Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

BIOLOGICAL CONTROL

Laboratory and Field Evaluation of *Metarhizium anisopliae* var. *anisopliae* for Controlling Subterranean Termites

A Hussain[1], S Ahmed[2], M Shahid[3]

[1]College of Natural Resources and Environment, South China Agricultural Univ, Guangzhou, China
[2]Dept of Agricultural Entomology, [3]Dept of Chemistry and Biochemistry, Univ of Agriculture, Faisalabad, Pakistan

Keywords
Microtermes obesi, *Odontotermes obesus*, field application, sugarcane, diesel oil, thiamethoxam, repellency

Correspondence
Abid Hussain, College of Natural Resources and Environment, South China Agricultural University, Guangzhou, 510642, P.R. China; solvia_aah@yahoo.com

Edited by Italo Delalibera Jr – ESALQ/USP

Received 01 August 2009 and accepted 11 December 2010

Abstract

The efficacy of the *Metarhizium anisopliae* strain ARSEF 6911 was determined in the laboratory and field against two sugarcane pests, *Microtermes obesi* Holmgren and *Odontotermes obesus* Rambur (Termitidae: Isoptera). The susceptibility of both termite species to different conidial suspensions (1×10^{10}, 1×10^{8}, 1×10^{6} and 1×10^{4} conidia/ml) was determined in laboratory. All conidial suspensions were able to induce mortality. Termite mortality caused by the fungal suspensions was dose dependent. There were no significant differences in the LT_{50} values between species. Field evaluation of *M. anisopliae* alone or in combination with diesel oil and thiamethoxam was carried out in two growing seasons (autumn 2005 and spring 2006) at two sites located in Punjab, Pakistan. Dipping the sugarcane setts in these suspensions was tried to determine their effects on germination and percentage of bud damage to sugarcane setts. All treatments significantly reduced termite infestation compared to the untreated control. The combined treatment of *M. anisopliae* and diesel oil significantly reduced insect damage by attaining higher germination > 55% and lower bud damage < 5.50% at both sites in both seasons. The results suggest that the application of *M. anisopliae* and diesel oil in combination might be a useful treatment option for the management of termites in sugarcane.

Introduction

The termite fauna of Pakistan consists of 50 species, and nine of them belong to Termitidae and are recorded as pests of agricultural crops (Akhtar & Shahid 1993). The most important termite species causing damage to sugarcane in Pakistan are *Microtermes obesi* Holmgren, *M. mycophagus* Desneux and *Odontotermes obesus* Rambur (Sattar & Salihah 2001, Ahmed *et al* 2006).

Termites attack sugarcane when setts are placed in furrows and are covered with soil. Termite's infestation at this time results in low or no germination of seedlings at all. After cane formation, attack by termites may cause lodging or production of a cane with poor quality for milling. Thus, insecticides are applied to setts to protect them from termite's infestation in three ways: (i) application to setts at the time of placing in furrows, (ii) dipping setts in insecticide solutions and then placing in furrows, or (iii) applying insecticides at the time of first irrigation, but this is the least preferred (Ahmed *et al* 2006).

Management of subterranean termites has relied heavily on chemicals such as soil treatment, baiting and dusting. Soil termiticide treatment creates a chemical barrier for exclusion of subterranean termites (Bläske *et al* 2003), and has been a popular termite management

tool over the last 50 years (Su *et al* 1997). In the past, persistent organo chlorine insecticides were available for termite control, but were later replaced by many of the current available insecticides. Chlorpyrifos, bifenthrin, imidacloprid and thiamethoxam have proven to be effective against many species of termites. But the shortcomings associated with the use of insecticides in recent years have prompted policy makers and scientists to evaluate the potential of biological control strategies to suppress subterranean termite populations (Culliney & Grace 2000).

Biological management has been investigated as an alternative to conventional insecticide use for a number of termite species, and laboratory data on the efficacy of fungal pathogens are available against *Reticulitermes* spp. (Ramakrishnan *et al* 1999), *Coptotermes* spp. (Ahmed *et al* 2009, Hussain *et al* 2010a), *Nasutitermes* spp. (Milner 2003), damp wood termites (Rosengaus & Traniello 1997), *Heterotermes tenuis* Hagen (Moino *et al* 2002) and *Cornitermes cumulans* Kollar (Neves & Alves 1999).

The studies carried out in a number of laboratories have focused on the possible avoidance behavior of termites against repellent entomopathogenic fungi. Recent studies by Mburu *et al* (2009) on *M. anisopliae* and *B. bassiana* suggested that termite response towards entomopathogenic fungi is directly related to the potential harm these fungi can inflict on the insect and that the virulent strains are more likely to be recognized from some distance and avoided. The repellent action of conidia has also been utilized to protect maize crops from termites, which significantly reduce maize plant logging and increased grain yield in Kenya (Maniania *et al* 2002). There are also some reports on the successful control of termites by direct blowing of conidia of less virulent strains into termite galleries, resulting in a successful control of only those termites which were directly hit by the conidia (Milner 2000). However, no attempt has been made to test their susceptibility to fungal pathogens against many species of *Odontotermes*, *Microtermes* and *Macrotermes,* prevalent in agricultural lands in Pakistan, which have been found attacking sugarcane, wheat, maize and gram.

The present study was initiated in order to evaluate the virulence of a strain (ARSEF 6911) of *M. anisopliae* to *M. obesi* and *O. obesus* workers. Further, chemicals were added to enhance the biocontrol potential of this entomopathogen. Although the idea is not novel, there is limited data on this tactic for pest control, especially in field conditions. Hence, mass produced spores of *M. anisopliae* ARSEF 6911 alone or in combination with different chemicals were evaluated by dipping sugarcane setts in their solutions in order to implement a practical approach to control termites in sugarcane; tests were performed in two successive growing seasons (autumn 2005 and spring 2006) at two different sites. The overall objective was to demonstrate the potential of the entomopathogenic fungus *M. anisopliae* ARSEF 6911 in order to develop an inexpensive IPM strategy for termites control in sugarcane.

Material and Methods

Insects

Subterranean termites such as *O. obesus* and *M. obesi* were collected from the sugarcane fields within damaged canes and from the corrugated cardboard in PVC traps installed at different places at the experimental areas of the University of Agriculture, Faisalabad, Pakistan.

Fungal isolate

Metarhizium anisopliae var. *anisopliae* strain ARSEF 6911 was obtained from the USDA, the Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF). Strain ARSEF 6911 was originally isolated from *Coptotermes formosanus* Shiraki in Lake Charles, Louisiana, USA and was cultured on Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) at 27 ± 2 °C in complete darkness. The isolate selection was based on its pathogenicity to *O. obesus* and *M. obesi* workers found in preliminary studies (data not given).

Laboratory bioassay

Conidial suspensions. Conidia of *M. anisopliae* were harvested from 28-day-old sporulating culture into 0.03% Tween 80 (Sigma-Aldrich). The conidial suspension was sieved through a piece of cheese cloth (Mesh: 36 × 36 threads/cm^2) to remove clumps of mycelia and the suspension was adjusted to concentrations of 1×10^{10}, 1×10^{8}, 1×10^{6} and 1×10^{4} conidia/ml. The viability of conidia was determined by inoculating PDA plates (90 × 15 mm) with 10 µl of 1×10^{6} conidia/ml at 25°C for 24h. Plates were then observed for germination of 100 conidia randomly selected in three separate fields of the Petri dishes using a compound microscope (×400). The viability was 94.3 ± 1.5%.

Laboratory experimental set up. The infectivity of the isolate was evaluated by directly applying conidial suspensions at different concentrations to individual termite workers. A Precision (Microprocessor Controller) PAX100-3 Microapplicator (Burkard, UK) was used to dispense from 1 µl to 10 µl per individual termite. Workers of *M. obesi* and *O. obesus* were placed separately in Petri dishes containing sterilized moistened Whatman No. 1 filter paper. The

Petri dishes were covered and then placed over ice to lower termite activity. Individual workers were held from their heads and 5 µl were applied to the ventral side of their abdomen. After drying, forty workers/ treatment were transferred to Petri dishes (90 × 15 mm) that contained 10 g sifted sterilized soil and a strip of sugarcane (10 × 60 mm), dampened with sterile distilled water. The dishes were sealed with laboratory film (Parafilm® Pechiney Plastic Packaging; Menasha. WI. US) and incubated at 27 ± 2°C in complete darkness. The mortality was recorded daily until all termites were dead. At each observation, dead termites were counted and removed from the Petri dishes. At each observation, dead termites were removed, counted and placed in dishes (90 × 15 mm) lined with wet filter paper and maintained at 25°C in growth chamber for mummification and sporulation to prove that the insects died because of the fungi used.

Data analysis. The Abbott's formula (Abbott 1925) was applied to correct for the percent mortality in the control. The data on dose-mortality were subjected to probit analysis to estimate the LT_{50}. All the values obtained were subjected to one way analysis of variance (ANOVA) and means were compared by using the Student Newman-Keuls test (SAS Institute 2000). The comparisons between termite species at all the concentrations were performed on LT_{50} values using the *t*-test.

Field experiments

Production of conidia on rice. One milliliter of a suspension of *M. anisopliae* ARSEF 6911 at a concentration of 1×10^6 conidia/ml was poured into a 250 ml flask containing 100 ml of the growth medium described in Table 1. Flasks were then put on a rotary shaker at 150 rpm for four days at 27 ± 2°C in complete darkness. Content of flasks were then inoculated at a rate of 150 ml per kg of autoclaved parboiled rice with the help of a syringe. The bags of inoculated rice were then incubated for 18 days at room conditions (24 ± 2°C, 75-85% RH). Rice bags with conidia were then allowed to dry for seven days at 30°C, before conidia were separated from the rice by sieving through a 300 µm mesh. Plastic sheeting was taped around both the top and bottom edges of the sieve and sealed at the top. A collecting vessel, such as a bucket was fitted to the plastic sheeting at the bottom of the sieve to create a funnel into the collecting vessel. The sieve was shaken until all the loose conidial powder had been removed from the rice and had been collected in the vessel below. The conidial powder was then further sieved using a 106 µm sieve to separate the larger rice dust particles from the conidial powder. Dry conidia were then stored at 4°C until used in field experiments (35-day-old conidia were used, with >94.5% germination after 24h on PDA). The compatibility of diesel oil and thiamethoxam was checked in preliminary studies. The viability of conidia in diesel oil and thiamethoxam showed >89% after 24h on PDA.

Field experimental set up. Sugarcane (*Saccharum officinarum*) variety HoSG 559 was planted in 3.35 × 7.32 m plots with a distance between rows of 1.22 m at the Ashabah Farm Shakarganj Sugar Mills Jhang, Punjab, Pakistan (site 1, 31° 09' 38" N, 72° 21' 55" E, 154 m elevation) and the Postgraduate Agricultural Research Station (PARS), University of Agriculture Faisalabad, Pakistan (site 2, 31° 23' 3" N, 73° 01' 4" E, 177 m elevation), in the autumn 2005 and in spring 2006. Sugarcane setts were inoculated by dipping them in the solutions presented in Table 2. The compatibility of chemicals with the fungal isolate was evaluated on PDA. The chemicals used had no negative effect on conidial germination of the studied strain of *M. anisopliae*. After setts had been treated, they were planted and each plot was irrigated separately when needed. Treatments were arranged in a randomized complete block design with three replicates.

The number of buds were counted before and two months after planting to determine the percentage of germination of sugarcane setts for each treatment. The presence of bud damage was also assessed for two months after planting by randomly choosing five $1m^2$ areas from each plot. Sugarcane setts were carefully removed from the soil and the number of buds damaged by termites were counted.

Data analysis. The data on germination and bud damage percentage were angularly transformed before analysis. A two way factorial analysis consisting of two seasons and six treatments was conducted for germination and bud damage percentage in order to investigate the main effect of these two factors. Significant differences among

Table 1 Composition of fungal liquid growth medium.

Ingredients	CSL	SM	$CaCl_2\ 2H_2O$	KCl	$MgCl_2\ 6H_2O$	$MgSO_4\ 7H_2O$	$NaHCO_3$	$NaH_2PO_4\ H_2O$
Concentration (%)	1.00	2.66	0.06	0.28	0.16	0.20	0.03	0.10

CSL = Corn steep liquor (Rafhan Maize Products Co. Ltd. Faisalabad, Pakistan)

SM = Sugar molasses (Shakarganj Sugar Mills Jhang, Punjab, Pakistan)

Liquid Medium preparation was finalized by adjusting the pH to 6.2, using sterilized solutions of HCl (0.1%) and NaOH (10%).

Table 2 Treatments in field experiment.

Treatments	Chemical sources			
	M. anisopliae suspension conidia/ml	Thiamethoxam g/5l	Diesel oil %	Tween 80 %
M. anisopliae	1×10^{12}			0.03
M. anisopliae × thiamethoxam	1×10^{8}	0.15625		0.03
M. anisopliae × diesel oil	1×10^{8}		2.5	0.03
Thiamethoxam		0.3125		
Diesel oil			5	
Control				0.03

*Thiamethoxam (Syngenta Pakistan Limited)
*Diesel oil (Shell Pakistan Limited)

means were separated by the Tukey's Honestly Significant Difference Test (SAS Institute 2000).

Results

Laboratory bioassay

Infectivity of *M. anisopliae* differed significantly when *M. obesi* ($F = 54.71$; $df = 3$; $P < 0.05$) and *O. obesus* ($F = 133.37$; $df = 3$; $P < 0.05$) workers were directly exposed with different conidial concentrations in the laboratory. Both termite species were susceptible to *M. anisopliae* in a dose-dependent manner. With decreasing conidial concentrations, LT_{50} values tended to be longer for both termite species (Table 3). At 1×10^{10} conidia/ml, the LT_{50} values were 2.82 and 3.08 days for *M. obesi* and *O. obesus*, respectively. No significant differences were observed between *M. obesi* and *O. obesus* workers exposed to *M. anisopliae* suspension at the concentrations of 1×10^{10}, 1×10^{8} and 1×10^{6} conidia/ml ($P > 0.05$). The variation in LT_{50} values was statistically significant ($P \leq 0.02$), when workers of both species were exposed to the lowest concentration (1×10^{4} conidia/ml) (Table 3).

Table 3 Comparison of LT_{50} values between *Microtermes obesi* and *Odontotermes obesus* workers at different conidial concentrations of *Metarhizium anisopliae*.

Concentrations (conidia/ml)	*M. obesi*	*O. obesus*
	mean ± SE (days)	
1×10^{10}	2.82 ± 0.15^{c}	3.08 ± 0.11^{d}
1×10^{8}	4.51 ± 0.20^{b}	4.57 ± 0.13^{c}
1×10^{6}	5.31 ± 0.21^{b}	5.95 ± 0.16^{b}
1×10^{4}	6.39 ± 0.24^{a}	7.51 ± 0.17^{a}

Values are the means of three replicates (n = 3). Means ± SE values with different letters within a column are significantly different. (Student Newman Keuls test, $P \leq 0.05$).

Field experiment

The percent germination of sugarcane recorded after two months post-treatment differed significantly among treatments at site 1 (Ashabah Farm Shakarganj Sugar Mills Jhang, Punjab, Pakistan) ($F = 28.08$; $df = 5, 22$; $P < 0.01$) and site 2 (Postgraduate Agricultural Research Station, University of Agriculture Faisalabad, Pakistan) ($F = 30.02$; $df = 5, 22$; $P < 0.01$). Mean percent germination of sugarcane setts in controls were low, ranging from 23.7-24.6% (site 1) and 22.0-26.4% (site 2). All treatments except diesel oil had significantly higher germination than the control in both cropping seasons and at both sites (Fig 1). The combined effect of *M. anisopliae* conidia with diesel oil during autumn 2005 and spring 2006 at both sites resulted in significantly higher percent germination over that caused by diesel oil alone, but it was similar to *M. anisopliae* conidia alone. When thiamethoxam was applied with *M. anisopliae* conidia, germination of sugarcane was not improved if compared with these treatments alone. There was no significant difference in the germination of sugarcane setts between autumn 2005 and spring 2006 at site 1 ($F = 2.80$; $df = 1, 22$; $P > 0.10$) and site 2 ($F = 2.49$; $df = 1, 22$; $P > 0.12$).

There was no significant difference in percentage of bud damage of the sugarcane setts between autumn 2005 and spring 2006 at site 1 ($F = 0.89$; $df = 1, 22$; $P > 0.05$) and site 2 ($F = 1.134$; $df = 1, 22$; $P = 0.297$), but there was a significant difference among treatments in comparison with the control at site 1 ($F = 54.87$; $df = 5, 22$; $P < 0.01$) and site 2 ($F = 66.28$; $df = 5, 22$; $P < 0.01$). The lowest bud damage < 5.5% was observed following exposure with conidia + diesel oil at both sites in autumn 2005 and spring 2006, except that this treatment was not statistically different from *M. anisopliae* + thiamethoxam and thiamethoxam alone in autumn 2005 and *M. anisopliae* + thiamethoxam in spring 2006 (Fig 2). Higher percentages of bud damage occurred in untreated control plots (> 35%). Treatment efficacy to prevent termite damage in sugarcane increased upon combination of

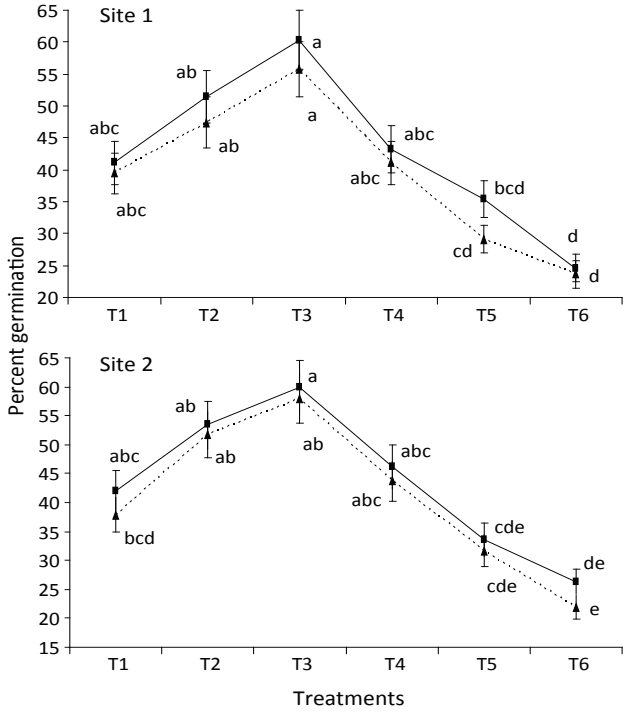

Fig 1 Germination of sugarcane setts at site 1 (Ashabah Farm Shakarganj Sugar Mills Jhang, Punjab, Pakistan) and site 2 (Postgraduate Agricultural Research Station, University of Agriculture Faisalabad, Pakistan) during two seasons (--▲-- autumn 2005 and —■— spring 2006) after dipping with the following treatments: 1 - *M. anisopliae* (1×10^{12} conidia/ml) + Tween (0.03%); 2 - *M. anisopliae* (1×10^{8} conidia/ml) + thiamethoxam (0.15625 g/5l) + Tween (0.03%); 3 - *M. anisopliae* (1×10^{8} conidia/ml) + Diesel (2.5%) + Tween (0.03%); 4 - thiamethoxam (0.15625 g/5l); 5 - Diesel (5%) and 6- 0.03% Tween (Control). Means ± SE values among the bars for the same field site sharing the same letter are not significantly different (Tukey's Honestly Significant Difference Test; P > 0.05).

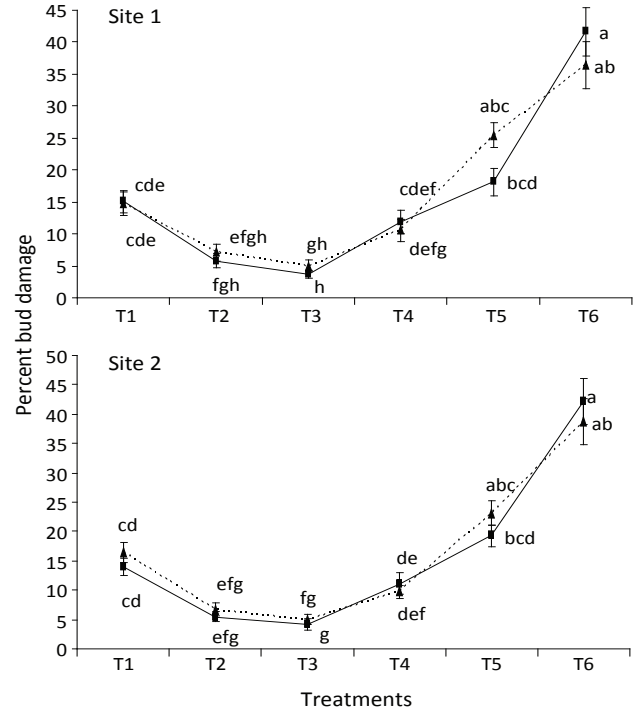

Fig 2 Percent bud damage of sugarcane setts at site 1 (Ashabah Farm Shakarganj Sugar Mills Jhang, Punjab, Pakistan) and site 2 (Postgraduate Agricultural Research Station, University of Agriculture Faisalabad, Pakistan during two seasons (--▲-- autumn 2005 and —■— spring 2006) after dipping with the following treatments: 1 - *M. anisopliae* (1×10^{12} conidia/ml) + Tween (0.03%); 2 - *M. anisopliae* (1×10^{8} conidia/ml) + thiamethoxam (0.15625 g/5l) + Tween (0.03%); 3 - *M. anisopliae* (1×10^{8} conidia/ml) + Diesel (2.5%) + Tween (0.03%); 4 - thiamethoxam (0.15625 g/5l); 5 - Diesel (5%) and 6- 0.03% Tween (Control). Means ± SE values among bars for the same field site sharing the same letter are not significantly different (Tukey's Honestly Significant Difference Test; P > 0.05).

conidia and diesel oil as compared to the each one of these treatments alone. This effect was not observed for thiamethoxam.

Discussion

The LT_{50} values obtained demonstrated that *M anisopliae* is highly pathogenic to *M. obesi* and *O. obesus*. Significant differences in LT_{50} values between both studied termite species at the lowest concentration (1×10^{4} conidia ml^{-1}) were observed, which could reflect different host susceptibility at this concentration. However, there were no significant differences in the susceptibility of both species at higher concentrations.

Application of mass-produced conidia on sugarcane setts in combination with diesel oil and thiamethoxam yielded encouraging results as compared to the other treatments. The germination and bud damage to sugarcane setts at our study did not vary considerably between seasons at both sites. The application of *M. anisopliae* with diesel oil proved to be a promising alternative to insecticides against termites in sugarcane by producing higher germination with the lowest bud damage < 5.5%. The effectiveness of entomopathogenic fungi in oil formulations is consistent with previous work reported by Bateman *et al* (1993) against locusts. Furthermore, the findings of Prior *et al* (1988) also suggested that Hypocreales fungi are more infectious when applied in oil formulations. Entomopathogenic fungi in oil formulations maintain their viability for longer periods than those in aqueous formulations (Daoust *et al* 1983).

Diesel oil + *M. anisopliae* conidia had the most pronounced effect in reducing bud damage when compared to treatments where only *M. anisopliae* conidia (13.99-16.40%) or diesel oil (18.13-25.45%) were used, suggesting that the synergistic effect of *M. anisopliae* + diesel oil may create a barrier that could

offer more effective protection to the sugarcane setts than causing disease to the subterranean termites at the time of planting the sugarcane crop. The nature of fungal repellency by the species within the order Isoptera has been widely assessed in the laboratory (Staples & Milner 2000, Hussain *et al* 2010b). This type of avoidance behavior is very important for the management of insect pests like termites residing in difficult-to-reach locations, such as in underground nests. Similarly, Sun *et al* (2008) found that organic mulches supplemented with *M. anisopliae* significantly repelled foraging *C. formosanus* and reduced mulch consumption by up to 71%.

Our work has also provided evidence that suggests that the variation in effectiveness among different treatments is due to the alteration in sensory perception of termites. The impregnation of sugarcane setts in oil-fungal formulation is helpful tool for the management of insects living in concealed habitats with millions of individuals by changing the volatile gaseous profile of the host. Otherwise, this habitat makes it impractical to inoculate fungal spores directly onto most colony members whose galleries may stretch more than 100 m. The repellent action of conidia has also been utilized to protect other crops such as maize from termites. Maniania *et al* (2002) concluded that the repellency of entomopathogenic fungi significantly reduced the maize plant logging and ultimately increased the grain yield in Kenya.

In our study, the neonicotinoid thiamethoxam tested alone was not effective against termites, resulting in 41.08-46.20% germination. However, the combined formulation or application of *M. anisopliae* + thiamethoxam was moderately effective and showed 47.43-53.40% germination of sugarcane setts and gave better results in lowering bud damage by 5.49-7.06% as compared to the untreated control. Studies on the compatibility of thiamethoxam with *M. anisopliae* showed that it did not affect the inoculum of *M. anisopliae* when applied in the field (Filho *et al* 2001). Similarly, the findings of Neves *et al* (2001) also explained the integration of insecticides acetamiprid, imidacloprid and thiamethoxam with fungal conidia on the basis of compatibility in order to enhance the biocontrol potential of entomopathogenic fungi.

In summary, our study indicates that the addition of diesel oil to *M. anisopliae* has pronounced positive effect in the control of termites at the time of sugarcane planting by increasing germination and reducing sugarcane bud damage.

Acknowledgments

We thank Dr Richard A Humber (USDA-ARS) for providing *Metarhizium anisopliae* var. *anisopliae* strain ARSEF 6911. Special thanks to Prof. Tian MY (South China Agricultural University, Guangzhou, P. R. China) for reading and providing useful comments on the earlier draft of the manuscript.

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18: 265-267.

Ahmed S, Ashraf MR, Hussain A, Riaz MA (2009) Pathogenicity of isolates of *Metarhizium anisopliae* from Gujranwala (Pakistan) against *Coptotermes heimi* (wasmann) (Isoptera: Rhinotermitidae). Int J Agric Biol 11: 707-711.

Ahmed S, Mustafa T, Riaz MA, Hussain A (2006) Efficacy of insecticides on subterranean termites in sugarcane at Bhakkar. Int J Agric Biol 8: 508-510.

Akhtar MS, Shahid AS (1993) Termites as pest of agricultural crops in Pakistan. *Pak* J Zool 25: 187-193.

Bateman RP, Carey M, Moore D, Prior C (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. Ann Appl Biol 122: 145-152.

Batista Filho A, Almeida JEM, Lamas C (2001) Effect of thiamethoxam on entomopathogenic microorganisms. Neotrop Entomol 30: 437-447.

Bläske VU, Hertel H, Forschler BT (2003) Repellent effects of isoborneol on subterranean termites (Isoptera: Rhinotermitidae) in soils of different composition. J Econ Entomol 96: 1267-1274.

Culliney TW, Grace JK (2000) Prospects for the biological control of subterranean termites (Isoptera: Rhinotermitidae), with special reference to *Coptotermes formosanus*. Bull Entomol Res 90: 9-21.

Daoust RA, Ward HG, Roberts DW (1983) Effect of formulation on the viability of *Metarhizium anisopliae* conidia. J Invertebr Pathol 41: 151-160.

Hussain A, Tian MY, He YR, Lei YY (2010a) Differential fluctuation in virulence and VOC profiles among different cultures of entomopathogenic fungi. J Invertebr Pathol 104: 166-171.

Hussain A, Tian MY, He YR, Bland JM, Gu WX (2010b) Behavioral and electrophysiological responses of *Coptotermes formosanus* Shiraki towards entomopathogenic fungal volatiles. Biol Control 55: 166-173.

Maniania NK, Ekesi S, Songa JM (2002) Managing termites in maize cropping system**s** with the entomopathogenic fungus, *Metarhizium anisopliae*. Insect Sci Appl 21: 41-46.

Mburu DM, Ochola L, Maniania NK, Njagi PGN, Gitonga LM, Ndung'u MW, Wanjoya AK, Hassanali A (2009) Relationship between virulence and repellency of entomopathogenic isolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the termite *Macrotermes michaelseni*. J Insect Physiol 55: 774-780.

Milner RJ (2000) Current status of *Metarhizium* as a mycoinsecticide in Australia. BNI 21: 47-50.

Milner RJ (2003) Application of biological control agents in mound building termites (Isoptera: Termitidae) – Experiences with *Metarhizium* in Australia. Sociobiology 41: 419-28.

Moino JA, Alves SB, Lopes RB, Oliveira PM, Neves J, Pereira RM, Vieira SA (2002) External development of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in the subterranean termite *Heterotermes tenuis*. Sci Agric 59: 267-273.

Neves PJ, Alves SB (1999) Associated control of *Cornitermes cumulans* (Kollar 1832) (Isoptera: Termitidae) with *Metarhizium anisopliae*, *Beauveria bassiana* and imidacloprid. Sci Agric 56: 305-311.

Neves PMOJ, Hirose E, Tchujo PT, Moino Jr A (2001) Compatibility of entomopathogenic fungi with neonicotinoid insecticides. Neotrop Entomol 30: 263-268.

Prior C, Jollands P, Patourel GL (1988) Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). J Invertebr Pathol 52: 66-72.

Ramakrishnan R, Suiter DR, Nakatsu CH, Humber RA, Bennett GW (1999) Imidacloprid-enhanced *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) susceptibility to the entomopathogen *Metarhizium anisopliae*. J Econ Entomol 92: 1125-1132.

Rosengaus RB, Traniello JFA (1997) Pathobiology and disease transmission in dampwood termites [*Zootermopsis angusticollis* (Isoptera: Termopsidae)] infected with the fungus *Metarhizium anisopliae* (Deuteromycotina: Hypomycetes). Sociobiology 30: 185-195.

SAS Institute (2000) SAS user's guide: statistics. SAS Institute, Cary, North Carolina.

Sattar A, Salihah Z (2001) Detection and control of subterranean termites. Technologies for sustainable agriculture. Proceedings of the National Workshop held at Faisalabad, Pakistan, p. 195-198.

Staples JA, Milner RJ (2000) A laboratory evaluation of the repellency of *Metarhizium anisopliae* conidia to *Coptotermes lacteus* (Isoptera: Rhinotermitdae). Sociobiology 36: 133-146.

Su NY, Chew V, Wheeler GS, Scheffrahn RH (1997) Comparison of tunneling responses into insecticide-treated soil by field population and laboratory groups of subterranean termites (Isoptera: Rhinotermitidae). J Econ Entomol 90: 503-509.

Sun JZ, Fuxa JR, Richter A, Ring D (2008) Interactions of *Metarhizium anisopliae* and tree-based mulches in repellence and mycoses against *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Environ Entomol 37:755-763.