

Pathogenicity of the Entomopathogenic Nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, against the Black Cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae)

Ghoneim, K.

Faculty of Science, Al-Azhar University, Cairo, Egypt

Tanani, M.

Faculty of Science, Al-Azhar University, Cairo, Egypt

Hassan, H. A.

Institute of Plant Protection Research,
Agricultural Research Center, Giza, Egypt

Bakr, N. A.

Faculty of Science, Al-Azhar University, Cairo, Egypt

ABSTRACT

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is worldwide in distribution. It is a polyphagous insect attacking nearly all vegetables and many economic field crops in the world. The objective of the present study was to investigate the pathogenicity of two entomopathogenic nematodes (EPNs), *Steinernema carpocapsae* (Weiser) and *Heterorhabditis bacteriophora* (Poinar) against 4th and 5th instar larvae of *A. ipsilon*. The newly moulted larvae were infected with five concentrations of each EPN and the data were recorded at 24, 48, 72 & 96 hr post-infection. The most important results could be summarized as follows. After infection of the 4th or 5th instar larvae with five concentrations of *S. carpocapsae* or *H. bacteriophora*, a dose-dependent course of the total mortality rate of infected larvae was recorded. After infection of larvae with *S. carpocapsae* or *H. bacteriophora*, the mortality rate of infected larvae increased with the increasing concentration of infective juveniles (IJs), at certain times of exposure. After infection of the 4th or 5th instar larvae with *S. carpocapsae*, the mortality rate of infected larvae did not increase with the increasing time interval of exposure. On the other hand, infection of the 4th or 5th instar larvae with *H. bacteriophora* resulted in an increasing mortality rate with the increasing time interval of exposure. After infection of 4th instar larvae, LC₅₀ values were 16 IJs/ml and 48 IJs/ml, for *S. carpocapsae* and *H. bacteriophora*, respectively. After infection of 5th instar larvae, LC₅₀ values were 21 IJs/ml and 62 IJs/ml, for *S. carpocapsae* and *H. bacteriophora*, respectively. Thus, *S. carpocapsae* was more pathogenic against *A. ipsilon* larvae than *H. bacteriophora*. Also, the 4th instar larvae were more susceptible to the nematode pathogenicity than the 5th instar larvae. A few nematode-infected 4th instar larvae could pupate at the lower two concentrations of *S. carpocapsae* and *H. bacteriophora*. A similar result was recorded after infection of 5th instar larvae with *S. carpocapsae*. Moreover, the infected larvae with *H. bacteriophora* successfully pupated at all concentrations except the highest one. In conclusion, *S. carpocapsae*

may be an effective microbial control agent in the integrated pest management program against the destructive insect pest, *A. ipsilon*.

Keywords: biocontrol, infectivity, mortality rate, pupae, symbiotic bacteria, virulence.

INTRODUCTION

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is globally distributed (Kononenko, 2003; Harrison, and Lynn, 2008; Binning et al., 2015) including tropical and subtropical regions throughout North, Central and South America, Europe, Asia, Australasia, Oceania, Africa, and the Middle East (Harrison, and Lynn, 2008; Binning et al., 2015; Mishra, 2020; Rodingpuia and Lalthanzara, 2021; CABI, 2021). It is a migrant and polyphagous species known to feed on many economically important plants (Picimbon, 2020; Moustafa et al., 2021) causing serious losses, especially in industrial plants and vegetables worldwide (Mahmoud et al., 2016; Abdel-Hakim and El-Mandarawy, 2017; Muştu et al., 2021; Ren et al., 2023). In addition to its obligate migratory behavior and strong flight performance, *A. ipsilon* has high reproductive capacity (Mustu et al., 2021). This pest is a nocturnal insect and larvae remain buried in the ground during the day, which hinders its viewing field is difficult (Bento et al., 2007). Therefore, *A. ipsilon* is one of the most challenging economic pests when it comes to control and management (Andersch and Schwarz, 2003; Zhang et al., 2022).

Although the IPM strategies are increasingly being developed in different parts of the world (Veres et al., 2020), the majority of treatments for pest insects still rely exclusively on the application of traditional insecticides (Jeschke et al., 2011; Meslin et al., 2021). The intensive use of many broad-spectrum currently marketed insecticides usually causes serious toxicological problems to the ecosystems (Ibarra et al., 2006; Tiryaki and Temur, 2010; Gill et al., 2012; Chowański et al., 2014) and drastically affect the natural enemies, allowing an exponential increase of pest populations (Demok et al., 2019) as well as adverse effects on human health and domestic animals (Vattikonda and Sangam, 2017; Shahzad et al., 2020).

In Egypt, the control strategy of this insect pest depends mainly on the application of conventional insecticides, particularly organophosphates, carbamates and pyrethroids (Vattikonda and Sangam, 2017; Abd-El-Aziz et al., 2019; Ismail, 2021), to which this pest quickly develops resistance and cross resistance (Yu et al., 2012; Fahmy, 2014; Mahmoud et al., 2016; Ahmed et al., 2022). Also, the chemical insecticides are often not effective and remain inadequate for the control of *A. ipsilon* because of its larval hiding behavior during the daylight hours causing hidden damage in the fields (Capinera, 2001; Takeda, 2008). Moreover, the latter instar larvae remain hidden in cracks and crevices during most of the life cycle, so chemical control is often ineffective (Kumar et al., 2022). However, soil-applied insecticides can cause serious ecological problems (Kumari et al., 2002; Mosleh et al., 2003) and, obviously, have no effect on migrating adults (Zhang et al., 2022).

Therefore, various researchers and research institutions in the world have searching for new control tools as alternatives to synthetic insecticides (Laznik and Trdan, 2012; Glare et al., 2016). These alternative agents should be eco-environmentally safe (Liao et al., 2017; Kunbhar et al., 2018), effective at low concentrations (Walkowiak et al., 2015) and biodegradable into harmless compounds (Tiryaki and Temur, 2010; Li et al., 2017). One of the eco-friendly

management strategies is the biological control of insect pests by natural enemies (parasitoids, predators and pathogens). It is highly promising (Amutha et al., 2021; Devi et al., 2021) because these agents are safe for humans and the environment. They have little or no effect on other non-targeted organisms (Jagodič et al., 2019).

Among the biological control agents, entomopathogenic nematodes (EPNs) have broad potential to kill the cutworms in soil itself (Kumar et al., 2022). They are natural enemies of soil insect pests whose effects as a biocontrol agent against many harmful pests have been recorded by many studies in the world (Peçen and Kepenekci, 2022). The parasitism of these beneficial nematodes leads to the suppression of the immune system of the insect host (Lewis and Clarke, 2012; Shapiro-Ilan and Brown, 2013; Lacey et al., 2015; Kaliaskar et al., 2022). Moreover, EPNs have an association with certain symbiotic bacteria which are carried in the intestine of infective juveniles (IJs) of EPNs (Arthurs et al., 2004; Lewis and Clarke, 2012; Chaston et al., 2013). The EPNs can be used individually as a biological control agent or in combination with other biocontrol agents, such as entomopathogenic bacteria and fungi in order to improve their efficacy for controlling the insect pests (Laznik et al., 2012). For reviews, see Vashisth et al., 2013; Sujatha and Jeyasankar, 2018; Jagodič et al., 2019; Askary and Abd-Elgawad, 2021; Kumar et al., 2022; Shaurub, 2023).

Out of 23 EPNs families, Steinernematidae and Heterorhabditidae are the two most famous families living naturally in the soil (Kumar et al., 2015) and used as biocontrol agents against diverse insect pests (Lacey and Georgis, 2012; Abd-Elgawad, 2020; Koppenhöfer et al., 2020; Yüksel et al., 2022). Their association with symbiotic bacteria is found to be the primary cause of insect mortality (Leonar et al., 2022). There are the genera *Xenorhabdus* and *Photorhabdus* associating with the families Steinernematidae and Heterorhabditidae, respectively, which produce natural products with insecticidal potential (Vicente-Díez et al., 2021) for the suppression of the immune system of the insect host (Lewis and Clarke, 2012; Shapiro-Ilan and Brown, 2013; Lacey et al., 2015; Kaliaskar et al., 2022). To date, more than 100 *Steinernema* and 16 *Heterorhabditis* have been described in the world (Shapiro-Ilan et al., 2017; Koppenhöfer et al., 2020; Bhat et al., 2020).

In general, EPNs have been reported to exhibit pathogenicity against many Lepidopteran insect pests, such as the tobacco cutworm *Spodoptera litura* (Rajkumar et al., 2003; Yadav et al., 2017; Thakur et al., 2022; Javed et al., 2022), the diamondback moth *Plutella xylostella* (Somvanshi et al., 2006; Kumar et al., 2022), the pink bollworm *Pectinophora gossypiella* (Shairra et al., 2016), the cotton bollworm *Helicoverpa armigera* (Vashisth et al., 2019; Yan et al., 2021; Thakur et al., 2022; Srivastava et al., 2022), the greater wax moth *Galleria mellonella* (Khashaba et al., 2020; Ali et al., 2022), the cabbage butterfly *Pieris brassicae* (Pervez and Rao, 2020; Tomar et al., 2022), the tomato leaf miner *Tuta absoluta* (Dlamini et al., 2020), the eggplant fruit borer *Leucinodes orbonalis* (Hussaini et al., 2002), the fall armyworm *Spodoptera frugiperda* (Caccia et al., 2014; Acharya et al., 2020; Lalramnghaki et al., 2021; Yan et al., 2021; Fallet et al., 2022; Mohamed and Shairra, 2023), and the Egyptian cotton leafworm *Spodoptera littoralis* (Yağcı et al., 2022; Abd El Azim, 2022; Yağcı et al., 2022; Abd El Azim, 2022).

Also, EPNs have been reported to exhibit pathogenicity against different insects of various orders, such as the Mediterranean fruit fly *Ceratitis capitata* (Diptera)(Malan et al., 2006; Shaurub et al., 2021), the silverleaf whitefly *Bemesia tabaci* (Hemiptera)(Qiu et al., 2008), the

tortoise beetle *Cassida vittata* (Coleoptera)(Saleh et al., 2009), the willow flea beetle *Crepidodera aurata* (Coleoptera)(Yaman and Erturk, 2016), the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera)(Manzoor et al., 2017), the super worm, *Zophobas morio* (Coleoptera)(Navarez et al., 2021), the white grub *Temnorhynchus baal* (Coleoptera)(Adly et al., 2022), the desert locust *Schistocerca gregaria* (Orthoptera)(Shairra, 2009), the prickly pear cochineal *Dactylopius opuntiae* (Hemiptera)(El Aalaoui et al., 2022), the wheat stink bug *Aelia rostrata* (Hemiptera)(Peçen and Kepenekci, 2022), and the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus* (Diptera) (Thanwisai et al., 2022).

In Egypt, the early research work on EPNs began in the 1970s when El-Kifl investigated the biological control potential of *Neoaplectana* (= *Steinernema*) *carpocapsae* on *A. ipsilon* (El-Kifl et al., 1972). Several surveys carried out in Egyptian soils revealed that species of family Heterorhabditidae were more prevalent than species of family Steinernematidae for biological control of some insect pests (Aashaq et al., 2020). Many studies had been conducted in order to evaluate the pathogenicity, virulence and control potential of EPNs species or their isolates against *A. ipsilon* larvae (Mathasoliya et al., 2004; Fetoh et al., 2009; Seal et al., 2010; Ebssa and Koppenhöfer, 2011; Khattab and Azazy, 2013; Hassan et al., 2016; Yuksel and Canhilal, 2018; Sobhy et al., 2020; Devi et al., 2021; Nouh, 2021, 2022). In this context, the present study was conducted aiming at the investigation of virulence of two EPNs, *Steinernema carpocapsae* (Weiser) and *Heterorhabditis bacteriophora* (Poinar) against the 4th and 5th instar larvae of *A. ipsilon*.

MATERIALS AND METHODS

Experimental Insect

A culture of the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) was established under constant conditions (27±2°C and 65±5% R.H.) at Department of Zoology and Entomology, Faculty of science, Al-Azhar University, Cairo, Egypt. It was originally started with a sample of eggs from the susceptible strain culture maintained for several generations in Plant Protection Research Institute, Doqqi, Giza, Egypt. Rearing technique was carried out according to Abdin (1979) with improvement of El-Shershaby (2010). The eggs were kept in wide-mouth plastic jars (1000 ml) fitted with filter paper until hatching. Newly hatched larvae were kept into new jars and provided with clean castor bean leaves *Ricinus communis* as a food every day. At reaching the 4th instar, larvae were reared in few numbers, in separate jars, to avoid crowding and cannibalism. These jars were covered with pieces of cloth for preventing the escape of larvae. Sawdust and fresh castor bean leaves were renewed daily until pupation. The pupae were then placed in plastic jars (10 x 25 cm) covered with muslin and fitted with filter paper, as an oviposition site for the future moths. After the adult emergence, a piece of cotton wool soaked in 10% sugar solution was suspended from the top of each jar and renewed every 48 hrs for feeding moths.

Rearing and Production of Selected Entomopathogenic Nematodes (EPNs)

Imported EPN species (Nematoda: Rhabditida), *Heterorhabditis bacteriophora* (Poinar) (Steinernematidae) and *Steinernema carpocapsae* (Weiser) (Heterorhabditidae) were supplied by Dr. El-Sadawy, National Research Centre, Doqqi, Giza, Egypt, for the mass culturing of each EPN. The last (7th) instar larvae of the greater wax moth *Galleria mellonella* were used as hosts according to (Shamseldean et al., 2008). Five live *G. mellonella* larvae were placed in a Petri dish

with approximately 100 live EPNs, 20 EPNs/ml, with a few drops of deionized water for each tested EPN. The infective juvenile stages (IJs) of each EPN species will enter and infect the larvae through their natural openings. Symbiotic bacteria carried within guts of the EPNs were released after they penetrate their hosts. Toxins produced by the bacteria caused blood poisoning of the larvae usually resulting in their death within 72 hours. The EPNs complete one to three generations before they emerge from the dead larvae (cadavers). Petri dishes were stored for a week in a dark place at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After six days, check larvae for infection. The successfully infected larvae will appear beige to dark red.

Upon successful infection, Petri dish containing nematodes, larvae and filter paper were placed within another larger Petri dish. The outer Petri dish halfway was filled with deionized water and covered with an opaque lid for three weeks. **IJs** will emerge from the host and swim into the water within one to three weeks. The **IJs** were verified still alive using a microscope. Nematodes can be stored in darkness in a container that provides a sufficient amount of air to nematodes by using shallow containers for approximately one month at 5°C .

EPN Infection of the *A. ipsilon* Larvae

For infection of the *A. ipsilon* larvae, a series of concentrations of each EPN was prepared as follows: *H. bacteriophora*: 200.0, 100.0, 50.0, 25.0 and 12.0 IJs/ml, and *S. carpocapsae*: 100.0, 50.0, 25.0, 12.0 and 6.0 IJs/ml. The EPN experiment was carried out in plastic cups (4 X 5 cm) filled with 50 gm of sterilized sand and moistened with 20% water (v/w). Nematodes suspensions were prepared in serial concentrations of different IJs/ml/cup. The newly moulted 4th and 5th instar larvae of *A. ipsilon* were placed in separate plastic cups. Sixty cups for each instar, for each concentration and control were conducted for each treatment. All cups were covered and kept at $25 \pm 2^{\circ}\text{C}$ and checked after 24, 48, 72 and 96 hr to record mortality and some metamorphosis criteria.

Statistical Analysis of Data

Data obtained were analyzed by the student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means using GraphPad InStat® v. 3.01 (1998).

RESULTS

Pathogenicity of EPNs against the 4th Instar Larvae of *A. ipsilon*

After infection of the 4th instar larvae of *A. ipsilon* with the EPN *Steinernema carpocapsae* at 5 concentration rates (100, 50, 25, 12 & 6 IJs/ml), data of the nematode virulence at different time intervals (24, 48, 72 & 96 hr post-infection) were distributed in **Table (1)**. Depending on these data, the total mortality rate of infected larvae was found in a dose-dependent course (93.3, 83.3, 60.0, 46.6 & 23.3% mortality, at nematode rates 100, 50, 25, 12 & 6 IJs/ml, respectively). Also, the mortality rate of infected larvae increased with the increasing concentration of infective juveniles (IJs), at different times of exposure, except the 96 hr post-infection. As clearly seen, the maximum mortality rate (93.3%) was recorded for infected larvae at the highest concentration, while the minimum rate (23.3%) was found at the lowest concentration of *S. carpocapsae*. Data of the same table revealed that the mortality rate of infected larvae did not increase with the increasing time of exposure, indicating a strong acute toxicity or high initial killing power of this EPN against larvae. The concentration-mortality relationship of *A. ipsilon* larvae to the EPNs was typically expressed as LC_{50} (median lethal

concentration) which as estimated for *S. carpocapsae* against 4th instar larvae in 16 IJs/ml (see **Figure 1**). Only a few nematode-infected larvae could pupate at the lower two concentrations (6.7 & 13.3% pupation, at 12 & 6 IJs/ml, respectively).

After infection of 4th instar larvae of *A. ipsilon* with 5 concentrations (200, 100, 50, 25 & 12 IJs/ml of the EPN *Heterorhabditis bacteriophora*, data of the nematode virulence were arranged in **Table (2)**. Depending on these data, the total mortality rate of infected larvae was found in a dose-dependent trend (86.7, 70.0, 46.7, 33.3 & 16.7% mortality, at 200, 100, 50, 25 & 12 IJs/ml, respectively). Also, the mortality rate increased with the increasing nematode concentration, only at 24 & 48 hr, not at 72 & 96 hr post-infection. As obviously shown, the maximum mortality rate (86.7%) was recorded at the highest concentration, while the minimum mortality rate (16.7%) was reached at the lowest concentration. In the light of data assorted in the previously mentioned table, the mortality rate of infected larvae increased with the increasing time interval of exposure, at each of 100 IJs/ml (10.0, 22.4, 33.1 & 35.0% mortality, at 24, 48, 72 & 96 hr, respectively), 25 IJs/ml (3.3, 10.4, 11.1 & 12.5% mortality, at 24, 48, 72 & 96 hr, respectively) and 12 IJs/ml (0.0, 0.0, 6.7 & 10.7% mortality, at 24, 48, 72 & 96 hr, respectively). LC₅₀ was calculated in 48 IJs/ml (See **Figure 2**).

Only, a few nematode-infected larvae could pupate, at the lower two concentrations (13.3 & 16.7% pupation, at 25 & 12 IJs/ml, respectively).

On the basis of LC₅₀ values of the tested two EPNs, *S. carpocapsae* was more pathogenic against 4th instar larvae of *A. ipsilon* than *H. bacteriophora* (see tables 1 & 2 and Figs 1 & 2).

Pathogenicity of EPNs Against the 5th Instar Larvae of *A. ipsilon*

After infection of the 5th instar larvae of *A. ipsilon* with the EPN *S. carpocapsae* at 5 concentration rates (100, 50, 25, 12 & 6 IJs/ml), data of the nematode virulence at different time intervals (24, 48, 72 & 96 hr post-inoculation) were assorted in **Table (3)**. According to these data, the total mortality rate of infected larvae was found in a dose-dependent course (86.7, 73.3, 56.7, 33.3 & 20% mortality, at 100, 50, 25, 12 & 6 IJs/ml, respectively). As clearly shown, the mortality ranged between 86.7% (maximum), at the highest concentration, and 20.0% (minimum), at the lowest concentration. On the other hand, the mortality rate increased with the increasing concentrations only on 72 hr post-infection, but a similar trend was not observed on other time intervals of exposure to *S. carpocapsae*. Data of the same table displayed that the mortality rate did not increase with the increasing time interval of exposure indicating a strong acute pathogenicity of this EPN species or its strong initial killing power against larvae. The concentration-mortality relationship of *A. ipsilon* larvae to the EPNs was typically expressed as LC₅₀ which was calculated as 21 IJs/ml, see **Figure 3**). Only, a few nematode-infected larvae could pupate at the lower two concentrations (10.0 & 16.7% pupation, at 12 & 6 IJs/ml, respectively).

After infection of 5th instar larvae of *A. ipsilon* with 5 concentrations (200, 100, 50, 25 & 12 IJs/ml of the EPN *H. bacteriophora*, data of the nematode pathogenicity were arranged in **Table (4)**. On the basis of these data, the total mortality rate was recorded in a dose-dependent course, with maximum mortality (80%) at the highest concentration, while the minimum mortality (10%) at the lowest concentration. Also, the mortality rate of infected larvae

increased with the increasing concentrations of *H. bacteriophora*, at all-time intervals of exposure. Depending on the data listed in the same table, mortality rate of infected larvae increased with the increasing time interval of exposure to the highest concentration (13.8, 27.3, 37.2 & 50.6% mortality, at 24, 48, 72 & 96 hr post-infection, respectively). Also, similar positive relation of mortality rate- exposure time was recorded after infection of larvae with concentrations of 100, 50 & 12 IJs/ml. In other words, the susceptibility of larvae increased with prolonged time of exposure. As seen in the aforementioned table and **Figure (4)**, LC_{50} of *H. bacteriophora* was 62 IJs/ml. Depending on data summarized in tables 3 & 4, *S. carpocapsae* was more infective against the 5th instar larvae of *A. ipsilon* than *H. bacteriophora* (LC_{50} values = 21 & 62 IJs/ml, respectively).

In addition, the determined LC_{50} values of the tested two EPNs, in the present study, revealed that the 4th instar larvae of *A. ipsilon* were more susceptible or sensitive to the nematode pathogenicity than the 5th instar larvae (LC_{50} values = 16 & 48 IJs/ml of *S. carpocapsae* and *H. bacteriophora*, against 4th instar larvae, respectively; as well as 21 & 62 IJs/ml of the same EPNs, against 5th instar larvae, respectively). Moreover, LC_{50} values indicated that *S. carpocapsae* was more virulent than *H. bacteriophora*, against both larval instars of *A. ipsilon*.

Table 1: Pathogenicity (mortality%) of EPN *Steinernema carpocapsae* against 4th instar larvae of *A. ipsilon* at different time intervals.

Nematode Conc. (IJs/ml)	Time interval					Pupation %	LC_{50}
	24 hr	48 hr	72 hr	96 hr	Total mortality		
100	43.3	70.0	66.7	0.0	93.3	0.0	16
50	23.3	48.8	41.7	44.4	83.3	0.0	
25	20.0	33.7	16.7	8.3	60.0	0.0	
12	13.3	23.1	13.9	6.7	46.6	6.7	
6	0.0	13.3	11.6	0.0	23.3	13.3	
Control	0.0	0.0	0.0	0.0	0.0	100.0	

Conc.: concentration.

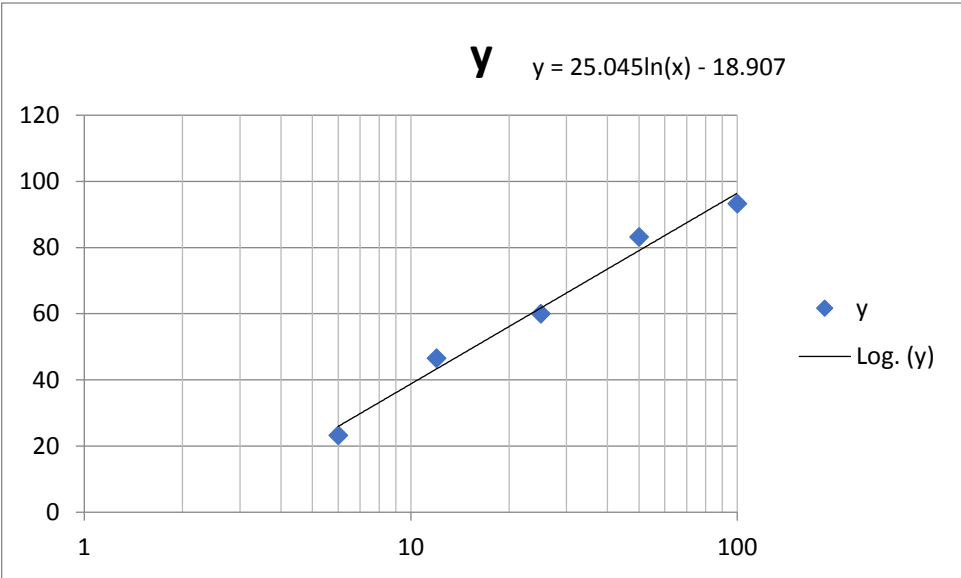


Figure 1: Regression line to calculate LC₅₀ of *S. carpocapsae* against *A. ipsilon* after infection of the 4th instar larvae.

Table 2: Pathogenicity (mortality%) of EPN *Heterorhabditis bacteriophora* against 4th instar larvae of *A. ipsilon* at different time intervals.

Nematode Conc. (IJs/ml)	Time interval					Pupation%	LC ₅₀
	24 hr	48 hr	72 hr	96 hr	Total mortality		
200	40.0	61.3	27.8	16.7	86.7	0.0	48
100	10.0	22.4	33.1	35.0	70.0	0.0	
50	6.7	14.1	25.0	11.4	46.7	0.0	
25	3.3	10.4	11.1	12.5	33.3	13.3	
12	0.0	0.0	6.7	10.7	16.7	16.7	
Control	0.0	0.0	0.0	0.0	0.0	100.0	

Conc.: see footnote of Table (1).

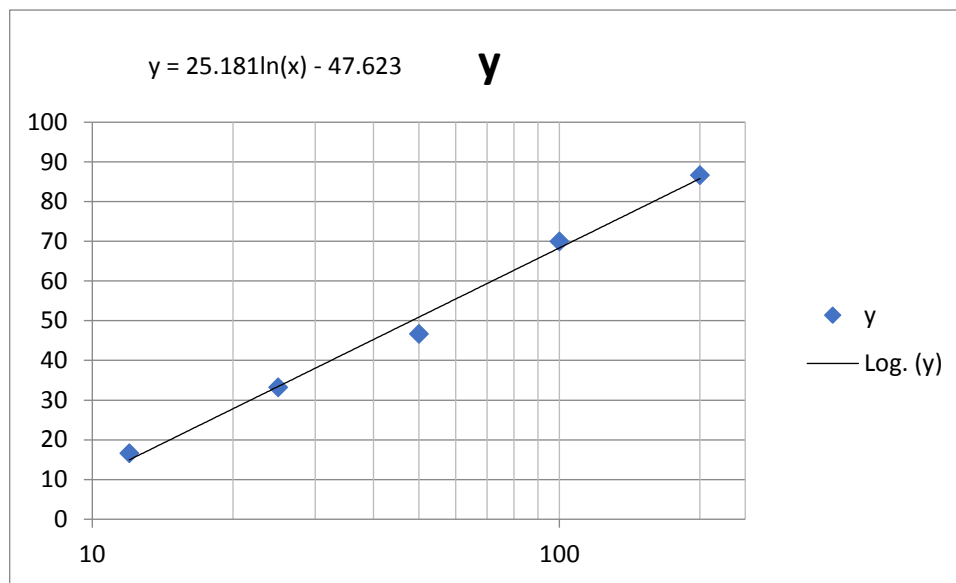


Figure 2: Regression line to calculate LC_{50} of *H. bacteriophora* against *A. ipsilon* after treatment of the 4th instar larvae.

Table 3: Pathogenicity (mortality%) of EPN *S. carpocapsae* against 5th instar larvae of *A. ipsilon* at different time intervals.

Nematode Conc. (IJs/ml)	Time interval					Pupation%	LC ₅₀
	24 hr	48 hr	72 hr	96 hr	Total mortality		
100	13.3	35.2	52.4	44.5	86.7	0.0	21
50	16.7	36.1	30.0	25.0	73.3	0.0	
25	10.0	19.1	16.9	26.2	56.7	3.3	
12	3.3	10.0	15.3	9.5	33.3	10.0	
6	0.0	3.3	3.7	14.2	20.0	16.7	
Control	0.0	0.0	0.0	0.0	00.00	100.0	

Conc.: see footnote of Table (1).

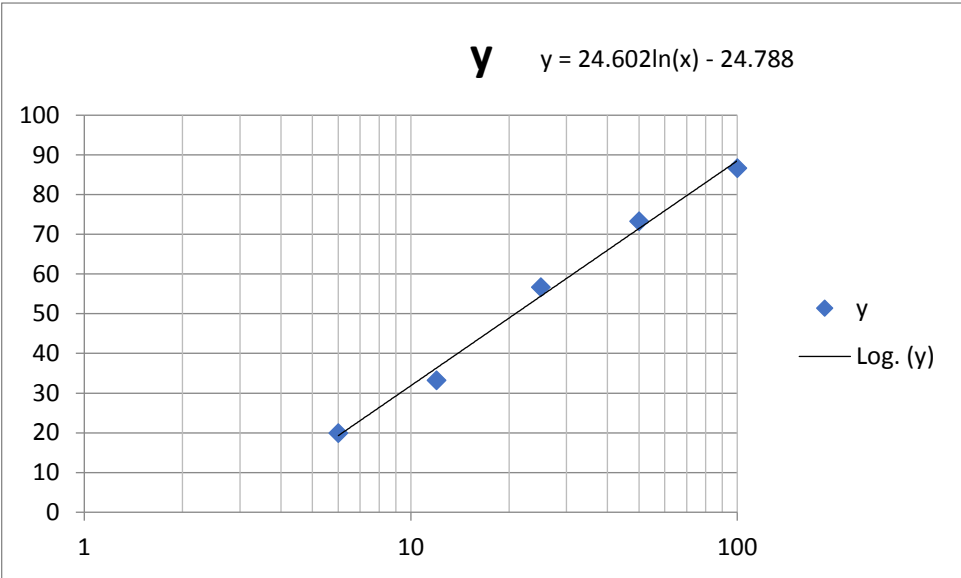


Figure 3: Regression line to calculate LC₅₀ of *S.carpocapsae* against *A. ipsilon* after treatment of the 5th instar larvae.

Table 4: Pathogenicity (mortality%) of EPN *H. bacteriophora* against 5th instar larvae of *A. ipsilon* at different time intervals.

Nematode Conc. (IJs/ml)	Time interval					Pupation%	LC ₅₀
	24 hr	48 hr	72 hr	96 hr	Total mortality		
200	13.8	27.3	37.2	50.6	80.0	0.0	62
100	13.3	18.9	23.8	38.9	66.7	6.7	
50	3.3	10.4	11.1	22.0	40.0	13.3	
25	0.0	6.7	3.3	14.8	23.3	20.0	
12	0.0	0.0	3.3	6.7	10.0	20.0	
Control	0.0	0.0	0.0	0.0	0.0	100.0	

Conc.: see footnote of Table ().

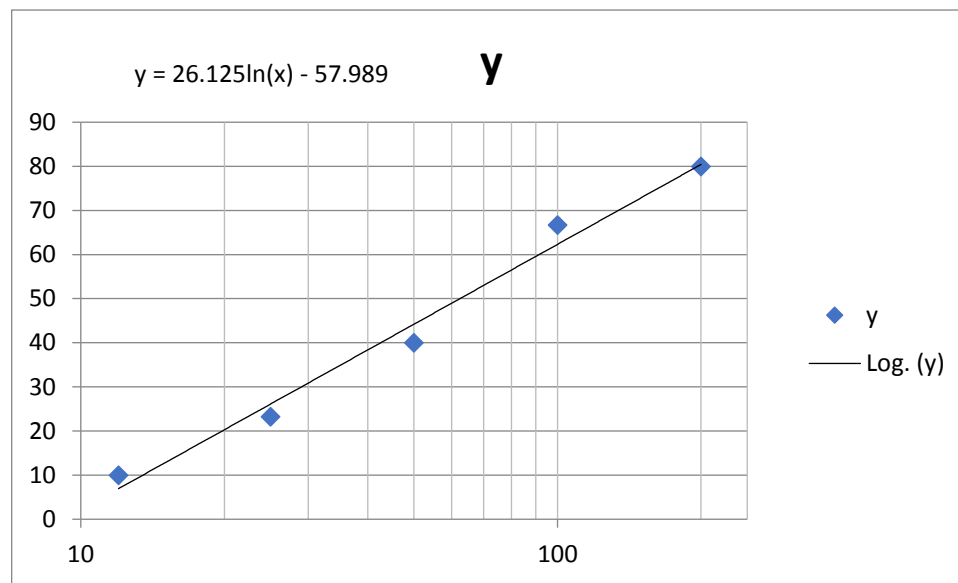


Figure 4: Regression line to calculate LC_{50} of *H. bacteriophora* against *A. ipsilon* after treatment of the 5th instar larvae.

DISCUSSION

Many studies have been conducted in order to evaluate the virulence and control potential of different entomopathogenic nematodes (EPNs) species, or their local isolates, against larvae of the black cutworm *Agrotis ipsilon* in different parts of the world (Mathasoliya et al., 2004; Fetoh et al., 2009; Seal et al., 2010; Ebssa and Koppenhöfer, 2011; Lemma and Albrecht, 2012; Khattab and Azazy, 2013; Yuksel and Canhilal, 2018; Sobhy et al., 2020; Devi et al., 2021; Nouh, 2022). The majority of these studies only investigated one larval instar: 3rd instar (Shapiro-Ilan et al., 2005; Shapiro-Ilan et al., 2009), 4th instar (Koppenhofer and Fuzy, 2003), 5th instar (Morris et al., 1990) or 7th instar (Capinera et al., 1988). Also, many studies investigated the infectivity of only one EPN species (Baur et al., 1997). Comparisons between studies, even where the same instar or EPN species was used, are more complicated by the use of different techniques, substrates, nematode rates and exposure time intervals (Koppenhofer and Fuzy, 2003). Also, some studies used mixtures of consecutive instars: 2nd and 3rd instars (Kunkel and Grewal, 2003) or 4th and 5th instars (Kaya et al., 1993; Kunkel and Grewal, 2003) or 6th and 7th instars (Epsky and Capinera, 1994), even though instars may differ significantly in susceptibility to different EPN species may differ among instars (Koppenhofer and Fuzy, 2003). In addition, several of the previously mentioned studies used only Petri dishes with filter paper as experimental arenas (Kunkel and Grewal, 2003) although nematode virulence against *A. ipsilon* can remarkably vary with substrate and the overall effect can vary with the nematode species (depending on foraging strategy) (Shapiro et al., 1999; Ebssa and Koppenhöfer, 2012; Hassan et al., 2016).

Pathogenicity of the selected EPNs Against *A. ipsilon*

In the present study, infection of the 4th instar larvae of *A. ipsilon* with five concentrations of *Steinernema carpocapsae* (Steinernematidae) or *Heterorhabditis bacteriophora* (Heterorhabditidae) caused larval mortality ranging between 93.3% and 23.3%, for *S. carpocapsae* and between 86.7% and 16.7%, for *H. bacteriophora*. Also, infection of 5th instar

larvae with these EPNs resulted in larval mortality ranging between 86.7% and 20.0%, for *S. carpocapsae* and between 80% and 10%, for *H. bacteriophora*.

The present results could be understood in the light of various reported results of pathogenic efficacies of different EPNs against the same insect, *A. ipsilon*. For example, the EPNs *Steinernema abbasi* and *H. bacteriophora* had been tested against the 4th instar larvae of *A. ipsilon* by Shoeb et al. (2006) who reported that *H. bacteriophora* caused 49, 53 and 73% mortality rates, at 25, 50, and 100 IJs/ larva, respectively, 48 h post-treatment. Depending on the study of Ebssa and Koppenhöfer (2012), the mortality rates of *A. ipsilon* larvae at the 2nd day exposure time were found 30 and 83% for *H. bacteriophora* and 18 and 55% for *S. carpocapsae*, at 30 and 100 IJs/larva, respectively. The concentration of 63 IJs per larva of *S. carpocapsae* GSN1 strain (ScG) killed >90% of 3rd instar *A. ipsilon* within 3 days (Lee and Potter, 2015). EPNs had been applied by Mahmoud et al. (2016) against the last instar larvae of *A. ipsilon* who recorded the mortality rates for *S. carpocapsae* and *H. bacteriophora* as 100% and 90%, respectively, at the 3rd day post-treatment.

Some years later, Yuksel and Canhilal (2018) applied the concentration 100 IJs/larva/Petri of *S. carpocapsae* and *H. bacteriophora* against *A. ipsilon* larvae and recorded the highest mortality rate of larvae as 85% and 45%, respectively, at the 3rd day post-treatment. Also, Giannasi et al. (2018) evaluated the potential of Brazilian nine isolates of EPNs against *A. ipsilon*. Depending on their study, isolate CH3 (*Steinernema* sp.) caused 100% mortality of *A. ipsilon* and the isolates GL (*H. amazonensis*), IBCBn-02 (*S. carpocapsae*) and IBCB-n05 (*H. indica*) caused only 90 % mortality. The efficacies of *S. monticolum* and *H. bacteriophora* against larvae of *A. ipsilon* had been studied under laboratory conditions. The mortality percentage was 100% at concentration 200 IJs/dish (Sobhy et al., 2020). The lowest potato tuber damage by *A. ipsilon* was recorded at 5.0×10^9 IJs/ha of *H. bacteriophora*, followed by the concentration 2.5×10^9 IJs/ha (Devi et al., 2021). The EPN *Hexameris arvalis* was reported in the U.S. infecting 60% of *A. ipsilon* larvae and killing them (Rodingpuia and Lalthanzara, 2021). In a semi-field experiment, *Heterorhabditis* TAN5 and Hb88 strains were effective against 4th instar larvae and 3-day old pupae of *A. ipsilon* at a high concentration of each nematode (Nouh, 2022).

Apart from *A. ipsilon*, our results were, to a great extent, corroborated with several reported results of the EPN infectivity against various lepidopteran pests. For examples, the concentration 40 IJs/ml of *S. carpocapsae* and *H. bacteriophora* caused 100% mortality of the larval instars of the Egyptian cotton leafworm *Spodoptera littoralis* (Reyad, 2001). Yağcı et al. (2022) recorded high mortality rate of last (6th) instar larvae of *S. littoralis* (98.81%) by *S. feltiae*, followed by *S. carpocapsae* (95.24%) and then *H. bacteriophora* (90.47%), at the highest concentration (1000 IJ/ml). EPN isolate of *H. taysearae* was tested against *S. littoralis*. At 150 IJs/larvae, 100% mortality rate was recorded, followed by 90% at 120 IJs/larvae and 60% at 60 IJs/larvae, 72 h post-treatment (Abd El Azim, 2022).

After treatment of 4th instar larvae of the tobacco cutworm *Spodoptera litura* with *S. carpocapsae*, the maximum (100%) mortality larvae were recorded at 400 IJs/Petri plate and the minimum (55%) mortality was observed after 96 hrs at 100 IJs (Yadav et al., 2017). At the concentration 350 IJs/ml of *S. pakistanense*; *S. siamkayai*, *S. ceratophorum*, *S. bifurcatum* and *H. indica*, Javed et al. (2022) found 95, 78, 74, 90 and 87% mortality of *S. litura* larvae, respectively.

With regard to the fall armyworm *Spodoptera frugiperda*, Fallet et al. (2022) reported that Rwandan *S. carpocapsae* strain (RW14-G-R3a-2) caused rapidly 100% mortality in 2nd and 3rd instars, but the rate decreased somewhat to 75% in 6th instar. Sayed et al. (2022) recorded the highest mortality rate (100%) in both 3rd and 5th instar larvae after 3–4 days of irradiated *S. carpocapsae* infection but mortality rates were 72.2 and 77.8%, respectively, for the tested instars with un-irradiated *S. carpocapsae* strains. Recently, Mohamed and Shairra (2023) found that *S. carpocapsae* was more virulent and effective against all larval instars, causing 100% mortality after 48–72 h post-exposure at different concentrations (150 - 2400 IJs), while *H. indica* caused 100% mortality in the early larval instars only after 96 h, but later larval instars required a longer time extending to 120–188 h.

In respect of the cotton bollworm *Helicoverpa armigera*, application of *H. bacteriophora* (HRJ) against 3rd instar larvae caused 73.3% mortality at 96 hr exposure time, while *H. indica* caused 80.0% mortality (Vashisth et al., 2019). As recorded by Srivastava et al. (2022), *S. abbasi* isolate CS-39 had high virulence to larvae since its concentration 200 IJs / larva caused 100% mortality at 36 h, whereas 100 IJs / larva caused 100% mortality, at 60 h. Also, Thakur et al. (2022) investigated the bio-efficacy of indigenous EPNs *H. bacteriophora* against the 3rd and 4th instar larvae of *H. armigera*, *S. litura* and the turnip moth *Agrotis segetum*. The highest mortality rate was recorded for both instars of all insects after 120 h of exposure.

EPNs *H. bacteriophora* and *S. carpocapsae* caused the highest larval mortality of *A. segetum* larvae at 100 IJs/insect (Goudarzi et al., 2015). After treatment of the tomato leaf miner *Tuta absoluta* 4th instar larvae with EPNs, *S. yirgalemense* and *S. jeffreyense*, mortality rates of 93% and 58.8% had been recorded (Dlamini et al., 2020). Complete mortality of the last instar larvae of the greater wax moth *Galleria mellonella* was achieved by the concentration 40 IJs/L of M.22 and M.313 isolates of *S. affine* from the Syrian coastal region, at 72 h of exposure (Ali et al., 2022). The highest concentration of *H. bacteriophora* (local isolate) (2000 IJs/ml) caused the maximal larval mortality of the cabbage butterfly *Pieris brassicae* (Tomar et al., 2022).

In addition to Lepidoptera, many studies revealed infectivity of different EPNs against several insect pests of various orders, as concisely shown in the following examples. In Diptera, the EPN *S. ceratophorum* caused high mortality of larvae of the chive gnat *Bradysia odoriphaga* (Ma et al., 2013a, b). In a study conducted by Mokrini et al. (2020), efficacy of five Moroccan EPN strains had been evaluated against 3rd instar larvae of the Mediterranean fruit fly *Ceratitis capitata*. Results of this study revealed that the highest larval mortality rates were found for *S. feltiae*- strain (MOR9) (96%), followed by *H. bacteriophora*-MOR7 and *S. feltiae*-MOR10 strains, 90 and 83% mortality rates, respectively. Yağcı et al. (2021) obtained a high mortality rate of last instar larvae of *C. capitata* (94%) by *H. bacteriophora* (11 KG), followed by *H. bacteriophora* (TOK-20) (91%), *S. carpocapsae* (85%) and *S. feltiae* (Tokat-Emir) (71%), at the highest concentration (200 IJ/larvae).

In Hemiptera, EPN *S. carpocapsae* exhibited a high virulence against the nymphs and adults of the earwig *Labidura riparia* (Reyad, 2012). In Turkey, Peçen and Kepenekci (2022) used some of the native isolates of *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* against adults of the wheat stink bug *Aelia rostrata*. They found the Black Sea isolate as the strongest pathogenic causing the highest mortality of the bug adults. In Morocco, El Aalaoui et al. (2022) evaluated the efficacy of two native EPN isolates of *S. feltiae* and *H. bacteriophora* (applied at 25, 50, and 75

IJs cm⁻²) against the prickly pear cochineal *Dactylopius opuntiae* nymphs and young adult females. They recorded the *S. feltiae* as more effective, causing higher mortality of nymphs and adult females (98.8% and 97.5%, respectively) after 8 days of exposure, while, *H. bacteriophora* caused lower mortalities (83.8% for nymph and 81.3% for adult females).

In Orthoptera, EPN *S. glaseri* killed almost 65% of the 5th instar nymphs of desert locust *Schistocerca gregaria* within 72 hr post-infection (Shairra, 2009). In Coleoptera, *S. ceratophorum* caused high mortality of larvae of the bark beetle *Dendroctonus valens* (Jian et al., 2002). The maximal and minimal mortalities of 3rd and 10th larval instars of the red palm weevil *Rhynchophorus ferrugineus* were 96.5% and 88.17% by *S. carpocapsae*, 85.75% and 74.4% by *H. bacteriophora*, and 38.68% and 35.35% by *S. feltiae* (Manzoor et al., 2017). At the concentration 4000 IJs/larva of both *S. glaseri* and *H. bacteriophora*, mortality percentages of the white grub *Temnorhynchus baal* larvae were calculated in 96 and 88%, respectively (Adly et al., 2022).

As clearly shown in the present study on *A. ipsilon*, different concentrations of IJs of both *S. carpocapsae* and *H. bacteriophora* caused various mortality rates of larvae, at different time intervals of exposure. To explicate these larval mortalities, it is important to point out that EPNs in the families of Steinernematidae and Heterorhabditidae are lethal endoparasites of insects, which could secrete insecticidal active substances, including toxins, proteases, and so on, contributing to the lethal effect on infected host insects (Brown et al., 2006; Ffrench-Constant et al., 2007; Toubarro et al., 2009). In addition to these lethal effects of EPNs themselves, the primary cause of the host death has been usually reported to the associating symbiotic bacteria carried in their intestines (Trdan et al., 2009; Laznik et al., 2010). Also, IJs kill their insect hosts with the aid of symbiotic bacteria carried in the nematode gut (*Photorhabdus* spp. bacteria are associated with *Heterorhabditis* spp. and *Xenorhabdus* spp. bacteria are associated with *Steinernema* spp.) (Martens et al., 2003; Lewis and Clarke, 2012; Chaston et al., 2013). This nematode / bacterium complex works together and is highly virulent as a biological control unit to kill an insect pest (Lacey et al., 2001). The relationship between nematode and symbiotic bacterium is a type of symbiosis, where both benefit from this association. After entering the insect body, the nematode penetrates through the gut wall, and regurgitates its symbiotic bacteria into the insect haemocoel cavity (Mastore et al., 2015). Regarding the mechanism, the bacteria break down the host tissues, and provide food sources for the nematode, which feeds and multiplies on bacterial cells and degrading host tissues. In other words, EPNs act by feeding on the bacteria and the damaging host tissue, they multiply, develop and reproduce (Griffin, 2012). However, the bacteria provide the nematode, and themselves a protected niche by producing antibiotics, that suppress the competition from other microorganisms (for review, see Mahmoud, 2016; Kumar et al., 2022; Shaurub, 2023).

For some detail, when EPNs are inside the insect haemocoel, symbiotic bacteria are released and the subsequent death of the insect is mainly due to bacteria-induced septicaemia in the late phase of infection (Bowen et al., 1998, Ffrench-Constant et al., 2007a,b). In addition to the action of bacteria, physiological interactions between the EPN and its insect host must be considered, since the life-cycle of an endoparasite is strongly related to its ability to evade and/or to suppress the host immune responses in the early phase of infection (Castillo et al., 2011; Toubarro et al., 2013). Both nematode and bacteria overcome the insect immune system

causing death of the insect within 48 hours post-infection (Adams and Nguyen, 2002). In this respect, another point of interest is the act of EPNs themselves, because shortly after infection, and before the bacteria is released from the gut of the invading nematode, there is a significant reduction in the total insect hemocytes, suggesting that the nematode itself is capable of suppressing the host immune system, to the benefit of its endosymbiotic bacteria (Bobardt et al., 2020; Dowds and Peters, 2003; Shapiro-Ilan et al., 2018).

In addition to the variation of mortality rates of *A. ipsilon* larvae, in the current investigation, after infection with different concentrations of IJs of *S. carpocapsae* and *H. bacteriophora*, many studies on the same insect, or other insect species, reported various percentages of mortality. These differences in mortality rates may be attributed to the number of larvae used for each study, since some researchers (Shoeb et al., 2006; Fetoh et al., 2009; Mahmoud et al., 2016) used 15, 25 or 20 larvae in their studies, while Yuksel and Canhilal (2018) used a total of 40 larvae for each concentration. Mortality rates might be, also, affected by the foraging strategies of EPNs, since *S. carpocapsae* is considered to be more effective than other species for controlling the moving insects due to its foraging behavior, and the Petri dishes might have created an environment that is more favorable to *S. carpocapsae* (Campbell and Gaugler, 1993). Also, *S. feltiae* possess an intermediate forage strategy (Griffin et al., 2005), whereas *H. bacteriophora* displays a cruise strategy (Bal and Grewal, 2015). These differences may be, also, attributed to variations in the experimental methods (Barbosa-Negrisoni et al., 2009). However, Shapiro-Ilan et al. (2018) reported that EPN foraging strategies consist of a continuum from ambusher (sit and wait) to cruiser (active search strategy) behaviors, yet a number of species display intermediate foraging strategies between the two extremes.

Increasing Mortality Rate of *A. ipsilon* Larvae with the Increasing EPN Concentration

After infection of 4th instar larvae of *A. ipsilon* with *S. carpocapsae*, in the present study, the mortality rate of infected larvae increased with the increasing concentration of infective juveniles (IJs), at some times of exposure. A similar mortality-concentration relationship was recorded after infection with *H. bacteriophora*, only at certain time intervals. After infection of 5th instar larvae with *S. carpocapsae*, the mortality rate increased with the increasing concentrations only on 72 hr post-infection, while the positive mortality- concentration relationship was observed at all time intervals of exposure, after infection with *H. bacteriophora*. Thus, the predominant trend was a positive relation between mortality rate and the EPN concentration in the present study. This result was in agreement with the reported results of many studies on *A. ipsilon* after treatment with different EPN species (Fetoh et al., 2009; Shoeb et al., 2006; Bélair et al., 2013; Mahmoud et al., 2016; Yuksel and Canhilal, 2018). For insects other than *A. ipsilon*, Shairra (2000) reported that the mortality percentages of some lepidopterous larvae increased with the increasing of IJs dose of *H. indicus* and *H. bacteriophora*. El-Bishry et al. (2002) found that the pathogenicity of *Heterorhabditis* spp. against the rice moth *Corcyra cephalonica* larvae was dose-dependent. Also, the pathogenicity of *H. bacteriophora* and *S. glaseri* against *S. gregaria* nymphs was found depending upon IJs concentration (Shairra, 2007, 2009). Depending on results of Shairra et al. (2016), mortality percentage of the pink bollworm *Pectinophora gossypiella* larvae increased with the increasing concentration of *S. riobrave* and *H. bacteriophora*. The mortality percentage of *S. littoralis* larvae increased with the increase of the concentrations of *S. feltiae*, *S. riobrave* and *H. bacteriophora* (Ahmed et al., 2014). Shaurub et al. (2021) assessed the effects of some EPN strains on *C. capitata* and found that the

mortality of 3rd instar larvae proportionally increased with the nematode density (infective juveniles per cm²). Recently, Mohamed and Shairra (2023) applied different concentrations of *S. carpocapsae* against all larval instars of *S. frugiperda* and calculated the LC₅₀ values in 3rd instar larvae as 170.415 IJs/larva (48 h); followed by the 4th instar (318.252 IJs/larva), but the 5th instar was the highest value (LC₅₀= 567.108 IJs/larva). Thus, mortality rate increased as the larval instar increased.

In the current study on *A. ipsilon*, the dose-dependent mortality rate might be due to the fact that, the success of initial penetration of some IJs individuals of *S. carpocapsae* and *H. bacteriophora* opens pathways for other IJs. The death of treated larvae is caused mainly by the effect of the nematode associated symbiotic bacteria. Thus, it could be suggested that the higher concentrations of nematodes may elaborate much more bacteria which in turn multiply rapidly producing huge number of bacteria and finally kill the insect larvae more rapidly (Salem et al., 2007).

Increasing Mortality Rate of *A. ipsilon* larvae with Increasing Time Interval of Exposure to EPNs

After infection of the 4th or 5th instar larvae of *A. ipsilon* with *S. carpocapsae*, in the current study, the mortality rate of infected larvae did not increase with the increasing time interval of exposure. Whereas, the infection of 4th or 5th instar larvae with *H. bacteriophora* resulted in an increasing mortality rate with the increasing time interval of exposure, regardless the nematode concentration. This result was found compatible with many reported results of increasing mortality rate of *A. ipsilon* larvae with increasing time interval of exposure to different EPN species, such as *S. carpocapsae* and *H. bacteriophora* (Mahmoud et al., 2016); *S. glaseri* and *H. bacteriophora* (Hassan et al., 2016); local EPNs isolates of *S. carpocapsae* and *H. bacteriophora* (Yuksel and Canhilal, 2018).

Also, our result was in corroboration with many reported results of increasing mortality rate in various insects with increasing time interval of exposure to different EPN species, such as *Heterorhabditis* spp. against *S. litura* and the diamondback moth *Plutella xylostella* (Salem et al., 2007); *H. indica* against larvae of the dipteran *Bradysia mabiusi* (Leite et al., 2007); *H. indica* against the rice leaf folder *Cnaphalocrosis medinalisthan* (Sankar, 2009); *S. carpocapsae* against 3rd and 5th instar nymphs and adults of *S. gregaria* (Youssef, 2014); *S. riobrave* and *H. bacteriophora* against *P. gossypiella* (Shairra et al., 2016); *H. bacteriophora* and *S. glaseri* against *S. litura* (Safdar et al., 2018); *H. bacteriophora*, individually or in combination with the Entomopathogenic fungus *Beauveria bassiana*, against *Rh. ferrugineus* (Mehdi et al., 2018); *Oscheius rugaoensis* against *G. mellonella* (George et al., 2019); Egyptian local isolates of *H. indica* against *G. mellonella* (Khashaba et al., 2020), *S. glaseri* against the six instars of *S. frugiperda* (Meka et al., 2020) and *S. feltiae* HR1 and *H. bacteriophora* HR2 against *P. brassicae* (Kasi et al., 2021).

Similar results had been reported for *S. littoralis* larvae by *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* (Yağcı et al., 2022), *H. armigera* larvae by *S. abbasi* isolate CS-39 (Srivastava et al., 2022), the coconut rhinoceros beetle *Oryctes rhinoceros* larvae by *S. carpocapsae* (CPCRI-Sc1) and *S. abbasi* (CPCRI-Sa1) (Sujithra et al., 2022), M.22 and M.313 isolates of *S. affine* from the Syrian coastal region against the last instar larvae of *G. mellonella* (Ali et al., 2022),

some isolates of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* against *S. littoralis* larvae (Yağcı et al., 2022) and *Steinernema abbasi* isolate CS-39 against *H. armigera* larvae (Srivastava et al., 2022).

In the current study, the mortality rate of *S. carpocapsae*-infected larvae of *A. ipsilon* did not increase with the increasing time interval of exposure. This might indicate a strong acute pathogenicity of this EPN species or its stronger initial killing power against larvae than *H. bacteriophora*. On the other hand, mortality rate of *A. ipsilon* larvae increased with the increasing time interval of exposure to *H. bacteriophora* in the current investigation. This result might indicate that the susceptibility of these larvae to this EPN species increased with the prolongation of exposure time. Also, it could be attributed to the smaller size of IJs, which facilitates penetration of IJs through natural openings of larvae, i.e. spiracles, mouth, and anus (Adams and Nguyen, 2002). Moreover, IJs of *H. bacteriophora* can use their dorsal teeth to penetrate through the host cuticle (Bedding and Molyneux, 1982). Similar findings were, also, reported by some authors (Lalramliana, 2009; Gorgadze et al., 2018). However, some authors (Shairra and Nouh, 2014; Sobhy et al., 2020) reported that higher concentrations of EPNs caused an acute effect, while the latent effect was observed in the case of lower ones. Also, time passed for the larval mortality was prolonged with the increase in inoculum dose of IJs, but when the size of larva increased, the time passed for the larval mortality also was prolonged (Mantoo and Zaki, 2014).

The Medium Lethal Concentrations (LC₅₀) of the Selected EPNs AGAINST *A. ipsilon*

In insects, the concentration-mortality relationship of larvae to the tested EPNs was typically expressed as LC₅₀ (median lethal concentration required to kill 50% of the larvae). According to the currently available literature, various LC₅₀ values had been determined for different EPNs after infection of different insects. For examples, LC₅₀ values of four locals Turkish EPNs isolates against the 4th instar *A. ipsilon* larvae ranged from 52 to 142 IJs in the Petri dish experiment and from 17 IJs to 24 IJs in the plastic container experiment (Yuksel and Canhilal, 2018). LC₅₀ values of two strains of *Heterorhabditis* spp. TAN5 and PGN6 against *A. ipsilon* larvae were 1285.527 and 1560.747 IJs/cup, respectively (Nouh, 2021). Also, LC₅₀ values for *H. bacteriophora*, *S. riobrave* and *S. feltiae* against *S. littoralis* larvae were 5.01, 8.57 and 16 IJs/larva, respectively (Ahmed et al., 2014). LD₅₀ values for *S. glaseri* against *S. gregaria* 5th instar nymphs were recorded as 1505.13, 393.16 and 812.63 IJs/ml over the exposure periods of 48, 72 and 96 hr, respectively (Shairra, 2009). The most virulent EPN against larvae of the yellow sawfly *Hoplocampa flava* was *H. bacteriophora* because LD₅₀ was calculated for 6.51 IJs, while the least virulent EPN was *S. carpocapsae* Tur-S4 because LD₅₀ was calculated for 16.617 IJs (Ulu et al., 2015). LC₅₀ values against larvae and adults of the Elm leaf beetle *Xanthogaleruca luteola* were 167.59 and 6.73 IJ·mL⁻¹ for *S. feltiae*, while LC₅₀ values were 218.23 and 204.09 IJ·mL⁻¹ for *S. carpocapsae*, and 338.66 and 70.29 IJ·mL⁻¹ for *H. bacteriophora*, respectively (Fard et al., 2020). The LC₅₀ of local *H. indica* (PIGCD1), isolated from peanut in Philippines, against *S. litura* under laboratory conditions was 7.13±1 (IJs/larva) (Dichusa et al., 2021). According to Chaneiko et al. (2021), LC₅₀ values of the South American fruit fly *Anastrepha fraterculus* larvae and adults were 427.2 IJs/ml and 1551.4 IJs/ml of the isolated *S. carpocapsae* IBCB 02, respectively. As recorded by Bhairavi et al. (2021), two EPNs species (isolated locally from Assam, India) were assessed against an important subterranean termite *Odontotermes obesus*. LD₅₀ for *H. bacteriophora* was 13.054 IJs/termite, and LD₅₀ for *S. aciari* was 42.040 IJs/termite.

In view of the previously reported results, LC₅₀ values of *A. ipsilon* larvae, in the present study, were 16 IJs/ml and 48 IJs/ml, for *S. carpocapsae* and *H. bacteriophora*, respectively, after infection of 4th instar larvae while LC₅₀ values were 21 IJs/ml and 62 IJs/ml, after infection of 5th instar larvae, for *S. carpocapsae* and *H. bacteriophora*, respectively. These results were, to some extent, in corroboration with reported LC₅₀ values for different EPNs against various insects, in some of recent studies. For examples, a laboratory bioassay revealed LC₅₀= 83.21 IJs of the *S. abbasi* isolate CS-39 against *H. armigera* larvae after 24 h of exposure (Srivastava et al., 2022); LC₅₀ values of M.22 and M.313 isolates of *S. affine* against the last instar larvae of *Galleria mellonella* were calculated as 11.7 IJs/L at 33.47 min. and 11.5 IJs/L at 32.43 min., respectively (Ali et al., 2022). According to Sujithra et al. (2022), LC₅₀ values of *S. carpocapsae* Sc1, *S. abbasi* Sa1 and *H. indica* Hi1 strains were 1078, 1663 and 9780 IJs larva⁻¹, respectively, at 72 h of incubation of *O. rhinoceros* larvae. As recorded by Thakur et al. (2022) for indigenous EPNs *H. bacteriophora*, LC₅₀ values of *H. armigera* 3rd and 4th instar larvae were 60.14 IJs/larvae and 57.90 IJs/larvae, respectively. LC₅₀ values of *S. litura* were 59.95 and 50.91 IJs/larvae in 3rd and 4th instar larvae, respectively. LC₅₀ values of the 3rd and 4th instar larvae of *A. segetum* were 54.86 and 57.90 IJs/larvae, respectively. In a recent study to evaluate the virulence of *H. indica* against larvae (3rd, 4th and 5th instars) and pupae of citrus fruit piercing moth *Eudocima maternal*, LC₅₀ of 3rd (14.43 IJs larva⁻¹), 4th (17.08 IJs larva⁻¹), 5th (23.63 IJs larva⁻¹) and LC₅₀ of pupae was 85.91 IJs pupa⁻¹, after 48 h post-exposure, respectively (Kumar et al., 2022). At 72 h of incubation of *O. rhinoceros* larvae, the LC₅₀ values of *S. carpocapsae* Sc1, *S. abbasi* Sa1 and *H. indica* Hi1 strains were 1078, 1663 and 9780 IJs larva⁻¹, respectively (Sujithra et al., 2022).

In Egypt, Adly et al. (2022) treated the larvae of *T. baal* with *S. glaseri* and *H. bacteriophora* and calculated LC₅₀ values in 937.44 and 1026.58 IJs/larvae, respectively. In Egypt, also, Nough (2022) assessed the infectivity of *H. bacteriophora*, *S. glaseri* and *S. carpocapsae* against larvae of three insects. According to her results, LC₅₀ values against 4th instar larvae of the potato tuber moth *Phthorimaea operculella* were 127.1496 and 148.5994 IJs/larva, respectively, while the LC₅₀ value of *S. glaseri* was 247.2687 IJs/larva. The LC₅₀ values of *S. glaseri* were 1073.640 and 1330.312 IJs/larva against 2nd and 3rd instars larvae of the white grub *Pentodon bispinosus*, respectively. LC₅₀ values of *H. bacteriophora* and *S. carpocapsae* against the 3rd and 5th instar larvae of *S. littoralis* were (38.489 and 40.791) and (42.149 and 51.932) IJs/larva, respectively, while LC₅₀ values of *S. glaseri* were 70.106 and 95.295 IJs/larva, respectively.

In conclusion, LC₅₀ values usually depend on the susceptibility of the infected insect and its stage or size, virulence of the tested EPN and its concentration rates, infective juvenile age, time of exposure and different experimental factors affecting penetration of EPN species.

Sensitive Larval Instars of *A. ipsilon* to the Selected EPNs

In the present study, the determined LC₅₀ values of the tested two EPNs, *S. carpocapsae* and *H. bacteriophora*, revealed that the 4th instar larvae of *A. ipsilon* were more susceptible or sensitive than the 5th instar larvae to the nematode pathogenicity. This result was, to some extent, in agreement with some reported results of 4th larval instar as the most sensitive one to different EPNs. For examples, the 4th larval instar of *A. ipsilon* was the most susceptible instar to the same EPNs (Ebssa and Koppenhöfer, 2012). The same larval instar of *A. ipsilon* was found more sensitive than 2nd instar larvae to *S. carpocapsae* GSN1 strain (ScG) (Lee and Potter, 2015). The 4th larval instar of the cigarette beetle *Lasioderma serricorne* (Coleoptera) was found more

sensitive than 3rd instar to nine isolates of *H. bacteriophora* (Giannasi et al., 2018). The 4th instar nymphs of the American palm planthopper *Haplaxius crudus* (Hemiptera) were the most susceptible to some EPNs (Guerrero and Pardey, 2019). The 4th instar larvae of *P. brassicae* were more susceptible than 2nd instar larvae to the two EPNs local strains; *S. feltiae* HR1 and *H. bacteriophora* HR2EPN strains (Kasi et al., 2021). Recently, the 4th instar larvae of *A. segetum* were more susceptible to infective juveniles (IJs) of all tested Turkish native EPN isolates at the concentration of 100 IJs/cm² after 72 hrs of exposure (Yüksel et al., 2022). In *H. armigera*, *S. litura* and *A. segetum*, the 4th instar larvae were more sensitive to the pathogenicity of *H. bacteriophora* than 3rd instar larvae (Thakur et al., 2022).

On the contrary, the present result was inconsistent with different reported results of sensitive larval instars other than 4th instar of some insects to various EPNs, such as the 3rd instar nymphs of *S. gregaria* seemed to be the most susceptible to *S. carpocapsae*, followed by the 5th nymphal instar (Youssef, 2014; Gaber et al., 2018); the *C. capitata* 3rd instar larvae were more susceptible to the native strains (*Heterorhabditis* sp. LPP14 and *Heterorhabditis* sp. LPP17) than to the exotic strain (*Heterorhabditis baujardi* LPP7) (Minas et al., 2016); 2nd, 3rd and 4th larval instars of *T. absoluta* were found more sensitive than 1st larval instar to some EPNs (Husin, 2017); the 2nd instar of both *S. littoralis* and *A. ipsilon* was of higher susceptibility to *S. monticolum* and *H. bacteriophora* (HP88) than other larval instars (Sobhy et al., 2020); 2nd and 3rd instar larvae of *P. brassicae* were more sensitive than 4th instar to *H. pakistanensis* (Askary and Ahmad, 2020); and the 1st, 2nd and 3rd instars were more sensitive than 4th, 5th and 6th instar larvae of *S. frugiperda* to *S. glaseri* (Meka et al., 2020). Recently, Wattanachaiyingcharoen et al. (2021) reported that the 2nd larval instar was more sensitive than 5th instar of *S. frugiperda* to two Thai indigenous EPNs isolates of *H. indica* and *S. siamkayai*. The 3rd instar larvae of *S. littoralis* were more sensitive than other larval instars to *S. feltiae* (100% mortality) (Yağcı et al., 2022). Depending on the mortality bioassay conducted by Mohamed and Shairra (2023), the 2nd and 3rd larval instars of *S. frugiperda* were highly susceptible to *H. indica* infection than 5th and 6th instar larvae.

The sensitivity of certain larval instar (s) to the EPNs could be attributed to the variation in genetics (Gaugler et al., 1989), infectivity (Griffin and Downes, 1991), physiology (Fitters et al., 1999), climatic adaptation (Solomon et al., 1999), and morphology of different nematode strains (Stock et al., 2000).

One EPN Species is More Pathogenic than Another Species Against *A. ipsilon*

On the basis of LC₅₀ values of the tested EPNs, in the present study, *S. carpocapsae* was more pathogenic than *H. bacteriophora* against both 4th and 5th instar larvae of *A. ipsilon*. The present result was agreeable to some reported results of *S. carpocapsae* as more virulent than other EPNs against the present insect. For examples, *S. carpocapsae* performed better than *H. bacteriophora* against *A. ipsilon* with a high speed of kill after 4 days under golf course conditions (Ebssa and Koppenhofer, 2011). Some Petri dish studies recorded that *S. carpocapsae* was found more virulent than *H. bacteriophora* against the *A. ipsilon* larvae (Bélair et al. 2013; Mahmoud et al., 2016).

Apart from *A. ipsilon*, Shapiro-Ilan and Cottrell (2006) reported that both *S. feltiae* and *S. carpocapsae* performed better than *H. bacteriophora* against the lesser peach tree borer *Synanthedon pictipes*. Also, *S. feltiae* was higher pathogenic than *H. bacteriophora* against the

olive fruit fly *Bactrocera oleae* larvae (Sirjani et al., 2009). *S. glaseri* was more effective than *H. bacteriophora* against the 3rd larval instar of *T. baal* (Atwa and Hassan, 2014). Full-grown larvae of *P. gossypiella* were more susceptible to *S. riobrave* than to *H. bacteriophora* (Shairra et al., 2016). EPN *S. glaseri* was more pathogenic than *H. indica* against 3rd instar larvae of the brinjal ash weevil *Myloccerus subfasciatus* (Nithiskarani et al., 2019). As reported by Rohde et al. (2020), both EPNs *S. carpocapsae* ALL and *H. amazonensis* JPM4 killed *C. capitata* larvae and pupae, but *S. carpocapsae* ALL was the most pathogenic EPN. According to results of Mazurkiewicz et al. (2020), the *Steinernema* strains were more pathogenic than *H. megidis* against the cabbage moth *Mamestra brassicae* larvae.

Recently, Pratisoli et al. (2021) evaluated the pathogenicity of *H. indica* and *S. carpocapsae* on pre-pupae of the fruit borer moth *Neoleucinodes elegantalis*. They found *S. carpocapsae* as more pathogenic than *H. indica*. In a study conducted by Peçen and Kepenekci (2022) on wheat stink bug *Aelia rostrata* adults, *S. carpocapsae* (Black sea isolate) was the most pathogenic among the three nematodes tested including *S. feltiae* and *H. bacteriophora* isolates. Also, larvae of *T. baal* were more susceptible to the infection with *S. glaseri* than *H. bacteriophora*, at the highest concentration (4000 IJs/larva)(Adly et al., 2022).

In contrast to our results, some studies reported that *Heterorhabditis* spp. were more virulent than *Steinernema* spp. against some insects. For example, Hussaini et al. (2005) found the isolates of *H. indica* more virulent than the isolate of *S. carpocapsae* against the *A. ipsilon* larvae. Also, *H. bacteriophora* was more pathogenic than *S. riobrave* and *S. feltiae* to *S. littoralis* larvae (Ahmed et al., 2014). EPNs *H. bacteriophora* strains were found more virulent than *S. carpocapsae* stains against late 3rd instar larvae of *C. capitata* (Shaurub et al., 2015). Ulu et al. (2015) recorded *H. bacteriophora* as more virulent than *S. carpocapsae* against larvae of the yellow sawfly *Hoplocampa flava*. Al-Ghnam and Heikal (2017) studied the activity of the EPNs, *S. carpocapsae* and *H. bacteriophora* against *P. operculella* larvae and pupae. Their results revealed that *H. bacteriophora* was more pathogenic than *S. carpocapsae* all concentrations. Depending on results of Gomaa et al. (2020), *H. bacteriophora* was more virulent than *S. carpocapsae* against 3rd instar larvae of *S. littoralis*. In their study, Bhairavi et al. (2021) concluded that *H. bacteriophora* displayed more virulence than *S. aciari* toward the subterranean termite *Odontotermes obesus* and *A. ipsilon*. Taha (2021) studied the susceptibility of the 5th instar larvae of *S. littoralis* to *S. carpocapsae*, and *H. bacteriophora*. Their data revealed that *H. bacteriophora* was the higher effective than *S. carpocapsae* on larvae causing 100% mortality after 48 h post-treatment. In addition, some surveys in the Egyptian soils revealed that species of family Heterorhabditidae were more prevalent than species of family Steinernematidae for biological control of some insect pests (Aashaq et al., 2020; Nouh, 2021).

As previously mentioned in the current study, *S. carpocapsae* was more pathogenic than *H. bacteriophora* against 4th and 5th instar larvae of *A. ipsilon*. To understand this result, it is very important to point out that mortality rates might be affected by the foraging strategies of EPNs. *S. carpocapsae* is considered to be more effective than other species in controlling moving insects due to its foraging behavior, and Petri dish might have created an environment that is more favorable to *S. carpocapsae* (Campbell and Gaugler, 1993; Shapiro et al., 1999; Ebssa and Koppenhöfer, 2012; Hassan et al., 2016). Also, smaller nematodes were more infective and

induced mortality more quickly (Shannag et al., 1994). The present result could be, also, explained by variations in strategies of the host or from the entrance of IJs in the insect body due to penetrate the interior of the insects occur through the mouth, anus, blow holes and track cuticle (Lewis et al., 2006; Chaneiko et al., 2021).

Some EPN-Infected Larvae of *A. ipsilon* Could Successfully Transform into the Next Stage

In the present study, a few EPN-infected 4th instar larvae of *A. ipsilon* could pupate at the lower two concentrations of *S. carpocapsae* and *H. bacteriophora*. In the present study, also, a similar result was recorded after infection of 5th instar larvae with *S. carpocapsae*. Moreover, the infected larvae with *H. bacteriophora* successfully pupated at all concentrations except the highest one (6.7, 13.3, 20.0 & 20.0% pupation, at 100, 50, 25 & 12 IJs/ml, respectively). As far as our literature survey could ascertain, no information was available on the successful metamorphosis of EPN-treated insect larvae into pupae. Unfortunately, there is no scientific explanation of our result right now!!

However, some studies reported the capability of EPN-treated pupae to transform into adults although the pupal stage is less conducive for EPNs (Malan et al., 2011; Odendaal et al., 2016; du Preez et al., 2021; Steyn et al., 2021). Whatever, treatment of Tobacco thrips, *Frankliniella fusca*, pupae (the soil dwelling stage) with *H. bacteriophora* (Fl1-1), *S. feltiae*, *S. riobrave* and *S. rarum* resulted in reduction of the adult emergence (18.3–75.0%)(Gulzar et al., 2021). When employing the high IJ concentrations of four EPN species (*S. feltiae*, *S. carpocapsae*, *S. riojaense* and *H. bacteriophora*) against pupae of the European grapevine moth *Lobesia botrana*, adult emergence was significantly blocked (Vicente-Díez et al., 2021). In a study of Filgueiras and Willett (2021), the presence of EPNs can alter the developmental times and changes in the risk of death of the non-susceptible pupal stage of the onion fly *Delia antiqua*.

CONCLUSION

As shown in the present study, neither *Steinernema carpocapsae* nor *Heterorhabditis bacteriophora* caused 100% mortality of *A. ipsilon* larvae. However, both ENPs exhibited high insecticidal activity, *in situ* or associated with their symbiotic bacteria, against these larvae. EPN *S. carpocapsae* was more pathogenic than *H. bacteriophora*. Therefore, *S. carpocapsae* may be effective microbial control agent in the integrated pest management program against *A. ipsilon*.

Acknowledgement

The authors deeply thank Dr. El-Sadawy, National Research Centre, Doqqi, Giza, Egypt, for kindly providing imported EPN species, *H. bacteriophora* and *S. carpocapsae*.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

Funding

Not applicable

Author Contributions

Ghoneim, K.: planning and writing the manuscript; Tanani, M.: statistical analysis of data; Hassan, H.A. & Bakr, N.A.: conduction of the experiments. All authors have read and agreed to the manuscript.

References

- Aashaq, H.B., Ashok, K.C., Tarique, H.A., 2020. Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. *Egyptian Journal of Biological Pest Control*, 30(1):1–15. <https://doi.org/10.1186/s41938-020-0212-y>
- Abd El Azim, A.M., 2022. Efficacy of the entomopathogenic nematode isolate *Heterorhabditis taysearae* to control the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 32:4 <https://doi.org/10.1186/s41938-022-00503-z>
- Abd-El-Aziz, H.S., Abd El Mageed, E.N.I., Salama, M.A.S., 2019. Field Evaluation of some insecticides for controlling black cutworm, *Agrotis ypsilon* and their effect on some histological aspects. *Egyptian Academic Journal of Biological Sciences*, 11(2): 57- 68.
- Abd-Elgawad, M.M.M., 2020. Can rational sampling maximise isolation and fix distribution measure of entomopathogenic nematodes? *Nematology* 22(8): 907–916. <https://doi.org/10.1163/15685411-00003350>
- Abdel-Hakim, E.A., El-Mandarawy, M.B., 2017. Effects of juvenile hormone mimic on growth, morphogenesis and morphology of hemocytes of the black cutworm, *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae). *Current Science International*, 6(3): 662-669.
- Abdin, M.I., 1979. Standard technique for mass rearing of the black cutworm, *Agrotis ipsilon*. M.Sc. Thesis, Faculty of Agriculture, Al-Azhar University, Egypt.
- Acharya, R., Hwang, H.-S., Mostafiz, M.M., Yu, Y.-S., Lee, K.-Y., 2020. Susceptibility of various developmental stages of the fall armyworm, *Spodoptera frugiperda*, to entomopathogenic nematodes. *Insects*, 11: 868, 13pp. [doi:10.3390/insects11120868](https://doi.org/10.3390/insects11120868)
- Adams, B.J., Nguyen, K.B., 2002. Taxonomy and systematic. In: "Entomopathogenic Nematology" (Gauglar, R. (ed.). CABI, New York, NY, pp: 1-34.
- Adly, D., Nouh, G.M., Eid, A.E., 2022. Application of entomopathogenic nematodes to control larvae of *Temnorhynchus baal* (Reiche and Saulcy) under laboratory conditions and in strawberry fields. *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, 15(3):67-77. DOI: 10.21608/EAJBSA.2022.256736
- Ahmed, N.F., Maklad, A.H.M., Yassin, S.A., Abolmaaty, S.M., 2014. Biochemical effects of *Steinernema feltiae*, *Steinernema riobrave* and *Heterorhabditis bacteriophora* on *Spodoptera littoralis* larvae. *Egyptian Academic Journal of Biological Sciences*, 6: 23-34.
- Ahmed, A.A.I., Khalil, S.S.H., Sahab, A.F., 2022. Identification and evaluation of isolated entomopathogenic fungus from Egyptian soil against the black cutworm larvae of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 32:67 <https://doi.org/10.1186/s41938-022-00564-0>
- Al-Ghnam, H.A.A., Heikal, G.A.M., 2017. Evaluating the role of entomopathogenic nematodes for the biological control of the potato tuber moth, *Phthorimaea operculella* under laboratory conditions. *Journal of Plant Protection and Pathology*, Mansoura University, Egypt, 8 (11): 577-580.
- Ali, M., Allouf, N., Ahmad, M., 2022. First report of entomopathogenic nematode *Steinernema affine* (Nematoda: Steinernematidae) in Syria and its virulence against *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Egyptian Journal of Biological Pest Control* 32:101 <https://doi.org/10.1186/s41938-022-00602-x>
- Amutha, V., Vengateswari, G., Shivakumar, M.S., 2021. Entomopathogenicity of nematode *Panagrolaimus* spp. (Rhabditida: Panagrolaimidae) against lepidopteran pest *Spodoptera litura*, *International Journal of Pest Management*, 67(4): 320-327, DOI: 10.1080/09670874.2020.1776415

- Andersch, W., Schwarz, M., 2003. Clothianidin seed treatment (PonchoReg.) - the new technology for control of corn rootworms and secondary pests in US-corn production. (Pflanzenschutz Nachrichten Bayer), 56(1):147-172.
- Arthurs, S., Heinz, K.M., Prasifka, J.R., 2004. An analysis of using entomopathogenic nematodes against above-ground pests. Bulletin of Entomological Research, 94(4): 297-306.
- Askary, T.H., Abd-Elgawad, M.M.M., 2021. Opportunities and challenges of entomopathogenic nematodes as biocontrol agents in their tripartite interactions. Egyptian Journal of Biological Pest Control (2021) 31:42, 10pp. <https://doi.org/10.1186/s41938-021-00391-9>
- Askary, T.H., Ahmad, M.J., 2020. Efficacy of entomopathogenic nematodes against the cabbage butterfly (*Pieris brassicae* (L.) (Lepidoptera: Pieridae) infesting cabbage under field conditions. Egyptian Journal of Biological Pest Control, 30:39, 7pp. <https://doi.org/10.1186/s41938-020-00243-y>
- Atwa, A.A., Hassan, S.H., 2014. Bioefficacy of two entomopathogenic nematodes against *Spodoptera littoralis* Boisduval (Lepidoptera) and *Temnorhynchus baal* Reiche (Coleoptera) larva. Journal of Biopesticides, 7(2):104-109.
- Bal, H.K., Grewal, P.S., 2015. Lateral dispersal and foraging behavior of entomopathogenic nematodes in the absence and presence of mobile and non-mobile hosts. PLoS ONE 10(6), e0129887. <https://doi.org/10.1371/journal.pone.0129887>.
- Barbosa-Negrisoni, C.R.C., Garcia, M.S., Dolinski, C., Negrisoni, A.S. Jr., Bernardi, D., Nava, D.E., 2009. Efficacy of indigenous entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae), from Rio Grande do Sul Brazil, against *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) in peach orchards. Journal of Invertebrate Pathology, 102: 6–13. <https://doi.org/10.1016/j.jip.2009.05.005>.
- Baur, M.E., Kaya, H.K., Tabashnik, B.E., 1997. Efficacy of a dehydrated steinernematid nematode against black cutworm (Lepidoptera: Noctuidae) and diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology, 90(5): 1200-1206.
- Bedding, R.A., Molyneux, A.S., 1982. Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). Nematologica, 28: 354-359. doi:10.1163/187529282X00402
- Bélair, G., Simard, L., Dionne, J., 2013. Canadian entomopathogenic nematode isolates: virulence against black cutworm (Lepidoptera: Noctuidae). Phytoprotection, 93(1): 43–46.
- Bento, F.M.M., Magro, S.R., Fortes, P., Zério, N.G., Parra, J.R.P., 2007. Biology and fertility life table of *Agrotis ipsilon* on artificial diet. Pesquisa Agropecuária Brasileira, 42: 1369-1372. DOI: 10.1590/S0100-204X2007001000001
- Bhairavi, K.S., Bhattacharyya, B., Devi, G., Bhagawati, S., Das, P.P.G., Devi, E.B., Manpoong, N.S., 2021. Evaluation of two native Entomopathogenic nematodes against *Odontotermes obesus* (Rambur) (Isoptera: Termitidae) and *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 31:111, 8pp. <https://doi.org/10.1186/s41938-021-00457-8>
- Bhat, A.H., Chaubey, A.K., Askary, T.H., 2020. Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. Egyptian Journal of Biological Pest Control, 30:31. <https://doi.org/10.1186/s41938-020-0212-y>
- Binning, R.R., Coats, J., Kong, X., Hellmich, R.L., 2015. Susceptibility to *Bt* proteins is not required for *Agrotis ipsilon* a version to *Bt* maize. Pest Management Science, 71: 601–606. doi:10.1002/ps. 3901PMID: 25186105

Bobardt, S.D., Dillman, A.R., Nair, M.G., 2020. The two faces of nematode infection: Virulence and immunomodulatory molecules from nematode parasites of mammals, insects and plants. *Frontiers in Microbiology*, 11:577846, 13pp. doi: 10.3389/fmicb.2020.577846

Bowen, D., Rocheleau, T.A., Blackburn, M., Andreev, O., Golubeva, E., Bhartia, R., French-Constant, R.H., 1998. Insecticidal toxins from the bacterium *Photorhabdus luminescens*. *Science*, 280: 2129-2132.

Brown, S.E., Cao, A.T., Dobson, P., Hines, E.R., Akhurst, R.J., East, P.D., 2006. Txp40, a ubiquitous insecticidal toxin protein from *Xenorhabdus* and *Photorhabdus* bacteria. *Applied and Environmental Microbiology Journal*, 72: 1653-1662.

CABI (Centre for Agriculture and Biosciences International), 2021. Invasive species compendium, *Agrotis ipsilon* (black cutworm). Retrieved January 9, 2022, from <https://www.cabi.org/isc/datasheet/3801>

Caccia, M.G., Del Valle, E., Doucet, M.E., Lax, P., 2014. Susceptibility of *Spodoptera frugiperda* and *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae) to the entomopathogenic nematode *Steinernema diaprepesi* (Rhabditida: Steinernematidae) under laboratory conditions. *Chilean Journal of Agricultural Research*, 74: 123-126.

Campbell, J.F., Gaugler, R., 1993. Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour*, 126(3): 155-169.

Capinera, J.L., 2001. Hand book of vegetable pests. Academic Press, New York, 729 pp.

Capinera, J.L., Epsky, N.D., Menout, G.S., Pelissier, D., 1988. Control of black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae), with entomogenous nematodes (Nematoda: Steinernematidae, Heterorhabditidae). *Journal of Invertebrate Pathology*, 52(3):427-435. [https://doi.org/10.1016/0022-2011\(88\)90055-9](https://doi.org/10.1016/0022-2011(88)90055-9)

Castillo, J.C., Reynolds, S.E., Eleftherianos, I., 2011. Insect immune responses to nematode parasites. *Trends of Parasitology*, 27(12): 537-547. <https://doi.org/10.1016/j.pt.2011.09.001>

Chaneiko, S.M., de Brida, A.L., Bernardi, D., Leite, L.G., Garcia, F.R.M., 2021. Biological activity of entomopathogenic nematodes on *Anastrepha fraterculus* (Diptera: Tephritidae). *Bioscience Journal*, 37: e37047. <https://doi.org/10.14393/BJ-v37n0a2021-53895>

Chaston, J.M., Murfin, K.E., Heath-Heckman, E.A., Goodrich-Blair, H., 2013. Previously unrecognized stages of species-specific colonization in the mutualism between *Xenorhabdus* bacteria and *Steinernema* nematodes. *Cell Microbiology*, 15: 1545-1559.

Chowański, S., Kudlewska, M., Marciniak, P., Rosiński, G., 2014. Synthetic Insecticides- is there an alternative? *Polish Journal of Environmental Studies*, 23(2): 291-302.

Demok, S., Endersby-Harshman, N., Vinit, R., Timinao, L., Robinson, L.J., Susapu, M., Makita, L., Laman, M., Hoffmann, A., Karl, S., 2019. Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Papua New Guinea. *Parasite Vectors*, 12(1): 333. doi:10.1186/s13071-019-3585-6

Devi, G., Saikia, M., Bhagawati, S., Bhattacharyya, B., 2021. Bioefficacy of entomopathogenic nematode, *Heterorhabditis bacteriophora* against cutworm, *Agrotis ipsilon* damaging potato under field condition. *Journal of Entomology and Zoology Studies*, 9(3): 323-325.

Dichusa, C.A., Ramos Jr, R., Aryal, S., Sumaya, N.P.D., Sumaya, N.H., 2021. Survey and identification of entomopathogenic nematodes in the province of Cotabato, Philippines, for biocontrol potential against the tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 31:60, 10pp. <https://doi.org/10.1186/s41938-021-00390-w>

- Dlamini, B.E., Dlamini, N., Masarirambi, M.T., Nxumalo, K.A., 2020. Control of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae in laboratory using entomopathogenic nematodes from subtropical environment. *Journal of Nematology*, 52, 8pp. DOI: 10.21307/jofnem-2020-013
- Dowds, B.C.A., Peters, A., 2003. Virulence mechanisms. In: "Entomopathogenic Nematology" (Gaugler, R., ed.). CABI Publishing, New Brunswick, New Jersey, USA. pp. 79–90.
- Du Preez, F., Malan, A.P., Addison, P., 2021. Potential of *in vivo* and *in vitro* cultured entomopathogenic nematodes to infect *Lobesia vanillana* (Lepidoptera: Tortricidae) under laboratory conditions. *PLoS ONE* 16(8):e0242645. <https://doi.org/10.1371/journal.pone.0242645>
- Ebssa, L., Koppenhöfer, A.M., 2011. Efficacy and persistence of entomopathogenic nematodes for black cutworm control in turf grass. *Biocontrol Science and Technology*, 21(7):779-796.
- Ebssa, L., Koppenhöfer, A.M., 2012. Entomopathogenic nematodes for the management of *Agrotis ipsilon*: effect of instar, nematode species and nematode production method. *Pest Management Science*, 68(6): 947-957.
- Ehlers, R.U., 2001. Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology and Biotechnology*, 56(5-6): 623-633. <https://doi.org/10.1007/s00253002530100711>.
- El Aalaoui, M., Mokri, F., Dababat, A.A., Lahlali, R., Sbaghi, M., 2022. Moroccan entomopathogenic nematodes as potential biocontrol agents against *Dactylopius opuntiae* (Hemiptera: Dactylopiidae). *Scientific Reports*, 12: 1–17. <https://doi.org/10.1038/s41598-022-11709-4>
- El-Ashry, R.M., Hegab, M.A.M., 2021. Compatibility of native and imported entomopathogenic nematodes with different applications of insecticides for controlling the Cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae). *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, 14(4):19-34. Doi: 10.21608/EAJBSA.2021.198979
- El-Bishry, M.H., El-Assal, F.M., Abd El-Rahman, R.M., 2002. Factors affecting penetration rate of the entomopathogenic nematodes *Heterorhabditis* spp. *Egyptian Journal of Biological Pest Control*, 12(1): 25-38.
- El-Kifl, A.H., Nasr, E.A., Moawad, M., 1972. Effect of host plants on various stages of *Agrotis ipsilon* (Hufnagel). *Bulletin of the Entomological Society of Egypt*, 56: 103–111.
- El-Shershaby, M.M.A., 2010. Toxicity and biological effect of *Capparis* leaves extracts to the black cutworm, *Agrotis ipsilon* (Hufn.). *Egyptian Academic Journal of Biological Sciences (F. Toxicology & Pest Control)*, 2(1): 45-51. doi: 10.21608/eajbsf.2010.17462
- Epsky, N.D., Capinera J.L., 1994. Invasion efficiency as a measure of efficacy of the entomogenous nematode *Steinernema carpocapsae* (Rhabditida, Steinernematidae). *Journal of Economic Entomology*, 87: 366-370. <https://doi.org/10.1093/jee/87.2.366>
- Fahmy, A.R., 2014. Toxicological, biological and biochemical impact of some chitin synthesis inhibitors on the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) (Hufn.). *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, 7(2): 119-128. Doi: 10.21608/EAJBSA.2014.13145
- Fallet, P., De Gianni, L., Machado, R.A., Bruno, P., Bernal, J.S., Karangwa, P., Kajuga, J., Waweru, B., Bazagwira, D., Degen, T., Toepfer, S., 2022. Comparative screening of Mexican, Rwandan and commercial entomopathogenic nematodes to be used against invasive fall armyworm, *Spodoptera frugiperda*. *Insects*, 13(2): 205. doi: 10.3390/insects13020205.
- Fard, N.Z., Abbasipour, H., Saeedizadeh, A., Karimi, J., 2020. Laboratory assay of entomopathogenic nematodes against the elm leaf beetle, *Xanthogaleruca luteola* Müller (Col.: Chrysomelidae). *Journal of Forest Science*, 66(12): 524–531. <https://doi.org/10.17221/89/2020-JFS>

Fetoh, A.S., Khaled, A.S., El-Nagar, T.F.K., 2009. Combined effect of entomophagetic nematodes and biopesticides to control the greasy cut worm, *Agrotis ipsilon* (Hufn.) in the strawberry fields. *Egyptian Academic Journal of Biological Sciences*, 2: 227-236. Doi: 10.21608/EAJBSA.2009.15718

Ffrench-Constant, R.H., Dowling, A., Waterfield, N.R., 2007a. Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. *Toxicon*, 49: 436–451. doi: 10.1016/j.toxicon. 2006.11.019.

Ffrench-Constant, R.H., Eleftherianos, I., Reynolds, S.E., 2007b. Nematode symbionts shed light on invertebrate immunity. *Trends in Parasitology*, 23: 514-517. <https://doi.org/10.1016/j.pt.2007.08.021>

Filgueiras, C.C., Willett, D.S., 2021. Non-lethal effects of entomopathogenic nematode infection. *Scientific Reports*, 11: 17090. <https://doi.org/10.1038/s41598-021-96270-2>

Fitters, P.F.L., Patel, M.N., Griffin, C.T., Wright, D.J., 1999. Fatty acid composition of *Heterorhabditis* sp. during storage. *Comparative Biochemistry and Physiology*, 124: 81-88. doi:10.1016/S0305-0491(99)00102-9.

Gaber, M.A.M., Shamseldean, M.S.M., Ibrahim, N.M., Rabia, M.M., 2018. Impact and biochemical changes of Egyptian and imported entomopathogenic nematodes on the desert locust *Schistocerca gregaria* (Forsk., 1775) (Orthoptera: Acrididae). *Middle East Journal of Agricultural Research*, 7(4): 1528-1544.

Gaugler, R., Campell, J.F., McGuire, T.R., 1989. Selection for host-finding in *Steinernema feltiae*. *Journal of Invertebrate Pathology*, 54: 363-372. doi:10.1016/0022-2011(89)90120-1.

George, J., Devi, G., Bhattacharyya, B., 2019. Survival and infectivity of entomopathogenic nematode *Oscheius rugaoensis* in different formulations against wax moth, *Galleria mellonella*. *Journal of Entomology and Zoology Studies*, 7(3): 241-244.

Giannasi, A.de O., Brambila, C.R., Zart, M., Guide, B.A., Alves, V.S., 2018. Assessment of entomopathogenic nematodes in *Agrotis ipsilon* (Lepidoptera: Noctuidae) under laboratory and greenhouse conditions. *Revista Colombiana de Entomología*, 44(1): 25-31. DOI: 10.25100/socolen.v44i1.6533

Gill, R.J., Ramos-Rodriguez, O., Raine, N.E., 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature*, 491(7422): 105-108. DOI: 10.1038/nature11585

Glare, T.R., Gwynn, R.L., Moran-Diez, M.E., 2016. Development of biopesticides and future opportunities. In: "Microbial-Based Biopesticides: Methods and Protocols" (Glare, T.R. and Moran-Diez, M.E., eds.). New York: Springer Science+Business Media BV: 211-221.

Gomaa, Sh.I., Halawa, S.M., Shalaby, F.F., Abdel-Hafez, H.F., 2020. Toxicological and biochemical parameters of microbial preparations on the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Egyptian Journal of Plant Protection Research Institute*, 3(1): 204-214.

Gorgadze, O., Bakhtadze, G., Nebieridze, D., 2018. Efficacy of local entomopathogenic nematodes against *Pieris brassicae* (L, 1758) (Lepidoptera: Pieridae). *International Journal of Development Research*, 8: 23900–23903.

Goudarzi, M., Moosavi, M.R., Asadi, R., 2015. Effects of entomopathogenic nematodes, *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser), in biological control of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae). *Turkish Journal of Entomology*, 39(3): 239-250. doi: 10.16970/ted.43220

GraphPad InStat® v. 3.01, 1998. GraphPad Software, Inc. 7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA. Available online at: <http://www.graphpad.com/scientific-software/instat/>

Griffin, C.T., 2012. Perspectives on the behavior of entomopathogenic nematodes from dispersal to reproduction: traits contributing to nematode fitness and biocontrol efficacy. *Journal of Nematology*, 44(2):177-184.

- Griffin, C.T., Downes, M.J., 1991. Low temperature activity in *Heterorhabditis* sp. (Nematoda: Heterorhabditidae). *Nematologica*, 37: 83-91. doi:10.1163/187529291X00088.
- Griffin, C.T., Boemare, N.E., Lewis, E.E., 2005. Biology and behavior. In: "Nematodes as Biocontrol Agents" (Grewal, P.S. et al., eds.), 1st ed., (CABI Publishing, Wallingford, 2005), PP: 47-59.
- Guerrero, M.R., Pardey, A.E.B., 2019. Selection of entomopathogenic nematodes to control nymphs of *Haplaxius crudus* (Van Duzee) (Hemiptera: Cixiidae). *American Journal of Entomology*, 3(1): 24-29. doi: 10.11648/j.aje.20190301.14
- Gulzar, S., Usman, M., Wakil, W., Wu, Sh., Oliveira-Hofman, C., Srinivasan, R., Toews, M., Shapiro-Ilan, D., 2021. Virulence of entomopathogenic nematodes to pupae of *Frankliniella fusca* (Thysanoptera: Thripidae). *Journal of Economic Entomology*, 114(5): 2018- 2023. <https://doi.org/10.1093/jee/toab132>
- Harrison, R.L., Lynn, D.E., 2008. New cell lines derived from the black cutworm, *Agrotis ipsilon*, that support replication of the *A. ipsilon* multiple nucleopolyhedrovirus and several group I nucleopolyhedroviruses. *Journal of Invertebrate Pathology*, 99: 28-34. doi: 10.1016/j.jip.2008.02.015.
- Hassan, H.A.; Shairra, S.A. and Ibrahim, S.S. (2016): Virulence of entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora* Poinar (HP88strain) against the black cutworm, *Agrotis ipsilon*. *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, 9(1): 33-48. Doi: 10.21608/EAJBSA.2016.12853
- Husin, B.A.T.O., 2017. Biological control of tomato leaf miner *Tuta absoluta* using entomopathogenic nematodes. Ph.D. Thesis, Newcastle University, available at: <http://hdl.handle.net/10443/3845>
- Hussaini, S.S., Singh, S.P., Nagesh, M., 2002. *In vitro* and field evaluation of some indigenous isolates of *Steinernema* and *Heterorhabditis indica* against shoot and fruit borer, *Leucinodes orbonalis*. *Indian Journal of Nematology*, 32(1): 63-65.
- Hussaini, S.S., Shakeela, V., Dar, M.H., 2005. Influence of temperature on infectivity of entomopathogenic nematodes against black cutworm, *Agrotis ipsilon* (Hufnagel) and greater wax moth, *Galleria mellonella* (Linnaeus) larvae. *Journal of Biological Control*, 19(1): 51-58.
- Ibarra, J.E., Castro, M.C.D.R.m Galindo, E., Patino, M., Serrano, L., Garcia, R., Carrillo, J.A., Pereyra-Alferez, B., Alcazar-Pizana, A., Luna-Olvera, H., 2006. Microorganisms in the biological control of insects and phytopathogens. *Revista Latinoamericana de Microbiología*, 48(2):113-120.
- Ismail, S.M., 2021. Field persistence of certain new insecticides and their efficacy against black cutworm, *Agrotis ipsilon* (Hufnagel). *Bulletin of the National Research Center*, 45:17, 7pp. <https://doi.org/10.1186/s42269-020-00481-y>
- Jagodič, A., Trdan, S., Laznik, Ž., 2019. Entomopathogenic nematodes: can we use the current knowledge on belowground multitrophic interactions in future plant protection programmes?- Review. *Plant Protection Science*, 55(4): 243- 254. DOI: 10.17221/24/2019-PPS
- Javed, S., Khanum, T.A., Ali, A., 2022. Storage and efficacy of entomopathogenic nematode species as a biocontrol agent against the armyworm, *Spodoptera litura* (Fabricius)(Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 32:6 <https://doi.org/10.1186/s41938-022-00505-x>
- Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. *Journal of Agriculture and Food Chemistry*, 59: 2897-2908. <https://doi.org/10.1021/jf101303g>
- Jian, H., Yang, X., Liu, Z., Yang, H., Miao, Z., 2002. Infectivity of entomopathogenic nematode *Steinernema ceratophorum* to bark beetle *Dendroctonus valens* larvae. *Scientia Silvae Sinicae*, 38:157-159.

Kaliaskar, D., Shibaeva, A., Zhappar, N., Shaikhutdinov, V., Asherbekova, L., Bekbulatov, S., Kalyaskarova, A., 2022. The efficiency of aboriginal entomopathogenic nematodes from semi-arid zone against Tenebrionidae larvae with comparison to commercial bio-insecticides. *AGRIVITA Journal of Agricultural Science*, 44(3): 526-536. <http://doi.org/10.17503/agrivita.v44i3.3760>

Kasi, I.K., Singh, M., Waiba, K.M., Monika, S., Waseem, M.A., Archie, D., Gilhotra, H., 2021. Bio-efficacy of entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the Cabbage butterfly (*Pieris brassicae* [L.]) under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 31:125, 7pp. <https://doi.org/10.1186/s41938-021-00469-4>

Kaya, H.K., Bedding, R.A., Akhurst, R.J., 1993. An overview of insect-parasitic and entomopathogenic nematodes. In: "Nematodes and the Biological Control of Insect Pests" (Bedding, R.A.; Akhurst, R.J. and Kaya, H.K., eds.). Australia, pp. 1-10

Khashaba, E.H.K., Moghaieb, R.E.A., Abd El Azim, A.M., Ibrahim, S.A.M., 2020. Isolation, identification of entomopathogenic nematodes, and preliminary study of their virulence against the great wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Egyptian Journal of Biological Pest Control*, 30:55, 7pp. <https://doi.org/10.1186/s41938-020-00257-6>

Khattab, M., Azazy, A.M., 2013. Efficacy of entomopathogenic nematodes as bait formulations for controlling the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 23(2): 255-259.

Kononenko, V.S., 2003. Noctuidae. In: "Keys to the insects of the Russian Far East" (Ler P.A., ed.). V. 5(4). Trichoptera and Lepidoptera. Vladivostok: Dal.nauka. 688pp. (In Russian with English summary).

Koppenhofer, A.M., Fuzy, E.M., 2003. Ecological characterization of *Steinernema scarabaei*, a scarab-adapted Entomopathogenic nematode from New Jersey. *Journal of Invertebrate Pathology*, 83:139-148. doi: 10.1016/s0022-2011(03)00056-9.

Koppenhöfer, A.M., Shapiro-Ilan, D.I., Hiltbold, I., 2020. Entomopathogenic nematodes in sustainable food production. *Frontiers in Sustainable Food Systems*, 4:125. <https://doi.org/10.3389/fsufs.2020.00125>

Kumar, P., Ganguly, S., Somvanshi, V.S., 2015. Identification of virulent entomopathogenic nematode isolates from a countrywide survey in India. *International Journal of Pest Management*, 61(2): 135-143.

Kumar, D., Kumari, P., Kamboj, R., Kumar, A., Banakar, P., Kumar, V., 2022. Entomopathogenic nematodes as potential and effective biocontrol agents against cutworms, *Agrotis* spp.: present and future scenario. *Egyptian Journal of Biological Pest Control*, 32:42 <https://doi.org/10.1186/s41938-022-00543-5>

Kunbhar, S., Rajput, L.B., Ahmed, G.A., Akber, C.G., Sahito, J.G.M., 2018. Impact of botanical pesticides against sucking insect pests and their insect predators in brinjal crop. *Journal of Entomology and Zoology Studies*, 6: 83-87.

Kunkel, B.A. and Grewal, P.S. (2003): Endophyte infection in perennial ryegrass reduces the susceptibility of black cutworm to an entomopathogenic nematode. *Entomologia Experimentalis et Applicata*, 107: 95-104.

Lacey L.A., Georgis R., 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology*, 44(2): 218-225.

Lacey, L.A., Frutos, R., Kaya, H.K., Vail, P., 2001. Insect pathogens as biological control agents: do they have a future? *Biological Control*, 21: 230-248. <https://doi.org/10.1006/bcon.2001.0938>

Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S., 2015. Insect pathogens as biological control agents: back to the future. *Journal of Invertebrate Pathology*, 132: 1-41. <https://doi.org/10.1016/j.jip.2015.07.009>

Lalramliana, Y.A.K., 2009. Laboratory evaluation of the pathogenicity of three entomopathogenic nematodes against larvae of cabbage butterfly, *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae). *Science Vision*, 9: 166-173.

Lalramnghaki, H.C., Lalramliana, Y.A.K., Lalremsanga, H.T., Vanlalhlhlimpuia, Lalramchuani, M., Vanramliana, 2021. Susceptibility of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), to four species of Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from Mizoram, North-Eastern India. *Egyptian Journal of Biological Pest Control*, 31:110, 11pp. <https://doi.org/10.1186/s41938-021-00453-y>

Laznik, Z., Trdan, S., 2012. Entomopathogenic nematodes (Nematoda: Rhabditida) in Slovenia: from tabula rasa to implementation into crop production systems. In: "Insecticides Advances in Integrated Pest Management" (Perveen F, ed.), pp: 627-656. DOI: 10.5772/29540

Laznik, Ž., Tóth, T., Lakatos, T., Vidrih, M., Trdan, S., 2010. Control of the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) on potato under field conditions: a comparison of the efficacy of foliar application of two strains of *Steinernema feltiae* (Filipjev) and spraying with thiametoxam. *Journal of Plant Diseases and Protection*, 117: 129–135. <https://doi.org/10.1007/BF03356348>

Laznik, Ž., Vidrih, M., Trdan, S., 2012. The effects of different fungicides on the viability of entomopathogenic nematodes *Steinernema feltiae* (Filipjev), *S. carpocapsae* Weiser and *Heterorhabditis downesi* Stock, Griffin and Burnell (Nematoda: Rhabditida) under laboratory conditions. *Chilean Journal of Agricultural Research*, 72:62-67.

Lee, D.W., Potter, D.A., 2015. Biological control of the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) with the Korean entomopathogenic nematode, *Steinernema carpocapsae* GSN1 strain (Rhabditida: Steinernematidae) in turfgrasses. *Weed Turfgrass Science*, 4(1): 58~64. <http://dx.doi.org/10.5660/WTS.2015.4.1.58>

Leite, L.G., Tavares, F.M., Bussóla, R.A., Amorim, D.S., Ambrós, C.M., Harakava, R., 2007. Virulence of entomopathogenic nematodes (Nemata: Rhabditida) against larva of the fungus gnat *Bradysia mabiusi* (Iane, 1959) and persistence of *Heterorhabditis indica* Poinar et al. 1992 on organic substrates. *Arquivos do Instituto Biológico* 74:337–342.

Lemma, E., Albrecht, M.K., 2012. Entomopathogenic nematodes for the management of *Agrotis ipsilon*: effect of instar, nematode species and nematode Pest Management Science, 68 (6): 947-957.

Leonar, A.L., Nimkingrat, P., Aryal, S., Martinez, J.G., Bhat, A.H., Sumaya, N.H., 2022. Natural association of the entomopathogenic nematode *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) from the Philippines with the non-symbiotic bacterium *Ochrobactrum anthropi* (Proteobacteria: Brucellaceae). *Egyptian Journal of Biological Pest Control*, 32:83 <https://doi.org/10.1186/s41938-022-00576-w>

Lewis, E.E., Clarke, D.J., 2012. Nematode parasites and entomopathogens. In: "Insect Pathology" (Vega, F.E. & Kaya, H.K., eds.), 2nd ed., Amsterdam: Elsevier pp: 395-424.

Lewis, E.D., Campbell, J., Griffin, C., Kaya, H., Peters, A., 2006. Behavioral ecology of entomopathogenic nematodes. *Biological Control*, 38: 66-79. <https://doi.org/10.1016/j.biocontrol.2005.11.007>.

Li, Y.-M., Kai, Z.-P., Huang, J., Tobe, S.S., 2017. Lepidopteran HMG-CoA reductase is a potential selective target for pest control. *PeerJ*, 5:e2881. doi: 10.7717/peerj.2881

Liao, M., Xiao, J.J., Zhou, L.J., Yao, X., Tang, F., Hua, R.M., Wu, X.W., Cao, H.Q., 2017. Chemical composition, insecticidal and biochemical effects of *Melaleuca alternifolia* essential oil on the *Helicoverpa armigera*. *Journal of Applied Entomology*, 141: 721–728 . <https://doi.org/10.1111/jen.12397>

Liu, Y.Q., Fu, X.W., Feng, H.Q., Liu, Z.F., Wu, K.M., 2015. Trans-regional migration of *Agrotis ipsilon* (Lepidoptera: Noctuidae) in north-East Asia. *Annals of the Entomological Society of America*, 108: 519-527. <https://doi.org/10.1093/aesa/sav050>

Ma, J., Chen, S., Moens, M., De Clercq, P., Li, X., Han, R., 2013a. Characterization in biological traits of entomopathogenic nematodes isolated from North China. *Journal of Invertebrate Pathology*, 114: 268-276. doi: 10.1016/j.jip.2013.08.012.

Ma, J., Chen, S., Moens, M., Han, R., De Clercq, P., 2013b. Efficacy of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the chive gnat, *Bradysia odoriphaga*. *Journal of Pest Science*, 86: 551-561. <https://doi.org/10.1007/s10340-013-0497-7>

Mahmoud, M.F., 2016. Biology and use of entomopathogenic nematodes in insect pests biocontrol, a generic view. *Cercetări Agronomice în Moldova XLIX (4)(168)*: 85-105. DOI: 10.1515/cerce-2016-0039

Mahmoud, M.F., Mahfouz, H.M., Mohamed, K.M., 2016. Compatibility of entomopathogenic nematodes with neonicotinoids and azadirachtin insecticides for controlling the black cutworm, *Agrotis ipsilon* (Hufnagel) in Canola plants. *International Journal of Research in Environmental Science*, 2(1): 11-18.

Malan, A.P., Nguyen, K.B., Addison, M.K., 2006. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the southwestern parts of South Africa. *African Plant Protection*, 12(1): 65-69. <https://hdl.handle.net/10520/EJC87795>

Malan, A.P., Knoetze, R., Moore, S.D., 2011. Isolation and identification of entomopathogenic nematodes from citrus orchards in South Africa and their biocontrol potential against false codling moth. *Journal of Invertebrate Pathology*, 2011; 108: 115–25. <https://doi.org/10.1016/j.jip.2011.07.006>

Mantoo, M.A., Zaki, F.A., 2014. Biological control of cabbage butterfly, *Pieris brassicae* by a locally isolated entomopathogenic nematode, *Heterorhabditis bacteriophora* SKUAST-EPN-Hr-1 in Kashmir. *SKUAST Journal of Research*, 16: 66–70.

Manzoor, M., Ahmad, J.N., Sharif, M.Z., Majeed, D., Kiran, H., Jafir, M., Ali, H., 2017. Comparative effectiveness of entomopathogenic nematodes against red palm weevil (*Rhynchophorus ferrugineus*) in Pakistan. *Journal of Entomology and Zoology Studies*, 5(5): 756-760.

Martens, E.C.; Heungens, K. and Goodrich-Blair, H. (2003): Early colonization events in the mutualistic association between *Steinernema carpocapsae* nematodes and *Xenorhabdus nematophila* bacteria. *Journal of Bacteriology*, 185: 3147-3154.

Mastore, M., Arizza, V., Manachini, B., Brivio, M.F., 2015. Modulation of immune responses of *Rhynchophorus ferrugineus* (Insecta: Coleoptera) induced by the entomopathogenic nematode *Steinernema carpocapsae* (Nematoda: Rhabditida). *Insect Science*, 22: 748–760. Doi: 10.1111/1744-7917.12141

Mathasoliya J.B., Maghodia A.B., Vyas R.V., 2004. Efficacy of *Steinernema riobrave* against *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) on potato. *Indian Journal of Nematology*, 34(2): 177-179.

Mazurkiewicz, A., Tumialis, D., Jakubowska, M., 2020. Biological control potential of native entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) against *Mamestra brassicae* L. (Lepidoptera: Noctuidae). *Agriculture*, 10: 388, 9pp. doi: 10.3390/agriculture10090388

- Mehdi, M.Z., Wakil, W., Jan, H., Raza, M.M., Shah, Q., Zia-ul-Haq, M., 2018. Evaluation of entomopathogenic nematode and fungi alone and their combination against red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Journal of Entomology and Zoology Studies, 6(2): 2038-2042.
- Meka, M., Anita, B., Vetrivelkalai, P., Muthukrishnan, N., 2020. Infectivity of entomopathogenic nematode, *Steinernema glaseri* on fall army worm (FAW), *Spodoptera frugiperda* (Smith, 1797) in Maize (*Zea mays*). Journal of Entomology and Zoology Studies, 8(6): 1023-1028. <https://doi.org/10.22271/j.ento.2020.v8.i6n.7971>
- Minas, R.S., Souza, R.M., Dolinski, C., Carvalho, R.S., Burla, R.S., 2016. Potential of entomopathogenic nematodes (Rhabditida: Heterorhabditidae) to control Mediterranean fruit fly (Diptera: Tephritidae) soil stages. Nematoda 3, e02016. <https://doi.org/10.4322/nematoda.02016>.
- Mishra, V.K., 2020. Insect pests of cumin and their management. In "Management of Insect Pests in Vegetable Crops: Concepts and Approaches" (Vishwakarma, R. and Kumar, R. eds.), p. 73, 1st ed., 344pp.
- Mohamed, H.O., Shairra, S.A., 2023. Pathogenicity of entomopathogenic nematodes against the new invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 33:24, 10pp. <https://doi.org/10.1186/s41938-023-00669-0>
- Mokrini, F., Laasli, S.-E., Benseddik, Y., Joutei, A.B., Blenzar, A., Lakhal, H., Sbaghi, M., Imren, M., Özer, G., Paulitz, T., Lahlali, R., Dababat, A.A., 2020. Potential of Moroccan entomopathogenic nematodes for the control of the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). Scientific Reports, 10: 19204, 11pp. <https://doi.org/10.1038/s41598-020-76170-7>
- Moroney, M.J., 1956. Facts from figures. (3rd ed.). Penguin Books Ltd., Harmondsworth, Middlesex, 228 pp.
- Morris, O.N. Converse, V., Harding, J., 1990. Virulence of entomopathogenic nematode-bacteria complexes for larvae of noctuids, a geometrid, and a pyralid. Canadian Entomologist, 122(3-4): 309-319. DOI: <https://doi.org/10.4039/Ent122309-3>
- Mosleh, Y.Y., Paris-Palacios, S., Couderchet, M., Vernet, G., 2003. Acute and sublethal effects of two insecticides on earthworms (*Lumbricus terrestris* L.) under laboratory conditions. Environmental Toxicology, 18:1-8. doi: 10.1002/tox.10095.
- Moustafa, M.A.M., Awad, M., Amer, A., Hassan, N.N., Ibrahim, E.S., Ali, H.M., Akrami, M., Salem, M.Z.M., 2021. Insecticidal Activity of Lemongrass Essential Oil as an Eco-Friendly Agent against the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). Insects, 12, 737. <https://doi.org/10.3390/insects12080737>
- Muştu, M., Aktürk, M., Akkoyun, G., Çakır, S., 2021. Life tables of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Lepidoptera: Noctuidae) on different cultivated plants. Phytoparasitica, 49: 21-31. <https://doi.org/10.1007/s12600-020-00868-7>
- Navarez, M.L., Sangcopan, R., Aryal, S., Sumaya, N.P.D., Bhat, A.H., Sumaya, N.H., 2021. Native Philippine *Heterorhabditis indica* isolates from banana and rice fields and preliminary results of their virulence against the larvae of super worm (*Zophobas morio* Fabricius Coleoptera: Tenebrionidae). Egyptian Journal of Biological Pest Control, 31:46, 10pp. <https://doi.org/10.1186/s41938-021-00388-4>
- Nithiskarani, M., Anita, B., Vetrivelkalai, P., Nelson, S.J., 2019. Biocontrol potential of entomopathogenic Nematodes, *Steinernema glaseri* (Steiner, 1929) and *Heterorhabditis indica* (Poinar, Karunakar & David, 1992) against brinjal ash weevil (*Mylocerus subfasciatus*). Journal of Entomology and Zoology Studies, 7(3): 160-163.
- Nouh, G.M., 2021. Efficacy of the entomopathogenic nematode isolates against *Spodoptera littoralis* (Boisduval) and *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 31:34, 5pp. <https://doi.org/10.1186/s41938-021-00374-w>

Nouh, G.M., 2022. Effect of temperature and soil moisture on the efficacy of indigenous and imported strains of the entomopathogenic nematode, *Heterorhabditis* sp. against the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 32:28 <https://doi.org/10.1186/s41938-022-00507-9>

Odendaal, D., Addison, M.F., Malan, A.P., 2016. Control of diapausing codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), in wooden fruit bins using entomopathogenic nematodes. *Biocontrol Science and Technology*, 26: 1504-1515. DOI: <https://doi.org/10.4001/003.023.0224>

Peçen, A., Kepenekci, İ., 2022. Efficacy of entomopathogenic nematode isolates from Turkey against wheat stink bug, *Aelia rostrata* Boheman (Hemiptera: Pentatomidae) adults under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 32:91 <https://doi.org/10.1186/s41938-022-00590-y>

Pervez, R., Rao, U., 2020. Eco-friendly management of lepidopteran insect pests through entomopathogenic nematodes. *Journal of Biological Control*, 34(1): 66-72. DOI: 10.18311/jbc/2020/23012

Picimbon, J.-F., 2020. Interpopulational variations of odorant-binding protein expression in the black cutworm moth, *Agrotis ipsilon*. *Insects*, 11(11): 798. doi: 10.3390/insects11110798

Poinar, G.O., Grewal, P.S., 2012. History of entomopathogenic nematology. *Journal of Nematology*, 44(2):153-161.

Pratissoli, D., Damascena, A.P., Fragoso, D.F.M., de Carvalho, J.R., Túler, A.C., Faria, L.V., de Araujo Junior, L.M., 2021. Performance of Entomopathogenic nematodes on *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae). *Acta Ambiental Catarinense-Unochapecó*, 18(1): 205-215. Doi: <http://dx.doi.org/10.24021/raac.v18i1.5575>

Qiu, B.L., Mandour, N.S., Xu, C., Ren, S.X., 2008. Evaluation of entomopathogenic nematode *Steinernema feltiae* as biological control agent of white fly *Bemisia tabaci*. *International Journal of Pest Management*, 54(3): 247–253. <https://doi.org/10.1080/09670870802044228>

Rajkumar, M., Parihar, A., Siddiqui, A.U., 2003. Effect of entomopathogenic nematodes, *Heterorhabditis* sp. against *S. litura*. *Annals of Plant protection Sciences*, 11(2): 369-410.

Ren, M., Lu, J., Li, D., Yang, J., Zhang, Y., Dong, J., Niu, Y., Zhou, X., Zhang, X., 2023. Identification and functional characterization of two chitin synthases in the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, toac193. <https://doi.org/10.1093/jee/toac193>

Reyad, N.F., 2001. Abundance and control studies of *Spodoptera littoralis* (Boisd.) infecting certain vegetables. M.Sc. Thesis, Faculty of Science, Cairo University, Egypt.

Reyad, N.F., 2012. Comparative studies on the effect of four entomopathogenic nematodes on the protein profile in *Labidura riparia* (Pallas) (Dermaptera: Labiduridae). *Egyptian Academic Journal of Biological Sciences*, 5(1): 89-98. Doi: 10.21608/EAJBSA.2012.14912

Rodingpuia, C., Lalthanzara, H., 2021. An insight into black cutworm (*Agrotis ipsilon*): A glimpse on globally important crop pest. *Science Vision*, 2021(2): 36-42. <https://doi.org/10.33493/scivis.21.02.02>

Rohde, C., Mertz, N.R., Junior, A.M., 2020. Entomopathogenic nematodes on control of Mediterranean fruit fly (Diptera: Tephritidae). *Revista Caatinga*, 33(4):974–984. <https://doi.org/10.1590/1983-21252020v33n412rc>

Safdar, H., Javed, N., Khan, S.A., Arshad, M., 2018. Reproduction Potential of Entomopathogenic Nematodes on Armyworm (*Spodoptera litura*). *Pakistan Journal of Zoology*, 50(2): 771-774.

- Saleh, M.M.E., Draz, K.A.A., Mansour, M.A., Hussein, M.A., Zawrah, M.F.M., 2009. Controlling of sugar beet beetle *Cassida vittata* with entomopathogenic nematodes. *Journal of Pest Science*, 82(3): 289-294.
- Salem, S.A., Abdel Rahman, H.A., Zebitz, C.P.W., Saleh, M.M.E., Ali F.L., El-Kholy, M.Y., 2007. Evaluation of entomopathogenic nematodes in control ling some cabbage pests. *Journal of Applied Science Research*, 3(4): 323-328.
- Sankar, M., 2009. Investigation on an indigeneous entomopathogenic nematode *Heterorhabditis indica* as a potential biocontrol agent of insect pests in rice. Ph.D. Thesis, Osmania University, Hyderabad, pp. 77-82
- Sayed, R.M., Ibrahim, S.S., El-Gepaly, H.M.K.H., 2022. Susceptibility of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), larvae to un-irradiated and gamma-irradiated entomopathogenic nematodes. *Egyptian Journal of Biological Pest Control*, 32:119, 7pp. [https://doi.org/ 10.1186/s41938-022-00619-2](https://doi.org/10.1186/s41938-022-00619-2)
- Seal, D.R., Jha, V.K., Liu, T.X., 2010. Potential of various strains of entomopathogenic nematodes in combination with insecticides for suppression of black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae). *Annals of Plant Protection Science*, 18(2): 293-300.
- Shahzad, M., Qu, Y., Zafar, A., Ur Rehman, S., Islam, T., 2020. Exploring the influence of knowledge management process on corporate sustainable performance through green innovation. *Journal of Knowledge Management*, 24(9): 2079-2106. DOI:10.1108/JKM-11-2019-0624
- Shairra, S.A., 2000. Studies on the effects of some entomopathogenic nematode isolates on different host species. M.Sc. Thesis, Fac. Sci., Cairo Univ., Egypt, 108 pp.
- Shairra, S.A., 2007. Effects of entomopathogenic nematodes and some pharmaceutical inhibitors of eicosanoid biosynthesis on the desert locust *Schistocerca gregaria* (Forsk.) (Lepidoptera: Noctuidae). Ph.D. Thesis, Fac.Sci., Cairo Univ., Egypt, 65 pp.
- Shairra, S.A., 2009. Parasitism of locust by entomopathogenic nematode in relation to insect microaggregation inhibitor. *Egyptian Academic Journal of Biological Sciences (A. Entomology)* 2 (2): 221- 230. Doi:10.21608/EAJBSA.2009.15445
- Shairra, S.A., Noah, G.M., 2014. Efficacy of Entomopathogenic nematodes and fungi as biological control agents against the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera; Noctuidae). *Egyptian Journal of Biological Pest Control*, 24(1): 247-253.
- Shairra, S.A., El-Sharkawy, M.A.A., Hassan, K.A., Ahmed, D.A., 2016. The efficacy of entomopathogenic nematodes on the pink bollworm, *Pectinophora gossypiella*. *Egyptian Academic Journal of Biological Sciences (F. Toxicology & Pest control)*, 8(2): 103-113. Doi: 10.21608/EAJBSF.2016.17123
- Shamseldean, M.M., Ibrahim, A.A., Zohdi, N.M., Shairra, S.A., Ayaad, T.H., 2008. Effect of the Egyptian entomopathogenic nematode isolates on controlling some economic insect pests. *Egyptian Journal of Biological Pest Control*, 18(1): 81-89. DOI: 10.13140/2.1.5146.8805
- Shannag, H.K., Webb, S.E., Capinera, J.L., 1994. Entomopathogenic nematode effect on pickleworm (Lepidoptera: Pyralidae) under laboratory and field conditions. *Journal of Economic Entomology*, 87:1205-1212. <https://doi.org/10.1093/jee/87.5.1205>
- Shapiro, D.I., Lewis, L.C., Obrycki, J.J., Abbas, M., 1999. Effects of fertilizers on suppression of black cutworm (*Agrotis ipsilon*) damage with *Steinernema carpocapsae*. *Journal of Nematology*, 31(4, Supplement): 690-693.
- Shapiro-Ilan D.I., Cottrell T.E., 2006. Susceptibility of the lesser peach tree borer (Lepidoptera: Sesiidae) to entomopathogenic nematodes under laboratory conditions. *Environmental Entomology*, 35: 358-365. <https://doi.org/10.1603/0046-225X-35.2.358>

Shapiro-Ilan, D.I., Brown, I., 2013. Earthworms as phoretic hosts for *Steinernema carpocapsae* and *Beauveria bassiana*: Implications for enhanced biological control. *Biological Control*, 66(1): 41–48. <https://doi.org/10.1016/j.biocontrol.2013.03.005>

Shapiro-Ilan, D.I., Stuart, R.J., McCoy, C.W., 2005. Characterization of biological control traits in the entomopathogenic nematode *Heterorhabditis mexicana* (MX4 strain). *Biological Control* 32(1): 97-103. <https://doi.org/10.1016/j.biocontrol.2004.08.004>

Shapiro-Ilan, D.I., Mbata, G.N., Nguyen, K.B., Peat, S.M., Blackburn, D., Adams, B.J., 2009. Characterization of biocontrol traits in the entomopathogenic nematode *Heterorhabditis georgiana* (Kesha strain), and phylogenetic analysis of the nematode's symbiotic bacteria. *Biological Control*, 51: 377-387. <https://doi.org/10.1016/j.biocontrol.2009.07.009>

Shapiro-Ilan, D.I., Hazir, S., Glazer, I., 2017. Basic and applied research: entomopathogenic nematodes. In: "Microbial control of insect and mite pests: from theory to practice"(Lacey, L.A., ed.). Amsterdam (The Netherlands; Boston, MA, USA): Academic Press, pp: 91-105.

Shapiro-Ilan, D.I., Hiltbold, I., Lewis, E.E., 2018. In: "Ecology of Invertebrate Diseases"(Hajek, A.E. and Shapiro-Ilan, D.I., eds.) 415–440 John Wiley & Sons.

Shaurub, E.H., 2023. Review of entomopathogenic fungi and nematodes as biological control agents of tephritid fruit flies: current status and a future vision. *Entomologia Experimentalis et Applicata*, 171:17–34. DOI: 10.1111/eea.13244

Shaurub, E.H., Soliman, N.A., Hashem, A.G., Abdel-Rahman, A.M., 2015. Infectivity of four entomopathogenic nematodes in relation to environmental factors and their effects on the biochemistry of the Medfly *Ceratitis capitata* (Wied.) (Diptera: Tephritidae). *Neotropical Entomology*, 44: 610-618. <https://doi.org/10.1007/s13744-015-0332-3>.

Shaurub, E.H., Soliman, N.A., Sabbour, M.M., 2021. Development of the soil-inhabiting stages of *Ceratitis capitata* infected with entomopathogenic nematodes: insights on survival. *Invertebrate Reproduction & Development*, 65(3): 155-162, Doi:10.1080/07924259.2021.1920482

Shoeb, M.A., Atalla, F.A., Matar, A.M., 2006. Pathogenicity of the Entomopathogenic nematodes; *Steinernema abbasi* and *Heterorhabditis bacteriophora* to certain economic insect pests. *Egyptian Journal of Biological Pest Control*, 16(1/2): 99-102. <https://doi.org/10.1186/s41938-022-00566-y>

Sirjani, F.O., Lewis, E.E., Kaya, H.K., 2009. Evaluation of Entomopathogenic nematodes against the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). *Biological Control*, 48: 274–280. <https://doi.org/10.1016/j.biocontrol.2008.11.002>.

Sobhy, H.M., Abdel-Bary, N.A., Harras, F.A., Faragalla, F.H., Hussein, H.I., 2020. Efficacy of entomopathogenic nematodes against *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (H.) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 30: 73, 8pp. <https://doi.org/10.1186/s41938-020-00265-6>

Solomon, A., Paperna, I., Glazer, I., 1999. Desiccation survival of the entomopathogenic nematode *Steinernema feltiae*: induction of anhydrobiosis. *Nematology*, 1: 61-68. doi:10.1163/156854199507983.

Somvanshi, V.S., Ganguly, S., Paul, A.N., 2006. Field efficacy of the entomopathogenic nematode *Steinernema thermophilum* Ganguly and Singh (Rhabditida: Steinernematidae) against diamondback moth (*Plutella xylostella* L.) infesting cabbage. *Biological Control*, 37(1): 9-15. <https://doi.org/10.1016/j.biocontrol.2005.11.017>

Srivastava, S., Heena, Chaubey, A.K., 2022. Studies on biological control potential of *Steinernema abbasi* CS-39 against *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae) by adopting the regression probit model. *Egyptian Journal of Biological Pest Control*, 32:9 <https://doi.org/10.1186/s41938-022-00510-0>

- Steyn, V.M., Malan, A.P., Addison, P., 2021. Efficacy of entomopathogens against *Thaumatotibia leucotreta* under laboratory conditions. *Entomologia Experimentalis et Applicata*, 169: 449–461. <https://doi.org/10.1111/eea.13044>
- Stock, S.P., Mráček, Z., Webster, J.M., 2000. Morphological variation between allopatric populations of *Steinernema kraussei* (Steiner, 1993) (Rhabditida: Steinernematidae). *Nematology*, 2:143–152. doi:10.1163/156854100509033
- Sujatha, P.C.K., Jeyasankar, A., 2018. Entomopathogenic nematode as a biocontrol agent- recent trends - a review. *International Journal of Entomology and Nematology Research*, 2(1): 10-24.
- Sujithra, M., Rajkumar, M., Mhatre, P.H., Guru-Pirasanna-Pandi, G., 2022. Biocontrol potential of native entomopathogenic nematodes against coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 32:88 <https://doi.org/10.1186/s41938-022-00587-7>
- Taha, E.H., 2021. Influence of Different Species of *Steinernema* and *Heterorhabditis* on *Galleria mellonella* and *Spodoptera littoralis*. *Egyptian Journal of Agronomatology*, 20(2): 128- 139.
- Takeda, M., 2008. Current research of pest insects of vegetables in last decade. *Annual Report of the Kansai Plant Protection*, 50: 39-44.
- Thakur, N., Tomar, P., Kaur, S., Kumari, P., 2022. Virulence of native entomopathogenic nematodes against major lepidopteran insect species of tomato (*Solanum lycopersicum* L.). *Journal of Applied Biology & Biotechnology*, 10 (Suppl 1): 10-16. Doi:10.7324/JABB. 2022.10s102
- Thanwisai, A., Muangpat, P., Meesil, W., Janthu, P., Dumidae, A., Subkrasae, C., Ardpairin, J., Tandhavanant, S., Yoshino, T.P., Vitta, A., 2022. Entomopathogenic Nematodes and Their Symbiotic Bacteria from the National Parks of Thailand and Larvicidal Property of Symbiotic Bacteria against *Aedes aegypti* and *Culex quinquefasciatus*. *Biology*, 11, 1658, 19pp. <https://doi.org/10.3390/biology11111658>
- Tiryaki, D., Temur, C., 2010. The fate of pesticide in the environment. *Journal of Biological and Environmental Sciences*, 4(10): 29-32.
- Tomar, P., Thakur, N., Sharma, A., 2022. Infectivity of entomopathogenic nematode against the cabbage butterfly (*Pieris brassicae* L.) in polyhouse and in field condition. *Egyptian Journal of Biological Pest Control*, 32:38 <https://doi.org/10.1186/s41938-022-00535-5>
- Toubarro, D., Lucena-Robles, M., Nascimento, G., Costa, G., Montiel, R., Coelho, A.V., Simões, N., 2009. An apoptosis-inducing serine protease secreted by the entomopathogenic nematode *Steinernema carpocapsae*. *International Journal of Parasitology*, 39:1319-1330. <https://doi.org/10.1016/j.ijpara.2009.04.013>
- Toubarro, D., Martinez, Avila, M., Montiel, R., Simoes, N., 2013. A pathogenic nematode targets recognition proteins to avoid insect defenses. *PLoS ONE*, 8(9): e75691. doi: 10.1371/journal.pone.0075691
- Trdan, S., Vidrih, M., Andjus, L., Laznik, Ž., 2009. Activity of four entomopathogenic nematode species against different developmental stages of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). *Helminthologia*, 46: 14–20.
- Ulu, T.C., Sadic, B., Susurluk, I.A., Aksit, T., 2015. Virulence of four entomopathogenic nematode species for plum sawfly, *Hoplocampa flava* L. (Hymenoptera: Tenthredinidae). *Invertebrate Survival Journal*, 12: 274-277.
- Vashisth, S., Chandel, Y.S., Sharma, P.K., 2013. Entomopathogenic nematodes - a review. *Agricultural Reviews*, 34(3):163-175. DOI: 10.5958/j.0976-0741.34.3.001

Vashisth, S., Chandel, Y.S., Chandel, R.S., 2019. Comparative efficacy of indigenous heterorhabditid nematodes from north western Himalaya and *Heterorhabditis indica* (Poinar, Karunakar & David) against the larvae of *Helicoverpa armigera* (Hubner), *International Journal of Pest Management*, 65(1): 16-22. Doi:10.1080/09670874.2018.1453099

Vattikonda S.R., Sangam S.R., 2017. Effect of forskolin on the growth and differentiation of the ovary of *Papilio demoleus* L. (Lepidoptera: Papilionidae). *International Research Journal of Environmental Science*, 6: 13-17.

Veres, A., Wyckhuys, K.A.G., Kiss, J., Tóth, F., Burgio, G., Pons, X., Avilla, C., Vidal, S., Razingar, J., Bazok, R., 2020. An update of the Worldwide Integrated Assessment (WIA) on systemic pesticides. Part 4: Alternatives in major cropping systems. *Environmental Science and Pollution Research International*, 27: 29867-29899. <https://doi.org/10.1007/s11356-020-09279-x>

Vicente-Díez, I., Blanco-Pérez, R., Chelkha, M., Puellas, M., Pou, A., Campos-Herrera, R., 2021. Exploring the use of entomopathogenic nematodes and the natural products derived from their symbiotic bacteria to control the grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae). *Insects*, 12, 1033, 14 pp, <https://doi.org/10.3390/insects12111033>

Walkowiak, K., Spochacz, M., Rosinski, G., 2015. Peptidomimetics - A new class of bioinsecticides. *Postepy Biologii Komorki*, 42(2): 235-254.

Wattanachaiyingcharoen, W., Lepcha, O., Vitta, A., Wattanachaiyingcharoen, D., 2021. Efficacy of Thai indigenous entomopathogenic nematodes for controlling fall armyworm (*Spodoptera frugiperda*) (J. E. Smith)(Lepidoptera; Noctuidae). *Egyptian Journal of Biological Pest Control*, 31:149, 7pp. <https://doi.org/10.1186/s41938-021-00497-0>

Yadav, S., Patil, J., Sharma, H.K., 2017. Bio-efficacy of *Steinernema carpocapsae* against *Spodoptera litura* under Laboratory Condition, *International Journal of Pure and Applied Biosciences*, 5(2): 165-172. doi: <http://dx.doi.org/10.18782/2320-7051.2864>

Yağcı, M., Fırat, T.A., Erdoğan, F.D., Şahin, M., 2021. Virulence of four entomopathogenic nematode against different stages of the Mediterranean fruit fly, *Ceratitidis capitata* Wiedemann (Diptera: Tephritidae). *Egyptian Journal of Biological Pest Control*, 31:126, 5pp. <https://doi.org/10.1186/s41938-021-00472-9>

Yağcı, M., Yücel, C., Erdoğan, F.D., Benk, G., Kepenekci, İ., 2022. Biological control potential of local entomopathogenic nematodes against the different stage larvae of cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 32:59 <https://doi.org/10.1186/s41938-022-00558-y>

Yaman, M., Erturk, O., 2016. Isolation, identification and insecticidal effects of entomopathogenic bacteria from the willow flea beetle, *Crepidodera aurata* (Coleoptera; Chrysomelidae). *Progress in Plant Protection*, 56(2): 225-229.

Yan, X., Chen, G., Chen, Y., Sun, B., Gu, X., Ruan, W., Han, R., 2021. Virulence of *Steinernema ceratophorum* against different pest insects and their potential for *in vivo* and *in vitro* culture. *Journal of Nematology*, 53: 12pp. DOI: 10.21307/jofnem-2021-046

Youssef, N.A., 2014. Effect of certain entomopathogenic fungi and nematode on the desert locust *Schistocerca gregaria* (Forsk.). *Annals of Agricultural Science, Faculty of Agriculture, Ain Shams University, Egypt*, 59(1): 125-131.

Yu, W., Du, J., Hu, Y., Shen, R., Mu, W., 2012. Toxicity of six insecticides to black cutworm *Agrotis ypsilon* (Rottemberg) and safety evaluation to oil organisms. *Acta phytophylacica sinica*, 39: 277-282.

Yuksel, E., Canhilal, R., 2018. Evaluation of local isolates of entomopathogenic nematodes for the management of black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 28: 82, 7pp. <https://doi.org/10.1186/s41938-018-0087-3>

Yüksel, E., Imren, M., Özdemir, E., Bozbuğa, R., Canhilal, R., 2022. Insecticidal effect of entomopathogenic nematodes and the cell-free supernatants from their symbiotic bacteria against different larval instars of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 32:54 <https://doi.org/10.1186/s41938-022-00555-1>

Zhang, Q., Liu, Y.Q., Wyckhuys, K.A.G., Liang, H.S., Desneux, N., Lu, Y.H., .2021. Lethal and sublethal effects of chlorantraniliprole on *Helicoverpa armigera* adults enhance the potential for use in 'attract-and-kill' control strategies. Entomologia Generalis, 41:111–120. DOI: 10.1127/entomologia/2020/1104