Sporulation Characteristics and Virulence of *Metarhizium anisopliae* Against Subterranean Termites (*Coptotermes formosanus*)

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**Abstract:** In the present study, four fungal isolates (*Metarhizium anisopliae*) were isolated and examined for their sporulation characteristics and virulence against *Coptotermes formosanus*. Initially fungal strains were isolated through insect bait (*Galleria*) method. The mortality was significantly varied between 2nd to 4th days. In this study, the mortality of termites was observed after two days after post-inoculation of *Metarhizium* spp. (Tk 4) which was a high virulent strain identified showing 86.6% mortality rate on 4th day post inoculation. The sporulation occurred after a week, while the white mycelial coverage followed by green conidia had appeared after 5-7 days on the cadavers.

**Key words:** *Metarhizium anisopliae* • *Coptotermes formosanus* • *Galleria mellonella* • pathogenicity

**INTRODUCTION**

Entomopathogenic fungi (EPF) belonging to the Hyphomycetes group have been isolated from termite body or nest material and many of these are highly virulent to termites [1,2]. 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 [3,4]. Termites are important urban pests which can cause a tremendous amount of damage to homes and structures. SUBTERRANEAN TERMITES, including the Formosan subterranean termite, *Coptotermes formosanus* prevention and control costs about $1 billion in the United States annually [5]. The Formosan subterranean termite is becoming the predominant termite pest species through out the world. Current Formosan subterranean termite control methods involve slow-acting, non-repellent termiticides and baits [6]. Foraging workers carry these termiticides back to the nest and spread them. Prevention of termite damage has been challenge because of their large populations and cryptic behavior. Various methods in termite control were explored in the past including physical, cultural, chemical and biological methods [7]. Extensive use of chemicals can cause environmental hazards and induce resistance. Biological control with pathogenic fungi might provide long-lasting insect control hazard up to environment or non-target organisms [8-10] and a promising alternative to chemical control against the Formosan subterranean termite, *Coptotermes formosanus* [11,12]. Hence, Insect pathogens are attractive candidates for baiting because of their self-replicating nature and safety to non-target animals.

*Metarhizium anisopliae* causes natural mortality of insects level in the field is increasing attention worldwide as a promising mycoinsecticide against pests such as froghoppers, scarabs, locusts and termites. A crucial initial step is the selection of a virulent isolate for further development. Frequently, the most effective isolate is obtained from an epizootic in the target pest under natural conditions [2]. Virulence and repellency are two factors that must be addressed to improve the efficacy of *M. anisopliae*. A high level of virulence is essential to control termites with minimum cost and with highest efficacy. Low repellency of the fungal conidia or a non-repellent fungal conidial formulation is necessary to induce a continuous infection to termite colonies. However, it is often true that highly virulent fungal strains...
are also highly repellent [13]. Most studies addressing termites control is focused on *Metarhizium* sp. and *Beauveria* sp. Recently, Eilenberg, Hajek and Lomer [14] patented *Paecilomyces* sp. for controlling subterranean termites. Many microorganisms have been associated with termite nests; however, only a few are potential pathogens. 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*.

This paper reports on twenty new *Metarhizium anisopliae* strains isolated from Indian sub-region at Theni districts and comparison of their virulence and pathogenecity against *C. formosanus* and *Galleria mellonella* under the laboratory conditions.

**MATERIALS AND METHODS**

Test isolates of the entomopathogenic fungus used in this study was isolated from agricultural soil samples of Theni district, Tamil Nadu. The conidia of *Metarhizium* spp., was collected by growing the fungus on SDAY plates, which were incubated at room temperature (27±2°C) for 14 days. Conidia were harvested by flooding each plate with 3-5 ml of sterile distilled water and 0.005% Tween 80 and the surface of the plate was scraped with glass plate and the resulting solution was filtered through Whatmann No.1. The conidial suspensions were determined using a haemocytometer and adjusting to concentrations of 1×10^7 conidia/ml.

**Preparation of Conidial Suspension and Bioassay:** An initial pathogenicity test of the four isolated *Metarhizium* spp. was carried out at a concentration of 1×10^7 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 1min. The contaminated workers were transferred aseptically to another sterile Petri-plates with small pieces of rubber wood, which were placed in plastic bags containing moist cloth and incubated at 26°C in the dark condition. Four replicates of each isolate, each with 10 individual workers, were maintained and mortality was observed at intervals (every 24 h) up to 6 days. Dead insects were incubated in a humid chamber for mycosis to confirm growth of the fungus. A batch of uninfected termites was maintained as a control. The bioassay was repeated three times.

**RESULTS**

Entomopathogenic fungus *M. anisopliae* was recovered from 20 (16.6%) out of 150 soil samples from agricultural fields in Theni region using a *Galleria* bait method. The occurrence of fungal species depends on moisture, pH, temperature and different agriculture field condition and vegetation type such as Banana, rice, Coconut and sugarcane. *Metarhizium* spp., *Beauveria* spp., *Paecilomyces* spp., *Aspergillus* spp. and * Fusarium* spp. were mostly recovered entomopathogenic fungus from soil. In our observation, the infected *Galleria* larvae of *Metarhizium* spp. turned from gray to brown or black in color with the white mycelia covering cadaver were finally produces green color conidial mass. Further, the time duration of insect infectivity of EPF was about 5-7 days and mycosis about 10-14 day (Fig. 1).

Since the highest number of isolates on EPF was recovered from red soil type of agriculture field. In our observation, *Metarhizium* spp. was found be positive in red soil type and soil samples that were characterized such as pH ranges between 6.54-7.55%, organic content 0.72 1.27 % and moisture content 14-31%.(Table1).

EPF was evaluated for its efficacy and pathogenicity *G. mellonella* larvae was used as a model insect (Koch’s postulate method). All four potential *Metarhizium* sp. strains were selected from preliminary screening,
Table 1: Isolation of entomopathogenic fungi *Metarhizium anisopliae* and their physico-chemical characterization of soil sample

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Host infectivity (%)</th>
<th>Time duration of infection (days)</th>
<th>Sporulation (days)</th>
<th>Nature of infected host</th>
<th>Physico-chemical characterization of soil</th>
<th>Organic content (%)</th>
<th>Electric Conductivity (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tk-4</td>
<td>90</td>
<td>4-6</td>
<td>4-5</td>
<td>Red soil</td>
<td>25-30</td>
<td>6.5-7.59</td>
<td>0.72</td>
</tr>
<tr>
<td>Tk-5</td>
<td>70</td>
<td>4-7</td>
<td>4-5</td>
<td>Sandy soil</td>
<td>25-30</td>
<td>6.8-7.42</td>
<td>0.75</td>
</tr>
<tr>
<td>Ot-4</td>
<td>65</td>
<td>4-6</td>
<td>4-6</td>
<td>Sandy soil</td>
<td>27-30</td>
<td>7.0-7.68</td>
<td>0.57</td>
</tr>
<tr>
<td>Md-1</td>
<td>40</td>
<td>4-7</td>
<td>4-7</td>
<td>Sandy soil</td>
<td>26-30</td>
<td>6.8-7.55</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Table 2: Pathogenicity of EPF against *C. formosanus*

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>% of mortality against <em>C. formosanus</em> (1 × 10^7/termite)</th>
<th>Sporulation (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tk-4</td>
<td>23.3±0.7</td>
<td>48hr</td>
</tr>
<tr>
<td>Tk-5</td>
<td>20.4±4.6</td>
<td>72hr</td>
</tr>
<tr>
<td>Ot-4</td>
<td>22.1±0.4</td>
<td>96hr</td>
</tr>
<tr>
<td>Md-1</td>
<td>23.3±0.9</td>
<td></td>
</tr>
</tbody>
</table>

which produced approximately 100% mortality among the twenty recovered isolates. The potential isolates of *Metarhizium* spp. was assessed for pathogenicity against subterranean termites *Coptotermes formosanus* soldiers. The concentration of 1×10^7 conidia/ml suspension caused an apparent repellency to termites. The potential isolates (Tk-4 (86.6±1.7), Md-1 (83.3±1.3) Tk-5 (79.5±2.5), Ot-4 (80.5±5.7) were produced mortality rate on 4th day post inoculation and exhibited exposed mycelial development (Table 2). *Metarhizium* sp. has been produced conidia on spots on the cadaver surface and it was completely with mycelial growth. Significantly mortality rate were difference between in four potential strains controlling to termites. *Metarhizium* sp. causing a high virulent and epizootic disease caused against *Coptotermes formosanus* termites. Several authors have reported that the conidia of virulent strains of *M. anisopliae* are repellent to termites.

**DISCUSSION**

This study provides the general information about the diversity and distribution of entomopathogenic fungi in different crop ecosystems of Theni district. The occurrence of EPF both *Metarhizium* spp. and *Beauveria* spp. in soil samples was about 32%, collected from different crop fields and trapped using a *Galleria* bait method. The abundance of EPF depends upon the soil environment, both micro and macro climate conditions and insect availability conditions. [15]. In this study, 13.3% of *Metarhizium* spp. and 8.0% of *Beauveria* spp. were isolated from Red soil of the Banana field.

The preliminary screening for virulence against *G. mellonella* shows that four strains were highly virulent and infectivity within short span of time. Therefore, sporulation is very important for reproduction and proliferation for EPF for insect control strategies. The four potential strains of *M. anisopliae* were tested against *C. formosanus* soldiers. *M. anisopliae* strains Tk 4 and Md 3 were highly virulent producing approximately 86.6±1.7% and 83.3±1.3 % mortality rate, reached on 4th day of post inoculation. In earlier, C4-B a *M. anisopliae* strain is highly virulent against *C. formosanus* and causing a 100% mortality rate on 14th day as reported by [15].

Recent reports have suggested that naturally occurring EPF *M. anisopliae* is a highly potent agents for controlling of termite populations. Isolates of *Metarhizium* vary in their pathogenicity for specific hosts; the most effective isolates are often obtained from naturally infected target hosts [16] The library of nearly 100 isolates was established as part of the termite control project in Australia contained hardly any collected directly from termites and those found in termite workings were thought to have originated from surrounding soil rather than being associated specifically with termites [2]. The isolates were typical *M. anisopliae* and did not represent a specific pathogenic type.

The effective exploitation of entomopathogenic fungi for the biological control of pests in agricultural fields has been limited by the humid warm condition required for infection. The isolation of entomopathogenic fungi provides an opportunity to develop them as bio-control agents for agricultural pests in the future.
The pathogenicity was demonstrated that these isolates might be used to control the termite populations and pest insects.

*M. anisopliae* were developed and successfully used as microbial agents against pest insects. The search for effective insect pathogens that can be used in insect control operations has been ongoing for several decades. Both laboratory and field studies of those fungi that appeared to have potential for operational use, have been evaluated. If promising, this was followed by development of methods for mass production and finally implementation in operational control programs, although few fungal species have reached this latter stage of development.

Sporulation of *M. anisopliae* may have a greater effect than virulence on laboratory transmission in *C. formosanus* in our experiment. Fungal isolates producing more conidia or producing them more quickly may simply counteract the advantage of a fungal isolate with greater virulence by getting more conidia to the target termite [17].

This study identified virulent isolates of *Metarhizium* sp. which could serve as good candidates for development as insecticides against insect model. Fungal spores of these isolates could be formulated with non-repellent or attractant constituents or baits to strengthen their efficacy in insect 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. Research should target three areas that need improvement: application, storage and production.

REFERENCES