Research Article



Development and Laboratory Evaluation of a Slow Release Formulation of Fipronil against Subterranean Termites (*Odontotermes obesus* Rambur)

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Abstract | Subterranean termites Odontotermes obesus (Isoptera: Termitidae) are economically important agricultural and structural pests. A wide range of insecticides with different modes of action are being used against these termite pests including organochlorines, organophosphates and pyrethroids. However, pesticidal control of termites is usually not persistent and long-lasting due to rapid decomposition and loss of insecticidal molecules active against target pests. This study was aimed to develop and evaluate a slow-release formulation (SRF) of a persistent insecticide (fipronil) against O. obesus. Technical grade insecticide fipronil was applied in the form of cellulose-made pellets made up of compressed powdered sugarcane (Saccharum officinarum L.) bagasse and maize (Zea mays L.) cobs. This pellet formulation was evaluated against worker individuals of O. obesus under laboratory conditions using soil macrocosms. Mortality of termites was determined at different time intervals after their exposure to formulated pellets treated macrocosm soils. Results revealed that fipronil formulated with Z. mays substrate remained more effective for longer period of time against subterranean termites as compared to that formulated with S. officinarum bagasse material. The maximum mortality of termites was observed at 15 days of treatment application in T3MPF (maize cob powder plus fipronil) treatment after 48 h of bioassay. It was concluded that fipronil can be used as a slow acting toxicant by formulating it with some cellulose-based material such as powdered maize cobs in order to attract and kill the subterranean termites.

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Keywords | Subterranean termites, Odontotermes obesus, Fipronil, Slow-release formulation, Cellulose bait, Maize cob matrix

Introduction

Termites are considered ecological engineers owing to their ability to breakdown the organic matter and recycling nutrients in tropical and subtropical ecosystems (Davies *et al.*, 2003; Brauman *et al.*, 2015; Duran-Bautista *et al.*, 2020). However, many species of subterranean termites have been destructive agricultural and infrastructural pests wreaking considerable damage to crops, forest plantations and wooden infrastructures all over the world (Su and Scheffrahn, 2000; Rust and Su, 2012).



These invertebrates are crypto-biotic and are usually hard-to-control pests due to their obscure feeding and foraging activities beneath the soil and within mud galleries (Thorne *et al.*, 1999; Bulmer and Traniello, 2002; Vargo and Husseneder, 2009).

Mostly liquid formulations of insecticides such as bifenthrin, cypermethrin, deltamethrin, chlorpyrifos, dichlorvos, chlorfenapyr, chlordane and fipronil are used against termite infestations (Hu, 2005; Ahmed *et al.*, 2006; Akbar *et al.*, 2019). However, one of the major drawbacks of using these liquid formulations is that they are highly susceptible to get off the target pests or surfaces either by evaporation losses or by leaching down or running off from the treated site (Fernández-Pérez, 2007). Secondly, these liquid formulations get readily decomposed by the climate extremities including temperature, light and humidity and by soil microbes if applied to soil (Singh, 2016; Huang *et al.*, 2018).

One of the solutions to this pesticidal drawback is the development of more target-oriented and longlasting formulations which can give the control of target pests for longer period of time with the minimum and slow rate of losses of active ingredients. These formulations are usually termed as slow-release release formulations (SRFs) (Gerstl et al., 1998; El-Nahhal et al., 2000; Undabeytia et al., 2000; Hermosin et al., 2001; Celis et al., 2002; Liu et al., 2011; Gautam et al., 2012). Due to low volatilization, less leaching and longer active periods, slow or controlled release formulations (SRFs) are far better than the conventional pesticides (Fernandez-Perez et al., 1999; Dailey, 2004). Many SRFs have been tested successfully against a wide range of insect pests including subterranean termites and other edaphic insect pests including ants, cockroaches, coleopterous grubs and fleas (Collins and Callcot, 1998; Overmyer et al., 2005; Gautam et al., 2012; Peters et al., 2019).

Nevertheless, research on SRFs has mainly focused on the release kinetics of pesticide and the use of sorbents in these formulations (Garrido-Herrera *et al.*, 2006; Li *et al.*, 2012). For controlled or slow release of pesticides, different polymers such as starch, alginate and silicate are used in the formulation matrix (Fernandez-Perez, 2007; Singh *et al.*, 2009; Chen *et al.*, 2017; Ashitha and Mathew, 2020). In many studies of pesticide formulations, urea is used as an additive which not only affects the release kinetics but

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also improves the properties of formulation (Wang and Wu, 2003; Cao *et al.*, 2005). Moreover, SRFs against subterranean termites become more effective when baited with cellulose material (Su, 2005; Zhang *et al.*, 2009; Dhang, 2011; Eger *et al.*, 2012; Peters *et al.*, 2019).

The objective of this study was to develop and evaluate a slow-release bait formulation of fipronil against subterranean termite *Odontotermes obesus* (Isoptera: Termitidae) which are economically important agricultural and structural pests in Indo-Pak regions (Ahmed *et al.*, 2007; Manzoor and Mir, 2010; Akbar *et al.*, 2019). Fipronil is a non-repellent broadspectrum phenylpyrazole insecticide and is used to control a wide range of chewing and sucking insect pests (Scott and Wen, 1997; Collins and Callcot, 1998; Elbert *et al.*, 1998; Overmyer *et al.*, 2005; Iqbal and Evans, 2017).

Materials and Methods

Collection and laboratory maintenance of termites

An intact colony of a healthy and active colony of subterranean termites was dug out and collected from a sugarcane (*Saccharum officinarum* L. var. BF-237) field ($32^{\circ}07'58''N$; $72^{\circ}41'32''E$) in the vicinity of College of Agriculture, University of Sargodha. It was ensured that there was no insecticide application at the collection site for last two months. The collected termite species was identified as *O. obesus* Ramb. In order to acclimatize the termite individuals to laboratory conditions, this colony was maintained for about two weeks in a transparent rearing box under controlled conditions (at $27\pm2^{\circ}C$, 65% relative humidity and 16:8 h light-dark photoperiod). Only healthy and active worker termites were used in all bioassays.

Preparation of fipronil pellets

Technical grade (99.5% pure) fipronil was procured from FMC United (Pvt.) Ltd. Pakistan. Slow-release formulation was prepared in the form of compressed pellets. For this purpose, sugarcane (*S. officinarum* L.) bagasse and maize (*Zea mays* L.) cobs were used as sources of cellulose and attractant for subterranean termites. These were collected and dried in sunlight and then in shade for 48 hours. After drying, these materials were ground to powder form separately with the help of an electric grinder. Some other additives were also used to enhance the properties of formulation and discharge kinetics of insecticide from the slow-release formulation. These additives were starch, sodium silicate, sodium alginate and urea. Four types of pellets were formulated *viz*; treatment 1 (T1SPF) containing sugarcane bagasse powder mixed with fipronil (5%), treatment 2 (T2SPC) containing sugarcane bagasse without fipronil (control), treatment 3 (T3MPF) containing maize cob powder mixed with fipronil (5%) and treatment 4 (T4MPC) containing maize cob powder without fipronil (control). The detailed composition of these pellets is given Table 1. The dimensions and average weight of each formulated pellet were 20×14 mm and 4 g, respectively.

Table 1: Composition of ingredients used for the preparation of slow-release formulation pellets.

Ingredients	Treat- ment 1 (T1SPF)	Treat- ment 2 (T2SPC)	Treat- ment 3 (T3MPF)	Treat- ment 4 (T3MPC)
Fipronil (TG)	5g	-	5g	-
Sugarcane bagasse powder	70g	75g	-	-
Maize cob powder	-	-	70g	75g
Sodium alginate	3g	3g	3g	3g
Sodium silicate	2g	2g	2g	2g
Urea	2g	2g	2g	2g
Starch	18g	18g	18g	18g

TG: technical grade; T1SPF: sugarcane bagasse powder + fipronil; T2SPC: only sugarcane bagasse powder; T3MPF: maize cob powder + fipronil; T4MPC: only maize cob powder.

Bioassay protocol

For determining the efficacy of Slow-release formulation (pellets) of fipronil against *O. obesus* termites, macrocosms ($210 \times 150 \text{ mm Pyrex}^{TM}$ borosilicate glass beakers with capacity of 3 L) were half-filled with sterilized soil. One formulated pellet was introduced in the soil at a depth of 5 cm in the centre of each macrocosm. After that, tap water was applied by hand-sprinkler in the morning and in the evening to moisten the soil till the end of the experiment. Due to moisture, the fipronil present in the pellets was assumed to be relocated and disturbed gradually and slowly in the surrounding soil.

The effectiveness of fipronil released in the soil profile of each macrocosm was tested against subterranean termites by exposing them to the treated soil taken from macrocosm at 3, 7, 15, 30 and 60 days postapplication. In brief, treated soil was taken from the immediate surroundings of the pellet and was put in glass Petri-dishes (100×15 mm). Then, ten healthy and active worker termites were released into each Petri-dish and data regarding their mortality were recorded at 6, 12, 24 and 48 hours post-exposure of termites to soil. Similar bioassays were conducted at 7, 15, 30 and 60 days after the pellets application in the soil. All these experiments were performed at $27^{\circ}C\pm 2$ and 70% relative humidity. Four independent replications were maintained for each treatment.

Statistical analysis

Statistical interpretation of the data was performed using Statistix[®] version 8.1 analytical software (Statistix, Tallahassee, FL). Data was subjected factorial one-way analysis of variance (ANOVA) using treatment (pellet formulation type) and time (post-application exposure time) as factors. Following ANOVA, the treatment means were compared using Fisher's least significant difference (LSD) posthoc test at standard level of significance (P = 0.05). Moreover, median lethal time (LT⁵⁰) values were calculated by probit analysis using POLO-PC[®] (LeOra Software, 1987) regression software.

Results and Discussion

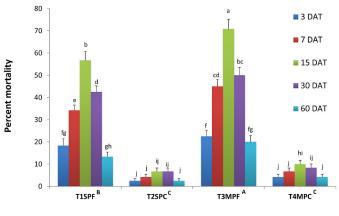
Results of these laboratory bioassays revealed a significant effect of both factors, *i.e.* the treatments (F $_{(3,300)}$ = 297.64, p < 0.001) and time intervals (F $_{(4,300)}$ = 79.45, p < 0.001), on the percent mortality of O. obesus termite individuals (Table 2). Similarly, the interaction of treatments and time intervals (F (12.300) = 17.05, p < 0.001) also exhibited a significant effect on percent mortality of termites (Table 2). Maximum average termite mortality (70.83 ± 4.31%) was recorded for maize cob pellets containing 5% fipronil (T3MPF) recorded at 15 DAT, followed by the treatment containing sugarcane bagasse and 5% fipronil (T1SPF) exhibiting 56.67 ± 4.06% average mortality of termites (Figure 1). Average termite mortality recorded at 30 and 60 DAT were 42.50 ± 2.68 and 13.33 ± 2.11 for T2SPF and 50.25 ± 3.44 and 20.67 ± 2.72 for T3MPF, respectively. Minimum average termite mortality was recorded for the treatments without fipronil insecticide (*i.e.* $2.50 \pm$ 1.03% and 4.17 ± 1.24% for T2SPC and T4MPC, respectively) recorded at 3 DAT (Figure 1).

Similar trend of termite mortality was recorded for individual bioassays conducted at 3, 7, 15, 30 and 60





DAT. For each DAT bioassay, fipronil mixed formulations (*i.e.* T1SPF and T3MPF) showed significantly high mortality of *O. obesus* termite individuals as compared to the pellets without insecticide (Figure 2). Similarly, termite mortality increased along with the exposure time for all bioassays. Maximum termite mortality (76.67 and 90% for T1SPF and T3MPF, respectively) was recorded at 48 h post-exposure at 15 DAT, while minimum mortality (3.33 and 10% for T1SPF and T3MPF, respectively) was found at 6 h post-exposure time intervals at 3 DAT. There was no or negligible mortality of termites recorded at 6 and 12 h post-exposure for both T2SPC and T4MPC treatments (Figure 2).



Treatments

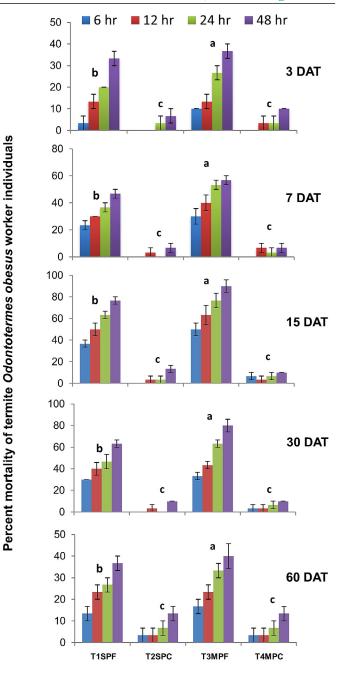
Figure 1: Percent mortality (mean \pm SE; sample size n = 16) of worker individuals of subterranean termite Odontotermes obesus in response to different treatments recorded at different post-exposure time intervals. Treatments included different formulated pellets containing sugarcane bagasse powder with (T1SPF) and without (T2SPC) fipronil, and maize cob powder with (T3MPF) and without (T4MPC) fipronil. Different lettersindicate the significant difference among treatments (factorial (two factor) one-way ANOVA at $\alpha = 0.05$).

Table 2: Analysis of variance table regarding the impact of different treatments on the mortality of termite Odontotermes obesus individuals bioassayed at different post-exposure time intervals.

Source	DF	SS	MS	F-value	P-value
Treatment	3	83837	27945.7	297.64	< 0.001
Time	4	29839	7459.7	79.45	< 0.001
Treatment×Time	12	19210	1600.9	17.05	< 0.001
Error	300	28167	93.9		
Total	319	161054			
GM / CV	21.46 /	45.16			

Factorial one-way ANOVA followed by least significant difference (LSD) post-hoc test at $\alpha = 0.05$; DF: Degree of freedom; SS: Sum of squares; MS: Mean sum of squares; F: F-statistic; GM: Grand mean; CV: Coefficient of variation.

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Treatments

Figure 2: Percent mortality (mean \pm SE; sample size n = 4) of worker individuals of subterranean termite Odontotermes obesus in response to different treatments recorded at different post-exposure time intervals. Treatments included different formulated pellets containing sugarcane bagasse powder with (T1SPF) and without (T2SPC) fipronil, and maize cob powder with (T3MPF) and without (T4MPC) fipronil. Different letters indicate the significant difference among treatments (factorial (two factor) one-way ANOVA at $\alpha = 0.05$). DAT: days after treatment.

Regarding, median lethal time (LT_{50}) values determined for *O. obesus* termite worker individuals exposed to the soils treated with different fipronil formulations was shown in Table 3. After 15 days of treatment application, treatment T3MPF appeared to be most toxic with minimum LT_{50} value (5.76 h)



followed by T1SPF (11.90 h). Similarly, treatment T3MPF was most effective with minimum LT_{50} value (22.86 h) followed by T1SPF (33.39 h) recorded after 30 days of treatment application (Table 3).

In the pest management industry, although termite control primarily relies on the application of liquid formulations, baiting techniques appear recently as promising tactics particularly in situations where liquid applications are unsuccessful on sustained basis (Kistner and Sbragia, 2001). For effective long-term control of subterranean termites with a minimized risk of offsite pesticide movements, slowreleases insecticidal bait formulations offer promising solutions. Many studies on bait and SRF systems have proved that they can completely eliminate the entire colony of the subterranean termites (Grace et al., 1996; Su and Scheffrahn, 1996; Tsunoda et al., 1998; Peters and Fitzgerald, 1999; Prabhakaran, 2001; Evans, 2010; Neoh et al., 2011; Osbrink et al., 2011; Eger et al., 2012). Huang et al. (2006) demonstrated that fipronil baited with white sugar and straw pulp was highly effective against the colonies of Odontotermes formosanus in the field.

The present study encompassed a preliminary attempt to develop and evaluate a slow-release formulation of fipronil, a well-known synthetic contact insecticide, against subterranean termite *O. obesus*. Small pellets (4 g 20×14 mm) were formulated by compressing mixtures of cellulose-material, starch, sodium alginate, sodium silicate and urea with and without 5% technical grade fipronil. Cellulose-based materials used in formulation were sugarcane (*S. officinarum* L.) bagasse and maize (*Z. mays* L.) cobs which were used to provide adsorbing matrix for fipronil molecules. Termite mortality was bioassayed by exposing them to treated soils taken from the macrocosms at 3, 7, 15, 30 and 60 days of slow release pellets application.

In this study, fipronil was found effective against the subterranean termites (O. obesus) individuals as a slow acting toxicant even till two months post-application of the formulated pellets applied in the soil. Many previous studies have been conducted regarding the development and evaluation of different types of slow-release insecticidal baits for controlling subterranean termites (Haverty et al., 2010; Neoh et al., 2011; Peters et al., 2019). Different formulations of bifenthrin, fipronil, hexaflumuron, thiamethoxam and imidacloprid showed significant suppression of many termite species (Sheets et al., 2000; Delgarde and Rouland-Lefevre, 2002; Ahmed et al., 2005; Remmen and Su, 2005; Rashid et al., 2012; Saljoqi et al., 2014) evaluated different insecticides including fipronil, pyriproxyfen, chlorpyrifos, hexaflumuron, imidacloprid and indoxacarb against subterranean termites and showed that fipronil was the most effective slow acting toxicant for controlling termites followed by imidacloprid and indoxacarb. Our results are in line with previous studies showing that fipronil is very effective even at a very low concentration (Kaakeh et al., 1997; Valles et al., 1997; Durier and Rivault, 2000).

Table 3: Median lethal time (LT_{50}) values for the worker individuals of subterranean termite Odontotermes obesus exposed to soils treated with different formulations of fipronil and bioassayed at different post-exposure time intervals.

Treatments	DAT	LT ₅₀ (hr)	Lower and Upper 95% Confidence Limits (hr)	X ² (df = 14)*	P-value	Slope ± SE	Intercept ± SE
T1SPF (sugar- cane bagasse plus fipronil)	3	88.31	61.37 - 164.47	33.44	0.002	1.40±0.12	2.73±0.17
	7	66.73	47.61 - 115.36	9.56	0.793	0.71±0.10	1.29±0.13
	15	11.90	10.29 - 13.52	17.60	0.225	1.18±0.10	1.27±0.12
	30	33.39	24.04 - 58.90	22.64	0.66	0.66±0.10	1.00±0.12
	60	255.66	109.10 - 2557.07	42.06	0.000	1.00±0.13	2.40±0.17
T3MPF (maize cob plus fipronil)	3	98.92	72.77 – 154.79	11.24	0.667	1.04±0.11	2.09±0.15
	7	24.72	18.83 - 35.89	26.56	0.022	0.80±0.10	1.11±0.12
	15	5.76	2.60 - 8.50	78.73	0.000	1.28±0.11	0.97±0.13
	30	22.86	19.65 - 27.22	11.70	0.630	0.97±0.10	1.19±0.12
	60	111.71	72.68 - 236.13	24.15	0.044	1.10±0.11	2.25±0.15

*Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.



Secondly, material used in baits also have considerable effects on the target insects' mortality. Many studies have payed attention towards the cardboard and wood baiting systems and few have focused to check the difference between the effectiveness of different materials used in baiting systems (Lenz et al., 2011; Wang and Henderson, 2012). According to our results, maximum mortality of termites for longer period of time was observed in treatment containing maize cob powder which proved that maize cob powder have more ability to hold-on or retain pesticidal (fipronil) molecules for longer period of time than sugarcane bagasse. Nevertheless, Wang and Henderson (2012) using different choice and no-choice experiments have demonstrated that subterranean termites prefer maize cob material over cardboard or wooden chips. According to the findings of Azubuike et al. (2011) and Shogren et al. (2011), it is evaluated that agricultural by product/waste such as maize cob contains reasonable amount of cellulose. Together with our results, these findings suggest that maize cob powder as baiting matrix would be with triple advantages for developing a controlled-release pesticidal formulation in future to be tested under field conditions. On one hand, it can retain pesticide active ingredients for long period of time, and on the other hand, it could be an effective attractant for termites as it contains more cellulose, aminoacids and sugar contents (Chen and Henderson, 1996; Lenz and Evans, 2002; Wang and Henderson, 2012). Moreover, it is also cost-effective as maize cobs are easily available and abundant waste material of agricultural products (Varvel and Wilhelm, 2008; Wilaipon, 2008).

A study conducted by Li *et al.* (2001) showed that sugarcane bagasse powder is not effective and attractant for subterranean termites (*Coptotermes formosanus* and *Reticulitermes flavipes* species) until unless it is infected with *Gloeophyllum trabeum* fungi. This also corroborates our study results about effectiveness of maize cob powder as compared to sugarcane bagasse powder.

Conclusions and Recommendations

In conclusion, this laboratory study showed that fipronil mixed with maize cob substrate remained more effective for longer period of time against subterranean termites as compared to sugarcane bagasse material. The maximum mortality (90%) of termites and minimum median lethal time (LT_{50}) of 5.76 h observed at 15 days post-treatment suggest that fipronil can be used as a slow acting toxicant by formulating it with some cellulose-based material such as powdered maize cobs in order to attract and kill subterranean termites for longer period of time. However, lack of HPLC determination of the release dynamics of fipronil and its metabolites into the surrounding soil of macrocosms treated with fipronil formulated pellets and lack of field evaluation of this formulation with different concentrations of fipronil as active ingredient render this study as a pilot preliminary study. Although these determinations were envisaged but could not be performed due to unavoidable reasons, these two deficiencies, indeed, encompass the future perspectives of this study.

Novelty Statement

This laboratory study validated that the insecticidal efficacy of fipronil can be enhanced and prolonged in the soil (up to two months) against subterranean termites (*O. obesus*) when baited with the matrix of maize (*Z. mays*) cob powered as cellulose source.

Author's Contribution

SAS and MZM conceived the idea and planned the experiment. SAS and MZM performed experiments and wrote the first draft of the manuscript. SNO performed statistical analyses. ML prepared graphs. MAR technically revised the manuscript. SA provided the technical assistance and proofread the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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