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

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ABSTRACT

The subterranean sand termite, *Psammotermes hybostoma* (Desn.) (Isoptera: Rhinotermitidae) is the most economic termite species in Egypt. Biological control with pathogenic fungi is a promising alternative against termite. The present study focused on pathogencity of the entomopathogenic fungus *Beauveria bassiana* against *P. hybostoma* workers. Termite workers were exposed to the pathogen by walking over harvested fungal conidia. 100% mortality obtained 3 and 4 days post treatment through termite workers maintained on water agar medium or sterile sand. Ability of termite workers to serve as fungal vector was estimated. Distribution of the fungus through healthy termites causing spread disease apparent even with a few numbers of vectors. Statistical analysis indicated that there were significant differences between the three tested treatments (5, 10 and 20 carrier termites) and control 16 days post treatment. But insignificant differences were found between 5 and 10 carriers as well as 5 and 20 carrier termites when they were mixed with healthy termites.

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

INTRODUCTION

Termites are among the oldest known pests in Egypt and in the Middle East (Ali, 1980). *Psammotermes hybostoma* (Desneux) is the most economic termite species causing considerable damage to house as subterranean termites, occurring in middle and upper Egypt and was collected from Giza, El-Fayoum, Minia, Assuit and Aswan governorates as well as from Kharga, Dakhla, Baharia and Ismailia Oases. (Kaschef & El-Sherif, 1971 and Ahmed, 1997).

Chemical insecticides have traditionally been the primary method of termite control (Grace, *et al.* 1999; Cabrera & Thoms, 2006 and El Naggar & Abd El-Latif, 2007). However, chemical control not only causes environmental hazard but also termite problems remains unchanged. Thus, avoiding insecticides by termite movement from one zone to another (Zoberi, 1995 and Abebe, 2002), focused on development of new biological control strategy for management of the subterranean termite. Local isolates of fungi pathogenic to termites appear to be promising biological control agents (Zoberi, 1995; Myles, 2002a & b and Krutmuang & Mekchay, 2005). The termite lifestyle has attributes which are suitable properties for fungal infection (Shimizu and Yamaji, 2003). Entomopathogenic fungus *B. bassiana* has the potential to infect termites (Jones, *et al.* 1996; Padmaja, 2001 and Susumu & Motoko, 2002).

The present paper aimed to evaluate the pathogencity and efficacy of the entomopathogen *B. bassiana* against the subterranean termite *P. hybostoma*. Termites aptitude as a carrier of the entomopathogen was approached.

MATERIALS AND METHODES

Termites

P. hybostoma specimens were collected from areas highly infested with termites at Ibshawai district, El-Fayoum governorate using corrugated cardboard traps. Collected cardboard rolls were transferred to the laboratory where they were carefully unrolled and examined to classify castes. Every trap contained healthy workers were kept into separate Petri dish together, 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.

Fungus

The entomopathogenic fungus *Beauveria bassiana* was isolated from unknown aphid species in Fayoum Governorate, Egypt by Dr. Nahla A. Ezz, Scale Insects and Mealybugs Department, PPRI. Fungal isolate 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.

Experimental Design and Assay

To establish fungal pathogencity and efficacy against termites, 100 healthy termite workers had been allowed to crawl over sterile Petri dish contained harvested conidia of the fungus for 3 min. Groups of 20 termite workers contaminated with fungal conidia were gently transferred to 9 cm Petri dish. Dishes contained 7 cm Whatman no.1 filters paper placed flat on the surface of 15 ml water agar medium or 20 g sterile damp sand with a piece of moistened corrugated cardboard. Filter papers and corrugated cardboard served as food sources. Treated termites were incubated at 27 ± 1 °C and dead insects were recorded daily for four days and compared to untreated termites incubated under similar conditions. Both bioassays were replicated three times as well as check control.

To evaluate ability of termite as a pathogen carriers and effect of the carrier numbers on the infection rates, three tests were carried out. One hundred healthy termite workers were placed in 9 cm Petri dish containing 20 g damp sand with a piece of moistened corrugated cardboard paper. Healthy termite workers were contaminated by walking on fungal propagules harvested in sterile Petri dish. After 20 minuets, groups of 5, 10 or 20 contaminated termite workers were gently added to 100 untreated termites. Treatments were replicated nine times. Additional nine Petri dishes, each containing 100 untreated termite workers were used as control. All treatments were incubated at 27±1°C and mortality was recorded every 48 hours. Mortality percentages were computed according to the general linear models and significant differences between treatment means were calculated by Duncan test at probability 0.05 using SPSS v. 8.0 computer program.

RESULTS AND DISCUSSION

Obtained data declared the pathogencity of the entomopathogenic fungus *B. bassiana* against *P. hybostoma* termite workers (Fig. 1). The mortality of termite workers exposed to the pathogen was low in sterile sand treatment (3%) within first 24 h compared with water agar medium treatment (45%). However, 100% mortality occurred 3 days post treatment through termite workers maintained on water agar medium. Complete death rates were recorded 4 days post treatment when termites maintained on sterile sand (Table 1).

Ability of termite workers to serve as fungal carrier was estimated. Transfer period of termite workers contaminated with conidia of *B. bassiana* through healthy population increased mortality rates (Fig.2). Statistical analysis indicated that there were significant differences among the three tested application treatments 5, 10 and 20 carrier termites and check control 16 days post treatment (Table 2). It has been observed that distribution of the fungus through healthy termites spread the disease apparent even with a few numbers of carriers. No significant differences were found between treated groups with 5 and 10 carriers as well as between treated groups with 5 and 20 carriers when they were mixed with healthy termite workers (Table 2).

Current study indicated pathogencity of *B. bassiana* tested isolate against *P. hybostoma* termites. Jones *et al.* (1996) and Padmaja (2001) remarked the virulence of *B. bassiana* different strains to termites. Development of indigenous fungal isolates adapted with the local conditions is successful and promising in Egyptian biological control strategies (Ezz, 2004). From the present data, it can be concluded that the pathogen caused rapid and significant mortality within short period. This also was clear in the test performed in sand symmetrical to field conditions. The same was concluded by Zoberi (1995), who found a similar pattern of activity with *Metarhizium anisopliae*.

Present work affirms capability of termite workers to serve as fungus vector. In agreement with Myles,

Table (1): Cumulative daily mortality of the termite, *Psammotermes hybostoma* workers treated with the entomopathogen *Beauveria bassiana* and maintained on water agar medium and sterile sand

Condition	Daily cumulative percent mortality			
	1	2	3	4
Water agar medium	45.67	74.33	100.00	100.00
check	3.33	6.00	6.00	6.00
Sterile sand	3.00	64.00	76.00	100.00
check	0.00	0.00	0.00	2.00

Table (2): Cumulative daily percent mortality of the termite, *Psammotermes hybostoma* workers mixed with 0, 5, 10 and 20 *Beauveria. bassiana* carrier termites

Treatment	Cumulative percent mortality through incubation periods in days							
	2	4	6	8	10	12	14	16
A	4.97	10.37	28.57	58.31	63.49	79.05	87.51	91.75^{bc}
B	5.76	12.42	25.45	55.66	62.93	72.93	82.22	89.19^{b}
C	11.20	31.11	58.70	85.46	86.76	95.74	96.67	97.78^{c}
Check	1.78	2.89	3.55	4.44	5.33	6.78	9.30	10.22^{a}

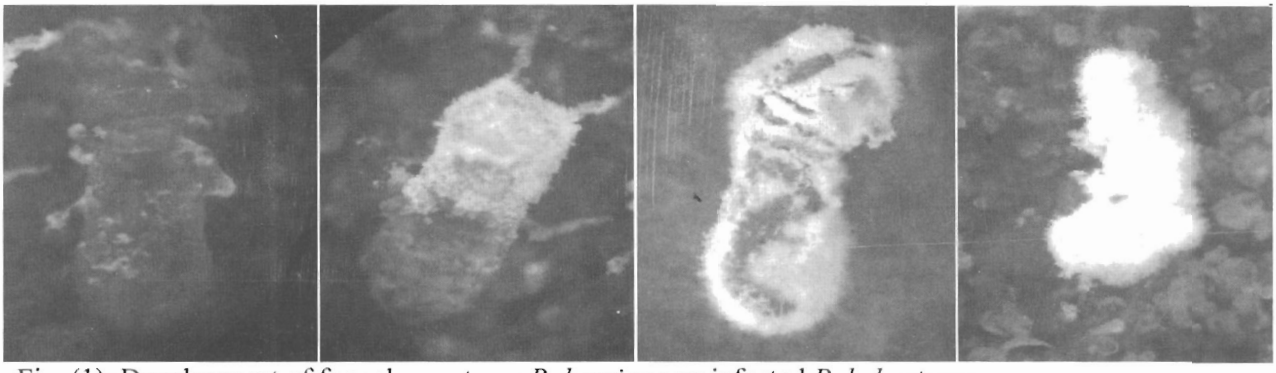

Fig. (1): Development of fungal symptoms *B. bassiana* on infected *P. hybostoma.*

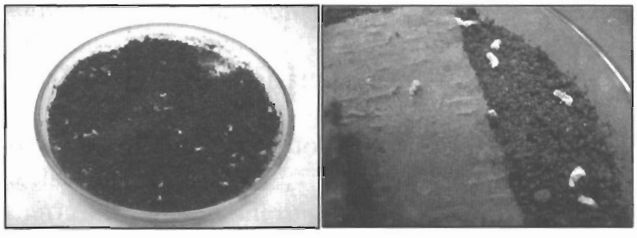

Fig. (2): Distribution of the fungus *Beauveria bassiana* through healthy termite population served as carrier termites.

(2002 a), the alive termites at all Petri dishes transmission assays acted as carriers of conidia and killed healthy termites. That appeared from progressive mortality rates which reached peak 16 days post treatment. Accordingly, Zoberi (1995) found 25 termite vectors caused complete mortality among 500 healthy individuals within 15 days. Disagreement with the pervious author, insignificant effect of carrier *P. hybostoma* numbers on infections ratios was found. The efficacy transfer and distribution of the fungal pathogen through healthy termites spread the disease with a few numbers of carrier termites after a period of 16 days. Performance of termites as a fungal carrier should be estimated under field conditions. This criterion also reflected powerful of the entomopathogenic fungus to disperse through termites community starting from the vectors. That can be emulated in the natural environment of castes when those entomopathogens extrapolate. Felicitous of the entomopathogenic fungi is not only due to their virulence against termites, but also to their effect against obstacles in termites ecosystem. These blockages could be presumable as avoiding, attacking and killing, burying living unhealthy colony members or cannibalism (Jones *et al.*, 1996 and Myles, 2002 a). Even with these actions, the fungal conidia could be spread allowing nestmates contamination by direct contact or by infected termites habitat. Moreover, the residual fungus from sporulated cadavers continued in contaminating sand and effectively could kill the fresh groups of healthy termites (Zoberi, 1995). Also, termites fed with fungus source could spread the spores via faeces (Padmaja, 2001). Mutual grooming behavior could remove conidia from termites cuticle (Shimizu and Yamaji, 2003). Thus, ongoing search to identify pathogenic and virulent isolates with faster penetration is needed (Moino *et al.*, 2002). Additionally, termites ecosystem has attributes which are suitable properties for fungal growth (Padmaja, 2001).

In conclusion, laboratory testes established certain ferocious of the indigenous *B. bassiana* isolate against subterranean sand termite *P. hybostoma*. Tests performed in sand where the conditions resembled those in fields declared capability of termite works to serve as a pathogen vector. Distribution and spread of the disease among healthy termites was not dependent on carrier numbers. It is recommended to use this isolate of *B. bassania* as baits for termite control where it is expected destroy the termite colony in nature. In general, developing assay techniques for testing fungi-termites relationships should be carried out under field conditions.

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