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EFFICACY OF ENTOMOPATHOGENIC NEMATODES AGAINST THE GREASY CUTWORM *AGROTIS IPSILON* (HUFN.)(LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT : Two entomopathogenic nematodes *Heterorhabditis indicus* (native and imported strains) were evaluated as biological control agents of cutworm *Agrotis ipsilon* (Hufn.). In Petri dish bioassays, all strains were pathogenic to last instar larva and prepupa of *A. ipsilon*. The LC_{50} values of treated 6^{th} instar larvae were 17 and 19 infective juveniles (IJs) per insect for native and imported strains, respectively. The LC_{50} values of treated prepupa were 13 and 20 IJs per insect for native and imported strains, respectively. Significant differences ($P > 0.01$) in mortality were recorded between different concentrations of the two strains for 6^{th} instar larva, prepupa and pupa. Significant differences ($P > 0.05$) were observed in the average weight of pupae treated as 6^{th} instar larvae or prepupae by two nematode strains. Whereas, the pupation period of larvae treated as 6^{th} instar by imported nematode strain increased significantly ($p > 0.01$) at higher concentrations. There were significant differences ($P > 0.05$) between various concentrations and untreated insects for the two strains of nematodes in adult emergence, number of deposited eggs/female and egg hatchability percent but not significant between different nematode strains.

INTRODUCTION

Larvae of the black cutworm, *A. ipsilon* (Hufn.) (Lepidoptera : Noctuidae), feed on grasses and other agricultural crops at or near the soil surface (Tashiro, 1987). The early instars feed on foliage, but the later instars may tunnel to a soil depth between 2.5 and 5 cm, cut the plants at the soil surface at night, and pull them into their burrows in the ground before feeding (Rings and Musick, 1976).

Entomopathogenic nematodes from the Heterorhabditidae and Steinernematidae are promising biological alternatives to chemical insecticides (Ishibashi and Choi, 1991; Kaya 1985 and Poinar 1986). These nematodes can penetrate and kill many economically important pests within 24-48 hours (Poinar, 1986). In some cases, nematode treatments provide levels of insect control equivalent to those of chemical insecticides (Georgis, 1990). Nematode species of the genus *Hetrorhabditis* are obligate pathogens of insects. The nematodes are symbiotically associated with the bacterium *Photorhabdus luminescens* (Boemare *et al.*, 1993). Cells of the bacterium are transmitted into haemocoel of host insects by a developmentally arrested third juvenile stage, the infective dauer juvenile (DJ). Reaching the host haemocoel, the DJ initiate development and release their symbiotic bacteria. The host dies within 3 days after infection (Johnigk and Ehlers, 1999). The nematodes complete two to three generations within the host, after which free-living infective juveniles emerge to seek new host (Poinar, 1990). These nematodes are exempt from government registration in the United States (Gaugler, 1988). Our objective was to test the infectivity of local and foreign strains of *Heterorhabditis indicus*, against different stages of cutworm *Agrotis ipsilon*.

MATERIALS AND METHODS

This work was done at the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University. The greasy cutworm *A. ipsilon* (Hufn.) (Lepidoptera: Noctuidae), larvae were collected from infected fields and reared in the laboratory on castor-oil leaves according to the method described by (Fahmy *et al.*, 1973). The nematode *Heterorhabditis indicus* (native and imported strains LN2 (USA); SAA2 (Egypt)) was propagated in larvae of the greater wax moth, *Galleria mellonella*, according to Dutky *et al.*, (1964), then stored in phosphate-buffered tap water (pH 7.2) at 6°C for at least 1 day to a maximum of 4 weeks. Prior to application, dead and inactive DJs were removed by placement of the nematode suspension on a 30 μm sieve. Five hours later DJs which had actively passed through the sieve were collected.

Nematode: *Heterorhabditis indicus*

Bacteria: *Photorhabdus luminuscens*

Strain: LN2 (USA) and SAA2 (Egypt)

Reference: Poinar *et al.*, 1992 Mona Hussein, (2004)

Bioassay of nematodes on Agrotis ipsilon:-

Forty replicates were used for each nematode concentration tested. During nematode exposure, the larvae were fed on castor-oil leaves. Concentrations of 5, 10, 20, 50 and 100 DJs in 100 μl sterilized distilled water were applied to the agar Petri dishes in direct vicinity to the insects. The experiment was repeated three times. Assuming that a positive relationship exists between nematode concentration and host mortality, probit analysis has been used to analyze data from dose-response tests and to calculate LC_{50} values (Glazer, 1991). For the nematode activity, a random sample of 8 to 15 larvae or prepupae was dissected and the nematodes inside the larvae or prepupae were observed.

All data were subjected to analysis (ANOVA) and mean values were separated by the least significant difference (LSD) procedure (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Mortality percentages of 6^{th} instar larvae, prepupae and pupae of the cutworm *A. ipsilon* (Hufn.) exposed to different concentrations of native and imported entomopathogenic nematode, *H. indicus*, are given in table (1). Significant differences

($P > 0.01$) were found between different concentrations of native and imported nematode strains, and untreated check for 6th instar larvae, prepupae and pupal stages of *A. ipsilon*. The LC_{50} values of treated 6th instar larvae were 17 and 19 infective juveniles (IJs) per insect for native and imported strains, respectively. The LC_{90} values of *A. ipsilon* treated as 6th instar larvae were 81 and 81 IJs per insect for native and imported strains, respectively. No significant differences ($P > 0.01$) between entomopathogenic nematode strains at larval, prepupal and pupal stages mortality were detected. The effect of *H. indicus* native and imported strains on prepupae of the cutworm *A. ipsilon* was recorded in table (2). Low mortality was observed among insects infected with *H. indica* (native and imported strains) at lower concentrations (5 and 10 IJs/prepupae). High mortality was recorded among prepupae infected with local and foreign *H. indicus* at higher concentrations (50 and 100 IJs/prepupae). The LC_{50} values of treated prepupae were 13 and 20 IJs per insect for native and imported strains, respectively. The LC_{90} values of *A. ipsilon* treated prepupae were 51 and 70 IJs per insect for native and imported strains, respectively. Significant differences ($P > 0.01$) were observed between different concentrations of *H. indica* strains against cutworm *A. ipsilon* treated as prepupae. We can conclude from these results that, the native strain is more effective against *A. ipsilon* especially when treated as prepupae. These results agree with Saleh (1995) who cleared the effect of *Heterorabditis spp* as biocontrol agent against the black cutworm *A. ipsilon* and cabbage worm *Pieris rapae* in bioassay testes and the concentrations of 5-100 nematodes/larva induced 30-100 and 55-100% larval mortality within 24 and 48 h. respectively . The respective mortalities caused by *Heterorhabditis sp*. SAA1 against apple bores, *Zeuzera pyrina* were 35.41 - 76.57 % when applied by spray at 1000 IJs/ml (Saleh and Abbas, 1998).These results also agree with Mogahed and El-kifl (1992) who reported the efficacy of two entomogenous nematode species against prepupal and pupal stages of the cotton leafworm, *S. littoralis* in soil under greenhouse conditions. Their results indicated that the nematode *H. heliothidis* was more effective than *H. bacteriophora*. Prepupal stage was more susceptible to infection with the two parasitic nematodes than the pupal stage.

The obtained data also agree with Bedding (1990) who cleared that the local strain of nematode are expected to be suitable for management of local insect pests because of their adaptation to local climate and population regulators.

The effect of *H. indicus*, native and imported strains, on average weight of pupae resulted from treated 6th instar larvae and prepupae of cutworm *A. ipsilon* reported in table (3). Significant differences $P > 0.05$) were observed in pupal weight resulted from treated 6th instar larvae and prepupae of *A. ipsilon* by different concentrations of *H. indica* (LN2 and SAA2 strains). No significant effect on pupal weight between two tested strains was obtained.

Data in table (4) demonstrated that, the pupation period of cutworm *A. ipsilon* treated as 6th instar larvae by imported nematode strain increased significantly (P > 0.01)at higher concentration. No significant differences (P > 0.05) in pupation period were found between different concentrations and untreated check when cutworm treated as prepupae. The pupation periods were decreased (10.0 ± 0.81 and 10.50 ± 2.04 days) at lower concentrations (5 and 10 IJs/larva) but increased (12.0±0.81 and 11.16±1.04 days) at higher concentrations (50 and 100 IJs/larva) when treated as 6th instar larvae by native nematode strain (SAA2). No significant difference (P > 0.05) were observed at pupation period for cutworm *A. ipsilon* treated as prepupae by native nematode strain (SAA2). The conclusion is there were significant difference between different concentrations on pupation periods for cutworm *A. ipsilon* when treated as 6th instar larvae by both nematodes (native and imported strains) but not significantly differed (P > 0.05) were noted at pupation periods between two nematode strains.

Percentages of adult emerged of cutworm *A. ipsilon* from 6th instar larvae and prepupae - treated nematodes decreased gradually by increasing the concentrations of tested nematode strains (LN2 and SAA2) treatment (tables, 5 and 6). The number of deposited eggs/female decreased in different concentrations compared with untreated check. The decrease number of deposited eggs after females infection by *H. indica* (LN2, SAA2) and the lower hatchability percent may be attributed to the effect of the nematodes and their symbiotic bacteria *P. luminescens* on the insect larvae and prepupae which resulted in reducing adult fertility and, of course, the lower fecundity compared to control. The haemolymph of *A. ipsilon* released anti-bacterially tic factors and the bacterially tic activities increased from 4 to 48 hr post-infection subsequently, the haemolymph became clear of bacteria (Abu El-Magd and El-kifl 1993). And they also showed clear changes in the relative numbers of circulating haemocytes following *H. heliothidis* infection. *A. ipsilon* showed decrease in prehaemocyte numbers, while granulocyte and coagulocyte numbers increased during the course of infection (the larvae did not die after that) and the dominant cell type was the coagulocyte. It was cleared that changes in the differential haemocyte counts may gave some clues to the function of different insect haemocyte population.

Table(1). The effect of *Heterorhabditis indicus*, foreign and local strains, on cutworm *Agrotis ipsilon* treated as 6th instar larvae.

Conc.	Nematode strain (LN2)				Nematode strain (SAAs)			
	L.M.% *	Prep.M %**	P.M.% ***	T.M.% ****	L.M.% *	Prep.M %**	P.M.% ***	T.M.% ****
0.0	0.0	0.0	12.0± 1.0	12.0± 1.0	0.0	0.0	12.66± 4.16	12.66± 4.16
5	4.33± 1.52	4.66± 1.69	11.67± 1.52	20.66± 5.03	12.33± 7.50	8.33± 6.50	4.67± 3.05	25.33± 17.03
10	3.66± 1.15	20.33± 2.51	19.66± 2.86	43.66± 7.02	16.66± 2.08	0.0	24.0± 11.0	40.66± 13.01
20	8.33± 1.52	19.67± 7.50	32.33± 3.51	60.33± 12.50	20.66± 3.05	24.67± 13.01	20.67± 5.03	66.00± 21.07
50	24.0± 1.0	12.0± .44	44.66± 12.01	80.66± 14.01	36.33± 6.50	12.33± 6.50	28.33± 16.50	77.0± 26.51
100	55.6± 3.05	16.33± 1.80	21.33± 11.05	93.26± 2.0	32.67± 4.04	40.66± 9.01	20.33± 7.50	93.66± 5.68
LSD								
0.05	2.82	3.16	4.81	5.62	2.69	7.41	8.09	9.03
0.01	2.92	4.61	6.84	7.98	3.91	11.23	11.50	12.83

* Larvae mortality ** Prepupae mortality *** pupae mortality **** Total mortality

Table (2). The effect of *Heterorhabditis indicus*, foreign and local strains, on cutworm *Agrotis ipsillon* treated as prepupae.

Conc.	Nematode strain (LN2)			Nematode strain (SAAs)		
	Prep.M.% **	P.M.% ***	T.M. ****	Prep.M.% **	P.M.% ***	T.M. ****
0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	4.33±2.05	16.33±8.50	20.66±11.01	4.0±0.81	4.66±2.49	8.66±4.04
10	12.0±1.63	24.33+3.68	36.33±6.50	8.33±6.50	16.0±1.63	24.33±6.94
20	20.66±2.49	36.66±1.69	57.32±5.13	8.66±0.94	44.33±1.24	52.99±2.64
50	16.66±1.69	46.66±4.09	81.32±6.11	36.33±2.05	44.66±8.02	80.998.01
100	92.33±1.52	0.0	98.33±1.521	74.0±12.16	20.33±2.05	94.33±9.81
LSD 0.05	1.45	3.8	4.50	8.07	3.04	9.01
LSD 0.01	2.11	5.82	6.55	11.74	4.43	13.1

= Prepupae mortality * pupae mortality **** Total mortality

Table (3). The effect of *Heterorhabditis indicus*, foreign and local strains, on average weight (mg) of pupae resulted from treated 6th instar and prepupae of cutworm *Agrotis ipsillon*.

Conc.	Nematode strain (LN2)		Nematode strain (SAAs)	
	larvae	Prepupae	larvae	prepupae
0.0	300.33±6.50	291.0±	291.33±3.51	293.33±1.52
5	356.33±3.51	405.33±11.0	292.66±2.08	302.33±3.51
10	410.66±2.08	412.0±7.50	298.66±7.02	362.0±2.64
20	472.33±2.08	426.0±1.0	345.66±6.65	355.33±2.52
50	370.33±2.51	450.0±4.0	295.33±5.03	375.33±5.51
100	280.0±5.0	---	282.0±2.51	---
LSD 0.05	1.73	8.13	2.51	1.75
LSD 0.01	2.47	11.82	3.58	2.55

Table (4). The effect of *Heterorhabditis indicus*, foreign and local strains, on the pupation period of cutworm *Agrotis ipsillon* treated as 6th instar larvae and prepupae.

Conc.	Nematode strain (LN2)		Nematode strain (SAAs)	
	6th instar larvae	prepupae	6th instar larvae	prepupae
0.0	11.0±0.81	11.5±1.47	12.0±1.41	11.5±1.47
5	11.33±1.63	11.5±2.2	10.0±0.81	11.33±1.08
10	13.0±1.0	11.33±0.47	10.50±2.04	11.0±0.81
20	13.0±1.63	11.66±2.05	12.0±2.16	11.33±2.16
50	13.50±1.08	11.66±1.69	12.0±0.81	11.0±2.44
100	13.33±2.44	11.33±1.69	11.16±1.04	11.5±0.40
LSD 0.05	0.93	0.89	0.91	0.78
LSD 0.01	1.33	1.2	1.29	1.1

Table (5). The effect of *Heterorhabditis indicus*, foreign and local strains, on adults emergence resulted from treated 6th instar of cutworm *Agrotis ipsillon*.

Conc.	Nematode strain (LN2)			Nematode strain (SAAs)		
	Adult Emergence. %	No. of Eggs/ ♀	Hatchability %	Adult Emergence . %	No. of Eggs/ ♀	Hatchability %
0.0	80.0±1.24	668.3±123.52	69.27	87.33±1.52	680.33±155.22	66.19
5	75.33±1.24	300.66±11.84	21.32	72.66±0.94	239.5±62.27	25.95
10	53.66±2.86	250.0±13.52	41.17	59.0±1.52	149.7.0±29.39	24.33
20	38.0±1.24	120.0±2.0	62.88	33.33±1.42	100.33±8.17	32.61
50	19.0±1.24	95.46±33.03	14.33	22.0±2.08	90.0±7.87	27.75
100	3.33±1.52	75.33±21.22	8.57	5.66±1.69	85.66±30.34	19.84
LSD 0.05 0.01	3.09 4.40	228.12 324.29		1.09 1.55	80.28 114.12	

Table (6). The effect of *Heterorhabditis indicus*, foreign and local strains, on adults emergence resulted from treated prepupae of cutworm *Agrotis ipsillon*.

Conc.	Nematode strain (LN2)			Nematode strain (SAAs)		
	Adult Emergence. %	No. of Eggs/ ♀	Hatchability %	Adult Emergence. %	No. of Eggs/ ♀	Hatchability %
0.0	99.66±0.57	671.33± 44.41	63.30	96.0±3.26	678.0± 108.59	70.10
5	78.66±1.69	245.0± 19.97	31.84	90.66±2.05	238.66± 12.09	36.21
10	62.0±2.0	212.0± 11.43	58.33	72.33±1.52	213.0± 2.64	43.53
20	41.33+1.52	108.6+ 3.39	50.0	45.00+1.0	112.66+ 4.02	29.39
50	17.33±1.24	97.0± 2.94	42.85	18.0±0.81	86.66± 3.68	45.04
100	0.0	0.0	0.0	0.0	0.0	0.0
LSD 0.05 0.01	18.24 26.54	27.76 40.38		1.47 2.15	67.89 98.75	

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كفاءة بعض سلالات من النيماتودا الممرضة للحشرات علي الدودة القارضة اجروتيس ابسيلون (الليليات- حرشفيات الأجنحة)

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تم تقييم سلالتين من نيماتودا هيتيرو رابديتيس انديكس الممرضة للحشرات إحداهما مستوردة والأخرى محلية كوسيلة مكافحة بيولوجية للدودة القارضة اجروتيس ابسيلون .وقد وجد أن كلا السلالتين كانتا ممرضتان في حالة معاملة العمر اليرقي الأخير وكذلك طور ما قبل العذراء، وكانت قيم الـ LC50في حالة العمر اليرقي الأخير المعامل ١٧ ، ١٩ فرد نيماتودا / يرقة لكل من السلالتين المحلية والمستوردة علي التوالي. بينما كانت قيم الـ LC50 في حالة معاملة طور ما قبل العذراء ١٣ ، ٢٠ فرد نيماتودا/ حشرة . وجدت فروق معنوية بين نسب الموت لمختلف التركيزات للسلالتين في حالة العمر اليرقي الأخير وكذلك طوري ما قبل العذراء والعذراء للحشرة، كما وجد فروق معنوية بالنسبة لوزن العذارى في حالة معاملة العمر اليرقي الأخير وما قبل العذراء بكلا السلالتين، بينما كان هناك زيادة معنوية في عمر العذاري للحشرة في حالة عدوي العمر اليرقي الأخير بالسلالة المستوردة في التركيزات العالية. وجدت فروق معنوية بين مختلف التركيزات والكنترول عند المعاملة بكلا السلالتين بالنسبة لخروج الفراشات وعدد البيض الموضوع للأنثى الواحدة ونسبة الفقس في البيض الموضوع في حين لا توجد معنوية بالنسبة للفرق بين تأثير السلالتين.