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Combinatorial Potential of bait matrix against subterranean termites under lab and field conditions

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Abstract

Two bait matrices with different treatments were evaluated against two termite species i.e. *Odontotermes obesus* and *Coptotermes heimi* both under laboratory and field conditions. Mean wood consumption in laboratory bioassays was investigated for 2, 4 and 6 weeks with maximum consumption after 4 weeks. While, field experiments were conducted for 24 weeks and there was greater consumption of the loosely bound bait matrix compared to the tightly bound matrix. However, feeding was comparatively high in combinations with attractants i.e. feeding stimulants (agar and sugarcane bagasse). Overall, treated colonies experienced a 90-95% decrease in population size after 24 weeks of baiting. The queen in the royal chamber of the mound was found dead.

Introduction

Termites are economically important pests because they destroy wood products of human homes, building materials, forests, agriculture crops and other commercial products (Monica et al., 2009). Baiting has been promoted as a desirable method of termite pest control. It is lauded as environmentally sound as it uses very small amounts of insect specific toxicants that are administered in localized baits that are targeted at the pest species (i.e. not large amounts of toxicants spread over large areas around a house). However, in order for baiting to work successfully, termites must find and consume the bait matrix and for the toxicant contained therein to be transferred back to the nest.

Since the introduction of baiting systems for managing subterranean termites in many parts of the world, various approaches have been used to evaluate the bait matrix and transfer the toxicant back to the nest. These approaches, among others, included the incorporation of additives, attractants, and phagostimulants into the bait matrices. Attractants such as fungal extracts (Esenther et al., 1961; Cornelius et al., 2004; Su, 2005) and carbon dioxide at10-12 mmol/mol (Bernklau et al., 2005) has been shown to improve the attractiveness of bait to termites. As a means to increase consumption, phagostimulants that made the bait more palatable, such as urea (Waller, 1996) amino acids (Chen & Henderson, 1996), and sugars (Morales-Ramos & Rojas, 2003a; Waller & Curtis, 2003; Saran & Rust, 2005, 2008), were also explored. Termites fed more on baits with additives than those without. Nutritionally enhanced bait such as a commercially available product, Summon Preferred Food Source, was more preferred by *Coptotermes formosanus* (Shiraki) than standard cardboard disks. Summon bait aggregated more termites, which resulted in higher consumption compared with standard cardboard disks (Cornelius & Lax, 2005). There are different types of baits that were made from cellulose material like wood, cardboard or tissue which attract termites and used for forecast infestation or control. However, not all termites' species prefer the same bait matrix. This may be due to the nature of matrix which is not preferred by these termite species.

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Baiting technology involves placing in-ground monitoring stations that contain a cellulose material in the soil at regular intervals along the perimeter of the structure. These stations are checked at regular time intervals for foraging termites. There are commercially available baits that eliminate the pre-baiting period by initially installing in-ground stations that contain active ingredient. Individual termites consume the bait and share it with other individuals within the colony after through feeding and grooming behaviors, by which the bait is distributed and eliminate the colony.

In social insects, horizontal transfer usually involves interactions where both the donors and the recipients are alive and actively interacting. Key among these mechanisms is trophallaxis, which facilitates the spread of bait toxicants (Hu et al., 2005), and mutual grooming which facilitates the spread of liquid spray insecticides (Soeprono & Rust, 2004). Control of subterranean termite was transfigured when first termite bait product was registered by Dow AgroSciences LLC (formerly known as ~ow~lanco) (Su, 1994). Su (1994) was the pioneer to use hexaflumuron as baits in field trials, which lead to the development of sentriconB. Termite baiting takes the advantage of social nature and foraging behaviour of subterranean termites where food sharing among the workers and nestmates via trophallaxis could enable the transfer of slow-acting toxicant to the whole colony (Lee & Chung, 2003). A new generation of baits includes high-moisture-content matrices formulated to be nutritionally attractive to termites. One of these bait matrices, developed by USDA-ARS researchers, was based on the feeding preferences and nutritional requirements of the Formosan subterranean termite (Morales-Ramos & Rojas, 2003a; Rojas & Morales-Ramos, 2001; Rojas et al., 2003). The purpose of this formulation was to increase bait consumption by subterranean termites in order to enhance assimilation of active ingredients within colonies, consequently reducing the time required by treated termites to attain lethal doses (Morales-Ramos & Rojas, 2003b; Rojas, 2002a,b; Rojas & Morales-Ramos, 2003). These bait matrix formulations in combination with feeding stimulants and masking agents (Rojas et al., 2004) allow incorporation of less palatable but more widely available active ingredients, such as diflubenzuron, without compromising bait consumption by the termites (Morales-Ramos & Rojas, 2003a).

The current research focusing on the effect of bait design and applications by employing combinatorial treatments on the survival and consumption of wood by highly destructive Pakistani termites *Odontotermes obesus* and *Coptotermes heimi*.

Materials and Methods

Collection of Termites

O. obesus and *C. heimi* were collected from different areas of district Lahore including Changa Manga forest, Jallo Park, Wagha border, Ravi siphon, Jinnah garden, agricultural crops and fallen wooden logs infested under natural conditions. They were also collected through the installation of different woods as stakes and supplemented by sugarcane stalks to aggregate the maximum number of termites. Collection was made by using bucket traps /wetted toilet rolls/ cardboards packed in plastic bottles with small holes at the base to permit the entry of termites, as they visit to the feeding stations.

Augmentation of termites

The workers and soldiers of *O.obesus* and *C. heimi* which were collected from different locations were established in a transparent test apparatus made up of acrylic sheet with specific dimensions (Length× height× width: 30× 35×30cm) supplied with sterilized moist soil, cellulose powder, pieces of moistened corrugated cardboards and tissues rolls which serves as source of food for termites, under laboratory conditions. The termites were handled with a moist paint brush. All the cages were then covered with black polythene/cloth to minimize the effects of light and placed in the controlled room at 26±1°C, RH 75%. Moisture in the containers was kept judiciously and checked twice a month.

2.3. Isolation and extraction of fungal toxins

Metarhizium anisopliae was isolated from different sources. Cultures were maintained on Potato Dextrose Agar (PDA). Conidial suspensions were prepared by lightly scraping the surface and suspending the conidia in 100mL distilled sterile along with 0.01% of Tween 80. The conidial concentration of the suspensions was determined using a haemocytometer. Isolates of *M. anisophilae* were cultured in 500 mL Erlenmeyer flasks containing 250 ml of sterile potato dextrose liquid medium The flasks were incubated separately for 7-10 days in the dark at 27-30^{o}C without agitation. Twenty five mL of chloroform were added to lyse the cells in order to recover mycelia and allow it to rotate for 10 min on a shaker. The flasks contents were filtered (Whatman no. 1) and the filtrate was used for toxin extraction. The filterate was transferred quantitatively to a separatory funnel and extracted successively with 100 mL of chloroform to partition the chloroform and aqueous layers. The procedure was repeated three times with lower transparent chloroform layer collected in a new flask. The chloroform was evaporated at 100^{o}C by a vaccum rotatory evaporator to obtain the crude extract of each fungus according to Mallek et al. 1993. The extracts were finally weighed and kept in refrigerator at 4^{o}C until further use.

Chemicals

Commercial formulation of two insecticides used for the bioassays were, fipronil (Fiprostar® 25 EC Starlet International,Pakistan) and imidacloprid (Mirage® 5% SC Ali Akbar Enterprises, Pakistan) at lower concentration of 0.3ppm.

Bait Application under Lab and Field Conditions
Locations for bait Installation

This experiment was designed to test whether bait station design affected bait matrix removal, not whether bait design affected bait discovery. Therefore it was decided that bait stations should be placed onto active foraging sites and thereby be encountered immediately. Two locations were selected for bait installation which was previously monitored for termite foraging activity. Mounds/nests at Wagha border were selected for *O. obesus* and Lahore canal bank in the vicinity of FC College dominated by *P. euramericana* plantation was selected for *C. heimi* (Fig 1 (A-B).

Bait Matrix

The most palatable wood, *Populus euramericana* (Manzoor et al., 2009; Aihetasham & Iqbal, 2012) was selected as bait matrix. The other components of bait matrix including: Agar, sugarcane molasses, fungal conidial suspension ($1*10^7$) and 0.3ppm of Imidacloprid and Fipronil. 100ml of suspension is prepared for each treatment. Wood blocks (Length× height× width: 30× 35×30cm) were soaked for 72 hours in each treatment in order to achieve leaching of suspension in wood blocks. The formulation was evaluated against *O. obesus* and *C. heimi* workers separately under laboratory conditions. All wood blocks were pre-weighed before start of experiment (Fig1(C-E).

Bait Treatments under laboratory and field bioassay

Five treatments were prepared for bait application under lab and field conditions which are as follow:
Control (*Populus euramericana*)
(a) *Populus euramericana* + $1*10^7$conidia/ml
(b) *Populus euramericana* + 0.3ppm of fipronil
(c) *Populus euramericana* + 0.3ppm of Imidacloprid
(d) Combinatorial bioassay using *Populus euramericana* + $1*10^7$ conidia/ml + 0.3ppm of fipronil + feeding stimulantants (agar 2g/100ml + 2ml sugarcane bagasse)
(e) Combinatorial bioassay using *Populus euramericana* + $1*10^7$ conidia/ml + 0.3ppm of Imidacloprid + attractant (agar 2g/100ml + 2ml sugarcane bagasse)

For laboratory bioassay all these treated combinations of wood blocks were conducted in three replicates i.e. in three Petri dishes with 148 worker and 2 soldiers of either *Odontotermes obesus* and *Coptotermes heimi* with a control parallel to determine the feeding on wood blocks under no choice experiment for two, four and six weeks. The lab no choice study was done by calculating the weight of each Petri plate alone and with the wood sample+matrix being given to the termites and as well as the bait is provided in a form of matrix so it was weighted along with wood sample and all the calculations were done before the placement of final value in the table. Treatment and control materials were preweighed prior to the introduction of termites. The mortality data was added in the paper as it was calculated but not added in article because we think we are more concern with consumption as well as field data was also related to consumption that's why we skip it but now it is a part of updated paper as well as control data was also added.

However, for field bioassay all these treated blocks of *P. euramericana* were tied with copper wire into a bundle separately with three replicates of each and placed into mound buried up to 30 cm at different active places of the nest. Control with untreated woods was also driven into the nest for comparison. The bioassay duration lasted for 24 weeks which is effective from Ist July 2014 -31st Jan 2015, and follow up studies for the remaining period up to March 2015. Monthly inspection of stations will be carried out. Three replicates (n=3 i.e. indicates "to repeat" (a scientific experiment) to confirm findings or ensure accuracy) of each bait treatment were prepared both for laboratory and field trial. While, five termites colonies were selected in field for each treatment.

Statistical Analysis

When no-choice laboratory bioassay was conducted, data on wood consumption (%) were subjected to the analysis of variance (one-way ANOVA). Data obtained from laboratory and field bioassays were statistically analyzed using Tukey's test.

Results

Bait Treatments under lab conditions using no-choice bioassay

Different bait treatments were used to observe the consumption by *O. obesus* and *C. heimi* workers and soldiers under no choice laboratory bioassay for 2, 4 and 6 weeks. In both termites highest consumption was noted on combinatorial treatments with feeding stimulants.

In case of *O. obesus*, after 4 weeks the baits with feeding stimulants showed significantly higher consumption i.e. 38.5±0.1 mg and 45.4±0.2 mg in comparison to other treatments and control which gives 33.6±0.1 mg respectively. However, the rate of mortality after 4 weeks was 38.5±0.1 and 45.4±0.2% in contrast to control which showed 35.7±0.4% respectively.

Consumption of bait after 2 and 6 weeks were not significantly different from each other. After 2 weeks maximum consumption was 23.5±0.3 and 26.5±0.1mg with 27.3±0.1 and 30.2±0.2% mortality whereas, 27.5±0.2 and 29.4±0.1mg consumption and 55.2±0.1 and 56.2±0.3% mortality were observed after 6 weeks. However the minimum consumption was noted in PE+MA treatment 13.5±0.1, 15.4±0.2 and 14.9 ±0.3mg with 36.3±0.2, 40.3±0.1 and 62.2±0.1% mortality was noted after 2, 4 and 6 weeks respectively (Table 1).

Same results were observed for *C. heimi* where maximum consumption was noted after 4 weeks. 30.5±0.1 and 40.2±0.3 mg consumption was observed in bait with feeding stimulants however, the rate of mortality was 42.4±0.1 and 44.2±0.2%

Fig 1 (A-B). Showing bait matrix treated blocks tied with copper wire (A-B) site selection at Wagha border and installation of bait delivery matrix inside mounds of Odototermes obesus under no choice field trials (C-E) Queen of Odontotermes obesus with physogastry (F) (Magnification=100X).

respectively as compare to control and other treatments which showed 39.8±0.2 mg consumption with 37.8±0.2% mortality after 4 weeks. After 2 and 6 weeks, high rate of mortality was indicated with low consumption of bait. 24.4±0.4 and 36.4± 0.3 mg consumption and 28.3±0.2 and 31.8±0.1% mortality was measured after 2 weeks whereas, at the end of 6 weeks bait with feeding stimulants was consumed to be 26.5±0.5 and 38.5±0.3 mg with 54.2±0.2 and 56.3±0.1% death rate respectively. While the lowest consumption was detected on PE+MA treatment i.e.14.6±0.1, 19.4±0.3 and 17.2±0.2 mg with33.8±0.3, 52.1±0.1 and 64.2±0.1% mortality at various weeks (Table 2).

Bait treatments under field conditions under no choice bioassay

The mean wood consumption by *O. obesus* and *C. heimi* at the end of the experiment is an important measure against which all bait treatments should be compared under no choice field bioassay for 24 weeks. Combinatorial treatments with feeding stimulants showed higher consumption in comparison to other treatments. As well as, bait which was loosely bound indicates greater termite activity as compared to tightly bound.

In case of *O. obesus*, bait with feeding stimulants gives 42.2±0.2 and 44.2±0.1mg in tightly bound wood blocks whereas,

Table 1. Shows mean wood consumption and mortality (±S.D) under no choice laboratory bioassay by *Odontotermes obesus* for 2, 4 and 6 weeks. (n=3)

Treatments	Mean wood consumption (mg)		
	2 weeks	**4 weeks**	**6 weeks**
CONTROL(PE)	21.8±0.1^{c}	33.6±0.1^{b}	22.30.2^{a}
PE+MA	13.5±0.1^{e}	15.4±0.2^{e}	14.9±0.3^{e}
PE+FIP	15.4±0.4^{e}	18.3±0.5^{d}	16.7±0.3^{de}
PE+IMI	18.2±0.2^{d}	22.4±0.1^{c}	19.6±0.2^{d}
PE+FIP+MA+ATT	23.5±0.3^{c}	38.5±0.1^{b}	27.5±0.2^{c}
PE+IMI+MA+ATT	26.5±0.1^{c}	45.4±0.2^{a}	29.4±0.1^{c}
	Mean Mortality (%)		
CONTROL(PE)	20.2±0.1^{a}	35.7±0.4^{c}	50.4±0.2^{b}
PE+MA	36.3±0.2^{c}	40.3±0.1^{b}	62.2±0.1^{b}
PE+FIP	38.3±0.4^{c}	58.9±0.4^{a}	80.2±0.3^{c}
PE+IMI	40.2±0.3^{c}	60.7±0.3^{cd}	85.5±0.2^{d}
PE+FIP+MA+ATT	27.3±0.1^{d}	40.5±0.2^{a}	55.2±0.1^{c}
PE+IMI+MA+ATT	30.2±0.2^{e}	41.2±0.1^{ab}	56.2±0.3^{e}

Note: Means followed by different letters within a column differ significantly at 0.05 (P < 0.05).
Populus euramericana + 1*10^{7} conidia/ml (*Metarhizium anisopliae)* (PE+MA), *Populus euramericana*+0.3ppm of fipronil (PE+FIP), *Populus euramericana* + 0.3ppm of Imidacloprid (PE+IMI), *Populus euramericana* +1*10 conidial/ml (*Metarhizium anisopliae)* + 0.3ppm of fipronil + Feeding stimulants (agar 2g/100ml + 2ml sugarcane bagasse) (PE+MA+FIP+ATT) and *Populus euramericana* + 1*10^{7} conidial/ml (*Metarhizium anisopliae)* + 0.3ppm of Imidacloprid + Feeding stimulants (agar 2g/100ml + 2ml sugarcane bagasse) (PE+MA+IMI+ATT).

Table 2. Shows mean wood consumption and mortality (±S.D) under no choice laboratory bioassay by *Coptotermes heimi* for 2, 4 and 6 weeks. (n=3)

Treatments	Mean wood consumption (mg)		
	2 weeks	**4 weeks**	**6 weeks**
CONTROL(PE)	24.6±0.1^{a}	39.2±0.2^{b}	32.30.1^{c}
PE+MA	14.6±0.1^{d}	19.4±0.3^{c}	17.2±0.2^{cd}
PE+FIP	15.4±0.2^{d}	20.3±0.5^{c}	19.6±0.5^{c}
PE+IMI	18.5±0.5^{c}	28.2±0.2^{b}	22.4±0.1^{bc}
PE+FIP+MA+ATT	24.4±0.4^{b}	30.5±0.1^{ab}	26.5±0.5^{b}
PE+IMI+MA+ATT	36.4± 0.3^{a}	40.2±0.3^{a}	38.5±0.3^{a}
	Mean Mortality (%)		
CONTROL(PE)	28.4±0.1^{a}	37.8±0.3^{c}	52.2±0.2^{b}
PE+MA	33.8±0.3^{c}	52.1±0.1^{c}	64.2±0.1^{b}
PE+FIP	41.2±0.1^{a}	62.9±0.3^{c}	83.2±0.1^{bc}
PE+IMI	45.1±0.1^{e}	63.8±0.2^{c}	85.5±0.1^{d}
PE+FIP+MA+ATT	28.3±0.2^{c}	42.4±0.1^{cd}	54.2±0.2^{a}
PE+IMI+MA+ATT	31.8±0.1^{a}	44.2±0.2^{ab}	56.3±0.1^{a}

Note: Means followed by different letters within a column differ significantly at 0.05 (P < 0.05).
Populus euramericana + 1*10^{7} conidia/ml (*Metarhizium anisopliae)* (PE+MA), *Populus euramericana*+0.3ppm of fipronil (PE+FIP), *Populus euramericana* + 0.3ppm of Imidacloprid (PE+IMI), *Populus euramericana* +1*10 conidial/ml (*Metarhizium anisopliae)* + 0.3ppm of fipronil + Feeding stimulants (agar 2g/100ml + 2ml sugarcane bagasse) (PE+MA+FIP+ATT) and *Populus euramericana* + 1*10^{7} conidial/ml (*Metarhizium anisopliae)* + 0.3ppm of Imidacloprid + Feeding stimulants ttractant (agar 2g/100ml + 2ml sugarcane bagasse) (PE+MA+IMI+ATT).

76.4±0.1 and 80.4±0.3 mg was observed in loosely bound bait matrix as compared to control which showed 40.1±0.2 and 75.3±0.1 mg consumption respectively. However, in other treatments wood consumption in tightly and loosely bound wood blocks are 39.6±0.3, 20.4±0.1 and 28.5±0.2 mg and 50.7±0.2, 32.4±0.3 and 43.8±0.4 mg, respectively (Table 3).

Table 3. Shows mean wood consumption (±S.D) for tightly and loosely bound wood blocks under no choice field bioassay by *Odontotermes obesus* for 24 weeks.

Treatments	Mean wood consumption (mg)	
	Tightly bound	**Loosely bound**
CONTROL(PE)	40.1±0.2^{a}	75.3±0.1^{bc}
PE+MA	39.6±0.3^{c}	50.7±0.2^{ab}
PE+FIP	20.4±0.1^{d}	32.4±0.3^{c}
PE+IMI	28.5±0.2^{cd}	43.8±0.4^{b}
PE+FIP+MA+ATT	42.2±0.2^{b}	76.4±0.1^{a}
PE+IMI+MA+ATT	44.2±0.1^{b}	80.4±0.3^{a}

Note: Means followed by different letters within a column differ significantly at 0.05 (P < 0.05).
Populus euramericana + 1*10^{7} conidia/ml (*Metarhizium anisopliae)* (PE+MA), *Populus euramericana*+0.3ppm of fipronil (PE+FIP), *Populus euramericana* + 0.3ppm of Imidacloprid (PE+IMI), *Populus euramericana* +1*10 conidial/ml (*Metarhizium anisopliae)* + 0.3ppm of fipronil + Feeding stimulants (agar 2g/100ml + 2ml sugarcane bagasse) (PE+MA+FIP+ATT) and *Populus euramericana* + 1*10^{7} conidial/ml (*Metarhizium anisopliae)* + 0.3ppm of Imidacloprid + Feeding stimulants (agar 2g/100ml + 2ml sugarcane bagasse) (PE+MA+IMI+ATT).

While with respect to *C. heimi* similar results was analyzed bait treatments with feeding stimulants showed maximum consumption in loosely bound wood blocks i.e. 80.7±0.1 and 86.6±0.2 mg in contrast to tightly bound which showed 43.4±0.3 and 45.2±0.1 mg. However, control treatment signifies 42.2±0.2 and 78.3±0.3 mg consumption in tightly and loosely bound woods respectively. Similarly, in other treatments the mean wood consumption observed was 38.5±0.1, 22.7±0.4 and 32.4±0.5 mg in tightly bound and 54.3±0.2, 43.3±0.1 and 47.8±0.3 mg was calibrated in loosely bound bait matrix (Table 4).

Changes recorded in mound/ nest after bait application

After 24 weeks the mounds of *O. obesus* and nests of *C. heimi* were dissected. Over the course of the experiment, all bait stations were infested by termites. In general, termites covered the top surface of the bait station and termite trap with mud whereas, bait matrix was consumed. The consumption data under controlled as well as treated conditions were analyzed. Both in mound and nest of termites revealed interesting results. In case of mound, although king caste was unable to be detected, whereas, queen of the mound was present in the royal chamber found dead (Figure 1f).

Similarly, in nest of *C. heimi* we could not locate both of reproductive castes, however, different workers and soldiers were found in aggregated form as dead in the dissected nest. A strong malodor/smell was emitted from the inner nest

Table 4. Shows mean wood consumption (±S.D) for tightly and loosely bound wood blocks under no choice field bioassay by *Coptotermes heimi* for 24 weeks.

Treatments	Mean wood consumption (mg)	
	Tightly bound	**Loosely bound**
CON(PE)	42.2±0.2[a]	78.3±0.3[a]
PE+MA	38.5±0.1[b]	54.3±0.2[ab]
PE+FIP	22.7±0.4[d]	43.3±0.1[b]
PE+IMI	32.4±0.5[c]	47.8±0.3[b]
PE+FIP+MA+ATT	43.4±0.3[b]	80.7±0.1[a]
PE+IMI+MA+ATT	45.2±0.1[b]	86.6±0.2[a]

Note: Means followed by different letters within a column differ significantly at 0.05 (P < 0.05).
Populus euramericana + 1*10^7 conidia/ml (*Metarhizium anisopliae)* (PE+MA), *Populus euramericana*+0.3ppm of fipronil (PE+FIP), *Populus euramericana* + 0.3ppm of Imidacloprid (PE+IMI), *Populus euramericana* + 1*10 conidial/ml (*Metarhizium anisopliae)* + 0.3ppm of fipronil + Feeding stimulants (agar 2g/100ml + 2ml sugarcane bagasse) (PE+MA+FIP+ATT) and *Populus euramericana* + 1*10^7 conidial/ml (*Metarhizium anisopliae)* + 0.3ppm of Imidacloprid + Feeding stimulants (agar 2g/100ml + 2ml sugarcane bagasse) (PE+MA+IMI+ATT).

and mound due to the presence of dead and decaying termite cadavers in a number of spots inside, especially near the nursery zone. Small numbers of workers and soldiers were observed, whereas none of the immature castes (e.g., larvae and nymphs) were found inside the nest. Overall, treated colonies experienced a 90-95% decrease in population size after 6 months of baiting. Fast-growing fungus was found growing on the carton material in the nursery zone or growing on termite cadavers. The queen was dark yellow in color, flaccid and physogastric.

Discussion

Results from this study show that bait systems can be used effectively to reduce subterranean termite population's area wide. Consumption by termites on treated baits in ascending order was documented as: PE+MA= PE+FIP <PE+IMI <PE+ FIP+MA+ATT = PE+IMI+MA+ATT. Consumption of the PE+MA bait matrix was significantly higher than other bait treatments by *O. obesus* and *C. heimi* at 6 and 8 weeks. Reduced consumption seen on treatments containing imidacloprid and fipronil only, is due to the mortality occurring more rapidly as compared to treatments with feeding stimulants. The substrate is the same between the PE+MA bait and other baits matrices, this shows that the matrix can have an influence on the amount of termite consumption. Termite species-specific behaviors such as speed and amount of consumption may influence the time to mortality after consumption of the bait (Wood, 1978; Delaplane & LaFage, 1989). Mortality was achieved over time, and the slightly delayed toxicity may provide additional time for trophallaxis to occur, which in turn may lead to better overall colony elimination by keeping insecticides in the colony longer. For a bait to be successful, it must be palatable and toxicologically active across termite species.

Eger et al. (2012) and McKern-Lee et al. (2010) studied that durable bait matrix will reduce the costs associated with frequent monitoring, reduce the disturbance for sensitive species and potentially provide the opportunity for baiting across large areas. Hamm et al. (2013) worked on different termites and observed that consumption of the blank durable bait matrix was significantly higher than consumption of a blank preferred textured cellulose matrix (PTC) when both contained the active ingredient noviflumuron. All bait treatments resulted in significant mortality relative to the untreated controls.

Agar as major components of termite bait, agar has been used by a number of researchers (Spragg & Fox, 1974; Patton & Miller, 1980; Spragg & Paton, 1980; Su et al., 1982; Easey, 1983, 1985; French & Robinson, 1984; Holt & Easey, 1985; Easey & Holt, 1988, 1989; Miller, 1990; Su, 1994). Maximum bait consumption and termite tunneling activities recorded with 3% concentration. This may be due to the fact with the increasing agar concentrations, the bait becomes more solid and termites prefer semi solid medium than soft medium. Baiting systems may provide long lasting control by suppressing termite activity. Studies testing the efficacy of different bait materials in managing O. obesus proved that sugarcane bagasse was more attractive to O. obesus and also rendered the colony weak (Rajavel et al., 2007). Su et al. (1984) while evaluating insecticides noted that higher concentration of agar stimulated tunneling behavior in termites. Huang et al. (2006) used fipronil, which is a neurotoxin and normally considered to be fast acting, in baits against *Odontotermes formosanus* in Wuhan, China. The baits 'suppressed' termite populations in 3-4 months, which is not particularly fast (NB 'suppressed' meant no active termites in their stations for 10-12 months in two of three sites, which other authors consider to be 'eliminated'). The longer time to control *O. formosanus* is probably because it is a fungus growing termite, and delivery of toxicants in food to these termites is more complex than for the wood-feeding rhinotermitids.

Of the two attributes tested in the field study, related to compaction were consistently important in affecting bait consumption. More of the wood that binds loosely was consumed more as compared with that which was tightly bound. Highest consumption in different bait treatments by *O. obesus* was observed in combinatorial treatments (PE+FIP+MA+ATT and PE+IMI+MA+ATT) with feeding stimulants (42.2mg) and (44.2 mg) in tightlty bind and 76.4 and 80.4mg in loosely bind treated woods; same results were obtained in case of *C. heimi* for these treatments with 43.4 and 45.2mg for tightly, 80.7 and 86.6mg loosely bound woods. However in other treatments the rate of consumption was comparatively low.

The success of loosely bound wood has one simple explanation; the greater surface area of wood immediately available for gnawing. The results of the present study showed that changes in the presentation of materials in the bait station can efficiently effects on bait matrix consumption. Evidently, much more work on food and foraging preferences is required to design more efficacious baiting protocols. According to Evans et al. (2006) for wood-eating termites, bait stations should be designed to encourage termite presence and to maximize their consumption of bait matrix in order to expedite control in minimal time. They examined the effect of bait size, compaction, and composition on termite presence for four months and suggested that all three factors were significant, with bait size the most important factor, followed by compaction and then composition.

Wang and Henderson (2012) studied the consumption efficiency of two commercially used termite bait materials, southern yellow pine wood and cardboard, and one potential bait material, maize (*Zea mays*) cob, against the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae), under laboratory conditions. They observed that in no-choice test, the consumption of wood and cob was similar and significantly more than cardboard while in the two-choice test, the consumption was cob > wood, wood > cardboard, cob = cardboard whereas, in the three-choice test, no significant difference was detected in consumption.

It is difficult to compare previous baiting studies as these normally had the aim of demonstrating that elimination was possible, whereas the current study aimed to determine the time to elimination. The high variability in time to control (24 weeks) reported in previous studies would depend on toxicants, concentrations, termite species, colony sizes, environment geography, season, and so forth; for example, wood eating *Reticulitermes* or *Coptotermes* species (family: Rhinotermitidae) in single structures or smaller areas (Su, 1994; Forschler & Ryder, 1996; Tsunoda et al., 1998; Su et al., 2000; Cabrera & Thomas, 2006), to multiple structures in larger areas (Su et al., 2002; Su & Hsu, 2003; Rojas & Morales-Ramos, 2003; Ripa et al., 2007) to fungus culturing *Odontotermes* and *Macrotermes* species (family Termitidae, subfamily Macrotermitinae) termites in dams (Huang et al., 2006; Wang et al., 2007).

The current study indicates the efficacy of termite baits against rhiniotermitids and termitids. Peters et al. (2008) reported successful colony suppression and elimination of termitids including a higher termite *Macrotermes gilvus* with chlorfluazuron baits. In the current study, on the average *O. obesus* and *C. heimi* ingested toxicants with different treatments both under laboratory and field conditions for six months baiting period, but one of colony of *O. obesus* was completely suppressed and eliminated and *C. heimi* colony was found suppressed. But with regards to field consumption data, contact activity of toxicants is also important with the elimination or suppression of colony.

This finding demonstrates the complexity and challenges faced when trying to manage fungus growing termites using baiting systems. Although a maximum amount of bait was removed by termites, but toxicant was not shared entirely and digested among nest mates in case of *C. heimi* colony. The bait matrix (*P. euramericana*) was detected in food stores and fungus comb, indicating that toxicants were integrated into the food processing pathway of termites. The bait matrix was picked up by termites approximately a week and subsequently excreted as fecal pellets. This lag of toxicants distribution to the termite castes explains why imidacloprid especially take longer to affect termites than wood feeding rhinotermitids. The colonies were moribund in termitids colony lost their reproductive capacity, as evidenced by the unhealthy and dead queen and absence of newly produced off springs. This could happen as early as a month after the baiting began, as large number of workers in the combs was observed at this point. Two possible explanations may account for the low worker output in the colony. Firstly, toxicant along with other treatment combination could have indirectly caused the worker population decline over time. Furthermore, foraging activity of workers declined because of number of workers present was insufficient to initiate foraging activity. Second, the queen appeared unhealthy. This likely reflects the fact that queen receives less food, nursing, and grooming by workers following the drastic decrease in the worker population, which in turn reduced their reproductive capacity. This finding is also reported by other termite investigators in other parts of the world (Peppuy et al., 1998; Rajos & Morales-Ramos, 2004; Haagsma & Rust, 2005). Nevertheless, we don't deny the possibility of toxicants eliminating or suppressing termites both lower and higher colonies if the test colonies are given more time to accumulate up to lethal level. The results help explain the mixed performance of bait against termites both rhinotermitids and termitids group. One of the factors that limit the widespread collective impact is the unique caste developmental pathways and incorporation of other effects (fungus, additives like agar and attractants) pose a great challenge in termite management programs. Our results suggested that colonies require intensive baiting efforts to allow sufficient active ingredient diffuse into the colony, either by installing more bait stations or extending the baiting period. In addition, it is also believe that bait toxicants and soil treatment to find out ways to target both the immature stages and the worker population. Such treatments are needed badly to increase the chances of successful termite colony elimination in lower as well as higher termites.

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