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Infectivity of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) isolates to the arboreal termite *Odontotermes* sp. (Isoptera: Termitidae)

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Abstract. Infectivity of the entomopathogenic fungus *Metarhizium anisopliae* against workers of the arboreal termite *Odontotermes* sp. was assayed under laboratory conditions. Test isolates were collected from different sources, including soil from varied locations and insect hosts from the orders Lepidoptera, Coleoptera, Hemiptera and Orthoptera. All the 23 isolates tested and the standard (ARSEF 7413) were pathogenic to the workers of *Odontotermes* sp. at a concentration of 10^7 conidia/ml, with mean mortality ranging from 57.5 to 100%. Two of the isolates (Ma2, Ma13) and the standard caused 100% mortality in the termite species. A detailed bioassay was subsequently conducted with the five most promising isolates, namely Ma1, Ma2, Ma13, Ma16 and Ma17, at concentrations ranging from 10^4 to 10^7 conidia/ml. The lethal concentrations (LC_{50}) of these isolates ranged from 0.01 to 0.46×10^5 conidia/ml. The average survival time (AST) for the termites treated with the most virulent isolate (Ma2) varied from 4.2 to 5.7 days across the four spore loads, while AST with the standard isolate ranged from 5.3 to 6.3 days. Two of the isolates, Ma2 and Ma13, were found to be significantly more pathogenic to *Odontotermes* sp. workers than all the others, including the standard.

Key words: arboreal termite, *Odontotermes* sp., fungus, *Metarhizium anisopliae*, pathogenicity, mycotermiticide, biological control

Introduction

Termites provide critical ecosystem services by recycling dead woody material and contributing to soil formation and turnover (Watson and Gay, 1991). However, some termite species are also major agricultural, forestry and household pests. They can cause severe economic damage to timber and wood products, standing trees, seedlings and even plastics (Rath, 2000). Wiseman and Eggleton (1994) estimated that termites cause around US$ 40,000 million in damage per year to buildings and are responsible for substantial losses to forestry and agriculture. Arboreal termites, especially *Odontotermes* spp., are common in timber plantations in India. Their management, using insecticides like chlorpyriphos, is possible only for short periods at a time (Remadevi *et al.*, 1998).

Termites could be effectively controlled using pathogenic microbes. Their habitats, which are humid and of a relatively constant temperature,

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are ideal for microbial growth, while crowded conditions and social interaction in termite nests encourage transmission of the fungus (Delante *et al.*, 1995; Creffield, 1996).

Many micro-organisms have been associated with termite nests; however, only a few are potential pathogens. Entomopathogenic fungi belonging to the Hyphomycetes group have been isolated from termite body or nest material, and many of these are highly virulent to termites (Zoberi and Grace, 1990; Milner *et al.*, 1998). Entomopathogenic fungi invade their host through the integument and cause death through depletion of host metabolites, production of toxins, destruction of vital tissues or a combination of the three (Yendol and Paschke, 1965; Bao and Yendol, 1971).

Several reviews have been published on the pathology and ecology of the two most widely studied species of entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* (e.g. Almedia *et al.*, 1997; Ferron, 1981; McCoy *et al.*, 1988; Goettel, 1992).

The primary objective of this study was to identify promising native isolates of *M. anisopliae* effective against *Odontotermes* sp. Development and application of formulations of the identified isolates could supplement existing termite control methods and reduce dependence on synthetic chemicals.

Materials and methods

Fungal culture

Test isolates of the fungus *M. anisopliae* used in this study were recovered and purified from soil from different locations (cultivated and fallow land, lake sides, pasture, orchards and forest plantations) and insects from the orders Lepidoptera, Coleoptera, Hemiptera and Orthoptera (Table 1). A selective medium containing 1% glucose, 1% peptone, 1.5% oxgall, 3.2% agar, 10 μg/ml dodine (Sigma Aldrich), 250 μg/ml cyclohexamide and 500 μg/ml chloramphenicol was used for the isolation of the fungus. The isolates were then grown on a potato dextrose agar medium (Hi Media, Mumbai, India) fortified with 1% yeast extract at 26 ± 1 °C in the dark and then stored at 4 °C. The isolates were regularly passed through their respective hosts and re-isolated to maintain virulence. In total, 23 isolates were prepared and tested together with the standard, ARSEF 7413 (USDA-ARS Collection of Entomopathogenic Fungal Cultures).

Table 1. *Metarhizium anisopliae* isolates collected from different sources and localities in India and their mortality effects against *Odontotermes* sp.

Isolate	Insects host or substrate (habitat)	Site and origin	% Mortality (mean ± SE)
ARSEF 7413 (standard)	*Kalotermes* sp. (Isoptera)	Sydney (Australia)	100.0 ± 0.0a (88.5)
Ma1	Mummified larvae (Lepidoptera)	Karnataka, 2006	90.0 ± 8.1b (73.7)
Ma2	Mummified larvae (Lepidoptera)	Karnataka, 2007	100.0 ± 0.0a (88.5)
Ma3	Mummified larvae (Lepidoptera)	Tamil Nadu, 2007	77.5 ± 5.0defg (61.7)
Ma4	*Eutectona machaeralis* (Lepidoptera)	Karnataka, 2007	75.0 ± 5.7efg (60.0)
Ma5	Adult Coleoptera	Karnataka, 2007	67.5 ± 5.0ghi (55.2)
Ma6	Adult Orthoptera	Karnataka, 2007	77.5 ± 5.0defg (61.7)
Ma7	*Eutectona machaeralis* (Lepidoptera)	Karnataka, 2007	67.5 ± 5.0ghi (55.2)
Ma8	Soil (non-cultivated)	Tamil Nadu, 2006	72.5 ± 5.0efgh (58.3)
Ma9	Soil (teak tree orchard)	Tamil Nadu, 2006	70.0 ± 8.1fgh (56.8)
Ma10	*Nilaparvata lugens* (Hemiptera)	Kerala, 2007	62.5 ± 9.5hi (52.2)
Ma11	Soil (termite mound)	Karnataka, 2007	75.0 ± 5.7efg (60.0)
Ma12	Coleoptera	Karnataka, 2007	57.5 ± 9.5i (49.3)
Ma13	Soil (termite mound)	Karnataka, 2007	100.0 ± 0.0a (88.5)
Ma14	*Spodoptera* sp. (Lepidoptera)	Karnataka, 2006	77.5 ± 5.0defg (61.7)
Ma15	Adult Coleoptera	Kerala, 2007	77.5 ± 9.5def (62.0)
Ma16	Adult Coleoptera	Kerala, 2006	87.5 ± 5.0bc (69.4)
Ma17	Soil (forest)	Kerala, 2006	85.0 ± 5.7bcd (67.4)
Ma18	Soil (eucalyptus tree orchard)	Karnataka, 2007	80.0 ± 8.1cde (65.2)
Ma19	Soil (non-cultivated)	Karnataka, 2007	77.5 ± 9.5def (62.0)
Ma20	Dead larvae (Lepidoptera)	Kerala, 2006	75.0 ± 5.7efg (60.0)
Ma21	Soil (termite mound)	Kerala, 2007	75.0 ± 5.7efg (60.0)
Ma22	Soil (termite mound)	Kerala, 2006	80.0 ± 0.0cdef (63.4)
Ma23	Soil (termite mound)	Tamil Nadu, 2006	72.5 ± 5.3efgh (58.3)

Values in parentheses are angular transformed. Means were separated by least significant difference, where standard error of difference = 3.2874, critical difference (CD) at 5% = 6.5533, CD at 1% = 8.6981. Means followed by different letters differ significantly ($P \leq 0.05$, ≤ 0.01).

Termite culture

Workers and soldiers of *Odontotermes* spp. along with soil from mud galleries on termite-infested trees and termite mounds were collected in plastic boxes (10 cm × 5 cm) and brought to the laboratory. They were kept in glass jars and maintained in a biochemical oxygen demand incubator at 25 ± 1 °C. A glass jar (21 cm diameter × 9 cm height) was filled with soil up to 4 cm height. Soil from the infested tree bark was also mixed in with the soil in the glass jar. Two small pieces of rubber wood were partly buried into the soil to serve as a food source. Fifty worker and twenty soldier termites were placed in the glass jar, whose mouths were then covered with muslin cloth. During establishment of the culture, the soil in the glass jar was periodically moistened to provide humidity for the termites.

Bioassays

An initial pathogenicity test of the 23 *M. anisopliae* isolates and the standard was carried out at a concentration of 10^7 conidia/ml. A 1 ml suspension of each fungal isolate was poured into a sterile Petri plate and allowed to dry partially, and worker termites were allowed to walk on the partially dried fungal suspension for 1 min. The contaminated workers were transferred aseptically to sterile Petri plates with small pieces of rubber wood, which were in turn placed in plastic bags containing moist cloth and incubated at 26 ± 1 °C in the dark. Four replicates of each isolate, each with 10 individual workers, were maintained, and mortality was observed at 24 h intervals for up to 6 days. Dead insects were incubated in a humid chamber to confirm growth of the fungus on their cadavers. A batch of uninfected termites was maintained as a control.

The five most effective isolates were selected, as judged from their lethal concentration (LC_{50}) values, and a multiple-concentration assay was conducted using four concentrations of these isolates (10^4, 10^5, 10^6 and 10^7 conidia/ml) using the method described above. The bioassay was repeated three times.

Data analysis

Mortality observed in the controls was used to correct mortality in the treated groups using Abbott's formula (1925). The data were subjected to arc sine transformation and analysed using one-way analysis of variance. The least significant difference test was used to compare the means. The median LC_{50} for the five isolates was estimated by probit analysis (Finney, 1971). The cumulative mortality response for each concentration of the isolates across the assessment period was analysed by Kaplan–Meier survival analysis. All the analyses were carried out using SPSS 11.0 for Windows. The relative potency for each isolate was calculated by dividing the LC_{50} value of each test isolate by that of the standard (Houping *et al.*, 2002).

Results and Discussion

Single-concentration assay

In the single-concentration assay (10^7 conidia/ml), the mortality of *Odontotermes* sp. ranged from 57.5 to 100% after 6 days of treatment (Table 1). Two of the *M. anisopliae* isolates, Ma2 and Ma13, and the standard were severely pathogenic (100% mortality) to *Odontotermes* sp., while Ma1, Ma16 and Ma17 gave more than 80% mortality.

Milner and Staples (1996) tested over 90 isolates of *M. anisopliae* against *Nasutitermes exitiosus* and *Coptotermes* spp. workers and found that many isolates induced over 80% mortality. Khan (1991) reported that among several mycopathogens, *M. anisopliae* was more virulent to *Odontotermes brunneus*. Gunner *et al.* (1994) tested 21 isolates of *M. anisopliae* against *Reticulitermes flavipes* workers and observed 80–100% mortality within 3 days of treatment. Changjin *et al.* (2009) found that conidia from *M. anisopliae* var. *dcjhyium* were highly virulent to *Odontotermes formosanus*, causing approximately 100% mortality after 3 days post-inoculation at a concentration of 3×10^8 conidia/ml.

Multiple-concentration assay

In the multiple-concentration assay, Ma2 and Ma13 were observed to be more virulent than the standard isolate, with significantly lower LC_{50} values (0.01×10^5 and 0.02×10^5, respectively). The LC_{50} of Ma1 (0.08×10^5 conidia/ml) was equal to that of the standard, whereas the LC_{50} values of Ma16 and Ma17 were lower than that of the standard (Table 2). χ^2 values were not significant ($\alpha = 0.05$), indicating a good fit of the regression lines, with regression coefficients for the isolates varying from 1.7 to 2.5. Although in general higher slope values indicate higher virulence, some of the isolates with higher slope values showed lower virulence. This could be due to differences in the nature of the isolates.

Potency indices for the five isolates calculated based on the LC_{50} values ranged from 0.12 to 5.75. A lower potency index was correlated with lower LC_{50} values for all the test isolates (Table 2). Isolates Ma2 and Ma13 had the lowest potency indices (0.12 and 0.25). The average survival time (AST) for the termites treated with the most virulent isolate (Ma2) varied from 4.2 to 5.7 days across the

Table 2. Mortality of *Odontotermes* sp. workers exposed to *Metarhizium anisopliae* isolates

Fungal isolate	No. of workers	Slope ± SEM	$LC_{50} \times 10^5$	95% CI $\times 10^5$	χ^2	Potency index
ARSEF 7413 (standard)	480	2.3 ± 0.60	0.08	0.0–0.24	2.4	—
Ma1	480	1.7 ± 0.56	0.08	0.0–0.03	1.1	1.00
Ma2	480	2.2 ± 0.96	0.01*	0.0–0.05	3.0	0.12
Ma13	480	2.3 ± 0.81	0.02*	0.0–0.09	2.0	0.25
Ma16	480	2.5 ± 0.55	0.46**	0.1–1.1	1.1	5.75
Ma17	480	2.2 ± 0.53	0.44**	0.8–1.2	4.1	5.50

LC_{50}, lethal concentrations; ARSEF 7413, standard isolate with which all test isolates were compared.
* LC_{50} value significantly lower than that of the standard ($\alpha = 0.05$).
** LC_{50} value significantly higher than that of standard ($\alpha = 0.05$).

four spore loads, while in the standard isolate AST ranged from 5.3 to 6.3 days (Table 3).

Pathogenicity by source of isolate

Mortality induced by the seven isolates from lepidopteran hosts ranged from 67.5 to 100%, among which Ma1 and Ma2 caused 90 and 100% mortality, respectively (Table 1). Isolates from coleopteran hosts recorded 57.5–87.5% mortality. The isolate from an orthopteran host (Ma6) caused 72.5% mortality and that from Hemiptera caused 62.5%. Of the six isolates obtained from termite mounds, Ma13 and Ma22 induced more than 80% mortality. The isolate from forest soil (Ma17) caused 85% mortality, while two obtained from tree orchards (Ma9 and Ma18) induced 70 and 80% mortality, respectively. Isolates from non-cultivated

Table 3. Kaplan–Meier survival analysis for time–mortality response of *Odontotermes* sp. workers exposed to *Metarhizium anisopliae* isolates

Fungal isolate	Concentration	AST (mean ± SE)	95% CI	MST	95% CI
ARSEF 7413 (standard)	10^7	5.3 ± 0.4	4.9–5.7	5.0	—
	10^6	5.4 ± 0.3	4.9–5.8	5.3	4.6–5.2
	10^5	6.1 ± 0.2	5.6–6.3	6.0	5.7–7.1
	10^4	6.3 ± 0.2	5.8–6.8	6.5	5.9–7.1
Ma1	10^7	5.3 ± 0.4	4.9–5.7	5.0	—
	10^6	5.4 ± 0.4	5.0–6.2	6.0	5.7–7.3
	10^5	5.9 ± 0.9	5.0–6.3	6.0	5.7–7.3
	10^4	6.1 ± 0.4	5.5–6.9	7.0	—
Ma2	10^7	4.2 ± 0.1	3.9–4.5	4.0	—
	10^6	4.2 ± 0.3	3.9–4.6	4.0	—
	10^5	5.2 ± 0.2	4.8–5.6	5.0	—
	10^4	5.7 ± 0.3	5.1–6.3	5.8	4.5–7.1
Ma13	10^7	4.8 ± 0.4	4.0–5.1	4.5	3.8–4.7
	10^6	5.3 ± 0.2	5.0–5.4	5.0	—
	10^5	5.9 ± 0.3	5.0–5.7	5.5	5.0–6.1
	10^4	6.2 ± 0.4	5.5–6.9	7.0	—
Ma16	10^7	6.0 ± 0.2	5.2–6.4	6.0	—
	10^6	6.0 ± 0.2	5.2–6.4	6.0	—
	10^5	6.8 ± 0.2	6.4–7.2	7.0	—
	10^4	6.8 ± 0.2	6.3–7.2	7.0	—
Ma17	10^7	6.1 ± 0.8	5.9–6.7	7.0	—
	10^6	6.6 ± 0.2	6.2–7.1	7.0	—
	10^5	6.8 ± 0.2	6.2–7.1	7.0	—
	10^4	6.8 ± 0.2	6.3–7.2	7.0	—

AST, average survival time. AST evaluation was limited to 7 days. $\alpha = 0.05\%$ according to the logrank test. MST, mean survival time.

soil (Ma8 and Ma19) induced 72.5 and 77.5% mortality, respectively. There was significant difference in virulence by source of the isolate.

It has been hypothesized that most virulent fungal strains are generally isolated from the test organism or a closely related species (Latch, 1965, 1976; Soares *et al.*, 1983; Poprawski *et al.*, 1985). However, no apparent relationship between pathogenicity and origin of isolates was observed in the present study. Isolates Ma2, Ma13 and Ma1 performed well, although they were not originally isolated from *Odontotermes* sp. The most pathogenic isolate, Ma2, was from a mummified lepidopteran larva collected from a forest locality. Jones *et al.* (1996) also observed similar LC_{50} values from three isolates of *M. anisopliae* recovered from three different species of scarab larvae in Australia, assayed against *Coptotermes formosanus*. Milner and Staples (1996) tested more than 90 isolates of *M. anisopliae* against *N. exitiosus* and *Coptotermes* spp. workers, and found that many of the isolates induced over 80% mortality. In a separate study, Milner *et al.* (1998) also observed only small differences in the mortality caused by several isolates from different sources in these two species.

Applicability in termite control

The tested isolates of *M. anisopliae* demonstrated high levels of virulence against *Odontotermes* sp. under laboratory conditions. Biological control with pathogenic fungi, already in use in agriculture, could also provide a long-lasting solution for termite control in areas like urban dwellings or forests. An isolate with low LC_{50} and short lethal time (LT_{50}) would be more economical, as smaller quantities would be required.

Dissemination strategies for pathogenic fungi are an important consideration. Social interaction, dark and damp habitats and a high relative humidity within a termite colony provide an ideal microenvironment for the germination of fungal spores on infected insects and also their spread to healthy members of the population. The dynamic behaviour of termites also favours infection under field conditions, as workers spend much time moving with soldiers in the nests and galleries. Although some behavioural characteristics of termites help in the removal of fungal spores from the body of contaminated individuals to result in random and low-level infections, grooming is not always effective in removing all fungal conidia and can, indeed, cause further cross-contamination. Intensive applications, however, could assure the spread of fungal infection despite these types of social behaviour.

Conclusion

This study identified virulent isolates of *M. anisopliae* which could serve as good candidates for development as mycotermiticides against *Odontotermes* sp. Fungal spores of these isolates could be formulated with non-repellent or attractant constituents or baits to strengthen their efficacy in termite control. This study also indicates that a screening exercise for potential pathogenic isolates against a pest insect should not be limited to isolates from the pest or closely related species. In addition, cultural characteristics and the effects of varying environmental conditions on the pathogen's growth and sporulation should be evaluated before its development into a mycotermiticide.

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