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RESEARCH ARTICLE - TERMITES

Efficacy of 1% fipronil dust of activated carbon against subterranean termite *Coptotermes formosanus* Shiraki in laboratory conditions

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Abstract

Toxicity and transmission of 1% fipronil dust of activated carbon were measured using the subterranean termite *Coptotermes formosanus* Shiraki in laboratory conditions. 1% fipronil dust of activated carbon has delayed toxicity towards *C. formosanus* compared with 0.5% fipronil dust of Talcum powder; knockdown times KT_{50} and KT_{90} were delayed by >9 and >15 h respectively. Furthermore, 1% fipronil dust of activated carbon showed excellent primary and secondary transfer levels. In primary transfer, recipient mortalities reached 100% by 24, 48 and 72 h at donor-recipient ratios of 1:1, 1:5 and 1:10, respectively. High transfer efficacies were also found if donor-recipient ratios were greatly increased: mortality reached 100% at 9 d at ratio 1:25 and >90% at 12 d at 1:50. In secondary transfer, the toxicant transmitting ability of *C. formosanus* was greater when the primary transfer ratio was lower, and the highest transfer efficacy was found with a donor-recipient ratio of 1:1 - recipient mortalities reached 100% at 5 d and 11 d, respectively. Application of 1% fipronil dust of activated carbon overcomes the problem that too high a concentration kills termites before they can contaminate their nestmates, while a lower concentration may not supply a sufficient dose for effective transfer from treated to untreated termites. The results showed that 1% fipronil dust of activated carbon was non-repellent and readily transferred from treated to untreated termites. As the post-exposure time and doses increased, the mortalities of both donors and recipients increased. And it has delayed toxicity to control *C. formosanus*.

Introduction

Many termite species are distributed in latitude 40 degrees south of the region in China and accounts for about 40% of the whole country. The main disaster area is located in the south of the Yangtze River. Su et al. (2012) reported that the economic losses caused by termites amounted to more than 200 million RMB in 2004. For a long time, the termites control mainly relied on chemical pesticides, such as chlordane, mirex. The production of the two above mentioned pesticides reached more than 100 tons from 2000 to 2003 (Anonymous, 2005). However, controlling of termites is facing a great challenge after the Stockholm Convention on POPs (Persistent Organic Pollutants), which the development of termiticides with high efficiency, low toxicity and environmentally friendly is imperative, especially development and improvement in the formulation. Currently, termites are controlled by applying three formulations of termiticides: non-repellent liquid, dust and bait in China. Insecticidal dusts against termites has been practiced for decades (Madden et al., 2000). Dust formulations (such as avermectin dust, borate dust and fipronil dust) are candidates for successful localized treatment to eliminate termite colonies (Esenther 1985; Lin et al., 2011; Grace, 1991; Zhao et al., 2012). However, 0.5% fipronil dust is the only powder-form termiticide that is registered for use in China; it has high activity and excellent transfer efficacy (i.e. from treated to

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untreated termites) (Ibrahim et al., 2003; Mao et al., 2011; Shelton & Grace, 2003; Remmen & Su, 2005; Gautam et al., 2012; Song & Hu, 2006; Ma et al., 2015; Gautam et al., 2014). In 2008, a new dust of microllose-base formulated with 0.5% fipronil (Termidor[â] Dry) was developed by BASF, which was highly effective to kill *C. formosanus* and had excellent transfer level (BASF. 2008).

Because of high toxicity and rapid effect on termites, only low concentrations of fipronil dust can be used for termite control. Ma et al. (2015) reported that the recommended dose of fipronil dust is ≤0.5%. Otherwise, termites will be killed rapidly and may not have sufficient time to carry and transfer a lethal dose to nestmates before they die. It can be difficult to obtain good transmission results away from the treatment site. Low concentrations of fipronil dust have some control effect on termites, but may not supply a sufficient dose for contaminated termites to transfer a lethal dose to unexposed termites. Shelton and Grace (2003) emphasized the importance in dose reporting of the need to consider lethal transfer of fipronil and imidacloprid from donors to recipients. Ma et al. (2015) reported that the transfer efficacy of low concentrations of fipronil dust are relatively poor compared with high concentrations. The combination of these effects greatly limits the further use and promotion of fipronil dust in the control of termites.

The key to improving transmission of the insecticide is increasing the concentration of fipronil dust while prolonging the time it takes to kill termites. By screening many experimental materials, we found that 1% fipronil dust of activated carbon (i.e. activated carbon powder infused with 1% fipronil) meets these conditions. The present paper reports on the insecticidal activity towards *C. formosanus* and potential for transmission of 1% fipronil dust of activated carbon in laboratory conditions.

Materials and Methods

Termites

Two colonies were used: field colony was collected from the campus of Shanghai Jiao Tong University by using underground traps as previously described (Hu & Appel, 2004) and were kept in the laboratory for <1 month before testing at a temperature of 26 ± 1°C and 85 ± 10% relative humidity. Laboratory colony was maintained in the laboratory for 10 years without exposure to any insecticides.

1% fipronil dust of activated carbon

Activated carbon (a kind of absorptive particle, which has strong adsorption capacity) and talcum powder (a kind of lubricant, which is helpful to the dispersion of solid pesticide) were purchased from Sinopharm Chemical Reagent (Shanghai) Co., Ltd. and Shanghai Junqian Chemical Co., Ltd., respectively. Both materials were sieved through 200 mesh and then sterilized in an oven at 100°C for 24 h. 1 g activated carbon or 0.5 g talcum powder was added to 4 ml 0.25% fipronil acetone solution (1 g activated carbon or 0.5 g talcum powder was added to 4 ml acetone solution as control), the mixture was vigorously vortexed and then incubated at 25 ± 1°C or 50 ± 1°C, respectively, for 2 h in 50 ml tubes. Lids of the tubes were opened and they were placed under a fume hood for 2 d to completely evaporate the acetone.

Lethal concentration bioassay

Bioassays were performed using filter paper method as described (Su et al., 1987) with slight modifications. Briefly, 0.8 ml insecticide-acetone solutions of different concentration were pipetted onto Whatman No. 1 filter papers that were placed in Petri dishes (9 cm diameter) after evaporating the acetone completely (or 0.8 ml acetone as control). Thirty worker termites were placed on each treated filter paper. Experimental mortality was recorded after 24, 48 and 72 h. At least three independent replicates were tested for each colony.

Fipronil dust bioassay

Thirty workers were placed in a Petri dish (9 cm diameter) containing 15 mg 1% fipronil dust of activated carbon (15 mg activated carbon as control) or 0.5% fipronil dust of talcum powder (15 mg talcum powder as control) evenly spread (by gently shaking back and forth) at the bottom of the dish. Exposure was for 15 min. Termites were then transferred to a clean Petri dish containing a filter paper moistened with 0.2 ml distilled water. The number of termite deaths was recorded every 1 h until they were all dead. At least five independent replicates were tested for each colony.

Transfer bioassay

Before the experiment, untreated workers were marked by feeding filter papers dyed with 0.1% Nile blue A (Aldrich, Milwaukee, WI) for 24 h. Blue termites (healthy) were carefully collected using soft brushes and put in Petri dishes in preparation for transfer bioassays for each colony.

(1) Primary transfer: to produce donors, thirty workers were allowed to be freely active for 15 min in a Petri dish (9 cm diameter) containing 15 mg 1% fipronil dust of activated carbon spread evenly at the bottom of the dish. Treated termites (donors) were then transferred to a clean Petri dish and allowed to walk for 3 min. Donors were then added to a Petri dish containing a filter paper moistened with 0.2 ml distilled water and untreated blue termites (recipients) at donor-recipient ratios of 1:1, 1:5, 1:10, 1:25 and 1:50. The number of termites in each dish was 50 ± 5. The number of termite deaths was recorded every 8 h for 24 h and then at 1 d intervals for 12 d.

(2) Secondary transfer: Treated non-dyed workers (as primary donors) were placed in a Petri dish containing untreated blue termites (as primary recipients) at donor-recipient ratios of

1:5 and 1:10. After 8 h (active), the blue termites (as secondary donors) were placed in a Petri dish containing untreated non-dyed workers (as secondary recipients) at donor-recipient ratios of 1:1, 1:5 and 1:10, respectively. The number of termites in each dish was 50 ± 5. The number of termite deaths was recorded every 8 h for 24 h and then at 1 d intervals for 12 d.

All experimental termites were kept in polyethylene containers (30 × 30 × 20 cm) and incubated at 25 ± 1°C and 85 ± 10% relative humidity in total darkness. At least five independent replicates were tested. There were 10% soldiers in each dish.

Statistical Analysis

All data are expressed as the mean value ± SE of independent experiments. Analysis of all data used SPSS 13.0 software and significant differences of mortality between activated carbon and talcum powder and between 25°C and 50°C were determined by Tukey's honestly significant difference test following ANOVA. Mortality was corrected using Abbott's formula (Abbott, 1925).

Results

Table 1 showed toxicity of fipronil to *C. formosanus*. LC_{50} and LC_{90} values were 11.05, 6.18, 4.46 mg/ml and 29.54, 17.13, 10.31 mg/ml after exposure for 24, 48 and 72 h, respectively. Fipronil exhibited moderate to high toxic to *C. formosanus*.

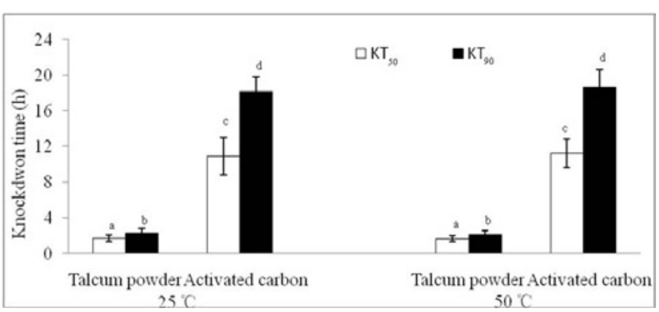

Fig 1. Average knockdown time (± SE) of *C. formosanus* by 0.5% fipronil dust of talcum powder and 1% fipronil dust of activated carbon with 25°C and 50°C incubation. Means with different lowercase letters show significant differences ($P < 0.05$).

0.5% fipronil dust of talcum powder has a quick knockdown time for *C. formosanus*: KT_{50} and KT_{90} were 1.57 and 2.16 h at 25°C and 1.56 and 2.10 h at 50°C, respectively. Termites did not appear poisoning symptoms within 6 h after treatment of 1% fipronil dust of activated carbon. Less termites (about 10%) have decreased activity capacity, such as slow-moving, dying kicks after 8 h, and mortality rate reached 100% in 23-24 h. KT_{50} and KT_{90} of 1% fipronil dust of activated carbon were both significantly prolonged compared with 0.5% fipronil dust of talcum powder ($P < 0.05$); the KT_{50} and KT_{90} were delayed by >9 h (25°C, F = 116.73, df = 1, $P < 0.0001$; 50°C, F = 178.11, df = 1, $P < 0.0001$) and >15 h (25°C, F = 333.33, df = 1, $P < 0.0001$; 50°C, F = 378.90, df = 1, $P < 0.0001$), respectively, at both temperatures (Fig 1). There were no significant effects of temperature on the capacity for adsorption of fipronil applied via activated carbon (KT_{50}, F = 0.14, df = 1, P =0.74; KT_{90}, F = 0.21, df = 1, P =0.67) or talcum powder (KT_{50}, F = 0.09, df = 1, P =0.75; KT_{90}, F = 0.17, df = 1, P =0.66). For each colony, the results showed that 1% activated carbon and 0.5% Talcum powder had no toxic to termites.

For each colony, 1% fipronil dust of activated carbon was excellent in primary transfer. Recipient mortalities reached 100% at 24, 48 and 72 h at donor-recipient ratios of 1:1, 1:5 and 1:10, respectively. Interestingly, high transfer efficacies were also found as the donor-recipient ratio was greatly increased: mortality reached 100% at 9 d at ratio 1:25 and >90% at 12 d at a 1:50 ratio (Fig 2).

In the secondary transfer experiment, the toxicant (1% fipronil dust of activated carbon) transmitting ability of *C. formosanus* was greater with a primary transfer donor-recipient ratio of 1:5 than with a primary transfer ratio 1:10 at secondary donor-recipient ratios 1:1, 1:5 and 1:10 (Fig 3). The highest transfer efficacy was found with a donor-recipient ratio of 1:1 - recipient mortalities reached 100% at 5 d and 11 d, respectively. Recipient mortalities were significantly reduced as donor-recipient ratios increased to 1:5 and 1:10 (58% , 29% and 27% , 19% at 12 d for ratios 1:5 and 1:10 in the primary transfer, respectively). Mortality of recipients negatively correlated with donor-recipient ratios.

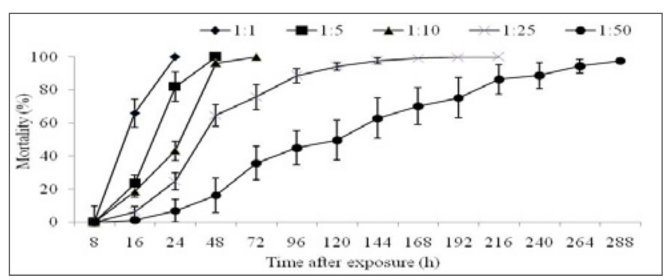

Fig 2. Average mortalities (± SE) of recipient *C. formosanus* at different donor-recipient ratios in the primary transfer of 1% fipronil dust of activated carbon.

Discussion

In the present study, mortalities reached 100% at 3 h after treatment of *C. formosanus* with 0.5% fipronil dust of talcum powder (Fig 1). It is evident that 0.5% fipronil dust of talcum powder kills *C. formosanus* too quickly and so cannot be used to eliminate or suppress the nest of termites. We found that fipronil exhibited moderate to high toxic to *C. formosanus* (Table 1). These results are consistent with previous reports (Ibrahim et al., 2003; Mao et al., 2011). Saran and Rust (2007) found that *C. formosanus* became less mobile 1 h after exposure to fipronil-treated sand and transfer was limited to the vicinity of the treated sites. Similarly, in a laboratory study of *C. formosanus*, they did not walk more than 5 m after

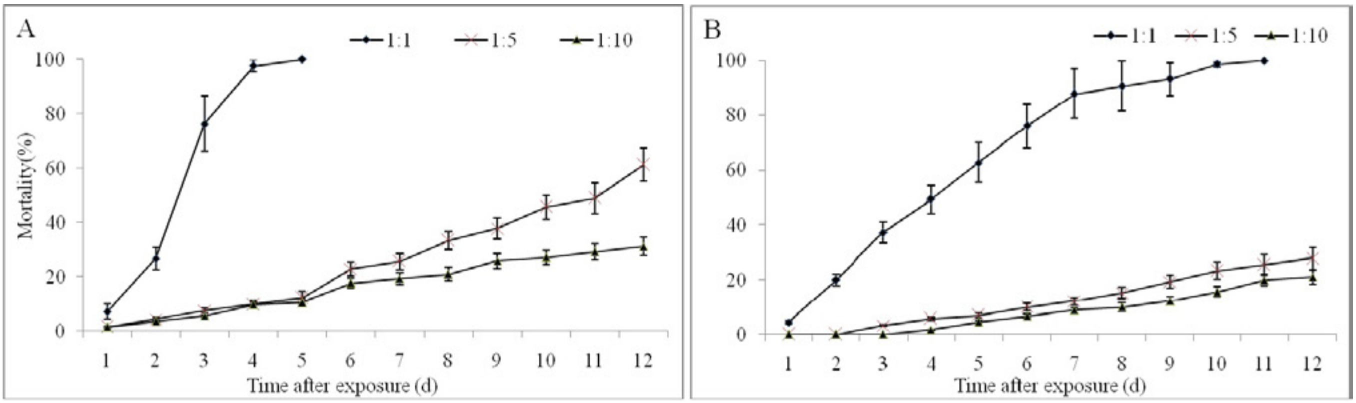

Fig 3. Average mortalities (± SE) of recipient *C. formosanus* at different donor-recipient ratios in the secondary transfer of 1% fipronil dust of activated carbon. A, primary transfer donor-recipient ratio 1:5; B, primary transfer donor-recipient ratio 1:10.

contacting fipronil-treated soil (Su 2005), whereas Saran and Rust (2007) found that termites exposed to lethal amounts of fipronil did not walk more than 1.5 m from the treated zone. Quarcoo et al. (2012) reported that tunneling and working ability of intoxicated termites are important factors that affect their potential to transfer toxicants to untreated nestmates, along with the insecticide exposure duration and dose, the donor-recipient ratio (Ibrahim et al., 2003; Saran & Rust, 2007; Shelton & Grace, 2003; Hu 2005; Song & Hu, 2006), the temperature (Spomer et al., 2008), substrate type and caste structure (Gautam & Henderson, 2011). However, 1% fipronil dust of activated carbon makes up for this shortcoming since the KT_{50} value is delayed by >9 h compared with 0.5% fipronil dust of talcum powder; i.e., this preparation greatly delayed the death time of *C. formosanus*.

1% fipronil dust of activated carbon has an excellent effect on *C. formosanus*. When the primary donor-recipient ratios were 1:1, 1:5 and 1:10, the recipient mortalities were 100% within 24-72 h. Shelton and Grace (2003) reported that untreated termites had a significantly greater mortality after a 15-day interaction at a high donor-recipient ratio (1:19). We found a similar situation in our experiments: mortalities reached 100% at 9 d at donor-recipient ratio 1:25 and >90% at 12 d for a 1:50 ratio. There are few previous reports about the secondary transfer level of fipronil to *C. formosanus*. In the present study, the mortalities of recipients (secondary mortality of recipient) were determined at different donor-recipient ratios in the secondary transfer. Recipient mortalities (secondary donor-recipient ratio 1:1) reached 100% at 5 d and 11 d after primary transfer at donor-recipient ratios of 1:5 and 1:10, respectively. There was a negative correlation between recipient mortality and the donor-recipient ratio for both primary and secondary transfer.

In our experiments, we used 200 mesh sieved activated carbon, i.e. fine particles. Such particles adhere easily to the surface of termites. Because of the strong adsorption of activated carbon, much of the toxic agent is absorbed in the particles, so treated termites are not killed immediately but instead are able to transfer a lethal dose to other (untreated) termites before they die. This may impact upon the entire colony. In addition, we observed a black substance in the intestines of donor termites, which may be due to the grooming ritual and the intake of activated carbon following primary transfer; this phenomenon is not obvious in recipients. The transfer of fipronil among termites can be caused by body contract, mutual grooming and trophallaxis (Song and Hu 2006; Saran and Rust 2007). The transmission mechanism of 1% fipronil dust of activated carbon is unknown and requires further study.

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Table 1. Toxicity of fipronil towards *C. formosanus* after exposure for 24, 48 and 72 h.

Exposure time	Slope±SE	LC_{50} (μg/ml)	95% CL	LC_{90} (μg/ml)	95% CL	χ^2(df)
24 h	3.07±0.09	11.05	8.14-15.57	29.54	20.26-62.10	26.46(3)
48 h	2.97±0.23	6.18	4.92-7.92	17.13	12.81-27.95	14.37(3)
72 h	3.80±0.12	4.46	4.44-5.02	10.31	9.50-11.37	1.53(2)

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