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Research Article

Application of Bait Treated with the Entomopathogenic Fungus *Metarhizium anisopliae* (Metsch.) Sorokin for the Control of *Microcerotermes diversus* Silv.

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Microcerotermes diversus Silvestri (Isoptera, Termitidae) is considered to be the most destructive termite in Khuzestan province (Iran), and its control by conventional methods is often difficult. Biological control using entomopathogenic fungi could be an alternative management strategy. Performance of a bait matrix treated with the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin, Strain Saravan (DEMI 001), against *M. diversus* was evaluated in this paper. The highest rate of mortality occurred at concentrations of 3.7×10^7 and 3.5×10^8 (conidia per mL). There was no significant difference between treatments, in the rate of feeding on the bait. The fungal pathogen was not repellent to the target termite over the conidial concentrations used. The current results suggest potential of such bait system in controlling termite. However the effectiveness of *M. anisopliae* as a component of integrated pest management for *M. diversus* still needs to be proven under field conditions.

1. Introduction

Currently, species in the genera, *Amitermes* and *Microcerotermes* (Termitidae), *Anacanthotermes* (Hodotermitidae), and *Psammotermes* (Rhinotermitidae), are the most important termites in Iran [1]. Majority of termites in the Khuzestan province belong to the subterranean termite group [2]. Studies show *Microcerotermes diversus* is the most destructive termite in Khuzestan province. It has a wide foraging area and is able to form secondary colonies in walls, ceilings of buildings, and in trees. This termite is also prevalent in other parts of Iran and in Iraq, Kuwait, Oman, United Arab Emirates (UAE), and Saudi Arabia and is one of the most important pests of date palms (*Phoenix dactylifera* L.) in Iran, Iraq, and Saudi Arabia [3]. Current management of subterranean termites in Iran involves the application of soil insecticides [1]. However, continuous use of chemical pesticides in the environment is a concern [4–6], especially in areas with a high groundwater table, as in the city of Ahvaz [7]. Biological control has been suggested as an alternative strategy to the widespread application of chemical pesticides. Following this interest in the use of entomopathogenic fungi to combat insect pests has increased. Application of entomopathogenic fungi against termites has the minimum negative impact on the environment [8]. There have been a number of studies evaluating the efficacy of the hypocrealean Hyphomycete, *Beauveria bassiana* (Bals.) Vuillemin, against subterranean termites [9]. Similarly Ascomycete, *Metarhizium anisopliae* (Metsch.) Sorokin, present in the soil also acting as a causal agent for "green muscardine" of insects, is an important pathogen for the biological control of pests [10, 11]. This study investigates the efficiency of cellulose bait treated with conidia of *M. anisopliae* against *M. diversus*.

2. Materials and Methods

2.1. Collection of Termites. Termites were collected from blocks of beech wood (*Fagus orientalis* Lipsky) by embedding the blocks in soil adjacent to nests in the Ahvaz region. Collected termites were then transported to the laboratory. The termites were maintained in a dark incubator at temperature

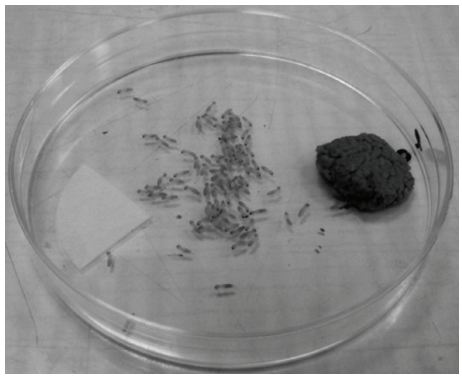

Figure 1: Petri dish-based test system to examine the response of *M. diversus* to *Metarhizium*-treated bait (BMet) versus UFP.

of 28 ± 1°C and 85 ± 5% relative humidity and kept on beech blocks (3 × 6 × 20 cm) before bioassays. Only mature worker termites were used for the test.

2.2. Fungal Isolate. *M. anisopliae* Strain Saravan (DEMI 001) from the collection maintained at Iranian Research Institute of Plant Protection was used. The fungus was cultured on Sabouraud Dextrose Agar with 1% yeast extract. Petri dishes were maintained in a dark incubator at a temperature of 28±1°C and 85±5% relative humidity. Two-to three-week-old fungal cultures were used for this experiment.

2.3. Preparation of Fungal Suspension. Conidial suspensions were prepared by lightly scraping the surface of fungal cultures with a sterile wooden spatula and suspending the conidia in 100 mL distilled sterile 0.01% of polysorbate monooleate (Tween 80). The conidial concentration of the suspensions was determined using a haemocytometer.

2.4. Bait Preparation. The bait was prepared the following way: 0.5 g of agar and 0.5 g of sugarcane molasses were poured into 25 mL of fungal conidial suspension and shaken for around 30 min until the mixture was uniform. Then 75 g of cellulose powder (SIGMA) was added and mixed well by hand. Concentrations of 1.1×10^5, 2.7×10^6, 3.7×10^7, and 3.5×10^8 conidia per mL were used, based upon preliminary experiments.

2.5. Bait Test

(A) Conidia-Treated Bait versus Untreated Filter Paper. In the first experiment, the test unit included a bait treated with *M. anisopliae* conidia (BMet) and untreated filter paper (UFP). Four grams of BMet was placed at one side of a 100 mm wide plastic Petri dish together with pieces of filter paper (Whatman No. 1001; 42 mm diameter, cut into two halves) at opposite sides of the dish (Figure 1). The filter paper was moistened with sterile distilled water. In the control, the same bait matrix treated with a solution of 0.01% Tween 80 (BCon) instead of the conidial suspension was offered. Each treatment was replicated four times. One hundred termite workers were added to each Petri dish. Units were then housed/placed in a dark incubator at 28 ± 1°C and 85 ± 5% relative humidity. Termite mortality was recorded daily for 14 days.

Table 1: LC_{50} and LC_{90} in both experiments.

Baits	LC_{50} (conidia per mL) (95% Fiducial limits)	LC_{90} (conidia per mL) (95% Fiducial limits)
BMet + UFP*	2.1×10^6 (7.3×10^5–6.1×10^6)	3.2×10^7 (1×10^7–3.2×10^8)
BMet + BCon**	3×10^6 (1.4×10^6–6.3×10^6)	7.3×10^7 (2.9×10^7–3.1×10^8)

*Bait with *Metarhizium* conidia and untreated filter paper.
**Bait with *Metarhizium* conidia and untreated bait.

(B) Bait with (BMet) and without (BCon) Metarhizium Conidia. The second experiment aimed to explore whether the presence of untreated bait (BCon) affected the consumption of bait treated with *Metarhizium* conidia (BMet). In this test, 4 g of BMet was placed on one side of a Petri dish and 4 g of BCon at the opposite side. Both baits were again placed on top of sections of filter paper as described above.

2.6. Statistical Analysis. Mortality data was subjected to angular transformation and analyzed using analysis of variance (ANOVA). PROC MIXED was used in the SAS software (SAS Institute, 2000). Mean was compared by the least significant difference (LSD) at $\alpha = 0.05$ after ANOVA (SAS Institute, 2000). Corrected mortality from fungal treatments was calculated using the formula by Abbott (1925). Graphs were plotted using Excel 2007 software.

3. Results

(A) Conidia-Treated Bait (BMet) versus UFP. In the experiment comparing treated bait (BMet) and untreated filter paper (UFP), there was a significant dose effect on *M. diversus* mortality (ANOVA $F = 29.75$, $df = 14$, $P < 0.0001$). The LC_{50} and LC_{90} values (Table 1) were 2.1×10^6 and 3.2×10^7 conidia per mL, respectively. Table 2 shows values of LT_{50} and LT_{90} for the same test. The highest and lowest levels of LT_{50} and LT_{90} were observed at the concentrations of 1.1×10^5 and 3.5×10^8 conidia per mL, respectively. At concentrations of 3.7×10^7 and 3.5×10^8 conidia per mL, the rate of mortality was highest with 100%. There was no significant difference between the two lower concentrations of 1.1×10^5 and 2.7×10^6 conidia per mL; both gave less than 40% mortality (Figure 2). However, the rate of mortality was significantly different from the mortality in the controls at all concentrations (ANOVA $F = 85.44$, $df = 4$, $P < 0.001$).

The feeding rate on untreated filter paper in the presence of BMet is shown in Figure 3. Only the rate of feeding on cellulose compound with a concentration of 3.5×10^8 conidia per mL was significantly less than that for the other treatments, except for the next lowest dose, 3.7×10^7 conidia per mL (ANOVA $F = 0.67$, $df = 4$, $P = 0.62$).

Table 2: LT_{50} and LT_{90} in both experiments.

Concentration (conidia per mL)	Baits	LT_{50} (day) (95% Fiducial limits)	LT_{90} (day) (95% Fiducial limits)
1.1×10^5	BMet + UFP*	—	—
	BMet + BCon**	—	—
2.7×10^6	BMet + UFP	11.12 (9.93–12.85)	—
	BMet + BCon	—	—
3.7×10^7	BMet + UFP	1.33 (1.28–1.39)	2.24 (2.14–2.36)
	BMet + BCon	4.22 (3.42–4.95)	12.71 (10.41–17.01)
3.5×10^8	BMet + UFP	1.01 (1–1.12)	1.54 (1.24–1.65)
	BMet + BCon	1.47 (0.99–1.91)	2.37 (1.83–4.08)

*Bait with *Metarhizium* conidia and untreated filter paper.
**Bait with *Metarhizium* conidia and untreated bait.
The high values of LT_{50} and LT_{90} are not reported.

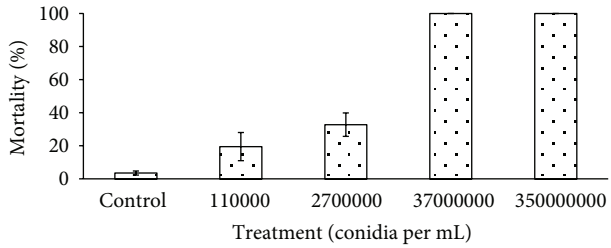

Figure 2: Effect of conidial concentration in the bait (BMet) on *M. diversus* mortality in the presence of UFP. Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

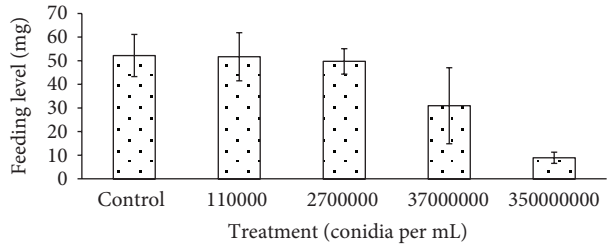

Figure 3: Effect of conidial concentration on mean *M. diversus* feeding rate (mg dry weight) on untreated filter paper in the presence of fungus-treated cellulose compound, as affected by conidial concentration. Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

Figure 4 shows the effect of conidial concentration on the mean feeding rate on BMet. Feeding on BMet was not significantly different from that of BCon and the same for all four conidial concentrations.

(B) Bait with (BMet) and without (BCon) Metarhizium Conidia. The values of LC_{50} and LC_{90} for BMet versus BCon against *M. diversus* is represented in Table 1. The rate of LC_{50} and LC_{90} was achieved at 3×10^6 and 7.3×10^7 conidia per mL respectively (ANOVA $F = 57.92$, $df = 14$, $P < 0.0001$). Table 2 shows the rate of LT_{50} and LT_{90} for the same test. The highest and the least level of LT_{50} and LT_{90} belonged to concentrations of 1.1×10^5 and 3.5×10^8 conidia per mL respectively.

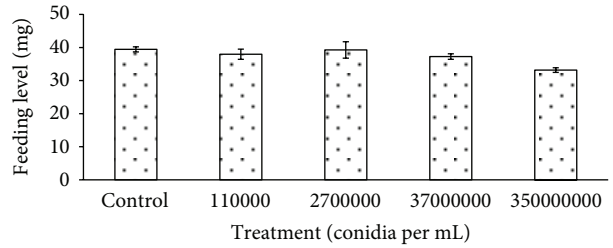

Figure 4: Effect of conidial concentration on the mean *M. diversus* feeding rate on *Metarhizium*-treated cellulose compound in the presence of UFP. Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

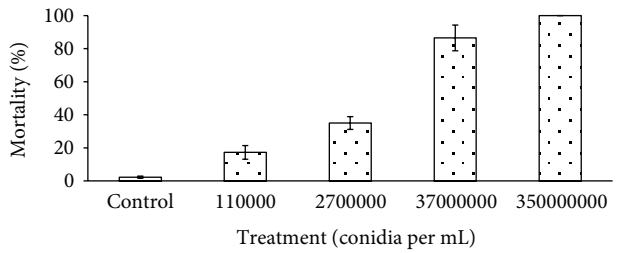

Figure 5: Effect of conidial concentration in bait (BMet) on mortality of *M. diversus* in the presence of untreated bait (BCon); same letter above the bars indicates absence of a significant difference at $P = 0.05$.

The comparison of mean mortality is shown in Figure 5. Overall, there was a significant difference in the rate of mortality between treatments. The maximum rate of mortality was observed at concentration of 3.5×10^8 conidia mL^{-1} (ANOVA $F = 99.76$, $df = 4$, $P < 0.0001$).

Figure 6 shows the comparison of mean consumption rates on BCon. The feeding rate did not differ between treatments (ANOVA $F = 2.08$, $df = 4$, $P = 0.3996$). Figure 7 shows the comparison of the mean feeding rate. The feeding rate did not show any significant difference across treatments (ANOVA $F = 0.41$, $df = 4$, $P = 0.7962$).

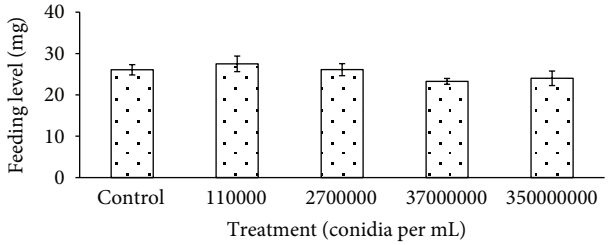

Figure 6: Effect of conidial concentration in bait (BMet) on *M. diversus* feeding on BMet in the presence of untreated bait (BCon). Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

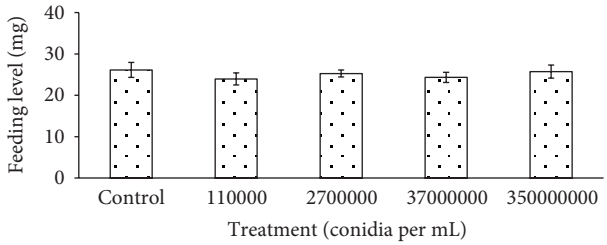

Figure 7: Effect of conidial concentration on the mean feeding rate of *M. diversus* on fungus-treated cellulose compound in the presence of untreated cellulose compound. Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

4. Discussion

The results obtained in this experiment show best values of LC_{50} and LC_{90} were obtained when BMet was offered with UFP than when offered with BCon. The same was true for LT_{50} and LT_{90} values in both experiments. The type of untreated component in the chosen experiments has shown to have caused this difference. Filter paper was the least attractive food compared to the matrix of BMet, making them feed more on BMet and hence had higher exposure to conidia. But when offered with BMet and BCon at the same time, their overall exposure to conidia was reduced since they had chosen to feed on both substrates.

The overall mortality rate increased with higher concentrations of conidia. The means of bait consumption did not show any significant differences between treatments. Hence, the conidia of the *M. anisopliae* isolate used in our study were not repellent to *M. diversus*. Significantly reduced feeding on the bait matrix at the highest conidia dose (Figure 3) is due to high mortality of workers.

Bayon et al. also observed that conidia of *M. anisopliae* were not repellent for *Reticulitermes santonensis* Feytaud and hence could be added readily to baits [8]. Effective concentrations of *M. anisopliae* were also not repellent in cellulose powder baits that Wang and Powell offered to *Reticulitermes flavipes* Kollar and *Coptotermes formosanus* Shiraki [12]. Their baits with conidia eliminated groups of termite *in vitro*. In addition, it was stated that more attractive bait formulations may be required for increasing impact of *M. anisopliae* against their target species.

The results obtained from this study show good potential for using baits with entomopathogenic fungus as an active ingredient in controlling pest termites. Irrespective of many issues cited in the literature, methods are available to improve the efficiency of entomopathogenic fungi against termites. One of the avenues is to develop a suitable matrix as carrier of fungal pathogens that is readily acceptable and consumed by termites over other food items. Ramakrishnan et al. showed that a very targeted use of pesticides such as Imidacloprid in sublethal doses together with fungal pathogen can enhance performance of the fungi [13]. Also Hussain et al. used a pesticide formulation containing entomopathogenic fungi as well against termites [14]. The compatibility of an entomopathogenic fungus formulated for use with another toxicant must be tested in any effort to integrate control methodologies.

Acknowledgments

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