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Efficacy of Entomopathogenic nematodes on some Lepidopteran larvae

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ABSTRACT

Experiments were conducted at the Biological Control Laboratory of the Economic Entomology and Agricultural Zoology Department, Faculty of Agriculture, Menoufia University, Shebin Elkom, Egypt to determine the productivity and effect of entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on some instar larvae of greater wax moth, *Galleria mellonella*, cotton leafworm, *Spodoptera littoralis* and black cutworm, *Agrotis ipsilon* under laboratory conditions. As for *G. mellonella* larvae *the* grand mean average revealed that the highest mortality was recorded with the concentration of 1600 IJs (68.8%), while the lowest mortality percentages was registered with the concentration of 50 IJs (33.0%) of *S. carpocapsae*, and the highest mortality was recorded with 1600 IJs (94.9%), while the lowest mortality was registered with 50 IJs (58.8%) of *H. bacteriophora*. As for *S. littoralis* larvae, the highest mortality was recorded with the concentration of 1600 IJs (67.7%), while the lowest was registered with the concentration of 50 IJs (33.0%) for *S. carpocapsae* , while the highest mortality was recorded with 1600 IJs (95.6%) , and lowest was registered with the concentration of 50 IJs (58.8%) for *H. bacteriophora*. As for *Agrotis ipsilon* larvae, the highest mortality was recorded with the concentration of 1600 IJs (63.8%), and the lowest mortality percentages was registered with the concentration of 50 IJs (15.4%) for *S. carpocapsae*, while the highest mortality percentages was recorded with the concentration of 1600 IJs (77.8%) , and the lowest mortality was registered with the concentration of 50 IJs (43.02%) for *H. bacteriophora*. It could be concluded that the use of entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in the control of lepidopteran larvae i.e., the greater wax moth, *Galleria mellonella,* cotton leafworm, *Spodoptera littoralis* and black cutworm, *Agrotis ipsilon* registered good results, but it needs more studies.

Key words: Entomopathogenic Nematodes, Greater Wax Moth, Cotton Leafworm, Black Cutworm, Biological Control.

INTRODUCTION

In recent years it has seen an increased attention for non-chemical methods of insect control protection, including biological control of field pests (Arbogast, 1984, Brower *et al*., 1996, Schoeller *et al*.,1997, Adler, 1998, Cox &

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Wilkin, 1998, Schoeller, 1998). Entomopathogenic nematodes have not been previously tested against stored-product insects in environments such as empty grain bins or food processing and warehouse facilities, but their effectiveness at finding and infecting hosts in other cryptic habitats has been demonstrated. The use of entomopathogenic nematodes in the control of some economic field insects is a new field. Recently, there are a few articles were published in this direction i.e. Ramos-Rodriguez, *et al.* (2007) who reported that persistence of stored-product insects in hidden refuge and their subsequent movement into stored commodities resulting in product infestation contributes to their pest status and represents a potential target for biological control agents. The effect of three insecticides commonly used in Arizona, Navarro, *et al.* (2014), dinotefuran, indoxacarb, and imidacloprid, was evaluated on two Arizona-native entmopathogenic nematodes (EPN), *Heterorhabditis sonorensis* (Caborca strain) and *Steinernema riobrave* (SR-5 strain), using *Helicoverpa zea* (Lepidoptera: Noctuidae) as the insect host, and assessed their effect on EPN survival and fitness (virulence and reproduction). The results showed that infective juvenile (IJ) survival of *S. riobrave* and *H. sonorensis* was not significantly affected by the application of the selected insecticides. Indoxacarb had an ambiguous effect on the *S. riobrave* life cycle showing a synergistic effect in the virulence of this nematode but reducing its progeny production by two-fold. Similar results were observed for nematode progeny production when *H. sonorensis* and indoxacarb were applied simultaneously. All combinations of imidacloprid were antagonistic to the virulence of *S. riobrave* but additive with respect to the virulence of *H. sonorensis*, Navarro, *et al.* (2014). In laboratory bioassays, *Steinernema riobrave* reduced survival of red flour beetle, *Tribolium castaneum*, larvae, pupae and adults from 77.9 ± 3.2% in the controls to 27.4 ± 2.5% in treatments. Shahina and Salma (2009) tested seven Pakistani strains of entomopathogenic nematodes belonging to the genera *Steinernema* and *Heterorhabditis* against last instar larval and adult stages of the pulse beetle, *Callosobruchus chinensis*. Athanassiou, *et al*. (2010) examined the insecticidal effect of *Heterorhabditis bacteriophora* Poinar, *Steinernema carpocapsae* (Weiser), and *Steinernema feltiae* (Filipjev) against Mediterranean flour moth, *Ephestia kuehniella* (Zeller) larvae, lesser grain borer, *Rhyzopertha dominica* adults, rice weevil, *Sitophilus oryzae* adults, and confused flour beetle, *Tribolium confusum* adults and larvae under laboratory conditions in wheat, *Triticum aestivum*. Laznik, and Trdan, (2010) tested the efficacy of three strains (B30, B49 in 3162) of *Steinernema feltiae* to control adults of rice weevil, *Sitophilus oryzae* Shahina, and Salma (2010) tested the virulence of 7 indigenous entomophilic nematodes viz., *Steinernema pakistanense* (Ham 10 strain), *S. asiaticum* (211 strain), *S. abbasi* (507 strain), *S. siamkayai* (157 strain), *S. feltiae* (A05 strains), *Heterorhabditis bacteriophora* (1743 strain) and *H. indica* (HAM-64 strain) against the adult and pupa of rice weevils, *S. oryzae* in laboratory bioassays. Shrestha and Gyun (2010) reported that the two entomo-pathogenic bacteria, *Photo-rhabdus temperata* sub sp. *temperata* (Ptt) and *Xenorhabdus nematophila* (Xn), are symbiotically associated with the nematodes, *H. megidis* and *Steinernema carpocapsae*, respectively, and found that a significant difference in pathogenicity was observed between these two bacteria against the red flour beetle, *Tribolium castaneum*, in which *P. temperata* sub sp. *temperata* exhibited more than six times higher pathogenicity than *Xenorhabdus nematophila*. In Egypt Sweelam *et al*., (2010) controlled red palm weevil, *Rhynchophorus ferrugineus* Oliver by entomopathogenic nematode species.

From these points of view, this research was conducted to throw a light on the possibility of using entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in the control of some lepidopteron pests.

MATERIALS AND METHODS

Experiments were conducted at the laboratories of the Economic Entomology and Agricultural Zoology Department, Faculty of Agriculture, Menoufia University, Shebin Elkom, Egypt.

Rearing of entomopathogenic nematodes:

Two species of entomopathogenic nematodes: ***Heterorhabditis bacteriophora*** Poinar (Nematoda: Heterorhabditidae), and ***Steinernema carpocapsae*** (Filipjev) (Nematoda: Steinernematidae) were extracted from the soil of the mango trees of the Experimental Station of the Faculty of Agriculture Shebin El-Kom, Minoufiya University. The greater wax moth, ***Galleria mellonella*** were used for culturing of both entomopathogenic nematodes. They starved for 2 hours before being infect with nematodes. Modified white traps (White, 1927) were used in large numbers to obtain sufficient numbers of nematodes for the experiments. Collected nematodes were stored in plastic tubes (50 ml) in a refrigerator adjusted to 10 °C temperature until used.

Tested insects:

1- **Greater wax moth, *Galleria mellonella***
2- **Cotton leafworm, *Spodoptera littoralis***
3- **Black cutworm, *Agrotis ipsilon***

The tested larval insect species were friendly obtained from Syngenta Agro Egypt S.A.E.

Application of nematodes on the stored insects:

Second instar larvae of insects were subjected to different concentrations of

50, 100, 500, 1000 and 2000 IJs (Infective juveniles) of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* nematodes to determine their effects against tested insects.

Ten insects were kept in Petri dishes, each 5-cm diameter containing 2 moist filter papers where insects were put between them and exposed to entomopathogenic nematodes. Every nematode concentration was sprayed on the insects as 5 ml distilled water containing nematodes. At control treatment, insects were sprayed with 5 ml distilled water without nematodes. Each treatment was replicated three times. Mortality was checked after 24, 48, 72, 96 hours for all concentrations of the two tested nematode species, and percentage of mortality was calculated for each nematode species at different concentrations using Abbott's formula (1925). Corrected mortality percentage was corrected by Schneider-Orelli's formula (Püntener W., 1981).

$$\textbf{Corrected \%} = \left(\frac{\textbf{M. \% in treated – M. \% in control}}{\textbf{100 – M. \% in control}} \right) * 100$$

M. = mortality

The obtained data were subjected to analysis of variance (ANOVA) one way direction with LSD 5% using CoStat Software, Version 6.4 (2008).

RESULTS

1- Efficacy of third immature stages of entomopathogenic nematode, *Steinernema carpocapsae* on greater wax moth, *Galleria mellonella* larvae under laboratory conditions:

The obtained data in Table (1) show the susceptibility of *G. mellonella* 4th , 5th and 6th instar larvae to the infection with the nematode *S. carpocapsae*, 24, 48 and 72 h after the inoculation with the concentrations of 50, 100, 200, 400, 800, and 1600 IJs/10 larvae / dish under laboratory conditions of 21 ± 4 °C and 72± 5 RH%.

As for 4th instar larvae of *G. mellonella*, the results indicated that the average mortality percentages after 3 days of treatment were: 45.8, 46.6 ,51.2 , 62.2, 66.6 and 70.7% at the concentrations of 50, 100, 200, 400, 800, and 1600 *S. carpocapsae* IJs /dish, respectively.

As for 5th instar larvae of *G. mellonella*, the results indicated that the average mortality percentages after 3 days of treatment were: 29.4,41.4 ,51.8 , 62.9, 67.4 and 67.4% at the concentrations of 50, 100, 200, 400, 800, and 1600 *S. carpocapsae* IJs /dish, respectively

Regarding to 6th instar larvae of *G. mellonella*, the results indicated that the average mortality percentages after 3 days of treatment were: 23.8, 36.4, 37.4, 58.6, 62.8, 68.4% at the concentrations of 50, 100, 200, 400, 800, and 1600 *S. carpocapsae* IJs /dish, respectively.

The grand mean average revealed that the highest mortality percentages was recorded with the concentration of 1600 IJs (68.8), while the lowest mortality percentages was registered with the concentration of 50 IJs (33.0%).

Table (1): Efficacy of 6 concentrations of entomopathogenic nematode, *S. carpocapsae* against larval instars of *G. mellonella* under laboratory conditions.

larvae Instars of	Post-exposure times	Mean mortality % of larval instars at six concentrations after 3 post-exposure times					
		Nematode concentrations IJs/ 10 larva/dish					
		50	**100**	**200**	**400**	**800**	**1600**
4th instar	24 h	02.2	11.2	15.6	17.8	16.8	17.8
	48 h	60.0	46.6	57.8	73.4	93.4	94.4
	72 h	75.6	82.2	80.0	95. 4	100.0	100.0
	Average	45.8	46.6	51.2	62.2	66.6	70.7
5th instar	24 h	02.2	06.6	17.8	26.6	30.2	34.8
	48 h	27.9	42.2	55.6	64.44	72.1	67.44
	72 h	58.2	75.6	82.2	97.8	100.0	100.0
	Average	29.4	41.4	51.8	62.9	67.4	67.4
6th instar	24 h	04.8	06.6	09.6	24.4	35.8	42.8
	48 h	21.4	35.4	38.2	60.0	52.6	61.8
	72 h	45.2	66.6	64.2	91.2	100.0	100.0
	Average	23.8	36.4	37.4	58.6	62.8	68.4
Grand mean average		33.0 c	41.4 b	46.8 b	61.2 a	65.6 a	68.8 a
LSD 5%		7.1					

Duncan's multiple-range test have no significant differences across means with the same letter at $p<0.05$.

2- Efficacy of third immature stages of entomopathogenic nematode, *Heterorhabditis bacteriophora* on greater wax moth, *Galleria mellonella* larvae under laboratory conditions:

The obtained data in Table (2) show the susceptibility of *G. mellonella* 4th, 5th and 6th instar larvae to infection with the nematode *H. bacteriophora*, 24, 48 and 72 h after the inoculation with the concentrations of 50, 100, 200, 400, 800, and 1600 IJs/10 larvae/ dish under laboratory conditions of 21 ± 4 °C and 72± 5 RH%.

As for 4th instar larvae of *G. mellonella*, the results indicated that the average mortality percentages after 3 days of treatment were: 63.8, 67.4, 77.4, 96.4, 100.0 and 100.0 % at the concentrations of 50, 100, 200, 400, 800, and 1600 *H. bacteriophora* IJs /dish, respectively

As for 5th instar larvae of *G. mellonella*, the results indicated that the average mortality percentages after 3 days of treatment were: 65.8, 77.4, 90.6, 100.0, 100.0 and 100.0 % at the concentrations of 50, 100, 200, 400, 800, and 1600 *H. bacteriophora* IJs /dish, respectively.

Regarding to 6th instar larvae of *G. mellonella*, the results indicated that the average mortality percentages after 3 days of treatment were: 46.8, 44.4, 63.0, 70.4, 77.7 and 84.6% at the concentrations of 50, 100, 200, 400, 800, and 1600 *H. bacteriophora* IJs /dish, respectively

The grand mean average revealed that the highest mortality percentages was recorded with the concentration of 1600 IJs (94.9%), while the lowest mortality percentages was registered with the concentration of 50 IJs (58.8%).

Table (2) Efficacy of different concentrations of the entomopathogenic nematode, *H. bacteriophora* against *G. mellonella* larvae under laboratory conditions

Instars of larvae	Post-exposure times	Mean mortality % of larval instars at six concentrations after 3 post-exposure times					
		Nematode concentrations IJs/ 10 larva/dish					
		50	**100**	**200**	**400**	**800**	**1600**
4th instar	24 h	17.8	29.2	60.0	89.0	100.0	100.0
	48 h	73.4	73.4	71.2	100.0	100.0	100.0
	72 h	100.0	100.0	100.0	100.0	100.0	100.0
	Average	63.8	67.4	77. 4	96.4	100.0	100.0
5th instar	24 h	20.0	31.8	70. 6	100.0	100.0	100.0
	48 h	77.8	100.0	100.0	100.0	100.0	100.0
	72 h	97.8	100.0	100.0	100.0	100.0	100.0
	Average	65. 8	77.4	90.6	100.0	100.0	100.0
6th instar	24 h	22.4	13.4	22.4	24.6	48.8	53.8
	48 h	48.8	35.6	66.6	86.8	84.4	100.0
	72 h	69.0	84.4	100.0	100.0	100.0	100.0
	Average	46.8	44.4	63.0	70.4	77.7	84.6
Grand mean average		58.8 d	63.0 d	77.0 c	88.9 b	92.6 ab	94.9 a
LSD 5%		7.2					

Duncan's multiple-range test have no significant differences across means with the same letter at p<0.05.

3- Efficacy of third immature stages of entomopathogenic nematode, *Steinernema carpocapsae* on cotton leafworm, *Spodoptera littoralis* larvae under laboratory conditions:

The obtained data in Table (3) show the susceptibility of *S. littoralis* 4th , 5th and 6th instar larvae to the infection with IJs *S. carpocapsae*, 24, 48 and 72 h after the inoculation with the concentrations of 50, 100, 200, 400, 800, and 1600 IJs/10 larvae/ dish under laboratory conditions of 21 ± 4 °C and 72± 5 RH%.

As for 4th instar larvae of *S. littoralis*, the results indicated that the average mortality percentages after 3 days of treatment were: 46.0, 46.6, 51.2, 62.2, 66.8 and 67.4% at the concentrations of 50, 100, 200, 400, 800, and 1600 *S. carpocapsae* IJs /dish, respectively.

As for 5th instar larvae of *S. littoralis*, the results indicated that the average mortality percentages after 3 days of treatment were: 29.4, 41.4, 51.8, 63.0, 67.4 and 67.4% at the concentrations of 50, 100, 200, 400, 800, and 1600 *S. carpocapsae* IJs /dish, respectively

Regarding to 6th instar larvae of *S. littoralis*, the results indicated that the average mortality percentages after 3 days of treatment were: 23.8 ,36.4, 37.4, 58.6, 62.8 and 68.4 % at the concentrations of 50, 100, 200, 400, 800, and 1600 *S. carpocapsae* IJs /dish, respectively

The grand mean average revealed that, the highest mortality percentages was recorded with the concentration of 1600 IJs (67.7%), while the lowest mortality percentages was registered with the concentration of 50 IJs (33.0%).

Table (3) Efficacy of different concentrations of the entomopathogenic nematode, *S. carpocapsae* against larval instars of *S. littoralis* under laboratory conditions.

Instars of larvae	Post-exposure times	Mean mortality % of larval instars at six concentrations after 3 post-exposure times					
		Nematode concentrations IJs/ 10 larva/dish					
		50	**100**	**200**	**400**	**800**	**1600**
4th instar	24 h	02.2	11.2	15.6	17.8	06.6	17.8
	48 h	60.0	46.8	57.8	73.4	93.4	84.4
	72 h	75.6	82.2	80.0	95.6	100.0	100.0
	Average	46.0	46.6	51.2	62.2	66.8	67.4
5th instar	24 h	2.4	06.8	17.8	26.8	30.4	35.0
	48 h	28.0	42.2	55.6	64.6	72.2	67.4
	72 h	58.2	75.6	82.2	97.8	100.0	100.0
	Average	29.4	41.4	51.8	63.0	67.4	67.4
6th instar	24 h	04.8	06.8	09.6	24.4	35.8	42.8
	48 h	21.4	35.6	38.2	60.0	52. 6	62.0
	72 h	45.2	66.8	64.4	91.2	100.0	100.0
	Average	23.8	36.4	37.4	58.6	62.8	68.4
Grand mean average		33.0 c	41.4 bc	46.8 b	61.2 a	65.6 a	67.7 a
LSD 5%		8.3					

Duncan's multiple-range test have no significant differences across means with the same letter at $p<0.05$

4- Efficacy of third immature stages of entomopathogenic nematode, *Heterorhabditis bacteriophora* on cotton leafworm, *Spodoptera littoralis* larvae under laboratory conditions:

The obtained data in Table (4) show the susceptibility of *S. littoralis* 4th , 5th and 6th instar larvae to the infection with IJs *H. bacteriophora*, 24, 48 and 72 h after the inoculation with the concentrations of 50, 100, 200, 400, 800, and 1600 IJs/10 larvae/ dish under laboratory conditions of 21 ± 4 °C and 72± 5 RH%.

As for 4th instar larvae of *S. littoralis*, the results indicated that the average mortality percentages after 3 days of treatment were: 63.8, 67.4, 77.4, 96.6, 100.0 and 100.0 % at the concentrations of 50, 100, 200, 400, 800, and 1600 *H. bacteriophora* IJs /dish, respectively. As for 5th instar larvae of *S. littoralis*, the results indicated that the average mortality percentages after 3 days of treatment were: 65.8,77.4 ,90.2, 100.0, 100.0 and 100.0 % at the concentrations of 50, 100, 200, 400, 800, and 1600 *H. bacteriophora* IJs /dish, respectively

Regarding to 6th instar larvae of *S. littoralis*, the results indicated that the average mortality percentages after 3 days of treatment were: 46.8 ,44.6, 63.0, 70.4, 84.0 and 86.9% at the concentrations of 50, 100, 200, 400, 800, and 1600 *H. bacteriophora* IJs /dish, respectively

The grand mean average revealed that the highest mortality percentages were recorded with the concentration of 1600 IJs (95.6%), while the lowest mortality percentages was registered with the concentration of 50 IJs (58.8%).

Table (4): Efficacy of different concentrations of the entomopathogenic nematode, *H. bacteriophora* against *S. littoralis* larvae under laboratory conditions.

Instars of larvae	Post-exposure times	Mean mortality % of larval instars at six concentrations after 3 post-exposure times					
		Nematode concentrations IJs/ 10 larva/dish					
		50	**100**	**200**	**400**	**800**	**1600**
4th instar	24 h	17. 8	29.0	60.0	88.8	100.0	100.0
	48 h	73.4	73.4	71.2	100.0	100.0	100.0
	72 h	100.0	100.0	100.0	100.0	100.0	100.0
	Average	63.8	67.4	77. 4	96.6	100.0	100.0
5th instar	24 h	20.0	31.8	70. 6	100.0	100.0	100.0
	48 h	77.8	100.0	100.0	100.0	100.0	100.0
	72 h	97.8	100.0	100.0	100.0	100.0	100.0
	Average	65. 8	77.4	90.2	100.0	100.0	100.0
6th instar	24 h	22. 2	13.4	22.2	24.4	49.0	60.8
	48 h	48.8	35.6	66.8	86.6	100.0	100.0
	72 h	69.0	84.6	100.0	100.0	100.0	100.0
	Average	46.8	44.6	63.0	70.4	84.0	86.9
Grand mean average		58.8 c	63.1 c	76.8 b	89.0 a	94.6 a	95.6 a
LSD 5%		8.6					

Duncan's multiple-range test have no significant differences across means with the same letter at p<0.05.

5- Efficacy of third immature stages of entomopathogenic nematode, *Steinernema carpocapsae* on black cutworm, *Agrotis ipsilon* larvae under laboratory conditions:

The obtained data in Table (5) show the susceptibility of *A. ipsilon* 4th , 5th and 6th instar larvae to the infection with IJs *S. carpocapsae*, 24, 48 and 72 h after the inoculation with the concentrations of 50, 100, 200, 400, 800 and 1600 IJs/10 larvae / dish under laboratory conditions of 21 ± 4 °C and 72± 5 RH%.

As for 4th instar larvae of *A. ipsilon,* the results indicated that the average mortality percentages after 3 days of treatment were: 28.2, 54.0, 51.8, 69.6, 77.8 and 88.8% at the concentrations of 50, 100, 200, 400, 800 and 1600 *S. carpocapsae* IJs /dish, respectively

As for 5th instar larvae of *A. ipsilon*, the results indicated that the average mortality percentages after 3 days of treatment were : 3.2,17.8 ,30.4, 31.2, 45.8 and 51.6% at the concentrations of 50, 100, 200, 400, 800 and 1600 *S. carpocapsae* IJs /dish, respectively

Regarding to 6th instar larvae of *A. ipsilon*, the results indicated that the average mortality percentages after 3 days of treatment were: 14.8, 17.2, 28.2, 33.4, 27.2 and 51.2% at the concentrations of 50, 100, 200, 400, 800 and 1600 *S. carpocapsae* IJs /dish, respectively

The grand mean average revealed that, the highest mortality percentages was recorded with the concentration of 1600 IJs (63.8%), while the lowest mortality percentages was registered with the concentration of 50 IJs (15.4%).

Table (5) Efficacy of different concentrations of the entomopathogenic nematode, *S. carpocapsae* against larval instars of *A. ipsilon* under laboratory conditions.

Instars of larvae	Post-exposure times	Mean mortality % of larval instars at six concentrations after 3 post-exposure times					
		Nematode concentrations IJs/ 10 larva/dish					
		50	**100**	**200**	**400**	**800**	**1600**
4th instar	24 h	04.6	22.2	28.8	37.8	47.8	71.2
	48 h	33.3	66.7	44.4	75.6	95.6	95.6
	72 h	46.7	73.3	82.2	95.6	100	100.0
	Average	28.2	54.0	51.8	69.6	77.8	88.8
5th instar	24 h	00. 0	00. 0	00. 0	02.2	02.8	11.4
	48 h	02.2	20.0	37.8	35.6	58.2	61.4
	72 h	06.6	33.4	53.4	55.6	76.8	81.8
	Average	03.2	17.8	30.4	31.2	45.8	51.6
6th instar	24 h	00.0	00.0	00.0	06.8	16.4	20.4
	48 h	17.8	22.2	35.6	37.8	25.8	58.4
	72 h	26.6	28.8	48.8	55.6	41.8	81.6
	Average	14.8	17.2	28.2	33.4	27.2	51.2
Grand mean average		15.4 e	29.6 d	36.8 c	44.7 b	50.2 b	63.8 a
LSD 5%		6.4					

Duncan's multiple-range test have no significant differences across means with the same letter at p<0.05.

6- Efficacy of third immature stages of entomopathogenic nematode, *Heterorhabditis bacteriophora* on black cutworm, *Agrotis ipsilon* larvae under laboratory conditions:

The obtained data in Table (6) show the susceptibility of *A. ipsilon* 4th , 5th and 6th instar larvae to the infection with IJs *H. bacteriophora*, 24, 48 and 72 h after the inoculation with the concentrations of 50, 100, 200, 400, 800 and 1600 IJs/10 larvae/ dish under laboratory conditions of 21 ± 4 °C and 72± 5 RH%.

As for 4th instar larvae of *A. ipsilon,* the results indicated that the average mortality percentages after 3 days of treatment were: 55.6, 52.4, 64.6, 63.8, 70.4 and 74.4% at the concentrations of 50, 100, 200, 400, 800 and 1600 *H.* bacteriophora IJs /dish, respectively. As for 5th instar larvae of *A. ipsilon*, the results indicated that the average mortality percentages after 3 days of treatment were: 31.8, 47.6, 60.6, 63.8, 79.8 and 81.4 % at the concentrations of 50, 100, 200, 400, 800, and 1600 *H. bacteriophora* IJs /dish, respectively.

Regarding to 6th instar larvae of *A. ipsilon*, the results indicated that the average mortality percentages after 3 days of treatment were: 41.7 ,43.2, 51.8, 61.4, 70.4 and 77.8 % at the concentrations of 50, 100, 200, 400, 800and 1600 *H. bacteriophora* IJs /dish, respectively

The grand mean average revealed that, the highest mortality percentages was recorded with the concentration of 1600 IJs (77.8%), while the lowest mortality percentages was registered with the concentration of 50 IJs (43.02%).

Table (6) Efficacy of different concentrations of the entomopathogenic nematode, *H. bacteriophora* against *A. ipsilon* larvae under laboratory conditions.

		Mean mortality % of larval instars at six concentrations after 3 post-exposure times					
Instars of larvae	Post-exposure times	Nematode concentrations IJs/10 larva/dish					
		50	**100**	**200**	**400**	**800**	**1600**
4th instar	24 h	09.0	18.2	28.8	17.8	26.2	35.6
	48 h	73.4	47.8	64.4	73.4	95.4	86.8
	72 h	84.4	90.8	100.0	100.0	100.0	100.0
	Average	55.6	52.4	64.6	63.8	70.4	74.4
5th instar	24 h	15.6	6.8	27.4	26.8	58.2	44. 8
	48 h	31.2	52.2	54. 4	64. 4	81.8	100.0
	72 h	48.8	84.0	100.0	100.0	100.0	100.0
	Average	31.8	47.6	60.6	63.8	79.8	81.4
6th instar	24 h	16.2	13.4	17.8	24.4	46.6	42.8
	48 h	40.8	35.6	40.0	60.0	64.4	90.4
	72 h	68.4	80.2	97.8	100.0	100.0	100.0
	Average	41.7	43.2	51.8	61.4	70.4	77.8
Grand mean average		43.02 c	47.7 c	59.0 b	63.0 b	73.5 a	77.8 a
LSD 5%		8.5					

Duncan's multiple-range test have no significant differences across means with the same letter at $p<0.05$.

DISCUSSION

The obtained results are in harmony with those obtained by Ramos-Rodriguez, *et al*., (2007), Shahina Fayyaz and Salma Javed (2009), Athanassiou, *et al*., (2010), Shahina, and Salma (2010) and Shrestha and Gyun (2010) who used *Heterorhabditis bacteriophora* and *Steinernema feltiae* in the control of the rice weevil, *Sitophilus oryzae* , the red flour beetle, *Tribolium castaneum*, the lesser grain borer, *Rhyzopertha dominica*, the Mediterranean flour moth, *Ephestia kuehniella* and the pulse beetle, *Callosobruchus chinensis* (L.).

Recently, Hassan *et al*. (2020) evaluate the efficacy of the Entomopathogenic nematode EPNs against the larvae of Egyptian cotton leaf worm *Spodoptera littoralis* (Boisduval) and the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) in vitro before in vivo study. The susceptibility of both larval species to the entomopathogenic nematode species, *Steinernema monticolum* and *Heterorhabditis bacteriophora*, was evaluated under laboratory conditions. Yağci *et al*. (2021) reported that, the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) is an important pest of apple in Turkey and other apple producing countries in the world. They reported that Entomopathogenic nematode (EPNs), for example, can be used as a potential alternative to chemical insecticides to control codling moth larvae in the soil as eco-friendly management their hosts that can actively find them in cryptic locations. Bingjiao Sun *et al.* (2021) In the present study, a survey of EPNs using the *Galleria*-baiting technique was conducted in 2017 and 2018 throughout the entire Yunnan province. In total, 789 soil samples were collected from 232 sites, of which 75 samples were positive for EPNs. Tarique and Abd-Elgawad (2021) reported the complex including entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* and their mutualistic partner, i.e., *Xenorhabdus* and *Photorhabdus* bacteria, respectively possesses many attributes of ideal biological control agents against numerous insect pests as a third partner. Asiye Uzun *et al.,* (2021) tested the virulence of four different concentrations of the entomopathogenic nematode, *Steinernema feltiae* on adults of the rose weevil, *Mecorhis ungarica* under laboratory conditions, and found different concentrations of *S. feltiae* were effective on adults of rose weevil. It is thought that entomopathogenic nematodes may be an alternative and promising biological control strategy to reduce the risk of pesticide residues in oil-bearing rose production areas. Salma Javed *et al*., (2022) conducted biocontrol evaluation of four species of Steinernematidae; *Steinernema pakistanense*, *S. siamkayai*, *S. ceratophorum* and *S. bifurcatum*, and one species of Heterorhabditidae; *Heterorhabditis indica*, against the armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). At 350 IJs/ml *S. pakistanense*; *S. siamkayai*, *S. ceratophorum*, *S. bifurcatum* and *H. indica* , showed 95, 78, 74, 90 and 87% mortality, respectively.

It could be concluded that the use of entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in the control of Lepidopteran larvae i.e. the greater wax moth, *Galleria mellonella,* cotton leafworm, *Spodoptera littoralis* and cotton leafworm, *Agrotis ipsilon* registered good results, but it needs more studies.

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