Potential of the Entomopathogenic Fungus *Beauveria bassiana* (Bals.) Vuill. for Infection the Sand Subterranean Termite, *Psammotermes hybostoma* (Desn.) (Isoptera: Rhinotermitidae)

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ABSTRACT

Sand termite, *Psammotermes hybostoma* (Bals.) Vull. (Isoptera: Rhinotermitidae) is an important common economically termites in Egypt. Three techniques for termites control using the entomopathogenic fungus, *Beauveria bassiana* were evaluated. Assays indicated that mortality percentages were 99.98 using cadavers of fungal infected termite and 100%, with cadavers of fungal infected *Galleria mellonella* and contaminated sand treatments 10 and 12 days post treatment. Mortality rate was 99.95 %, 10 days post treatment using one cadaver of *G. mellonella* / 2000 healthy termite workers reflecting fungal potential to spread through dense posses. Termites mortality was 98.00% 14 days post treatment and 100% 35, days post treatment employing 3 and 5 containers laboratory test units, respectively. These results showed that employment of fungal-killed cadavers as baits could be dependable.

Key Words: Termite, *Psammotermes hybostoma*, *Beauveria bassiana*, Entomopathogenic fungi, termite control.

INTRODUCTION

Sand termite, *Psammotermes hybostoma* (Desn.) is a true desert creature and it is the most common economically important termite in Egypt, causing high economic losses annually, especially, in the border land of the delta, new Valley, upper and middle Egypt (EL-Hemaesy, 1976; Khalil *et al.* 1982 and Ahmed, 1997). Conventional controls of termites depend on the use of pesticides (Grace *et al.*, 1999 and Thorne & Breisch, 2001). Extensive use of chemicals causes environmental hazards; it may also develop termite resistance against these chemicals. Moreover, termites can also avoid the chemicals and can move from one zone to another (Zoberi, 1995). Therefore, need for another treatment option when a traditional termiticide treatment was not successful in protecting a structure is apparent (Abebe, 2002). Entomopathogenic fungi such as *Beauveria bassiana* have a potential to infect termite (Padmaja, 2001; Shimizu & Yamaji, 2003 and Neves & Alves, 2004). Using these pathogens alone or associated to new active ingredients has proved to be an efficient and environmentally favorable method for control of termite (Moino *et al.*, 2002). Additionally, entomopathogenic fungi proved capability for subterranean termites management, they have been isolated through termite surroundings (Jones, *et al.*, 1996 and Myles, 2002).

The present study aimed to discuss the potential of the entomopathogenic fungus *B. bassiana* against *P. hybostoma* and the efficacy of termite workers in disseminating the pathogen among colony individuals using a laboratory choice test units with different techniques, similar to nesting system in nature.

MATERIALS AND METHODES

Insect maintenance

1-Termites

P. hybostoma specimens necessary for bioassay tests were collected from areas highly infested with termites at Ibshawai district, El-Fayoum governorate using cardboard traps. Collected cardboard rolls were transferred to the laboratory, where they were carefully unrolled and examined to classify castes. Every trap with healthy workers were kept into separate Petri dishes, with small pieces of moistened corrugated cardboard paper as food and moisture source. Petri dishes were maintained at 27±1 °C and 90 % R.H for 7 days at least for acclimatization.

2- *Galleria mellonella*

Larvae of the greater wax moth, *G. mellonella* kindly provided by Dr. M. Zen-Elabedeen, Applied center of Entomonematodes, Faculty of Agriculture, Cairo Univ.

Fungus

The entomopathogenic fungus, *Beauveria bassiana* isolated from unknown aphid species in Fayoum Governorate, Egypt by Dr. Nahla A. Ezz, Scale Insects and Mealybugs Department, PPRI was used in this study. Fungus was cultured and maintained on Sabouraud dextrose agar medium

(SDA) (10 g mycopeptone, 40 g dextrose, 15 g agar and 1000 ml distilled water). The fungus was incubated at 25 ± 1 C in the dark for 14 days.

Inoculants

Healthy termite workers or larva of *G. mellonella* were inoculated with *B. bassiana* by allowing them walking over conidiospores harvested in sterile Petri dish. After 10 minute of exposure, insects were transferred to 9 cm Petri dish with 7 cm Whatman no.1 moistened filter papers. Contaminated insects provided with food sources then incubated at 27 ± 1 °C till death. Thereafter, the dead individuals were placed in new Petri dishes containing filter papers and incubated at 27 ± 1 °C till the cadavers completely coated with fungal spores.

Experimental Design and Assay

A laboratory choice test was carried out to assess efficacy of *B. bassiana* to *P. hybostoma* and to demonstrate different inoculation methods. Six assays were performed. In the first four, a laboratory choice test unit consisted of two plastic boxes (15x10x5cm) half filled with damp sand connected with 20 cm plastic tube inserted into 9 mm diameter hole near the base of the containers were used (Fig. 1a). Termite workers were allowed to move freely between the two boxes. For the first two assays, 300 healthy termite workers were added in first container. Thirty termites or one *G. mellonella* fungus-killed cadaver were used as an inoculum source. They were placed in the second container with wetted piece of corrugated cardboard as a food source. At the third assay, another 300 healthy termite workers were placed in container linked with box contained contaminated moistured sand with *B. bassiana* that previously used in first treatments with a piece of moisture cardboard paper. Additional experiment was carried out to demonstrate capability of one sporulated cadaver of wax moth larva to infect large termite numbers. Two thousand untreated termites were added in first container. The second container contained one cadaver of *G. mellonella* fungus-killed and a piece of corrugated cardboard provided as a food source.

In the fourth experiment, a laboratory test unit consisted of three plastic boxes (15x10x5cm), half filled with damp sand were used. In first container, 300 healthy workers were added, while moistured piece of corrugated cardboard as a food source were placed at the third container. One sporulated fungal-killed cadaver of *G. mellonella* was placed in middle container. The workers were allowed to move freely among the three boxes through two plastic tubes (20 cm each) inserted into 9 mm diameter hole near the base of the containers (Fig. 1b). For all previous experiments, each consisted of three replicates and units for control purpose used without cadavers or contaminated sand. All systems were incubated at 27± 1°C in dark, and mortality rates were recorded from the 6th day every 48 hr.

In general, the nest of *Anacanthotermes ochraceus* (Burm.) consists of an assemblage of chambers excavated into the soil at different levels joined to each other by galleries of various lengths. Excavated chambers are of two distinct types; the storage chambers and the dwelling chambers. The storage chambers occur in the soil at a depth of 5-30 cm below soil surface. Most of dwelling chambers occur at depths of 30-120 cm below soil surface. The galleries are cylindrical in shape with very smooth inner walls, mainly in the range of 5-9 mm in diameter. Galleries connecting chambers are 10 to 40 cm long (Abd El latif, 2003).

To overall actual effect of the pathogen on population, laboratory choice test unit was used which was similar to nest in nature. Unit consisted of central plastic box and 4 secondary boxes, same diameters as previously mentioned and half filled with damp sand. Whole boxes connected together with 4 pieces of plastic tubes (20 cm each) where they were inserted into holes near the boxes bottoms. The four secondary boxes were provided with pieces of moistured cardboard as a food source, and one cadaver of *G. mellonella* heavily sporulated with the pathogen was added to one secondary box. Three hundred healthy termite workers were placed into the central box and allowed to move freely among the whole boxes (Fig. 1c). Three units were used as replicates and incubated at 27° ± 1C in dark, other three units without *G. mellonella* cadavers served as control. Observations were conducted for undisturbed termites simulated habitat till supposing complete mortality. That was 19, 26 and 35 days post treatment.

RESULTS AND DISCUSSION

Conducted experiments declared acceptability and pathogenicity of *B. bassiana* to foraging *P. hybostoma*. The cumulative percent mortality values were increased through incubation period till it reached the peak. These values were 99.89% using termite fungus-killed cadavers and 100% with *G. mellonella* fungus-killed cadaver and contaminated sand treatments, respectively (Table 1). These percentages were recorded 10 and 12 days post treatment. Exposing higher numbers of termite workers (2000 individuals) to one sporulated cadaver

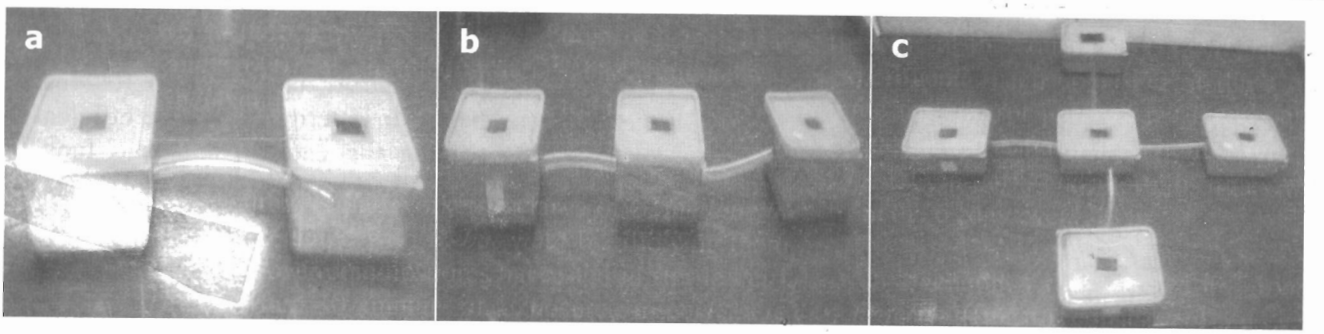

Fig. (1): Laboratory choice test units. (a) two containers, (b) three containers, (c) five containers.

Table (1): Percentage mortality of *P. hybostoma* workers infected with *B. bassiana* using three different inoculation methods under laboratory conditions.

Treatment	Cumulative Percent mortality through incubation period in days			
	6	8	10	12
Termite sporulated cadavers	59.22	84.56	97.00	99.89
G. mellonella sporulated cadavers	86.78	98.11	99.67	100.00
Contaminated sand	97.11	99.33	100.00	100.00
Check control	0.11	0.11	0.22	0.22

Table (2): Percentage mortality of 2000 *P. hybostoma* workers exposed to *G. mellonella* fungal sporulated cadaver.

Treatment	Cumulative Percent mortality through incubation period in days		
	6	8	10
G. mellonella sporulated cadaver	92.53	98.82	99.95
Check control	1.20	1.20	1.20

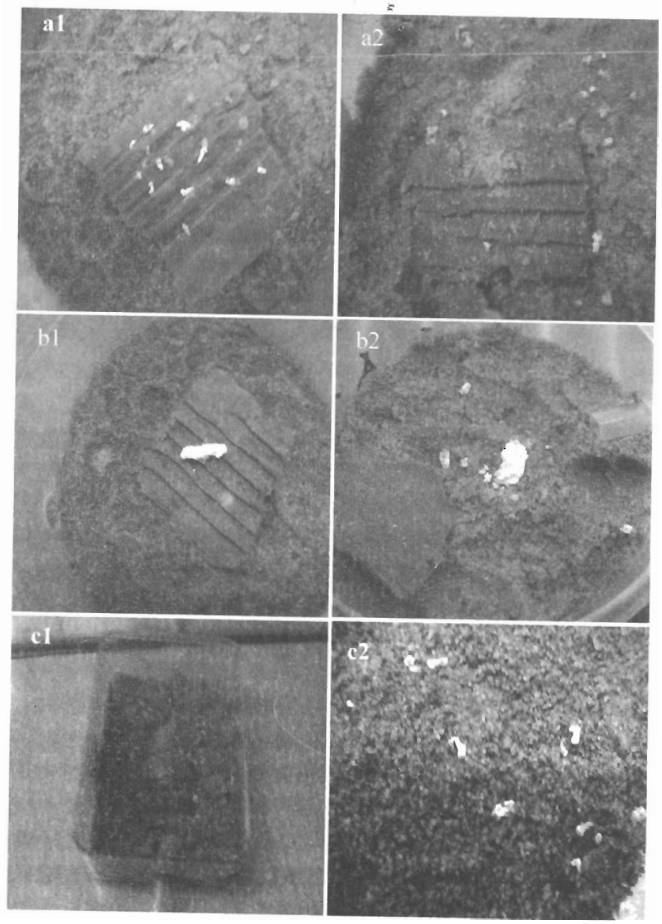

Fig. (2): Three inculcation methods of *B. bassiana* against *P. hybostoma* and fungal symptoms after termites death. (a1&2) infected termite cadavers. (b1&2) infected *G. mellonella* cadaver. (c1&2) contaminated sand.

Table (3): Mortality of *P. hybostoma* workers incurrence to *G.mellonella* sporulated cadaver using 3 containers laboratory test unit

	Cumulative Percentage mortality through incubation period in days				
	6	8	10	12	14
Treatment	36.44	79.33	92.11	97.56	98.00
Check control	0.00	0.00	0.11	0.11	0.11

Table (4): Mortality of *P. hybostoma* workers incurrence to *G. mellonella* sporulated cadaver using 5 containers laboratory test unit

	Cumulative Percentage mortality through incubation period in days		
	19	26	35
Treatment	81.00	97.56	100.00
Check control	0.11	0.11	0.11

of wax moth larva showed approximately same mortality percentage values at the same period (99.95 %, 10 days post treatment) (Table 2).

Fig.2 (2a, 2b and 2c) illustrated the symptoms of infection with *B. bassiana* on foraging workers through boxes searching for food as result of contacting with treated cadavers or contaminated sand.

Data present in Table (3 and 4) indicated that termite workers were contaminated with fungal conidia from the sporulated *G. mellonella* cadaver throughout their movement to food source in the 3 - containers laboratory test units. Cumulative percent mortality reached 98.00% after 14 days of treatment (Table 3). However, external observation to 5- containers laboratory test unit simulating nest in nature showed absence of termite movement inside the tubes or tunnels in sand. Internal demonstration appeared 81.00 and 97.56 % mortality, 19 and 26 days post treatment, respectively (Table 4). Living termites were lethargic and motionless. Furthermore, 100% mortality was recorded, 35 days post treatment. Mortality among untreated control termites in both previous treatments were 0.11%. Termites death casually occurred during workers passage with fungus-killed cadavers within food searching.

Results indicated that *B. bassiana* isolate spread among healthy termites causing significant mortality. Where the infection provided through mobility of workers searching for food and contacting with treated cadavers or sand. Tested exposure methods testified pathogenicity and acceptability of termites to fungal infection. Approximately 100% mortality resulted, 10 and 12 days post treatment. Zoberi (1995) and Ramakrishnan *et al.* (1999) indicated that same termite mortality percentages were observed after 15 and 21 days of direct exposure to contaminated soil with *Metarhizium anisopliae*. Zoberi (1995) recorded 100% mortality in termite population using *M. anisopliae*-killed termites as inoculation source after 5 days, but this percentage were caused by direct exposure in Petri dishes.

Present results revealed efficacious use of *G. mellonella* cadaver bearing by the pathogen conidia to control termites even within dense posses. Approximately, the same mortality percentages were recorded in both small and large termite posses at the same period post treatment in spite of the great difference in insect numbers in both groups when one wax larva with sporulated cadaver was used. That may pertain to a large quantity of conidia provided by one infested cadaver. Furthermore, Kramm *et al.* (1982) and Abebe (2002) reported that the grooming behavior in termites provide efficient means for spreading the entomopathogenic fungi among the colony.

It is apparent from current data that obligatory passage of termites to sporulated cadaver toward food sources arranged opportunity to contamination by fungal spores that almost complete death rates in termite posses within two weeks. Additionally, significant response to *G. mellonella* fungus-killed cadaver was apparent when simulated laboratory unit to nest in nature was employed. This declares that even with termites disjunctive traveling to pathogen bait source, the disease will be spread throughout termite gallery system. Successful fungal pathogen in producing an epizootic should cause mortality after responsible lag period (Jones *et al.* 1996). In the current study, complete mortality was achieved 35 days post treatment. Dusting termites environmental may not prove good enough since the inoculum probably does not reach the target (Padmaja, 2001). Furthermore, absence of termite behavioral response to *B. bassiana*-killed cadavers (leaving them in the open without buried in substrate) should encourage the spread of the pathogen leading to epizootic (Jones *et al.*, 1996). With the criteria and the high mortality rates caused using *G. mellonella* fungal–killed cadavers, it would be interested to know how far this bait can be employed in field.

Efficacy and acceptability of *B. bassiana* to savaging *P. hybostom* was clear. The tested isolate performed high mortality rates in reasonable periods, and it can be used as a remedial control against *P. hybostom*. Employment fungal-killed cadavers as a bait could be dependable. Moreover, when judging laboratory assay results, it is important to consider the termites behavior. The common grooming behavior can enhance the control potential with entomopathogenic fungi among cross contamination. In addition, an appropriate environmental conditions for termites extended as moderate temperature, humid and populous nest are ideal for fungal growth. Generally, emphasis should be given to develop more reliable assay techniques for testing fungus-growing termites, or the assays should be carried out as much as possible under field conditions.

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