



RESEARCH ARTICLE - TERMITES

Evaluation of formic acid toxicity to subterranean termite, *Reticulitermes chinensis* Snyder

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Abstract

This study examined formic acid contact and fumigation toxicity to *Reticulitermes chinensis* in the laboratory. In the contact toxicity bioassay, the LD₅₀ values ranged from 267.86 to 287.68 µg adult⁻¹ for workers, 279.09 µg adult⁻¹ for alates (male and female) and 223.08 µg adult⁻¹ for soldiers after 24 h, respectively. In the fumigation bioassays, the LC₅₀ values ranged from 0.84 to 1.08 µg ml⁻¹ for workers, 1.19 µg ml⁻¹ for alates (male and female) and 0.57 µg ml⁻¹ for soldiers after 24 h, respectively. At the concentration of 2.50 µg ml⁻¹, the KT₅₀ value of formic acid ranged from 25.38 to 34.75 min for workers, from 42.21 to 45.62 min for alates (male and female), from 32.18 to 36.37 min for soldiers. Although formic acid was significantly less toxic to subterranean termite than bifenthrin, but higher toxic to many other pest insects. The findings of this study provide important confirmation of formic acid with fumigation toxicity against termite. It may be worth investigating the use of formic acid for managing subterranean termite.

Introduction

Formic acid is the simplest organic acid, present in many organisms. It is found in most plant species, including *Polygonum hydropiper*, *Urtica dioica*, *Urtica urens* (Hutchens 1992, Chopra & Chopra 2006, Rahmatullah et al. 2010). In animal, formic acid is the major component of ants from the subfamily Formicinae. The produced formic acid concentration in such ant produced substances can reach up to 54% (Hölldobler & Wilson 1990). Formic acid also occurs in carabid beetles (Will et al. 2010, Rossini et al. 1997, Attygalle et al. 1992) and notodontid caterpillars (Weatherston et al. 1979, Attygalle et al. 1993).

Formic acid was reported to possess significant insecticidal activity. It has been used as a fumigant to control the varroa mite, *Varroa destructor* Anderson (Sharma et al. 1983, Underwood & Currie 2004, 2005, 2007, vanEngelsdorp et al. 2008, Calderone 2010, Giovenazzo & Dubreuil 2011), and the tracheal mite, *Acarapis woodi* Rennie (Hoppe et al. 1989, Wilson et al. 1993, Nelson et al. 1994), and red imported fire

ants, *Solenopsis invicta* Buren (Chen et al. 2012), and stored product insects, *Sitophilus oryzae* and *Rhyzopertha dominica* (Chaskopoulou 2007), and the mosquito larvicide, *Culex quinquefasciatus* and *Aedes aegypti* (Welling & Paterson 1985), and *Drosophila* and houseflies (Song & Scharf 2008a, b, 2009).

Termites are world-wide pests threatening agriculture and the urban environment (Verma et al. 2009) worldwide. They cause over 3 billion dollars worth of damage to wooden structures annually throughout the U.S. (Su & Scheffrahn 1998). Control and repair costs due to termites in China, have been increasing annually. *Reticulitermes chinensis* Snyder (Isoptera: Rhinotermitidae) is a species of termites with broad distribution that damages the wooden structures of buildings and the xylems of living old trees in China (Wei et al. 2007). Over the past two decades, the control of termite was usually accomplished by using organochlorines and organophosphates, which have been banned owing to environmental and human health concerns (Potter 1997). At the present time, several termiticides, including bifenthrin, chlorfenapyr, cyper-



methrin, fipronil, imidacloprid and permethrin, were registered for termite control around the world (UNEP Report 2000). However, the persistent use of synthetic chemicals to control termites raises several concerns related to environment and human health. In order to reduce the negative impact of pesticides on the environment and to minimize the development of insecticide resistance in target pest species, as a result, the search for good efficacy and environmentally friendly insecticide is essential. As a safer or 'green' alternative, plant derived compounds have been used in termite management as contact insecticides or repellents, such as β -Thujaplicin, Thujopsene, T-muurolol, Cinnamaldehyde and Nootkatone (Nakashima & Shimizu 1972, Yoshida et al. 1998, Maistrello et al. 2001, Chang & Cheng 2002, Cheng et al. 2004). Another potential source of natural toxins against termite is the defensive/offensive chemicals in ants. Some genus such as *Leptogenys*, *Centromyrmex*, *Termitopone*, *Megaponera* and *Pheidole* are all obligate predators of termites (Hölldobler & Wilson 1990). In addition, Kenne et al. (2000) observed that *Myrmecaria opaciventris* can quickly subdued *Macrotermes bellicosus* soldier with its pretarsus and abdominal thorn, thereby attacking the workers. Nascimento (2001) reported that the *Pheidole pallidula* preyed on *Reticulitermes lucifugus*, which play an important role in suppressing the establishment of termite colonies. *Pachycondyla analis*, living in the dry forests of the Tanzanian coast and feeding on termites, can attack the termite nest (Bayliss & Fielding 2002). Ke et al. (2008) found that *Diacamma rugosum*, *Harpegnathos venator*, *Pachycondyla astuta* and *Polyrhachis dives* had strong agonistic behavior and attacking capabilities to *C. formosanus*, and also observed the ant nest adjacent to the *O. formosanus* nest and the ant predation behavior to termite in the wild. The defensive chemicals of ants how to affect termite is not well understood, let alone the utilization of those chemicals in termite control. Therefore, the aim of present work was to study the contact and fumigation toxicity of formic acid to *R. chinensis* in the laboratory.

Methods

Termite

The termite samples of *R. chinensis* colonies were collected from Huazhong Agriculture University, Wuhan, China. Colonies has been reared on wood pieces in a glass container (70 cm \times 40 cm \times 30 cm) in dark at $26 \pm 1^\circ\text{C}$ and $80 \pm 5\%$ relative humidity for more than six months.

Contact toxicity

Contact toxicity of formic acid (98%, Sinopharm chemical Reagent Co., Ltd. China) to *R. chinensis* workers, alates (male and female) and soldiers was determined. For the ease of handling and obtaining uniform body weight, large

workers were selected in the bioassay. Acetone was used as solvent, and the solution was applied with a 1.0 μl capillary tube. For workers, alates (male and female) and soldiers, doses of 100, 150, 200, 250, 300 and 350 $\mu\text{g adult}^{-1}$ were applied. Controls were treated with acetone. Twenty adults of *R. chinensis* workers, alates (male and female) and soldiers were used for each concentration and control, and the experiment was replicated 3 times. Four colonies were used for workers. For alates (male and female) and soldiers, only one colony was tested. The contact toxicity of bifenthrin (98%, Huangma Agrochemicals Co., Ltd, Jiangsu, China) to *R. chinensis* workers was also estimated using 2 colonies. Doses of 3.125, 6.25, 12.5, 25.0 and 50.0 ng adult^{-1} were used. Bifenthrin is the most widely used commercial termiticide as positive control. Each dose was replicated 3 times, and each replicate consisted of 20 workers. Both treated and control insects were then transferred to glass Petri dishes, and kept under the same environmental conditions described for the rearing. Mortality percentages were recorded after treatment for 24 h and LD_{50} values were calculated according to Finney (1971).

Fumigation toxicity

To determine the fumigant toxicity of formic acid and the median effective time to cause mortality in 50 % of the workers from four different colonies (LT_{50} values), filter papers (Whatman No 1, cut into 2 cm diameter pieces) were impregnated with an appropriate concentration of 1.25, 2.5, 5.0, 10.0 and 20.0 $\mu\text{g ml}^{-1}$, respectively. The impregnated filter paper was then attached to the undersurface of the 1000 ml glass jar's (10 cm in diameter \times 12.5 cm in height) screw cap, respectively. The cap was tightly screwed onto the jar, which contained 20 termites. In addition, workers from two other colonies were tested only at 2.5 $\mu\text{g ml}^{-1}$. Alates (male and female) and soldiers were also tested at 2.5 $\mu\text{g ml}^{-1}$. Two colonies were used for alates (male and female) and soldiers. Each concentration and control was replicated three times. Mortality was counted every minute after the formic acid had been delivered into the tube until all termites were dead. When no leg or antennal movements were observed, insects were considered dead.

Another experiment was designed in order to determine the 50% lethal concentration. For the mortality bioassay, 20 workers, 20 alates (male and female) or 20 soldiers were placed in the bottle. The number of dead termites was counted after the formic acid had been delivered into the bottle for 24 h. Six concentrations were tested, including 0.20, 0.40, 0.60, 0.80, 1.0 and 1.2 $\mu\text{g ml}^{-1}$ for workers, alates (male and female) and soldiers, respectively. Three colonies were used for workers, and only one colony was used for alates (male and female) and soldiers. For each concentration \times colony combination, there were three replicates. All bioassays were conducted at $26 \pm 1^\circ\text{C}$ and $80 \pm 5\%$ relative humidity. The fumigation toxicity of bifenthrin (Huangma Agrochemicals

Co., Ltd, Jiangsu, China) on workers was also measured. Six concentrations were tested, including 0.125, 0.25, 0.625, 1.25 and 2.5 ng ml⁻¹. Two colonies were used, and there were three replicates for each colony.

Data analysis

Polo Plus v.1.0 (LeOra Software, Petaluma, CA) was used to estimate LD₅₀, KT₅₀ and LC₅₀ with 95% confidence intervals (CIs). The relative toxicity ratio with their upper and lower 95% confidence limits was used to evaluate the significance of the difference between LD₅₀, KT₅₀ and LC₅₀ values. The significance was set at the P = 0.05 probability level. If the 95% confidence interval of the ratio between two LD₅₀, KT₅₀ or LC₅₀ values included 1, these were not considered to be significantly different (Robertson et al. 2007).

Results

Toxicity of formic acid against *R. chinensis* was shown in Table 1. The LD₅₀ values of formic acid were ranging from 267.86 to 287.68 µg adult⁻¹ for workers, which were larger than those of bifenthrin with LD₅₀ values ranging from 7.16 to 10.39 ng adult⁻¹, and no significant difference was also found in LD₅₀ values of formic acid for workers between colony A and the other three colonies. Alates and soldiers were more sensitive to formic acid than workers. The LD₅₀ values for alates (male and female) and soldiers were at 279.09 and 223.08 µg adult⁻¹, respectively.

Table 1- LD₅₀ values of formic acid by contact against workers, alates and soldiers of *R. chinensis*.

Chemical	Colony	Caste ^a	Slope (±SE)	LD ₅₀	95% CI	
Formic Acid	A	W	2.36(±0.31)	287.68 (µg adult ⁻¹)	250.02-347.80	
		B	2.09(±0.29)	286.24 (µg adult ⁻¹)	245.04-322.94	
		A	2.91(±0.35)	279.09 (µg adult ⁻¹)	248.91-250.48	
	C	W	2.54(±0.32)	273.77 (µg adult ⁻¹)	241.01-322.79	
		D	2.21(±0.29)	267.86 (µg adult ⁻¹)	232.15-322.98	
		E	2.93(±0.31)	223.08 (µg adult ⁻¹)	200.83-250.48	
	Bifenthrin ^b	E	W	2.52(±0.26)	7.16 (ng adult ⁻¹)	5.99-8.41
		F	W	2.46(±0.24)	10.39 (ng adult ⁻¹)	8.82-12.20

^a W: works; A: alates (male and female); S: soldiers.

^b Positive control.

As can be seen from Table 2, the LC₅₀ values ranged from 0.84 to 1.08 µg ml⁻¹ for workers, 1.19 µg ml⁻¹ for alates (male and female) and 0.57 µg ml⁻¹ for soldiers after treatment for 24 h, respectively. The LC₅₀ values of a well-known commercial pesticide used as a positive control in this study, bifenthrin, were 0.40 and 0.42 ng ml⁻¹, respectively (Table 2). At a concentration of 2.50 µg ml⁻¹, the KT₅₀ value of formic acid ranged from 25.38 to 34.75 min for workers, from 42.21 to 45.62 min for alates (male and female), from 32.18 to 36.37 min for soldiers (Table 3). Meanwhile, the higher the concentration, the smaller the KT₅₀ value was. Within A colony, the KT₅₀ value of workers was always significantly smaller than that of alates (male and female), and soldiers.

Table 2- LC₅₀ values of formic acid applied as fumigant for 24 h against workers, alates and soldiers of *R. chinensis*.

Chemical	Colony	Caste ^a	Slope (±SE)	LC ₅₀	95% CI
Formic Acid	A	W	6.71(±0.76)	0.95 µg ml ⁻¹	0.88-1.03
		C	4.56(±0.52)	0.84 µg ml ⁻¹	0.77-0.93
	B	W	4.39(±0.59)	1.08 µg ml ⁻¹	0.98-1.23
		A	4.72(±0.73)	1.19 µg ml ⁻¹	1.07-1.38
		S	4.77(±0.54)	0.57 µg ml ⁻¹	0.49-0.65
Bifenthrin ^b	C	W	3.06(±0.29)	0.40 ng ml ⁻¹	0.32-0.50
	D	W	2.80(±0.26)	0.42 ng ml ⁻¹	0.35-0.50

^a W: works; A: alates (male and female); S: soldiers.

^b Positive control.

Discussion

When applied topically, formic acid was significantly less toxic to subterranean termite than bifenthrin, but higher toxic to many other pest insects. The LC₅₀ value of formic acid for rice weevil, *Sitophilus oryzae*, was 6.03-7.60 µg ml⁻¹, and for the mosquito, *Culex quinquefasciatus*, was 3.67 µg ml⁻¹ (Welling & Paterson 1985). Formic acid displayed low toxicity against yellow fever mosquito, *Aedes aegypti*, at 6.00 µg ml⁻¹ (Chaskopoulou 2007). As demonstrated recently by Chen et al. (2012), the LC₅₀ value of formic acid was 0.26-0.70 µg ml⁻¹ for red imported fire ants. This indicated that the toxicity of formic acid to subterranean termite, *R. chinensis*, was higher than that of the insects studied so far, but similar to red imported fire ants.

Table 3- KT₅₀ values of formic acid applied as fumigant against workers of *R. chinensis*.

Colony ID	Caste ^a	Dosage (µg/ml)	Slope (±SE)	KT ₅₀ (min)	95% CI	
A	W	1.25	4.32(±0.77)	91.67	82.27-109.12	
		2.5	7.87(±0.75)	26.71	25.47-28.01	
	W	5.0	9.06(±1.00)	16.79	15.80-17.82	
		10.0	9.63(±1.43)	8.74	7.78-9.34	
	W	20.0	5.10(±0.58)	5.14	3.87-5.48	
		B	W	1.25	2.32(±0.28)	107.45
	W		2.5	8.45(±0.81)	32.85	31.61-34.25
	W		5.0	9.47(±0.88)	19.70	18.97-20.49
	W		10.0	6.24(±0.64)	9.55	9.05-10.05
	W		20.0	7.15(±0.72)	4.74	4.42-5.03
A	2.5		7.77(±1.06)	45.62	43.34-48.79	
S	2.5		4.19(±0.51)	36.37	33.21-41.01	
C	W		1.25	9.84(±0.84)	84.12	81.25-86.94
	W	2.5	7.95(±0.63)	25.38	24.21-26.51	
	W	5.0	11.32(±0.96)	19.63	19.05-20.20	
	W	10.0	14.18(±1.37)	7.74	7.44-8.04	
	W	20.0	15.62(±1.63)	5.25	5.08-5.42	
	D	W	1.25	14.05(±1.35)	79.53	76.53-82.67
W		2.5	12.03(±1.13)	29.74	28.49-31.07	
W		5.0	6.02(±0.66)	17.90	16.54-19.72	
W		10.0	8.56(±0.79)	6.31	5.94-6.68	
W		20.0	5.72(±0.64)	3.79	3.01-4.54	
E		W	2.5	12.94(±1.24)	34.75	33.52-36.02
		A	2.5	9.40(±1.47)	42.21	40.14-45.72
		S	2.5	8.30(±1.44)	32.18	30.30-35.30
		F	W	2.5	6.95(±0.62)	28.25

^a W: works; A: alates (male and female); S: soldiers.

The action mode of formic acid was to inhibit the activity of mitochondrial cytochrome c oxidase (Nicholls 1975, Petersen 1977). Recently, formic acid has been reported to have a significant excitatory effect on the nervous system of housefly larvae (Song & Scharf 2008a, b). Song & Scharf (2009) reported the formic acid have a significant excitatory effect on the nervous system of *Drosophila melanogaster*. It may explain why formic acid has such a low contact toxicity

compared with fumigation.

Fumigation has been extensively applied as building treatment in termite management programs. Fumigants include methyl bromide, sulfuryl fluoride, dichloroethane and dibromoethane. However, compared to contact insecticides, fumigation has lower toxicity. Therefore, the high efficacy of the conventional synthetic contact insecticides may have slowed down the development of any alternatives.

Using formic acid directly as fumigant against subterranean termite may not be suitable for its own acidity and corrosiveness. However, formate ester is much less corrosive than formic acid and should be much easier to handle. Scharf et al. (2006) and Nguyen et al. (2007) also found the low molecular weight formate esters are volatile compounds with fumigant insecticidal activity. Similarly, Chaskopoulou et al. (2009) reported the volatile low molecular weight compounds against *Aedes aegypti* and *Culex quinquefasciatus*. Previous research reported some formate esters had neurological activity on *Drosophila melanogaster* Meig and *Musca domestica* L, owing to their hydrolyzed metabolite, formic acid (Song & Scharf 2008a, b). Previously, Haritos & Dojchinov (2003) reported some formate esters exert toxicity in the stored product beetle *Sitophilus oryzae* L. for its hydrolyzed metabolite, formic acid, which causes mitochondrial impacts. Therefore, formate esters may also be as good candidates for subterranean termite, particularly as fumigant in treatment. However, the prerequisite is that formic acid can be liberated from formate esters in termite bodies. This is definitely worth for further investigation.

The formic acid fumigation bioassay to termite was carried out in an enclosed environment for termite control, such as house, storage spaces and timber. In order to make fumigation effectively, the enclosed environment must be intact during the application. Simultaneously, other factors such as temperature, humidity, the concentration and time of formic acid applied all should be considered. These are the key of successful application of formic acid in the termite control. In addition to considering their potential fumigant effect, like other commercial fumigant, the more important concern of formic acid application is its toxicity to humans and the environment. The current Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) is 3 mg m⁻³ as the 8 h time-weighted average (TWA) concentration for DDVP, 9 mg m⁻³ for formic acid, 80 mg m⁻³ for methyl bromide, 250 mg m⁻³ for methyl formate and ethyl formate is 300 mg m⁻³ (Chen et al. 2012). Therefore, the effect of formate esters against termite is more worth to investigate, as it seems that formate esters may be more practical to use than formic acid.

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