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Dissemination of *Metarhizium anisopliae* infection among the population of *Odontotermes obesus* (Isoptera: Termitidae) by augmenting the fungal conidia with attractants

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ABSTRACT

Long-term biocontrol strategies for termite management have limited success as the behavioral response exhibited by termites in the presence of entomopathogenic fungi was different. To minimize these responses, a study was conducted to attract the termites towards the treated area by augmenting fungal conidia with a mixture of attractants such as sugarcane bagasse, sawdust and cardboard powder. In laboratory experiments, mortality due to the horizontal transmission of *Metarhizium anisopliae* infection among *Odontotermes obesus* workers and soldiers was 50 to 98% and 16 to 78% for the five isolates tested. The foraging activity of workers and soldiers increased from 23 to 58% for IWST-Ma13 when conidia were mixed with attractants. In a field study, the weight loss of stakes treated with conidial attractant decreased to 10.9 g compared to dry conidial treatments (19.3 g) for IWST-Ma13 after five months. Similarly, the width of mud galleries covering the tree bark was reduced when treated with conidial baits and gunny bags containing conidial attractant. In treated mounds, as the Acoustic emission signal voltage decreased from the threshold voltage level, the relative magnitude signal (RMS) remains low after five months of treatments with that of the control. From this study it was observed that the mixing of conidia with attractants could augment the dissemination potential of fungal infection among the termites. By adapting attractant based *Metarhizium* baits and dusting of conidial attractant mixture inside mounds may augment the dissemination potential in epizootic transmission of fungal infection.

Introduction

Termites are one of the most problematic pests and there are over 2800 described species of termites with approximately 185 considered pests (Lewis, 1997). They are abundant in tropical and subtropical environments. They become economically important pests when they started to destroy the wood and wooden products of human homes, building materials, forests, agriculture crops and other commercial products (Monica et al., 2009). The major mound building termite species like *Odontotermes obesus* Rambur, *O. redemanni* Wasmann, *O. wallonesis* Wasmann, *O. horni* Wasmann, *Heterotermes indicola* Wasmann, *Coptotermes kishori* Roonwal & Chhotani, *C. heimi* Wasmann, *Microtermes obesi* Holmgren, *Trinervitermes biformis* Wasmann and *Microcerotermes beesoni* Snyder attack the bark and heart wood of standing trees such as *Butea monosperma* (Lam.) Taub., *Dipterocarpus indicus* Bedd., *Eucalyptus* sp., *Pterocarpus marsurpium* Roxburgh, *Santalum album* L., *Shorea robusta* Roth., *Terminalia bellirica* (Gaertn.) Roxb. *Swietenia macrophylla* King., *Dalbergia sissoo* Roxb., *Pinus wallichiana* A. B. Jacks., *Tectona grandis* Linn., *Toona cilita* M. Rome., *Haldina cordifolia* (Roxb.) Ridsdale etc. (Rajagopal, 2002; Remadevi et al., 2005).

Although termites are excellent decomposers of dead wood and other sources of cellulose, they become a serious problem when they attack standing trees, logs, and crops. Therefore, effective control methods such as physical, chemical and biological methods were extensively studied and exploited by many researchers. Toxic physical barriers (Chlorfenapyr) and non-toxic physical barriers (sand or gravel aggregates, metal mesh, or sheeting) have been used to prevent subterranean termite attacks on wooden structures (Rust and Saran, 2006; Monica et al., 2009). Chemical treatment measures are one of the various techniques used to reduce the infestation of termites. Several termiticides containing active ingredients: bifenthrin, chlorfenapyr, cypermethrin, fipronil, imadaclopride and permethrin are registered for termite control around the world under various brand names (Ahmed et al., 2006; Monica et al., 2009). Although physical and chemical control is a proven means of protection from termites, its excessive use is economically

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high and harmful to the environment. Hence, new eco-friendly control methods like plant derived natural products (Park and Shin, 2005; Mao et al., 2006; Cheng et al., 2007), entomopathogenic fungi (Monica et al., 2009), nematodes (Weeks and Baker, 2004) and bacteria (Devi et al., 2006) are being developed by researches as alternative control methods for the management of termites.

Fungal entomopathogens are diverse and globally ubiquitous natural enemies of arthropods, which have been of considerable research, focusing on their potential as microbial control agents (Eilenberg et al., 2001; Hajek et al., 2005). Although entomopathogenic fungi are widely applied for several pest management practices, their sensitivity is considered to be limited especially in the management of termites. The complex social behavior such as pathogen alarm behavior (Rath, 2000; Mburu et al., 2009), avoidance of treated area (Rath, 2000) and burial of fungus killed nest mates (Rath, 2000) exhibited by termites are found to be a barrier in causing epizootic among the nest mates. In order to understand this complex behavior exhibited by termites many researchers studied the behavioral responses of the termites to the conidia and volatile compounds of entomopathogenic fungi (Mburu et al., 2009; Hussain et al., 2010) and found that virulent strains are more likely to be recognized from some distance and avoided. Such bondages reduce the development of effective long term control strategies for the management of termites. However, the other social behaviors such as the grooming of nest mates and proctodeal trophallaxis are helpful in the spread of infection among termites that can be effectively used for the entomopathogenic fungi control of termites (Rath, 2000). With this background in the present investigation we have successively minimized the repellency behavior of the termites to the fungal conidia or treated area and render them non-repellent by mixing the dry conidia with the cellulose based components such as sugarcane bagasse, cardboard powder and sawdust.

Materials and methods

Fungal cultures

Five isolates of *Metarhizium anisopliae* were collected and isolated from different insect orders. Among which, four test isolates, two isolates from the order Lepidoptera-mummified larvae (IWST-Ma1 and IWST-Ma2), one each from Coleoptera-*Oryctes rhinoceros* Linnaeus (IWST-Ma13) and Isoptera-*Coptotermes* sp. (IWST-Ma16) were used. One isolate ARSEF 7413 (*Kalotermes* sp.) that was collected from Agriculture Research Service for Entomopathogenic Fungi (ARSEF) was used as the standard for comparative studies with the native isolates of *M. anisopliae*. The strains were maintained at Sabouraud dextrose medium (Hi-Media) fortified with 1% yeast extract at 26 ± 0.3 °C in complete darkness.

Harvesting of the fungal conidia

The fungal isolates were mass multiplied on the solid substrate using rice grains. Approximately 1 kg of broken rice (variety IR-20 and IR-50) was soaked in distilled water up to 12 h and 100 g of dried rice was transferred separately into ten polythene bags (HiDispo Bag™; size — 40 cm h × 20 cm b). The mouth of the bags was covered by inserting the tip of the bags into plastic pipes (5 cm h × 3 cm b). The neck of the bags was plugged with cotton and autoclaved. A spore suspension of 10 ml was inoculated into the rice bags separately and incubated at 26 ± 0.3 °C in the dark. After twelve days of incubation, the sporulated grains were removed from the bags and dried in the biosafety cabinet for 18–24 h to remove excess moisture. The dry spores were harvested in sterile glass vials using a MycoHarvester (Model-MH5; Make-UK) and stored at −20 °C.

Preparation of dust and bait mixture

About 10 g of harvested spores was mixed in 100 g of mixture containing sugarcane bagasse (50 g), cardboard (20 g) and sawdust (30 g). To make the bait mixture, 30 ml of starch water was added and mixed in a Speed Mixer at 3000 rpm for 5–10 min to obtain a semi-solid paste. The mixture was poured into the ice-cube trays for the preparation of bait cubes.

Horizontal transmission of fungal infection

The horizontal transfer of fungal infection among the termite *O. obesus* was studied for five isolates of *M. anisopliae*, where twenty workers and twenty soldiers were allowed to walk over the partially dried fungal suspensions of 10^8 conidia ml^{-1} separately for 1 min. The treated twenty workers and twenty soldiers were transferred separately to plastic trays of size 60 cm (l) × 30 cm (b) × 15 cm (h) containing eighty workers (untreated) and eighty soldiers (untreated) with small pieces of rubber wood (*Hevea brasiliensis*) and pieces of fresh fungal combs. The trays were covered with a dark moist cloth and incubated at 26 ± 0.3 °C in darkness. For each isolate, five replicates, each with twenty (treated) and eighty (untreated) individual workers and soldiers were maintained separately, and mortality was recorded at 24 h intervals for ten days. A batch of 100 workers and soldiers of infected termites was maintained separately as the control. Dead insects were incubated in a humid chamber to confirm the growth of the fungus on cadavers.

Foraging activity of termites to dry conidia and conidia mixed with attractant

Repellency of the workers and soldiers of *O. obesus* to the five isolates of *M. anisopliae* was evaluated by modifying the method described by Mburu et al. (2009) and Hussain et al. (2010). Plastic trays (size: 90 cm (l) × 60 cm (b) × 30 cm (h)) were filled with mound soil (up to 15 cm). Each tray was separated equally into two compartments A and B with nylon gauze (60-mesh size) where compartment A served as the releasing site for the termites. In the treatments, the first set contains (A — termite comb and rubber wood; B — dry conidia 0.1 g, termite comb and rubber wood), the second set contains (A — termite comb and rubber wood; B — bait without conidia, termite comb and rubber wood) and the third set contains (A — termites comb and rubber wood; B — bait with conidia, termite comb and rubber wood). About 100 workers and 50 soldiers were released in compartment A, where the releasing site was illuminated with a florescent bulb (220 V, 13 A, AC) for 3 min. The remaining portion of the trays was shielded with a dark moistened cotton cloth. The combination of brightness and darkness acted as a "push–pull" set of visual stimuli to induce the termites to move away from the release area towards the treated compartments. The number of termites foraging in the treated and control compartments together with those in their respective arms was recorded at an interval of 10 min up to 90 min to give nine readings for each replicate and mortality and mycosis was evaluated after 10 days of incubation at 26 ± 0.3 °C.

Stake test

The field efficacy of two effective isolates (IWST-Ma13 and ARSEF 7413) was carried out using rubber wood stakes. The stakes of 30.5 cm long and 3.18 cm square in the cross-section as per the Indian Standard (IS: 4833-1968); were treated with the dry conidia, conidia mixed with attractant and attractant alone by spreading the mixture over the stakes. Six testing sites in the plantation of sandal wood that was infected by *O. obesus* were selected for laying out the specimen for tests. Six replications of each treatment and in each sample along with three controls (untreated) were buried half below and half exposed above the ground in horizontal and vertical rows of 60 cm apart. The mortality, mycosis of termites sticking on the stakes and weight loss of the stakes were observed for five months by monthly intervals.

Tree treatments

The sandal tree bark covered with mud galleries of *O. obesus* was selected around the plots. The experiments were set up as follows; the mud galleries were gently removed up to a diameter of 30 cm in the middle of the infected stem (from base two 60 cm height) leaving the other part intact. The gunny bags (30 cm) dusted with dry conidia, conidia mixed with attractant and only attractant were tied separately over the 30 cm area between the mud plasters on the stem. The mortality, mycosis and width of galleries roofed over the gunny bags were observed at an interval of (4, 7, 14, 28, 42, 56, 70, 84 and 98 days) for a period of three months. The control trees were treated with gunny bags containing attractants only. Five replicates of twenty-five trees each for each treatment were maintained.

Soil treatments

The soils were removed up to a depth of 15 cm around 30 cm away from the center of the stem of sandal tree. The mean average height of the mud galleries on each branch of the stem was measured and marked before starting the experiments. A quantity of eight bait cubes was buried around the stem of each plant. The width of the mud plaster covering the branches, mortality and mycosis was observed in an interval of (4, 7, 14, 28, 42, 56, 70, 84 and 98 days) for a period of three months. The control trees were treated with only baits without conidia. Five replicates of twenty-five trees each for each treatment were maintained.

Mound treatments

Two to three year old active colonies of *O. obesus* were selected fifteen days before the experiment by asking the farmers/forest guards about the age of each mound in the sandal plots. The activity of each mound was determined at the time of selection using a “hole repair method” where, rectangular holes on each side of the active mounds were dug down to the level of the termite comb. The volume of the mound material removed was measured and the size of hole was measured after 24 and 48 h. Mounds with holes sealed within 48 h were considered as active mounds. Conidial dust (100 g) mixed with the attractant was applied by blowing them through ventilation holes at the center (top) of the mound, with a small container connected to a bicycle pump and rubber hose. During the operation, cardboard was used to cover the hole to prevent the spore dust from puffing out of the artificial hole. After applications, the holes were covered with plastic sheets to prevent rainwater from entering the holes. The activity of termite colonies in the mound was measured by an AED-2000L (Acoustic Emission Consulting) Insect pest detection kit at an interval of 0, 1st, 3rd and end of 5th months. About twenty five readings per mound were measured and the average was graphically represented as the RMS value. The mounds were cut open to observe mortality and mycosis after five months of treatments.

Data analysis

Mortality observed in the controls was used to correct mortality in the treated groups using Abbott's formula ($P = C - T/C \times 100$) $P = \%$ of corrected mortality, $C =$ no. of. insects alive in the control, $T =$ no. of insects alive in the treatment (Abbott, 1925). The data from the mortality, mycosis and sporulation estimates were subjected to arc sine transformation and analyzed using analysis of variance. The least significant difference test was used to compare the means. All the analyses were carried out using SPSS 11.0 for Windows. The activity of termite colonies in the mound was measured by an AED-2000L (Acoustic Emission Consulting) Insect pest detection kit. The relative potency for each treatment was calculated by dividing the weight loss of the stake with each test isolate (native isolate) by that of standard isolate (ARSEF strains) (Houping et al., 2002).

Results

Horizontal transmission of fungal infection

The workers of *O. obesus* exposed to the concentration of 1×10^8 conidia ml^{-1}, were groomed more frequently than unexposed control workers. The mortality and mycosis of the workers were highest when groomed with the individuals exposed to isolates IWST-Ma13 (98 and 90%) ($P = 0.275$, d_1-4, d_2-35 and $F = 1.339$) and ARSEF-7413 (90 and 87%) ($P = 0.649$, d_1-4, d_2-35 and $F = 0.623$) (Fig. 1a). A mortality of 16 to 78% ($P = 0.033$, d_1-4, d_2-35 and $F = 2.958$) and mycosis of 12 to 72% ($P = 0.345$, d_1-4, d_2-35 and $F = 1.161$) (Fig. 1b) were observed in the soldier populations. The transfer of fungal infection by IWST-Ma2 among the workers and soldiers was less with the lowest mortality of 56 and 32% and mycosis of 47 and 28% respectively.

Repellency of O. obesus in the presence of dry conidia and conidia mixed with attractant

The foraging activity of workers and soldiers in the presence of dry conidial powder and conidial attractant differed significantly among the population of *O. obesus*. In the dry conidial and conidial attractant applications the termites foraged were ranged from 0 to 39% ($P = 0.635$, d_1-4, d_2-35 and $F = 2.314$) and 15 to 78% ($P = 0.894$, d_1-4, d_2-35 and $F = 2.472$) (Figs. 2a and b) respectively. In the dry conidial application, foraging activity of the termites remained constant for 10 min and subsequently increased up to 90 min for all the isolates tested. However, for the two isolates IWST-Ma13 and ARSEF 7413 the activity started after 30 min and reduced significantly after 90 min intervals (Fig. 2a). The highest foraging activity of 39% was observed for IWST-Ma16 in contrast with the most virulent isolates IWST-Ma13 and ARSEF 7413 where the foraging activity was (23 and 22%) after 90 min intervals. In contrast, when the conidia were mixed with attractant, the foraging activity was significantly increased from 10 min to 90 min with the highest being 74% for IWST-Ma16 followed by IWST-Ma2 (Fig. 2b). Similarly, mortality and mycosis of termites in the foraging arenas were ranged from 22 to 68% ($P = 0.039$, d_1-4, d_2-35 and $F = 2.827$) and 10 to 79% ($P = 0.096$, d_1-4, d_2-35 and $F = 2.145$) (Fig. 2c) for dry conidial application. Though, there was no significant difference in mortality caused between the dry conidial and conidial attractant applications, the mycosis increased significantly with a range of 20–85% ($P = 0.066$, d_1-4, d_2-35 and $F = 2.436$) (Fig. 2d) for all the isolates tested.

Stake treatments

The mortality and mycosis of *O. obesus* from the stakes treated with dry conidia and conidial attractant were ranged from 13 to 78% ($P = 0.148$, d_1-3, d_2-28 and $F = 1.926$) and 10 to 56% ($P = 0.699$, d_1-3, d_2-28 and $F = 2.189$) (Table 1) after five months of treatment. When the stacks were treated with conidial attractant of IWST-Ma13 and ARSEF 7413, the mortality and mycosis of *O. obesus* increased significantly over a period of five months. Similarly, weight loss in the stacks increased significantly up to five months of treatments ($P = 0.036$, d_1-5, d_2-42 and $F = 2.645$) (Table 2). The lowest weight loss of 3.9, 5.6, 7.8, 8.8 and 10.9 g, respectively, after the 1st, 2nd, 3rd, 4th and 5th months was observed in the stacks treated with conidial attractant of IWST-Ma13. The relative potency indices

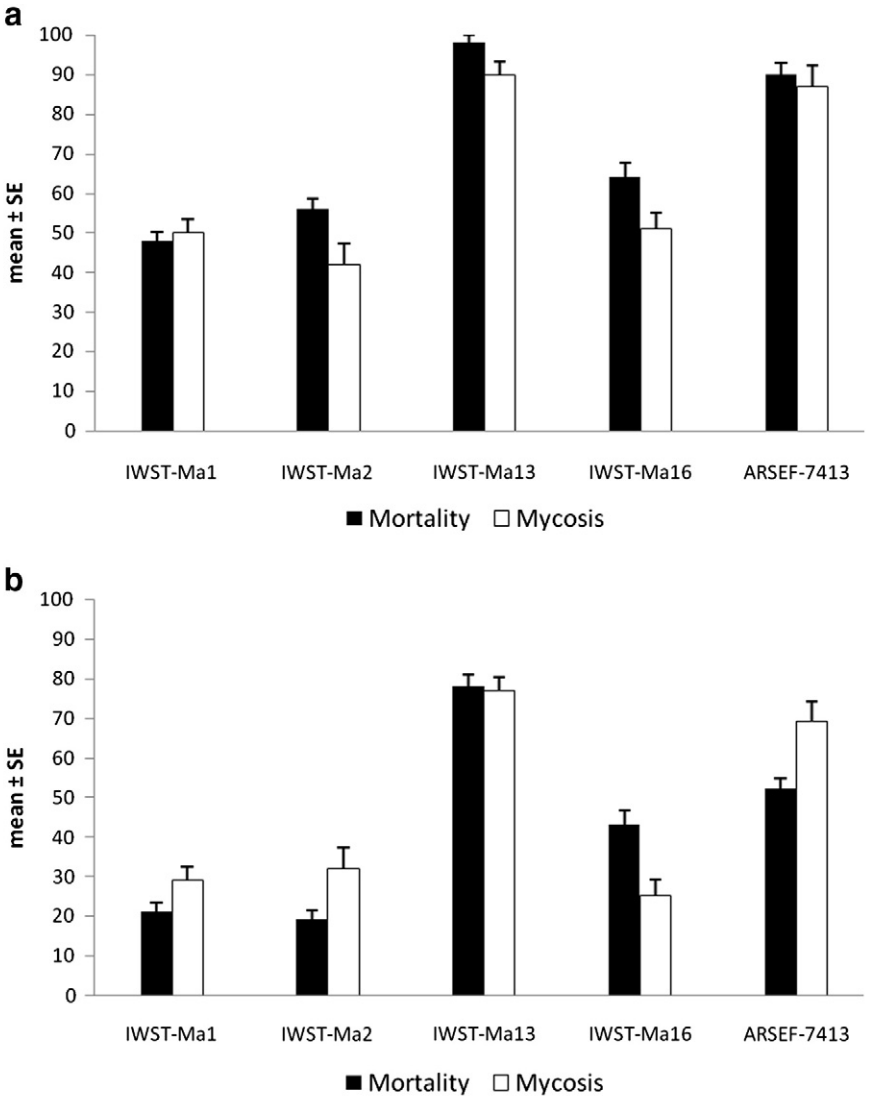

Fig. 1. a—Percentage mortality and mycosis of *Odontotermes obesus* workers, when 80% of the untreated workers were exposed to 20% of five *Metarhizium anisopliae* infected workers. The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$. b—Percentage mortality and mycosis of *Odontotermes obesus* workers, when 80% of the untreated soldiers were exposed to 20% of five *Metarhizium anisopliae* infected soldiers. The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$.

were lowest when the conidial attractant of IWST-Ma13 was applied (Fig. 3).

Tree treatments

The mortality and mycosis of termites caused by two isolates IWST-Ma13 and ARSEF 7413, when gunny bags were applied with dry conidia and conidial attractants, ranged from 0 to 12% and 26 to 33% ($P = 0.042$, d_1—4, d_2—25 and $F = 2.915$) and 0 to 51% and 72 to 89% ($P = 0.021$, d_1—4, d_2—25 and $F = 3.527$) after 98 days of treatment (Figs. 4a and b) respectively. The mortality and mycosis increased significantly after 98 days of treatment when the fungal conidia were mixed with attractants. Although the mortality was reduced after 42 days when conidia mixed with attractants were applied, the mycosis remained above 70% with a maximum of 89% for the isolate IWST-Ma13. In general, the mortality and mycosis for all treatments increased from 4 days to 42 days and significantly decreased after 42 days when dry spores were applied. Similarly, the width of mud galleries covered over the gunny bags was lowest at 15 cm for the IWST-Ma13 conidial attractant treatment and differed significantly with control gunny bags of 28 cm (treated only with attractant) ($P = 0.857$, d_1—5, d_2—32 and $F = 1.161$) (Fig. 4c). In contrast when the conidia were mixed with attractant, the width of mud galleries covered over gunny bags was significantly low (16 to 19 cm) for both the isolates tested after 98 days of treatment.

Soil treatments

The mortality of termites in the soil treated with baits of IWST-Ma13 and ARSEF 7413 ranged from 18 to 53% ($P = 0.673$, d_1—4, d_2—35 and $F = 2.864$) and mycosis from 10 to 67% ($P = 0.587$, d_1—4, d_2—35 and $F = 1.258$) after 98 days of treatment (Fig. 5a) with highest mortality and mycosis on the trees treated with baits of IWST-Ma13. Similarly, the width of the mud plasters covered over the branches ranged from 21 to 48 cm after 98 days of treatments ($P = 0.057$, d_1—5, d_2—35 and $F = 1.238$) (Fig. 5b). The width of mud plasters covered by the termites on the branches treated with baited conidial preparation was significantly different from dry conidial treatments.

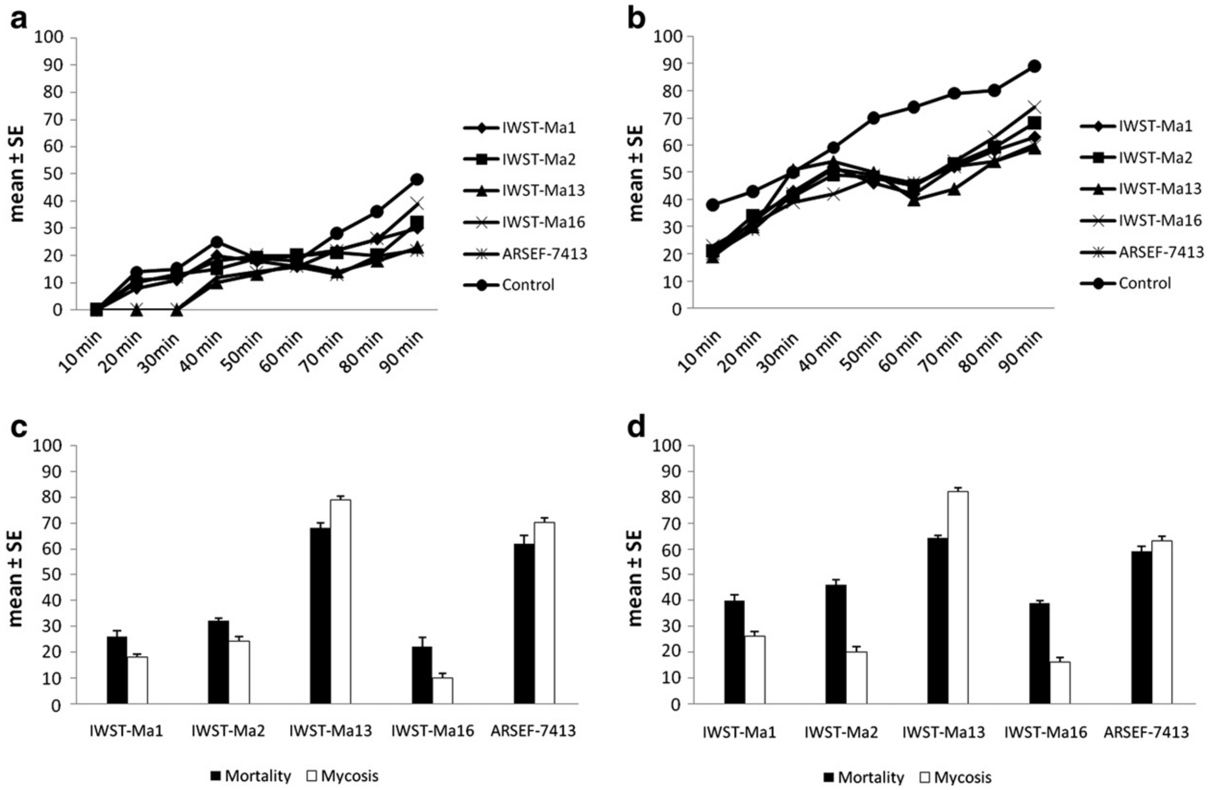

Fig. 2. a—Percentage of *Odontotermes obesus* foraged in treated and controls compartment applied with dry conidia of *Metarhizium anisopliae* together with those in their respective arms were recorded at an interval of 10 to 90 min. The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$. b—Percentage of *Odontotermes obesus* foraged in treated and control compartments applied with conidial attractant of *Metarhizium anisopliae* together with those in their respective arms were recorded at an interval of 10 to 90 min. The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$. c—Percentage mortality and mycosis of *Odontotermes obesus* foraged in treated and control compartments applied with dry conidia of *Metarhizium anisopliae* together with those in their respective arms were recorded at an interval of 10 to 90 min. The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$. d—Percentage mortality and mycosis of *Odontotermes obesus* foraged in treated and control compartments applied with conidial attractant of *Metarhizium anisopliae* together with those in their respective arms were recorded at an interval of 10 to 90 min. The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$.

Mound treatments

The 'head banging sound' emitted by the termite populations in the mound before treatments were measured as an Acoustic emission signal voltage. As this signal exceeds the threshold voltage level 0.5 V constantly in the control mound the relative magnitude signal expressed as a root mean square (RMS) remains maximum for 0 and 5 months after treatment (Fig. 6a). The ratio plotted for Volts and time showed that the peaks are high at 0 and 5 months, indicating that the ratio of the termite population (soldiers and workers) was high for a period of 20 min. As the activity of the termite populations decreased in the treated mound, the initial threshold voltage reading was zero for the 1st, 3rd and 5th months at 4, 12 and 16 min. In the treated mounds the threshold voltage level (RMS) values decreased initially for 0 to 4, 0 to 12 and 0 to 16 min for one, three and five months after treatment (Fig. 6b). Though the relative magnitude signal (RMS) value exceeds the threshold voltage level for a limited time after three months of treatment, the reduction in the threshold voltage level to 0.2 V even for 20 min was observed after five months in the treated mounds.

Discussion

Horizontal transmission of fungal infection among termites

Horizontal transmission is an important component to be considered in the development of termite control strategies as in many species; most of the colony and nest are not accessible for direct treatment. In the present study, the epizootic of *M. anisopliae* infection was horizontally transferred among the untreated population of *O. obesus*, when the treated population of termites was released at the feeding sites. Similar studies conducted by Jones et al. (1996) and Rosengaus and Traniello (1997) found that 49–100% mortality was caused in groups

Table 1

Percentage mortality and mycosis of *Odontotermes obesus* on rubber wood stakes treated with dry conidia and conidial attractants and buried inside the soil as per Indian Standard (IS: 4833-1968), in plantation of sandal wood for five months by monthly intervals. The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$.

Treatments	Mean % mortality ± SE at monthly intervals					Mean % mycosis ± SE at monthly intervals				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
IWST-Ma13 (dry conidia)	42.8 ± 3.2b	26.4 ± 1.0bc	0 ± 0.0	0 ± 0.0	0 ± 0.0	38.3 ± 3.4bc	62.7 ± 1.9a	0 ± 0.0	0 ± 0.0	0 ± 0.0
IWST-Ma13 (with attractant)	78.0 ± 1.2a	63.1 ± 2.3a	71.4 ± 0.3a	43.0 ± 1.3a	49.7 ± 2.4a	52.8 ± 2.4a	48.6 ± 1.8b	56.3 ± 1.6a	44.7 ± 1.3a	39.5 ± 2.5a
ARSEF 7413 (dry conidia)	26.3 ± 1.1c	19.7 ± 2.6c	13.2 ± 0.4c	0 ± 0.0	0 ± 0.0	37.4 ± 1.7bc	33.5 ± 1.4b	10.4 ± 1.4b	0 ± 0.0	0 ± 0.0
ARSEF 7413 (with attractant)	37.7 ± 1.0bc	33.9 ± 1.0b	39.4 ± 1.2b	29.9 ± 2.6b	17.1 ± 3.1b	23.7 ± 1.6c	19.6 ± 1.2c	22.7 ± 1.5b	9.4 ± 2.9b	0 ± 0.0

Table 2
Weight loss in rubber wood stakes treated with dry conidia and conidial attractants and attractant buried inside the soil as per Indian Standard (IS: 4833-1968), in plantation of sandal wood that was infected by *Odontotermes obesus* for five months by monthly intervals. The weight loss of the stakes was compared by LSD (α = 0.05).

Treatments	% weight loss of rubber wood pickets ± SE				
	1st month	2nd month	3rd month	4th month	5th month
Control	11.9 ± 3.2cd	19.8 ± 1.2cd	22.7 ± 1.2c	28.7 ± 1.4cd	36.1 ± 2.2d
Only attractant	17.4 ± 1.2d	24.0 ± 1.2e	27.6 ± 1.3d	30.2 ± 1.2d	35.6 ± 2.1d
IWST-Ma13 (dry conidia)	7.3 ± 2.4ab	13.0 ± 1.6bc	15.7 ± 1.4b	19.6 ± 1.0bc	19.3 ± 2.0b
IWST-Ma13 (conidia + attractant)	3.9 ± 2.1a	5.6 ± 1.2a	7.8 ± 1.2a	8.8 ± 1.2a	10.9 ± 2.4a
ARSEF 7413 (dry conidia)	10.9 ± 3.0cd	20.9 ± 1.0d	22.3 ± 2.2c	23.7 ± 1.4c	29.8 ± 2.0cd
ARSEF 7413 (conidia + attractant)	7.4 ± 2.4ab	8.6 ± 1.0ab	11.9 ± 1.3ab	15.7 ± 1.1b	17.6 ± 2.3b

of *C. formosanus* when spores of three strains of *B. bassiana* and *M. anisopliae* were applied as dust to 20% of the worker populations. The grooming termite becomes contaminated but most likely only around the mouth area which may groom another individual causing transmission of infection to spread in the colony. As the infected workers and soldiers migrate inside the galleries along with healthy nest mates, this ultimately leads to the spread of infection among the healthy termites. Lai (1977) demonstrated that workers of *C. formosanus* moved more than 110 m through their galleries in 48 h. However, the residual effectiveness of the fungus inoculums will depend on the efficiency of spore dispersal through the frass and proctodeal trophollaxis. Though the treated workers were attacked, dismembered or buried alive, the attacking termites would be likely to be contaminated by the exposure to the treated termites and so some horizontal transmission could occur.

Repellency of termites in the presence of fungal pathogens

In addition to direct mortality as a consequence of contact with the dry conidia of *M. anisopliae*, the spores of some isolates of *M. anisopliae* were found to be repellent to *O. obesus*. However, repellent spores of *M. anisopliae* may have less horizontal transmission potential from infected or contaminated termites to other nest mates. The nature of repellency may be due to the conidia, the dead or mummified cadavers, or even the high level of foreign particulate material associated with the contamination of workers. Kramm et al. (1982) found that healthy workers of *R. flavipes* actively avoid nest mates which had died from *M. anisopliae* infection. Rath and Tidbury (1996) showed that, the workers of *C. acinaciformis* foraged less on the treated cardboard and only 2.3% of workers were found to forage on the spore treated cardboard over a three day period, compared to 33% of workers which foraged on the untreated cardboard. A similar observation was observed in this study, as the complex social and defensive interaction between workers and soldiers in the colony was different they elicited a different avoidance response and foraged less when the dry conidia were applied. Rath (2000) also described a similar response in *R. flavipes* and also observed that an alarm behavior was exhibited by untreated termites. To avoid these responses by the termites, spores of *M. anisopliae* could either disguise or render them non-repellent or could be made attractive to termites. In this study the spores were made attractive to termites by mixing them with an attractant such as sawdust, sugarcane bagasse and cardboard powder that was rich in cellulose materials. Theses baits showed a positive response with the termites where the maximum percentage of workers was attracted towards the treated area that favored the epizootic transmission of fungal infection among the population. Rath and Tidbury (1996) demonstrated that 15% more workers of *C. acinaiformis* foraged on wetted cardboard treated with *M. anisopliae* and a proprietary masking component than on treated wetted cardboard.

Management of termite attack in plantations

A major limiting factor, in the control of termites in urban pest control programs, is whether the termites' population is suppressed or their nest is eliminated when treatment has been undertaken. Widely used techniques which allowed for the estimation of termite populations, such as the mark release–recapture protocol have been found to be inaccurate and unreliable (Evans et al., 1998). Lack of activity at treatment points of monitoring stations is not a proof of elimination or suppression of the population. Untreated control sites are not used in the studies on termite control in urban areas, as the pest control operators and pesticide companies of home owners are not willing to have continued termite activity with liability of increased property damage.

In the present study, four different methods were adapted to test the efficacy of the fungal pathogen in the field. In stake treatments,

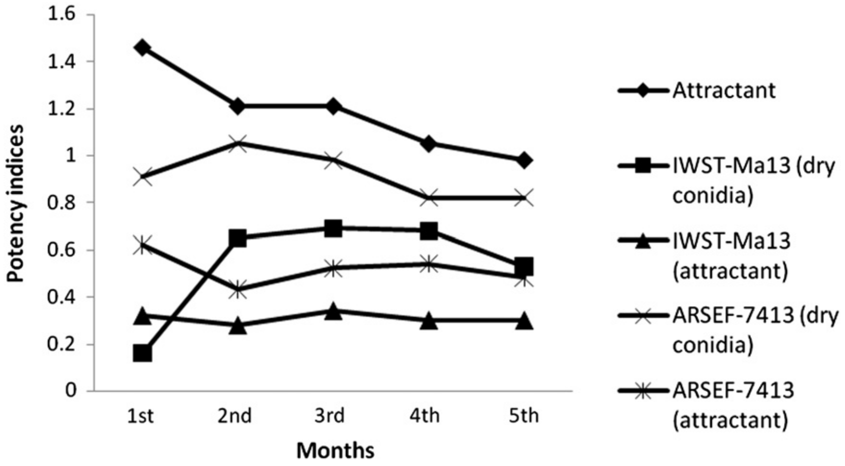

Fig. 3. Relative potency indices of weight loss in rubber wood stakes treated with dry conidia and conidial attractants and buried inside the soil as per Indian Standard (IS: 4833-1968), in plantation of sandal wood that was infected by *Odontotermes obesus* for five months by monthly intervals. The relative potency for each treatment was calculated by dividing the weight loss of stake with each test isolate by that of standard.

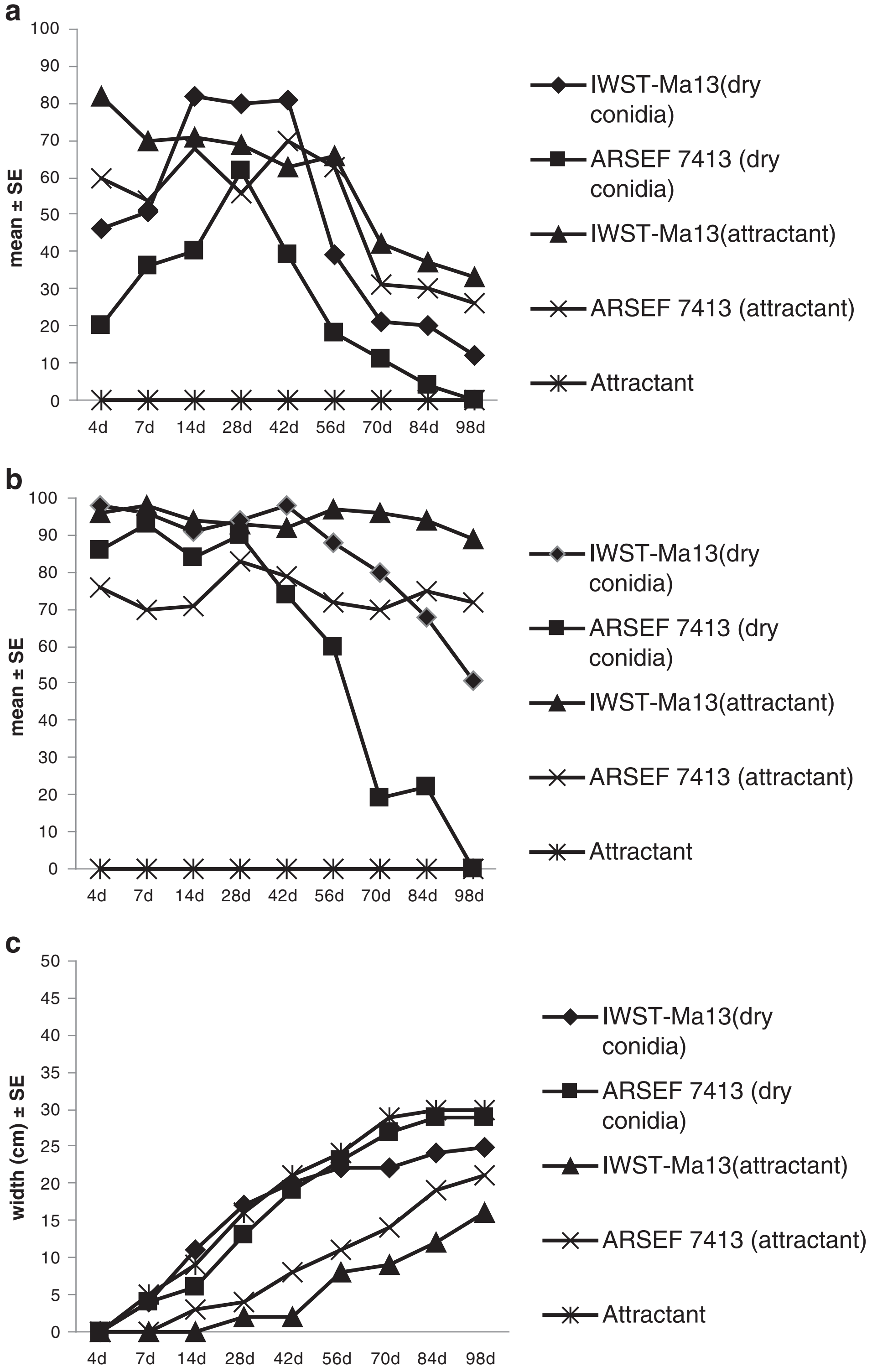

Fig. 4. a: Percentage mortality of *Odontotermes obesus* on gunny bags dusted with two isolates of *Metarhizium anisopliae* dry conidia, conidial attractants and attractant on the bark of sandal trees, for a period of three months. The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$. b: Percentage mycosis of *Odontotermes obesus* on gunny bags dusted with two isolates of *Metarhizium anisopliae* dry conidia, conidial attractants and attractant on the bark of sandal trees, for a period of three months (98 days). The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mycosis was corrected in the treated groups using $P = C - T/C \times 100$. c: Width of mud plasters covered on the gunny bags (30 cm) intacted on the bark of sandal trees treated with two isolates of *Metarhizium anisopliae* dry conidia, conidial attractants and attractant for a period of three months (98 days). The mean width of the galleries was compared by LSD ($\alpha = 0.05$).

infestation rate and weight loss of stakes decreased when treated with conidial attractant. However, control stacks were severely infested after five months of treatments. The termite colonies did not avoid the treated area. Rather they reduced their activity near the treated area by nullifying the efficacy of fungus conidia. A similar response was observed by Krueger et al. (1995) where the reinspection of the Bioblast™ treated sites in USA displays that 60% of the wooden structure was free of termites, 15% still had partial activity, 5% had no reduction in activity and 20% were still to be inspected. Wherever the termites were eliminated, the structures had remained uninfected for a period ranging from 6 to

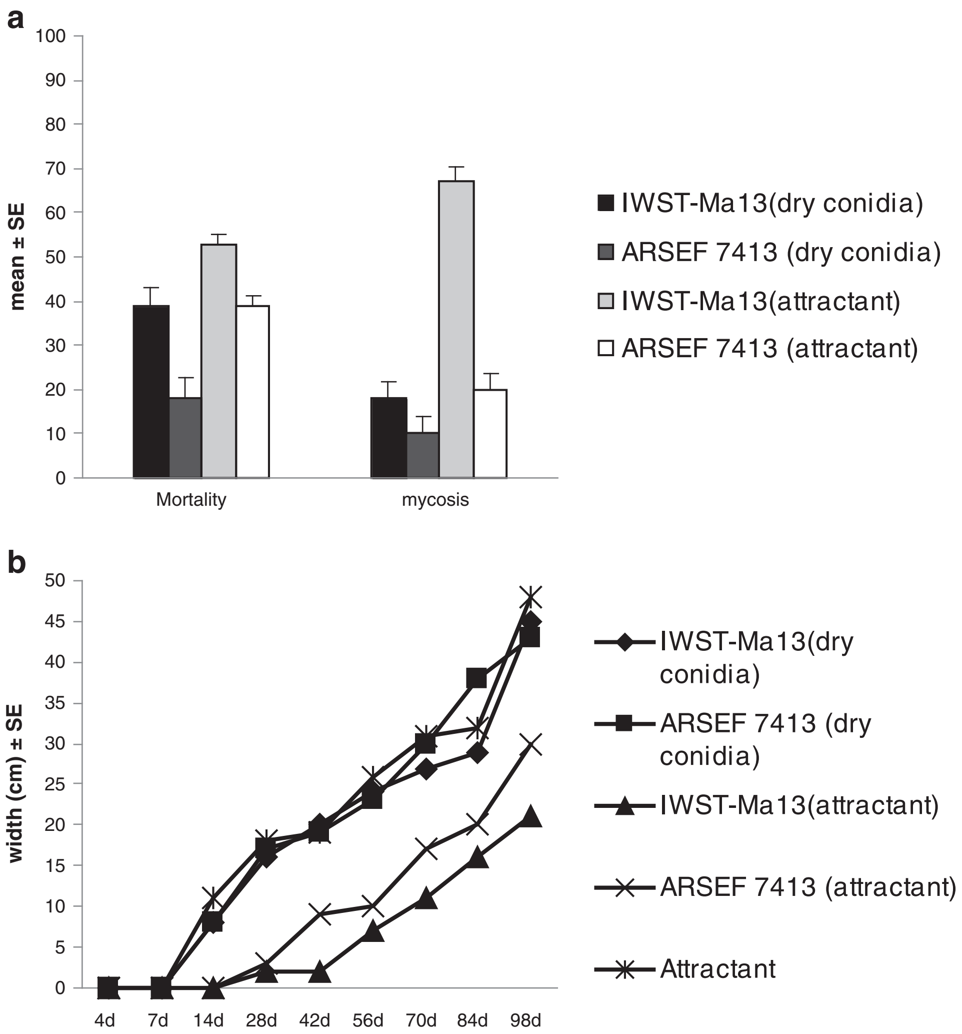

Fig. 5. a—Percentage mortality and mycosis of *Odontotermes obesus* when fungal baits (8 bait cubes each of 1 × 10^8 cfu/tree) and dry conidia were applied around the tree base for a period of three months (98 days). The means were angularly transformed and compared by LSD ($\alpha = 0.05$). Control mortality was corrected in the treated groups using $P = C - T/C \times 100$. b—Width of mud plasters covered on the gunny bags (30 cm) intacted on the bark of trees treated with fungal baits (8 bait cubes each of 1 × 10^8 cfu/tree) and dry conidia for a period of three months (98 days). The mean width of the galleries was compared by LSD ($\alpha = 0.05$).

15 months. Rath (1995) observed from his studies, that there was no indication whether the success of the treatments was due to colony elimination or suppression, or due to the active avoidance of treated areas. Although the learned avoidance helps the termites avoid the treated area and prevent epizootic, complete coverage of conidial attractant over the stakes was effective when compared to dry conidia application.

In tree treatments, activity of termites on the treated trees is reduced, when the trees were treated with conidial attractants. The mud galleries over the gunny bags, mortality and mycosis signify the reduction in the activity of termites. Although the mortality and mycosis was initially high for up to forty days when the trees are treated with highly virulent strains of dry conidia, the mud galleries covered over the gunny bags were high. This signifies that the termites' activity was initially minimized, but later the sensitivity of the strain was reduced by healthy nest mates by burring the infected nest mates. However, when the fungus conidia were mixed with attractant there are fewer possibilities for the healthy termites to recognize the infected individuals before it transfers the learned avoidance signals among the other nest mates. This will augment the transfer of infection by the highly virulent strains faster than the pathogen responses emitted by the healthy nest mates. In one way burring of the nest mates could reduce the transfer of infection among the population, but the buried cadaver that develops the fungal spore over their surface as well as the grooming of the mouth areas of infected termites could still have the potential to spread the infection among the populations. The use of *M. anisopliae* as a repellent in the field has been proposed by Ko et al. (1982) who found a correlation between the level of *M. anisopliae* present in soils and the mortality of *C. formosanus*. Localities in which the fungi were commonly present had never reported *C. formosanus* infestations. The author could conclude that the presence of the fungi in soil was providing protection against termite attacks. Milner et al. (1997) showed that treating soil with *M. anisoplaie* damage to wood can be lessened but not eliminated. They suggested that a minimum dose rate of 1 × 10^8 spores g^{-1} was effective. Also, Ko et al. (1982) found that soil with 9 × 10^5 spores g^{-1} resulted in 100% mortality of *C. formosanus* after 14 days at 15 °C in the laboratory. Milner and Staples (1996) reported that their treatments repelled termites rather than killed them and treatments gave up to 3 year protection of timber under cool dry conditions but less than 6 months under tropical conditions.

In the bait treatments, termite populations were suppressed when baits were applied around the trees and the spread of infection among the nest mates could ultimately suppress the termite populations. In this study, conidia of a highly virulent strain were mixed with attractants such as cardboard powder, sugarcane bagasse and saw dust to reduce the possibilities of recognization of pathogens by termites and the presence of attractant could attract the termites towards the treated

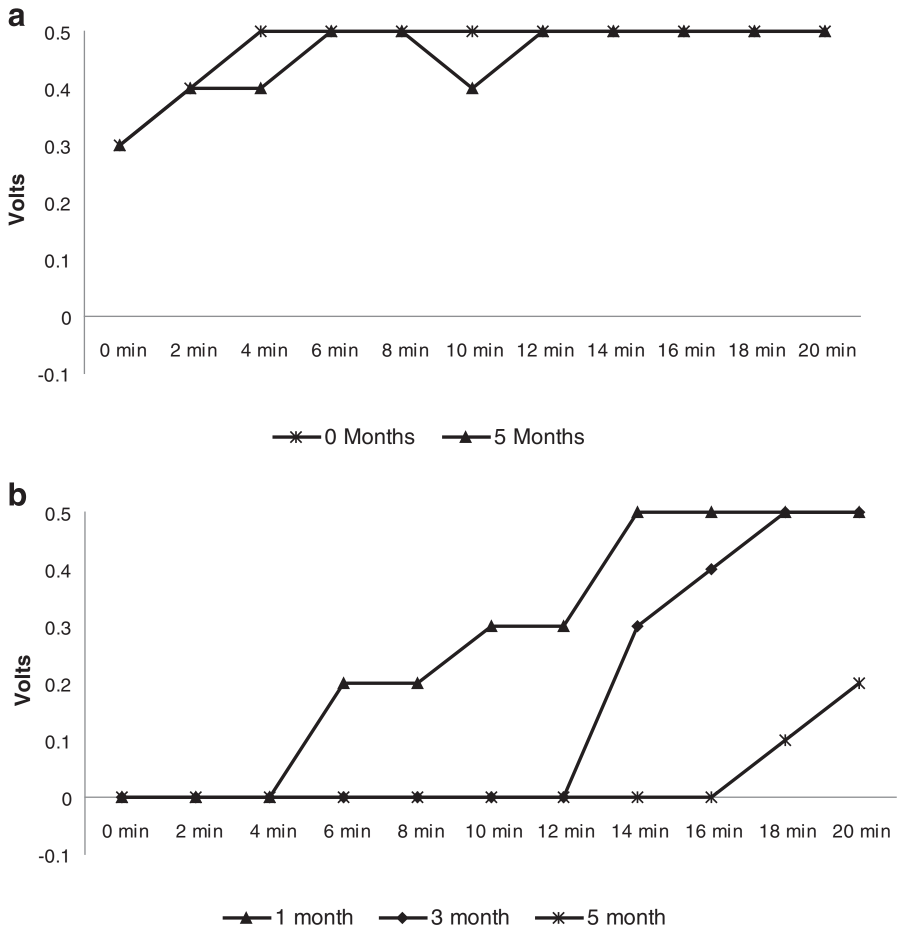

Fig. 6. a: Activity of *Odontotermes obesus* colonies in the control mounds was measured by AED-2000L (Acoustic Emission Consulting) Insect pest detection kit with a fixed threshold of 0.5 V at an interval of 20 min for 5 months. Twenty five readings per mound was measured and the average RMS values were graphically represented as Volts. b: Activity of *Odontotermes obesus* colonies in the treated mounds was measured by AED-2000L (Acoustic Emission Consulting) Insect pest detection kit with a fixed threshold of 0.5 V at an interval of 20 min for 5 months. Twenty five readings per mound were measured and the average RMS values was graphically represented as Volts.

area. Similar studies by Deka et al., 1999 observed that, with different formulations of insecticides (fenvalerate 0.4% dust, malathion 10% dust and sugarcane press mud) against *O. obesus*, a 10% formulation of malathion was effective. Singh and Singh (2002) in their field evaluation of neem-based formulations found that, Nimbicidine and Nemactin were effective for up to two months and Rakshak, Multineem, Neemgourd and Vanguard were effective for up to one month. Similarly, antifeedant activity of Thiamethoxam formulation (ACTARA 25 WG) against the African termites viz., *Trinervitermes trinervius* and *O. smeathmani*, showed that the products are consumed by the termites rather than repelled (Huang et al., 2005). The foraging behavior of *O. rmosanus* studied on pure baits such as pine powder; sugarcane powder; millet powder; powder of log of cultivated *Lentinula edodes*, *Artemisia argyi* powder and potato powder showed that, the consumption rate, mud sheet area and mud sheet covering rate in the case of millet powder were high and all the additives significantly improved the phagostimulating effect on *O. formosanus* (Huang et al., 2006a). Field studies on the use of attractive toxicants in controlling ground and deadwood termites were effective in controlling *O. formosanus* populations in the field (Huang et al., 2006b). 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).

In the mound treatment, direct dusting of large quantities of conidia mixed with attractants into the galleries of the mound was attempted to specifically suppress the colonies. We found that the activity of the termites reduced gradually over a five month period when compared to that of control mounds. This could be due to the rapid transmission of the fungal infection among the population of termites inside the treated galleries. Our study also indicated that by direct dusting inside the mound galleries, the termite's population could be suppressed. The patent of Milner et al. (1997) which corners the control of termites with *M. anisopliae* described few field treatments. Their treatments appeared to have been described specifically to eliminate colonies by direct nest treatments of mound building and tree nesting termite species. They found that treatments which consisted of applying small doses of conidia to termites repairing the damaged part of mounds were unsuccessful as the treated regions were walled off and the colonies continued to grow in other parts of the nest. Milner and Staples (1996) found that colonies could only be killed when large quantities of pure dry conidia were blown directly into the nursery region. They found success in several hundred colonies of five different species of termites with nests in mounds or trees. Fernandes and Alves (1991) found that either *M. anisopliae* or *B. bassiana* resulted in 100% mortality of *Cornitermes cumulance* colonies within 10 days of application of 5 g of dust to nests. Milner and Staples (1996) also claim that treatments of mound nests by damaging small sections and applying *Metarhizium* based dusts are ineffective as the termites wall-off the treated area. Hanel and Watson (1983) treated both the mound nests and the feeding sites of *Nasutiteermes exitiosus* with *M. anisopliae*. They were not as successful as Fernandes and Alves (1991) but concluded that disease can spread through a field colony from conidia applied by dusting or spraying on a few of its members in the mound or away from it at feeding sites.

Conclusion

Grooming and social interactions of termites are seen to have the potential to spread the fungus through the colony. From our study, we could find a cumulative effect on the suppression of the colony by adapting the attractant based fungal baits and direct dusting of termite nests. Hence these approaches could be used as one among a number of possible tools in the long term control strategies of the termites in forest and agriculture areas. However, factors such as avoidance of the fungus conidia, removal and burial of fungus killed termites, together with the defensive secretion and inhibitory components in frass and the possibility of humoral resistance may limit the spread of the diseases in the colony. The mixing of conidia with attractants like sugarcane bagasse, sawdust and cardboard powder could augment the dissemination of the fungal pathogens among the population that causes epizootic. Even if *Metarhizium anisopliae* cannot routinely eliminate termite colonies in forest areas, there will still be a place for products based on theses fungi as termite control is becoming more focused on the suppression of colonies.

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