IMPACT OF ENTOMOPATHOGENIC NEMATODES ON DIFFERENT STAGES OF THE PUMPKIN FLY, *DACUS CILIATUS* (LOEW) AS A NEW APPROACH IN ITS BIOLOGICAL CONTROL

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ABSTRACT

The infectivity of two steinernematid entomopathogenic nematodes, namely *Steinernema carpocapsae* All and *Steinernema riobravae* (Weiser,1955) Poinar and one species of heterorhabdit nematode , *eterorhabditis bacteriophora* Poinar on the leaping larvae, pupae and adults of the pumpkin fly, *Dacus ciliatus* (Loew) (Diptera: Tephritidae) was evaluated. The obtained data demonstrated that *D. ciliatus* larvae were very susceptible to nematodes, especially for *S. riobravae* than *S. carpocapsae* or *H. bacteriophora.* The parasitism rate followed by death ranged between 9.93% to 93.33%. *D. ciliatus* pupae and adults in the contrary were susceptible to *H. bacteriophora* than the other two steinernematid nematodes *S. riofravae* and *S. carpocapsae*.The parasitism rate followed by death ranged from 6.33% to 90.00% and from 11.10% to 91.13% for pupae and adults, respectively.

Key words: *Dacus ciliatus,* Biological control, Nematodes.

INTRODUCTION

In Egypt, the pumpkin fly or the cucurbit fruit fly, *Dacus ciliatus* (Loew) was recorded for the first time by **Azab and Kira (1954)** as a serious pest on cucurbitaceous fruits, which had continued nearly till 1980 and then disappeared. However, it reappeared again after nearly 25 years, it, in most cases, causing damage attaining 100% loss in the yield **(Fetoh, 2003).** Control of this pest was mainly occurred by using chemical insecticides. Although, insecticides give noticeable success in the pest control, continuous use of such chemical materials contaminates the environment. Also, it has accumulating effect inducing pest resistance. Moreover, chemical control against insect stages in soil is very difficult or may be useless due to burrowing habits of the pest in soil. In addition, the pupae are enclosed by very hard chitinous puparium which protect them from the hazard of insecticides **(Zayed *et al.*, 1997).**

The present work is the first attempt to use and evaluate the entomopathogenic nematodes as biological control agents for controlling *D. ciliatus*, especially it pupate in soil. No reports have been found in the literature dealing with such point in Egypt or elsewhere.

MATERIALS AND METHODS

A culture of adult flies of *D. ciliatus* was maintained in the laboratory at 25±1°C and 65±5%RH; fed on sugar, protein hydrolyzate and water **(Vargas *et al.*, 1993).** Larvae were reared on small marrow fruits. Leaping larvae and pupae were obtained by sieving from sandy layer in the bottom of

rearing containers (10x15x30cm), covered with cotton fabric and tied with elastic threads.

Infective third stage juveniles (IJs) of two steinernematid nematodes species, (*Steinernema carpocapsae* (Sc) and *S. riobravae)* (Sr),and one species of heterorhabdit nematode *Heterorhabditis bacteriphora* (Hb) were used in this study. They were cultured on the last instar larvae of the wax moth, *Galleria mellonella* **(Dutky *et al*., 1964)** under laboratory conditions.

Application of nematodes on:

1- Leaping larvae and pupae:

1350 individuals of larvae or pupae of insect were used to evaluate the effect of nematodes. The tested individuals of each stage were divided into 15 groups, each had 3 replicates and each replicate had 30 individuals which were kept in small plastic cups (6x9x9 cm). Each cup contained 50 gm of clean dry sand soil at the bottom which was thoroughly moistened with 5ml distilled water. The tested nematodes were introduced at level 150, 300, 600, 1200 and 2400 IJs/cup i.e. 5, 10, 20, 40 and 80 IJs/ individual. In addition, chek treatment received distilled water only.

2- Adults:

Also, 1350 adult flies were used, and each concentration of the nematodes as mentioned before, was added to sugar solution in small glass vials (10 ml) containing cotton wick piece, used as a source of food and drink. Adult flies and the prepared glass vials were put in large glass pots (250 ml), covered with cotton fabric and then tied with elastic threads. Check treatment had only sugar solution.

After seven days all the cups were provided with clean and dry sand and also the vials with sugar solution free from nematodes. Daily inspection was carried out for two weeks for monitoring alive and dead insects. Dead individuals were dissected to detect nematode infection. Mortality resulted from nematode parasitism was calculated and corrected according to Abbott's formula **(Abbot, 1925)** log-probity lines for different concentrations of nematodes were determined according to **Finney (1971)** using a detected software program. Relative toxicity between different nematode species was determined using the same software. Duncan's multiple range test **(Duncan, 1955)** was used to differentiate between the means.

RESULTS AND DISCUSSION

The results indicated that all examined entomopathogenic nematode species successfully controlled leaping larvae, pupae and adults of *D. ciliatus*. Variations in LC_{50}, LC_{90} and mortality rates among treatments were pronounced. The obtained data in Tables (1) and (2) show that all nematode strains had highly significant effect on leaping larvae of *D. ciliatus*. Mortality was dose–dependent i.e. increasing nematodes was accompanied by increasing the parasitism rate and death. Based on the LC_{50} values and log – dose probity lines Fig. (1) the steinernematid *S. riobravae* was more virulent than *S. carpocapsae* and *H. bactriophora*, respectively. LC_{50} for Sr was 17.05, LC_{50} for Sc was 19. 68 and Lc_{50} for Hb, was 21.43, while LC_{90} for Sr was 85.82, Lc_{90} for Sc was 72.00, and LC_{90} for Hb was 123.83. Parasitism rate followed by death ranged between 9.93% and 93.33%. Furthermore, data in Table (3) and (4) and Fig. (2) indicate that *H. bacteriophora* had highly significant effect than *S. riobravae* or *S. carpocapsae* on pupae of *D.ciliatus.* LC_{50} for Hb was 17.0b3, LC_{50} for Sr was 21.27 and LC_{50} for Sc was 25.50;

while LC_{90} for Hb was 73.94, LC_{90} for Sr was 113.05 and for Sc was 160.90. The parasitism rate followed by death was ranged between 6.33% - 90.00%.

Table (1): Efficacy of three entomopathogenic nematode species in biological control of the leaping larvae of pumpkin fly *dacus ciliatus*.

Nematode species and Concentrations	%Parasitism rate followed by death				
	5IJs	10IJs	20IJs	40IJs	80IJs
Sr	12.23±2.3^{fg} (10.0-16.7)	36.67±2.7 ^{d} (33.3-40.0)	64.47±5.7^{c} (56.7-70.0)	66.67±7.2^{c} (56.7-73.3)	90.00±2.7^{ab} (86.7-93.3)
Sc	9.93±2.7^{g} (6.7-13.3)	24.43±4.2^{e} (20.0-30.0)	43.33±2.8^{d} (40.0-46.7)	85.57±4.2^{ab} (80.0-90.0)	93.33±2.7^{a} (90.0-96.7)
Hb	12.93±4.4^{etg} (6.7-16.7)	23.33±2.7^{et} (20.0-26.7)	65.57±4.2^{c} (60.0-70.0)	63.33±2.7^{c} (60.0-63.3)	78.87±4.1^{b} (73.3-83.3)

F value = 108.04*** , $L.S.D._{0.01}$ = 11.21
Numbers with the same letters are not significantly different.

Table (2): LC_{50} and LC_{90} of three entomopathogenic nematode as biological control agents for the leaping larvae of the pumpkin fly, *Dacus ciliatus*.

Namatode species	LC_{50}	LC_{90}	Folds	Index	Slope
Sr	17.052	85.82	1.257	100.00	1.826
Sc	19.677	72.002	1.089	86.66	2.275
Hb	21.426	123.829	1	79.59	1.667

Index compared with *Steinernema riobravae* and number of folds compared with *Heterorhabditis bacteriophora*.

Table (3): Efficacy of three entomopathogenic nematode species in biological control of the pupae of the pumpkin fly, *Dacus ciliatus*.

Nematode species and Concentrations	%Parasitism rate followed by death				
	5IJs	10IJs	20IJs	40Ijs	80Ijs
Hb	11.13±4.2^{h} (6.7-16.7)	36.67±2.7 ^{ef} (33.3-40.0)	60.00±2.6^{d} (56.7-63.3)	75.57±4.1^{b} (70.0-80.0)	90.00±2.6^{a} (8607-9303)
Sr	6.33±3.2 ^{h} (2.3-10.0)	25.57±4.2^{g} (20.0-30.0)	61.13±4.1^{d} (56.7-66.7)	71.13±4.1^{bc} (66.7-76.7)	76.67±2.7^{b} (73.3-80.0)
Sc	10.00±2.7^{h} (6.7-13.3)	28.90±1.6^{fg} (26.7-30.0)	44.43±4.2^{e} (40.0-50.0)	63.33±2.7^{cd} (60.0-66.7)	76.67±2.3^{b} (73.3-80.0)

F value = 143.38*** , $L.S.D._{0.01}$ = 9.06
Numbers with the same letters not significantly different.

Table (4): LC_{50} and LC_{90} of three of three entomopathogenic nematode species as biological control agents for the pupae of pumpkin fly, *Dacus ciliatus*.

Namatode species	LC_{50}	LC_{90}	Folds	Index	Slope
Hb	17.032	73.939	1.497	100.00	2.001
Sr	21.274	113.049	1.198	80.06	1.767
Sc	25.491	160.976	1	66.82	1.601

Index was compared with that of *Heterorhabditis bacteriophora* and number of folds were compared with that of *Steinernema carpocapsae.*

It is obvious from the presented data in Tables (5) and (6) that *D. ciliatus* adults were susceptible to the heterorhabdit nematode than those of the steinernematid nematodes. Parasitism rate followed by death ranged between 11.10% and 91.13%. Based on the LC_{50} values derived from log-dose probity lines (Fig. 3), the tested nematodes can be arranged descendingly according to their infectivity as: *H. bacteriaphora, S. riobravae* and finally *S. carpocapsae*. LC_{50} was 14.42 and LC_{90} was 64.86 for Hb, LC_{50} was 17.25 and LC_{90} was 98.73 for Sr and finally was 26.72 and 179.77 for Sc, respectively.

These results emphasized that the entomopathogenic nematodes can be used successfully in IPM program to control the pumpkin fly. This is in the same trend of **Gaugler and Kaya (1990)** who mentioned that entomopathogonic nematodes of genera *Heterorhabditis* and *Steinernema* (Nematoda: Rhabditide) have emerged as excellent insect biological control agents. Also, it agree with other attempts concerning the infectivity of entomopathogenic nematodes (EPN) on other dipterous flies like: house fly, *Musca domestica* **(Geden *et al*., 1986),** *Parasarcophage dux* and *Cephalonica titillator* infesting farm animals **(El-Sadawy, 1994),** stomach botfly, *Gasterophilus intestinalis* **(Zayed *et al*., 1997)** and the peach fruit fly, *Bactrocera zonata* **(Attala and Eweis, 2002).**

Table (5): Efficacy of three entomopathogenic nematode species in biological control of the adults of the pumpkin fly, *Dacus ciliatus*.

Nematode species and Concentrations	%Parasitism rate followed by death				
	5IJs	10IJs	20IJs	40IJs	80IJs
Hb	18.87 ± 4.2^{hi} (13.3-23.3)	33.33 ± 2.3^{g} (30-36.7)	64.43 ± 4.1^{e} (60.0-70.0)	84.43 ± 4.2^{ab} (60.0-70.0)	91.13 ± 4.6^{a} (86.7-96.7)
Sr	13.33 ± 2.3^{i} (10.0-16.7)	30.00 ± 2.6^{g} (26.7-33.3)	71.13 ± 4.1^{de} (66.7-76.7)	74.43 ± 4.2^{cd} (70.0-80.0)	80.00 ± 2.7^{bc} (76.7-83.3)
Sc	11.10 ± 1.5^{i} (10.0-13.3)	26.67 ± 2.7^{gh} (23.3-30.0)	43.33 ± 2.3^{f} (40.0-46.7)	63.33 ± 2.7^{e} (60.0-66.7)	74.43 ± 4.1^{cd} (70.0-80.0)

F value = 173.90 *** , $L.S.D._{0.01} = 8.25$

Number with the same letters are not significantly different.

Table (6): LC_{50} and LC_{90} of three entomopathogenic nematode species as biological control agents for the adults of the pumpkin fly, *Dacus ciliatus*.

Namatode species	LC_{50}	LC_{90}	Folds	Index	Slope
Hb	14.417	64.859	1.853	100.00	1.962
Sr	17.253	98.725	1.548	83.562	1.692
Sc	26.716	179.772	1	53.964	1.548

Index was compared with that of *Heterorhabditis bacteriophora* and number of folds were compared with that of *Steinernema carpocapsae.*

Fig. 1

Fig. 2,3

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"تأثير النيماتودا الممرضة للحشرات كوسيلة للمكافحة البيولوجية للأطوار المختلفة من ذبابة المقات "

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تم دراسة فاعلية نوعين من جنس ستشنير نيما ونوع من جنس هيتيروربتيتديس من النيماتودا الممرضة للحشرات لأول مرة بنجاح علي الأطوار المختلفة من ذبابة المقات (اليرقات القافزة والعذارى والأطوار الكاملة)، وقد أظهرت النتائج المتحصل عليها أن اليرقات كانت أكثر حساسية لجنس ستشنير نيما عن جنس هيتيروربتيتديس وكانت نسبة تطفل النيماتودا المتبوعة بموت بين ٩.٩٣% و٩٣.٣٣%، أما كلا من العذارى والأفراد الكاملة فكانت أكثر تأثرا بجنس

هيتيروربتيتديس عن جنس ستشنير نيما، وكانت نسبة التطفل المتبوعة بموت بين ٦.٣٣% و٩٠.٠٠% للعذارى، وبين ١١.١٠% و٩١.١٣% للأفراد الكاملة علي التوالي.

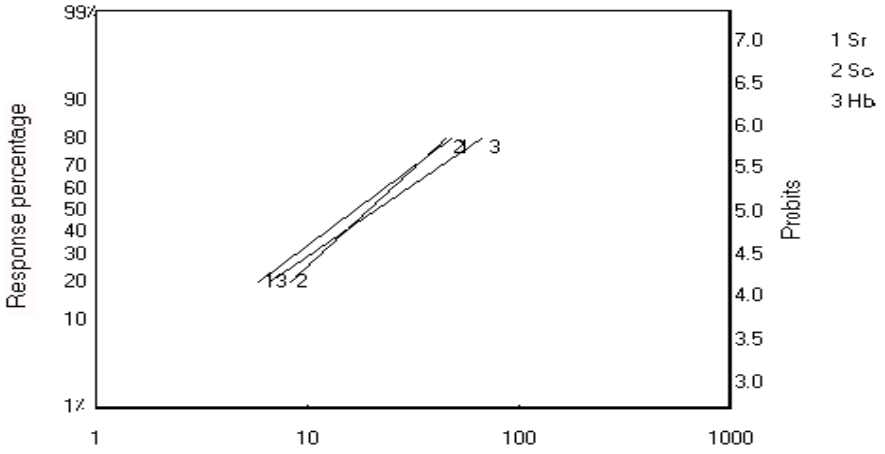

Fig (1): Log-Probity curve of predict percent of entomopathogenic nematode species on larvae of *Dacus ciliatus*.

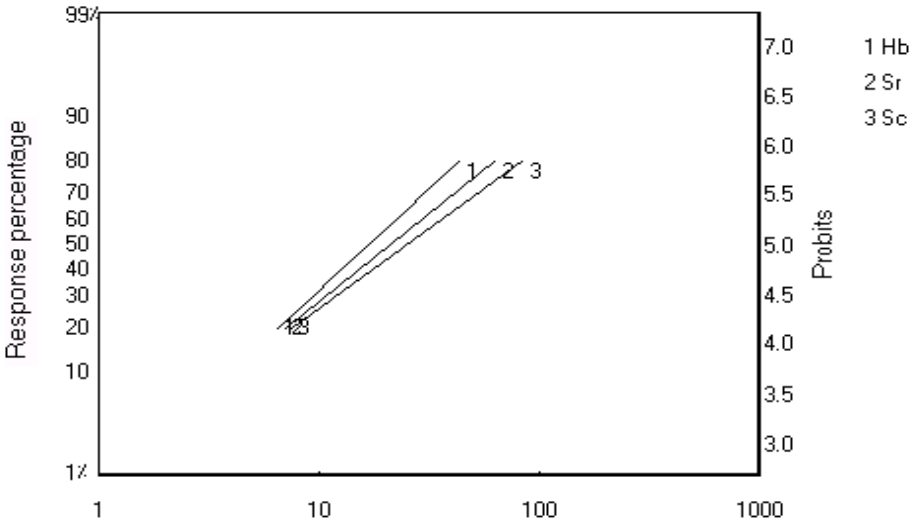

Fig (2): Log-Probity curve of predict percent entomopathogenic nematode species on pupae of *Dacus ciliatus*.

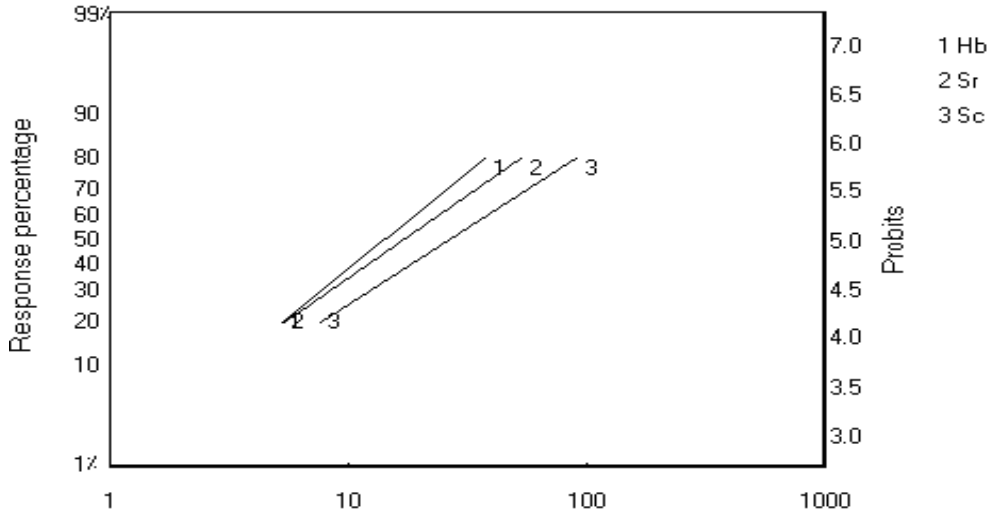

Fig (3): Log-Probity Curve of predict percent entomopathogenic nematode species on adu lts of *Dacus ciliatus.*