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A Laboratory Study to Control Subterranean Termites, *Psammotermes hybostoma* (Desn.) (Isoptera: Rhinotermitidae) using Entomopathogenic Nematodes

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ABSTRACT

Four nematode species, two belong to the genus *Steinernema* (*S. riobrave* and *S. carpocapsae*), and the other two belong to *Heterorhabditis* (*Heterorhabditis* sp. and *H. bacteriophora*) were tested for their ability to kill the workers of subterranean termite (*Psammotermes hybostoma* Desn.) in the laboratory. The results indicated that mortality percentages increased with increasing nematode concentration and the highest mortality rate was obtained by using 500 infective juvoniles (IJs)/ Petri dish. The present study indicated also that the susceptibility of termites collected from Ismailia and Fayoum Governorates to nematode was different. In general, the mortality percentage was much higher in Ismailia samples compared with Fayoum samples at all nematode species used. Based on the present results, nematodes can provide adequate termite control depending on the species used. Nematode species differed in terms of their survivability. The results suggest that *H. bacteriophora* HB88 and *S. carpocapsae* proved to be effective against the subterranean termite and are recommended to be tested for controlling termite under field conditions.

Key Words: Termites, *Psammotermes hybostoma*, Entomopathogenic nematode, Control.

INTRODUCTION

Nematodes are ubiquitous roundworm of the Phylum Nematode found in nearly all environments throughout the world. Of the nearly 40 nematode families that are associated with insects, only two of these families, Steinernematidae and Heterorhabditidae, are widely used in biological control (Gaugler and Kaya 1990). These nematodes are obligate insect parasites (Poinar 1979) associated with bacterial symbionts, *Xenorhabdus* spp. and *Photorhabdus* spp. (Boemare *et al.* 1993 and Forst *et al.*, 1997). Nematodes have several distinct advantages over other forms of control in that they are easy to produce, are easy to store, have a high degree of safety among vertebrates and other non-target organisms, and reduce or eliminate the use of chemicals around a structure that needs treatment.

The sand subterranean termite, *P. hybostoma* (Desn.) is one of the most common important termites in Egypt, causing remarkable economic losses annually especially in the border land of the delta, new valley, upper and middle Egypt. Chemical control of termites is the most common method over the entire world, depends on insecticides (El Naggar and Abd El-Latif 2007).

Few studies have addressed the use of nematodes in termite control. Trudeau (1989) recorded high mortalities of termites using nematodes. Insect susceptibility to entomopathogenic nematodes is influenced by nematode species, strain, and an assembly of abiotic and biotic factors.

Entomopathogenic nematodes have received considerable attention recently as biological agents, particularly because of their availability for testing, ability to search for and kill hosts rapidly, ease of application, and safety to mammals and plants (Gaugler, 1988). El-Sebay and El-Bishry (1994) studied the efficacy of the nematodes *Steinernema carpocapsae* and *S. glaseri* and a commercial preparation of *Bacillus thuringienis* against the subterranean termites, *Anacanthotermes ochraceus* in the laboratory. The results revealed morality rates ranged from 58.49 to 100%, *S. carpocapsae* was the most effective bioagent followed by *S. glaseri*. The lowest mortality rates were obtained in case of *B.t.* treatment. Mixing *B.t.* with *S. carpocapsae* coating the diet with agar as moistening material increased the efficacy of *B.t.* and *S. glaseri*, but this treatment gave negative effects with S. *carpocapsae*.

In laboratory studies, Mix (1985) found that *Steinernema feltiae* eliminated colonies of *Reticulitermes flavipes* when both were placed in the same container. Epsky and Capinera (1988) studied the potential of entomopathogenic nematode, *Steinernema feltiae* Filipjev (*Neoaplectana carpocapsae* Weiser), Breton Strain, for control of subterranean termite, *Reticulitermes tibialis* (Banks). They found a significant difference between untreated and treated baited traps in field trials at rate of 1 x 10^7 IJs per m^2, and protection was provided for 2-3 weeks.

Nguyen and Smart (1994) isolated a new nematode species *(Neosteinernema longicurvicoda)* from the termite *Reticulitermes flavipes*. Wang *et al.* (2002) found that nematode, *Rhabditis* sp. associated with three subterranean termite species *Reticulitermes flavipes, R. virginicus* and *Coptotermes formosanns*. Percentages of *R. flavipes, R. virginicus* and C. *formosanns* parasitized by nematodes were 67.9, 38.8, and 3.3%, respectively. The nematodes were found mainly in the termite heads (85.8% in *R. flavipes , R. virginicus*). The infectivity of four species of entomopathogenic nematodes *Steinernema carpocapsae* (Breton Strain), *S. riobrave* (Weslaco strain), *Heterorhabditis bacteriophora (*HP88 strain), and *H. indica* (Coimbatore strain) was examined in the laboratory against two subterranean termites *Reticulitermes flavipes* and *Coptotermes formosanus* (Wang *et al.*, 2002). In Petri dish tests, they were all effective against *C. formosanus* at 400 nematodes per termite. *S. riobrave* had no detectable effect against *R. flavipes* even at a rate of 2000 nematodes per termite. The length of repellency varied with the nematode concentration. Nematodes were able to reproduce from *R. flavipes* and *C. formosanus*.

In the present study, four nematode species, *Steinernema riobrave*, *S. carpocapsae*, *Heterorhabditis* sp. (ISK-2) and *H. bacteriophora* were tested against the subterranean termites (*Psammotermes hybostoma*) under laboratory conditions.

MATERIALS AND METHODS

Collection of termites:

Corrugated cardboard traps were used to obtain the termite from two locations, commonly infested with *P. hybostoma*; El- Hashatra village, Ibshawai district, El-Fayoum Governorate. and Ismailia regional research station, Ismailia Governorate. The collected termites were maintained in Petri dishes for one week to obtain healthy individuals for further laboratory experiments.

Application methods:

Two methods of inoculation with the four nematode species (*S. riobrave*, *S. carpocapsae*, *Heterorhabditis* Sp (ISK-2) and *H. bacteriophora* HBbb) were used as follows:

1- **Direct exposure:**

In this method, the nematode suspension was directly applied on the soil surface before *P. hybostoma* workers were placed in the same Petri-dish containing 20 g of soil. Twenty termite workers were introduced, together, into each dish, with a small piece of moistened corrugated cardboard paper as a food and moisture source. Each nematode species was used with six concentrations (0,100,200,300,400 and 500 IJs/Petri-dish). Each treatment was replicated three times. Nematode suspension in one milliliter of distilled water was applied to the soil surface of each Petri-dish. All dishes were kept under constant laboratory conditions of 25±2°C and were examined daily for 7 days to count the dead workers and calculate the percentages of mortality. Data were statistically analyzed according to the method previously described by Snedecor and Cochran (1956).

2- In A choice test:

A laboratory test unit (Fig.1) consisted of two plastic containers (15 x 10 x 10 cm each.) half filled with the soil was used. A plastic tube (15 cm. long and 9 mm internal and external diameters) connecting the two containers as shown in the figure (1). 100 termite workers were placed in the first plastic container with a small piece of cardboard paper and allowed to feed for 7 days before connection with the second container. After this period, the connection was allowed and two nematode species (*S. carpocapsae* and *H. Bacteriophora*) were separately added to the second container with also a small piece of cardboard paper.

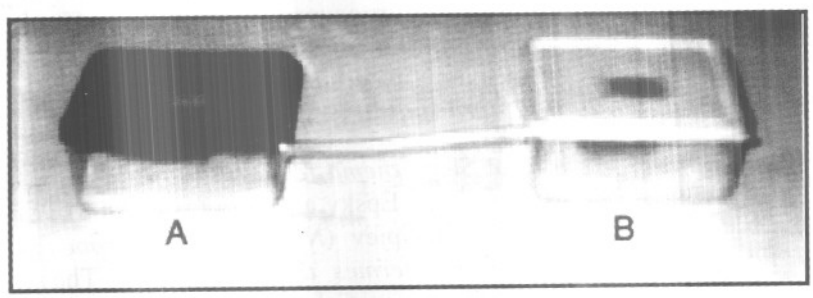

Fig. (1): A laboratory test unit. A: a container half filled with the soil, B: a container half filled with the soil treated with nematode

Each species was used with the concentrations of 0, 1000, 2000, 3000 and 4000 IJs/container. Each treatment was also replicated three times and three additional units (un inoculated with nematodes) were used as a control. All units were examined daily to determine the dead termites. After 2 weeks in all test units, surviving termites were counted to calculate percentage of survival (mortality).

RESULTS AND DISCUSSION

The data indicated that in the sample collected from Ismailia region mortality percentage increased with increasing nematode concentration and the highest mortality percentages was obtained by using 500 IJs/ Petri dish. Similar results were obtained from the termites collected from Fayoum (Fig. 2).

Statistical analyses of the obtained data indicated differences between tested nematode species and highly significant between concentrations (Table 1).

The data agree with those reported by Azazy and Ahmed (2006) in which, the optimum concentration to kill termite was 25IJs/insect.

The present study indicates that the susceptibility of termites collected from Ismailia and Fayoum Governorates to nematode was different. In general, the mortality percentage was much higher in Ismailia samples compared with Fayoum samples at all nematode species used. *H. bacteriophora* showed a higher mortality percentage (50 and 45% in Ismailia and Fayoum samples, respectively), followed by *S. carpocapsae* (42.16 and 37%) then *S. riobrave*(35.6 and 31%) while the *Heterorhabditis sp.* gave the lowest mortality (27.6 and 22%) compared with the other nematode species used (Fig 3).

Based on these results, the two nematode species (*H. bacteriophora* HB88 *S. carpocapsae*) were selected and tested against the termites collected from Ismailia using a choice test method. Data indicated that *H. bacteriophora* showed higher mortality percentages after 72 hrs post treatment compared with *S. carpocapsae*. The survival percentages decreased with increasing nematode concentration up to 4000IJs/ container (Table 2).

Table (1): Efficacy of four nematode species at different concentrations against the workers of subterranean termite *P. hybostoma*.

S.V	*df*	Ismailia (MS)	Fayoum (MS)	$F_{0.05}$
Treatments	4	518.68**	954.93**	3.26
Nematode species	3	652.05**	382.58**	4.49
Error	12	9.01	16.79	
Total	19			

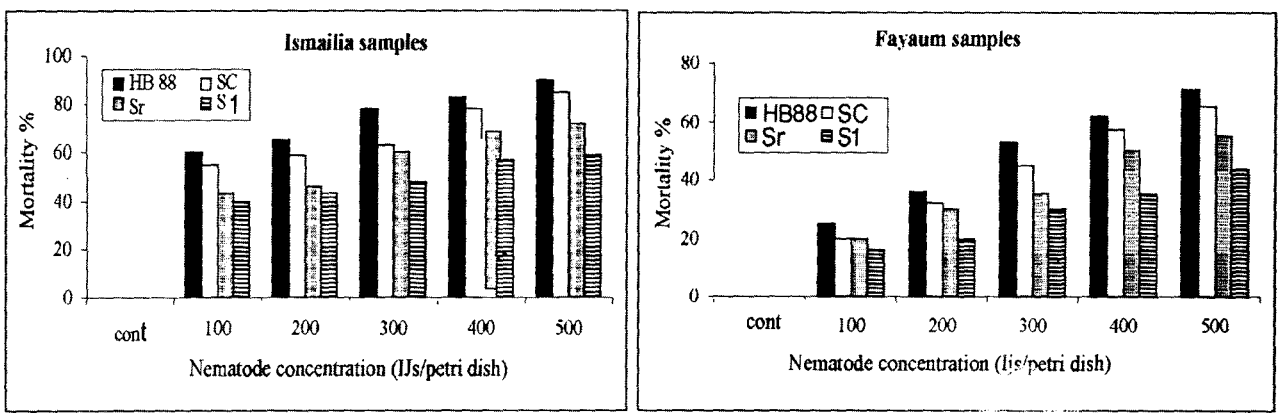

Fig. (2): Mortality percentages of four nematode strains applied to the soil surface with different concentrations against workers of subterranean termite *P. hybostoma* samples collected from two different locations.

Table (2) Corrected mortality and survival percentages of the workers of subterranean termites treated with two nematode strains using a choice test after 48 and 72 hrs.

Nematode strain	Conc. IJs/ container	No. of treated insect	48 hrs after treatment	72 hrs after treatment
			Mortality%	Mortality%
H. bacteriophora	0	100	0.0	5
	1000	100	33	43
	2000	100	35	57
	3000	100	60	70
	4000	100	63	80
S. carpocapsae	0	100	0.0	0.0
	1000	100	15	28
	2000	100	22	42
	3000	100	31	46
	4000	100	40	67

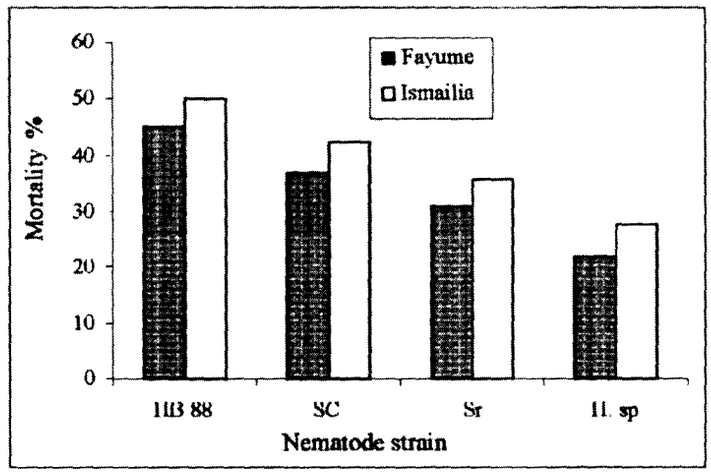

Fig (3): Mortality percentages of different nematode species against the workers of subterranean termite *P. hybostoma* collected from two different locations in Egypt.

The results indicated that entomopathogenic nematodes can provide adequate termite control depending on the species used. Nematode species differ in terms of their survival ability. Temperature, pH, substrate and other environmental factors may play a role in nematode survival. The results suggest that some nematode species such as *H. bacteriophora* and *S. carpocapsae* proved to be more effective against the subterranean termite *P. hybostoma* and are recommended to be tested for controlling the termite in the field.

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الملخص العربى

مكافحة النمل الأبيض التحت أرضى
Psammotermes hybostoma (Desn.) (Isoptera: Rhinotermitidae)
تحت الظروف المعملية باستخدام النيماتودا الممرضة للحشرات

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فى هذه الدراســـة تم استخدام أربعةأنواع من النيماتودا الممرضة للحشرات ،نوعين يتبعا الجنس Heterorhabditis (*H. bacteriophora* و نوعين يتبعا الجنس Steinernema (*S. riobrave, S. carpocapsae*) لمعرفة مدى قدرتهم على قتل شغالات النمل الأبيض تحت الظروف المعملية ، أوضحت النتائج أن نسبة الموت المتسببة عن النيماتودا تتزايد بازدياد تركيز نوع النيماتودا وذلك مع جميع أنواع النيماتودا المستخدمة وقد حقق التركيز ٥٠٠ فرد معدى من النيماتودا / طبق بترى أعلى نسبة موت داخل جمهور الحشرات . كما أوضحت الدراسة اختلاف نسبة الموت المتحققة داخل حشرات النمل الأبيض المعزول من محافظة الاسماعلية عنها فى الحشرات المعزولة من محافظة الفيوم فىجميع أنواع النيماتودا المستخدمة ،كما أوضحت النتائج أن كلا من النوعين أكثر فعالية ضد النمل من النوعين الأخرين. *H. bacteriophora* ، *S. carpocapsa* طبقا للنتائج المتحصل عليها فانه يتضح مدى امكانية الاعتماد على بعض أنواع النيماتودا الممرضة للحشرات المختبرة كعامل من عوامل المكافحة الحيوية ضمن برنامج المكافحة المتكاملة لحشرات النمل الأبيض تحت الظروف الحقلية.