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Laboratory evaluation of the entomopathogenic fungus, *Metarhizium anisopliae* var. *major* against the subterranean termite, *Odontotermes guptai* Roonwal and Bose

P. R. SWARAN and R. V. VARMA
Division of Entomology
Kerala Forest Research Institute
Peechi, Thrissur 680653, Kerala, India
E-mail: swaranpr@yahoo.com

ABSTRACT: **Infectivity of the soil mixed conidia of the fungal pathogen *Metarhizium anisopliae* var. *major* was evaluated against the termite, *Odontotermes guptai* Roonwal and Bose under laboratory conditions. Four concentrations, *viz.*, $0.8x10^7$, $1.2x10^7$, $1.6x10^7$, and $2x10^7$ conidia/g soil caused complete mortality of the test termite in 192, 156, 132 and 120 hours, respectively. The LC_{50} value at 120h was calculated as $9.07x10^6$ conidia/g soil. The LT_{50} value for the most effective dose was 86.84h. However, the test termites became less active within 48 hours of incubation.**

KEY WORDS: Eucalyptus, *Metarhizium anisopliae*, *Odontotermes guptai*, termite

INTRODUCTION

Termite control is largely dependent on chemical insecticides and the possibilities of alternative methods are being explored worldwide. Among the biological agents reported against termites, entomopathogenic fungi are considered the most suitable (Jones *et al.*, 1996) due to various reasons including the humid habitat and interacting social behaviour of the termites, which are conducive for the multiplication and spread of the pathogen. The two fungal pathogens, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauvaria bassiana* (Balsamo) Vuillemin (Dueteromycotina: Hyphomycetes) have greater potential against termites, and have received more attention in recent times. *B. bassiana* is reported to have poor survival in the soil (Ferron, 1978) and also less pathogenic to termites compared to *Metarhizium* (Sajap and Jan, 1990; Grace, 1991; Jones *et al.*, 1996). Screening of isolates and testing of pathogenicity of *M. anisopliae* against different species of termites have been reported earlier (Hanel and Watson, 1983; Sajap and Jan, 1990; Milner *et al.*, 1998). In most of these studies the fungal conidia was directly applied on to the test termite and in the few field trials, conidia were applied to the termite mound (Hanel and Watson, 1983).

Subterranean termites cause serious damage to young forest plantations. In eucalyptus, the loss of out-planted seedlings due to termite attack goes up to 80 per cent (Nair and Varma, 1981). Infectivity of the soil mixed conidia, once proved would be helpful in exploring the possibility of its use against subterranean termites. The termite species used in the bioassay, *Odontotermes guptai* is widely distributed in India and is known to attack roots

and bark of young eucalyptus (Nair and Varma, 1985). The purpose of the present study was to investigate the potential of soil mixed conidial suspension of *M. anisopliae* var. *major* against termites under laboratory conditions.

MATERIALS AND METHODS

Preparation of conidial suspension

A pure culture of *M. anisopliae* var. *major* was obtained from the Central Plantation Crops Research Institute (CPCRI), Regional Station, Kayamkulam, Kerala. In order to maintain the virulence, the fungus was re-isolated from termites infected with conidial application in the laboratory. The re-isolated conidia was subcultured in Petri-plates with Potato Dextrose Agar (PDA) medium. Fungal conidia were collected from 10-14 day-old cultures by scrapping off with a glass rod. A homogeneous conidial suspension was prepared in sterile distilled water by adding a few drops of the wetting agent Tween-20. The conidial concentration of the suspension was determined using an improved Neubauer Haemocytometer. A stock solution of $4x10^7$ conidia per ml was prepared from which serial dilutions were made.

Laboratory bioassay

Four doses of the conidial suspension, ranging from $0.8x10^7$ to $2x10^7$ conidia per gram soil were evaluated in the bioassay. Four conidial concentrations, *viz.*, $1.6x10^7$, $2.4x10^7$, $3.2x10^7$, $4x10^7$ conidia per ml were prepared as follows. Four volumes, namely, 2, 3, 4 and 5ml each were drawn from the stock solution ($4x10^7$) and the lower volumes were made up to 5ml with distilled water to equalize the effective application volume. Each of the four resulting doses was mixed well with 10g of sterilized soil in Petri-dishes of 10cm diameter. Thus the effective concentration becomes $0.8x10^7$, $1.2x10^7$, $1.6x10^7$ and $2x10^7$ conidia per gram soil, respectively.

The termite species used in the bioassay was *Odontotermes guptai* Roonwal and Bose (Isoptera: Termitidae). Field collected worker termites, maintained in the laboratory were released in twenty numbers each to the Petri-plates containing conidia treated soil. A filter paper of 10cm diameter was placed above the soil without disturbing the termites and covered with a glass plate permitting air circulation. Same number of termites, released into the soil treated with distilled water served as control. There were three replicates per each dose. Water was sprinkled at regular interval to maintain high humidity for the survival of the termites. Observations were taken at 12h interval and dead termites were removed and kept on moist filter paper in Petri-dishes for mycelial growth and sporulation.

The time mortality relation was analysed using Probit analysis (Finney, 1972). A software POLO-PC (©LeOra Software, 1987), based on Finney (1972) was used for the analysis of the replication-wise data. The angular transformed values of the percentage mortality were statistically analysed using one way Analysis of Variance (ANOVA) to find significance of variation between the treatments at various incubation periods using SPSS version 10.

RESULTS AND DISCUSSION

Complete mortality of the termites was observed from 120 to 192h post inoculation with different doses. A dose of $2x10^7$ conidia/g soil was the most effective, which resulted in complete mortality of the test termite within 120h of incubation. From the 60h observation onwards the conidial treatments showed significant difference over the control in termite mortality. From 96h onwards there was significant difference within the various doses and from 120h, all the treatments were significantly different from each other (Table1). Presence of the fungal hyphae in the smear made from cadavers confirmed the cause of mortality. Though some mortality was observed in the control (26.5%), the cause could not be determined. The mortality-time relation with respect to different conidial concentrations showed an increase in mortality with increase in the conidial concentration (Fig.1). The lethal concentrations (LC_{10}, LC_{50} and LC_{90}) at 120h obtained are given in Table 2. The median lethal concentration (LC_{50}) was calculated as $9.074x10^6$ conidia/g soil. The median lethal time (LT_{50}) for the most effective dose ($2x10^7$conidia/g

Table 1. Percentage mortality of termites in the laboratory bioassay

Incubation period (h)	Mean mortality of termites (%)*					LSD
	NIL	0.8 x 10^7 conidia/g soil	1.2 x 10^7 . conidia/g soil	1.6 x 10^7 conidia/g soil	2 x 10^7 conidia/g soil	
60	0^a	$3.3(10.45)^{ab}$	$9.6\ (18.05)^b$	$5.6\ (13.74)^b$	$10\ (18.44)^b$	13.13
72	$1.1\ (6.15)^a$	$9.6(18.05)^b$	$23\ (28.67)^b$	$24.6(29.73)^b$	$24.9\ (29.92)^b$	11.90
84	$8.2(16.60)^a$	$24.9(29.92)^b$	$34.9(36.24)^c$	$40\ (39.21)^c$	$40(39.21)^c$	5.6
96	$8.2(16.60)^a$	$29.9(33.16)^b$	$43.3(41.15)^c$	$48.3(44.03)^c$	$60\ (50.79)^d$	6.24
108	$11.6(19.89)^a$	$45(42.12)^b$	$61.7(51.76)^c$	$60.3(50.96)^c$	$75.1\ (60.08)^d$	7.79
120	$14.8(22.60)^a$	$53.3(46.91)^b$	$71.7(57.86)^c$	$86.8(68.66)^d$	$100\ (90.00)^e$	4.37
132	$21.6\ (27.71)^a$	$65.2(53.82)^b$	$83.4(65.95)^c$	$100\ (90.00)^d$	-	5.58
144	$21.6(27.71)^a$	$68.5(55.85)^b$	$93.1(74.81)^c$	-	-	16.26
156	$23.3(28.85)^a$	$79.3(62.91)^b$	$100\ (90.00)^c$	-	-	10.54
168	$24.9(29.92)^a$	$89.8(71.39)^b$	-	-	-	11.79
180	$24.9(29.92)^a$	$97.6(81.15)^b$	-	-	-	17.78
192	$26.5(30.99)^a$	$100(90.00)^b$	-	-	-	4.35

Figures superscripted by same letters across a row are homogeneous at P=0.05.

Figures in parentheses are the angular transformed Values.

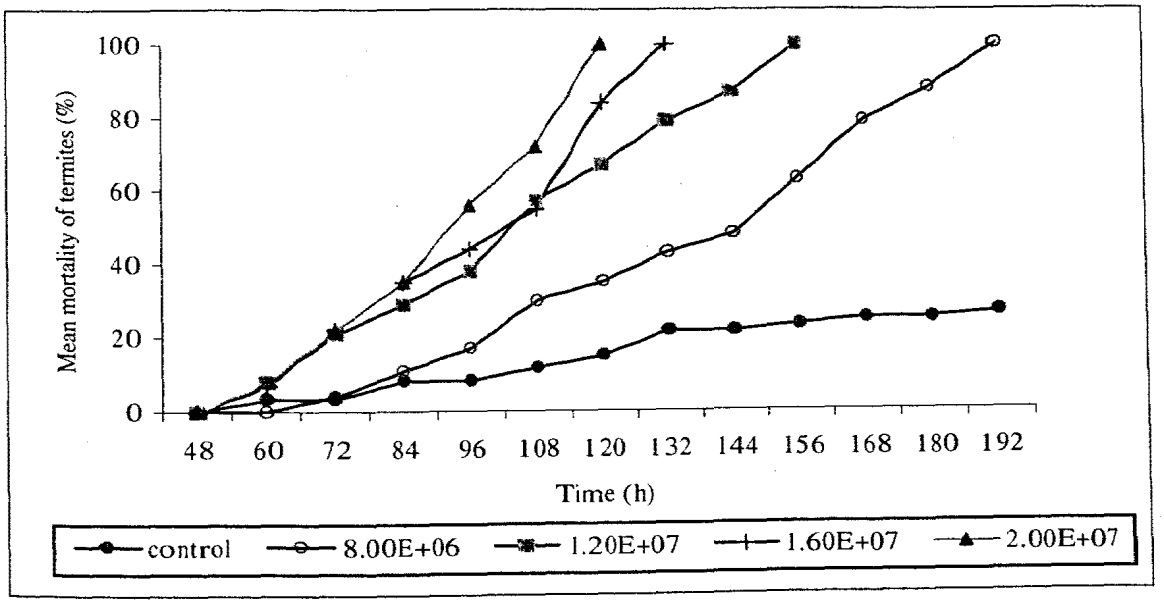

Fig. 1.Time - mortality relationship in the bioassay

Table 2. Lethal concentration (LC) values at 120h

Lethal concentration	Dosage (Conidia /g soil)	Confidence	Slope limits (95%)	Potency of estimation (95% CL)
LC_{10}	$4.96x10^6$	$3.18x10^6$ to $6.27x10^6$	4.881±.81	0.106
LC_{50}	$9.07x10^6$	$7.56x10^6$ to $1.02x10^7$		
LC_{90}	$1.66x10^7$	$1.47x10^6$ to $2.02x10^7$		

'With almost all good sets of data, potency of estimation will be substantially smaller than 1.0, and seldom greater than 0.4." (Finney 1972)

Table 3. Median lethal time (LT_{50}) for the different doses

Dose (conidia / g soil)	LT_{50} (h)	Confidence limits (95%)	Slope	Potency of estimation (95% CL)
$0.8x10^7$	113.079	108.78 to 117.39	6.662±.412	0.015
$1.2x10^7$	95.826	92.065 to 99.604	7.306±.505	0.018
$1.6x10^7$	91.228	87.822 to 94.734	8.491±.676	0.024
$2.0x10^7$	86.841	83.704 to 90.090	9.497±.808	0.028

soil) was found to be 86.84h. LT_{50} values calculated for the different doses are given in Table 3. Ferron (1981) reported that mixing 10^5 to 10^8 spores/g soil would normally develop the disease in insects under laboratory conditions.

The bioassay showed that conidial suspension of *M. anisopliae* var. *major* when mixed with soil is pathogenic to *O. guptai* and can cause complete mortality of the termites within a week. However, the infectivity of the different conidial doses in the bioassay could be related to the number of conidia received per individual termite, which can vary since the conidia were mixed with soil and not applied directly on the termites. Several other factors, including the behaviour of the test termite would also determine the spread of conidia. It is generally held that there could be variation in virulence depending on the isolate from various hosts and/or geographical regions. Ignoffo and Garcia (1985), opined that microbes undergo selective recombination and mutation in nature depending on the ecological situation which influences their genetic make up which will also reflect on the virulence of the microbes. Although the pathogen used in the present study was originally from a different host (*Oryctes rhinoceros* L. (Coleoptera; Dynastidae)), it was infective to termites. There was a marked decrease in the overall activity of treated termites from the second day of bioassay.

The infectivity of soil mixed conidia is a pre-requisite for the successful field application of the pathogen. It is reported that the infective conidia of *M. anisopliae* can survive in soil up to 90 days (Anonymous, 2000). A field evaluation of the conidia is being carried out for the management of termites attacking eucalypt plantations and the observations so far is promising. One major requirement for survival of the fungus is the high levels of relative humidity and the forest plantation soil offers this

ideal condition. Milner (2000) observed that the conidial treated areas of termite mound as being walled-off by the workers. This behaviour is significant in field application of the microbial agent as it can probably tempt the termites to keep away from the conidia treated soil. There are also reports on variations in the repellent behaviour exhibited by termites with different isolates of *Metarhizium* under laboratory conditions (Staples and Milner, 2000), which requires detailed investigations.

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