of *O. formosanus* was 27.8 times that of *R. flaviceps* and 19.3 times that of *C. formosanus*. Meanwhile, the LD_{50} values for the three species tended to stabilize 72 hr after the topical treatment because the 95% confidence intervals partly overlapped from 72 to 120 hr (Table 1).

2. Toxicity of Acetamiprid-Treated Sandy Soil

When the water-based solution of 20% acetamiprid SP was applied to the soil, acetamiprid was highly toxic to the three species of termites tested. After 24 hr exposure to the sandy soil containing 120 ppm of acetamiprid, there was 100% mortality among the workers of *C. formosanus* and *O. formosanus* and >90% mortality among the workers of *R. flaviceps*. After 120 hr, 100% of the workers of *R. flaviceps* and *C. formosanus* and >90% of the workers of *O. formosanus* died from the exposure to the sandy soil with 4.8 ppm of ac-

Table 2. Toxicity of acetamiprid-treated sandy soil among workers of three species of termites

Species	Period of treatment (hr)	LC_{50} (95% Confidence interval) (ppm)
R. flaviceps	24	26.5 (17.1–44.4)
	48	7.58 (5.20–11.33)
	72	1.32 (1.08–1.85)
	96	0.877 (0.682–1.170)
	120	0.675 (0.517–0.903)
C. formosanus	24	3.87 (3.18–4.71)
	48	3.30 (2.73–3.99)
	72	2.12 (1.82–2.47)
	96	1.35 (1.16–1.56)
	120	1.05 (0.90–1.23)
O. formosanus	24	3.23 (2.13–4.86)
	48	0.795 (0.450–1.423)
	72	0.449 (0.255–0.797)
	96	0.159 (0.085–0.308)
	120	0.067 (0.038–0.128)

etamiprid.

The termites exposed to the acetamiprid-treated sandy soil showed similar responses to those treated topically. The LC_{50} value for all three species decreased as the period of exposure increased. However, there were some differences in the susceptibility to acetamiprid between the soil-based treatment and the topical treatment. *O. formosanus* showed greater susceptibility to acetamiprid than *R. flaviceps* and *C. formosanus*. The LC_{50} value of the former was as low as 10.1–15.7 times that of the latter after 120 hr exposure (Table 2). Possible reasons for this phenomenon are (1) the workers of *O. formosanus* were more vivacious than those of *R. flaviceps* and *C. formosanus* in the Petri dish, the former moved ceaselessly over the sandy soil, and this behavior may have resulted in them receiving more acetamiprid than the latter; and (2) the acetamiprid may have more easily penetrated the body wall of *O. formosanus* than that of *R. flaviceps* or *C. formosanus* when formulated with 20% SP, but no data are available on this at present.

3. *Anti-Tunneling Effect of Acetamiprid on Worker Termites*

The data in Tables 3 and 4 indicated that *R. flaviceps* and *C. formosanus* would dig through 10 cm of the soil pillar without chemicals within 5 days, but could build tunnels just 3.5±2.5 cm and 2.0±0.2 cm long in the loam with 2.0 ppm of acetamiprid after 30 days, respectively. Notably, when the concentration of acetamiprid in the soil reached 8.0 ppm, 10 cm of toxic soil could retard completely the penetration by *R. flaviceps* and *C. formosanus*.

DISCUSSION

Although >470 species of termites have now been recorded in China, only about 10 species cause serious economic damage. *R. flaviceps*, *C. formosanus* and *O. formosanus* are most representative of the species of concern and distribute broadly in the south and southeast area of China.[11)]

Table 3. Tunnel length of *Reticulitermes flaviceps* in loam soil pillar treated with acetamiprid (mean±SE cm)

Period of treatment (days)	Concentration of acetamiprid in loam soil (ppm)					
	Control	0.5	1.0	2.0	4.0	8.0
5	10.0±0 a	2.5±1.2 bc	1.8±0.5 cd	0.5±0.5 de	0.1±0.2 de	0±0 e
17		10.0±0 a	8.0±1.8 a	2.5±1.3 b	3.0±0.8 b	1.3±0.5 b
30			9.0±1.7 a	3.5±2.5 b	3.2±1.1 b	2.1±1.6 b

* Means within each row followed by the same letter are not significantly different ($P<0.05$; ANOVA).

Table 4. Tunnel length of *Coptotermes formosanus* in loam soil pillar treated with acetamiprid (mean±SE cm)

Period of treatment (days)	Concentration of acetamiprid in loam soil (ppm)					
	Control	0.5	1.0	2.0	4.0	8.0
4	10.0±0.0 a	2.8±0.3 b	1.8±0.2 bc	1.5±0.5 bcd	1.3±1.1 cd	0.3±0.4 d
30		8.3±2.4 a	4.5±1.8 b	2.0±0.2 bc	1.3±1.0 bc	0.4±0.4 c

* Means within each row followed by the same letter are not significantly different ($P<0.05$; ANOVA).

The traditional method of controlling subterranean termites was to apply a liquid termiticide in the soil to form a chemical barrier around the structure in order to block all possible routes of entry. Any termites attempting to penetrate the treated soil were either killed or repelled.[14] Several chloronicotinyl insecticides such as imidacloprid and thiamethoxam have been studied extensively for the control of subterranean termites.[15,16]

Boucias *et al.*[17] reported that imidacloprid treatment caused workers of *R. flavipes* to become sluggish, inhibited grooming and tunneling, and eventually caused death. In our tests, workers of *R. flaviceps*, *C. formosanus* and *O. formosanus* treated with acetamiprid showed similar symptoms of poisoning. Meanwhile, these three species were very susceptible to acetamiprid and the LD_{50} values at 72 hr after topical treatment were all below 0.0015 μg/termite. *Reticulitermes dabieshanensis* is also sensitive to acetamiprid, the LD_{50} value at 24 hr after exposure being just 7.00×10^{-5} μg/termite.[7]

For three species of African termites, *Trinervitermes trinervius* Rambur, *Odontotermes smeathmani* Fuller, and *Amitermes evuncifer* Silvestri, thiamethoxam caused 100% mortality within 2–8 days at a concentration of 0.3 ppm. Meanwhile, it clearly reduced the tunneling activity of *T. trinervius* and *A. evuncifer*, but had little effect on the tunneling activity of *O. smeathmani*.[15] A 5.0-cm thick barrier of soil treated with imidacloprid at a concentration $\geq$10 ppm protected wooden sticks from attack by *R. flavipes* in laboratory tests.[17] Meanwhile, termites suffered 100% mortality when exposed to 100 ppm of imidacloprid in soil, but just >75% mortality at 10 ppm of imidacloprid.[18] Wei *et al.*[19] also reported that when the concentration of imidacloprid in soil was $\geq$150 ppm, exposed *C. formosanus* workers died within 7 days, and the distance they tunneled in the soil was just 0.2–1.5 cm. Our tests showed that 4.8 ppm of acetamiprid in sandy soil caused 100% mortality among the workers of *R. flaviceps* and *C. formosanus* and >90% mortality among the workers of *O. formosanus* after 120 hr, while 4.0 ppm of acetamiprid in loam prevented tunneling by *R. flaviceps* and *C. formosanus*. Teng *et al.*[7] reported that *R. dabieshanensis* workers exposed to filter-paper with 20 ppm of acetamiprid died after 144 hr. In soil treated with 4.0 ppm of acetamiprid, workers of *R. parvus* exhibited 100% mortality at 96 hr and when the concentration was 1.6 ppm or more, the workers could not pass through 8.5 cm of soil.[8] It is suggested that acetamiprid is more toxic than imidacloprid but less toxic than thiamethoxam among termites.

Imidacloprid, whose trade name is Premise, is used extensively to control termites in several countries.[19–22] Besides being directly lethal to termites, imidacloprid has sublethal effects on the behavior[23] and feeding[24] of termites. Meanwhile, imidacloprid could be transferred among individuals of *C. formosanus*.[25] The mechanism by which acetamiprid acts on insects is similar to that of imidacloprid.[26] Zhang *et al.*[10] reported that sublethal concentrations of acetamiprid had no effect on the cellulase activity of *R. flaviceps* workers. However, it was not clear if acetamiprid has sublethal effects on the behavior and feeding of termites. If acetamiprid could be used for termite control, undoubtedly, it would play a very important role in eliminating the use of chlordane in China. In order to promote its application, studies should be conducted that reflect field application procedures, determine efficacy in various types of soil and investigate the transfer of acetamiprid between individual termites.

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