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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 8.5×10^3 and 0.2×10^2 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

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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 successe 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 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, taker 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

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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 (10^1, 10^2, 10^3, 10^4 and 10^6 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 LC_{50} values for *A. vilis* and *M. gabrielis* after treatment with *M. anisopliae*. The LC_{50} value of entomopathogenic fungus against *M. gabrielis* (0.2×10^2 spores ml^{-1}) was lower than that of *A. vilis* (8.5×10^3 spores ml^{-1}).

Table 1 LC_{50} values of *Amitermes vilis* and *Microcerotermes gabrielis* after treatment with *Metarhizium anisopliae* DEMI 001 isolate.
*Probability

Specie	No. insects	No. concentrations	Slope (± SE)	Intercept (± SE)	$LC_{50(CL95\%)}$	X^2	Pr.*
A. vilis	210	5	1.10 ± 0.27	-4.32 ± 1.10	8.5×10^3 (3×10^3 - 2×10^4)	1.75	0.62
M. gabrielis	500	5	0.79 ± 0.09	-1.05 ± 0.20	0.2×10^2 (0.1×10^2 - 0.3×10^2)	7.42	0.05

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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.

Species	Concentration (spores ml^{-1})	Correlation coefficient	(LT_{50} ± SE) in days
M. gabrielis			
	10^1	0.99	6.84 ± 2.61
	10^2	0.99	5.84 ± 1.39
	10^3	0.99	2.08 ± 0.39
	10^4	0.99	1.52 ± 0.08
	10^6	0.99	0.77 ± 0.11
A. vilis			
	10^1	0.94	13.62 ± 3.80
	10^2	0.97	9.12 ± 2.65
	10^3	0.99	6.34 ± 2.67
	10^4	0.99	4.76 ± 0.66
	10^6	0.99	2.64 ± 0.90

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 7days 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***.**

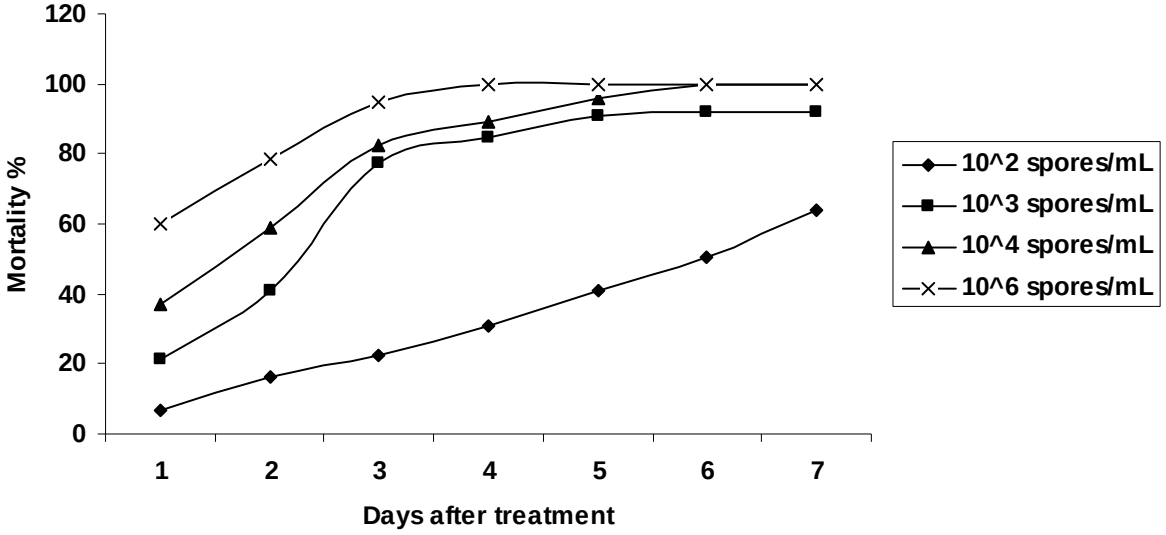

Figure 1 Percentage mortality of *Microcerotermes gabrielis* after treatment with *Metarhizium anisopliae* DEMI 001 isolate for 7 days.

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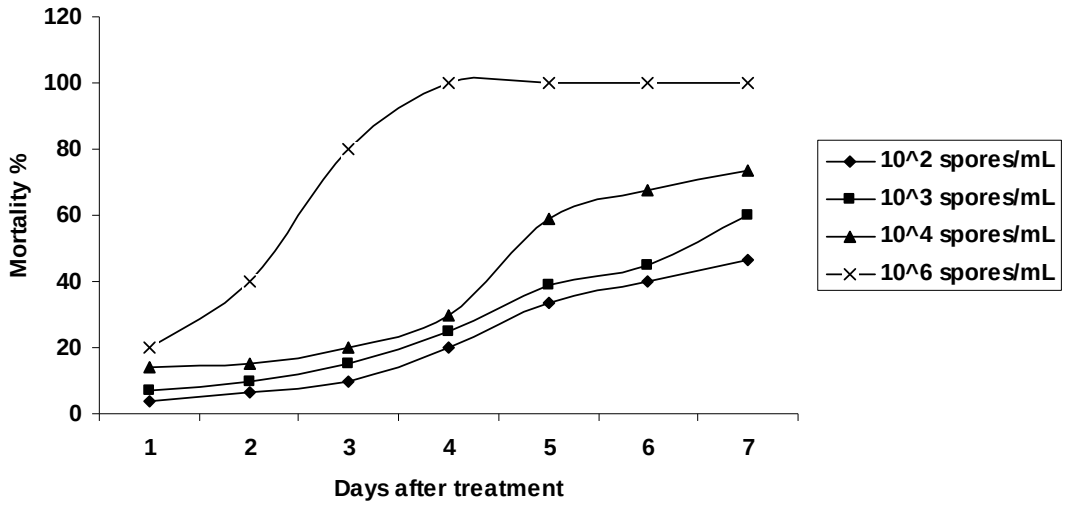

Figure 2 Percentage mortality of *Amitermes vilis* 7 days after treatment with *Metarhizium anisopliae* DEMI 001 isolate.

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* (2.2×10^8 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 funus 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

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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 1×10^7 conidia ml^{-1} within 3 days of post-inoculation. However, isolate TA caused 100 % mortality at 1×10^7 conidia ml^{-1} within 6 days of post-inoculation. Additionally, at the lowest concentration of conidial suspension, 1×10^6 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.

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ارزیابی اثرات کنترل کنندگی قارچ *Metarhizium anisopliae* (Metschnikoff) روی دو گونه موریانه‌ی *Amitermes vilis* (Hagen) و *Microcerotermes gabrielis* (Weidner) در آزمایشگاه

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چکیده: موریانه‌های زیرزمینی یکی از مهمترین آفات ساختمان‌ها، ابنیه تاریخی و محصولات کشاورزی در برخی از نقاط ایران هستند. استفاده از قارچ‌های بیمارگر به عنوان حشره کش‌های میکروبی، معمولاً بخشی از کنترل بیولوژیک و مدیریت حشرات آفت محسوب می‌شود. بیماریزایی قارچ بیمارگر *Metarhizium anisopliae* (Metschnikoff) Sorokin (DEMI 001) که از گونه *Rhynchophorus ferrugineus* (Oliver) جداسازی شد، روی دو گونه موریانه‌ی *Amitermes vilis* (Hagen) و *Microcerotermes gabrielis* (Weidner)تحت شرایط آزمایشگاهی، انجام و نتایج حاصل از آن با هم مقایسه گردید. سوسپانسیون‌های اسپورهای قارچ در پنج غلظت $۱۰^{۱}$,$۱۰^{۲}$، $۱۰^{۳}$، $۱۰^{۴}$ و $۱۰^{۶}$ اسپور در میلی‌لیتر به منظور محاسبه LC_{50} و LT_{50} آنها تهیه و جهت تعیین LC_{50} و LT_{50} *M. anisopliae*، آزمایش زیست سنجی روی کارگر هر دو گونه موریانه انجام شد. مقدار LC_{50} اندازه گیری شده پس از تیمار دو گونه موریانه‌ی *A. vilis* و *M. gabrielis* با قارچ *M. anisopliae* به ترتیب $۸/۵ \times ۱۰^{۳}$ و $۰/۲ \times ۱۰^{۲}$ اسپور در میلی‌لیتر و LT_{50} گونه *M. gabrielis* نیز در پنج غلظت آزمایش شده کمتر از *A. vilis* مشاهده گردید. براساس نتایج حاصل از زیست سنجی، قارچ *M. anisopliae* بیشترین تاثیر را در کنترل گونه‌ی *M. gabrielis* در مقایسه با موریانه‌ی گونه‌ی *A. vilis* داشت.

واژگان کلیدی: LT_{50}، LC_{50}، *Metarhizium anisopliae*، *Microcerotermes gabrielis*، *Amitermes vilis*

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