Laboratory evaluation of *Metarhizium anisopliae* (Metschnikoff) for controlling *Amitermes vilis* (Hagen) and *Microcerotermes gabrielis* (Weidner) (Isoptera: Termitidae)

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Abstract: Subterranean termites are one of the most important pests of buildings, historic monuments and agricultural crops in some parts of Iran. Using entomopathogenic fungi as microbial insecticides is usually a part of biological control and insect pest management. The pathogenicity of entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin (DEMI 001) isolated from *Rhynchophorus ferrugineus* (Oliver) was compared against two subterranean termites, *Amitermes vilis* (Hagen) and *Microcerotermes gabrielis* (Weidner) under laboratory conditions. Suspensions of the fungus spores at five concentrations of $10^1$, $10^2$, $10^3$, $10^4$, $10^6$ spores ml$^{-1}$ were prepared to define LC$_{50}$ and LT$_{50}$. To determine LC$_{50}$ and LT$_{50}$ of *M. anisopliae*, bioassays were carried out on worker casts of both termite species. LC$_{50}$ values for *A. vilis* and *M. gabrielis* were $8.5 \times 10^3$ and $0.2 \times 10^2$ spores ml$^{-1}$, respectively. LT$_{50}$ value for *M. gabrielis* was shorter than that of *A. vilis* at all five concentrations tested. According to the results of the bioassay, *M. anisopliae* was more effective for controlling *M. gabrielis* than that for *A. vilis*.

Keywords: *Amitermes vilis*, *Microcerotermes gabrielis*, *Metarhizium anisopliae*, LC$_{50}$ and LT$_{50}$.

Introduction

Termites have an important economic role in economic entomology, with the cost of damage to the buildings, especially in developed countries in America and Asia, amounting to millions of pounds. In developing countries they have even more adverse effect, destroying local cottages and crops of poor subsistence farmers. Villages in India and Egypt have been destroyed by termites and the residents forced to move to other regions.

In Asia, ancient temples have also been attacked (Pearce, 1997).

In agriculture, termites’ attacks occur at different development stages of crops, particularly at seedling and maturity stages. In general, damage is greater in rain-fed than irrigated crops and during dry periods than periods of regular rainfall (Cowie and Wood, 1989).

Termites are also important urban pests, which can cause a tremendous amount of damage to homes and structures. Prevention of termites’ damage has been a challenge due to their large populations and cryptic behavior. Different methods for control of termites have been investigated in the past.
Pathogenicity of Metarhizium anisopliae for termites

including physical, cultural, chemical, and biological control methods (Pearce, 1997). Among them, the biological control is one that has received great interest among researchers (Milner and Staples 1996, Grace 1997). Biological control, particularly using entomopathogenic fungi, is an essential part of integrated pest management (IPM) strategies for reducing the population density of many pests. Therefore, conservation of entomopathogens that occur naturally, or are introduced for insect control, should be considered in plant protection programs (Oliveira et al., 2003). Using synthetic chemicals to control soil insects, like termites, has led to many problems such as ground water contamination, insecticide resistance in pests, pest resurgence, undesirable toxic effects to natural enemies, toxic residues in crops and other environmental concerns. In the last decade, many entomopathogens such as viruses, bacteria, protozoa and fungi have been tested against insect pests such as subterranean termites with considerable success. Among these, fungi have been widely examined in an attempt to control termites (Kramm and West, 1982; Milner et al., 1998) and among various entomopathogenic fungi used for controlling termites, many studies were focused on Metarhizium anisopliae (Metschnikoff) Sorokin and Beauveria bassiana (Balsamo) Vuillemin (Culliney and Grace, 2000; Grace, 1997; Rath, 2000). Both species are widely distributed in soil and have very broad host ranges, they have generally proven to be effective against termites in laboratory studies but have had little success in field trials (Hanel and Watson, 1983; Lai, 1977; Milner and Staples, 1996). M. anisopliae is an important entomopathogenic fungus which has been a long-standing model for the study of biological control of insect pests by fungi. This fungus is known to infect and kill a range of species of termites under laboratory conditions (Kramm and West, 1982; Milner et al., 1998). M. anisopliae is a ubiquitous fungus that can be found globally in most soils (Huxham et al., 1989).

Disease transmission is considered to be very important in termite control because in many species major parts of the colony and the nest are not accessible to direct treatments (Rath, 2000).

In the past, there were no studies on entomopathogenic fungus; M. anisopliae to control A. vilis and M. gabrielis in Iran. Therefore, the aim of this research was to evaluate pathogenicity of M. anisopliae isolated from Rhynchophorus ferrugineus (Oliver) against A. vilis and M. gabrielis.

Materials and Methods

Collection and preparation of termites

The population of A. vilis was collected from a termite-infested monument (Saveh Jameh mosque) in Iran using a trapping technique. The population of M. gabrielis was collected using cardboard bait buried in soil in the region of Dasht Ahmad located near Qom province of Iran. The traps consisted of five thin slices (25 × 6 × 0.5 cm) of wood wrapped in cardboard rolls, encircled by a PVC pipe with a lid to protect the trap from rain. Trapped termites were placed in plastic boxes, taken to the laboratory in Iranian Research Institute of Plant Protection located in Tehran. They were provided with fresh moistened cardboard, and were held at 25 °C for further use. Distilled water was sprayed on the inside walls of the container to keep the relative humidity above 80 %. Mature worker and soldier termites were separated from logs.
or nest debris by breaking and tapping materials into plastic trays containing moist paper towels. Termites were then sorted using a soft bird feather and were used for bioassay within one hour of extraction and segregation.

**Entomopathogenic fungus**

*M. anisopliae* isolate DEMI 001 (isolated from *R. ferrugineus*) was used in this study. The fungus was cultured on Sabouraud’s Dextrose Agar (Merck, Darmstadt, Germany) with 1 % yeast extract (SDAY) in Petri dishes (9 cm in diameter), and incubated for 2-3 weeks at 25 ± 1 °C, under a 16 : 8 h (L : D) photoperiod and 60 ± 5 % RH. A suspension of the fungus sposs was prepared, its concentration was determined using an improved haemocytometer and adjusted to five concentrations (10¹, 10², 10³, 10⁴ and 10⁶ spores ml⁻¹).

**Inoculation**

Before each test, conidia of *M. anisopliae* were examined under a phase contrast microscope to check for their germinability. With five ml of conidial suspension Of each concentration was pipetted onto filter paper placed in the sterile plates. Three replicates of 10 termites were used for each conidial concentration of *M. anisopliae*. Controls were treated in a similar manner, but the conidia suspensions were replaced with plain water. The plates of treated termites were kept in a dark chamber maintained at an average temperature of 28 °C with a range of 26-30 °C and an average ambient relative humidity of 92-96 %. Sterile distilled water was sprayed at the inner side of the plate covers at 2 days intervals to maintain the humidity. The termites were checked daily and their mortality was recorded for seven days. Only the mortality data on 7th day were used for Abbott’s formula (Abbott, 1925). Mortality data were transferred to Probit for analysis. The corrected mortality was calculated by the difference between total death in the treatment and the control.

**Results**

Table 1 shows LC₅₀ values for *A. vilis* and *M. gabrielis* after treatment with *M. anisopliae*. The LC₅₀ value of entomopathogenic fungus against *M. gabrielis* (0.2 × 10² spores ml⁻¹) was lower than that of *A. vilis* (8.5 × 10³ spores ml⁻¹).

<table>
<thead>
<tr>
<th>Specie</th>
<th>No. insects</th>
<th>No. concentrations</th>
<th>Slope (± SE)</th>
<th>Intercept (± SE)</th>
<th>LC₅₀(CL95%)</th>
<th>X²</th>
<th>Pr. *</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. vilis</em></td>
<td>210</td>
<td>5</td>
<td>1.10 ± 0.27</td>
<td>-4.32 ± 1.10</td>
<td>8.5 × 10³ (3 × 10³ - 2 × 10⁴)</td>
<td>1.75</td>
<td>0.62</td>
</tr>
<tr>
<td><em>M. gabrielis</em></td>
<td>500</td>
<td>5</td>
<td>0.79 ± 0.09</td>
<td>-1.05 ± 0.20</td>
<td>0.2 × 10² (0.1 × 10² - 0.3 × 10²)</td>
<td>7.42</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Probability
According to the results, LT$_{50}$ value of M. anisopliae for M. gabrielis at highest concentration (10$^6$ spores ml$^{-1}$) was shorter (0.77 ± 0.11 days) than that of A. vilis (2.64 ± 0.90 days). At the lowest concentration (10$^1$ spores ml$^{-1}$), LT$_{50}$ value of M. gabrielis was 6.84 ± 2.61, whereas LT$_{50}$ value for A. vilis was 13.62 ± 3.80. The results of the present study revealed that the LT$_{50}$ value for M. gabrielis at all concentrations used was shorter than LT$_{50}$ value for A. vilis (Table 2).

**Table 2** LT$_{50}$ values (in days) for Microcerotermes gabrielis and Amitermes vilis after treatment with Metarhizium anisopliae DEMI 001 isolate.

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration (spores ml$^{-1}$)</th>
<th>Correlation coefficient</th>
<th>LT$_{50}$ ± SE in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. gabrielis</td>
<td>10$^1$</td>
<td>0.99</td>
<td>6.84 ± 2.61</td>
</tr>
<tr>
<td></td>
<td>10$^2$</td>
<td>0.99</td>
<td>5.84 ± 1.39</td>
</tr>
<tr>
<td></td>
<td>10$^3$</td>
<td>0.99</td>
<td>2.08 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>10$^4$</td>
<td>0.99</td>
<td>1.52 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>10$^6$</td>
<td>0.99</td>
<td>0.77 ± 0.11</td>
</tr>
<tr>
<td>A. vilis</td>
<td>10$^1$</td>
<td>0.94</td>
<td>13.62 ± 3.80</td>
</tr>
<tr>
<td></td>
<td>10$^2$</td>
<td>0.97</td>
<td>9.12 ± 2.65</td>
</tr>
<tr>
<td></td>
<td>10$^3$</td>
<td>0.99</td>
<td>6.34 ± 2.67</td>
</tr>
<tr>
<td></td>
<td>10$^4$</td>
<td>0.99</td>
<td>4.76 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>10$^6$</td>
<td>0.99</td>
<td>2.64 ± 0.90</td>
</tr>
</tbody>
</table>

Pathogenicity tests of *M. anisopliae* against *A. vilis* and *M. gabrielis* indicated that the percentage of mortality increased with increasing the fungus concentrations. The percentage of mortality of these termite species after 7 days exposure to *M. anisopliae* is presented in Figs. 1 and 2. The highest mortality of the termites was observed on 7th day of the treatment. At all three concentrations used (10$^2$, 10$^3$ and 10$^4$ spores ml$^{-1}$), the percentage of mortality was 46.66, 60.00 and 73.33 %, respectively for *A. vilis* and 64.00, 92.00 and 100 %, respectively for *M. gabrielis* on 7th day. At the highest concentration (10$^6$ spores ml$^{-1}$), the percentage of mortality of both *A. vilis* and *M. gabrielis* was 100% on 7th day of the treatment. The results indicated that *M. anisopliae* was more effective for controlling *M. gabrielis* than for *A. vilis*.

![Figure 1](image-url)  
*Figure 1* Percentage mortality of *Microcerotermes gabrielis* after treatment with *Metarhizium anisopliae* DEMI 001 isolate for 7 days.
**Discussion**

Fungi are a frequent and often important natural mortality factor in insect populations under natural conditions (Milner, 2000). The entomopathogenic fungus, *M. anisopliae* as a mycoinsecticide is recommended for practical management of termites because of its safety to humans and domestic animals, causing infection to all species of termites tested, easy to formulate and store, and long conidial survival time (more than 18 months) in termite nests (Milner and Staples, 1996). Comparing the LC$_{50}$ of *M. anisopliae* against two termites tested in this study indicated lower LC$_{50}$ for *M. gabrielis* than *A. vilis*, suggesting that the termiticidal efficacy of *M. anisopliae* against *M. gabrielis* was higher than *A. vilis*. Pathogenicity experiments of *M. anisopliae* to termites, *Coptotermes* sp. and *Microcerotermes* sp. indicated that the percentage of mortality of the termites was dependent on the concentration of the conidial suspension used and varieties of *M. anisopliae*, suggesting *M. anisopliae* var. *anisopliae* was more lethal than var. *majus* (Krutmuang and Mekchay, 2005).

As can be seen in Table 2, the LT$_{50}$ values showed a dose-dependent mortality of the examined termites. On the other hand, the mean time required for killing half of the workers *A. vilis* and *M. gabrielis* was influenced by the spore concentration of the fungus to which they were exposed. These findings are in agreement with the results of Liu et al. (2002) and Wright et al. (2005), who reported that the susceptibility of termites to fungal infection was often dose dependent. Furthermore, Rosengaus et al. (1999) stated that higher spore concentration of *M. anisopliae* ($2.2 \times 10^8$ spores ml$^{-1}$) was more lethal to termite’s population. According to the results of Milner et al. (1998), *M. anisopliae* has been known as the most effective fungal pathogen against termites. This fungus species is easy to mass produce and has been successful in field colony control of mound building termites (Milner and Staples, 1996). Additionally, the termite’s social behavior, trophallaxis and allogrooming are often thought to help the spread of pathogens such as *Metarhizium* sp. (Milner, 2000).

The percentage mortality of *A. vilis* and *M. gabrielis* at the highest concentration used ($10^6$ spores ml$^{-1}$), was 100% on 7$^{th}$ day of the
treatment. This finding is not in agreement with the results of Kramm and West (1982), who reported a 100% mortality of *Reticulitermes* sp. within one day of exposure to whole culture of *M. anisopliae*. Meanwhile, Hoe et al. (2009) reported that the isolate LR2 of *M. anisopliae* was the most pathogenic, causing 100% mortality at $1 \times 10^7$ conidia ml$^{-1}$ within 3 days of post-inoculation. However, isolate TA caused 100% mortality at $1 \times 10^7$ conidia ml$^{-1}$ within 6 days of post-inoculation. Additionally, at the lowest concentration of conidial suspension, $1 \times 10^6$ conidia ml$^{-1}$, none of the isolates produced 100% mortality even after one week of post-inoculation (Hoe et al., 2009).

Some possible reasons for such disagreements between our research and above-mentioned researches are the differences in the examined termite species, variation in *M. anisopliae* isolate and different concentrations of conidial suspensions used.

According to the results achieved from the bioassay, the entomopathogenic fungus *M. anisopliae* was more effective in controlling *M. gabrielis* than *A. vilis*. It would be concluded that *M. anisopliae* has the potential to be developed as a mycoinsecticide for control of *M. gabrielis* in the Integrated Pest Management system. Further studies will be required to investigate the termiticidal potential of *M. anisopliae* against dominant species of termites especially *M. gabrielis* and *A. vilis* in the field conditions.

**Acknowledgements**

This research was supported by grants from the University of Tehran and Iranian Research Institute of Plant Protection, which is greatly appreciated.

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ارزیابی اثرات کنترل کندگی فارج Metarhizium anisopliae (Metschnikoff) بر روی دو گونه Microcerotermes gabrielis (Weidner) و Amitermes vilis (Hagen) در آزمایشگاه

علاوه بر رحمی‌زاده، مرعشی رشید، علی‌خانی گرجان، بیژن ناصری

جهت کنترل اثرات آزمایشگاهی، انجام و تأثیر حاصل از آن بر هم مقایسه گردید. سوسپنژ‌های Amitermes vilis و Microcerotermes gabrielis در شرایط تجربی کنترل، مقدار نهایی شده پس از تیمار دو گونه موریانه M. gabrielis به ترتیب 107 × 2 و 107 × 0.1 سپهر در میلی‌لیتر و M. anisopliae به ترتیب 107 × 3 و 107 × 5 سپهر در میلی‌لیتر بود. به‌عنوان نهایی شده کنترل، مقدار نهایی شده پس از تیمار دو گونه M. gabrielis در مقایسه با موریانه D. vilis داشت.

چکیده: موریانه‌های زیرزمینی یکی از مهم‌ترین آفات ساختاری‌های بسیار پیچیده و بسیار مشترک است. استفاده از تیمارهایی که به عنوان حشره‌کشی به‌کار می‌رود، معمولاً به‌عنوان کنترل Metarhizium anisopliae (Metschnikoff) Sorokin (DEMI 001) و Rhyynchophorus ferrugineus (Oliver) استفاده می‌شود. این تحقیق به‌منظور تعیین تیمار به‌کار بردن Amitermes vilis و Microcerotermes gabrielis، در مقایسه با M. anisopliae انجام شد. نتایج نشان داد که این تیمارهای به‌عنوان کنترل اثرات آزمایشگاهی، انجام و تأثیر حاصل از آن بر هم مقایسه گردید.

واژگان کلیدی: M. anisopliae, M. gabrielis, A. vilis, LC50, LT50