SUSCEPTIBILITY OF EGYPTIAN SUBTERRANEAN TERMITE TO SOME ENTOMOPATHOGENIC NEMATODES

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Abstract

The present work has been conducted to study the possibility of using entomopathogenic nematodes *Heterorhabditis baujardi* and *Heterorhabditis indica* against subterranean termite *Psammotermes hypostoma* (Desn.), and *Anacanthotermes ochraceus* (Burm.), under laboratory conditions as a preliminary test for using them under the field conditions. Data showed that, the using of tested pathogenic nematodes *H. baujardi* and *H. indica* were effective on the two tested termites. The bioagent *H. baujardi* strain was highly effective for control of the two tested subterranean termites, but the subterranean termite *P. hypostoma* was highly susceptible than *A. ochraceus*. In addition to the highly rate of mortality which started in the 3rd day in all treatments approximately, decreased gradually to reach of stability in 6th or 7th day. Overall, the mortality increased as the nematodes concentrations increased and vice versa.

INTRODUCTION

The termites feed mostly on anything containing cellulose component, and represented one of the most important pests causing great losses to houses and foundations in Egypt most of these losses due to the subterranean termite species, as well as the expensive cost of its chemical. Several studies resulted that, the economic losses caused by subterranean termites in Egypt was more than 6 million LE El-Sebay (2004), also El-Sebay and Ahmed (2006), estimated about 4.3 million LE losses at Fayoum, and 755110 LE for termites control at North Sinai. In USA, Johnston (1967), counted that, the losses may be reaching to 62 million dollars yearly, and Baker (1972), estimated damage from 400 to 500 million dollars/year. Chemical control against termite already caused dangerous environmental harmful to the world life, therefore, the biological control methods become pressing need for environment protection. Nematodes are a ubiquitous roundworm of the Phylum Nematoda found in nearly all environments throughout the world. Of the nearly 40 nematode families that are associated with insects, only 2 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, Forst *et al.* 1997).The infective juvenile stage of the nematode is a free living stage that remains in the soil until it can invade the body of a potential host on contact with a

susceptible insect. After infection of the insect host, symbiotic bacteria are released into the insect hemocoel, causing septicemia and death (Kaya and Gaugler 1993). Nematodes have several distinct advantages over other forms of control in that they are easy to produce, don't require registration by the USEPA, 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 is needing treatment (Woodring and Kaya, 1988). With the general public becoming increasingly concerned about pesticide usage, the use of nematodes for termite control is a potentially promising market. Few studies have addressed the use of nematodes in termite control, and the insect susceptibility to entomopathogenic nematodes in the family Heterorhabditidae. Thus far no studies have been undertaken on nematode effects on *P. hypostoma* and *A. ochraceus*. Entomopathogenic nematodes also differ in their abilities to survive in different environmental conditions. Ability to penetrate soil, method of host attack, and ability to handle environmental extremes may favor the use of a particular nematode over another (Kaya and Gaugler 1993).

In this study we tested two nematode species, *Heterorhabditis baujardi* and *Heterorhabditis indica* belonging to family Heterorhabditidae for their ability to induce mortality for two subterranean termites *Psammotermes hypostoma* and *Anacanthotermes ochraceus* under laboratory conditions.

MATERIALS AND METHODS

Termite collections

El-Sebay modified trap, (El-Sebay Y. 1991), was used in subterranean termite collection. The collection of *P. hypostoma* (Desn.), termites were obtained from the infested area of Experimental Res. at Ismailia Governorate, while the harvester termite *A. ochraceus*, were collected from an infested home at Sangha village, Kafr Sakr, Sharqia Governorate. Termites were separated by small brush from the traps and kept in Petri-dishes (1×9.5cm height and diameter, respectively) provided with moistened corrugated card-boards as a source of cellulose and moisture for seven days, then, the healthy workers were used in the test.

Nematode source

Two Egyptian *Heterorhabditis* species were used in the present study. The first species *Heterorhabditis indica,* was defined as a first record by Abd El-Rahman (2001), and the second species was *Heterorhabditis baujardi*, was defined as a first record by Abd El-Rahman (2006), the two species were defined according to the morphometric measurements.

Treatments

Stock suspension at concentrations of 1000, 2000 and 4000 infective juvenile (IJs/ml) was prepared for test against subterranean termites. Concentrations were chosen on that basis and the micropipette 1ml was used to pulling of suspensions as follow; dose of 1ml, 2ml and 4ml were pull from the three suspensions to equal conc. of 10, 20 and 40 IJs/insect, for 1st, 2nd and 3rd suspension, respectively. The used Petri-dishes (1×9.5cm height and diameter, respectively) were sterilized in an oven at 100C° for 24 hours after treatments, and 100 healthy workers of subterranean termites were placed in the Petri-dish. Treatments were replicated five times to equal 500 termites/treatment (conc.). The concentration was added to a piece of jelly agar (15gm) as a source of moisture and suitable media of nematodes, placed beside a piece of corrugated card-board 5x5cm as a source of cellulose for termite. Elbassiouny (2001), used the baits of agar media treated with nematodes against subterranean termites, *P hypostoma*. Treatments were kept under a constant temperature 27+1C° in an incubator. Mortality was recorded daily for seven days. Control was contained a piece of corrugated card-board (5x5cm) and a piece of jelly agar (15gm), treated with 1ml of distilled water. Mortality was corrected according Abbott's formula, Abbott (1925).

Statistical analysis

Data was subjected to analysis of variance (ANOVA) and the means were compared by LSD test at 0.05 level, using SAS program (SAS, 1988).

RESULTS AND DISCUSSION

1. The effect of *Heterorhabditis baujardi*

a. Laboratory evaluation of the efficiency of *H. baujardi* against subterranean termite *P. hypostoma*:

In Table (1), when used the pathogenic nematode *H. baujardi* against subterranean termite *P. hypostoma*; Data revealed that, the action of nematodes conc of 1000 IJs/ml recorded average of 12.6 mortality after three days increased gradually to 40.4 after seven days, while the action of pathogenic nematode conc 2000 IJs/ml recorded average 23.2 after three days, increased gradually to 63.6 after seven days. In the case of using pathogenic nematode conc 4000 IJs/insect, data recorded 28.4 after three days, increased gradually to reach 95.8 after seven days. Throughout the three concentrations mortality began from the 3rd day, and increased gradually to be stable in the 6th or 7th day. The highest of average was 1.8 mortality in control for three concentrations.

Table 1. Numbers and average of dead termites, *P. hypostoma* affected by *H. baujardi* treatments.

Conc. IJs/ml	Replicates	Mortality in days						
		1	2	3	4	5	6	7
1000	1	1	1	14	23	29	30	30
	2	0	1	11	31	27	28	28
	3	0	2	16	45	50	51	51
	4	1	1	13	26	31	38	39
	5	2	3	9	25	42	54	54
	Average	٠.8	1.6	12.6	30	35.8	40.2	40.4
2000	1	0	3	18	26	42	42	42
	2	0	5	22	35	52	75	57
	3	1	3	16	40	61	64	65
	4	0	10	34	68	77	100	100
	5	0	1	26	47	53	54	54
	Average	0.2	4.4	23.2	43.2	57.0	67.0	63.6
4000	1	0	0	23	38	74	90	90
	2	0	0	32	53	74	100	100
	3	0	5	45	71	92	96	100
	4	0	1	22	45	60	89	89
	5	1	2	20	63	70	100	100
	Average	0.2	1.6	28.4	54.0	74.0	95.0	95.8
Control	1	0	0	2	3	3	3	3
	2	0	0	0	0	0	1	1
	3	1	2	0	0	0	0	0
	4	0	2	5	5	5	5	5
	5	0	0	0	0	0	0	0
	Average	0.2	٠.8	1.4	1.6	1.6	1.8	1.8

1000 IJs/ ml = 10 IJs/ insect

2000 IJs/ ml = 20 IJs/ insect

4000 IJs/ ml = 40 IJs/ insect

b. Laboratory evaluation of the efficiency of *H. baujardi* against subterranean termite *A. ochraceus*

Data in Table (2), reveal the effect of pathogenic nematode *H. baujardi*, against subterranean termite *A. ochraceus*, and data recorded average 9.2 mortality by using of first conc 1000 IJs/ml after three days, increased gradually to reach 32.6

after six day, also the effect of pathogenic nematode conc 2000 IJs/ml; data recorded 17.2 after three days, increased gradually to reach 57.8 after seven day. In case of using pathogenic nematode conc 4000 IJs/ml; data gave average 23.6 after three days, increased gradually to 81.2 after seven days. Throughout the three concentrations mortality began from the 3rd day to be stable in 6th or 7th day. With comparison between the used concentrations, mortality represented of average 32.6 - 81.2 at low and high level, respectively. The highest of average was 0.6 mortality in control for three concentrations.

Epsky and Capinera (1988), in United States, studied the potential of entomopathogenus nematode, *Steinernema feltiae* Filipjev = *Neoaplectana carpocapsae* (Weisner), Breton strain, for control of a subterranean termite, *Reticulitermes tibalis* (Banks); they showed that, the termite workers were susceptible to nematodes in laboratory tests, but large numbers of nematodes were required for mortality. LD_{50} was estimated as 1.5 $x10^4$ nematodes per termite in standard filter paper assays. Nematodes were applied to the soil directly beneath baited traps at rate of $1x10^7$ per m2 in field trials, and the data revealed that there was a significant difference between treated and untreated traps in number of termites per trap, and protection was provided of 2-3 wk. Termites attacking traps on treated sites and entered traps at a corner or from the top. They suggested that, termites might be avoiding contact with entomogenous nematodes. This study suggests either nematode application should be made frequently or that the entire colony rather than only the feeding site must be treated. El-Sebay and El-Bishry (1994), tested the entomopathogenic nematodes *Steinernema carpocapsae* and *Steinernema glaseri,* against subterranean termites *A. ochraceus*, they found, the *S. carpocapsae* alone was the most effective bioagent followed by *S. gloseri.* Mixing *B. T.* with *S. gloseri* enhanced the mortality; the opposite was evident with *S. carpocapsae*, coating the diet with agar as moistening materials increased the efficacy of *B. T.* and *S. gloseri*, while *S. carpocapsae* was negatively affected. Nematode development inside dead termites was detected only in case of *S. gloseri*, while *S. carpocapsae* failed to complete its life cycle. Elbassiouny (2001), tested entomopathogenic nematode *Steinernema carpocapsae* to control of termite *P. hypostoma*, and found that the termites didn't avoid contact with nematoda baits, and fed upon the treated agar; data showed highly morality percentages when termites treated with the highly conc = 4 IJs/insect started with 31.4 mortality% in the second day and reach its maximum 100% in the sixth day. LT_{50} was 7.32, 3.21 and 2.23 days for the three conc; 1 IJs/insect, 2 IJs/insect and 4 IJs/insect respectively. The symptom of death of the contaminated termites was suffering, sluggish, tissue bodies are ragged and the

juveniles emerged from the body cavity of dead individuals after 5-7 days. Juveniles emerged from soft body regions, especially prothorax, lateral abdominal regions, anus and the head pits. The termites workers were more susceptible to nematodes than alates and soldiers.

Table 2. Numbers and average of dead termites, A. ochraceus affected by H. baujardi, treatments.

Conc. IJs/ml	Replicates	Mortality in days						
		1	2	3	4	5	6	7
1000	1	0	0	7	18	32	35	35
	2	0	1	10	24	25	25	25
	3	0	0	9	17	33	37	37
	4	0	0	11	21	36	37	37
	5	0	1	9	16	28	29	29
	Average	0.0	0.4	9.2	19.2	30.8	32.6	32.6
2000	1	0	0	13	23	35	37	37
	2	0	0	21	33	46	56	56
	3	0	0	12	43	52	60	60
	4	0	0	22	38	57	73	73
	5	0	0	18	41	42	62	63
	Average	0.0	0.0	17.2	35.6	46.4	57.6	57.8
4000	1	0	2	19	30	65	90	91
	2	0	0	28	44	80	84	84
	3	0	1	33	40	77	89	89
	4	0	0	21	39	54	72	72
	5	0	0	17	42	59	68	70
	Average	0.0	0.6	23.6	39.0	67.0	80.6	81.2
Control	1	0	0	0	1	2	2	2
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	1	1	1	1
	5	0	0	0	0	0	0	0
	Average	0	·	0	0.4	0.6	0.6	0.6

1000 IJs/ ml = 10 IJs/ insect

2000 IJs/ ml = 20 IJs/ insect

4000 IJs/ ml = 40 IJs/ insect

c. Relative toxicity of *H. baujardi* on tested termites

Data presented in Tables (2&4) and graphically illustrated as toxicity lines in Figs (1 and 2) show that tabulated data indicate, the potency of nematode *H. baujardi* was varied tremendously due to the used concentrations. As a general trend, the higher concentration gave the rate of mortality and vice versa. According to the obtained data, different mortality percentages were recorded when the nematode *H. baujardi* were treated against subterranean termite *P. hypostoma*. The LC_{50} and LC_{90} were 15.03 and 361.53 with slop 0.92 in 3rd day, and decreased gradually to reach 1.30 and 3.49 with slop 2.9 in 7th day, respectively. When tested nematode *H. baujardi* treated against termite species *A. ochraceus*, the LC_{50} and LC_{90} were 20.26 and 398.59 with slop 0.99 in 3rd day, decreased gradually to reach 1.60 and 6.09 with slop 2.21 in 7th day, respectively.

Table 3. LC values (IJs/ml) of *H. baujardi* on the termite *P. hypostoma* under laboratory conditions.

Days	LC_{50}	LC_{90}	Slope
3 days	15.034	361.535	0.928
4 days	3.107	53.745	1.035
5 days	1.621	9.459	1.673
6 days	1.253	2.969	3.421
7 days	1.303	3.496	2.9

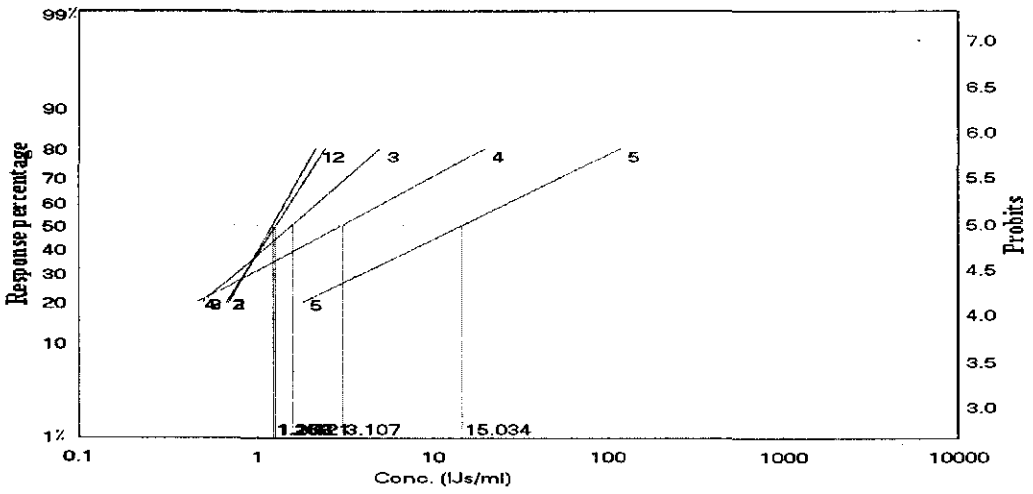

Fig. 1. LC values (IJs/ml) of H. baujardi on the termite P. hypostoma under laboratory conditions.

Table 4. LC values (IJs/ml) of *H. baujardi* on the termite *A. ochraceus* under laboratory conditions.

Days	LC_{50}	LC_{90}	Slope
3 days	20.26	398.598	0.99
4 days	6.725	148.684	0.953
5 days	2.158	14.253	1.563
6 days	1.618	6.269	2.179
7 days	1.609	6.097	2.215

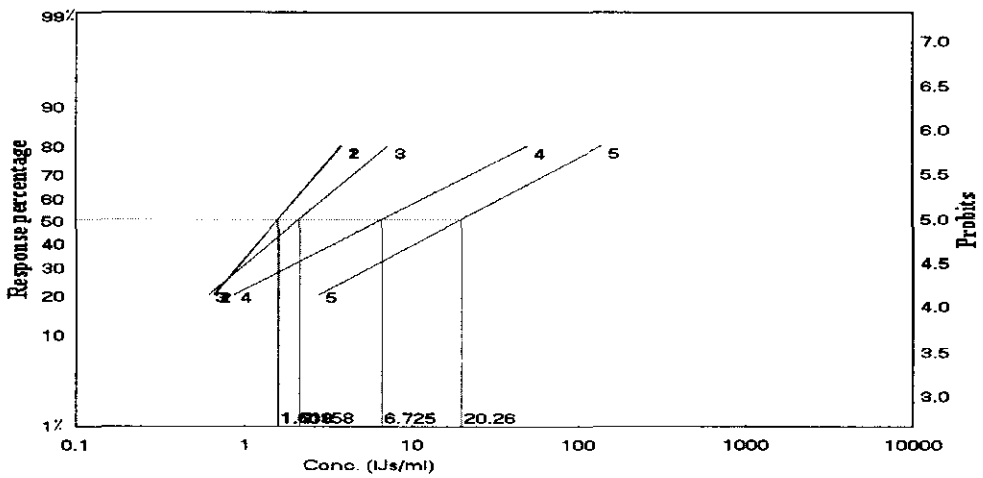

Fig. 2. LC values (IJs/ml) of H. baujardi on the termite A. ochraceus under laboratory conditions.

2. The effect of *Heterorhabditis indica*

a. Laboratory evaluation of the efficiency of *H. indica* against subterranean termite *P. hypostoma*

Data in Table (5), resulted that, the average of mortality were 12.4 after three days, increased gradually to reach 38.2 after seven days, when use of conc 1000 IJs/ml by pathogenic nematode *H. indica*, against subterranean termite *P. hypostoma*; while was 19.0 after three days, increased gradually to 55.4 after seven days, when use of the conc 2000 IJs/ml, also the effect of pathogenic nematode conc 4000 IJs/ml; data recorded 23.4 after three days, increased to reach 78.2 after seven days. Throughout the treatments, mortality began from the 3rd day to be stable in the 6th or 7th day. With comparison between three concentrations, mortality represented 38.2 - 78.2 at the low and high level respectively. The highest of average was 1.2 mortality in control for three concentrations.

b. Laboratory evaluation of the efficiency of *H. indica* against subterranean termite *A. ochraceus*

Data in Table (6), showed that, the action of pathogenic nematode *H. indica* against subterranean termite *A. ochraceus*; and data calculated, the average of mortality was 18.4 after four days, increased gradually to reach 32.8 after seven days, when use of conc 1000 IJs/ml, also the effect of pathogenic nematode conc 2000 IJs/ml; data recorded average 14.6 after three days, increased gradually to 48.0 after seven days. In case of using pathogenic nematode conc 4000 IJs/ml; data recorded 20.0 after three days, increased gradually to 70.0 after six days. Throughout the three concentrations mortality began from the 3rd day, to be stable in the 6th or 7th day. With comparison between the three concentrations mortality represented 32.8 - 70.0 at the low and high level, respectively. The highest of average was 1.4 mortality in control for three concentrations.

Table 5. Numbers and average of dead termite P. hypostoma affected by H. indica treatment.

Conc. IJs/ml	Replicates	Mortality in days						
		1	2	3	4	5	6	7
1000	1	0	0	10	18	23	27	27
	2	0	1	16	31	35	39	40
	3	0	4	14	20	34	47	47
	4	0	1	9	26	43	51	52
	5	0	7	13	22	22	25	25
	Average	0.0	2.6	12.4	23.4	31.4	37.8	38.2
2000	1	0	1	13	21	35	35	35
	2	0	0	17	38	43	54	54
	3	2	2	9	22	41	56	58
	4	0	3	26	32	39	39	42
	5	1	1	30	44	63	87	88
	Average	0.6	1.4	19.0	31.4	44.2	54.2	55.4
4000	1	0	6	17	53	72	72	72
	2	0	3	21	48	61	70	73
	3	0	2	33	54	100	100	100
	4	0	2	31	51	54	54	55
	5	0	0	15	44	68	84	91
	Average	0.0	2.6	23.4	50.0	71.0	76.0	78.2
Control	1	0	0	1	1	1	2	2
	2	0	1	1	1	1	1	1
	3	0	0	0	0	0	0	0
	4	0	0	2	3	3	3	3
	5	0	0	0	0	0	0	0
	Average	0.0	·.2	0.8	1.0	1.0	1.2	1.2

1000 IJs/ ml = 10 IJs/ insect
2000 IJs/ ml = 20 IJs/ insect
4000 IJs/ ml = 40 IJs/ insect

Table 6. Numbers and average of dead termite A. ochraceus affected by H. indica treatment.

Conc. IJs/ml	Replicates	Mortality in days						
		1	2	3	4	5	6	7
1000	1	0	0	7	12	27	28	30
	2	0	0	4	22	31	31	31
	3	0	0	10	16	25	29	29
	4	0	0	8	23	32	32	33
	5	0	0	11	19	40	41	41
	Average	0	0	8.0	18.4	31.0	32.2	32.8
2000	1	0	0	10	17	31	43	43
	2	0	3	13	26	40	60	60
	3	0	2	11	28	38	38	40
	4	0	0	17	29	41	42	42
	5	0	1	22	33	45	53	55
	Average	0	1.2	14.6	26.6	39.0	47.2	48.0
4000	1	0	0	15	47	65	68	68
	2	0	1	20	39	63	70	70
	3	0	0	23	51	74	74	74
	4	0	0	25	43	60	62	62
	5	0	0	17	38	70	76	76
	Average	0	0.2	20.0	43.6	66.4	70.0	70.0
Control	1	0	0	1	1	1	1	1
	2	0	0	0	1	1	1	1
	3	2	3	3	3	3	3	3
	4	0	0	0	0	0	0	0
	5	1	2	2	2	2	2	2
	Average	0.6	1.0	1.2	1.4	1.4	1.4	1.4

1000 IJs/ ml = 10 IJs/ insect

2000 IJs/ ml = 20 IJs/ insect

4000 IJs/ ml = 40 IJs/ insect

Generally, in all treatments for the two tested nematodes and termites, the small workers (nymphs) of termites were more susceptible and their death occurred faster than the large workers (adults), whereas the small workers (nymphs), represented the most mortalities in the 1st and 2nd day approximately. From the 2nd

day, the contaminated termites with pathogenic nematodes were sluggish and the body tissues were ragged.

Epsky and Capinera (1988), mentioned that, the *Reticulitermes tibalis,* workers were susceptible to nematodes *S. carpocapsae*. Mauldin and Beal (1989), found that, termites survival rates in the nematode treatment and in the untreated control did not differ significantly. Amarasingh and Homonick (1993a&b), found that, *S. carpocapsae* were better in control of termite *psteletrotermes militaris* than other isolates tested. Nguyen and Smart (1994), mentioned that using of genus *Steinernema* as a parasite of termites and resulted that Juveniles nematodes are found out side the termite cadaver, and the diagnosis of Nematodes was accommodate to the termite. Elbassiouny A. R. (2001), tested earthen nematodes *Steinernema carpocapsae* to control of termite *P. hypostoma*, he found that, nematode are able to control of subterranean termite in lab and the nematode can be developed inside dead termites.

c. Relative toxicity of *H. baujardi* on tested termites

According to obtained data in tables (7 and 8), different mortality percentages were recorded, when the nematode *H. indica* were treated against termite species *P. hypostoma*, the comparison between the effects of tested nematode showed that, the LC_{50} and LC_{90} were 40.80 and 2748.45 with slope 0.70 in 3rd day, then decreased gradually to reach 1.54 and 8.08 with slope 1.77 in 7th day, respectively, when the tested nematode treated against termite species *A. ochraceus*, the LC_{50} and LC_{90} were 31.59 and 792.07 with slope 0.91 in 3rd day, decreased gradually to reach 1.97 and 12.37 with slope 1.60 in 7th day, respectively. In general, the slope of line is useful to evaluate the ability of tested different concentrations of nematodes species. Overall, the slope increased in regression as the nematodes concentrations increased.

Table 7. LC values (IJs/ml) of H. indica on the termites P. hypostoma under laboratory conditions.

Days	LC_{50}	LC_{90}	Slope
3 days	40.808	2748.455	0.701
4 days	4.292	48.394	1.218
5 days	2.08	11.551	1.721
6 days	1.596	9.245	1.68
7 days	1.54	8.089	1.779

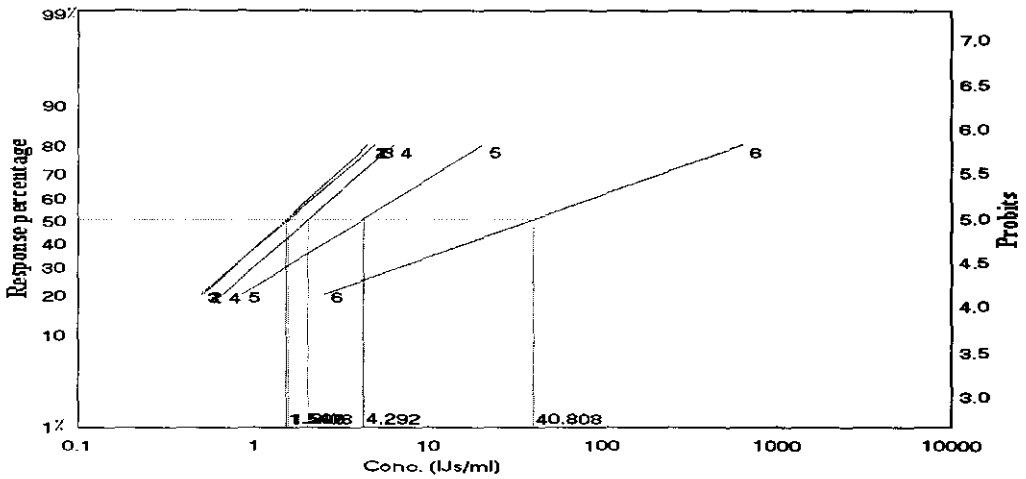

Fig. 3. LC values (IJs/ml) of H. indica on the termites P. hypostoma under laboratory conditions

Table 8. LC values (IJs/ml) of *H. indica* on the termites *A. ochraceus* under laboratory conditions

Days	LC_{50}	LC_{90}	Slope
3 days	31.596	792.074	0.916
4 days	5.673	61.083	1.242
5 days	2.397	16.466	1.531
6 days	2.013	12.208	1.637
7 days	1.977	12.378	1.609

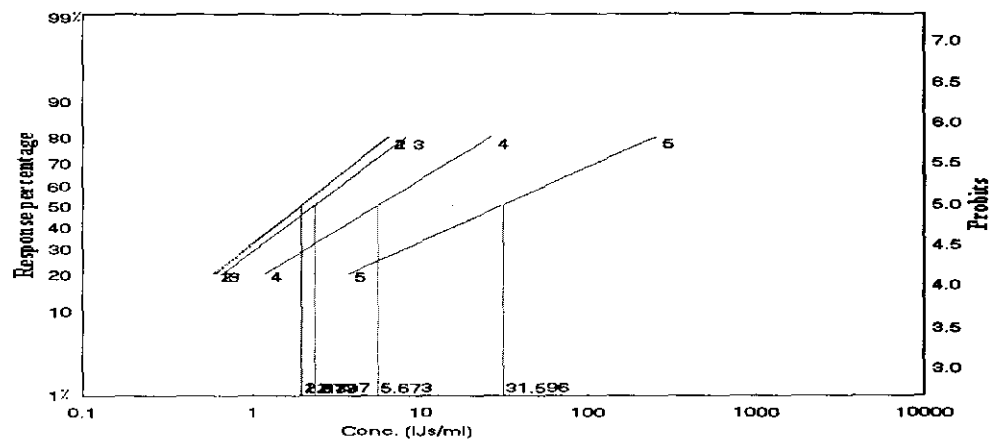

Fig. 4. LC values (IJs/ml) of H. indica on the termites A. ochraceus under laboratory conditions

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دراسة على حساسية النمل الأبيض تحت أرضى فى مصر بفعل النيماتودا الممرضة للحشرات

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أجريت هذه الدراسة بغرض إمكانية إستخدام الممرضات من النيماتودا الأرضية " هيتيرورابديتس بايوجارديا " و " هيتيرورابديتس إنديكا " ضد النمل الأبيض تحت أرضى " ساموترمس هيبوستوما " و " أناكانثوترمس أوكريشيس " تحت الظروف المعملية . وقد أثبتت البيانات أن إستخدام نوعى النيماتودا كان له فاعلية ضد أنواع النمل المختبر ، و عند مقارنة نوعى النيماتودا ، أثبتت البيانات أن نوع النيماتودا هيتيرورابديتس بايوجارديا كان الأكثر فاعلية فى مقاومة نوعى النمل الأبيض المختبر ، ولكن نوع النمل الأبيض "ساموترمس هيبوستوما" كان الأكثر حساسية من النوع " أناكانثوترمس أوكريشيس" . بالإضافة إلى أن نسبة الموت كانت قد بدأت فى اليوم الثالث تقريبا فى كل المعاملات ثم تناقصت تدريجيا حيث ثبتت تقريبا فى اليوم السادس أو السابع . أيضا كان من الطبيعى أن تزداد النسبة المئوية للموت مع زيادة التركيز والعكس صحيح .